Standard Gas Addition: A Calibration Method for Handling Temporal Drifts of Mass Spectrometry-Based Sensors

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This work describes a new method for the correction of signal drift of a MS-based sensor by standard gas addition (SGA). It consists of introducing a gaseous He–Xe mixture continuously and independently of the carrier gas into the mass spectrometer source. To mimic the disturbances generated by periodical tuning of the mass detector, three series of adjustments were made to the main acquisition parameters. The influence of the SGA signal correction on the discriminating power of the data was evaluated from the analysis of three batches of cheeses by dynamic headspace coupled to mass spectrometry. SGA afforded a good correction of the main types of drift classically observed in mass spectrometry.

The use of rapid characterization systems based on fingerprint recognition relies heavily on the availability of robust, permanently valid databases. Robustness strongly depends on the experimenter (e.g., choice of large enough representative samples), whereas the permanence of databases is often undermined by the instability of instrument performance. New systems of characterization by mass spectrometry (MS-based sensors) have recently shown promise for rapid analysis, in particular in the agrifood sector. However, the question of signal stability still arises for such systems, as signal drift can occur for many different reasons during the life of the mass detector, for example: (i) gradual fouling of the source; (ii) maintenance operations (e.g., opening and cleaning of the source, changing of the electro- or photomultiplier, replacement of the filament and repeller); (iii) impaired vacuum quality (quality of the carrier gas, introduction of too much material, etc.)

Depending on its origin, the drift can be gradual or sudden, linear or nonlinear, and very often difficult to predict. Thus, although various tuning procedures exist, if the signal goes uncorrected during a campaign of analyses, the resulting data are weakened or even made valueless. Accordingly, it is important to have procedures to monitor the state of the mass detectors and help correct drift.

Little work has so far been published on these issues. In a recent study, Marsili advocate using an internal standard to overcome serious instrument problems in solid-phase microextraction-mass spectrometry (SPME-M). However, although the use of such a standard has proved effective for milk samples, it is difficult for the analysis of solid samples. Haugen et al. proposed a correction algorithm to rectify the drift observed with gas sensors. In practice, this method is achieved by inserting reference samples at regular intervals during the analysis sequence. The variation in the response to these references makes it possible to characterize the drift, which in this case was modeled by a linear function. This method has produced satisfactory results and is readily applicable to rapid mass spectrometry characterization systems generally. However, the procedure entails carrying out an increased number of analyses (generally 3 reference samples for 8–10 test samples) and can be disturbed by memory effects between successive analyses. Last, Goodacre and Kell proposed a method of rectification by neuron networks for the correction of drift in Curie point pyrolysis-mass spectrometry spectra. Reference samples were analyzed at two different periods to set up a neuron network that would then correct the potential drift of the signal of each mass fragment between these two periods. This method proved satisfactory for lysozyme assay in glycerol. However, like the preceding method it requires analysis and management of reference samples, which is sometimes difficult, especially in the agrifood sector (choice of a reference sample, physical and chemical stability, storage, etc.).

Here, a new method for the correction of signal drift of a MS-based sensor by standard gas addition (SGA) is described. The type of MS-based sensor used in this work comprised a dynamic...
headspace device coupled to a mass spectrometer (DHS-MS). To mimic the disturbances generated by periodical tuning of the mass detector, three series of adjustments were made to the main acquisition parameters. The three tuning operations carried out induced variations in the sensitivity of the mass detector. The influence of the signal correction by SGA on the discriminating power of the DHS-MS was evaluated from the analysis of three batches of Camembert-type cheeses.

**MATERIALS AND METHODS**

**Origin and Storage of Products.** The three cheeses analyzed were commercially available products of the “Camembert” type (ripening time 30 days). Two of the cheeses were made from raw milk (Rm1 and Rm2) and the third was from heat-treated milk (Cou). The cheeses were cut into equal 25-g portions, which were wrapped in aluminum foil, vacuum-packed in polyethylene bags, and stored at −25 °C.

**Preparation of Samples.** The packed samples were thawed at ambient temperature before analysis. To generate the headspace, 5 g of cheese was introduced into a 20-mL flask (Interchim, Montluçon, France) and immediately sealed with a butyl Teflon septum and crimped aluminum closure (Interchim).

