Chromatography
Separation Techniques

Gineys et al., J Chromatogr Sep Tech 2015, 6:7
DOI: 10.4172/2157-7064.1000299

Research Article Open Access

# Simultaneous Determination of Pharmaceutical and Pesticides Compounds by Reversed Phase High Pressure Liquid Chromatography

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## Abstract

A reversed phase HPLC method has been developed for the simultaneous separation and quantification of a mixture, constituted by a total of thirteen selected pollutants: pharmaceutical products and others compounds including one hormone, a pesticide, a natural marker and solvents. For each pollutant, the calibration curve shows a good linearity ($r^2 \geq 0.998$) over the concentration range of 1.0 × 10-5 – 5 × 10-8 mol/L. The limit of detection at 230 nm is ranging from 5.0 × 10-6 to 4.0 × 10-5 mg/mL, whereas the limit of quantification is in the range 1.8 × 10-5 - 1.2 × 10-4 mg/mL depending on the selected pollutant. The separation of the complex mixture was performed on a Hypersil Gold column 100 mm × 2.1 mm (dp=3 µm) using a multi-step linear gradient at 210, 230 and 280 nm. This method is well adapted for the detection of pharmaceutical compounds or micropollutants at low concentrations as a routine analysis but also as an alternative method to sophisticated ones like HPLC-MS or HPLC-fluorescence.

**Keywords:** Pharmaceutical compounds; Hormones; Pesticides; HPLC-UV (DAD) method validation

**Abbreviations:** ACB: Clofibric acid; ASA: Acetylsalicylic acid; BPA: Bisphenol A; CAF: Caffeine; CBZ: Carbamazepine; DAD: Diode Array Detector; DFN: Diclofenac sodium salt; HPLC: High Pressure Liquid Chromatography; IBP: Ibuprofen; LOD: Limit of detection; LOQ: Limit of quantification; MCP: Mecoprop; 4NP: 4-Nonylphenol; OES: β-Estradiol; OFX: Ofloxacin; PCP: Pentachlorophenol; POL: Acetaminophen; SA: Salicylic acid

## Introduction

Pharmaceuticals are widely used for health applications and ingested by humans and animals at high doses levels. Additionally, antibiotics are overused in breeding to minimize diseases distribution and epidemic threats. However, pharmaceuticals are not easily metabolized by the living organisms and therefore found in stools or urines. Others pollutions sources like industrial rejects, resulting from matter loss during fabrication processes or agricultural practices, are responsible for the dissemination of numerous compounds in the environment, like dyes, pesticides, solvents, chemical additives or organic pharmaceutical precursors and residues. Finally, the wastewater treatments technologies actually available are not able to remove efficiently all of these contaminants that are therefore detected at very low concentrations, in the range of several µg/L to several ng/L in water after treatment steps. These noxious compounds are found in many environmental compartments, as such as surface water, soil, sediment, ground water. Unlike hormones and pesticides, whose noxious effects on human health are no more to be proved, pharmaceutical products are actually still not considered by the REACH implementation. However, due to their regular occurrence in the environment, and potential risks on human health (antiobioresistance, carcinogenic properties) and ecosystems (fish feminization), these compounds are major targeted pollutants to be controlled and eliminated in a near future [1-3].

The recent developments in analytical technology allow setting up new normalized methods for the detection and the quantification of pharmaceuticals products, at very low concentrations and even trace levels in many water samples. For example, recently (February 2013) a normalized method XP T 90-223 for the assay of pharmaceutical products and their metabolites in water - dissolved fraction by liquid chromatography with tandem mass spectrometry coupled with a solid phase extraction, was developed. This method is efficient at a very low concentration level with a quantification limit ranging from 1 to 25 ng/L and shows a good relevancy on different water samples as ground water, surface water or water for human consumption [4]. Several studies have reported the separation and the quantification of a mixture of pharmaceuticals products (Carbamazepine, Diclofenac), hormones and pesticides with liquid chromatography coupled with fluorescence [5] or mass spectrometry for the detection phase [6]. Excellent results were obtained with quantification limits in the range of ng/L (10 to 1000 ng/L). This method is indeed rather sophisticated and technically heavy and therefore presents a high analysis cost. HPLC analytical methods using UV equipped with diode array detector (DAD) is well developed because of its easier accessibility, handling and lower cost as compared to mass or fluorescence detectors. DAD detection is less sensible but indeed permits to reach intermediate sensitivity levels, and to bring quick and cheap analyses. Many studies on pharmaceutical compounds (Diclofenac, Ofloxacin, Aspirine) using HPLC-UV method were achieved on different matrices (water, urine, plasma sample, tablet or drugs) with detection and quantification limits ranging from µg/L to mg/L [7-10]. If DAD detection is well adapted for known mixtures of water contaminants at intermediate concentration (to µg/L), for real matrices representative of treatment plants successive extraction, clean up and preconcentration steps were required. Recently, Zhou developed a hollow-fiber-supported ionic liquid microextraction method coupled with HPLC-UV in order to detect and quantify four endocrine disrupting compounds (bisphenol A, 17-β-estradiol, estrone and diethylstilbestrol) present in water surface samples. After extraction optimization, the proposed method allowed to reach a good

