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Guidelines for LC–MS identifications of flavouring substances in nature, made by the Working Group on Methods of Analysis of the International Organization of the Flavor Industry (IOFI)

IOFI Working Group on Methods of Analysis*

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The IOFI Working Group on Methods of Analysis (WGMA) evaluates the validity of identifications of flavouring substances in nature, and has developed and published guidelines to assist authors and reviewers of publications concerning such identifications.[1] These guidelines were limited to gas chromatography coupled with mass spectrometry (GC–MS), because most of the flavour molecules discovered until recently were sufficiently volatile to be analysed by this technique.

In recent years more and more molecules that are of higher molecular weight or more polar character, and which have potential use in flavourings, have been found in nature. Hence, liquid chromatography coupled with mass spectrometry (LC–MS) is becoming a routine technique in flavour research. The WGMA therefore felt that it had become necessary to develop additional guidelines for the use of LC–MS and to define criteria for valid identifications using this technique.

LC–MS generates highly method-dependent information. There are various MS experiments that generate different MS spectra according to the experimental set-up. Most often only a few ions are observed, which makes the analyte identification more difficult than in the case of GC–MS in scan mode.

Since an ion has a given occurrence probability in an MS spectrum,[2] the simultaneous occurrence of several ions in a spectrum has a lower probability, and then a higher specificity. Matching the abundance ratios of a target spectrum also increases the specificity of the analyte recognition. This is the basis of the 'identification point' (IP) concept that has also been extended to the specificity of MS^n transitions, exact mass measurements, etc.

LC–MS on its own is generally inappropriate to yield a sufficient number of IPs. In addition, in contrast to GC–MS, the retention time in LC–MS is not a selective criterion, due to the lower resolution of conventional LC compared to GC. Therefore, it cannot currently count for an IP, but is nevertheless a prerequisite to any LC–MS identification.

The detection of compounds occurring in trace amounts does not always allow the recording of full MS spectra (e.g. SIM detection). Therefore, their positive identification requires collecting a minimum of four IPs, according to the Commission Decision, dated 12 August 2002, of the European Communities.[3] This is a minimum requirement and will be evaluated on a case-by-case basis by plausibility. Whenever possible, more than four IPs will be preferred, as it will strengthen the analyte identification. Below are some recommendations corresponding to the most frequent cases related to flavour analyses, and a practical case embodying these can be found in a recent publication.[4]

For low-resolution MS, MS–MS spectra of the unknown should be compared to those of the authentic compound, taking into account the following criteria.

1. Choice of the Transition

The choice of the parent ion, and especially the transition between precursor and product ions, should be structurally characteristic and preferably unambiguous for the molecule under investigation and should be explained in the text. Guidelines are given in Tables 5 and 6 of the Commission Decision mentioned above, and some examples are given below.

Examples (Non-exhaustive List)

Triple quadrupole.

- 1 precursor ion + 2 product ions + 1 ratio (product 1:product 2) → 4 IPs.
- The precursor:product ratio should not be used due to possible interferences of isobaric ions with the precursor.

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Ion-trap.

- *Either* a full spectrum from an MS–MS experiment exhibiting at least 1 precursor + 2 product ions + 1 ratio → 4 IPs.
- *Or* an MS–MS–MS experiment: 1 precursor + 2 product ions from MS–MS + 1 product ion from MS–MS–MS + 1 ratio (product ion:precursor) → 5.5 IPs.

Triple quadrupole linear ion-trap.

- *Either* a full spectrum from an MS–MS experiment exhibiting at least 1 precursor ion + 2 product ions + 1 ratio → 4 IPs.
- *Or* an MS–MS experiment: 1 precursor + 2 product ions + 1 ratio (product ion : precursor) and an MS-MS-MS experiment: + 1 product ion from MS-MS-MS → 5.5 IPs.

Hybrid systems including a high resolution MS [resolution > 5000 FWHM (full width at half maximum)].

- 1 precursor in low resolution + 2 product ions in high resolution + 1 ratio → 6 IPs.

Note: Two transitions measured on the same LC peak, one in positive mode, another in negative mode, can be considered (five IPs). In such a case, the switching time between both modes must be quick enough to achieve both measurements in a significant part of the LC peak. Alternatively, the sample may be injected twice in positive and negative modes, if the LC system provides a strict repeatability of retention times.

2. Tolerances for the Abundance Ratios

Relative intensity (% of base peak)	Accepted deviation (%)
>50	± 20
20–50	± 25
10–20	± 30
<10	± 50

In the case of quantitative analysis with triple quadrupoles or ion-trap detectors, the limit of detection should be adjusted on the less sensitive transition. Quadrupoles should be set at a unit mass resolution.

3. Blanks

To ensure that no cross-contamination has given rise to a false positive, blank experiments must be performed as much as possible (e.g. using the same matrix or a very similar matrix free of the target compound):

- A blank experiment should be performed before and after any positive experiment to show the absence of the target compound.
- Conversely, a positive experiment at the limit of detection should be performed before and after any negative experiment.

If such a 'blank' matrix does not exist, the analyst is invited to provide all necessary evidence that there was no possible contamination.

Members of the WGMA (March 2009)

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