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Improved Calibration for the Analysis of Emerging Contaminants in Wastewater Using Ultra High Performance Liquid Chromatography and Time-of-Flight Mass Spectrometry

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Abstract : The focus of this paper is to present techniques to overcome certain difficulties in quantitative analysis with a time-of-flight mass spectrometer (TOF-MS). The method is based on conventional solid-phase extraction, followed by reversed-phase ultra high performance liquid chromatography of the extract, and mass spectrometric analysis. The target compounds included atenolol, atrazine, caffeine, carbamazepine, diclofenac, estrone, ibuprofen, naproxen, simazine, sucralose, sulfamethoxazole, and triclosan. The matrix effects caused by high concentrations of organic compounds in wastewater are especially significant in electrospray ionization mass spectroscopy. Internal-standard calibration with isotopically labeled standards corrects the results for many matrix effects, but some peculiarities were observed. The problems encountered in quantitation of carbamazepine and triclosan, due to nonlinear calibration were solved by changing the internal standard and using a narrower mass window. With simazine, the use of a quadratic calibration curve was the best solution.

Keywords : emerging contaminant, time-of-flight mass spectrometry, method development, municipal wastewater, internal standard calibration

Introduction

Several methods are available for the analysis of emerging contaminants (ECs) in wastewater (WW) or surface water.[1-5] Mostly ECs are analyzed using triple quadrupole mass spectrometers because of their excellent sensitivity and broad dynamic range. Here, the aim was to develop a method for both quantitative analysis of ECs in wastewater and for qualitative analysis of the transformation products of them. Therefore, method that is based on ultrahigh-performance liquid chromatography and time-of-flight mass spectrometry (UPLC-TOF-MS) was developed.

The information in the literature was used as the starting point in our method development. However, we found that many modifications were needed. Here, we describe some of the problems encountered in calibration of the method and the solutions we found. In most reports of method development, such problems are not discussed, but only the final method is presented.

This method was successfully applied in MOTREM project to determine degradation of ECs after advanced oxidation processes.[6] The compounds studied included atenolol (ATN), atrazine (ATZ), caffeine (CFN), carbamazepine (CBZ), diclofenac (DCF), estrone (EST), ibuprofen (IBP), naproxen (NAX), simazine (SMZ), sucralose (SCL), sulfamethoxazole (SMX), and triclosan (TCS).

Experimental

Ultrapure water (18.2 MΩ/cm) was prepared in the laboratory with an Elga® Purelab Ultra water purification system. Solvents were all of LC-MS quality. The purity of the analytical standards was >98% in most cases but sucralose-D_6 was 95% pure. The standard compounds were weighed and dissolved in methanol to make a set of dilutions for calibration. Carbamazepine-D_{10} was purchased as a solution.

Solid-phase extraction (SPE) of WW samples was carried out using Oasis HLB cartridges (500 mg/6 ml; Waters Inc., Milford, MA, USA). An internal-standard mixture (40 ng or 400 ng of each isotope-labeled target compound per sample) was added to each WW sample (100-500 ml) and the samples were shaken for an hour before extraction. The SPE cartridges were eluted with methanol and after evaporation with a stream of nitrogen the final sample was dissolved in

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1 ml of 20% methanol and filtered with a 0.2-μm PTFE filter (VWR International).

LC separation of the components was done with a Waters Acquity UPLC, using a Waters Acquity UPLC HSS T3 column (1.8 μm, 2.1×100 mm; Waters Inc.). In positive-ion mode, eluent A consisted of 5% methanol in water with 0.1% formic acid and eluent B of 100% methanol with 0.1% formic acid. In negative-ion mode, eluent A consisted of 5% methanol in water with 1 mM ammonium fluoride and eluent B of 100% methanol with 1 mM ammonium fluoride. The use of NH_4F was suggested in the literature to improve signal response of several ECs.[7] Gradient elution was used for both modes: 1 min 100% A, 30 min from 100% A to 100% B, 8 min 100% B, and 3 min 100% A. The flow rate was 0.2 ml/min and the injection volume 20 μl.

The TOF-MS was a Waters/Micromass LCT Premier XE (Waters Inc./Micromass®, Manchester, UK) with a dual electrospray ionization (ESI) source. Leucine-enkephalin solution (used as the lock mass) was monitored every 50 scans, using the reference sprayer. The instrument was controlled with MassLynx V4.1 software (Waters Inc., Manchester). The data were processed with TargetLynx, one of the application managers of MassLynx (also from Waters). The peak area was used for quantitation.