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**Received** September 11, 2015; **Accepted** October 05, 2015; **Published** October 12, 2015

**Citation:** Gineys M, Kirner T, Cohaut N, Béguin F, Delpeux-Ouldriane S (2015) Simultaneous Determination of Pharmaceutical and Pesticides Compounds by Reversed Phase High Pressure Liquid Chromatography. J Chromatogr Sep Tech 6: 299. doi:10.4172/2157-7064.1000299

linearity range (0.15-100 µg/L) and reproducibility but also to achieve very low detection limits: 0.03, 0.05, 0.10, 0.05 µg/L for bisphenol A, 17-β-estradiol, estrone and diethylstilbestrol respectively [11].

The aim of this study was therefore to develop an easy method able to perform the separation and the quantification of a wide diversity of targeted water pollutants as such as pharmaceuticals, pesticides and hormone molecules, in the range of µg/L using a reversed phase chromatography technique equipped with UV detection.

## Materials and Methods

### HPLC instrumentation

The HPLC system consists of a Dionex Ultimate 3000 equipped with a PDA detector (Accela 80 Hz, cell of 5 cm), coupled to a computer with Chromeleon 6.8 software package.

### Reagents and chemicals

HPLC grade acetonitrile and orthophosphoric acid 85% were purchased from VWR international. Acetaminophen (POL), Caffeine (CAF), Ofloxacin (OFX), Acetylsalicylic acid (ASA), Carbamazepine (CBZ), Bisphenol A (BPA), β-Estradiol (OES), Clofibric acid 97% (ACB), Diclofenac sodium salt (DFN), Ibuprofen (IBP), Pentachlorophenol (PCP) were purchased from Sigma-Aldrich. Mecoprop (MCP) and 4-Nonylphenol (4NP) were purchased from Fluka.

### Preparation of the mobile phase

100 µL of orthophosphoric acid 85% was mixed with 1000 mL of ultrapure water ($\sigma_{water}$=0,055 µS.cm$^{-1}$), stirred and was finally filtered using 0.2 µm disk (hydrophilic membrane). The pH of the solution is controlled and fixed to 2.9.

### Preparation of standard solutions

The mixture was composed of different pharmaceuticals products as an analgesic (Acetaminophen), non sterodial anti-inflammatory (Diclofenac, Ibuprofen, and Acetylsalicylic acid), antibiotic (Ofloxacin), neuroleptic (Carbamazepine) and an anti-cholesterol (Clofibric acid). Additionally, the mixture contains other compounds as endocrine disruptor (Bisphenol A, 4-Nonylphénol), hormone (β-Estradiol), pesticide (Mecoprop), natural marker (Caffeine) and a solvent (Pentachlorophenol). The chemical formulas of these pollutants were drawn in Figure 1. All of these pollutants have been selected because of their nature and their regular occurrence in water treatment plants at increasing concentration [12,13]. A standard solution was directly prepared without intermediate steps by dissolving the appropriate mass of each pollutant in ultrapure water ($\sigma_{water}$=0.055 µS.cm$^{-1}$) to get a concentration of $1.0 \times 10^{-5}$ mol/L per pollutant; the total pollutant concentration being equal to $1.3 \times 10^{-4}$ mol/L. The weighted mass corresponding to the standard concentration ($1.0 \times 10^{-5}$ mol/L) was lower than the compounds water solubility whatever the molecules (Table 1). Appropriate dilutions of the $1.0 \times 10^{-5}$ mol/L initial solution were realized to obtain eight calibration points in the concentration range of $1.0 \times 10^{-5}$-$5.0 \times 10^{-8}$ mol/L, i.e., $5.0 \times 10^{-8}$, $2.5 \times 10^{-7}$, $5.0 \times 10^{-7}$, $1.0 \times 10^{-6}$, $2.5 \times 10^{-6}$, $5.0 \times 10^{-6}$, $7.5 \times 10^{-6}$ and $1.0 \times 10^{-5}$ mol/L. A linear regression analysis was carried out at the concerned wavelength.

