

Operational options to reduce matrix effects in liquid chromatography–electrospray ionization–mass spectrometry analysis of aqueous environmental samples

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Abstract

Matrix effects like signal enhancement or suppression can severely compromise quantitative analysis of environmental samples with liquid chromatography–electrospray ionization–mass spectrometry (LC–ESI–MS). Several operational options were studied to reduce such matrix effects in the determination of polar organic trace contaminants from water, like non-steroidal anti-inflammatory drugs, among them ibuprofen, diclofenac and naproxen, lipid regulators like bezafibrate and clofibrac acid and industrial chemicals (2-substituted benzothiazoles). A step-wise removal of organic matrix from a wastewater sample by ultrafiltration showed that the majority of matrix effects in that sample was due to low molecular weight compounds <1 kDa. For such wastewaters samples size-exclusion, as in restricted access material (RAM), is not a useful clean-up strategy. Reducing the eluent flow entering the ESI interface by post-column splitting increased instrumental sensitivity and reduced matrix effects. The flow optimum was analyte-dependent and ranged from 20 to 100 $\mu\text{L}/\text{min}$. Sensitivity in the positive ion mode increased up to nine-fold upon flow-reduction for some analytes detected in the positive ion mode. At low flow rates matrix effects are reduced by 45–60% on average. If moderate matrix effects occurred, post-column splitting may allow obtaining reliable quantitative data even with external calibration.

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1. Introduction

During the last decade high-performance liquid chromatography (HPLC) coupled to mass spectrometry (LC–MS) via atmospheric pressure ionization (API) interfaces (electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI)) has become the analytical technique of choice for the determination of polar environmental pollutants [1–3].

However, a critical aspect in quantitative analysis with LC–MS is the occurrence of matrix effects that may lead to a

significant difference in the response of an analyte in a sample as compared to a pure standard solution [2,4]. These matrix effects are attributed to those organic and/or inorganic components of a sample that co-elute with an analyte. Since the nature and the amount of these co-eluting matrix compounds can be rather variable between samples matrix effects in a series of samples can also be highly variable and difficult to predict. The mechanism by which co-eluting compounds interfere with each other during the electrospray ionization process is still not very clear [5] and is closely related to the more general debate on the mechanisms involved in the generation of gas phase ions in electrospray ionization [6].

Matrix effects may be compensated by using internal standards. But as all sample constituents are subjected to

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chromatographic separation, the sample matrix and thus, the matrix effects can strongly depend upon the chromatographic retention time and, therefore, more than one internal standard may be needed [7]. The ideal internal standards are those isotopically labelled, but these are expensive and not regularly available. Therefore, finding a suitable internal standard for each single analyte can be a rather difficult task. Alternatively, standard addition of each analyte into each sample may be used to compensate matrix effects, but this leads to a significant increase in analysis and processing time [8].

While these approaches may compensate matrix effects and result in a quantitatively accurate result they cannot avoid the loss of sensitivity that is accompanied by signal suppression and the variability in method sensitivity that may occur between samples of a series. This can only be achieved by eliminating matrix interferences, for example by an improved sample clean-up prior to the LC–MS analysis. One approach into this direction is the use of restricted access materials (RAM) for sample enrichment, as these sorbents exclude high molecular weight materials with a nominal mass above 15 kDa from enrichment [9]. Some authors have found RAM useful to reduce matrix effects related to humic substances, from model groundwater or sediment extracts [10,11].

It has also been reported that APCI is less matrix sensitive than ESI [5,12,13] but this may not always be true and, moreover, APCI cannot serve all the highly polar and ionic analytes that are well amenable to ESI [14]. Therefore, ESI will remain the interface of choice for many environmental applications despite its potentially higher sensitivity against matrix effects.

