Simultaneous enantiomeric analysis of non-steroidal anti-inflammatory drugs in environmental samples by chiral LC-MS/MS: A case study in Beijing, China

Ma R.[1,2], Qu H.[2], Wang B.[2,*], Yu G.[2], And Yu Y.[1]

[1] State Environmental Protection Key Laboratory of Environmental Pollution Health Risk Assessment, Center for Environmental Health Research, South China Institute of Environmental Sciences, MEP, Guangzhou, 510535, China

[2] Beijing Key Laboratory of Emerging Organic Contaminants Control, State Key Joint Laboratory of Environmental Simulation and Pollution Control, Collaborative Innovation Center for Regional Environmental Quality, School of Environment, Tsinghua University, Beijing, 100084, China

*corresponding author: WANG B.

e-mail: thuwb@tsinghua.edu.cn

Abstract

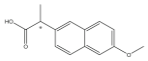
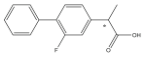
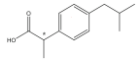
A method for directly simultaneous enantiomeric determination of frequently used non-steroidal anti-inflammatory drugs (ibuprofen, naproxen and flurbiprofen) was firstly achieved on Chiralpak AD-RH by UHPLC-MS/MS. The mobile phase composition, pH values, flow rates, and column temperatures were optimized to give high sensitivity and resolution. The overall performance was satisfactory in terms of linearity, precision, accuracy and LODs for environmental analysis. The present method was sensitive, simple and efficient for chiral analysis in environment with MQLs of single enantiomers ranging from 1.2 to 37 ng/L and runtime within 20 min, which are lower and faster than many reported methods. The proposed methodology is successfully applied for monitoring of pharmacologically active compounds (PhACs) at enantiomeric level in environmental samples and superior for its simple and safe system of mobile phase compatible with MS detector under reverse phase mode. Besides, the method was based on a more universal chiral selector that could be adapted for other co-existing chiral PhACs analysis in environment. Furthermore, a monitoring survey in surface water in Beijing, China was conducted to evaluate the pollution of PhACs in Beijing, and for the first time gain an insight into the spatiotemporal variation and chiral characteristics of these emerging pollutants in China.

Keywords: Enantioseparation, Chiral analysis, Pharmaceutical active compounds, LC-MS/MS, River water

1. Introduction

In addition to understanding variable physicochemical and biological characteristics of chiral pharmaceutically active compounds (PhACs) during environmental processes, there is an emerging interest in the developing of enantioselective analysis of PhACs during the last decade [1,2]. Chiral PhACs, which represent 56% of the pharmaceuticals in current use [3], have been receiving an increasing attention in the enantiospecific ecological effects due to their stereoselective pharmacological characteristics [4-6], Thus determination of enantiomeric composition of chiral PhACs in environment is of significance to understand and predict the environmental fate, and accurately evaluate their ecotoxicological potencies. Non-steroidal anti-inflammatory drugs (NSAIDs) consist of many chiral pharmaceuticals prescribed in high quantities worldwide, and most of them are marketed as racemates [7]. These pharmaceuticals were frequently detected in surface water and in WWTP effluents due to the inefficient removal by WWTP treatment [8-11]. Despite this, only limited research has been undertaken on the enantiomeric analysis of NSAIDs in environment [12-15]. Such information is still scarce partly due to the limitation of analytical techniques. Only a few methods have been reported to determine enantiomeric composition of profens in the environment. Most of them were based on gas chromatography or GC-MS followed by the derivatization steps [16]. The main disadvantages of these GC-based methods are the requirement of complicated and time-consuming pretreatment, which may cause analyte losses and affect the repeatability [17,18]. Compared to GC-based methods, LC can serve as a very important tool for enantioseparation of a wide range of chiral compounds. Moreover, reversed-phase (RP) HPLC has shown higher potential than normal-phase HPLC for superiority in compatible with mass analysis. Among the commercially available chiral stationary phases (CSPs), macrocyclic antibiotic and derivatized polysaccharide selectors have the broadest enantioselectivity [19,20]. However, the currently reported HPLC methods for NSAIDs chiral analysis showed clear disadvantages with regard to the low resolution and sensitivity and incompatible with MS detector [21-23]. Considering the trace levels in environment, developing direct, fast and sensitive analysis methods compatible with MS detection urgently needs to be addressed and has remained a challenge. The published methods for analyzing chiral PhACs in environment by LC-MS have mostly involved β-blockers, antidepressants or illicit drugs, few was focused on profens [24-27]. As far as we know, no comprehensive evaluation of

