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Enantioselective simultaneous analysis of selected pharmaceuticals in environmental samples by ultrahigh performance supercritical fluid based chromatography tandem mass spectrometry

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Abstract

In order to assess the true impact of each single enantiomer of pharmacologically active compounds (PACs) in the environment, highly efficient, fast and sensitive analytical methods are needed. For the first time this paper focuses on the use of ultrahigh performance supercritical fluid based chromatography coupled to a triple quadrupole mass spectrometer to develop multi-residue enantioselective methods for chiral PACs in environmental matrices. This technique exploits the advantages of supercritical fluid chromatography, ultrahigh performance liquid chromatography and mass spectrometry. Two coated modified 2.5 μ m-polysaccharide-based chiral stationary phases were investigated: an amylose tris-3,5-dimethylphenylcarbamate column and a cellulose tris-3-chloro-4-methylphenylcarbamate column. The effect of different chromatographic variables on chiral recognition is highlighted. This novel approach resulted in the baseline resolution of 13 enantiomers PACs (aminorex, chloramphenicol, 3-N-dechloroethylifosfamide, flurbiprofen, 2-hydroxyibuprofen, ifosfamide, imazalil, naproxen, ofloxacin, omeprazole, praziquantel and tetramisole) and partial resolution of 2 enantiomers PACs (ibuprofen and indoprofen) under fast-gradient conditions (<10 min analysis time).

The overall performance of the methods was satisfactory. The applicability of the methods was tested on influent and effluent wastewater samples. To the best of our knowledge, this is the first feasibility study on the simultaneous separation of chemically diverse chiral PACs in environmental matrices using ultrahigh performance supercritical fluid based chromatography coupled with tandem mass spectrometry.

Keywords: supercritical fluid based chromatography, polysaccharide-based chiral stationary phases, enantiomer resolution, wastewater.

1. Introduction

Enantiomers of chiral pharmacologically active compounds (cPACs) have become an important group of emerging micropollutants in modern environmental research. They often exhibit different pharmacokinetics and pharmacodynamics that can result in enantiomer dependent toxicity, due to the inherent stereospecificity of biological processes. Despite enantiomers of the same PAC exhibiting different levels of biological activity, it is worrying that most of them are still commercialized as “racemates” or “racemic mixtures”. The relative proportion of a pair of enantiomers is commonly expressed in terms of the enantiomeric fraction (EF) (0.5 for racemic mixtures) and provides an insight of the compound’s history, as well as pointing to the nature and sources of environmental pollution [1,2].

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Unfortunately there is limited understanding of environmental fate and effects of cPACs due to the lack of robust analytical methods allowing for simultaneous chiral recognition of cPACs. To date, enantioselective separations of cPACs remain a significant challenge, especially for environmental samples. High performance liquid chromatography (HPLC) has been the preferred choice for chiral separations using chiral stationary phases (CSP), over gas chromatography (GC), capillary electrophoresis (CE) or supercritical fluid chromatography (SFC) for environmental samples [1–11]. Nevertheless, nowadays the latter has attracted growing attention.

Enantioselective SFC provides fast and high chromatographic efficiency with significantly lower solvent consumption than conventional HPLC. SFC makes use of supercritical or subcritical CO₂-based mobile phases modified with small proportions (1–50 %, v/v) of a co-solvent that improves the elution strength of the mobile phase for cPACs. The use of CO₂, which is a nontoxic and nonreactive solvent with low viscosity, allows reduced analysis times and lower pressures without posing a great impact in the efficiency of the CSP performance. The recent introduction of 2 μm-particle size CSPs and the instrumental improvements have led to the emergence of ultrahigh performance supercritical fluid based chromatography (UHPSFC) that merges the advantages of SFC and ultrahigh performance liquid chromatography technology, scarcely investigated for chiral analysis [11–13]. Furthermore, the addition of tandem mass spectrometry (MS/MS) detectors offers high sensitivity and selectivity, strongly needed for environmental analysis. Traditionally though, methods capable of enantiomeric separations have used ultraviolet detectors with non-volatile buffers or normal phase solvents incompatible with MS detectors. However, this trend is changing and more analytical methods using compatible-MS mobile phases are emerging [5,10,14,15].

The aim of this study was to develop a fast, selective and sensitive enantioselective method for the simultaneous analysis of 23 veterinary and human cPACs, belonging to 7 therapeutic groups (anthelmintic, antibacterial, antifungal, antihistamine, anti-inflammatory, cytostatic, and gastrointestinal drugs), in environmental samples (influent and effluent wastewater samples) using the novel approach of UHPSFC-MS/MS. In addition, the enantioselectivity of two modified polysaccharide-based CSPs with 2.5 μm particle size, an amylose tris-3,5-dimethylphenylcarbamate (AMY) and a cellulose tris-(3-chloro-4-methylphenylcarbamate (CEL) column, was compared. The influence of mobile phase composition on the resolution of the enantiomers was also studied.

2. Experimental

2.1 Chemicals and reagents

Table 1 shows the structures of cPACs. A set of 23 structurally different cPACs, covering acidic and basic compounds, was used as a model for the verification of chiral recognition with two modified polysaccharide-based CSPs. Selection of the cPACs was based on their high human and veterinary use worldwide and frequency of detection in the aquatic environment, possibility of chiral inversion during biological processes and commercial availability as racemic mixtures. Moreover, some chiral metabolites have also been selected. Please note that S-(+)-O-desmethylnaproxen is sold in its enantiomerically pure form.

HPLC-grade acetonitrile (≥99.9%), methanol (≥99.9%) and isopropanol (≥99.9%), ammonium acetate (≥99.0%), ammonium hydroxide solution (28–30% NH₃ basis), and formic acid (≥96.0%) were supplied by Sigma-Aldrich. R/S(±)-Aminorex, R/S(±)-carboxyibuprofen (mixture of diastereomers), R/S(±)-carprofen, S-(+)-O-desmethylnaproxen, R/S(±)-fenoprofen calcium salt hydrate, R/S(±)-2-hydroxyibuprofen, R/S(±)-ibuprofen, R/S(±)-ifosfamide, R/S(±)-imazalil (as sulphate), R/S(±)-indoprofen, R/S(±)-ketoprofen, R/S(±)-omeprazole, R/S(±)-ofloxacin, R/S(±)-2-phenylpropionic acid, R/S(±)-praziquantel, R/S(±)-tetramisole hydrochloride, S-(-)-tetramisole hydrochloride (known as levamisole), S-(-)-ofloxacin (known

as levofloxacin) and 1R,2R(-)-chloramphenicol were purchased from Sigma-Aldrich (Gillingham, UK). R/S(±)-3-N-dechloroethylifosfamide, R/S(±)-dihydroketoprofen (mixture of diastereomers), R/S(±)-naproxen and S-(+)-naproxen were obtained from Toronto Research Chemicals Inc. (Ontario, Canada). R/S(±)-Fexofenadine hydrochloride, R/S(±)-cetirizine dihydrochloride and 1S,2S-(+)-chloramphenicol were supplied by LGC Standards (Teddington, UK). R/S(±)-Flurbiprofen and S-(+)-ibuprofen were purchased from Fisher.

Surrogate/internal standards (IS): R/S(±)-ibuprofen-d₃, R/S(±)-ifosfamide-d₄, R/S(±)-ketoprofen-d₃, R/S(±)-naproxen-d₃, R/S(±)-praziquantel-(cyclohexyl-d₁₁) and R/S(±)-tetramisole-d₅ hydrochloride were purchased from LGC Standards (Teddington, UK) and Sigma-Aldrich (Gillingham, UK).

All standards were of high purity grade. Stock solutions of each compound (1 mg mL⁻¹) were prepared in methanol and stored at -16°C. Working solutions were prepared by diluting stock solution in methanol and stored at -16°C. All glassware was deactivated with dimethylchlorosilane (5% DMDCS in toluene, Sigma-Aldrich) [16]. Oasis HLB (6cc, 200 mg, 30 μm) and MAX (6cc, 200 mg, 30 μm) cartridges were bought from Waters (Milford, MA, USA).

2.2 Sample collection, preparation and solid-phase extraction

24-h composite influent and effluent wastewater samples were collected from a wastewater treatment plant in Western Europe in June 2015. In addition, 24-h composite influent and effluent wastewater samples from a wastewater treatment in Northern Europe were collected during 5 consecutive days in August 2015. All samples were collected in high density polyethylene sample bottles (Fisher, UK). Samples were frozen (-20°C) immediately after collection and stored until analysis.

2.3 Solid-phase extraction

Solid-phase extraction (SPE) was used according to previously published protocols [10]. Some variations to the method were introduced. Briefly, Oasis HLB-MAX cartridges were set up in tandem and conditioned with 4 mL of methanol and equilibrated with 2 mL of deionized water at pH 7.5. Samples (50 mL of influent and effluent wastewater were spiked with a mixed solution of deuterated ISs (1 μg L⁻¹). Samples were filtered and percolated through the cartridges at a flow rate of 6 mL min⁻¹. SPE cartridges were washed individually: Oasis HLB with 2 mL water and Oasis MAX with 2 mL of 5% ammonium hydroxide; dried and connected in series afterwards. Analytes were eluted with four aliquots of 1 mL methanol at a flow rate of 1 mL min⁻¹, and additionally 2 aliquots of 1 mL methanol (2% formic acid) were passed through Oasis MAX cartridges. Eluates were combined, evaporated to dryness (40°C) under a gentle stream of nitrogen, reconstituted with 0.5 mL of methanol and centrifugated at 21100 x g for 10 min. A 5 μL of the sample was injected into the UHPSFC instrument. A flow chart of the SPE method can be found in Fig. 1.

2.4 UHPSFC-MS/MS

An UHPSFC instrument from Waters (Milford, MA, USA) equipped with an ACQUITY UPC² column manager, and ACQUITY UPC² convergence manager (for automatic back pressure regulation), an ACQUITY UPC² sample manager, an ACQUITY UPC² binary solvent pump, and a Waters 515 compensation pump for post-column addition of a make-up flow, was used for this study.

Separation was carried out using a modified polysaccharide amylose tris-3,5-dimethylphenylcarbamate column (AMY, ACQUITY UPC² Trefoil) and a cellulose tris-(3-chloro-4-methylphenylcarbamate column (CEL, ACQUITY UPC² Trefoil), both with the same dimensions (150 x 2.1 mm, i.d. 2.5 μm) (Waters, UK) (Fig. S1 in Supplementary Material).

Column temperature was held at 30°C and the flow-rate of the mobile phase was 1.5 mL min⁻¹. The active back pressure regulator was set at 1800 psi. The post-column flow was mixed with the make-up flow, containing methanol and 0.1% formic acid in positive ionization mode and methanol with 0.2% ammonium hydroxide in negative ionization mode, pumped at 0.3 mL min⁻¹. Sample manager was kept at 15°C. The screening step in positive and negative ionization mode was performed in a gradient elution programme in the following conditions: 0 min – 95 % CO₂, 1 min – 95 % CO₂, 6 min – 40 % CO₂, 8.5 min – 40% CO₂, 8.7 min – 95 % CO₂, 10 min – 95% CO₂. For AMY and CEL CSPs the optimized chromatographic conditions are as follow: separation was carried out using solvent A (CO₂) and solvent B (methanol/acetonitrile/isopropanol, 1:1:1, v/v with 10mM ammonium acetate and 0.1% ammonium hydroxide) under a gradient program (0 min – 85 % A, 1 min – 85 % A, 5 min – 40 % A, 7.5 min – 40% A, 7.8 min – 85 % A, 9 min – 85% A) in positive ionization mode. For cPACs detected in negative ionization mode the final optimized chromatographic conditions using AMY are as follow: gradient elution programme using solvent A (CO₂) and solvent B (methanol with 0.1% ammonium hydroxide) (0 min – 95% A, 3.5 min – 95% A, 10 min – 40% A, 13.5 min – 40% A, 13.8 min – 95% A, 16 min – 95% A). A flow chart is shown in Fig. 1. A volume of 5 µL was injected into the system.

MS analyses were performed with a triple quadrupole mass spectrometer (Quattro Premier XE Mass Spectrometer, Waters Corp, Milford USA), equipped with an electrospray ionization source (ESI). Analyses were done in positive and negative modes. Ionization of analytes was carried out using the following settings: MS capillary voltage = 3 kV (ESI+) and 1.7 kV (ESI-), desolvation temperature = 400°C, cone gas flow = 100 L h⁻¹ and desolvation gas flow = 550 L h⁻¹. Nitrogen was used as a nebulising and desolvation gas and argon was used as the collision gas. MassLynx 4.1 (Waters, UK) software was used to collect and analyse the obtained data. Mass spectrometry analyses were performed in multiple reaction monitoring (MRM) mode. For each compound, two MRM transitions were monitored, except for O-desmethylnaproxen, dihydroketoprofen, flurbiprofen, 2-hydroxyibuprofen, ibuprofen, ibuprofen-d₃, naproxen-d₃, and 2-phenylpropionic acid for which only one MRM transition was chosen due to poor fragmentation. Details of the optimal TQD-MS conditions for each compound are summarized in Supplementary Material (Table S1).

2.5 Method validation

The performance of the analytical method was assessed with respect to linearity and range, precision, accuracy and sensitivity.

To assess linearity and range of the analytical method, calibration curves of the target compounds were constructed at different concentrations, in the range of 0.005 to 1000 µg L⁻¹ for positive ionization mode and up to 8000 µg L⁻¹ for negative ionization mode (after extraction) for each racemic mixture, injected in triplicate and prepared in stock standard solvent. The IS method was used for quantification.

Precision was evaluated as the relative standard deviation (RSD) of replicate measurements. Instrumental intra- and inter- day precisions were determined through triplicate injections of standard solutions covering five concentrations: 0.5, and covering four concentrations: 2.5, 25, 50 and 250 µg L⁻¹ for each enantiomer with AMY CSP, on the same day for intra-day precision and on different days for inter-day precision. Method intra-day precision was estimated by recovery experiments in triplicate in influent and effluent wastewater fortified at three concentration levels (0.125, 2.5 and 5 µg L⁻¹ in wastewaters for each enantiomer).

Method accuracy (expressed as recovery percentage) was estimated from recovery experiments at three different concentration levels in spiked influent and effluent wastewater. Absolute recoveries were calculated as the ratio of the peak areas of spiked samples before extraction (the peak area of analyte in unspiked sample extract was subtracted) to those of a standard

solution at the same concentrations level after extraction and concentration. Relative recoveries were calculated by comparing cPACs concentrations of spiked samples before extraction (the concentration in unspiked samples was subtracted) with cPACs concentrations in standard solutions prepared at the initial concentration level.

Matrix effects were evaluated by the comparison of the peak areas of spiked wastewater extracts ($A_{\text{spiked extract}}$), to which the peak areas corresponding to the native analytes present in the sample were subtracted (A_{unspiked}), with the peak areas in the mobile phase spiked with target analytes ($A_{\text{mobile phase}}$) at the same concentration level.

$$\text{Matrix effect (\%)} = 100 - \frac{(A_{\text{spiked extract}} - A_{\text{unspiked}}) \times 100}{A_{\text{mobile phase}}}$$

Quantification was performed by the internal standard approach. Selection of ISs for those compounds for which deuterated or C13 analogues were not available commercially or in our laboratory was based on structural similarity, degree and type of matrix effects and elution time to account for possible signal suppression/enhancement, in those cases their analysis can only be considered semi-quantitative.

