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Novel calibration model maintenance strategy for solving the signal instability in quantitative liquid chromatography–mass spectrometry

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ABSTRACT

In this contribution, a multiplicative effects model with a parameter accounting for the variations in overall sensitivity over time was proposed to reduce the effects of signal instability on quantitative results of LC–MS/MS. This method allows the use of calibration models constructed from large standard sets without having to repeat their measurement even though variations occur in sensitivity and baseline signal intensity. The performance of the proposed method was tested on two proof-of-concept model systems: the determination of the target peptide in two sets of peptide digests mixtures and the quantification of melamine and metronidazole in two sets of milk powder samples. Experimental results confirmed that multiplicative effects model could provide quite satisfactory concentration predictions for both systems with average relative predictive error values far lower than the corresponding values of various models investigated in this paper. Considering its capability in solving the problem of signal instability across samples and over time in LC–MS/MS assays and its implementation simplicity, it is expected that the multiplicative effects model can be developed and extended in many application areas such as the quantification of specific protein in cells and human plasma and other complex systems.

1. Introduction

Due to its inherent selectivity and sensitivity, tandem mass spectrometry coupled to liquid chromatography (LC–MS/MS) has been widely applied to the analysis of samples ranging from small molecules [1,2] to large protein mixtures [3,4]. However, its application suffers from ion suppression that electrospray ionization response of organic bases decreased with an increase in concentrations of other organic bases [5]. Moreover, 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 drifting of baseline signal intensity over time. All these factors affect the overall sensitivity and signal stability of LC–MS/MS and hence limit its use in routine quantitative analysis.

Recently, the emergence of proteomics saw increasing application of LC–MS/MS to quantitative analysis of proteins [6]. Quantification of proteins by LC–MS/MS can be classified in two major approaches: the label-free methods such as spectral counting [7–11] and peptide ion intensity [12], and the labeling techniques including cysteine labeling [13], proteolytic ^{18}O labeling [14], metabolic labeling [15] and tandem mass tagging [16,17] as well. However, quantification of proteins by LC–MS/MS is still challenging, largely because of the above mentioned ionization suppression among coeluting species [5] and instability factors associated with mass spectrometry. Barnidge et al. quantified the tryptic cleavage product of native ROS from two experiments run on two different days using the same standard curve (i.e. calibration model) made from dilutions of a synthetic version of the tryptic peptide. Their results showed that the coefficient of variation values for these two experiments were quite different, with a value of 0.4% for experiment 1 and a value of 28% for experiment 2 [18]. These results fully demonstrate that LC–MS/MS as a quantitative tool for proteins has the problem of long term signal instability. Therefore, the long term stability of a calibration model established for routine quantitative application of LC–MS/MS is questionable. With a view to enable the routine quantification of analytes (e.g. proteins) in complex systems by LC–MS/MS, a multiplicative effects model was proposed in this contribution to reduce the effects of signal instability on quantitative results, and hence to achieve accurate routine quantification assays using LC–MS/MS.

2. Experimental

2.1. Chemicals

Synthetic peptides, GGSERGSSGDREGSDQDKSEDG and TTVSKTETSQVAPA(>98% purity), were purchased from ChinaPeptides Co.,

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Ltd. (Shanghai, China). Acetonitrile (HPLC grade) and methanol (HPLC grade) were obtained from Oceanpak Alexative chemical Co., Ltd. (Beijing, China). Formic acid (HPLC grade), ammonium bicarbonate (analytical grade), hydrogen chloride (analytical grade), sequencing grade modified trypsin, melamine (analytical grade), metronidazole (analytical grade), chloramphenicol (analytical grade), ammonium acetate (analytical grade) and ammonia solution (analytical grade) were purchased from Aladdin Reagent (Shanghai, China). Milk powder was obtained from Nestle Shuangcheng Ltd. All chemicals were used as received without any further purification. Ultrapure water (18.25 MΩ cm) was used throughout this study.

