EURACHEM 2019 workshop
20th and 21st of May, Tartu, Estonia

"Validation of targeted and non-targeted methods of analysis"

BOOK OF ABSTRACTS

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Validation of targeted methods: Where we are?

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To support laboratories in ensuring the validity of their measurement results Eurachem published the first version of the guide "*The Fitness for Purpose of Analytical Methods: A Laboratory Guide to Method Validation and Related Topics*" in 1998. Over the following decades, a vast technological development has taken place, giving new challenges to the aim of securing valid methods for the many fields of application. The Eurachem Working Group on Method Validation (MVWG), reacted to this by initiating a long and meticulous process of revising the guide, ending in the issue of version 2 in 2014.

AND - there is still a lot of new tasks for the MVWG as some of the old challenges are still present (e.g. in the light of the end-users having higher demands in relation to reliability of the results), and along with that a number of new challenges has appeared as a result of the technological development in the various fields of using testing methods.

So where are we in 2019?
As for the well-known subjects related to method validation, the MVWG has just launched two supplementary documents:

- *Blanks in Method Validation* - as the WG saw the need for giving better and more detailed guidance on the understanding of the many types of blanks and their use in the validation processes
- *Planning and Reporting Method Validation Studies* - which is a very practically oriented guideline on setting up the protocol for a validation study (based on the principles in the "Fitness for Purpose" Guide)

The WG has also been working intensely on other potential supplements, "Handling of Bias", "Linearity in Method Validation" and "Selection of an appropriate Test Kit", which may be issued separately or just end up as annexes to the next version of the guide, for which the review work has already started.

The new subjects related to method validation, coming on the agenda of the MVWG have actually very much been related to some of the issues being in focus during this workshop

- bioanalytical methods (often "wrapped up" in a rapid method or a biosensor)

- computers for handling vast amounts of data

- more sensitive detection principles, also giving new challenges with regard to interferences

One of the fields developing rapidly in these years is exactly the field of multiparameter and non-targeted methods (mostly based on some of the new biotechnological principles). As we shall hear during this workshop, these methods have especially be found very relevant in relation to dealing with food fraud and recently a new CEN Technical Committee has been suggested for working horizontally across the existing, vertical CEN/TCs (on specific product groups). The new proposed Technical Committee will be responsible for developing standardization of validated analytical methods for detection of food authenticity and, in addition, quantitative analyses, as appropriate. A part of the work programme should be focusing on "..standardization of validation concepts for non-targeted methods regarding food authenticity"

This will also be an important outcome of this workshop: Taking ideas and good practices back to the ongoing work in the Eurachem MVWG – which obviously will be kept active also in the future.

No doubt, that the outputs from this workshop will give a lot of input to the MVWG – and everybody are welcome to join us.

Time trends as a prioritization strategy in non-target analysis

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INTRODUCTION

Over the past decade, nontarget screening has emerged as a promising new technique for detecting many hundreds of chemicals simultaneously in a sample. Despite the enormous potential of this technology, the large quantity of data produced by non-target methods remains challenging to interrogate. Prioritization strategies which filter and isolate important features for further identification are vital to the success of these methods.[1]

Time trend-based prioritization involves flagging features which display interesting trends within a time series. For example, features displaying an increasing time trend in human blood may represent emerging bioaccumulative contaminants which should be prioritized for further investigation. Recent examples of the successful application of time trend-based prioritization include identification of transformation products in biodegradation experiments[2] and identification of emerging contaminants in dated sediment cores[3] and sludge[4] samples.

The current work focuses on the application of time-trend-based prioritization to non-target human biomonitoring. Advantages and limitations of this approach, along with areas for future research will be discussed.

EXPERIMENTAL METHODS

Proof of principle

Artificial time trends were prepared by fortifying human whole blood with isotopically labelled standards. Two different time trends were created, each at 3-different fortification levels: a) a continuously increasing trend; and b) an initial increase followed by steady state. Thereafter, samples were extracted and analysed using methods described in reference 5.

Two statistical approaches were tested on the data obtained from this experiment: The first involved determination of a 'time trend ratio (TTR)', which was based on comparison of average intensities in time points 7-9 vs 1-6; the second approach involved calculation of the Spearman's rank correlation coefficient (ρ).

Application to real samples

Anonymized human whole blood was obtained from the German Environmental Specimen Bank (Münster, Germany). A total of six samples were randomly selected every fourth year from 1983 to 2015, resulting in nine different years and 54 samples in total. Analysis was carried out on both individuals and yearly pools using methods described in reference 6.

Following time trend-based data reduction, shortlisted features were a) searched against the KemI Market List database and b) subjected to additional MS/MS experiments followed by mzCloud and MetFrag searches. Finally, standards were obtained for a short list of 11 tentatively identified substances.

RESULTS AND DISCUSSION

Proof of principle

The TTR-based approach was particularly effective at ranking spiked compounds which were only detectable in two to three of the latest time points in the time series. While this calculation is thus an efficient method to filter
out substances appearing in recent years (i.e., emerging
bioaccumulative contaminants); it is somewhat sensitive to the choice of time points used in the calculation. In contrast, Spearman's ρ was effective at prioritizing compounds displaying an increasing trend in three or more time points. Compared to initial peak lists, this combined strategy reduced the dataset by 80-85 %.

Application to real samples

Following alignment, peak detection, grouping, and gap filling, up to 14,460 features were obtained. This number was reduced to ≤716 using TTRs and Spearman's ρ to identify features which increased over the 32-year time series. Further prioritization and tentative identification using the KemI market list database, along with MS/MS experiments + MetFrag/mzCloud searches yielded a total of 64 unique features. Among these tentatively identified

substances, 7 were confirmed using authentic standards.

CONCLUSION

This work demonstrates that time trends are an effective prioritization strategy for nontarget human biomonitoring data. Further work is needed to improve MS databases, in particular with regards to MS/MS data for environmental contaminants.

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ACKNOWLEDGEMENTS

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KEYWORDS (5 keywords)

Non-target analysis, time trends, high resolution mass spectrometry

Detection of a multitude of (unknown) components in complex samples Criteria for identification

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INTRODUCTION

Identification of unknown compounds in complex samples increases in popularity in a modern research era. Nowadays studies of large sample pools (N>1000) are a common occurrence. Not only samples are getting more complex, but also a requirement to analyse more samples in a shorter span of time becomes more vital for research success. Therefore, significance of accurate balancing between throughput and selectivity is increasing. Additionally, effectiveness of instrumentation plays more significant role than ever.

EXPERIMENTAL

One of the most efficient ways to increase throughput is deploying of shorter more efficient columns at the maximum of their performance. That results in increased performance requirements presented for chromatographic systems and mass spectrometers. Data acquisition strategies must be considered for better coverage and identification effectiveness. Mass spectrometers should be capable of handling fast gradients with narrow peaks (2-5 sec). Data acquisition method should allow for sufficient amount of points per peak in MS^1 for proper integration and sufficient points of high spectral quality in MS^2 for structural information. Addition of orthogonal separation approaches gives a distinctive advantage when dealing with complex samples.

DISCUSSION

Mass accuracy, mass resolution and isotopic distribution allow for initial molecular formula elucidation. In $MS^2(^n)$, fragmentation pattern allows for molecular formula confirmation with additional structural information. For generation of fragmentation pattern a collision energy ramp is often used for more efficient fragmentation of different compound classes. Ionisation and fragmentation modes can allow for supplementary information acquisition. Chromatographic and orthogonal (ion mobility) separations allow for further distinct criteria that should be used for compound identification. However, in pursuit of higher throughput reproducibility has to remain a priority.

CONCLUSION

A balanced approach for identification criteria selection is a key to a successful study. Acquisition of sufficient amount of data in the shortest amount of time is one of the most important factors for consideration. Depending on the complexity of the sample and desired throughput some selectivity could be sacrificed, provided reproducibility remains unaffected.

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ACKNOWLEDGMENTS

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KEYWORDS

Identification, mass spectrometry, chromatography, selectivity, throughput

Tracebility and uncertainty of qualitative targeted and non-targeted analysis

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INTRODUCTION

Many decisions with relevant socioeconomic impact are based on qualitative chemical analysis. Sometimes qualitative analysis precedes the measurement of the identified component and in fact represents the most demanding stage of the determination.

In many research fields and in advanced monitoring of environment and food products, items are screened for the presence of non-specified components. Instead of just checking the presence of a list of components, items are screened for the presence of any other component relevant for the purpose of the analysis. These two types of analyses are designated targeted and non-targeted analyses.

Qualitative analysis is only fit for the intended use when based on adequate references and associated with adequate uncertainty.

For instance, the identification of a compound by GC-MS is better performed by using a reference mass spectrum obtained in equivalent instrumentation and conditions. Therefore, it is relevant for any interested party on the result to know how the used reference was obtained. When identified compound level is very low, the evidences of compound presence becomes weak. Therefore, it is also important to measure how strong collected evidences of the reported nominal property are.

DISCUSSION

This presentation discusses the reporting of qualitative analysis results traceability and uncertainty to make sure that no decision will be based on inadequate references and weak evidences of the determined property.

This discussion is illustrated with examples of targeted and non-targeted analysis.

The reporting of qualitative result traceability involves presenting all details relevant to adequately characterise the reference. That details depend on the principles of the qualitative analysis and complexity of analysed item.

It is discussed the evaluation of qualitative analysis uncertainty by false results rates and Bayesian theorem's metrics such as the posterior probability, posterior odds or likelihood ratio[1-3]. The determination of the likelihood ratio does not requires knowledge of how likely the occurrence of the studied property is.

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KEYWORDS (5 keywords)
Qualitative Analysis, Traceability, Uncertainty, Reference, Likelihood ratio

Validation of non-targeted methods in the food area

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Abstract

Food fraud is an issue that has received much attention recently. To combat food fraud, thorough document checks need to be accompanied with analytical checks. These analytical checks can be either targeted or non-targeted. Non-targeted methods are usually multivariate and might be one-class or multiclass methods. There are plenty of non-targeted methods available, and all base their decisions on a database of previous samples with known attributes. True validation for such a method can be translated as establishing the performance of such a method on future samples. We suggest to pre-define the future capabilities of the method before starting the method development. Then, the training and test sets should be evaluated on coverage of the true population it stands for. We argue that the results from the multivariate classification algorithm results should be handled probabilistically or as relevant measure of class distance, not as binary classification results. As the cross-validation and external validation contain variation on the within-database and the variance on unseen samples, we argue that this forms a basis for estimating the uncertainty of the model for future samples. We suggest a way to combine both results into a 'worst-case' prediction distribution, from which performance of the model for future samples can be extracted.

Creating Reliable Data – a Challenge for Non-target Screening

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INTRODUCTION

The ability of non-target screening (NTS) to detect and identify emerging contaminants, and subsequently trigger exposure mitigation measures has been demonstrated[1]. For efficient use in chemical management, such as prioritising chemicals for monitoring programmes, evaluation of treatment technologies and environmental quality assessment, harmonized NTS protocols and minimum quality requirements are needed. First drafts of national guidelines are available for example in Germany (German Chemical Society), with a specific focus on surface water monitoring. The NORMAN network of reference laboratories, research centres and related organisations for monitoring of emerging environmental substances aims to provide a more general guideline based on the experiences gained through different collaborative trials and other activities for water, indoor dust and biota.

