Using calibration approaches to compensate for remaining matrix effects in quantitative liquid chromatography/electrospray ionization multistage mass spectrometric analysis of phytoestrogens in aqueous environmental samples

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Received 19 August 2007; Revised 9 October 2007; Accepted 11 October 2007

Signal suppression is a common problem in quantitative liquid chromatography/electrospray ionization multistage mass spectrometric (LC/ESI-MSn) analysis in environment samples, especially in highly loaded wastewater samples with highly complex matrix. Optimization of sample preparation and improvement of chromatographic separation are prerequisite to improve reproducibility and selectivity. Matrix components are reduced if not eliminated by optimization of sample preparation steps. However, extensive sample preparation may be time-consuming and risk the significant loss of some trace analytes. The best way to further compensate matrix effects is the use of an internal standard for each analyte. However, in a multi-component analysis, finding appropriate internal standards for every analyte is often difficult. In this present study, a more practical alternative option was sought. Matrix effects were assessed using the post-extraction addition method. By comparison of three different calibration approaches, it was found that matrix-matched calibration combined with one internal standard provides a satisfactory method for compensating for any residual matrix effects on all the analytes. Validating experiments on different sewage treatment plant (STP) influent samples analyzing for a range of phytoestrogens showed that this calibration method provided satisfactory results with concentration ratio 96.1–105.7% compared to those by standard addition. Copyright © 2007 John Wiley & Sons, Ltd.
in the multi-component analysis of environmental samples, such as highly loaded wastewater samples. In many cases it is not possible or practical, particularly if one has a well-developed method that needs to be used on a large number of samples which may vary considerably in their background matrix components. To these samples, after optimized sample preparation, the best way to further compensate matrix effects is the use of an appropriate internal standard for each analyte. However, appropriate internal standards for every analyte in a multi-component analysis are often not available.

The aims of the present experiments were to evaluate different calibration methods by assessing the extent of matrix effects in the quantitative analysis of phytoestrogens in aqueous environmental samples and find a practical and effective calibration approach to compensate for remaining matrix effects on the analysis, after extensive optimization of the method and sample preparation.

EXPERIMENTAL

Materials

Daidzein (DAID, 98% purity), genistein (GEN, 98% purity), formononetin (FORM, 99% purity), biochanin A (BIA, 97% purity), glycitein (GLY, 97% purity), enterodiol (END, 95% purity), enterolactone (ENL, 95% purity), and coumestrol (COUM, 97.5% purity) were purchased from Sigma-Aldrich (Sydney, Australia). Deuterated genistein (3',5',6,8-d4) (98% purity, 95% isotopic enrichment) was purchased from Cambridge Isotope Laboratories (Andover, MA, USA). Acetonitrile and methanol, both HPLC grade, were supplied by Crown Scientific (Sydney, Australia). Milli-Q water (Milli-Q plus 185, Australia) was used for to make all aqueous solutions. Standard stock solutions of each compound were prepared at a concentration of 100 µg/mL in either acetonitrile or acetonitrile/methanol (80:20 v/v). Concentrated working solutions were prepared in acetonitrile/water (1:3 v/v) and obtained by tenfold dilution of the stock to a concentration of 10 µg/mL. Working solutions were then prepared in acetonitrile/water (1:3 v/v) by successive tenfold dilutions to concentrations of 100 to 1 ng/mL. All these solutions were stored in the dark at −20°C. The cartridges employed for solid-phase extraction (SPE) were 6 mL, 200 mg Oasis HLB cartridges, purchased from Waters (Sydney, Australia).

Apparatus

An optimized method for the sample extraction and LC/ESI-MSn analysis was developed as outlined below. All analyses were carried out on a ThermoElectron Finnigan LTQ linear ion trap mass spectrometer (ThermoFinnigan, San Jose, CA, USA) equipped with an ESI source. A ThermoElectron Surveyor HPLC system was interfaced to the mass spectrometer for automated LC/MS analysis.