2.2. Sample preparation for quantification of peptide in synthetic peptide digests

Stock solutions of synthetic peptide digests were prepared as follows. 1 mg of each synthetic peptide was dissolved in 1 mL of 100 mM ammonium bicarbonate buffer, and then boiled for ten minutes. After the solution was cooled down to room temperature, sequencing-grade trypsin at an enzyme: peptide ratio of 1:50 was added. The mixture was incubated at 37 °C for 6 h, and then an additional amount of trypsin (1:50) was added. The mixture was continually incubated at 37 °C for another 16 h. Stock solutions of synthetic peptide digests were further diluted by 9:1 (v/v) water/acetonitrile solution and mixed together to prepare seven mixture samples comprising 0.1% acetic acid, 0.1 μg/mL TTVSKTETSQVAPA (internal standard) and GGSERGSSGDREGSDQDKSEDG (target analyte) with concentration ranging from 0.100 μg/mL to 30.0 μg/mL (referred to as "experiment 1"). Fifteen days later, twelve peptide digests mixture samples comprising 0.1% acetic acid, 0.100 μg/mL TTVSKTETSQVAPA and GGSERGSSGDREGSDQDKSEDG with concentration varying from 0.100 μg/mL to 30.0 μg/mL were prepared and analyzed in the same way as the above seven samples (referred to as "experiment 2"). The detailed experimental designs of the above two experiments are listed in Table S1 (Supporting Information).

2.3. Sample preparation for quantification of melamine and metronidazole in milk powder

Stock solutions (100 μg/mL) of melamine, metronidazole and chloramphenicol were prepared by dissolving appropriate amount of each compound in methanol and treated with an ultrasonic bath for 5 min, respectively. Working standard solutions of the three chemical compounds were obtained by diluting the corresponding stock solutions with appropriate amount of methanol, respectively.

Eight milk powder samples (referred to as "experiment 1") were prepared by precisely weighing 0.200 g of milk powder and mixing it with 1.5 mL of water and certain volumes of standard solutions of melamine, metronidazole and chloramphenicol in 10 mL centrifuge tubes. The mixture samples were treated with an ultrasonic bath for 5 min. Subsequently, 1% ammonia/acetonitrile solution was added to bring each mixture sample up to a total volume of 5 mL. After being treated with an ultrasonic bath for another 30 min, each mixture sample was then centrifuged at a rate of 10,000 r/min for 15 min at room temperature. The supernatant (ca. 1 mL) was then transferred into a HPLC vial for further LC–MS/MS analysis. Twenty-five days later, another six mixture samples were prepared and analyzed in the same way as the above eight samples (referred to as "experiment 2"). The concentrations of melamine and metronidazole in the mixture samples were in the ranges of 0.100–7.00 μg/mL and 0.200–14.0 μg/mL, respectively. Chloramphenicol was served as an internal standard. Its concentration was therefore kept at 1.00 μg/mL. The detailed experimental designs of the above two experiments are listed in Table S2 (Supporting Information).

2.4. Peptide identification conditions

Peptide identification was carried out on an EASY-nano LC1000 coupled online to an ESI-LTQ-Orbitrap Velos mass spectrometer (Thermo Fisher Scientific). 4 μL of peptide digests of GGSERGSSGDREGSDQDKSEDG (10.0 μg/mL), TTVSKTETSQVAPA (10.0 μg/mL), and mixture comprising of GGSERGSSGDREGSDQDKSEDG (10.0 μg/mL) and TTVSKTETSQVAPA (10 μg/mL) were eluted through a trap column (column size: C18, 75 μm × 2 cm, bead size: 3 μm, pore size: 100 Å) and an analytical column (column size: C18, 50 μm × 15 cm, bead size: 2 μm, pore size: 100 Å) running under gradient elution mode with linear decrease of 100% eluent A (0.1% formic acid in water) to 60% eluent B (0.1% formic acid in acetonitrile) within 15 min. Mass spectra were acquired in a positive mode using the data-dependent automatic (DDA) survey MS scan. Each DDA consisted of a survey scan of the m/z range 200–2000 with a resolution of 60,000. Subsequently, tandem mass spectra (MS/MS) acquisition of the 4 most intense ions was carried out in the linear trap quadrupole (LTQ) using the collision-induced dissociation.