Another interesting approach to reduce matrix effects is to decrease the flow that is delivered to the ESI-interface. This strategy has not gained much attention, yet, although it has been shown some years ago that decreasing the flow directed into the ESI to as low as 0.1 $\mu\text{L}/\text{min}$ resulted in a substantial reduction of signal suppression [15,16]. For these low flow rates, a nano-ESI interface is required. Contrary to a sample clean-up reducing the flow into the ESI interface does not shift the ratio between analyte and co-eluting matrix but it significantly reduces the amount of organic material that requires ionization at a given period of time. Moreover, the droplet size decreases with decreasing flow and the droplet surface area increases substantially. Then target analytes and sample matrix components may not need to compete with each other any more during desolvation and ionization in the electrospray process [17].

In this paper, operational options to reduce matrix effects are investigated. One of these options was the use of a size-exclusion process as a clean-up to reduce matrix components and the second option was to decrease the flow rate during ESI–MS. Benzothiazoles and pharmaceuticals were selected as model compounds as these analytes cover different chromatographic methods (RPLC and IPLC) and both, positive and negative mode ESI–MS.

2. Experimental

2.1. Reagents and chemicals

Benzothiazole (BT), 2-hydroxybenzothiazole (OHBT), pharmaceuticals (piroxicam, ketorolac, clofibrac acid, naproxen, ketoprofen, bezafibrate, fenoprofen, ibuprofen, diclofenac, and meclofenamic acid), 2,5-dichlorobenzoic acid and fenoprop were obtained from Sigma–Aldrich (Steinheim, Germany). 2-Mercaptobenzothiazole (MBT) from Merck (Darmstadt, Germany), 2-aminobenzothiazole (ABT) was purchased from Fluka (Buchs, Switzerland) and 2-methylthiobenzothiazole (MTBT) and 2-methylbenzothiazole (MeBT) from Ferak (Berlin, Germany). Benzothiazole-2-sulfonic acid (BTSA) was kindly provided by Heleen De Wever (Vito, Mol, Belgium). Stock solutions at 2 g/L were prepared in methanol, stored in the dark at 4 °C, and diluted to the desired concentration with ultrapure water.

Ultrapure water was obtained by an ELGA Maxima HPLC ultrapure water system (ELGA, Ubstadt-Weiher, Germany). Methanol, acetone, formic acid, ammonium acetate and acetic acid, all HPLC grade, were supplied by J.T. Baker (Deventer, Holland) and tri-*n*-butylamine (TrBA) was purchased from Fluka (Steinheim, Switzerland).

2.2. Samples

Composite samples (24 h) of the influent and the effluent of a municipal wastewater treatment plant were collected in March 2004. All samples were filtered through 0.45 μm membrane filters (cellulose acetate; Sartorius, Goettingen, Germany) and stored at $-20\text{ }^{\circ}\text{C}$ until analysed.

2.2.1. Ultrafiltration of samples

A membrane filtered raw wastewater sample was split and three aliquots were subjected to ultrafiltration at three different nominal cut-offs (1, 3 and 10 kDa). Dead-end ultrafiltration was carried out by pressurising stirred cells of 200 mL volume (Amicon System) that were equipped with ultrafiltration membranes YM1, YM3 or YM10 of 63.5 mm diameter (Amicon/Millipore, Bedford, MA) with 3 bar of nitrogen. Ultrafiltration permeates and the original membrane filtered sample were parallelly extracted and matrix effects determined as described below.

2.2.2. Determination of dissolved organic carbon

DOC-determination of the samples was performed with a high TOC analyzer (Elementar, Hanau, Germany).

2.3. Solid-phase extraction (SPE) of pharmaceuticals

Sample preparation of pharmaceutical compounds has been previously described [18]. In brief, sample pH was adjusted to 2–2.5 with 1N HCl prior to solid phase extraction (SPE), which was performed with an Autotrace SPE Workstation (Zymark, Hopkinton, MA). Extraction cartridges (Oasis

HLB, 60 mg; Waters, Milford, USA) were sequentially conditioned with 5 mL MeOH and 5 mL ultrapure water adjusted to pH 2–2.5. Samples (50 mL) were then passed through at a flow rate of 10 mL/min, the cartridge dried for 30 min and finally eluted with three fractions of 2 mL of MeOH. The combined extracts were finally concentrated in a Turbopap II nitrogen concentrator (Zymark) down to ca. 0.3 mL, spiked with 100 μ L of IS solution (1 μ g/mL) and diluted to a final volume of 1 mL with ultrapure water.