### HPLC method

The chromatographic separation was performed in the reversed phase mode using a Hypersil Gold $C_{18}$ column at 25°C (100 × 2.1 mm with a particle size of 3 µm). This column permitted to obtain during the separation thin and symmetric peaks and consequently an optimized resolution and sensibility. Furthermore, it can be used in a wide pH range, particularly at very low pH. The low activity of the silanol groups indeed reduced the peak trailing of the molecules, particularly in the case of basic compounds. The eluents were water (A) at pH 2.9 through acidification by orthophosphoric acid 0.01% (v/v) and acetonitrile (B). The following multi-step linear gradient was applied: from 10% B to 80% B in 25.45 min (slope of 2.75 mL/min), followed by a plateau for 2 min then a decrease from 80% B to 10% B in one minute and a final plateau of 3 min at the initial conditions. The flow rate was set to 0.25 ml/min and the volume of injection to 50 µL. The Accela PDA detector allowing to follow three simultaneous wavelengths, a compromise was established to obtain the highest sensitivity for a maximum of pollutant and so optimized answers. The overlap of the pollutant UV spectra, extracted from PDA data, allowed selecting three wavelengths: 210, 230 and 280 nm (Figure 2). In the mixture, peaks identification was accomplished comparing the retention time and UV spectra of each peak and the ones obtained for each selected pollutant injected separately with the same analytical method.

## Results and Discussion

### Analysis of pharmaceutical, hormone and pesticide products

The typical chromatogram of the mixture obtained with the reversed phase method described here, is illustrated in Figure 3. The thirteen compounds constituting the mixture were separated in thirty minutes with a good resolution and return to baseline for each peak. All of the pollutants were fully recovered except Acetylsalicylic acid (ASA), almost completely hydrolyzed in acidic medium in Salicylic acid (SA) and Acetic acid. System suitability tests were performed and chromatographic parameters such as retention time, resolution ($R_s$), selectivity (α), capacity factor (k') and asymmetry for each pollutant are reported in Table 2. The pollutants elution depends on the molecule affinity for the mobile and the stationary phase. Within the few first minutes of elution, the more hydrophilic compounds like Acetaminophen, Caffeine or Ofloxacine (Log D=1.1, -0.8 and -2.0 respectively) which present a better affinity for the polar mobile phase (water - acetonitrile, 80/20), are separated and eluted more quickly than the more hydrophobic compounds like Pentachlorophenol and 4-Nonylphenol (Log D=4.4 and 5.4 respectively). The increase of the organic solvent ratio along the analysis permits to improve the affinity of the hydrophobic compounds for the less polar mobile phase and therefore to promote their elution. Presenting a better affinity for the $C_{18}$ stationary phase, the hydrophobic compounds are more strongly retained and as a consequence eluted more slowly from the column, after 23.0 and 28.7 min respectively. The resolution $R_s$, represents the column ability to separate two components. For a resolution lower than 1.5, the components are considered as not totally separated, whereas for a resolution higher than 1.5, the separation is complete. The resolution parameters are ranging from 2 to 20, therefore showing a good separation for almost all the pollutants studied here. Nevertheless, in the chromatogram, the trio Bisphenol A, β-Estradiol and Clofibric acid seems to be co eluted. At a higher magnification, it appears finally that the return to baseline is suitable (Figure 4). In order to demonstrate that the separation was complete, a purity test using PDA data has been performed. The PPI (Peak Purity Index) were drawn on each peak of the chromatogram. The PPI representation has a rectangular shape in the case of a pure eluted molecule and becomes curved in the case of an impure peak. In this study, the PPI has a rectangular shape whatever the compound of the mixture, proving that the chromatographic separation is fully accomplished. Furthermore, it demonstrated that no co elution with others compounds takes place, particularly in the

