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# Current issues involving screening and identification of chemical contaminants in foods by mass spectrometry

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ABSTRACT

Although quantitative analytical methods must be empirically validated prior to their use in a variety of applications, including regulatory monitoring of chemical adulterants in foods, validation of qualitative method performance for the analytes and matrices of interest is frequently ignored, or general guidelines are assumed to be true for specific situations. Just as in the case of quantitative method validation, acceptable method performance criteria should be established for qualitative analysis purposes to suit the analytical needs for given applications, and empirical method validation should be conducted to demonstrate the qualitative performance capabilities the method. This critical review article is intended to describe and discuss recent developments with of respect to qualitative aspects in mass spectrometry, and to make recommendations for validation of qualitative methods that meet common needs for monitoring of chemical contaminants in foods.

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## 1. Introduction

In 2008, Lehotay et al. wrote an extensive critical review article about the "identification and confirmation of chemical residues in food by chromatography-mass spectrometry and other techniques" [1]. In the article, the authors highlighted many aspects pertaining to qualitative analysis by mass spectrometry (MS) coupled to chromatography, and, to gain a better understanding of the issues, we suggest that the reader should refer to that article and its citations before reading the updated information in this article. We only refer to a few papers cited in the previous *TrAC* article, and instead, our aims in writing this critical review article include to:

1) discuss qualitative aspects for screening, identification, and confirmation using MS-based techniques;
2) review and suggest practical qualitative method validation guidelines and acceptable performance criteria; and,
3) summarize and update recent developments in the literature related to analysis of food contaminants.

For the sake of brevity, we present only applications involving targeted analytes and contemporaneously analyzed reference standards. Analysis of unknowns and non-targeted compounds is a much more complicated subject, but our view is that all identifications require comparison to a reference standard of the analyte run at the same conditions within the same sequence. Although we mainly discuss regulatory analysis applications involving food contaminants, this article may also be appropriate for similar regulatory applications in other fields, such as environmental, clinical drug testing and forensic analyses.

### 1.1. Indications, determinations, identifications and confirmations

Analytical chemists should take care to use defined terms in qualitative analysis, and a few essential terms are worth refining from the 2008 critical review [1]. Table 1 defines some terms as we use them in this article. Screening methods tend to provide yes/no responses that provide little quantitative value, and we use the term "indication" to express results from qualitative screening analyses. The quality of the screening method is assessed by the rates of false-positive and false-negative indications depending on purpose-defined acceptability criteria (e.g., ≤10% false negatives at an action level with ≤5% false positives for blanks or other designated analyte concentrations in matrices of interest). In the case of quantitative results (reported concentrations) that lack sufficient qualitative assessment (e.g., gas chromatography with element-selective detection), then we use the term "determination."

Qualitative "identification" involves detection by selective MS-based techniques that yield highly diagnostic information (e.g., molecular formula, structural features) for targeted analytes, and that meet defined criteria expected to minimize false positives (e.g., 5%, 1%, 0.01%, or lower, depending on the purpose of the analysis). The distinction between indication and identification by MS analysis depends on the criteria to be met, which typically relate to the selectivity of the method, quantitative aspects, and the type/degree of validation required. For current purposes, we use chromatographic retention time ($t_R$) as a criterion for analyte identification, but other measurable parameters that exclude chemical interferences may be demonstrated to be equally (or more) justifiable.

Table 2 lists current identification criteria used by several different organizations for MS-based techniques coupled to gas and liquid chromatography (GC and LC).

A frustratingly common mistake in scientific reports and oral communications is the treatment of "identification" and "confirmation" as interchangeable terms, which they are not. By definition, "confirmation" requires agreement in the results of at least two analyses of the same sample, preferably involving re-analysis of a different test portion using different chemical mechanisms (orthogonally selective) in sample preparation and/or analysis. We ask the reader to please make those distinctions in their communications, and we stress the importance of fit-for-purpose considerations in all respects [15]. For example, we add the caveat for regulatory purposes that confirmation must include both identification and determination for an analyte, so indication and determination are insufficient.

### 1.2. Detectability, limit of identification and reporting level

Another important, but frequently confused, term in analytical chemistry is "sensitivity", which is often used interchangeably with regards to "limit of detection" (LOD). By definition, "sensitivity" is the relationship between response *versus* analyte amount or concentration, and in practice, sensitivity is the slope of the calibration curve. This does not account for noise or selectivity, whereas LODs depend on signal/noise (S/N) ratios. Thus, higher sensitivity does not necessarily lead to lower LODs. Rather than using "sensitivity" in relation to "detection limit," we prefer the subjective term "detectability," and we invite the reader to refer to previous discussions on this topic [16,17].

Mass spectrometrists have learned not to trust analytical figures of merit or instrument-performance specifications based solely on sensitivity, or even S/N ratios in the absence of real-world matrix components. For example, the use of solvent-only standards in the

**Table 1**
Definitions of several terms used in this article

| Term | Definition |
|---|---|
| Confirmation | Agreement in result(s) from at least two analyses, preferably involving different sample test portions and chemical mechanisms in sample preparation and/or analysis. |
| Detectability | Subjective term to express the relative ability of a method or technique to achieve low detection limits (high S/N ratios). |
| Determination | Result (concentration) from a quantitative method of analysis. |
| Identification | Qualitative result from a method providing structural information (e.g., MS) which is in agreement with contemporaneous reference data of the analyte and meets acceptable criteria for the purpose of the analysis. |
| Indication | Qualitative yes/no result in a screening method. |
| Reporting Level (RL) | Fit-for-purpose threshold concentration above which the laboratory reports the presence of an analyte in the sample. |
| Screening | Qualitative approach that yields a result to indicate the presence or absence of the analyte(s) with respect to a threshold level. |
| Selectivity | Subjective term to express the relative ability of a method or technique to distinguish the analyte(s) from other chemicals. |
| Sensitivity | Signal of an analyte *vs.* its concentration or amount. |
| Specificity | Theoretical ability of a method or technique to distinguish the analyte with 100% confidence from all sources of noise. |
| Spurious Error (Gross Error) | Non-random and non-systematic errors, predominantly due to human mistakes, including decision-making. |
| Limit of Detection (LOD) | Concentration at which S/N = 3, preferably for the weakest diagnostic ion in MS-based methods. |
| Limit of Identification (LOI) | Lowest concentration at which the identification criteria for a method of analysis are met. |
| Limit of Quantification (LOQ) | Concentration at which S/N = 10 for quantification ion. |