VALIDATION IN ANALYTICAL SCIENCE
Current Practices and Future Challenges

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BOOK OF ABSTRACTS
Welcome to the fascinating world of Method Validation.

Method Validation is a concept which was introduced in the early 90’es. Before that, it was “just” stated in e.g. the European Standard, EN 45001 “General criteria for the operation of testing laboratories” that “…The testing laboratory shall have adequate documented instructions on the use and operation of all relevant equipment, on the handling and preparation of test items (where applicable), and on standard testing techniques, where the absence of such instructions could jeopardize the efficacy of the testing process” [1]. This was the background requirement for accredited laboratories in Europe in those days.

In 1994 the International Conference On Harmonisation Of Technical Requirements For Registration Of Pharmaceuticals For Human Use (ICH) issued the so-called Harmonised Tripartite Guidelines, Q2A: Text on Validation of Analytical Procedures [2] and Q2B: Validation of Analytical Procedures: Methodology [3]. And maybe it was inspired by this initiative within the pharmaceutical industry, that in the mid 90’es some European accreditation bodies started requesting analytical laboratories to perform something called “validation” of their methods although the concept didn’t exist in the standard for accreditation (EN 45001). The “World of Method Validation” was created and has been expanding ever since.

For most people in our modern society, this is an unknown “Underground World” – as authorities, patients, consumers, patients etc. who are relying on trustworthy results for setting up (and monitoring) regulations, making a diagnosis or judging on the quality of a product, don’t know (mostly) about this amazing world of LODs, measuring ranges, intermediate precision, robustness etc.. They leave it to the laboratories to secure, that results sent out for making any kind of decisions are reliable.

However, in the laboratories everybody are very much aware of the fact, that an analytical method is not just a “cook-book” taken down from the shelf to follow when performing the often complex work of applying a number of activities on a sample to finally come up with a number, which we can trust being useful information about the content of a sample. We know that the method must be scrutinized in many details to secure that it is fit for the purpose for which it is applied. And we also know that since 2000 accreditation is based on the standard ISO/IEC 17025 [4], which includes detailed requirements on method validation and how validation studies should be planned, performed and evaluated.

The presentations given (orally or by poster, for which the abstracts can be found in this booklet) during this workshop illustrates very well how many forms and levels of details in the work of method validation the skilled people in various types of laboratories have to consider and tackle in dealing with validation studies.

During the 2-day workshop we shall hear and see about everything from validation of method for analysis of phthalates in toys to the challenges of replacing traditional microbiological methods for bacteria determination by modern bioanalytical techniques (PCR). From handling of multiparameter methods in the clinical laboratories to new possibilities of designing validation studies and use of chemometrics for data-evaluation. From dealing with validation of automated analytical systems to attempts on applying the principles of method validation to semi-quantitative and qualitative methods.

Indeed a fascinating world, the ever-growing “World of Method Validation”.

Enjoy the tour together with a lot of good colleagues from all around the world...

Welcome ☺

Lorens P. Sibbesen
Chair of Eurachem Method Validation Working Group
and member of the Workshop Scientific Committee.

[1] EN 45001 “General criteria for the operation of testing laboratories”, CEN/CENELEC 1989
[3] Q2B: "Validation of Analytical Procedures: Methodology", Approved by ICH Steering Committee meeting on 6 November 1996 [Note: In 2005, Q2A and Q2B were combined to one guideline, Q2(R1) ]
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INTERNATIONAL GUIDANCE - AN OVERVIEW

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The concept of Method Validation – at least dedicated studies for the purpose – became more and more prevalent during the 90’es, ending with the words being spelled out directly for the first time as a requirement to accredited laboratories in the new standard ISO/IEC 17025, General requirements for the competence of testing and calibration laboratories in 2000 [1].

However, already in 1994 the International Conference On Harmonisation Of Technical Requirements For Registration Of Pharmaceuticals For Human Use (ICH) issued the so-called Harmonised Tripartite Guidelines, Q2A: Text on Validation of Analytical Procedures [2] and Q2B: Validation of Analytical Procedures: Methodology [3] (later, 2005, combined to one guideline, Q2(R1) ). Primarily for use in the pharmaceutical industry.

As the requirements for doing something on the validity of methods applied among accredited analytical laboratories grew through the 90’s (without the exact requirement being expressed in the standard yet), Eurachem decided to elaborate the well-known guideline The Fitness for Purpose of Analytical Methods – A Laboratory Guide to Method Validation and Related Topics, which was issued in it’s first version in 1998 [4].

Since then a multitude of guidelines and recommendations on how to plan, perform and evaluate a validation study has been elaborated. Some of them generic in their approach, some of them more sector specific (e.g. on food contaminants or forensic analysis). Some of them following generally accepted approaches and concepts, some of them elaborating their own (e.g. the concepts of $\text{CC}_\alpha$ and $\text{CC}_\beta$ for food contaminants instead of the generally used LOD/LOQ).

Over the years the challenges to method validation have also increased in line with increased requirements to the methods, new technology, new areas of application etc. and the need for user-friendly guidance seems to be never ending.

The Eurachem Guide came out in it’s 2nd version in 2014 and is aiming at having a generic approach to the methodology of method validation, and it’s relevance has already been proven by the many downloads from the Eurachem website plus the fact, that it has already been translated into to other languages (Farsi, Ukrainian and Spanish – and more are under way ) for securing understanding and implementation in the daily work in the laboratories around the world.

But the working group behind the guide (MVWG) is already working on an expansion, trying to follow up on the many challenges – and need for guidance internationally – the analytical laboratories are facing.

[2] Q2A: Text on Validation of Analytical Procedures, Approved by ICH Steering Committee meeting on 27 October 1994
[3] Q2B: Validation of Analytical Procedures: Methodology, Approved by ICH Steering Committee meeting on 6 November 1996
K02

SETTING THE REQUIREMENTS FOR A METHOD (INCLUDING ASPECTS OF MU)

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The validation of measurement methods is crucial for assuring the quality of the results. In 2014 Eurachem released a second edition of its Guide on method validation, devised to aid laboratories in demonstrating the fitness for purpose of test methods.

In order to demonstrate fitness for purpose we first need to set requirements for the test results based on the client’s requirements. The client may need to 1) compare two different treatments of a product under development, 2) assess whether the process control shows that the production is in control 3) assess whether a production batch meets specifications, 4) decide whether a legal limit has been exceeded, e.g. whether an athlete is doped, the annual emission of a restricted compound from a plant is within limits or a driver has a blood alcohol concentration above the national limit.

Based on the client’s requirements we need to develop a sampling plan, choose a test and set the requirements. Last year Eurachem released a Guide providing guidance to analysts, regulators and other end-users of analytical information on setting the target measurement uncertainty. In cases 3) and 4) above the key analytical requirement is the uncertainty but in case 1) and 2) the key requirement is only the precision. In case 1 the key requirement is the repeatability providing that all tests can be performed the same day. In case 2 the key requirement is the intermediate precision which in this case is the within-laboratory reproducibility. The interrelation between these requirements is shown here in a ladder where measurement uncertainty includes all steps. Based on the client’s needs the requirement can be set on a 95 % confidence (19 cases out of 20) but in the legal sector the confidence is usually well above 99 %.

The presentation will address how to set the analytical requirements in the typical cases 1 to 4 described above.
K03

EXTENT OF VALIDATION/VERIFICATION STUDIES & EXAMPLES OF EFFICIENT PLANNING OF VALIDATION STUDIES.

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Two questions are always asked when planning validation studies; “how many performance characteristics should be studied?”, and “how many samples and replicates are needed for sound validation?”. Often, the answers to these question are followed rapidly by a further question: “How can get all the necessary information with less work?”

This presentation will review present and developing guidance on the extent and scale of validation studies. It will draw on existing guidance on the range of performance characteristics to be studied in different circumstances, including that from Eurachem [1], IUPAC [2] and the International Council on Harmonisation (ICH) [3]. Typical guidance on the scale of validation studies will be reviewed and methods for establishing sufficient sample size will be considered.

Examples of efficient use of resources will also be given, including simultaneous determination of key performance characteristics from single experiments and the use of experimental design approaches to maximise the information obtained from studies.


METHOD VALIDATION IN CLINICAL CHEMISTRY

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Method validation in clinical chemistry follows the established standards and procedures accepted by all disciplines of chemical metrology. Sampling, measuring and interpreting measurement results from living organisms including humans, introduces unique challenges e.g. regarding fitness of purpose estimations. Furthermore, humans over time usually visit a number of different laboratories using different measurement systems. The patients themselves also sometimes perform their own measurements. Single laboratory- or single measurement system validation therefore commonly needs to be widened into full method validation which preferably includes all measurement systems a sample from a particular patient is likely to encounter over extended periods of time.

Humans maintain the concentrations of measurands in their organisms within more or less narrow probability densities around individual “biological setting points”. The concentrations of measurands important for e.g. nerve conduction (K⁺ or Ca²⁺) are tightly regulated, whereas others, including enzymes have wider probability densities. This variation is called “biological variation” and should be subdivided into within- and between subject variation. The biological variation is commonly double the analytical variation, underscoring the importance of extra-analytical factors when interpreting measurement results.

Pre- and postanalytical variations are other components of the extra-analytical variation. Preanalytical variation includes e.g. the preparation of the patient before sampling, the quality of the sampling itself, and all aspects of handling and transport of the sample to the laboratory. Postanalytical variation is caused by interpretation of the results in the clinical context by the laboratory or by healthcare personnel, transmission of the information from the laboratory to the user including the ability to notify the users of critical results.

