Can lame cows be detected with automatic milking systems data?

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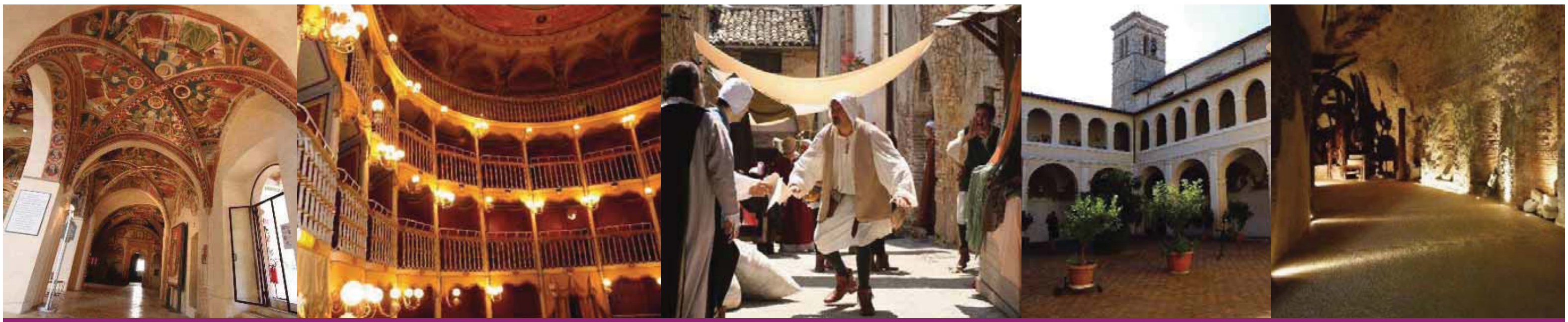

June 30th - July 4th, 2013 Bevagna, Italy

VIII Colloquium Chemometricum Mediterraneum


...dubito, ergo indago...

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Data transformation methods
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Beata Walczak

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Preprocessing of instrumental signals is a crucial step of data analysis. Until now, it is, however, its main bottleneck also. The preprocessing step usually involves preprocessing of individual signals (signals enhancement via signals de-nosing and background elimination), as well as preprocessing of the signal set (signal transformations, signals alignment, etc.). There are no strict rules concerning data preprocessing, because the choice of the methods is data dependent. Problems associated with data preprocessing will be demonstrated, based on the SIMS signals from biological material. What is typical of the SIMS signals, it is their huge dynamic range and a fact that the raw SIMS data follows the Poisson counting statistics. Different types of signal transformations can be used at different stages of data preprocessing to deal with data specificity. The influence of different data transformations on the data correlation structure and on the biomarkers identification is going to be demonstrated for the simulated data sets as well. The main accent will be laid on the data normalization methods, applied for the removal of the so-called size effect. Additionally, a comparison of data analysis based on the peak table with that based on the entire fingerprints, taking into the account final identification of significant features, will be made (the term ‘features’ is used for both, peaks and the original variables). A comparison of these two approaches is performed for simulated data, because only in this case, all necessary details about sample components and significant features are known.

APPLICATIONS OF CHEMOMETRICS IN DRUG DESIGN

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In the past, drug design has been considered as an inventive process mostly relying on trial-and-error and serendipity paradigma. However, the accumulated evidence of the flop in preclinical or clinical trials of promising candidate drugs and the need to minimize the cost-to-market prompted the advent of chemometrics-based strategies enhancing the potential of rational drug design by accelerating the drug pipeline progression. In this respect, the authors will emphasize why and how chemometrics comes into play and in particular will focus on the role of non-deterministic and multi-objective optimization techniques as strategies with a real-life impact on the derivation of QSAR as well as ligand- and structure-based models.

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ANALYSIS OF CHEMICAL DATA FROM A COMPOSITIONAL POINT OF VIEW

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Many practical data sets in chemistry and chemometrics are in fact compositional data because only the ratios between the variables are informative. An example are concentrations of chemical elements, measured e.g. in mg/kg: an increase of the concentration in one component has an effect on the other components, since the overall sum is constrained.

Compositional data are represented in the Aitchison geometry on the simplex, and for applying statistical methods designed for the Euclidean geometry they need to be transformed first. The isometric logratio (ilr) transformation has the best geometrical properties, but usually the results are difficult to interpret because the ilr coordinates are formed by non-linear combinations of the original variables. We show for different multivariate statistical methods how the ilr transformation can be sucessfully used for interpretation.

Based on real data examples we compare results from a standard approach and from a compositional data approach.

DATA FUSION IN FOOD AUTHENTICATION

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The safety and quality of food commodities are of major concern for consumers, who increasingly demand reliable and high-quality products. This increasing interest for quality has driven the attention towards the techniques used for authentication of food and beverage products. In recent years, rapid and reliable sensory, spectroscopic and chromatographic techniques have been developed which, together with chemometric analysis, have improved the quality control process by reducing the time of analysis and providing higher quality results. In this progression of more and better information, the next step is to combine the outputs of different multivariate instrumental techniques to increase the reliability of a classification or prediction regarding a single analytical technique. From the literature review, the combination (fusion) of data from several techniques with the help of chemometrics appears to be promising for the authentication of food and beverage products. However, data fusion is not exempt from problems and limitations, which in turn pose a challenge for chemometricians. The objective of this presentation is to provide a general overview of the different data fusion techniques and strategies, focusing on the critical points. Issues such as data preprocessing, scaling, normalization, variable selection and outlier detection will be covered and applications from the field of food and beverage authentication will be critically discussed.

IMPROVING SPATIAL RESOLUTION OF HYPERSPECTRAL IMAGES: THE SUPER-RESOLUTION CONCEPT.

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Hyperspectral imaging has become a major tool for the characterization of complex samples. Although molecular characterization at micron level is achieved for many applications, it usually fails producing chemical images of micron size samples as expected in chemical, environmental and biological analysis.

The main objective of the presented work is to introduce the potential of the super-resolution concept in vibrational spectroscopic imaging [1]. This original chemometrics approach uses several low resolution images of the same sample (observed from different point of views) in order to generate a higher resolution chemical image. It is thus possible to overcome in a certain way some physical and instrumentals limitations.

We will illustrate the concept with different results in Raman, Near-Infrared and Mid-infrared imaging [2-5].

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SPECTROSCOPY COUPLED TO CHEMOMETRICS FOR VINEYARDS AND GRAPES QUALITY INTENSIVE MAPPING

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Production of high quality wines requires a permanent monitoring of the entire winemaking process. Grapes composition is the result of the "terroir", which is utmostly influenced by the soil composition and climatic conditions [1]. Soil impact on grapes quality and ripening processes is well known. However. Current strategy for analyzing soils (pedology) is based on wet chemistry methods, which are often laborious, expensive, time-consuming and of limited use during harvesting campaigns. But, is it possible to use efficiently an high-throughput analytical method for estimating the impact of soil quality, tillage and thinning on the grapes quality? Near infrared spectroscopy (NIRS) proved to be the most efficient tool for direct in-situ analysis of soils, leaves and grapes [2,3]. In this context, multivariate or chemometric analysis of the near-infrared signal is of upmost importance and multiple approaches are required depending on the specific target [4]. This communication presents results obtained from directly monitoring vineyards in four Portuguese locations (wine appellation regions "Alentejo", "Dão", "Douro" and "Vinhos Verdes") using a FieldSpec 4 portable near-infrared spectrometer (ASD, Boulder, CO). Soil characterized vineyards were monitored according to designed sampling grids accounting for the specifities of soils and varieties. Measurements of soils (at different dephts), plant leaves and berries were performed on different stages of the ripening period. Spectral analysis was performed with chemometric methods, essentially data-mining and regression (classification) methods. Strategies to ensure models robustness and statistical significance were particularly accounted. This monitoring approach revealed to be an excellent tool to support the process of vineyards micro-zoning [5,6]. It is also excellent as a quick methodology to calibrate hyperspectral satellite images increasing substantially their usefulness. Globally, the major and ultimate deliverable of this approach is a cost-effective NIRS based technology for winemakers in order to add product value, by increasing the efficiency of vineyards mapping, not only in terms of soil characterization, but also on soil/climate impact on grapes quality and ripening processes.

Acknowledgments

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CAN LAME COWS BE DETECTED WITH AUTOMATIC MILKING SYSTEM DATA?

RECONSIDERING THE PRE-PROCESSING PROCESS

J. Engel, J. Gerretzen, L.M.C. Buydens

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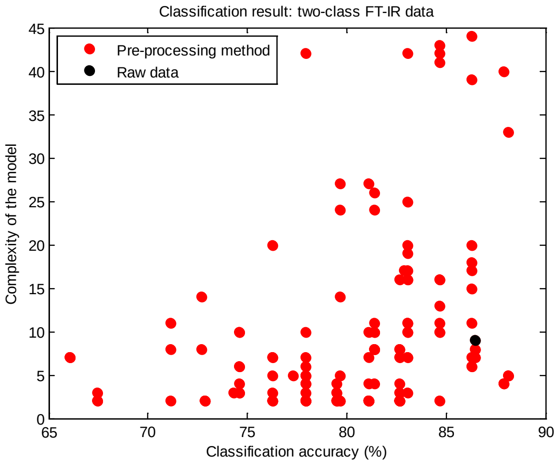
Many research areas are confronted with an ever increasing amount of measurement data, often originating from multiple advanced analytical technologies. Data analysis is therefore crucial to many different fields. Pre-processing – removal of data artifacts and making the data suitable for (multivariate) data analysis – is recognized as a pivotal step in the full data analysis chain by chemometricians. Choosing the right pre-processing technique for a particular problem is currently based on trial and error, visual inspection or by data quality parameters. We will shake these pre-processing fundamentals by showing that all three approaches easily lead to wrong choices.



As an example, consider the figure on the right, that shows the effect of different pre-processing techniques on FT-IR data for a two-class problem. Classifying the raw data (i.e. *no* pre-processing) already leads to 86% correct classification – see the black dot. Many pre-processing methods clearly worsen the classification accuracy (by up to 20%) or increase the complexity of the model. The choice of a pre-processing technique thus has a very large influence on the outcome of the data analysis. In this case, it might even be advisable to not pre-process at all, which is completely contradictory to current practices. All current approaches on selecting the optimal pre-processing technique for a particular problem have major difficulties in doing so, as we will extensively show in this lecture

APPLICATION OF NEAR INFRARED AND PLS-DA TO CLASSIFY ALMONDS WITH RESPECT TO THEIR BITTERNESS

Eva Borràs[a], José Manuel Amigo[b], Frans van den Berg[b], Ricard Boqué[a], Olga Busto[a]

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The almond (*Prunus amygdalus*) is a member of the family *Rosaceae* and the fruit is classified as a drupe. There are two different species as function of the kernel bitterness: the bitter and the non-bitter/sweet almonds. The bitterness of the almond is a consequence of the presence of cyanogenic glucosides, such as amygdalin and prunasin, concentrated in the kernel. When the seed tissue is damaged an enzymatic hydrolysis occurs that produces benzaldehyde (that confers the bitter flavor), sugars and hydrogen cyanide (HCN), which is potentially toxic. Since amygdalin is not found in sweet almonds its detection might be a specific indicator of bitterness in the almonds. This work shows a simple, fast, non-destructive and robust methodology to discriminate bitter and sweet almonds by the *in situ* measurement of the Near Infrared (NIR) spectra of the kernel surface without any sample pretreatment. NIR spectra are formed by broad absorption bands and, therefore, can be difficult to interpret directly. For this reason their analysis is inherently linked to the use of multivariate data analysis methods. In this case principal component analysis (PCA) and partial least-squares discriminant analysis (PLS-DA) models have been performed to develop a reliable method to separate bitter from sweet kernels. Different sets of both sweet and bitter almonds were measured in different days. After pre-processing of the spectra (standard normal variate scaling and mean centering), the calibration model was developed using the almonds measured on the first day by leave-one-out cross validation with good results (Fig.1). Using this calibration model, the other measurement sets (hence, almonds measured on different days) were used as external prediction set. No significant differences between days were observed indicating a robust calibration/prediction model (results not shown). Figure 2 shows the high levels of sensitivity and specificity obtained for both classes, with over 95% of the samples correctly classified, as well as low classification errors. Raman spectroscopy was used as reference technique to validate and confirm the results obtained by NIR. Using a number of selective bands in the Raman spectra and working with PLS-DA classification, we obtained the same classification results as for the NIR analysis (results not shown).

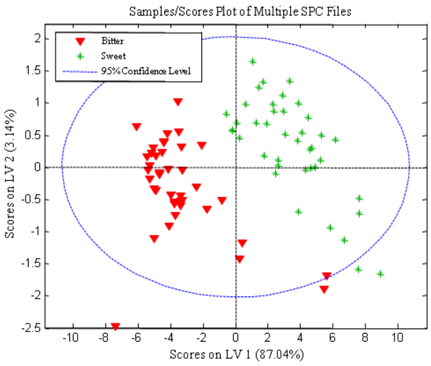
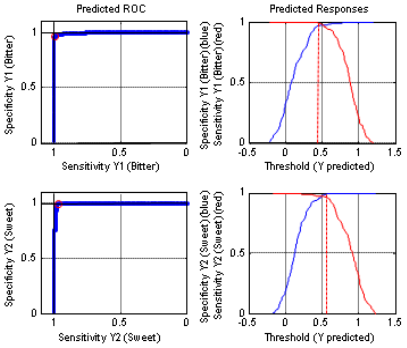


Figure 1. Scores plot of the PLS-DA model for the NIR spectra

Statistics for each y-block column:

Modeled Class:	Bitter	Sweet
Sensitivity (Cal):	0.950	1.000
Specificity (Cal):	1.000	0.950
Sensitivity (CV):	0.950	1.000
Specificity (CV):	1.000	0.950
Sensitivity (Pred):	0.992	0.967
Specificity (Pred):	0.967	0.992
Class. Err (Cal):	0.025	0.025
Class. Err (CV):	0.025	0.025
Class. Err (Pred):	0.021	0.021



Confusion Matrix:

Class:	TP	FP	TN	FN
CV:				
Bitter	0.950	0.000	1.000	0.050
Sweet	1.000	0.050	0.950	0.000
Prediction:				
Bitter	0.992	0.033	0.967	0.008
Sweet	0.967	0.008	0.992	0.033

Confusion Table:

	Actual Class	
	Bitter	**Sweet**
CV:		
Predicted as Bitter	38	0
Predicted as Sweet	2	40
Prediction:		
Predicted as Bitter	117	4
Predicted as Sweet	1	117

Figure 2. PLS-DA model results. Top-left, figures of merit. Top-right, predicted ROC curves. Bottom-left, confusion table for cross-validation and prediction. Bottom-right, confusion matrix for cross-validation and prediction.

As main conclusion we present success in the development of a fast, non-destructive and robust methodology to separate between sweet and bitter almonds by combining NIR and PLS-DA. This new procedure could be used as an automated and simple method in industry, suitable to be implemented for quality assurance and control of raw material or final product, i.e. final packaged almonds or almonds intended for baking or other secondary products.

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UNTARGETED VERSUS TARGETED MODELING IN A FOOD ADULTERATION PROBLEM

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Spectroscopic techniques together with chemometrics have turned into a powerful tool for food authenticity, quality and adulteration studies [1, 2]. In the recent past, food fraud has become more sophisticated due to the use of unconventional or synthetic adulterants. So, the every-increasing range of analytes which may be used in fraud together with the impossibility of cover them all makes difficult to model all possible adulterants.

The most common classifiers are based on discriminant or modeling ability. At least two or more classes must be defined in the first group whereas the second group can be used in problems where there is only one class of interest [3]. Despite the potential of class-modeling techniques, their use is not extended for one-class modeling approach.

The aim of this study is to investigate the feasibility of NIR spectroscopy coupled with class modeling to determine adulteration considering, as a case study, hazelnut pastes adulteration. In this work, we have taken into account two different adulterants; (1) a similar product such as almond paste and (2) an unexpected one as chickpea flour.

We have developed and compared two approaches, summarized in Fig. 1. The first one was based on modeling only the unadulterated class. When the adulterated samples were predicted in that model 3.6% of samples was wrongly classified in the unadulterated class whereas the rest was considered as ‘not in the model’.

The second approach considered the modeling of the adulterant class as well as the unadulterated one. The samples used to build the model were the ones adulterated with almond paste whereas the rest of samples adulterated with chickpea flour were used in predictions. The results show that the modeled adulterated samples were mainly classified in their own class (90.9%). On the other hand, samples with chickpea were mainly predicted in the adulterated class or in ‘not in any model’, 67.3% and 30.9%, respectively.

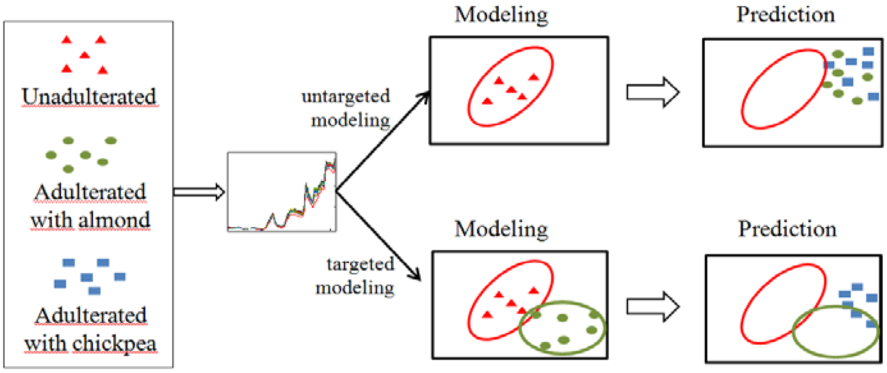

Fig. 1 Schematic summary of the two approaches used in this study.

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GEOGRAPHICAL TRACEABILITY AND AUTHENTICITY OF EXTRA VIRGIN OLIVE OIL BY CHEMOMETRIC TECHNIQUES AND CHROMATOGRAPHIC FINGERPRINT

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In recent years, special attention was posed on the issues related to food quality and safety. In this context, the identification and traceability of foods play a key role, as a defense, both for the producers and the consumers. Indeed, at present, only label and production records guarantee the effective traceability of foodstuff. Therefore, the necessity to develop new analytical methods that allow, a posteriori, to define the correct geographical origin, emerged. PDO Extra virgin olive oil is one of the foods made in Italy with the highest added value, because it is related to a delimited area of production. However, it is too often subjected to frauds and imitation that are difficult to detect, especially if control relies simply on reading the label. The aim of this work was therefore to develop a new analytical method that could allow the identification of PDO extra virgin olive oil, and in particular, which could allow to discriminate the Sabina PDO from other olive oil, extra virgin or not. To achieve this objective, a complex chemical and chemometric analysis were carried out. In fact, univariate analysis of some parameters, like the free acidity, peroxide number and the total content of polyphenols, is restrictive and inadequate, allowing only the distinction between different kinds of vegetable oils. On the other hand, studies in the literature suggest that the quality and quantity of polyphenols present in olive oils and multivariate analysis can be valid instruments for the geographical classification of the product. At first, through an experimental design protocol, the extraction of polyphenolic components was optimized in terms of recovery, time and cost. The identification of thecompounds was performed through the use of mass spectrometry while multivariate analysis was conducted on the entire chromatogram of the phenolic fraction, recorded at 254, 280 and 340nm. The chromatographic profile of each sample was considered as a fingerprint of olive oil and with the application of chemometric methods, it was possible to extract useful chemical information for the classification and determination of the geographical origin.

Before applying classification methods, it was necessary to pretreat the chromatographic data to eliminate the variability due to variations of the baseline and the shift of the retention times of the analytes. For the correction of the baseline, the algorithm "Penalyzed Asymmetric Least Squares"[1] was used. After correcting the baseline, it was necessary to pretreat further chromatographic signals to ensure that the peaks of the analytes were aligned. Operatively, the alignment of the chromatograms was performed using iCoshift algorithm, which divides the chromatograms into sever parts and for each part identifies the best alignment [2]. The chromatographic profiles of extra virgin olive oils extracts (27 Sabina PDO and 50 other origins) after being "pretreated", have been used as data for the construction of the classification model. Specifically, the method applied for discriminant classification was Partial Least Squares Discriminant Analysis (PLS-DA) [3]. The predictive capability of a multivariate classification model can be affected by the presence of a large number of variables, in our case, not all the points that constitute the chromatographic profile carry discriminant information, and a selection of portion of the chromatogram was necessary. For this purpose, the technique Backwards Interval PLS (Bi-PLS) coupled to a procedure based on Genetic Algorithms (GA) [4] was used. Once calibrated, the classification model (PLS-DA after Bi-PLS-GA) has been validated, and tested for its predictive capacity on external extra virgin olive oil samples and 90% of these were correctly classified. Of 27 samples of extra virgin olive oil (Sabina PDO and not) 24 were classified in the appropriate class of perfectly.

In conclusion, the analytical-method developed, being based on the chemometric processing of the results of chemical analysis on the finished product, doesn’t rely on label and can allow detecting imitations and falsifications of Sabina PDO. Furthermore, it was demonstrated that the chromatographic fingerprint of the phenolic fraction of extra virgin olive oil may be a possible indicator of product traceability. In the future,models of traceability, similar to this, can be built for other extra virgin olive oil with the appellation of origin to revealing fraud.

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NIR SPECTROSCOPY AS A TOOL FOR VINEYARDS SOIL CHARACTERIZATION

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The wine industry has always been particularly interested in the influence of the *terroir* characteristics on the features of a wine, but over the past few years a growing interest has spurred on the mechanisms by which a particular soil influences: growth of the vineyard, grape variety characteristics and ultimately wine quality [1]. Near-infrared Spectroscopy (NIRS) is a rapid, non-destructive, inexpensive and accurate analysis technique and its use in soil evaluation as a fast method for discriminating different types of soil and also for determining different soil constituents is rapidly increasing [2]. In this work, a total of 14 sampling points with different soil compositions, previously characterised by geological reference methods, were chosen from a vineyard in the Dão Delimited Region, centre of Portugal. Wet and dried (Fig. 1) samples were scanned on a Fourier Transform Near-infrared Spectrometer (FTLA 2000, ABB, Québec, Canada) in diffuse reflectance mode. The spectra were further processed through chemometric tools, namely Principal Component Analysis (PCA) and Partial Least Squares Discriminant Analysis (PLS-DA). Preliminary results, through PLS-DA, indicate that this method is able to discriminate between the different soil types collected with an accuracy of 85% and 90% for wet and dry samples respectively. The objective of this work is to realise if NIRS technology is suitable to correlate, complement and eventually replace costly, time-consuming geological reference methods.

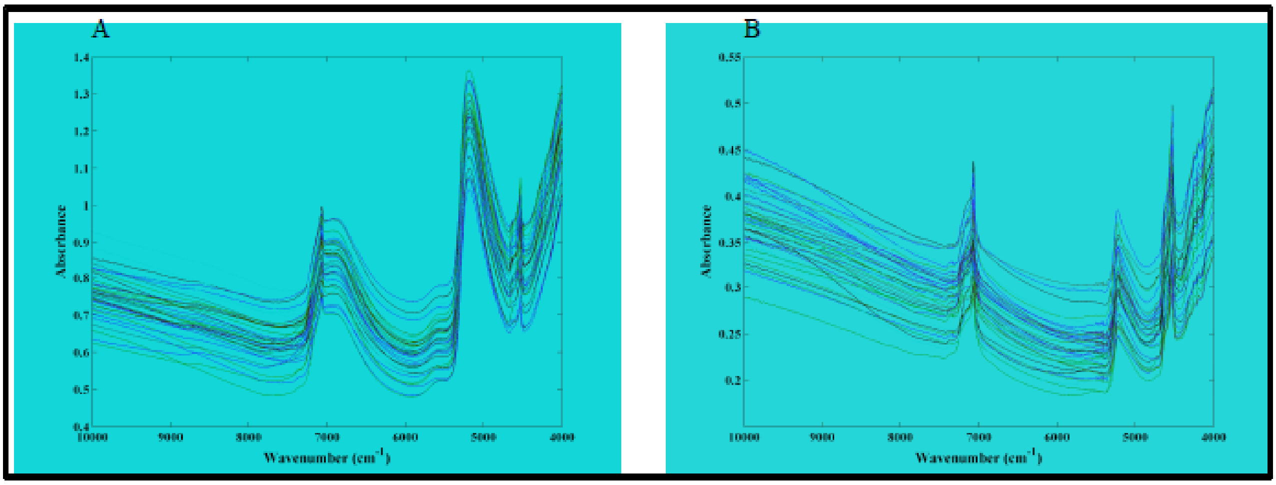

Figure 1. Raw spectra of wet (A) and dry (B) soil samples

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STATISTICAL METHODS FOR IMPROVING AUTHENTICATION OF WINES BASED ON STABLE ISOTOPE RATIOS

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Wine derives its economic value partly from its geographical origin and production technologies. Authenticity evaluation involves a check of several features like declaration of origin, year of vintage, and also the evaluation of any addition of unpermitted substances during the production process like water and sugar.

In Italy, at least 400 reference samples (collecting a particular number of samples from every region) every year are used to build the isotope ratio databank ($(D/H)_1$, $(D/H)_2$, R, $\delta^{18}O$, $\delta^{13}C$).

In this work, we evaluate several methods based on this unique data repository for checking authenticity claims (wine origin, detection of unpermitted sugar and water addition). The results show that using multivariate methods false claims of origin as well as unpermitted sugar and water addition can be discovered much easier [1].

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A QSPR APPROACH FOR ULTRA-FAST ESTIMATION OF DFT-CALCULATED MOLECULAR PROPERTIES

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The rapid access to intrinsic physicochemical properties of molecules is required to derive interpretable molecular descriptors, and process thousands or millions of structures in data mining explorations. Quantum chemistry calculations provide estimations of several properties with increasing accuracy, e.g. by Density Functional Theory (DFT), but are still too computationally expensive for large-scale uses. This work explores the possibility of pre-calculating molecular properties by DFT for thousands of structures, and make machine learning (ML) algorithms extract knowledge from the data with QSPR approaches. Once trained, the ML models can be applied to new structures and yield fully-automated ultra-fast estimations of DFT calculations.

Here we present the first implementations of this concept in our lab, for the estimation of bond dissociation energies (BDE), partial atomic charges, molecular orbital energies and electrophilicity index [1] Collections with thousands of molecular structures were retrieved from the ZINC database, [2] and atomic/bond/molecular properties were calculated by DFT methods with the GAMESS program. [3] For the QSPR protocol, atomic and bond descriptors were designed to encode atoms and bonds in molecules, while molecular descriptors were used from the CDK open-source package. [4]

An example is detailed for the prediction of bond energies. Homolytic bond dissociation energies were calculated by B3LYP/6-311++G(d,p)//DFTB for >12,000 non-ring bonds in a highly diverse set of compounds with elements C, H, O, N, or S. Bonds were represented by topological bond descriptors designed to encode atom types and connectivity in the 2D topological environment of the bonds. Machine learning methods, such as random forests and neural networks, were trained with the goal of predicting BDEs from the bond descriptors. The best model, a neural network ensemble based on 85 bond descriptors, was able to predict the (DFT-calculated) BDE of 887 bonds in an independent test set (covering a range of 17.67 - 202.30 kcal/mol) with RMSD of 5.29 kcal/mol, mean absolute deviation of 3.35 kcal/mol, and R^2=0.953. The predictions were compared with semi-empirical PM6 calculations, and were found to be superior for all types of bonds, except for O-H, N-H, and N-N bonds. An experimental web service and the database of the calculated BDEs are available at http://joao.airesdesousa.com/bde.

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INVESTIGATING SAR LANDSCAPES

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According to the congenericity principle, structurally similar compounds are assumed to be associated with similar activities. However, the activity landscape of QSAR datasets is not always as smooth as it is expected to be. Similar molecules may have very different activities leading to discontinuous SAR landscapes or activity cliffs. It is defined by the ratio of the difference in activity of two compounds to their "distance" of separation in the chemical space.[1]

Activity cliffs ratio was first described in terms of the Structure-Activity Landscape Index (SALI) by Guha and Van Drie.[2] Later different studies have been published about activity cliffs and ways of calculating it.

This work is aiming to make a comparison study between the different approaches from the literature and to introduce a new approach based on "multi-centered" Mahalanobis distance to assess SAR landscapes. In addition to activity cliffs, this approach has the advantage to equally consider structural cliffs that, correspondingly are the result of dissimilar compounds with similar activity. These two properties are extracted from a specific distance matrix designed for the purpose. Unlike the Euclidean distance matrix, it is dissymmetric and obtained from distances between a couple of data points calculated centering the covariance matrix on each one of them.

This approach was tested and compared with the published ones available in the literature. The analysis was conducted on three datasets; a simulated one that consisted of 49 data-points, a dataset of 82 polycyclic aromatic hydrocarbons [3], and the Selwood dataset consisting of 53 compounds [4]. The proposed approach showed promising results in terms of visualization of SAR landscapes to better detection of continuous/ discontinuous SAR regions and thus the corresponding compounds to each region.

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RECEPTOR-BASED 3D-QSAR UNDER THE MULTIPLE-INSTANCE PARADIGM

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Modern medicinal chemistry relies heavily on 3D structural information about therapeutic target; whenever such information is available, it is used to understand target mechanisms and experimental ligand binding data to guide the search of new ligands, being in many cases the key for successful drug design [1, 2]. Among the structure-based design methodologies, docking techniques are based on computational description of the binding site and provide for each investigated ligand (generally a relatively small molecule) a pool of structural solutions (called *poses*), due to the degree of both conformational and roto-translational freedom within the binding site of the receptor. Under the assumption that only a pose is involved in the receptor's recognition, one of the most critical issue is the selection of that single solution (frequently indicated as the *bioactive* conformation) representative of the main stereo-electronic features of the ligand responsible for the intermolecular interaction. This solution should provide the basic information on which three-dimensional descriptors, used in the further development of a statistical quantitative structure-activity relationships (QSAR) model, are calculated. It follows that the results of this studies can be strongly biased by the selection's criteria used for the reference conformation for each ligand. Moreover the calculation of the interaction forces among the ligands and the receptors are approximate in nature and the maximum interaction energy principle should not be used routinely.

In this work we suggest a new method to address the issues related to the *pose* selection, under a supervised multiple-instance learning framework [3]; according to this paradigm and related semantics, each ligand represents an *object* described by *multiple instances* of the object itself, i.e. the conformations. Each instance is described in our case by *a real-valued features vector* of 3-D descriptors. All instances of an object constitute a *bag*, which is in turn *real-value labelled* with the binding affinity data. After that suitable flexible-ligand docking calculations are performed and a pool of conformations is selected for each ligand, the task is to find the best 3D-QSAR model. In our approach, the quantitative model is built using the Multiple Linear Regression (MLR) method, in combination with an iterative sequence of steps as follows: *i)* select a n-dimensional descriptor space in which the *instances* of each *bag* are projected, *ii)* define the list of the *primary instances* extracting the "centrotype" conformation for each *bag* and *iii)* calculate the MLR model which correlate the primary instances' features with the bags' labels. This procedure is done iteratively under the supervision of a feature selection genetic algorithm optimizing the robustness and the predictive ability of the model using a Leave-More-Out cross-validation protocol. Furthermore the cross-validation procedure is extended to the *centrotype* selection method in order assess the sensitivity of the model robustness to the distribution of the available *instances* in each *bag*. The proposed procedure for deriving 3D-QSAR models was applied to several case studies: a) using a data set of ligands for the Acetylcholinesterase receptor adopting the WHIM molecular descriptors and b) using two datasets of Matrix Metalloproteases inhibitors where energy-based descriptors derived from the ligand-receptor interactions of each *pose* were used. The validation of the proposed procedure was also assessed comparing the selected conformations entering in the best models with the available crystallographic data of the corresponding ligand-receptor complexes.

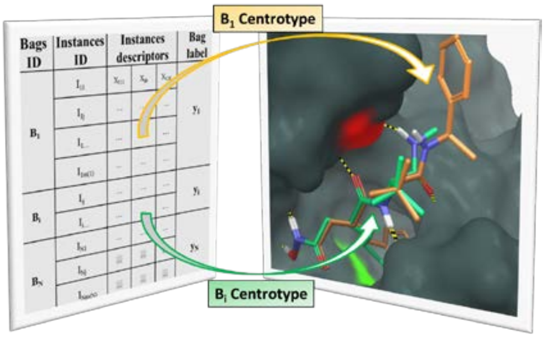

Figure 1: multiple instances dataset and docking solutions relationship in the multiple-instance framework.

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QUANTIFICATION OF API ENANTIOMERIC EXCESS IN DRUGS BY NIR SPECTROSCOPY AND CHEMOMETRICS

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Individual enantiomers often differ considerably in their pharmacological activity (something one being even toxic whereas the other is active and having desired properties): accordingly, in 1992 FDA issued a directive requiring drug manufacturers to evaluate the effects of individual enantiomers and then check the purity of chiral molecules produced. In this framework, our study focuses on the possibility of rapidly and non-invasively determining the enantiomeric excess of two commonly marketed APIs, ibuprofen and epinephrine, by using of NIR spectroscopy and chemometrics.

Solid mixtures at different enantiomeric compositions of ibuprofen and epinephrine were prepared by adding the active enantiomer and the racemate in opportune proportions. Matrix matched standard were also prepared by adding the APIs at different enantiomeric excess with commonly used excipients. The NIR spectra (between 1000-2500 nm) for each prepared sample was acquired and the calibration models relating the spectral profiles to the enantiomeric excess of the APIs were built on the entire spectral range at first, while successively both interval-PLS and genetic algorithm were used, to have more easily interpretable results and higher predictive accuracy in cases where the interference of excipients could be more marked. In all cases, to reduce the impact of undesired variability on the signals, different spectral preprocessing were tested.

