

The effect of signal suppression and mobile phase composition on the simultaneous analysis of multiple classes of acidic/neutral pharmaceuticals and personal care products in surface water by solid-phase extraction and ultra performance liquid chromatography–negative electrospray tandem mass spectrometry

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Abstract

A new multi-residue method for the determination of 25 acidic/neutral pharmaceuticals (antibiotics, anti-inflammatory/analgesics, lipid regulating agents, diuretics, triazides, H₂-receptor antagonists, cardiac glycosides and angiotensin II antagonists) and personal care products (sunscreen agents and preservatives) in surface water with the usage of a new technique: ultra performance liquid chromatography–negative electrospray tandem mass spectrometry (UPLC–MS/MS) was developed and validated. The novel UPLC system with 1.7 μm particle-packed column allowed for good resolution of analytes with the application of low mobile phase flow rates (0.05 mL min^{−1}) and short retention times (from 4.7 min to 13.3 min) delivering a fast and cost-effective multi-residue method. SPE with the usage of Oasis MCX strong cation-exchange mixed-mode polymeric sorbent was chosen for sample clean-up and concentration. The influence of mobile-phase composition, matrix assisted ion suppression and SPE recovery on the sensitivity of the method was identified and quantified. The instrumental limits of quantification varied from 0.2 $\mu\text{g L}^{-1}$ to 30 $\mu\text{g L}^{-1}$. The method limits of quantification were at low nanogram per litre levels and ranged from 0.3 ng L^{−1} to 30 ng L^{−1}. The instrumental and method intra-day and inter-day repeatabilities were on average less than 5%. The method was successfully applied for the determination of PPCPs in River Taff. Thirteen compounds were determined in river water at levels ranging from a single to a few hundred nanograms per litre. Among them were ten pharmaceuticals (aspirin, salicylic acid, ketoprofen, naproxen, diclofenac, ibuprofen, mefenamic acid, furosemide, sulfasalazine and valsartan) and three personal care products (methyl- and ethylparaben and 4-benzophenone).

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1. Introduction

Pharmaceuticals and personal care products (PPCPs) are a group of emerging, potentially hazardous contaminants, which have, to date, received limited attention, although interest in this area is increasing considerably and the need for further investiga-

tion in this field is continuously emphasised by different research groups [1–6]. PPCPs are found in surface water and wastewaters at levels of up to a few $\mu\text{g L}^{-1}$ [1,2,6–30]. They enter the aquatic environment mainly through treated (or raw) sewage from domestic households and hospitals, waste effluents from manufacturing processes and runoff. Domestic animals are the main direct source of the environmental disposal of many PPCPs such as veterinary pharmaceuticals. The other direct route from which PPCPs enter the environment is recreational activities. Many PPCPs are ubiquitous and persistent in the environment. Some are capable of bioconcentration and many of those investi-

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gated are biologically active compounds. Some are suspected, or are recognised to be, endocrine disruptors, which could potentially influence environmental and human health. Additionally, they are continuously introduced into the environment; therefore even compounds of a low persistence might cause adverse effects in human and aquatic life. The other issue is the synergic effect of different PPCPs on aquatic life, through their combined non-targeted action with many other biologically active compounds present in the environment [1,2]. A few pharmaceuticals and personal care products have been determined in drinking water, which poses a direct risk to humans [2] and raises the issue of contaminated water sources and especially water reuse.

Due to growing concern regarding the presence, fate and effects on the environment and humans, there is an obvious need for fast and sensitive multi-residue methods for the determination of low levels of PPCPs in the environment. Traditional gas chromatography is of limited value in the case of polar (non-volatile) compounds as it requires time consuming derivatization procedures. Liquid chromatography–mass spectrometry (LC/MS) using mainly ESI (electrospray ionisation) is the method of choice for the analysis of polar compounds in complex matrices. So far, a few multi-residue analytical methods for the determination of PPCPs in the aqueous environment have been established [31–36]. These methods utilise solid-phase extraction as a sample preparation method and almost exclusively liquid chromatography coupled with electrospray ionisation tandem mass spectrometry for separation and quantification of up to 30 compounds on C18 column with up to 50 min elution gradient time and average mobile phase flow rate of 0.2 mL min^{-1} .

Here a new fast and sensitive method for the determination of a broad range of pharmaceuticals and personal care products is presented. The method uses a single SPE method and single LC/MS/MS method utilising UPLCTM (ultra performance liquid chromatography) coupled with triple quadrupole tandem mass spectrometry. UPLC is a novel technology providing significant improvements in resolution, speed and sensitivity due to the exploitation of a $1.7 \mu\text{m}$ particle-packed column.

This paper presents a novel method for the analysis of 25 acidic/neutral pharmaceuticals and personal care products in surface water with the utilisation of SPE/UPLC/ESI-(negative ionisation)-MS-MS and demonstrates its application in the Welsh environment. The group of PPCPs of interest have never previously been studied in the Welsh aqueous environment, to the best knowledge of the authors. A previous paper published by the authors [37], presented another novel multi-residue method for the analysis of a further 28 basic/neutral pharmaceuticals and illicit drugs in surface water by means of SPE/UPLC/ESI+(positive ionisation)-MS-MS. The overall methodology published in the form of the two papers allows for the analysis of almost 50 PPCPs in surface water. The rationale for the preparation of two separate methods for the analysis of all PPCPs resulted from their different structures and physico-chemical properties influencing both the composition of mobile phase used for analytes separation in LC and ionisation mode used in ESI-MS. Additionally, the paper identifies and quantifies

the influence of matrix components on the performance of the analytical methods and presents possible ways to solve it.

2. Experimental

2.1. Chemicals and materials

Reference standards (>95% purity) were purchased from Sigma–Aldrich (Gillingham, UK) and Sequoia Products Research Limited (Pangbourne, UK). All solvents used as mobile phases and their additives were of LC/MS quality. Surrogate/internal standards (IS): clofibric-d4 acid (4-chlorophenyl-d4), 3,4-dichlorobenzoic (2,5,6-d3) acid, bisphenol A-d16 and 4-chlorophenol (2,3,5,6-d4) were purchased from QMX Laboratories Limited (Essex, UK). All standards used as both surrogate and internal standards were added to the samples before extraction and were also used for the quantification of the samples.

Stock solutions of PPCPs ($0.5\text{--}1 \text{ g L}^{-1}$) were prepared in methanol and stored in the dark at 0°C . Working solutions were prepared fresh daily by diluting stock solution with methanol stored at 0°C . Ultrapure water was obtained using Neptune, Purite (MJ Patterson Scientific Ltd., UK). For method development and validation both HQ water (ultrapure water) and BB water (surface water collected from the source of the River Taff in Brecon Beacons National Park, which is not affected by PPCPs) were used. The average dissolved organic carbon of BB water was $4.5 \text{ mg DOC L}^{-1}$.

All glassware used was deactivated with 5% DMDCS (dimethylchlorosilane) in toluene to minimise sample loss through absorption of polar compounds onto –OH sites present on glass surfaces.

2.2. Sample preparation and solid-phase extraction

All samples were collected in 1 L silanized bottles with teflon faced phenolic caps (Wheaton, USA), acidified with 31% HCl to pH 2.0 and vacuum filtered through a $0.7 \mu\text{m}$ glass fibre filter GF/F (Whatman, UK).

The SPE method was optimised through several preliminary experiments involving the following variables: type of adsorbent, pH value of the sample, elution conditions and eluting agents. Out of all sorbents used (Oasis HLB, MCX, MAX, WCX and WAX (60 mg, Waters, UK), Chromabond C18ec (200 mg, Anachem, UK) and Isolute ENV+ and HCX (100 mg and 200 mg, respectively, Kinesis, UK)) Oasis MCX and HLB were found to give the best recoveries for most PPCPs and therefore they were used for further analysis.

SPE Gilson, Aspec XL4 (Anachem, UK) was utilised for solid-phase extraction. The SPE cartridges were conditioned with 2 mL of MeOH and equilibrated with 2 mL of water acidified with HCOOH (2% HCOOH; pH 2.1) at a rate of 3 mL min^{-1} . One litre of acidified and filtered water sample spiked with 200 ng of surrogate/internal standards was passed through the cartridge at a rate of 4 mL min^{-1} . The cartridges were subsequently washed with acidified water (2 mL 2% HCOOH/ H_2O ; flow rate, 3 mL min^{-1}). After drying, SPE cartridges were wrapped in aluminium foil and stored in a

freezer until eluted. PPCPs were extracted with 2 mL of MeOH and 1 mL of 5% NH_4OH in MeOH at a rate of 1 mL min^{-1} . The extracts were directly collected into a 6 mL collection tube and were evaporated to dryness with TurboVap evaporator (Caliper, UK; 40°C , N_2 , 5–15 psi) and finally reconstituted in 0.5 mL of HQ water modified with TrBA (tributylamine) and CH_3COOH (mobile phase, 100% A: 79.5% H_2O , 20% MeOH, 0.5% CH_3COOH and 10 mM TrBA). All reconstituted extracts were filtered through $0.2 \mu\text{m}$ PTFE filters (Whatman, Puradisc, 13 mm) and transferred to maximum recovery deactivated vials with PTFE septa (Waters, UK).

