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Feature

Strategies for Drawing Quantitative Conclusions from Nontargeted Liquid Chromatography−High-Resolution Mass Spectrometry Analysis

This Feature aims at giving an overview of different possibilities for quantitatively comparing the results obtained from LC−HRMS-based nontargeted analysis. More specifically, quantification via structurally similar internal standards, different isotope labeling strategies, radiolabeling, and predicted ionization efficiencies are reviewed.

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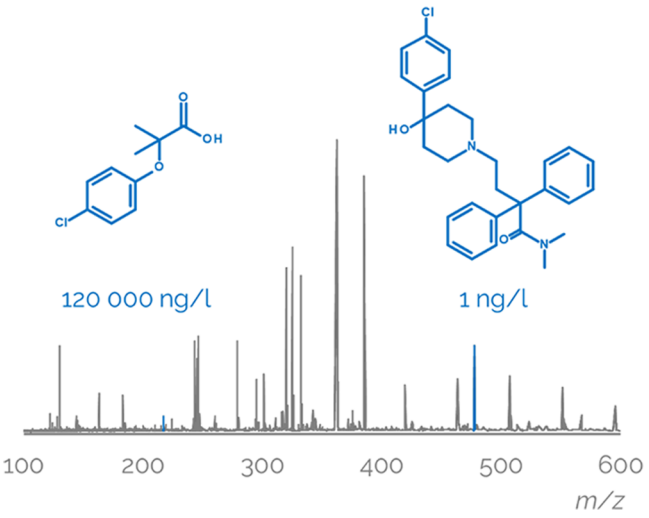

About 10 years ago, most of the liquid chromatography (LC) electrospray (ESI) mass spectrometry (MS) analysis of environmental, metabolomics, and pharmaceutical samples were predominantly carried out as targeted analysis. Targeted analyses allow detection and quantification a few selected analytes with the aid of standard substances. Today, the center of gravity is shifting toward nontargeted methods which utilize high-resolution mass spectrometry (HRMS). Furthermore, the targeted and nontargeted methods are merging into each other. LC−HRMS-based nontargeted methods allow detecting compounds recovered from the sample preparation and ionizing in the electrospray ionization (ESI) source. New possibilities arising from applying the machine learning tools to LC−HRMS data have already transformed the process of identifying the compounds. The computer-aided identification process is not compatible with a traditional calibration graph-based quantification methods. The main obstacle arises from the fact that in ESI, different compounds ionize to a very different extent. Differences up to 100 million times have been reported. This phenomenon results in a vastly different response of different compounds at the same concentration and complicates the quantification for compounds without standard substances. However, decision making is hindered without quantitative information. Therefore, the need to obtain quantitative information from the nontargeted analysis is triggering an emerging field of research.

NONTARGETED SCREENING WITH LC−HRMS

Targeted liquid chromatography−mass spectrometry (LC−MS) methods have been used for several decades to quantify defined compounds from various samples, such as pesticides from fruits and vegetables, pharmaceuticals from medical samples, known metabolites from biofluids and tissues.[1] These methods are an important part of quality control in all areas from medicine to food production. Unfortunately, these robust analytical methods focus only on the compounds for which the method has been developed. This has resulted in a "Matthew Effect" in the chemical analysis: researchers target only the compounds detected by previous studies and compounds that are not included in the targeted lists are ignored.[2]

To overcome this problem, researchers in the field of metabolic research[3,4] and environmental monitoring[5] are developing new suspect and nontargeted screening methods. In the suspect analysis, the compounds are identified based on the suspected compounds list.[6] In the case of nontarget analysis, the compounds are identified without any previous list of targeted or suspect compounds. The identification is based on the information retrieved from the analysis: retention time, exact mass, isotope pattern, as well as fragmentation spectra.[6] The accuracy and efficiency of assigning a structure to the LC−

