Journal of Chromatography A, 1445 (2016) 112–117

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Generalized multiple internal standard method for quantitative liquid chromatography mass spectrometry

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ARTICLE INFO

Article history:
Received 16 November 2015
Received in revised form 1 April 2016
Accepted 3 April 2016
Available online 4 April 2016

Keywords:
Liquid chromatography–mass spectrometry
Signal instability
Colorant
Generalized multiple-Internal-Standard method
Quantitative analysis

ABSTRACT

In this contribution, a multiplicative effects model for generalized multiple-internal-standard method (MEM_{GMIS}) was proposed to solve the signal instability problem of LC–MS over time. MEM_{GMIS} model seamlessly integrates the multiple-internal-standard strategy with multivariate calibration method, and takes full use of all the information carried by multiple internal standards during the quantification of target analytes. Unlike the existing methods based on multiple internal standards, MEM_{GMIS} does not require selecting an optimal internal standard for the quantification of a specific analyte from multiple internal standards used. MEM_{GMIS} was applied to a proof-of-concept model system: the simultaneous quantitative analysis of five edible artificial colorants in two kinds of cocktail drinks. Experimental results demonstrated that MEM_{GMIS} models established on LC–MS data of calibration samples prepared with ultrapure water could provide quite satisfactory concentration predictions for colorants in cocktail samples from their LC–MS data measured 10 days after the LC–MS analysis of the calibration samples. The average relative prediction errors of MEM_{GMIS} models did not exceed 6.0%, considerably better than the corresponding values of commonly used univariate calibration models combined with multiple internal standards. The advantages of good performance and simple implementation render MEM_{GMIS} model a promising alternative tool in quantitative LC–MS assays.

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1. Introduction

Liquid chromatography–mass spectrometry (LC–MS) is one of the most popular tools for the analysis of complex systems. It has been widely applied to the analysis of many complex systems [1–8]. Nevertheless, it has its own weak points. In mass spectrometry, the presence of less volatile compounds in samples to be analyzed can change the efficiency of droplet formation or droplet evaporation, and hence affects the amount of charged ions in the gas phase that ultimately reaches the detector, which is commonly referred to as ion suppression effects [9]. There are many possible sources for ion suppression, including matrix effect [10,11], co-eluting analytes [12,13], and stable-isotope-labeled internal standards [14], etc. Ion suppression can result in variations in the overall sensitivity and signal stability of LC–MS and compromise interpretation of mass spectral data. In most of the cases, the signal intensity is reduced, although sometimes signal enhancement could also be detected. In addition to ion suppression, the gradual fouling of the ion source, vacuum instability, and aging of the ion multiplier and the headspace sampler might also lead to changes in sensitivity and gradual shifting of baseline signal intensity over time.

Many methods were developed to solve the problem of signal instability of LC–MS. For instances, Moragues et al. developed a method consists of an aqueous extraction and a two-step clean-up to decrease ion suppression for the determination of beta-agonists in animal liver and urine [15]. D'Autry et al. coated a gold layer on the ion source to improve signal stability [16]. ^{13}C labeled internal standards were used by Berg et al. to minimize ion suppression effects on UPLC–MS method for the determination of amphetamine and methamphetamine in urine [13]. Calibration transfer methodology has also been proposed for solving the problem of signal instability in quantitative headspace-mass spectrometry [17].

Among the existing methods developed for improving the accuracy and precision of quantitative LC–MS, the simplest but effective ones might be the univariate and multivariate methods based on internal standards [18–21]. Ideally, an appropriate internal standard should have a similar structure to the analyte of interest with the most appropriate being the isotopically tagged analyte analogue. Due to the limited availability of isotopically labeled analogues of analytes, substances with retention times close to that of

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http://dx.doi.org/10.1016/j.chroma.2016.04.008
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the analytes of interest are often used as internal standards. Dual or multiple internal standards are needed when several analytes are to be simultaneously determined in the same complex sample [22–26]. The use of multiple internal standards can generally ensure better quantitative results than the usual use of one standard. However, it also poses a problem for analytes, i.e. among the multiple internal standards used, which one should be selected for the quantification of each of the analytes of interest? At present, the optimal internal standard for each of the analytes of interest is determined by scrutinizing the performance of all the internal standards used. This way of using multiple internal standards does not take the comprehensive advantages provided by the multiple internal standards, and therefore its results may not be optimal.

In this contribution, a generalized multiple internal standard methods was proposed for quantitative LC–MS assays. The proposed method takes the comprehensive advantages of multiple internal standards, and hence avoids the trouble of selecting an optimal internal standard from multiple ones for the quantification of each of the analytes of interest.

2. Generalized multiple-internal-standard method for quantitative LC–MS analysis

For quantitative LC–MS analysis with the problem of signal instability, the use of multiple internal standards can generally ensure better quantitative results than the usual use of one internal standard. However, the conventional multiple-internal-standard methods based on the ratios between the peak heights (or peak areas) of the target analytes and the corresponding values of the internal standards suffer from the problems of background interferences, baseline shifting and the selection of an optimal internal standard for the quantification of a specific target analyte from multiple internal standards. In order to avoid the above problems, the following multiplicative effects model for generalized multiple-internal-standard method (MEM_{GMIS}) was proposed for quantitative LC–MS analysis. For simplicity, the case of double internal standards was used to drive the MEM_{GMIS} model. First of all, let's construct LC–MS data ($\mathbf{x}_k$) of the k-th sample according to Eq. (1).

$$\boldsymbol{x}_k = \boldsymbol{x}_{Targ,k} + \boldsymbol{x}_{IS1,k} + \boldsymbol{x}_{IS2,k}; \quad k = 1, 2, \cdots, N \tag{1}$$

Here, $\mathbf{x}_{Targ,k}$, $\mathbf{x}_{IS1,k}$, and $\mathbf{x}_{IS2,k}$ represent the mass spectra (either the whole mass spectra or partial mass spectra measured in multiple reaction monitoring mode) of the target analyte and the two internal standards in the k-th sample recorded at the apexes of their chromatographic elution curves, respectively (Fig. 1); K represents the number of samples.