2.4. LC–MS analysis

2.4.1. Instrumentation

A HP1100 (Agilent Technologies) liquid chromatographic system consisting of a membrane degasser, binary high-pressure gradient pump, autosampler and column thermostat was used. The system was interfaced to a Quattro LC triple-stage quadrupole mass spectrometer (Micromass, Manchester, UK) equipped with a Z-spray electrospray interface. Nitrogen was provided by a nitrogen generator (Model 75-72, Whatman, Haberville, USA) and used as drying and nebulising gas. Argon (99.999%) was used as collision gas. The system was controlled with Masslynx 3.3 software.

2.4.2. Analyses

The pharmaceuticals are separated on a 150 mm \times 2.0 mm Luna phenyl-hexyl 3 μ m column (Phenomenex, Eschborn, Germany) by ion-pair chromatography with a MeOH/water gradient, containing 10 mM TrBA and 0.5% acetic acid, and detected by ESI operating in the negative mode. Details have been given elsewhere [18]. During the first 4.5 and the last

5 min of a chromatographic run the column effluent was directed to waste by means of a post-column switching valve built into the column thermostate. This column switching reduced the delivery of involatile highly polar (inorganic salts) and high molecular weight organic compounds to the electrospray interface.

Benzothiazoles were analyzed in two different HPLC–MS/MS runs. BTSA, OHBT and MBT were separated with a C18 reversed phase column using ammonium acetate as inorganic modifier. These analytes were ionized in the negative ESI mode. For ABT, BT and MTBT also a C18 column was used but with formic acid as modifier and the analytes were detected in the positive ionization mode. Multiple reaction monitoring of two transitions per analyte was used for quantification. Further details have been reported before [19]. An overview on the analytes used in this study, their retention times and the respective ionization modes is provided by Table 1.

2.4.3. Post-column splitting

The LC column effluent was splitted via a T-piece directly in front of the ESI-probe to feed the ESI source with a reduced flow. This flow was adjusted by selecting PEEK tubings of appropriate internal diameter and length for the waste line. For the analysis of acidic pharmaceuticals the flow was reduced from 200 to 50 μ L/min. For the determination of benzothiazoles, the chromatographic flow of 500 μ L/min was routinely reduced to 20 μ L/min in positive ion mode and to 90 μ L/min in negative ion mode. Additionally, flows of 500 (no split), 155, 90 and 20 μ L/min were selected to study the influence of ESI flow rates on ESI–MS signal intensity.

Table 1
Investigated analytes

Compound	Acronym	Retention time (min)
Acidic pharmaceuticals, ion-pair chromatography; ESI negative mode [18]		
2,5-Dichlorobenzoic acid (IS)	1	9.8
Piroxicam	2	14.7
Ketorolac	3	15.5
Clofibric acid	4	17.5
Naproxen	5	18.9
Ketoprofen	6	19.0
Bezafibrate	7	21.1
Fenoprofen	8	23.6
Fenoprop (IS)	9	24.7
Ibuprofen	10	24.9
Diclofenac	11	26.6
Meclofenamic acid (IS)	12	27.6
Benzothiazoles, reversed phase chromatography, ESI positive mode [19]		
2-Aminobenzothiazole	ABT	3.0
Benzothiazole	BT	6.1
Methylthiobenzothiazole	MTBT	8.6
Benzothiazoles, reversed phase chromatography, ESI negative mode [19]		
Benzothiazole-2-sulfonic acid	BTSA	3.9
2-Mercaptobenzothiazole	MBT	8.1
2-Hydroxybenzothiazole	OHBT	8.2

2.5. Evaluation of matrix effects

Standard addition into SPE extracts was performed in order to investigate matrix effects in the determination of pharmaceuticals. A 50 μL volume of standard solutions containing increasing concentrations of the analytes (Table 1) was added to 200 μL aliquots of each extract, resulting in spike concentration levels of 0, 20, 60 and 200 $\mu\text{g/L}$. Matrix effects were evaluated by comparing the slopes of linear calibration curves from these standard addition experiments with that obtained from pure aqueous standards at the same concentration levels.