Table 1. Physicochemical properties and MS parameters of the target analytes

Compound	Chemical structure [a]	pK_a	MRM [b]	DP	EP	CE	CXP
(R)/(S)-Naproxen		4.85	229.05>170.01	-10	-2.5	-18	-4
(R)/(S)-Flurbiprofen		4.33	243.10>199.10	-15	-2.0	-8	-4
(R)/(S)-Ibuprofen		4.91	205.10>161.10	-20	-3.0	-6	-2

a The asterisks denote the chiral centre

b Only one MRM transition was chosen for the compounds in negative mode

the polysaccharide derivatives CSP utilizing LC-MS/MS for simultaneous enantioselective analysis of representative NSAIDs in environment has been published to date. This study aims to develop a novel and reliable direct analytical method for three frequently-used NSAIDs (naproxen, ibuprofen and flurbiprofen) at enantiomeric level in surface water using a polysaccharide-based chiral column by UHPLC-MS/MS. The mobile phase composition, flow rates, column temperatures and MS parameters were optimized to obtain high sensitivity, selectivity and satisfactory resolution. The performance of proposed method was evaluated and compared with reported studies. It has also been extended for applications on enantiomeric determination of profens in surface water in Beijing.

2. Experimental

2.1. Chemical and reagents

HPLC-grade solvents were purchased from Fisher Scientific (Loughborough, Leicestershire, UK). Analytical grade formic acid (FA) and ammonium acetate were purchased from Fluka (Buchs, Switzerland). (+)/(-)-ibuprofen, (+)/(-)-naproxen and (+)/(-)-flurbiprofen were supplied by Dr.Ehrenstorfer (Augsburg, Germany). The isotopically labelled (R)/(S)-ibuprofe-d3, (R)/(S)-flurbiprofen-d3 and (R)/(S)-naproxen-d3 as internal standards (IS) were obtained from TRC (Toronto, Canada). SPE cartridges were Oasis HLB (200mg/6mL) purchased from Waters Corporation (Milford, MA, USA).

Individual stock standard and IS solutions were dissolved in acetonitrile (1 mg/mL) and stored at 4 ℃ in the dark. Working standard solutions (1 mg/L) were prepared freshly by serial dilution.

2.2 Sample collection and extraction

Surface water samples were collected from the Beiyun Rivers, Beijing, China in July and November, 2016. All samples were stored in dark glass containers, transported refrigerated at 4 ℃ to the lab, and pretreated within 48 h. Samples were filtered through 0.7 μm glass fibre filter GF/F (Whatman, UK). After filtration, a 500 mL portion was adjusted to pH 7 and spiked with mixed internal standard (50 ng of each compound). Oasis HLB cartridges (Waters, U.K.) were used for concentration and clean-up. The elutes were reconstituted in 0.5 mL of mobile phase and filtered prior to instrumental analysis.

2.3 UHPLC-MS/MS conditions

Chiral determination was performed with an ultra-high performance liquid chromatography (Ultimate3000 HPLC system, Dionex, USA) coupled to tandem mass spectrometry (ESI-MS/MS, API3200, AB Sciex, USA) operated in the negative mode. Enantioseparations were carried out on a CHIRALPAK AD-RH (150 × 4.6 mm, 5 μm) column procured from Daicel Chemical Industries Ltd. (Tokyo, Japan). The optimized mobile phase consisted of an aqueous solution containing 10 mM ammonium acetate buffer (pH=5, formic acid adjusted)–acetonitrile (65:35, v:v). The flow-rate was 0.4 mL/min and the column temperature maintained at 25 ℃. The optimized mass spectrometric conditions were as follows: CUR (N2) 20, CAD 5, IS -4500 V, TEM 475 ℃, GS1 60 and GS2 70.

