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Technical Note

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Real Time Online Correction of Mass Shifts and Intensity Fluctuations in Extractive Electrospray Ionization Mass Spectrometry

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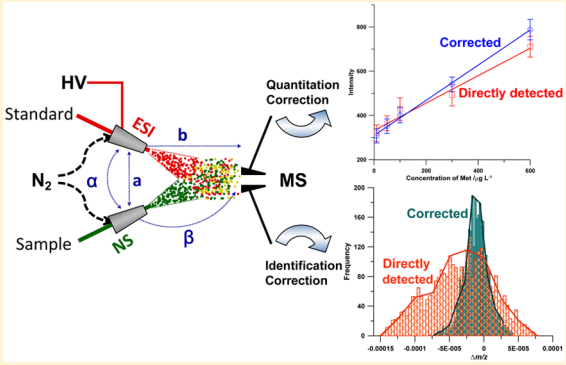
Supporting Information

ABSTRACT: Real time online calibration of mass shift and intensity fluctuation to improve the accuracy of measurements for identification and quantitation in trace mass spectrometric analysis was demonstrated using extractive electrospray ionization mass spectrometry (EESI-MS). The signals of authentic compounds (e.g., lysine (Lys), proline (Pro), and histidine (His)) spiked into the extractive solution for the EESI process were used as the references to calibrate the signal of analytes (e.g., methionine (Met)) in the untreated sample solution. The intensity of the analyte signal was recorded simultaneously with the reference signals. The analyte signals at a given time point were calibrated on the basis of these correlation factors and real time signal response of the reference. The calibrated signal of Met at 10 μg L^{-1} was improved with a better signal-to-noise ratio (S/N from 2.3 to 4.3), better linearity (R^2 from 0.9758 to 0.9980), and reduced relative standard deviation (RSD from 9.8% to 6.0%). The shift of mass-to-charge ratio of Met signal between the detected and theoretical values was decreased from 247 ± 133 to −7 ± 167 ppm for 50 min of detection using a linear ion trap mass analyzer and was reduced from −0.27 ± 0.60 to −0.12 ± 0.23 ppm for 50 min of detection using an Orbitrap mass analyzer (P = 95%). This method has been validated using a certified standard amino acids solution (GBW(E)100062) and applied for quantitative detection of amino acids in chicken feed, urine, nutritional drink, and facial mask samples, showing that the method is useful to improve the accuracy of mass spectrometric analysis.



Mass spectrometry (MS) is increasingly used for trace detection in multiple disciplines such as element analysis,[1] clinical chemistry,[2] metallomics,[3] proteomics,[4] metabolomics,[5] lipidomics,[6] etc. due to its unparalleled capability for identification and quantification of the analytes. Normally, efforts have been spent to improve the sensitivity and resolution power of MS instruments to acquire more accurate and reliable results.[7−9] To date, most commercial mass spectrometers are routinely calibrated prior to use for sample analysis to guarantee the accuracy and precision of the measurement,[10] especially for cases (e.g., analysis of biological, geological, and environmental samples) where matrix effects are notably detected. Because the spectral baseline shift is normally encountered,[11,12] the long-term accuracy and reliability of the analytical results can be affected after a long operation time. This problem is particularly serious for biological analyses using large amount of samples, e.g., metabolomics,[11,13] causing misinterpretation of experimental data.

External calibration, standard addition, and internal standards are commonly used to obtain quantitative results.[12] However, the instrument drift is hardly corrected using these methods in practical applications.[14] External calibration performed with MS instruments with high frequency should be able to reduce the instrument shift, but it is too laborious to carry out, especially in cases where online real time monitoring or a large batch of sample analysis is required.[10] Isotope labeled internal standards are also widely used to correct the signal variation caused by differences in sample composition and instrument drift.[15,16] Unfortunately, the isotope labeled compounds are usually expensive and not commonly available for all analytes. For certain compounds, the naturally occurring isotopes of the analyte and nonlinear signal response would also influence the signal of the internal standard.[11,17,18] Eckers et al.[19] and Wolff et al.[20] developed the LockSpray for accurate mass measurements. By switching the reference and sample spray alternately into MS, the mass value of the analytes could be calibrated by the reference for both MS and tandem mass spectrometry (MS/MS) measurements.[19−22] However, the mass intensity correction by two sprayers was not reported to our knowledge. Therefore, it is highly desirable to develop a new method for