2.3. Ultra performance liquid chromatography–tandem mass spectrometry

Waters ACQUITY UPLCTM system (Waters, Manchester, UK) consisting of ACQUITY UPLCTM binary solvent manager, ACQUITY UPLCTM sample manager and ACQUITY UPLC BEH C18 column ($1.7 \mu\text{m}$; $1 \text{ mm} \times 100 \text{ mm}$) (Waters, UK) was used for the separation of analytes. Several mobile phases (H_2O , MeOH and acetonitrile) and their additives were studied for an improvement of compounds separation in LC and an improvement of ESI performance in negative ionisation mode. Among the mobile phase additives studied were basic additives: ammonia, ammonium formate and acetate, primary amines (methyl-, ethyl- and butylamine), secondary amines (dimethyl-, diethyl- and dibutylamine), tertiary amines (trimethyl-, triethyl- and tributylamine) at concentrations in mobile phase ranging from 1 mM to 50 mM and/or acidic compounds: formic and acetic acid at concentrations ranging from 0.05% to 0.5%.

After initial analyses the following composition of mobile phases was chosen:

- mobile phase A (pH 3.9): 79.5% H_2O , 20% MeOH, 0.5% CH_3COOH , 10 mM TrBA;
- mobile phase B (pH 5.5): 5% H_2O , 94.5% MeOH, 0.5% CH_3COOH , 10 mM TrBA.

The gradient program was as follows:

$$100\% \text{A} \xrightarrow{0.1 \text{ min}} 100\% \text{A} \xrightarrow{3.4 \text{ min}} 50\% \text{A} \xrightarrow{1.5 \text{ min}} 50\% \text{A} \xrightarrow{5 \text{ min}} 20\% \text{A} \xrightarrow{2 \text{ min}} 0\% \text{A} \xrightarrow{2 \text{ min}} 0\% \text{A} \xrightarrow{2 \text{ min}} 100\% \text{A} \xrightarrow{4 \text{ min}} 100\% \text{A}$$

Ten microlitres of the sample was injected into the system. The column was kept at 22°C and the temperature in the sample manager was kept at 6°C . The flow rate of mobile phase was 0.05 mL min^{-1} , which gave an average initial pressure of 6500 psi.

A Quatro Micro triple-quadrupole mass spectrometer (Micro-mass, Manchester, UK) equipped with an electrospray ionisation source was used for PPCPs identification and quantification. The analyses were performed in negative mode with a capillary voltage of 3 kV, a source temperature of 120°C and a desolvation temperature of 350°C . A cone gas flow of 20 L h^{-1} and desolvation gas flow of 400 L h^{-1} were used. Nitrogen, used as a nebulising and desolvation gas, was provided by a high purity

nitrogen generator NM 30LA 230VOC (Peak Scientific Instrument Ltd., UK). Argon (99.999%) was used as a collision gas. The mobile phase at a flow rate of 0.05 mL min^{-1} was directly introduced into the ion source from LC, without splitting. Mass-Lynx 4.1 (Waters, UK) software was used to collect and analyse the obtained data.

Mass spectrometry analyses were performed in the multiple reaction monitoring (MRM) mode, measuring the fragmentation of the deprotonated pseudo-molecular ions of each pharmaceutical and personal care product. A dwell time of 200 ms per ion pair was used. The choice of fragmentation products for each substance based on the most intense signal and the optimisation of cone voltages, energy collisions and other instrument parameters was done individually for each compound in a continuous-flow mode through a direct infusion of standard solutions at concentrations of 1 mg L^{-1} into the stream of the mobile phase. For optimisation of precursor ion/product ion transitions QuanOptimise software (Waters, UK) was used.

Signal suppression of analytes in ESI source was estimated for each PPCP as a percentage decrease in signal intensity in a sample matrix versus in deionised water using the following equation:

$$\text{Signal suppression (\%)} = \left(1 - \frac{I_{\text{BB}}}{I_{\text{HQ}}}\right) \times 100 \quad (1)$$

where I_{BB} was the PPCP peak area in BB water extract spiked after extraction with $500 \mu\text{g L}^{-1}$ of each PPCPs, and I_{HQ} was the PPCP peak area in HQ water extract spiked after extraction with $500 \mu\text{g L}^{-1}$ of each PPCP. No PPCPs were present in extracts of both HQ and BB water before their enrichment with PPCPs.

2.4. Quantification and method validation parameters

Quantification of PPCPs was carried out by means of MRM, using the highest characteristic precursor ion/product ion transitions and recording one to two transitions simultaneously. The following surrogate/internal standards (SS/IS) were used: clofibric-d4 acid (4-chlorophenyl-d4), 3,4-dichlorobenzoic (2,5,6-d3) acid, bisphenol A-d16 and 4-chlorophenol (2,3,5,6-d4) for the quantification of compounds analysed. The usage of only four internal standards is a limitation of the method due to the variability of chemical structure/properties between PPCPs studied and chosen internal standards. The choice of only four IS resulted from both the very high cost of isotope labelled compounds and difficulty with their purchase.

Detailed discussion concerning validation of the method is presented in authors' previous paper [37]. All instrumental validation parameters were determined for HQ water (containing 0.5% CH_3COOH , 20% MeOH and 10 mM TrBA) spiked with known concentrations of PPCPs. Method quantification and detection parameters were determined for BB water spiked with known concentrations of PPCPs before extraction.

Twelve-point multi-component internal standard calibration curves for the HQ water and BB water extract spiked with PPCPs before extraction ($0\text{--}1200 \text{ ng L}^{-1}$) were applied for quantifica-

tion of PPCPs with the utilisation of QuanLynx software (Waters, UK).

Quantitation and detection limits were determined using both signal-to-noise approach and were calculated using Eq. (2). HQ water standard solutions were used for instrumental detection and instrumental quantification limits determinations ($IDL_{S/N}$ and $IQL_{S/N}$ respectively). BB water extract spiked with PPCPs before extraction were used for the determination of method detection and method quantification limits ($MDL_{S/N}$ and $MQL_{S/N}$ respectively). The quantitation limit ($QL_{S/N}$) was estimated for the concentration of compound that gave a signal-to-noise ratio of 10:1. The detection limit ($DL_{S/N}$) corresponded to the concentration that gave a signal-to-noise ratio of 3:1. For confirmation purposes method quantification limits (MQL_{calc}) were also calculated using the following equation [38]:

$$MQL_{calc} = \frac{IQL_{S/N} \times 100}{Rec \times CF} \quad (2)$$

where $IQL_{S/N}$ is the instrumental quantification limit ($ng L^{-1}$), Rec is the absolute recovery of the analyte (%) and CF is the concentration factor, which in this method denotes 2000.

Linearity and range of the analytical procedure were performed by serial dilution of a stock solution of PPCPs ($10 mg L^{-1}$). Accuracy of the method was evaluated as the percentage of deviation from the known added amount of analyte in the sample. Precision was evaluated as the relative standard deviation (R.S.D.) of replicate measurements. Instrumental

intra-day precision and intra-day precision of the analytical method were verified under the same operating conditions over a short interval of time. Nine determinations covered respectively three concentrations ($50 \mu g L^{-1}$, $500 \mu g L^{-1}$ and $1000 \mu g L^{-1}$) of acidified HQ standards and BB water extract spiked with PPCPs before extraction. Instrumental inter-day precision and inter-day precision of the analytical method were verified by determinations that covered three concentrations ($50 \mu g L^{-1}$, $500 \mu g L^{-1}$ and $1000 \mu g L^{-1}$) of HQ standards solutions and BB water extract spiked with PPCPs before extraction, three replicates each undertaken on three different days.

3. Results and discussion

Seventeen pharmaceuticals and eight personal care products were the subject of the research (Table 1). The choice of pharmaceuticals was mainly based on the prescription data in Wales and England [39,40] and the metabolism routes of pharmaceuticals, mainly excretion as parent compounds and active main metabolites. The choice of PCPs was based on their high annual usage in wide range of household products and concern over their possible effect on human and aquatic organisms [41].

