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General strategies to increase the repeatability in non-target screening by liquid chromatography-high resolution mass spectrometry

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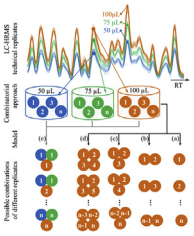
HIGHLIGHTS

- Combinatorial approach for validation of the data evaluation in non-target screening.
- Replicates decrease false negative and false positive findings.
- Signal fluctuations emerged as powerful filter criteria.
- Data processing increases repeatability.
- Screening method and data evaluation in general applicable at trace levels.

GRAPHICAL ABSTRACT



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ABSTRACT

This article focuses on the data evaluation of non-target high-resolution LC-MS profiles of water samples. Taking into account multiple technical replicates, the difficulties in peak recognition and the related problems of false positive and false negative findings are systematically demonstrated. On the basis of a combinatorial approach, different models involving sophisticated workflows are evaluated, particularly with regard to the repeatability. In addition, the improvement resulting from data processing was systematically taken into consideration where the recovery of spiked standards emphasized that real peaks of interest were barely or not removed by the derived filter criteria. The comprehensive evaluation included different matrix types spiked with up to 263 analytical standards which were analyzed repeatedly leading to a total number of more than 250 injections that were incorporated in the assessment of different models of data processing. It was found that the analysis of multiple replicates is the key factor as, on the one hand, it provides the option of integrating valuable filters in order to minimize the false positive rate and, on the other hand, allows correcting partially false negative findings occurring during the peak recognition. The developed processing strategies including replicates clearly point to an enhanced data quality since both the repeatability as well as the peak recognition could be considerably improved. As proof of concept, four different matrix types, including a wastewater treatment plant (WWTP) effluent, were spiked with 130 isotopically labeled standards at different concentration levels. Despite the stringent filter criteria, at 100 ng L^{-1} recovery rates of up to 93% were reached

Abbreviations: A, peak area; *C*, number of combinations; C_{max}, maximum achievable number of combinations; *cps*, counts per second; *CV*, coefficient of variation; *DDA*, data-dependent acquisition; *FWHM*, full width at half maximum; *H*, peak height; *HRMS*, high-resolution mass spectrometry; $\bar{I}$, mean improvement factor; *k*, number of samples taken from *n* to form a subsample; *n*, number of technical replicates; *N*, number of variables, i.e. features or standards; *R*, retention time; *RKI*, river kilometer index; *r*, number of remaining replicates; *RR*, rate of recognition; $\overline{RR}$, mean rate of recognition; *u*, number of different injection levels; *V*, injection volume; *W*, peak width.

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in the positive ionization mode. The proposed model, comprising three technical replicates, filters less than 5% and 2% of the standards recognized at 100 and 500 ng L^{-1}, respectively and thus indicates the general applicability of the presented strategies.

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1. Introduction

Recent developments in high-resolution mass spectrometry coupled to liquid chromatography (LC-HRMS) have initiated new possibilities for the analysis of micropollutants without having any a priori information available [1]. Modern HRMS instruments provide accurate mass data while combining sufficient selectivity and sensitivity, which allows the determination of trace substances in complex environmental matrices [2–5]. LC-HRMS has emerged as a powerful tool as it enables the detection of thousands of compounds within a single run and does not require a reference standard during the method set-up [1,6] as it is usual for triple quadrupole instruments. In contrast, conventional targeted analytical methods only allow to monitor a tiny fraction of known contaminants per run and therefore miss unknowns, such as transformation products and chemicals which were initially not anticipated regardless of how high their concentrations might be [7]. While the untargeted data acquisition offers a variety of advantages, sophisticated processing strategies are needed to handle the wealth of data and to extract the significant information. In the first step of most untargeted workflows, peak finding algorithms are used to extract peaks (features) from the existing data set [8,9]. Throughout a large number of studies, it became evident that the peak finding step, independently of the software used, reveals type I (false positive) and type II (false negative) errors. The intensity threshold to differentiate noise from real peaks is one of the most important parameters in non-target screening. Setting a low intensity threshold favors type I errors whereas higher thresholds will cause real peaks of interest to be missed. Many authors found that false positives, i.e. noise or matrix background recorded as peaks, as well as false negatives, i.e. true peaks which were not recognized, represent the main challenge especially if dealing with low abundant signals [6,10,11]. Consequently, different strategies have been developed in order to distinguish real peaks from noise signals while still keeping a chosen set of known peaks, i.e. spiked standards [7,12–15]. Many approaches attempt to emphasize the temporal, spatial or process-based variances among different samples [16,17] using multivariate statistics. This, however, requires that the variance is attributed to true differences between the samples [14]. Problems in peak recognition between different samples appear largely random and therefore hamper the elucidation of discriminating features as real differences are superimposed by apparent ones created during the peak finding.