Taken together all causes of variation in the clinical laboratory (biological, analytical, preanalytical and postanalytical) need to be taken together when making diagnoses and monitoring treatment effects, and also especially when estimating fitness for purpose, which in the *lingua franca* is called “analytical quality specifications”. Strenuous efforts are currently being taken for reducing pre- and postanalytical variation and efforts are also being made to get better estimates of within- and between individual biological variation.
K05

VALIDATION IN AN ACADEMIC ENVIRONMENT: ACHIEVING QUALITY IN ANALYTICAL DATA

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Does analytical method validation exist in an academic environment? Yes, it does! Achieving high-quality analytical data is a prerequisite when competing in the academic society. The current state of academic research bodies requires validated analytical results. The scientific community does not longer take single graphs with few validation data-points for granted; full validation reports including measurement uncertainty are the state-of-art and are requested by scientific high-impact journals. Nowadays, students, following a science-related education, are confronted in their early bachelors with the topic of method validation; also, PhDs are trained to perform method validation in a proper way. The question appears: how do academic laboratories handle the topic of method validation and what references and protocols are they using to perform method validation?

The Laboratory of Food Analysis at Ghent University is an ISO/IEC 17025 accredited laboratory (BELAC 049-TEST) for the analysis of mycotoxins in food and feed for third parties. A quality management system has been established since 1996. However, research is the Laboratory’s main focus, and the development of new analytical techniques is one of the major research lines in the Laboratory. Therefore, the aspects of the quality management system in terms of standard operating procedures were recently extrapolated to research. One of the main goals was to implement ‘method validation’ in research. This presentation will highlight the points mentioned in this abstract and will give an overview of the challenges of accreditation and method validation in research.
CURRENT PRACTICES AND FUTURE CHALLENGES IN METHODS VALIDATION – NEW AREAS OF APPLICATION

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During the last 10 years, the role of the laboratory as “conformity assessment body” has considerably been reinforced, especially in the regulated sectors: the laboratory may not only be requested to provide a reliable test result but also a conclusion of compliance of the tested product with respect to national regulations or act as subcontracting party of a notified body in the framework of the European harmonization legislation. The expectations of the marketplace in terms of confidence in results provided by the laboratories reflect the importance of these results for economy and trade.

Since the publication of the ISO/IEC 17025 standard, the role of validation as a tool to increase the level of confidence in test results has been highlighted by the laboratories themselves but also by the accreditation bodies when they assess their competence. The recent revision of ISO 15189 has confirmed this trend for the medical laboratories too.

Validation was originally focused on the (final) measurement step within the testing process but has progressively developed to the concept of validation of the final test result: this includes both the validation of the testing method as a whole and the determination of its level of performance as well as ensuring the quality of the test results on a continuous basis.

The standardization bodies as well as scientific or professional groups (including EURACHEM) have developed rules or provisions on validation methodology covering especially quantitative testing and these recommendations are now largely implemented. However, the evolution of the testing needs introduces new challenges for the laboratories community.

From an accreditation point of view, the implementation of the validation concept in some specific technical areas still raises questions. Achieving an harmonized level of requirement during assessments, not only at the national but also at the international level remains an open issue.

The development of guidelines is desirable in particular (but not limited to) with respect to the following situations:

- Qualitative testing (including the “pass-fail” cases): the result is not expressed with a number which prohibits the recourse to a classical statistical approach for validation.
- The use of non-standardized test methods: what is expected from a laboratory that implement test methods (slightly) derived from standardized ones or fully developed by the laboratory itself?
- Accreditation according to a flexible scope: how to design a partial / complementary validation pattern when the laboratory implements a new test method linked to the already accredited (validated) group of methods?
- Destructive testing: repetition of tests may not be technically or economically feasible (e.g. fire testing).
- Analytical sectors where validation may not be straightforward (e.g molecular biology testing in forensics or medical labs – role of biostatistics).
K07

VALIDATION OF MICROBIOLOGICAL METHODS FOR WATER AND ENVIRONMENT: REVISION OF ISO 13843

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The ISO/IEC 17025:2005 standard requires the use of standardized methods which are validated following the development process or the implementation of a proper validation procedure for in-house developed methods [1]. For water and environmental fields, ISO/TR 13843 (2000): water quality – Guidance on validation of microbiological methods is a rough guide [2], which describes how to apply the common characteristics of analytical methods performances to the specificity of the microbiological determinations:

- The analyte is a living microorganism which is taxonomically defined, or in some cases defined by a group designation less accurate than taxonomic definitions (i.e.: coliforms)
- The particle nature of microorganisms and their random distribution even in perfectly mixed waters, lead to peculiar statistical considerations and inexorably limit the enumeration precision
- The measurand (Colony-Forming-Unit) is based on the cultivability of the microorganisms which can be in a large panel of different physiological states depending on the matrix (disinfectant stress in chlorine water, nutrient depletion in oligotrophic waters...)

After the publication of ISO 29201:2012, which gave the baselines of the uncertainty of measurement of microbiological enumeration methods [3], and the revision of ISO 17994:2014 for the comparison of the relative recovery of two microbiological methods [4], the challenging revision of ISO/TR 13843 aims to give a more precise determination of the parameters useful for the characterization of microbiological methods in order to achieve an ISO standard status.

In water and environmental microbiology, the characterization is seen as an exploratory process with the aim of establishing the likely set of performance characteristics of a new or a modified method, under a specific set of circumstances. The sensitivity, specificity and selectivity are determined by verifying presumptive counts compared to confirmed counts. The linearity is seen as an aspect of trueness, it is studied with a view of determining the maximum upper limit of counting. At the other extreme of the working range, the definitions of the detection level and the limit of determination need to take into account the random distribution properly, using appropriate statistical models. The recovery is always relative as the true value of the measurand remains unknown. As to precision parameters, repeatability and reproducibility of the methods can be assessed following the ISO 5725-2:1994 guidelines with some adaptations as the basic principles originally applied to continuous data and not to discrete data such as colony counts.

TRENDS IN INTER-LABORATORY METHOD VALIDATION

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Standardised international test and measurement methods are important for a wide range of trade and regulatory purposes. International implementation of standard procedures requires that the procedures are reliable when used in a wide range of different laboratories. Adoption of such methods therefore relies heavily on inter-laboratory studies for the final stage of evaluation and approval. These inter-laboratory validation studies are typically carried out following well-established standard protocols (for example, ISO 5725 part 2 [0] and the IUPAC protocol [0]) to ensure that the performance results are reliable and can be compared from one study to another.

Although these protocols have been in use for many years, they have limitations. For example, the statistical methods used rely on techniques developed in the early to mid-20th century, and have not adapted to improvements in statistical methodology. The handling of outliers is far from ideal, and advances in robust statistics have seen relatively little uptake for interlaboratory validation studies.

A further issue is the perceived expense of interlaboratory study and the growth in proficiency testing. Several organisations (for example, the UK Analytical Methods Committee [0]) have proposed that proficiency testing data might be of use in establishing the performance of standardised test methods, reducing the need for separate validation studies.

This presentation reviews current practice briefly and discusses emerging developments in inter-laboratory validation. Particular attention will be given to current proposals for the development of ISO 5725, including some developments in statistical methodology. Proposals for the use of proficiency testing data to supplement or replace formal inter-laboratory validation studies will be discussed.


Implementing Principles of Quality by Design (QbD) in Validation Context

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Analytical method performances have to be specified by the analyst through the definition of the “Analytical Target Profile (ATP)”, as proposed by the regulatory bodies. In the specific context of the pharmaceutical industry, regulatory authorities have recently imposed the assessment and management of risk throughout the entire product lifecycle. This includes the analytical procedure and consequently its own lifecycle.

The development step of an analytical method is still largely addressed using a “Changing One Separate Factor a Time (COST)” approach (also known as the “Quality-by-Testing (QbT)” approach). This strategy can lead to a suitable method for assessing the risk of routine use, even where the experimental domain is not examined. However, in order to consider an experimental domain rather than a set of specific experimental conditions during the development phase, a multivariate approach must be considered: the “Quality-by-Design (QbD)” strategy. This strategy allows the definition of a “Design Space (DS)” by means of design of experiments (DoE). This DS, computed considering critical method parameters, allows the analyst to focus on the main objective of an analytical method: obtaining reliable results using a robust method. In the course of a specific case study, the benefits of the QbD strategy in terms of managing the qualitative part of the analytical process were highlighted.

Working in the context of analytical procedure, the validation step is a major part of the analytical method lifecycle. Indeed, the objective of analytical method validation is to demonstrate that this method is suited for quantifying the target analytes with an established and suitable level of accuracy, as defined by the “ATP”. This is sometimes called the “fit-for-future-purpose” concept. The decision regarding the validity of a method based on prediction can be achieved by using the “β-expectation tolerance interval” (accuracy profile). The capability of this approach to manage the quantitative part of the analytical procedure is nowadays largely illustrated in scientific literatures.

Considering the assessment and management of risk throughout the analytical lifecycle, a global strategy allowing the unification of the development and validation phases in a single step was considered. With this innovative approach, a strategy allowing the management of global analytical risk (i.e., for both qualitative and quantitative part of the analytical method) was proposed. Indeed, the developed strategy allows validating an entire experimental domain by means of the accuracy profile rather than a single set of specific experimental conditions. With this strategy, the DS is no longer simply the place where qualitative performances are obtained, but also the space where quantitative performances of the analytical procedure are assessed and managed.
SHORT ORAL PRESENTATION
O01

CHEMOMETRICS IN METHOD VALIDATION - WHY?

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Chemometrics is a tool that many scientists are reluctant or afraid to use; why? Is it because it is considered a "black box" difficult to use? It is certainly a useful and powerful tool worth discovering. Many analytical methods (e.g. HPLC, GC-MS, ICP-MS) seem to be validated. However, are we sure that the best working conditions were identified and applied? Was the method properly optimised? A lot of time and money is spent applying the classical approaches described in the literature. "Design of Experiments" (DoE) may reduce the number of attempts and samples to be analysed while providing additional information related to interactions between the various experimental parameters investigated. Unfortunately, this information is not readily available in traditional approaches. Such an optimised method may proceed to the validation study. Several examples of DoE applications will be presented in the poster, related to Asymmetric Field Flow Fractionation (nanotechnology), GC x GC-MS, Raman and Supercritical fluids.