Sample preparation

Samples were collected from different sources: surface water from Mullet Creek (Kanahooka, NSW, Australia), effluent and influent from Wollongong sewage treatment plant (STP) (Wollongong, NSW, Australia) and Gerringong - Gerroa STP (Gerringong, NSW, Australia). Amber glass bottles rinsed with Milli-Q water were used for this purpose. Before SPE, samples were adjusted to pH ~5 with dilute H2SO4 and vacuum filtered through GF/D and GF/F filters (Whatman, UK). Each aliquot for SPE was 100 mL for STP influent, and 600 mL for STP effluent and creek water. The SPE cartridges, which were placed on a vacuum manifold, were preconditioned with 5 mL methanol and 10 mL Milli-Q water. Subsequently, the samples were drawn through SPE cartridges at a flow rate <10 mL/min. After that, the cartridges were washed with 15 mL of Milli-Q water and 1 mL of a methanol/Milli-Q water (10:90, v/v) solution. The cartridges were then vacuumed dry for a few minutes. The retained components were then slowly eluted with 8 mL methanol. The extracts were evaporated to dryness under a gentle stream of nitrogen at 40°C and reconstituted with 250 µL mixed solvent of water/acetonitrile (3:1 v/v).

HPLC/MSn system

The ThermoElectron Surveyor HPLC system, including an autosampler and micro-syringe pump, was controlled using Xcalibur 1.3 software (ThermoElectron). An X Terra MS C18 column (2.1 × 150 mm, 3.5 µm; Waters) and guard column (2.1 × 20 mm, 3.5 µm; Waters) were maintained at 26°C inside a column oven. A triple solvent mobile phase system was used: 2% formic acid in water as solvent A, Milli-Q water as solvent B, and acetonitrile as solvent C. The gradient program started at 20% of solvent C, 75% of solvent B, and 5% of solvent A (this last component was kept in this proportion during the entire run). The sample was injected at time t = 0 min. After 5 min, solvent C was gradually increased to 45% by 30 min. Then solvent C was sharply increased to 90% and kept at constant for 8 min to wash all retained organics from the column. Solvent C was then decreased back to 20% and kept running at this composition for 8 min to re-equilibrate the column. The total running time was 48 min. The flow rate of the mobile phase was controlled at 200 µL/min and sample injection volume was 5 µL. The flow from the HPLC system was fed directly into an LTQ linear ion trap mass spectrometer, via an ESI source. All mass spectra were acquired in negative ion mode with a spray voltage of 3.61 kV, capillary voltage at −11.83 V, and a capillary temperature of 274.8°C. Nitrogen was used as both the sheath and auxiliary gas at 29 and 3 arbitrary units, respectively. Helium was used as damping and collision gas at a partial pressure of 0.1 Pa. The analytes were monitored by selected MS2 or MS3 transitions: DAID, m/z 252.9 → 224.8; GLY, m/z 282.9 → 267.8 → 239.8; END, m/z 301.0 → 253.0; GEN-d4, m/z 272.9 → 184.9; GEN, m/z 268.9 → 180.9; COUM, m/z 266.8 → 238.8 → 210.8; ENL, m/z 296.9 → 252.9 → 194.9; FORM, m/z 266.9 → 251.9 → 222.9; BIA, m/z 282.9 → 267.8 → 238.9. The normalized collision energies were from 36% to 49% for each analyte, respectively.

Recovery, post-extraction addition, and calibration experiments

Recovery experiments were carried out by adding 100 µL of a standard working solution (which contained 1 µg/mL of DAID, GEN, GLY, END, ENL, and 0.30 µg/mL of FORM, BIA, COUM) into the sample before SPE. The same amount
of analyte standard was also spiked into the replicate extracts after SPE. For a better comparison, a fixed amount of internal standard (100 ng/mL of GEN-d4) was spiked into all sample extracts before injection. The recoveries were calculated by comparing the peak area ratios for each analyte relative to the internal standard in samples that had been spiked before SPE (pre-spiked), with analogous ratios for samples in which the same levels of the analytes were added post-extraction (post-spiked). The peak area ratios obtained from the unspiked samples were subtracted from the pre-spiked and the post-spiked samples.30,31

The post-extraction addition experiments for assessment of any remaining matrix effects were arranged as follow: the replicate sample extracts from each sample were spiked with different concentration levels of analyte standards (5, 10, 25, 50, 100 ng/mL for surface water extracts and STP effluent extracts; and 10, 25, 50, 100, 500 ng/mL for STP influent extracts) and a fixed amount of the internal standard (100 ng/mL GEN-d4). The same levels of the analytes and internal standard were also spiked into solvent solutions. The original existence of the analytes in the analyzed samples was simultaneously quantified by the standard addition. In order to minimize the possible instrument variations, the spiked real sample extracts, the blanks, and the standard solutions were run alternately.

