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Evaluation of trace analyte identification in complex matrices by low-resolution gas chromatography – Mass spectrometry through signal simulation

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ABSTRACT

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

1. Introduction

The monitoring of environmental and food contaminants, and the development of new biologically active compounds depend on the ability of identifying and quantifying trace level of these compounds or their metabolites in complex matrices. The quantification of these compounds is performed after confirming their presence. Therefore, the reliability of the identification is particularly important in these studies. According to the latest edition of the International Vocabulary of Metrology [1], the determination of a nominal property (i.e. property not described by a magnitude) is performed through an examination. This term is also introduced, together with many others, in an IUPAC provisional vocabulary for qualitative assessments [2]. In this work, the term examination is used to refer to generic characteristic of qualitative tests or identification to discuss specificities of the studied type of examination.

The ability to correctly identify a compound in a matrix decreases as compound level decreases and matrix complexity increases. In some cases, the identification reliability close to defined limits for quantitative assessments, such as the limit of detection (LOD) or quantification (LOQ), is not enough to ensure that the signal used in the quantification is produced by the studied analyte. If analyst is not aware of examination limitations next to LOD or LOQ there is the risk of the false presence of studied quantity (i.e. false positive result) or even the false absence of the analyte (i.e. false negative result) being reported. This scenario is particularly critical for the analysis of trace levels of organic compounds in complex matrices and results from the lack of tools to quantify examination reliability in these situations.

In this manuscript, the general term quantity is used when different types of quantities such as mass concentration, molar concentration or mass fraction are applicable.

In some analytical fields, such as the analysis of pesticide residues in foodstuffs, extensive research has been developed to allow a harmonised and reliable definition of identification criteria [3–7]. More recently Mol et al. [8] assessed the identification criteria currently proposed in SANCO guidelines [9] using results of over 135,000 chromatogram obtained in five laboratories. This work concluded that predefined criteria should be revised but,

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ideally, identification criteria and identification performance should be set and assessed experimentally respectively.

Bayes' theorem has been used to quantify the quality of examination results based on collected single or independent evidences [10–13]. This theorem can be used to report the examination result with the examination uncertainty as a likelihood ratio, LR, or as the probability, $P(A|e)$, of the examination result, A, being correct given one or various evidences, *e*. One of the most interesting features of these metrics is the combination in a single metric of the most relevant quantitative values of examination quality: true positive and false positive results rates. The $P(A|e)$ has the additional advantage of being readily interpretable by anyone interested in the result, independently of his or her background in Bayesian statistics, but requires knowing the probability of A occurrence in the studied population, $P(A)$, known as prior probability since it must be defined before the examination. The LR quantifies examination quality without the need for a prior knowledge of the studied population but its interpretation is not straightforward. In some fields, conversion tables of LR into a verbal expression of examination quality are used to support the interpretation of LR. In the following section Bayes' theorem metrics are presented.

The most challenging task of examination quality (i.e. uncertainty) evaluation is the quantification of false positive results rates (FP) since, in most cases, this rate is extremely low requiring an extremely high number of experimental tests for its reliable determination. A FP (e.g. probability of being identified analyte in a matrix free of analyte) of 0.1% is only quantifiable in more than 1000 examinations of matrices free of analyte.

GC–MS^n and LC–MS^n are the most popular instrumental methods of analysis for the identification and quantification of trace levels of organic compounds in biological matrices. The identification of compounds depends on the agreement of retention time (RT) or relative retention time (RT'), and the abundance ratio of characteristic analyte fragments of the mass spectrum (AR) between analyte peaks of calibrators and sample peaks. If identification criteria is defined from observed agreement of analyte signals of different injections and adequate statistical models, the true positive rate, TP, can be estimated from the confidence level of the identification criterion. If a *P* confidence level is considered, the TP is *P*. The probability of defined identification criteria producing false positive results (FP) is more difficult to estimate.

In this work adequate statistical models for RT and AR are built to estimate identification criteria for a specific confidence level. Since RT is normally distributed, normal statistics is used in these cases. The possible deviations to normality of AR distributions are studied through Monte-Carlo simulations based on pairs of abundance mean and standard deviations, and respective linear correlation. The 1% and 99% percentile of simulated results are used to set the identification criteria for a confidence level of 98%. The identification criteria are estimated for the analyte signal in foodstuff matrix.