3.1. Liquid chromatography and mass spectrometry

3.1.1. Mobile phase and additives

Methanol and water were chosen as mobile phases for PPCPs separation. Ionic compounds are in general weakly retained in

Table 1
Chosen PPCPs and their properties

Group	Properties				
	Compound	CAS no.	Molecular formula	MW	pK _a
Pharmaceuticals					
Antibiotics	Sulfamethoxazole	723-46-6	C ₁₀ H ₁₁ N ₃ O ₃ S	253.28	5.8
	Chloramphenicol	56-75-7	C ₁₁ H ₁₂ Cl ₂ N ₂ O ₅	323.13	11.0
Anti-inflammatory/analgesics	Ibuprofen	15687-27-1	C ₁₃ H ₁₈ O ₂	206.28	4.9
	Diclofenac	15307-86-5	C ₁₄ H ₁₁ Cl ₂ NO ₂	296.15	4.2
	Ketoprofen	22071-15-4	C ₁₆ H ₁₄ O ₃	254.28	4.5
	Naproxen	22204-53-1	C ₁₄ H ₁₄ O ₃	230.26	4.2
	Aspirin	50-78-2	C ₉ H ₈ O ₄	180.16	3.5
	Salicylic acid (aspirin metabolite)	69-72-7	C ₇ H ₆ O ₃	138.12	3.0
	Mefenamic acid	61-68-7	C ₁₅ H ₁₅ NO ₂	241.29	4.2
Lipid regulating agents	Clofibrate	882-09-7	C ₁₀ H ₁₁ ClO ₃	214.65	–
	Bezafibrate	41859-67-0	C ₁₉ H ₂₀ ClNO ₄	361.82	0
	Pravastatin	81093-37-0	C ₂₃ H ₃₆ O ₇	424.53	–
Diuretics	Furosemide	54-31-9	C ₁₂ H ₁₁ ClN ₂ O ₅ S	330.75	3.9
Triazides	Bendroflumethiazide	73-48-3	C ₁₅ H ₁₄ F ₃ N ₃ O ₄ S ₂	421.42	8.5
H2-receptor antagonists	Sulfasalazine	599-79-1	C ₁₈ H ₁₄ N ₄ O ₅ S	398.39	–
Cardiac glycosides	Digoxin	20830-75-5	C ₄₁ H ₆₄ O ₁₄	780.94	–
Angiotensin II antagonists	Valsartan	137862-53-4	C ₂₄ H ₂₉ N ₅ O ₃	435.52	3.7
Personal care products					
Sunscreen agents	Benzophenone-1	131-56-6	C ₁₃ H ₁₀ O ₃	214.22	–
	Benzophenone-2	131-55-5	C ₁₃ H ₁₀ O ₅	246.22	–
	Benzophenone-3	131-57-7	C ₁₄ H ₁₂ O ₃	228.24	–
	Benzophenone-4	4065-45-6	C ₁₄ H ₁₂ O ₆ S	308.31	–
Preservatives	Methylparaben	99-76-3	C ₈ H ₈ O ₃	152.15	–
	Ethylparaben	120-47-8	C ₉ H ₁₀ O ₃	166.17	8.3
	Propylparaben	94-13-3	C ₁₀ H ₁₂ O ₃	180.20	–
	Butylparaben	94-26-8	C ₁₁ H ₁₄ O ₃	194.23	8.5

reversed phase chromatography with aqueous-organic mobile phases. Basic additives were added to the mobile phase in order to increase retardation of acidic compounds through the formation of ion-pairs with charged groups of analytes, which resulted in subsequent suppression of their dissociation and allowed for an interaction of analytes with the hydrophobic stationary phase. Longer retention of analytes also allowed for the gradient elution to start with a higher content of organic solvent in the mobile phase, which is known to be beneficial for the ionisation of analytes in ESI-MS detector. Although non-volatile additives (e.g. tetraalkylammonium salts or phosphate buffer) generally provide better retention and subsequent separation, in this case only volatile additives were studied as this is a well known limitation of ESI-MS due to a possible deposition and contamination of ESI source with non-volatile salts. An additional issue concerns strong bases/acids used as additives. These additives (e.g. trifluoroacetic acid) can form strong ion pairs with analytes that cannot be broken and as a result they prevent ionisation of analytes. Ammonia and ammonium salts as well as alkylamines are a good compromise providing sufficient separation of analytes and sensitive ESI-MS detection.

Ammonium cation (in the form of ammonia), the weakest ion-pairing agent, and tributylamine (TrBA), a stronger, more hydrophobic basic additive, were found to be the most effective additives for the studied PPCPs. However, TrBA was chosen for this method as it provided higher sensitivity (with the exception of digoxin), better peak shapes and longer retention times resulting in better separation of all analytes (Fig. 1). On average a twice as high peak area of analytes (dissolved in HQ) was observed in the case of the method using TrBA as the mobile phase additive when compared to NH_4OH (e.g. salicylic acid, sulfasalazine and valsartan had respectively 3.5, 2.8 and 2.3 times higher signal intensity in the presence of TrBA than NH_4OH ; see Fig. 1). Because high concentrations of mobile phase additives, despite good separation of analytes, may reduce, due to suppression

effect, the sensitivity of ESI-MS detection, concentrations of mobile phase additives were kept to minimum: 10 mM TrBA or 5 mM NH_4OH . Acetic acid at the concentration of 0.5% was also added to mobile phases containing basic additives to lower the pH of mobile phase from above 10 to below 6. An increase of response of anions of acidic compounds containing carboxyl groups in the presence of alkylamines can be explained by a sodium displacement mechanism [42]. Alkylammonium cation displaces the sodium cation in the anionic groups of carboxylic analyte. The formed ion-pairs dissociate during the ionisation process taking place in ESI-MS and as a result an increase of the signal of molecular anion of the acid, when compared to sodium adduct, is observed [42]. This theory explains an increase of ionisation of the following investigated compounds containing carboxyl groups: salicylic acid, furosemide, sulfasalazine, aspirin, pravastatin, ketoprofen, clofibric acid, naproxen, bezafibrate, valsartan, diclofenac, ibuprofen and mefenamic acid. The presence of TrBA also increased the ionisation of parabens and benzophenones and therefore these two groups of PPCPs were also analysed with the proposed method. It was, however, observed that the presence of matrix components extracted from BB water together with PPCPs causes higher suppression of some analytes, especially those characterised by short retention times (eluting with mobile phase of a high water to methanol ratio), when TrBA was used as a mobile phase additive when compared to NH_4OH (Fig. 1). Despite the higher suppression of some analytes caused by TrBA, this mobile phase additive was used in the analysis of acidic/neutral PPCPs as it resulted in an increase of retention of analytes in C18 column, an improvement of the shapes of the peaks, and their better separation, as is shown in Fig. 1.

3.1.2. UPLC/MS/MS—the method

Chromatograms of SPE extract of BB water spiked with PPCPs before extraction are presented in Fig. 2. Utilisation of

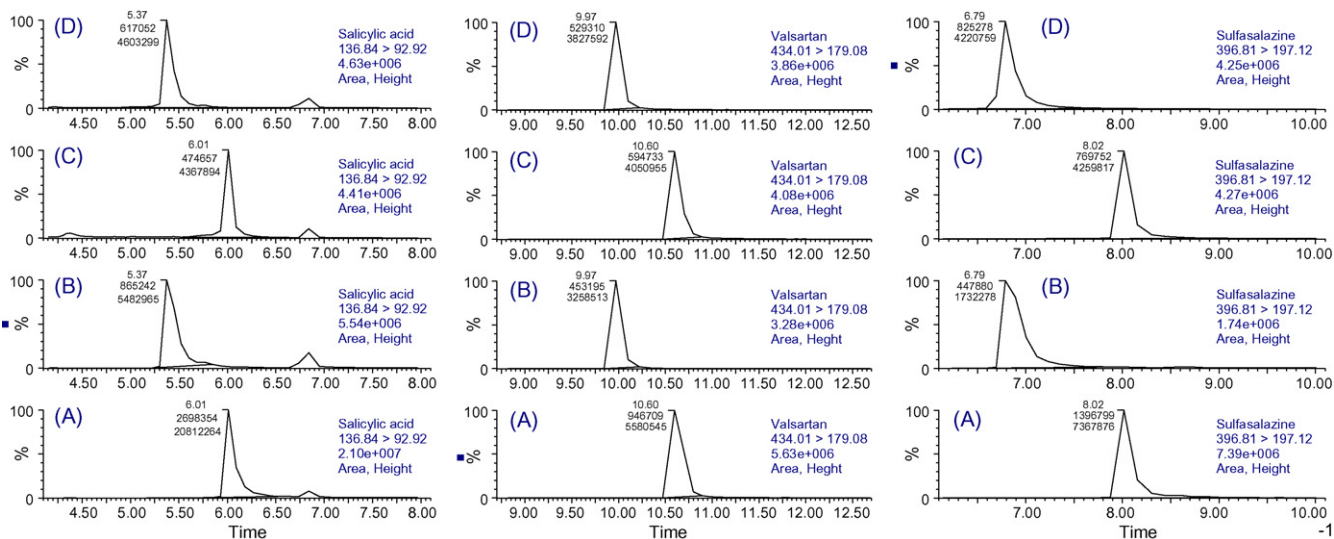


Fig. 1. UPLC/MS/MS separations for salicylic acid, sulfasalazine and valsartan (concentration of PPCPs, $500 \mu\text{g L}^{-1}$; (A) PPCPs spiked into HQ water (mobile phase, 100%A) and analysed using TrBA as a mobile phase additive; (B) PPCPs spiked into HQ (mobile phase, 100%A) water and analysed using NH_4OH as a mobile phase additive; (C) PPCPs spiked into BB water extract and analysed using TrBA as a mobile phase additive; (D) PPCPs spiked into BB water extract and analysed using NH_4OH as a mobile phase additive).

