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IOFI recommended practice for the quantitative analysis of volatile flavouring substances using coupled gas chromatography/mass spectrometry with selected-ion monitoring (SIM)

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ABSTRACT: The present recommended practice concerns the quantification of volatile flavouring substances by coupled gas chromatography/mass spectrometry with selected-ion monitoring (SIM). This technique is especially suitable for unresolved peaks in classical gas chromatography (e.g. quantification in a complex chromatogram) and is more sensitive than the use of the mass spectrometer in the full-scan mode. Copyright © 2012 John Wiley & Sons, Ltd.

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Principle

Instead of recording a full scan of ions as does a mass spectrometer in conventional applications, in the selected-ion monitoring (SIM) mode only a few selected ions are monitored for each peak. The analyser successively allows each chosen mass to reach the detector for a short duration (the 'dwell time'), and chromatograms based on these selected ions are obtained. The resulting peaks are then integrated to give quantitative results, using the selected ions of a suitable internal standard.

Equipment

Gas chromatograph equipped with a capillary column according to the IOFI guidelines for the quantitative gas chromatography of volatile flavouring substances.[1]

Mass spectrometer equipped with a quadrupole or a magnetic analyser; the use of ion-trap detectors for quantification should be avoided. Inter-laboratory testing has shown that results from the latter are unreliable for the quantification of volatile flavouring substances.

A computer control for the system, equipped with suitable software for integration of the chromatographic peaks.

Reagents

For quantification with a stable-isotope labelled molecule, the isotopic purity of the internal standard must be accurately known.

For quantification using non-labelled internal standards, their purity must be at least 98%.

Procedure

Choice of the Internal Standard(s)

Three possibilities are described, in decreasing order of applicability.

A stable isotopic isomer (isotopomer) of the molecule to be quantified. The molecule can be labelled with one or several atom(s) of deuterium, carbon-13, nitrogen-15, etc. When labile hydrogen atoms exist in the molecule, any possibility of D/H exchanges must be checked and avoided.

A molecule having one or several ions in common with the molecule(s) to be quantified (e.g. an isomer, or a compound in a homologous series). It must elute in a region of the chromatogram where the selected ions are not subject to interference with those from other molecules.

A molecule without an ion in common with the one under study. This method will require additional ion switching during the data acquisition.

In each of the three cases above, the sample must be checked for the absence of molecules having ions in common with the internal standard in the area of its elution. Furthermore, the molecule and the standard should ideally have retention times in close proximity.

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Add the internal standard to the sample before subsequent preparation or concentration, in order to account for losses which may be incurred during these steps.
When multiple analytes are concerned in the same gas chromatography (GC) run, covering a range of elution times and responses, more than one internal standard may be necessary.

Choice of the Ions to Be Monitored

Choose abundant ions in the mass spectrum. Preferentially monitor uncommon ions in order to optimize the signal-to-noise ratio.

Monitor simultaneously at least three ions for the internal standard(s) and for each of the compounds to be quantified. One ion is the quantifier, the others are the qualifiers. The ratios of each individual qualifier-ion abundance (not their sum) to that of the quantifier ion for a given molecule are essential to confirm its identification. Avoid the simultaneous monitoring of numerous ions which decreases the dwell time for each of them and reduces the measurement accuracy. It is preferable to monitor fewer ions at any one time and switch to another group of ions before another compound of interest elutes.

Mass Spectrometer Parameters

The ionization mode can be electron impact, negative or positive chemical ionization. The two latter modes produce less fragmentation and more abundant ions at higher masses, and hence often lead to a better signal to noise ratio.

Optimize the mass spectrometer tuning for SIM analysis.

If a high sensitivity is required, decrease the resolving power between two adjacent mass peaks (so-called de-resolve mode). If an isotopomer only differs by 1 Da from the unlabelled molecule (one D, ^{13}C or ^{13}N, etc.) de-resolving can lead to mass-peak overlapping and should be avoided. This will decrease the selectivity. For a resolving power of 1000 and masses less than 500 Da, choose a 0.5 Da mass window.

Adjust the dwell time to obtain at least 10–12 data points for each ion for a given chromatographic peak.

Since the scanning mode does not allow sufficient detection time for each mass, the extraction of selected ion chromatograms from a full scan acquisition should not be used in quantification due to poor accuracy and low sensitivity.

The possibility provided by some instrument manufacturers of performing SIM/scan simultaneously should be used with care, since the number of data points in SIM mode may be insufficient over an eluting peak.

Results

Peak Identification

To avoid confusion with other peaks (especially in trace analysis), the peak identity must be verified in each quantitative analysis by using two criteria:

First, the comparison of the analyte retention time to that of calibration solutions of authentic standards, or using its retention index

Second, the comparison of the abundance ratios between at least three ions per analyte with the corresponding ratios of a reference compound recorded on the same instrument and using the same conditions; this comparison can be achieved in two ways:

- using the tolerances recommended by the European Union:[2]

Relative intensity (% of base peak)	% Tolerance (relative)
>50	±10
>20 to 50	±15
>10 to 20	±20
≤10	±50

- using the calculation of the *Q*-value proposed by one instrument manufacturer:[3]

$$Q = 100 - \frac{\sum_{i=1}^{i=n} \left(100 \times \left|r_i - r_i'\right|\right)\left(\ln[100r_i + 1]\right)^2}{21.3 \times \sum_{i=1}^{i=n} r_i}$$

The *Q*-value should be higher than 89 for a positive recognition of the peak.

Quantitative Determination

All selected-ion chromatograms are integrated as for a normal chromatogram. Quantities are calculated from the peak areas according to the IOFI guidelines for the quantitative gas chromatography of volatile flavouring substances.[1]

When an isotopomer of the molecule to be quantified is used as an internal standard, the area of the latter could be altered by the natural abundance of the non-labelled molecule (e.g. normally the abundance of unlabelled vanillin at *m/z* 153 is about 8.8% of the *m/z* 152 ion. If the internal standard is only labelled with one carbon-13, the area of its molecular ion must be corrected for the *m/z* 153 abundance of the unlabelled molecule). This correction may be computed or determined from a standardization curve.

Expression of Results

See IOFI guidelines for the quantitative gas chromatography of volatile flavouring substances.[1] Calculate the levels of the individual analytes by reference to the calibration lines obtained from the calibration levels described above, making sure that the result falls within the range of the calibration used, taking account of dilution factors. Express the result in terms of mg/kg of the flavouring or raw material. For each substance and each sample, the values of the recognition criteria should be reported or be available for inspection.

Members of the WGMA

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