Matrix-matched standard calibrations curves, consisting of six concentration levels (0, 5, 10, 25, 50, 100 ng/mL for surface water and STP effluent extracts; 0, 5, 25, 50, 100, 500 ng/mL for STP influent extracts), were set up by spiking these different amounts of analyte with the internal standard (100 ng/mL GEN-d4) into sample extracts. Blanks (samples with zero addition of the analytes) were simultaneously quantified using the standard addition, and the levels of analyte present in the sample were subtracted. To evaluate the performance of the calibration curves, eight STP influent samples (collected independently from Gerrington Gerroa STP and Wollongong STP) were analyzed using a standard addition method with four levels of analyte standards (0, 5, 50, 100, 500 ng/mL) and a fixed amount of the internal standard spiked into the sample extracts. Spiked sample extracts and blanks were run consecutively in the instrument.

RESULTS AND DISCUSSION

Method optimization: sample preparation and chromatography

Optimising sample preparation is essential to reduce matrix component and thus to improve reproducibility and accuracy of the analysis. In this study, an extraction of phytoestrogens from the water samples was developed and optimized using Oasis HBL SPE cartridges.30,32–34 Alternatives such as liquid-liquid extraction (LLE) may sometimes provide clean final extracts, but the recovery, particularly for polar analytes, may be very low.35 Sample preparation is an important aspect of any analytical method development and optimising the SPE was a critical step in the sample preparation. To avoid exceeding the retention capacity of the SPE cartridge and thus reducing the recoveries of the analytes, the sample loading volume for each single cartridge was trialled by experiment. For those highly concentrated samples such as WWTP influent, 100 mL as aliquot volume proved optimal for SPE, whilst for the lower concentration samples, 600 mL of creek water and STP effluent were found to be suitable. Although Oasis HBL cartridges can be used over a wide range of pH, some studies has shown that for some compounds the extraction efficiency is affected by the pH.21,36 There has been research that has shown that coextraction of humic and fulvic acids from water is influenced by the pH of the sample extract applied for SPE; they declined rapidly at neutral pH versus extraction at acidic pH.37 The pH of the samples is important as this would affect the state of the analyzed compounds and the interaction between the analyte and SPE cartridge packing material. Therefore, prior to extraction, the acidity of samples was adjusted to an appropriate pH range. For our analytes, phytoestrogens, which possess phenolic or diphenolic groups, the acidic level at pH ~5 was selected. Keeping this acidity could be helpful in reducing inferences from some alkaline organic compounds and also for the repeatability of the SPE. To reduce the inorganic and some organic interference on the SPE cartridge, the cartridge sorbent was washed with an appropriate solvent before elution. The selection of washing solvent in SPE balanced removal of unwanted compounds and retaining as much of the trace analytes as possible on the SPE cartridge. In this experiment, 15 mL of Milli-Q water was used to wash off salts and other very polar chemicals, followed by 1 mL of a methanol/Milli-Q water (10:90 v/v) solution to remove comparatively more polar organics. The recovery control experiments showed that this small amount of methanol did not wash off significant amounts of the analytes from the cartridge. The recovery experiments showed good accuracy and repeatability (see Table 1).

Although complete separation by HPLC seems not necessary when using selective MS detection, it still plays an important role in improving both detectability and reliability. This is because high resolution in chromatographic separation reduces the level of compounds coeluting with the analyte of interest and therefore results in reduced ion suppression. HPLC separation of phytoestrogenic compounds has usually been carried out on a reversed-phase column with a mobile phase of methanol or acetonitrile and water.5,6 In this study, both methanol and acetonitrile were tested for their separation performance; it was found that the retention time of the analytes changed slightly, but better peak shape was obtained using acetonitrile with the Xterra MS C18 column. The phytoestrogenic compounds analyzed in our work all contain phenolic hydroxy groups, which exhibit a weak acidic nature. Therefore, it would be expected that the use of an acidic modifier would allow the analytes to become dissociated in the solvent system, thus enhancing the chromatographic separation and improvement of peak shapes.5,6 To confirm this, three types of mobile phase of acetonitrile/water systems (added 0.1% formic acid, added 0.1% ammonia, and no modifier added) were tested for their chromatographic performance. It was found that better peak shapes were obtained by the addition of 0.1% formic acid. The chromatographic separation was carried out maintaining the column temperature at 26°C so that stable retention
times were obtained. The gradient program for the mobile phase was optimized by experiment. In the gradient program enough time was given to re-equilibrate the column prior to the next injection. Figure 1 shows the SRM chromatogram obtained from surface water extracts spiked with 100 ng/mL of each analyte standard.