Internal standard (ISTD) calibration technique with isotopically labeled compounds was used. The concentration of internal standards in standard mixtures was 20 μg/l for ATZ, EST, SMZ, and TCS, and 200 μg/l for the other compounds. The highest concentration used for calibration was 180 μg/l for ATZ, EST, SMZ, and TCS, and 1800 μg/l for the other compounds. In this study, atenolol, atrazine, caffeine, carbamazepine, estrone, naproxen, simazine, and sulfamethoxazole were quantitated, using the positive-ion mode, and diclofenac, ibuprofen, sucralose, and triclosan using the negative-ion mode. The linearity of calibration was assessed by analyzing a set of standards from 0.5 μg/l to 180 μg/l or from 5 μg/l to 1800 μg/l. In the calculations the origin was excluded and a weighting factor 1/x was used. The calibration was considered to be linear when the square of the correlation coefficient (R^2) was 0.99 or better.

Results and discussion

Matrix-related signal suppression or enhancement causes calibration problems in wastewater analysis. Matrix-matched standards cannot be prepared since there is no such matrix available and, in addition, the matrix may vary over time. Synthetic wastewater has been used to overcome this problem,[8] but such matrices are never the same as real WW. Standard addition calibration is one way to correct for matrix effects,[9] but it is a very laborious technique. We believe that the best way to overcome calibration problems is to use isotope-labeled internal standards for each compound. Correct internal-standards compensate for matrix effects and also possible losses during sample treatment.

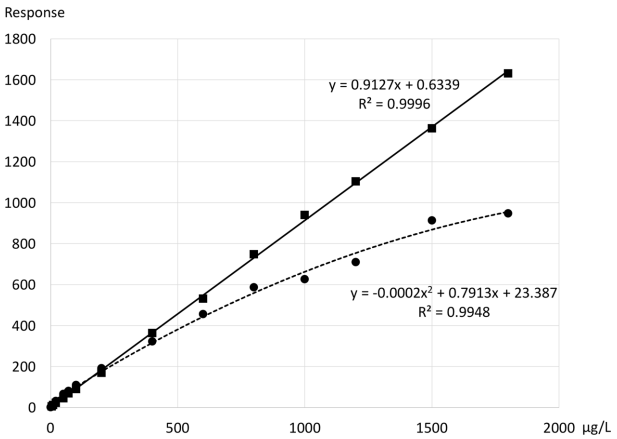

Figure 1. Calibration curve of carbamazepine with carbamazepine-D_{10} as the internal standard ● and linear calibration with carbamazepine-$^{13}C_6$ as the internal standard ■. Response =$Area_{CBZ}$*$Concentration_{ISTD}$/$Area_{ISTD}$.

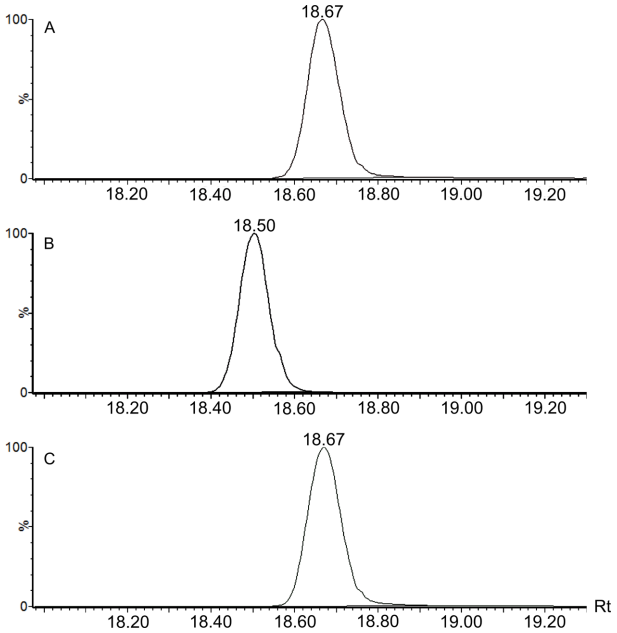

Figure 2. Narrow-width extracted-ion chromatograms of A) carbamazepine, B) carbamazepine-D_{10}, and C) carbamazepine-$^{13}C_6$.

For most compounds, the calibration was linear over the range studied when the criterion for linearity was set as $R^2 \geq 0.99$. However, in some cases the linear range was very narrow and the calibration was studied in further detail. One of these compounds was carbamazepine with a linear range only up to 70 μg/l. However, a much better linear calibration (up to 1800 μg/l) was obtained when the internal standard was changed from carbamazepine-D_{10} to carbamazepine-$^{13}C_6$ (Figure 1).