When taking the presence of baseline shifting, background interferences, and variations in sensitivity caused by ion suppression and other factors into consideration, $\mathbf{x}_k$ can then be decomposed as follows:

$$\boldsymbol{x}_k = b_k \cdot c_{Targ,k} \cdot \boldsymbol{s}_{Targ} + b_k \cdot \sum_{j=1}^{2} c_{IS,j} \cdot \boldsymbol{s}_{IS,j} + \boldsymbol{d}_k; \quad k = 1, 2, \cdots, N \tag{2}$$

Here, $c_{Targ,k}$ and $c_{IS,j}$ denote the concentrations of the target substance in the k-th sample and the j-th added internal standard, respectively. $\mathbf{s}_{Targ}$ and $\mathbf{s}_{IS,j}$ are the pure mass spectra of the target substance and the j-th added internal standard per unit concentration. Parameter b_k accounts for the multiplicative confounding effect on signal intensities caused by changes in variables other than analytes' concentrations in the k-th sample, such as ion suppression across samples, the gradual fouling of the ion source, vacuum instability, and aging of the ion multiplier and the headspace sampler. The multiplicative parameters b_k ($k = 1, 2, \cdots, N$) for N calibration samples can be estimated out by the optical path length estimation and correction method and its modified version developed by Chen et al. [27,28]; $\mathbf{d}_k$ is a composite term that represents the signal contributions of possible baseline shifting and background interferences in the k-th sample.

The quantification of the target analyte using MEM_{GMIS} model can be implemented according to the procedure described in reference [20]. Briefly, two calibration models ($b_k = \alpha_1 + \boldsymbol{x}_k \cdot \boldsymbol{\beta}_1$ and $c_{Targ,k} \cdot b_k = \alpha_2 + \boldsymbol{x}_k \cdot \boldsymbol{\beta}_2$) are built by multivariate linear calibration methods (e.g. partial least square regression, PLS). Once the model parameters α_1, $\boldsymbol{\beta}_1$, α_2, and $\boldsymbol{\beta}_2$ are estimated by PLS, the concentration of the target analyte in a test sample can then be accurately predicted from its LC–MS data $\mathbf{x}_{test}$ ($\boldsymbol{x}_{test} = \boldsymbol{x}_{Targ,test} + \boldsymbol{x}_{IS1,test} + \boldsymbol{x}_{IS2,test}$) through dividing the prediction of the second calibration model by the corresponding prediction of the first calibration model. It is quite clear that the information carried by multiple internal standards has been fully utilized by the above MEM_{GMIS} model during the quantification of the target analyte. For the convenience of readers, a detailed description of the procedure of implementing MEM_{GMIS} model was provided in Supporting information.

3. Experimental

3.1. Chemicals and reagents

Brilliant blue (Bb, 85%), acesulfame-k (Ac-k, 98%), sunset yellow (Sy, 87%), ammonium acetate (AA, 99%), polyamide (200–400), and amaranth (Ama) were purchased from Aladdin Reagent (Shanghai, China). Methanol (HPLC grade) was obtained from Oceanpak Alexative Chemical Co., Ltd. (Beijing, China). Tartrazine (Tar, 85%) was purchased from Titan Chem Co., Ltd. (Shanghai, China). Allura red (Ar, analytical grade) and chloramphenicol (Chl, 97%) were obtained from Yuanye Bio-Technology Co., Ltd. (Shanghai, China). Formic acid was purchased from Guangfu Chemical Research Institution (Tianjin, China). Ethanol (analytical grade) was purchased from Xilong Chemical Co., Ltd. (Guangdong, China). RIO peach cocktail drink and RIO orange cocktail drink were purchased from local supermarket in Changsha. All chemicals were used as received without any further purification. Ultrapure water (18.25 MΩ) was used throughout the experiment

3.1.2. Sample preparation

Stock solutions of Bb, Ama, Sy, Tar, Ar, Ac-k (internal standard), Chl (internal standard) were prepared in ultrapure water and stored in refrigerator at 4 °C. Eight calibration samples were prepared by mixing appropriate amounts of the stocking solutions with ultrapure water (Table S1, Supporting Information).

Edible artificial pigments were extracted from two kinds of cocktail drinks, i.e. RIO orange cocktail drink (containing Tar and Sy) and RIO peach cocktail drink (containing Ar) according to China national standard (GB/T 5009.35-2003). Briefly, 35 mL of RIO peach cocktail drink/RIO orange cocktail drink taken directly from the corresponding drink bottles was firstly adjusted to pH 6 using ammonia water (13%), and heated to 60 °C for 30 min to wipe off ethanol and CO_2. The pretreated cocktail drink was mixed with 2 g polyamide dispersed in small volume of water. The mixture was stirred for 3 min at 60 °C and then underwent filtration. The polyamide obtained after filtration was sequentially washed with 40 mL citric acid solution (pH = 4) and 40 mL formic acid- methanol solution (formic acid:methanol = 4:6) to remove possible natural colorants in cocktail drinks absorbed on the surfaces of polyamide, and then with ultrapure water until neutral. Subsequently, edible artificial pigments were desorbed from polyamide by washing polyamide with 30 mL desorption solution (ethanol: 0.5% ammonia

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