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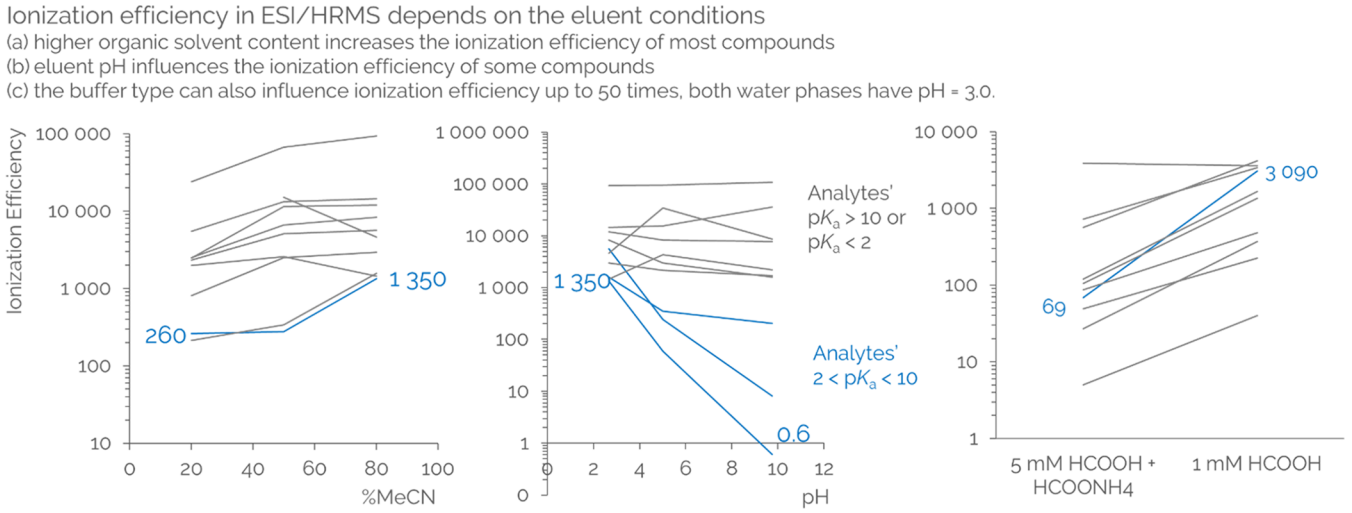

Figure 1. Ionization efficiency of compounds depends on the organic solvent content in the eluent (left), pH of the buffer (middle), and buffer type (right). All studies are in ESI positive mode. The graph has been redrawn based on the data from Liigand et al.[31] and Ojakivi et al.[34]

HRMS data are increasing and the popularity of nontargeted methods for exploration as well as regulatory purposes is growing.[6] If possible, standard substances are used to confirm or reject the identity of the compound detected in the final stage of the analysis. Nevertheless, this step depends on the availability of standard substances. For both newly discovered metabolites and emerging contaminants standard substances are often unavailable and need to be synthesized.

The key difference between targeted and nontargeted methods is that for the (preliminary) structural assignment the standard substances are not used in the nontargeted analysis, which makes the detection of the compounds without preselection of the analytes feasible. This also implies that the obtained results are qualitative in nature, which is currently one of the most important limitations of the nontargeted analysis. The quantification is essential to understand the significance of the detected compounds and to communicate the significance of the results in a clear manner.[7] Quantification is also required to transfer the conclusions from one laboratory to another and to compare the results obtained in different laboratories.

IONIZATION IN ESI

The difficulty in quantifying compounds in LC−HRMS arises from vastly different responsiveness of the compounds. At the same concentration, two compounds may yield very different LC−HRMS signals: the signal of some compounds may be saturated while the signal of other compounds may be barely over the detection limit.[8] Additionally, the signals registered on one instrument are not directly comparable to the signals measured on the other instrument as the intensity scales strongly depend on the instrumentation used.

EFFECT OF THE STRUCTURE OF THE COMPOUND ON THE IONIZATION EFFICIENCY

The different responsiveness of compounds arises from the vastly different efficiency of the generation of gas-phase ions from different compounds. This effect is called ionization efficiencies, and the vastly different ionization efficiencies were first noticed by Kebarle and Tang[9] who described how the size of the cations influences their response. In 1997, Prof. C. G. Enke[10] presented a fundamental idea about the ionization process. He proposed that ionization in the ESI droplets can be seen as a partitioning of the ion between the neutral interior of the droplet and the charge carrying surface of the droplet. From the droplet's surface, the ions can escape to the gas phase and become detected with MS. This model does not account for specific processes that need to take place for the compounds that are not charged in the solution phase under given conditions.

Since then, measuring and quantifying ionization efficiency has been used to understand the ionization process better.[8,11] The response or the relative response of the compound is measured and correlated with the physicochemical properties.[8,11−17] Most often, the effect of log P,[18−20] molecular mass,[21,22] pK_a or pK_b,[20,23] gas-phase acidity (GA) or gas-phase basicity (GB)[20] have been studied. Ion evaporation rate,[9,17] log P,[18,19,24] hydrophobicity (carboxylic acid chain length for aliphatic compounds and number of fused rings for aromatic compounds),[25] retention times of small peptides in reversed-phase LC,[26] nonpolar surface area,[13] gas-phase proton affinity,[16,27] pK_a,[23,24,28] as well as surface area[24] have been shown to correlate with ionization efficiency. Interestingly, sometimes contradicting results have been observed in different studies. For example, it has been generally agreed that hydrophobicity of the compound increases ESI ionization efficiency; however, in a number of studies[8,11,20] no significant correlation between the ESI-MS response and log P has been observed.