Instrumental detection limits (IDL) and instrumental quantification limits (IQL) were calculated using signal-to-noise approach in standards prepared in mobile phase as the concentrations of each compound corresponding to signal-to-noise ratio of 3:1 and 10:1, respectively. Method detection limits (MDL) and method quantification limits (MQL) for wastewater were calculated as previously described in [10]. MDL and MQL were estimated from IDL and IQL, respectively, after taking into account recovery factor of each compound in each type of matrix and the concentration factor.

2.6 Evaluation of the chromatographic system

Chromatographic parameters, retention factor (k'), selectivity factor (α) and resolution (R_S) were calculated to evaluate the retention, enantioselectivity and separation power, respectively (Supplementary Material Tables S2-S3):

The enantiomeric fraction (EF) was calculated with both absolute and relative (normalised with IS) peak areas as follows:

$$EF = \frac{E1}{E1 + E2} \text{ or } \frac{(+)}{(+)+(-)}$$

where E1 and E2 are the peak areas (or concentrations) of the first and the second eluting enantiomer, respectively and (+) and (-) enantiomers if the elution order is known. EF equals 1 or 0 in the case of single enantiomer form and 0.5 in the case of racemate.

3. Results and discussion

3.1 Screening of factors influencing enantioseparations in UHPSFC-MS/MS

The nature of the CSP and the composition of the mobile phase are the factors that have the highest impact on chiral recognition. The screening strategy consisted on diverse experimental trials to achieve the best conditions to resolve the highest number of cPACs simultaneously.

3.1.1. Nature of CSP

Two modified coated polysaccharide-based CSPs with an amylose and cellulose base were thoroughly studied in terms of efficiency, retention and resolution of enantiomers.

The mechanism of chiral discrimination of the polysaccharide CS involves a combination of attractive forces, such as hydrogen bonding between the ester and carbamate moieties of the CS and the corresponding functional groups of the enantiomers. Moreover, hydrophobic, dipole-dipole and π - π interactions and the geometric configuration of the CSP and the cPACs play an important role in chiral recognition [17].

CEL CSP exhibited, under tested conditions, optimal selectivity for the following pairs of enantiomers: aminorex, chloramphenicol (1R,2R and 1S,2S), 3-N-dechloroethylifosfamide, omeprazole, praziquantel and tetramisole (Fig. S2). In the case of ifosfamide, imazalil and ofloxacin their chiral resolution was dependent on the mobile phase composition (section 3.1.2). None of the anti-inflammatory drugs were resolved using this CSP. What this group of cPACs have in common with the rest of non-resolved compounds (cetirizine and fexofenadine) is the presence of a carboxylic group in their structure (Table 1) (except ofloxacin). As stated in previous studies, the substituents on the phenyl moiety of the cellulose phenylcarbamate derivatives greatly affect chiral discrimination [17]. The presence of an electron withdrawing substituents (chloride group in position 3) affects the electron density of the CS, also increasing the acidity of the NH proton of the carbamate group [18].

In contrast to CEL CSP, AMY CSP exhibited an optimal selectivity for the two pairs of enantiomers of ifosfamide and its metabolite, while the resolution of the rest of cPACs (aminorex, carprofen, chloramphenicol, fenoprofen, flurbiprofen, 2-hydroxyibuprofen, ibuprofen, naproxen, omeprazole, praziquantel and tetramisole) were dependent on the mobile phase composition (section 3.1.2) (Fig. S3).

As reported previously, amylose benzoates showed lower enantiomer recognition ability than cellulose benzoates, due to the lower conformational stability of the amylose derivatives [18]. Moreover, the presence of an electron-withdrawing group and an electron-donating group attached to the phenylcarbamate group in CEL CSP, in contrast to two electron-donating groups (alkyl groups in position 3 and 5) in AMY CSP would increase the enantiomer resolving ability of the former CSP. However, in this study it was observed that by further optimization of the mobile phase composition the success rate was higher with the AMY CSP for the target cPACs.

3.1.2. Mobile phase optimization

A mobile phase composed of CO₂ provides normal-phase-like selectivity, so when polar compounds, such as cPACs are involved, it is necessary to add co-solvents and additives to increase the polarity of the mobile phase in order to elute all analytes and reduce analysis time. The content and nature of the organic modifier (co-solvent) can critically modulate chiral recognition as well as retention. According to literature [19] the presence of alcohols in the mobile phase yielded better resolutions with polysaccharide-based CSPs. In this study enantioseparations under CO₂-based mobile phases modified with methanol, 2-propanol (both hydrogen bond donor/acceptor solvents) and acetonitrile (hydrogen bond acceptor/dipole solvent) and their mixtures were studied. As the concentration of the co-solvent is increased the polarity of the mobile phase increases resulting in reduced retention times. Co-solvent mixtures containing higher percentages of methanol resulted in lower retention times than when isopropanol or acetonitrile were the main components.

For CEL CSP, the co-solvent mixtures gave the same success rate as when only single co-solvents were used (Fig. S2). Regarding AMY CSP, resolution of most of the target analytes needed an optimization of the co-solvent composition. Fig. S3 shows that using ternary mixtures the number of successful enantioseparations increased in comparison to single co-solvents alone. For example, a fraction of acetonitrile in the mobile phase was essential for the successful enantioseparation of omeprazole. Most of the cPACs eluted in 3.5 and 5 minutes, when the co-solvent content represented the 35-60% of the mobile phase.

As in other chromatographic techniques, small quantities of additives can be used to improve peak shapes and/or resolution of the separation. They can modify the stationary phase surface or act as ion pairs changing selectivity of the target compounds. By combining different additives and their ratio, enantioseparations can be optimized, although not always in a predictable way [20]. Usually, for basic racemates a basic additive, such as diethylamine,

ammonia or ammonium hydroxide may be necessary to mask acidic sites on the CSP surface and to suppress the ionization of the basic analytes, thus increasing their interaction with hydrophobic moieties part of the CSP [20]. In the case of acidic racemates an acidic additive (acetic, formic or trifluoroacetic acid) is recommended. In this study, additives must be compatible with MS detectors; therefore the addition of a salt (ammonium acetate), an acid (formic acid), a base (ammonium hydroxide) and their combination was investigated. No significant effects on t_R and R_s were observed when the ammonium acetate content was increased from 10 mM to 20 mM (Fig. S3). However, the presence of ammonium acetate in the mobile phase was decisive for the chiral recognition of tetramisole in AMY CSP.

Due to the “acidic” nature of CO₂, it was expected that the use of formic acid would not favourably alter enantiomer selectivity compared with the addition of ammonium hydroxide. When additives were present in the mobile phase R_s of chloramphenicol, 3-N-dechloroethylfosfamide, flurbiprofen, 2-hydroxyibuprofen, ifosfamide, omeprazole, praziquantel and tetramisole increased (Fig. S3). However the addition of formic acid caused the loss of R_s of aminorex unlike when ammonium hydroxide was used. After the benefits of adding ammonium hydroxide to the mobile phase were realized, an increase of its concentration (from 0.1% to 0.25%) was investigated. However, an increase of base concentration negatively altered the enantiomer selectivity (Fig. S3) and led to lower signal-to-noise ratios.

In summary, a mobile phase consisting of CO₂ as primary eluent and a ternary co-solvent mixture formed by methanol/isopropanol/acetonitrile (1:1:1, v/v/v) with 10 mM ammonium acetate and 0.1% ammonium hydroxide yielded the best simultaneous resolution for the highest number of compounds on AMY and CEL CSP in the positive ionization mode (Fig. 2A and 2B). The selection of the same chromatographic conditions for both CSPs would allow running them in parallel, simplifying the process, reducing running times and as a consequence increasing the economic benefits. The same screening approach was performed for each CSP also in the negative mode. Unlike AMY CSP, CEL CSP did not show any enantiomer selectivity for cPACs monitored in negative mode and therefore no further method development was undertaken for this CSP. On the other hand, AMY CSP and a mobile phase composed by CO₂ as primary eluent and methanol modified with 0.2% ammonium hydroxide, combined with a softer gradient (section 2.4), resulted in excellent chiral recognition for: carprofen ($R_s=2.3$), chloramphenicol ($R_s=1.2$), flurbiprofen ($R_s=2.3$), 2-hydroxyibuprofen ($R_s=1.8$), ibuprofen ($R_s=0.7$) and naproxen ($R_s=1.5$) (Fig. 2C). The verification of the elution order of studied cPACs, under the final conditions, was possible only in the case of chloramphenicol, ibuprofen, naproxen, ofloxacin and tetramisole as standards of single enantiomers were commercially available for only these compounds.

For the following cPACs: carboxyibuprofen, cetirizine, dihydroketoprofen, fexofenadine, indoprofen, ketoprofen, mandelic acid and 2-phenylpropionic acid no enantiomer selectivity was observed under any of the chromatographic conditions studied with both CSPs in both ionization modes and therefore AMY and CEL CSPs are not considered appropriate for those cPACs.

3.3 Method validation

The analytical performance of the proposed method was evaluated in terms of linearity, limits of detection and quantification, precision and accuracy. Method validation data for cPACs is presented in Tables 2-3 and Tables S4-S9 (Supplementary Material).

Linearity was studied at 15 different concentration levels (ranging from 0.005 to 1000 µg L⁻¹ for positive ionization mode and up to 8000 µg L⁻¹ for negative ionization mode, for each racemic mixture). Good linearity of the analytical response was observed with global mean correlation coefficients equal or higher than 0.995 (Tables S4 and S5).

Precision was within the acceptable limits ($\leq 20\%$). Both instrumental intra-day and inter-day precision (assessed over three days across the range of concentrations) were in general lower than 10% and 15%, respectively (Tables S4 and S5). The overall method intra-day precision, estimated from recovery experiments in influent and effluent wastewater at three concentration levels was on average below 15% (Supplementary Material Tables S6-S9).

Method accuracy (expressed as recovery percentage) was evaluated by recovery experiments of target compounds in influent and effluent wastewater samples, spiked in triplicate at three fortification levels (0.125, 2.5 and 5 $\mu\text{g L}^{-1}$ for each enantiomer) (Supplementary Material Tables S6-S9). In general average recoveries were satisfactory at three fortification levels which were typically $>70\%$ (Fig. 3 and 4) for both CSPs. Low recoveries were obtained for 3-N-dechloroethylifosfamide, ofloxacin and omeprazole. As it has been stated in previous studies [16,21–23] retention of PACs in SPE sorbents is strongly affected by the pH of the eluent, therefore the chosen conditions may not be the optimal to recover these cPACs. In fact, this is one of the limitations of multi-residue analytical methods, where a compromise on the final analytical conditions has to be reached to achieve the best conditions for the highest number of cPACs. As can be seen in Tables S6-S9, recoveries at low, medium or high spike levels were not provided for some cPACs in the non-spiked influent (cetirizine and fexofenadine) and effluent wastewater samples (cetirizine, fexofenadine, 2-hydroxyibuprofen, ketoprofen and naproxen) because they were present at high concentration levels in the non-spiked wastewater samples or because the spike concentration level was close or lower than their IDL.

The presence of interfering substances can lead to inaccurate results due to matrix effects. Several strategies to reduce this detrimental effects have been reported in LC-MS/MS (e.g. purification of the sample prior to analysis and/or the use of suitable calibration approaches [24,25], but just a few studies concerning determination of matrix effects in SFC-MS/MS have been reported so far [26]. Matrix effects were evaluated by comparison of the signal of each compound spiked in matrix after SPE with the signal of the compound in mobile phase. Results are displayed in Fig. 5. Signal suppression was observed for most of the enantiomers. Average matrix effects ranged from -89 % (2-hydroxyibuprofen E2) to 28 % (carprofen E1) in influent wastewater and from -48 % (2-hydroxyibuprofen E1) to 14 % (flurbiprofen E1) in effluent wastewater with the AMY CSP. Regarding CEL CSP, matrix effects ranged from -89% (omeprazole E1) to 96% (R-(-)-naproxen) in influent wastewater and from -40% (praziquantel E1) to 4% (N-dechloroethylifosfamide E1) in effluent wastewater. As expected matrix effects were more pronounced with an increase of the complexity of the matrix. Differences in matrix effects between enantiomers of the same compound were also observed, especially when AMY CSP was involved. The results showed that the signal of the second eluted enantiomer was more suppressed than the first eluted enantiomer (e.g.: aminorex, carprofen, 3-N-dechloroethylifosfamide and fenoprofen) in AMY CSP, independently of the matrix. Whereas, the same trend was also observed for 3-N-dechloroethylifosfamide in CEL CSP, the pair of enantiomers of indoprofen showed the reversed pattern, with the first eluted enantiomer showing higher suppression than the second one.

The instrumental limits of detection and quantification were estimated using the signal-to-noise ratio approach. The results are summarized in Table 2 and 3 for all the compounds studied. The results indicated a wide variety of MDLs. Compounds ionized in negative mode showing the highest MDLs. No significant differences were found between the results obtained for influent and effluent wastewater samples. The most sensitive cPACs included: aminorex, 3-N-dechloroethylifosfamide, fexofenadine (with CEL CSP), ifosfamide, imazalil, indoprofen, praziquantel and tetramisole with MDLs of less than 10 ng L^{-1} with both CSPs. This group of cPACs contains amino groups (in the rings) and also chlorine, sulphur or fluorine atoms in their chemical structure. For the rest of cPACs their MDLs depended on the CSP used. Regarding CEL CSP, ketoprofen, ofloxacin and omeprazole had a MDL $< 100 \text{ ng L}^{-1}$ and naproxen < 300

ng L⁻¹. In the case of AMY CSP: fenoprofen, flurbiprofen, ketoprofen and ofloxacin were in the range between 150 and 500 ng L⁻¹ and other cPACs had MDLs > 500 ng L⁻¹. The majority of compounds with high MDLs corresponded to the anti-inflammatory group which were monitored in negative mode and had hydroxyl or carboxylic groups present in their chemical structure.

3.4 Application to environmental samples

To ascertain its applicability, the validated UHPSFC-MS/MS method was applied to wastewater samples. Ten 24-h composite influent and effluent wastewater samples, collected during 5 consecutive days from a wastewater treatment plant situated in Northern Europe and two additional 24-h composite influent and effluent wastewater samples from Central Europe were analyzed.

The results indicated low concentrations of targeted analytes in samples from Northern Europe, however two compounds were quantified (fexofenadine and ketoprofen). Fexofenadine was present at quantitative concentration levels in all samples. Concentrations of fexofenadine ranged from 0.66 to 0.95 µg L⁻¹ and from 0.60 to 0.77 µg L⁻¹ in influent and effluent wastewater samples, respectively. Ketoprofen was detected in all influent wastewater samples (<MQL-1.09 µg L⁻¹) but only in two effluent wastewater samples (<MDL-0.68 µg L⁻¹). Whereas the mean removal rate of fexofenadine was lower than 10%, the mean removal rate of ketoprofen was >80%.