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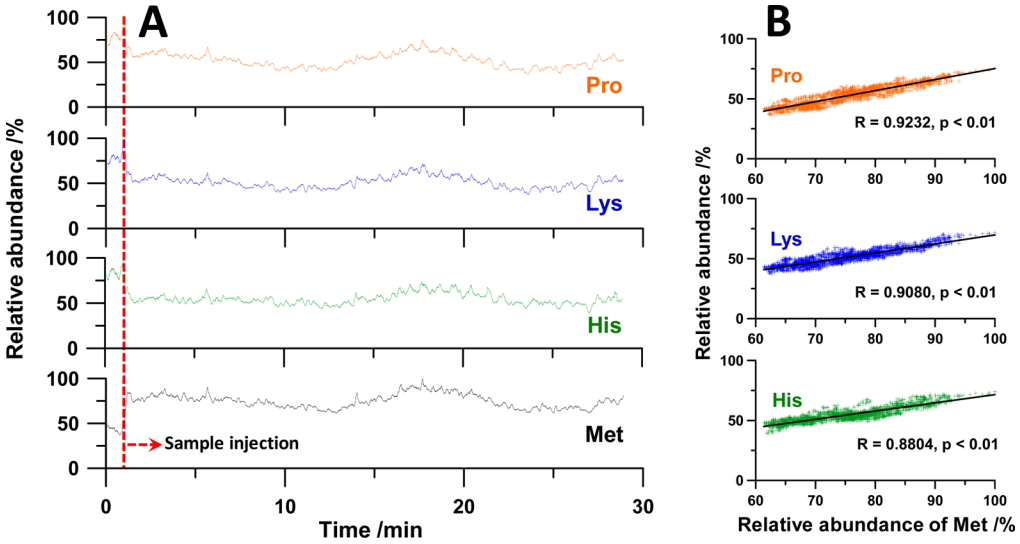

Figure 1. Signal correlation between the references and the analyte. The Pearson correlation coefficients between the Pro-Met, Lys-Met, and His-Met were 0.9231, 0.9080, and 0.8804 ($p < 0.01$), respectively. (A) The relative abundance of ESI standard solution (Pro, Lys, and His) and sample solution (Met) generated by EESI-LTQ-MS; (B) Pearson correlations between relative abundance of ESI standard solution (Pro, Lys and His, 10 μg L^{-1}) and Met (100 μg L^{-1}).

online real time quantitative detection of trace analytes in complex matrices using MS.

Recently, rapid MS analyses of liquids,[23−25] viscous heterogeneous mixtures,[26−28] solids,[29−31] and aerosol samples[32] have been successfully demonstrated using extractive electrospray ionization mass spectrometry (EESI-MS), showing high tolerance toward complex matrices. Owing to the unique configuration of EESI, raw sample is introduced to the charged plume separately from the electrospray solvent.[33] Therefore, the reference compounds can be directly spiked into the ESI solvent at constant concentrations, providing the stable reference signals to calibrate the analytes signals. Theoretically, the mass shift and the intensity fluctuation of all analytes can be notably reduced after calibration, resulting in a more reliable method for both qualitative and quantitative analysis. To demonstrate this strategy, three amino acids, lysine (Lys), proline (Pro), and histidine (His), were selected as the standard reagents to compensate for the signal variations of the analyte (e.g., methionine, Met) in different matrices, allowing more reliable detection for the trace amount of Met with improved analytical performance.

■ EXPERIMENTAL SECTION

Instrumentation and Reagents. The details of the instruments and reagents were shown in the Supporting Information (reagents, instruments, Figures S1 and S2).

Data Processing. All the recorded experimental data were imported to Microsoft Office Excel for data processing. The moving average with an interval of 20 data points was performed for the signal intensities of the ESI spray and the selected analyte ions. The IBM SPSS Statistics program was use to test the linear correlations between ESI and sample signals (the Pearson correlation coefficients and p values).