In this presentation, quality assurance challenges in NTS and the related consequences for producing reliable data and for harmonization of NTS will be discussed using illustrative examples.

EXPERIMENTAL METHODS

All examples are based on measurements using liquid chromatography coupled with electrospray ionization to tandem high resolution mass spectrometry with Orbitrap technology.

RESULTS AND DISCUSSION

The following steps along the NTS workflow will be discussed, regarding quality assurance using examples of surface, groundwater and wastewater monitoring studies.

- Challenges for pre-processing such as in-source fragmentation, grouping, blank subtraction
- Requirements for and application of suspect screening lists
- Criteria for false positive and negative assignments including retention time prediction
- Specification of the widely accepted level system[2] for communication of identification confidence (e.g. match levels, number of fragments)
- Quantification estimation without reference standards
- Challenges in data evaluation using statistical methods

CONCLUSION

Most probably, harmonization of NTS workflows together with appropriate infrastructure and trained personnel in both the research and regulatory communities will facilitate implementation of NTS in European regulatory processes within the next decade.

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KEYWORDS (5 keywords)

LC-HRMS/MS, identification, in-source fragmentation, suspect screening, false positives and negatives

The use of high resolution mass spectrometric dereplication as a chemical approach for fungal identification and classification

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ABSTRACT

Filamentous fungi are a rich pool of secondary metabolites, some of them are being toxic and hence called mycotoxins. Combining several approaches such as chemical profiling, morphological and molecular approaches provide an accurate way for classification of mycotoxins producing fungi. Thanks for the current technology represented in high resolution mass spectrometry devices that allow performing targeted and un-targeted analysis of the compounds of interest. Dereplication is a rapid screening of previously identified fungal compounds without reference standards. This procedure is also a crucial step to the fast discovery of novel natural products. In the present work, we aimed at developing an in-house screening library with a validated UHPLC–TOF-MS profiling method that can screen for hundreds of mycotoxins. Results of chemical profiling will be matched with the morphological and molecular results for an accurate identification and classification of many mycotoxigenic fungal species isolated from sugarcane juice sold in Egypt.

Quality Control in LC-MS based metabolomics

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INTRODUCTION

Metabolomics represents a rapidly expanding field. However after almost two decades of development, LC-MS based metabolomics is still not technologically mature in comparison e.g. to genomics[1-4]. Quantitation of endogenous metabolites in biological fluids and particularly in blood-derived samples has to overcome several issues. This is due to pragmatic reasons, such as the lack of analyte-free matrix (blank samples) or Certified Reference Materials (CRMs). Comprehensive method validation that includes matrix effect and recovery is rarely described[5].

EXPERIMENTAL METHODS

LC-MS was applied as the analytical tool for various projects in our group for the analysis of blood, urine and other biological samples. Raw data was treated by special software for data pre-treatment and then was imported to multivariate statistics software (Simca). Targeted metabolomics data was generated in HILIC-MS/MS mode with a lab built method[6] and data was treated by TargetLynx, Excel, R scripts and various other software. Untargeted data was generated from RPLC or HILIC coupled to TOF-MS analysis. Data was treated by XCMS, R scripts and other software tools.

RESULTS AND DISCUSSION

The presentation will discuss necessary measures for the Quality control of LC-MS based metabolomics. Potential issues that may hinder application in life sciences will be highlighted. Pre- to post- analytical aspects will be discussed, with paradigms from the application of untargeted metabolomics in the analysis of urine and quantitative analysis of human blood. Discussion includes Quality Control measures and protocols developed by the authors as well as other researchers active in the field.

CONCLUSION

LC-MS metabolomics is still method dependent. Efforts are needed to promote the field toward application in wide scale. Implementation of quality control protocols is necessary[8]

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KEYWORDS (5 keywords)
Metabolomics, LC-MS, validation ,QC

Increasing coverage and throughput in metabolomics by chromatography

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As a matter of fact, discoveries based on non-targeted global metabolic fingerprints, intrinsically depend on the number of significant mass spectrometric features and on the accuracy of fold change determination between the sample groups. Both aspects are determined by the metabolite coverage and selectivity of the applied analytical methods. Chromatographic separations are indispensable, especially where high-resolution mass spectrometers are challenged by isobaric metabolites and in-source fragments. On the other hand, in order to obtain a representative metabolic/lipidomic snapshot, analytical workflows have to consider a vast chemical diversity. As a consequence, comprehensive analysis requires multiple analytical runs using fractionated sample extraction and orthogonal separation methods. The development of streamlined workflows in order to increase the analytical throughput while maintaining high coverage of the metabolome is a topical research theme. Our group addressed this challenge by on-line combinations of orthogonal chromatographic separations with high resolution mass spectrometry. Different solutions of dual-LC and heart-cut LC-HRMS will be discussed with regard to their analytical figures of merit.

Performance characteristics and other quality control parameters for non-target methods

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INTRODUCTION

The presentation focuses on parameters for the characterization of the performance and discriminatory power of non-targeted methods in the case that few samples are available so that the reliability of classical performance characteristics such as sensitivity and specificity is compromised.

EXPERIMENTAL METHODS

Many qualitative classification methods are based on quantitative classification scores. We propose to use this quantitative output in order to develop quantitative assessment parameters such as measures of intermediate and reproducibility variability. This allows a characterization of method performance on the basis of both in-house and interlaboratory studies.

The new approach is illustrated with mass spectrometry data corresponding to the analysis of 190 *Staphylococcus aureus* isolates by MALDI TOF. The method's purpose is the classification of the isolates as either resistant or nonresistant. Each isolate was analyzed twice, once in 2018, and once in 2019.

The new approach was also applied to other data sets, such as mass spectrometry data corresponding to the analysis of 96 fish samples by LC-ESI-MS/MSMS for the assignation to different species.

RESULTS AND DISCUSSION

Various intermediate and reproducibility standard deviations were computed for the *S. aureus* and the fish data, corresponding to different data preparation steps. It was shown that this approach to method characterization is very responsive to relatively slight changes in the method, and thus constitutes a promising avenue for method performance assessment. Results were also compared with more traditional approaches such as sensitivity and specificity parameters and ROC curves.

CONCLUSION

The new approach is shown to be particularly beneficial in the case of low sample numbers, when sensitivity and specificity are highly unreliable and ROC curves are practically indistinguishable, and in relation to the question of the identification of outliers among the isolates/samples.

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None

KEYWORDS (5 keywords)

TOF, non-target method, performance characterization, variability, classification

The development of small molecule profiling technologies for the detection of complex food fraud

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Food fraud is an issue that has been around for many centuries, from the Babylonians worrying about the watering down of beer to the substitution of saffron with marigold petals in medieval times. In both cases the death penalty was deemed a suitable punishment, such was seriousness of the crime. In recent years there have been many high profile cases of food fraud such as the substitution of horsemeat for beef in 2013 or the melamine inclusion in milk in China in 2008.

Like any criminal activity, the true value of the problem is not fully known. However, estimates have put the cost of global food fraud between $10 and $40 billion per year. As there are limited punishments for individuals who engage in this activity, it is becoming increasingly attractive to organised criminal gangs. In recent years there has been a drive to detect more subtle frauds that misrepresent foodstuffs to add value to a product illegally, rather than simple substitutions.

The paper trail of a foodstuff is the traditional way to detect any illegal activity. As supply chains grow there is a need to develop specific tests to detect the authenticity of the product. This talk will focus on the non-targeted small molecule profiling methodologies that have been developed to detect subtle food fraud. It will describe the quality systems used to ensure the validity and robustness of the data, alongside key frauds that have been investigated.

HALOSEARCH: Searching for Unknown Halocarbons in the Atmosphere

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INTRODUCTION

Over 100 halogenated compounds are known in the atmosphere[1], manufactured mostly as refrigerants, foams or blowing agents. They are potent greenhouse gases and many are ozone-depleting substances. Together they may contribute up to 20% to anthropogenic global warming by 2050.
New compounds are continuously commercialised and subsequently emitted to the atmosphere[2], at trace levels (pmol/mol or below).

The aims of the HALOSERCH project are:

- To perform suspect and non-target search for new trace compounds in the atmosphere;
- For this, to develop machine learning tools for the automated identification of unknown compounds detected by GC-TOFMS (gas chromatograph, time of flight mass spectrometry).
- Once compounds are identified and time series are measured, to automatise source pattern recognition, because substances emitted during the manufacturing process may be emitted alongside the main molecule, producing similar time series.

EXPERIMENTAL METHODS

The air measurements are based on the robust experimental system developed within the AGAGE network[1] (Advanced Global Atmospheric Gases Experiment): a pre-concentration unit trapping 2 L of air and coupled to a gas chromatograph (GasPro column, Agilent). Here, the novelty is to use an electron-impact ionisation source (EI) coupled to a TOF-MS as detector (Tofwerk). Our TOF is tuned to detect masses between 2 and 300 u, with a mass resolution of ~4000. After internal mass calibration, the mass accuracy is better than 10 ppm.

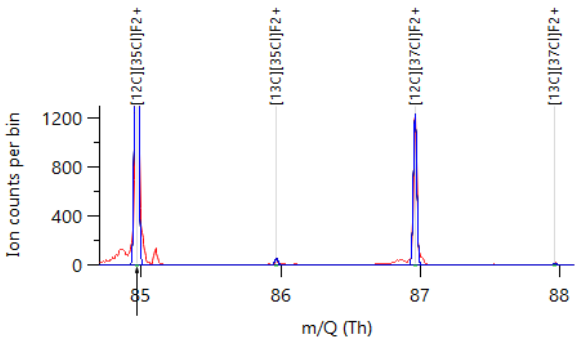

Figure 1: Isotopologues of $CClF_2$, a fragment of $CBrClF_2$, as measured by our TOFMS. Four isotopologues are detectable.

RESULTS: DEVELOPEMENT OF THE ALGORITHM

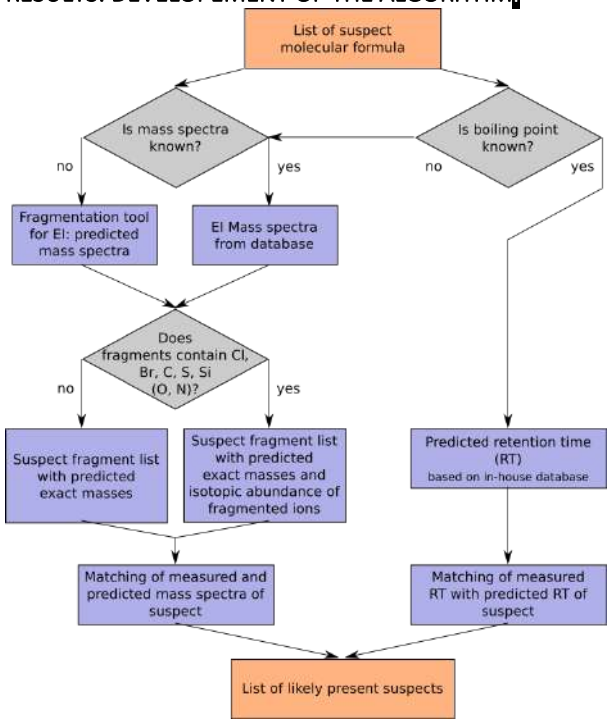

Figure 2: Workflow for suspect screening for EI-TOFMS.