2.4 Method development and validation

The composition of mobile phase, effect of pH, flow rate and column temperature were optimized to obtain the best chromatographic resolution for simultaneous enantiomeric determination within reasonable analysis time and enough sensitivity for MS spectrometry. Linearity was determined using a 7- point calibration curve for each enantiomer of 10.0, 20.0, 50.0, 100, 200, 500 and 1000 μg/L, injected in triplicate. Inter-day and intra-day precision and accuracy was determined by repeated injection within one day and 3 consecutive days, respectively. Instrumental detection limits (IDL) and instrumental quantification limits (IQL) were calculated as 3 and 10 times the signal to noise (S/N) ratios for each enantiomer, respectively. Method detection limit (MDL) and method quantification limit (MQL) evaluated with surface water were calculated using IDL or IQL divided by 10 times of recovery. Resolution was calculated using the following equation,

$$R_s = \frac{2(tr2 - tr1)}{w1 + w2}$$

where R_s is the resolution of two enantiomers, tr_1 and tr_2 are the retention times, w1 and w2 are the base widths of the two enantiomers. $R_s \geq 1.5$ means a baseline separation,

Table 2. Method validation parameters of the three pairs of enantiomers

Compound	R^2	t_r	R_s	IDL (μg/L)	IQL (μg/L)	MDL (ng/L)	MQL (ng/L)	Recovery (%) (mean±SD)	EF	Matrix effect (%)
R-(-)-Naproxen	0.995	9.28		0.34	1.1	0.38	1.2	89.9±3.1		1.5
S-(+)-Naproxen	0.995	10.05	1.0	0.36	1.2	0.41	1.4	89.7±3.7	0.50±0.01	1.8
S-(+)-Flurbiprofen	0.996	11.16		2.1	7.0	2.4	7.9	87.6±5.3		-3.9
R-(-)-Flurbiprofen	0.994	13.40	2.3	2.9	9.7	3.3	11	86.9±5.9	0.51±0.01	-4.6
R-(-)-Ibuprofen	0.995	14.71		8.5	28	9.6	32	88.1±4.1		-7.7
S-(+)-Ibuprofen	0.991	15.70	1.1	9.9	33	11	37	88.6±4.8	0.53±0.02	-8.3

and $R_s \geq 1$ was deemed adequate for quantification. Enantiomeric fraction (EF) was calculated using the equation below:

$$EF = \frac{E(+)}{E(+)+E(-)}$$

where E(+) is the peak area of (+)-enantiomer, E(-) for (-)-enantiomer.

Matrix effects (ME) were determined by comparing the responses of post-extraction spiked with ISs with responses of neat IS standards (in solvent), where a negative value of ME indicates signal suppression occurred and vice versa. The matrix-matched IS at the same concentration were also prepared for calculation of recovery.

3. Results and discussion

3.1 Optimization of UHPLC-MS/MS method

Acetonitrile presented lower column pressure and gave better resolution than methanol. Decreasing acetonitrile content would increase retention time and enantiomeric resolution, and this contribution was significant even with very small amplitude of change. Nevertheless, this effect was compound-dependent. Both retention and resolution were greatly improved with ACN decreasing from 40% to 30%, while ibuprofen was not that sensitive with minor fluctuation of Rs around 1.3. However, decreasing of acetonitrile also resulted in reduction of the S/N ratio. The content of ACN was optimized to be 35% and was not further reduced because of the unfavorable ionization at a high proportion of aqueous part in mobile phase. The column temperature was tested from 15 ℃ to 30 ℃, and showed little influence on enantiomeric resolution (see Fig. 1A). The resolutions were slightly higher at low temperature, but the retention time increased with decreasing temperature, thus the column temperature was maintained at room temperature of 25℃. By comparison, effect of flow rate was compound-dependent. The resolution of flurbiprofen dramatically decreased with the increasing flow rate while resolutions of naproxen and ibuprofen were not much affected. Finally, 0.4 mL/min was optimized in terms of peak width and MS ionization efficiency. Most of the reported methods on enantiomeric analysis of NSAIDs on the same CSP were performed under acid condition, commonly with phosphoric acid or trifluoroacetic acid (TFA) in mobile phase, which was incompatible with MS detector or unrecommended for strong ion suppression in negative mode. To overcome this problem, formic acid and ammonium acetate were introduced for helping ionization. The pH value of buffer solution was found to be a main influencing factor. The signal intensities would be insufficient for environmental determination under low pH conditions. Both the resolution and retention time decreased with the increasing pH (Fig.1). When the pH of aqueous buffer at or below 5.0, the Rs of the three analytes could all exceed 1.0, which was adequate for quantification. NH_4Ac concentration did not exert significant influence on signal intensity, so the frequently used 10 mM was applied. From the above, the condition for LC-MS/MS were optimized to provide the best enantioselectivity, satisfactory analysis times (within 20 min) and sensitivity.