Identification criteria based on signal models at the limit of detection, *LOD*, limit of quantification, *LOQ*, and two times both these levels (i.e. 2*LOD* and 2*LOQ*) are developed since these are the most critical levels for examinations. Models of ion abundance at *LOD*, 2*LOD*, *LOQ* and 2*LOQ* are also developed to define signal thresholds for evaluations next to these levels.

Signals from matrix free of analyte are collected to develop worst-case models of signal noise capable of misleading about analyte presence. Since signal noise will not produce negative peaks, collected signals are used to feed a model based on a truncated *t*-distribution.

Signal noise simulations, ion abundance signal thresholds at studied quantity levels (*LOD*, 2*LOD*, *LOQ* and 2*LOQ*) and estimated identification criteria are used to predict the probability of signal noise producing a false positive, i.e. the FP.

This assessment allowed defining models of FP variation with a target analyte quantity. These models and the defined TP were subsequently used to estimate examination uncertainty as a LR or probability collected evidence of analyte presence being correct.

The minimum analyte quantity capable of producing "extremely strong" evidences of analyte presence is designated Limit of Examination.

All correlation and dispersion estimations and Monte Carlo simulations were performed in user-friendly MS-Excel files made available with this manuscript as Electronic Supplementary material.

The developed approach was successfully applied to the identification of chlorpyrifos-methyl and malathion in QuEChERS method extracts of foodstuffs of vegetable origin with high water content by GC–MS. The developed tools can be applied to the analysis of other analytes in other matrices by GC–MS or LC–MS.

2. Bayes' theorem

2.1. Probability of an event given the respective evidence

The examination uncertainty can be quantified by Bayes' theorem metrics. The following equation presents the simplest version of Bayes' theorem illustrated for the identification of an analyte, A, in a matrix supported in an evidence e. In identifications performed by GC–MS, evidence e is observed when RT and AR are within acceptance intervals for these parameters. Eq. (1) presents the quantification of the probability of A presence in the matrix, indicated by evidence e, being correct, $P(A|e)$ (i.e. the probability of analyte being present given evidence e).

$$P(A|e) = \frac{P(A \cap e)}{P(e)} = \frac{P(A)\cdot P(e|A)}{P(e)} \quad (1)$$

where in the second term of the equation $P(A \cap e)$ is the probability of the simultaneous occurrence of A and e (i.e. the probability that the analyte presence agrees with the evidence e), and $P(e)$ is the probability of e being observed in the studied matrices population. The $P(A|e)$ is the portion of cases where given an evidence e, A is in fact present. For instance, if 498 matrices with and 474 matrices without analyte A are tested for the presence of analyte and the identification results are the ones summarised in Table 1, the probability an evidence e being correct, $P(A|e)$, is 92.5% ($0.925 = 0.457/0.494 = 444/480$). Fig. 1 presents a graphical representation of results from Table 1.

The $P(A \cap e)$ can also be estimated as the probability of the simultaneous occurrence of two independent events: the analyte presence, $P(A)$, and the evidence of the presence, e, when analyte is present, $P(e|A)$, (i.e. the probability of the evidence e given A; also known as the true positive rate, TP). The probability of the simultaneous occurrence of two independent events is estimated by multiplying respective probabilities, therefore $P(A \cap e) = P(A) \cdot P(e|A)$ (see Eq. (1)). In the example of Table 1 and Fig. 1, $P(A)$ and $P(e|A)$ are estimated from the same sample of 972 items ($P(A)+P(\neg A) = P(e)+P(\neg e) = 972$) but both independent probabilities (i.e. $P(A)$ and $P(e|A)$) can be estimated from different sources of data. The $P(e|A)$ is the portion of A cases where e is observed and the equivalence $P(e|A) = P(A \cap e)/P(A)$ can be used to convert the third in the second term of Eq. (1).

Eq. (1) can also be described by the updating of available prior test information with additional one. The $P(A)$ is designated *a priori* probability since it represents the probability of A occurrence independent of identification test outcome. After updating $P(A)$ with information about the probability of an evidence e being produced by the presence of A ($P(e|A)/P(e)$) (Eq. (2)), the *a posteriori*