A major challenge is the development of a data analysis algorithm for an automated identification of the measured compounds, given that many get fragmented in EI-MS to a degree where the parent molecule may not be present. Such an algorithm, presented as a workflow, is show for suspect screening in Figure 2.

CONCLUSION/FUTURE WORK

The decision tree for suspect screening is tested using our in-house database of halogenated substances as training set. Machine learning tools are being developed to optimise the algorithm and especially the combination of different information sources, e.g. Bayesian classification framework. The method will then be generalised for non-target analysis.

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KEYWORDS

Halogenated compounds, GC-TOF-MS, machine learning, non-target analysis, compound identification.

Making non-targeted LC/HRMS screening quantitative

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ABSTRACT

Target screening with LC/MS has found its solidified status in the field of analytical chemistry for research, industry, and legislative purposes. At the same time, the applicability of non-target screening is still in its infancy. The most significant obstacles for non-target LC/HRMS screening today are the scarcely defined scope of the method and the inability to provide quantitative information. This is well shown by the data. Currently, the Human Metabolome Database contains 114 100 compounds and only 3383 (<3%) of these have been both detected and quantified.

The inability to provide quantitative data for non-targeted screening with LC/ESI/HRMS originates from the vastly different ionization efficiencies of different compounds in ESI source. The overall scale of ionization efficiencies covers 6 orders of magnitude in both ESI positive and negative mode. For example, the positional isomers 2- and 4-nitrophenol yield a 40 times different response at equal concentrations. Additionally, different LC conditions also affect the ionization efficiencies.

The only possibility to still obtain quantitative information is to predict ionization efficiencies. We have measured more than 2500 ionization efficiency values in ESI positive mode and more than 1000 in ESI negative mode. These measurements have been conducted in vastly different solvent (60 combinations); we have used eluents with both methanol and acetonitrile with 0 to 100% content and covered pH range from 2.0 to 10.7 with all common LC/MS buffers. The ionization efficiency scale covers more than 7 orders of magnitude in both modes.

Here we will present a possibility to predict ionization efficiencies based on the 2D descriptors obtained from PaDEL. We use random forest regression to relate the 2D descriptors with the ionization efficiency values. The average miss prediction is below 4 times. The accurate result obtained for ionization efficiency predictions has encouraged us to test this approach also for predicting the concentration. We have tested the approach on the screening for food contaminant in cereal samples (positive mode) and metabolites in green tea samples (negative mode) non-targeted LC/MS methods. Both analysis have been carried out in the gradient elution mode. In the cereal samples the average prediction error was below 5 times; while, for the metabolites it was less than 3 times. For both samples the concentrations on the detected compounds ranged over more than five orders of magnitude (10-8 to 10-4 M).

Additionally, we gathered more than 1100 data point from 62 literature sources (both ESI positive and negative mode) and carried out the ionization efficiency predictions for these data. The average prediction error was 2.7 times and after transforming all values to the same scale the ionization efficiency values ranged over 3 orders of magnitude.

Comparison of ionization (ESI) of different biomass based anhydrosugars

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INTRODUCTION

As oil plays a significant role in the global economy, the rapidly growing consumption of fossil energy resources and the overall decline in oil reserves lead to the global energy crisis [1]. As a result, many studies are based on the use of biomass as the most valuable renewable resource in the production of liquid fuels, materials and chemicals [2]. Bio-oil is a complex mixture of compounds containing alkenes, aromatic compounds, phenols, furans, esters, aldehydes, ketones, sugars and acids [3-5]. Carbohydrate derivatives, such as 1,6-Anhydro-beta-glucopyranose (or levoglucosan, or LG) and it's ketone - (1S,5R)-6,8-Dioxabicyclo[3.2.1]oct-2-en-4-one (or levoglucosenone, or LGO) are among the main products of thermochemical degradation of cellulose and are valuable platform chemicals.

The LG and LGO are mainly determined using GC or HPLC [6]. The UHPLC used in this study yields better results – higher resolution, and shorter analysis time. UV or RI detection is used primarily for LGO assays because LGO and its by-products contain chromophore groups [7]. But for LG analysis UV detector cannot be used because LG does not absorb in UV/VIS spectrum. This work focused on the use of mass spectrometry, to determine whether both anhydrosugars have similar ionization. In literature the ionization of LGO in electron spray ionization (ESI) conditions has not been described.

EXPERIMENTAL

In this study we analysed LG and LGO standards and also pyrolysis liquid samples, which were prepared, using lignocellulose obtained from hydrolysed birch (Betula pendula) chips. Two separate UHPLC methods were used: BEH amide column (1.7 µm, 2.1 × 100 mm) for LG analysis and CSH Fluoro-Phenyl (1.7 µm, 2.1 x 100 mm) for LGO analysis. In both methods the mobile phase was 0,1 % NH_4OH in water/acetonitrile (40:60) with flow rate 0.15 mL/min. The ionization of LGO and LG in the positive and negative electroionisation mode and the effect of a mobile phase additive on LG, LGO and its degradation product ionisation have been evaluated.

RESULTS AND DISCUSSION

Optimal conditions for ionization of LG are negative ionization mode, cone voltage 5 V and capillary voltage 0.8 kV. In these conditions ion with *m/z* 161 Da corresponds to the molecular ion of LG $[M-H]^-$, but the ion with a *m/z* 323 Da is a dimer of LG, which forms in ion source $[2M-H]^-$. Analysing LGO in both positive and negative ionization modes ions with *m/z* 95 and 125 Da were determined, but 125 Da ion is more intense. Ion with *m/z* 125 Da theoretically corresponds to the LGO molecular ion $[M-H]^-$. The difference between 125 and 95 Da is 30 Da, which could correspond to formaldehyde cleavage. There is evidence, that spontaneous degradation product of LGO elutes with the same retention time and is ionised instead of LGO itself. For the degradation product observed typical molar mass of LGO 125 Da $[M-H]^-$ and 95 Da $[M-CH_2O-H]^-$ as well as 143 Da which corresponds to added water molecule in degradation process.

CONCLUSIONS

According to the MS results, we can conclude that LGO does not ionizes by utilising ESI system, while its degradation product does. In comparison, biomass based anhydrosugar LGO ionizes using ESI system in negative ionization mode. So, we can conclude that there is a significant difference in ionization between these two anhydrosugars. The differences of ionization can be explained by structural differences of these compounds – ketones poorly ionise in the ESI source.

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ACKNOWLEDGMENTS

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KEYWORDS

BIOMASS, ANHYDRO-SUGARS, LEVOGLUCOSAN, LEVOGLUCOSENONE, MASSPECTROMETRY

Analytical methods and results in PTSs for phthalates in wine and spirits

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Phthalates are widely used as additives in the production of plastic materials as well as in certain food packaging materials. Some of these compounds are considered powerful endocrine disruptors, which recently led to a change in regulations concerning their use in materials destined to be in contact with foodstuffs. Wines and spirits produced, handled and/or transported in contact with plastic material could be contaminated by phthalates and exposed to a refusal of marketing authorization. This is the reason why the request of analyses of phthalates in wines and spirits has gradually increased in recent years, leading laboratories to improve their analytical performances in term of detection and quantification of these molecules. The OIV recommended the GS/MS method (OIV-MA-AS323-10 and OIV-MA-BS-33), but several laboratories perform these analyses by HPLC/UV, UPLC/UV or develop a specific analytical procedure. Proficiency-testing schemes are being organized by BIPEA since October 2015 proposing wines and spirits spiked with phthalates to allow laboratories to monitor the reliability of their results. The aim of this work is to compare the results obtained by laboratories using different methods for the analyses of phthalates in wines and spirits.

Determination of relative potency as the ratio of effective concentration estimated from sigmoidal-response curves and respective measurement uncertainty

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INTRODUCTION

Bioassays are *in vivo*, *ex vivo* or *in vitro* assays often used for the determination of the activity or relative potency (ρ) of drug products, which may be applied during process development, product development or product release testing. Bioassays are typically performed using a parallel-assay or a sigmoidal curve assay. Assuming that the standard (S) and test (T) samples are biologically similar, the test sample can be expected to behave like a dilution of the standard ($d_T = \rho \times d_S$). When sigmoidal curve assays are adopted, the relative potency is calculated as the ratio of effective concentration of test sample and standard ($\rho = EC50\%_T / EC50\%_S$).[1] The standard uncertainties of the effective concentrations of test ($u_{EC50\%_T}$) and standard ($u_{EC50\%_S}$) is used to calculate the combined uncertainty of the relative potency (u_ρ). However, $EC50\%_T$ and $EC50\%_S$ may be correlated due to shared relevant experimental conditions, which may affect the measurement uncertainty of the relative potency (u_ρ).[2] The aim of this work was to propose a methodology for the measurement uncertainty evaluation of the relative potency determined using a smartphone-based colorimetric assay and sigmoidal-response curves.

EXPERIMENTAL METHODS

Aliquots of standard and test samples in a range from 100 to 0.2 µg·mL^{-1} was transferred to a 96-well microtiter plate, followed by the addition of tryptic soy broth (TSB) previously inoculated with 10^5-10^6 CFU·mL^{-1} of *Staphylococcus aureus* (ATCC 6538 – 10^5-10^6 CFU·mL^{-1}) and resazurin solution. Microtiter plate was incubated at 37 ± 1ºC for 90 minutes. After incubation, the microbial growth inhibition was measured using a smartphone camera device and a colour analyser app (RGB - Red-Green-Blue).

RESULTS AND DISCUSSION

A 4-parameter logistic regression model was used to explain the microbial inhibition growth ($Y\%$) as function of the logarithm of the antibiotic ($ln(d)$), as presented in the equation below:

$$Y\% = A + \frac{(D - A)}{1 + e^{B \times (ln(C) - ln(d))}}$$

The upper and lower asymptotes (A and D, respectively), the slope (B) and the inflection point (C) are expected to be the same for both standard (S) and test (T) sigmoidal curves, since the standard and test samples are assumed to be to be biologically similar, as can be seen of **Figure 1**.

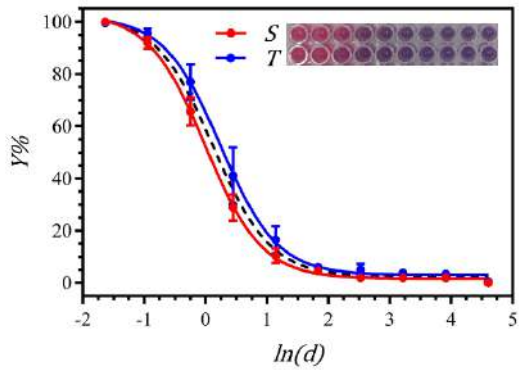

Figure 1. Sigmoidal curves for standard (S) and test (T) samples.

$EC50\%_T$ and $EC50\%_S$ values were found to be 1.18 and 1.16 µ·mL^{-1} and the respective uncertainty factors (U_F) were 1.065 and 1.061, respectively. In addition, $EC50\%_T$ and $EC50\%_S$ quantity values were significantly correlated (r = 0.83), due to shared experimental conditions. Kragten spreadsheet method[3] were used to estimate the measurement uncertainty associated with the relative potency calculate as the ratio of effective concentration of test sample and standard. Considering the correlation between $EC50\%_T$ and $EC50\%_S$ quantity values, the relative potency was found to be 98.5% *,/ 1.036. While the relative potency was found to be 98.5% *,/ 1.091, when the correlation between $EC50\%_T$ and $EC50\%_S$ quantity values were not considered. The target uncertainty factor (U_F^{target}) was found to be 1.052, considering the specification range from 90 to 135% for relative potency.