In contrast to samples from Northern Europe, wastewater samples from Western Europe contained much higher concentrations of cPACs and frequency of detection (Table 4).

Out of the 22 target compounds monitored, 10 were detected in influent wastewater samples (aminorex, carboxyibuprofen, dihydroketoprofen, fexofenadine, 2-hydroxyibuprofen, ibuprofen, imazalil, naproxen, ofloxacin and tetramisole) and 3 in effluent wastewater samples (fexofenadine, naproxen and tetramisole).

The highest concentration levels of cPACs in influent wastewater samples corresponded to the anti-inflammatory cPACs and their metabolites. Only the S-enantiomer of ibuprofen was present with a concentration level that reached up to 5.24 µg L⁻¹. Although ibuprofen is marketed as a racemic mixture, an excess of S-(+)-ibuprofen is excreted in the urine, due to an extensive chiral inversion of the inactive R-(-)-ibuprofen during metabolism. S-(+)-ibuprofen was found to be predominant in both influent and effluent wastewater, which is in accordance with published results [27–29]. Ibuprofen metabolites were detected at higher concentration levels than the parent compound. R/S(±)-carboxyibuprofen and 2-hydroxyibuprofen E2 reached 11.1 and 13.3 µg L⁻¹, respectively, whereas the concentration of the first eluted enantiomer of 2-hydroxyibuprofen reached 2.81 µg L⁻¹ in influent wastewaters. The EF of 2-hydroxyibuprofen was therefore 0.2. These concentration levels were higher than those reported previously [10]. Unlike ibuprofen, naproxen is marketed as the enantiomerically pure S-enantiomer, due to the well-known negative effects of R-naproxen. S-(+)-naproxen was detected in influent and effluent wastewater at concentration levels of 4.75 and 0.95 µg L⁻¹, respectively, what represented a removal rate up to 83 %. The EF of naproxen (1.0) did not change during wastewater treatment. Although the concentration of R/S(±)-ketoprofen was <MQL, its main metabolite was present in influent wastewater at 0.42 µg L⁻¹.

Tetramisole enantiomers were detected at 0.05 (R-enantiomer) and 0.03 (S-enantiomer) µg L⁻¹ in influent wastewater and at 0.09 (R-enantiomer) and 0.05 (S-enantiomer) µg L⁻¹ in effluent wastewater. The EF remained unaltered during wastewater treatment, with an excess of the R-enantiomer. Information regarding concentration levels of this veterinary cPAC in the environment remains scarce. Concentration levels of tetramisole enantiomers are similar or higher than those reported previously in effluent wastewaters from UK [10]. Due to the anthelmintic activity of tetramisole residing in its S-enantiomer (levamisole), this cPAC is

marketed as enantiomerically pure S-enantiomer. Therefore the presence of both enantiomers in influent wastewater suggests that chiral inversion took place during human and animal metabolism. It is also possible that the racemic mixture of tetramisole was used as an adulterant in illicit cocaine samples [30,31]. The metabolites of tetramisole were also quantified. Aminorex E1 was present at $0.02 \mu\text{g L}^{-1}$ and E2 was quantified at $0.01 \mu\text{g L}^{-1}$ in influent wastewater.

R/S(\pm)-fexofenadine, imazalil E2 and S(-)-ofloxacin also detected in wastewater samples. R/S(\pm)-fexofenadine was detected in both matrices at concentration levels similar to those reported for the samples from Northern Europe. In addition, no removal of this compound was observed, which indicates that fexofenadine is recalcitrant. In the case of the antifungal cPAC imazalil, there was an excess of the second eluted enantiomer in influent wastewater samples, with total concentration of $0.028 \mu\text{g L}^{-1}$, whereas its concentration in effluent wastewater was below MDL. Imazalil is employed as a racemic mixture and the general population can be exposed to it through contaminated food. A previous study has shown that the binding of imazalil to human proteins, specifically human serum albumin, is enantioselective [32], what could suggest that imazalil is excreted with an excess of one enantiomer or that chiral inversion took place, but this effect has been scarcely studied so there is not enough information available regarding this effect. Moreover, recent published data showed that the S(-)-enantiomer degrades quicker than the R(+)-enantiomer [33,34], what could explain the presence of only one enantiomer in influent wastewater samples.

Finally, S(-)-ofloxacin (levofloxacin) was found to be readily removed during wastewater treatment. It was present in influent wastewater at concentration levels of $0.539 \mu\text{g L}^{-1}$. Ofloxacin is a potent quinolone antibacterial agent marketed as enantiomerically pure S(-)-ofloxacin since 1995, although it can be marketed as the racemic mixture too. It has been reported that the S(-)-enantiomer has antibacterial activity up to 2 orders of magnitude greater than that of the R(+)-enantiomer due to the greater binding potency of the S(-)-enantiomer to the enzyme-DNA complex [35]. S(-)-ofloxacin is excreted from the body unchanged and up to date no chiral inversion has been reported in the environment.

4. Conclusions

Two novel enantioselective analytical methods, based on SPE before analysis by UHPSFC-MS/MS, were developed for the simultaneous analysis of several cPACs in wastewater samples. Baseline resolution (13 cPACs) and partial resolution (2 cPACs) for the pair of enantiomers of 23 cPACs belonging to 7 different therapeutic groups was achieved in less than 10 min.

Enantioseparation was achieved with two modified polysaccharide-based CSPs which exhibited complementary separation properties. The nature and concentration of the co-solvent was the most important parameter to achieve successful enantioseparation of the target compounds. The validation data confirmed satisfactory analytical performance in terms of mean recoveries, linearity, accuracy, precision, sensitivity and selectivity. Although pronounced matrix effects were encountered for some cPACs in these complex environmental samples, the internal standard approach proved to be a practical option. If increased sample throughput and short analyses are required, it is of high significance to use a plausible approach, such as an effective sample preparation, in order to avoid inaccurate quantification in real samples due to matrix effects.

Under the optimized conditions, the developed methods were successfully applied to the analysis of cPACs in real wastewater samples. Out of 22 target compounds monitored, 11 (aminorex, carboxyibuprofen, dihydroketoprofen, fexofenadine, 2-hydroxyibuprofen, ibuprofen, imazalil, ketoprofen, naproxen, ofloxacin and tetramisole) were detected in influent and effluent wastewaters and not always as racemic mixtures. Tracing chemically diverse

cPACs at enantiomeric level in environmental matrices is vital for a proper understanding of the possible environmental risks posed by these compounds. This study demonstrates a suitable alternative and environmentally friendly method to existing complex and time consuming methods, to simultaneously identify and quantify a large variety of cPACs in environmental samples.

Acknowledgments

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Conflict of interest

The authors declare no conflict of interests.

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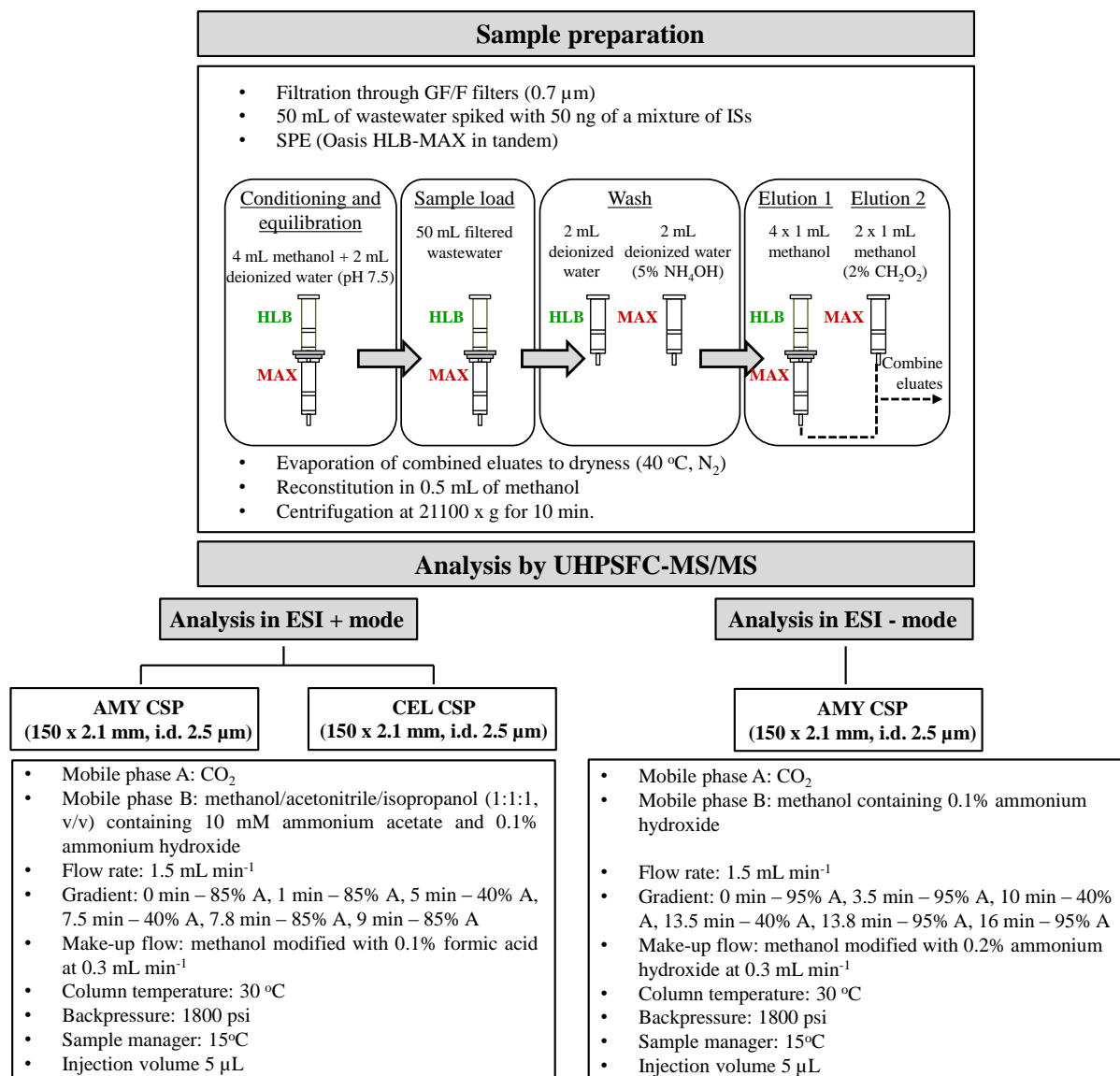


Fig. 1. Overview of analytical protocol used to determine target cPACs in wastewater by UHPSFC-MS/MS

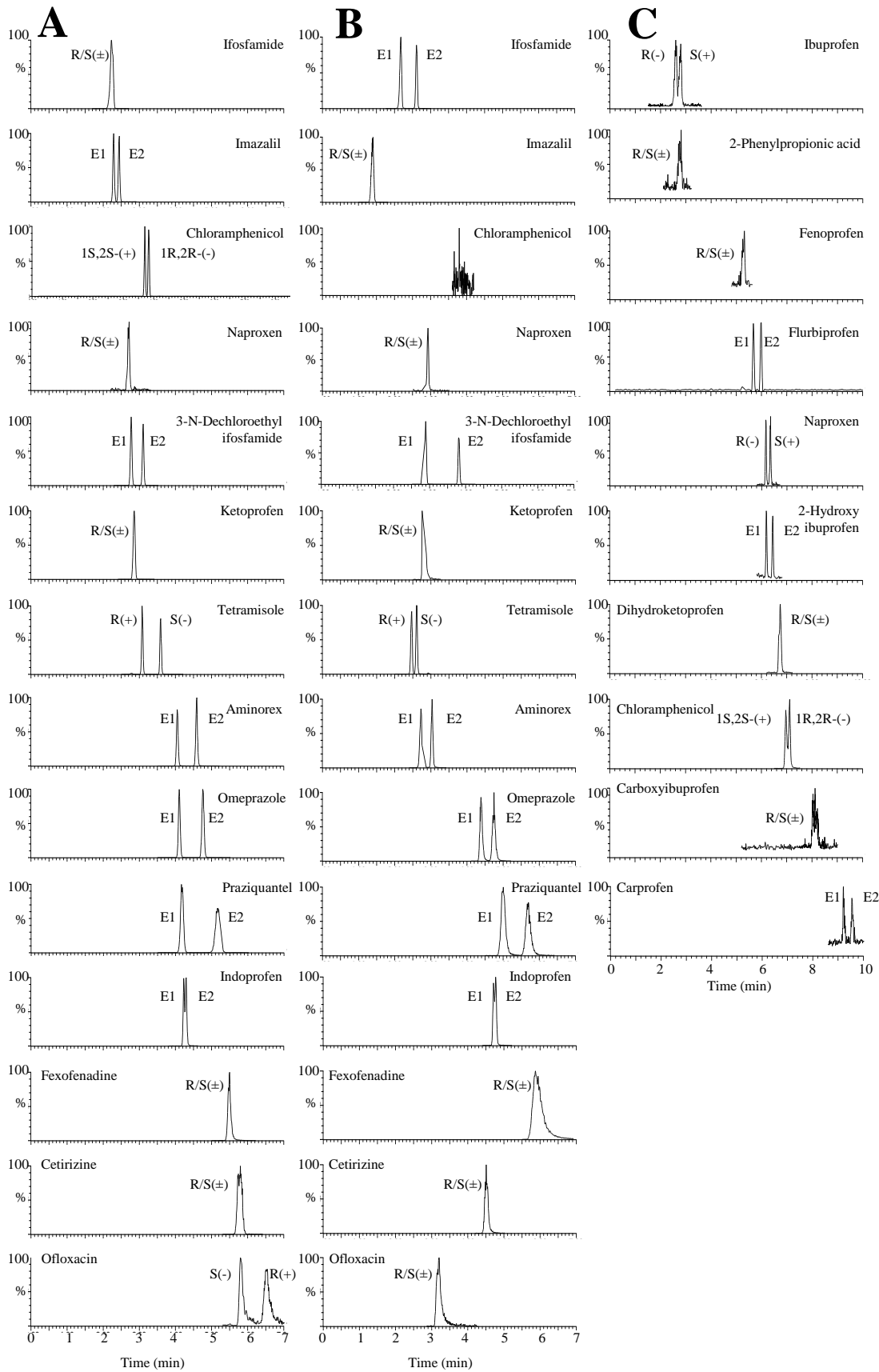


Fig. 2. Chromatograms of cPACs in standard solution (250 ng/mL) in CEL (A) and AMY (B) in positive ionization mode and in AMY (C) in negative ionization mode

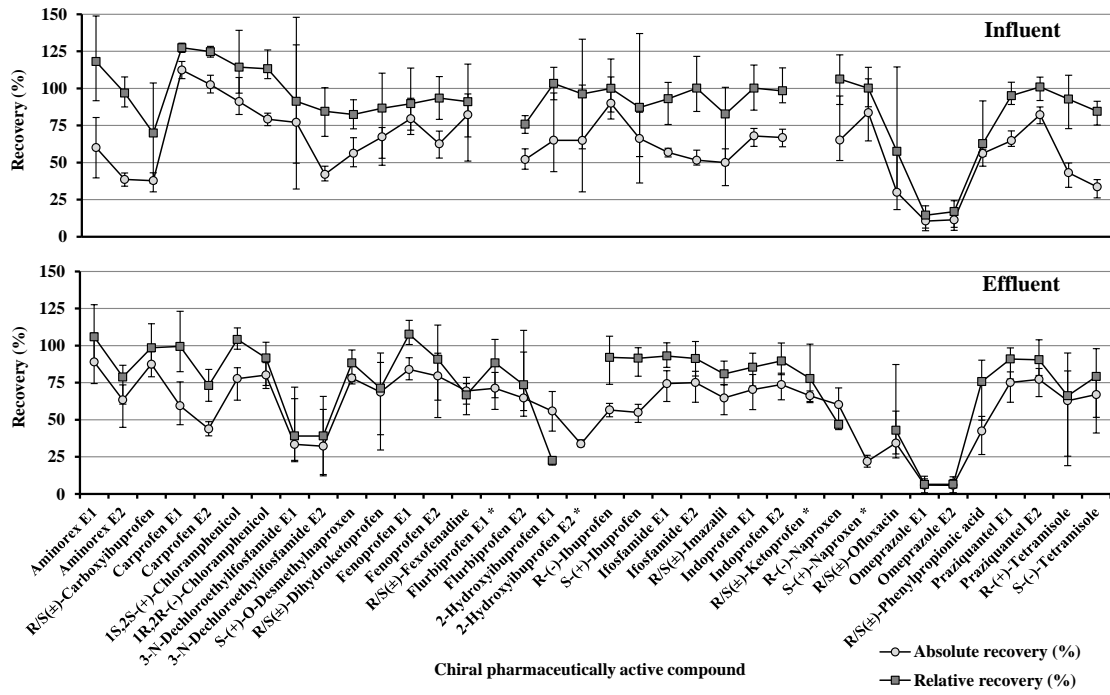


Fig. 3. Mean Absolute and relative recoveries (%) of cPACs in influent and effluent wastewater with AMY CSP. For cPACs marked with * at least one recovery value is not available.