**Dynamic Headspace-Mass Spectrometry.** The apparatus for the extraction/concentration of the volatile compounds was composed of an automated dynamic headspace system (Compact Desorber 1, INRA/SRV, Theix, France) coupled to an autosampler (HP19395A, Hewlett-Packard, Palo Alto, CA) with a capacity of 21 flasks. The system functioned sequentially: (i) stabilization of the headspace in the flask, equilibration for 20 min at 30 °C; (ii) extraction/trapping, purging of the headspace of the cheeses for 2 min by a helium stream at 120 mL min⁻¹ (U quality, purity 99.995% Air Liquide) toward an adsorbent trap (0.1 g of Tenax TA, stainless steel tube of length 18 cm, and inside diameter of 2.5 mm (Interchim); (iii) desorption/injection, ohmic heating of the trap at 220 °C for 3 min under a stream of helium carrier (He N55, purity 99.9995% Air Liquide) corresponding to an applied pressure at the trap head of 0.8 bar. The material thus desorbed was injected in splitless mode into the source of a mass spectrometer (MD800, Fisons Instruments) through a deactivated silica transfer line (length 0.5 m, inside diameter 0.15 mm; SGE) heated at 180 °C in the oven of a chromatograph (GC 8060, Fisons Instruments). The m/z range covered was from 33 to 150 atomic mass units (amu) The total ionic current obtained under these conditions took the form of an asymmetrical peak of width ~0.5 min (Figure 1a). The mean abundance values of mass fragments recorded between 0.3 and 0.8 min were used in calculations (Figure 1b); (iv) cooling of the trap for 2 min (return to ambient temperature) before the following sample. Under these conditions, the autosampler provided a throughput of one sample every 7 min (extraction/trapping, 2 min; desorption/injection, 3 min; cooling of trap, 2 min), the equilibration step taking place during the preceding analytical sequences.

**Standard Gas Addition.** A He–Xe gas mixture (He 98%–Xe 2% Air Liquide) was introduced into the mass spectrometer source continuously and independently of the carrier gas. This mixture was introduced by means of an interface consisting of a restrictor and a deactivated silica capillary column of length of 50 cm and inside diameter of 0.15 mm (Supelco, Bellefonte, PA). The pressure applied at the head of the SGA device was 1 bar, and the flow rate of gas introduced continuously into the source was 0.2 mL min⁻¹.

**Disruptive Factors of MS Tunings.** Three different tuning settings corresponding to normal conditions of use of a mass spectrometer were programmed (main parameters given in Table 1). To observe the effects of these tuning settings on the signal, each of the three cheeses was analyzed five times after each tuning. In all, 45 analyses were spread over 3 days (one tuning operation per day). The cheeses were analyzed in random order.
Preprocessing of Data. Selection and Filtering of Mass Fragments. The mass fragments lighter than 45 amu were not counted owing to their multiple nonspecific origins (ambient air, carrier gas). In addition, only those mass fragments with abundance values above the background noise threshold of the apparatus (in arbitrary units of abundance (aua), determined at 1500 for tuning A, 2500 for tuning B, and 5000 for tuning C) were counted. These fragments were then used in calculations, either as they were (noise-filtered spectra) or after one of the two correction procedures presented below.

Internal Normalization. Each mass fragment was expressed as a percentage of the sum of the mass fragments.

SGA Normalization. For each recording, the abundance of each mass fragment was divided by the abundance of $^{129}\text{Xe}$. The isotope $^{129}\text{Xe}$ was chosen as reference in this study because of its intensity and its unequivocal origin (for the cheeses analyzed).

Data Analysis. Principal component analysis (PCA; Statistica software)\(^\text{(19)}\) and factorial discriminating analysis (FDA; SAS software)\(^\text{(20)}\) were carried out for each preprocessing step. These analyses respectively visualized the structure of the data and described their ability to discriminate between the cheeses irrespective of the tuning settings.

For the FDA, only the five most relevant variables ($p < 0.05$ in and out, forward stepwise procedure) were kept. The discriminating power of the data according to the pretreatment applied was determined by the percentages of well-classified individuals by cross-validation ("leave-one-out") and by the sum of the Mahalanobis distances, which characterize the spatial separation of the groups (ratio of inter- to intragroup distances).

RESULTS AND DISCUSSION

Desportion Profile and Spectral Fingerprints. Figure 1 shows a desorption profile obtained by DHS-MS along with the corresponding mass spectrum. Comparison with the results obtained in previous studies\(^\text{(21)}\) shows that the addition of a standard gas does not modify the overall shape of the signal. However, owing to the continuous introduction of xenon into the spectrometer source, the level of the baseline is slightly higher. Xenon was chosen because it is an inert nontoxic, nonpolluting gas that is monatomic and so easily identified. The signal group corresponding to the five major isotopes of xenon shows up distinctly in the mass spectrum (Figure 1b) between 129 and 136 uma

Table 1. Principal Tuning Settings

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<th>Parameter</th>
<th>Tuning a</th>
<th>Tuning b</th>
<th>Tuning c</th>
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<td>340</td>
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<td>Extender (V)</td>
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<td>6</td>
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<tr>
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<td>4</td>
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<tr>
<td>Lens2 (V)</td>
<td>50</td>
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<td>60</td>
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<tr>
<td>Miscellaneous</td>
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<td>pump down 30 min before tuning</td>
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\(^{a}\) PM voltage, photomultiplier voltage.