SOLVENT EFFECT ON THE IONIZATION PROCESS IN ESI

In addition to the compound's structure, the ionization efficiency is also influenced by the properties of the eluent, both by the organic solvent, its content and the buffer.[29,30] The ionization efficiency of the compounds depends on the speed the ESI droplets dry and form smaller droplets from which the ion evaporation can occur. The eluent that contains more organic solvent obeys higher ionization efficiencies (Figure 1a), as these droplets tend to dry faster.[31−33]

While the effect of organic solvent content on ionization efficiency is relatively straightforward, the effect of buffer type and pH has been very hard to understand (Figure 1b). In the ESI source, gas-phase ions are also formed of compounds that are neutral in normal solution conditions, such as phthalates. Therefore, the droplets formed in ESI are called "super acidic".[35]

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Even for compounds with a pK_a in the studied pH range, the effects are not straightforward. For example, it has been observed that the ionization efficiency of 2,6-diaminopyridine (pK_a of 8.7) is constant in the pH range of 1.4−10.3 (measured in pure water), while the ionization efficiency, and therefore the signal, of 4-methoxy-*N*,*N*-dimethylaniline (pK_a of 5.6) changes more than 200 times in the same pH range.[34] Therefore, the interaction of several compound properties is expected to be relevant,[36] and it is obvious that the processes occurring in the droplets generated in ESI are much more complicated than bulk solution phase.

In addition, different buffers with the same pH result in different ionization efficiencies. Ojakivi et al.[34] proposed an idea that the acidic properties of the ESI droplets result from the specific nature of the ion located on the surface of the ESI droplets. For example, if protonated water H_3O^+ is responsible for the charge, the droplets behave as a strongly acidic medium, while NH_4^+ cations are significantly less acidic and, therefore, result in lower ionization efficiencies (Figure 1c).

INSTRUMENT INFLUENCES THE IONIZATION EFFICIENCY

Another important factor is the instrument. Different instruments use different ionization source geometries and parameters. For example, the nanospray sources have been claimed to ionize all of the compounds with almost 100% recovery.[37] On the other hand, in the nebulizer gas-assisted ESI sources, only a small fraction of the molecules present in the solution reach the gas phase. Additionally, in the heated electrospray, the additional heated nebulizer gas around the spray plume influences the ionization behavior. The organic solvent content effect on ionization efficiency is almost negligible if heated ESI is used.[32]

As a result, the ionization efficiency values are not constant from instrument to instrument. Nevertheless, a good correlation of ionization efficiency values between different instruments has been observed.[38] The slope and intercept of these correlation plots depend on the instruments; however, based on these correlations, it has become obvious that the ionization efficiency predictive model developed on one instrument can be transferred to another instrument by taking advantage of this correlation.[39]

EFFECT OF THE SAMPLE TYPE

Another very important factor in the analysis of any sample with LC−ESI-HRMS is the effect of the sample components coeluting with the compounds of interest, also known as the matrix effect. Mostly, suppression of the signal of the analyte has been reported; rarely though, ionization enhancement has also been observed.[40,41] The mechanism of the suppression is understood much better than the mechanism of enhancement. To be ejected to the gas phase, the compounds need to move to the surface of the ESI droplets; therefore, the most obvious suppression mechanism is the competition for the surface charge.[10] The compound with a higher affinity toward surface charge and ionization efficiency suppresses the compound with lower surface affinity.[32] The compound with lower surface affinity will have only a minimal effect on the ionization of compound with higher surface affinity.[32] Our group has lately shown that in spite of strong ionization suppression, the ionization efficiency values measured in the extracts of biological matrixes are in good correlation with the ionization efficiency values measured in pure solvent.[42]

STRATEGIES TO QUANTIFY RESULTS OF NONTARGETED ANALYSIS

In order to obtain quantitatively meaningful results without standard substances, a number of different strategies have been developed. Some of the most extensively used approaches are summarized below, namely, (1) using peak areas directly or in combination with statistical data treatment, (2) isotope dilution, (3) radiolabeling, (4) using structurally similar compounds for quantitation, and (5) quantitation based on the predicted ionization efficiencies. The summary of each of the strategies is given in Table 1.