CONCLUSION

The correlation between $EC50\%_T$ and $EC50\%_S$ quantity values reduced significantly the uncertainty factor for relative potency, which is smaller than the target uncertainty factor.

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ACKNOWLEDGMENTS

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KEYWORDS

Bioassays, relative potency, measurement uncertainty, multiplicative uncertainty factor, correlation.

Potential of three years old plantation willow bark as a source of proanthocyanidins

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INTRODUCTION

Proanthocyanidins, known also as condensed tannins, are complex class of polyphenols that mainly consist of epicatechin and gallocatechin units. The proanthocyanidins obtained in significant amounts from different Latvian wood resources (pine, oak, goat willow) are confirmed as a potential ingredient in antioxidant additives for food, cosmetic and health industries[1], as a component of adhezives[2] and in many other areas of industrial application. Willows - fast growing, easily cultivable trees from the genus Salix are widespread in cold and moderate temperate regions of the Northern Hemisphere. Willows are known as a source of such important compounds as phenolic glycosides, namely, salicin, and their esters (tremulacin or salicortin), as well as proanthocyanidins. The aim of the present work was detection and characterization of proanthocyanidins in the bark of plantation willow (exemplified by *Salix Klara sp.*) and comparison of data obtained with well-known rich-in proantocyanidins pine (*Pinus Sylvestris*) bark.

EXPERIMENTAL METHODS

Salix "Klara" bark was acquired from plantation in Skriveri municipality in Latvia, from 3 years old trees. Pine bark was acquired from Ogres municipality in Latvia, from 76 years old tree. Bark samples were dried on open air, milled using Retch (≤2mm). Soxhlet extraction with acetone during 8 hours[3] and accelerated solvent extraction (ASE) using 60% ethanol water extraction after removal of lipophilic compounds with hexane were performed. Total proanthocyanidin content in the extracts was analysed using acid – butanol assay[1].

Individual compounds in the extracts were identified using UHPLC-qToF-MS/MS (Waters) system with UPLC CSH C18 column (100 x 2,1 mm, 1, µm). Mobile phases were ultra-pure water with 0,1 % formic acid (A) and acetonitrile (B).

RESULTS AND DISCUSSION

The yields of proanthocyandin extracts obtained from plantation willow "Klara" bark were 22.3 % and 17.8 % for Soxhlet and ASE, correspondingly (Fig.1.). Meanwhile the yield of extractives from pine bark was slightly lower: 18.1 % for ASE and 14.2 % for Soxhlet extraction. Results from acid – butanol assay show that in these extracts proanthocyanidins content varies from 17.6 % in pine bark to 28.7 % in "Klara" bark. Respectively, the total yield of proanthcyandins from willow bark investigated is higher than obtained from pine bark (Fig. 2.).

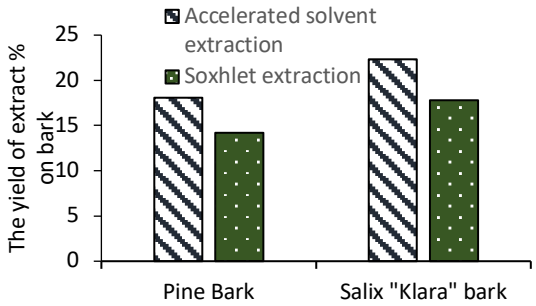

Fig. 1. The yield of extractives obtained by Soxhlet and ASE from bark of pine and willow

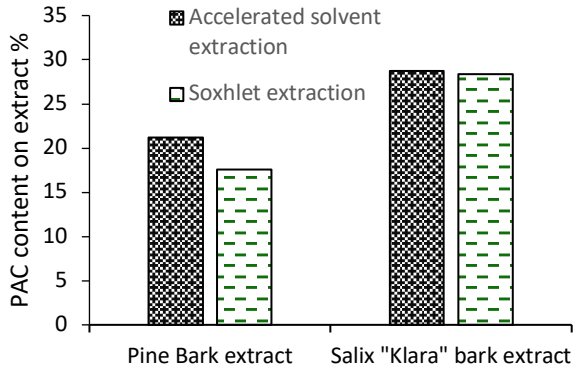

Fig.2. The total content of proanthocyanidins (PACs) in the extracts of Klara and pine bark

Qualitative composition of willow extracts determined using UPLC-MS/MS showed the presence of the following PAC monomers – epicatechin, catechin and gallocatechin, as well as dimers: ((E)C-GC and (E)C-(E)C), trimers: ((E)C-(E)C-(E)C and (E)C-(E)C-GC), and tetramers: ((E)C-(E)C-(E)C-(E)C, (E)C-(E)C-(E)C-GC and ((E)C-(E)C-GC-GC). Both A type and B type linkages between monomeric units were estimated. Such variety in composition opens different opportunities for these extracts application.

CONCLUSION

From acquired results, it's possible to conclude that willow "Klara" bark could be considered as a valuable potential source of proanthocyanidins rich extracts.

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KEYWORDS

Plantation willow, Pine, bark, Proanthocyanidins

Standardisation of non-targeted methods - new initiatives of the official authorities in Germany

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INTRODUCTION

As coordination office for § 64 LFGB (German Food and Feed Act), the unit "General affairs and method standardisation" at the Federal Office of Consumer Protection and Food Safety (BVL) has among other things the statutory obligation to keep up-to-date the Official Collection of Methods of Sampling and Analysis (ASU) in Germany. In this context, the potential of modern non-targeted methods is being considered, e.g. for species identification and for checks on the geographic origin or method of production [1, 2, 3].

In order to meet the requirements of the official authorities responsible for food surveillance in Germany and Europe, these methods must be validated and included in the Official Collection of Methods of Sampling and Analysis (ASU). Furthermore, they must be conveyed to the Comité Européen de Normalisation (CEN). Therefore, the BVL is constituting new working groups consisting of experts from the field of non-targeted methods.

RESULTS AND DISCUSSION

Due to its multiplexing capacity, mass spectrometry is gaining increasing attention from food surveillance. In recent years, several methods have been developed that use liquid chromatography coupled to mass spectrometry (LC-MS) to identify species-specific marker peptides in food. These marker peptides can be used to verify food authenticity. Although these methods have great potential, none of them have been standardised or validated in inter-laboratory studies so far. In order to achieve this goal, the BVL has, as the first of its kind, constituted a new working group for the peptide-based mass spectrometric analysis of food and agricultural products [2]. The purpose of this group is to identify and validate methods based on LC-MS, which can then be used to control and enforce regulations dealing with food authenticity.

Further methods used for authenticity testing are matrix-assisted laser desorption and ionisation (MALDI) time-of-flight (TOF) MS, next generation sequencing (NGS), nuclear magnetic resonance (NMR) spectroscopy, isotopic ratio mass spectrometry (IRMS) and other so-called untargeted methods incorporating a multivariate classification. The constitution of new working groups regarding MALDI-TOF, NGS, NMR and IRMS is organised at the BVL.

CONCLUSION

On the European level, the ASU coordination office supports the CEN coordination group "Food Authenticity" (FACG). After taking an inventory of the methods that need to be standardised, the methods will be prioritised. The German Institute for Standardisation (DIN) got the order from the CEN to establish a Technical Committee (TC) on "Food Authenticity" and to host its secretariat. The methods standardised and validated in the § 64 working groups will then be introduced into this TC.

The first meeting of the DIN working group NA 057-08-02 AA „Lebensmittelauthentizität" (Food authenticity) was on 28 February 2019 and the first meeting of the CEN/TC 460 "Food authenticity" will be on 14 June 2019 in Berlin

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ACKNOWLEDGMENTS

Members and stakeholders of the new working groups.

KEYWORDS

Official Collection of Methods; non-targeted methods; mass spectrometry: MALDI-TOF; NGS

Compound Databases in Official Food Control - Thoughts and Challenges for Validation and Verification

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INTRODUCTION

In recent years, new analytical methods with high potential have reached the food surveillance sector. The new methods include, among others, peptidomics with liquid chromatography (LC) mass spectrometry (MS), fingerprinting with matrix-assisted laser desorption and ionisation (MALDI) time-of-flight (TOF) MS, or nuclear magnetic resonance (NMR) spectroscopy. In order to harness the new methods for official food control, the coordination office for § 64 LFGB (German Food and Feed Act) of the Federal Office of Consumer Protection and Food Safety (BVL) is currently constituting several new working groups (WGs) for standardisation of the new methods[1,2]. Since the new methods generally target more than one parameter for analysis or even use an untargeted approach, intentional deception of the methods is particularly difficult for food fraudsters.

Due to the large number of parameters, usage of compound databases is often mandatory for the new methods. However, available databases do not usually meet the requirements of official food control, which, according to VO (EU) 2017/625, have to be accredited regarding ISO/IEC 17025 and need legally binding results. For this reason, the validity of databases is an ongoing topic in the new § 64 WGs during the process of method standardisation. The purpose of this manuscript is to identify possible challenges regarding validation and verification of databases for official food control. Moreover, first ideas and thoughts of the new WGs on this topic will be presented.

RESULTS AND DISCUSSION

Nowadays, the compound databases utilised by the new food forensic methods mainly divide into two groups; free-access, relatively open databases, such as UniProt[3] for peptidomics; and restricted, commercially licensed databases, such as the Bruker Biotyper databases for MALDI-TOF. Since the latter group often generates high costs and dependencies on commercial providers, usage of such databases is not feasible for official food control. Moreover, commercial databases are usually not transparent (the so-called black boxes), as providers keep their source code and algorithms secret for economic reasons. Nevertheless, in some areas, commercial databases are still widely used even in official laboratories, simply because there are no alternatives.

The non-commercial, open group of databases offers high transparency in their source codes and algorithms. The database entries, however, are often not traceable and valid, as usually anyone can make new entries into the databases. Moreover, there is still a lack of non-commercial databases for food commodities. Assembling such databases for food control is a difficult task due to the need for reference and authentic materials. In regards to official food control, there is another problem that has to be considered in the development of open databases. If the databases are accessible to anyone, which can help expand the database, potential food fraudsters also have access to the database and can use the information to make their adulterations less detectable.

CONCLUSION

There is consensus in the new § 64 WGs about the need for verified and validated databases, in order to successfully apply the new food forensic methods in official food control. Ideally, the databases should be provided by official authorities to ensure transparency and verified database entries and prevent potential fraudsters from accessing the databases. One idea even suggests the founding of a national reference centre that provides data storage and sharing capabilities. However, the officials would have to factor the costs and amount of work and time to accomplish such a project.

Since in some areas the use of commercial databases is currently unavoidable, another validation approach has been developed by the official food control laboratories of the state of Baden-Württemberg (BW). Instead of validating the whole databases, only the database entries needed for the laboratory are validated individually by verifying the entries with a set of authentic samples and a negative control sample set, therefore determining

sensitivity and selectivity of the database entry. The validation procedure has been accepted by the German accreditation bodies and is currently valid for all official food control laboratories in the state of BW.

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Contributors and members of the new WGs.