The objective of this study was to illustrate the problematics of false positive and false negative findings on the basis of a systematic evaluation of multiple technical replicates of different matrix types. The improvement in feature detection by comparative evaluation of different processing strategies involving various models is shown. The features' frequency of recognition was therefore adopted as a measure for the repeatability of the method, which was determined using a combinatorial approach that is exemplified in the supplementary video. Starting with very poor repeatability, pointing to the above mentioned problems of type I and type II errors, the rate of recognition is successfully increased by applying different models and the involved filter criteria. Moreover, the question of how many replicates are needed to provide a representative result will be discussed.

Supplementary video related to this article can be found at http://dx.doi.org/10.1016/j.aca.2016.06.030.

2. Materials and methods

2.1. Chemicals

The standard substances combined in the stock solutions (supplemental table S1 and S2) were purchased from various suppliers (supplemental table S3) Isotopically labeled standards (supplemental table S4) were provided by the Swiss Federal Institute for Aquatic Science and Technology (Dübendorf, Switzerland) as a multi-component standard. Water (Rotisolv® Ultra LC-MS), acetonitrile (Rotisolv® ≥ 99.95%, LC-MS-Grade) and methanol (Rotisolv® ≥ 99.95%, LC-MS-Grade) were purchased from Carl Roth (Karlsruhe, Germany) while formic acid (for mass spectrometry ~ 98%) was supplied by Sigma-Aldrich (Steinheim am Albuch, Germany). For mass calibration, the APCI positive and negative calibration solutions were delivered by Sciex (Framingham, USA).

2.2. Sample preparation

Except spiking the samples with different stock solutions, in general, no prior sample preparation was performed to avoid discriminating against certain substances. In non-target analysis, we believe this to be very important even though the analytical method might suffer from the lack of pretreatment. Samples that obviously contained suspended matter were centrifuged for 5 min at 5000 rpm before the analyses.

2.2.1. Stock solutions

An exact amount of each pure substance was dissolved in methanol, stored at −18 °C and, shortly before the analyses, multi component standards were produced by diluting numerous standard solutions to the required concentration. Based on this procedure, stock solution I (supplemental table S1) covering 32 pharmaceutical drugs as well as stock solution II (supplemental table S2) comprising 263 various substances from different classes (e.g. pesticides, biocides, industrial chemicals and corrosion inhibitors) were produced. Stock solution III (supplemental table S4) containing 130 isotopically labeled substances was received fully prepared and thus only had to be diluted to the desired concentration. Note that reference substances were selected to be compatible with the method applied whereas special attention was given to adequately cover a relevant polarity range (logP ≈ −1 to 5).

2.2.2. Samples for the comprehensive evaluation

Sample - A - Stock solution II was prepared and diluted with ultrapure water to a final concentration of 500 ng L^{-1}; Sample - B - Stock solution I was prepared and diluted with ultrapure water to a final concentration of 1000 ng L^{-1}; Sample - C - River water sample which was collected from the Danube River at RKI 2568 (24 h composite sample, DOC ≈ 2.7 mg L^{-1}) spiked with stock solution I to reach a final concentration of 100 ng L^{-1}; Sample - D - Stagnant

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