2.5. Peptide quantification conditions

Peptide quantification was performed on an 1290 HPLC system (Agilent Technologies) equipped with a 2.0 mm × 150 mm C18 reversed-phase column with a bead size of 5-μm and a pore size of 300-Å (Jupiter, phenomenex, Guangzhou, China). The column was maintained at 30 °C. The mobile phase consisted of water containing 0.1% formic acid (eluent A) and acetonitrile containing 0.1% formic acid (eluent B). Gradient elution was performed with linear decrease of 95–72% A within 12 min. The flow rate was set to 0.1 mL/min. For each sample, a volume of 10 μL was loaded onto the column via an autosampler from a 96-well sample tray. The column was re-equilibrated at initial conditions for 6 min before the next analysis. Each sample in both experiment 1 and experiment 2 was analyzed nine and three times, respectively. LC–MS/MS data of samples was collected by an Agilent G6460 Triple Quadrupole mass spectrometer (Agilent Technologies) with electrospray interface (ESI) operated in the positive mode using the following settings: nebulizer pressure = 15 psi, fragmentor voltage = 135 V, capillary voltage = 4000 V, drying gas flow rate = 11 L/min; and drying gas temperature = 300 °C, electron multiplier voltage = 200 V. The flow-rate of collision gas (high purity N_2) was set at an appropriate value such that the high vacuum pressure was 2.3×10^{-5} Torr. The MS detector was operated in MS2 scan mode (200–910 amu) at a rate of 2.25 cycle/s. The scan time of each scanning was 200 ms.

2.6. LC–MS/MS conditions for quantification of melamine and metronidazole in milk powder

Quantification of melamine and metronidazole in milk powder samples was performed on the same LC–MS/MS system as used in peptide quantification experiments. The column was maintained at 25 °C. The mobile phase consisted of water containing 0.04% formic acid and 60 mM ammonium acetate (eluent A) and methanol (eluent B). Gradient elution was performed with linear decrease of 90–10% A within 7 min. The flow rate was set to 0.2 mL/min. For each sample, a volume of 10 μL was loaded onto the column via an autosampler from a 96-well sample tray. The column was re-equilibrated at initial conditions for 4 min before the next analysis. Each sample was analyzed three times. The mass spectrometer was operated in the positive mode for both melamine and metronidazole, and in the negative mode for chloramphenicol using

the same settings as used in peptide quantification experiments. The MS detector was operated in multiple reaction monitoring (MRM) at a rate of 2.25 cycle/s. The scan time of each scanning was 200 ms. The collision energy, qualitative and quantitative ion pairs for melamine, metronidazole and chloramphenicol were provided in Table S3 (Supportive Information).

2.7. Data analysis

For the quantification of peptide in peptide digests mixtures, the calibration set consisted of LC–MS/MS data of four samples from experiment 1 and two samples from experiment 2 (Table S1, Supportive Information). The calibration set for the quantification of melamine and metronidazole in milk powder also contained two samples from experiment 2 (Table S2, Supportive Information). The reason for adding two samples from experiment 2 into the calibration sets was to account for the possible differences between the background interferences in the two experiments. In order to mitigate the effects of ionization suppression and signal instability on quantitative results, the following novel data structure and multiplicative effects model for mass spectroscopy (MEM_{MS}) were adopted for the analysis of LC–MS/MS data.

$$\mathbf{x}_i = \mathbf{x1}_i + \mathbf{x2}_i, \quad i = 1, 2, \ldots, N \tag{1}$$

where, for MS detector operated in MS2 scan mode, $\mathbf{x1}_i$ and $\mathbf{x2}_i$ represent the mass spectra of the target analyte and the internal standard in the i-th sample recorded at the peaks of their chromatographic elution curves, respectively (Fig. S1, Supporting Information); If the MS detector was operated in MRM mode, $\mathbf{x1}_i$ and $\mathbf{x2}_i$ represent the chromatograms of the target analyte and the internal standard in the i-th sample at their quantitative ions, respectively. N is the number of samples.

Considering the presence of background interferences and variations in overall sensitivity caused by ion suppression, $\mathbf{x1}_i$ and $\mathbf{x2}_i$ can then be decomposed as follows:

$$\mathbf{x1}_i = b_i \cdot c_{targ,i}\mathbf{s}_{targ} + \mathbf{f1}_i \ldots \mathbf{x2}_i = b_i \cdot c_{stand,i}\mathbf{s}_{stand} + \mathbf{f2}_i \tag{2}$$