Assessment of remaining matrix effects

Any remaining matrix effects were assessed using the post-extraction addition method. The concept of quantitative assessment of matrix effect used in this paper was adapted from Matuszewski et al.,38 where matrix effect (ME %) was calculated by comparing the peak area of known amount of a standard solution (A) with that from a sample extract spiked with the same amount of analyte after extraction (B). The ratio \( \frac{B}{A} \times 100 \) is defined as absolute matrix effect (ME %). In the current experiments, because the analytes were already present in the samples, it was not possible to obtain a real control matrix sample without any of the analytes. Therefore, the presence and identity of the analytes in the samples was verified and the amount quantified by standard addition methods. The response factor \( RF = \frac{\text{peak area}}{\text{concentration}} \) was used to represent the response of an analyte in a certain matrix;27 thus the matrix effect (ME %) was calculated by comparing the RFs in the real samples with the RFs in the standard solution:

\[
\text{ME} \% = \left( \frac{RF(\text{analyte})_{\text{sample}}}{RF(\text{analyte})_{\text{solvent}}} \right) \times 100.
\]

Table 1. Recoveries of analyzed phytoestrogens in the spiked real samples

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Surface water</th>
<th>STP effluent</th>
<th>STP influent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average recovery (%)</td>
<td>RSD (%)</td>
<td>Average recovery (%)</td>
</tr>
<tr>
<td>Daidzein (DAID)</td>
<td>92</td>
<td>5.9</td>
<td>97</td>
</tr>
<tr>
<td>Genistein (GEN)</td>
<td>89</td>
<td>6.1</td>
<td>97</td>
</tr>
<tr>
<td>Formononetin (FORM)</td>
<td>93</td>
<td>6.5</td>
<td>89</td>
</tr>
<tr>
<td>Biochanin-A (BIA)</td>
<td>95</td>
<td>6.0</td>
<td>98</td>
</tr>
<tr>
<td>Glycitein (GLY)</td>
<td>98</td>
<td>6.6</td>
<td>97</td>
</tr>
<tr>
<td>Enterodiol (END)</td>
<td>94</td>
<td>5.1</td>
<td>96</td>
</tr>
<tr>
<td>Enterolactone (ENL)</td>
<td>91</td>
<td>3.9</td>
<td>96</td>
</tr>
<tr>
<td>Coumestrol (COUM)</td>
<td>96</td>
<td>6.6</td>
<td>91</td>
</tr>
</tbody>
</table>

*Average recoveries were determined by measuring five replicate aliquots, each aliquot was tested four times. RSD (%) of average recoveries calculated by measure results of the five independent replicate aliquots.

Three different types of sample (surface water, STP effluent, and STP influent) were used to evaluate the matrix effects on phytoestrogenic compounds in quantitative LC/ESI-MS\textsuperscript{n} analysis. These were the same matrices used in the method development. These real sample extracts were spiked with a series of concentrations of the analyte standards (5, 10, 25, 50, 100 ng/mL for surface water extracts and STP effluent extracts; and 10, 25, 50, 100, 500 ng/mL for

Figure 1. SRM chromatogram obtained from surface water spiked with 100 ng/mL of each analyte standard.
STP influent extracts). The presence and true concentration of the analytes in the samples was simultaneously quantified by standard addition. Samples at each concentration level were run five times and, in order to minimize the possible instrument variations, the spiked real sample extracts and the standard solutions were run alternately. The ratio of RF obtained in the spiked real sample extract to the RF obtained in the standard solution reflected the degree of matrix effects occurring with the analyte in this type of matrix sample and is represented by the value ME %. Figure 2 shows the signal suppression range of the internal standard (GEN-d$_4$) in the three types of spiked real sample extract compared with that in the standard solution made with water/acetonitrile (3:1 v/v) as solvent. The data shows the extent of signal suppression by the three sample matrices. For the control solvent sample, the mean ME % is 100% by definition. The grey box in this instance represents the reproducibility of the measurement for 25 replicate injections (5 runs × 5 concentration levels).