On the basis of the method of least-squares, the solver program in Microsoft Office Excel was used to calculate the linear correlations (slope and intercept) between ESI and sample signals, including signals intensities of Pro-Met, Lys-Met, and His-Met.[34] The sums of the signal intensity variations were counted by eqs 1−3, and the values of slope (k) and intercept (b) were accordingly set as the decision variables and initialized with a random value at first. To avoid the signal instability caused by introducing the analyte solution at the beginning of EESI detection, the data recorded between the acquisition time of 1.0 to 1.5 min was not used for calculating the k and b. For example, the intensity data recorded from 1.5 to 4.5 min (R^{Pro}, R^{Lys}, R^{His}, and R^{Met}) were used to solve the minimum of the eqs 1−3, the values of k and b in the present study. When solving the minimum of the f, the k and b were calculated by the solver program. In reverse, the k, b, and the ESI signals were used to calculate the corrected R^{Met} by fixing f equal to 0.

$$f(R^{Pro}) = \sum ((k^{Pro}R_n^{Pro}+b^{Pro}) - R_n^{Met})^2 \quad (1)$$

$$f(R^{Lys}) = \sum ((k^{Lys}R_n^{Lys}+b^{Lys}) - R_n^{Met})^2 \quad (2)$$

$$f(R^{His}) = \sum ((k^{His}R_n^{His}+b^{His}) - R_n^{Met})^2 \quad (3)$$

Correction of the mass shifts was performed directly in Microsoft Office Excel by using eqs 4−6, in which, $\Delta m/z_{Pro}$, $\Delta m/z_{Lys}$, and $\Delta m/z_{His}$ was the m/z difference of Pro, Lys, and His between the detected and their theoretical values, respectively.

$$m/z_{Met}^{Corrected} = m/z_{Met}^{Detected} + \Delta m/z_{Pro} \quad (4)$$

$$m/z_{Met}^{Corrected} = m/z_{Met}^{Detected} + \Delta m/z_{Lys} \quad (5)$$

$$m/z_{Met}^{Corrected} = m/z_{Met}^{Detected} + \Delta m/z_{His} \quad (6)$$

■ RESULTS AND DISCUSSION

Signal Correlation for the Reference Compounds and the Target Analyte. Without introducing the analyte solution, the ESI solution containing Pro, Lys, and His generated a mass spectrum showing dominant peaks at m/z 116, m/z 147, and m/z 156 (Figure S3A), corresponding to the protonated Pro, Lys, and His, respectively. The detection of these reference compounds was confirmed by the CID mass spectra (Figure

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S3B−D), providing characteristic fragments for each protonated precursor ions. Once the Met solution of 100 μg L^{-1} was introduced as the sample spray, typical EESI-MS mass spectra of Met were recorded as shown in Figure S4A. The analyte signal, i.e., protonated Met ($[M + H]^+$, m/z 150) was successfully detected and confirmed by the CID experiments (Figure S4B), through which the precursor ions (m/z 150) generated major fragments of m/z 133, m/z 132, m/z 104, and m/z 88 by loss of NH_3, H_2O, [HCOOH], and [NH_3, COOH], respectively. The fragmentation pathways were all in good agreement with the molecular structure of Met and the previous studies.[35]

The signal of Met and each reference compound are presented in Figure 1. The relative abundances were subjected to moving averaging with an interval of 20 scans to better visualize the variation trends of the selected ion intensities. As Met was injected into the EESI source at 1 min after data acquisition, the relative abundance of Met (m/z 150) was notably increased after 1 min while the signal of ESI solution (Pro, m/z 116; Lys, m/z 147; His, m/z 156) was decreased at the same time (Figure 1A). The signal decrease for the reference compounds ionized by ESI could be explained by the EESI mechanism through which the protons transferred from the ESI plume to the analytes.[24,33] To better visualize the correlation between the signal characteristics of Met and reference signals, the Pearson correlations analysis was performed. The correlations of Pro-Met, Lys-Met, and His-Met are shown in Figure 1B. The p values were less than 0.01, and the coefficient of correlation (R) was 0.9231, 0.9080, and 0.8804 for Pro-Met, Lys-Met, and His-Met, respectively. These results confirmed the synchronizm between the reference and the sample signal fluctuations, showing the linear relationship between the signal intensities of analytes and reference compounds.