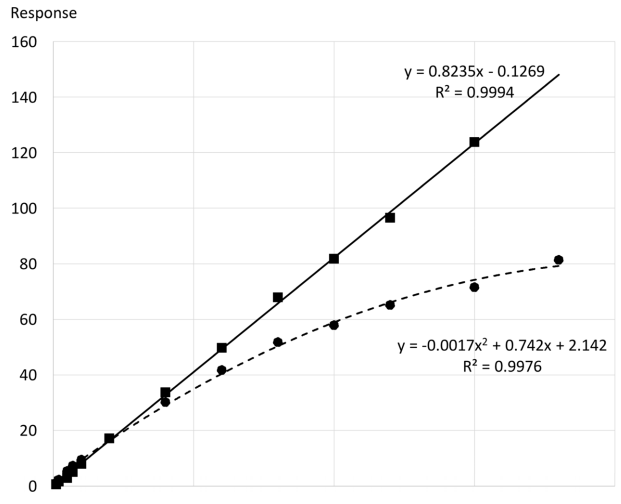

Figure 3. Calibration of triclosan with standard parameters for the internal standard (triclosan-D_3, 50 mDa mass window, m/z 289.9622), ●, and with a narrow mass window (10 mDa) and ^{37}Cl isotope peak (m/z 291.9592) ■. Response= $Area_{TCS}$*$Concentration_{TCS\text{-}D3}$/$Area_{TCS\text{-}D3}$.

The nonlinear calibration with carbamazepine-D_{10} may have been due to the small difference in retention time (Figure 2) and the corresponding difference in the composition of the mobile phase.[10] The retention time of carbamazepine-$^{13}C_6$ is exactly the same as that of the parent compound and, therefore, the matrix interferences are compensated. Nonlinear calibration has also been noted for carbamazepine in an application note.[11] The author suspected that this was due to "the nature of the compound" and suggested the use of a quadratic calibration equation.

The linear range for triclosan calibration was initially only up to 20 μg/l (Figure 3). We noticed that the peak size of the internal standard (triclosan-D_3) increased with increasing analyte concentration, although the triclosan standard contained no deuterated triclosan. The reason was found to be the ion $[M\text{-}H]^-$ = 289.9437 of triclosan corresponding to the elemental composition $C_{11}(^{13}C)H_7Cl_2(^{37}Cl)O_2$ -H. This ion interfered with the molecular ion of triclosan-D_3, $[M\text{-}H]^-$ = 289.9622 when the normal mass window of 50 mDa was used for peak detection.

The linearity of calibration was improved when the ion 291.9592 was used as the target ion for TCS-D_3. This is the first ^{37}Cl isotope peak of TCS-D_3 and the parent compound (mass peak 291.9408; $[C_{11}(^{13}C)H_7Cl(^{37}Cl_2)O_2$ -H] does not affect its intensity markedly. In addition, a narrower mass window (10-20 mDa) was used to minimize that interference. This could be done since the mass accuracy was very good, -0.1 mDa (-0.3 ppm) for triclosan (Table 1). The mass resolution of the spectrometer was typically about 11 000 (FWHM; W optics setup).

Table 1. Mass accuracy of the internal standards.

	Theoretical mass $[M+H]^+$	Measured mass $[M+H]^+$	Error mDa	Error ppm
ATN-D_7	274.2148	274.2147	0.1	0.4
CFN-$^{13}C_3$	198.0983	198.0977	0.6	3.0
SMX-D_4	258.0850	258.0840	1.0	3.9
NAX-D_3	234.1209	234.1212	-0.3	-1.3
SMZ-D_{10}	212.1487	212.1482	0.5	2.4
CBZ-$^{13}C_6$	243.1229	243.1230	-0.1	-0.4
ATZ-D_5	221.1330	221.1331	-0.1	-0.5
EST-D_2	273.1824	273.1815	0.9	3.3
	$[M\text{-}H]^-$	$[M\text{-}H]^-$		
SCL-D_6	401.0444	401.0440	0.4	1.0
DCF-$^{13}C_6$	300.0290	300.0291	-0.1	-0.3
IBP-D_3	208.1417	208.1414	0.3	1.4
TCS-D_3	289.9622	289.9623	-0.1	-0.3

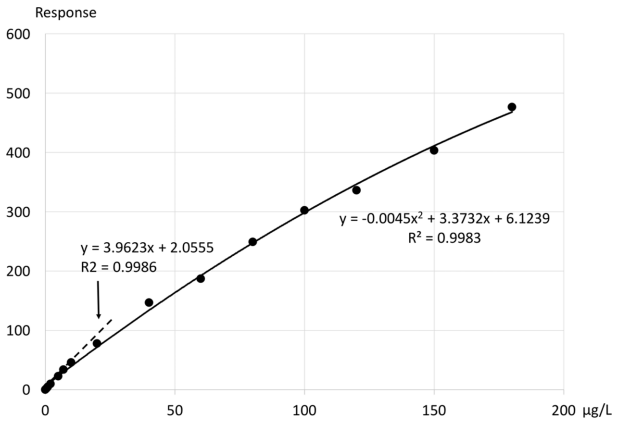

Figure 4. Calibration of simazine; the linear range was only up to 10 μg/l. Response = $Area_{SMZ}$*$Concentration_{SMZ\text{-}D10}$/$Area_{SMZ\text{-}D10}$.

