NORMAN 2020 JOINT PROGRAMME OF ACTIVITIES (JPA 2020)

INTERLABORATORY COMPARISON ON STRATEGIES FOR SEMI-QUANTITATIVE NON-TARGETED LC-ESI-HRMS

HELLENIC REPUBLIC
National and Kapodistrian University of Athens

LC-ESI-HRMS RESPONSE

The difficulty in quantifying compounds in LC-ESI-HRMS arises from vastly different responsiveness of the compounds. At the same concentration, two compounds may yield very different signals due to the differences in the ionization efficiency of the compounds. Generally, it is known that the response of the compound depends on the hydrophobicity of the compound, acid-base properties, hydrogen bonding, etc.[1] However, the quantification is complicated as even structural isomers may have response factors that differ by orders of magnitude.[1,2] The response factor also depends on the mobile phase used in the LC separation.[3] Generally, the acidic mobile phase provides higher response factors for analysis in positive ionization mode;[4] however, all compounds are not affected in the same magnitude due to the differences in acid-base properties. Higher organic modifier content results in higher response factors;[4] therefore, the gradient program and chromatographic separation are highly important factors influencing the response factor of the compounds. All these factors make quantification without the analytical standards challenging. At the same time, quantification is essential to understand the significance of the detected compounds and to communicate the significance of the results in a clear manner to the stakeholders.

In order to obtain quantitatively meaningful results without analytical standards, different strategies have been developed. These include using peak areas directly or in combination with statistical data treatment, isotope dilution, radiolabelling, using structurally similar compounds for quantitation, and quantitation based on the predicted ionization efficiencies. Though isotope dilution and radiolabelling are very accurate and well applicable in other non-targeted screening applications, these methods are not applicable in the context of environmental screening. Therefore, the currently accessible approaches focus on (1) applying structurally similar compounds for the quantification and (2) predicting the response of the compounds in LC-HRMS. These strategies together with sub-strategies will also be incorporated in this interlaboratory comparison.

SCOPE OF THE STUDY

The interlaboratory comparison focusses on comparing the semi-quantification methods applicable in environmental screening.

SAMPLES

Water samples, tap water and surface water fortified with 37 compounds will be distributed. The list of the compounds present in the samples will be sent to the participants together with the samples. Additionally, we will send a standard mix of 30 compounds which can be used as analytical standards in all methods and a mixture of 3 isotopically labelled internal standards.

SEMI-QUANTIFICATION METHODS

The methods included in this interlaboratory comparison are described below. For all methods, a calculation platform will be provided.

LC-HRMS CONDITIONS

Labs are encouraged to use the conditions that are normally used for non-targeted screening in their laboratories. The information about the methods will be collected together with the results.

TIME PLAN

31. AUG 2020	feedback to the current guide
30. OCT 2020	end of registration for the interlaboratory comparison
31. NOV 2020	samples are sent to all participants
NOV 2020	web training on the semi-quantification methods
NOV 2020	progress update on the General Assembly
31. JAN 2021	labs submit the results of the analyses
SPRING 2021	results of the interlaboratory comparison are analysed
SUMMER 2021	first draft of the paper on the results is available for comment

TAP AND SURFACE WATER SAMPLES WITH 37 CONTAMINANTS

DATA TREATMENT

COMPONENTIZATION

The peak areas returned by different data treatment software may have a different meaning. Sometimes the area corresponds only to the mono-isotopic peak of the parent ion, sometimes the whole isotope pattern of the parent ion, and sometimes all peaks (isotope peaks, fragments, adducts) are summed up. These differences need to be taken into account by the semi-quantification strategy. For example, the predicted response factors (see page 8) correspond to the protonated or deprotonated species of the compounds and incorporate all isotope peaks. In this interlaboratory comparison, we do not focus on the differences caused by the software; therefore, all laboratories are suggested to use the peak area of the most abundant monoisotopic peak for both protonated species and observed in-source fragments. Labs are also suggested to report the *m/z* used for integration.

SIGNAL RANGE

It is very important to assure that the signal of the compound is in the linear ranges as all of the quantification methods assume a linear relationship between the signal and the concentration of the contaminant. Ideally, this assumption should be validated by measuring the sample on several dilution factors and comparing the predicted concentrations. If measurements are performed in the linear range and no ionization suppression occurs the results of the two dilutions should match. However, if the dilutions do not agree, the results from the more diluted sample are usually more accurate as it is more likely to be in the dynamic range and also ionization suppression is reduced with the dilution. Therefore, we suggest running the samples at least two dilutions (e.g. undiluted and a 10 fold dilution) and submitting the results for both dilutions.