In the case of spectroscopic methods (e.g. Raman, infrared) where spectra are acquired, a multivariate approach may be appropriate to use the wealth of data available through the whole wavelength range (instead of a single/fixed wavelength). An example of Raman will be presented.

Even when the optimised method is validated, chemometrics is to be applied for multi-dimensional analytical methods, such as GCxGC-MS, to assess and correct for potential co-elution effects.

By now you know that chemometrics is useful; just give it a try.
Eurachem (www.eurachem.org) is a network of organisations within Europe designed to a) establish a system for the international traceability of chemical measurements and b) promote good quality practices in analytical sciences. Currently represented in 32 European countries, Eurachem aims to provide a forum for analytical scientists, laboratory staff and those interested in using the results of analytical measurements to discuss common problems and develop informed and considered approaches to both technical and policy issues. Eurachem members and stakeholders meet once a year at the Eurachem General Assembly. An Executive Committee and several topical Working Groups pursue the organisation’s stated goals throughout the year, often in cooperation with other organisations. Participation is open and channelled through national representatives. Eurachem’s main output is authoritative guidance documents, promoted through dedicated events which are also designed to provide opportunities for collecting feedback. Beside the guides, Eurachem publishes information leaflets, i.e. short briefing documents on specific topics usually intended to inform a wide audience, including laboratory staff, managers and laboratory customers. This poster aims to summarise current Eurachem activities, inform readers about the available guidance and attract active participation.
Eurachem is a network of organisations within Europe that focuses on promoting the reliability of measurement results in analytical sciences and fostering good laboratory practices. To this aim, Eurachem brings together expert working groups to identify needs, pool expertise and propose authoritative guidance. This is accomplished by producing information leaflets and guides and organising or contributing to workshops and other events to promote the exchange of experience and views within the analytical community.

The validation of test methods is a key issue in ensuring the quality of analytical results, and as such is specifically addressed in standards underpinning the requirements for testing and calibration laboratories. The Eurachem Method Validation Working Group (MVWG) was set up to address the issues involved in this topic. This poster summarises the terms of reference for the MVWG, its current activities and main outcomes, among which is the recent release of the second edition of the Eurachem Guide addressing method validation as the tool to demonstrate the fitness for purpose of analytical methods [1].

THE FITNESS FOR PURPOSE OF ANALYTICAL METHODS: A LABORATORY GUIDE TO METHOD VALIDATION AND RELATED TOPICS

Marina Patriarca\(^a\) and Piotr Robouch\(^b\) on behalf of the Project Group, Vicki Barwick,\(^c\) Pedro P. Morillas Bravo,\(^a\) Stephen L. R. Ellison,\(^c\) Joakim Engman,\(^e\) Elin L. F. Gjengedal,\(^f\) Ulla Oxenbøll Lund,\(^g\) Bertil Magnusson,\(^h\) Hans-Thomas Müller Mersin,\(^i\) Barbara Pohl,\(^j\) Lorens P. Sibbesen,\(^k\) Elvar Theodorsson,\(^l\) Florent Vanstapel,\(^m\) Isabelle Vercruysse,\(^n\) Aysun Yilmaz,\(^o\) Perihan Yolci Ömeroglu,\(^p\) and Ulf Örnemark\(^q\)

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Method validation is an important requirement in the practice of analytical sciences. Most analytical chemists are aware of its importance, but why it should be done and when, and exactly what needs to be done, is not always clear. The purpose of the Eurachem Guide \cite{1} is to discuss the issues related to method validation and increase readers’ understanding of what is involved and why it is important, also giving practical guidance on how it can be accomplished. The aim of method validation is to demonstrate that the test results are fit for their intended use. From this, it follows that a clear statement of the analytical requirements underpinning the fitness for purpose of the test results represents the first step of the validation. Translating the customer’s stated or implied needs into analytical requirements is part of the job of the analyst. For example, if the customer’s request is to determine the cadmium content in chocolate to state compliance to existing legal limits, it is the analyst’s duty to assess the maximum allowable measurement uncertainty and other related analytical requirements. The extent of validation required may vary, however, in general, it is necessary to test several characteristics of a test method. Limit of quantification, precision and trueness will almost always be among the parameters assessed. The Guide provides the reader with the rationale behind the determination of the various parameters and quick reference tables to help put this knowledge to work. A wealth of information is produced during a validation study. The Guide indicates how to record and report it in the best way, to support the statement of “fitness for purpose”. In addition, the validation study is the best source of information on which to base the on-going monitoring of the method performance in routine use. The Guide provides support to analysts on how to make the best use of these data for setting up an Internal Quality Control plan. Last, but not least, several reference documents are mentioned in the bibliography. The Method Validation Working Group also provides input to the Eurachem “Reading list”, a bibliography of documents related to several aspects of the quality of tests and measurements, updated yearly.

POSTER 4

GAS QUALITY CONTROL: AN INTERLABORATORY STUDY TO ASSESS THE PERFORMANCE OF FIVE LABORATORIES FOR SAMPLING AND ANALYSIS OF BIOMETHANE SAMPLES

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The development of renewable energy is a major challenge all over the world, in response to various environmental, geopolitical and economic issues. Biomethane (methane produced after treatment of biogas) to be injected into the grid is one answer. The French Energy Agency (ADEME) developed a roadmap for biomethane that suggests the production of 30 TWh by 2030 (about 1400 biomethane injection plants).

By the end of 2015 in France, 14 biomethane plants have GRDF (French Distribution System Operator) authorization to inject into the grid. According to French specifications, 8 parameters need to be controlled before the injection into the gas grid: ammonia (NH₃), total sulfurs and mercaptans, mercury (Hg), chloride (Cl), fluoride (F), carbon monoxide (CO) and hydrogen (H₂).

As there is no international reference method for biomethane, CRIGEN is one of the only French laboratories to have developed specific methods for quantification of biomethane samples. They have been validated in laboratories and on field. For many laboratories (specialized on biogas or air quality monitoring), sampling and analysis of biomethane is a new challenge because of the level of concentrations, difference of matrices with their own developments (biogas or air quality).

In 2015, an interlaboratory study was carried out by CRIGEN for French DSO in order to identify laboratories able to sample and quantify trace compounds in biomethane with reliable results. This study aimed at assessing laboratories and methods to quantify these parameters in biomethane samples. That is why, gas reference sample in cylinders was employed for this proficiency testing. Each laboratory had one gas sample containing the same gas blend but with blinded contents. Then Z-scores were estimated according to ISO 13528 through the maximum permissible error as the standard deviation of the proficiency assessment.

The study shows that the original scope was too ambitious to be performed in one step: on one hand evaluate sampling skills of the laboratories, on the other hand evaluate analysis skills for quantification. Indeed French laboratories do not manage biomethane sampling (materials of the samplers, gas volumes to be sampled, sampling and analysis of gas under pressure, etc.), whereas this step impacts highly the quantification of the target compounds.

As a conclusion, this study allowed to define the current situation of laboratories skills mainly for gas sampling. Different improvements need to be managed by laboratories to master the sampling step. Then a second step should be done to evaluate only the analytical methods for biomethane samples. These interlaboratory studies are an interesting way to define the best practices, both for biomethane sampling and biomethane analysis. It can be helpful for any standardization process for the quantification of parameters in biomethane.
POSTER 5

IN-HOUSE METHOD VALIDATION OF A METHOD FOR THE DETERMINATION OF CERTAIN PHTHALATES IN TOYS, CHILDCARE AND TEXTILE ARTICLES

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Toys, plastic childcare and coated and printed textile childcare articles, containing certain phthalates in a concentration greater than 0.1\% m/m of the plasticised material, shall not be placed on the market. Phthalates such as bis (2-ethylhexyl) phthalate (DEHP), dibutyl phthalate (DBP), benzyl butyl phthalate (BBP), are restricted by the entry 51 in Annex XVII of the Regulation (EU) 1907/2006. Validated methods for the analysis of phthalates in these consumer products are an effective tool with which the market surveillance authorities can guarantee the compliance in the European framework of the Directive 2001/95/EC.

A gas chromatographic - mass spectrometric (GC-MS) method was implemented for the simultaneous determination of DEHP, DBP and BBP in poly(vinyl chloride) PVC matrices, considering the methodology described in CPSC-CH-C1001-09.3\textsuperscript{[1]} and ISO 14389\textsuperscript{[2]}. The aim of this study is to describe the procedure for in-house method validation following the methodology described in the EURACHEM Guide\textsuperscript{[3]}. Two types of plastics matrices were used; one powdered, a certified reference material, CRM-PVC 001 Phthalates in Poly(vinyl chloride), from SPEXOrganics\textsuperscript{®} and other semi rigid, a proficiency testing material, #11012, provided by the Institute for Interlaboratory Studies from Netherlands. Recovery values ranged from 85.56 to 119.9\%.

In parallel, the method was validated by participating in the Laboratory Comparisons Tests (LCT3) for REACH regulated phthalates in PVC organized by the working group II of the Joint Action on Cooperation between PROSAFE and the General Administration of Quality, Supervision and Quarantine (AQSIQ) of the People’s Republic of China in the Field of Consumer Product Safety.