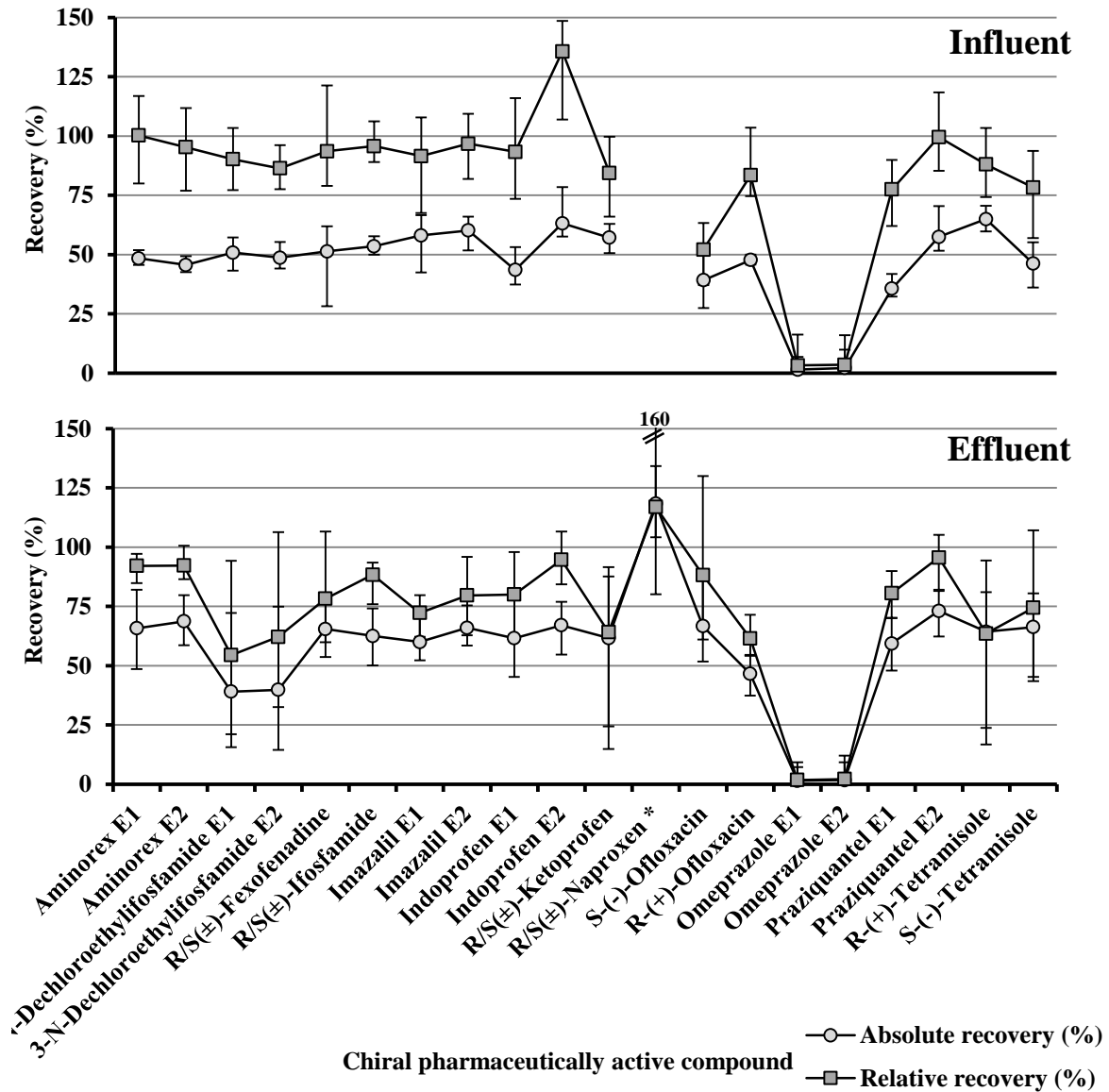
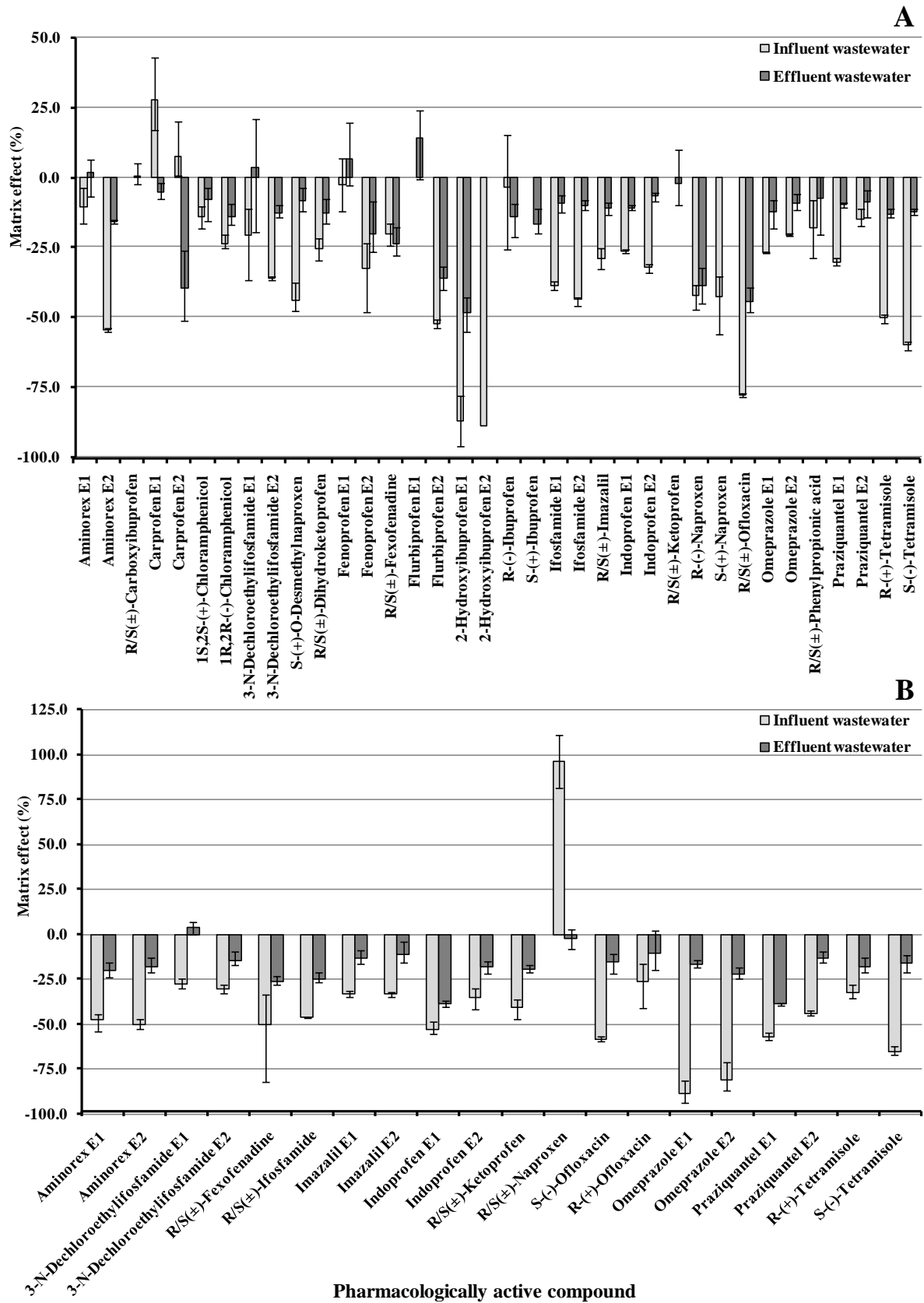
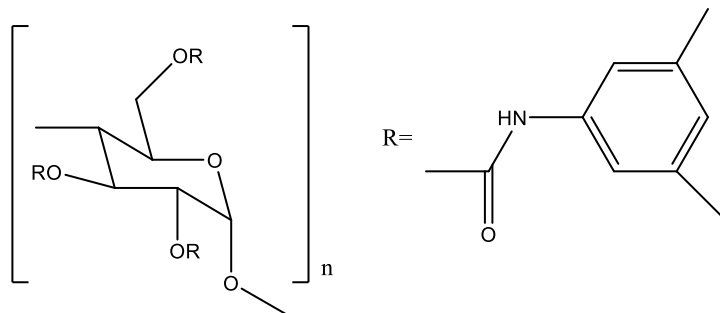
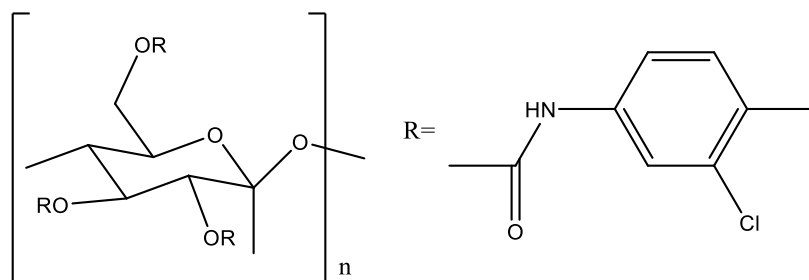


Fig. 4. Mean Absolute and relative recoveries (%) of cPACs in influent and effluent wastewater with CEL CSP. For cPACs marked with * at least one recovery value is not available.





Amylose tris-(3,5-dimethylphenylcarbamate) (AMY)



Cellulose tris-(3-chloro-4-methylphenylcarbamate) (CEL)

Fig. S1. Structure of the AMY and CEL chiral selector

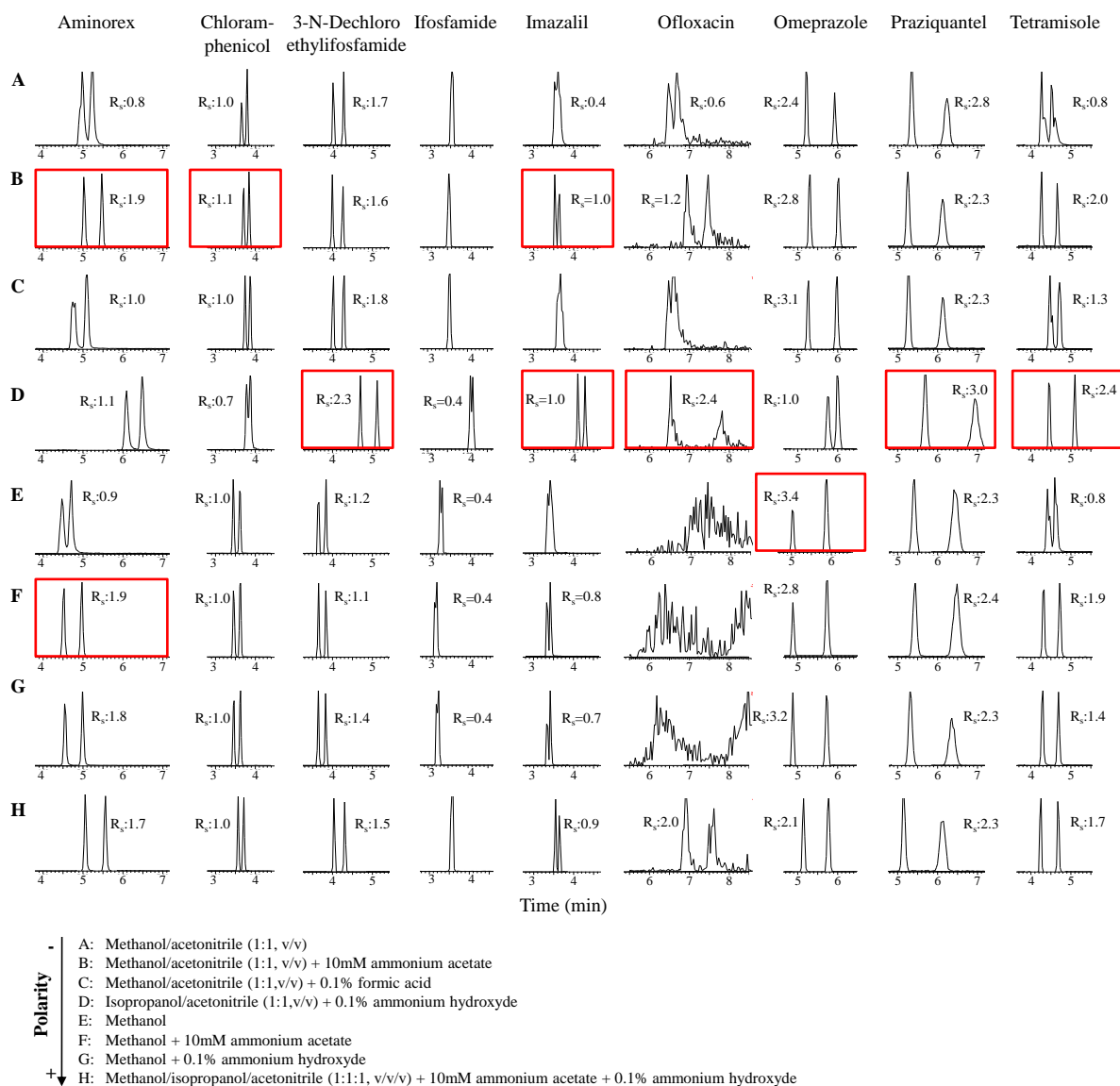


Fig. S2. Effects of mobile phase composition on the enantioseparation of several cPACs in CEL (chromatographic conditions: mobile phase: CO₂ (A')/co-solvent (A-H) (B'); gradient: 0 min – 95 % A', 1 min – 95 % A', 6 min – 40 % A', 8.5 min – 40% A', 8.7 min – 95 % A', 10 min – 95% A'; flow: 1.5 mL/min; ABPR: 1800psi; make-up flow: methanol (0.1% formic acid) for positive ionization and methanol (0.2% ammonium hydroxide) for negative ionization at 0.3 mL min⁻¹)



Fig. S3. Effects of mobile phase composition on the enantioseparation of several cPACs in AMY (chromatographic conditions: mobile phase: CO₂ (A')/co-solvent (A-K) (B'); gradient: 0 min – 95 % A', 1 min – 95 % A', 6 min – 40 % A', 8.5 min – 40% A', 8.7 min – 95 % A', 10 min – 95% A'; flow: 1.5 mL/min; ABPR: 1800psi; make-up flow: methanol (0.1% formic acid) for positive ionization and methanol (0.2% ammonium hydroxyde) for negative ionization at 0.3 mL min⁻¹)

Table S1Optimized MRM transitions for the target cPACs by UHPSFC-MS/MS and pK_a values.