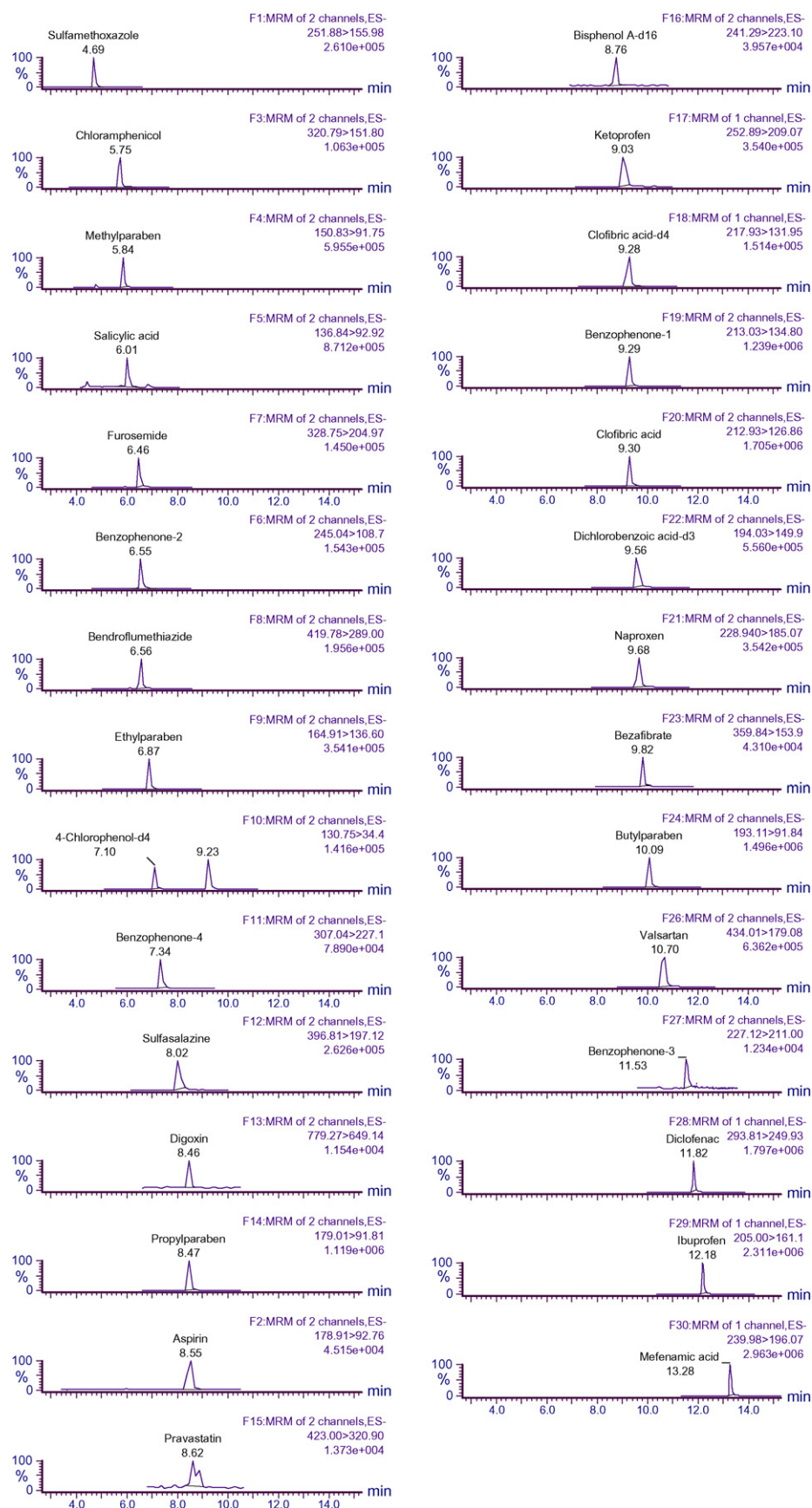


Fig. 2. UPLC/MS/MS separations for chosen PPCPs spiked into BB water and extracted by SPE (concentration of PPCPs, 100 ng L^{-1} ; IS, 200 ng L^{-1}).

Table 2

Optimised MRM conditions for the analysis of chosen PPCPs by UPLC/MS/MS (CV: cone voltage (V); CE: collision energy (eV))

Compound	CV/CE	MRM1 (quantification)	CV/CE	MRM2 (confirmation)
Sulfamethoxazole	30/17	251.9 > 156.0	30/25	251.9 > 91.9
Chloramphenicol	27/15	320.8 > 151.8	27/15	320.8 > 256.0
Methylparaben	34/20	150.8 > 91.8	20/14	150.8 > 135.8
Salicylic acid	30/15	136.8 > 92.9	30/30	136.8 > 64.9
2-Benzophenone	26/20	245.0 > 108.7	26/15	245.0 > 135.1
Furosemide	30/20	328.8 > 205.0	30/15	328.8 > 284.9
Bendroflumethiazide	45/25	419.8 > 289.0	45/25	419.8 > 327.8
Ethylparaben	20/14	164.9 > 136.6	26/20	164.9 > 91.9
4-Benzophenone	44/24	307.0 > 227.1	42/35	307.0 > 211.1
Sulfasalazine	35/25	396.8 > 197.1	35/25	396.8 > 240.0
Digoxin	50/45	779.3 > 649.1	50/45	779.3 > 475.9
Propylparaben	34/25	179.0 > 91.8	20/16	179.0 > 136.0
Aspirin	12/20	178.9 > 92.8	12/6	178.9 > 136.9
Pravastatin	30/16	423.0 > 320.9	30/15	423.0 > 302.9
Ketoprofen	20/8	252.9 > 209.1	–	–
1-Benzophenone	36/20	213.0 > 134.8	34/25	213.0 > 90.8
Clofibric acid	20/15	212.9 > 126.9	20/10	212.9 > 84.9
Naproxen	15/8	228.9 > 185.1	15/15	228.9 > 170.1
Bezafibrate	30/19	359.8 > 153.9	30/30	359.8 > 273.9
Butylparaben	34/25	193.1 > 91.8	40/16	193.1 > 136.0
Valsartan	35/25	434.0 > 179.1	35/20	434.0 > 350.1
3-Benzophenone	30/20	227.1 > 211.0	34/24	227.1 > 183.9
Diclofenac	22/13	293.8 > 249.9	–	–
Ibuprofen	20/8	205.0 > 161.1	–	–
Mefenamic acid	30/15	240.0 > 196.1	–	–
Clofibric-d4 acid	18/15	217.9 > 132.0	–	–
3,4-Dichlorobenzoic-d3 acid	25/15	194.0 > 149.9	–	–
Bisphenol A-d16	40/18	241.3 > 223.1	–	–
4-Chlorophenol-d4	32/16	130.8 > 34.4	–	–

the novel ultra performance liquid chromatography system with 1.7 μm bridged ethylsiloxane/silica hybrid (BEH) particles and 1 mm internal diameter column allowed for the establishment of sufficient separation of 25 analytes using much lower mobile phase flow rates (0.05 mL min^{-1}) and shorter retention times (from 4.7 min to 13.3 min) when compared to methods established with the usage of conventional HPLC, where higher flow rates of 0.2 mL min^{-1} and longer method times of up to 50 min are applied to separate up to 30 compounds [31,34]. Additionally, a new column technology allowed for an application of very short column equilibration times (4 min). As a result, a sensitive, fast and cost-effective method was developed. Due to low column temperature (22°C), which was optimal for the best separation of 25 analytes, a high initial pressure of 6500 psi was observed. This, however did not affect the performance of the method as a new technology implemented in the UPLC system is capable of pumping mobile phase at pressures up to 15,000 psi.

The mass spectrometry parameters are presented in Table 2. The most intensive product ion from each precursor ion was selected for quantification (MRM1). Retention time was the other primary criterion for identification of compound. A less sensitive secondary transition (MRM2) was used as the second criterion for confirmation purposes. In the case of ketoprofen, diclofenac, ibuprofen and mefenamic acid no secondary transition was observed. All of the compounds showed maximum sensitivity in the negative ionisation mode, although the degree of ionisation of PPCPs in ESI varied significantly and depended

mainly on the functional groups present in the molecule. The highest response was observed for: salicylic, mefenamic and clofibric acids, diclofenac, ibuprofen and parabens. Digoxin and benzophenone-3 showed the lowest degree of ionisation, although it was sufficient enough to undertake environmental analysis (Fig. 2).

3.2. Solid-phase extraction and signal suppression

Out of eight different sorbents studied for the multi-residue analysis of investigated PPCPs (see Section 2), Oasis MCX and HLB were found to give the highest SPE recoveries for the studied PPCPs at acidic pH (pH 2.0). Oasis HLB is a hydrophilic–lipophilic water-wettable reversed-phase sorbent containing two monomers: hydrophilic *N*-vinylpyrrolidone and lipophilic divinylbenzene. It is a universal sorbent for acidic, neutral and basic compounds. Oasis MCX is a strong cation-exchange mixed-mode polymeric sorbent, which is capable of both ion-exchange and reversed-phase interactions. MCX sorbent is built upon HLB copolymer. The additional presence of sulfonic groups allows for cation-exchange interactions. Therefore, MCX adsorbent is designed for the extraction of basic and neutral compounds. Acidic pH of the solution is required in order to ionise basic compounds and neutralise acidic compounds.