POSTER 6

COMPARISON OF Cronobacter sakazakii DETECTION BY USING RAPID METHOD (PCR) WITH CLASSICAL CULTURAL METHOD IN INFANT FORMULA

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Cronobacter sakazakii which was reclassified in 2007, is an opportunist pathogenic bacteria belongs to Enterobacteriaceae family and causes meningitis, septicaemia and necrotic enterocolitis in newborns and immunosuppressive babies. The bacteria can be isolated from various food and environmental samples. However, because of the several Cronobacter sakazakii endemics of babies, isolation from infant formulas prepared in new-born units of the hospitals, received a great scientific attention.

Cronobacter sakazakii was mostly isolated from dehydrated infant formula which are used for babies which cannot receive breast milk. The bacteria has a severe prognosis and several cases has resulted with death (mortality rate is 42%).

The main cause of high pathogen contamination risk in dehydrated formula is that, these products are not heat processed after hydrating and needs a significant care during preparation.

In our country 20% (approximately 1 million) of the 0 to 3 year old kids consume infant or advanced formula. This forces the research area to develop and validate sensitive and reliable methods for detection of Cronobacter sakazakii.

Although legal authorities generally rely on classical cultural methods, PCR is consider as an alternative method because of its high accuracy and sensitivity.

Making a decision for choosing an appropriate alternative method is based on the evaluation results of the verification/validation of the methods. For this, performance of an alternative method should be evaluated in reference to an international standard method.

The aim of our study was to verify the performance of the alternative rapid PCR method (BAX\textsuperscript{®} System) with classical cultural method for detection of Cronobacter sakazakii in infant formula containing single and multiple competitive flora, in compliance with the requirements of ISO 16140.

For this, infant formula samples were divided into two groups. The first group was spiked with Cronobacter sakazakii (ATCC 29544) and E.coli (ATCC 25922) to form a single competitive flora while the other group was spiked with Cronobacter sakazakii (ATCC 29544), E.coli (ATCC 25922), C.freundii (ATCC 8090), S.enteriditis (ATCC 13076) and S.epidermitidis (ATCC 12228) to form a multiple competitive flora. The spiking of Cronobacter sakazakii was made in three different levels. The samples were then analyzed with two methods and validation parameters of LoD, false negative, false positive, relative specificity and sensitivity of the two method were calculated.
POSTER 7

INTERNATIONAL VALIDATION STUDY OF A NIRS SCREENING METHOD

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Near infrared reflectance spectroscopy (NIRS) is a very fast non-destructive and environmentally friendly analytical technique. This method has proved to be very effective for a simultaneous determination of some soil constituents in soils, with practically no sample pre-treatment. Calibration equations reflect the relationship between the constituents of the sample as determined by a suitable reference method and the NIRS spectral information.

The Central Institute for Supervising and Testing in Agriculture (UKZUZ) has developed and optimized the NIRS method for determining oxidizable carbon (Cox), total carbon (Ctot) and total nitrogen (Ntot) in soils and prepared this method for international standardization in ISO TC 190 Soil quality. The main task in the validation study was to avoid as much as possible the influence of the variability of the reference methods and to determine only the NIRS variability. Therefore, not only samples for a validation study, but also samples for the NIRS calibration were send to the participants. The calibration samples were provided with the results received by reference methods in one laboratory. Soil samples were prepared for a direct measurement. Two calibration sample sets (60 soil samples from arable land and grasslands and 60 forest soil samples) were selected to obtain an evenly distributed coverage of the basic soil properties and most of the possible spectral variability. Six soil samples were selected for the validation study. Mineral soils with lower content of organic matter (three samples) and soils with higher content of organic matter (three samples). All soil samples were air dried and sieved (<2mm fraction). Finally, 126 boxes with soil samples were shipped to each participant.

The participants prepared their own calibrations for both concentration ranges by using the two calibration sample sets and the results from the reference methods provided by the organizer. Then they determined the measurands in the six validation samples. The NIRS measurement method, calibration procedure and instrument conditions were flexible.

Ten independent result data sets from nine participating laboratories from five countries were reported. The data were obtained from five different types of instruments. There were very few outliers reported by the participants. Repeatability was very good in both concentration ranges. Reproducibility of the method was fairly good for higher concentrations of the measurand and acceptable also for low concentrations. The number of samples in the calibration set was found to be acceptable for a screening method. Nevertheless the results could be improved if more samples were used for calibration of the instruments.

The results of the validation study were accepted by ISO TC 190, they were included into the text of the standard and the final standard was published (ISO 17184:2014, Soil quality – Determination of carbon and nitrogen by near-infrared spectrometry).
POSTER 8

CHEMOMETRICS IN METHOD VALIDATION - WHY?

Jone Omar, Ana Boix, Christoph von Holst

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Chemometrics is a tool that many scientists are reluctant or afraid to use; why? Is it because it is considered a "black box" difficult to use? It is certainly a useful and powerful tool worth discovering.

Many analytical methods (e.g. HPLC, GC-MS, ICP-MS) seem to be validated. However, are we sure that the best working conditions were identified and applied? Was the method properly optimised? A lot of time and money is spent applying the classical approaches described in the literature. "Design of Experiments" (DoE) may reduce the number of attempts and samples to be analysed while providing additional information related to interactions between the various experimental parameters investigated. Unfortunately, this information is not readily available in traditional approaches. Such an optimised method may proceed to the validation study. Several examples of DoE applications will be presented in the poster, related to Asymmetric Field Flow Fractionation (nanotechnology), GC x GC-MS, Raman and Supercritical fluids.

In the case of spectroscopic methods (e.g. Raman, infrared) where spectra are acquired, a multivariate approach may be appropriate to use the wealth of data available through the whole wavelength range (instead of a single/fixed wavelength). An example of Raman will be presented.

Even when the optimised method is validated, chemometrics is to be applied for multi-dimensional analytical methods, such as GCxGC-MS, to assess and correct for potential co-elution effects.

By now you know that chemometrics is useful; just give it a try.
OPTIMIZATION AND VALIDATION OF AN UHPSFC METHOD FOR THE QUALITY CONTROL OF VITAMIN D3 RAW MATERIAL

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Nowadays, cholecalciferol or vitamin D3 arouses an increasing interest among the scientific community. Indeed, it is known to be involved in many health benefits such as supporting the bone health, immune system and lowering occurrence of chronic diseases \cite{1}. However, despite these advantages, a worldwide deficiency is currently recognized for the large extent of the population - adults and children comprised- due to the small exogenous intakes and the low endogenous synthesis. Therefore, to obtain satisfying levels of vitamin D, supplementation with medicines or pharmaceuticals is necessary. In order to ensure the quality of these products, it is mandatory to proceed to quality control of the raw material used for their manufacturing. Among the panel of tests proposed by pharmacopeias, Normal Phase Liquid Chromatography (NPLC) is used to assess the purity of cholecalciferol. However, given the large consumption of toxic solvents and the long runtimes usually required, alternatives to such analytical technique may be appreciated and considered for intensive work.

In the context of green analytical chemistry, Supercritical Fluid Chromatography (SFC) is often suggested as an alternative to NPLC \cite{2}. Indeed, modern SFC provides fast, efficient and green separations \cite{3}. So, given the current interests devoted to vitamin D3 and green analytical chemistry, the quantitative performances of a modern UHPSFC method were challenged on a real-life case study: the Quality Control (QC) of vitamin D3 as a raw material.

A rapid and green UHPSFC method was optimized thanks to the Design of Experiment-Design Space (DoE-DS) methodology. Robust method with a high quality separation of the compounds of interest in 2 minutes was obtained using a gradient of ethanol as co-solvent of the carbon dioxide. The analytical method was then fully validated according to the total error approach, demonstrating the compliance of the method to the specifications of U.S. Pharmacopeia (USP: 97.0 – 103.0%) and European Pharmacopeia (EP: 97.0-102.0%) for an interval of [50 – 150\%] of the target concentration. In order to allow quantification of impurities with vitamin D3 as an external standard in SFC-UV, correction factors were determined and confirmed during method validation. Thus, accurate quantification of impurities was demonstrated at the specified levels (0.1 and 1.0\% of the main) in a 70.0 – 130.0\% dosing range. This work demonstrates the validity of an UHPSFC method for the QC of vitamin D3. Therefore, the present study clearly supports the interest of the switch to a greener and faster alternative to NPLC in the pharmaceutical industry.

\cite{1} M. Wacker, M.F. Holick, Vitamin D-effects on skeletal and extraskeletal health and the need for supplementation, Nutrients. 5 (2013) 111–148. \url{http://dx.doi.org/10.3390/nu5010111}
\cite{2} L. Taylor, Past, Current, and Future Directions in Supercritical Fluid Chromatography, LC GC North America. 31 (2013) s44–s49.
\cite{3} A. Tarafder, Metamorphosis of supercritical fluid chromatography to SFC: An Overview, TrAC Trends in Analytical Chemistry. (2016) in press. \url{http://dx.doi.org/10.1016/j.trac.2016.01.002}
POSTER 10

VALIDATION PROCEDURE OF PESTICIDES SUCH AS α - BHC, γ - BHC, β - BHC, DDE, DDD AND DDT IN FRUITS AND VEGETABLES GROWN IN AZERBAIJAN

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Fruits and vegetables are an important part of a healthy diet because of their high nutrient content and minerals. Most fruits and vegetables contain various vitamins such as B group vitamins, carotenoids (α, β carotenoids, lutein), vitamin C, etc. However, at the same time they can also be the source of toxic substances such as pesticides. Pesticides are unhealthy residues, which are commonly used in agriculture to protect the plants, crops, and different insects and diseases. The use of pesticides has increased because they have quick action, decrease toxins produced by forth infecting organisms and are less labor intensive than other methods.

Approximately 30 - 40 years ago, in the Soviet period, pesticides such as α - BHC, γ - BHC, β - BHC, DDE, DDD, and DDT were used intensively in the cotton plant fields in Azerbaijan. Then the use of these pesticides was prohibited. But despite this, these pesticides can remain in the soil for a long time. Therefore, investigation and controlling of MRL (maximum residual level) were important in Azerbaijan for the protection of health of consumers.