CHALLENGES DATA TREATMENT

SAMPLES & DATA

DATA TREATMENT

PROVIDED TO THE PARTICIPANTS'

We provide to the participants the following standards and samples:
1. Water samples with different complexity. Samples contain 37 contaminants over a wide concentration range. The list of the contaminants is provided together with the samples. Additionally, 30 compounds with known concentrations have been spiked into this sample that can be used as calibrants in the semi-quantification approaches.
2. An internal standard mix with 3 isotopically labelled compounds.
We also provide access to computational resources required for testing the semi-quantification strategies.

THE PARTICIPANTS SUBMIT

The participants are expected to submit:
1. Instrumental parameters used. This includes LC parameters (mobile phase, column, flow rate, gradient program) and mass spectrometry parameters (ion source parameters, mass range, etc.).
2. Integrated results (peak area, retention times, and corresponding *m/z* values) for all of the detected compounds for both samples and standards for each sample and standard run.
3. The calculated concentration for each of the samples and information about any manipulations done to the sample, including dilutions
4. Raw chromatogram files of the data files that will be uploaded through the provided link.

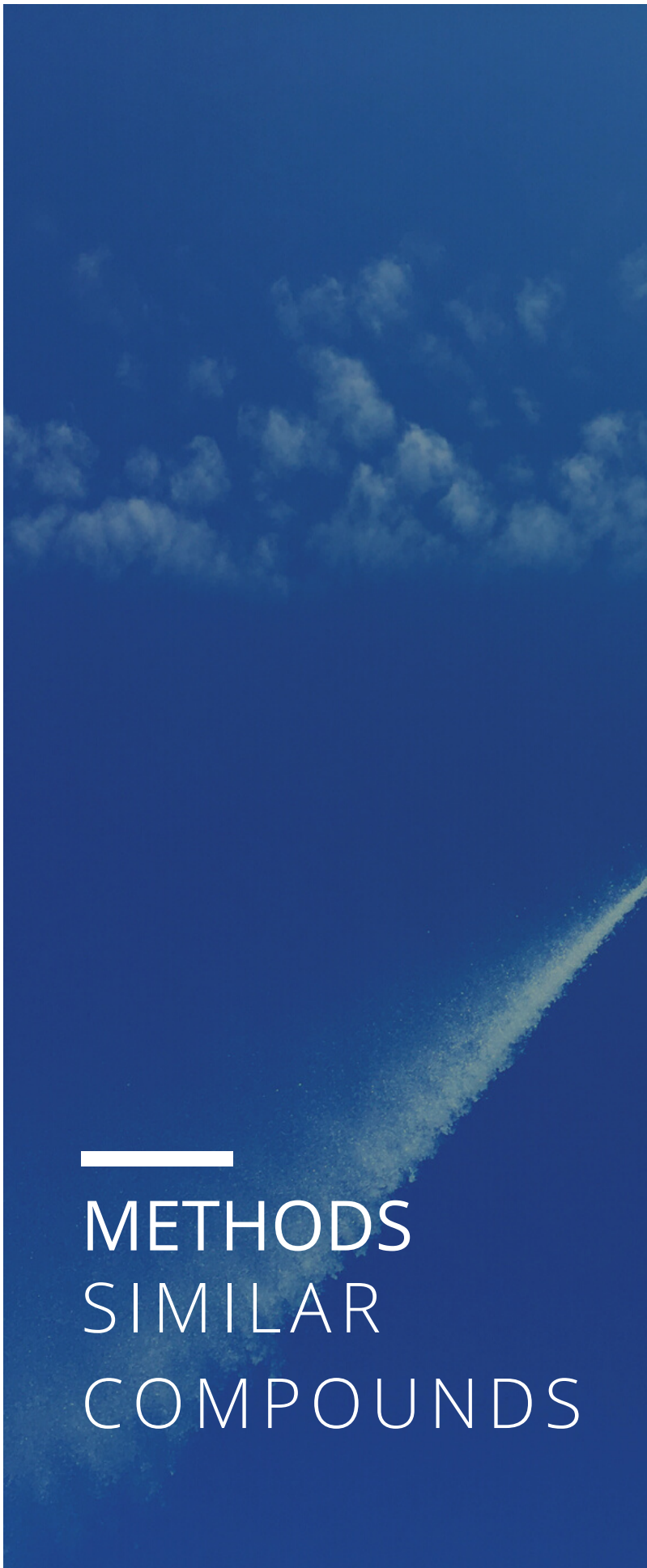
METHODS

STRUCTURALLY SIMILAR STANDARDS

The first possibility is to use the standard addition calibration of the structurally similar compound for quantification of the tentatively identified compound. In this method, the standard addition calibration or even single-point calibration is suggested. The 2D-based chemical similarity is used to find the most similar analytical standard compound. To find the most similar standard, the 2D-linear fragment descriptors based on the atom pairs and atom sequences are calculated and the Tanimoto coefficient is used as the similarity distance function. The online tool for finding the structurally most similar compound against NORMAN SusDat database is available at http://dsfp.chem.uoa.gr/semiquantification. The calibration graph or one-point calibration of the most similar compound is used for quantification of the suspected compound. Additionally, for each compound, the similarity percentage is reported by the tool and the similarity score is used as a measure of the accuracy of the semi-quantification. In the collaborative trial, we will provide a ready-made excel file indicating the compounds with the highest similarity from the calibration standard mix.