here, $c_{targ,i}$ and $c_{stand,i}$ are the concentrations of the target analyte and the internal standard in the i-th sample, respectively. $\boldsymbol{s}_{targ}$ and $\boldsymbol{s}_{stand}$ represent the pure mass spectra (or chromatograms) of the target analyte and the internal standard per unit concentration. $\mathbf{f1}_i$ and $\mathbf{f2}_i$ denote the signal contributions of background interferences to the mass spectra (or chromatograms) of the target analyte and the internal standard in the i-th sample, respectively. The multiplicative parameter b_i accounts for the variations in overall sensitivity caused by ionization suppression and signal instability across samples. Therefore, $\mathbf{x}_i$ can be re-expressed as follows:

$$\mathbf{x}_i = b_i \cdot c_{targ,i}\mathbf{s}_{targ} + b_i \cdot c_{stand,i}\mathbf{s}_{stand} + \mathbf{f1}_i + \mathbf{f2}_i \tag{3}$$

Since the concentration of the internal standard ($c_{stand,i}$) is kept constant across samples, the multiplicative parameter vector ($\mathbf{b} = [b_1; b_2; \cdots; b_N]$) for calibration samples can be estimated out by the modified optical path length estimation and correction method ($OPLEC_m$) developed by Chen et al. [19,20]. Define $\mathbf{X}_{cal} = [\mathbf{x}_1; \mathbf{x}_2; \ldots; \mathbf{x}_N]$ and $\mathbf{c}_{targ} = [c_{targ,1}; c_{targ,2}; \ldots; c_{targ,N}]$, the following two calibration models can be built by multivariate linear calibration methods (e.g. partial least square regression, PLS).

$$\mathbf{b} = \alpha_1\mathbf{1} + \mathbf{X}_{cal}\boldsymbol{\beta}_1; \quad diag(\mathbf{c}_{targ})\mathbf{b} = \alpha_2\mathbf{1} + \mathbf{X}_{cal}\boldsymbol{\beta}_2 \tag{4}$$

here, $diag(\mathbf{c}_{targ})$ denotes the diagonal matrix in which the corresponding diagonal elements are the elements of $\mathbf{c}_{targ}$. 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 $\mathbf{X}_{test}$ ($\mathbf{x}_{test} = \mathbf{x1}_{test} + \mathbf{x2}_{test}$) through dividing the prediction of the second calibration model by the corresponding prediction of the first calibration model.

For comparison purpose, PLS calibration models were also built between $\mathbf{c}_{targ}$ and the raw data ($\mathbf{X}_{cal}$) or the preprocessed data by multiplicative signal correction (MSC) [21], standard normal variate (SNV) [22] and extended inverted signal correction (EISC) [23]. The optimal MEM_{MS} and PLS calibration models were determined by leave-one-out cross validation procedure. For the convenience of presentation, thereafter, PLS calibration models built on the raw and preprocessed $\mathbf{X}_{cal}$ by MSC, EISC and SNV are denoted by PLS_{raw}, PLS_{MSC}, PLS_{EISC} and PLS_{SNV}, respectively.

3. Results and discussion

3.1. Quantification of peptide in synthetic peptide digests

3.1.1. Peptide identification

The total ions chromatogram of peptide digests of GGSERGSSGDREGSDQDKSEDG (Fig. S2a, Supporting Information) shows three distinctive peaks at 5.48, 5.50 and 6.15 min, respectively; while that of TTVSKTETSQVAPA has one main peak at 10.46 min (Fig. S2b, Supporting Information). Corresponding peaks can be observed in the total ions chromatograms of peptide digests of mixture comprising of GGSERGSSGDREGSDQDKSEDG and TTVSKTETSQVAPA (Fig. S2c, Supporting Information). Based on the sequences of the peptides identified automatically by LTQ orbitrap velos pro (Thermo Fisher Scientific) equipped with SEQUEST program (Table S4, Supporting Information), the mass spectra at the peaks of the chromatographic elution curves of peptides $[EGSDQDKSEDG]^{2+}$ (at 5.50 min) and $[TETSQVAPA]^{2+}$ (at 10.46 min) were taken as $\mathbf{x1}_i$ and $\mathbf{x2}_i$, respectively.