In the food testing laboratory, validation of pesticides was carried out using the method BS EN 15662:2008 - Fruits of plant origin, Determination of pesticide residues using GC-MS and/or LC-MS/MS following acetonitrile extraction/partitioning and clean-up by dispersive SPE, QuEChERS-method. A matrix was used without any pesticides including above mentioned substances. Calibration standard solutions were prepared at five concentration levels 10, 20, 50, 100, and 500 ppb for each pesticide. The validation plan is two analysts in same and different periods (for estimation repeatability and reproducibility) carried out 10 times testing together and separately on spiked sample in three concentrations (10 ppb, 100 ppb, and 500 ppb) using GC-MS equipment. The recovery of analysis for each pesticide in three concentrations was 76.23 – 125%. The CVr were among 9,37 – 26%. For monitoring the validity of the method, the laboratory participated in proficiency tests and the results were within the acceptable Z score. As a result, recoveries, RSDr, and RSDa values, calibration curve, specificity, LOG, trueness (bias), matrix effect for each pesticide were within the acceptable range according to the criteria specified in SANCO/12571/2013 19 November 2013 rev. 0. During the validation procedure, expanded uncertainty for each analysed pesticide was estimated in different concentrations. There were varies between 0,199 ppb to 0,787 ppb.

[1] BS EN 15662:2008 - Fruits of plant origin, Determination of pesticide residues using GC-MS and/or LC-MS/MS
POSTER 11

DETERMINATION OF NITROSAIMINES IN FOOD USING LC-MS/MS

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Nitrosamines are formed as food is heated through the reaction of amines with nitrites, which are generally used as a preservative [1]. Nitrosamines are known as carcinogens and these compounds potentially appear in cured meat, dairy products particularly in cheese, beer and fishery products. Nitrosamines have shown the potential of being carcinogenic to some animal species and are likely to be related to human cancer [2]. Therefore, there is an increasing interest in determination nitrosamine compounds in food by several analytical techniques. In this study, an analytical method was developed based on liquid chromatography coupled with triple quadrupole mass spectrometry to determine the analytes in different food groups, which are fish, meat, cheese and beer. In the extraction stage, the quechers method was modified [1,2]. Five nitrosamines compounds (N-nitroso piperidine, N-nitroso diphenylamine, N-nitroso di-N-propylamine, N-nitroso di-N-buthylamine and N-nitroso pyrolidine), which are mainly formed in food products were determined in four different food groups. The limits of quantification for all nitrosamine compounds in all matrixes groups were determined as 0.1 mg/kg. Recovery values were between 84.9% and 102.8%.

VALIDATION OF AN UPLC METHOD FOR THE DETERMINATION OF AMINO ACIDS IN FEEDS

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An effective monitoring of different feed materials in the scope of amino acids involves using a rapid and specific method like a reverse phase ultra-performance liquid chromatography (UPLC). The aim of the present study was to validate an UPLC method for quantification of 17 amino acids (histidine, serine, arginine, glycine, aspartic acid, glutamic acid, threonine, alanine, proline, lysine, tyrosine, valine, isoleucine, leucine, phenylalanine, cystine, methionine) in feeds, and to compare obtained results with performance characteristics of the official ion-exchange chromatographic method published in the regulation (EC) No 152/2009 [1]. Feeds used in the study were: rye grains, porcine hemoglobin and feed mixture, with different content of amino acids. Samples were hydrolyzed with hydrochloric acid, filtrated, evaporated and dissolved in water. The 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC) was used as pre-column derivatizing reagent. Cystine and methionine were oxidized to derivatives – cysteic acid and methioninesulfone, respectively. Products of pre-column derivatisation were analyzed chromatographically with using Waters ACQUITY UPLC system. The reversed phase separation occurs on the AccQ·Tag Ultra column and detection at 260 nm. For each amino acid LOQ, repeatability, within-laboratory reproducibility, recovery and expanded uncertainty were determined. LOQ for analyzed amino acids were from 0.2 g/kg (for methionine) to 2.2 g/kg (for glutamic acid). The precision and accuracy parameters as well as uncertainty for some amino acids are summarized in the Table 1 below. Expanded uncertainty (k=2) was quantified as duplicate within-laboratory reproducibility (intermediate precision). Expanded uncertainties were also calculated using proficiency testing data and for selected amino acids amounted: 22% for methionine, 11% for threonine, 16% for lysine and 16% for cystine.

Table 1. Some performance parameters of the UPLC method compared to the official method for the chosen amino acids

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Range, g·kg⁻¹</th>
<th>UPLC, repeat.%</th>
<th>UPLC, int.prec.%</th>
<th>UPLC, Recovery,%</th>
<th>U (k=2) %</th>
<th>Repeat. 152/09,%</th>
<th>Reprod. 152/09,%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thr</td>
<td>0.4-31.0</td>
<td>0.6-1.9</td>
<td>1.1-2.3</td>
<td>98.2-102.0</td>
<td>6.8-9.6</td>
<td>1.9-2.7</td>
<td>3.8-5.2</td>
</tr>
<tr>
<td>Lys</td>
<td>0.6-77.0</td>
<td>1.5-2.2</td>
<td>2.6-3.2</td>
<td>92.8-95.4</td>
<td>6.0-9.6</td>
<td>2.1-2.8</td>
<td>3.0-5.4</td>
</tr>
<tr>
<td>Cys</td>
<td>0.5-7.0</td>
<td>1.5-3.7</td>
<td>4.0-5.7</td>
<td>93.0-96.6</td>
<td>8.6-10.4</td>
<td>2.6-3.3</td>
<td>8.8-12.3</td>
</tr>
<tr>
<td>Met</td>
<td>0.2-9.0</td>
<td>0.8-1.9</td>
<td>2.2-6.8</td>
<td>87.1-97.0</td>
<td>8.4-10.4</td>
<td>2.2-3.4</td>
<td>7.0-13.0</td>
</tr>
</tbody>
</table>

Accuracy of the UPLC method was confirmed in the proficiency testing organized by AGES, Austria: IAG - Feedingstuffs 2014 and satisfying results were obtained (mean z-score equal to 0.37; from -1.1 to 0.7). The UPLC method is characterized by validation parameters similar to the official method and can be used for determination of amino acids in feeds as well as a method for official control purpose.

POSTER 13

OPTIMIZATION AND VALIDATION OF AN ANALYTICAL METHOD FOR THE DETERMINATION OF ISOTHIOCYANATES AND GLUCOSINOLATES IN BROCCOLI USING HPLC ANALYSIS.

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Broccoli is a vegetable of the family Brassicaceae. It is an edible vegetable that is rich in vitamins and secondary metabolites. Because of the biological activity of its secondary metabolite sulforaphane, an isothiocyanate with antioxidative effects, broccoli became a popular food supplement. However, broccoli also contains the compounds progoitrin and goitrin, which can enlarge the thyroid gland. To avoid these effects, the Royal Decree of August 29, 1997 limits the daily intake of progoitrin and goitrin with 20 and 5 mg respectively [1]. Here, we describe two optimized and validated analytical methods for the quantification of progoitrin and goitrin in broccoli powder. The ICH guidelines on the validation of analytical methods were used for the validation of both methods [2].

The method for the analysis of progoitrin was based on the validated method for the analysis of glucosinolates in watercress, which was previously developed in the research group NatuRA (UAntwerp). Glucotropaeolin was used as internal standard. The standard curve of glucotropaeolin was linear in the range of 17.9 – 537 µg/mL. The precision of the method for time and concentration gave relative standard deviation (RSD) values higher than 5% (6.55% and 6.56% respectively) but is still accepted because of the complexity of sample preparation. The broccoli powder that was tested contained an average of 1.27 mg/g progoitrin.

For the analysis of goitrin, the sample preparation described by Wang et al. (2013) was used [3]. The standard curve of goitrin was linear in the range 1 µg/mL – 400 µg/mL. The precision of the method for time (3 days) and concentration (3 levels: 10%, 100%, 200%) was tested by spiking broccoli powder with goitrin, where the 100% level was 5.0 mg/g broccoli powder. The precision of the method with respect to time and concentration is accepted with RSD values of 4.3% and 3.5% respectively. The recovery of the method was determined to be 99.1%.

POSTER 14

OPTIMIZATION AND VALIDATION OF AN ANALYTICAL METHOD FOR THE DETERMINATION OF POLYMETHOXYLATED FLAVONOIDS IN CITRUS SINENSIS.

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*Citrus sinensis* is a fruit, commonly known as orange or sweet orange. It belongs to the family of the *Rutaceae.* *Citrus sinensis* is used in the traditional medicine in several countries. It is used for a.o. diabetes mellitus, relief of stomach pains, nausea, vomiting and gastrointestinal infections. The interest in its activity against many disorders is high, since *C. sinensis* has a wide range of compounds. One specific group of compounds is called the polymethoxylated flavonoids (PMF). PMFs appear to have a broad spectrum of activities, such as cardiovascular, anti-inflammatory and chemopreventive activities[1].

Many PMFs have already been identified, the two most common PMFs in *Citrus* are nobiletin and tangeretin. Since PMFs are often used for bio-activity studies, there was a need for an analytical method. We developed and optimized an analytical method for the quantification of the polymethoxylated flavonoids nobiletin and tangeretin in dried orange peel powder. In the final method nobiletin and tangeretin were extracted out of the dried orange peel powder using methanol 70% and the samples were analyzed on an HPLC-DAD-system. Validation of the method was done conform to the ICH guidelines on the validation of analytical methods [2]. A calibration model of both nobiletin and tangeretin standards were made in the concentration range from 1.8 µg/mL to 18.4 µg/mL and 0.4 µg/mL to 3.5 µg/mL respectively. The method was validated for the intermediate precision (3 days) and linearity (3 concentration levels). The recovery of the method was determined as 101.2% for nobiletin and 99.8% for tangeretin. For nobiletin, an average amount of 406.5 µg/g dried orange peel powder was found, with a RSD of 3.5%. For tangeretin, the average amount was 93.6 µg/g dried orange peel powder with a RSD of 0.96%.