KEYWORDS (5 keywords)

Databases; Validation concepts; § 64 LFGB; Method standardisation; New food forensic methods

Purification of Carrageenases from Cultured Marine *Cellulophaga* Species

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INTRODUCTION

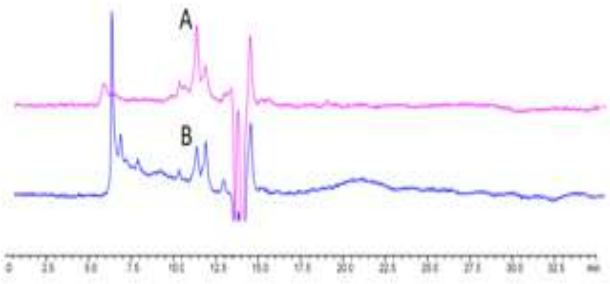
Carrageenans are gel-forming linear sulfated galactans extracted from certain marine red algae. Due to their lower molecular weight compared to native ones, oligosaccharides obtained from cleaving process of carageenans can show potential anti-tumor activities, anticoagulation, anti-inflammation, anti-oxidation, anti-thrombosis, and viral inactivation[1,2].Different chemical and physical methods included enzymatic and acid hydrolysis, and ultra-sonication are applied to degrade carageenans. Oligosaccharides produced by the action of microbial enzymes found to be more invaluable than produced by other methods because enzymes are highly specific to their substrates thus they can generate oligo-derivatives with uniform molecular weights[3]. The present study was undertaken in an attempt to purify and characterize carrageenases produced by marine *Cellulophaga* species.

EXPERIMENTAL METHODS

In this study, the extracellular carrageenase was isolated from the cell-free medium of a culture of marine bacterium *Cellulophaga* species grown on marine broth with 0.16% furcellaran. A single active peak of carrageenase was separated and purified from the mixture by ultrafiltration, ammonium sulfate precipitation, Sephadex G-200 gel filtration chromatography, and size-exclusion chromatography. Molecular weight of the purified carrageenase was estimated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). As each enzyme is a very specific compound, the enzymatic activity of enzymes against different substrates as carrageenans, agars, and alginates was measured by reducing sugar and total proteinassays. High-performance Liquid Chromatography with pulsed amperometric detector (HPLC-PAD) was to identify and determine oligosaccharides resulted from the enzymatic hydrolysis process.

RESULTS AND DISCUSSION

The carrageenase was purified by a series of purification procedures and finally yielded a significantly high activity against carrageenan polysaccharides. The result of SDS-PAGE showed that the purified enzyme was a single protein band with molecular weight of about 40 kDa. Depending on results obtained by size exclusion chromatography and enzymatic activity assay, the ammonium sulphate precipitation proceed in two steps first by 40% salt followed by second precipitation step with 70% ammonium sulphate gave the most active enzyme peak(Fig1).



Retention time (min)

Fig1.Sizeexclusion chromatography of extracellular carrageenase. A) Obtained after ultrafiltration and two steps40% and 70%) of ammonium sulfate precipitation. B) Obtained after centrifugation process only.

CONCLUSION

In this study, an enzyme with high activity toward carrageenan polysaccharides was successfully isolated and purified from marine *Cellulophaga* species cultivated in marine broth. The molecular weight of the enzyme was estimated to be around 40 kDa, and it is specific of cleaving the β-1,4 linkage.

KEYWORDS

Carrageenases, *Cellulophaga,* Purification, Oligosaccharides, Sulfated galactans.

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Comparison of Derivatization Methods for the Quantitative Gas Chromatographic Analysis of Oils

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INTRODUCTION

Determination of fatty acid composition using quantitative gas chromatographic (GC) analysis is commonly used of characterising fats and oils in food industry, conservation studies and other fields. A wide variety of derivatization methods have been developed to enable the GC analysis of non-volatile oil components. However, there has been no systematic comparison of these methods in truly quantitative terms.

EXPERIMENTAL METHODS

In this study, for the first time, a comprehensive quantitative comparison of four derivatization methods are discussed: (1) m-(-trifluoromethyl)phenyl-trimethylammonium hydroxide (TMTFTH) methylation, (2) two-step derivatization with sodium ethoxide (NaOEt) and N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA), (3) two-step derivatization with KOH and BSTFA and (4) acid-catalyzed methylation (ACM).

For these derivatizations three different oils were used - analytical grade canola oil standard, commercially available clarified linseed oil and Olivia canola oil as real-life samples for testing the quantitative approach. Also, a mixture of triacylglycerides (TAGs) with known contents was prepared for the validation of the quantitative procedures. The comparison of the results obtained with both mass spectrometric (MS) detector and flame ionization detector (FID) is mainly based on derivatization efficiency (absolute quantification with internal standard calibration) and intermediate precision (within-lab reproducibility) over several weeks.

RESULTS AND DISCUSSION

Table 1. Average derivatization efficiency (yield) of the four derivatization methods. Pooled standard derivation is presented in the brackets.

TMTFTH	KOH + BSTFA	ACM	NaOEt + BSTFA
96 (2) %	95 (7) %	83 (3) %	64 (2) %

Table 1 shows the results of the absolute quantification where it can be seen that the TMTFTH method has the highest derivatization efficiency and stable results.

The qualitative comparison (Table 2) of the four derivatization methods showed that the TMTFTH method also had the most advantages.

Table 2. Qualitative comparison of the four derivatization methods.

Method	Advantages	Drawbacks
TMTFTH	· Operator time 1h · One-step deriv. · No sample transfers · Easy procedure · Determination of degrad. products	· The most expensive chemicals
KOH + BSTFA	· Operator time 4h · Determination of degrad. products	· Two-step deriv. · Labor-intensive
Acid-catalyzed methylation	· One-step deriv. · Determination of degrad. products	· Operator time 7h · Labor-intensive
NaOEt + BSTFA	· Operator time 4h · Differentiating between free and bound fatty acids	· Two-step deriv. · Multiple derivatives

CONCLUSION

The results indicate that out of the examined methods the TMTFTH derivatization is the least work-intensive and the most accurate – both in terms of reproducibility and derivatization efficiency. Therefore, it is the preferred method for the determination of the absolute quantities of fatty acids in oil samples.

ACKNOWLEDGMENTS

This research was supported by the Personal Research Funding PUT1521 and the Institutional Fundings IUT20-14 and IUT20-15 from the Estonian Research Council and by the EU through the European Regional Development Fund (TK141 “Advanced materials and high-technology devices for energy recuperation systems”). We are thankful to PhD Siim Salmar for helping with the GC-MS/FID system, also to PhD Ester Oras and PhD Agnes Kütt for their advice on the practical aspects and to Prof. Klaas Jan van den Berg for the useful discussions. This work was carried out using the instrumentation at the Estonian Center of Analytical Chemistry (AKKI, www.akki.ee).

KEYWORDS

Gas chromatography, Derivatization, Quantitative analysis, m-(-trifluoromethyl)phenyltrimethyl-ammonium hydroxide, N,O-bis(trimethylsilyl)tri-fluoroacetamide

Features of Measurement Method Validation of harmful Substances Concentration in the Air of the working Area

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INTRODUCTION

Today in the Republic of Belarus, there is an acute problem of measurement methods validation of harmful substances concentration in the air of the working area in order to assessment of workplaces. Enterprises use long developed measurement procedures that lack information about performance characteristics and is contained outdated measurement equipment. The work that has been done was to develop a unified approach to the organization and implementation of validation of such measurement procedures within one laboratory, followed by application of the obtained data for quality control and measurement uncertainty evaluation.

MEASUREMENT METHOD

Spectrophotometric measurement methods were studied. Samples were taken through an aspirator to the filter or into the absorption solution. The substance concentration was determined by the calibration curve with recalculation to the air sample volume that has been taken and reduced to a temperature of 20 ºC and an atmospheric pressure of 101.3 kPa.

VALIDATION DESIGN

The test sample is air. Such matrix is not possible to model. Therefore, the experimental data were obtained on the real air samples with different substance concentrations in interest that has been taken (to establish detection limits and precisions). Simultaneous sampling under similar conditions was carried out by two operators for establishing precision in intermediate conditions. Each operator took at least 10 samples on each concentration level in interest. Model samples with a known concentration of the analyte were also prepared (by spiked pure substance to a clean filter or absorption solution) in order to study the laboratory bias of the analytical stage of the measurement procedure. The number of prepared model samples was not less than 10 for each concentration level in interest. Samples were taken or prepared at least on three concentration levels from the measurement range of the procedure including the value of the maximum permissible concentration to establish the dependence of accuracy parameters on the measured value level. The data treatment and the evaluation of the performance characteristics of measurement procedure was carried out in accordance to EURACHEM Guide[1] and ISO 5725[2].

RESULTS AND DISCUSSION

Based on validation data of various measurement methods of harmful substances concentration in the air of the working area, it can be noted:

- the intermediate precision standard deviation (arithmetic means of two parallels measurements, obtained by two operators) in the whole investigated range of measurement procedures is numerically comparable with the repeatability standard deviation (results obtained on two parallels by one operator);
- analytical laboratory bias for the whole range of concentrations of measurement procedures is insignificant relative to the precision;
- sampling, calibration, and precision are the main sources of uncertainty.

CONCLUSION

The proposed validation design has applied on the enterprises of the Republic of Belarus and, in the opinion of the authors, it is optimal for the purposes of intralaboratory validation of measurement methods of harmful substances concentration in the air of the working area.

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1. EURACHEM Guide "*The Fitness for Purpose of Analytical Methods. A Laboratory Guide to Method Validation and Related Topics*", Second edition, 2014
2. ISO 5725:1994 *Accuracy (trueness and precision) of measurement methods and results* (Parts 1-3)

ACKNOWLEDGMENTS

The authors acknowledge to the enterprises of the Republic of Belarus for their cooperation and provision of experimental data.

KEYWORDS

Method validation,
harmful Substances Concentration, occupational Safety and Health, Air of the working Area

Risk analysis during method validation

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INTRODUCTION

During the transition towards compliance with the new version of ISO / IEC 17025, laboratories need to perform the risk analysis throughout the entire test process. This paper presents the methodology applied for risk analysis in the validation or confirmation stage of test methods to verify the adequacy and improve the available control actions (portfolio) defined into the laboratory.

EXPERIMENTAL METHODS

The tool requires, in the first instance, the identification of the process stages to be evaluated (selection or design of the method, method validation/confirmation and use of the method in routine analysis). In a second stage, requirements to be met must be identified, those related with ISO / IEC 17025: 2017 standard, analytical, legal and customer requirements and those related with the laboratory management system. Risks related with non-fulfillment of these requirements, that could affect the quality in the result or service are identified. Based on the portfolio of actions, the probability and magnitude of the risks is evaluated. The risk significance is based on the parametric of the following table:

Usually					
Probable					
Possible					
Unlikely					
Rare					
Probability / Consequencce	Insignificant	Minor	Moderate	Significant	Mayor

Significance	Actions to avoid / minimize risk
Extreme risk	Monitor and take immediate action to change current controls / actions
High risk	Monitor and change controls / current actions
Moderate risk	Monitor and evaluate if change of controls / current actions is necessary
Low risk	Control measures / current actions are sufficient

Based on the expected test quality result, the portfolio of action stablished into the laboratory and the risk level to be assumed, action plans are defined for monitoring and improvement.

