Recommended Practice

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Guidelines for the quantitative gas chromatography of volatile flavouring substances, from 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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Introduction

The present guidelines concern the investigation of flavouring substances by gas chromatography (GC), with the objective being the quantitative determination of specific volatile substances in flavourings and other complex mixtures such as essential oils. Limits of detection and of quantification are not discussed in these guidelines.

Principle

Quantitative data for individual volatile substances or whole aromatic fractions, contained in foodstuffs or flavourings, are calculated based on comparisons between GC signals. The signal of a known quantity of standard (internal or external, or addition of the analyte itself) is used to determine the level of the analyte.

Quantitative data can be obtained from essential oils, solvent extracts, headspace sampling (static or dynamic), or sorptive extraction, e.g. solid-phase micro-extraction (SPME). It should be borne in mind that sample preparation techniques influence the recovery of an analyte.

In the case of indirect headspace injection (e.g. SPME, dynamic headspace), results need to be examined critically and interpreted according to the many variables in the experimental parameters. Guidelines for the use of SPME in the analysis of volatile flavouring substances have recently been published.[1]

The following requirements should be met with respect to the analyte to be measured:

- No irreversible adsorption on the stationary phase
- No degradation during measurement
- No co-elution with other analytes (no interfering detector signal)
- Separation at the baseline from other peaks.

Equipment

Appropriate Gas Chromatograph

Injectors

Splitless or on-column injectors are recommended, especially for headspace determination. Particular precautions must be taken with injection in split mode, as it is known that quantitative discriminations between analytes may be introduced.

The appropriate injection liner should be used, depending on different parameters, mainly the injection mode and the nature of the solvent.

Detectors

Different types of detectors (selective or not) can be used to generate signals. Relationships between detector responses and analyte quantities must be known and be precisely controlled before measurements, within the range of use, are made. The most common detectors are non-selective, e.g. the flame ionisation detector (FID) and thermal conductivity detector (TCD).

Selective/specific detectors can also be used, such as the flame photometric (FPD), electron capture (ECD), nitrogen phosphorus (NPD), mass spectrometric in selected-ion mode (SIM), chemiluminescence and atomic emission (AED) detectors.

Computers equipped with software for GC or GC-MS data treatment are commonly used. Integration needs to be performed on peaks separated at the baseline. Measurement of peak heights is not recommended for the generation of quantitative data.

Columns and carrier gas

Columns should be made of suitable inert material, e.g. fused silica. The most commonly used phases are polysiloxane or polyethyleneglycol derivatives, but other phases can be used. The carrier gas, preferably helium or hydrogen, should be highly purified and free from oxygen, water and hydrocarbons.

Chromatographic parameters

It should be possible to operate the column either under a constant temperature and/or pressure or under a temperature and/or pressure program. The operating temperature range must be within the supplier's recommendations for the column in use.

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Reagents

Choice of Solvent

The choice of solvent depends on factors such as sample matrix, anticipated interference with analytes (e.g. formation of acetals from carbonyl compounds if ethanol is used as solvent), chromatographic performance, etc. Final dilutions of calibration solutions and of the sample solution must be carried out in the same solvent.

Standards

Requirements for standards

Standards should be of known purity as determined by appropriate techniques. By definition, non-volatile impurities should be absent, and any volatile impurities should be taken into account in the calculations.

Stability of standards

Standards need to be stable within the range of chromatographic temperatures. They must not react chemically with the stationary phase or with any other part of the chromatographic system, nor with any of the analytes, and they must be soluble in the solvent in which the analyte is dissolved.

Quantification

Determination of the Linearity Range

Quantification must be made within the linear range of response of the analysing system, the limits of which should be determined experimentally. Whereas integration systems have wide ranges, some specific detectors have limited linearity (e.g. flame photometric detectors). During method development and the construction of the calibration lines, the analyst must ensure that in the case of the external standard method, the analyte levels do not fall outside these linearity limits. Likewise, both analyte and internal standard concentrations must be within these limits for the internal standard method to yield valid results.

As a measure of the calibration-line linearity, the coefficient of determination, R^2, obtained by regression analysis, should be equal to or greater than 0.995.

Absolute Response Factor, *R*

The absolute analyte response factor, R_a, for any compound a is defined as:

$$R_a = \frac{A_a}{m_a} \tag{1}$$

where m_a is the mass of compound a, and A_a is the corresponding peak area of compound a.

External Standardization

A set of standard solutions containing solely the analyte at known concentrations in a suitable solvent (Choice of Solvent) is prepared, and from these solutions a series of corresponding chromatograms is obtained. From the peak areas of the analyte, a calibration line relating peak areas and concentrations is then constructed. It is essential that the range of concentrations covers the expected levels of the analyte in the sample matrix. Dilution of the sample may be necessary to achieve this.

A chromatogram of the sample is then obtained under identical conditions to those used to run the standards. Particular attention must be paid to the reproducibility of the injection volume, and the use of an auto-sampler is essential. In fact for syringe injection internal standardization is preferable to external standardization, although the latter is very useful for some other injection techniques, such as SPME.

The following expression is used for obtaining the result from a single-point calibration, otherwise the result may be obtained directly from the calibration line:

$$c_a = \frac{c_{std} \times A_a}{A_{std}} \tag{2}$$

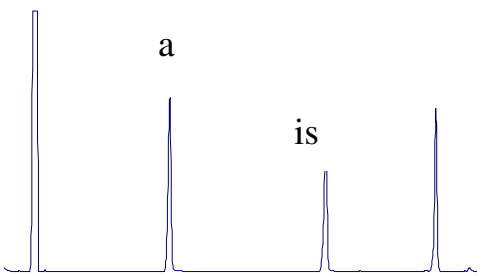
where

c_a is the concentration of analyte a in the sample
c_{std} is the concentration of analyte a in the standard chromatogram
A_a is the area of analyte a from the sample chromatogram
A_{std} is the area of analyte a from the standard chromatogram.