In the case of ibuprofen, the best model on full spectrum was obtained after pretreatment with SNV and 2nd derivative, and resulted in very good performances on the external test set (almost no bias, and RMSEP=1.71). However, when the optimized model was used to predict the enantiomeric excess in commercial drugs, it was not possible to reach the same accuracy due to the presence of excipients. In this respect, the use of variable selection techniques, allowed to increase the predictive accuracy on real samples and to obtain more robust and easily interpretable models.

On the other hand, the best spectral pretreatment for epinephrine resulted to be SNV followed by detrending, which lead to almost no bias in the prediction of validation samples and a RMSEP of 1.91. In this case, since commercial epinephrine is sold only in solution and the control on solid epinephrine is carried out only during the production stage, where no excipients are added yet, further modeling steps were not needed.

BRAZILIAN SOIL CLASSIFICATION USING RAMAN SPECTROSCOPY WITH VARIABLE SELECTION AND CHEMOMETRIC TOOLS

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The classification allows adequate soil preparation for cultivation, important for a country that exports various agricultural commodities. In Brazil, the system adopted is the Brazilian System of Soil Classification, which uses chemical parameters, physical and morphological changes; however, some analyzes are time-consuming, mainly because of the stage of sample preparation [1]. The aim of this study was to use Raman spectroscopy coupled with chemometric techniques of pattern recognition, as a tool for classification of Brazilian soils.

124 samples of the five Brazilian soils were provided, without identification, pulverized and sieved in airtight bags, Embrapa Solos SA (Rio de Janeiro, Brazil). The samples were analyzed as received. After being analyzed by a Raman spectrophotometer, samples were divided into two sets of calibration and validation, using the algorithm Kennard-Stone. The calibration and validation sets were built with 75 and 49 samples, respectively.

Several chemometric tools were used to classify the soils samples using Raman spectra data like as SIMCA, HCA, PLS-DA, DA-SVM and iPLS-DA. The tested methods produced unsatisfactory results with larger classification errors in calibration and validation sets, however, when the genetic algorithm or intervals were used as variable selection with PLS-DA or SVM-DA; the soil samples were correctly classified. Table 1 shows a summarized results with figures of merit.

Therefore, the variable selection coupled with chemometric techniques is a powerful tool to classify soil samples using Raman spectra data.

Table 1 - Summary of misclassification and sensitivity obtained by the methods of classification with variable selection.

Subset	**Calibration**					**Validation**				
Group	Soil 1	Soil 2	Soil 3	Soil 4	Soil 5	Soil 1	Soil 2	Soil 3	Soil 4	Soil 5
iPLS-DA										
N[a]	15	15	15	15	15	10	9	10	10	10
N[b]	0	1	0	0	3	0	0	0	0	0
ME(%)[c]	0.0	6.7	0.0	0.0	20.0	0.0	0.0	0.0	0.0	0.0
TP[d]	1.000	0.933	1.000	1.000	0.800	1.000	1.000	1.000	1.000	1.000
FP[e]	0.017	0.033	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000
TN[f]	0.983	0.967	0.983	1.000	1.000	1.000	1.000	1.000	1.000	1.000
FN[g]	0.000	0.067	0.000	0.000	0.200	0.000	0.000	0.000	0.000	0.000
Sens[h]	1.000	0.933	1.000	1.000	0.800	1.000	1.000	1.000	1.000	1.000
GA-PLS-DA										
N[a]	15	15	15	15	15	10	9	10	10	10
N[b]	0	1	0	0	2	0	0	0	0	0
ME(%)[c]	0.0	6.7	0.0	0.0	14.3	0.0	0.0	0.0	0.0	0.0
TP[d]	1.000	0.933	1.000	1.000	0.857	1.000	1.000	1.000	1.000	1.000
FP[e]	0.017	0.034	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
TN[f]	0.983	0.966	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
FN[g]	0.000	0.067	0.000	0.000	0.143	0.000	0.000	0.000	0.000	0.000
Sens[h]	1.000	0.933	1.000	1.000	0.857	1.000	1.000	1.000	1.000	1.000
GA-SVM-DA										
N[a]	15	15	15	15	15	10	9	10	10	10
N[b]	0	1	0	0	0	0	0	0	0	0
ME(%)[c]	0.0	6.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
TP[d]	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
FP[e]	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
TN[f]	0.983	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
FN[g]	0.000	0.067	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Sens[h]	1.000	0.933	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

Where: N[a]: number of samples in each soil; N[b]: number of misclassified soils; ME(%)[c]: Misclassification Error, in %; TP[d]: proportion of positive cases that were correctly identified; FP[e]: proportion of negatives cases that were incorrectly classified as positive; TN[f]: proportion of negatives cases that were classified correctly; FN[g]: proportion of positive cases that were incorrectly classified as negative; Sens[h]: Sensitivity.

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ALTERNATIVE METHODS FOR MULTI-OBJECTIVE OPTIMIZATION OF RADIAL BASIS FUNCTION – SUPPORT VECTOR MACHINES FOR CLASSIFICATION (RBF-SVM-C)

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In discrimination, one tries to find a relation between an m x n data matrix **X**, consisting, for example, of m spectra or chromatograms containing each n variables, and an m x 1 response vector **y**, which is categorical, i.e. containing the class labels of the m samples. Various discrimination techniques have been developed. One technique, called Support Vector Machines for classification (SVM-C), can model complex non-linear boundaries between classes through the use of adapted kernel functions, such as the often applied Radial Basis Function (RBF) kernel [1,2]. Using this kernel function, two parameters should be optimized, i.e. the regularization parameter C and the RBF kernel parameter γ.

For their optimization, different approaches were compared [3], i.e. a grid search with logarithmic steps, a grid search with equal steps, an experimental design optimization [4], and a variable-size simplex optimization [5]. For a given data set, in all approaches, the same ranges for the parameters were examined, i.e. 1 till 10^6 for C, and 10^{-4} till 1 for γ. The responses considered to determine the optimum parameter settings were the percentage of correct classification rate (%CCR) for the calibration set with five-fold cross-validation (CV) (%CCR_cal_cv), and the total number of Support Vectors (nSV_{TOT}). For all approaches, Derringer's desirability functions [6] were used to optimize both responses simultaneously.

Three different data sets were used. All data sets contain NIR spectra of manufactured textiles of various compositions. The classification problem consists in the determination of the level of a physical property that can take two or three discrete values for data sets 1 and 2/3, respectively. Data set 1 contains spectra with 480 NIR wavelengths and was divided in 192 training and 96 test set samples. Data sets 2 and 3 contain 13 variables or 2800 NIR wavelengths, respectively, and were divided in 132 training and 91 test set samples. Both data sets were preprocessed using Standard Normal Variate and column centering.

For NIR data set 1, similar %CCR_cv_cal, %CCR_cal, %CCR_test, % specificity for each class, and % sensitivity for each class were obtained for all approaches. Also similar nSV_{TOT} and SV's were selected. For NIR data set 2, adequate and more or less similar %CCR_cv_cal, %CCR_cal, %CCR_test, % specificity for each class, and % sensitivity for each class were obtained for all approaches. Also rather similar nSV_{TOT} and SV's were selected. For NIR data set 3, except the variable simplex method, all approaches allowed optimizing the RBF-SVM-C parameters γ and C and obtaining adequate models. The simplex approach failed as it got stuck in a local optimum. When comparing the time needed for the optimization, the experimental design approach was found to be the fastest for all considered data sets, followed by both grid search methods and the variable simplex approach.

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FUNCTIONAL DENSITY BASED INVERSE REGRESSION FOR CALIBRATION

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Statistical calibration plays a crucial role in many areas of technology such as pharmacology, neuroscience and chemometrics. The calibration problem can be described as follows. An observable random variable X is related to a variable of interest Y according to a statistical model specified by a conditional probability density $f(X/Y)$. The density of Y may be imposed by the researcher (controlled experiments) or given by nature (natural experiments). A sample D of independent observations $(x_1,y_1),\ldots,(x_n,y_n)$ of (X,Y) is available. Given a new (future) observation x_0 of X that corresponds to an unknown value y_0 of Y, the problem is to make statistical inferences about y_0 on the basis of the given statistical model, the data D and x_0.

The practical motivation that leads us to study the above problem comes from chemometrics, specifically from spectroscopy, where some chemical variable Y (e.g., concentration of a substance) needs to be predicted from a digitized function X (e.g., an absorbance spectrum). In this setting, such conditional density $f(X/Y)$ ("inverse model") represents the physical data generation mechanism in which the output spectrum X is determined by the input chemical concentration Y, plus some random perturbation mainly due to the measurement procedure. Then, given an observed spectrum x_0, that corresponds to a new substance, it is desired to obtain an estimate of its concentration y_0, based on (past) observations of pairs of spectra and concentrations (x_i,y_i), $i = 1,\ldots,n$.

Hereafter, we restrict ourselves to cases where the variable of interest Y takes real values (e.g., only the concentration of one substance is considered). In this framework, different calibration setups arise, depending on *a)* the space in which the random variable X takes values (univariate, multivariate or functional calibration), and *b)* the kind of design: fixed design (the Y values are not random but set by the researcher) or random design (Y is a random variable as well as *X*). A review of the literature on this subject, for both univariate and multivariate calibration, can be found in (Osborne 1991).

This paper is concerned with the setting of functional calibration, and specifically, the focus is put on the case of random design (we refer the reader to (Cuevas et. al. 2002) for an approach on functional calibration under fixed design). A widely used criteria for calibration in case of random design is the mean squared error, which is minimized by the regression function $E(Y/X = x_0)$. This can be estimated by means of functional regression methods in which the response *Y* is a real random variable and the explanatory variable *X* has functional nature. A number of such methods have been proposed (Ramsay 2005, Ferraty 2006).

However, none of these approaches for predicting *Y* makes use of the specific structure of density $f(X/Y)$ ("inverse" regression model *X vs Y*), which plays a major role in the calibration context. On the contrary, their probabilistic assumptions are made about the densities $f(Y/X)$ and $f(X)$. Note that in the calibration setting, the model $f(X/Y)$ represents the data generation mechanism, and so it is natural that the probabilistic assumptions refer to this model. In this generating mechanism, the response variable actually is *X*, and *Y* is the explanatory variable. Hence, contrary to standard prediction in regression problems, the variable to be predicted, *Y*, is not the response variable of the generating statistical model, $f(X/Y)$, but its explanatory variable.

In this paper, a new functional calibration approach, named Functional Density-Based Inverse Regression (DBIR), is introduced. This method makes it possible to incorporate knowledge on the ("inverse") regression model $f(X/Y)$ for the prediction of a scalar variable *Y* on the basis of a functional data *X*, so taking into consideration the just mentioned specificities of the prediction problem in the calibration setting.

The performance of the functional DBIR approach has been corroborated through a simulation study. DBIR was also test on a real-world benchmark data set named Tecator showing promising results.

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CHEMOMETRICS AND DIFFERENTIAL SCANNING CALORIMETRY FOR THE CHARACTERIZATION OF THE POLYMORPHIC BEHAVIOR OF PRULIFLOXACIN

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Chemometrics was successfully applied in combination with spectroscopic techniques (FTIR and XRPD) for the characterization of the solid state of pharmaceuticals [1,2]. Differential Scanning Calorimetry (DSC) is a very sensitive technique for investigating the polymorphic behavior and stability of pharmaceutical active ingredients. Moreover, information can be obtained on the thermodynamic relationships between different polymorphs and also on the transformation rates if traces of other crystalline forms, not detectable with spectroscopic techniques, are present [3]. Polymorphic impurity in fact could adversely compromise both the stability and the performance of the final products.

In our study we describe an interesting and useful approach where DSC and hyper-DSC are combined with statistical projection methods for characterizing the polymorphic behaviour of prulifloxacin, an active pharmaceutical ingredient (API) having a high level of polymorphism. A fine DSC-based classification of API is proposed to predict potential, unexpected crystalline form transformations that may occur due to trace levels of other polymorphs. Ninety production batches of prulifloxacin drug substance characterized by a high crystalline purity (> 95% by XRPD) and well defined particle size were collected and analyzed. Differences observed on the basis of the DSC thermogram profiling of the collected lots were investigated after a suitable data pre-treatment by Principal Component Analysis, O2PLS and Hierarchical Cluster Analysis [4,5]. Three main types of DSC profile were identified (type A, B and C). A further thermal stress study (70°C for 40 days, in the dark) was performed in order to evaluate if the type of profile could be related to a different behavior of the lot under thermal stress. As result, 21% of the lots having one of the three main profiles (profile A) showed modification in their DSC profile while for the other lots the DSC profile was unchanged. The analysis of the hyper-DSC profiles after thermal stress highlighted that most of the lots having DSC profile similar to that showing modifications exhibited a characteristic hyper-DSC profile. Then, a predictive O2PLS-DA/Naïve Bayes classifier was built with the aim to suggest in advance potential instability. The classifier was successfully used to elucidate the anomalous behavior of some lots of prulifloxacin employed in the development of the drug product production process.

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CONSTRUCTION OF SPACE FILLING DESIGNS USING WSP ALGORITHM: CONSTRUCTION AND APPLICATION

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1. Introduction

Experimental designs help the research scientist to establish optimal experimental strategies with regard to the expected targets and the available tools. For most of the classical designs, as factorial design or Doehlert design or D-optimal design for example, the selected experiments are suited to the underlying mathematical model and are generally positioned on the boundaries of the experimental domain. When there is no prior model or when the experimental domain is very large, as in numerical experiments, these designs are no more appropriate and we need specific designs that aim at "filling" or "covering" the numerical space.

2. Space Filling designs

Space-filling designs (SFD) that spread the points at which the response is observed evenly throughout the domain, are more suitable and are increasingly used in computer experiments. The most known space-filling designs are Latin Hypercubes (random, orthogonal, optimized) and low discrepancy sequences. But, simulation codes becoming more and more complex, high dimensional optimal designs are needed to study a high number of parameters (more than 20 parameters) and the construction proves difficult. We propose to explore a construction method of new space-filling designs for high dimensional spaces. After a short presentation of the criteria considered to quantify the intrinsic quality of the uniform designs, the generation of these designs using WSP algorithm is presented [1]. As the first step consists in generating a set of candidate points, the influence of the initial set of points is investigated and the final designs are compared to classical space-filling designs. Then, designs are proposed in dimension 20, 30, 40 and 50 and the study of the intrinsic quality of these new space-filling designs highlights the robustness of this generation method in high dimensional spaces.

3. Applications

We present several application cases, in function of the objectives. Indeed, these designs built from WSP algorithm can be used for different situations:

- Sensitivity analysis : when the number of factors is very high, it is essential to determine the most important factors to include in a metamodel simpler but realistic, but the classical screening methods are not adapted when the domain of variation is very large.
- Modelling : when phenomena are complex, specific modeling algorithms as Support Vector Machines regression or krigging are often used in order to approximate very complex and chaotic responses surfaces with irregularities, and well-spread experiments are needed in order to fit the phenomena (training step).
- Quantitative Structure-Activity Relationship: the presented algorithm proposes a selection of a representative subset of compounds among a set of candidate points in the descriptors space.

4. Conclusion

The new space filling designs based on WSP algorithm propose a set of points uniformly spread in the experimental space. These designs are easy to built even in high dimensional space and are very useful in different application domains.

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ADDRESSING, INCORPORATING AND VISUALIZATION OF RISK IN QBD

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The principles of Quality by Design (QbD) and Design Space are gaining increasing attention in the DOE community. Despite the need for incorporating the ability to assess and visualize risk, associated with experiments used to support and confirm development in applications with complex processes such as pharmaceutical development and production, this is rarely done, in part because it has been difficult to accomplish a risk estimate with conventional DOE tools and software. The presentation will address this weakness by enabling an easy visualization and estimation of risk. With advanced optimization tools in combination with Monte Carlo Simulations, graphs that can be interpreted as spaces or regions of factor settings where all result specifications are fulfilled and with low risk of failure. Design Space tools as the Probability Contour Plot present the operational region that meets the specifications according to a risk analysis, which will guide engineers in determining how likely it is that their experiments truly identify the safest operating region. These principles underline that the engineer must incorporate a risk estimate in the interpretation of the results from designed experiments.

- Extended tools for Design Space estimation — Probability Contour Plot
- The effect of uncertainties in factor settings can be studied
- Risk analysis based on the user specification

Tools that will be discussed and interpreted:

Contour Plot: Problems and interpretations for QbD.

Sweet Spot: An overlay Contour Plot with multiple responses on top of each other.

Probability Contour Plot: The low risk region is significantly smaller than the corresponding classical sweet spot or contour plot region.

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SELECTION OF NEARLY ORTHOGONAL BLOCKS IN AN 'AD-HOC' EXPERIMENTAL DESIGN

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In the most usual context, optimization refers to the searching of experimental conditions to optimize several analytical responses of interest. In general, this has to be approached from an experimental perspective. Consequently, whether these responses are individually or jointly optimized, the reliability of the optimal solutions is dependent on a proper experimental design.

For some experimental procedures, above all when there are several experimental factors, the number of experiments in a standard design may be unaffordable. From this perspective, optimization refers to the selection of the experimental design itself, based on its best characteristics for the given problem. However, there are several criteria to measure the quality of an experimental design [1] (for example, variance inflation factors for the coefficients, values of the variance function for the prediction, and related to them the so-called alphabetic criteria), so that the search of a reduced design that maintains the required quality is theoretically inside the multicriteria optimization. The approach to deal with this multicriteria setting is to compute the Pareto-optimal front, POF, that is, the set of different experimental designs that are optimal in at least one of the criteria being handled, while losing the least possible in the others. This approach has proven useful [2] for simultaneously minimizing the VIFs, the volume of the joint confidence region for the coefficients (D-criterion) or the prediction variance in the whole experimental domain (G- criterion), and both in the context of factorial or response surface designs –discrete or continuous domains.

In this work, we deal with the problem of arranging experimental designs into blocks, i.e, blocking experimental designs. Sometimes it is not possible to conduct all the experiments at once (in a single session, or maintaining the experimental conditions). In such cases, the possible effect of these modifications must be 'separated' from the effect of the factors on the responses. One alternative to do it is to add a dummy variable in the proposed model that accounts for shifts in the responses, the so-called block variable whose significance would be related to a significant bias between the responses in the two blocks. In order to correctly interpret the significance of the block, the estimate of the coefficient of the blocking variable should be independent of the other coefficients.

To measure the ‘degree’ of independence between the coefficient of the blocking variable and the remaining ones, we use directly the corresponding covariance factors. That means that there are as many values as coefficients in the model (minus two), that should be, all of them, as near to zero as possible. Nevertheless, the blocking of the design may change the variance and covariance of the estimates of the remaining coefficients (the ‘treatment’ coefficients) - this is measured somehow in [3] in the form of a weighted average of the efficiency index of all the coefficients. To take into account this effect we add the D-criterion to the covariance factors in the definition of the multicriteria problem and compute the Pareto-optimal front with the aim of describing the trade-offs among criteria and helping in the decision about the design to choose.

Some comparison with more standards designs are made as well as comparison to other criteria for blocking designs reported in the literature [e.g. 3].

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SENSITIVITY ANALYSIS WITH SPACE FILLING DESIGN: ISTHME (IMPROVED SENSITIVITY THROUGH MORRIS EXTENSION) METHOD

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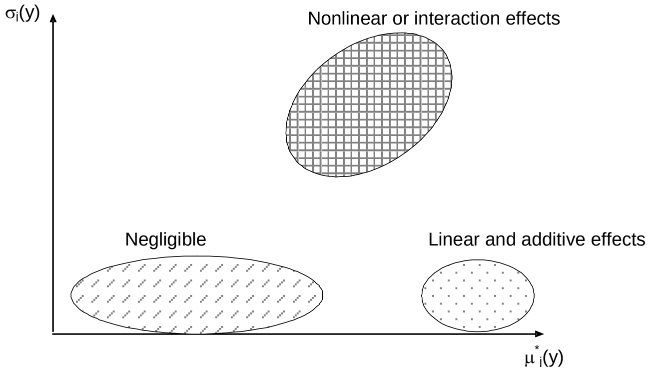
In the last decade, industrial phenomena (oil industry, nuclear,...) are often studied using numerical simulation. These simulation models are increasingly complex with a large number of input parameters and consequently a long time of calculation. Therefore, it becomes essential to determine the most important factors to include in a metamodel, simpler but realistic, by using screening or sensitivity analysis. The classical screening methods as Plackett and Burman designs, supersaturated designs or sequential bifurcation are not adapted when the variation domains are very large since the points are mainly located on the periphery of the domain.

Space filling design The purpose of these designs is to spread the points evenly throughout the variable's space. These designs are called Space-Filling Designs (SFD) and the most known are Latin Hypercubes (random, orthogonal, optimized) and low discrepancy sequences or designs based on algorithms as WSP [1]. These designs are well-adapted for sensitivity analysis when the relationship between the response and the inputs is unknown and allow global analysis in order to detect the irregularities of the phenomenon.

Sensitivity study: Morris's method. The method proposed by Morris [2] provides a global sensitivity measure to identify factors effects. For that, a design composed of individual randomized One-At-a-Time (OAT) designs is built in order to determine, for each factor X_j, the elementary effects $d_j(y)$. Considering L different trajectories, a statistical analysis of these elementary effects provides the mean $\mu^*_j(y)$ which assesses the global influence of the factor X_j and the standard deviation $\sigma_j(y)$ which indicates the presence of higher order effects and measures the non-linearities or the interactions of the j^{th} factor with others factors.

According to the values of $\mu^*_j(y)$ and $\sigma_j(y)$, Morris shows that studied factors can be classed into three groups, factors having:
- negligible effects,
- linear and additive effects,
- nonlinear or interaction effects.

For an easier interpretation, the values of $\mu^*_j(y)$ and $\sigma_j(y)$ can be plotted as shown on the opposite figure.



Nevertheless, this method which allows the determination of the main effects and gives indication on nonlinearities or interactions requires many simulations without the possibility of using the simulations for a subsequent study.

Sensitivity study: ISTHME method [3]. A new sensitivity analysis method named ISTHME based on the principles of Morris's method without the construction of randomized one-at-time (OAT) design was developed. This method can be applied on any experimental design and more particularly on Space Filling Designs. This specificity is very interesting in terms of time and calculation economy. Indeed, we can use a universal design, which is adapted to sensitivity analysis as well as optimization (response surface methods or/and kriging) without any supplementary simulation. In the ISTHME method, the first step consists in the construction of constellations using points of a space filling design. The number of these constellations depends on two parameters, *l* (length of the segments) and α (the angle between two segments). The study of these two parameters and several application examples will be presented. In all cases, the ISTHME method allows a correct classification of the factors.

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FEATURE SELECTION STRATEGY ON EXPERIMENTAL DESIGN SIMULATION OF GASEOUS MIXTURE SPECTRA

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The development of new miniaturized, low cost sensing systems for the detection of substances difficult to handle implies the need to perform preliminary data analysis on simulated spectra. This challenging task can be solved following different strategies. In this work, since there was the need to consider a high number of substances (33 different gases including 4 target molecules, 20 interfering species and 9 air components), we developed an approach [1,2] that starts from literature databases spectra. They are used as "building blocks" in order to simulate the spectral profile that would be measured using an External Cavity-Quantum Cascade Laser Photo Acoustic Sensor (EC-QCLPAS) [3] sensing system, planned to cover a 200 cm^{-1} range within the 1000-2500 cm^{-1} spectral range. Firstly, literature spectra imported from different literature databases were standardized, in order to obtain unit concentration spectra over the desired spectral range, at the desired resolution. Then, each single spectrum was denoised using a Fast Wavelet Transform (FWT) [4,5] based algorithm. Three concentration matrices, for target molecules, interfering species and air components, respectively, were built separately by means of Experimental Design techniques, along with a randomization strategy. Subsequently, the three matrices were merged into a single concentration matrix. This was then multiplied by the denoised spectra to give the corresponding mixtures spectra. To this aim, since the simple matrix multiplication gives unrealistic results, due to the presence of the background, a sigmoidal transfer function has been developed and optimized ad-hoc, in order to preserve the correct signal shape. The final matrix of simulated spectra was obtained by adding to the denoised mixture spectra the noise structure characteristic of the EC-QCLPAS spectra. This was estimated by means of FWT and of robust regression models, using sample spectra measured with a prototypal version of the EC-QCLPAS. To select the optimal 200 cm^{-1} wavenumber range and the single wavenumbers therein contained, a thoughtful pre-selection (SMARTGRID, in-house developed procedure) was carried out on the whole range, in order to cancel out the regions containing sharp and intense absorption peaks. This allowed us to avoid problems related to the hypothesized final instrument precision in repositioning, which is rather low when compared to the extremely narrow bandwidth. The optimal 200 cm^{-1} wavenumber range was then selected by maximizing the Classification Efficiency values calculated with PLS-DA for each position of a moving window with 200 cm^{-1} size , covering the whole spectral range of the laser. Finally, the optimal wavenumber values were identified within the selected range using Genetic Algorithms (GA) [6] and resampling, performing a series of random subsamplings from a training set, and applying GA to each subsample for the classification of each target molecule considered separately. The global frequency of selection in correspondence to each wavenumber was calculated, and the spectral variables were then ranked accordingly. Finally, starting from the most frequently selected wavenumber and adding each time a further variable, a series of PLS-DA models was calculated using all the samples of the simulated spectral database (3000 spectra in the training set and 2000 in the test set).The optimal number of single wavenumbers to be kept was defined on the basis of the maximum value of the overall Classification Efficiency estimated in cross-validation.

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MULTI-STEP SENSORY-BASED APPROACH FOR THE MONITORING OF RED WINE ALTENATIVE AGING PROCESSES

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Maturation of red wines in oak barrels is an ancient art steeped in tradition aimed at improving wine quality and contributing to the enhancement of its sensory characteristics. However, the traditional aging system not only requires long time periods but it is also very costly as oak barrels are highly demanding in terms of space requirements, labor and maintenance. For this reason, simpler and more affordable aging practices such as the use of pieces of oak wood in combination with the application of a controlled micro-oxygenation (MO) have gained increased significance in the wine industry in the last decade.

Taking into account that the purpose of alternative aging systems is to mimic the positive effects of traditional aging on wine sensory quality but shortening the time and costs, the development of a methodology for monitoring changes in sensory properties during accelerated aging processes could provide a very useful tool for process supervision, control and optimisation to ensure that the final products meet the desired quality standards.

In this work, a multi-step sensory-based approach was proposed for monitoring the impact of accelerated aging treatments on sensory quality development. Two young red wines from D.O. Navarra (Spain) were separately subjected to accelerated aging in tanks with oak chips added and micro-oxygenation. Wine samples were collected during each process and were analyzed by a sensory panel according to 17 descriptors. The comprehensive stepwise strategy applied attempted to lump together two important needs in sensory analysis: the quality control of sensory panels, and the analysis of individual sensory profile data to handle differences between assessors and to derive on their basis a compromise solution. Panel performance analysis was carried out both at a global and at an individual level thanks to the joint application of several methods (including mixed model ANOVA, Tucker-1 and Manhattan plots, and one-way ANOVA based F and MSE plots). The STATIS (Structuration des Tableaux À Trois Indices de la Statistique) method was subsequently applied to find an optimal compromise solution for the significant attributes describing each process.

Significant product effects were found for 7 and 6 attributes in the first and second aging series, respectively. Although panel performance was acceptable in both cases, moderate differences between assessors were detected, stressing the need for adopting a consensus approach to properly handle them. The STATIS compromise provided a reliable estimation of the sensory profiles of wine samples through the aging period studied for each maturation process. The assignment of individual weights was consistent with previous findings on assessors' agreement The first principal component of the compromise matrix, which accounted for 86.5% and 90.9% of the variance in each trial data, revealed a sample trend directly related to the time-course evolution of each process, and contrasted the attributes fruit and herbaceous with the descriptors related to the wood–wine interaction.

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STUDY OF THE CHARACTERIZATION AND QUANTITATION OF PROTEINACEOUS BINDERS IN RED MEDIEVAL PAINTS BASED ON FOURIER TRANSFORM INFRARED SEPCTROSCOPY AND CORRELATION CONSTRAINED MULTIVARIATE CURVE RESOLUTION ALTERNATING LEAST SQUARES METHOD.

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For a better conservation of the cultural heritage, detailed information about the artwork materials used, such as the pigments and the binding media, which were used to carry the pigment, is needed. Previous studies of the materials of Portuguese medieval paints revealed that proteinaceous binders such as parchment glue, egg white and egg yolk or mixtures of them might have been consistently used [1-2]. This work aims at characterizing and quantifying the binding media formulation used to produce red medieval paints based on the combined use of Fourier transform infrared (μ-FTIR) spectroscopy and correlation constrained multivariate curve resolution alternating least squares (correlation constrained MCR-ALS). The proposed correlation constrained MCR-ALS is a modification of the classical MCR-ALS that introduces a new constraint to establish internal calibration models for quantitative analysis [3-5].

First, correlation constrained MCR-ALS has been applied to synthetic binder mixtures of bs in order to check for the possibility to design an appropriate calibration model to obtain good quantitative estimations of the three binders (parchment glue, egg white and egg yolk). This first exploratory step is relevant given the high similarity among the spectra of some of these binders. Best calibration models have been finally obtained calibrating simultaneously all binders in the same MCR-ALS analysis and using either SNV or MSC with Savitzky–Golay (SG) filter with 1st derivative as FTIR spectra pretreatment. In these models, RMSEC values around 0.025-0.115 and correlation coefficients above 0.92 have been obtained. The major difficulty has always been distinguishing between parchment glue and egg white, since these are the binders with the largest spectral similarity. Finally, classical MCR-ALS has been first applied in the set of historical samples to check for the presence/absence of these binders. In this first analysis, parchment glue and egg white have been often found separately or in mixtures of different proportions, whereas the binder egg yolk seems to be generally absent in all samples (or in very minor amount in few of them). Correlation constrained MCR-ALS has been used in multisets combining the binder mixtures (used for calibration) and the historical samples to provide quantitative values about the binding media formulation used to produce red medieval paints. The quantitative values in historical samples, however, should be considered carefully since there is no clear information about the possible ageing processes of the binders.

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COELUTING COMPOUNDS WITH SHARED IONS: A CHALLENGE FOR ANALYTICAL CHEMISTRY SOLVED BY MEANS OF THREE-WAY TECHNIQUES

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Several compulsory regulations on the control of pesticides [1], migrants [2] and veterinary residues in food [3,4] demand that the analytical procedures aimed at the identification and quantification of these substances must fulfil some strict requirements, also considered in the technical document about anti-doping [5] and the ISO Standard 22892 [6]. These guidelines propose the use of chromatographic techniques coupled to multivariate detectors (usually MS), so identification will be performed according to both the relative retention time (RRT) and the mass spectrum recorded in full scan or SIM mode. The RRT must not exceed a pre-established limit, while particular requirements for mass spectrometry identification are generally given. The maximum permitted tolerances for the relative peak intensities of selective ions (m/z) are the more common ones, although they are not fully agreed in different documents. They are expressed as percentages of the base peak intensity (relative abundance) depending on the relative intensities. Furthermore, the number of mass peaks taken into account during identification depends on which MS technique is used and on the type of compounds (either forbidden or with a permitted limit). The relationship between the effective number of ions (identification points, IP), and the technique type is considered (1 IP per ion in MS^1 or per precursor ion in MS^n and 1.5 IP per product ion in MS^n). The concept of IP came into being not long ago [3]. When data come from chromatographic techniques and various characteristic ions are acquired for every analyte in several samples at different elution times, a three-way tensor, $\underline{\mathbf{X}}$, is obtained. If $\underline{\mathbf{X}}$ fulfils the second-order property, PARAFAC (or PARAFAC2) will prove to be adequate for the identification of the unique factor related to every analyte [7]. This work displays several cases of coeluting compounds with shared ions solved using the second-order property of PARAFAC. Case 1: For the determination of primary aromatic amines, regulated by [2], by means of HS-SPME-GC-MS, a previous derivatization reaction via treatment with iodine after diazotization is necessary. In spite of the removal of the by-products and the excess of the reagents, an unidentified compound coelutes and shares some ions with iodobenzene (derivative of aniline). Case 2: For the determination of bisphenol A (BPA) by PTV-GC/MS, its deuterated compound, BPA-d_{16}, is used as IS, so they elute close. When analysing samples from the migration test of BPA from polycarbonate tableware, several interferents coelute. Case 3: Unequivocal identification of several acetyl-chlorophenols and chloroanisoles determined by means of HS-SPME-GC/MS. Trichloro-anisole (TCA) and its deuterated TCA-d_5 coelute and share the base peak of the latter, so it is critical to distribute correctly the fraction of the two ions that corresponds to each analyte. Case 4:.In the determination of pesticides by PTV-GC/MS, a co eluent appears with 3,5-dichlorobenzonitrile (IS of dichlobenil) both sharing an ion. Nevertheless, another compound coelutes with this IS when spiked onion samples are considered; this new interferent and 3,5-dichlorobenzonitrile share four ions, among others, the base peak of the latter.

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EXTENDED MULTIVARIATE CURVE RESOLUTION FOR QUADRILINEAR AND FOR INCOMPLETE DATA SETS

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MCR-ALS has been shown to be adapted easily to diferente posible multiset data structures, including multiway data sets (1,2), and to higher complexity models, including multilinear models and mixed multilinear models. We have shown recently two extensions of MCR-ALS, one to four way data sets fulfilling quadrilinear models (3) and another one, to incomplete data multisets (4) fulfilling also mixed bilinear and multilinear models.

In the first case, the algorithm used is similar to the one developed for the analysis of three-way data fulfilling a trilinear model with results similar to those obtained by other multiway methods like PARAFAC or TLD. The extension of MCR-ALS to four-way data fuldilling a quadrilinear model (3) allows for the analysis of mixed models ranging from bilinear, trilinear and quadrilinear data structures and also independently for every resolved componente (this feature is not presented in other methods). An application of this new cosntraint is shown for environmetal data acquired to monitor river water quality parameters, at differnent locations, and sampling periods at different months and years (four data ways or modes in total).