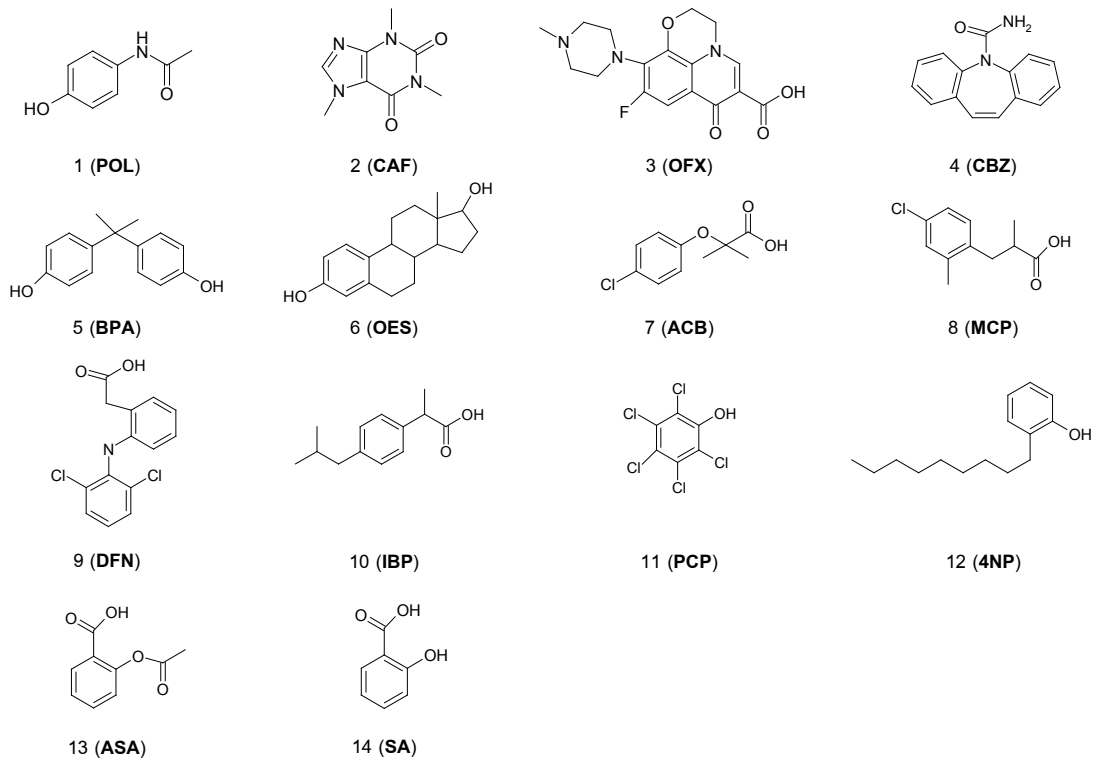


**Figure 1:** Structure of compounds constituting the selected mixture - 1: Acetaminophen, 2: Caffeine, 3: Ofloxacin, 4: Carbamazepin, 5: Bisphenol A, 6: β-Estradiol, 7: Clofibric acid, 8: Mecoprop, 9: Diclofenac, 10: Ibuprofen, 11: Pentachlorophenol, 12: 4-Nonylphenol, 13: Acetylsalicylic acid, 14: Salicylic acid.