Internal Standardization

The internal standard must be absent as a component of the samples to be measured except in the special case of the standard addition method where a component peak may be assigned this function (see Standard Addition Method). In general, the internal standard should be similar in physico-chemical properties (e.g. boiling point and polarity), particularly in functional group type, to the analyte but must not co-elute with it or another component of the sample. When specific detectors are used (e.g. GC/MS in SIM mode) co-elution is permissible and the use of an isotopically labelled analogue of the analyte as a standard is ideal.[2]

Calculation of the relative response factor, RRF



From equation 1:

$$\text{RRF} = \frac{R_a}{R_{is}} = \frac{A_a}{A_{is}} \times \frac{m_{is}}{m_a} \tag{3}$$

where:

m_a is the (known) amount of analyte a in a standard solution
m_{is} is the amount of internal standard (is) introduced into the standard solution
A_a is the area of analyte a peak from the chromatogram
A_{is} is the area of internal standard is from the chromatogram.

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For an actual sample, after addition of the internal standard, the amount of analyte in the sample is given by:

$$m_a = \frac{A_a}{A_{is}} \times \frac{m_{is}}{RRF} \quad (4)$$

In practice, it is usual to construct calibration lines for each analyte using a series of solutions of varying nominal concentrations containing each analyte. An identical amount of internal standard is added to each solution and the corresponding chromatograms obtained.

Calibration is achieved by plotting the ratio of analyte peak area to internal standard peak area against analyte concentration.

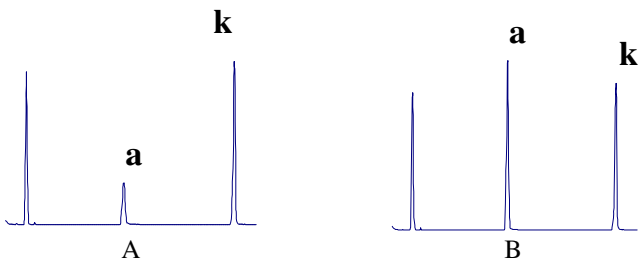
The percentage of analyte a in the sample is calculated as follows:

$$\%\ \text{analyte} = \frac{m_a}{m_{sample}} \times 100 \quad (5)$$

where m_{sample} is the mass of sample taken before addition of the internal standard.

Standard Addition Method

This method is of use if the analyte is present at relatively low levels in a complex mixture. Although the retention time of the analyte may be known, matrix effects could cause uncertainty in the identification of the analyte peak.



1. A chromatogram (A) is obtained of the sample and the analyte peak, a, is identified if possible. A second peak, k, is chosen as a reference and both peak areas noted. This reference peak should be close to and of similar area to the analyte. If an existing reference peak is not available then a suitable internal standard may be added.
2. A measured amount of pure analyte is added to a known amount of the test sample, in such a way that dilution of the sample is negligible.
3. A second chromatogram (B) is obtained from the mixture described in step 2; the addition of pure analyte will result in an increase of the peak area of the analyte to be quantified, without significant change in its retention time.
4. The steps 2 and 3 may be repeated by the further addition of pure analyte to the sample to afford sufficient data for a graph of response against peak-area ratios to be drawn.
5. The amount of analyte a in the original sample may then be determined by reverse extrapolation.
6. Alternatively, for a single addition of analyte, the approach given in ISO standard 7609 may be used.[3]

Internal Normalization

This is a general approach to the quantification of complex (or simple) chromatograms where it is unnecessary or impossible to use an internal standard method.

The use of internal normalization, or the expression of results as area% (see below), may lead to the over-estimation of the analytes in a mixture, if material not recorded by the detector is present. Only the internal-standard method gives a reliable figure for the true concentration of an analyte.

If the response factors of all analytes in the sample can be determined, the following equation can be applied, making use of the respective absolute response factors, R:

$$\%\ \text{analyte} = \frac{R_a A_a}{\Sigma(R_i A_i)} \times 100 \quad (6)$$

where $\Sigma(R_i A_i)$ is the sum of the all the 'corrected' peak areas in the chromatogram.

Expression of results as area%

If not all response factors can be determined, the following simplified expression for the percentage of analyte a can be used, which assumes all response factors to be unity:

$$\%\ \text{analyte} = \frac{A_a}{\Sigma A_i} \times 100 \quad (7)$$

where ΣA_i is the sum of all the peak areas in the chromatogram.

In this last case, the results should not be considered as quantitative in absolute terms.

Report

The report should contain at least the following information:

- identification of the sample
- the details of the method used
- the result(s), expressed with a number of significant figures corresponding to the precision of the method
- the purity of the standards
- the units, as appropriate: abbreviations such as ppm and ppb should not be used
- any deviations from the procedure
- any unusual features observed
- the date of the test.

Members of the WGMA

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References

1. IOFI. Guidelines for solid-phase micro-extraction (SPME) of volatile flavour compounds for gas-chromatographic analysis. *Flavour Fragr. J.* **2010**, *25*, 6.
2. IOFI. Analytical procedure for a general quantitative method using coupled capillary gas chromatography/mass spectrometry with selected ion monitoring (SIM). *Z. Lebensm.-Unters.-Forsch.* **1997**, *204*, 395.
3. ISO. Essential oils – Analysis by gas chromatography on capillary columns – General methods. International standard ISO 7609, International Organization for Standardization, Geneva, **1985**.

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