The mean absolute and relative (relative to the recovery of surrogate/internal standard) recoveries PPCPs in both HQ and BB water are presented in Table 3. A maximum or close to

Table 3
SPE recovery for studied PPCPs (concentration, 500 ng L⁻¹)

Compound	Sorbent	Recovery (%) (<i>n</i> = 3)			
		HQ water		BB water	
		Absolute	Relative ^a	Absolute	Relative ^a
Sulfamethoxazole	MCX	21.6 ± 0.5	25.6 ± 1.1 ^b	18.1 ± 1.9	27.2 ± 4.5 ^b
Chloramphenicol	MCX	93.8 ± 8.5	97.9 ± 4.9 ^d	27.2 ± 1.3	69.5 ± 4.7 ^d
			92.4 ± 4.7 ^b		37.0 ± 1.4 ^b
Methylparaben	MCX	127.8 ± 14.2	133.7 ± 8.4 ^d	61.9 ± 8.4	163.8 ± 13.8 ^d
			121.1 ± 1.4 ^c		106.1 ± 2.4 ^c
Salicylic acid	MCX	118.1 ± 14.2	122.9 ± 7.6 ^d	28.1 ± 4.0	75.7 ± 5.1 ^d
			116.6 ± 7.7 ^b		39.9 ± 2.3 ^b
2-Benzophenone	MCX	109.2 ± 8.2	114.4 ± 3.0 ^d	32.7 ± 3.6	86.8 ± 10.4 ^d
			111.1 ± 9.1 ^c		56.4 ± 2.3 ^c
Furosemide	MCX	88.0 ± 4.7	92.4 ± 1.3 ^d	33.3 ± 2.9	89.0 ± 2.9 ^d
			87.0 ± 1.0 ^b		47.4 ± 2.3 ^b
Bendroflumethiazide	MCX	78.3 ± 5.4	82.1 ± 2.4 ^d	19.9 ± 1.7	52.7 ± 3.1 ^d
			77.6 ± 3.0 ^b		28.4 ± 0.6 ^b
Ethylparaben	MCX	132.2 ± 11.5	138.4 ± 7.2 ^d	48.1 ± 4.7	127.1 ± 6.1 ^d
			134.4 ± 10.3 ^c		82.5 ± 2.4 ^c
4-Benzophenone	MCX	15.3 ± 4.1	16.0 ± 3.5 ^d	6.6 ± 0.7	17.7 ± 3.1 ^d
			15.6 ± 4.2 ^c		11.7 ± 2.6 ^c
	HLB	94.8 ± 10.1	109.5 ± 6.8 ^d	11.9 ± 2.2	70.1 ± 6.5 ^d
			120.4 ± 18.3 ^c		37.2 ± 6.2 ^b
Sulfasalazine	MCX	80.8 ± 6.3	84.5 ± 2.9 ^d	33.2 ± 1.4	85.4 ± 3.4 ^d
			79.8 ± 1.3 ^b		47.4 ± 3.2 ^b
Digoxin	MCX	45.7 ± 3.6	46.5 ± 3.7 ^c	25.3 ± 3.5	46.5 ± 9.3 ^c
			43.9 ± 3.3 ^b		37.9 ± 5.3 ^b
	HLB	76.7 ± 4.5	87.5 ± 4.8 ^c	19.3 ± 5.3	113.2 ± 18.8 ^d
			84.3 ± 4.7 ^b		58.8 ± 10.3 ^b
Propylparaben	MCX	124.8 ± 8.4	130.9 ± 3.1 ^d	44.8 ± 4.1	119.3 ± 14.1 ^d
			126.9 ± 8.4 ^c		77.2 ± 3.7 ^c
Aspirin	MCX	133.9 ± 1.7	138.7 ± 2.6 ^d	63.5 ± 3.8	146.5 ± 3.7 ^d
			132.6 ± 7.8 ^b		90.4 ± 6.5 ^b
Pravastatin	MCX	8.4 ± 2.4	8.6 ± 2.4 ^c	5.5 ± 0.2	9.5 ± 1.3 ^c
			8.3 ± 1.8 ^b		7.8 ± 0.9 ^b
	HLB	51.0 ± 1.0	64.7 ± 1.8 ^c	9.9 ± 1.5	30.9 ± 1.3 ^c
			56.9 ± 3.4 ^b		30.5 ± 5.0 ^b
Ketoprofen	MCX	114.1 ± 5.6	113.0 ± 4.8 ^b	38.0 ± 1.8	54.3 ± 3.5 ^b
			113.3 ± 3.5 ^b		52.5 ± 4.6 ^c
			117.8 ± 2.6 ^d		98.1 ± 3.3 ^d
			115.9 ± 3.7 ^c		65.8 ± 4.6 ^c
1-Benzophenone	MCX	104.6 ± 6.1	109.6 ± 1.0 ^d	41.4 ± 2.9	109.9 ± 9.2 ^d
Clofibric acid	MCX	100.0 ± 3.0	99.2 ± 4.1 ^b	59.3 ± 2.9	84.7 ± 6.3 ^b
Naproxen	MCX	107.0 ± 7.2	105.7 ± 0.9 ^b	64.1 ± 4.6	91.4 ± 8.5 ^b
Bezafibrate	MCX	107.4 ± 11.0	106.0 ± 2.0 ^c	79.3 ± 6.1	104.8 ± 2.5 ^c
			106.0 ± 4.5 ^b		112.9 ± 4.0 ^b
Butylparaben	MCX	112.1 ± 9.6	117.5 ± 4.3 ^d	76.5 ± 4.7	203.3 ± 12.2 ^d
			107.9 ± 0.9 ^c		132.4 ± 8.5 ^c
Valsartan	MCX	98.3 ± 8.5	99.8 ± 5.6 ^c	54.2 ± 2.6	96.3 ± 2.2 ^c
			97.1 ± 2.4 ^b		77.4 ± 5.1 ^b
3-Benzophenone	MCX	76.1 ± 9.0	79.7 ± 5.8 ^d	46.6 ± 2.4	124.7 ± 14.4 ^d
Diclofenac	MCX	104.0 ± 7.0	102.8 ± 1.1 ^b	71.5 ± 2.3	102.1 ± 7.8 ^b
Ibuprofen	MCX	82.8 ± 4.1	81.9 ± 1.2 ^b	66.7 ± 1.8	95.4 ± 7.0 ^b
Mefenamic acid	MCX	97.0 ± 3.5	96.0 ± 2.8 ^b	101.7 ± 9.0	138.0 ± 10.5 ^b
Clofibric-d4 acid	MCX	101.1 ± 6.5	–	70.3 ± 7.3	–
	HLB	89.6 ± 3.7	–	32.7 ± 3.4	–
3,4-Dichlorobenzoic-d3 acid	MCX	101.2 ± 9.1	–	77.2 ± 7.0	–
	HLB	83.6 ± 6.5	–	36.2 ± 5.2	–
Bisphenol A-d16	MCX	95.2 ± 4.6	–	39.2 ± 4.9	–
	HLB	86.4 ± 4.1	–	17.4 ± 4.8	–
4-Chlorophenol-d4	MCX	98.4 ± 4.0	–	58.0 ± 6.0	–
	HLB	79.2 ± 4.0	–	32.0 ± 3.7	–

^a Recovery relative to surrogate/internal standard.

^b Clofibric-d4 acid (4-chlorophenyl-d4).

^c 3,4-Dichlorobenzoic (2,5,6-d3) acid.

^d Bisphenol A-d16.

^e 4-Chlorophenol (2,3,5,6-d4).

maximum recovery was observed for almost all PPCPs dissolved in HQ water both in the case of HLB and MCX adsorbents. MCX gave generally better recoveries than HLB with the exception of 4-benzophenone, digoxin and pravastatin. As can be observed from Table 3 the recoveries of 4-benzophenone, digoxin and pravastatin were respectively only 15.3, 45.7 and 8.4% in HQ water in the case of MCX and 94.8, 76.7 and 51.0% in the case of HLB sorbent. Therefore for these compounds the usage of HLB sorbent is strongly recommended.

A significant decrease of the absolute recovery of some PPCPs was observed in BB water due to the presence of the matrix components in the sample and is believed to result from both a reduction of sorption efficiency of SPE cartridges and also signal suppression in the electrospray interface due to the presence of matrix impurities. The second factor was found to be the dominant phenomenon affecting sensitivity of the method as it is discussed below. HLB sorbent, apart from giving lower PPCPs recovery than MCX sorbent in the case of HQ water, was also found to be much more affected by the presence of matrix components in BB water than MCX sorbent and therefore MCX adsorbent was chosen for final method development and validation purposes. It is suggested that HLB, because of its universal nature, is less selective than MCX sorbent and capable of sorption of many more matrix components, resulting in lower SPE recovery or more likely higher ion suppression in ESI source.

Signal suppression is the main disadvantage of electrospray mass spectrometry and results from the fact that ESI-MS is susceptible to organic and inorganic components that are present both in the sample together with analytes and in the mobile phase. In this paper signal suppression of analytes resulting from both matrix components (that were extracted together with analytes) and mobile phase additives was studied (see Section 3.1.1). Table 4 presents signal suppression observed for the analysed PPCPs dissolved in SPE extract of BB water. Chloramphenicol, salicylic acid, bendroflumethiazide, furosemide, methylparaben, ethylparaben, 2- and 4-benzophenone were found to be the most susceptible to matrix components. No or only a slight signal suppression was observed for diclofenac and ibuprofen. A low signal enhancement was observed in the case of mefenamic acid.