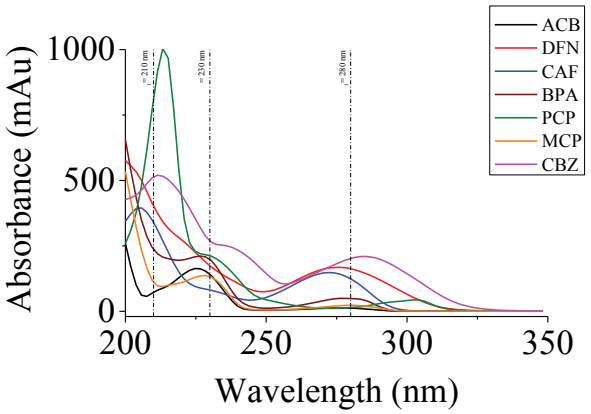


**Figure 2:** UV spectra using PDA detector of some compounds constituting the selected mixture.

case of the trio Bisphenol A, β-Estradiol and Clofibric acid. The 1.5 $R_s$ value indeed demonstrates that the separation is efficient and sufficient enough to perform the quantitative analysis of the three components of the peak. The capacity factor k' is usually used to describe the migration rate of an analyte on the column. For high retention factor value, greater than 20, it is established that the elution time is too high and that the separation conditions needs to be optimized. In our case, the last eluted compound, 4-Nonylphenol, shows a capacity factor lower than 20. The developed method therefore permits to achieve a complete and fast separation of the thirteen pollutants within thirty minutes. Furthermore the selectivity factor α represents, like the resolution, the column capacity to separate two compounds. It allows to calculate the separation power of the column for each pair of pollutant (α>1). In our case, the selectivity (α) and the number of theoretical plates (N) which were always higher than 1 and 2000 respectively proved that the Hypersyl Gold column was adequate and efficient to perform the pollutants mixture separation. However, with a selectivity factor α near to one, the separation of the massive peak constituted by BPA, OES and ACB appears to be at the limit of acceptance. The peak asymmetry permitted to evaluate the column quality. Ideally, the asymmetry of a perfectly gaussian peak is equal to one. Experimentally, the asymmetry was ranging from 1.2 to 5, where quantification is considered as less and less precise. In our case, the peak asymmetry was ranging from 1.1 to 1.5 (except Ofloxacin with an asymmetry of 5.6), reflecting the good quality of the column. Furthermore, the tailoring factor values were less than 2 and demonstrated consequently that the system suitability requirement was reached (Table 2). In the case of OFX, the high asymmetry value is explained by the interaction between the molecule and the residual silanol groups of the chromatographic column. Such interactions between the cationic adsorbate and dissociated silanol groups appear specifically in the chromatogram through an increasing peak trail. In order to reduce the peak trailing of this molecule, addition of triethylamine in the mobile phase could be considered.

## Linearity

The linearity of the method was evaluated using eight calibration points, in the concentration range from $1.0 \times 10^{-5}$ to $5.0 \times 10^{-8}$ mol/L. The calibration curve was obtained by plotting peak area *versus* the concentration of standard solutions. The linear regression equation *y=(Slope)x+Offset*, is presented in Table 3 for each pollutant. The obtained standard calibration curves possess an acceptable degree of

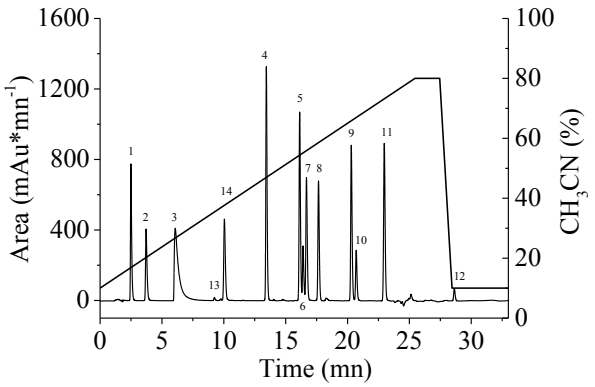


**Figure 3:** Chromatogram of a selected pharmaceutical, hormones and pesticides containing standard solution (1.0 × $10^{-5}$ mol/L) using PDA detector at 230 nm.

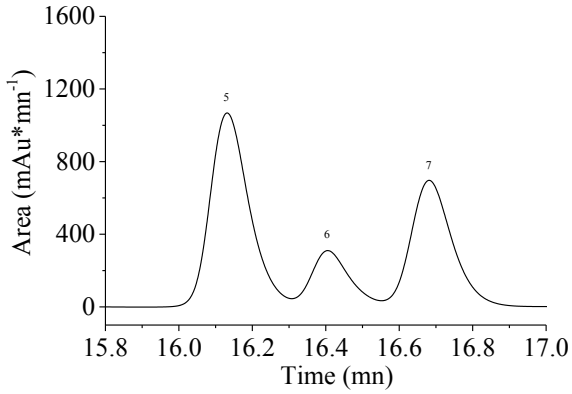


**Figure 4:** Chromatogram of the trio Bisphenol A, β-Estradiol and Clofibric acid at a higher magnification (1.0 × $10^{-5}$ mol/L) using PDA detector at 230 nm.

| Pollutants | $pK_A$ | Speciation* | Log D* | solubility * (mg/mL) | solubility** (mg/mL) | $[Pollutant]_{mixture}$ (mg/mL) |
|---|---|---|---|---|---|---|
| **POL** | 9.5 | Neutral | 1.1 | 11.10 | 11.10 | 1.5 × $10^{-3}$ |
| **CAF** | 0.6 | Neutral | -0.8 | 43.80 | 43.70 | 1.9 × $10^{-3}$ |
| **OFX** | 5.5 / 8.2 | Cationic | -2.0 | 347.30 | 4.40 | 3.6 × $10^{-3}$ |
| **SA** | 3.5 | Neutral | 0.9 | 3.7 | 280.30 | 1.8 × $10^{-3}$ |
| **CBZ** | 2.3 / 13.9 | Neutral | 3.2 | 0.05 | 0.05 | 2.4 × $10^{-3}$ |
| **BPA** | 9.8 | Neutral | 4.3 | 0.16 | 0.16 | 2.3 × $10^{-3}$ |
| **OES** | 10.3 | Neutral | 3.7 | 0.03 | 0.03 | 2.7 × $10^{-3}$ |
| **ACB** | 3.4 | Neutral | 2.4 | 0.32 | 81.10 | 2.1 × $10^{-3}$ |
| **MCP** | 3.5 | Neutral | 3.6 | 0.18 | 7.10 | 2.1 × $10^{-3}$ |
| **DFN** | 4.0 | Neutral | 3.9 | 0.02 | 1.16 | 3.2 × $10^{-3}$ |
| **IBP** | 3.8 | Neutral | 3.8 | 0.06 | 0.70 | 2.1 × $10^{-3}$ |
| **PCP** | 5.0 | Neutral | 4.4 | 0.01 | 0.04 | 2.7 × $10^{-3}$ |
| **4NP** | 10.3 | Neutral | 5.4 | 0.01 | 0.01 | 2.2 × $10^{-3}$ |