PARENT COMPOUND AND TPs

Another possibility of using structurally similar compounds is feasible for transformation products of pharmaceuticals, pesticides, and similar. In these cases, the analytical standard of the parent compound is often available and can be used for quantification of the transformation product. However, this strategy is applicable only for the transformation products. In the dataset included in this collaborative trial, the parent compound is in most cases also the structurally most similar compound according to the previous semi-quantification method.



METHODS

CLOSE ELUTING STANDARDS

Another possibility is to use the calibration graph of the internal standard with retention time closest to the compound of interest for quantification. This approached is based on the assumption that compounds with similar ionization efficiency also elute close in time from LC. The calibration graph or one-point calibration of the close eluting analytical standard is used for quantification of the suspected compound. The closest eluting standard can be found either manually or by using the ready-made excel file or R scrip provided in this collaborative trial.
Using the standard with retention time similar to the suspect has a significant advantage: the full identification of the structure of the contaminant is not required and all detected compounds can be quantified. At the same time, the compound eluting closest to the contaminant does not necessarily have to be most similar in structure.

CHEMICAL SIMILARITY AND PROPERTIES

Chemical similarity measures often fail to show the correlation between the chemical functional groups and instrumental response factor in ESI.[1] Therefore, this method utilizes several correction factors for the structurally similar standards method. The major focus is given on the chemical similarity based on the maximum common substructure overlap (MCSO) and Jaccard index as well as the inclusion of retention time data calculated from retention time indices.[5] Based on the quantification approach and instrumentation used, the final score is modified for searching a pair reference standard to semi-quantify an analyte.[6] The chemical similarity is calculated with respect to the structurally annotated MS/MS fragmentation where the specific substructure or ionisable moiety are dominant. Therefore, the compounds are compared based on their important substructure rather than other common moieties. Similar to the structural similarity method, this method provides a top hit list of target compounds which can be used to semi-quantify the suspect compound. The calibration graph or one-point calibration of the most similar compound is then used for quantification of the suspected compound. An online platform is under development to help the computational part of this work at www.rti.chem.uoa.gr. An R package, called “semi-quant”, enabling automatic application of this method is under development. More details about any further developments will be informed.