The results presented in Tables 3 and 4 lead to the conclusion that the effects of signal suppression and low SPE recovery, both resulting from the presence of matrix interferences, are the main factors affecting the sensitivity of the analytical method. Among the compounds characterised by the highest ion suppression in ESI source are chloramphenicol, salicylic acid, bendroflumethiazide, furosemide, methylparaben, ethylparaben and 2-benzophenone. Therefore, for these compounds, the lower absolute SPE recoveries (Table 3) are probably due to the suppression of the signal during electrospray ionisation (Table 4). It is evident that the presence of the higher percentage of H₂O in the mobile phase contributes to signal suppression of analytes, as the above compounds elute within the retention time of 5.7–7.3 and mobile phase gradient from 50 to 35% of mobile phase A containing 79.5% of H₂O.

The loss of method sensitivity for the majority of the PPCPs studied, which resulted both from low SPE recovery and sig-

Table 4

Signal suppression of PPCPs in BB water spiked after extraction (concentration, 500 µg L⁻¹)

Compound	Signal suppression (%)	
	Absolute	Relative ^a
Sulfamethoxazole	41.3 ± 1.9	11.1 ± 3.6 ^b
Chloramphenicol	88.0 ± 0.5	73.7 ± 0.3 ^d
Methylparaben	81.7 ± 0.4	59.8 ± 1.4 ^d
Salicylic acid	80.7 ± 0.5	57.6 ± 1.6 ^d
2-Benzophenone	72.1 ± 0.9	38.8 ± 0.2 ^d
Furosemide	76.0 ± 0.8	47.4 ± 1.5 ^d
Bendroflumethiazide	83.7 ± 0.5	64.1 ± 0.3 ^d
Ethylparaben	79.3 ± 0.6	54.6 ± 0.5 ^d
4-Benzophenone	75.0 ± 0.9	45.1 ± 1.5 ^d
Sulfasalazine	41.3 ± 0.9	-29.1 ± 2.6 ^d
Digoxin	67.2 ± 0.8	15.3 ± 1.8 ^e
Propylparaben	62.4 ± 1.5	17.4 ± 3.4 ^d
Aspirin	57.8 ± 2.1	-0.9 ± 4.6 ^d
Pravastatin	62.1 ± 1.0	2.0 ± 3.9 ^e
Ketoprofen	67.0 ± 2.1	50.1 ± 2.5 ^b
		22.9 ± 4.2 ^c
		14.8 ± 6.8 ^d
		27.6 ± 3.2 ^e
1-Benzophenone	61.6 ± 1.6	15.6 ± 2.0 ^d
Clofibric acid	58.3 ± 1.3	37.0 ± 2.5 ^b
Naproxen	45.4 ± 1.1	17.5 ± 1.6 ^b
Bezafibrate	65.4 ± 1.7	30.8 ± 2.9 ^c
Butylparaben	47.9 ± 1.5	-14.7 ± 6.4 ^d
Valsartan	43.6 ± 3.0	2.0 ± 5.3 ^e
3-Benzophenone	32.7 ± 0.7	-47.8 ± 6.4 ^d
Diclofenac	17.6 ± 3.5	-24.7 ± 5.2 ^b
Ibuprofen	10.9 ± 3.2	-34.7 ± 4.9 ^b
Mefenamic acid	-16.0 ± 8.3	-76.2 ± 1.1 ^b
Clofibric-d4 acid	34.2 ± 1.7	-
3,4-Dichlorobenzoic-d3 acid	57.3 ± 0.5	-
Bisphenol A-d16	54.5 ± 1.5	-
4-Chlorophenol-d4	61.2 ± 0.8	-

^a Recovery relative to surrogate/internal standard.

^b Clofibric-d4 acid (4-chlorophenyl-d4).

^c 3,4-Dichlorobenzoic (2,5,6-d3) acid.

^d Bisphenol A-d16.

^e 4-Chlorophenol (2,3,5,6-d4).

nal suppression in ESI-MS, was in this work compensated by the usage of the appropriate surrogate/internal standards (Tables 3 and 4). It should be emphasised here that the choice of internal standard is crucial in terms of compensation for suppression effect, which is a common problem in MS-ESI. An influence of the type of IS/SS standard on relative recovery of ketoprofen is a good example (see Tables 3 and 4). The comparison of relative recoveries of ketoprofen (Table 3) clearly indicates that out of the four IS/SS standards studied only bisphenol-A-d16 compensates for suppression of ketoprofen because both compounds are similarly susceptible to suppression in ESI-MS (Table 4). Based on similarities in structures and susceptibility to suppression during ESI-MS analysis the following primary IS/SS were chosen for different groups of PPCPs:

- (a) clofibric-d4 acid: sulfamethoxazole, clofibric acid, naproxen, diclofenac, ibuprofen and mefenamic acid,

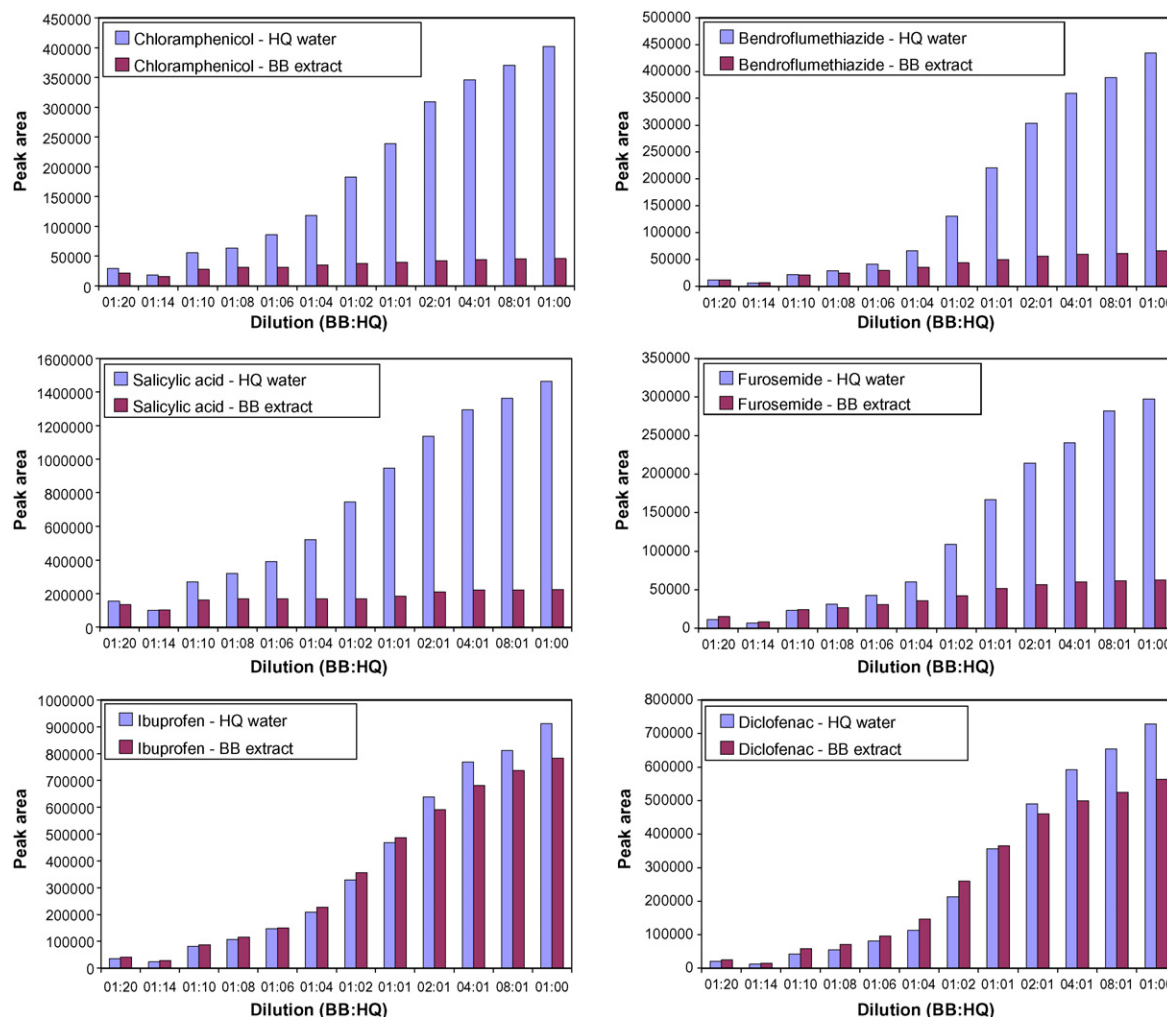


Fig. 3. Influence of BB extract dilution on the intensity of the signal of studied PPCPs (HQ water: mobile phase, 100%A spiked with $500 \mu\text{g L}^{-1}$ of PPCPs before dilution; BB extract: reconstituted with mobile phase, 100%A and spiked with $500 \mu\text{g L}^{-1}$ of PPCPs before dilution).