Compound	Precursor ion	Product ion	CE (eV)	CV (V)	Mode	pK _a ^a
R/S(±)-Aminorex (M)	163.0	120.0 ^b 103.0 ^c	15 20	20	ESI+	7.49
R/S(±)-Carboxyibuprofen (mixture of diastereomers) (M)	235.0	191.0 ^b 72.9 ^c	8 8	25	ESI-	4.77
R/S(±)-Carprofen	272.0	228.0 ^b 226.0 ^c	6 8	48	ESI-	4.42
R/S(±)-Cetirizine	389.1	201.0 ^b 166.0 ^c	40 21	32	ESI+	2.12, 3.60, 7.79
1R,2R(-)-Chloramphenicol + 1S,2S(+)-Chloramphenicol	323.0	274.8 ^b 304.8 ^c	10 10	20	ESI+	13.58
	320.8	151.8 ^b 256.0 ^c	15 15	27	ESI-	13.58
R/S(±)-3-N-Dechloroethylfosfamide (M)	198.9	170.9 ^b 77.9 ^c	15 30	30	ESI+	13.04
S-(+)-O-Desmethylnaproxen (M) ^d	215.0	170.5 ^b	10	20	ESI-	4.34, 9.78
R/S(±)-Dihydroketoprofen (M)	255.0	211.0 ^b	8	30	ESI-	4.30, 13.73
R/S(±)-Fenoprofen	241.0	197.0 ^b 93.0 ^c	25 20	50	ESI-	3.96
R/S(±)-Fexofenadine	500.1	456.1 ^b 378.1 ^c	14 19	33	ESI+	4.04, 9.01, 13.20
R/S(±)-Flurbiprofen	243.0	199.0 ^b	10	30	ESI-	4.42
R/S(±)-2-Hydroxyibuprofen (M)	221.0	177.0 ^b	6	30	ESI-	4.63
R/S(±)-Ibuprofen	204.9	161.5 ^b	6	26	ESI-	4.85
R/S(±)-Ifosfamide	261.0	92.0 ^b 154.0 ^c	28 22	40	ESI+	13.94
R/S(±)-Imazalil	297.0	158.9 ^b 201.1 ^c	20 18	40	ESI+	6.77
R/S(±)-Indoprofen	282.1	236.2 ^b 77.0 ^c	20 50	45	ESI+	3.74
R/S(±)-Ketoprofen	255.0	209.2 ^b 105.1 ^c	14 25	35	ESI+	3.88
R/S(±)-Naproxen	230.9	170.3 ^b 153.6 ^c	24 30	28	ESI+	4.19
R/S(±)-Ofloxacin	362.2	261.1 ^b 318.3 ^c	36 32	56	ESI+	5.45
R/S(±)-Omeprazole	346.1	198.1 ^b 151.0 ^c	10 20	20	ESI+	1.59, 4.77, 9.68
R/S(±)-2-Phenylpropionic acid (M)	149.0	105.0 ^b	5	20	ESI-	4.59
R/S(±)-Tetramisole	205.0	183.0 ^b 151.0 ^c	15 25	50	ESI+	6.98
R/S(±)-Praziquantel	313.1	203.0 ^b 83.0 ^c	15 35	40	ESI+	n.a.

CE: Collision energy; CV: Cone voltage; R/S(±): Racemic mixture; M: metabolite; ^aChemaxon predicted values; ^bQuantification; ^cConfirmation; ^dCompound sold as enantiomerically pure; n.a.: not available

Table S2

Chromatographic parameters for each cPAC obtained under different mobile phase composition with AMY CSP

Compound	Mobile phase																							
	A: MeOH/ACN (1:1, v/v) + 10mM NH ₄ OAc				B: MeOH/ACN (1:1, v/v) + 10mM NH ₄ OAc + 0.1% formic acid				C: MeOH				D: MeOH/IPA (1:1, v/v)				E: MeOH/IPA (1:1, v/v) * 0.1% formic acid				F: MeOH/IPA/ACN (1:1:1, v/v/v) + 0.1% formic acid			
	<i>k</i> ₁	<i>k</i> ₂	<i>α</i>	<i>R</i> _S	<i>k</i> ₁	<i>k</i> ₂	<i>α</i>	<i>R</i> _S	<i>k</i> ₁	<i>k</i> ₂	<i>α</i>	<i>R</i> _S	<i>k</i> ₁	<i>k</i> ₂	<i>α</i>	<i>R</i> _S	<i>k</i> ₁	<i>k</i> ₂	<i>α</i>	<i>R</i> _S	<i>k</i> ₁	<i>k</i> ₂	<i>α</i>	<i>R</i> _S
R/S(±)-Aminorex	-	-	-	-	-	-	-	-	0.93	0.94	1.02	1.00	-	-	-	-	-	-	-	-	-	-	-	-
R/S(±)-Carboxyibuprofen	- ^a	- ^a	- ^a	- ^a	-	-	-	-	- ^a	- ^a	- ^a	- ^a	-	-	-	-	-	-	-	-	-	-	-	-
R/S(±)-Carprofen	- ^a	- ^a	- ^a	- ^a	0.95	0.95	1.00	1.20	- ^a	- ^a	- ^a	- ^a	-	-	-	-	0.96	0.96	1.00	0.70	-	-	-	-
R/S(±)-Cetirizine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1S,2S-(+)-Chloramphenicol + 1R,2R-(-)-Chloramphenicol	0.94	0.94	1.00	0.84	0.94	0.94	1.00	0.92	0.94	0.95	1.00	0.89	-	-	-	-	-	-	-	-	0.94	0.94	1.00	0.80
R/S(±)-3-N-Dechloroethyl ifosfamide	0.94	0.95	1.01	6.30	0.94	0.96	1.01	6.55	0.94	0.95	1.01	3.94	0.94	0.95	1.01	3.56	0.94	0.95	1.01	3.85	0.94	0.95	1.01	5.40
S-(+)-O-Desmethylnaproxen	- ^a	- ^a	- ^a	- ^a	- ^a	- ^a	- ^a	- ^a	- ^a	- ^a	- ^a	- ^a	-	-	-	-	-	-	-	-	-	-	-	-
R/S(±)-Dihydroketoprofen	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
R/S(±)-Fenoprofen	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
R/S(±)-Fexofenadine	-	-	-	-	-	-	-	-	-	-	-	-	- ^a	- ^a	- ^a	- ^a	- ^a	- ^a	- ^a	- ^a	-	-	-	-
R/S(±)-Flurbiprofen	0.94	0.94	1.00	0.95	0.93	0.93	1.00	1.20	- ^a	- ^a	- ^a	- ^a	0.94	0.94	1.00	0.88	0.93	0.94	1.00	1.50	0.93	0.93	1.00	1.20
R/S(±)-2-Hydroxyibuprofen	- ^a	- ^a	- ^a	- ^a	0.94	0.94	1.00	1.10	- ^a	- ^a	- ^a	- ^a	0.94	0.94	1.00	0.56	0.94	0.94	1.00	1.00	0.94	0.94	1.00	1.13
R/S(±)-Ibuprofen	-	-	-	-	- ^a	- ^a	- ^a	- ^a	- ^a	- ^a	- ^a	- ^a	-	-	-	-	- ^a	- ^a	- ^a	- ^a	-	-	-	-
R/S(±)-Ifosfamide	0.93	0.94	1.00	1.00	0.93	0.94	1.00	1.60	0.93	0.94	1.00	1.00	0.93	0.94	1.00	1.20	0.93	0.94	1.01	1.55	0.93	0.94	1.00	1.92
R/S(±)-Imazalil	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
R/S(±)-Indoprofen	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
R/S(±)-Ketoprofen	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
R/S(±)-Naproxen	0.94	0.94	1.00	0.75	- ^a	- ^a	- ^a	- ^a	- ^a	- ^a	- ^a	- ^a	-	-	-	-	-	-	-	-	-	-	-	-
R/S(±)-Ofloxacin	- ^a	- ^a	- ^a	- ^a	-	-	-	-	- ^a	- ^a	- ^a	- ^a	- ^a	- ^a	- ^a	- ^a	- ^a	- ^a	- ^a	- ^a	- ^a	- ^a	- ^a	- ^a
R/S(±)-Omeprazole	0.96	0.96	1.00	0.64	0.96	0.96	1.00	0.92	0.96	0.96	1.00	0.82	-	-	-	-	-	-	-	-	0.96	0.96	1.00	0.96
R/S(±)-Phenylpropionic acid	-	-	-	-	- ^a	- ^a	- ^a	- ^a	- ^a	- ^a	- ^a	- ^a	-	-	-	-	- ^a	- ^a	- ^a	- ^a	- ^a	- ^a	- ^a	- ^a
R/S(±)-Praziquantel	0.96	0.97	1.01	3.00	0.96	0.97	1.01	6.00	- ^a	- ^a	- ^a	- ^a	0.96	0.97	1.01	3.00	0.96	0.97	1.01	2.02	0.96	0.96	1.00	1.84
R/S(±)-Tetramisole	-	-	-	-	0.94	0.94	1.00	0.75	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

MeOH: methanol; ACN: acetonitrile; IPA: isopropanol; NH₄OAc: ammonium acetate; -: no resolution; ^ano peak; *k*₁: retention factor of the first eluted peak; *k*₂: retention factor of the second eluted peak; *α*: selectivity factor; *R*_S: resolution

$$k' = (t_R - t_0) / t_0$$

$$\alpha = k'_2 / k'_1$$

$$R_S = 2(t_{R2} - t_{R1}) / (w_1 + w_2)$$

where *t*_R is the retention time of each enantiomer (first or second) and *t*₀ is the retention time of an unretained compound.

where *k*'₂ and *k*'₁ are the capacity factors of the second and first eluting enantiomers, respectively.

where *t*_{R1} and *t*_{R2} are the retention times of the first and the second eluting enantiomers, respectively, and *w*₁ and *w*₂ are the widths of their signals (peaks) at the base line.

Table S2 (Continued)

Compound	Mobile phase																			
	G:				H:				I:				J:				K:			
	MeOH/IPA/ACN (1:1:1, v/v/v) + 10mM NH ₄ OAc				MeOH/IPA/ACN (1:1:1, v/v/v) + 20mM NH ₄ OAc				MeOH/IPA/ACN (1:1:1, v/v/v) + 10mM NH ₄ OAc + 0.1% formic acid				MeOH/IPA/ACN (1:1:1, v/v/v) + 10mM NH ₄ OAc + 0.1% ammonium hydroxyde				MeOH/IPA/ACN (1:1:1, v/v/v) + 10mM NH ₄ OAc + 0.25% ammonium hydroxyde			
	<i>k</i> ₁	<i>k</i> ₂	<i>α</i>	<i>R</i> _S	<i>k</i> ₁	<i>k</i> ₂	<i>α</i>	<i>R</i> _S	<i>k</i> ₁	<i>k</i> ₂	<i>α</i>	<i>R</i> _S	<i>k</i> ₁	<i>k</i> ₂	<i>α</i>	<i>R</i> _S	<i>k</i> ₁	<i>k</i> ₂	<i>α</i>	<i>R</i> _S
R/S(±)-Aminorex	0.94	0.94	1.00	0.80	0.94	0.94	1.00	1.10	-	-	-	-	0.94	0.94	1.00	1.20	0.94	0.94	1.00	1.10
R/S(±)-Carboxybuprofen	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
R/S(±)-Carprofen	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
R/S(±)-Cetirizine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1S,2S-(+)-Chloramphenicol + 1R,2R-(-)-Chloramphenicol	-	-	-	-	0.94	0.94	1.00	0.75	0.94	0.94	1.00	0.78	0.94	0.94	1.00	0.61	-	-	-	-
R/S(±)-3-N-Dechloroethyl ifosfamide	0.94	0.95	1.01	4.13	0.94	0.95	1.01	7.12	0.94	0.5	1.01	5.93	0.94	0.95	1.01	5.10	0.94	0.95	1.01	4.31
S-(+)-O-Desmethylnaproxen	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
R/S(±)-Dihydroketoprofen	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
R/S(±)-Fenoprofen	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
R/S(±)-Fexofenadine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
R/S(±)-Flurbiprofen	0.94	0.94	1.00	0.72	0.94	0.94	1.00	1.00	0.93	0.93	1.00	1.10	0.94	0.94	1.00	1.22	0.94	0.94	1.00	1.10
R/S(±)-2-Hydroxybuprofen	0.94	0.94	1.00	0.70	0.94	0.94	1.00	1.00	0.94	0.94	1.00	0.95	0.94	0.94	1.00	0.84	0.94	0.94	1.00	0.95
R/S(±)-Ibuprofen	-	-	-	-	-	-	-	-	- ^a	- ^a	- ^a	- ^a	-	-	-	-	-	-	-	-
R/S(±)-Ifosfamide	0.93	0.94	1.00	1.60	0.93	0.94	1.01	1.90	0.93	0.94	1.01	2.32	0.93	0.94	1.01	2.16	0.93	0.94	1.01	1.70
R/S(±)-Imazalil	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
R/S(±)-Indoprofen	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
R/S(±)-Ketoprofen	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
R/S(±)-Naproxen	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
R/S(±)-Ofloxacin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	- ^a	- ^a	- ^a	- ^a
R/S(±)-Omeprazole	0.96	0.96	1.00	0.72	0.96	0.96	1.00	0.95	0.96	0.96	1.00	0.95	0.96	0.96	1.00	0.96	0.96	0.96	1.00	1.17
R/S(±)-Phenylpropionic acid	-	-	-	-	-	-	-	-	- ^a	- ^a	- ^a	- ^a	-	-	-	-	-	-	-	-
R/S(±)-Praziquantel	0.96	0.96	1.00	1.80	0.96	0.96	1.01	1.65	0.96	0.96	1.01	1.89	0.96	0.96	1.01	2.15	0.96	0.96	1.01	1.60
R/S(±)-Tetramisole	0.93	0.94	1.00	0.89	0.94	0.94	1.00	0.98	0.94	0.94	1.00	1.00	0.93	0.94	1.00	1.04	0.94	0.94	1.00	1.00

MeOH: methanol; ACN: acetonitrile; IPA: isopropanol; NH₄OAc: ammonium acetate; -: no resolution; ^ano peak; *k*₁: retention factor of the first eluted peak; *k*₂: retention factor of the second eluted peak; *α*: selectivity factor; *R*_S: resolution

$$k' = (t_R - t_0) / t_0$$

$$\alpha = k'_2 / k'_1$$

$$R_S = 2(t_{R2} - t_{R1}) / (w_1 + w_2)$$

where *t*_R is the retention time of each enantiomer (first or second) and *t*₀ is the retention time of an unretained compound.

where *k*'₂ and *k*'₁ are the capacity factors of the second and first eluting enantiomers, respectively.

where *t*_{R1} and *t*_{R2} are the retention times of the first and the second eluting enantiomers, respectively, and *w*₁ and *w*₂ are the widths of their signals (peaks) at the base line.