QUANTIFICATION BASED ON THE PEAK AREA OF A SINGLE COMPOUND

From above, it is clear that the peak area of a single compound in a single sample analyzed on one specific instrument with a specific LC gradient program and the eluent is not informative about the absolute concentration of this compound in this sample. However, if the analysis of several samples are carried out in the same lab with the same method on the same instrument and very close in time (e.g., within the same run), the change of the signal of one specific compound can be associated with the change of the concentration of this compound from sample to sample. Therefore, relative peak areas can be used to follow trends in metabolomics, environmental samples, and exposomics.

For example, Plassmann et al.[49] has used a time series in a human blood exposome study to prioritize detected compounds, as increasing intensities in the time series analysis may refer to bioaccumulating compounds or to compounds with increased exposure. A similar comparison has been done by Gago-Ferrero et al.[50] for the analysis of wastewater samples in Greece. The signal variation from sample to sample is also used to aid identification of relevant metabolites or biomarkers from the numerous compounds discovered with the suspect and nontargeted LC−HRMS analysis. The largest and most significant variations are detected with the aid of different statistical tools. The examples include discovering relevant chemicals for white tea,[51] chemical associated with fermentation of black tea,[52] coffee roasting,[53] as well as other food,[54] bacteria,[55] and environmental samples.[56] As a result, trends in relative peak areas are an important tool in discovering emerging contaminants as well as biomarkers. However, the limitation is that all samples need to be collected and analyzed together, which might be complicated if unstable compounds are of interest.

The most severe drawback for peak area-based methods is that they are seldom transferable between laboratories,[57] as the variation of the signals depends on instrument, method conditions, as well as cleanliness and age of the instrument. In this context, Naz et al.[58] has pointed out that if the aim of the nontargeted analysis is to "find statistically significant biomarkers through unbiased differential analysis of as many signals as possible", the results will be valid as long as all of the samples have been measured under strictly identical conditions. Therefore, the main advantage of using peak areas for quantitatively comparing results from nontargeted screening is feasibility for different methods and application areas.

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Table 1. Comparison of the Main Strategies to Quantify Results from Nontargeted Screening

Method	Concentration obtained	Application	Trueness of the concentration	Transferable between laboratories	Comment
Peak areas only	Relative between samples	All fields	Very low.	No	Applicable to all methods.
U-^{13}C	Relative to reference extract	Metabolomics studies	High. Up to 2 times.[43]	Yes, if commercial reference extract is used.	Applicable to metabolomics only.
Radiolabeling	Absolute	Primarily to pharmaceutical metabolism	High. Almost full recovery.[44]	Yes	High LOD. Expensive radiolabeled standards and scintillation materials required.
Structurally similar standards	Absolute	Depends on the standards	Low to high. Depends on the similarity of the compound and standard: from 1.4 to 100 times.[39,42,45,46]	Yes	Only for application where relatively similar compounds are detected.
Predicted ionization efficiencies	Absolute	All fields	Medium. Depends on the algorithm approximately 2 times.[39,47,48]	Yes	Applicable also retrospectively. Currently only for MS scan data.

■ ISOTOPE DILUTION AND ITS ALTERNATIVES

In targeted LC−ESI-MS, the isotope dilution has been used for quite a while to account for the various losses of the analyte during analysis, most notably for the incomplete extraction of the analyte during sample preparation and ionization suppression in the ESI source.[59] The main problem of the isotope labeled standards for targeted analysis is their relative unavailability which is naturally even more likely for nontargeted analysis. To overcome this Mashego et al.[60] proposed a mass isotopomer ratio analysis of U-^{13}C labeled extracts (MIRACLE) approach. In this approach, an isotopically labeled reference extract of the cell culture of interest is prepared by feeding the culture with a single fully ^{13}C labeled carbon source. As a result, all of the metabolites in the cell culture eventually become U-^{13}C labeled. The extract prepared can be used as an internal standard by adding it to each sample of interest prior to extraction and analysis. This allows quantification of each the metabolite relative to its U-^{13}C-labeled equivalent and improves accounting for the losses in the sample pretreatment.[61] MIRACLE allows following the quantitative changes in the metabolite abundance from one cell line to another, from one manipulation to another, and along with the time series analysis, as long as all of the metabolites of interest are present in U-^{13}C labeled culture. By now, first U-^{13}C-labeled cell culture extracts are also available commercially.[62] Furthermore, using the same U-^{13}C-labeled cell culture from lab to lab allows direct comparison of the results. The first interlaboratory comparison of the quantitative metabolite profiling has been carried out between two laboratories.[43] Generally, consistent results were observed (differences up to 2 times); however, for redox-sensitive amino acid methionine, major inconsistencies (difference of 20 times) were observed and it was concluded that isotope dilution prior to extraction should be preferred if possible.