- (b) 3,4-dichlorobenzoic-d3 acid: bezafibrate,
- (c) bisphenol A-d16: aspirin, chloramphenicol, salicylic acid, furosemide, bendroflumethiazide, sulfasalazine, ketoprofen, parabens and benzophenones,
- (d) 4-chlorophenol-d4: digoxin, pravastatin and valsartan.

A slight overestimation (>100%) was observed for the relative recoveries of these PPCPs, which were not significantly affected by matrix assisted signal suppression/low recovery (e.g. mefenamic acid). This phenomenon can be explained by the fact that signal suppression for the surrogate/internal standard is higher than for the analyte. In the case of the compounds that were characterised by good SPE recovery, but suffered high signal suppression (chloramphenicol, salicylic acid, furosemide and bendroflumethiazide) which was not compensated by the usage of IS/SS, two procedures can be undertaken. Firstly, the choice of a more suitable IS/SS, which is problematic, or the dilution of sample extracts as proposed by Gros et al. [34] and Gómez et al. [43]. It was observed that, on average a higher than 8-times dilution of BB extract with HQ water is necessary to avoid matrix assisted signal suppression for the studied

PPCPs: chloramphenicol, salicylic acid, furosemide and bendroflumethiazide (Fig. 3). For comparison, a minor influence of dilution of sample extracts on the intensity of the signal of compounds which are not significantly susceptible to signal suppression (diclofenac and ibuprofen) is also presented in Fig. 3. It should be emphasised, however, that too high dilution can also lead to a considerable decrease in sensitivity and this has to be taken into consideration when applying this approach.

The above discussion indicates that there is an obvious need for the application of a higher number of surrogate/internal standards to more accurately compensate for matrix assisted signal suppression and the low SPE recovery of the different groups of PPCPs studied. This is, however, very often impossible due to the lack of suitable surrogate/internal standards or their high cost. The other possibilities that could eliminate matrix effect involve selective extraction/better sample clean-up, time consuming standard addition or dilution of sample extracts as proposed by Gros et al. [34] and Gómez et al. [43]. In summary, there are several approaches that can be undertaken concerning quantification issues. In this study internal standard calibration is considered to be the right approach for PPCPs determination

Table 5

Performance data for pharmaceuticals (instrumental/method limits of detection and quantification; linearity— R^2)

PPCPs	t_R (min)	Instrumental parameters ^a			Method parameters ^b			
		IDL _{S/N} ($\mu\text{g L}^{-1}$)	IQL _{S/N} ($\mu\text{g L}^{-1}$)	R^2	MDL _{S/N} (ng L^{-1})	MQL _{S/N} (ng L^{-1})	MQL _{calc} (ng L^{-1})	R^2
Sulfamethoxazole	4.69	0.2	1.0	1.000	0.5	2.0	2.8	0.999
Chloramphenicol	5.75	0.05	0.2	0.999	0.5	2.0	0.4	0.999
Methylparaben	5.84	0.05	0.2	0.999	0.1	0.3	0.2	0.997
Salicylic acid	6.01	0.05	0.2	1.000	0.1	0.5	0.4	0.999
Furosemide	6.46	0.2	1.0	0.999	2.5	7.0	1.5	0.998
2-Benzophenone	6.55	0.1	0.4	0.997	0.1	0.5	0.6	1.000
Bendroflumethiazide	6.56	0.1	1.0	0.998	1.0	3.0	2.5	0.998
Ethylparaben	6.87	0.05	0.2	0.996	0.15	0.5	0.2	0.997
4-Benzophenone	7.34	0.05	0.2	0.997	1.5	5.0	1.5	1.000
Sulfasalazine	8.02	0.2	1.0	0.996	1.0	5.0	1.5	0.997
Digoxin	8.46	10.0	15	0.996	10.0	30.0	29.6	0.996
Propylparaben	8.47	0.1	0.4	0.997	0.1	0.3	0.5	1.000
Aspirin	8.55	0.2	1.0	0.999	0.1	0.5	0.8	1.000
Pravastatin	8.62	1.0	5.0	0.997	20.0	60.0	50.0	0.998
Ketoprofen	9.03	0.2	1.0	0.999	0.8	2.5	1.3	0.999
1-Benzophenone	9.29	0.05	0.2	0.997	0.1	0.3	0.2	1.000
Clofibrilic acid	9.30	0.1	0.4	0.994	0.1	0.5	0.4	0.997
Naproxen	9.68	0.2	0.6	0.997	0.5	1.5	0.9	1.000
Bezafibrate	9.82	2.0	10.0	0.996	2.5	10.0	6.3	1.000
Butylparaben	10.09	0.1	0.4	0.999	0.1	0.3	0.3	0.999
Valsartan	10.70	0.2	0.5	0.999	0.1	0.5	0.5	1.000
3-Benzophenone	11.53	10.0	30.0	0.996	10	30.0	32.2	0.999
Diclofenac	11.82	0.2	0.7	0.997	0.2	0.5	0.5	0.999
Ibuprofen	12.18	0.2	0.7	0.995	0.1	0.5	0.5	0.999
Mefenamic acid	13.28	0.3	1.0	0.997	0.1	0.5	0.5	0.999

^a HQ standards spiked with pharmaceuticals; concentration, 0–1200 $\mu\text{g L}^{-1}$.^b BB water spiked with pharmaceuticals before extraction; concentration, 0–1200 ng L^{-1} .

in environmental samples. For the compounds that IS/SS did not compensate for ion suppression, dilution of samples should be undertaken. This approach was also proposed by Gómez et al. [43].

3.3. Quantification and method validation parameters

The instrumental and method limits of detection and quantification are presented in Table 5. The instrumental limits of quantification varied from 0.2 $\mu\text{g L}^{-1}$ for chloramphenicol, methyl-, ethyl- and butylparaben, salicylic acid, and 1-, 4-benzophenones to 30 $\mu\text{g L}^{-1}$ for 3-benzophenone. The method limits of quantification were at low nanogram per litre levels and ranged from 0.3 ng L^{-1} for methyl-, propyl- and butylparaben and 1-benzophenone to 30 ng L^{-1} for digoxin and 3-benzophenone, which makes the method useful for the determination of very low levels of PPCPs in the aqueous environment such as surface waters. The values of MQL_{S/N} that were determined using a signal-to-noise approach were also confirmed with MQL_{calc} calculated using Eq. (2) (see Table 5).

The mean correlation coefficients (R^2) of the calibration curves, which are on average higher than 0.997 in both HQ water and BB surface water (Table 5), show good linearity of the method in the range of 0–1200 ng L^{-1} . The accuracy range was within the value of –30 to 20%. The instrumental intra- and inter-day repeatabilities as indicated by standard deviation

calculated from the analysis of three replicates were below 5%. The method intra- and inter-day repeatabilities were on average less than 5% (Table 6).

3.4. Environmental application

The multi-residue method was successfully applied to verify the presence of PPCPs in River Taff in South Wales, a region of the UK which, according to the best knowledge of the authors, has never been studied for the analysis of the chosen PPCPs. Several sampling points were chosen along River Taff:

1. Brecon Beacons National Park, the source of River Taff.
2. Merthyr Tydfil—23.5 km downstream, just after Merthyr Tydfil (population, 55,000).
3. Abercynon—12 km downstream of Merthyr Tydfil, just after Abercynon, 1 km upstream of a WWTP.
4. Pontypridd—2 km downstream of a WWTP, just before Pontypridd (population, 33,000).
5. Trefforest Estate—7 km downstream of Pontypridd.
6. Cardiff—18 km downstream of Trefforest Estate, the bay area of Cardiff (population, 320,000), where the river enters the Bristol Channel.

The results are presented in Table 7. The research proved that River Taff at its source, which is in Brecon Beacons National Park, is not contaminated with PPCPs. The first signs of con-

Table 6

Performance data for pharmaceuticals (inter- and intra-day repeatability)