POSTER 15

OPTIMIZATION AND VALIDATION OF ANALYTICAL RP-HPLC METHODS FOR THE ANALYSIS OF GLUCOSINOLATES AND ISOThIOCYANATES IN NASTURTIUM OFFICINALE R. BR.

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Nasturtium officinale R. Br. (watercress) is a plant that belongs to the Brassicaceae and is growing mainly in Europe and Asia. The plant contains a considerable amount of vitamins, minerals and secondary metabolites and is used in food and for its medicinal properties. These are mainly attributed to the glucosinolates, which are precursors of bioactive compounds such as the isothiocyanates. Glucosinolates are sulphur containing secondary metabolites, containing a β-D-thioglucose and an aglycon. The main glucosinolate in Nasturtium officinale R. Br. is gluconasturtiin. The main isothiocyanate in Nasturtium officinale R. Br. is phenylethylisothiocyanate (PEITC). Since the quality of a food supplement of watercress depends on the content of its glucosinolates and isothiocyanates, two quantitative methods were developed: one to analyse the glucosinolates and another one to analyse PEITC.

An existing method for the determination of gluconasturtiin [1,2] was optimized by changing the volume of the extraction solvent, extraction time and number of extraction steps. Sinigrinemonohydrate was used as internal standard. For the analytical method of PEITC we adapted a method described by Heyerick [2]. Both methods were validated conform the ICH guidelines on the validation of analytical methods [3]. The standard curve of sinigrinemonohydrate was linear in the concentration range of 33.2 – 166.2 µg/mL. The precision of the method with respect to time (3 days) and concentration (3 concentration levels) was respectively 9.74% and 8.96%, although relatively high, it was still accepted because of the complexity of the method. The mean concentration of gluconasturtiin was 8.5 mg/g lyophilized watercress. For PEITC a linear range was proven from 2.2 to 170.2 µg/mL. The precision of the method (3 days) was 12.67%. The precision on 3 different concentration levels was 7.77%. These values are high but acceptable due to the volatility of PEITC.

FULLY AUTOMATED DIRECT EXTRACTION AND ANALYSIS OF DRIED BLOOD SPOTS FOR THE DETERMINATION OF FOUR ANTI-EPILEPTIC DRUGS AND TWO ACTIVE METABOLITES

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Dosage adjustment of anti-epileptic drugs by therapeutic drug monitoring is very useful, especially for children. Considering the benefits of dried blood spots (DBS), this matrix could be an alternative to conventional venous sampling for this purpose. Since manual punching and off-line extraction slow down DBS analysis, an automated direct extraction and analysis of DBS can be advantageous. A method for quantifying four anti-epileptic drugs (AEDs) and two active metabolites was developed, including carbamazepine, valproic acid, phenytoin, phenobarbital, carbamazepine-10,11-epoxide and oxcarbazepine. To that end, we used a prototype on-line DBS-SPE device (Spark Holland) coupled to liquid chromatography (Shimadzu) and tandem mass spectrometry (AB SCIEX QTRAP® 5500) (LC-MS/MS).

For the LC-MS/MS method, a Chromolith® reversed phase (RP)-18 endcapped 100x4.60 mm column equipped with a 0.5 µm Krudkatcher classic HPLC in-line filter was chosen as it gave the best results in terms of compound separation. A mobile phase consisting of 5 mM ammonium acetate (A) and 5 mM ammonium acetate in acetonitrile/water (95/5, v/v) (B) at a flow rate of 1.0 ml/min turned out to be the best option, with the following proportions of solvent B in the 8 minute gradient elution program: 20% for 0.34 min, linearly increased to 49% in 3.21 min, followed by a short isocratic period of 49% for 0.55 min, a fast increase to 95% in 0.4 min, maintained for 1.0 min and finally, reversal to starting conditions. The QTRAP® 5500 was equipped with an ESI source (TurboIonSpray®) and detected all compounds, using an optimized multiple reaction monitoring (MRM) algorithm, operating in negative mode for valproic acid, phenytoin and phenobarbital and in positive mode for the other three compounds. For the on-line DBS-SPE system a HySphere resin GP, 7µm, 2x10 mm internal diameter cartridge (Spark Holland) was best suited for sample clean-up. The following SPE conditions turned out to be the best option: (1) preconditioning with 1 mL of methanol (MeOH); (2) equilibration with 1 mL of water; (3) elution of the DBS sample from the paper card directly to the SPE cartridge using 1 mL of water; (4) washing the cartridge with 1 mL of 5% MeOH in water and (5) elution of the sample from the cartridge using the LC pump gradient. In a next step, the eluent is directed to the Chromolith® column for LC-MS/MS analysis. Preliminary experiments on real-life patient samples readily demonstrated the applicability of the method. In a next step, the developed method will be validated based on U.S. FDA and European Medicines Agency (EMA) guidelines for bioanalytical method validation. This will encompass the evaluation of selectivity, carry-over, matrix effect, linearity, precision, accuracy, stability, recovery and hematocrit-effect. Carry-over will be investigated by analyzing blank blood DBS cards after the analysis of the highest calibrator and QC sample. Matrix effect and recovery will be tested by analyzing blood of different hematocrit levels. Accuracy and precision will be evaluated using QC samples at four concentration levels, analyzed on different days. Stability will be investigated by storing QC samples at four concentration levels at room temperature for 2 and 4h before spotting. To establish the stability on the DBS cards, QC samples at four concentration levels will be spotted onto DBS cards, stored at three different temperatures (room temperature, 4°C and -20°C) and tested on different days for several months. After completing the validation, the method will be applied on patient samples originating from developing countries, to demonstrate the benefits of DBS sampling of AEDs in pediatrics in developing countries. Automated direct extraction and analysis of DBS can open up new ways for TDM of AEDs, certainly in clinical routine.
POSTER 17

VALIDATION OF A NOVEL NON-CONTACT METHOD TO ESTIMATE THE HEMATOCRIT OF DRIED BLOOD SPOTS

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The hematocrit (Hct) effect is considered to be one of the most crucial issues in dried blood spot (DBS) analysis. Since the Hct of a blood sample affects blood viscosity and hence, the volume of blood contained in a fixed-size DBS punch, deviating Hct values can significantly impact DBS-based quantitation. To evaluate the extent of the Hct effect for a given DBS we previously developed a method that allows estimating the Hct of a DBS based on its potassium (K\textsuperscript{+}) content [1]. Additionally, using caffeine and paraxanthine as model compounds, it was shown that the K\textsuperscript{+} content could also be employed to introduce a Hct specific correction factor (utilizing a K\textsuperscript{+}-based correction algorithm) which alleviates the Hct effect [2]. Although this K\textsuperscript{+}-based method yielded good results when applied to patient samples, it also suffered from some practical drawbacks, as it consumed part of the DBS and required additional sample preparation. Therefore, we now developed a non-destructive method, which allows to predict a DBS’ Hct using non-contact diffuse reflectance spectroscopy. This way, mere scanning of a DBS suffices to derive its Hct.

This non-contact method was successfully validated based on FDA guidelines. A linear calibration model after log/log transformation best described the data. The bias, intra- and interday imprecision at low, mid and high Hct, as well as at the lower (0.20) and upper (0.67) limit of quantitation were always within 15%. Stability at ambient conditions after storage for up to five months and stability at 60°C for up to three days (the latter mimicking extreme transport conditions) were evaluated at three Hct levels. Additionally, DBS-specific aspects (i.e. the volcano effect and the volume effect) were evaluated during validation, again at three Hct levels. Although a slight volcano and volume effect were discerned, this was of no practical influence (i.e. within 15%).

In a next step, the non-contact Hct estimation method was applied to 233 patient DBS with varying Hct values (Hct = 0.20 - 0.50). The Hct of these patient DBS was estimated using the non-contact method, whilst the true Hct of the patient samples was determined on the corresponding venous whole blood samples using a Sysmex XE-5000 hematology analyzer. The non-contact method was found fit for purpose, since i) a good correlation was observed between the estimated and true Hct (r = 0.95), ii) the limits of agreement obtained with Bland and Altman analysis were very similar to those obtained with the K\textsuperscript{+}-based method and since iii) over 95% of the estimated Hct values were within 15% of the corresponding true Hct values. In addition, incurred sample reanalysis demonstrated the excellent reproducibility of the method, since, with a single exception of 5.4%, all reanalysis results were within 3.5% of the corresponding average of the original and reanalysis result.

POSTER 18

ANOVA: A TOOL FOR BETTER EXPERIMENTAL DESIGN AND EVALUATION OF METHOD VALIDATION

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Analysis of Variance (ANOVA) is a powerful tool for the estimation and separation of the different causes of variation. In the new edition of EURACHEM Guide “The Fitness for Purpose of Analytical Methods – A Laboratory Guide to Method Validation and Related Topics”, ANOVA is proposed as an alternative way for the simultaneous determination of intermediate precision and repeatability in a validation study. In literature have been also proposed nested ANOVA as another way for the estimation of trueness and uncertainty [1-3]. Moreover, through nested (hierarchical) experimental design, the source of variation (matrix, concentration or replicate) can be estimated that it helps the analyst to have a better view of methods characteristics. The aim of this work is to explore the capabilities of ANOVA in validation. Through an example of method validation for biogenic amines in food matrices, it will be described the estimation of precision, trueness and uncertainty with the same nested experimental design and it will be discussed the advantages and disadvantages for using ANOVA instead of classical approach.