RESULTS AND DISCUSSION

Identification of stages: although the tool is applicable in all stages for the analysis process, for this paper we consider exclusively the method validation requirements.

Identification of requirements: some of the validation stage requirements include documentation, personnel competence, statistical criteria / parameters / sources of
influence, traceability of measurements, equipment / supplies / conditions / information, among others.

Identification of risks: some of the risks identified in the validation stage are, refer to a method not documented /
updated / adequate, studies influenced by the results obtained, incomplete data or statistical significance not adequate / representative of the scope of the method in range , matrices and analytes, not considering all sources of influence to assess the need for control, interferences not identified in routine matrices, unknowledge of the analytical-client-legal and possible uses of the test result requirements, not clear responsibilities, use of not fit for purpose equipment, personnel not competent in the method and use of the result, environmental conditions not defined / under control.

Portfolio of actions: some of the actions to be taken to minimize the risks can be: have an updated database of methods and regulations applicable to client requests. Agree with the client in the contract review an evaluation of uncertainty and applicable regulations. Have competent personnel in the specific area. Have a validation plan that includes studies to be carried out and criteria to assure objective evaluations. Have an updated database of reference material and proficiency test providers that includes an assessment based on compliance with technical requirements and applicable reference standards. Control of uncertainty sources considering the validation data. Database that allows an overall controls evaluation (including at least, equipment controls, internal and proficiency testing quality controls, use of reference materials) to evaluate and prevent that changes in the quality and/or frequency of controls do not affect the confidence level of the result.

Evaluation of significance: the significance levels varies between laboratories, depending on the result and quality expected service. The evaluation of risks significance depends on the test type, the impact of the test result (health, environment, etc.) and risk frequency. The risks can be evaluated from low to extreme significance.

Decision and action plan: each laboratory define the risk levels to be assumed and managed, as well as the controls to be taken to reduce the frequency of occurrence and / or minimize the consequences thereof. Actions can vary from monitoring, changes in sample flow, improvement of staff competence, new control strategies, etc.

REFERENCES

ISO/IEC 17025:2017 General requirements for the competence of testing and calibration laboratories
ISO 31000:2018 Risk management – Guidelines

KEYWORDS

Risk, validation, uncertainty, ISO/IEC 17025, controls

Looking for variability sources in the measurement of grain Quality

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INTRODUCTION

This paper presents a study of the variability source related with tests to define de physic quality of grains. The main concern in this regard is the high data dispersion obtained in defects grains tests that impact mainly on the validation parameters accuracy and uncertainty of measurement. This makes it difficult and adds complexity to the product evaluation considering the actual quality product specifications recommended internationally and / or used for trade. We select two types of measurements: fermented grains in soybeans and damaged and broken grains in milled rice. Soybeans and rice samples are selected due to: the importance in world consumption of these products, they are national production crops, they are grains of different sizes (medium and small respectively) and finally they have different end use (for human consumption and as raw material for oil or proteins extraction). The different sources of error related with the measurements are analyzed, especially the errors due to the assumptions of statistical behavior of the data used for the estimation of the validation parameters, the random and systematic errors of the method, the error due stability and sampling.

EXPERIMENTAL METHODS

Measurement Methods

The analytical method procedure is segregating the defective grains from the test sample, weighing these defective grains and expressing the test results as a percentage of the original sample. Defects are defined as follows:

-Broken grains in rice (BK): Visual identification in function of their size and, in case of doubt, measure with caliber.

-Damaged (Stained) grains in rice (ST): Visual identification of stained grains.

-Fermented grains in soy (FR): Visual identification of fermented grains and, in case of doubt, cut the grain crosswise with a scalpel to identify the defect in the cotyledon.

Tests comply with ISO/IEC 17025:2017.

Statistical data processing

Exploratory and inferential statistical studies are done to identify data distribution and outliers. Data analysis of accuracy studies are made by appropriate statistical tests.

Accuracy and uncertainty studies

Accuracy is evaluated in term of precision and trueness of each of the methods through appropriate proficiency test studies and by expert comparisons. Performance studies are conducted in more than 15 laboratories in 11 rounds. Precision is evaluated as Relative Standard Deviation (RSD) under conditions of repeatability (RSD_{rep}), intermediate precision (RSD_{ip}) and reproducibility (RSD_R) based on the guidelines of ISO 5725-2:1994. Proficiency tests comply with ISO/IEC 17043:2010. Three independent uncertainty estimation methods are used in order to evaluate the dependence of these results with the mathematical assumptions assumed by each one: Monte Carlo Method, from validation data and by individual uncertainty sources.

Robustness studies

Robustness studies are carried out related to the size of the test sample and the stability of the measurand. Tests are carried out in different size samples and different days.

RESULTS AND DISCUSSION

Behavior data (Table 1) is compatible with a normal distribution. Precision data (Table 2) show high variability of the stained and fermented grain tests being smaller for the broken grain tests. This high variability seems to be characteristic of the method, probably because defects arise by chemical or biological reactions and the classification criterion is visual observation. Variability of the broken grain tests is lower, probably because this defect is associated with the industrial process and the classification criterion is related with instrument measurements. Robustness sample size studies suggest no significant differences related. Control samples for Stained and fermented grain defects are stable for six months and control samples for broken grain defect is stable for a year when rice is preserved with its husk. Uncertainty estimation demonstrates no significant differences between different approaches.

Table 1. Statistical data processing

Statistic/Defect	BR	ST	FR
Kurtosis	0.2	-1.1	0.3
Asymmetry	-0.1	0.5	0.5
Shapiro-Wilk	p>0.05	p>0.05	p>0.05
Outliers_Grubbs	no	no	no

Table 2. Precision data

Defect	RSD (%)
BK	RSD_{rep} (7.7) ≈ RSD_{ip} (9.4) < RSD_R (20)
ST	RSD_{rep} (31) < RSD_{ip} (47) ≈ RSD_R (48)
FR	RSD_{rep} (23) ≈ RSD_{ip} (29) < RSD_R (64)

CONCLUSION

Tests involved in this paper show high variability due to nature of the measurand. Proficiency testing studies seems to be an essential tool for validation purpose, improving the comparability of different laboratories and compatible with the stability of the test samples.

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2. Instrucao Normativa 11/2007 del MAPA de Brasil.

KEYWORDS (5 keywords)
Grains, defects, broken, damaged, fermented

Validation of Gas-Phase Acidity Measurements of Superacids

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INTRODUCTION

Gas-phase acidity (GA) of an acid HA is the Gibbs' free energy change on deprotonation of the acid, according to the following equilibrium:

$$HA \overset{GA}{\rightleftharpoons} A^- + H^+$$

GA values are independent of solvent effects and represent the intrinsic acidity of the compound. They are useful for describing both the acid in question as well as the effect solvents have on the acidities.

Acids that are more acidic than sulfuric acid in the same medium are superacids.[1] They are used e.g. as reactants and catalysts in organic synthesis and industrial processes.[1] The conjugate bases of superacids have high thermal and chemical stability, which makes them very useful for energy storing devices and ionic liquids.[2]

It is increasingly evident that the reported experimental GA values of several superacids differ significantly from the corresponding computational values.[3] There is thus necessity to validate or revise the previously reported GA values.

EXPERIMENTAL METHODS

GA measurement results are almost always carried out as relative measurements and presented as a ladder (Fig 1). The relative acidity (ΔGA) of any two acids can be obtained by combining different sets of ΔGA values. Comparing ΔGA values found by different "paths" is the key to validating the results.

An FT-ICR mass spectrometer with a 7T super-conducting magnet was used. The basic principle of the measurement method is described in detail by Leito *et al*.[3] Two gaseous acids were leaked into the ICR cell region and their anions were generated by low energy electron impact. The formed ions were trapped in the ICR cell and allowed to react with the neutrals in the gas-phase until equilibrium was reached. Partial pressures of the compounds (estimated from mass spectra), denoted below as $p(A_1H)$ and $p(A_2H)$, as well as the equilibrium-state intensities of the anions, $I(A_1^-)$ and $I(A_2^-)$, were used to calculate the equilibrium constant value (K) and ΔGA of the proton transfer reaction between acids A_1H and A_2H, using the following equations:

$$A_1H + A_2^- \overset{K}{\rightleftharpoons} A_1^- + A_2H$$

$$K = \frac{I(A_1^-)\, p(A_2H)}{I(A_2^-)\, p(A_1H)} \qquad \Delta GA = -RT \ln K$$

RESULTS AND DISCUSSION

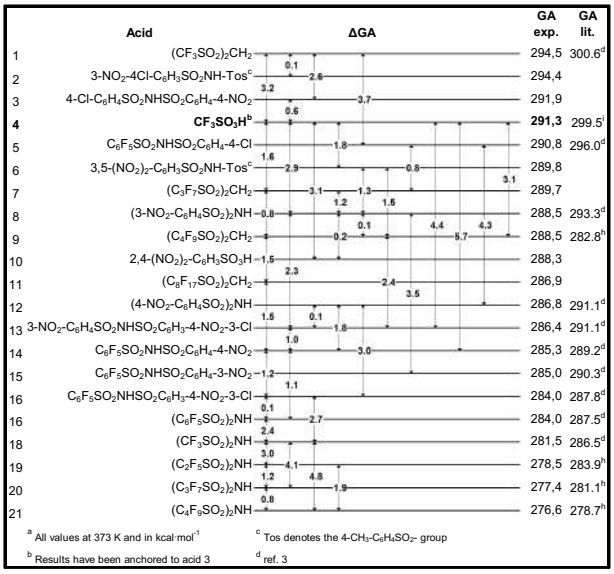

Figure 1: Self-consistent gas-phase acidity scale compiled in this work. Every arrow denotes one ΔGA measurement series.

The consistency standard deviation of the scale is 0.3 kcal mol^{-1}, indicating good consistency.

CONCLUSION

The gas-phase acidities measured in this work are in good agreement with each other and also with the reported computational values.

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ACKNOWLEDGMENTS

IUT20-14 from the Estonian Research Council, TK141 form the European Regional Development Fund.

KEYWORDS
Gas-phase acidity, superacid, FT-ICR-MS

Standard substance free quantification of LC/ESI/MS on the example of pesticides in cereal

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INTRODUCTION

LC/HRMS is increasingly applied for the analysis of environmental samples, especially in non-targeted analysis. As LC/HRMS can detect thousands of molecular features in a single run and the recent rapid developments in the identification of these molecular features scientists have a good overview of what can be detected in the environment. Up to now for the quantitative information standard substances are needed. For all the detected molecular features, unfortunately, standard substances are not available or affordable, especially, if we consider hundreds or even thousands of different molecular features in one sample. To overcome this issue, we propose a quantitation approach based on in silico predicted electrospray ionization efficiencies.

EXPERIMENTAL METHODS

Oat, barley, wheat, rye, rice and maize were spiked with 152 pesticides. A generic QuEChERS method was applied for sample pretreatment. The eluent consisted of 0.1% formic acid (A) and acetonitrile(B). A 7-minute linear gradient from 5% to 100% B was used. Two different mass spectrometric setups (Agilent 6495 triple quadrupole and Bruker Daltonics micro-TOFq) in two different laboratories were used to test the robustness of the method. Ionization efficiency values were predicted using a model based on random forest algorithm which was based on 2500 different ionization values of analyte (400 unique compounds) eluent combinations. A set of 10 compounds was used to calculate instrument specific
ionization efficiency values.