The extension of MCR-ALS to incomplete multiset data stuctures (4), allows extending the use of this method to situations where information acquired in the diferente modes could not be complete for diferente reasons. MCR-ALS algorithm has been adapted to cover this type of situations which can also be present when different types of data are fused sharing some common modes. An example is shown for environmental monitoring data where ozone and nitrogen oxide concentrations were acquired hourly, daily and yearly, although in some circunstance some of the full year data subsets were lacking

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DEALING WITH NON-LINEARITIES IN ULTRAFAST SPECTROSCOPY: MCR-ALS ALTERNATIVES

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Multivariate curve resolution by alternating least squares (MCR-ALS) is a powerful method to infer information about short-lived chemical intermediate states created during ultrafast chemical reaction from a series of time-resolved spectra. However, the application of MCR relates to the fulfilment of a low-rank bilinear model for the decomposition of the experimental data. In ultrafast time-resolved spectroscopy, due to the presence of vibrational relaxation, continuous spectral evolution and band broadening/narrowing are observed on top of spectral variations associated to transitions between excited states. The basic assumption mentioned above may thus sometimes be questioned.

In this presentation, a methodology based on partially constrained MCR-ALS where classical constraints such as non-negativity are relaxed for some components is extended to hard- and soft-MCR. These alternative models enable to describe deviations from ideal data bilinearity. Combined with the input of additional information available from the photophysics, both for hard-modeling constraint on the concentration profiles (kinetic rates constant for transitions) and for soft-modeling constraints (selective time domain for vibrational relaxation), this approach is of particular relevance for the investigation of ultrafast photoinduced processes. We demonstrate this on time-resolved UV-vis and infrared spectroscopy data for the characterization of the photodynamics of innovative photochromic compounds.

MULTIVARIATE CURVE RESOLUTION (MCR) IN PERSPECTIVE

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Multivariate Curve Resolution designs a family of methods devoted to decompose a data set into a simple bilinear model of chemically meaningful contributions. Born in the 70's with Lawton and Sylvestre's works, it has been continuously evolving until now and there are still clear directions for improvement.

Relevant milestones in MCR history were the discovery of the relevance of local rank into the feasibility to get unique and true solutions or the advent of the multiset extension of the method, which opened a wealth of new scenarios to be solved and largely decreased the uncertainty linked to MCR solutions.

The simple basic bilinear scaffold of the method, which allowed improvements in the nature of information accommodated and in the flexible implementation of constraints (by profile or by set), has made that MCR has surpassed the original definition of factor analysis method. As a result, the recent hybrid variants of the method interface clearly with other families of chemometric methodologies, such as hard-modelling, multi-way analysis, calibration or discrimination methods.

Reconsidering the definition of MCR contribution and profiting from the advantages above, MCR has entered in new challenging application domains, such as environmental data analysis, hyperspectral image analysis or –omic sciences.

New challenges may go in the direction of extending the kind of data structures to be analyzed, the fact of using domain-specific knowledge for the design of new constraints (based on spatial or morphological information in image analysis or in the inclusion of ontological and database information in –omic sciences) or the use of the MCR profiles, which are noise-filtered meaningful compressed representations of original (often massive) raw data as starting information for further data analysis or postprocessing procedures.

MULTISET CLASSIFICATION BY MCR-ALS DISCRIMINATION APPLIED TO WINE AUTHENTICATION

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Recently Multivariate Curve Resolution (MCR) methodology has been extended to regression tasks by implementation of the so-called correlation constraint [1], which performs an internal regression model that allows the prediction of analyte concentrations or other properties of interest. So far, this constraint has been applied for quantitative analysis [2,3]. This constraint, as any other one, is applied to some or all the profiles in the concentration matrix **C,** performing univariate calibration models per each compound, and to all or to some sets in a multiset structure. The present work is the first attempt to adapt the correlation constraint for discrimination purposes. It is important to note that, in this context, the typical MCR model, $\mathbf{D} = \mathbf{CS}^{\mathbf{T}}$ has to be reinterpreted. Thus, **D** rows contain the samples information that may allow distinction of classes, i.e., spectra, chromatographic fingerprints,…; **C** is the matrix of class membership profiles, which will contain class membership information in some profiles and variance unexplained by class-related information in the rest of profiles and $\mathbf{S}^{\mathbf{T}}$ contains the related qualitative class profiles, i.e., response profiles representative of the samples within a particular class, and some profiles with instrumental response variation unassigned to a specific class. For the application of the correlation constraint the reference values in the regression model hold class membership information. The method performs a regression model during the ALS iterations. Class membership is coded as 1/ 0 for the calibration samples and applies to the suitable profile in the **C** matrix in the MCR model. Univariate classification models are performed on the selected profiles in **C** between the **C-** values calculated at each ALS iteration and the reference class membership information. The model is then used to predict class membership for test samples, which are included in the same **C** matrix. Number of components in the MCR models can be assessed in this case looking at the variation in classification rate (also in CV) and the lack of fit.

Two frameworks are considered to test the performance of the method in the case that more than two classes have to be modeled: the use of separate MCR analysis with one model per each class or the use of a single MCR analysis with a single multiclass model, in analogy with the PLS1-DA/ PLS2-DA contexts.

The proposed methodology is useful to extract class information from multiset data, such as chromatography with a multi-wavelength or mass spectrometry detection. In this case, classical multiset MCR yields the resolution of the chromatographic peaks as a preliminary step to provide a reduced dataset represented by the matrix containing area values of the resolved peaks. This reduced matrix of peak areas is used as starting information for the discriminant MCR.

The potential of this method is tested on a problem of authentication of Lambrusco wines, i.e. different geographical production or different PDO denominations. The results provide class membership profiles and, equally important, chromatographic fingerprints associated with each one of the classes. The results obtained are compared with those coming from SIMCA and PLS-DA analysis.

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DETERMINATION OF DICHLOBENIL AND ITS MAJOR METABOLITE (BAM) IN ONIONS BY PTV–GC–MS AIDED BY PARAFAC2 DECOMPOSITION AND EXPERIMENTAL DESIGN METHODOLOGY

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Many papers have been published about determination of the herbicide dichlobenil and its main metabolite 2,6-dichlorobenzamide (BAM) using GC analysis in water [1]. Analysis of dichlobenil in food commodities have also been reported (in fish, shellfish, cabbage…), but applications are hardly found where both pesticide and metabolite are simultaneously determined in complex matrices by GC. For example, Pang et al. [2] determined both compounds in animal tissues together with many other pesticides by GC–MS, and reported LOQs for dichlobenil and BAM of 5 and 50 μg kg^{-1} respectively. Like most transformation products of pesticides, BAM is more polar and less volatile than dichlobenil, so it might require a previous derivatization step to increase sensitivity. But the derivatization reagent (trialkiylsilyl) can form unexpected derivatives as silylation artifacts resulting from reactions with itself, organic solvents, etc. [3] which not always can be avoided. These artifacts lead to unexpected components and to confusion about the unequivocal identification of the analytes.

Three-way techniques of analysis have been shown to be very useful in determining target compounds in food commodities [4] by solving problems with co-eluting interferents and with little shifts in the retention time. In this work, parallel factor analysis 2 (PARAFAC2) decomposition is used in the simultaneous determination of dichlobenil and BAM in onions by programmed temperature vaporization (PTV)–GC–MS. Target compound are extracted from onions with a QuEChERS modified procedure which involves a dispersive solid phase extraction (dSPE) step. Subsequently, extracts are derivatized with BSTFA (*N*,*O*–bis(trimethylsilyl)trifluoroacetamide) and injected into the chromatographic system.

Optimization of some experimental parameters is performed using the experimental design methodology. A central composite design and two D-optimal designs coupled to PARAFAC2 are used to select the best conditions of the derivatization (time, reagent volume and temperature), extraction (mixing and centrifugation times, rpm…) and PTV injection (inlet pressure, vent flow and time, PTV conditions, injection speed…) steps. The use of a PARAFAC2 decomposition allows unequivocal identification according to document SANCO/12495/2011 (in all cases, relative retention time and at least 3 relative ion abundances are within the corresponding tolerance intervals). The EU established a maximum residue level (MRL) of 50 μg kg^{-1} of dichlobenil in bulb vegetables as onion in Reg. (EC) No 149/2008. This Regulation shall continue to apply to products which were lawfully produced before 26 April 2013 when it will be amended by Reg. (EU) No 899/2012, which establishes a new MRL of 20 μg kg^{-1}. The detection limits (CCα) found are below the latter MRL.

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A WAVELET-BASED MULTIVARIATE IMAGE ANALYSIS APPROACH TO DETECT DEFECTS IN ORANGES

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The use of image analysis represents a fast and non-invasive low-cost methods to be applied on products and processes, thus presenting a growing appeal for on-line monitoring of food products, for a fast defects detection. Multivariate Image Analysis has its bases on the Multivariate Statistical Process Control paradigm: Normal Operating Conditions images are used to build a reference PCA model, then multivariate control charts are used for fault detection, with the possibility of locating the faulty pixels in the original image. This is particularly useful, as in this application, when different kinds of defects are present, and localization helps in the task of building reference models for each of them. In this presentation, the following procedures are considered: i) pixels-wise unfolded wavelet transformed images at different resolution are used to obtain multivariate control charts, thus maintaining defects localization; ii) a WT decomposition block at a given level which highlights some defects is used as an input image to which apply traditional MIA.

The basics of the WT-MIA approach are as follows: i) given a wavelet filter, the image is decomposed to a given level, L, by using the 2D-DWT, applied separately to each color channels; ii) each decomposition block is reconstructed to the original image domain, obtaining a reconstructed image for each block and level: a total of 4 (Approximation, Horizontal, Vertical and Diagonal coefficients) times L (number of decomposition levels) times N (color channels) images are obtained; iii) MIA is applied to the images, unfolded and united so that a data matrix of dimensions: pixels × (4×L×N) is obtained; iv) multivariate control charts for Hotelling-T^2 and residual sum of squares on the basis of one or few normal operating images (NOC) are built and defects are detected in faulty ones. This procedure represents a strong reduction of data matrix dimensions when compared to the Bharati and MacGregor's approach [1]. In the latter, data dimensionality depends on the number of neighbouring pixels to be considered, in general: N (channels) × $(2w+1)^2$, where w stands for the considered window/lag. [2]. Thus, a window of size 2 yields 25 variables per channel, which roughly corresponds to a 2D-WT decomposition at level 7 (28 variables per channel), a case in which a high resolution is achieved.

In the application, a preliminary step is performed to determine the best wavelet family to characterize the surface expression of an orange disease. To this aim, reference images representative of each kind of defect were selected to assess the best wavelet filter and decomposition scheme (DWT or SWT). The WT decomposition (in the example, a haar wavelet was used) and its PCA analysis gives an insight on which components are more related to the phenomenon of interest (score images) and which bands and decomposition images contribute to it (loadings). The extraction of the PCs of interest to obtain T^2 or residual images helps in the detection of the defects and of the area affected by the disease, as shown for instance in Figure 1, where the T^2 image is compared to the original one. The model can then be used to assess test images to highlight the defective areas.

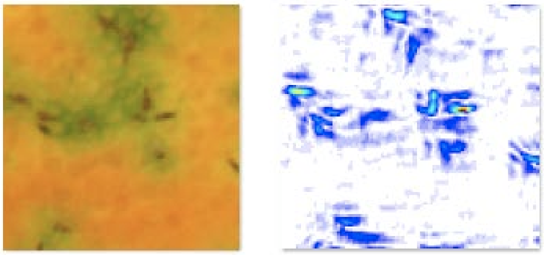

Figure 1: Original image and T^2 image based on the PC's of interest.

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MULTISET ANALYSIS OF MULTITECHNIQUE RAMAN AND IR HYPERSPECTRAL IMAGES

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Hyperspectral imaging is an active area of research that has grown quickly during the last decade. Hyperspectral imaging techniques based on Raman, infrared and fluorescence spectroscopy are useful methods in different areas, such as polymer research, materials science, biomedical diagnostic, pharmaceutical industry, analytical chemistry, process control and environmental analysis.

Multiset type of analysis has been extensively used in process analysis of different experiments monitored with the same technique (column-wise augmented matrices) or of a single experiment monitored with several techniques (row-wise augmented data matrix) [1]. In the context of hyperspectral images, there already exist examples where the simultaneous analysis of several images with the same acquisition system is performed, e.g., 3D multilayer images of multiple processes or in quantitative analysis In contrast, multiset analysis has been still seldom used for the analysis of multiple images obtained from the same sample, using different techniques (e.g. Raman/Infrared). Possible reasons for this are the difficulty to acquire joint data sets on the same spatial section (translation, /rotation differences among images) and the differences of spatial resolution (pixel size and definition) among different techniques.

From a data analysis point of view, multivariate resolution techniques are very suited for the simultaneous analysis of several images. The aim of hyperspectral image resolution methods is to provide distribution maps and pure spectra related to the image constituents of a sample from the information originally contained in the measured raw image. Multivariate Curve Resolution-Alternating Least Squares (MCR-ALS) is an iterative resolution method oriented to recover the underlying spectroscopic bilinear model, i.e., concentration profiles (folded back into distribution maps) and pure spectra, by applying constraints related to chemical or mathematical properties of the profiles to be resolved [2,3]. Single image analysis by MCR is often used, but it may be easily extended to the analysis of multiset structures formed by several images [2,4].

As mentioned above, few studies have been reported yet, the joint use of different spectroscopic imaging techniques to analyze the same sample. To apply this strategy, identification of preprocessing procedures envisioned at matching image contours and balancing the different spatial resolution (pixel size) of the coupled techniques are needed. To explore this multiset problem, images of pharmaceutical mixtures of the same sample acquired with different imaging systems (Raman and IR), are submitted to multiset image resolution to enhance the structural description of the image constituents. In doing this, the complementary spectral information obtained can help to differentiate more clearly between possible pharmaceutical polymorphs, e.g., with the Raman information, or to improve the modelling of less crystalline components, e.g., with the IR information, both problems more difficult to be solved when an image from a single acquisition system is used.

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HANDLING LARGE DATASETS OF HYPERSPECTRAL IMAGES

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In the context of HyperSpectral Imaging (HSI) techniques, the large file size of each acquired hyperspectral image represents the main issue in handling datasets composed by a wide number of samples. The size of each hypercube, which is a three-dimensional array consisting in more than one hundred of congruent images composed by tens of thousands of pixels, is generally greater than 50 MB. The compression of the useful information contained in each hyperspectral image into an optimized set of few parameter values is therefore essential for analysing datasets formed by a large number of images, as well as for enabling on-line monitoring. In order to solve this problem, a chemometric strategy is proposed to reduce significantly the dataset size, allowing to analyse at the same time from tens up to hundreds of hyperspectral images. This procedure is derived from the colourgrams approach, already developed for the elaboration of RGB images [1-3], and essentially consists in compressing the useful information contained in each hypercube into a one-dimensional signal, named hyperspectrogram, which can then be used as a compact set of descriptors for further blind analysis techniques. More in detail, hyperspectrograms are created by merging in sequence the frequency distribution curves of the scores, Q residuals and of the Hotelling T^2 vectors obtained from a PCA model calculated separately for each HSI. By adding at the end of the signal the PC loading vectors, the hyperspectrogram preserves also the most relevant spectral features of the hypercube data. Since the new hyperspectrogram dataset still conveys all the variability of the original hypercube dataset, it can be subjected to explorative analysis, enabling a complete overview of each dataset and allowing to easily identify possible outlier samples. In addition, this approach can also be applied for the calculation of robust calibration and classification models based on large number of samples. In this context, a further improvement both of data compression and of calibration/classification performances can be obtained by applying a proper variable selection method to the hyperspectrograms dataset. A visual evaluation of the correctness of the choices made by the feature selection algorithm can be achieved by representing the selected spatial features back into the original image domain. Likewise, the interpretation of the chemical information underlying the selected regions of the hyperspectrograms related to the loadings is enabled by projecting them in the original spectral domain. Examples of applications of the hyperspectrogram-based approach to face calibration and defect identification issues using hyperspectral images of food samples demonstrate the effectiveness of the proposed procedure. Further applications of the hyperspectrogram approach, which cover the possibility to monitor the instrumental stability over time as well as to investigate dynamic processes, are also presented.

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MULTIVARIATE DATA ANALYSIS OF A BATCH POLYMERIZATION REACTION WITH STABILITY PROBLEMS

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Batch process is nowadays widely diffuse in various fields such as pharmaceutical, alimentary and petrochemical industry. In versalis, the eni group chemical company, as an example batch process is used for production of Expandable Poly Styrene (EPS); EPS is a key material for building insulation. Styrene monomer conversion occurs in water suspension and critical stability is maintained by stabilizer (e.g. TCP, PES, PVA). A crucial aspect of EPS production is the collapse of small styrene particles dispersed in water that must be avoid. The decision to investigate both process data and water quality data with multivariate data analysis consented a better understanding of trouble occurred in EPS plant, not clearly explained by traditional approach. The study began in 2010, first step was collection of historical data, since 2005, and then starting with real time data, in the following months. The aim of the present case study was to establish a cause of trouble in a plant apparently in the same condition since ever.

Also water data, collected each day from treatment water plant, was analyzed. The overall data were analyzed by MVDA, the results obtained pointed a change of the water properties since 2005 as correlated with batch problems. These results were taken into account to project new treatment technology for the water feed to the EPS plant.

Results show the suitability of such a supervised strategy to identify human tumour tissue in, which can be used as a valuable tool in cancer diagnosis, complementary to traditional histopathological tissue examination.

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SOIL CLASSIFICATION FOR FORENSIC PURPOSE BY USING SCANNING ELECTRON MICROSCOPY WITH X-RAY ANALYZER, COLOR ANALYSIS AND CHEMOMETRIC TOOLS

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Soil forensic evidence samples are very difficult to process, due to the great number of general and individual characteristics present at the same time, and the low discriminative information that the surface layer has, which is the one that is collected in crime scene or in shoes and other objects belonging to a suspects.

The purpose of this paper is to demonstrate the feasibility and benefits of the Scanning Electron Microscopy with X-ray Analyzer coupled (SEM-EDS) for the analysis and chemical characterization of soil samples of one municipality of Havana City, and the construction of an automatic classification model for soil samples discrimination, in correlation with the present-day genetic classification existing of this place, in order to predict the origin and soil type in unknown samples related with a case, using Chemometrics tools for the multivariate processing of the data.

Data from the SEM-EDS analysis was submitted to an exploratory analysis using Principal Component Analysis (PCA) and Hierarchical Clusters Analysis (HCA), demonstrating the feasibility and differentiation of 6 types of soils of the 9 presents, when the color parameter is introduced. The elements Mg, Si, K, Ca, Ti, Fe and Al were the most significant elements in the discrimination. From these results a model for the automatic classification for 6 types of soil samples was constructed using a Support Vector Machines (SVM) classifier. The model was validated with external samples not present in the training set with 97 % of accuracy. Another model was built from the data obtained with SEM-EDS, the analysis of color with the Munsell charts, and the color parameters of the Image Editor of Windows Adobe

Photoshop 7.0 (R, G, B, C, M, And, K, H, S, B1, L, a, b), achieving a differentiation in 9 types of ground by means of PCA and HCA, with the construction of an automatic classification model of the 9 classes obtained, having as best classifier the SVM. The model was validated with external samples with 100 % of accuracy.

CLASSIFICATION AND PREDICTION METHODS FOR COMPREHENSIVE GAS CHROMATOGRAPHY MASS SPECTROMETRY

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Comprehensive gas chromatography mass spectrometry (GC×GC-MS) enables separation of hundreds of organic compounds in complex samples. Fig. 1 exemplifies a typical dataset measured on pyrolysis oil from peat visualized by a bubble plot. Each bubble represents a chemical compound and the bubble diameter is proportional to the area of the peak. In addition, the electron impact mass spectrum is available for each compound. Although the chromatogram is structured by distinct areas populated by certain compound classes, interpretation of the whole 3D-datasets remains a tedious task and needs to be automated by means of appropriate chemometric methods.

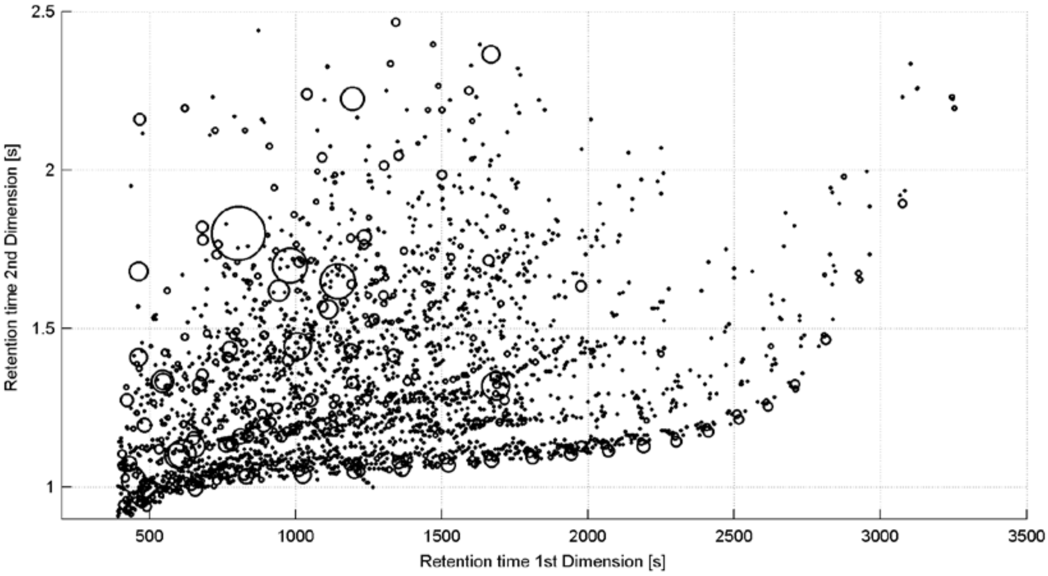

Fig. 1 Comprehensive chromatogram of a pyrolysis oil from peat

To model the appearance of structurally related compounds in the separation plane *polygons* have been proposed [1]. Their use, however, is limited if compound classes overlap as is to be expected in complexly composed samples. In this work we exploit the similarity of mass spectra within a given compound class in order to model and classify the individual compounds. The classification model is based on 69 reference compounds of different chemical structures. The peak lists of chromatograms of the reference compounds with their retention times, peak areas and mass spectra are imported into Matlab. Then, the mass spectra are transformed into different features, such as, *modulo-14 summation* [2]. The resulting matrix is decomposed into principal components and the scores from PCA are modeled on the compound classes by *linear discriminant analysis*. Prediction of unknown compounds is feasible after pretreatment of the peak and mass spectral information in the same way as for the reference compounds. The *likelihood* of class assignments is calculated from Bayesian statistics as the posterior probability [3]. The classification results will be discussed in dependence on the posterior probability threshold as well as the number and area of peaks that can be classified in relation to the total number of peaks and their area, respectively.

In order to predict separation areas for new compound classes, quantitative structure property relationships (QSPR) were developed. The chemical compounds are coded by molecular descriptors calculated by the software Dragon 6 [4] and regressed on retention times in the two GC-dimensions. Algorithms for selection of features from the almost 5000 descriptors are discussed together with the results of predicting the retention behavior of new compound classes.

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MOVING TOWARDS CONTINUOUS MANUFACTURING: SUCCESSFUL REAL -TIME MONITORING AND EARLY FAULT DETECTION.

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Multivariate (MV) analysis methods are powerful tools for characterizing process performance and linking the connection between raw material quality, the manufacturing environment and the resulting product quality. This presentation discusses the application of multivariate analysis methods on continuous unit operations with case studies to identify and resolve production issues in real-time. The multivariate model of the process may be used to represent a design space for identification of abnormal operation. Optimization methods are applied to the multivariate models to identify target values for key process variables and mid-course correction of continuous process in real-time.

The map of the process from initiation to completion can be summarized into a multivariate signature, or fingerprint. Comparison with this historical fingerprint provides quality assurance and indicates the state of the process. These MVA models may be executed in real-time using online monitoring and for identifying the root-cause of problems as they occur in a continuous process.

Multivariate tools can assist in continuous process monitoring and final quality control by

i) Analyzing Variation

The final product quality depends on process conditions as well as on the variation of initial conditions such as raw materials due to vendor differences. Multivariate data analysis can visually summarize these variations and relate them to process and product quality more effectively than the traditional univariate acceptance criteria in use today.

ii) Identifying and Monitoring of Critical Quality Attributes

For processes of a continuous nature, identification of out-of-control (OOC) operation is performed at discrete snapshots in time. Even for large volumes of complex data, multivariate analysis tools can, in real time, identify key quality attributes.

iii) Real-time Process Monitoring with early Fault Detection

Traditionally you must wait until the end of a quality test to ascertain its quality. The final product quality (release testing) is determined in the QC lab. If a below spec result is detected, it is very difficult to discover the root cause and it is too late to correct the problem.

The four actionable takeaways from our presentation will be:

1. Prevent Product Loss:

2. Design & Control Space Monitoring

3.Final QC Prediction

4.Process Control

TRANSFERRING THE MANUFACTURING OF A PHARMACEUTICAL NANOPARTICLE PRODUCT BETWEEN DIFFERENT PLANTS

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In this work, the problem of transferring a product between different devices is considered for the manufacturing of pharmaceutical nanoparticles. The problem under investigation is the estimation of the operating conditions in a target device which ensure a product of assigned properties that has already been manufactured in a source device of different geometry.

Despite a limited historical database is available for the target device, a large historical database is available for the source device and can be exploited to support the transfer. One of the major issues in the transfer is that the difference in device geometries causes a completely different mixing behavior and performance in the devices. Furthermore, the product transfer problem is complicated by the fact that the target plant could only be run under a setup that is different from that under which the available historical dataset had been obtained.

A joint-Y projection to latent structures (JY-PLS) model inversion approach [1] is used to transfer the nanoparticle product from the source device to the target device. In particular, two specific problems are studied. In the first one, JY-PLS inversion is used to estimate the conditions in a target device with the new experimental setup to manufacture nanoparticles with an assigned mean size. In the second problem, JY-PLS inversion is used to design the experiments in order to obtain nanoparticles with mean size below an assigned threshold. The experiments confirmed the effectiveness of the proposed procedure in designing the target device operating conditions in such a way as to obtain nanoparticles of assigned size range.

Finally, since the inversion of the JY-PLS model generates an infinite number of solutions that all lie in the so-called null space [2], experiments are carried out to provide for the first time an experimental validation of the null space.

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MULTIVARIATE DATA ANALYSIS OF A BATCH POLYMERIZATION REACTION WITH STABILITY PROBLEMS

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Batch process is nowadays widely diffuse in various fields such as pharmaceutical, alimentary and petrochemical industry. In versalis, the eni group chemical company, as an example batch process is used for production of Expandable Poly Styrene (EPS); EPS is a key material for building insulation. Styrene monomer conversion occurs in water suspension and critical stability is maintained by stabilizer (e.g. TCP, PES, PVA). A crucial aspect of EPS production is the collapse of small styrene particles dispersed in water that must be avoid. The decision to investigate both process data and water quality data with multivariate data analysis consented a better understanding of trouble occurred in EPS plant, not clearly explained by traditional approach. The study began in 2010, first step was collection of historical data, since 2005, and then starting with real time data, in the following months. The aim of the present case study was to establish a cause of trouble in a plant apparently in the same condition since ever.

Also water data, collected each day from treatment water plant, was analyzed. The overall data were analyzed by MVDA, the results obtained pointed a change of the water properties since 2005 as correlated with batch problems. These results were taken into account to project new treatment technology for the water feed to the EPS plant.

A REVIEW ON THE APPLICATIONS OF PORTABLE NEAR INFRARED SPECTROMETERS IN THE AGRO-FOOD INDUSTRY

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Industrial purposes created the need for a cost-effective and non-destructive quality control analysis system [1]. This requirement increased the interest for NIR spectroscopy leading to the development and marketing of handheld devices, enabling new applications to be implemented in-situ at an industrial level [2]. Handheld NIR spectrometers are powerful instruments offering several advantages for non-destructive, on-line or *in-situ* analysis: small size; low cost; robustness; ease of analysis; sample user interface; portability and even ergonomic design. Several studies were made using NIR applications on-site: quality of meat; types of coffee and its content; octane; quality of olive oil; rice and milk protein and fat content; sugars and acidity of wine grapes; and soil analysis.

Chemometrics is an essential part of NIR spectroscopy, whose spectra may be complicated by wavelength dependent scattering effects; instrumental noise; ambient effects and other sources of variability. As a consequence it is difficult to assign specific absorption bands to specific functional groups. To achieve useful and meaningful results, multivariate statistical techniques (involving essentially regression techniques coupled with spectral preprocessing) are, therefore, required to extract the information hidden in the NIR spectrum. The NIR data calibration and spectra interpretation depend on chemometric methods, and this dependence has resulted in the co-evolution of the two fields.

The aim of this work is to review the evolution of portable NIR spectrometers with special emphasis on the application in the agro-food industry.

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CAN LAME COWS BE DETECTED WITH AUTOMATIC MILKING SYSTEM DATA?

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Lameness is a major production threat in dairy herds and may be of special interest in herds with automatic milking systems (AMS) due to decreased attendance to the milking robot. Undetected and thus untreated lame cows constitute a source of production losses – up to 570 Kg of milk/ cow/ lactation [1] – and can experience poor animal welfare due to chronic pain. The objective of the present study was to explore multivariate data collected in the AMS during milking process and build a classification model that could discriminate lame and not lame cows.

A Danish dairy farm with around 150 milking cows and two DeLaval VMS robotic milking units participated in the study. Gait scores of all milking cows were done at weekly intervals during 5 weeks in autumn 2012, inside the free-stall area by encouraging each cow gently to walk and in a random order. Non-milking and fresh cows (day 0 and 1) were excluded. Lameness was assessed using a 4-point scale adapted from DairyCo® scoring system [2]: score 1 - even, long and fluid strides (not lame); score 2 - uneven steps but the limbs favoured were not obvious (not lame); score 3 - one or more favoured limbs obviously detected (lame); score 4 - very reluctant to put weight on one or more limbs (lame). The variables obtained from the AMS were summarized week-wise. The mean or sum of 7 days was calculated for each variable and associated with the respective cow's lameness score at the end of each week. The variables included in the model were: average of days in milk, average of milking duration, average of milking interval, sum of milk yield, average of milk produced per hour, sum of kicks during milking, average of average flow and of peak flow (both variables on quarter level). Neither lactation number nor parity groups were included in the model. The classification models were developed by using Partial Least Squares-Discriminant Analysis method, including only the extreme lame scores 1 and 4 in the model in order to identify which variables could be more meaningful. All data was autoscaled prior analysis. Cross-validation was done by estimating the class of the available weekly scores of each cow at a time. The analysis was done by using the PLS-Toolbox v.7.0.2 (Eigenvector Research, WA, USA) working under MATLAB® v.7.14 (The Mathworks A.S, MA, USA) environment.

A PLS-DA model with 6 Latent Variables was selected which explained 84% of the variation. The variables associated with lame cows were milking duration, days in milk and milking interval in the first latent variable, while in the second latent variable were average flow and peak flow variables but also milking interval and milking duration. Opposite to this, not lame cows were associated with peak flow and average flow variables, weekly milk yield, milk produced per hour and number of kicks in the first latent variable; whereas in the second latent variable only the last three were associated with the not lame class. The sensitivity and specificity of the calibration model were 0.66 and 0.83, respectively; while the cross-validated sensitivity and specificity were 0.55 and 0.76, respectively. The cross-validated classification error was then 0.35.

In conclusion, milking related data seemed to retain information associated with lameness conditions and could be useful to automatically detect lame animals. A clear future challenge will be the inclusion of not only more cows and farms but also more information in the models about each individual cow, such as reproduction, production or activity parameters. This could help to improve the current results, namely achieving a farmer-friendly (higher) specificity which can minimize the number of false positives.

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D-OPTIMAL DESIGN AND PARALLEL FACTOR ANALYSIS: USEFUL TOOLS IN THE IDENTIFICATION AND DETERMINATION OF PRIMARY AROMATIC AMINES BY MEANS OF SPME-HS-GC/MS

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Primary Aromatic Amines (PAAs) are widespread chemicals in several industries. They are used in the manufacture of rubber chemicals, pesticides, dyes, pharmaceuticals and photographic chemicals. Their major use, however, is in the production of rigid polyurethanes and polyamide kitchenware. Several PAAs have been classified as ″possibly carcinogenic to humans″ by the International Agency for Research on Cancer (IARC). Hence, their presence in foodstuffs should be avoided. According to the present European Legislation, plastic materials and articles shall not release PAAs in a detectable quantity into food or food stimulant. This detectable quantity level is 0.01 mg of substance per kg of food or food simulant. This detection limit applies to the sum of primary aromatic amines released as it is specified in the COMMISSION REGULATION (EU) No 10/2011 of 14 January 2011 [1] on plastic materials and articles intended to come into contact with food.

A headspace solid-phase microextraction and gas chromatography coupled with mass spectrometry (HS-SPME-GC/MS) method for trace determination of primary aromatic amines was developed. The following analytes were investigated: aniline (A), 4,4’-methylenedianiline (4,4’-MDA) and 2,4-toluenediamine (2,4-TDA) using 3-chloro-4-fluoroaniline and 2-amimobiphenyl as internal~~s~~ standards. Prior to extraction the analytes were derivatized in the aqueous solution by diazotation and subsequent iodination [2]. The derivatives were extracted by SPME using a PDMS/DVB fiber.

A D-optimal design was used to study the parameters affecting the HS-SPME process and the derivatization step. Two experimental factors at two levels and one factor at three levels were considered: (i) reaction time, (ii) extraction temperature, and (iii) extraction time in the headspace.. The loadings in the sample mode estimated by a PARAFAC (PARAFAC2) decomposition for each analyte were the response used in the design because they are proportional to the amount of analyte extracted.