3.1.2. Peptide quantification

The effects of ionization suppression among coeluting peptide digests and signal instability across samples can distort the linear relationship between the intensity and the concentration of peptide digests, thereby deteriorating the prediction capability of multivariate linear calibration models built on the raw LC–MS/MS data. Fig. 1a shows the concentration of target peptide in both the calibration and test samples predicted by PLS calibration model (PLS_{raw}) on the raw $\mathbf{X}_{cal}$ with two underlying components. The significant deviations of the predictions of PLS_{raw} model from the expected values clearly demonstrated the impotency of PLS_{raw} model in accounting for the effects of ionization suppression and signal instability across samples in LC–MS/MS assay.

Compare with the PLS_{raw} model, MEM_{MS} model was purposely designed to mitigate the effects of signal instability across samples in LC–MS/MS assay. The application of MEM_{MS} involves the estimation of the multiplicative parameter vector $\mathbf{b}$ for the calibration samples. The multiplicative parameters estimated by $OPLEC_m$ for the calibration samples vary in the range of 1.0–1.78 (Fig. S3, Supporting Information) It is evident that different samples generally have rather different multiplicative parameter values, i.e. different overall sensitivities. After the estimation of $\mathbf{b}$, an optimal MEM_{MS} calibration model with seven underlying components was established on the raw $\mathbf{X}_{cal}$. The results displayed in Fig. 1b showed that MEM_{MS} model not only fitted the calibration samples quite well but more importantly provided considerably accurate predictions for the test samples. The predicted values of MEM_{MS} model are very close to their expected ones, which fully demonstrated the effectiveness of MEM_{MS} model in fulfilling its designated mission, i.e., solving the problem of signal instability of mass spectrometer.

For a more convincing comparison, the performance of the optimal MEM_{MS} and various PLS calibration models (i.e., PLS_{raw}, PLS_{MSC}, PLS_{EISC} and PLS_{SNV}) for the test samples were investigated. As shown in Table 1, the root-mean-square error of prediction (RMSEP) values of PLS_{raw} model were 0.6910 and 1.531 μg/mL for