RESULTS AND DISCUSSION

Each pesticide was studied on 10 different concentration levels from 4 nM to 350 µM. The average accuracy of pesticide concentration in cereal matrix was 5.5 times. The performance of ionization efficiency prediction with the aim of quantitation of pesticides in the cereal matrices is presented on Figure 1. Comparing the different concentration levels, the accuracy was consistent except the lowest concentration level, where it was slightly lower (7.7 times). For single compound, the highest accuracy of concentration was 1.2 times and the lowest 140 times. For 29 % of compounds, the accuracy was better than 2 times, for 78 % of pesticides the accuracy was better than 5 times and for 89 % of studied pesticides the accuracy was better than 10 times.

Comparing all the studied cereal matrices wheat had slightly better performance (4.4 times off on average) and barley and oats performed slightly worse (6.6 and 7.4 times off respectively) than rye, rice and maize which had on average misprediction of 5.5 times.

The 3 best-performing pesticides were tetraconazole (19 %), myclobutanil (20 % off) and fenamiphos-sulfoxide (26 %). It is seen, that model is universal for nitrogen as well as for oxygen bases. Additionally, compounds (propiconazole) present in training set perform better.

The worst performers were carbosulfan (51 times off), pirimicarb (16 times off) and fluometuron (15 times off). Carbosulfan has moiety N-S-N which is not present in the training set (400 compounds) of the model.

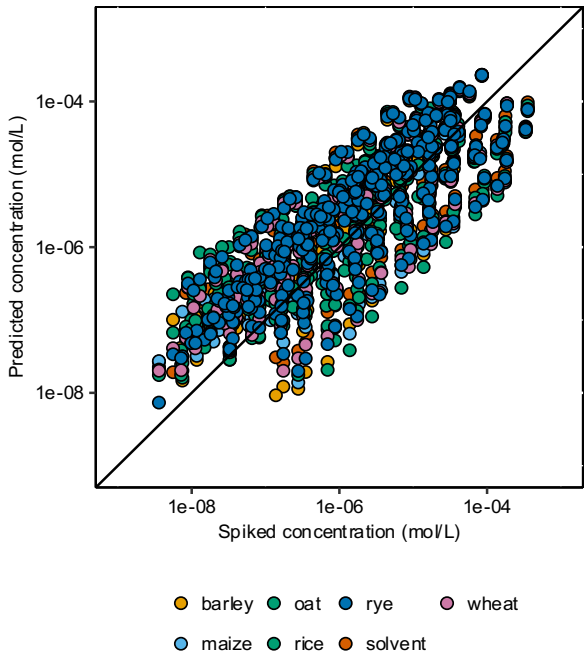

Figure 3 Performance of ionization efficiency prediction in example of pesticides in cereal

CONCLUSION

Quantitation of pesticides in cereal with LC/ESI/MS without authentic standards is feasible with the accuracy of 5 times off on average.

ACKNOWLEDGMENTS

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KEYWORDS (5 keywords)

Cereal, standard substance free quantification, food sample, pesticides, mycotoxins

Comparing Nontargeted LC-MS Methods by Co-visualizing Linear Dynamic Range and Chemical Coverage

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INTRODUCTION

Biological and environmental samples contain thousands of small molecule species that all vary in chemical properties and concentration range. Identifying and quantifying all these chemical entities remains a long-term goal in metabolomics and related systems approaches. Due to its broad selectivity, nontargeted LC-MS is usually the method of choice for broad chemical screening. Optimizing nontargeted LC-MS methods, however, is less straightforward than for targeted methods where sensitivity, specificity, linearity etc. serve as well-established performance criteria. We therefore investigated linear dynamic range (LDR) and chemical classification as alternative performance criteria to guide nontargeted method development.

EXPERIMENTAL METHODS

LDR was defined as the linear portion of a feature's response curve over multiple concentration levels[1]. Comparing the LDR of features across methods can be expected to be significantly more robust than comparing signal intensities for a single concentration. To determine LDR for all features, a computational workflow was implemented in the R programming language. For estimating the linear portion of a curve, several mathematical approaches including linear, non-linear and piecewise linear regression were evaluated. Chemical classification was based on *ClassyFire*, which computes chemical classes for a given structure. To avoid false classifications for incorrectly annotated compounds, we took the following statistical approach. For each compound, multiple likely annotation hypotheses were derived using a recently described workflow[2]. All annotation hypotheses were submitted to *ClassyFire* and obtained classifications were ranked by frequency. The most frequently suggested class was kept for further analysis. Finally, LDR and chemical classes were visualized together on a molecular network, which was constructed using the well-established MS/MS similarity approach.

RESULTS AND DISCUSSION

For technical validation of the workflow, several hundred curve fits obtained from the different regression models were reviewed visually. Piecewise linear regression performed the most reliably with respect to the heterogeneous curve shapes of 'real-life' features. Validation of chemical classification was performed against a compound library, which showed that 90% of ~450 library compounds were correctly classified using the described approach. Two liquid chromatography methods (HILIC, RPC) as well as two electrospray ionization variants (low/high-temperature ESI) applied to urinary metabolomics were exemplarily studied to test the workflow. Molecular network visualization indicated that of all analytical setups, HILIC/high temperature ESI performed best in terms of high LDR achieved over a wide range of compound classes. Despite one order of magnitude lower sensitivity, HILIC/low temperature ESI showed similar chemical coverage, except for organic nitrogen compounds that were underrepresented compared to high-temperature ESI. Both RPC setups were inferior to the HILIC setups in terms of high-LDR features, supporting previous findings for the given matrix. The higher relative representation of benzenoids and lipids in RPC demonstrated that the workflow successfully captured expected selectivity differences between chromatographies.

CONCLUSION

When comparing nontargeted LC-MS methods for optimization purposes, ideally all available quantitative and qualitative information should be integrated. The present workflow follows this idea. Visualizing LDR and chemical classes of all features on a molecular network quickly indicated differences in method selectivity that were otherwise difficult to spot. As an automated approach, it is easily applied to repeated optimization steps, enabling effective optimization strategies.

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KEYWORDS

liquid chromatography-mass spectrometry; nontargeted approach; method development; chemical coverage; linear dynamic range

P-15

Non-targeted chemical analysis: Total Organic Carbon (TOC) analysis as a powerful diagnostic tool in safeguarding public health and in environmental monitoring

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Instrumental analysis has always been at the forefront of environmental and forensic-type investigations providing valuable data for decision purposes. In addition, this is also used in safeguarding public health and enables efficient monitoring of critical process parameters. Although test results normally relate to specific parameters that can be interpreted on an individual basis, it seldomly happens that scientists investigating emergency situations or even routine activities need to have access to more generic parameters. This introduces the concept of non-targeted chemical analysis in the sense that the analyte's concentration does not refer to a specific element and/or substance. Total Organic Carbon (TOC) is attracting increasing attention in a wide range of industrial and Health and Safety applications. These relate to process optimisation, water treatment, monitoring of drinking water supplies, European pharmacopoeia water quality standards in the health care sector and environmental baseline investigations. Our laboratory has been extensively involved in projects where TOC is used as a diagnostic tool in decision making regarding regulatory compliance and/or process optimisation. Specifically, TOC is used to appraise and monitor drinking and haemodialysis water quality, process efficiency in wastewater treatment plants (WWTP), pollution levels in coastal waters and the magnitude of organic load in dams and surface waters. Valuable data relating to above applications that have been accumulated during the past decade will be presented and discussed together with appropriate fitness-for-purpose justification.

The Statistical Evaluation and Analysis of Proficiency Test Data on Plant Protection Products

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INTRODUCTION

In 2017, the first Proficiency Test (PT) was organized among laboratories all over Europe on plant protection products available on the Italian market. The aim of the trial was to find out the quantity of active ingredient on the different formulation of the plant protection products. Nine Italian laboratories and fifteen ones from the rest of European Union, who routinely deal with pesticides, were invited to participate. Laboratories are not obligated to take part in the PT; all the European laboratories and seven from Italy sent their results. All laboratories obtained data with acceptable values of z-score within the limits, except for two of them who got higher than 3.5 z-score values for the active substance Cymoxanyl. All the laboratories enjoyed taking part at this trial so another one is planned for the 2019.

EXPERIMENTAL METHODS

The statistical evaluation of the results was performed applying the Jarque-Bera test for the verification of the hypothesis of normality. To use the Jarque-bera test is need to calculate asymmetry and curtosi check. These data are used to verify the acceptability in $\chi 2$ distribution at 95th percentage with the Jarque-Bera formula:

[(GdL^(asymmetry;2)/6)+(GdL1)^(curtosi;2)/(GdL+1)]

After this verification, the z-score values was calculated for each participant in each sample with the following formula: Zi = 0,6745 * (Xi - Median)/MAD.

RESULTS AND DISCUSSION

Based on the statistical evaluation, for Cymoxanil every laboratories gave acceptable z-score values within the range of -1.96 and + 1.96 and only one laboratory gave a questionable value within -3.5 and -1.96.

For Methomyl, all the laboratories gave acceptable z-score values within the range of -1.96 and + 1.96 and only two laboratories gave unacceptable values under -3.5 and over +3.5.

For Oxamyl, all the laboratories gave acceptable z-score values within the range of -1.96 and + 1.96.

CONCLUSION

The outcome of the ITPT2018 can be considered satisfactory due to the first PT organized by Italy.

The participation of the Italian and European laboratories was good. For Italy, nine laboratories were distributed as three in the north, four in the central and two in the south. The European laboratories were fifteen, excluding Italy, distributed as one in the south, ten in the centre and four in the north.

The performance of the laboratories expressed in terms of modified z-score was satisfactory by almost all participants for all substances. Only for Methomyl two laboratories obtained outlier data and these laboratories should analyse the reason of their results.

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KEYWORDS (5 keywords)

Proficiency test, Plant protection product, z-score, normality, performance

Non-Targeted Analysis Based on Objective Mosaic Scoring Framework

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INTRODUCTION

Analysis of complex histopathological samples is an extremely difficult task. The sample matrices in most cases are complex, therefore traditionally tracing analytical/microscopic methods have been specifically developed for a certain type of sample and group of cells. The traditional *targeted* approach provides good sensitivity and possible reliable identification and quantification of the target cells. Nevertheless, such approach presents an important drawback because in most of cases it misses not selected compounds, which could be untargeted substances/cells/tissues even in high concentrations or with high clinical significance. Moreover, in many cases the presence of the target compounds/cells/tissues are not high enough to support the toxic effects of samples. As consequence, *non-target* screening methods became important tools for clinical chemistry. The microscopic techniques present the advantage of detecting specific sample's characteristics ranging from structural features and morphological differentiations to cell types. However, as any other analytical investigation tool, the microscopy methods are coming with some disadvantages, namely: the high subjectivity related to the experimental data reporting and the necessity to apply to highly qualified and trained researchers. During last years, a lot of efforts have been directed towards development of software programs able to address the mentioned issues. In the presented work is introduced a new methodology for evaluating the bright filed microscopy image mosaics based on an algorithm which allow the objective mosaic scoring framework.