A calibration based in a PARAFAC decomposition provided the following values of CCα: 1.66, 1.74, and 2.36 μgL^{-1} for A, 2,4-TDA 4,4'-MDA respectively for a probability of false positive set at 5%. Also, the accuracy (trueness and precision) of the procedure is assessed. PARAFAC decomposition made it possible to identify unequivocally (according to the maximum permitted tolerances for relative ion abundances) and quantify each analyte. This identification, based on mass spectra and retention times guaranteed the specificity of the procedure.

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[1] COMMISSION REGULATION (EU) No 10/2011 of 14 January 2011

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ACTUATOR PERFORMANCE COMPARISON BY DOE AND PCA

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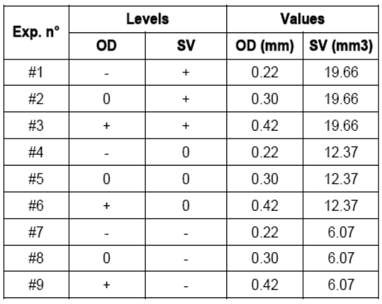
Goal of the study: The medication contained in a Pressurized Metered Dose Inhaler (pMDI) is delivered to the patient through an actuator that provides the spray atomization. The evaluation of the pMDI efficiency in the atomization process is carried out by performance tests. These tests allow to quantify the amount of the medication reaching the small peripheral airways of the lungs. Geometrical actuator parameters, such as orifice diameter (OD) and sump volume (SV), could strongly affect the atomization process. OD is the diameter of the exit orifice where the atomization takes place while the sump is a small expansion chamber before OD. The goal of this work was to determine the effect of OD and SV on the different responses obtained by the performance tests.

Experimental: The variables were set according to the Face Centered Design (FCD) summarized in Table 1. The nine experiments were carried out in triplicate, in random order. The chosen performance tests were the Delivered/Metered Dose tests evaluated by Unit Spray Collection Apparatus (USCA) at 28.3 L/min and Aerosol Particle Distribution evaluated by Next Generation Impactor (NGI) at 60 L/min. Both tests were evaluated by validated HPLC/UV methods, on a pMDI under development, leading to 15 responses. On the resulting data set (27 rows by 15 columns) a Principal Component Analysis (PCA) has been performed.

Table 1: FCD matrix



Exp. n°	Levels		Values	
	OD	SV	OD (mm)	SV (mm3)
#1	-	+	0.22	19.66
#2	0	+	0.30	19.66
#3	+	+	0.42	19.66
#4	-	0	0.22	12.37
#5	0	0	0.30	12.37
#6	+	0	0.42	12.37
#7	-	-	0.22	6.07
#8	0	-	0.30	6.07
#9	+	-	0.42	6.07

Discussion and conclusions: The collected NGI responses were: FPM (Fine Particle Mass, 1), that is the amount of particles with aerodynamic diameter ≤5µm, FPF (Fine Particle Fraction, 2) that is the percentage of FPM on the amount of drug delivered to the patient, the depositions on the impactor stages such as Actuator (3), Induction Port (4), Cup 1 to Filter (from 5 to 12) and MMAD (13) that represents the calculated mass median aerodynamic diameter. The USCA responses were Actuator deposition (14) and Delivered Dose (15). Figure 1 shows the loading plot. The first two components explain about 76% of the total variance. It is possible to highlight a group of responses with high positive loading on the first component (group A, namely 1, 2, 7-12), opposite to response 4 (group C). This confirms that the greater the Induction Port deposition (response 4) the lower the responses of group A. Moreover, this shows that the measurement of the deposition at each single cup is redundant, because all these responses are strictly correlated. The second component highlights a correlation among responses 5, 6 and 13 (group B), opposed to response 3 (group D). Responses 14 and 15 have lower loadings on both components, this meaning that they give a very poor contribution to the system description. Since they are the two responses obtained by the USCA analysis, this test can be considered useless to differentiate the performance of the actuators. In the score plot shown in Figure 2 the experiments are coded according to the levels reported in Table 1. It can be seen that the first variable (OD) has a clear linear effect on the first component, since its increase corresponds to lower scores on it (and therefore higher values of response 4 and lower values of the responses of group A). It also has a quadratic effect on the second component, with the experiments with an intermediate OD having the highest scores, this meaning higher values of responses 5, 6 and 13 and lower values of response 3. The second variable (SV) has no effect on the first component, whereas the experiments with larger SV have lower scores on the second

Figure 1: Variable loadings on Components 1-2 (76% of variance)

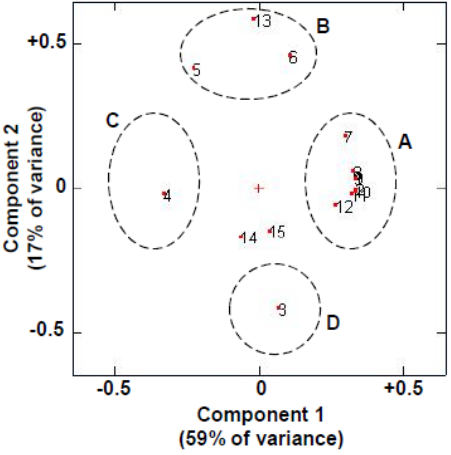


Figure 2: Object scores plot on Component 1-2 (76% of variance)

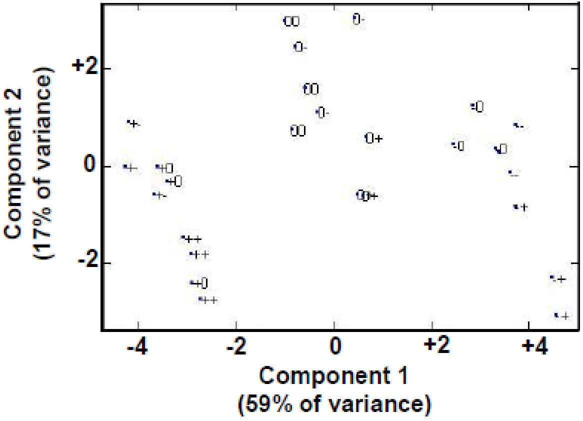


Figure 2: Variable loadings
on eigenvectors 1-2 (76% of total variance)

component, this corresponding to lower values of responses 5, 6 and 13 and larger values of response 3. In other words, the reduction of SV did not affect significantly the actuators performances that are, on the contrary, largely affected by the OD size.

APPLICATION OF CURVILINEAR COMPONENT ANALYSIS FOR CLUSTER VISUALIZATION IN HIGH-DIMENSIONAL EXPERIMENTAL DESIGNS

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1. Introduction

In domains such as oil industry, astronomy, optics, etc... experiments are expensive and time consuming. Therefore, phenomena are often studied using numerical simulations, but the time of calculation can be very long because the models are increasingly complex, involving a large number of coefficients. In this case, designs of experiments could be useful to select the simulations to run but we need specific experimental designs that explore the whole domain in a reasonable number of simulations. The Space-Filling Designs (SFD) propose a uniform distribution of the points in the space and are well adapted to numerical simulations. However it is known that all space-filling designs are not equivalent in terms of quality criteria (as mindist, coverage ...) which characterize the uniformity of the points distribution. Moreover, in high dimension, some designs can present clusters or holes which could be penalizing for the modelling step.

2. Curvilinear Component Analysis

Thus, we need methods in order to visualize data by reducing dimensionality while keeping the maximum of information. Classically, this reduction could be obtained from a linear method such as Principal Component Analysis (PCA)[1] but this method doesn't consider dependence between variables. Therefore we suggest using a non linear method called Curvilinear Component Analysis (CCA)[2, 3] based on the Kohonen Self-Organizing Maps (SOM)[4]. This method brings improvements: the output space isn't a grid fixed *a priori* but a continuous space able to take the best adapted shape to the data. The objective is to reproduce initial space in a low dimensional mapping through a neural network. The theory is based on respect of local topology where short-range output distances are respected, that allows the detection of two close points and therefore the localisation of clusters.

The principle is to minimize a criterion characterizing topology differences between initial space and projection space.

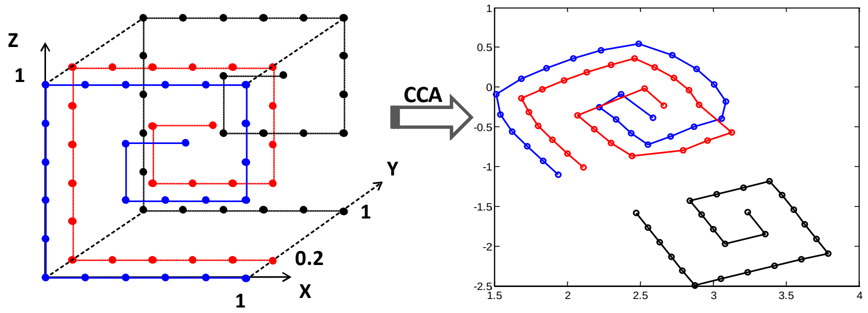
$$E_{CCA} = \frac{1}{2}\sum_{i}\sum_{i\neq j}\left(d_{ij}^{n} - d_{ij}^{p}\right)^{2} F_{\lambda}\left(d_{ij}^{p}\right)$$

where, d_{ij}^{n} (respectively d_{ij}^{p}) is Euclidean distance between x_i and x_j vectors in original space in n dimensions (respectively p dimensions).

Generally[4], $\boldsymbol{F}$ is chosen as a monotonic positive decreasing function, in order to favor local topology conservation. The most widely used is:

$$F_{\lambda}(d_{ij}^{p}) = \begin{cases} 1, & d_{ij}^{p} \leq \lambda \\ 0, & d_{ij}^{p} > \lambda \end{cases}$$

For example, data are a set of points describing three spirals in the plane (x, z) spaced on the third axis y. Blue and red spirals are respectively on $y = 0$ and $y = 0.2$ planes whereas black spiral is on $y = 1$.



By using CCA, the closest spirals are mixed whereas the farthest spiral is isolated. By this example, we show that CCA allows the unfolding of this set of points by remaining low distances, and therefore close points.

3. Study case: comparison of Space Filling Designs in 20 dimensional space

A new criterion calculated from results obtained after CCA allows the comparison of high-dimensional experimental designs quality, in terms of uniform distribution. An example in 20 dimensions will be presented and several designs as random distribution, WSP design and low discrepancy sequences as Sobol' and Faure will be studied by detecting and locating the clusters.

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MULTIVARIATE OPTIMIZATION OF HYDRIDE GENERATION FOR DETERMINATION OF SELENIUM IN EGG SAMPLES BY HG-AFS

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In the optimization of an analytical procedure, there is a need to adjust the variables in establishing the best conditions to perform the analyzes. [1]. The experimental design is an important statistical tool, and because of its simplicity, is being increasingly used by analytical chemists for different samples and purposes that may confer or not the hypotheses of the research [2]. The response surface methodology (RSM) is an optimization technique for complete procedure, which is a multivariate technique that fits, mathematically, the experimental domain studied in the theoretical design by use of a response function. In the most current research, there are several second-order designs that can be employed for this purpose, such as the Central Composite Design (CCD), Box–Behnken Design (BBD) or Doehlert matrix [2]. They have been used to determine the real function established among analytical response and the factors chosen for experimental evaluation.

In this paper, the factorial and Doehlert designs were applied to optimise the process pre-reduction and hydride generation to determine overall selenium in in samples of eggs by HG-AFS. The factorial and Doehlert designs were applied to optimise the process variables, pre-reduction time, pre-reducing volume, concentration of hydrochloric acid and concentration of sodium tetrahydroborate (Figure 1 and 2).

Using this method, was determined that the optimal experimental conditions were: 20 min. pre-reduction time, 1 mL de pre-reducing, 5.3 mol L^{-1} hidrochloric acid and 2.6 % (w v^{-1}) sodium tetrahydroborate. The samples were decomposed using a system acid digestion with block digestor and cold finger.

For pre-reduction of Se (VI) to Se (IV), were added to 3.0 ml sample, 3.0 ml of 5.3 mol L^{-1} hydrochloric acid, 1.0 mL pre-reducing 10% (w v^{-1}) potassium bromide and 20 min pre-reduction.

The accuracy of the proposed methodology was verified by analyzing a certified reference material (NIST 1566b), testing the addition of analyte and recovery range was 96 to 109% and comparing the data obtained by ICP-MS analysis. The values are agreement at 95% confident level. The LOD and LOQ values obtained were 0.2 and 0.7 ng L^{-1}, respectively, and relative standard deviations were in general lower than 4.7%. The proposed method was applied to samples of chicken eggs, red and white, quail and duck. The results were from 0.35 ± 0.01 to 0.88 ± 0.03 µg g^{-1}. The selenium content was higher in the yolks of eggs in most samples analyzed.

Figure 1: Pareto chart

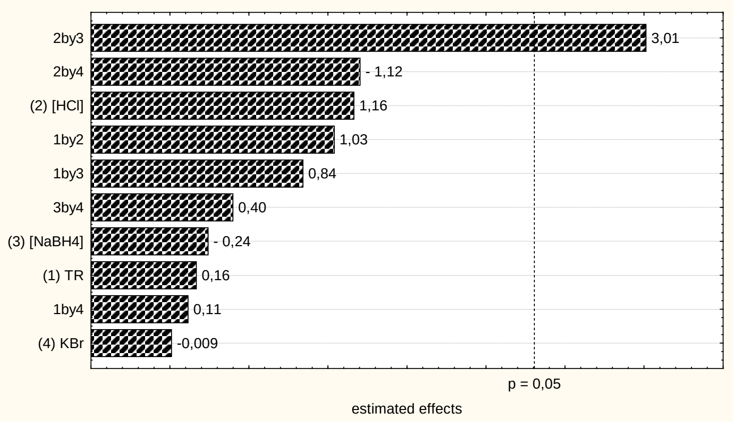
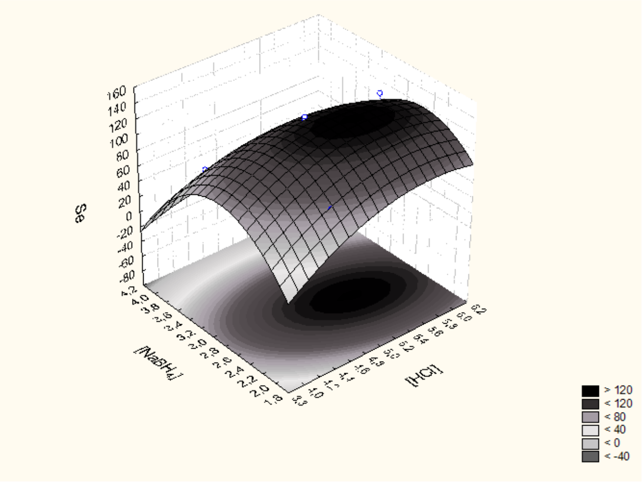


Figure 2: Response surface



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FACTORIAL DESIGN AND DOEHLERT MATRIX FOR OPTIMIZATION OF METHOD FOR DETERMINATION OF AS SPECIES IN CANNED FOODS BY HG AFS

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The arsenic is considered toxic because it has no essential and beneficial characteristics for humans, produces disastrous effects even in trace amounts. This work is based on the determination of arsenic by atomic fluorescence spectrometry with hydride generation (HG AFS) in canned foods (corn and olives). The samples of corn and olives were ground in a knife mill. Then the corn samples were sieved at 500 mesh and olive taken directly for digestion. The procedure for acid decomposition, block digester consists in weighing 0.2 g of the sample followed by addition of 2.0 mL of concentrated HNO_3, 1.5 ml of H_2O_2 30% v.v^{-1} and 1.0 mL of HCl 6.0 mol L^{-1}. The mixture was heated at 120 °C for 3 hours. The conditions for the pre-reduction of As (V) to As (III) and formation of arsine were optimized using full factorial design at two levels (2^4). The variables and their respective optimum levels were: pre-reduction time (10-30 min) HCl concentration (2 - 4 mol L^{-1}), concentration of sodium borohydride (1 to 3% w v^{-1}) and volume of the pre-reducing potassium iodide (0.5 to 1.5 mL). By analyzing the Pareto chart generated by factorial design, we found that two variables were significant: concentration of HCl and sodium borohydride. For the significant variables applied design Doehlert and response surface methodology in order to obtain optimum values. In accordance with the generated surface, the optimal values were obtained: [HCl] = 4.7 mol L $^{-1}$ and [$NaBH_4$] = 3 % w v^{-1}. The statistically significant effects of the variables were studied, and interactions among them were evaluated by applying analysis of variance

(ANOVA) using *Statistica* 7.0 software. All experiments were performed in a random sequence. The Table 1 below shows the concentration of arsenic (ng mL^{-1}) in some samples.

Table 1. Concentration of total arsenic and arsenic (III), in ng mL^{-1}.

Packing	**Samples**	**Total As**	**As (III)**
Olives - Direct Analysis of Net Conserva			
glass	AVRV	14.08 ± 0.27	13.44 ± 0.12
plastic	AVDS	7.44 ± 0.18	6.82 ± 0.03
Olives - Digestion of Food			
glass	AVRV	0.14 ± 0.01	0.03 ± 0.01
plastic	ALVS	0.17 ± 0.01	0.10 ± 0.01
Corn - Digestion of Food			
tin	MGVL	0.15 ± 0.03	0.07 ± 0.04
tetra pak box	MQUC	< LOQ	< LOQ
glass	MQUV	< LOQ	< LOQ

Multivariate optimisation was shown to be an appropriate tool for the optimisation for procedure of pre-reduction and hydride generation; thus it is a suitable method for the determination of arsenic in samples of corn and olives.

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Acknowledgements: PRONEX, FAPESB, CNPq, CAPES.

PLANNING OF MIXTURES FOR DETERMINATION OF PESTICIDES IN SEDIMENT SAMPLES USING HPLC-DAD

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The pesticides include a wide variety of chemicals with different functional groups, modes of action, biotransformation and elimination. Due to intensive and sometimes inappropriate, these pesticides have become very persistent pollutants and contaminants in environmental compartments such as water, air and soil. In the rural area of the Todos os Santos Bay, Bahia, are developed agricultural activities near rivers that bathe this region, where applications are made of various pesticides. Therefore, this paper proposes the use of planning of mixture for three components in the development of analytical procedure for determination of pesticides carbendazim, carbaryl, carbofuran and carbosulfan in sediment samples from rivers Jaguaripe, Mataripe, Paraguaçu and Subaé, of the Todos os Santos Bay, using HPLC-DAD. A planning mixtures of three components (Figure 1) was used to optimize the proportions of the extraction phase of the sediments of suspensions of pesticides in order to determine by HPLA-DAD. The optimized conditions were: sample weight 0.5 g; extraction mixture consisting of methyl alcohol PA (methanol) and hydrochloric acid 0.1 mol L^{-1} (1:1); sonication time of 20 minutes, and time and speed centrifugation 10 minutes and 4000 rpm, respectively. The method was applied to samples of sediments of the Todos os Santos Bay and the results are shown in Table 1.

Figure 1. Composition of mixtures.

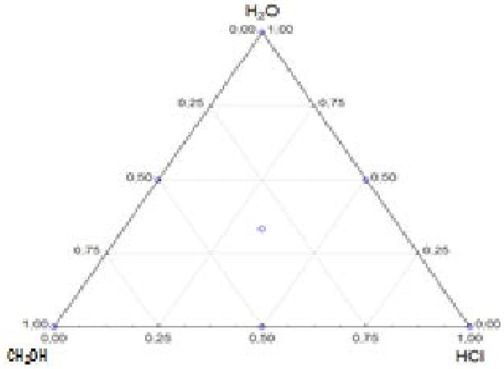

Table 1 - Concentration of pesticides (ppm) determined in sediment samples from Todos os Santos Bay, HPLC-DAD.

	Sediment (river)			
Analytes (ppm)	**Jaguaripe**	**Mataripe**	**Paraguaçu**	**Subaé**
Carbaryl	26,76±1,35	27,28±1,85	27,99±0,85	24,73±1,03
Carbendazim	31,01±3,18	21,88±1,61	25,96±3,21	29,50±1,33
Carbofuran	ND	ND	ND	ND
Carbosulfan	ND	ND	ND	ND

The use of planning of mixtures allowed to select the best extractor in the optimization of an analytical procedure for the determination of pesticides in sediment samples from Todos os Santos Bay.

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QUALITY BY DESIGN APPROACH FOR THE COMPUTER ASSISTED METHODS DEVELOPMENT IN REVERSED PHASE LIQUID CHROMATOGRAPHY

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Computer assisted methods development in liquid chromatography has been evolving along the last four decades to a mature state by providing chromatographers with computer tools helping the development of better separation procedures both in terms of resolution and runtime. The basic mechanism in all these tools is to have a mathematical model of the retention for peaks and the use this model to predict (simulate) chromatograms under a variety of conditions. These chromatograms can be judged in terms of separation quality either by the chromatographer or by any appropriate algorithm thus enabling the selection of optimal conditions for the given separation.

The computer-aided optimized chromatogram should of course be tested experimentally to verify that separation effectively performs as expected and to demonstrate that separation expectancies are really fulfilled. Now the validation of the separation procedure start and the robustness of the procedure is a critical piece of that validation procedure. More recently, the idea of using the concepts of quality by design (QbD) in the development of chromatographic separations have gained importance and actually is accepted in the pharmaceutical and other industries subject to strict performance and operation rules.

Here a computer-assisted methods development tool for reversed-phase liquid chromatography based on QbD principles will be shown with applications to separations developed at high temperatures, transfer processes of developed procedures between chromatographic systems and design space determination.

D – OPTIMAL DESIGN FOR SIMULTANEOUS METHOD DEVELOPMENT ON SILICA, CYANO AND DIOL COLUMN IN HYDROPHILIC INTERACTION LIQUID CHROMATOGRAPHY

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The retention behaviour of substances in hydrophilic interaction liquid chromatography (HILIC) is extremely complex and poorly studied since it includes various mechanisms such as partition, adsorption and ion exchange depending on the analytes, choice of stationary and mobile phase. The aim of this study was simultaneous development of methods for the analysis of the model mixture of six antidepressants on three different columns by changing the percentage of acetonitrile in the mobile phase, pH of the water phase and the buffer type (formic and acetate). The experimental plan created by D – optimal design included the examination of the influence of the chosen factors through 12 experiments and 3 replications. Using the experimental design methodology, the chromatographic behaviour of the analysed mixture was presented with second order polynomials describing its dependence on the percentage of acetonitrile and pH value for all three columns and each of the buffers. Complexed criteria of separation which simultaneously estimates all of the selectivity factors between adjacent peaks, as well as the overall time of the analysis were followed as the system responses. The model was optimized graphically. The adequate separation was shown to be impossible on cyano column, while optimal conditions were established on silica and diol columns, which was proven by the experimantal verification. D – optimal design enabled the resolution of the defined chromatographic problem through only 12 experiments which represents a huge improvement compared to the full factorial design where 54 experiments were necessary. It has been shown that D – optimal design provides reliable development of the complexed chromatography methods in a very efficient way.

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MODELING OF CHROMATOGRAPHIC RESPONSES BY INTERPOLATION POLYNOMIAL WITH DIVIDED DIFFERENCES

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A novel approach to mathematical modeling of chromatographic responses based on interpolation polynomial with divided differences for a function of multiple variables is presented. The proposed technique significantly decreases the modeling error especially when indirect modeling of complex chromatographic responses is performed. It allows accurate description of factors-responses relation in situations where commonly used linear or quadratic models are not applicable: when the nature of dependence is more complex or the investigated factors intervals are broad. The novel technique is incorporated in Design of Experiments methodology for systematical development and optimization of hydrophilic interaction liquid chromatographic method for the analysis of model mixture of five antidepressants. The retention factors of investigated substances are modeled directly by interpolation polynomial with divided differences and the obtained correlation coefficients were equal 1.00 in all cases. Further on, the overall quality of chromatograms was estimated by calculation of composite objective function which enables simultaneous estimation of separation quality and total analysis duration. Finally, grid point search was applied for the optimum location. High agreement between theoretically and experimentally obtained chromatograms in the identified optimal point is proven. The advantages of novel modeling technique are highlighted comparing to the results obtained by traditionally applied modeling

by quadratic function and least squares method. This technique provided lower coefficients of correlation for individual retention factors modeling so the errors of these individual models accumulated in objective function and located extremely suboptimal results. It is shown that interpolation polynomial with divided differences can be significant alternative in the modeling of chromatographic responses.

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MULTIVARIATE OPTIMIZATION OF METHOD BASED ON THE CHEMICAL GENERATION OF NO FOR THE DETERMINATION OF AMMONIUM BY HR-CS MAS

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In this work was developed a new method based on the chemical generation of NO for the determination of ammonium by high-resolution source continuum molecular absorption spectrometry (HR-CS MAS). Initially, ammonium ion is oxidized to nitrite by hypochorite in basic media in the presence of bromide as catalyst. Afterwards, the nitrite formed is reduced to nitric oxide (NO) by ascorbic acid in acidic media. The NO gas generated is then transported by a stream of argon carrier gas to a quartz cell positioned in the light path of the instrument. The measurement of molecular absorption of NO was performed at 214.803 nm. Two-level full factorial design was applied for evaluates the factors involved in the oxidation reaction of ammonium to nitrite: concentration of NaClO, concentration of NaOH and concentration of KBr. The curvature effect was significant in the experimental domain studied. This way, the conditions of the central point were established for the reaction: 0.0015 mol L^{-1} NaClO, 0.02 mol L^{-1} NaOH and 0.06% (m/v) KBr. A second two-level factorial design was applied for evaluates the factors involved in the chemical generation of NO: concentration of ascorbic acid, concentration of HCl and volume of reducing solution. Only the volume of reducing solution was significant for this process in the experimental domain studied being then further studied by univariate methodology. The experimental conditions established were: 1.5% (m/v) ascorbic acid, 1.0 mol L^{-1} HCl and 3.0 mL of reducing solution.

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MULTIVARIATE OPTIMIZATION AND VALIDATION OF AN ANALYTICAL METHOD FOR DETERMINATION OF MN IN ETHANOL FUEL SAMPLES USING GF AAS

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The determination of metallic species in ethanol fuel is one of the parameters to assess the quality of automotive fuels. The fuel contamination with metals can occur in the manufacturing process itself as well as in transport and storage tanks [1]. The presence of these species can affect the quality of fuel, besides causing environmental impact with the vehicle emissions [2]. This work aimed to employ multivariate optimization in association with graphite furnace atomic absorption spectrometry (GF AAS) for direct determination of Mn in ethanol fuel samples. The effects of the following variables were studied simultaneously through a full two-level factorial design 2^3: atomization temperature, pyrolysis temperature and pyrolysis time. The factors studied and their respective value ranges are shown in Table 1. The minimum and maximum values for each of the investigated factors were chosen in accordance with previously reported data or experiments. All of these experiments were performed in a random order.

The matrix of the factorial design and the responses of the analytical signal (integrated absorbance peak area for Mn) are shown in Table 1.

Table 1: Matrix of the full two-level factorial design 2^3.

Experiment	Pyrolysis Temperature (ºC)	Pyrolysis time (s)	Atomization Temperature (ºC)	Integrated absorbance (s)
1	-1 (800)	-1 (10)	-1 (1800)	0.00951
2	1 (1400)	-1 (10)	-1 (1800)	0.00847
3	-1 (800)	1 (30)	-1 (1800)	0.00656
4	1 (1400)	1 (30)	-1 (1800)	0.00884
5	-1 (800)	-1 (10)	1 (2400)	0.10450
6	1 (1400)	-1 (10)	1 (2400)	0.12210
7	-1 (800)	1 (30)	1 (2400)	0.11000
8	1 (1400)	1 (30)	1 (2400)	0.10570
9	0 (1100)	0 (20)	0 (2100)	0.10030
10	0 (1100)	0 (20)	0 (2100)	0.11670
11	0 (1100)	0 (20)	0 (2100)	0.12090

Data are consistent with results obtained by conventional method (univariate) using 5 mg of modifier (Pd + Mg) and 30 s of pyrolysis time .

The significance of each parameter was evaluated by analysis of variance (ANOVA) using P-value significance levels. The results were used to generate the Pareto charts of interactions and effects (Fig. 1). Figure 1 demonstrates that only the temperature of atomization was significant for Mn determination in ethanol fuel. An increase in the values of the temperature of atomization from the lowest (-1) to the highest (+1) level leads to an increase in the analytical signal. This result indicates that the temperature of atomization has a positive effect on the analytical signal; the signal increases with an increase in the temperature of atomization.

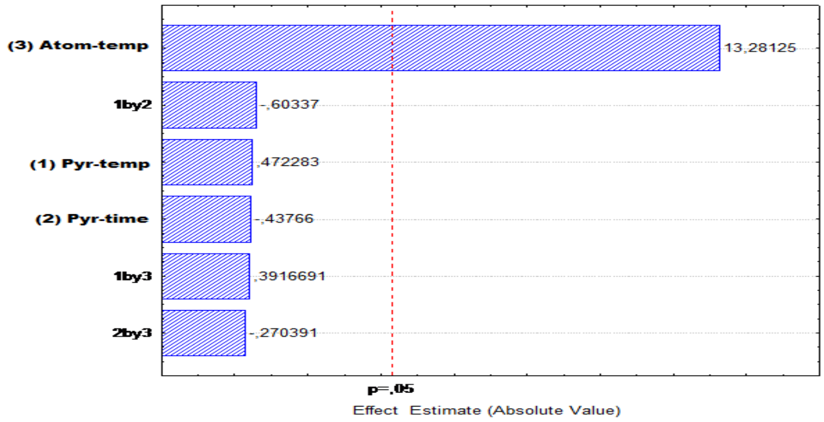

Figure 1. Pareto chart for the two-level factorial design

The proposed method was applied to Mn determination in hydrated ethanol fuel samples collected from different gas stations in Salvador, Brazil. The results indicated that the technique of factorial design describes adequately the relationships between the variables involved and the answers, being efficient in optimizing the GF AAS method.

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DETERMINATION OF THE MINERAL COMPOSITION OF CAIGUA (C*yclanthera pedata*) AND EVALUATION USING MULTIVARIATE ANALYSIS

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Caigua (in Brazil "*maxixe do reino")* is a fruit that is generally consumed either cooked or even raw as salad. This fruit has been used as a food and also in folk medicine. In this work, the mineral composition of Caigua was determined for the first time. Twenty-nine samples from five farms located in the southwestern region of Bahia, Brazil were acquired and analyzed using inductively coupled plasma optical emission spectrometry. The elements determined in this fruit included calcium, magnesium, sodium, potassium, phosphorus, manganese, iron, zinc, copper and vanadium. Principal component analysis (PCA) and hierarchical cluster analysis (HCA) were applied to evaluate the obtained results. The dominant variables for the first principal component (PC1) are phosphorus and the micronutrients copper, iron, sodium, zinc and manganese because these variables represent 46.13% of the total variance. All these six elements contribute to the major variability shown in the samples, and they are positively correlated. The second principal component (PC2) accounts for 21% of the total variance, with calcium and magnesium as the dominant variables. The HCA results correlate well with other results from PCA.

The average concentrations of the determined elements (expressed as mg kg^{-1}) were as follows: 9.09 for sodium, 1519 for potassium, 194 for phosphorus, 119 for calcium, 84 for magnesium, 0.74 for manganese, 2.11 for iron, 0.13 for copper, 1.27 for zinc and 0.15 for vanadium.

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VALIDATION OF A QUALITY OF LIFE QUESTIONNAIRE FOR CHRONIC HEPATITIS B

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Nowadays, about two billion people have been infected by hepatitis B virus (HBV) and about 350 million remain infected. Annually more than 500.000 people die due to diseases related to HBV infection. Individuals with Chronic Hepatitis B (CHB) are at increased risk of developing liver cirrhosis and hepatocellular carcinoma (fifth most common cancer worldwide, killing approximately 400.000 people per year), significantly affecting the quality of life of patients with the disease. CHB is a chronic debilitating condition that can lead to progressive impairment of physical and mental health as the disease progresses.

The World Health Organization (WHO) states that ‘health is a state of complete physical, mental and social well-being’ (subjective perception of an individual’s state of living, which has a similar concept as quality of life). In the context of health services, the focus is on health related quality of life (HRQOL) in an attempt to quantify the net consequence of a disease and its treatment on the patient’s perception of his/her ability to live a useful and fulfilling life. HRQOL can provide additional information on the effectiveness, quality of care and should be considered an important outcome measure for assessing the impact of CHB and the effectiveness of treatment.

The goal of healthcare is to maintain, restore and improve health of patients and, in particular, to evaluate health related quality of life in CHB patients *the Hepatitis B Quality of Life Instrument, version 1.0* (HBQOL v1.0) can be used. Furthermore, the effect of an intervention on HBQOL has become a very important topic for both consumers and providers of health services. Understanding the impact of illness on quality of life can make health care more responsive to patients' needs.

The identification and definition of symptom clusters in patients with cancer or chronic disease has the potential to impact on their treatment and pharmacotherapy. Although it is a very effective tool in assessing the overall quality of life in patients with various diseases, there is the need of additional statistical mechanisms to assess evaluate the different aspects of HBQOL and not only a single summary score for each patient.

Thus, techniques such as multivariate statistics are necessary in order to identify the most important parameters in the evaluation; in other words, in this case which are the more relevant questions to define and separate the individuals with high or low quality of life.

So, the role of the pharmacist researcher may be more effective as it is possible to make a more direct intervention on each patient in order to have a more effective response in maintaining a good quality of life, or to improve more rapidly those who are in the low quality of life group.

The aim of this study was to validate and culturally adapt the HBQOL, version 1.0 in Chronic Hepatitis B patients. Furthermore, using multivariate exploratory data analytical methods, e.g. Principal Component Analysis, it could be possible to extract additional information about what are the most important needs among patients with similarly defined clinical severity to prioritize them – information that cannot be easily found by traditional clinical outcomes.

The study protocol, conformed to the ethical guidelines, was approved by UFPR - Human Research Ethics Committees. Patients with Chronic Hepatitis B were recruited from Clinical Hospital – UFPR, informed about the study by a researcher and invited to participate. Those who consented were visited at hospital by an interviewer.