As all the real sample extracts contained no internal standard (GEN-d$_4$) before it was spiked, the deduction in RFs thus may be attributed to the matrix effect only. The results shown in Fig. 2 confirmed that the sample matrix for this analyte caused signal suppression in the three environmental samples, and that the extent of signal suppression was dependent on the sample background. The more concentrated and complex samples (such as STP influent) suffered greatest signal suppression. The matrix effects for the eight analytes are clearly depicted by the plots in Fig. 3 and the same trend in signal suppression is observed; the signal suppression was found to be not equal for all the analytes, with the more polar analytes (which eluted earlier) suffering the most signal suppression. This was especially so at higher analyte concentrations and more complex matrix background samples, such as STP influent. These results are in agreement with the previous literature.\textsuperscript{12,13,18,39}

Comparison of three calibration approaches
The best method to further compensate for any remaining matrix effects after method development due to sample background variation is the use of an internal standard for each analyte.\textsuperscript{8,13,40} However, in a multi-component analysis, finding appropriate internal standards for every analyte may be rather difficult or impossible. Potential alternatives, such as the standard addition method, do not require any internal standards, but this method is very time-consuming in nature and the laborious manipulation involved makes the method often impractical for routine analysis particularly with large batches of samples. By contrast, external matrix-matched calibration may only effectively compensate for matrix effects from moderately loaded samples with uniform matrix.\textsuperscript{27,29} In this present study, a better, more practical alternative option was sought. In order to achieve this, a combination of internal standard with matrix-matched calibration was applied.
In order to evaluate this calibration method, the matrix-matched calibration curves were set up by spiking a series of standards using the three different types of real sample extracts (5, 10, 25, 50, 100 ng/mL). Because of the high concentrations of some analytes in influent sample extracts, an additional 500 ng/mL standard was used in the calibration. Figure 4 shows calibration curves of GEN (Fig. 4(a)) and DAID (Fig. 4(b)); they were obtained by using a single internal standard (GEN-d4) in different matrices. The calibration slopes and the correlation coefficients for all analytes are listed in Table 2.

In Fig. 4(a) calibration curves for GEN obtained from the real sample extracts nearly coincide with the calibration curve from the standard solution, indicating that the internal standard GEN-d4 can successfully compensate for remaining matrix effects from the three different matrix backgrounds. However, in Fig. 4(b) the calibration slopes from the real sample extracts were lower than that from the standard solution; this means only a single internal standard cannot compensate any remaining matrix effects for all analytes and that matrix-matched calibrations are required for quantitation in different matrices. As Fig. 4(b) and Table 2 show, the internal standard matrix-matched calibration curves were determined on the specific sample matrix; therefore, any variation in the signal suppression across the different analytes in the sample matrix, such as the severe signal suppression of the three early eluting analytes, has already been taken into account in their calibration curves. With one internal standard to correct the variations of different batches of samples, this calibration method could be an easily practical approach to further compensate the signal suppression from the real samples.

To evaluate the performance of matrix-matched standard calibration with one internal standard in compensating matrix effects in real samples, eight independent STP influent samples were analyzed and the results were compared to those obtained by standard addition. STP influent samples were selected for this assessment because they suffered the most signal suppression. In this assessment, it was assumed that standard addition provided the most accurate value of the analyte concentration and the most effective way to eliminate any matrix effect.27 The concentrations of the analytes in blank sample extracts were quantitated by three different calibration methods: (i) solvent standard calibration with one internal standard, (ii) external matrix-matched standard calibration, and (iii) matrix-matched standard calibration with one internal standard. The concentrations obtained by each calibration curve were compared to the concentrations obtained by standard addition. The ratios [concentration(analyte) by calibration / concentration(analyte) by standard addition] were calculated; they represented the performance of the three standard cali-

Table 2. Slope and the square of the correlation coefficient (R²) of the internal standard calibration curves for the analyzed phytoestrogens in the solvent and in matrix-matched sample extracts