EXPERIMENTAL METHODS

The hundreds of images (over 200) have been acquired in bright filed by help of a microscope LEICA DM 3000 LED with MC 190 HD camera. The mosaics were generated using image tiles of 3648x2736 pixels, and the overlapping degree of adjacent tiles was 25-35%. The studied samples consisted from a tissue fragment from a patient diagnosed with moderately differentiated invasive cancer. The tumor grade was evaluated applying Nottingham scoring system. The samples have been fixed in buffered formalin and then processed through histopathological methods using paraffin embedding, with a 3 µm section and hematoxylin-eosin staining. From the common applied full reference quality algorithms there have been applied 13 FR-IQA have been used for a hierarchy. For the development of quality assessment algorithm mosaics methodology implies the generation of a set of image mosaics with a controlled hierarchy. The quality mosaic framework assessment algorithms is considered based on the followings: tile-mosaic, tile-tile, tile-degradation. The chosen parameters for tile degradation were: exposure, duration and gamma.

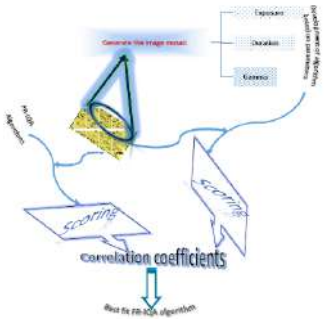

Figure 1. Experimental overview.

RESULTS AND DISCUSSION

The score of mosaics as was considered for the developed algorithm is equal with the sum of the assigned scores for the studied areas. The calculations have been performed using N_i' vectors specific for each subset. The applicability was verified through comparison with most used FR-IQA algorithms. The results recorded showed a correlation coefficient of 0.9979 related to the MS-SSIM which is considered the gold standard in assigning the correct image evaluations.

Figure 2. Mosaic simulation.

CONCLUSION

Over the past decades new analytical tools with its corresponding software have been developed, transforming the non-target screening approach more realistic nowadays. The developed methodology could be successfully applied to assign reference quality scores to histopathology slide image mosaics thus allowing a reliable image correlation metrics.

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KEYWORDS *non-target microscopy analysis; image mosaics; image stitching; objective scoring system; image quality*

Photodegradation Study of Irinotecan and Identification of Transformation Products in Water Samples by UHPLC-MS/MS

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Irinotecan (CPT-11) is a water-soluble anticancer drug widely used to treat several types of cancer such as colon, small lung, ovarian, brain, gastric, cervical and pancreatic cancers. Nevertheless, not all administered CPT-11 drug is metabolized, but an amount around 45-63% is excreted as parent drug by the human body and enter the sewerage system ultimately reaching ground and surface waters. Even if the metabolites of CPT-11 are well-known and investigated, very limited information is present in the literature about the formation of photodegradation products that can naturally originate from sunlight irradiation when the drug is released in aqueous systems.

In the present study, CTP-11 solutions at 10.0 mg L^{-1} were irradiated by simulated sunlight through a solar box (Co.fo.me.gra 3000e, Milan, Italy) utilizing Xe lamp at 600 W m^{-2} and temperature of 35 °C. Exactly 28 mL solutions of the drug were placed in cylindrical quartz cuvettes and they were irradiated in the solar box for a maximum of 13 days. In the course of the photodegradation process, sample aliquots of about 3 mL were withdrawn after irradiation at prefixed time intervals. In order to monitor the progress of the photodegradation process, the sample aliquots were subsequently analysed by UV/Vis spectrophotometer (Jasco V-550, Milan, Italy). The intensity of CPT-11 decreased by 90% after 7.5 days of irradiation and no significant reduction of absorbance values was observed after 12 days.

A sensitive UHPLC-MS/MS method was developed employing a hybrid triple quadrupole/linear ion trap mass spectrometer (Nexera UHPLC-MS/MS, Shimadzu, Tokyo, and 3200 Qtrap, Sciex, Canada), that is able to work in data-dependent acquisition mode, in order to automatically obtain information about the unknown species formed by irradiation and to build a reaction monitoring method with the MS/MS fragmentation pattern of the species previously investigated. The method was validated obtaining for CPT-11 LOD and LOQ values of 0.02 and 0.05 ng mL^{-1}, respectively and MDL and MQL in river water of 0.03 and 0.10 ng mL^{-1}. Eight photodegradation products were identified and five of them for the first time. The total ion chromatogram (TIC) given in Fig. 1 shows the formation of the photodegradation products for the first five irradiation time points. Based on the MS and MS/MS fragmentation, the chemical structures of the degradation products are proposed. Hydrolysis experiments were also carried out on the same solutions preserved in the dark, but no formation of other species was highlighted. The method was applied to several real samples, among which river water, but neither CPT-11 nor any of its photodegradation products were found. The outcomes of this study may be useful for updating the pollutant screening in water samples.

Keywords: CPT-11; degradation; irinotecan; mass spectrometry; photodegradation products; transformation products; UHPLC-MS; water.

Advantages of ultra performance liquid chromatography –high resolution mass spectrometer for the analysis of cyanotoxins in water for human consumption

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INTRODUCTION

Cyanobacteria (also called blue-green algae) are a group of prokaryotic and photosynthetic organisms.
They are widespread in lakes, rivers, and oceans, potentially as a consequence of eutrophication and they are able to produce, in particular conditions, toxins that cause adverse effects on animal and human health[1].
In this research, 21 cyanotoxins of different classes (including 12 Microcystins, 5 Microginins, 2 Cyanopeptolins, and 2 Anabaenopeptins) were simultaneously detected in water samples for human consumption by two different mass spectrometric techniques (UPLC-HRMS/MS and LC-MS/MS) and the results were compared.

EXPERIMENTAL METHODS

Two different methods were compared in this study:

- LC-MS/MS method: a HPLC system Ultimate 3000 (Dionex Corporation, Sunnyvale, CA, USA), equipped with an Alltima C18 column (2.1 × 250 mm, ID 5 µm, Alltech, Sedriano, Italy) thermostated at 40 °C and coupled with a triple-quadrupole mass spectrometer API 3000 (Applied Biosystems, Darmstadt, Germany). This method is an update of a previous method[2] to which it has been added Cyanopeptolins, Anabeanenopeptins, and Microginins as analytes, optimizing it in terms of instrumental response and extraction efficiency.
- UPLC-HRMS/MS method: an UPLC system Acquity (Waters Corp., Milford, MA, USA) equipped with Acquity UPLC BEH C18 column (2,1 mm ID x 100 mm, 1,7 µm, Waters Corp., Milford, MA, USA) thermostated at 40 °C and interfaced with a XEVO G2S Q-TOF mass spectrometer (Waters Corp., Milford, MA, USA). 10 µL of the extracted sample were injected into the UPLC-QTOF system.
  The chromatographic gradient was (t in min): t0, A=90%; t4, A=70%; t8, A = 30%; t10, A= 0%; t12, A= 0%; t13, A=90%; t16, A = 90%.
  The experiments were acquired in polarity ES+, Sensitivity Mode with a source temperature of 130°C, a desolvation temperature of 500 °C and a desolvation gas flow of 1000.0 l/Hr. Two different acquisition mode were employed: MS mode with a full scan 50-1200 without collision energy to observe the molecular ion signal as MH^+ and a MS/MS mode to observe the fragmentation signals applying specific collision energies.

Both the chromatographic gradients were employed using water 10 mM formic acid (A) and acetonitrile 10 mM formic acid (B) as mobile phases.

RESULTS AND DISCUSSION

The LC-MS/MS method was robust with a reproducibility better than 17% and LODs were in the range of 0.003– 0.032 µg/L for all the analytes,
Good linearity was achieved, with correlation coefficients in the range $0.9925 \leq R^2 \leq 0.9998$.
The UPLC-QTOF method has proven to be robust, precise and accurate with recovery percentages above 85%, with relative standard deviations ≤16% and LODs between 0.001 and 0.047 µg/l for the intended purposes at the concentrations of interest.
A calibration curve was obtained analyzing water samples spiked at four different concentration levels (0.1, 0.5, 1.0 and 2.0 µg/l) and good linearity was achieved, with correlation coefficients in the range $0.9902 \leq R^2 \leq 0.9999$.

CONCLUSION

A good resolution has been obtained with both methods.
The advantages obtained by UPLC-HRMS/MS method are the shorter analysis times (16 minutes vs 27 minutes) and a lower injection volume (10 µl vs 50 µl).
Furthermore, this method allows the simultaneous identification of target and non-target compounds, allowing to detect the presence of other compounds potentially harmful to human health.

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KEYWORDS (5 keywords)

Eg. Cyanotoxins, drinking water, Q-TOF, microcystin, water sample

Quantification of beta-lactam antibiotics in human plasma by HPLC-MS method. A validation study.

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β-lactam antibiotics are the cornerstone of antibacterial treatment and frequently prescribed drugs, especially in the intensive care units (ICU) of hospitals. Contemporary β-lactam antibiotic dosing is debatable in severely ill patients, since the occurrence of pathophysiological changes in critical illness can result in great inter-individual variability. Therapeutic drug monitoring (TDM) is a commonly used dosing strategy to optimize exposure and thereby minimize toxicity and maximize the efficacy. Currently, TDM of β-lactam antibiotics is rarely performed, due to poor availability in clinical practice. We describe here simple and rapid HPLC–MS method for the determination of ampicillin, amoxicillin, cefepime, ceftazidime, imipenem, meropenem, cilastatin and piperacillin in human plasma. This method involves simple sample preparation step and was comprehensively validated according to EMA guidelines [1]. For all analytes, mean accuracy and precision values were within the acceptance value. The lower and upper limits of quantification were found to be sufficient to cover the therapeutic range for all antibiotics. Finally, in collaboration with the North Estonia Medical Centre the feasibility of the analytical procedure was demonstrated in routine clinical practice. This simple, sensitive and rapid assay requires small-volume samples and can easily be implemented in clinical laboratories to promote the TDM of β-lactam antibiotics.

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Methodological Framework Design to sustain integration of non-target screening into French national water monitoring programs

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INTRODUCTION:

Non-target analysis for the identification of contaminants of emerging concern or transformation products in the environment are increasingly popular and of great interest in terms of research areas as well as for improvement of regulatory monitoring, such as WFD. AQUAREF, the French Reference Laboratory for Water and Aquatic Environment has defined and implemented a technical program of actions in order to identify and propose some solutions to the main technical and operational locks.

DISCUSSION:

Actions are focused on suspect screening data treatments

The objectives of these actions aiming operational and regulatory applications are:

- Harmonization of practices and establishment of best practive/guidance
- Transfer and Teaching activities to end-users (eg accredited laboratories) and stakeholders (water agencies, environmental agencies)

For that, technical actions (state of the art, methodological comparative studies and also interlaboratory comparison,) have been undertaken since 5 years.

In parallel, an important demonstration exercise has been established in the frame of the National Prospective Monitoring Network in order to:

- Demonstrate on large scale campaigns the new opportunities and better level of information of NTS
- Create a digital samples database of water samples for retrospective investigations

The proposal will present on overview of these different actions and the first results of demonstration exercise.

ACKNOWLEDGMENTS

The proposal will present French works that are currently performed by AQUAREF consortium (French Reference Laboratory for Water and Aquatic Environment) in the frame of the National Prospective Monitoring Network and funded by French Agency for Biodiversity.

KEYWORDS

Water samples, suspect screening, monitoring survey