Data were analyzed with the Statistical Package for Social Sciences (SPSS 17.0) software and MATLAB software, version 7.13 (The Math-Works, Natick, USA) - PLS Toolbox, version 6.5 (Eigenvector Technologies, Manson, USA).

EVIDENCING THE AGGREGATION/ASSOCIATION PROCESSES OF INSULIN BY MEANS OF MCR-ALS ANALYSIS

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Insulin is a protein that has a natural propensity to self-assemble, resulting in the association and/or aggregation processes. Medium conditions play a key role in this process, because they can favour the occurrence of one process over the other [1-2]. The present work presents a chemometrics approach based on experimental design techniques and multivariate curve resolution-alternating least squares (MCR-ALS) to simultaneously assess the effect of acidity, temperature and ionic strength in the processes. A factorial design 2^3 was employed using the aggregation time (Table 1), detected by the PCA of the infrared spectra monitored at each experimental condition, as the studied response (Figure 1).

Table 1. Experimental plan for a 2^3 design

Experiment number	T (ºC)	pH	I (mM)	time (min)
1	35	3	0.3	67
2	45	3	0.3	35
3	35	7	0.3	43
4	45	7	0.3	14
5	35	3	1.5	35
6	45	3	1.5	33
7	35	7	1.5	48
8	45	7	1.5	28

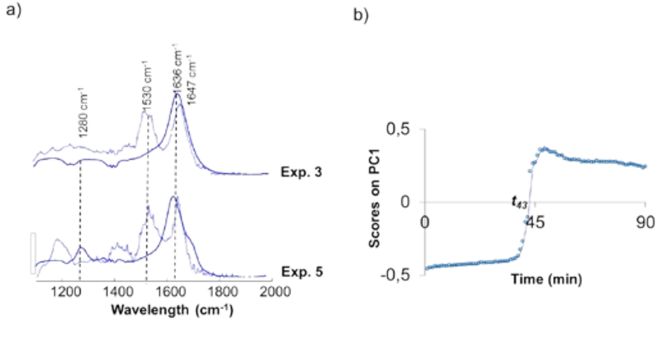

Figure 1. (a) The initial (solid line) and final (dotted line) spectra of experiment 3 and 5. (b) Scores plot for the first principal component of experiment 3.

The influence of medium conditions on the aggregation/association pathway was determined by means of multivariate curve resolution alternating least squares (MCR-ALS). Three or four forms were detected by singular value decomposition, based on the pH value: three at neutral pH whereas four forms at acid pH. The spectral and concentration profiles of the forms involved in each set of experiments were recovered by multivariate curve resolution-alternating least squares (MCR-ALS) (Figure 2). Depending on the pH of the medium, two different processes were detected. The association occurred at high pH, while aggregation took place at acidic pH. A more accurate interpretation of the results was provided by applying MCR-BANDS method in the recovered results. The uncertainty of the solutions was detected in the first stage of the processes and therefore this ambiguity does not affect to the final conclusions for either processes.

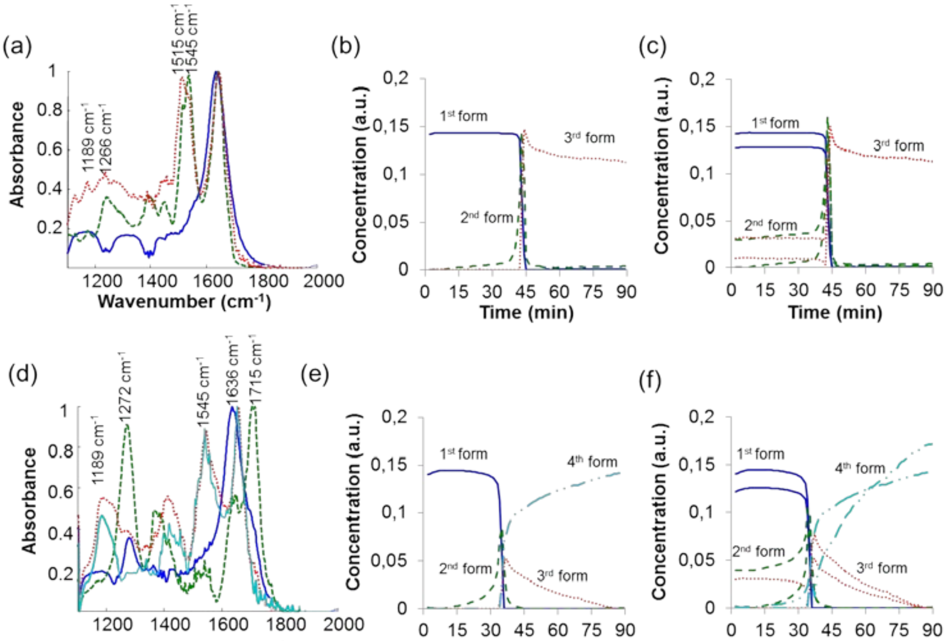

Figure 2 Experiment 3: (a) spectral profiles, (b) concentration profiles and (c) feasible bands for the concentration profiles. Experiment 5: (d) spectral profiles, (e) concentration profiles and (f) feasible bands for the concentration profiles (solid line: first forms, dashed line: second forms, dotted line: third forms and dashed/dotted line: fourth forms)

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TEMPTATIVE DECONVOLUTION OF SCATTERING AND ABSORPTION SPECTROSCOPIC CONTRIBUTIONS USING MCR-ALS

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Spectroscopy on suspensions or opaque media is particularly complicated, especially due to the huge differences in the light scattering phenomenon which can be observed even for chemically identical samples. These variations sometimes represent the major part of the total variance described by the data and can show themselves as baseline shifts or other so-called nonlinearities. In general, the scattering properties of the analyzed samples are too complex to be interpreted and this is the main reason why spectral preprocessing algorithms are often required to remove scatter contributions from pure absorbance spectra. On the contrary, very few works [1] have been devoted to attempt to separate these two different kinds of information and use the first one to study, for instance, the morphology of a substrate.

Multivariate Curve Resolution-Alternating Least Squares (MCR-ALS) has been used for qualitative and quantitative analysis of multicomponent spectroscopic datasets. It constitutes a basic chemometric tool to extract spectral and concentration profiles of individual components involved in unknown spectroscopic mixtures. So, since the measured signal for a turbid sample can be considered primarily as a combination of the scattering and the absorption contributions, the main aim of this work is to check the feasibility of a chemometric MCR-based approach to deconvolve them from a multi-experiment dataset made up of UV-Vis spectra collected at different time during the synthesis of organic photochromic nanoparticles performed by laser ablation. As the particle size decreases and the number of absorbing molecules rises during the process, the scattering and the absorption contributions can be assumed to evolve together with respect to time, which makes the dataset suitable for data resolution by MCR-ALS.

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RAMAN MICRO-SPECTROSCOPY IMAGING COMBINES WITH MCR-ALS FOR CHOCOLATE ANALYSIS

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Raman investigations of constituents and final food products have been carried out by obtaining spectra at individual points[1]. Recently, Raman mapping (imaging) was proposed for food analysis too. Raman mapping can give a wealth of information about the spatial distribution, amount and identity of constituents within a complex sample[2]. Due to the huge size of mapping data, chemometric methods are a good choice to extract the information included in the measured images. In this work, MCR-ALS was assessed to analyze the constituents in white chocolate[3] using Raman micro-spectroscopic imaging.

Multivariate Curve Resolution (MCR) methods have been shown to be very powerful methods for spectral resolution[4]. These methods allow solving the mixture analysis problem in general and at a pixel level in the analysis of spectroscopic images. The potential of hyperspectral imaging systems coupled with Multivariate Curve Resolution Alternating Least Squares (MCR-ALS) was applied for the resolution of hyperspectral images [5, 6].

In this work, MCR-ALS was used to extract the contribution and distribution of the different constituents in samples of white chocolate. Constituents of white chocolate were partially resolved, indicating that sucrose was mixed with fat in the constituent particles. Pure spectra of sucrose and fat constituents of white chocolate were obtained and compared with reference profiles. Pure spectra of whey and lactose were obtained too, but more work is needed to improve the accuracy of these profiles. Milk and black chocolate samples were analyzed too but, in these cases, it was more difficult to obtain good chemical images because of the strong fluorescence interference from cocoa solids in them.

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MULTIVARIATE CURVE RESOLUTION OF INCOMPLETE DATA MULTISETS

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Multivariate Curve Resolution Alternating Least Squares method (MCR-ALS) has been applied to incomplete data multisets. The problem of incomplete data sets is ubiquitous in many application areas like network traffic analysis, bibliometrics, social network analysis, chemometrics, computer vision, etc. Imputation of missing values can be useful as long as the amount of missing data is small, however performance degrades for large amounts of missing data or when this is systematically produced. The problem of incomplete data can be very tricky for chemometric analysis, and here we have developed a new Multivariate Curve Resolution method to analyse incomplete data multisets where some of the data subsets (full data matrices) are missing. The MCR method presented in this work allows analyzing incomplete data multisets fulfilling or not, or partly, multilinear models.

The experimental incomplete data multiset studied in this work is taken from a previous multiannual atmospheric monitoring study of the changes of ozone and nitrogen oxide concentrations in an air quality sampling station located in the city of Barcelona, in which some of the individual data subsets were missing. Based on the preliminary results obtained in this study, new data multisets, complete and incomplete, with different levels of noise were simulated and analyzed by a new variant of the MCR-ALS method which optimizes a combined error function including all possible complete data subsets derived from the original incomplete data multiset. Conclusions are drawn about the effects of data completeness on the obtained results for different noise levels and for the application or not of trilinear models.

DLLME AND MCR-ALS FOR THE RESOLUTION AND DETERMINATION OF PHENOLIC ACIDS IN VOO BY RRLC-DAD

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Phenolic compounds are part of the minor fraction of compounds in virgin olive oil (VOO) which constitutes approximately 2 % by weight of total. The interest in the study and analysis of them is related to the fact that they act as natural antioxidants and may contribute to the prevention of human disease. In addition, they also contribute to the sensory properties of VOO as well as to its stability [1]. This work presents the development of a rapid resolution liquid chromatography method coupled to UV-visible detection (RRLC-DAD) combined with multivariate curve resolution-alternative least-squares (MCR-ALS) approach to carry out the resolution and quantification of a mixture of five phenolic acids in VOO samples, employing a reversed phase dispersive liquid–liquid microextraction (RP-DLLME). The modeling strategy involves the building of a single MCR-ALS model composed of matrices augmented in the temporal mode, namely spectra remain invariant while time profiles may change from sample to sample. 4-Hydroxybenzoic (4HB), gentisic (GEN), vanillic (VAN), caffeic (CAF) and syringic (SY) acids have been chosen as a representative group of phenolic compounds. All of them present different absorption spectra and overlapped time profiles with the olive oil matrix interferences and between them. So MCR-ALS is used to cope the coeluting interferences on account the second order advantage inherent to this algorithm. Initially, a set of 9 calibration samples were randomly prepared, in a concentration range from 0.5 to 5 $\mu g \cdot mL^{-1}$ for all phenolic acids. The validation was firstly carried out using standard mixtures of these acids in ethanol:water (60:40, v/v) and, secondly, in real VOO samples getting good results in both cases.

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CHEMOMETRIC TOOLS EMPLOYED TO DISTINGUISH RAMAN SPECTRA OF BLUE BALLPOINT PEN INKS.

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The development of methods able to differentiate pen inks is of great importance in the field of forensic science because it can help to solve crimes that involve questioned document. Ballpoint pen inks are composed of a wide variety of chemical compounds, for example dyes, pigments, solvents, resins, biocide, lubricant, viscosity adjusters, antioxidants and surfactants [1]. Because of this complex mixture, the analysis of such sample is difficult. Another factor that further complicates the analysis of pen inks is that the questioned documents should preferably be analyzed by non-destructive methods because the physical integrity of the crime evidences should not be compromised during analysis. In this context, Raman spectroscopy has been used in this work because it is noninvasive and does not require sample treatment.

Commercial blue ballpoint pens of different brands and free pen samples from different origin were used in this study. Whenever possible, several pens of the same brand were acquired to obtain a more representative data set and to check for the within-brand variability. Replicate ink lines from the same pen were written on A4 sulfite and on recycled paper to study the effect of the type of paper on the distinction of ink samples. All the ink lines and papers used were analyzed by Raman spectroscopy using a Horiba Jobin Yvon LabRam instrument.

Punctual Raman spectra will be first corrected to suppress fluorescence contributions and further analyzed by Principal Component Analysis to assess whether this technique can distinguish different ink types and to find relevant spectral features useful for ink differentiation.

Preliminary studies on Raman hyperspectral images of different inks on paper will also be analyzed by Multivariate Curve Resolution to see whether the simultaneous presence of different inks in the same sample can be detected and spectrally and spatially modeled [2].

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SELECTIVITY RATIO (SR) EXTENSION TO N-WAY ARRAY

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Variable selection techniques are increasingly used in Multi-way analysis because the identification of the most "important" variable in prediction, simply based on the examination of regression coefficients, is not so straightforward often due to the unreadable complex patterns associated [1], as in the case of spectral data.

We propose here an extension of the original formulation of Selectivity Ratio (SR) parameter [2] to multi-way arrays, close to SR definition for bilinear data, in order to highlight the most relevant features to predict the studied dependent properties either for interpretative purposes or to orient feature selection.

As in the original code the SR index is calculated by using the ratio of explained variance by the target component to the residual variance of each x-variable. A high value means that the variable is relevantly correlated with the Y.

In the n-way implementation of SR the target component is obtained by a one component PARAFAC model of $\underline{\mathbf{X}}$ calculated as a projection on mode 1 of $\mathbf{Y}$ through the normalized regression coefficient b_{NPLS}. The SR is defined for all the variables/conditions modes of the $\underline{\mathbf{X}}$ array.

Here we present application of the method described to three different datasets, considering regression (NPLS) as well as discrimination framework (NPLS-DA). The data sets are related to optimization of food processing and authentication issue for products with protected denomination of origin. Moreover, the relevance of variables resulting form SR analysis is compared with VIP scores for n-way models, recently implemented by us [3].

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EXPLORATORY ANALYSIS OF SECONDARY METABOLITES EXTRACTED FROM GREEN TEA USING HPLC-DAD AND CHEMOMETRICS METHODS

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Tea plant of Camellia sinensis is the most widely consumed beverage in the world after the water. Based on processing or harvested leaf development, tea varieties are black, green, oolong and white. Many beneficial effects of green tea on health have been reported, including prevention of cancer and cardiovascular diseases, and anti-inflammatory, among others.

In this work a simplex centroid mixture design was used to investigate varying proportions of binary, ternary and quaternary mixtures of ethanol (e), ethyl acetate (a), dichloromethane (d) and chloroform (c) for the extraction of secondary metabolites of samples of green tea from a first and second harvest. These extracts were submitted to high performance liquid chromatography analysis with a diode array detector (HPLC) using a Hilic Kinetex column appropriate for the separation of the more polar metabolites. Due to the size of the matrix generated and to the large amount of samples, Principal Components Analysis (PCA) and Multivariate Curve Resolution-Alternating Least Squares (MCR-ALS) were used to identify possible differences between extractions of first and second harvest and to identify what metabolites could be responsible for this differentiation.

According to components 1 and 3, in Figure 1a, sample 2d from second harvest extracted with dichloromethane pure was well separated from the rest of samples. In Figure 1b the corresponding loading 1 and 3 are shown.

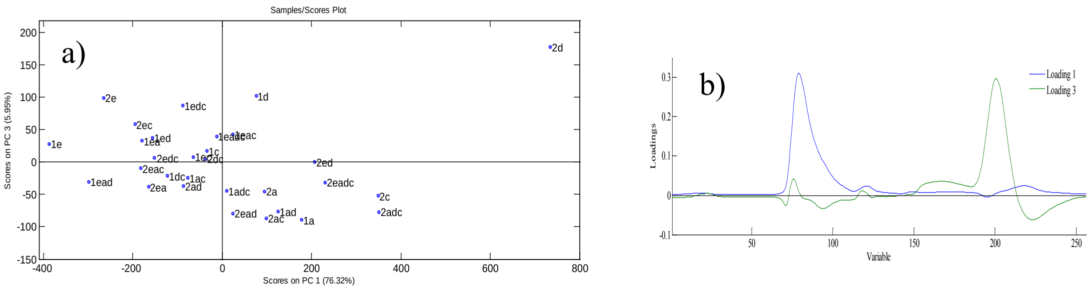

Figure 1 – a) score graph of factors 1and 3. b) loading graph of factors 1 and 3

The green peak region in Figure 1b, was selected from the chromatograma of all samples and a new data matrix was obtained and analyzed by MCR-ALS. Figures 2a and b show the MCR-LS resolved augmented concentration profiles of the coeluted components (for all the simultaneously analyzed samples) under the selected chromatographic peak and the corresponding resolved UV-vis (pure) spectral profiles, respectively, with a data lack of fit (% LOF) of only 0.45%.

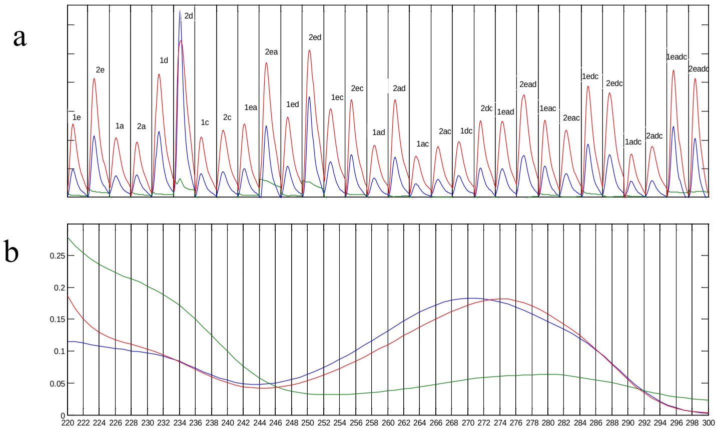

Figure 2 – Simultaneous MCR-ALS resolution to peaks chromatographic isolated a) concentration profiles. b) related pure spectra

Results obtained by MCR-ALS showed that the sample extracted from the second harvest of pure dichloromethane (2d) again outstands from the rest of samples due to the relative concentrations of its coeluted components, and specially in the relative contribution of one of them (blue line) which corresponds to the component with pure spectrum with its maximum at 271 nm. For the other samples, the component with higher concentration refers to the one (red line) with a spectrum maximum at 275 nm, as resolved by MCR-ALS. These are preliminary results and a deeper investigation of them is being performed at present.

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CHEMOMETRIC EVALUATION OF *Saccharomyces cerevisiae* METABOLIC PROFILES USING LC-MS

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Under stress conditions, the organism cells try to maintain the homeostasis and the metabolic control by varying the biological fluids composition. This involves adjusting the concentration of hundreds or thousands of metabolites to maintain cells characteristics. These metabolic balances are expressed by the metabolic profiles; and the profiles can be used for the study of dynamic effects. High pressure liquid chromatography coupled to electrospray mass spectrometry (HPLC-ESI-MS) has emerged as a powerful tool for the acquisition of the metabolic fingerprints, it enables the separation and detections of thousands of ions in biofluid samples [*A Roux et al.*, 2011]. The complex data generated in the HPLC-ESI-MS profiling experiment is analysed by multivariate statistical tools [*J v d Greef and A K Smilde*, 2005; *J Trygg and T Lundstedt*, 2007].

Saccharomyces cerevisiae is proposed as an eukaryotic model [*J I Castrillo and S G Oliver*, 2006] for evaluating the metabolic profiles under stress conditions using chemometric techniques. The influence of culture temperature (30 and 42 ºC) on a population is studied. An appropriate number of samples of the investigated system are taken at each culture temperature. The extraction procedure of intracellular metabolites is optimized considering their nature and polarity [*B Gonzalez et al.*, 1997]; and the extracts are analysed through HPLC-ESI-MS.

The LC-MS profiling data is exported to Matlab for preprocessing (interpolation, noise filtering, alignment and scaling) and statistical analysis. Principal Component Analysis (PCA) is used for the total ion current (TIC) chromatograms exploration. Then, Multivariate Curve Resolution-Alternating Least Squares (MCR-ALS) [*R Tauler*, 1995] is applied to the three-way data sets using column wise augmented matrices. The profiles as a function of the temperature are estimated for the metabolites present in the cultures. A comparative study using profiles estimations showed that some of the metabolites follow different behaviour for the different cultures. The corresponding pure spectra estimations are used for metabolite identification. The Yeast Metabolome Database (YMBD) [*T Jewison et al.*, 2012] and the MassBank database [*H Horai et al.*, 2010] are used for the search and the identification of the compounds.

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APPLICATION OF THIRD ORDER ALGORITHMS TO DETERMINATION OF CARBARYL, NAPHTOL AND PROPOXUR BY KINETIC SPECTROSCOPIC MEASURES.

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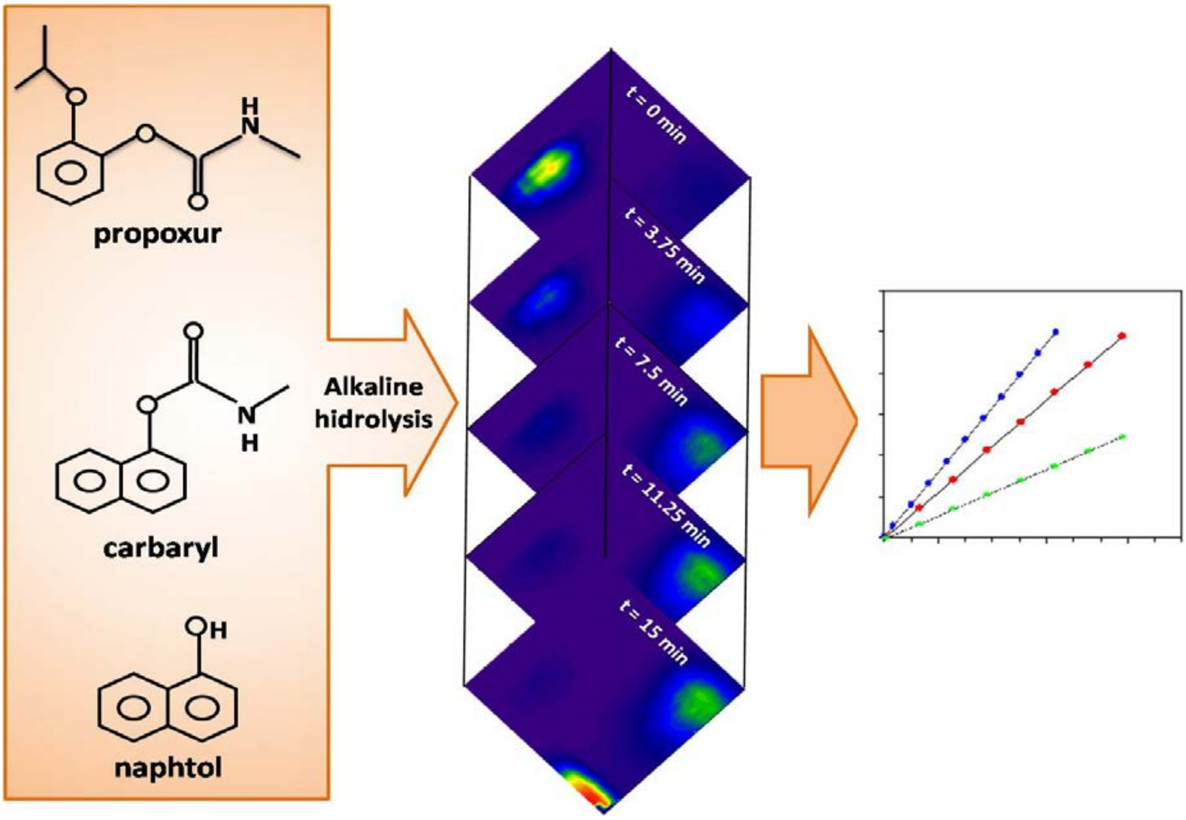
In the present work is presented a new application of third order algorithms to quantify carbaryl, naphtol and propoxur using kinetic spectroscopic data.

It was measured time evolution of fluorescence data matrix to follow the alkaline hydrolysis of pesticides mentioned above [1]. The data was analyzed by three different methods, Parallel Factor Analysis (PARAFAC), Unfolded Partial Least Squares (U-PLS) and Non-Unfolded Partial Least Squares (N-PLS) [2-5], theses last two method was assisted with Residual Trilinearization (RTL) [6] to model the presence of inspected signals no included in the calibration step. This experimental system has the additional complexity that one of the analites is the product of reaction of other analite, this present problems of linear dependency between concentrations.

The ability of different algorithms to predict concentrations was checked with validation samples. Also was prepared samples with unexpected components, tiabendazole and carbendazim, and additional water samples of a creek was extracted to addition with the three analites for check the recovery concentrations.

The better results were obtained by U-PLS/RTL and N-PLS/RTL because these two method are more flexible than PARAFAC. In Figure 1 is showed a representation of data flow, there are the structures of all analites and the data of a typical sample with carbaryl.

Figure 1



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CHEMOMETRIC MODELING OF ORGANIC CONTAMINANT SOURCES IN SURFACE WATERS OF A MEDITERRANEAN RIVER BASIN (CATALONIA) DISTRICT

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Chemometric methods are applied to the analysis and interpretation of large multivariate data sets obtained in environmental monitoring studies [1-3]. The concentrations of multiple organic compounds were measured in river samples taken from several sampling sites, at various geographical locations, during a number of campaigns and/or sampling time periods. Samples were collected and analyzed as part of an extensive multi-annual monitoring program. The water samples were taken from a mediterreanea river basin (in Catalonia, at the northeast of Spain), while historical data were available from the Water Catalan Agency. The identification of the main contamination sources and the description of their temporal and spatial distributions were achieved using both chemometric and geostatistical methods. Due to the great amount of data in these databases and to their complexity, chemometric modeling [principal components analysis (PCA) [4] and multivariate curve resolution with alternating least-squares (MCR-ALS) [5]] coupled to appropriate mapping representations is proposed to evaluate the environmental quality of the studied rivers. The results achieved in this study are intended to be a contribution to quality assessment and evaluation of the global situation of the contamination of surface waters in Catalonia, and to support public policies of environmental control and protection in the region under study.

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DEVELOPMENT OF A MULTIWAY ANALYTICAL METHOD TO HANDLE THE QUENCHING EFFECT IN THE DETERMINATION OF CARBAMATE PESTICIDES IN LIME FLOWER TEA BY MEANS OF EXCITATION-EMISSION MOLECULAR FLUORESCENCE

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Nowadays, it is of great importance to provide simple and inexpensive methods for the determination of toxic residues and pollutants in fields under legislation.

In fluorescence measurements, the magnitude of the signal and the highly overlapping spectra have a marked influence on the detection capability, so that worst results are obtained for highly overlapped and slightly fluorescent analytes. Moreover, this could make their identification difficult.

In this work, the determination of two carbamate pesticides (carbaryl and carbendazim) and the degradation product of carbaryl (1-naphthol) was carried out using molecular fluorescence spectroscopy (with excitation-emission data) and Parallel Factor Analysis (PARAFAC). This analysis was performed in synthetic samples, using pure methanol as solvent, and in a vegetal product such as lime flower tea. In lime flower tea samples, after the extraction with ethyl acetate and subsequent reconstitution in methanol, a matrix with a complex fluorescent signal that shows quenching effect was obtained. To be able to quantify ternary mixtures of the three analytes, an experimental strategy in two stages has been followed: 1) Determine the adequate dilution of the lime flower tea extract to minimize the quenching effect and to obtain the characteristic excitation-emission profiles of the lime flower tea extract, 2) Use the standard addition method to prepare a calibration based on a D-optimal design. From the total of the mixtures used, 5 pure standards of increasing concentrations for each analyte, selected as protected points of the design (13 samples), and 12 ternary mixtures formed the 25 calibration standards (three test samples and five spiked samples were also measured for validation). Once the background (matrix fluorescent components) was subtracted from the previous signals, the PARAFAC decomposition of the new tensor (with dimensions 33 × 206 × 11, where 33 corresponds to the sample mode, 206 to the emission mode and 11 to the excitation mode) showed a CORCONDIA index equal to 100.

Carbendazim was very little fluorescent compared to the other analytes studied in this work and it was totally overlapped with them and with the matrix. This made difficult its determination and the results were worse for this analyte than for the rest. Better decision limit values (CCα), in $x_0 = 0$ with probabilities of false positive and false negative fixed at 0.05, were obtained for the calibration done in pure solvent: 2.97 μg L^{-1} for 1-naphthol, 3.74 μg L^{-1} for carbaryl and 23.25 μg L^{-1} for carbendazim; while these values in matrix were 3.38, 12.5 y 37.58 μg L^{-1} respectively. The trueness was established by the regressions "predicted concentration *versus* true concentration" in all cases. Furthermore, the three analytes were unequivocally identified by the correlation between the pure spectra and the PARAFAC spectral loadings. In all cases, the correlation coefficients were greater than 0.97 for the emission and excitation profiles.

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CORROSION EVALUTION OF CHROME ELECTROPLATED SHEETS BY IMAGE ANALYSIS

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In general, a good quality chrome electrodeposit has a high corrosion resistance. Nowadays, the most common technique to evaluate corrosion on metal electroplated surfaces is through electrochemical techniques and the electrochemical impedance module obtained at a concrete frequency. The higher this module value is, the higher the corrosion resistance.

In the present communication image analysis techniques are proposed in order to evaluate surface corrosion on chrome electrodeposit. Several microscopic images have been obtained in a confocal microscope after keeping the chrome deposits in a NaCl media at room temperature along time in intervals of 30 and 60 minutes and 3, 6, 9 and 24 hours. The MIA (Multivariate Image Analysis) strategy by shifting and stacking each R,G,B channel of a multivariate image [1-2] has been applied in order to quantify the surface affected by corrosion. This method allows simultaneous spectral and textural information of an image. Unlike the common used electrochemical techniques, information about the distribution of the corrosion over the surface can be obtained. The percentage of the surface affected by corrosion over the whole electroplated surface can be also quantified by pixel computation.

The Figure 1 shows the area of a chrome deposit affected by corrosion and the percentage over the whole surface after 3 (top) and 24 hours (bottom). In order to speed up the algorithm computation images resolution has been reduced.

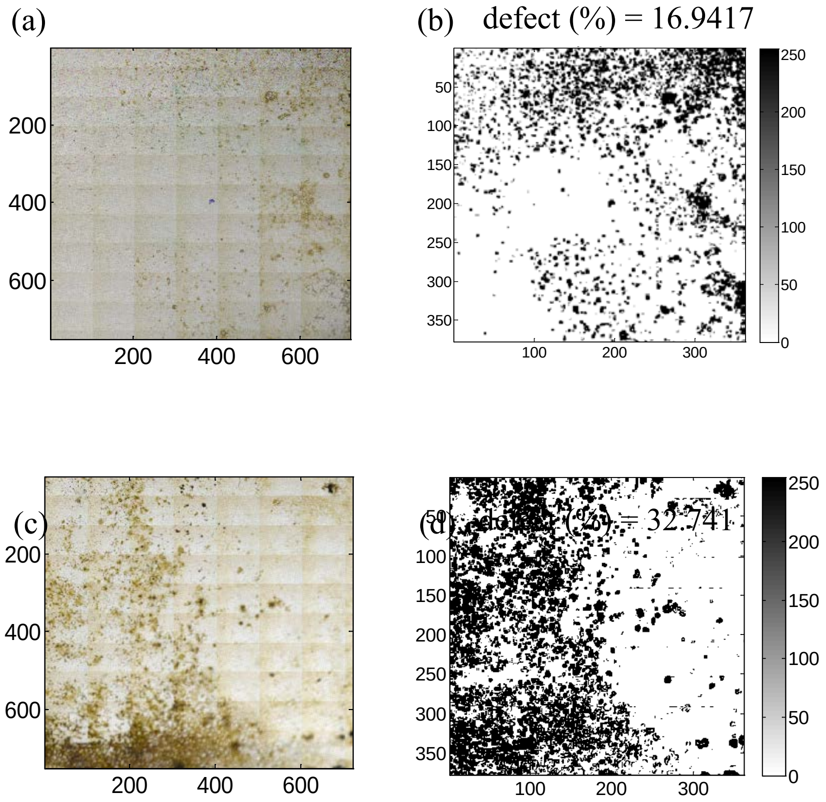

Figure 1. Original images (a, c) of chrome electrodeposits and extracted information (b,d) after corrosion procedures. 3-hours period (a,b); 24-hours period (c,d).

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IMAGE PROCESSING METHODS FOR AUTOMATIC IDENTIFICATION OF DRUGS BY THIN LAYER CHROMATOGRAPHY

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The use of computational methods combined with the thin layer chromatography (TLC), for the identification of drugs of abuse brings significant improvements in the speed and accuracy of the identification results. The computational processing is performed on images of the resulting plates, specifically analyzing the position, shape and color of the spots corresponding to each substance.

The identification of the drug of abuse through the TLC technic leads to qualitative and quantitative analysis of the traces of each substance resulting from the chromatographic separation process. This evaluation process which is normally done manually by the analyst is now carried out fully automatically through a proposed sequence of image processing algorithms. Those algorithms include the detection, cropping and straightening of the plate. Besides, it includes the detection of the lanes and base line, the location and segmentation of the spots, and the measurement of their characteristics, through the measuring of its R_f value, its shape, and its color. Finally the identification of the substance sample is performed by calculating a similarity value respect to previously stored patterns. The similarity value combine properly different distances related with the color, the shape [Flusser, Suk, et.al (2009)] and the R_f value with an adequate weight.

In order to obtain successfully results was necessary to use methods capable of dealing with the mistakes of the physical-chemical process. It was applied different filters in order to reduce or eliminate the noise, to normalize geometrically and photometrically the images and to judge which degree of confidence we should work with in accordance with the overall quality of the process. Overlooking to this latter purpose it has been introduced an evaluation step for evaluating the quality. This evaluation is based on the statistical values of kurtosis and skewness [Spiegel, Schiller, et.al (1970)] of the image histogram combined with a supervised learning approach.