Table S3

Chromatographic parameters for each cPAC obtained under different mobile phase composition with CEL CSP

Compound	Mobile phase															
	A: MeOH/ACN (1:1, v/v)				B: MeOH/ACN (1:1, v/v) + 10mM NH ₄ OAc				C: MeOH/ACN (1:1, v/v) + 0.1% formic acid				D: IPA/ACN (1:1, v/v) + 0.1% ammonium hydroxyde			
	<i>k</i> ₁	<i>k</i> ₂	α	<i>R</i> _S	<i>k</i> ₁	<i>k</i> ₂	α	<i>R</i> _S	<i>k</i> ₁	<i>k</i> ₂	α	<i>R</i> _S	<i>k</i> ₁	<i>k</i> ₂	α	<i>R</i> _S
R/S(±)-Aminorex	0.95	0.96	1.00	1.88	0.95	0.95	1.00	1.00	0.96	0.96	1.00	1.05	0.95	0.95	1.00	0.90
R/S(±)-Cetirizine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1S,2S-(+)-Chloramphenicol+	0.93	0.94	1.00	0.96	0.94	0.94	1.00	1.05	0.94	0.94	1.00	1.00	- ^a	- ^a	- ^a	- ^a
1R,2R-(-)-Chloramphenicol																
R/S(±)-3-N-Dechloroethylifosfamide	0.94	0.94	1.00	1.70	0.94	0.94	1.00	1.63	0.95	0.95	1.00	1.82	0.93	0.94	1.00	2.32
R/S(±)-Fexofenadine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
R/S(±)-Ifosfamide	-	-	-	-	-	-	-	-	-	-	-	-	0.94	0.94	1.00	0.42
R/S(±)-Imazalil	0.93	0.93	1.00	0.98	-	-	-	-	0.94	0.94	1.00	1.04	-	-	-	-
R/S(±)-Indoprofen	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
R/S(±)-Ketoprofen	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
R/S(±)-Ofloxacin	0.96	0.96	1.00	0.60	0.97	0.97	1.00	1.20	-	-	-	-	0.96	0.97	1.01	2.43
R/S(±)-Omeprazole	0.95	0.96	1.01	2.40	0.95	0.96	1.01	2.84	0.95	0.96	1.01	3.10	0.95	0.96	1.00	1.00
R/S(±)-Praziquantel	0.95	0.96	1.01	2.75	0.95	0.96	1.01	2.32	0.95	0.96	1.01	2.30	0.96	0.97	1.01	2.95
R/S(±)-Tetramisole	0.94	0.95	1.00	0.75	0.94	0.95	1.01	1.95	0.95	0.95	1.00	1.30	0.95	0.95	1.01	2.35
	H: MeOH/IPA/ACN (1:1:1, v/v/v) + 10mM NH₄OAc + 0.1% ammonium hydroxyde															
	E: MeOH				F: MeOH + 10 mM NH₄OAc				G: MeOH + 0.1% ammonium hydroxyde				H: 0.1% ammonium hydroxyde			
	<i>k</i> ₁	<i>k</i> ₂	α	<i>R</i> _S	<i>k</i> ₁	<i>k</i> ₂	α	<i>R</i> _S	<i>k</i> ₁	<i>k</i> ₂	α	<i>R</i> _S	<i>k</i> ₁	<i>k</i> ₂	α	<i>R</i> _S
R/S(±)-Aminorex	0.95	0.95	1.00	0.90	0.95	0.95	1.00	1.85	0.95	0.95	1.00	1.80	0.95	0.95	1.00	1.71
R/S(±)-Cetirizine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1S,2S-(+)-Chloramphenicol+	0.93	0.93	1.00	1.03	0.93	0.93	1.00	1.00	0.93	0.93	1.00	1.00	0.93	0.94	1.00	1.00
1R,2R-(-)-Chloramphenicol																
R/S(±)-3-N-Dechloroethylifosfamide	0.93	0.94	1.00	1.20	0.93	0.94	1.00	1.10	0.93	0.94	1.00	1.38	0.94	0.94	1.00	1.45
R/S(±)-Fexofenadine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
R/S(±)-Ifosfamide	0.93	0.93	1.00	0.40	0.93	0.93	1.00	0.40	0.93	0.93	1.00	0.40	-	-	-	-
R/S(±)-Imazalil	-	-	-	-	0.93	0.93	1.00	0.80	0.93	0.93	1.00	0.72	0.93	0.93	1.00	0.90
R/S(±)-Indoprofen	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
R/S(±)-Ketoprofen	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
R/S(±)-Ofloxacin	- ^a	- ^a	- ^a	- ^a	-	-	-	-	-	-	-	-	0.96	0.97	1.00	2.00
R/S(±)-Omeprazole	0.95	0.96	1.01	3.44	0.95	0.96	1.01	2.80	0.95	0.96	1.01	3.20	0.95	0.96	1.01	2.10
R/S(±)-Praziquantel	0.96	0.96	1.01	2.27	0.96	0.96	1.01	2.40	0.96	0.96	1.01	2.30	0.96	0.96	1.01	2.30
R/S(±)-Tetramisole	0.95	0.95	1.00	0.80	0.94	0.95	1.01	1.90	0.94	0.95	1.01	1.42	0.94	0.95	1.01	1.73

MeOH: methanol; ACN: acetonitrile; IPA: isopropanol; NH₄OAc: ammonium acetate; -: no resolution; ^ano peak; *k*₁: retention factor of the first eluted peak; *k*₂: retention factor of the second eluted peak; α : selectivity factor; *R*_S: resolution

Table S4

Linearity and range and inter- and intra-day precision (RSD %) of the studied cPACs with the AMY CSP

Compound	Linearity range ($\mu\text{g L}^{-1}$)	r^2	Intra-day precision (% RSD)												Inter-day precision (% RSD)			
			2.5 ($\mu\text{g L}^{-1}$)			25 ($\mu\text{g L}^{-1}$)			50 ($\mu\text{g L}^{-1}$)			250 ($\mu\text{g L}^{-1}$)			2.5 ($\mu\text{g L}^{-1}$)	25 ($\mu\text{g L}^{-1}$)	50 ($\mu\text{g L}^{-1}$)	250 ($\mu\text{g L}^{-1}$)
			D1	D2	D3	D1	D2	D3	D1	D2	D3	D1	D2	D3				
Aminorex E1	0.83 – 500	0.999	2.5	3.6	15.6	2.7	2.9	7.7	3.1	1.9	3.3	0.2	2.7	6.1	31.9	41.2	36.5	35.9
Aminorex E2	0.83 – 500	0.999	0.1	5.4	2.0	3.0	1.8	2.5	4.9	1.4	1.7	1.9	3.4	1.8	18.4	20.5	25.3	20.1
R/S(\pm)-Carboxybupropfen	408 – 4000	0.999	-	-	-	-	-	-	-	-	-	4.8	2.3	5.6	-	-	-	8.4
Carprofen E1	168 – 500	0.979	-	-	-	-	-	-	-	-	-	6.8	6.1	9.5	-	-	-	14.3
Carprofen E2	168 – 500	0.980	-	-	-	-	-	-	-	-	-	7.4	3.4	8.5	-	-	-	10.3
R/S(\pm)-Cetirizine	3.33 – 500	0.997	11.4	15.2	1.1	3.3	8.0	4.4	6.4	2.2	3.3	5.1	1.0	4.6	9.7	15.6	14.9	16.7
1S,2S-(+)-Chloramphenicol	16.7 – 500	0.998	-	-	-	4.5	4.9	4.1	5.7	5.0	1.6	5.1	1.7	2.6	-	9.7	4.7	6.0
1R,2R-(-)-Chloramphenicol	16.9 – 500	0.998	-	-	-	7.0	6.8	0.8	4.9	1.1	4.3	3.7	4.6	4.6	-	9.2	3.7	4.8
3-N-Dechloroethylifosfamide E1	0.17 – 50	0.998	2.7	3.9	n.a.	2.3	1.9	n.a.	6.9	1.5	n.a.	3.2	2.2	n.a.	63.9	60.1	69.0	68.6
3-N-Dechloroethylifosfamide E2	0.83 – 125	0.998	0.9	10.0	4.8	2.4	3.2	2.2	0.5	0.9	2.1	3.5	0.6	1.5	14.1	11.4	9.8	16.4
S-(+)-O-Desmethylnaproxen	173.5 – 1000	0.997	-	-	-	-	-	-	7.5	16.1	2.9	4.2	2.1	3.2	-	-	7.4	8.9
R/S(\pm)-Dihydroketoprofen	33.3 – 4000	0.998	-	-	-	3.6	9.0	4.9	2.9	3.3	4.7	0.8	4.1	1.0	-	6.4	7.2	7.2
Fenopropfen E1	171 – 4000	0.999	-	-	-	-	-	-	-	-	-	15.3	9.5	8.7	-	-	-	9.0
Fenopropfen E2	171 – 4000	0.994	-	-	-	-	-	-	-	-	-	11.8	3.9	9.2	-	-	-	9.5
R/S(\pm)-Fexofenadine	16.9 – 500	0.997	-	-	-	2.9	5.8	3.5	2.3	2.3	3.2	4.5	3.2	0.8	-	8.7	5.6	8.1
Flurbiprofen E1	83.8 – 2000	0.996	-	-	-	-	-	-	9.1	22.0	n.a.	5.6	14.3	n.a.	-	-	5.9	20.0
Flurbiprofen E2	83.8 – 2000	0.996	-	-	-	-	-	-	7.6	7.3	5.0	5.3	5.2	5.5	-	-	5.3	3.3
2-Hydroxyibuprofen E1	163.5 – 500	0.999	-	-	-	-	-	-	8.2	7.6	3.3	2.6	1.9	3.7	-	-	4.9	6.2
2-Hydroxyibuprofen E2	163.5 – 500	0.997	-	-	-	-	-	-	9.0	3.4	3.4	2.8	2.3	1.3	-	-	6.9	7.9
R-(-)-Ibuprofen	415 – 2000	0.997	-	-	-	-	-	-	-	-	-	9.2	10.4	7.1	-	-	-	13.0
S-(+)-Ibuprofen	415 – 2000	0.993	-	-	-	-	-	-	-	-	-	11.0	4.3	6.9	-	-	-	12.3
Ifosfamide E1	0.17 – 125	0.998	1.3	2.4	4.3	5.2	0.3	2.5	3.4	3.6	0.4	2.1	1.6	1.3	5.8	6.4	5.2	4.8
Ifosfamide E2	0.17 – 125	0.995	3.6	5.1	5.5	3.1	4.9	3.2	3.6	3.0	2.1	3.0	1.4	2.4	7.4	8.4	13.2	12.0
R/S(\pm)-Imazalil	0.87 – 1000	0.999	3.7	1.8	1.3	2.9	2.0	2.0	1.9	2.5	0.3	2.1	1.0	0.7	11.8	12.3	10.6	12.6
Indoprofen E1	0.85 – 250	0.998	13.6	4.9	9.0	8.7	2.0	3.1	6.8	4.5	1.6	3.0	3.1	0.4	11.0	9.1	10.9	5.3
Indoprofen E2	0.85 – 500	0.999	11.7	9.0	8.2	5.7	11.8	6.1	5.0	5.7	4.5	7.2	4.2	1.5	11.6	13.6	15.0	10.8
R/S(\pm)-Ketoprofen	33.2 – 1000	0.995	-	-	-	17.5	11.8	12.6	0.7	4.0	6.3	4.1	10.8	14.6	-	32.4	43.9	28.7
R-(-)-Naproxen	84.3 – 2000	0.998	-	-	-	5.6	7.0	7.5	9.1	14.6	6.5	2.7	5.0	3.3	-	9.2	11.5	5.1
S-(+)-Naproxen	84.3 – 2000	0.999	-	-	-	7.3	9.2	3.1	6.5	6.6	1.9	4.0	5.4	2.6	-	11.5	7.5	6.9
R/S(\pm)-Ofloxacin	32.8 – 1000	0.995	-	-	-	6.7	6.4	5.8	1.6	5.8	1.3	2.1	2.3	4.1	-	24.6	20.6	23.3
Omeprazole E1	0.82 – 125	0.998	7.9	4.0	3.8	0.8	1.7	3.3	3.5	5.7	1.2	2.6	2.9	1.1	21.4	12.9	14.6	14.3
Omeprazole E2	0.82 – 250	0.997	3.3	2.8	4.3	7.7	1.3	2.0	2.1	3.6	0.9	4.4	1.6	1.7	5.2	11.6	12.2	8.5
R/S(\pm)-Phenylpropionic acid	741.3 – 4000	0.999	-	-	-	-	-	-	-	-	-	12.6	3.4	6.7	-	-	-	9.6
Praziquantel E1	0.83 – 50	0.996	6.9	3.5	7.3	2.0	3.5	1.1	1.5	1.6	2.5	1.6	0.2	2.0	8.3	3.8	6.6	2.8
Praziquantel E2	0.83 – 50	0.998	3.6	6.2	7.0	3.1	1.8	4.6	0.6	2.9	3.4	1.2	0.3	0.9	7.7	5.8	4.4	5.1
R-(+)-Tetramisole	0.83 – 500	0.998	4.8	6.4	4.8	1.7	1.0	2.5	6.4	2.5	4.8	2.2	4.0	3.7	7.5	4.9	5.7	4.6
S-(-)-Tetramisole	0.83 – 500	0.997	2.4	1.2	8.9	5.1	2.6	4.0	9.8	2.4	4.0	3.8	2.3	5.6	10.5	6.8	6.1	7.2

D1, D2, D3: day 1, 2 and 3; n.a.: not available

Table S5

Linearity and range and inter- and intra-day precision (RSD %) of the studied cPACs with the CEL CSP