A similar strategy can be used for quantitative metabolite flux studies. In this case, an organism is fed with the compound of interested that is tagged with stable isotopes.[63] Some of the first studies involve lead metabolism[64] and have later been applied also for metabolomics studies.[65] The matrix effect has also been corrected with stable isotope labeling strategies.[66,67] However, the later does not allow quantification without the standard substances.

The main requirement of applying MIRACLE is that the metabolite of interest is present both in the sample as well as in the U-^{13}C labeled reference extract. As a result, concentrations relative to the reference sample can be achieved. Unfortunately, the usage of the same reference extract is currently limited by the commercial availability of the reference extracts. Also, this is applicable only to "living" systems, such as cells, plants, and even mammals. The application in nonliving systems, such as contaminant analysis for both living and nonliving systems, is impossible as U-^{13}C labeling strategies cannot be used. Lastly, the results yield relative concentrations, which are useful for understanding the faith of one compound under different conditions or in time series analysis but is of less relevance for engineering studies where absolute amounts are of relevance.

■ RADIOCHROMATOGRAPHY

One of the most accurate techniques to quantify drug metabolites is LC with a radioactivity detector. This technique is sometimes also called radiochromatography and is widely applied in the studies of the metabolism of pharmaceuticals. Usually, two detectors are used in parallel, HRMS for structural

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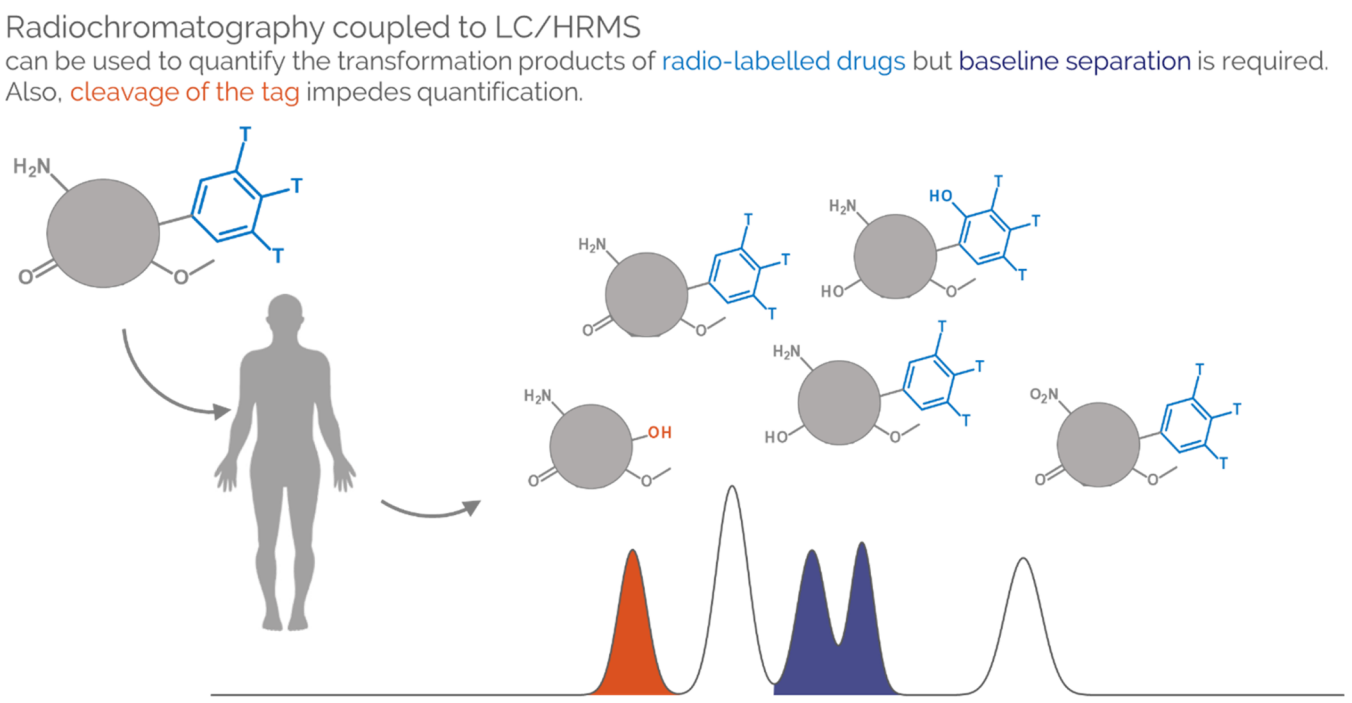