The used algorithms based on morphological operations [González and Woods (2001)] are capable of discriminating between spots when they are overlapped if the percent of overlapping does not reach a certain threshold, which facilitates the identification of substances whose spots have very close R_f values.

To achieve detection of spots whose contrasts are very small in relation to the background it has been incorporated an adaptive thresholding algorithm.

An internal standard for each plate has been introduced as a reference for the determination of the relative value of R_f [Sajewicz, Piętka, et al. (2005)]. This avoids its possible variation due to the particular conditions of the chemical process of TLC, such that a secure identification of drugs is achieved. The fact of the indirect participation of analysts in the measurement and evaluation of the parameters of each spot, avoids any variations inherent to manual processing.

The process consumes only a few milliseconds and gives as response the percentage value of similarity with respect to the stored patterns and the name of the identified drugs. The experiments were performed with 42 drugs of abuse, where was achieved a 99 % of identification rate, which shows the validity of the solution.

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HYPERSPECTRAL IMAGING COMBINED WITH PRINCIPAL COMPONENT ANALYSIS TO DETECT EDIBLE MUSHROOMS INFECTED BY MYCOPHILIC FUNGI

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Mycophilic fungi of anamorphic genus *Sepedonium* (telomorphs in *Hypomyces*, *Hypocreales*, Ascomycota) infect and parasitize the fruit bodies (or sporomata) of boleticolous species, forming conidia in the final stage of the infection process [1-3]. The preferred hosts are genera within *Boletales*, such as *Boletus*, which include the most and expensive edible wild-grown mushrooms, *Boletus edulis* and relative group. Infections lead to total necrosis and the formation of large quantities of yellow aleurioconidia.

Up to date, *Sepedonium* infections have been evaluated only through macroscopic and microscopic analysis by experts mycologists, but this method can be too expensive, in terms of time, as a routine methodology for industrial purposes. Up to now, no analytical technique has been proposed for the detection of *Sepedonium*, while different applications of hyperspectral imaging (HSI) and chemometrics for monitoring different aspects of mushroom quality have been reported [4-5].

In this study, the potential application of HSI and principal component analysis (PCA) for detection of *Sepedonium* infection on *Boletus edulis* and relative group was investigated.

Hyperspectral images were obtained using a pushbroom line-scanning HSI instrument, operating in the wavelength range between 400–1000 nm with 5 nm resolution. PCA was applied on a training dataset composed by 14 average spectra of normal samples and 14 average spectra of contaminated samples, which were obtained by selecting regions of mushrooms corresponding to the two typologies, respectively, at different points on the mushroom surfaces. To reduce the spectral variability caused by factors unrelated to *Sepedonium* infection such as scattering effects and differences in sample height, different spectral pre-treatments were applied. A supervised rule was then developed to assign spectra recorded on new test samples to each of the two classes, based on the PC scores. This allowed to visualize directly – within false-color images of test samples – which points of the samples were contaminated.

The results achieved might lead to the development of a non-destructive monitoring system for a rapid on-line screening of contaminated mushrooms.

Acknowledgement: DV-Optic Tecnologie d'Avanguardia (Padova, Italy) is gratefully acknowledged for having provided instrumentation use.

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INVESTIGATING THE POTENTIAL OF SPECTRAL AND HYPERSPECTRAL-IMAGING TECHNIQUES FOR DETECTING LACTIC ACID BACTERIA AS FOOD CONTAMINANTS

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Spoilage of meat products – in particular, packaged sliced ham – is generally accompanied by souring, slimy meat juice exudates and swelling of the pack due to gas production.

Such a spoilage usually results in sensory quality defects such as sour off-flavour, discolouration, gas production, and ropy slime formation and is usually caused by lactic acid bacteria (LAB). The most frequently isolated organisms from spoiled cooked meats are *Lactobacillus sakei*, *Lactobacillus curvatus* and *Leuconostoc mesenteroides* [1].

Non-destructive, rapid and sensitive analytical techniques are therefore required for early detection of LAB spoilage in products destined to markets, in order to guarantee the consumer a higher quality and safety level.

In the present study, we investigated the potential of a macroscopic hyperspectral scanner operating in the NIR (955-1700 nm) region and of a FTIR microscopy operating in the region 4000-675 cm^{-1} for providing information useful for detecting LAB spoilage.

In more detail, cultures of *Lactobacillus sakei* and *Lactobacillus curvatus* on agar medium were submitted to spectral and hyperspectral analysis. Application of suitable multivariate exploratory methods (such as principal component analysis – PCA and multivariate curve resolution – MCR), supervised methods (such as PLS-DA), and techniques for data reduction (such as hyperspectrograms) proved that information embodied in spectral and hyperspectral data was functional to characterise and identify the LAB species investigated responsible for ham spoilage.

Moreover, preliminary outcomes of analyses on sliced ham samples artificially spoiled with LAB indicate the possibility of detecting contamination directly on the food matrix.

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K-CONTRACTIVE MAP (K-CM) FOR CLASSIFICATION

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Supervised pattern recognition methods for classification are increasingly applied in several fields, such as food chemistry, analytical chemistry, metabonomics, process monitoring, medical sciences, pharmaceutical chemistry, chemical modelling, as well as social and economic sciences. Classification is one of the fundamental methodologies in multivariate analysis and consists basically in finding mathematical models able of recognizing the membership of samples to their proper class.

Among traditional classifiers, Artificial Neural Networks (ANNs) are increasing applications and nowadays can be considered as one of the most important emerging tools in multivariate analysis. One of the reasons of their success can be related to their ability of solving both supervised and unsupervised issues, such as clustering and modeling of both qualitative and quantitative responses.

In this work, we propose the application of a new ANN, K-Contractive Map (K-CM), which is able to handle non-linear classification issues. The classification performances of K-CM have been evaluated on ten benchmark datasets with different characteristics (number of samples, variables and classes). These different datasets were considered in order to significantly evaluate the classification performance of the considered algorithms in different scenarios. The classification performances were compared with those of traditional classifiers, such as K-Nearest Neighbor (KNN), Linear Discriminant Analysis (LDA), Partial Least Square Discriminant Analysis (PLS-DA) and Tree-based classification models (CART).

For each dataset, classification models were validated and classification performances of the classification methods were compared. K-CM showed the best classification performances in validation and, on average, over performed the other classification methods.

A COMPARATIVE STUDY ON DIFFERENT METHODS FOR APPLICABILITY DOMAIN ASSESSMENT

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With the growing popularity of using QSAR predictions towards regulatory purposes, such predictive models are now required to be strictly validated, an essential feature of which is to have the model's Applicability Domain (AD) defined clearly. Although in recent years several different approaches have been proposed to address this goal, no optimal approach to define the model's AD has yet been recognized.

In this study, an extended comparison among different AD methods is performed on several data sets with an aim to quantitatively evaluate their performance and similarities in characterizing the interpolation space of the model.

All the AD methods relevant to this study were also implemented into a MATLAB toolbox, which will be freely available for download in the near future.

RESHAPED SEQUENTIAL REPLACEMENT ALGORITHM (RSR) FOR VARIABLE SELECTION

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Variable selection (VS) is a key step in multivariate analysis for modelling purposes. It consists in the selection of optimal subsets of variables, in order to obtain parsimonious models and maximize their predictive power, also increasing interpretability. VS plays a crucial role in scientific fields which deal with a large number of variables, such as QSAR.

Here we propose a VS method, named Reshaped Sequential Replacement (RSR), which takes inspiration from the Sequential Replacement (SR) method of Miller[1]. In the SR method, starting from a randomly generated population of models, each variable is replaced at a time with all the remainders, searching if a better model is found. The new model is chosen after all variables have been replaced and the obtained models have been compared. The extensive exploration of possible combinations of variables is computationally very demanding, especially when dealing with high dimensional problems. The RSR algorithm shares the simplicity of SR method, being based on the same replacement procedure, but aims at reducing the computational time needed and increasing the probability of convergence upon optimal models. The principal functions able to "reshape" the SR method are: 1) Tabu list, which consists in the preliminary exclusion of variables not correlated with the response that can be re-included during a later replacement procedure if they provide a relevant improvement in the quality of the model; 2) Roulette Wheel for the initialization of the population, in which each variable is given a probability of entering the initial models proportional to a pre-defined fitness function; 3) QUIK rule[2], a statistical test used in regression during the replacement procedure, in order to reject *a-priori* models affected by high predictor collinearity.

The aim of the study was to: 1) understand how each of the new functions influences the performance; 2) compare the performances of SR and RSR algorithms; 3) compare the replacement methods with other VS methods. To this end, SR and RSR have been applied to 2 datasets for regression (using OLS) and 3 datasets for classification (using KNN) (Table 1). Models available in the scientific literature for each dataset and obtained by other VS methods were used as benchmark for the comparison. 2 of the 3 datasets for classification had a small number of variables and therefore some random variables were added, obtaining a total of 50 variables. Benchmark models were then found using Genetic Algorithms. Furthermore, 6 simulated datasets with different number of objects and different levels of noise were tested in regression (Table 2). A DoE was made for all the 11 datasets in order to understand the influence of the new functions on the performance. All models obtained were then evaluated and compared using several statistical tests and functions, such as Y-scrambling and R-function[2] based rules.

Table 1. Real datasets used: *n* = number of objects of training and test sets; *p* = number of variables.

Dataset	*Type*	*n train*	*n test*	*p*
LC$_{50}$[3]	Reg	408	57	899
Melting Point[4]	Reg	10000	2634	150
IRIS[5]	Class	150	-	50
ItalOils[6]	Class	572	-	50
Biodegradability[7]	Class	837	218	776

Table 2. Simulated datasets: *n* = number of objects; *p* = number of variables; *p'* = number of variables used to generate the response.

n	*p*	*p'*	*Noise %*
500	500	5	0, 10, 15
100	500	5	0, 10, 15

Results highlighted the capability of the replacement methods to converge upon optimal solutions even with a small initial population of models. For RSR, the principal function able to decrease the computational time with respect to SR is the Tabu list (up to about 10 times faster). Roulette Wheel allows a quicker convergence upon optimal solutions, reducing the total number of models generated to reach the best solution. The effect of QUIK rule is strongly dependent on the characteristics of the dataset. In the majority of cases, RSR outperformed SR or the performances were comparable, with the advantage of the first of being faster. Performance of RSR on real datasets is in most cases comparable with other VS methods, such as Genetic Algorithms and Random Forest. In about 50% of the cases RSR was able to reproduce the benchmark model.

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CHEMOMETRICS APPLIED TO THE ANALYTICAL STUDY OF THE CONFORMATIONAL EQUILIBRIA OF CYTOSINE-RICH DNA SEQUENCES

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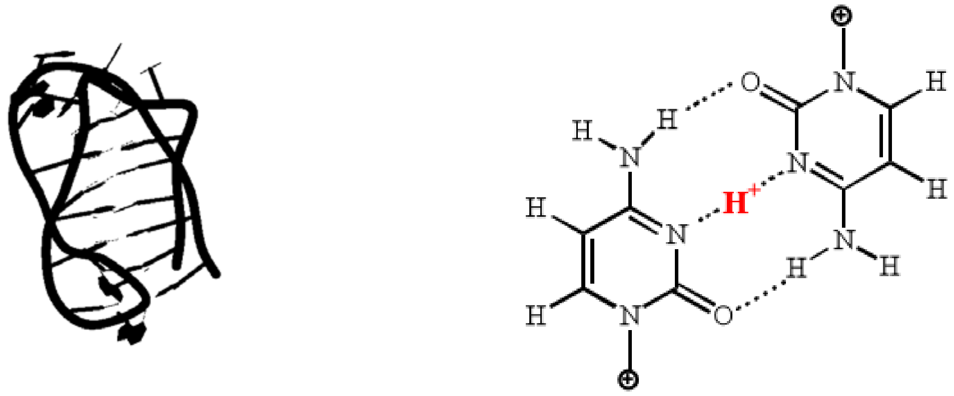
Cytosine-rich regions of DNA are capable of forming complex structures known as i-motif (see left pannel). The formation of such structures in vitro has been demonstrated in DNA sequences corresponding to the end of telomeres [1] and to the promoter regions of several oncogenes, such as c-kit, c-myc or bcl-2 [2]. The existence of these structures in vivo is still a matter of discussion because the stability of i-motifs is strongly dependent on pH , being maximal at pH values near the pKa of the cytosine base (~4.5, right pannel).



Left: intramolecular i-motif structure. Right: C+•C, the constituent block of i-motif

In the present study, we have focused our attention on the influence of bases, such as thymine, cytosine, adenine or guanine, located in the loops on the overall stability of the i-motif structures.

Circular dichroism and molecular absorption spectroscopies have been used to monitor the experiments (acid-base titrations and meltings) carried out. Multivariate data analysis based on soft- and hard-modeling methods has been used to recover qualitative and quantitative information about the species and conformations present in all experiments. The results show that the application of a multivariate approach allows the succesful resolution of systems involving i-motif structures.

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FAST VIRTUAL SCREENING OF ALKALOIDS FROM APOCYNACEAE WITH POTENTIAL ANTITRYPANOSOMAL ACTIVITY.

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Chagas' disease is a human tropical parasitic disease, caused by *Trypanosoma cruzi,* which occurs particularly in South America. The big social problem caused by this infection, the restricted number of drugs available, their serious side effects and, the emergence of new drug resistant forms, support the research for new antiprotozoal drugs. Secondary metabolites play an important role to propose new promising active lead structures, and several studies highlights antiprotozoal activities of alkaloids. In view of this, we performed a virtual screening in an in-house databank (SISTEMATX) of 469 indole alkaloids of Apocynaceae, corresponding to 861 botanical occurrences (number of times that a compound appears in different species) using fragment descriptors and Support Vector Machines (SVM). We select from CHEMBL database a diversity set of 550 agrochemicals, 2-aryl-5-aminomethyloxazoles and N-substituted pyridylamidoximes derivatives (document references CHEMBL1862790 and CHEMBL1857833), which were screened in vitro in a whole cell parasite assay against a *T. cruzi* strain (Tulahuen, Clone 4 – CHEMBL assay 5693). The SMILES code and respective values of activity were downloaded and compounds were classified using values of -logIC_{50} (mol/L) = pIC_{50}, being 385 actives (>7) and 165 inactive (<6). SMILES were used as input data in DRAGON program v. 6.0.30 to generate descriptors from Ring descriptors, Functional group-counts and Atom-centred fragments blocks. Descriptors with constant values, near constant values, standard deviation less than 0.0001, and at least one missing value were excluded, as well descriptors with pair correlation larger than or equal to 0.90. Knime 2.7.1 software was used to perform all analysis process described hereinafter. The 146 remaining descriptors and class variable were exported and data were divided in train and test set. Finally, 18 variables were selected by backward feature elimination method and all models were generated using the WEKA nodes which perform SVM using Sequential Minimal Optimization algorithm to classify the compounds, using fragment descriptors. **Table 1** summarizes the performed of the match rates of SVM model for the training, cross-validation (12 stratified groups according activity class variable). Receiver Operating Characteristics (ROC) plot was generated for test set with 0.895 area value under the curve.

Table 1. Summary of training and test and respective match results using SVM.

	Train			**Validation**		**Test**		
	Samples	**Match**	**%Match**	**Match**	**%Match**	**Samples**	**Match**	**%Match**
Active	308	305	99.0	303	98.4	77	77	100
Inactive	132	107	81.1	107	81.1	33	27	81.8
Overall	440	412	93.6	410	93.2	110	104	94.5

The SVM model selected only 5 similar alkaloids, as actives, from 2 species of *Aspidosperma* genus (*excelsum* and *marcgravianum*). The values of MLogP of these structures are between 3.6 to 5.3 and, two alkaloids, isolated form *A. excelsum* passed to the filter of Lipinski alert index and with values > 0.7 of Drug Like Score consensus. These structures can be an interesting start point of antitrypanossal studies of alkaloids from Apocynaceae. The methodology, adopted in this work, which was performed as part of the activities of ResNetNPND (http://www.uni-muenster.de/ResNetNPND/), showed to be rapid and can help to the process of virtual screening of larger natural products databases.

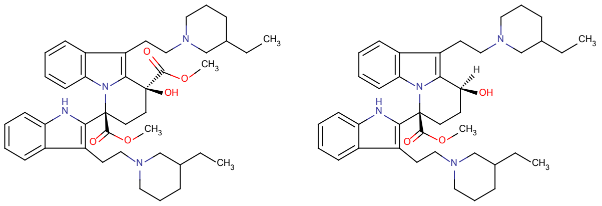

Figure 1. Selected structures with potential activity against *T. cruzi*

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EVALUATION OF LIGANDS FOR KETONE REDUCTION BY ASYMMETRIC HYDRIDE TRANSFER IN WATER BY ULTI-SUBSTRATE SCREENING

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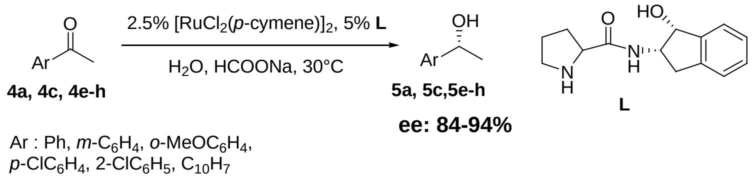
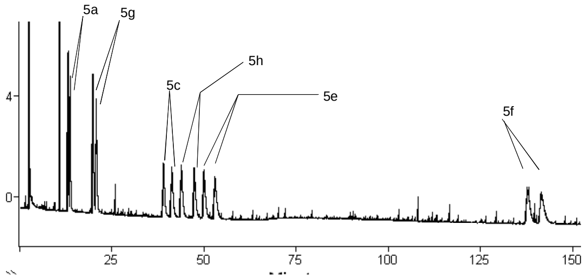
Nowadays a major concern for chemists is the design of new, low-cost and highly sustainable methodologies following green chemistry principles. [1].The use of water as solvent allows one to fulfill most of these requirements and catalysis in water represents a major area [2]. Asymmetric transfer hydrogenation in water is an alternative method to perform reactions under mild conditions with high enantioselectivities and have been widely employed for the preparation of chiral alcohols. In this area we have studied catalysts for ATH reduction of ketones in water and reported on ruthenium-based catalysts which are reusable both with the same substrate or changing the ketone for each reduction without a loss of enantioselectivity [3].Various ligands for the ruthenium-catalyzed enantioselective reduction of ketones in water have been investigated. Multi-substrate reactions have been carried out for the comparison of various proline amides and aminoalcohol ligands. Two sets of six aromatic ketones have been selected in order to evaluate the enantiomeric excesses of all the resulting alcohols by a single chromatographic analysis. The proline amide derivative prepared from (1R, 2S)-cis-aminoindanol revealed as the best ligand for most of the ketones used in the multi-substrate reductions. This ligand has been employed for the enantioselective reduction of a variety of other aromatic ketones and in all cases the enantiomeric excesses were improved compared to those obtained with phenylprolineamide used in our previous work.





Analyse du mélange des énantiomères des alcools 5a, 5c, 5e-h par colonne chirale GC

Keywords: asymmetric catalysis; hydride transfer; multi-substrate screening; reduction; ruthenium

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CRYSTALLOGRAPHIC ELUCIDATION OF PURELY STRUCTURAL, THERMAL AND LIGHT-INDUCED SPIN TRANSITIONS IN AN IRON(II) BINUCLEAR COMPLEX

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The dinuclear complex [{Fe(bpp)(NCS)$_2$}$_2$(4,4'-bipy)]2MeOH (where bpp=2,6-bis(pyrazol-3-yl) pyridine and 4,4'-bipy=4,4'-bipyridine) undergoes an incomplete (50%) spin crossover below 120 K, as shown in Fig. 1, as well as photo-induced spin crossover at low temperatures Such an incomplete transition may be viewed as the first step in a potentially 2-step spin crossover material, in which the lower temperature step may not be observed at ambient pressure The structural symmetry breaking is the result of torsion in the central 4,4'-bipyridine unit, going from a conformation in which the two rings are approximately planar at room temperature (the rings related to each other by an inversion centre) to one in which they are twisted around the C–C bridging bond below 161 K. Using evidence from DFT studies of 4,4'-bipy bridged systems it was argued that this twisting of the bipyridine rings to some extent ''switches off'' communication between metal centres, resulting in only the first step of a possible two-step spin crossover. No evidence was found of any relaxation of the 1 : 1 HS : LS state towards a full low spin state. The effect of pressure on the SCO phenomenon has been studied using a variety of techniques including Mossbauer, infra-red, Raman optical and X-ray spectroscopies, RPE spectroscopy, reflectivity, magnetometry and X-ray diffraction. Such investigations have enhanced understanding of how pressure influences SCO and led to the development of various theoretical models15 to describe the pressure-driven process.

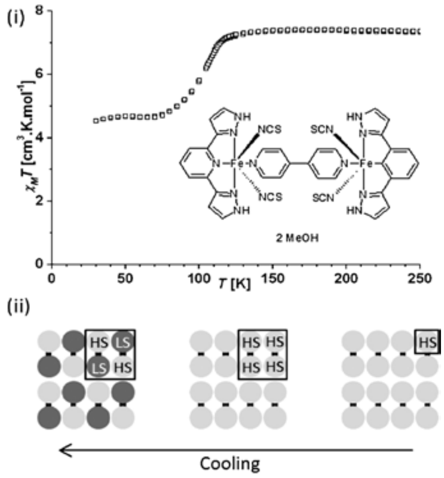

Figure 1

Keywords: dinuclear, iron (II), Mossbauer and magnetism, spin crossover.

IMPROVED VISUALIZATION USING OPLS WITH SENSORY AND QUALITY DATA.

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The intent with the presentation is to describe an alternative to PLS that is called OPLS. OPLS has certain model interpretation properties rendering the method more tractable than PLS; these properties will be highlighted. In so doing, we shall discuss OPLS from theoretical as well as practical perspectives. We will also clearly distinguish between single-Y and multi-Y OPLS models. Much like the standard applications using PLS, the current presentation will provide a walk-through of the OPLS method, containing examples of which parameters are available for model interpretation. Although the beam of light is directed onto OPLS, we will provide a comparison with PLS, so that the interpretational advantages of OPLS become clear. As example application sensory data from the food industry will be used.

Multivariate tools can assist in monitoring and quality by

i) Making the loadings more useful

In a multi component (A>1) PLS model interpretation is always difficult. This due to the fact that all component correlate to Y. The situation in very different for single-Y OPLS, where only the 1st component correlate to Y and later components are orthogonal to Y. This makes visualization and interpretation of the model clearer.

ii) Deciding the direction of your projection

We often use PCA as a projection tool for deeper data understanding. One property of the PCA model is that it r otates the model for maximum description. OPLS allows us to determine the horizontal direction. This linear direction is retained for both scores and loadings, and makes understanding of data easier.

iii) Discriminant analysis using OPLS (OPLS-DA)

Again, the group separation is on the first component (horizontal axis). This is the same for the loadings, where the discriminating variables are easily identified. Just as for PLS, but "rotated". The second component describes the common within group variation, orthogonal to the first, with its corresponding loadings. Excellent for both identifying similarities and differences

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PLS-DA OF GAS AND LIQUID CHROMATOGRAPHIC FINGERPRINTING FOR AUTHENTICATION OF PALM OIL

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Partial least squares-discriminant analysis (PLS-DA) is an appropriate class-modeling and prediction technique. It was applied to discriminate the geographical origin of palm oils from south eastern Asia, Africa and South America. The authentication was based on liquid and gas chromatographic fingerprints of triacylglycerols (TAGs), without either identification or quantification of the chromatographic peaks before building the chemometric models. The geographical origin of palm oils with the use of the raw chromatograms and chemometrics tools had not been studied yet in bibliography. However, it has been previously reported by the fatty acid (FA) and volatile organic compound (VOC) fingerprints [1].

The results show that it is possible to perform classification models for palm oil samples from either liquid or gas chromatographic raw data based on their TAG profile since they present different elution features The proposed method provides a rapid methodology for palm oil classification according to geographical origin and could serve as a traceability analytical tool to verify the labeling compliance of the oil. The rates of successful in prediction of the geographical origin of the 85 samples varied between 90 and 100%. The results for both kinds of data are compared.

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HANDLING TRACEABILITY ISSUES IN FOOD: A CHEMOMETRICS APPROACH FOR ENOLOGICAL PRODUCTS

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The interest toward the geographical origin of food has increased over the last years, mainly as a consequence of the globalization of food market. The link between the product and the territory of origin has been recognized by the European Union as a quality attribute, through the introduction of protected marks, such as the Protected Designation of Origin (PDO) and Protected Geographical Indication (PGI). In this context, the possibility to identify objective criteria for the geographical traceability of food represents a challenging target, useful for the valorization of food products.

The main research project, which this work belongs to, is focused on the development of geographical traceability models concerning Italian PDO wines, such as Lambrusco of Modena and Trentodoc. In particular, the following aspects are taken into consideration: 1) identification of the suitable traceability indicators, 2) optimization of the analytical methodologies for their determination, 3) planning of a representative sampling (for both soils and food) and 4) study of the indicator's behavior with respect to soil variability, plant uptake and winemaking chain influence.

A synergistic approach between analytical methods and multivariate data analysis is here presented to investigate some of the abovementioned aspects, in order to obtain information about the analyzed system. Given the extension of the investigated geographical areas, a pilot study was conducted on four farms, representative of the Lambrusco production areas and characterized by a complete enological chain. In particular, to obtain more detailed information on the soil sampling procedure, in terms of inter and intra site variability, sampling depth and seasonal variability, a fast screening approach of the soil samples was carried out by means of X-ray diffraction of powder and chemometrics analysis of the obtained signals. As processing step, noise reduction and background correction were achieved in wavelet domain; then the diffractograms were aligned and a block-scaling procedure was applied. Finally, Principal Component Analysis (PCA) and PARAFAC analyses were used as explorative data analysis tools [1].

On the basis of the obtained results, it was possible to reduce the number of soils to be sampled and further analyzed, while maintaining an exhaustive description of the investigated production areas. The $^{87}Sr/^{86}Sr$ isotope ratio was used as geographical tracer [2, 3] and thus measured in several matrices of the considered oenological chains, namely soil – branch – grape juice. PCA was used as exploratory analysis in order to investigate the potentiality of the investigated parameter. The results highlight a good match between the isotopic values monitored in the soil fractions mimicking the bio-available part and their respective grape juices for almost all the investigated geographical areas. The correlation with food matrices satisfyingly improves when the isotopic values of vine branches are considered.

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FOOD QUALITY CONTROL: APPLICATION OF NEAR INFRARED SPECTROSCOPY FOR DRIED EGG-PASTA CHARACTERIZATION

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Food quality control is not an optional extra in food processing; neither is it something done only by large manufacturers. It is an essential component of any food processing business. Quality control need not be time consuming or expensive, and the results of quality control tests should help save money in the long run. In general, quality control procedures should be as simple as possible and only give the required amount of information. Quality control is used to predict the quality of the processed food and then control the process so that the expected quality is achieved for every batch. This means that quality specifications must be written and agreed with suppliers or sellers, and control points must be identified in the process. Today pasta has become a dietary staple all over the world. Dried egg-pasta is important in the market, since the range of about 50 different dried egg pasta shapes reflect traditional regional Italian cuisine. The success is due to the unique characteristic that dried egg-pasta looks and tastes like home made and is available in many unusual shapes and sizes. When considering dried egg-pasta, three are the main parameters which can affect the quality of the final product, drying time and temperature, and the amount of eggs used. Indeed, on one hand, thermal processes, have an influence on the quality of pasta on a macromolecular level due to reciprocal interactions between proteins and starch. In particular, changes in dried and in cooked pasta structure were determined regarding protein solubility, thermal properties and digestibility of starch, microscopic and rheological measurements. On the other hand, the color, taste, flavor, texture and cooking properties of different dry pasta products are determined primarily, besides the quality of ingredients used, by the quantity of eggs added. Based on these considerations, in this study the possibility of using NIR spectroscopy as a rapid and non destructive tool to assess dried egg pasta quality was investigated, by determining the influence of the three main parameters (egg percent amount, drying temperature and drying time) on the spectroscopic fingerprint of the final product. Reference pasta samples were prepared with different egg percent concentration (20%, 22%, 25%, 27%, 30% and 33%), and different drying temperatures and times were tested. The results show that all of the three parameters have a relevant impact on the shape of the spectroscopic signal. Therefore, NIR spectroscopy appears a very promising tool to be applied *at-line* in pasta industry since it is able to monitor the modifications induced by the change of each considered parameter. A similar approach has never been reported in the literature, where only one study can be found and it is simply voted to the egg percent determination of few commercial samples.

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USING THE LC-FINGERPRINT OF STEROLS FRACTION TO DISCRIMINATE OLIVE OIL FROM OTHERS EDIBLE OILS.

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The main objective of this work is the discrimination of the olive oil from other vegetable oils (soybean, sunflower, rapeseed, canola, corn and pomace) through the fingerprint of the sterol fraction, obtained by Liquid Chromatography with Ultraviolet-Visible detection (HPLC-UV). Figure 1 shows, as an example, the chromatogram obtained after injecting 20 μL of the unsaponifiable extract of an oil sample analyzed using the operation conditions indicated. In the chemometric study, from the whole chromatograms obtained for 51 samples of different oils (6916 variables per chromatogram) a raw data matrix (51x2001) corresponding to the sterols fraction (obtained in a windows time of 7.5 to 9.5 minutes), has been selected as chromatographic fingerprint.

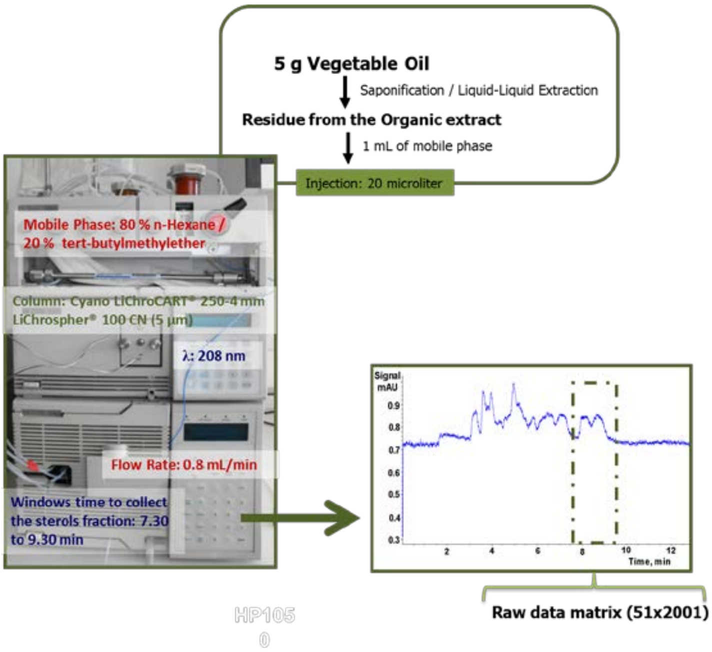
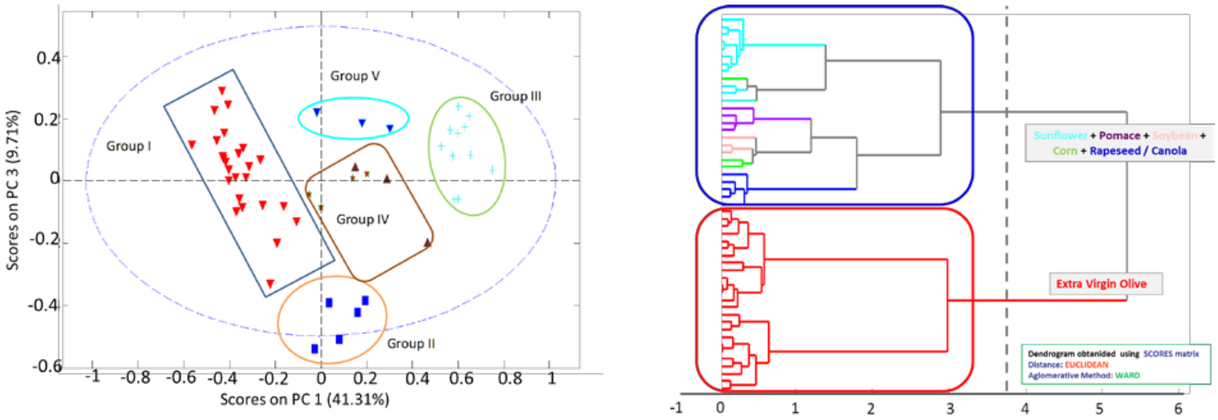


Figure 1. Procedure for obtaining the sterols fraction LC-fingerprint of a vegetable oil sample.

Previously to the development of any model, the raw data has been preprocessed using next steps: (i) correction of the baseline (in order to remove baseline contributions to the data); (ii) smoothing of the variables using a polynomial of second degree (Savitsky-Golay method); and (iii) data set mean centered.

In order to observe the natural grouping of the sterols LC-fingerprints, principal components (PCA) and hierarchical cluster (HCA) analysis have been used as unsupervised pattern recognition techniques, in both cases, the olive oil chromatographic fingerprint differs from the rest of vegetable oils (Figure 2). In the case of PCA, 4 PCs have been needed in order to explain a 78% of the total variance and five groups have been observed: Group I: extra-virgin olive; Group II: rapeseed/canola; Group III: sunflower; Group IV: pomace and corn, and Group V: Soybean. For HCA, the Euclidean distance of the scores obtained from PCA and Ward method have been used, using a $D_{linkage} = 0.66\ D_{max}$ two natural groupings have been observed, one for olive oil and one for the rest of edible oils analyzed.



(a) (b)

Figure 2. Natural groupings obtained from (a) PCA and (b) HCA.