**Table 1:** Adsorbate characteristics. *: at pH 2.9; **: at pH 5.9.

| Pollutants | Retention time (mn) | Retention factor (k') | Asymmetry | Tailoring factor | Theorical plates (N) | Pollutants pair | Resolution ($R_s$) | Selectivity (α) |
|---|---|---|---|---|---|---|---|---|
| POL | 2.5 | 0.8 | 1.5 | 1.7 | 3 690 | POL - CAF | 6.5 | 2.2 |
| CAF | 3.7 | 1.6 | 1.4 | 1.5 | 5 150 | CAF - OFX | 5.2 | 2.1 |
| OFX | 6.2 | 3.3 | 5.6 | 4.5 | 1 930 | OFX - SA | 8.3 | 1.8 |
| SA | 10.1 | 6.1 | 1.3 | 1.3 | 28 100 | SA - CBZ | 14.9 | 1.4 |
| CBZ | 13.5 | 8.4 | 1.2 | 1.3 | 93 340 | CBZ - BPA | 14.0 | 1.2 |
| BPA | 16.2 | 10.3 | 1.1 | 1.2 | 115 200 | BPA - OES | 1.5 | 1.0 |
| OES | 16.4 | 10.5 | 1.2 | 1.3 | 147 800 | OES - ACB | 1.5 | 1.0 |
| ACB | 16.7 | 10.7 | 1.3 | 1.3 | 110 580 | ACB - MCP | 4.9 | 1.1 |
| MCP | 17.7 | 11.4 | 1.2 | 1.2 | 119 500 | MCP - DFN | 12.5 | 1.2 |
| DFN | 20.4 | 13.2 | 1.2 | 1.2 | 169 150 | DFN - IBP | 1.9 | 1.0 |
| IBP | 20.7 | 13.5 | 1.2 | 1.2 | 169 730 | IBP - 4NP | 8.9 | 1.1 |
| PCP | 23.0 | 15.1 | 1.2 | 1.2 | 171 330 | PCP - 4NP | 21.4 | 1.3 |
| 4NP | 28.7 | 19.0 | 1.1 | 1.1 | 288 740 | | | |

**Table 2:** Chromatographic parameters for each compound of the mixture.

linearity in the selected concentration range, for each pollutant. More particularly, the higher correlation coefficient $r^2$ of 0.999 is observed at 230 nm, except for 4-Nonylphenol whose extinction coefficient is low whatever the wavelength [14,15].

## Precision and accuracy

The intra-day precision of the method was determined for each compound by measuring standard repeatability characteristics, at three concentration levels ($1.0 \times 10^{-5}$, $7.5 \times 10^{-6}$ and $5.0 \times 10^{-6}$ mol/L respectively) by making five repeated analysis performed on the same day [14,15]. The inter-day precision of the analytical method was determined at the same three concentration levels previously selected for the intra-day precision but repeated day by day over a period of five days. Precision is evaluated by the estimation of the relative standard deviation values (RSD) and the results are given in Table 4. The method was found to be precise with RSD value within 0.04-0.5% for intra-day experiment and RSD value within 0.07-3.6% for inter-day experiment. In both cases, % RSD values were found within 5% limit, indicating that the current method is repeatable for each pollutant. The RSD lower values correspond to the analytical method precision. Furthermore in the case of Ofloxacin, the quantification is less precise as compared to others pollutants with a RSD% value between 1 and 5%. Taking into account the OFX peak asymmetry of 5.6, the RSD value can be therefore linked to the peak asymmetry value, for which values higher than 5 lead also to a decrease of the quantification precision. The method accuracy was determined by comparing the experimental amount obtained from the calibration curve with the theoretical amount fixed in the standard solution prepared by weighting. The accuracy is ranging from 97.83 to 104.46% depending on the selected pollutant and the concentration level. The calculated values are much closed to the nominal values, suggesting that the analytical method possesses a good accuracy. The extremely high values can be directly correlated to the rigorous way for preparing the standards, especially the use of an accurate weighting scale (absolute accuracy of $10^{-5}$ g).