For the benzothiazoles wastewater treatment plant influent (untreated, raw wastewater) and effluent (treated wastewater) were directly spiked (no SPE) with the six analytes to concentration levels of 20 and 200 $\mu\text{g/L}$. The spiked and the non-spiked samples were analysed by LC–MS and the signal intensity compared to that obtained from pure aqueous solutions.

3. Results and discussion

3.1.1. Molecular size of disturbing matrix constituents

Clean-up processes based on size-exclusion may be suitable to reduce matrix effects, provided that the molecular size of the matrix constituents is sufficiently larger than that of the target analytes. Restricted access materials make use of this principle to exclude matrix components during the extraction. To test whether size-exclusion would be suitable for reducing matrix effects from wastewater samples one wastewater sample was subjected to ultrafiltration using three different membranes with nominal molecular mass cut-offs of 1, 3 and 10 kDa. The membrane permeates were extracted by SPE and aliquots of the extracts spiked with acidic pharmaceuticals at three concentration levels.

The response factors obtained from linear calibration in each of these extracts normalized to the response obtained from pure aqueous solution are shown in Fig. 1 for some representative compounds. For three of the four compounds shown in Fig. 1, the response factors increased slightly with the molecular weight cut-off of the ultrafiltration membrane decreasing from 10 to 1 kDa. However, for the whole set of analytes an average 27% of suppression or enhancement remained even after ultrafiltration with a nominal cut-off of 1000 Da, compared to an average 33% observed in the original sample matrix. The only exception is fenoprop, which seemed not to be affected by the matrix constituents at all (Fig. 1).

It can be concluded that most of the signal suppression in this sample was due to matrix components with a molecular mass below 1000 Da, which actually accounted for more than 70% of the DOC (Fig. 1). However, it must be noted that this raw wastewater samples was comparatively rich in low molecular weight organic material.

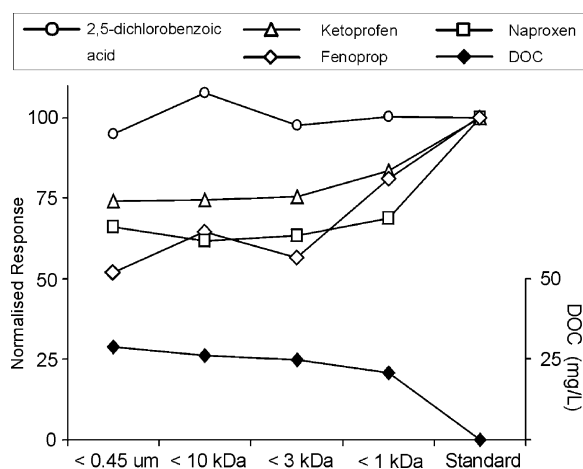


Fig. 1. Influence of the molecular size of the sample organic matter from a raw wastewater sample on the signal suppression during ESI–MS analysis of selected pharmaceuticals.

These results indicate that the use of RAM cartridges or precolumns with a molecular mass cut-off of 15 kDa would hardly be suitable to reduce matrix suppression from wastewater samples. RAM materials may be more promising when matrix components of high molecular weight like humic substances are present, such as in groundwaters or sediment extracts [10,11].

3.2. Post-column split

While enhanced clean-up may reduce the matrix load of water extracts, some operational options may limit matrix effects to occur in ESI–MS detection. For example, it has been shown previously that a drastic decrease of the column effluent flow directed to the ESI-interface to about 0.1 $\mu\text{L}/\text{min}$ can substantially decrease matrix effects [15,16]. This effect may be due to the reduction of the total amount of organic compounds that has to be ionized in a given period of time and to the reduced droplet size that provides increased droplet surface.