Finally, in order to discriminate among the edible oils, a partial least squares discriminant analysis (PLS-DA) as supervised pattern recognition technique has been used. According to HCA two classes were defined: Class I: Olive oil and Class II: Rest of the edible oils. The validation of the model has been made in two ways: (i) with a cross-validation using venetian blinds (with 7 splits), and (ii) externally using the 25% of the samples studied. In spite of natural grouping observed in PCA, and accordingly with HCA, the 5 LVs selected (explaining 83.04% of Block X and 95.31 % of Block Y variances of the classification set) permit to discriminate between two classes: olive oil and the rest of oils analyzed (soybean, sunflower, rapeseed, canola, corn and pomace).

FT-IR-PCA STUDY OF COMPOSITIONAL DIFFERENCES IN EXTRACTS OF SUNNY AND SHADED ARABIC COFFEE LEAVES PLANTED IN RECTANGULAR AND SQUARE ARRANGEMENTS

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Coffee is one of the most popular and consumed drinks in the world. Owing to its economic importance hundreds of scientific papers can be encountered in the literature focusing on species discrimination or compositional differences of raw and roasted coffee beans [1]. However no article has been found relating plant architecture and chemical digital fingerprints for Arabic coffee leaves. Plant architecture is directly or indirectly related to multiple plant functions, like light interception, photosynthesis and transpiration [2]. In plants, especially in medicinal and alimentary ones, the environmental conditions induce some physiological reactions [3] closely related to the biochemical changes observed in their metabolism that may be important for bean quality. The Agronomic Institute of Paraná (IAPAR), located in Londrina, Brazil contains a collection of 144 accessions of *C. arabica* collected in Ethiopia, region of the species' origin. This collection has been used as the basis for genetic improvements at IAPAR. Considering these facts the main objective of this work is to investigate compositional variations of different extracts of Arabic coffee leaves in order to discriminate vegetable architecture by Fourier Transform Infrared Spectroscopy (FTIR).

The coffee trees were planted in a rectangular arrangement of 3.0m x 0.41m with a space of 1.25 m^2 for each plant (8,000 plants ha^{-1}) and a square arrangement of 0.84 x 0.84 m^2 with a space of 0.71 m^2 for each plant (14,000 plants ha^{-1}). The leaves were collected from the vertical plant strata (shaded and sunny ones). To perform extractions, 2.50 g of dried arabica coffee leaves were weighed and macerated ultrasonically for 120 min in 60 mL of solvent extractor. Solvent mixtures followed a statistical simplex-centroid design consisting of ethanol (et), acetone (ac), dichloromethane (dc) and hexane (hx) pure solvents, six 1:1 binary mixtures of these solvents, four ternary mixtures in equal proportions and one quartenary 1:1:1:1 mixture [4]. The leaves were collected at the Agronomic Institute of Parana on April 10, 2010.

The score graphs of the first five principal components showed that ethanol:acetone and ethanol:dichloromethane mixtures discriminate the square and rectangular arrangements of the shaded plants. The ternary ethanol:dichloromethane:hexane mixture discriminated these arrangements in the sunny areas. New extracts were prepared in triplicate using the best separation conditions found and their infrared spectra were subjected to principal component analysis. Figure 1 shows this score graph of the first and fifth factors that explain 99,89% of the total data variance.

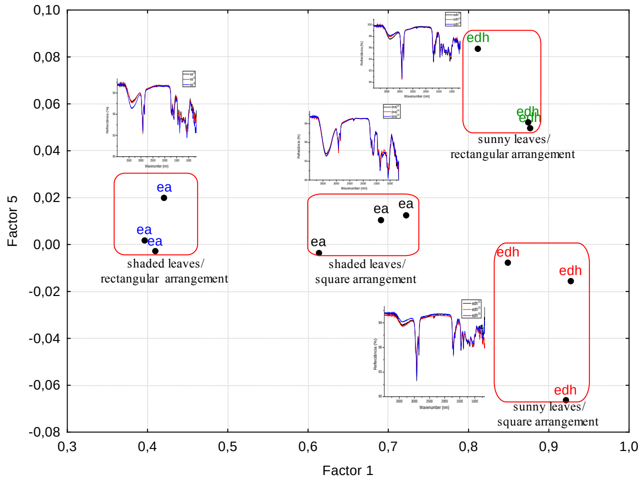

Figure 1. *Arabic coffee* L. samples from square and rectangular arrangements for extracts prepared with ethanol:acetone and ethanol: dichloromethane:hexane under sunny and shaded conditions.

The spectra of the ethanol:acetone extract for the shaded leaves of the rectangular arrangement on the left of the graph are discriminated from shaded leaves of the square arrangement owing to weaker reflectance bands in the 3037-3645 cm^{-1} region that can be attributed to phenols and also in the 664-1674 cm^{-1} region that is attributable to carbonyl compounds. The spectra of the ethanol:dichloromethane:hexane extracts how differences at 1649 cm^{-1} that can be related to different tertiary amide concentrations.

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INVESTIGATION ON THE POSSIBILITY OF CHARACTERIZING THREE MONOVARIETAL EXTRAVIRGIN OLIVE OIL FROM MARCHE BY SPECTROSCOPIC AND CHEMOMETRIC METHOD.

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There is a growing wealthy niche market for some extra virgin olive oil of special organoleptic characteristics related to the traditional cultivars and cultivation methods[1]. It is of special importance to develop analytical methods to recognize and protect these valuable oils.

Several authors studied the possibility to characterize olive oil by means of aspecific measurements with respect to the orchard location or the vegetal variety. NIR and MIR signals were analysed by LDA to assess the botanical variety of Italian monovarietal olive oil [2]. Ligurian oils were classified combining head-space mass spectrometry (electronic nose), UV–visible and NIR spectroscopy [3]. Forina and coworkers [4] investigated the possibility to compute QDA and SIMCA models from UV-Vis spectroscopy to classify the West Liguria PDO “Riviera Ligure-Riviera dei fiori” extra virgin olive oil.

In the present work we investigate the possibility to recognize the geographical and/or varietal origin of extra virgin olive oil (EVOO), obtained from three olive variety of Marche in central Italy, through instrumental methods and chemometric data treatments. Sampling were conducted in restricted areas typical for each olive variety, in particular we got oil of Mignola in the landscape near Cingoli, Piantone di Mogliano around Mogliano and Coroncina near Caldarola; all samples were provided by the farmers that guarantee the authenticity. Spectroscopic measurements were performed: UV-Vis spectra were acquired with a UV-vis Varian Cary 50 Scan spectrophotometer the IR spectra were obtained with a Perkin Elmer spectrum 100 FT-IR with ATR and the X-ray fluorescence was measured by a ED-XRF Shimadzu 800HS2 with a 10 mm collimator.

Some analyzing methods as PLS-DA, LDA, SIMCA and UNEQ were applied to the data to compare their performance. The considered spectroscopies were suitable to classify the samples, as shown in the figure, but they have different prediction ability. UV-Vis spectroscopy coupled to LDA or PLS-DA data treatment permitted both a good classification and prediction ability.

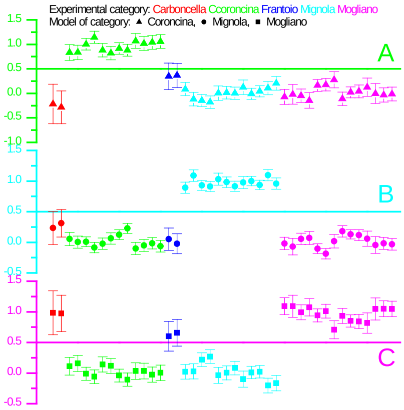

Figure 1: Prediction of all the samples using PLS-DA models with 11 latent variables computed on SNV plus autoscaled data UV-Vis measurements. Box A show prediction with respect to the model of Coroncina, box B versus model of Mignola and box C versus the model of Mogliano.

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PREDICTING SOLUBLE SOLID CONTENT IN INTACT JABOTICABA [MYRCIARIA JABOTICABA (VELL.) O. BERG] FRUIT USING NEAR-INFRARED SPECTROSCOPY AND CHEMOMETRICS

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The aim of this study was to evaluate the potential of near-infrared reflectance spectroscopy (NIR) as a rapid and non-destructive method to determine soluble solid content (SSC) in intact jaboticaba [*Myrciaria jaboticaba* (Vell.) O. Berg] fruit. Samples of jaboticaba with a SSC ranging from 12.1 to 23.5 °Brix were collected from Casa Branca – São Paulo State, Brazil. The NIR spectra were acquired in the 1000–2500 nm range. Multivariate calibration techniques were compared with pre-processed data and variable selection algorithms, such as partial least squares (PLS), interval partial least squares (iPLS), a genetic algorithm (GA), a successive projections algorithm (SPA) and nonlinear techniques (BP-ANN, back propagation of artificial neural networks; LS-SVM, least squares support vector machine) were applied to building the calibration models. The PLS model produced prediction accuracy (R^2=0.71, RMSEP=1.33 °Brix, and RPD=1.65) while the BP-ANN model (R^2=0.68, RMSEM= 1.20 °Brix, and RPD=1.83) and LS-SVM models achieved lower performance metrics (R^2=0.44, RMSEP=1.89 °Brix, and RPD=1.16). These results suggest that the PLS model is a promising method for optimizing the efficiency of NIR spectral calibration models to predict SSC in jaboticaba fruit.

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TOWARD SPECIES-INDEPENDENT FOODSTUFF AUTHENTICATION FROM SPECTRAL DATA: THE CASE OF FRESH AND FROZEN-THAWED FISH

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The substitution of fresh fish with frozen-thawed fish is a typical fraud that damages consumers from an economic point of view and causes health and safety issues (Pavlov, 2007). Several methodologies have been proposed for fresh fish authentication (Duflos et al., 2002; Bozzetta et al., 2012). However, the classification ability of the majority of them is strongly affected by the species under investigation, the integrity of the product, or by its shelf life.

In this work, three different strategies based on latent variable modeling techniques and nearinfrared spectroscopy are explored to develop a multi-species classifier in order to discriminate between fresh and frozen-thawed fish.

The first strategy is based on a Partial Least Squares Discriminant Analysis (PLSDA; Barker and Rayens, 2003) model built on spectra from samples of different species. The second strategy is a two-level PLSDA model: the first-level PLSDA splits the samples according to their species, and the second-level PLS-DA, tailored on each species, classifies the samples status (i.e., fresh or frozen-thawed). The third strategy uses Orthogonal Partial Least Squares Discriminant Analysis (OPLSDA; Trygg and Wold, 2002) to jointly remove the variability in the data that is not related to the fresh/frozen-thawed status and species-independently classify the samples.

The three strategies are tested on a database of spectra collected with two NIR instruments from more than 1200 samples of fish, using also samples of species not included in the calibration data.

This study demonstrates the effectiveness of NIR spectroscopy as a screening method for fresh/frozen-thawed fish authentication, and the possibility of working on a multi-species database

(considering also species not included in the calibration data) without the need of tailoring the classification model on a specific species.

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NIR APPLICATION TO UNMASKING ADULTERATION OF TAGGIASCA OLIVES IN BRINE

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Due to their appreciated sensory features associated with a low annual yield, commercial olives in brine of cultivar Taggiasca, a traditional product of Liguria, an Italian region, are potentially subject to falsifications, in particular by cultivars Leccino and Coquillo, morphologically very similar to Taggiasca olives but less expensive [1][2]. The objective of this study was to determine the amount of olive of different cultivar added in Taggiasca samples based on NIR spectra information.

Olive in brine samples provided by certified producers and representative of the production of Taggiasca olives were collected, together with a balanced number of samples of cultivars Leccino and Coquillo. They were washed with water, dried and stoned, then the pulp was ground and used for spectroscopic analysis. NIR measurements were performed in reflectance mode using a FT-NIR Buchi (NIRFlex N-500). After recording the spectra of all the pure olive samples, Taggiasca olives were blended with Leccino and Coquillo samples; the blending degree were between 5 and 50 w/t%.

Principal Component Analysis (PCA) was applied for visualising pure samples after data pre-treatment (SNV and first derivative). Partial least squares regression (PLS) was employed to build the models aimed at predicting the amounts of Leccino or Coquillo in Taggiasca samples. The optimal complexity – i.e., the number of latent variables to be used for building the models – was estimated by a cross-validation procedure. The prediction ability of PLS models, either on the full spectra or after variable selection, was evaluated on two independent external test sets. The predictive ability of the models after variable selection was similar to that of PLS applied on the whole spectral range. However, variable selection helped to determine the spectral regions most useful to identify the adulteration of Taggiasca olives with Leccino and Coquillo cultivars. All the models showed a satisfactory predictive ability.

The potential of NIR and multivariate analysis to detect the adulteration of Taggiasca olives products was demonstrated. NIR spectroscopy can be a promising procedure to be considered in future applications to detect and quantify different adulterants in Taggiasca olives products. Sample collection and analysis should be performed through a number of years, in order to account for variability factors closely related to the harvest and to obtain models characterized by a global applicability.

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PRELIMINARY STUDY OF THE EFFECT OF CLIMATE VARIABLES ON THE RELATIONSHIP BETWEEN AMINOACIDS IN GRAPE MUST AND AROMAS IN RED WINE OF THE TEMPRANILLO VARIETY

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The amino acids present in the grape must are the primary source of nitrogen for the yeast in the fermentation process to obtain the wine. Moreover, it is well known that the amino acid metabolic pathways lead to the formation of the majority of wine aromas. While it has been possible to establish well-defined routes for the formation of some specific aromas, it can be said that this is an intrinsically complex process where the final profile of aromas in wine is related to initial amino acid profile of the grape juice in a not well defined multivariate way.

Previous studies clearly demonstrate the impact of the amino acid profile on the characteristic flavors of some wine varieties; it has also been studied the effect of the controlled addition of some amino acids in the grape must and how this addition is reflected in the increase/decrease of concentration of aromas. However, a systematic study linking the amino acid profile of grapes with the wine aroma profile in relation to climatic variables is still missing.

In the context of a project to systematically study the effect of climate variables on grape and wine, in this communication we present the relationship of amino acids and major aromas in a Spanish grape: tempranillo. Data from two different vintages (both grapes and produced wines) and two areas of Spain, with a very distinct climatology (continental and mediterranean) are analyzed. As essential climate variables we have chosen the Growing Degree Day (GDD) and water supply (rain and irrigation). Preliminary results on the relationship between amino acids, aromas and climatology obtained from the analysis of PCA, PLS and canonical correlation are reported.

EFFECT OF CLIMATE CHANGE ON WINE AROMA PRECURSORS COMPOSITION OF *Vitis vinifera L.* cv. TEMPRANILLO GRAPES

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One of the best known effects of global warming associated with climate change is the increasing gap between the alcoholic and aromatic maturity. In the framework of a project aimed to study the influence of warming on different variables of both grape and wine, in this paper we show the evolution of the precursors of aroma, present at a very low concentration (µg/L grape juice), in grapes of the Tempranillo variety from a Spanish region characterized by a continental climate regime.

The experimental design consisted in sampling grapes at three levels of saccharimetric maturity (Brix degrees) from six plots (three irrigated and three non-irrigated, during three years (2009, 2010, 2011) also measuring a set of climate variables for each zone (rainfall, temperature, solar radiation, etc.). To facilitate interpretation, the 42 individual precursors detected in the three years have been grouped by families: terpenes, norisoprenoids, vainilles, volatile phenols, benzenes and miscellaneous.

The principal component analysis (PCA) of these data has allowed identifying the changes in the development of precursors of aromas with the maturity and irrigation. It has also allowed correlate these changes with the Growing Day Degree (GDD), which has proved to be the most significant climate variable in this study.

A very different behavior of terpenes by one hand and volatile phenols, benzenes vainilles by the other has been detected in relation to GDD and grape ripening.

EFFECT OF MICRO-OXYGENATION AND OAK CHIP TREATMENTS ON SENSORY PROPERTIES OF RED WINES

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Oak barrels have been traditionally used in aging wine to improve wine quality and contribute to its organoleptic characteristics. However, the use of this type of aging is an expensive practice since implies the use of high cost tanks. In addition, when the barrel is used for a long time, it might be infected with microorganisms, such as Brettanomyces, that can cause off-flavors that degrade the wine quality. Therefore, there is growing interest in developing alternative methods to simplify the ageing process, while ensuring that the wood-related volatile compounds are released into the wine and the colour is stabilised by a slow uptake of oxygen, without decreasing the quality of the wine produced. These possible alternative techniques that can be applied to ageing the wines are the micro-oxygenation and the addition of wood pieces, commonly name “oak chips”. The characteristics of the final wines depend on the peculiarity of the wood used (geographical origin, toasting degree), the contact time between wood and wine, the amount of wood added and the dose of oxygen applied.

Therefore, the objective of the present work was to present a multivariate attempt at evaluating the influential parameters of alternative ageing (based on the simultaneous application of micro-oxygenation and chips maceration) and their effect on sensory characteristics of the wines. The multivariate optimisation strategy used including experimental design and response surface methodology allowed a very interesting study that was not previously attempted.

To evaluate all possible influential factors and their effects on the sensory characteristics of the wines a hybrid design was applied. Once experimentation was performed, was possible to conclude that, best valued wines were those elaborated with chip doses next to 6 g/l combined with medium-high toasting degrees, with a blend of American and French chips, and oxygen doses of 6 ml/l/month and 2 ml/l/month, for 21 and 42 days of maceration, respectively. Moreover, thanks to the results obtained is possible to elaborate wines with specific sensory attributes according to consumer/tasters preferences. To our knowledge, this is the first time that experimental design methodology has been used as a tool to evaluate the sensorial effects that alternative ageing has in wines.

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CHARACTERIZATION OF RAW AND COOKED OKRA (*Abelmoschus esculentus L.*) ACCORDING TO THEIR MINERAL NUTRIENT COMPOSITION

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Okra (*Abelmoschus esculentus L.*) is a plant native to Africa (Jarret et al 2011). It was introduced in Brazil with the slave trade and became grown in all regions, including in the state of Bahia, northeastern Brazil. In this paper, the mineral composition of cooked and raw okra of conventional and organic cultives marketed in the state of Bahia was determined and the results were evaluated using multivariate analysis. The samples were digested in heating block using nitric acid and hydrogen peroxide and they were analyzed using inductively coupled plasma optical emission spectrometry (ICP OES). The accuracy of the method was confirmed by analysis of a standard reference material tomato leaves - NIST 1573a. The results expressed as milligrams of element per 100 g of sample demonstrated that the average concentration for raw and cooked okra varied, respectively, from 366 and 325, for Ca; from 0.102 and 0.052, for Cu; from 267 and 97.7 for K; from 45.3 and 18.3, for Mg; from 18.3 and 7.00, for Na; from 44.5 and 25.8, for P; from 0.233 and 0.094, for Zn.

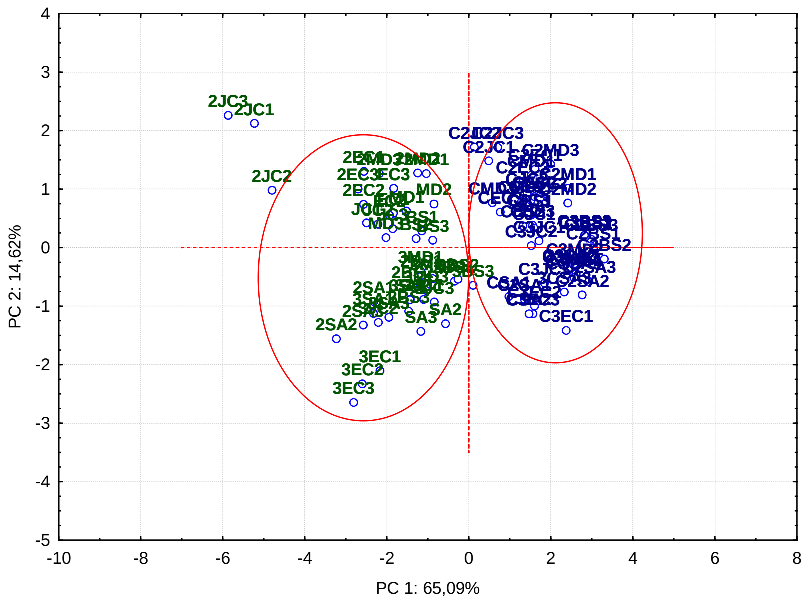

Fig. 1 Plot of the first principal component (PC1) v*ersus* the second principal component (PC2);, Legend: **raw samples** / **cooked samples**.

Comparing raw and cooked samples, it was noticed a tendency of separation in the principal component analysis (PCA) and hierarchical cluster analysis (HCA). The raw samples had the highest concentrations for the all elements analyzed. The raw samples had the highest concentrations for the all elements analyzed, indicating the loss of nutrients during the cooking process, possibly leached for the cooking water. The elements K, P, Mg, Cu, Na and Zn contribute to the major variability between raw and cooked samples and with minor contributions to Ca.

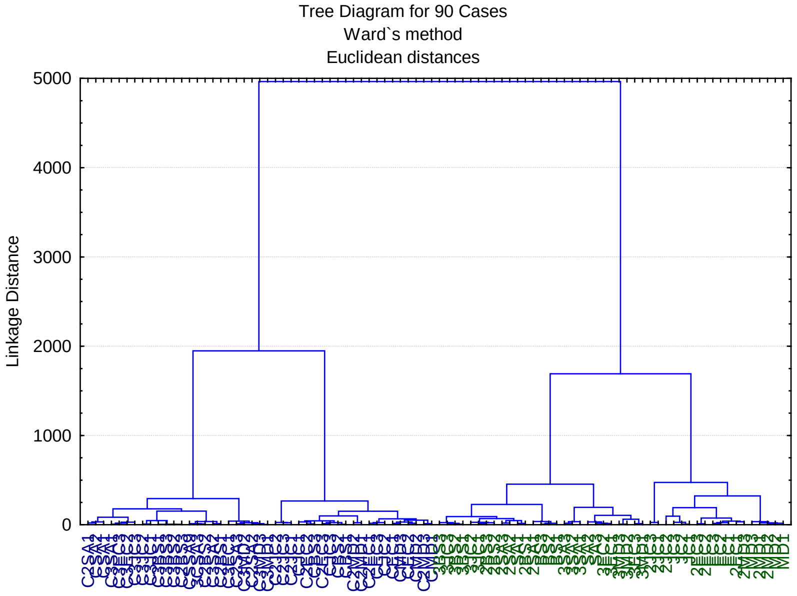

Figure. 2 Dendrogram for okra samples showing Ward’s method with Euclidean distances. Legend: **raw samples**/**cooked samples**.

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MID-IR REFLECTANCE (DRIFTS) FOR IDENTIFICATION OF PLANTS KNOWN AS "MALVA" IN BRAZIL

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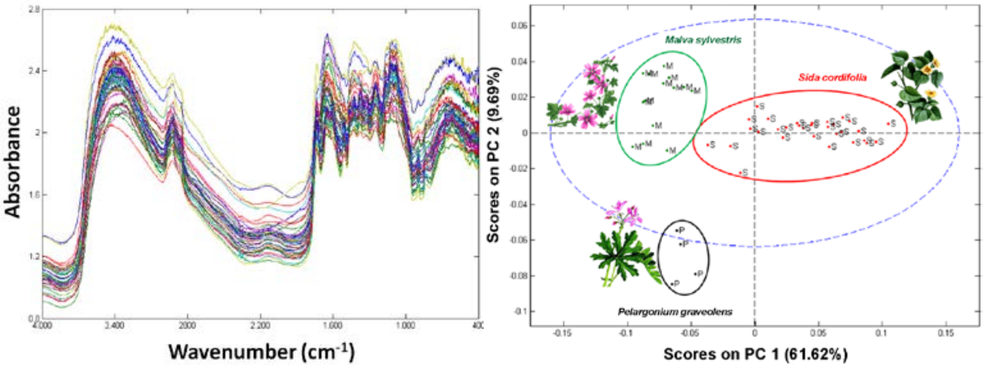
Among the medicinal plants commonly used to treat inflammatory disorders, *Malva sylvestris* L. (Malvaceae) has been used throughout the world since 3000 B.C. This species is effective against mouth and throat diseases and can relieve swelling and toothaches, thrush, gum inflammation and periodontitis [1]. In Brazil, the use of *M. sylvestris* (known as "malva") has been stimulated by government policies. Although the species *M. sylvestris* is described in the Brazilian Pharmacopoeia [2], 21 other plants are also known as "malva" in Brazil according to the local name, such as *Sida cordifolia* ("malva-branca") and *Pelargonium graveolens* ("malva-cheirosa"). This fact causes inaccuracy in medicinal use of the species, and thus it is necessary an adequate identification method to prevent that different species are provided as *M. sylvestris,* since the therapeutic effects are quite diverse [3,4].

Reflectance FT-IR spectroscopy coupled with chemometric analysis can be a useful method for the recognition of species, given that it is solvent-free and simpler and faster than the morpho-anatomical studies. Aim of the present study is to use this approach to characterize commercial samples of "malva" and to distinguish *M. sylvestris* from others species using PCA.

Commercial samples (dried leaves) of "malva" (n=49) were purchased from different regions of Brazil and were originally labeled as: *M. sylvestris* or "malva" (n=39), *S. cordifolia* or "malva-branca" (n=7) and *P. graveolens* or "malva-cheirosa" (n=3). The samples were milled and sieved through a 1190-microm sieve, homogenized and immediately analyzed in the FT-IR DRIFTS (Bruker Optik GmbH). The spectral data were recorded in three independent replicates, with an accumulation of 64 scans in 4000–375cm^{-1} range with a resolution of 4 cm^{-1} under controlled temperature (20.0±0.2ºC) and humidity (45-55%), done using software OPUS (version 6.0) for Windows. Chemometric analysis was done using PLS Toolbox, version 6.5 (Eigenvector Technologies, Manson) under MATLAB environment, version 7.13 (The Math-Works, Natick, MA).

The spectra showed no significant differences visually between samples (Figure 1-A). However, PCA analysis (Figure 1-B) on spectral data after first derivative and mean centering allowed identifying three clusters. The identity of samples of *M. sylvestris* cluster was confirmed by morpho-anatomical analysis, comparing the samples with standards properly identified. For the other two groups is considered the possibility of belonging to the genus *Pelargonium* and *Sida*, due to the macroscopic characteristics of the material. Among the 39 samples labeled as *M. sylvestris*, only 36% (n=14) are indeed *M. sylvestris*. The results show inadequate identification and commercialization of these species in commercial samples. This fact can lead to therapeutic failures, since the plant delivered to the patient may not have the expected pharmacological effects. Overall, this work demonstrates that application of FT-IR and chemometrics analysis can be used to differentiate species of "malva" and identify unconformity in commercial samples.



(A) (B)

Figure 1. Raw MIR spectra of powder samples (A) and scores of the first two principal components (B).

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DATA FUSION APPROACH FOR THE VARIETAL CLASSIFICATION OF LAMBRUSCO P.D.O. WINES

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Nowadays the necessity to reveal the hidden information from complex data sets is increasing due to the development of high-throughput instrumentation. The possibility to jointly analyze data sets arising from different sources (e.g. different analytical determinations/platforms) allows capturing the latent information that would not be extracted by the individual analysis of each block of data. Several approaches are proposed in the literature and are generally referred to as data fusion approaches. In this work a hierarchical data fusion is proposed [1,2] for the characterization of three varieties (Salamino di Santa Croce, Grasparossa di Castelvetro, Sorbara) of Lambrusco Wine, a typical P.D.O. wine of the District of Modena (Italy). 60 wine samples of the three different varieties were analyzed by means of HPLC-DAD of the phenolic compounds, Emission-Excitation Fluorescence Spectroscopy and ^{1}H-NMR.

Since the analytical outputs are characterized by different dimensionality (matrix and tensor), several multivariate analysis were applied (PCA, PARAFAC, MCR-ALS) in order to extract and merge, in a hierarchical way, the information present in each data set.

The results showed that this approach was able to well characterize Lambrusco samples giving also the possibility to understand the correlation between the source of information arising from the three analytical techniques.

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LOW AND MID-LEVEL FUSION STRATEGIES FOR THE DISCRIMINATION OF OLIVE OILS FROM DIFFERENT ORIGINS

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Multivariate classification has widespread use in the discrimination of olive oil origins. These methods are commonly based on single-instrument data and show different sensitivities and specificities depending on the type of raw data being used. It is interesting to test whether the combination of different data sources can improve the sensitivity and specificity, as well as offer better outlier detection capabilities and more confident classifications. Data are said to be combined (fused) at low level, mid (intermediate or feature)-level and high level (or decision level) depending on the processing the data have suffered before they are combined (1).

This study tests low level and mid level of data fusion of NIR and MIR data with partial-least squares discriminant analysis (PLSDA) for discrimination of olive oil samples from different olive cultivars.

Olive oil data were supplied by Casale et.al. (2), who studied the identification of 82 monovarietal extra virgin olive oil samples, from three different cultivars: Leccino, Frantoio and Casaliva (27 from Casaliva, 28 from Leccino, 27 from Frantoio). All samples were measured by both NIR and MIR. Data were preprocessed by SNV and 1st derivative. Data were then first fused at low level and modeled by PLSDA. For mid level fusion, PCA were applied to both datasets and then the scores were fused. Low and mid level fusion strategies improved the percentage of correct classification for Leccino samples in the training set. In general both training and test set percentages of correct classifications changed after fusion. Especially for Frantoio test samples, both fusion techniques improved the correct classification from 70.0% to 88.9% for low level fusion and to 75% for mid level fusion. For Casaliva and Leccino test samples, the percentage of correct classification was increased when the data was fused at mid level when compared to the individual models. Individual models resulted in 66.7% for NIR and 84.6% for MIR, for Casaliva and Leccino test samples, respectively. For mid level fusion, those rates were improved to 92.3 % for Casaliva class and 90.0 % for Leccino class.

Mid level fusion gave more promising results than low level fusion. Mid level fusion improved the correct classification percentages of the training set samples of the three classes more than low level fusion. In the mid level fusion case, the percentages of correct classification of the training sets were 85.7, 100, and 68.4%, whereas for low level fusion these rates were 81.2, 94.1, and 55.5% for Casaliva, Leccino and Frantoio classes, respectively.

For the test samples, Leccino and Casaliva class samples had higher percentages of correct classification, 90.0 and 92.3 % respectively, where percent correct classification for Frantoio samples is decreasing from 88.89 % in the low level fusion case to 75 % in the case of mid level fusion. In conclusion, the fusion of mid- and near-infrared (MIR and NIR) measurements improved the sensitivity and specificity of the classification when compared to classification models built separately on each technique.

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PLS DETERMINATION OF PHYSICOCHEMICAL PARAMETERS IN INSULATING OIL USING NIR, FLUORESCENCE AND NMR SPECTROSCOPIC FUSED DATA

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Transformers are components of great importance for the transmission and distribution of electrical energy. These devices have an insulation system, which consists of Kraft paper immersed in insulating mineral oil. Kraft paper is composed of cellulose, hemicelluloses and lignin. Cellulose degrades slowly with the ageing of the transformer and the polymer chains break down, releasing degradation products into the oil. Cellulose degradation processes involve breaking of glycosidic bonds that hold together the glucose rings. During the decomposition reaction of cellulose chains, water and furanic compounds, such as 5-methyl-2-furfural, 2-acetylfuran, 5-hydroxymethyl-2-furfural, furfuryl alcohol, and 2-furfural, are generated. These compounds change the oil physicochemical properties, such as color and interfacial tension. The interfacial tension is one of the most important parameters used for evaluating the degradation of the insulation system. It corresponds to an indirect measurement of polar substances, such as the furanic compounds and water, so the more the insulating system is degraded, the lower is the interfacial tension. The oil color and its aspect are very important visual characteristics for the oil analysis.The color parameter increases with increasing degradation of the insulation system and has a high correlation with the interfacial tension.

This work presents a rapid and non-destructive method to determine the interfacial tension and color parameters using the spectroscopic techniques FT-NIR, ^{1}H NMR and Fluorescence combined with multivariate calibration methods. One hundred insulating oil samples were obtained from CELG (*Centrais Elétricas de Goiás S. A.*), the electrical power company of the State of Goiás, which is located in the center-west region of Brazil. These transformers presented a wide variety of conditions, with their lifetimes varying from one to about thirty years. Interfacial tension water/oil of these samples was measured by using a torsion Krüss K8 tensiometer, according to Brazilian norm NBR 6234. The parameter color was measured by using a colorimeter Lovibond, according to Brazilian norm NBR 14483. The NIR spectra were obtained using a spectrometer Perkin Elmer 100N, with a reflectance accessory. Each spectrum was measured in the range from 830 to 2500 nm. While the fluorescence spectra were obtained in a Varian Cary Eclipse Spectrofluorimeter, using a 10.00 mm quartz cuvette, all the fluorescence spectral surfaces were obtained in the excitation range from 250 to 650nm (10 nm steps) and in the emission range from 270 to 700nm (2nm steps). Excitation wavelengths were 350 and 550 nm was selected. ^{1}H NMR spectra were recorded in a Bruker Avance III spectrometer operating at 11.75 Tesla with a working frequency of 500.13 MHz for ^{1}H, using $CDCl_3$ as solvent and TMS as internal standard and 64 scans. Data were treated in Matlab version 7.12 using PLS Toolbox version 6.2. The NIR spectra were preprocessed using base line correction and Multiple Scatering Correction (MSC) in the selected range from 1330 to 1530 nm. ^{1}H NMR spectra were preprocessed using base line correction, Savitzky–Golay smoothing filter with a window width of 15 points, wavelet transform and *i*coshift. Subsequently, the range from 7.5 to 6.4 ppm was selected.

PLS calibration models were generated in order to correlate the data set containing all the spectra for the 100 mineral oil samples to their interfacial tensions and color values. Eighty six samples were selected for the calibration set and the remaining 14 samples were used for the prediction set. The best PLS model was obtained with mean centering data, using four latent variables. The Table 1 shows the main characteristics of the PLS model for prediction of interfacial tension and color properties. As a result, the predicted error obtained by the proposed model is significantly lower than the one from the reference method. Moreover, the spectroscopic techniques open some perspectives for the quantification of furanic compounds.