Figure 2. Schematic workflow of radiochromatography with its possibilities and limitations.

characterization and radioactive detector for quantitation. In this technique, the parent drug is labeled with one or a few radioactive elements before dosing. The metabolites formed from the parent compound containing the radioactive element can be quantified by measuring the radioactivity (Figure 2). In order to measure the radioactivity of the compounds separated in LC, the so-called scintillation is used to convert radioactive irradiation to visible energy, i.e., photons.[44]

The advantage of radiochromatography is the high quantification accuracy, as the signal does not depend on the structure of the compound and results in absolute quantitation if run in isocratic mode.[68] Therefore, the validation guidelines, such as ICH, recognize it as a standard method for metabolism studies of drugs. Additionally, radiochromatography is known to have a low noise level.

The drawbacks of radiochromatography include a requirement for baseline separation, low sensitivity, and availability of the radiolabeled compounds. Low sensitivity has been tackled by increasing the volume of the flow through the cell[69] or by offline measurements.[70] However, these cause the peak broadening, reduce baseline separation, and increase inconvenience and analysis time. Additional limitations involve the availability of the radioactively labeled substances and the expense of the instrumentation. The most commonly used radioactive elements are ^{3}H and ^{14}C.[71−73] Lastly, the costs of applying radiochromatography are high. In addition to the extra equipment costs, the radioactively labeled substances are very expensive, and additional costs related to radioactive waste management occur.[68]

CALIBRATION WITHIN THE GROUP OF COMPOUNDS

One of the earliest approaches to overcome the lack of standard substances is to use a structurally similar compound to estimate the concentration of the detected compound. Most commonly, the calibration graph of the parent compound is used to quantify the metabolites of the compound. In order to characterize the accuracy of such an approach, Dahal et al.[46] measured a set of O-demethylation, N-demethylation, aromatic hydroxylation, and benzylic hydroxylation products of pharmaceuticals. They observed that assuming the same response for the metabolite and the substrate may lead to the error of up to 4 times even for relatively similar compounds.

Another possibility is to use the calibration graph of a structurally similar compound for the quantification of the identified compounds. Pieke et al.[45] evaluated this approach for the semiquantification of 17 compounds in food samples. The best prediction accuracy was observed when the marker compound with the closest retention time was used for quantifying the compound of interest. All studied compounds were relatively similar and, therefore, good accuracy was also observed (average prediction error of 2.08 times).

The choice of the standards, however, is crucial. We have previously observed that using structurally similar compounds for semiquantification of pharmaceuticals and pharmaceutical-alikes can be up to 660 times in ESI negative mode for the analysis of biofluids.[42]

In summary, the advantage of using structurally similar compounds for quantification is simplicity. Additionally, in occasions where the compounds are relatively similar to each other, sufficient accuracy may be observed. Nevertheless, the structurally similar standards need to be run together with the samples of interest and chosen before the structures of the compounds in the samples are identified. For applications, such as environmental analysis, where the structures of the compounds of interest vary significantly, it is challenging if not impossible to find a set of compounds that can serve as suitable standards.

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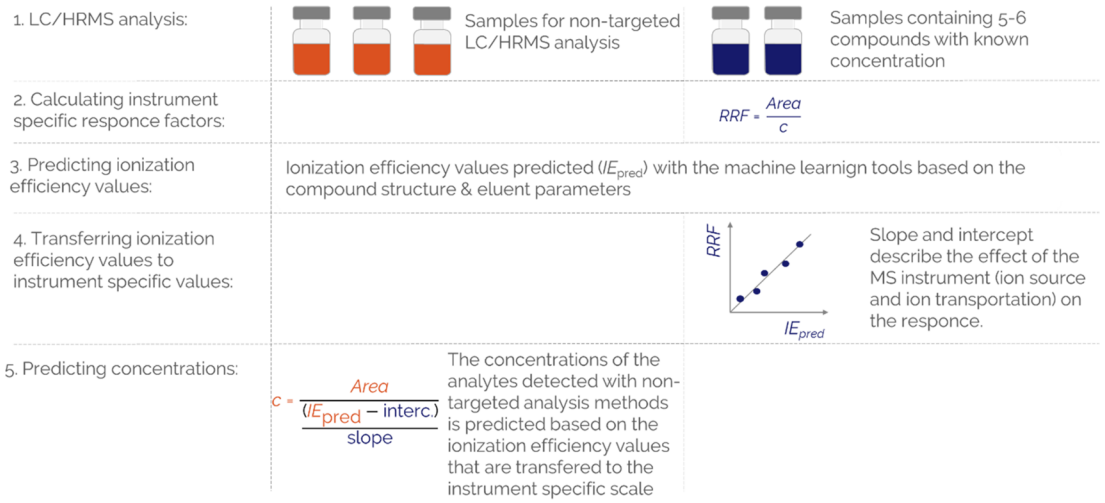

Figure 3. Workflow for using predicted ionization efficiencies for quantification of compounds discovered in nontargeted analysis: accounting for the structure of the compound, eluent composition, as well as instrument-specific aspects.