This way the linear range was up to 150 μg/l with an R^2 value of 0.9994. Petrie et al.[7] also observed nonlinear calibration for triclosan and used two different calibrations in quantitation, one in the range of 1-200 μg/l and another in the range of 100-1000 μg/l. The internal standard was not mentioned in that study.

In the case of simazine, the narrow linear range could not be expanded. The linear range was just up to 10 μg/l (Figure 4). Using another quantitation ion or narrower mass window did not improve linearity and the best solution was to use a quadratic calibration function.

Table 2 shows the linear calibration range and the correlation coefficient R^2 values of the target compounds. In general, the linear range was favorable (up to 150-180 μg/l or 1800 μg/l) for all compounds, except simazine. Ranges between 50 μg/l and 250 μg/l were reported in the literature for several pharmaceuticals, using a TOF-MS

Table 2. Linear range of the calibration of the target compounds (correlation coefficients $R^2 \geq 0.99$).

	Linear range µg/l	R^2
Atenolol	5–1800	0.9992
Caffeine	5–1800	0.9966
Sulfamethoxazole	5–1800	0.9969
Naproxen	5–1200	0.9914
Simazine	0.5–10	0.9986
Carbamazepine	5–1800	0.9996
Atrazine	0.5–150	0.9901
Estrone	0.5–180	0.9949
Sucralose	5–1800	0.9981
Diclofenac	5–1800	0.9993
Ibuprofen	5–1800	0.9986
Triclosan	1–150	0.9994

similar to the type employed in this study.[12]

The detection limits for wastewater samples are much lower than the concentrations of the calibration solutions since the samples are concentrated 100-500 times during the sample preparation. The method limit of quantitation (MLQ) for wastewater effluent samples varied from 4 ng/l (atrazine) to 66 ng/l (estrone; data not shown).

Conclusions

We found that the application of methods presented in the literature was complicated by several problems encountered during the validation process.

The use of isotopically labeled internal standards was considered essential in the calibration. Yet, certain adjustments had to be made to improve the calibration of some compounds. The narrow linear range was improved by selecting more suitable internal standards and ions for the calibration in the case of carbamazepine and triclosan.

Supporting Information

Supplementary information is available at https://drive.google.com/file/d/1eZz2TxPCe8amG6RQ0Jqb4xCYhayhAVAQ/view?usp=sharing.

Acknowledgments

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References

1. Ferrer, I.; Thurman, E. M. *Trends Anal. Chem*. **2003**, 22, 750.
2. Petrovic, M.; Gonzalez, S.; Barceló, D. *Trends Anal. Chem.* **2003**, 22, 685.
3. Fatta, D.; Nikolaou, A.; Achilleos, A.; Meriç, S. *Trends Anal. Chem.* **2007**, 26, 515.
4. Petrie, B.; Barden, R.; Kasprzyk-Hordern, B. *Water Res*. **2015**, 72, 3.
5. Lindholm, P. C.; Knuutinen, J. S.; Ahkola, H. S. J.; Herve, S. H. *BioResources* **2014**, 9, 3688.
6. Rodríguez-Chueca, J.; García-Cañibano, C.; Lepistö, R. -J.; Encinas, Á.; Pellinen, J.; Marugán, J. *J. Hazardous Mater. in press*. https://doi.org/10.1016/j.jhazmat.2018.04.044.
7. Petrie, B.; Youdan, J.; Barden, R.; Kasprzyk-Hordern, B. *J. Chromatogr. A* **2016**, 1431, 64.
8. Gómez, M. J.; Gómez-Ramos, M. M.; Malato, O.; Mezcua, M.; Férnandez-Alba, A. R. *J. Chromatogr. A* **2010**, 1217, 7038.
9. Nurmi, J.; Pellinen. J. *J. Chromatogr. A* **2011**, 1218, 6712.
10. Berg, T.; Strand D. H. *J. Chromatogr. A*. **2011**, 1218, 9366.
11. Meng, C. -K. Agilent Application Note 5989-5319EN; See https://www.agilent.com/cs/library/applications/5989-5319EN.pdf.
12. Al-Qaim, F. F.; Abdullah, M. P.; Othman, M. R.; Latip, J.; Afiq, W. *J. Braz. Chem. Soc.* **2014**, 25, 271.