METHODS CHEMICAL SIMILARITY

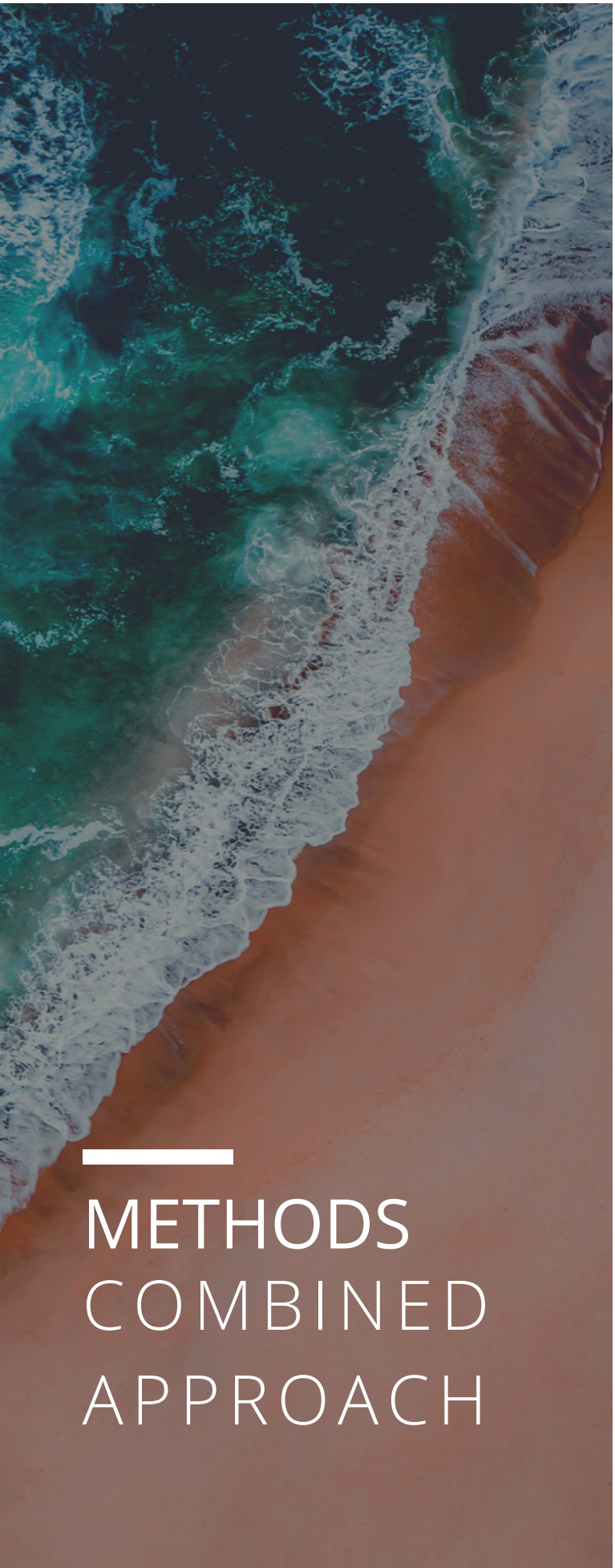
METHODS

IONIZATION EFFICIENCY

An alternative approach is to predict the ionization efficiency of the contaminant detected in LC-ESI-MS and to use this response factor to estimate the concentration of the contaminant. The response factor prediction needs to account for the structure of the compound and mobile phase composition (organic modifier composition, pH) at the retention time of the compound. Here we will use an automated tool based on the prediction of ionization efficiencies of the compounds with the aid of PaDEL 2D descriptors of the compound and parameters of the eluent. This machine learning tool is based on the previously measured ionization efficiency values and compound structure.

APPLICATION

1. Spike the sample with the mixture of the analytical standards and analyse the samples with your normal LC/HRMS non-targeted methods.
2. Compile the data about the structures, retention times, peak areas into one *.csv file. For the standards mix, add also the concentrations. These concentrations will be used to calibrate the response factor predictions of the machine learning algorithm to your analytical method.
NB! Include only the peak areas as a sum of the molecular ion and all observed fragments. At present, adducts can not be calculated.
3. Use the provided on-line calculator app.quantem.co to semi-quantify the compounds.



METHODS COMBINED APPROACH

METHODS

COMBINED APPROACH

Here the semi-quantification approach is based on the chemical similarity analysis (chemical fingerprints and MCSO), ionization efficiency, MS full scan spectrum, MS/MS spectrum and retention time indices.[7] The 18 compounds of the retention time indices mix alongside one isotopically labelled (IS) compound are used as the calibrants to harmonize the calibration curve parameters before establishing the ionization scale. A consensus ionization efficiency values from QSPR model based on support vector machine regression are used for quantification of the suspect and non-target screening data. This approach provides both quantitative (from harmonized log*IE*) and qualitative (hit list, target compounds that can be used to semi-quantify) outcome. An online platform is under development to help the computational part of this work at www.rti.chem.uoa.gr.

APPLICATION

1. Analyse the samples with your normal LC/HRMS non-targeted methods.
2. Run the retention time indices mix and IS together at a known concentration in the same sequence.
3. Compile a *.csv file including the chemical name, retention times, pH of the mobile phase, peak areas, ion type, concentration, dilution factor and SMILES of the RTI mix and IS. These data will be used to calibrate the developed models for the response factor of ESI to your analytical method. Moreover, based on the quality of the RTI calibration curve, different LC conditions will be comparable.
4. Compile a *.csv file which includes, chemical name, retention times, pH of the mobile phase, peak areas, m/z ion type, dilution factor and the SMILES for the suspects.
5. Use the provided on-line platform at www.rti.chem.uoa.gr to semi-quantify the compounds.

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ADDITIONAL DATA TREATMENT

In the case of diluting the samples, the labs should take into account the sample dilution factor. In this collaborative trial, we suggest running samples on two dilutions to guarantee that the measurements are carried out in the linear range and to estimate the possible impact of matrix effect.

Additionally, internal standards are spiked into all samples at the same concentration level. Therefore, the internal standard signals can be used to correct for small systematic variation in the signal from sample to sample. For similarity-based methods, the signals (i.e. peak areas) can be divided by the signals of the selected internal standard. However, for the methods 5.4 and 5.5 the signal of the internal standard should be used in the transformation of the ionization efficiency values to response factors and included in the *.csv files. The correction is done automatically by the software.

REFERENCES

REFERENCES

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