Quantifying the constituents of flavours, fragrances and essential oils

Technical Editorial
Quantifying the constituents of flavors, fragrances and essential oils

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Abstract
This technical editorial provides the authors with up-to-date information to assure the scientific reliability of quantitative data when preparing a manuscript. It summarizes the key points of procedures that have been previously detailed in the Guidelines and Recommended Practices cited hereafter. The reader is warmly encouraged to refer to these papers published in open access.

1. Choice of a sample preparation technique

The sample preparation / clean-up procedure must compatible with a quantification. *Pro memoria*, an experimental calibration is mandatory when using solid-phase microextraction (SPME), stir-bar sorptive extraction (SBSE), headspace-sorptive extraction (HSSE) and purge-and-trap headspace (P&T-HS).

2. Identification

Before quantification, the analytes should be unambiguously identified (see the related Technical Editorial[1]).

3. Quantification of volatile constituents

In general, the quantification of volatile constituents is achieved by a gas chromatograph (GC) or by a comprehensive two-dimensional gas chromatograph (GCxGC) equipped with a flame-ionization detector (FID) or a mass spectrometer (MS) operating in electron impact mode. Specific detectors are occasionally used, such as a flame photometric detector (FPD), or a thermal conductivity detector (TCD). The detector has to be used within its response linearity range[2].

3.1. Area percentages and semi-quantification

With an MS detection, the area percentages and the semi-quantification (*i.e.* all relative response equal to unity) factors are not repeatable over time. They are not accepted in this Journal.
With a flame ionization detector, the area percentages and the semi-quantification are inaccurate and therefore discouraged, except when comparing the composition of samples from identical matrices, submitted to the same sample preparation and under the same analytical conditions.

3.2. Experimental calibration

The most accurate quantification procedure is by calibrating the gas chromatograph with solutions of pure standards at known concentration. .

3.2.1. Liquid sample

When injecting a liquid sample, the quantification can be performed by external or internal standardization, internal normalization or standard addition, as described in the dedicated Recommended Practice[2]. External standardization is less accurate than the three other techniques and not recommended for a liquid injection with a manual syringe.
In case of a SPME or SBSE sampling, the use of an internal standard is inappropriate for GC-FID: an external calibration of each constituent in the same matrix as that of the investigated compound(s), is necessary. Quantification with GC-MS in liquid samples can also be carried out with an isotopically-labelled internal standard of each investigated constituent (stable

isotope dilution assay, "SIDA") after liquid injection, direct or HS-SPME-, SBSE- or HSSE-, P&T-HS- injection[3].

The GC-MS quantification by selected-ion monitoring (SIM) is described in another Recommended Practice[4]. With recent MS instruments, quantification in scan mode is also possible.

3.2.2. Headspace

Quantifying exclusively the constituents present in the headspace requires a calibration in the gas phase, which needs a complicate experimental set-up and falls outside the scope of the present Technical Editorial.

GC patterns after HS-SPME or HSSE sampling are not representative of the headspace composition.

Quantifying the volatiles constituents of a matrix can be achieved by HS-SPME, HSSE or P&T-HS, after an external calibration in the same matrix free of the investigated analyte, under the same sampling time, temperature and stirring rate[5]. The use of an internal standard is improper, except when isotopomers corresponding to each investigated analyte to be quantified are available. As an alternative, an "in-fiber standardization" can be used[6].

3.3. Rapid quantification

The volatile constituents can rapidly be quantified using predicted response factors relative to an internal standard. This approach is described in a specific recommended method[7], and does not require the authentic substances and any experimental calibration. It is exclusively applicable to GC-FID, with a mean accuracy of about 6%.

4. Quantification of non-volatile compounds

The analytes are usually separated using a liquid chromatograph (LC). They can be identified by a MS detector prior to quantification using a method suitable for spectra exhibiting only a few diagnostic ions (Recommended Practice[8]). However, their identities must be confirmed in the course of the quantification using the signal of the quantification run:

- UV/Vis detection: single wavelength detectors are not suitable to confirm the analyte identity. The full UV/Vis spectrum of the analyte should be compared to that of a reference substance, recorded under the same elution conditions, and using an appropriate algorithm. In general, such a detector is not suitable for low/trace concentrations.
- LC/MS spectra only exhibit a few ions, and their identification using the "match quality factors" generally applied for full scan-spectra is unsuitable. The use of the "identification points" concept is thus highly recommended[8,9].

WIth LC techniques, an external calibration provides a good accuracy. In case of a MS detection, the risk of matrix effect (ion suppression) has to be investigated.

5. Quantification of regulated constituents

Developing a quantification method for regulated constituents, such as allergens or bioactive aroma constituents, deserves a special attention because such results may impact the consumer health, and/or the compliance with the existing flavor and fragrance regulation.

- Experimental calibration using authentic substances (purity to be checked[10])
- Checking the identity of each analyte of the quantification run, using the identification points (IPs) or a similar technique[8-10]. The identity confirmation has to be performed using the data of the quantification run
- The reliability of the method should be evaluated using a validation procedure[10]. Validation may also be published in a separate article.

Variability

The composition of flavors, fragrances or essential oils determined from a single batch from a natural source (food, plant) has little interest, because of the intrinsic variability of natural products. Reporting the variability should take into account sampling and instrumental factors. The authors are therefore encouraged to analyze several batches and provide standard deviations to account for the range of variation of the different constituents. Whenever possible, the quantitative results should be submitted to a statistical analysis to correlate the variations with the parameters responsible for them.

The present Editorial only represents a brief overview of quantification techniques and does not report all possible alternatives (e.g. multiple headspace extraction, direct-injection mass spectrometry, etc.). FFJ editors will evaluate the manuscripts on a case-by-case basis.

Abbreviations

FPD	Flame photometric detector
GC	Gas chromatograph(y)
GCxGC	Comprehensive two-dimensional gas chromatograph(y)
HSSE	Headspace-sorptive extraction
LC	Liquid chromatograph
MS	Mass spectrometer
P&T-HS	Purge-and-trap headspace
SBSE	Stir-bar sorptive extraction
SIDA	Stable isotope dilution assay
SIM	Selected-ion monitoring
SPME	Solid-phase microextraction
TCD	Thermal conductivity detector
UV/Vis	Ultra violet / Visible

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