3.2 Method validation and application for surface water analysis

The method performance was evaluated and presented in Table 2. The IDLs and IQLs ranged from 0.34 to 9.9 μg/L and from 1.1 to 33 μg/L, respectively. The MDLs and MQLs in surface water ranged from 0.38 to 11 ng/L and from 1.2 to 37 ng/L, respectively. RSD was determined for intra-day and inter-day showing satisfactory with RSD below 20%. ME ranged from −8.3% to 1.8% and did not vary much between enantiomers of the same compound. Non-significant signal suppression/enhancement was observed for surface water samples. The recoveries were evaluated at three concentrations (10, 50, 200 ng/L) in triplicate. Good recoveries were obtained ranging between 87%-90%. The EFs of standards were monitored and the changes from racemic were within tolerance (0.50±0.05). The results demonstrated that the method was sensitive, robust and reliable, with good reproducibility and much lower MQL, and thus could be applied for simultaneous analysis of the target NSAIDs in environment at enantiomeric level.

3.3 Comparison the present study with existing methods

Comparison of the proposed method with other methods is summarized in Table 3. By comparison, the present method allows the achievements of direct and simultaneous enantiomeric determination of ibuprofen, naproxen and flurbiprofen and lower MQLs for environmental analysis. The methodology proposed is shown to be superior in the following aspects: (i) the usage of a CSP which has broad enantioselectivity for a wide range of physicochemical properties of compounds, and a

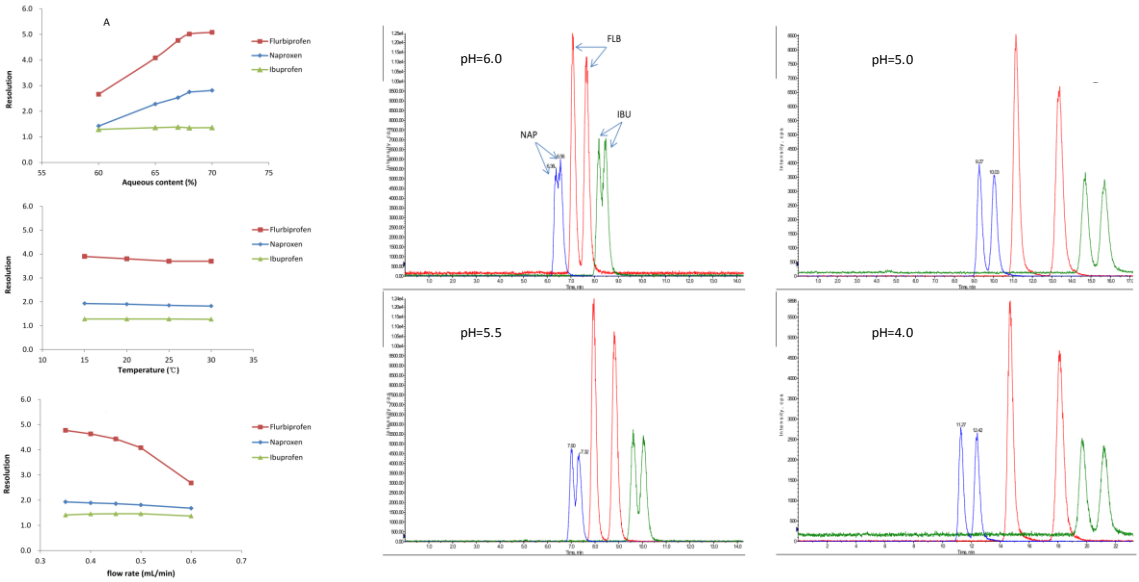


Fig. 1 Method development and effects of pH values on enantiomeric discrimination