The MS signal intensity for Met was corrected using the linear signal correlations between the reference compounds and the analyte. To achieve a more accurate linear correlation, the Solver program implemented in Microsoft Office Excel was used to calculate the slope (k) and intercept (b) for the signal intensities of Pro-Met, Lys-Met, and His-Met, respectively. For the results, the values of k^{Pro}, k^{Lys}, k^{His}, b^{Pro}, b^{Lys}, and b^{His} were calculated as 1.0041, 1.3082, 1.3093, 20.6009, 8.1150, and 8.9012, respectively. Using these linear correlations, the Met signal was fitted by the signals of Pro, Lys, and His (Figure S5). As the linear dependence between the signals of the ESI standard and sample solutions was established, the signal of the analyte at a given time could be calibrated with improved accuracy. The Met signal intensities acquired at different times could be calibrated by the same reference signal, which highly reduced the errors generated by the instrument shift during MS determination. As illustrated in Figure S5, based on 1.5 to 4.5 min of the correlation between reference and sample signals (training set), the fit signal of Met was close to the detected signals in 4.5 to 25 min (test set). The residual sum of squares (RSS) between fitted and detected signals was 0.00045 and 0.00064 for the training set and the test set. Because these RSSs were in the same level, this method could be used to reduce the intensity alteration that originated from the instrument drift during MS quantitation.

Reduction of Stochastic Error for Quantification of Met. A dynamic response curve of Met from 10 to 600 μg L^{-1} was obtained using the Met signals before and after intensity correction was applied. The signal correlations between Met signal at a given concentration level and the reference compounds were calculated by the Solver program as described in the Data Processing section. The signal levels of Met at given concentration level were calibrated with reduced stochastic errors, resulting in the improved analytical performance (Figure S6). For example, after calibration, the signal-to-noise ratio (S/N) for Met detection at 10 μg L^{-1} was increased from 2.3 to 4.3, and the linearity coefficient of determination (R^2) was increased from 0.9758 to 0.9980. The relative standard deviation (RSD) of signal intensity was reduced from 9.8% to 6.0% for detection of Met at 300 μg L^{-1}.

Quantification of Amino Acids in Standard Reference Solutions and Real Samples. The method established here was validated using a national standard amino acids solution, GBW(E)100062. As the standard solution contains various amino acids, including Met, Pro, Lys, His, and etc., a standard addition approach was necessary for quantitative detection of Met in the standard solution. After 1000 times dilution, Met at different concentrations (0 to 400 μg L^{-1}) was spiked into the solution, and the results were summarized in Table S1. Without applying the calibration, only Met of 0.89 ± 0.10 mmol L^{-1} was directly detected by EESI-MS. In contrast, the linearity of the calibration curve was also improved from 0.9949 to 0.9995 (R^2), and a total Met of 0.97 ± 0.04 mmol L^{-1} was detected in the standard amino acids solution (145.34 ± 5.03 μg L^{-1} of Met was detected in the diluted solution), showing a good agreement with the Met concentration (1.00 ± 0.03 mmol L^{-1}) marked on the standard sample solution. This result demonstrated the accuracy of EESI-MS is effectively improved by the present method.

A diluted chicken feed extract water solution was directly analyzed by EESI-MS with or without applying the calibration procedure. Without signal correction, the sample offered no reliable signal in either ESI-MS or EESI-MS due to the low concentration and highly complex matrices. With the proposed correct method, 0.263 ± 0.078 mg g^{-1} of Met was found in the chicken feed and a recovery of 100.4% was obtained for 100 μg L^{-1} Met spiked into the diluted chicken feed extracts (Table S1). Eleven amino acids in human urine, nutritional drink, and facial mask samples were also determined by EESI-MS with the calibration procedure. The results certified the applicability of the present method for multiple analytes determination in complex matrixes (Table S2).