## Limits of detection and quantification

The detection and quantification limits of an individual compound were determined at the three selected detection wavelengths by calculating signal/noise ratio (S/N=3) and (S/N=10), respectively for each compound (Table 5) [14,15]. The limits of detection differ depending on the selected pollutant and the wavelength used. For example, the limit of detection was ranging from $2.0 \times 10^{-6}$ to $5.0 \times 10^{-5}$ mg/mL at 210 nm, from $5.0 \times 10^{-6}$ to $4.0 \times 10^{-5}$ mg/mL at 230 nm and much higher at 280 nm. Owning a high extinction coefficient at 280 nm, Ofloxacin ($2.0 \times 10^{-6}$ mg/mL) and Caffeine ($1.0 \times 10^{-6}$ mg/mL) behave differently. In fact, for these two molecules sensitivities are higher and therefore it becomes possible to perform a quantitative analysis to lower concentration limits. The same behavior was observed for the limit of quantification. It was ranging from $5.0 \times 10^{-6}$ to $1.8 \times 10^{-4}$ mg/mL at 210 nm, from $2.0 \times 10^{-5}$ to $1.2 \times 10^{-4}$ mg/mL at 230 nm and much higher at 280 nm except for OFX ($1.0 \times 10^{-5}$ mg/mL) and CAF ($3.0 \times 10^{-6}$ mg/mL) whose quantification limits are much lower at that wavelength.

| Pollutants | Slope | Offset | $r^2$ |
|---|---|---|---|
| **POL** | 53.9 | 0.06 | 99.99 |
| **CAF** | 28.2 | 0.05 | 99.99 |
| **OFX** | 43.8 | -1.64 | 99.89 |
| **SA** | 48.7 | 0.03 | 99.99 |
| **CBZ** | 64.0 | 2.14 | 99.94 |
| **BPA** | 53.0 | 0.75 | 99.90 |
| **OES** | 10.6 | -0.13 | 99.97 |
| **ACB** | 39.5 | 0.59 | 99.97 |
| **MCP** | 42.0 | 0.50 | 99.98 |
| **DFN** | 37.5 | 0.16 | 99.97 |
| **IBP** | 17.3 | 0.11 | 99.99 |
| **PCP** | 47.4 | 0.27 | 99.96 |
| **4NP** | 5.5 | -0.45 | 94.75 |

**Table 3:** Regression analysis of the calibration data for each compound of the mixture at 230 nm.

| Pollutants | n | Intra-day | | | | Inter-day | | | | Accuracy (%) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $RSD_1$ (%) | $RSD_2$ (%) | $RSD_3$ (%) | RSD (%) | $RSD_1$ (%) | $RSD_2$ (%) | $RSD_3$ (%) | RSD (%) | $E_1$ | $E_2$ | $E_3$ |
| **POL** | 5 | 0.04 | 0.07 | 0.03 | 0.05 | 0.07 | 0.29 | 0.09 | 0.15 | 99.7 | 100.3 | 101.3 |
| **CAF** | 5 | 0.05 | 0.04 | 0.03 | 0.04 | 0.34 | 1.16 | 0.14 | 0.55 | 99.4 | 101.2 | 100.5 |
| **OFX** | 5 | 0.38 | 0.12 | 0.26 | 0.25 | 1.16 | 3.25 | 4.63 | 3.01 | 98.4 | 104.5 | 96.8 |
| **SA** | 5 | 0.08 | 0.03 | 0.04 | 0.05 | 1.02 | 2.08 | 0.34 | 1.15 | 100.8 | 98.4 | 101.1 |
| **CBZ** | 5 | 0.09 | 0.09 | 0.07 | 0.08 | 0.43 | 0.44 | 0.21 | 0.36 | 98.9 | 101.0 | 102.0 |
| **BPA** | 5 | 0.14 | 0.08 | 0.08 | 0.10 | 0.30 | 0.13 | 0.58 | 0.34 | 98.0 | 101.4 | 103.3 |
| **OES** | 5 | 0.51 | 0.34 | 0.40 | 0.42 | 1.42 | 0.89 | 1.88 | 1.40 | 97.8 | 99.6 | 98.6 |
| **ACB** | 5 | 0.16 | 0.11 | 0.10 | 0.12 | 0.39 | 0.44 | 0.51 | 0.45 | 99.0 | 100.5 | 101.8 |
| **MCP** | 5 | 0.05 | 0.05 | 0.03 | 0.04 | 0.07 | 0.32 | 0.16 | 0.18 | 99.5 | 100.7 | 101.7 |
| **DFN** | 5 | 0.05 | 0.05 | 0.08 | 0.06 | 0.75 | 0.26 | 1.12 | 0.71 | 99.0 | 100.8 | 100.9 |
| **IBP** | 5 | 0.16 | 0.12 | 0.13 | 0.14 | 2.22 | 3.61 | 0.32 | 2.05 | 100.9 | 97.0 | 101.0 |
| **PCP** | 5 | 0.08 | 0.05 | 0.04 | 0.06 | 0.82 | 0.48 | 1.63 | 0.98 | 99.0 | 101.0 | 101.6 |