QUANTIFICATION BASED ON PREDICTING THE IONIZATION EFFICIENCIES

In order not to be limited by the preselected structurally similar compounds, the ionization efficiency of the compounds can be predicted and used for estimating the concentration of the identified compounds. Several models to predict the ionization efficiency of the compounds have been developed.[8,11,13,21,74] Chalcraft et al.[21] observed an average prediction error of 49% for predicting the response of polar metabolites based on the molecular volume, log *P*, absolute ion mobility, and effective charge. Our group has previously proposed using a quantitative charge delocalization parameter (WANS or WAPS) and ionization degree in both ESI positive[8,75] and negative modes.[11] A higher charged delocalization degree and being charged in the eluent increase the ionization efficiency of a compound. These parameters allow achieving the average prediction error of 2.5 times. Also, the partial least-squares (PLS) method[16,17] and artificial neural networks (ANN)[24] have been used to predict the ESI-MS response. Unfortunately, the number of data points used for model development is very small.

Though the ESI-MS response has been modeled by several authors, it has still been rarely applied for concentration predictions. In spite of the first pioneering works, quantifying the compounds with the aid of predicted ESI-MS response or ionization efficiencies are available. Kamga et al.[47] have proposed to use the carbon number of the fatty acids to aid quantification of the acids for which standard substances are not available. For this, they used five long-chain fatty acids standards (n-C_{15}, n-C_{19}, n-C_{24}, n-C_{26}, and n-C_{30}) with different carbon numbers to establish the relationship between the ESI-HRMS response of the fatty acid and the carbon number. Later, this allowed one to determine the response factor of other fatty acids with a carbon number between 15 and 30. Though numerical validation data have not been given by the authors, the consistency with the GC/FID analysis for most of the acids is good (average error within 2 times). The approach proposed by Kamga et al.[47] does not account for solvent effects as all of the measurements were carried out in the infusion mode.

Lately, Wang et al.[76] has used molecular volume, log*P*, pK_a, pK_b, and polarizability to predict the response factors of pyrrolizidine alkaloids in honey samples. The studied pyrrolizidine alkaloids were relatively similar in structure and eluted very close in retention time; therefore, changes in eluent conditions were not accounted for.

Further development to account for eluent composition have been proposed by Wu et al.[48] and Kruve et al.[39] Wu et al.[48] quantified 25 organic acids via five parameters, and the R^2 of the response factor prediction was 0.89. The concentration prediction accuracies ranged from 65.8 to 124.2%. Kruve et al.[39] proposed using multilinear calibration in combination with a simple system of calibration compounds to evaluate the importance of each physicochemical parameter specific to the instrument and solvent used for the analysis.[39] This approach allowed achieving the mean prediction error of 3.5 times between predicted and actual concentration in the ESI negative mode and is applicable between different instruments.

Only recently, we have proposed a robust and automated tool obtaining quantitative information based on the prediction of ionization efficiencies of the compounds with the aid of 2D descriptors of the compound, parameters of the eluent, and machine learning tools in both ESI positive and negative mode.[77,78] The average prediction error of ionization efficiency was 2 times and has allowed estimating the concentration of compounds detected with LC−MS based on the measured peak areas and predicted ionization efficiency (Figure 3). This approach was tested on the screening for food contaminants in cereal samples (positive mode) with nontargeted LC−MS methods. The average prediction error for the concentrations was below 5 times. This means that if the pesticide concentration is estimated to be 1 ppm, then actually it would be between 0.2 and 5 ppm.

Currently, the quantification based on the predicted ionization efficiency values is applicable to compounds with a tentatively identified structure. In the case of suspect screening, the concentrations can be estimated for all of the possible candidates and prioritized based on the expected effect. This is convenient for (drug) metabolism studies where the number of possible changes to the structure is limited or for applications where large suspects libraries are available. However, for compounds with a completely unknown structure, the limitations remaining though the concentrations of these compounds are of great interest as well.

In short, quantification based on the predicted ionization efficiencies has a wide application scope as it allows extrapolating

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to compounds that are up to a certain extent different from the standards available in the laboratory. It can be theoretically applied to all detected compounds and yields absolute concentration. The applications in both positive and negative modes have been reported. Currently, the accuracy is lower than for the isotope labeling and radiochromatography but is expected to increase as more data are collected to improve the understanding of the ionization process.