![Figure 2](image)

Figure 2. PCA carried out from the following: (a) noise-filtered data; (b) data corrected by internal normalization; (c) data corrected by SGA normalization. Ti (i = a–c) designates a series of analyses carried out with tuning i. The numbers (1–5) designate the intratuning analysis order. Arrows D1 illustrate the modifications to the signal caused by the three tuning settings. Arrows D2 show the drift of the signal linked to the fall in the sensitivity of the system from one analysis to the next.

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\((19)\) Statistica A comprehensive system for statistics, graphics and application development; Statsoft, Maisons-Alfort, 1999.


Effect of Different Tuning Settings. The effect of the different tuning settings on the response of the mass detector was studied from the noise-filtered data. The three series of measurements corresponding to the three tuning settings (Ta, Tb, Tc) are separated in the main plane of the PCA (Figure 2a). The funnel effect observed in plane 1–2 of the PCA reveals the presence of two types of drift. The first drift (D1) is attributable to modifications of the signal caused by the three tuning settings (overall diminution of the abundance of mass fragments Tc to Ta, Figure 3a), while the second drift (D2) is linked to the intratuning analysis order. The time course of the abundance of the reference ion (129-Xe, injected at constant flow) provides information on the variations in the sensitivity of the apparatus that can be linked to gradual pollution of the source, a variation in the quality of the vacuum caused by a high analysis throughput, or a fall in the performance of the photomultiplier. The fall in the abundance of fragment 129 thus shows that the drift D2 is caused by a fall in the sensitivity of the mass spectrometer (Figure 3b).

The effects of the two drifts observed have repercussions on the discrimination between cheeses. Thus, with data that are only filtered (Figure 2a), Rm2 is distinguished from Rm1 and Cou only. Inspection of the other axes of the PCA does not afford any distinction between Rm1 and Cou. The classification of the cheeses obtained by FDA (Figure 4a) gave 93% well-classified individuals by cross-validation. Groups Rm1 and Cou are very close, with in particular a broad intragroup dispersion for Rm1, causing three Rm1 individuals to be confounded with the Cou group.

Evaluation and Comparison of Normalization Procedures: SGA versus Internal Normalization. The normalization procedure is a simple operation commonly used in data analysis to overcome effects due to variations in the intensity of the recorded signals (sensitivity, injected quantities). Internal normalization for a particular spectrum consists of expressing each mass fragment relative to the sum of all the mass fragments. The PCA carried out from the normalized data (Figure 2b) shows an appreciable improvement in the separation of the three batches...
of cheese relative to the results obtained from the noise-filtered data. The distinction between the groups is now clearly seen in the principal plane. This preprocessing overcomes the drift D1, but the correction remains incomplete because the drift D2 associated with the intratuning analysis order can still be observed. The results of the FDA presented in Figure 4b shows that the three groups of products are correctly distinguished (100% of well-classified individuals) despite a still large intragroup variability for batch Rm1.

The PCA carried out from data normalized using SGA (Figure 2c) also shows a considerable improvement relative to the results obtained from the noise-filtered data. The different tuning settings are no longer discernible, irrespective of the combination of axes chosen. In addition, unlike the internal normalization, the SGA corrected drift D2 associated with the intratuning analysis order of the cheeses. The improvement is immediate: the results of the FDA presented in Figure 4c show that the individuals are perfectly distinguished. The intragroup variability is weak and there is a noteworthy increase in the distance between batch Rm2 and the two other batches. The percentage of well-classified cheeses is 100% and the Mahalanobis distances are more than 4 and 2 times greater than those calculated from the noise-filtered and normalized data, respectively.

The improved results obtained from SGA relative to internal normalization are linked to the principle underlying the correction made. In internal normalization, the n individual variables have a high covariance due to the mode of expression (relative to the percentage of the sum total), which generates statistical links among all the fragments, some of which drift strongly (in particular ions with high abundance, e.g., m/z = 46, 47, 58, 62 amu). Thus, the fall in sensitivity is directly translated on the PCA by the presence of drift D2. Unlike internal normalization, SGA normalization is carried out relative to the 129Xe, which is independent of the other mass fragments. Hence there are no statistical coelutions between variables, and the FDA selects more relevant variables. Consequently, the discriminating power of the data is thereby improved.

CONCLUSION

The method of signal correction by SGA normalization shows that the controlled introduction of the standard gas 129Xe can correct effects arising from modifications made to the instrumental parameters of a mass spectrometer. This new technique will also correct for temporary fluctuations of ionization conditions that can arise during the direct injection of volatile compounds. SGA offers a better solution than internal normalization for the correction of spectral fingerprints obtained by DHS-M.S. In addition, the very small quantity of standard gas that needs to be introduced continuously into the source makes SGA a very inexpensive method of calibration. Technically, it can be generalized to all MS-based sensor systems.

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