However, flow rates of 0.1 $\mu\text{L}/\text{min}$ require special nanoflow ESI probes and are not amenable to conventional ‘high flow’ electrospray interfaces. Therefore, it was intended to test if a reduction of the flow directed to the ESI interface was beneficial in terms of matrix effects and if such a positive effect was to be obtained within the flow range applicable with a conventional ESI interface.

Starting with the non-split conditions that delivered the total column effluent to the ESI interface flow rates were reduced down to ca. 50 $\mu\text{L}/\text{min}$ for pharmaceuticals and 20 $\mu\text{L}/\text{min}$ for benzothiazoles. Lower flow rates were not suitable as they resulted in an unstable spray and, thus, fluctuating signal intensities of the MS.

3.2.1. Influence of reduced flow rate on signal intensities

The signal intensity of six different benzothiazoles that are detected either in the positive or in the negative ionization

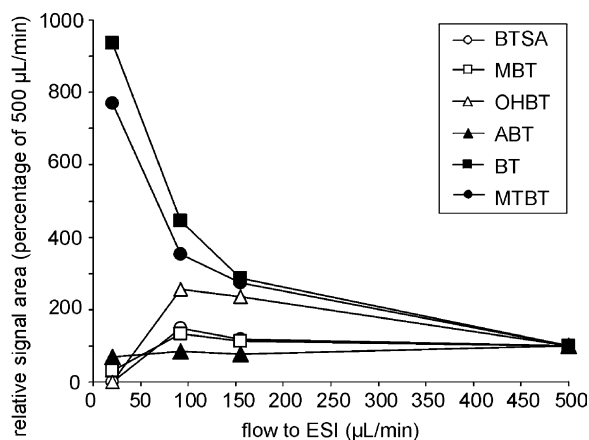


Fig. 2. Influence of the flow rate directed to the ESI interface on the peak areas obtained for the six benzothiazoles from standard solution (20 µg/L). Filled symbols: positive ion mode; open symbols: negative ion mode.

mode was determined from pure aqueous solutions at flow rates between 500 and 20 µL/min. There was no uniform reaction of the signal intensity on the flow reduction (Fig. 2). A continuous and drastic increase in signal intensity with decreasing flow down to 20 µL/min was observed for BT and MTBT, two of the analytes that are detected in the positive ion mode. While their signal increased by a factors of 8–10, the third compound detected in the positive ion mode, ABT, was largely unaffected by flow variation. When lowering the low flow rate below 20 µL/min retention times increased and some peak broadening occurred.

Those analytes detected in the negative ion mode (BTSA, MBT and OHBT) also showed increasing sensitivity with decreasing flow, but here an optimum appeared to exist around 100 µL/min of flow, below which the signal intensity dropped strongly. Maximum signal increase in this mode as compared to the 500 µL/min flow was by a factor of 2.5 (Fig. 2).