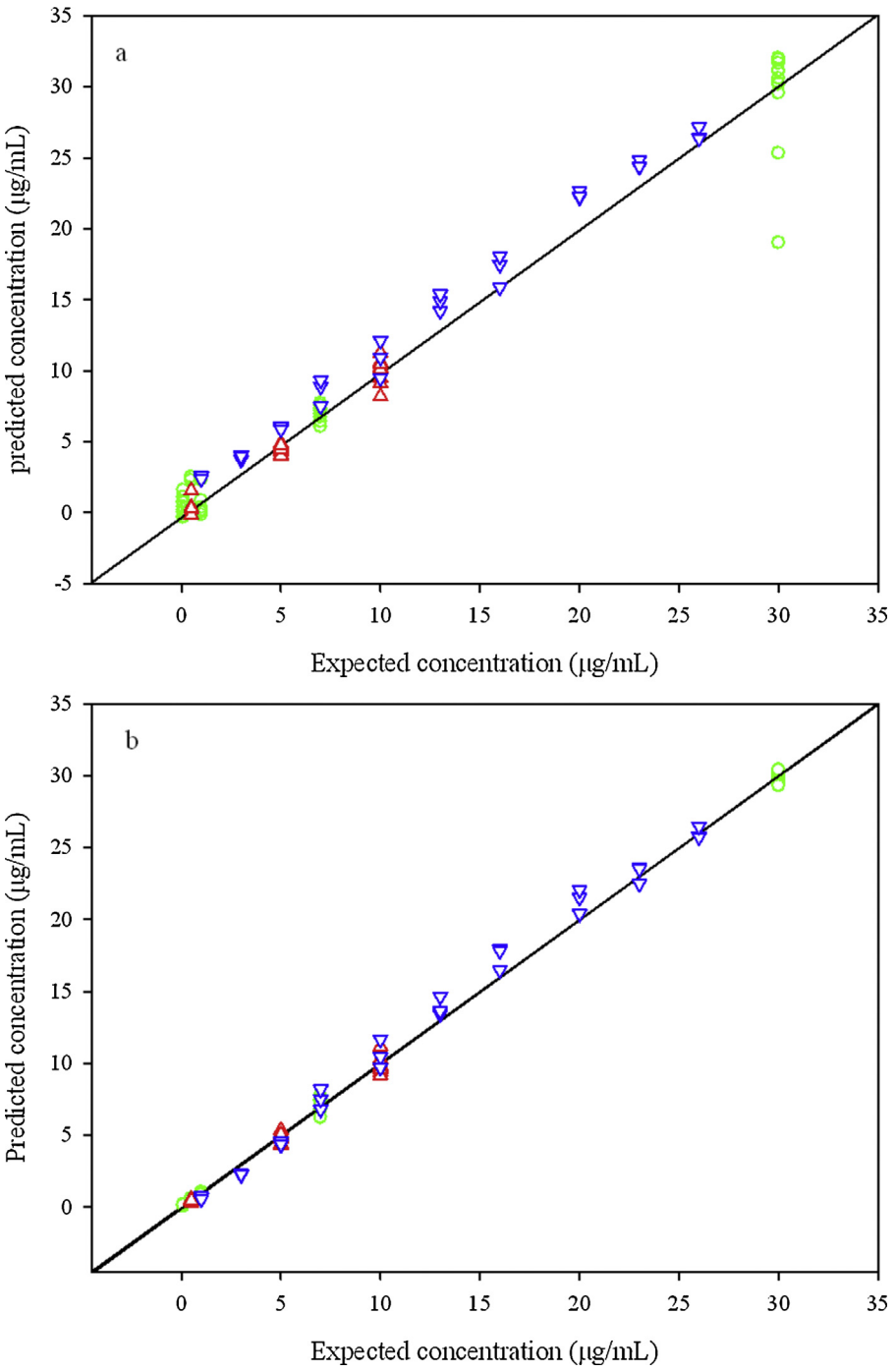

Fig. 1. Concentration of the target peptide in the calibration (green circle) and test (triangle) samples predicted by the optimal PLS calibration model (PLS_{raw}) on the raw $\mathbf{X}_{cal}$ (a) and the MEM_{MS} model (b) (red triangle up: the test samples from experiment 1; blue triangle down: the test samples from experiment 2). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Table 1
The predictive performance of different calibration methods for the test samples of peptide digests.

Method	Experiment 1		Experiment 2	
	RMSEP[a] (μg/mL)	ARPE[b] (%)	RMSEP (μg/mL)	ARPE (%)
PLS_{raw}	0.691	36.1	1.53	27.9
PLS_{EISC}	1.20	31.6	5.00	52.3
PLS_{MSC}	2.37	162.8	4.29	79.5
PLS_{SNV}	1.59	53.7	4.47	57.7
MEM_{MS}	0.388	8.4	0.938	11.2

[a] RMSEP: root mean square error of prediction ($RMSEP = \sqrt{\sum_{i=1}^{N}(\hat{c}_i - c_i)^2/N}$).

[b] ARPE: average relative predictive error ($ARPE = (1/N)\sum_{1=1}^{N}(\left|\hat{c}_i - c_i\right|/c_i) \times 100\%$).