Table 3. Comparison between the present study and the existing methods

Analytes	Method	CSP	Mobile phase conditions	Resolution and analysis time	Matrix application	LOQ	References
Ibuprofen, Naproxen, Ketoprofen	LC-MS/MS	Chirobiotic V	MeOH containing 4mM NH_4AC and 0.005% formic acid	Ibuprofen: Rs 1.27; Rt(min) 21.45/24.08 Naproxen and Ketoprofen unresolved	Surface water and wastewater	Not reported	[30]
Ibuprofen, Naproxen, Ketoprofen	LC-MS/MS	Sumichiral OA-2500	Tetrahydrofuran: ammonium acetate (50 mM) in MeOH (90:10 v:v)	Rs: 1.4(ibuprofen) 1.7(ketoprofen) 2.8(naproxen)	wastewater	0.5-1.2 ng/L	[31]
10 profens including metabolites	LC-MS/MS	Chiral-AGP	10 mM ammonium (pH 6.7): CH_3CN (99:1 v:v)	Rs: 1.0(ibuprofen) 1.6(naproxen) For flurbiprofen: no distinct peaks observed	Surface water and wastewater	8.49-94.81 ng/L (for ibuprofen and naproxen)	[29]
naproxen	LC-MS/MS	Chiralpak AD-RH	0.1% formic acid:CH_3CN (50:50, v/v);	Rs: not mentioned Analysis time within 30 min	River water	100 ng/L	[13]
Ibuprofen	LC-MS/MS	Chiralpak AD-RH	methanol: water (80:20, v/v), containing 0.1% phosphoric acid solution (pH=2); make-up liquid of 4.5% (w/v) NH_4OH aqueous solution	Rs=1.25	Human plasma	0.12 μg/mL	[28]
Ibuprofen and metabolites	LC-MS/MS	Chiralpak AS-H	Hexane: isopropanol: TFA (95: 5: 0.1 v:v: v); Post-column infusion with 10 mmol/L ammonium acetate in MeOH	Rs: not mentioned Analysis time within 25 min	culture medium	0.1 μg/mL for Ibuprofen	[32]
Ibuprofen	LC-MS/MS	Lux Cellulose-3	0.1% (v/v) acetic acid in mixture of methanol and water (90:10 v:v)	Rs=3 Rt(min) 9.88/10.74	Human plasma	100 μg/L	[33]
Flurbiprofen	HPLC	Chirobiotic V	Ammonium nitrate (100 mm, pH 5): Tetrahydrofuran (80:20)	Rs=4.67	Rat plasma	0.25 μg/mL	[34]
Ibuprofen, flurbiprofen and naproxen	LC-MS/MS	Chiralpak AD-RH	CH_3CN : buffer solution (10 mM NH_4OAC, pH 5.0, formic acid adjusted) (35: 65 v:v), 0.4 mL/min	Rs:2.3 (flurbiprofen); 1.1 (naproxen); 1.0 (ibuprofen) Analysis time within 25 min	Surface water	1.2-37 ng/L	Present study

simple and safe system of mobile phase for MS detector; (ii) a direct separation without derivatization or post-column makeup; (iii) an efficient runtime for chiral analysis with appropriate retention times and good resolutions of enantiomers

3.4 Method application

To further evaluate the method application, 34 samples of surface water from Beiyun River were collected in Beijing, China twice in 2016 (Table 4). Ibuprofen was the most abundant compound of the three, with detection frequency of 98%, followed by naproxen of 85%. Flurbiprofen was much less frequently detected probably due to the lower consumption. The concentrations were from ND (not detected) to 386.6 ng/L for ibuprofen and ND-42.8 ng/L for naproxen (sum of the two enantiomers). The EF values varied in a range of 0.69±0.12 for ibuprofen, and 0.92±0.07 for naproxen (flurbiprofen not available).

Ibuprofen is mostly sold as racemate, while it has been reported that R-ibuprofen undergoes chiral inversion during metabolism causing excess of the S-ibuprofen [2,5,14,31]. Naproxen was manufactured optically in form of S-enantiomer, but the R-naproxen could be detected in a few samples at trace level.

Table 4. Concentrations and EFs of profens in river water

Enantiomer	July, 2016		November, 2016	
	Con. (ng/L)	EF values	Con. (ng/L)	EF values
(S)-Naproxen	<MQL-35.8	0.92±0.07	1.7-42.8	0.93±0.04
(R)-Naproxen	ND-1.9		ND-1.2	
(S)-Flurbiprofen	< MQL	—	< MQL	—
(R)-Flurbiprofen	<MQL		< MQL	
(S)-Ibuprofen	ND-319.7	0.69±0.12	ND-196.6	0.71±0.09
(R)-Ibuprofen	ND-88.1		ND-100.2	

4. Conclusion

A method for directly simultaneous enantiomeric determination of three NSAIDs was firstly achieved on Chiralpak AD-RH by UHPLC-MS/MS. The proposed method was proven to be sensitive, simple and efficient for trace level of environmental samples with lower MQLs than many of the existing methods.

References

A.R. Ribeiro, P.M. Castro, M.E. Tiritan, Environ. Chem. Lett. 10 (2012) 239-253.

N.H. Hashim, S.J. Khan, J. Chromatogr. A 1218 (2011) 4746-4754.