PPCPs	Method parameters ^a , precision											
	Intra-day R.S.D.% (<i>n</i> = 3)									Inter-day R.S.D.% (<i>n</i> = 3)		
	50 (ng L ⁻¹)			500 (ng L ⁻¹)			1000 (ng L ⁻¹)			50 (ng L ⁻¹)	500 (ng L ⁻¹)	1000 (ng L ⁻¹)
	D1	D2	D3	D1	D2	D3	D1	D2	D3			
Sulfamethoxazole	1.3	1.1	0.4	1.0	0.2	2.1	1.6	1.0	1.5	3.9	4.8	5.4
Chloramphenicol	1.3	2.5	0.7	3.1	0.8	1.7	0.6	0.7	0.9	1.7	2.9	2.9
Methylparaben	3.1	2.6	0.8	3.8	1.2	1.1	1.0	1.5	2.2	4.6	6.4	5.5
Salicylic acid	0.9	1.4	0.4	3.3	1.0	1.4	2.2	1.6	1.0	3.3	3.3	5.4
Furosemide	1.0	1.5	0.5	1.5	1.6	1.0	1.4	1.7	0.5	4.6	4.9	3.2
2-Benzophenone	2.1	4.4	0.2	2.9	2.4	0.4	0.5	1.1	2.3	3.9	4.8	5.5
Bendroflumethiazide	2.7	2.1	0.8	1.0	2.0	1.6	3.0	1.2	1.1	1.8	2.7	5.1
Ethylparaben	2.3	3.9	1.2	1.0	1.8	1.8	4.8	0.2	1.4	2.5	5.6	4.0
4-Benzophenone	3.8	4.6	1.7	4.1	3.1	2.2	0.8	3.9	3.0	6.5	7.0	4.6
Sulfasalazine	2.7	1.6	0.6	3.3	0.7	1.3	0.6	0.8	0.5	2.0	1.8	2.9
Digoxin	13.7	1.9	3.9	4.2	3.8	6.6	4.7	0.6	3.4	8.3	8.1	8.9
Propylparaben	1.5	3.8	2.1	2.4	4.0	4.1	1.3	3.7	5.0	4.0	9.9	4.9
Aspirin	3.5	1.8	1.5	2.4	1.0	1.0	2.3	0.2	0.6	2.4	3.9	3.5
Pravastatin	–	–	–	4.1	3.7	1.0	1.0	0.3	4.4	–	7.9	5.0
Ketoprofen	2.2	1.4	1.2	2.5	1.2	1.0	4.2	1.1	0.6	2.8	2.9	3.0
1-Benzophenone	0.5	3.1	0.9	2.5	3.2	1.1	0.3	4.4	4.7	3.0	6.5	4.8
Clofibric acid	0.9	1.6	0.3	2.7	2.5	0.4	1.2	1.2	1.8	4.4	5.3	4.9
Naproxen	0.9	0.8	0.8	1.6	0.9	2.1	2.6	1.0	1.4	1.8	4.7	4.4
Bezafibrate	6.5	2.5	1.4	4.2	1.2	4.7	4.9	1.8	3.1	3.5	8.8	7.4
Butylparaben	0.4	2.6	1.6	1.3	1.4	2.8	1.5	2.6	1.2	2.2	5.2	4.7
Valsartan	4.5	2.2	1.2	4.8	2.4	2.3	1.6	0.7	0.4	4.3	4.7	3.2
3-Benzophenone	0.5	2.2	2.6	3.5	1.2	0.4	0.5	0.5	2.3	8.3	6.6	3.0
Diclofenac	1.5	0.5	0.5	1.0	0.6	1.0	2.1	0.8	0.7	2.6	3.2	3.6
Ibuprofen	1.7	0.6	1.0	1.3	1.4	1.2	2.2	1.0	1.4	3.2	4.8	4.4
Mefenamic acid	1.3	0.6	2.2	0.1	0.3	0.8	0.1	0.2	0.9	2.6	4.8	3.4

^a BB water spiked with pharmaceuticals before extraction; concentration, 0–1200 ng L⁻¹.

Table 7

Concentration of PPCPs in River Taff (two replicate samples)

Compound	Concentration (ng L ⁻¹)				
	Merthyr Tydfil	Abercynon	Pontypridd	Trefforest Estate	Cardiff
Sulfamethoxazole	<MQL	<MQL	<MQL	<MQL	<MQL
Chloramphenicol	<MQL	<MQL	<MQL	<MQL	<MQL
Methylparaben	30 ± 5	48 ± 2	26 ± 2	17 ± 2	10 ± 10
Salicylic acid	21 ± 2	33 ± 4	18 ± 3	14 ± 1	14 ± 1
2-Benzophenone	<MQL	<MQL	<MQL	<MQL	<MQL
Furosemide	<MQL	<MQL	57 ± 8	40 ± 2	7 ± 1
Bendroflumethiazide	<MQL	<MQL	<MQL	<MQL	<MQL
Ethylparaben	4 ± 0.2	7 ± 1	8 ± 1	4 ± 1	4 ± 1
4-Benzophenone	<MQL	54 ± 9	220 ± 20	187 ± 22	128 ± 18
Sulfasalazine	7 ± 0.1	12 ± 0.4	30 ± 3	14 ± 0.4	20 ± 1
Digoxin	<MQL	<MQL	<MQL	<MQL	<MQL
Propylparaben	<MQL	<MQL	<MQL	<MQL	<MQL
Aspirin	3 ± 2	9 ± 2	7 ± 1	6 ± 1	7 ± 1
Pravastatin	<MQL	<MQL	<MQL	<MQL	<MQL
Ketoprofen	<MQL	<MQL	6 ± 1	4 ± 1	6 ± 2
1-Benzophenone	<MQL	<MQL	<MQL	<MQL	<MQL
Clofibric acid	<MQL	<MQL	<MQL	36 ± 1	21 ± 1
Naproxen	3 ± 0.2	9 ± 1	34 ± 2	29 ± 3	20 ± 2
Bezafibrate	<MQL	<MQL	<MQL	<MQL	<MQL
Butylparaben	<MQL	<MQL	<MQL	<MQL	<MQL
Valsartan	1 ± 0.1	7 ± 1	21 ± 2	19 ± 2	15 ± 0.2
3-Benzophenone	<MQL	<MQL	<MQL	<MQL	<MQL
Diclofenac	<MQL	1 ± 0.4	12 ± 2	8 ± 1	8 ± 1
Ibuprofen	0.6 ± 0.3	20 ± 2	25 ± 3	18 ± 2	37 ± 2
Mefenamic acid	<MQL	<MQL	9 ± 6	5 ± 4	<MQL

tamination are observed in Abercynon, which might be due to a run-off or an uncontrolled discharge of untreated sewage from surrounding housing estates. A significant increase of PPCPs concentration takes place in Pontypridd, which is after a wastewater treatment plant (WWTP Cilfynydd treating mainly communal wastewater). A slight decrease of PPCPs concentration is observed in Cardiff, but it still remains high when compared to the quality of river water upstream of the WWTP, at the first three sampling points.

PPCPs were identified at concentrations of a few ng L^{-1} to a few hundreds ng L^{-1} . The highest concentrations were observed in the case of pharmaceuticals for anti-inflammatory/analgesics such as salicylic acid, naproxen and ibuprofen and other pharmaceuticals such as: furosemide and valsartan. Among personal care products 4-benzophenone and methylparaben were found at the highest concentrations.

4. Conclusions

This paper concerns the development and validation of a novel, fast and cost-effective multi-residue method for environmental monitoring of 25 acidic/neutral pharmaceuticals (antibiotics, anti-inflammatory/analgesics, lipid regulating agents, diuretics, triazides, H₂-receptor antagonists, cardiac glycosides and angiotensin II antagonists) and personal care products (sunscreens agents and preservatives) in the low nanogram per litre range. The method involved single solid-phase extraction with the usage of strong cation-exchange mixed-mode polymeric sorbent (Oasis MCX, 60 mg) and subsequent ultra performance liquid chromatography–negative electrospray ionisation tandem mass spectrometry. The usage of the novel ultra performance liquid chromatography system with 1.7 μm bridged ethylsiloxane/silica hybrid (BEH) particles and 1 mm internal diameter column allowed for good separation of analytes with the application of low mobile phase flow rates (0.05 mL min^{-1}) and short retention times (from 4.7 min to 13.3 min). High sensitivity, resolution and speed of analysis are some of the main advantages of the method when compared to other multi-residue methods using high-performance liquid chromatography combined with mass spectrometry.

The influence of mobile-phase composition, matrix assisted ion suppression and SPE recovery on the sensitivity of the method were evaluated. High recovery and reproducibility for MCX adsorbent was obtained for many of the pharmaceuticals studied. The effects of signal suppression and low SPE recovery, both resulting from the presence of matrix interferences, were found to be the main factors affecting the sensitivity of the established analytical method. Surrogate/internal standards were therefore added to the sample so as to compensate for losses of compounds during both the sample preparation procedure and resulting from matrix assisted suppression. For the compounds that IS/SS did not compensate for ion suppression, dilution of samples was undertaken.

The mean correlation coefficients (R^2) of the calibration curves, which are on average higher than 0.997 in both HQ water and BB surface water extract, showed good linearity of the method in the studied range of 0–1200 $\mu\text{g L}^{-1}$ and

0–1200 ng L^{-1} respectively. The instrumental limits of quantification varied from 0.2 $\mu\text{g L}^{-1}$ (chloramphenicol, salicylic acid, diclofenac, ibuprofen, methyl-, ethyl- and butylparaben, 1- and 4-benzophenone) to 30 $\mu\text{g L}^{-1}$ (3-benzophenone). The method limits of quantification were at low nanogram per litre levels and ranged from 0.3 ng L^{-1} (methyl-, propyl-, butylparaben and 1-benzophenone) to 30 ng L^{-1} (dioxin and 3-benzophenone). The instrumental and method intra-day and inter-day repeatabilities were on average less than 5%.

The method was applied for the determination of PPCPs in River Taff. The results confirmed its applicability in environmental monitoring. Thirteen compounds were determined in river water at levels ranging from a single to a few hundred nanograms per litre. Among them were 10 pharmaceuticals (aspirin, salicylic acid, ketoprofen, naproxen, diclofenac, ibuprofen, mefenamic acid, furosemide, sulfasalazine, valsartan) and 3 personal care products (methyl- and ethylparaben and 4-benzophenone). The highest concentrations were determined in river water samples collected after wastewater plants, which confirms the hypothesis that wastewater effluents are the main source of water contamination with PPCPs.

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