DRIED BLOOD SPOT-BASED ANALYSIS OF THE CYANOETHYLVALINE-ADDUCT OF HEMOGLOBIN FOR SMOKING ASSESSMENT

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Cigarette smoke contains more than 4000 chemicals, one of which is acrylonitrile. Acrylonitrile is a chemical substance widely used in industry for different purposes like fabrication of synthetic fibres, household articles and resins. Its toxicity is of major concern not only during chronic exposure but particularly also during acute poisoning. Smokers are exposed chronically, while acute poisoning can happen in disasters with spill-over of acrylonitrile, as was the case in the train disaster in Wetteren in 2013.

Here, we present a new dried blood spot (DBS)-based method to screen for exposure to acrylonitrile. When acrylonitrile enters the bloodstream, it binds covalently to hemoglobin, thus forming an adduct, the N-terminal cyanoethylvaline-adduct (CEV). In contrast to nicotine and cotinine, which only have a short half-life, this CEV adduct remains present for months, offering a wide window of detection. The use of DBS offers many benefits since it is a minimally invasive sampling approach for the patient, which is especially relevant for newborns.

Existing methods to screen for the CEV adduct are based on a modified Edman degradation. This procedure allows specific detachment and isolation of N-substituted N-terminal valine as a thiohydantoin derivative. However, these methods are lengthy (about 16 hours of derivatization) and require several milliliters of blood.

In our method, we implemented microwave-based on-spot derivatization of DBS and further optimized the degradation. In the optimized procedure fluorescein isothiocyanate (FITC) is added onto a 6 mm DBS punch and derivatization is performed for 15 minutes in the microwave at 300 W. Further sample clean-up is done by solid phase extraction. For the measurement of this adduct an LC-MS/MS/MS method was developed. In this MS/MS/MS method the motherion is FITC-CEV with m/z 542 which fragments to a daughterion of m/z 499, which in turn fragments to a new daughterion of m/z 374. This method was already successfully applied on genuine smoker samples.

Validation will be performed based on US FDA and EMA guidelines for bioanalytical method validation. This validation will comprise selectivity, carry-over, LLOQ, linearity, precision, accuracy, matrix effect, recovery, stability and incurred sample reanalysis. Carry-over will be analyzed by running blanks after samples with high concentrations of the analyte. The linearity will be examined by using a calibration curve made out of blank blood spiked with a custom-made CEV-peptide at different concentrations. Precision will be determined by analyzing the same sample several times. The matrix effect and recovery will be tested by analyzing blood of different hematocrits, while the stability will be tested at different temperatures (room temperature, 4 °C and -20 °C) for several months.

Following validation, this methodology will be applied on DBS from smokers, passive smokers and non-smokers, to evaluate the potential of this novel approach to assess (historical) smoking behavior.
CONFIRMATORY METHOD FOR THE SIMULTANEOUS DETERMINATION OF NITROIMIDAZOLES RESIDUES IN EGGS BY UPLC-MS/MS: VALIDATION ACCORDING TO THE COMMISSION DECISION 2002/657/EC

Maurizio Fiori, Emanuela Gregori, Marina Patriarca, Antonella Semeraro, Paolo Stacchini and Cinzia Civitareale

Nitroimidazoles (NMZs) are effective antiprotozoal agents and for this reason they are used in the prophylactic and therapeutic treatment of histomoniasis and coccidiosis in avian species. Studies suggested possibly mutagenicity and carcinogenicity of these compounds in humans. Due to their toxicological characteristics, the use of nitroimidazoles in food-producing animals was prohibited, therefore this drugs have been included in Table 2 of the Commission Regulation (EU) No 37/2010. In this Table there are compounds for which no Maximum Residue Levels can be fixed. However in 2007 the European Union Reference Laboratories published a Guidance Document setting recommended concentration/levels for prohibited substances without maximum residue limits. For NMZs and their metabolites CCα for confirmatory methods should be lower than 3 µg/kg in plasma, retina and eggs was fixed.

A very important tool to minimize and to control possible illicit use of nitroimidazoles in avian species is the availability of suitable and validated analytical methods.

The validation of a method for the simultaneous determination of residues of NMZs in whole eggs by UPLC-MS/MS is presented. This technique is able to combine the speed of a screening technique with the reliability of a confirmatory method.

The compounds considered in this validation study were carnidazole, dimetridazole, ipronidazole, metronidazole, ronidazole, ternidazole and tinidazole. In addition the metabolites 2-hydroxymethyl-1-methyl-5-nitroimidazole, hydroxy-ipronidazole (HMMNI) and hydroxy-metronidazole were considered. Labeled internal standards were used for quantification of analytes.

The validation study was performed according to the Commission Decision 2002/657/EC using the alternative matrix-comprehensive in-house validation approach based on designed InterVal Plus software (QuoData GmbH, Dresden, Germany).

Experiments were carried out at the concentration range of 0-20 µg/kg for all substances. The outcomes of experimental design were CCα, CCβ, recovery at CCα, reproducibility at CCα and measurement uncertainty.

The results of validation study showed that the method is able to identify and to quantify the presence of NMZs and their metabolites in eggs at the recommended level required in the official control of residues.
INTER-LABORATORY VALIDATION STUDY FOR DETERMINATION OF DEOXYNIVALENOL BIOMARKERS IN URINE

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The study was performed in the frame of a research project funded by EFSA, GP/EFSA/CONTAM/2013/04, Experimental study of deoxynivalenol biomarkers in urine. Deoxynivalenol (DON) belongs to a large group of mycotoxins named trichotheccenes, which represent the main group of Fusarium toxins commonly found in cereal grains.

The aim of the present study was to obtain representative data on the occurrence of DON and its metabolites in urine from European population groups, namely children, adolescents, adults, elderly, vegetarians and pregnant women and to get reliable information on the associations between concentrations of DON in urine and cereal-based food consumption data as provided by the investigated subjects, by using Food Frequency Questionnaires and 24h and 48h diary records.

Urine samples, collected in Italy, Norway and the United Kingdom, were assessed for free DON (un-metabolised form of the toxin), total DON (combined measurement of both free and DON-3-glucuronide (DON-3-Glc)) and de-epoxy deoxynivalenol (DOM-1) using HPLC coupled to mass spectrometry.

With the aim of guaranteeing both the maximum reliability of the results and the full harmonization of the performance among the three laboratories involved in the analytical work, an inter-laboratory validation of the method was performed according to the AOAC Part D: Guidelines for Collaborative Study Procedures to Validate Characteristics of a Method of Analysis [1]. The results obtained for the inter-laboratory validation were considered satisfactory, showing good performance across the range of investigated concentrations, i.e. 5 to 100 ng/mL for both, DON and DOM-1.

The evaluation of DON and DON metabolites in urine may constitute a valuable indicator of the dietary exposure that plays a pivotal role in the context of the risk assessment.

POSTER 22

QUANTITATIVE ANALYSIS OF GMO IN FOOD AND FEED: DIGITAL PCR VERSUS REAL TIME PCR METHOD

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The European legislation establishes that food and feed are labelled for their Genetically Modified Organism (GMO) content when the ingredients contain authorized GMOs and provides a tolerance thresholds of 0.9% for authorized GMOs in Europe and 0.1% (only in animal feed) for GMOs authorized in third countries. Therefore, the ability to quantify nucleic acids with accuracy and precision is fundamental to comply with EU legislation.

Currently, the most widely used technique to detect the presence of GMOs in food and feed is the real-time PCR that requires a standard curve to calculate the quantity of an unknown target sequence. Recently, the digital PCR was used as an alternative to real-time PCR in different analytical fields, including GMOs, given the major benefits that have been found in its use: absolute quantification free from standard curves and amplification efficiency of the reaction; high precision metrological use; high sensitivity in the detection of rare events; no inhibition due to the matrix effect; possibility to perform analysis in duplex and multiplex, so as to reduce the amount of reagents and the global analysis costs.

The present work consists in the validation of the method for the quantitative analysis of GM maize MON 810 and the assessment of applicability of the digital PCR method (absolute quantification using sealed-chip technology) in samples of highly processed food and feed.

During the digital PCR validation process, a quantitative analysis of the same reference material samples was simultaneously conducted using the real-time PCR platform. With the purpose of avoid biases when comparing the two platforms, we transferred the validated MON 810 specific assay for Real Time PCR to digital PCR with minimum adaptation (primers, probe, DNA concentration were the same). Linearity of the response, the limits of detection and quantification, trueness and repeatability of assays complied with international recommendations [1, 2]. The applicability with different matrices and the practicability of use for routine GMO testing were also evaluated. From the obtained results, it was assessed that the two methods were completely comparable even when the digital PCR method didn’t exceed the real-time PCR performance.

The digital PCR showed high performance and demonstrated to be a solid analysis method and the analytic data obtained from the work highlighted: high precision on low sample concentration levels; high analysis accuracy; applicability of the method on complex and highly processed matrix; transferability of validated real-time PCR methods to the digital PCR platform without major protocol changing; lower running costs than those of the standard quantitative PCR technology (it was assessed an average cost per sample of 15.67 Euros for digital PCR analysis and 19.75 Euros for real-time PCR analysis). Hence, it was concluded that digital PCR can be applied for routine quantification of GMOs, or any other field where quantitative analysis is required.


Poster 23
VALIDATION OF MICROBIOLOGICAL METHODS FOR WATER AND ENVIRONMENT: REVISION OF ISO 13843

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The ISO/IEC 17025:2005 standard requires the use of standardized methods which are validated following the development process or the implementation of a proper validation procedure for in-house developed methods [1].