B. Kasprzyk-Hordern, Chem. Soc. Rev. 39 (2010) 4466-4503.

H.-R. Buser, T. Poiger, M.D. Müller, Environ. Sci. Technol. 33 (1999) 2529-2535.

V. Matamoros, M. Hijosa, J.M. Bayona, Chemosphere 75 (2009) 200-205.

L.J. Fono, D.L. Sedlak, Environ. Sci. Technol. 39 (2005) 9244-9252.

S.K. Khetan, T.J. Collins, Chem. Rev. 107 (2007) 2319–2364.

J.B. Quintana, S. Weiss, T. Reemtsma, Water Res. 39 (2005) 2654-2664.

L.P. Padhye, H. Yao, F.T. Kung'u, C.-H. Huang, Water Res. 51 (2014) 266-276.

T. Poiger, H.-R. Buser, M.D. MüLLER, M.E. Balmer, I.J. Buerge, CHIMIA International Journal for Chemistry 57 (2003) 492-498.

N. Nakada, T. Tanishima, H. Shinohara, K. Kiri, H. Takada, Water Res. 40 (2006) 3297-3303.

S.L. MacLeod, C.S. Wong, Water Res. 44 (2010) 533-544.

T. Suzuki, Y. Kosugi, M. Hosaka, T. Nishimura, D. Nakae, Environ. Toxicol. Chem. 33 (2014) 2671-2678.

Z. Wang, Q. Huang, Y. Yu, C. Wang, W. Ou, X. Peng, Environ. Geochem. Health 35 (2013) 683-691.

S.J. Khan, L.L. Wang, N.H. Hashim, J.A. McDonald, Chirality 26 (2014) 739-746.

N.H. Hashim, L.D. Nghiem, R. Stuetz, S. Khan, Water Res. 45 (2011) 6249-6258.

G. Carlucci, M. Carlucci, M. Locatelli, Anti-Inflammatory & Anti-Allergy Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Inflammatory and Anti-Allergy Agents) 11 (2012) 96-112.

N. Hashim, S. Shafie, S. Khan, Environ. Technol. 31 (2010) 1349-1370.

D.W. Armstrong, B. Zhang, Anal. Chem. 73 (2001) 557 A-561 A.

A.A. Younes, D. Mangelings, Y. Vander Heyden, J. Chromatogr. A 1269 (2012) 154-167.

J. Ye, W. Yu, G. Chen, Z. Shen, S. Zeng, Biomed. Chromatogr. 24 (2010) 799-807.

H. Ates, D. Mangelings, Y. Vander Heyden, J. Chromatogr. B 875 (2008) 57-64.

X.W. Teng, S.W. Wang, N.M. Davies, J. Chromatogr. B 796 (2003) 225-231.

B. Kasprzyk-Hordern, V.V.R. Kondakal, D.R. Baker, J. Chromatogr. A 1217 (2010) 4575-4586.

V.K.H. Barclay, N.L. Tyrefors, I.M. Johansson, C.E. Pettersson, J. Chromatogr. A 1218 (2011) 5587-5596.

B. Kasprzyk-Hordern, D.R. Baker, Environ. Sci. Technol. 46 (2012) 1681-1691.

C. Caballo, M. Dolores Sicilia, S. Rubio, Anal. Bioanal. Chem. 407 (2015) 4721-4731.

P.S. Bonato, M.P.F. Del Lama, R. de Carvalho, J. Chromatogr. B 796 (2003) 413-420.

D. Camacho-Muñoz, B. Kasprzyk-Hordern, Anal. Bioanal. Chem. 407 (2015) 9085-9104.

R. López-Serna, B. Kasprzyk-Hordern, M. Petrović, D. Barceló, Anal. Bioanal. Chem. 405 (2013) 5859-5873.

C. Caballo, M.D. Sicilia, S. Rubio, Talanta 134 (2015) 325-332.

K.B. Borges, A.R. Moraes de Oliveira, T. Barth, V.A. Polizel Jabor, M.T. Pupo, P.S. Bonato, Anal. Bioanal. Chem. 399 (2011) 915-925.

N. Nakov, R. Petkovska, L. Ugrinova, Z. Kavrakovski, A. Dimitrovska, D. Svinarov, J. Chromatogr. B 992 (2015) 67-75.

F. Péhourcq, M. Matoga, C. Jarry, B. Bannwarth, Biomed. Chromatogr. 18 (2004) 330-334.