<table>
<thead>
<tr>
<th>Analyte standards</th>
<th>DAID</th>
<th>GLY</th>
<th>END</th>
<th>GEN</th>
<th>COUM</th>
<th>ENL</th>
<th>FORM</th>
<th>BIA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>In the solvent</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slope</td>
<td>1.4812</td>
<td>43.803</td>
<td>62.272</td>
<td>5.4695</td>
<td>3.5129</td>
<td>3.1828</td>
<td>31.668</td>
<td>33.689</td>
</tr>
<tr>
<td>R²</td>
<td>0.9996</td>
<td>0.9905</td>
<td>0.9991</td>
<td>0.9979</td>
<td>0.9826</td>
<td>0.9998</td>
<td>0.9987</td>
<td>0.9963</td>
</tr>
<tr>
<td><strong>In surface water extract</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slope</td>
<td>1.2440</td>
<td>38.510</td>
<td>52.867</td>
<td>5.3523</td>
<td>3.6807</td>
<td>3.5745</td>
<td>30.973</td>
<td>35.460</td>
</tr>
<tr>
<td>R²</td>
<td>0.9997</td>
<td>0.9992</td>
<td>0.9947</td>
<td>0.9991</td>
<td>0.9979</td>
<td>0.9986</td>
<td>0.9993</td>
<td>0.9984</td>
</tr>
<tr>
<td><strong>In STP effluent extract</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slope</td>
<td>1.1586</td>
<td>35.671</td>
<td>53.338</td>
<td>5.1512</td>
<td>3.5297</td>
<td>3.2825</td>
<td>29.638</td>
<td>33.177</td>
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<tr>
<td>R²</td>
<td>0.9990</td>
<td>0.9991</td>
<td>0.9979</td>
<td>0.9993</td>
<td>0.9959</td>
<td>0.9991</td>
<td>0.9904</td>
<td>0.9967</td>
</tr>
<tr>
<td><strong>In STP influent extract</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slope</td>
<td>1.0271</td>
<td>31.336</td>
<td>47.074</td>
<td>5.3487</td>
<td>3.3837</td>
<td>3.4157</td>
<td>32.424</td>
<td>35.774</td>
</tr>
<tr>
<td>R²</td>
<td>0.9999</td>
<td>0.9998</td>
<td>0.9998</td>
<td>0.9988</td>
<td>0.9952</td>
<td>0.9997</td>
<td>0.9999</td>
<td>0.9997</td>
</tr>
</tbody>
</table>

bration methods. Figure 5 shows the comparison of the three standard calibration methods in the analysis of eight different STP influent samples.

It can be seen from Fig. 5 that the concentrations quantitated by matrix-matched standard calibration with one internal standard were consistently similar to those obtained by standard addition, with the concentration ratios close to 100%. The means of the concentration ratios of all analytes in the eight different samples were 96.1–105.7% and the relative standard deviations (RSDs) for the analytes of the eight independent samples were 3.0–7.6%. These results demonstrate that matrix-matched standard calibration with one internal standard has effectively compensated for any remaining matrix effects in the selected samples.

Use of external matrix-matched standard calibration without the internal standard also achieved better compensation for matrix effects for all analytes compared to solvent standard calibration with a single internal standard, especially for the early eluted analytes DAID, GLY, and END, which had shown severe signal suppression in our earlier experiment (see Fig. 3). However, unfortunately, this method does not give satisfactory precision with the RSDs 7.6–21.3%. This result is not surprising given the lack of an internal standard which plays a very important role in compensating the variation from samples, instrument and manipulations.

As for the results obtained by use of solvent standard calibration with one internal standard, the attempts to compensate matrix effects were not satisfactory. The amounts of the early eluting analytes, DAID, GEN and END, were severely underestimated with the means of the concentration ratios in the eight samples being 70.2%, 68.7% and 76.0%. Although this calibration method provided fair precision (see error bars in Fig. 5), it is clearly deficient for the analysis of multiple analytes, especially in complex matrix samples such as STP influent.

In summary, by comparing the three standard calibration methods for compensating matrix effects, matrix-matched standard calibration with one internal standard has been shown to provide the best results. Although the validating samples in this study are limited, it strongly suggests that this calibration method may be an easily practical and effective approach to compensate matrix effects.

CONCLUSIONS

Previous experience has demonstrated that matrix effects are awkward problems that must be tackled to ensure reliable quantitation in LC/ESI-MS. Optimization of sample preparation and improvement of chromatographic separation are essential to improved reproducibility and selectivity of quantitation. Whilst it is desirable to eliminate completely matrix effects this is often not feasible particularly in dealing with environmental samples with trace levels of analytes and wide variation in background matrix. In these circumstances, however, a considerable degree of signal suppression in the real sample extracts, such as wastewater extracts, may be experienced and needs to be compensated by effective calibration approaches. Use of internal standards is known to be the best way to compensate matrix effects, but in a multi-component analysis one internal standard has been shown to be deficient in compensating the signal suppression on all analytes. That means that each analyte would require an internal standard of its own, but this is not always possible. We have clearly demonstrated in a systematic study that matrix-matched calibration with one internal standard could be a practical alternative option to compensate matrix effects in multi-component analysis of environmental samples.

Matrix-matched calibration with a single internal standard can retain the main advantages of both standard addition and internal standard methods; it saves laborious work in standard addition and renders unnecessary the requirement to find appropriate internal standards for every analyte in a multi-residue analysis.
Acknowledgements

The authors are grateful to Glen Austin from Sydney Water and Jeff Kydd from VEOLIA for their assistance in sampling.

REFERENCES