Reduction of Mass Drift for Low Resolution MS (LTQ-MS). Mass accuracy is often more important than the intensity accuracy, especially for exact mass measurements. In this study, a novel strategy has been proposed to correct the mass drift to achieve better qualitative analysis results. Similar to the intensity calibration, reference compounds such as Pro, Lys, and His were electrosprayed as the standard reagents and Met was introduced from the sample spray of the EESI. The differences of theoretical and experimentally detected values between the mass-to-charge ratio values (m/z) of Pro, Lys, and His were used for calibration of the mass shift for Met detection. Figure S7 illustrated the distribution of relative mass difference ($\Delta m/z$) of Met between the theoretical and the detected values for detection of Met with a data acquisition time of 50 min. To distinguish the major trend of the m/z excursion, the m/z data for both ESI and sample were moving averaged with an interval of 20 by Microsoft Office Excel. As shown in Figure S7, the m/z of Met was shifted 247 ± 133 ppm (average ± 1.96SD, P = 95%, n > 10 000) as compared to the theoretical m/z value (150.058). The mass shift correction was

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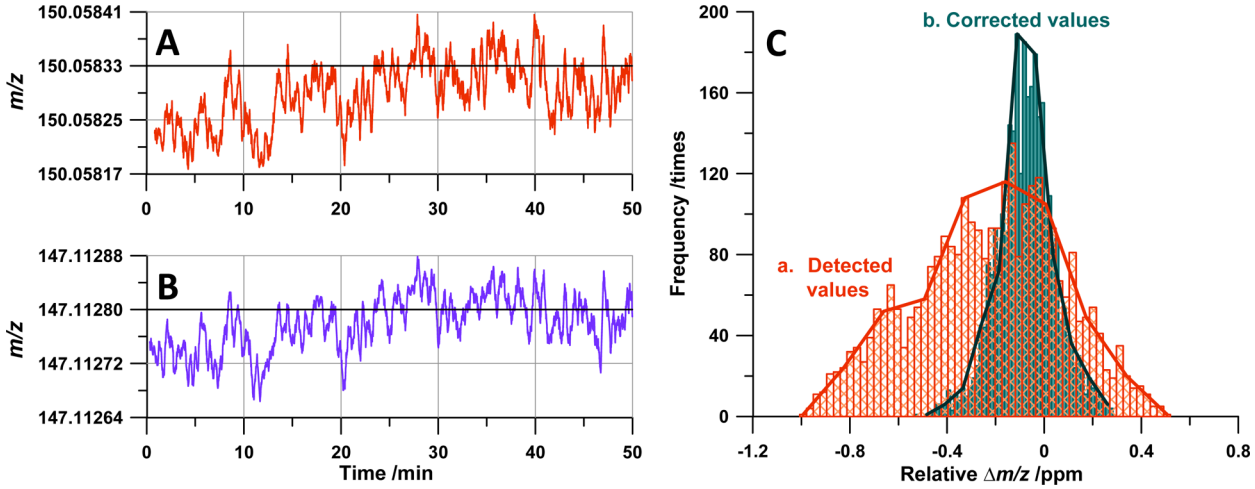

Figure 2. Calibration of mass shift of amino acids. (A) The molecular weight (MW) of Met ions ($[M + H]^+$) identified by EESI-MS in positive mode; (B) the MW of Lys detected by EESI-MS for correction MW of Met; (C) (a) the distributions of the mass-to-charge ratio difference ($\Delta m/z$) between theoretical and detected values; (b) the distributions of $\Delta m/z$ between theoretical values and the corrected values for 50 min of detection of Met by EESI-high-resolution MS (running average, $n = 5$). Met, 100 μg L^{-1} injected from sample sprayer; Lys, 10 μg L^{-1} injected from ESI sprayer.