Compound	Linearity		Intra-day precision (% RSD)															Inter-day precision (% RSD)				
	range ($\mu\text{g L}^{-1}$)	r^2	0.5 ($\mu\text{g L}^{-1}$)			2.5 ($\mu\text{g L}^{-1}$)			25 ($\mu\text{g L}^{-1}$)			50 ($\mu\text{g L}^{-1}$)			250 ($\mu\text{g L}^{-1}$)			0.5	2.5	25	50	250
			D1	D2	D3	D1	D2	D3	D1	D2	D3	D1	D2	D3	D1	D2	D3	($\mu\text{g L}^{-1}$)	($\mu\text{g L}^{-1}$)	($\mu\text{g L}^{-1}$)	($\mu\text{g L}^{-1}$)	($\mu\text{g L}^{-1}$)
Aminorex E1	0.83 – 500	0.998	14.5	11.8	6.0	6.2	3.0	5.8	0.9	4.3	0.9	3.2	0.4	2.6	0.5	2.5	1.2	16.7	12.1	11.3	10.1	11.7
Aminorex E2	0.83 – 500	0.996	3.9	18.4	7.8	3.0	1.6	2.7	1.9	2.9	1.6	2.0	1.7	1.6	1.5	2.4	2.6	14.6	9.8	11.5	10.3	10.3
R/S(\pm)-Cetirizine	16.4 – 250	0.997	-	-	-	20.8	18.5	18.6	2.7	4.2	1.1	4.3	3.4	1.7	3.0	3.7	2.4	-	22.8	21.9	19.1	17.9
3-N-Dechloroethylifosfamide E1	0.83 – 125	0.996	11.2	13.8	7.9	2.1	0.5	1.3	1.6	2.1	0.6	5.5	0.7	0.9	0.7	2.1	0.4	13.2	1.4	11.9	5.2	8.3
3-N-Dechloroethylifosfamide E2	0.83 – 125	0.999	13.8	6.3	9.5	5.2	6.3	2.9	3.4	1.0	2.9	1.1	2.3	1.7	1.0	2.9	0.8	10.3	8.4	3.5	1.7	2.4
R/S(\pm)-Fexofenadine	1.69 – 500	0.999	19.3	17.6	16.9	9.5	4.4	2.5	3.3	4.0	2.0	2.2	2.4	1.7	4.4	1.5	1.5	23.1	14.3	17.3	15.8	14.1
R/S(\pm)-Ifosfamide	1.69 – 100	0.999	7.3	3.6	5.2	4.5	8.3	3.4	0.8	1.6	3.6	1.3	1.4	0.6	0.9	0.7	1.2	6.7	8.8	3.2	5.1	4.9
Imazalil E1	1.74 – 500	0.998	9.4	13.2	18.3	6.5	5.1	9.2	5.4	1.8	3.0	1.2	2.8	5.8	3.8	1.5	1.8	21.5	17.1	13.3	14.4	13.0
Imazalil E2	1.74 – 250	0.997	24.0	24.5	13.5	7.7	17.7	6.5	4.3	3.3	6.7	3.5	1.4	1.8	4.4	1.1	1.2	23.6	16.8	13.0	14.4	12.2
Indoprofen E1	1.69 – 500	0.998	10.9	8.9	n.a.	2.4	8.5	2.4	8.6	6.6	2.6	7.2	4.2	5.6	1.0	2.5	4.1	18.5	7.8	12.8	12.3	6.9
Indoprofen E2	1.69 – 250	0.997	14.2	8.0	n.a.	2.5	4.6	4.9	1.7	6.0	4.4	1.9	3.8	0.5	1.9	1.5	1.2	21.4	14.3	15.5	13.7	11.9
R/S(\pm)-Ketoprofen	16.6 – 500	0.998	-	-	-	2.1	4.9	7.4	3.3	1.1	1.2	4.1	3.1	3.9	0.6	1.5	1.1	-	11.5	12.5	11.9	11.0
Ofloxacin E1	16.4 – 500	0.997	-	-	-	-	-	-	13.5	13.9	14.3	4.1	10.0	6.9	2.0	1.8	2.6	-	-	15.5	14.0	13.1
Ofloxacin E2	16.4 – 250	0.996	-	-	-	-	-	-	9.3	6.3	13.1	8.4	13.5	6.5	4.1	5.8	4.2	-	-	18.3	13.9	13.8
Omeprazole E1	1.63 – 500	0.995	14.2	12.3	n.a.	2.0	4.1	6.0	7.1	5.3	1.3	2.5	5.4	2.8	2.0	2.0	1.8	29.6	14.0	17.0	15.5	16.4
Omeprazole E2	1.63 – 500	0.993	9.7	10.4	n.a.	5.3	2.0	4.7	3.3	1.3	3.3	6.6	2.2	1.0	3.9	1.2	2.5	30.1	9.7	15.0	15.2	15.0
Praziquantel E1	1.67 – 500	0.999	11.5	n.a.	n.a.	2.7	3.1	2.4	1.8	2.1	2.7	0.7	1.3	0.6	1.2	0.4	0.7	11.5	7.1	9.7	10.0	8.7
Praziquantel E2	1.67 – 250	0.997	14.0	n.a.	n.a.	4.3	9.8	5.5	4.1	5.3	2.6	0.9	6.3	1.0	2.2	2.7	2.8	14.0	8.0	10.9	9.3	7.5
R-(+)-Tetramisole	0.83 – 250	0.994	4.6	11.7	16.9	0.5	6.1	6.4	4.7	0.8	4.8	4.7	2.6	2.3	2.3	2.2	3.9	12.3	5.5	10.4	9.6	11.2
S-(-)-Tetramisole	0.83 – 500	0.994	11.4	13.3	3.0	8.2	7.9	7.9	1.6	2.3	1.8	5.6	3.1	1.1	2.1	2.2	5.8	17.0	12.1	10.0	7.7	6.6

D1, D2, D3: day 1, 2 and 3; n.a.: not available

Table S6

Recovery (%), intra-day precision (RSD %) and enantiomeric fraction in influent wastewater with the AMY CSP

Compound	Low level (n=3)			Medium level (n=3)			High level (n=3)		
	RR (%)	AR (%)	EF±SD	RR (%)	AR (%)	EF±SD	RR (%)	AR (%)	EF±SD
Aminorex E1 ^a	102.4 (10.3)	45.5 (11.3)	0.38 ± 0.01	97.3 (n.a.)	79.0 (2.5)	0.49 ± 0.10	125.1 (6.4)	65.2 (3.7)	0.48 ± 0.01
Aminorex E2 ^a	98.1 (8.9)	34.9 (2.2)	-	97.3 (7.1)	40.6 (5.1)	-	95.2 (9.0)	40.5 (5.5)	-
R/S(±)-Carboxyibuprofen ^b	-	-	-	-	-	-	86.8 (27.4)	37.8 (17.7)	-
Carprofen E1 ^c	-	-	-	133.3 (8.2)	113.8 (1.7)	0.46 ± 0.01	127.4 (3.4)	111.4 (5.4)	0.46 ± 0.01
Carprofen E2 ^c	-	-	-	171.3 (3.6)	143.7 (0.3)	-	124.8 (4.1)	102.5 (5.9)	-
R/S(±)-Cetirizine ^e	_{-h}	_{-h}	-	_{-h}	_{-h}	-	_{-h}	_{-h}	-
1S,2S-(+)-Chloramphenicol ^b	117.8 (18.0)	97.8 (10.0)	0.59 ± 0.04	115.0 (5.1)	91.3 (4.9)	0.60 ± 0.03	110.2 (15.2)	84.2 (2.1)	0.55 ± 0.01
1R,2R-(-)-Chloramphenicol ^b	109.6 (2.8)	80.4 (3.1)	-	111.3 (1.4)	77.0 (3.3)	-	118.8 (6.4)	80.5 (3.1)	-
3-N-Dechloroethylifosfamide E1 ^d	167.6 (n.a.)	110.0 (n.a.)	0.50 ± 0.05	108.4 (20.0)	101.5 (19.9)	0.50 ± 0.05	74.2 (n.a.)	45.4 (n.a.)	0.50 ± 0.05
3-N-Dechloroethylifosfamide E2 ^d	78.7 (3.7)	43.8 (7.7)	-	77.6 (11.2)	44.7 (8.1)	-	91.5 (13.3)	39.4 (3.9)	-
S-(+)-O-Desmethylnaproxen ^b	-	-	-	87.9 (7.1)	60.6 (11.4)	-	76.8 (5.3)	51.9 (8.3)	-
R/S(±)-Dihydroketoprofen ^b	58.7 (15.5)	62.0 (19.9)	-	98.3 (9.2)	70.0 (5.7)	-	103.0 (8.4)	70.0 (3.8)	-
Fenopropfen E1 ^b	-	-	-	89.7 (15.1)	78.7 (11.7)	0.46 ± 0.06	89.7 (24.1)	80.4 (14.0)	0.42 ± 0.08
Fenopropfen E2 ^b	-	-	-	95.1 (15.4)	62.3 (14.6)	-	91.8 (11.6)	63.0 (8.7)	-
R/S(±)-Fexofenadine ^e	51.8 (2.3)	74.7 (9.7)	-	103.1 (4.8)	88.7 (7.5)	-	106.9 (9.9)	83.4 (7.7)	-
Flurbiprofen E1 ^b	-	-	-	_{-g}	_{-g}	-	_{-g}	_{-g}	-
Flurbiprofen E2 ^b	-	-	-	76.1 (8.0)	52.6 (11.3)	-	75.8 (5.7)	51.5 (10.2)	-
2-Hydroxyibuprofen E1 ^b	-	-	-	160.0 (n.a.)	77.0 (22.8)	0.22 ± 0.01	120.2 (26.0)	82.4 (34.3)	0.25 ± 0.02
2-Hydroxyibuprofen E2 ^b	-	-	-	90.3 (25.0)	116.2 (11.5)	-	96.3 (n.a.)	82.4 (34.3)	-
R-(-)-Ibuprofen ^c	-	-	-	88.4 (4.3)	82.2 (5.7)	0.75 ± 0.01	117.4 (2.9)	101.8 (8.3)	0.62 ± 0.02
S-(+)-Ibuprofen ^c	-	-	-	81.7 (1.7)	77.9 (7.7)	-	67.8 (28.7)	48.6 (36.0)	-
Ifosfamide E1 ^d	90.6 (15.5)	54.9 (3.9)	0.54 ± 0.03	99.7 (4.4)	57.7 (2.7)	0.57 ± 0.01	88.7 (7.3)	56.1 (5.5)	0.49 ± 0.04
Ifosfamide E2 ^d	99.5 (4.4)	55.5 (4.5)	-	87.9 (4.8)	50.7 (2.9)	-	112.9 (9.8)	48.7 (0.8)	-
R/S(±)-Imazalil ^d	66.3 (9.4)	36.4 (5.3)	-	98.5 (3.0)	57.1 (4.3)	-	89.4 (11.9)	56.6 (7.1)	-
Indoprofen E1 ^e	99.0 (15.6)	66.2 (6.8)	0.53 ± 0.01	106.9 (3.8)	71.2 (2.3)	0.53 ± 0.01	94.7 (3.8)	66.6 (4.8)	0.53 ± 0.001
Indoprofen E2 ^e	101.8 (11.6)	68.3 (5.6)	-	94.9 (4.9)	63.3 (4.5)	-	98.3 (5.3)	69.1 (6.0)	-
R/S(±)-Ketoprofen ^f	_{-h}	_{-h}	-	_{-h}	_{-h}	-	_{-h}	_{-h}	-
R-(-)-Naproxen ^b	-	-	-	108.4 (8.6)	57.8 (3.8)	0.80 ± 0.01	104.3 (16.2)	55.1 (6.6)	0.74 ± 0.02
S-(+)-Naproxen ^b	-	-	-	94.6 (4.5)	96.6 (16.1)	-	97.1 (13.7)	70.8 (7.5)	-
R/S(±)-Ofloxacin ^d	78.2 (6.2)	49.3 (18.1)	-	36.6 (13.7)	20.9 (11.0)	-	45.8 (15.2)	19.6 (5.9)	-
Omeprazole E1 ^e	19.1 (9.4)	14.8 (7.0)	0.46 ± 0.03	17.2 (16.3)	11.7 (17.0)	0.48 ± 0.01	7.3 (17.2)	5.3 (20.5)	0.48 ± 0.02
Omeprazole E2 ^e	22.9 (5.3)	15.3 (14.8)	-	19.3 (21.2)	12.9 (21.8)	-	8.6 (22.7)	6.1 (25.8)	-
R/S(±)-Phenylpropionic acid ^c	-	-	-	54.8 (12.9)	56.0 (4.7)	-	70.6 (25.8)	56.4 (2.2)	-
Praziquantel E1 ^e	99.2 (4.9)	66.9 (7.4)	0.52 ± 0.01	94.1 (5.7)	62.9 (4.4)	0.48 ± 0.02	92.0 (2.8)	64.7 (4.6)	0.48 ± 0.01
Praziquantel E2 ^e	98.2 (7.2)	81.1 (2.7)	-	101.4 (0.9)	85.3 (2.3)	-	103.2 (5.1)	80.3 (4.7)	-
R-(+)-Tetramisole ^a	76.1 (4.4)	36.3 (7.4)	0.54 ± 0.001	98.0 (10.9)	44.9 (1.1)	0.55 ± 0.04	104.1 (7.8)	48.3 (2.6)	0.56 ± 0.02
S-(-)-Tetramisole ^a	80.5 (5.8)	27.8 (5.2)	-	84.7 (7.2)	35.3 (2.7)	-	88.5 (2.8)	37.7 (2.3)	-

^aIS: Tetramisole-d5; ^bNaproxen-d3; ^cIbuprofen-d3; ^dIfosfamide-d4; ^ePraziquantel-d11; ^fKetoprofen-d3; ^gDistorted peak; ^hPresence in the unspiked at high concentration levels; n.a.: not available

Table S7

Recovery (%), intra-day precision (RSD %) and enantiomeric fraction in effluent wastewater with the AMY CSP