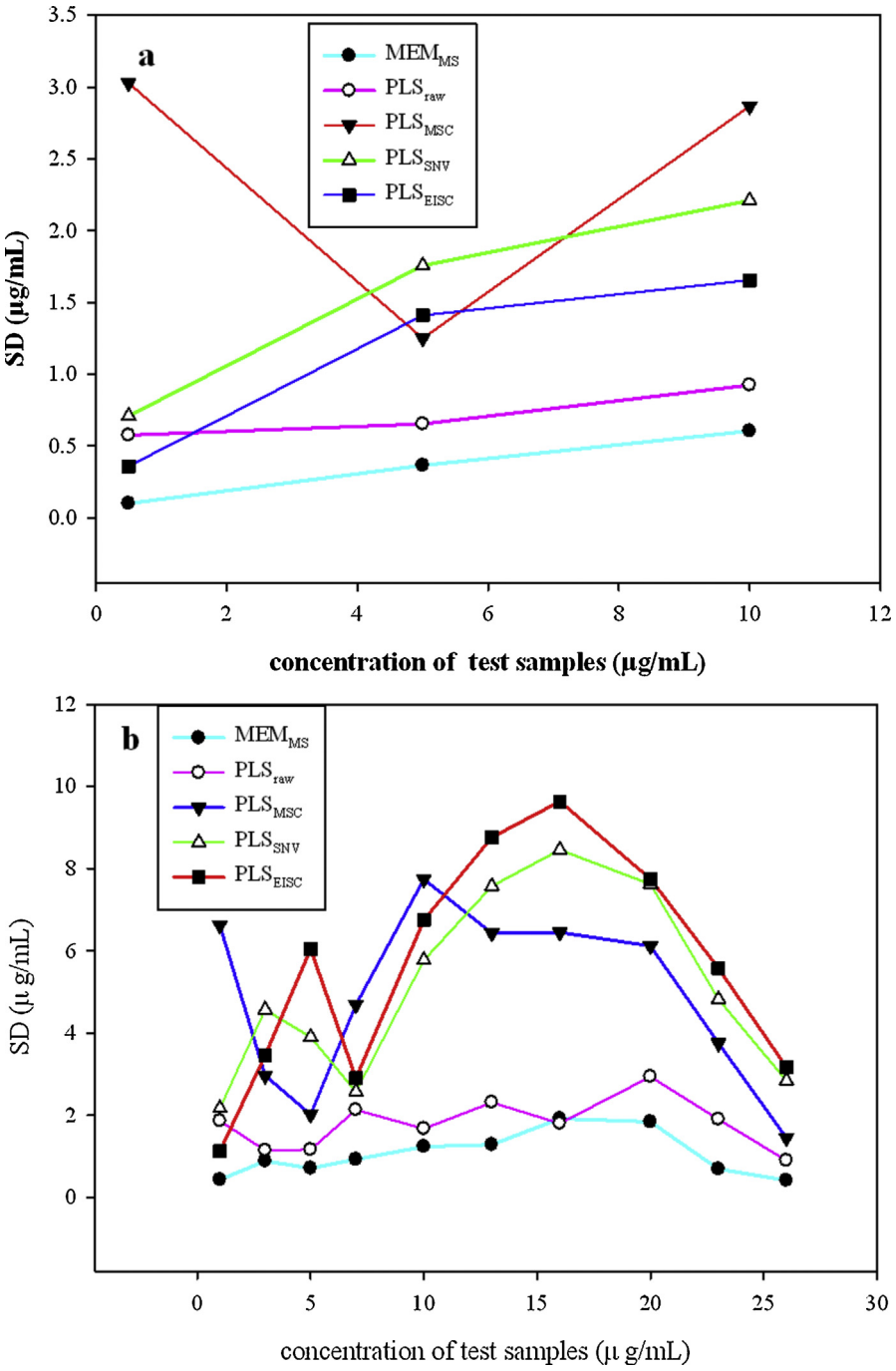

Fig. 2. The standard deviations ($SD = \sqrt{\sum_{i=1}^{I}(\hat{c}_i - c_{true})^2/(I-1)}$) of the predictions obtained by different calibration methods for the target peptide in the test samples from both experiment 1 (a) and experiment 2 (b).

Table 2
The predictive performance of MEM_{MS} and PLS_{raw} for metronidazole and melamine in the test powder milk samples.

Compound	Method	Experiment 1		Experiment 2	
		RMSEP (μg/mL)	ARPE (%)	RMSEP (μg/mL)	ARPE (%)
Metronidazole	PLS_{raw}	0.069	9.0	0.148	32.0
	MEM_{MS}	0.038	6.4	0.057	7.8
Melamine	PLS_{raw}	0.109	9.6	0.942	11.4
	MEM_{MS}	0.060	6.4	0.387	7.6

the test samples from experiment 1 and experiment 2, respectively. It clearly demonstrated that the variation in the overall sensitivity over time in LC–MS/MS assay could significantly deteriorate the predictive capability of calibration models. The application of spectral processing methods such as MSC, EISC, and SNV saw no improvement but rather significant deterioration in the predictive ability of PLS calibration models, which reveals the fact that spectral preprocessing methods MSC, EISC, and SNV are not effective options for the correction of overall sensitivity variation and signal instability in LC–MS/MS assay.

The optimal MEM_{MS} model achieved RMSEP values of 0.3878 and 0.9384 μg/mL for the test samples from experiment 1 and experiment 2, which are equivalent to average relative predictive error (ARPE) values of about 8.4% and 11.2% (far lower than the corresponding values of various PLS models), respectively. Furthermore, the standard deviations of the predictions obtained by MEM_{MS} model for the test samples from both experiment 1 and experiment 2 are consistently and significantly lower than those of the other methods (Fig. 2). These results confirmed that the variations in the total sensitivity cross samples and over time as well have little impact on the predictive capability of MEM_{MS} model.