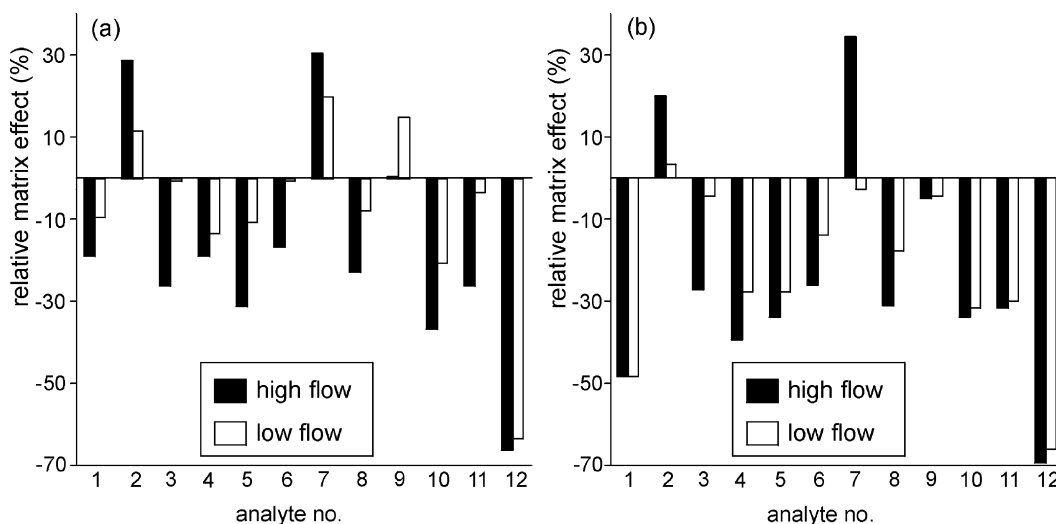


Fig. 3. Relative response of pharmaceuticals with ESI–MS/MS detection in the negative mode at high (200 µL/min) and low (50 µL/min) flow to the ESI source. (a, left): 1 kDa fraction of an untreated wastewater; (b, right): full untreated wastewater. (1) 2,5-Dichlorobenzoic acid; (2) piroxicam; (3) ketorolac; (4) clofibrac acid; (5) naproxen; (6) ketoprofen; (7) bezafibrate; (8) fenoprofen; (9) fenprop; (10) ibuprofen; (11) diclofenac; (12) meclufenamic acid.

These data show that even without the competition with matrix components a reduction of the flow directed towards the ESI interface can increase the instrumental sensitivity. This is consistent with a previous study, in which the authors intended to increase the flow directed to the ESI from 200 to 1200 µL/min and recognized that this was accompanied by a decrease of the instrumental sensitivity [20]. Likely, a lowering of the flow rate directed into the ESI interface leads to reduced droplet size and increased surface size, which is favorable for ion desorption into the gas phase.

3.2.2. Influence of reduced flow rate on matrix effects

It was, then, tested whether reducing the flow rate into the ESI interface also reduces matrix effects of co-eluting organic matrix constituents.

3.2.2.1. Acidic pharmaceuticals. A raw wastewater sample and its low molecular weight (<1 kDa) fraction obtained by ultrafiltration were investigated in parallel. As shown above (Fig. 1), the low molecular weight fraction was responsible for 50–80% of the suppression found in the full sample.

Standard solutions at different concentrations were added into aliquots of the extracts, the analytes determined by LC–MS at different flow rates directed into the ESI interface by post-column splitting and response factors calculated for the different flow rates by linear calibration. Response factors obtained for 200 and 50 µL/min flow into the ESI interface were compared with those obtained from pure aqueous solutions (Fig. 3). Although the pattern is not homogenous, the statistical evaluation (Student's *t*-test, 99% confidence interval) proved that matrix effects, whether signal suppression or enhancement, are significantly lower at 50 µL/min flow rate as compared to a flow rate of 200 µL/min. This was true for both samples (Fig. 3a and b).

Table 2
Average absolute matrix effects determined in various samples for the three groups of analytes

	Absolute difference to response from standard solution (%) ^a	
	High flow ^b	Low flow ^c
Pharmaceuticals (<i>n</i> = 12) ^d		
Whole raw wastewater	33	23
<1 kDa fraction	27	15
Benzothiazoles, positive mode (<i>n</i> = 3) ^d		
Raw wastewater	20	12
Treated wastewater	9	4
Benzothiazoles, negative mode (<i>n</i> = 3) ^d		
Raw wastewater	115	50
Treated wastewater	80	45

^a $|(RF_{\text{sample}} \times 100/RF_{\text{water}}) - 100|$. A value of 0 indicates no matrix effects.

^b No split applied: 200 $\mu\text{L}/\text{min}$ for pharmaceuticals, 500 $\mu\text{L}/\text{min}$ for benzothiazoles.