For water and environmental fields, ISO/TR 13843 (2000): water quality – Guidance on validation of microbiological methods is a rough guide [2], which describes how to apply the common characteristics of analytical methods performances to the specificity of the microbiological determinations:

- The analyte is a living microorganism which is taxonomically defined, or in some cases defined by a group designation less accurate than taxonomic definitions (i.e.: coliforms)
- The particle nature of microorganisms and their random distribution even in perfectly mixed waters, lead to peculiar statistical considerations and inexorably limit the enumeration precision
- The measurand (Colony-Forming-Unit) is based on the cultivability of the microorganisms which can be in a large panel of different physiological states depending on the matrix (disinfectant stress in chlorine water, nutrient depletion in oligotrophic waters...)

After the publication of ISO 29201:2012, which gave the baselines of the uncertainty of measurement of microbiological enumeration methods [3], and the revision of ISO 17994:2014 for the comparison of the relative recovery of two microbiological methods [4], the challenging revision of ISO/TR 13843 aims to give a more precise determination of the parameters useful for the characterization of microbiological methods in order to achieve an ISO standard status.

In water and environmental microbiology, the characterization is seen as an exploratory process with the aim of establishing the likely set of performance characteristics of a new or a modified method, under a specific set of circumstances. The sensitivity, specificity and selectivity are determined by verifying presumptive counts compared to confirmed counts. The linearity is seen as an aspect of trueness, it is studied with a view of determining the maximum upper limit of counting. At the other extreme of the working range, the definitions of the detection level and the limit of determination need to take into account the random distribution properly, using appropriate statistical models. The recovery is always relative as the true value of the measurand remains unknown. As to precision parameters, repeatability and reproducibility of the methods can be assessed following the ISO 5725-2:1994 guidelines with some adaptations as the basic principles originally applied to continuous data and not to discrete data such as colony counts.

Poster 24

VALIDATION OF MULTI-PARAMETER METHODS – REVISITING AN EU-COORDINATED
IN-HOUSE VALIDATION STUDY FOR NSAID COMPOUNDS IN BOVINE MILK

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In the framework of monitoring residues in food of animal origin, EU laboratories face the challenge of validating analytical methods for numerous (sometimes several hundred) analytes and matrices. It is thus highly desirable to propose an approach, which will reduce the associated workload while maintaining the required level of reliability. One such approach consists in identifying relationships between the different analytes in order to constitute groups within which the method can be expected to exhibit uniform validation characteristics. This poster examines one possible approach consisting in selecting a subgroup of analytes for the validation. This approach is tested on the basis of a “coordinated” in-house validation study for a multi-parameter LC-MSMS method for NSAID compound residues in bovine milk.

This validation study involving 14 EU laboratories was conducted in 2011. Each laboratory was asked to perform an in-house validation for a multi-parameter LC-MSMS method for 16 NSAID compounds in both lyophilised and fresh bovine milk. The main advantage of this confirmatory method is the simultaneous determination of different groups of NSAID compounds. Within each laboratory, measurements were conducted according to a factorial design (with factors such as operator, sample storage and SPE lot) in accordance with the CD 657-2002 alternative approach as extended by QuoData and implemented in the software package InterVAL PLUS. The results for all 14 laboratories then formed the basis for a combined validation across laboratories, i.e. in-house validation parameters such as the in-house repeatability, intermediate and reproducibility standard deviations and the critical concentration $C_{C\alpha}$ were determined on the basis of the results of all 14 laboratories. Similarly, the contributions to uncertainty corresponding to the different factors (including matrix) were quantified on the basis of the results of all 14 laboratories. Finally – and this is the decisive aspect here – it was possible to compare the laboratory-specific $C_{C\alpha}$ values. The following result was observed: on the one hand, for many laboratories, the method yielded satisfactory performance characteristics (e.g. $C_{C\alpha}$ values lying below the maximal admissible value according to CD 657-2002) for all compounds, while, for the remaining laboratories, the performance characteristics were unsatisfactory for several compounds.

These results suggest the following approach: First, identify all analytes which have caused difficulties in the past or which are known as “critical” for example in terms of concentration level or correct identification. Then select randomly at least 6 of the remaining analytes. A complete in-house validation is then conducted on the basis of this selection. For the other analytes, an individual “ad-hoc” validation could also be performed. This approach works very well with the available data set of the NSAID compounds in bovine milk. Further studies are required to investigate whether it is also successful for other compounds and matrices.
Poster 25

USER-FRIENDLY TUTORIAL AND SPREADSHEET FOR THE BOTTOM-UP EVALUATION OF LEAST-SQUARES CALIBRATIONS

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The top-down evaluation of the measurement uncertainty based on intralaboratory data is very popular since it only requires an adequate evaluation of measurement intermediate precision and trueness. However, in some cases, estimated performance data is not representative of performance over the analytical range due to precision and trueness variations in this range.

Since the top-down approach tends to overestimate the measurement uncertainty, small performance variations in the analytical range can be somehow masked by the uncertainty overestimation. However, in some cases and for some portions of the analytical range, the simplified evaluation of the measurement uncertainty can drive to bad estimates. Occasional and not explained bad performance in proficiency test can be caused by these inconsistencies. A careful bottom-up evaluation of the measurement uncertainty can more easily ensure reported uncertainty is adequate for the all analytical range.

In measurements based on an instrumental method of analysis, uncertainty is frequently underestimated in bottom-up evaluations due to the inadequate assessment of calibrators value uncertainty. If the Least-Squares regression model is used, calibrators quality must be adequate for the regression process and taken into account in the uncertainty budget. The frequently reported correlation between calibrators signals (i.e. the smaller deviation between signal replicates than to the regression line - Figure) results from poor calibrators quality.

The detailed validation of regression model assumptions can be useful for top-down evaluations of the measurement uncertainty since bias become controlled throughout the calibration range.

This communication presents a tutorial for the design, validation quality control of analytical calibrations. The tutorial includes the definition of the calibration range and calibrators preparation procedure, the assessment of regression model assumptions, the estimation of the limits of detection and quantification, the evaluation of the measurement uncertainty and calibration quality control. This tutorial is implemented in a user-friendly and validated MS-Excel spreadsheet.

The application of this tutorial to the quantification of nitrites in drinking water by molecular spectroscopy, between 0.1 mg L\(^{-1}\) and 0.4 mg L\(^{-1}\), with an expanded relative uncertainty ranging from 2.1 % to 3.1 % is presented. Measurement uncertainty estimation quality was checked through the analysis of four control standards distributed along the calibration range, in ten independent calibrations performed in different days summing up 40 controls. The metrological compatibility of estimated and reference values of control standards proved the adequacy of the measurement model.

EXAMINATION CRITERIA AND UNCERTAINTY OF TRACE LEVELS OF ORGANIC COMPOUNDS IN COMPLEX MATRICES BY GC-MS

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The identification of trace levels of compounds in complex matrices by conventional low-resolution gas chromatography hyphenated with mass spectrometry is based on the comparison of retention times and abundance ratios of characteristic mass spectrum fragments of analyte peaks from calibrators with sample peaks. Statistically sound criteria for the comparison of these parameters were developed based on the normal distribution of retention times and in the simulation of possible non-normal distribution of correlated abundances ratios (Figure). The confidence level used to set the statistical maximum and minimum limits of parameters define the true positive rates of identifications. The false positive rate of identification were estimated from worst-case signal noise models. The estimated true and false positive rate of identifications from one retention time and two correlated ratios of three fragments abundances were combined using simple Bayes statistics to estimate the probability of compound identification being correct designated examination uncertainty. Models of the variation of examination uncertainty with analyte quantity allowed the estimation of the Limit of Examination as the lowest quantity that produce “Extremely strong” evidences of compound presence. User friendly MS-Excel files are made available to allow the easy application of developed approach in routine and research laboratories. The developed approach was successfully applied to the identification of chlorpyrifos-methyl and malathion in QuEChERS method extracts of vegetables with high water content for which the estimated Limit of Examination is 0.14 mg kg\(^{-1}\) and 0.23 mg kg\(^{-1}\) respectively.

Figure: a – Distribution of simulated abundance ratios of fragments 117 m/z, g, and 127 m/z, α, in the identification of 0.25 mg L\(^{-1}\) malathion in solvent. The distribution of the ratio is asymmetric. b – Correlation of simulated g and α values (Pearson’s correlation coefficient of 0.9333).

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WHEN A GOOD RECOVERY FAILS TO ASSESS A TEST METHOD

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In order to monitor corrosion in the reactor coolant circuit, different metals are measured several times each month. Here the focus is on the determination of sodium (Na). The matrix in the reactor coolant is pure water with lithium (Li) concentration varying from 1 mg L\(^{-1}\) to 5 mg L\(^{-1}\) and boron (B) in the range from 20 mg L\(^{-1}\) to 2500 mg L\(^{-1}\). Sodium can be determined by several methods, flame AAS, flame AES, ICP-AES. In routine use the LOQ for flame AES is around 0.5 µg L\(^{-1}\), for flame AAS around 3 µg L\(^{-1}\) and for ICP-AES around 5 µg L\(^{-1}\). Upper concentration level is 100 µg L\(^{-1}\), but focus is between LOQ and 10 µg L\(^{-1}\). All three methods are used at Ringhals’ two process control laboratories.

In the validation study, the focus was on the performance characteristics LOD and recovery using matrices of varying boron concentration. In the validation of each method, the recovery was tested by spiking and a good recovery was obtained. After strict contamination control, the LOQ was mainly limited by the technique used for determining sodium.

When comparing results using the same test method, between the two laboratories on samples from the coolant circuit, one lab typically measured 4 µg L\(^{-1}\) and the other lab measured 15 µg L\(^{-1}\). Both laboratories in this case used flame AES. This discrepancy was investigated and found to be background emission from the boron matrix. The solution, to this interference on the background, was either using a cooler flame, or flame AAS. Also an emission technique with background correction, ICP-AES was found possible to use.