3.2. Quantification of melamine and metronidazole in milk powder

The total ion chromatogram of a milk powder sample containing of melamine, metronidazole and chloramphenicol in MS2 scan mode (Fig. S4a, Supportive Information) revealed the presence of significant baseline drift and background interferences in the LC–MS/MS data of milk powder samples. Furthermore, the chromatographic peaks were not well separated. These facts suggested that the LC–MS/MS data collected in MS2 scan model was not appropriate for accurate quantitative analysis of the target analytes. In contrast, there are only four chromatographic peaks in the total ion chromatogram of the same milk powder sample collected in MRM scan mode, and these chromatographic peaks are well separated (Fig. S4b, Supportive Information). Therefore, the calibration models for the quantitative analysis of melamine and metronidazole in milk powder samples were built on the LC–MS/MS data collected in MRM scan mode.

As shown in Table 2, the predictive results of the PLS_{raw} calibration models for both metronidazole and melamine in the test powder milk samples of experiment 1 were basically satisfactory, with ARPE values of 9.0 and 9.6%, respectively. However, deterioration in the predictive capability of PLS_{raw} calibration models for both metronidazole and melamine in the test powder milk samples of experiment 2 was observed. The ARPE values of the PLS_{raw} calibration models for melamine and metronidazole increased up to 11.4% and 32.0%, respectively, which demonstrated ionization suppression and signal instability across samples have different degrees of detrimental effects on different analytes. The PLS_{raw} calibration models could not deal with the detrimental effects caused by ionization suppression and signal instability across samples.

As expected, the MEM_{MS} calibration models achieved quite satisfactory predictive results (ARPE: 6.4%) for both metronidazole and melamine in the test powder milk samples of experiment 1. Ionization suppression and signal instability across samples seemed to have no significant effects on the prediction capability of MEM_{MS} calibration models for both metronidazole and melamine in the test powder milk samples of experiment 2 (Table 2). The ARPE values of the MEM_{MS} calibration models for metronidazole and melamine in the test powder milk samples of experiment 2 were 7.8% and 7.6%, respectively. These results further confirmed that MEM_{MS} calibration models were capable of modeling the variations in the total sensitivity cross samples and over time. With the application of MEM_{MS} model, long term accuracy and stability in quantitative LC–MS/MS assays can be realized by just adding as few as two new samples into the calibration model for each batch of test samples.

4. Conclusions

With a view to realize the routine use of LC–MS/MS for quantitative analysis, a novel data structure and multiplicative effects model for mass spectroscopy (MEM_{MS}) were proposed for the analysis of LC–MS/MS data. For MS detector operated in MS2 scan mode, the two mass spectra of the target analyte and the internal standard in a sample recorded at the peaks of their chromatographic elution curves were summed up to give a virtual spectrum, which was then subjected to quantitative analysis using MEM_{MS} model. If the MS detector was operated in MRM mode, the MEM_{MS} model was built on virtual chromatograms. Each virtual chromatogram was the summation of the chromatograms of the target analyte and the internal standard in a sample at their quantitative ions, respectively. In MEM_{MS} model, a multiplicative parameter was introduced to account for the variations in overall sensitivity caused by ionization suppression and signal instability across samples. Experimental results on two proof of concept model systems (i.e. the determination of the target peptide in peptide digests mixtures, and the quantification of melamine and metronidazole in milk powder samples) revealed that the variations in overall sensitivity and signal instability across samples in LC–MS/MS assay caused large predictive errors in the predictions of PLS calibration models. The application of empirical spectral preprocessing methods such as multiplicative signal correction, standard normal variate and extended inverted signal correction significantly deteriorated instead of improved the predictive accuracy of PLS calibration models. While in contrast, the results obtained by MEM_{MS} were highly satisfactory. Ionization suppression and signal instability across samples did not cause significant deterioration in the predictive capability of MEM_{MS} calibration models. Long term accuracy and stability in quantitative LC–MS/MS assays was realized by just adding as few as two new samples into the calibration sample set of MEM_{MS} model for each batch of test samples. Our future work is to further develop and extend MEM_{MS} into application areas such as the quantification of specific protein in cells and human plasma.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chroma.2014.02.036.

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