^c 50 $\mu\text{L}/\text{min}$ for pharmaceuticals, 20 $\mu\text{L}/\text{min}$ for benzothiazoles in positive mode and 90 $\mu\text{L}/\text{min}$ for benzothiazoles in negative mode.

^d For analyte details refer to Table 1.

For samples with a moderate matrix effect as represented by the <1 kDa fraction of the raw wastewater, the mean of matrix effects was reduced from 27 to 15% by reducing the flow from 200 to 50 $\mu\text{L}/\text{min}$ (Table 2). This corresponds to an average 44% reduction of the matrix effects. For 9 of the 12 analytes, the matrix effects were below 15%, whereas at high flow rates only one analyte had a matrix effect below 15% (Fig. 3a). Reducing the ESI flow by a post-column splitting may, thus, eliminate matrix effects from moderately loaded samples to such an extent, that external calibration is suitable for accurate quantification and that the standard addition procedure can be avoided. Moreover, one can expect that the variability of matrix effects between different samples of one series will also be reduced. This would also ease the quantification process [8]. However, not all analytes respond similarly to the flow reduction. In this particular example, no reduction of matrix effects was observed for meclufenamic acid (Fig. 3a, no. 12).

In the highly loaded raw wastewater with a DOC of 29 mg/L, the improvement by a flow reduction to 50 $\mu\text{L}/\text{min}$ was less clear (Fig. 3b). On average, the matrix effects have been reduced by one third from 33 to 23% compared to

a pure aqueous solution (Table 2). Hardly any improvement was visible for 2,5-dichlorobenzoic acid, ibuprofen, diclofenac and meclufenamic acid (nos. 1, 10, 11 and 12 in Fig. 3b). These compounds are the early and late eluting analytes (Table 1), respectively, and they are therefore expected to elute together with the largest amounts of matrix components, the most polar and poorly retained compounds together with 2,5-dichlorobenzoic acid and the least polar compounds together with meclufenamic acid.

3.2.2.2. Benzothiazoles. Similar studies were performed for the benzothiazoles, with previously optimized reduced flow rates of 20 $\mu\text{L}/\text{min}$ in the positive ion mode for detecting ABT, BT and MTBT and 90 $\mu\text{L}/\text{min}$ in the negative ion mode for BTSA, MBT and OHBT (see above). Here, 20 $\mu\text{g}/\text{L}$ concentrations of the analytes were spiked into raw wastewater with a high matrix load and treated wastewater with a low matrix load and compared to the response obtained from a pure aqueous solution. Again, reducing the flow to the ESI interface improved the signal integrity for both matrices, treated wastewater and untreated wastewater (Fig. 4a and b).

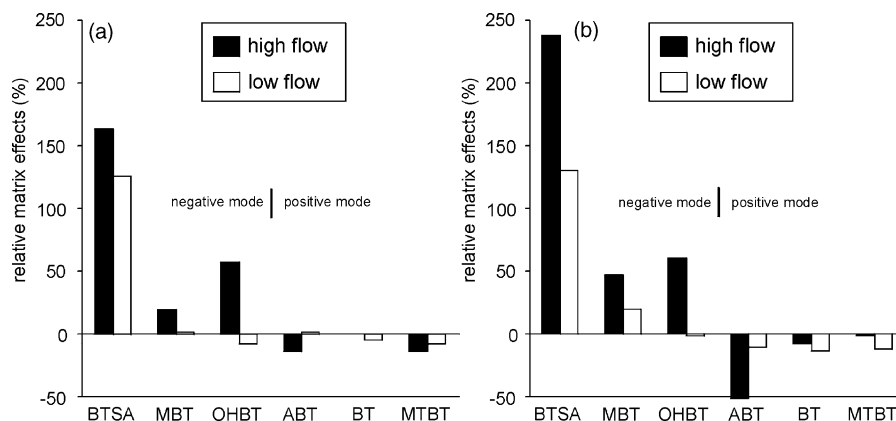


Fig. 4. Relative response of benzothiazoles with ESI-MS/MS detection at high (500 $\mu\text{L}/\text{min}$) and low (90/20 $\mu\text{L}/\text{min}$) flow to the ESI source. (a, left) treated wastewater; and (b, right) untreated wastewater.