**Table 4:** Precision and accuracy of the HPLC method.

| Pollutants | LOD × $10^5$ (mg/mL) | | | LOQ × $10^5$ (mg/mL) | | |
|---|---|---|---|---|---|---|
| | 210 nm | 230 nm | 280 nm | 210 nm | 230 nm | 280 nm |
| **POL** | 1.0 | 1.3 | 4.1 | 3.4 | 4.2 | 14 |
| **CAF** | 0.5 | 1.1 | 0.1 | 1.6 | 3.6 | 0.3 |
| **OFX** | 5.3 | 3.7 | 0.3 | 18 | 12 | 1.0 |
| **SA** | 0.2 | 0.9 | 1.8 | 0.5 | 3.0 | 6.1 |
| **CBZ** | 0.2 | 0.5 | 0.6 | 0.5 | 1.8 | 1.9 |
| **BPA** | 1.0 | 0.7 | 2.0 | 3.4 | 2.2 | 6.7 |
| **OES** | 1.9 | 3.0 | 4.8 | 6.5 | 9.9 | 16 |
| **ACB** | 3.0 | 1.0 | 8.0 | 10 | 3.2 | 27 |
| **MCP** | 2.1 | 1.0 | 2.6 | 7.0 | 3.2 | 8.5 |
| **DFN** | 0.8 | 0.7 | 0.7 | 2.6 | 2.5 | 2.2 |
| **IBP** | 1.5 | 1.6 | 220 | 5.0 | 5.3 | 740 |
| **PCP** | 0.6 | 2.5 | 18 | 2.0 | 8.3 | 60 |
| **4NP** | 3.0 | 1.7 | 4.8 | 9.8 | 5.7 | 16 |

**Table 5:** Analytical parameters of the proposed method (limit of detection (LOD) and limit of quantification (LOQ)) for each compound of the mixture.

## Conclusion

In this study, a wide variety of pollutants were investigated, pharmaceutical products, pesticides, hormone, and solvents; these contaminants being selected in relation with their occurrence in tertiary treatment plants. The wastewater treatments technologies actually available, as such as ozonation, membrane or adsorption processes, are indeed not able to remove efficiently all of these contaminants that are therefore detected at very low concentrations. Suitable analytical methods, allowing the detection and the precise quantification of these pollutants in water samples, need therefore to be developed. The HPLC-UV method described in this work appears as simple, precise, reproducible and sensitive in the low range of concentration i.e., µg/L. Furthermore, this method remains as an alternative to the more sophisticated methods like HPLC-MS or HPLC-fluorescence, in particular as far as intermediate concentrations are concerned (to µg/L). It can be used for routine analysis and is well adapted for many research studies, particularly concerning water treatment remediation. In our case, the developed method has been employed to study the adsorption of emerging pollutants on activated carbon cloths but also in order to evaluate the regeneration potentialities of the carbon material after loading using electrochemical techniques. This technique is well adapted for synthetic mixtures of water contaminants or samples containing an identified pollution. The limits of the method appear when considering real matrices and samples coming from treatment plants; the co-elution risks being more important as far as an increasing number of molecules, especially non-identified pollutants constituting the water matrix, are concerned. Considering its relatively weak sensibility, this method is however easy to handle and could find applications for the determination of composition and concentration of industrial or hospital effluents, particularly at high concentration levels (mg/L - µg/L).

### Acknowledgements

The authors thank ANR, the French National Research Agency, in particular the ECOTECH program for the financial support of the PARME project.

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