■ ACCURACY OF THE QUANTIFICATION

The choice of a quantification method for nontargeted data depends primarily on two factors (1) availability and (2) required accuracy. First, some of the approaches are unavailable in some cases due to limitations in time, money, materials, or instruments. Still, the approximate quantitative information may be an important tool for drawing further conclusions or prioritizing the next steps in the investigation. In such situations, the applicable tools should not be disregarded due to uncertainty that is higher compared to the classical targeted analysis. However, the uncertainty needs to be recognized and communicated to avoid misconception.

Second, the desirable uncertainty needs to be viewed within the boundaries of the application. For example, in environmental monitoring, it is desirable to combine the quantitative result with the toxicity predictions.[79] Toxicity predictions have very high uncertainty (~ an order of magnitude). As a result, the uncertainty of the concentration estimation has a negligible effect on the combined uncertainty as long as it is reasonably (~2 times) lower than the uncertainty of the toxicity predictions. At the same time, in metabolomics, the clinically relevant concentration change may be in ±20% from the healthy levels. Therefore, in these cases, the desired uncertainty needs to be much lower than the expected variation of 20%.

■ FUTURE CHALLENGES

As the popularity of nontargeted analysis is increasing also the need to obtain quantitative results is increasing. In the Solutions and Workflows in (Environmental) Molecular Screening and Analysis (SWEMSA) conference 2019, in Munich, the need to start obtaining quantitative results from the nontargeted analysis was stressed. It has been described that the general public is better accustomed to the way of reporting results for targeted methods, which also contain concentrations.

Each of the methods discussed above needs different improvements to become successful. For example, both quantifications with structurally similar compounds as well as quantification based on predicted ionization efficiency would greatly benefit from standardized workflows and increased data sharing. Several laboratories are making great efforts to increase the number of experimental MS and MS/MS spectra available in suspect screening databases. Adding simple metadata of the measurements, such as concentrations, solvent, and instrument, would allow evaluating the relative response of the compounds and to develop ionization efficiency predictions further.

However, for the development of all of the methods, a general benchmarking strategy is needed. This could be a data set against which different research groups could compare the performance of their proposed method, similar to Critical Assessment of Small Molecule Identification (CASMI)[80,81] that has been used to evaluate the performance of compound identification or a reference sample. Additionally, interlaboratory comparisons could yield field boosting effects. Several interlaboratory comparisons on the identification of a compound with nontargeted screening have already been organized by the NORMAN network (network of reference laboratories, research centers, and related organizations for monitoring of emerging environmental substances)[82,83] and the Environmental Protection Agency (EPA).[84] Now, the next step could be to move forward with the collaborative trials on semiquantification and some of the nontargeted analysis communities, e.g., the NORMAN network, are working toward such strategies.

■ CONCLUSION

The nontargeted analysis methods are becoming more popular, and the algorithms used for compounds identification are developing fast. Currently, the majority of the nontargeted analysis yield qualitative data. The next step in nontargeted screening is to increase the proportion of quantitative data.

The methods described in this Feature have different accuracies and scopes of application. Radiochromatography, quantitation with structurally similar compounds and with predicted ionization efficiencies, allows retrieving absolute concentrations while the extracts of isotopically labeled cell culture allow quantitation relative to the reference extract. The highest accuracy is possessed by the isotopically labeled standards and radiochromatography, but these strategies are application dependent. The quantitation with the aid of predicted ionization efficiencies possesses intermediate accuracy; the obtained concentration is up to a few times different relative to the actual concentration, but the method is theoretically universally applicable.

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Notes

The author declares no competing financial interest.

Biography

Anneli Kruve graduated from the University of Tartu in 2011. She carried out her postdoctoral studies in Technion and was a Humboldt fellow at Freie Universität Berlin. In 2018, she was selected to the Top 40 under 40 Power List by the *Analytical Scientist*. In 2019, she joined Stockholm University. The field of studies of Anneli Kruve is focused on mass spectrometry fundamentals and applications. Specifically, her group is working on establishing semiquantitative nontargeted analysis as well as measurement science issues both in targeted as well as nontargeted methods. The group of Anneli Kruve uses modelling and machine learning to understand ionization processes in electrospray (ESI) and connecting the LC and MS. Based on this knowledge, she is developing semiquantitative nontargeted analysis methodology, creating a quantitative understanding of the sources of contamination in environment and food, and improving LC−HRMS-based analysis methods.

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