Even in the treated effluent (Fig. 4a) with a comparatively light organic matrix (DOC = 14 mg/L) strong matrix effects were observed for BTSA (+160%) and OHBT (+60%) which are determined in the negative ion mode. The strong matrix effects on BTSA determination may, again, be due to the comparatively short retention time of this analyte (3.9 min, Table 1) that enhanced the risk of co-elution with polar matrix constituents. By reducing the ESI flow to 90 $\mu\text{L}/\text{min}$, these matrix effects could be eliminated for MBT and OHBT (to +2 to -7%) (Fig. 4a). The improvement was much weaker for BTSA, where an enhancement of +126% remained. Only weak matrix effects were discernible for those benzothiazoles determined in the positive ion mode (ABT, BT and MTBT) with an average suppression of 9% at high flow conditions. This was astonishing since positive ionization is considered less selective and competition would, thus, be expected to be more widely occurring. Owing to the low suppression occurring under high flow conditions a reduction of the ESI flow could not lead to a significant improvement with respect to matrix effects. However, it must be noted that the decrease in ESI flow led to an enormous sensitivity enhancement by a factor of 8–9 for BT and MTBT from pure aqueous solution (Fig. 2) as well as from real samples.

For the highly loaded untreated municipal wastewater (DOC = 57 mg/L), matrix effects in the negative ionization mode were stronger for all three analytes (Fig. 4b) with an average enhancement of 115% (Table 2). Again, reducing the flow directed to the ESI interface helped considerably and matrix effects could be diminished to about 50% in the negative ion mode as compared to those observed at high flow rates (Fig. 4b). In the positive ion mode, an average 20% of suppression occurred in raw wastewater with ABT being suppressed by 50%. Reducing the flow into the ESI interface to 20 $\mu\text{L}/\text{min}$ eliminated the suppression almost completely (Fig. 4b). An average 12% signal reduction remained for the three analytes ABT, BT and MTBT in untreated wastewater (Table 2).

4. Conclusions

The occurrence of matrix effects, i.e. suppression or enhancement of the analyte signal intensity due to the presence of co-eluting sample constituents, requires additional efforts to ensure reliable quantitation of environmental contaminants using LC–MS. If these efforts are not made matrix effects can severely compromise quantitative LC–MS data.

Using ultrafiltration for size separation of dissolved organics it could be shown, that the matrix effects in LC–MS analysis of acidic pharmaceuticals from wastewater was primarily due to low molecular weight compounds <1 kDa. Thus, clean-up based on size exclusion does not seem promising to reduce matrix effects from wastewater samples.

This study investigated whether operational modifications in the LC–ESI–MS coupling are suitable to reduce matrix

effects so that advanced clean-up or the necessity to perform standard addition can be avoided.

Reducing the flow directed to the ESI interface by a post-column T-piece proved helpful to increase the instrumental sensitivity and to reduce matrix effects. Flows down to 20–50 $\mu\text{L}/\text{min}$ could be used with a conventional ESI interface but lower flow rates resulted in an unstable spray, peak broadening and retention time shifts. With decreasing flow a strong sensitivity increase was obtained for many analytes in negative and positive electrospray ionization. For some compounds, sensitivity increased by almost one order of magnitude. The flow optimum and the sensitivity increase varied for the different ion modes. Furthermore, a flow reduction to the ESI interface can significantly reduce matrix effects. For many compounds, matrix effects could be nearly eliminated so that ‘conventional’ external calibration would be suitable for a reliable quantitation, while other analytes did not respond at all to a reduced flow. On average, a 45–60% reduction of matrix effects was achieved by flow decrease.

Post-column switching and column effluent splitting prior to the ESI interface appear to be useful measures to reduce the problems associated with sample matrices, to increase stability of LC–MS systems and to improve precision and accuracy of environmental analyses using LC–MS.

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