Compound	Low level (n=3)			Medium level (n=3)			High level (n=3)		
	RR (%)	AR (%)	EF±SD	RR (%)	AR (%)	EF±SD	RR (%)	AR (%)	EF±SD
Aminorex E1 ^a	102.0 (8.3)	82.8 (9.2)	0.46 ± 0.04	115.7 (10.5)	100.0 (8.9)	0.49 ± 0.04	92.7 (2.8)	84.3 (3.8)	0.40 ± 0.02
Aminorex E2 ^a	72.8 (16.0)	52.7 (13.5)	-	82.9 (4.1)	65.5 (0.3)	-	80.9 (5.2)	72.0 (2.5)	-
R/S(±)-Carboxyibuprofen ^b	-	-	-	107.5 (9.4)	89.5 (6.6)	-	89.7 (6.6)	85.6 (6.9)	-
Carprofen E1 ^c	-	-	-	86.3 (4.7)	49.0 (6.5)	0.52 ± 0.02	112.6 (9.6)	69.9 (11.1)	0.53 ± 0.02
Carprofen E2 ^c	-	-	-	71.5 (10.3)	42.9 (5.2)	-	74.6 (14.8)	44.8 (11.1)	-
R/S(±)-Cetirizine ^e	_{-h}	_{-h}	-	_{-h}	_{-h}	-	_{-h}	_{-h}	-
1S,2S-(+)-Chloramphenicol ^b	109.3 (3.4)	74.8 (14.7)	0.58 ± 0.03	99.8 (3.2)	76.6 (5.9)	0.50 ± 0.02	103.3 (5.7)	81.9 (3.6)	0.53 ± 0.04
1R,2R-(-)-Chloramphenicol ^b	93.6 (9.1)	79.6 (10.7)	-	95.1 (6.4)	78.8 (1.8)	-	86.4 (14.4)	82.2 (6.7)	-
3-N-Dechloroethylifosfamide E1 ^d	29.8 (7.8)	25.5 (5.8)	0.50 ± 0.05	37.0 (25.3)	29.7 (23.5)	0.50 ± 0.05	63.3 (19.6)	55.3 (22.9)	0.50 ± 0.05
3-N-Dechloroethylifosfamide E2 ^d	58.1 (18.7)	44.6 (39.6)	-	29.0 (0.1)	22.1 (0.8)	-	52.4 (20.7)	50.3 (10.6)	-
S-(+)-O-Desmethylnaproxen ^b	-	-	-	93.6 (5.7)	77.3 (2.3)	-	83.0 (9.4)	79.0 (1.9)	-
R/S(±)-Dihydroketoprofen ^b	35.1 (20.9)	43.9 (12.5)	-	91.6 (3.3)	77.8 (3.4)	-	87.1 (5.7)	84.4 (4.5)	-
Fenoprofen E1 ^b	-	-	-	102.7 (1.8)	81.3 (6.0)	0.52 ± 0.03	112.6 (5.1)	86.6 (5.7)	0.57 ± 0.001
Fenoprofen E2 ^b	-	-	-	104.5 (12.6)	84.3 (17.7)	-	100.7 (6.1)	91.0 (6.0)	-
R/S(±)-Fexofenadine ^e	_{-h}	_{-h}	-	60.8 (14.1)	63.8 (4.7)	-	72.7 (3.8)	75.1 (4.3)	-
Flurbiprofen E1 ^b	-	-	-	_{-g}	_{-g}	-	88.4 (23.0)	71.3 (18.1)	0.60 ± 0.02
Flurbiprofen E2 ^b	-	-	-	66.6 (0.7)	55.1 (5.2)	-	64.6 (18.3)	61.4 (13.9)	-
2-Hydroxyibuprofen E1 ^b	-	-	-	_{-h}	_{-h}	-	22.6 (12.2)	64.4 (7.6)	0.49 ± 0.02
2-Hydroxyibuprofen E2 ^b	-	-	-	_{-h}	_{-h}	-	-	33.8 (7.1)	-
R-(-)-Ibuprofen ^c	-	-	-	85.5 (12.6)	54.8 (5.5)	0.50 ± 0.03	98.6 (9.4)	58.4 (3.9)	0.50 ± 0.03
S-(+)-Ibuprofen ^c	-	-	-	85.8 (10.0)	50.8 (7.0)	-	97.1 (1.6)	59.1 (3.3)	-
Ifosfamide E1 ^d	89.0 (1.2)	66.0 (6.0)	0.52 ± 0.02	100.2 (2.0)	77.0 (0.8)	0.51 ± 0.03	89.9 (7.1)	80.3 (3.1)	0.54 ± 0.04
Ifosfamide E2 ^d	92.1 (7.6)	66.1 (10.3)	-	95.0 (12.5)	77.4 (0.8)	-	86.8 (7.4)	81.7 (2.0)	-
R/S(±)-Imazalil ^d	76.8 (0.3)	56.9 (6.0)	-	87.3 (4.1)	67.0 (2.8)	-	78.7 (8.1)	70.3 (3.9)	-
Indoprofen E1 ^e	79.1 (8.8)	61.3 (10.3)	0.54 ± 0.02	88.1 (5.5)	71.7 (2.2)	0.52 ± 0.01	89.2 (8.5)	78.0 (4.7)	0.53 ± 0.001
Indoprofen E2 ^e	82.4 (2.2)	64.2 (1.9)	-	95.7 (8.2)	77.9 (3.9)	-	90.9 (9.1)	79.2 (3.2)	-
R/S(±)-Ketoprofen ^f	_{-h}	_{-h}	-	_{-h}	_{-h}	-	66.3 (10.0)	66.2 (5.5)	-
R-(-)-Naproxen ^b	-	-	-	_{-h}	_{-h}	-	46.9 (6.8)	67.4 (6.9)	-
S-(+)-Naproxen ^b	-	-	-	_{-h}	_{-h}	-	_{-h}	_{-h}	-
R/S(±)-Ofloxacin ^d	70.3 (34.2)	49.8 (17.2)	-	37.8 (10.6)	30.5 (1.3)	-	29.7 (15.0)	27.7 (12.3)	-
Omeprazole E1 ^e	<10	<10	-	<10	<10	-	<10	<10	-
Omeprazole E2 ^e	<10	<10	-	<10	<10	-	<10	<10	-
R/S(±)-Phenylpropionic acid ^c	-	-	-	87.9 (3.2)	50.0 (4.0)	-	70.3 (15.1)	39.1 (15.9)	-
Praziquantel E1 ^e	86.4 (9.1)	68.0 (8.8)	0.50 ± 0.04	95.0 (3.7)	77.4 (2.4)	0.50 ± 0.01	91.6 (8.4)	80.0 (2.4)	0.51 ± 0.01
Praziquantel E2 ^e	94.0 (13.3)	71.6 (13.3)	-	90.4 (4.8)	78.1 (1.3)	-	86.9 (4.1)	82.0 (2.7)	-
R-(+)-Tetramisole ^a	25.3 (32.4)	32.4 (27.3)	0.54 ± 0.01	87.9 (8.1)	77.6 (6.1)	0.53 ± 0.03	85.4 (3.3)	78.7 (5.7)	0.50 ± 0.03
S-(-)-Tetramisole ^a	51.8 (0.2)	43.4 (7.6)	-	86.9 (5.2)	69.5 (1.1)	-	89.6 (8.3)	80.3 (1.2)	-

^aIS: Tetramisole-d5; ^bNaproxen-d3; ^cIbuprofen-d3; ^dIfosfamide-d4; ^ePraziquantel-d11; ^fKetoprofen-d3; ^gDistorted peak; ^hPresence in the unspiked at high concentration levels

Table S8

Recovery (%), intra-day precision (RSD %) and enantiomeric fraction in influent wastewater with the CEL CSP

Compound	Low level (n=3)			Medium level (n=3)			High level (n=3)		
	RR (%)	AR (%)	EF±SD	RR (%)	AR (%)	EF±SD	RR (%)	AR (%)	EF±SD
Aminorex E1 ^a	86.3 (10.1)	47.3 (5.4)	0.52 ± 0.01	107.6 (7.7)	48.3 (0.8)	0.52 ± 0.01	106.9 (13.1)	49.8 (4.2)	0.51 ± 0.001
Aminorex E2 ^a	86.2 (13.5)	45.8 (1.2)		98.6 (11.7)	44.1 (4.5)		101.2 (12.8)	47.1 (5.1)	
R/S(±)-Cetirizine ^a	- ^e	- ^e		- ^e	- ^e		- ^e	- ^e	
3-N-Dechloroethylifosfamide E1 ^b	95.5 (8.3)	55.3 (3.6)	0.51 ± 0.01	89.0 (7.5)	52.0 (9.0)	0.50 ± 0.001	86.0 (13.3)	45.4 (4.1)	0.50 ± 0.01
3-N-Dechloroethylifosfamide E2 ^b	90.5 (5.4)	52.5 (5.5)		83.5 (6.1)	48.7 (7.8)		85.1 (9.6)	45.0 (2.7)	
R/S(±)-Fexofenadine ^a	- ^e	- ^e		85.1 (6.4)	59.8 (3.5)		102.1 (16.6)	56.0 (5.6)	
R/S(±)-Ifosfamide ^b	97.4 (7.4)	55.3 (4.2)		90.3 (1.3)	52.5 (2.9)		99.7 (5.7)	52.8 (4.7)	
Imazalil E1 ^c	72.8 (9.8)	50.5 (17.1)	0.50 ± 0.01	102.0 (7.6)	63.4 (5.3)	0.50 ± 0.01	99.9 (9.8)	60.4 (6.9)	0.49 ± 0.01
Imazalil E2 ^c	88.9 (7.0)	57.6 (12.0)		101.1 (9.0)	62.5 (6.2)		100.2 (9.0)	60.5 (6.0)	
Indoprofen E1 ^a	102.7 (11.8)	50.2 (11.8)	0.41 ± 0.03	91.9 (18.2)	40.6 (11.1)	0.37 ± 0.03	85.5 (12.2)	39.7 (5.2)	0.38 ± 0.01
Indoprofen E2 ^a	145.4 (2.3)	70.9 (10.7)		128.8 (2.6)	59.5 (0.8)		127.8 (14.1)	59.2 (3.6)	
R/S(±)-Ketoprofen ^c	77.9 (19.6)	62.4 (1.1)		81.9 (6.4)	52.1 (3.4)		93.5 (6.1)	57.0 (4.2)	
S-(-)-Ofloxacin ^a	- ^e	- ^e	-	50.2 (19.9)	50.4 (11.8)	0.34 ± 0.02	61.6 (4.1)	35.6 (7.2)	0.45 ± 0.04
R-(+)-Ofloxacin ^a	- ^e	- ^e	-	78.1 (5.2)	47.8 (3.3)		88.9 (14.4)	47.6 (4.5)	
Omeprazole E1 ^a	<10	<10	-	<10	<10	-	<10	<10	-
Omeprazole E2 ^a	<10	<10		<10	<10		<10	<10	
Praziquantel E1 ^a	83.7 (6.6)	40.3 (3.5)	0.45 ± 0.03	75.7 (8.2)	32.8 (1.4)	0.44 ± 0.02	73.0 (13.0)	33.8 (2.5)	0.40 ± 0.03
Praziquantel E2 ^a	109.6 (8.1)	63.6 (12.1)		89.7 (4.2)	55.5 (2.6)		99.4 (15.1)	53.4 (3.2)	
R-(+)-Tetramisole ^d	84.0 (16.0)	62.5 (3.9)	0.53 ± 0.02	91.5 (11.3)	66.5 (5.4)	0.51 ± 0.02	88.6 (8.6)	66.0 (4.2)	0.49 ± 0.03
S-(-)-Tetramisole ^d	64.2 (9.8)	49.6 (9.8)		80.9 (1.9)	48.0 (4.6)		89.8 (4.4)	41.1 (4.4)	

^aIS: Praziquantel-d11; ^bIfosfamide-d4; ^cKetoprofen-d3; ^dTetramisole-d5; ^ePresence in the unspiked at high concentration levels

Table S9

Recovery (%), intra-day precision (RSD %) and enantiomeric fraction in effluent wastewater with the CEL CSP

Compound	Low level (n=3)			Medium level (n=3)			High level (n=3)		
	RR (%)	AR (%)	EF±SD	RR (%)	AR (%)	EF±SD	RR (%)	AR (%)	EF±SD
Aminorex E1 ^a	96.3 (1.5)	55.9 (11.6)	0.50 ± 0.01	89.7 (4.1)	66.7 (7.1)	0.49 ± 0.01	91.2 (2.5)	74.8 (8.6)	0.49 ± 0.01
Aminorex E2 ^a	95.1 (7.4)	55.9 (17.2)		90.8 (5.2)	67.9 (5.6)		90.8 (2.4)	74.6 (6.0)	
R/S(±)-Cetirizine ^a	_{-e}	_{-e}		_{-e}	_{-e}		_{-e}	_{-e}	
3-N-Dechloroethylifosfamide E1 ^b	47.1 (29.0)	29.4 (22.3)	0.53 ± 0.01	28.7 (33.4)	22.1 (25.6)	0.51 ± 0.01	66.3 (12.6)	84.5 (16.2)	0.53 ± 0.02
3-N-Dechloroethylifosfamide E2 ^b	43.8 (36.4)	27.2 (30.1)		28.8 (28.0)	19.6 (23.1)		102.6 (5.2)	74.1 (1.6)	
R/S(±)-Fexofenadine ^a	_{-e}	_{-e}		63.8 (5.4)	58.1 (9.6)		92.7 (15.7)	72.8 (13.0)	
R/S(±)-Ifosfamide ^b	84.0 (10.2)	55.0 (8.4)		89.9 (3.2)	61.8 (3.8)		91.2 (3.0)	70.9 (4.5)	
Imazalil E1 ^c	78.2 (1.9)	54.7 (1.1)	0.46 ± 0.001	73.6 (7.2)	57.9 (9.6)	0.48 ± 0.001	65.1 (5.1)	67.4 (9.6)	0.49 ± 0.01
Imazalil E2 ^c	92.9 (3.4)	65.5 (2.8)		80.9 (7.4)	63.6 (7.7)		65.2 (3.3)	68.9 (8.7)	
Indoprofen E1 ^a	70.2 (11.4)	41.1 (20.3)	0.39 ± 0.03	74.5 (9.3)	55.6 (6.3)	0.43 ± 0.01	95.5 (2.6)	78.1 (3.7)	0.47 ± 0.01
Indoprofen E2 ^a	103.3 (3.2)	59.7 (7.4)		92.0 (6.1)	68.9 (6.6)		88.7 (5.6)	72.7 (6.1)	
R/S(±)-Ketoprofen ^c	20.0 (41.5)	29.5 (22.4)		86.3 (3.3)	69.9 (9.6)		86.2 (10.4)	85.6 (3.0)	
S-(-)-Ofloxacin ^a	-	-	-	75.6 (6.7)	56.4 (8.8)	0.49 ± 0.02	66.1 (10.8)	54.3 (2.4)	0.45 ± 0.05
R-(+)-Ofloxacin ^a	-	-		62.4 (12.5)	47.2 (10.0)		60.6 (9.7)	50.3 (10.8)	
Omeprazole E1 ^a	<10	<10		<10	<10		<10	<10	
Omeprazole E2 ^a	<10	<10		<10	<10		<10	<10	
Praziquantel E1 ^a	85.1 (4.9)	51.2 (9.1)	0.45 ± 0.03	73.7 (5.2)	55.0 (5.5)	0.44 ± 0.01	84.3 (7.7)	69.2 (1.7)	0.48 ± 0.02
Praziquantel E2 ^a	91.4 (8.9)	68.8 (8.5)		94.6 (5.4)	73.4 (6.2)		100.9 (3.7)	77.1 (5.3)	
R-(+)-Tetramisole ^d	18.2 (10.8)	34.8 (5.7)	0.52 ± 0.02	78.9 (2.4)	73.6 (6.3)	0.49 ± 0.02	78.3 (4.9)	88.2 (7.0)	0.51 ± 0.01
S-(-)-Tetramisole ^d	48.5 (7.5)	49.7 (11.4)		95.9 (10.1)	74.1 (4.8)		78.8 (7.0)	75.2 (10.9)	

^aIS: Praziquantel-d11; ^bIfosfamide-d4; ^cKetoprofen-d3; ^dTetramisole-d5; ^ePresence in the unspiked at high concentration levels