Table 1. Results for the PLS models for determination of interfacial tension and color.

PLS Models	RMSEC	RMSEP	Prediction Errors (%)	r
Interfacial tension model	4.4	3.9	10.9	0.8435
Color model	0.3	0.3	18.9	0.9242

The proposed method showed to be useful for the determination of the interfacial tension and color in samples of the insulating mineral oils by the proposed spectroscopic techniques or even better using the three combined techniques. The method suggested is direct, rapid and non-destructive and does not generate chemical residues. Finally, this work opens perspectives for possible implementing a non-invasive on-line assessment of the conditions of transformer insulating systems.

LOCAL CLASSIFICATION: LOCALLY WEIGHTED – PLS-DA

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Classification problems occur ubiquitously in chemometrics, from traceability to process monitoring, passing through metabolomics or image analysis. However, the development and widespread availability of more and more efficient instrumental platforms resulted in a corresponding increase in the complexity of the problems and tasks chemists are called to solve. In the framework of pattern recognition, this consideration translates to the possibility that many sources of variation, other than the class belonging, affect the instrumental signal, resulting in a non-linear separability of the categories in the feature space. Whenever this occurs, the use of non-linear classification methods constitutes an obvious answer to this issue; however, involving an higher number of adjustable parameters, these kind of methods usually have strict requirements in terms of the samples to variables ratio, and are more prone to overfitting. One way of overcoming this limitations, which has already been extensively used also for calibration problems, is to implement the non-linearity through the training of locally linear classifiers [1].

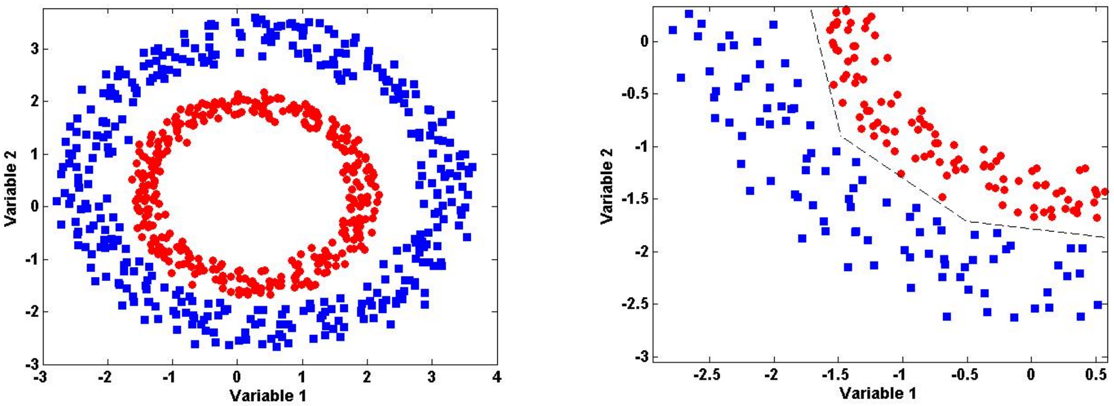

Figure 1 – Example of a non-linear classification task in two dimensions (left) and its transformation to a piecewise linearly separable problem (right).

In this study, the possibility of extending the Locally Weighted-PLS approach originally proposed by Centner and Massart [2], to deal with non-linear classification problem was thoroughly investigated. In this context, different weighting schemes (uniform weighting or distance-based weighting) and different criteria for selecting the local training set (*k* nearest neighbours, *k*-means clustering, identification of prototypes) were tested and compared. Additionally, the possibility of using Euclidean or Mahalanobis distances, or other dissimilarity measurements was also considered.

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MULTIVARIATE NIR CALIBRATION STRATEGIES USEFUL FOR QUALITY CONTROL OF PHARMACEUTICAL FORMULATIONS

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Near infrared spectroscopy (NIR) together with chemometrics data processing is considered one of the fastest and most versatile analytical methods for the determination of important parameters of pharmaceutical products in the final stage and throughout the manufacturing process. In order to quantify the concentration of the active principal ingredient (API) of two pharmaceutical formulations during different manufacturing steps such as granulation, compacting and coating, two multivariate calibration models have been constructed using partial least squares regression (PLS1). To obtain a calibration model with a good predictive ability, it is necessary to add the variability sources of the production samples to the calibration set. Therefore, two different calibration strategies have been used: **a) calculation and addition of the process spectrum[1],** which is a virtual spectrum that incorporates the variability added by the manufacturing process, to the calibration set. In this case, the difference between a production tablet and a powder sample prepared in the laboratory with of same composition to the calibration set, and in this sense a new spectra matrix containing the all the process variability is obtained. b) **The under and over dosage of industrial granulates** has been the second strategy used, in which a calibration set can be obtained that contains the physical variability due to the granulation process and a broad concentration range by the addition either of API or an excipient mixture (placebo). In this sense, the samples are spanned in a certain concentration range that allows for the quantification of the API in each industrial sample.

For both the powder laboratory samples and the under/over dosage samples, known quantities of API and placebos have been weighed, spanned in an API concentration range of ± 20% with respect to the nominal value present in the formulation. API nominal concentration value in each formulation is given as a) 9% (w/w), b) 10 mg/g aprox.

To build up the calibration model, four consecutive steps were followed: 1) spectral pretreatment 2) Selection of the calibration set 3) construction of the calibration model using the PLS1 regression algorithm and 4) the validation of the model.

The use of derivates combined with standard normal variate (SNV) has been the best spectral pretreatment that, on the one hand, allowed the increase of the differential between broad and overlapped bands characteristic of NIR, and on the other hand, corrects the scatter effects due to heterogeneous particle size.

Principal component analysis was used for the calibration set which should contain the maximum spectral variability and spanned in the all concentration range. Through calculation of the model using PLS1 regression, the correlation between the analytical signal and the concentration has been maximized and in this sense it has been possible to build up a calibration model with a good predictive analysis.

For the validation of the model, two different sample sets have been used: laboratory samples (powder or under/overdosage granulates mixtures) and industrial samples (granulates, uncoated (core) and coated tables). These models corroborate their usefulness in control quality processes for the pharmaceutical industry.

a) Model build up by calculation and addition of process spectra						
	Uncoated			**Coated**		
	CALIBRATION	PREDICTION		CALIBRATION	PREDICTION	
Spectral pretreatment	2^a Der (S.G - 25points; 3^o polynomial) + SNV					
Spectral range (nm)	(1000-1790) (1810 – 2500)					
concentration range (% w/w)	6.9 - 10.3					
Number of factors	4			4		
Y- explained variance (%)	98.8			99.5		
Type of sample	Lab+SP_Cores	Lab+SP_Cores	Cores	Lab+SP_Coated	Lab+SP_Coated	Coated
Number of samples	34	12	10	34	12	10
RSEC/RSEP (%)	1.26	1.96	3.74	0.78	1.22	3.93
RMSEC/RMSEP (% w/w)	0.10	0.17	0.10	0.06	0.11	0.10
Residual average		-0.05	0.22		-0.04	-0.04

a) Model build up under and overdosage of industrial granulates

	CALIBRATION	PREDICTION	
Spectral pretreatment	SNV+ 2ª Der (S.G - 25points; 3º polynomial)		
Spectral range (nm)	(1000-1749) (2051-2200)		
concentration range(mg/g)	8.0 – 12.0		
Number of factors	5		
Y- explained variance (%)	99		
Type of sample	Under/overdosage samples.	Under/overdosage samples.	Industrial Granulates
Number of samples	15	15(CV)	57
RSEC/RSEP (%)	1.23	4.93	3.92
RMSEC/RMSEP (mg/g)	0.12	0.50	0.38
Residual average		-0.10	0.04

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COMPARISON OF LIPIDIC PAINT BINDERS AGEING UNDER DIFFERENT CONDITIONS BY RAMAN SPECTROSCOPY AND CHEMOMETRICS

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Among the different organic substances used y artists in the past, traditional paint binders of lipid nature are represented by egg yolk in the tempera technique and drying or siccative oils in the oil technique. The chemical composition of a painting is a complex heterogeneous matrix changing over the course of time depending on factors like conservation conditions such as changes in temperature and humidity and exposure to natural and artificial light among others. The identification of the original substances and the characterization of the degradation processes affecting the binders are important for restoration purposes. Here we explore the capabilities of Raman spectroscopy in combination with chemometric techniques for this purpose.

Samples of egg yolk and the most commonly employed drying oils, namely linseed, poppy seed and walnut oils and mixtures of them were prepared and aged for six years under natural ambient conditions. Replicates of these samples were artificially aged for 960 hours in a UV accelerated test chamber. Particularly, irradiance was set at 765Wm^{-2}, and the maximum and minimum temperatures of the samples were maintained between 30–35 ◦C and 15–20% relative humidity. Figure 1 compares the Raman spectra of the pure binders aged naturally during 6-7 years and the same binders subjected to artificial ageing.

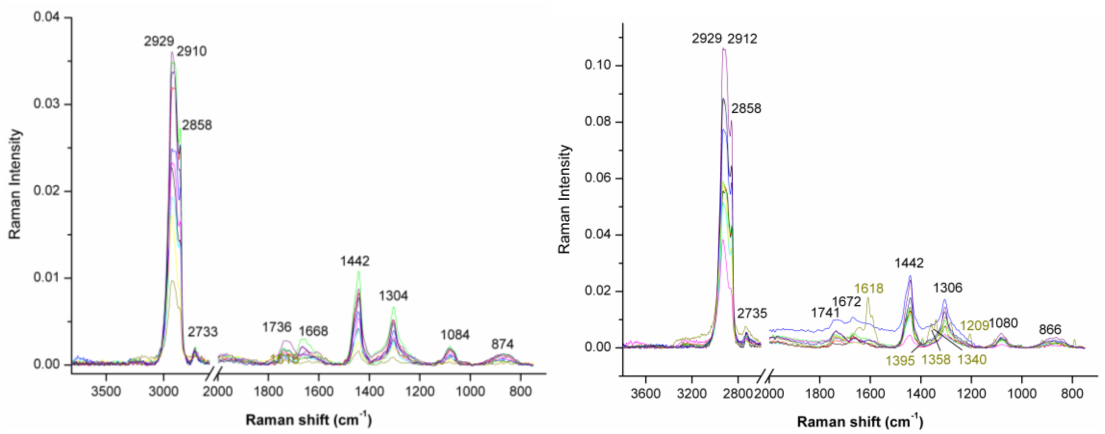

Figure 1 (a and b) show the Raman spectra of drying oils and egg mixtures

In all cases the most prominent bands are found in the C-H stretching region from 2840 to 2950 cm-1 and C-H bending region 1300-1450 cm-1. The broad bands in the region 1680-1740 cm-1 are due to the overlapping of the C=O stretching of the ester and the different carbonyl compounds formed by lipid oxidation. The spectral features are similar for all the binders with natural ageing and the spectra differ in the relative intensities of the bands. In the case of samples with additional artificial ageing it is remarkable the presence of several distinct Raman bands in the spectra of pure poppy seed oil (1618, 1395, 1358, 1340 and 1209 cm-1). Although their origin is still not completely clear they could be assigned to the formation of carboxylate salts.

Both Hierarchical cluster analysis (HCA) and Principal component analysis (PCA) were used to explore the Raman spectra and the results were consistent. When studying natural aged samples, a group can be defined for poppy seed oil samples whereas samples containing walnut and linseed oil form another cluster. Egg samples were widespread in both groups. PC1 with a 78.65 % of explained variance shows the same tendency that HCA. Furthermore, samples containing egg always show negative values for PC2 (10.12%).

Artificially aged samples show a different behaviour in HCA caused by the grouping of linseed and poppy seed oil pure samples separated from a group that can be also divided in two subgroups: one containing egg samples and another with linseed oil mixtures and walnut samples. PCA needs only two PCs to separate pure samples, while mixtures are more complicated to separate.

In order to get a discriminant model to classify unknown samples according to the presence of each drying oil, PLSDA was applied to both series of samples. Results were satisfactory for artificially aged samples with sensitivity and specificity values for prediction always close to

1.000 except for poppy seed oil samples. However the classification for natural aged samples is worse and only egg yolk is well classified in all the samples.

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AD-HOC MASS CLUSTERING TO STUDY TEMPORAL EVOLUTION OF MALDI-TOF-MS MAPPEPTIDE FINGERPRINTING OF MONOCLONAL ANTIBODY

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Monoclonal antibodies (mAbs) represent the faster growing class of human therapeutics. From a structural point of view they are tetrameric glycoproteins with high molecular weights (150 kDa). They are composed by two identical heavy chains and two identical light chains linked by a disulfide bond. Among the different isotypes of mAbs, IgG1 is the most used clinically due to its particular characteristics. Although IgG1 are relatively stable molecules, they are subject to a variety of degradation reactions that can occur during manufacturing, formulation, storage or when handled in hospital conditions. These modifications can decrease the therapeutic activity of the mAbs. Therefore many efforts are being made to detect and track these chemical changes. Among all analytical techniques used for mAbs characterization, mass spectrometry based approaches have led these investigations supplying information on amino acid sequences, higher order structures and conformations [1].

The objective of our study is to provide an algorithm to study the temporal evolution of peptide mass fingerprinting (PMF) of mAb recorded by matrix-assisted laser desorption/ionization time of flight mass spectrometry MALDI-TOF-MS. Previously, the mAb was trypsin digested.In this kind of PMF, a huge volume of MS data is often generated. Each MS spectra consist of two large vectors corresponding to mass to charge ratio (m/z) and intensity [2].

In our study, we obtain PMF at different days. Furthermore, on the same day, we obtain 5 different PMF, which we call *replicates*. The main problem when studying temporal peptide evolution is the following: given two samples $(x_1,y_1)_k^j$ and $(x_2,y_2)_{k'}^{j'}$ that were obtained respectively in replica *j* of day *k* and in replica *j'* of day *k'*, can we determine whether they correspond to the same peptide or not?

As it is well-known, experimental data of the mass of a given peptide are subject to random errors when using PMF. The magnitude of the error depends on the resolution of the equipment, but it is expected that two mass measurements very close to each other actually correspond to the same peptide. In this study, the idea is to group together peptide masses with enough similarity, thus obtaining, for each peptide, a mass interval that indicates the mass range corresponding to the peptide. The initial assumptions are that i) measurement errors are independent random variables with normal distribution and mass-dependent variance, and ii) the maximum measurement error admitted for a given mass is a percentage of the mass and enables us to distinguish between peptides. In order to determine the mass range of each peptide, we present a novel constrained clustering algorithm for peptide masses, in which the two assumptions mentioned before have been incorporated to the algorithm. The number of intervals to construct (i.e., number of peptides present in the data) is also determined by the algorithm as it is not known a priori.With these intervals, it is possible to know if a sample obtained at any day corresponds to a given peptide of interest or not, by checking whether the mass value falls inside the mass range of that peptide, which has been calculated by our algorithm.This allows tracking the temporal evolution of the peptide's occurrence.

In order to check long term stability of a marketed mAb, namely rituximab (RTX) when reconstituted and diluted in hospital conditions, peptides from complementarity determining regions (CDR) were tracked by applying our algorithm with successful results.

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HIGH-THROUGHPUT DETERMINATION OF CHYLOMICRON TRIGLYCERIDES CONTENT IN HUMAN BLOOD BY NMR METABOLOMICS

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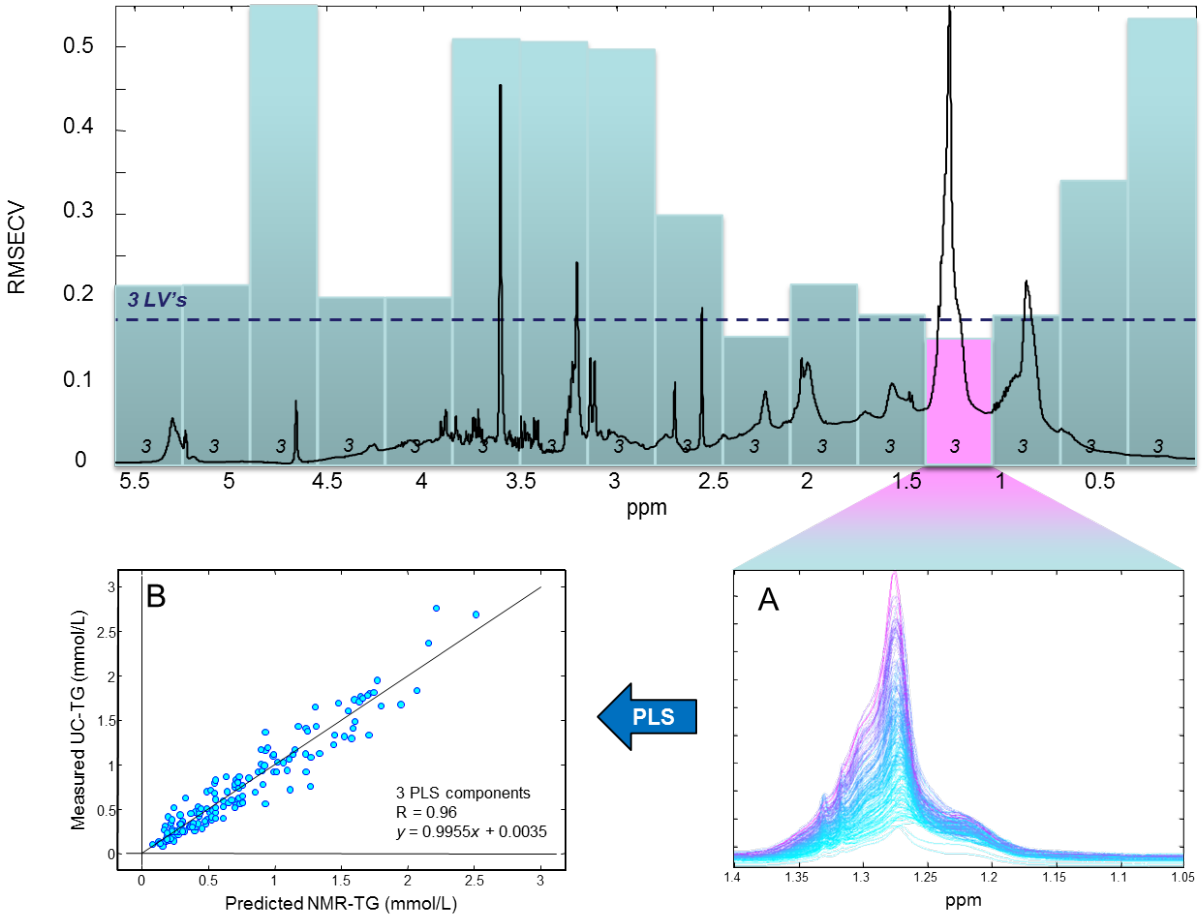
The lipid content of chylomicrons is a risk factor of cardiovascular diseases and a key biomarker for the understanding of obesity. A high-throughput metabolomics determination of chylomicrons in human blood serum is outlined here that overperforms the expensive and time consuming "gold standard" analytical method based on density gradient ultracentrifucation and colorimetric test kit. The new method, which uses a combination of Nuclear Magnetic Resonance (NMR) analysis and chemometrics, enables extraordinarily fast, inexpensive, and robust prediction of triacylglycerol (TAGs) content in chylomicrons. It is the position and shape of the complex lipid methylene band that determines the chylomicron TAG status [1]. The resulting method is a relatively simple iPLS [2] multivariate model that facilitates parsimonious and accurate prediction of chylomicron lipids from NMR spectra of blood plasma. The iPLS model predicts the chylomicron TAG content with a Pearson's correlation coefficient of 0.96 to density gradient ultracentrifugation data (see figure).

The presented method facilitates large scale clinical and nutritional trials with inclusion of diagnostics of chylomicron status and thus creates new opportunities for research in lifestyle diseases and obesity.



iPLS plot indicating the best performing NMR spectral region in predicting chylomicron TAGs content

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FOURIER TRANSFORMATION – A PERFORMANCE TOOL FOR THE CHROMATOGRAPHIC SIGNAL PROCESSING

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In the field of chromatography there are many characteristics available (mean, variance, autocorrelation function, autocovariance function, etc.) for describing signal-time functions, but these can't inform us about the frequencies and phases of the signal constituents. In reality the useful signals are always accompanied by unexpected, disturbing signals, by so-called noises. The quality of the analytical measurement (precision, detection limit, etc.) is curable by decreasing the noise. A good method for noise decreasing is Fourier transformation [1, 2], which can also be used for a subsequent improvement of an established signal, when the frequencies of the signal and noise deviate properly from each other. One of the demanding signal-smoothing method constitutes the curve fitting process, when curve is fitted to the measured digitized signals using a least square method [3]. In this work variation of the Fourier transform of a function due to peak shape changes was examined suitable for describing chromatographic peaks. A mathematical process had been elaborated, during which curve was fitted to the chromatographic peaks in frequency domain using a least square method. After curve fitting applying inverse Fourier transformation, we get back symmetrical chromatographic peaks. The process is presented at separation of phenol derivatives using real chromatograms. This method is suitable when the calculation of Fourier transform of the chromatogram is necessary because of other signal processing operations (noise filtering, interpolation, curve resolution, etc.)

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DETERMINATION OF PARACETAMOL AND IBUPROFEN IN TABLETS AND URINE USING SPECTROMETRIC DETERMINATION COUPLED WITH CHEMOMETRICS

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Paracetamol (PAR) and Ibuprofen (IBU) (Fig. 1) are among the most consumed drugs in world. PAR has an analgesic and antipyretic power similar to aspirin showing the advantage of no irritating the gastrointestinal mucosa. IBU is a non-steroidal antiinflammatory drug that also has analgesic and antipyretic power. Some pharmaceutical formulation has both compounds (Sena *et al*, 2007).

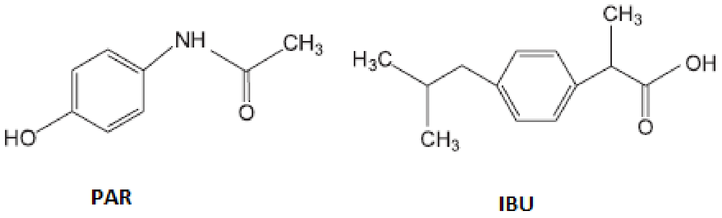

Fig. 1: PAR and IBU structures

Many methods were used for individual determination of each one of these drugs in tablets such as spectrophotometrics, chromatographics and potenciometrics for PAR or IBU. Official methods for determination of both species are based on HPLC with UV detection (Sena *et al*, 2007). Chromatographic methods are recommended by American Pharmacopeia. Other methods such as voltammetric or flow injection analysis can be used for accompaniment of reaction mechanisms (Vieira; Lupetti; Filho, 2003). Simões *et al* (2006) proposed a method for PAR determination in pharmaceutical formulations by using NIR spectroscopy coupled with variable algorithm selection (SPA – Successive Projection Algorithm). However, simultaneous determination of PAR and IBU is related in few articles. In one of them this determination is done using Nuclear Magnetic Ressonance (NMR), that is a expensive and not entirely accessible by most of laboratories. In other article was used spectrophotometry and multiple linear regression (MLR). For any of these methods was not estimated figures of merit. The fact of PAR and IBU present overlap spectra in UV it is not possible to perform direct and simultaneous determinations (Suarez *et al*, 2005).

Caused by that, the proposal of this paper is develop a spectrometric method using second order calibration techniques to perform the PAR and IBU spectra deconvolution and quantification of these species in tablets and urine and also determinates figures of merit for them what is not performed yet. Some chemometric techniques will be employed to verify which one of them will furnish the best results. These techniques are PARAFAC (Parallel Factor Analysis), U – PLS (Unfolded Partial Least Squares Regression), N – PLS (N-way Least Squares Regression), RBL (Residual Bilinearization). Preliminary tests were performed in aqueous standard such as best value of pH (was set in 10 – due to avoid overlap of spectra that is more evident in low pH values), the range of wavelength was set from 210 – 260 nm (step of 2 nm) for excitation and a range from 270 – 400 nm (step of 2 nm) for emission and the concentration range of samples (was fixed between 350 – 950 µg. L^{-1}) and were obtained good results. After fixed these parameters will be performed analysis in tablets containing different amounts of two species and after that this study will be extended to urine samples coupled with second order standard addition.

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SIMULTANEOUS DETERMINATION OF AFLATOXINS B_2 AND G_2 IN PEANUTS USING SPECTROFLUORIMETRY AND MULTIVARIATE CALIBRATION.

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Aflatoxins are secondary metabolites produced by fungi of the genus *Aspergillus* (*A. flavus*, *A. parasiticus* and *A. nomius*) present in foods such as peanuts, corn and wheat. Exhibit carcinogenic, mutagenic, teratogenic, and immunosuppressive [1].

The objective of this study is to compare chemometric tools for multivariate calibration of second order (PARAFAC, and N-PLS/RBL U-PLS/RBL) [2] the simultaneous determination of aflatoxins B_2 and G_2 present in peanut samples with post-column derivatization immunoaffinity analyzed by spectrofluorimetry. The sample extraction was performed with of 70% methanol v/v. The extract is filtered, diluted in water and applied to an affinity column containing monoclonal antibody specific for aflatoxin B_2 and G_2. Aflatoxins are isolated, purified, and concentrated in the column. The removal of the antibodies was done with methanol. The next step was to obtain the excitation-emission spectra of the samples by spectrofluorimetry and multivariate models through comparison of the figures of merit. A comparison of models for each aflatoxin, was performed by exactitude, expressed through the values of RMSEP. Furthermore, we compared the values of limit of detection and quantitation, sensitivity and the analytical sensitivity of a randomly chosen sample (in this case containing 20 ng mL^{-1} each aflatoxin).

The proposed methodology was adequate for the simultaneous determination of aflatoxins B_2 and G_2 for all three chemometric models studied (PARAFAC, and N-PLS/RBL U-PLS/RBL), whose RMSEPs were 0.375, 2.247 and 2.027 for aflatoxin B_2 and 1.012, 3.145 and 3.492, for aflatoxin G_2, respectively. Despite the PARAFAC model presenting the lowest RMSEP for both aflatoxins, the application of the test F (F_{crit}: 3,316) indicates no difference between the three methods for the quantification of both aflatoxin B2 (F_{calc}: 0.028) and aflatoxin G_2 (F_{calc}: 0.356). The limits of detection and quantification were below the maximum tolerated (LMT) 20 micrograms / kg for the sum of aflatoxins, according to the legislation (RDC. 274) [3].

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THE USE OF PROFICIENCY TESTING TO DETERMINE REPEATABILITY AND REPRODUCIBILITY OF NATURAL GAS COMPOSITION BY CHROMATOGRAPHY

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Interlaboratory proficiency testing presents itself as a valuable tool for laboratories to demonstrate their competence and seek mutual recognition, therefore adapting their technological infrastructure to international standards, overcoming technical barriers and increasing their integration in foreign trade [1-3]. Therefore, the participation of public and private laboratories in proficiency testing programs is important to ensure the reliability of analytical results provided by these laboratories.

Natural gas is a highly traded and valuable commodity. and is sold to consumers based on its heating value, that can be calculated by the determination of the molar fraction of each natural gas component present in the mixture. The most widely used instrument for measuring the composition of natural gas is the gas chromatograph (GC) equipped with thermal conductivity (TCD) and flame-ionization detectors (FID) described in the standard test methods such as ISO 6974-3, ASTM D 1945 and NBR 14903 [4-6].

The objective of this work was the performance evaluation of the participating laboratories by different statistical methods and the determination of the repeatability and the reproducibility precision parameters [7] for all components measured in natural gas mixture using gas chromatography technique .

Twenty-seven laboratories distributed in nine Brazilian states participated in this collaborative program to determine the components present in natural gas synthetic mixture. Two cylinders containing gas mixtures of known composition were used in this program. Initially, the cylinders were stored in the Inmetro's Laboratory of Gas Analysis (Labag) at laboratory climate conditions (20 ± 2) °C. Each participating laboratory received only one cylinder, properly identified, with a pressure regulator. The composition of the cylinders was analyzed by Labag during the study which confirmed that the composition of the cylinders did not change throughout this study. The analytical results submitted by the laboratories were evaluated for outliers using the statistical criteria according to Cochran, Dixon and Grubbs tests.

The performance evaluation was conducted by using the consensus values which were obtained from the mean data of each natural gas component informed by all participants, after the elimination of outliers. The performances of the laboratory were statistically evaluated using Z-score, Hierarchical Cluster Analysis (HCA) and Principal Component Analysis (PCA).

Afterwards, the calculations for repeatability and reproducibility values were performed by using the results from laboratories that were considered satisfactory for each natural gas component. The lowest values of standard deviation under repeatability and reproducibility conditions were 0.02 % and 0.13 % for the methane component, respectively, that are in agreement with the requirements reported in the ISO 6974-3 and ASTM D 1945.

Thus, it was possible to determine the parameters estimation of repeatability and reproducibility for the determination of the chemical composition of natural gas mixture by gas chromatography, according to standardization documents, as well as determine the performance of the participating laboratories for the proposed test.

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MACHINE LEARNING CLASSIFICATION OF NEAT AND BURNT IGNITABLE LIQUIDS FOR ARSON DETECTION

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Ignitable liquids (ILs) are frequently used to promote and accelerate the development of fire in arson cases. The chemical analysis of fire debris, usually by extraction followed by GC-MS analysis, can detect the presence of ILs, or identify patterns of volatile compounds resulting from their burning. This type of analysis can assist in the forensic investigation of a fire, to determine its origin and cause. However, fire debris always contain complex mixtures of volatile compounds, influenced by the burnt substrates in addition to possible ILs. The pattern of compounds to be observed also depends on the extent of burning and the delay between burning and sampling.

Chemometric methodologies have been applied to the pattern recognition of fire accelerants. Hardy et. Al [1] classified samples of five ASTM classes of accelerants, exposed to different types of wood or carpet, using PCA and SIMCA. For one type of carpet the effect of evaporation was studied, as well as the ability to classify samples after matrix charring. NicDaeid et. al.[2] [3] used principal component analysis (PCA), hierarchical cluster analysis (HCA), and self organizing maps (SOM) to classify samples of neat and evaporated refill lighter fluid samples from five different brands, and samples of neat/evaporated three medium petroleum distillate products (white spirit, paint brush cleaner, and lamp oil) from different brands. Baerncopf et. al.[4] classified samples of burnt carpet spiked with different ILs (gasoline, diesel, ultra pure paraffin lamp oil, adhesive remover, torch fuel, paint thinner) using PCA and Pearson product moment correlation (PPMC).

Here we present the chemometrics exploration of a new data set with new fire simulation/analytical procedures consisting of 41 samples of six ILs (gasoline, diesel, kerosene, white spirit, paint brush cleaner and charcoal starter) which were analysed neat, after burning to various extent, and after different delays between burning and sampling. Samples were extracted with activated charcoal strips (ACS) and analysed by GC-MS following the method previously published.[5] For each GC/MS data file, the total ion chromatogram (TIC) was divided into 412 equal time intervals and the TIC in each segment was summed to represent a variable in the data matrix.

Classification of samples was accomplished by decision trees, random forests, and self organizing maps, according to IL, gasoline brand, and burning extent. A functional tree could correctly classify 93% of the samples according to the six types of ILs in 10-fold cross-validation experiments. Correct classification of gasolines according to octane number was observed, as well as some ability to identify gasoline brands. The investigation of a larger data set will also be presented, including samples of simulated fire debris with different types of substrates.

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A WAVELET-BASED PROCEDURE TO DETECT SHARP PEAKS IN LASER PHOTOACOUSTIC SPECTRA OF GAS MIXTURES

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In the last decade there was a market growth of chemical agent and toxic materials sensor-devices, based on numerous detection techniques for several substances and related to different scenarios and applications, which are able to screen and identify specific molecules even at very low concentration at laboratory or in controlled environment. However, a portable system which would be simple to use, sensitive, compact, and capable of providing screening over a large number of compounds and discriminate them with low probability of false alarms with short response time scale is still demanded. In this context, the use of an External Cavity-Quantum Cascade Laser Photo Acoustic Sensor (EC-QCLPAS) [1] operating in the mid-IR spectral region seems very promising, since it offers a high sensitivity in a wide spectral range. However, in order to achieve also a high specificity for the detection of the target molecules, the EC-QCLPAS spectra must be properly preprocessed to cancel out the contribution of those signal regions where the sensor output shows a low reproducibility. In particular, the preprocessing procedure must be focused at discarding the signal portions containing sharp intense peaks of small interfering chemical components, since their contribution to the final mixtures spectra is highly irreproducible. At the same time, however, utmost attention must be paid to include all the neighboring regions, which could potentially bring useful information for the identification of the target molecules. The problem due to sharp peaks of small molecules, such as H_2O and CO_2, arises since in these spectral regions the measurement accuracy is heavily affected by the instrument precision in repositioning, which is rather low when compared to the extremely narrow bandwidth. For this reason, an algorithm [2] was developed *ad-hoc* in order to detect the extent, intensity and position of the sharp peaks deriving from all the small molecules included in a dataset of simulated gas mixtures spectra, and to discard all the corresponding spectral regions in an interval equal to the error in repositioning. The implemented approach makes use of a graphical interface based on the Fast Wavelet Transform (FWT) [3] to separate the high frequency content of the spectrum, corresponding to the sharp peaks, from the smooth variation due to the presence of large absorption bands and signal background. In order to quantify the position and the intensity of the sharp peaks, a sharpness function is then calculated using the approximations and the detail vectors. This function reflects how intense, for each single wavenumber, the sharp variations are with respect to the neighboring parts of the signal, i.e. with respect to the smooth variations, and weights this ratio by the corresponding molar absorptivity and by the maximum possible concentration of each considered molecular species. The overall sharpness function is then calculated as the mean of the sharpness functions of all the molecules generating sharp peaks, thus representing the positions and intensities of all the sharp peaks that can be present in the final mixtures. Finally, all the wavenumber values where the sharpness function assumes values higher than a fixed threshold are discarded, together with all the wavenumbers whose distance from these ones is lower than the estimated error in repositioning.

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