carried out by applying the m/z of ESI signals with eqs 4−6. The Met relative $\Delta m/z$ distributions of the corrected values were also illustrated in Figure S7 with different color lines. Thereby, the mass shift of Met could be corrected using Pro, Lys, and His reference signals. As the m/z of Lys is closer to Met, the mass shift after correction by Lys was much better than the other two references. The $\Delta m/z$ for Met detection with LTQ-MS was moved around to −7 ± 167 ppm (average ± 1.96SD, P = 95%, n > 10 000). This result indicated that a similar m/z of the standard reagent was preferred for the correction of the mass shift. Theoretically, by employing three amino acids as the standard reagents in the present method, mass shifts of various amino acids could be simultaneously corrected. Table S3 summarized the mass shift correction of 11 common amino acids analyzed by EESI-LTQ-MS. With the mass shift correction, the acquired m/z of the target amino acid was clustered more closely to the theoretical values. These results showed that the method is potentially useful to acquire high mass accuracy using a low resolution MS instrument for detection of multiple compounds.

Reduction of Mass Shift for High Resolution MS (LTQ-Orbitrap-MS). The identification of compounds by high resolution MS has become the most important approach in life science and other research areas. However, the stochastic error generated by the instrument drift and intensity fluctuate seriously misled the MS software to make a correct judgment in practical applications. In comparison with the theoretical molecular ion weight of Met ($[M + H]^+$, m/z 150.05833), the variation range of the mass accuracy of a linear quadrupole ion trap (LTQ)-Orbitrap mass spectrometer was from −1.6 to 1.1 ppm (P = 95%) for continual detection of Met for 50 min. Herein, the EESI correction strategy was applied to correct the mass shift effect commonly seen in the high resolution MS, and the results were summarized in Figure 2. After the Met injection at 1 min, the signals of Met and Lys were synchronized during the 50 min of analysis. Using the moving average with an interval of 20 data points, the m/z data of Met and Lys detected by EESI-LTQ-Orbitrap-MS were shown in Figure 2A,B. The molecular ion weight ($[M + H]^+$) of Met was between 150.05817 and 150.05841, and with the mass shift alteration, it ranged from −0.87 to 0.33 ppm (P = 95%) compared to the theoretical value. The maximum mass shift for Met occurred at 4.32, 11.65, and 20.42 min with m/z at 150.05818, 150.05817, and 150.05818, with the maximum mass error of 1.13, 1.20, and 1.13 ppm, respectively. At the same time, the signals of Lys also shifted to 147.11269, 147.11266, and 147.11268, respectively, below the theoretical value (m/z 147.11280). These results indicated that the m/z values of Lys were shifted by 0.75, 0.95, and 0.82 ppm at 4.32, 11.65, and 20.42 min. After applying the mass shift correction, the mass errors of Met were reduced below 0.27 ppm for these three points. The statistical results for the Met m/z shift, including the distribution of $\Delta m/z$ between the theoretical value and the detected or corrected value, were shown in Figure 2C. Without correction, the $\Delta m/z$ of Met centered around −0.27 ± 0.60 ppm (average ± 1.96SD, P = 95%, n > 3000), and the mass error of Met was in the range of −0.87 to 0.33 ppm. Corrected by Lys, the $\Delta m/z$ distribution of Met shifted to −0.12 ± 0.23 ppm (average ± 1.96SD, P = 95%, n > 3000), and the mass error was also decreased in the range of −0.35 to 0.11 ppm. Thus, this method also enabled mass correction of high resolution MS data.

■ CONCLUSIONS

Extractive electrospray ionization allows all the given reference compounds to be stably ionized by the electrospray without interference from the matrix of the sample. On the basis of this feature of EESI, a novel analytical strategy for the real time online calibration of signal intensity and mass accuracy has been developed, without using isotopically labeled compounds. The strategy has been validated using certified authentic samples and has been tested on both low resolution ion trap and high resolution Orbitrap mass spectrometers. As the EESI device is simple and the procedure described here can be easily implemented on commonly available commercial instruments, the strategy can be broadly applied for the more accurate trace molecular analysis by mass spectrometry.

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■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.analchem.5b04372.

Reagents and materials; instrumentation; sample pretreatment; additional figures and tables (PDF)

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Notes

The authors declare no competing financial interest.

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