Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 993 (2003) 29–37
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Simultaneous determination of neutral and acidic pharmaceuticals in wastewater by high-performance liquid chromatography–post-column photochemically induced fluorimetry

C. González-Barreiro, M. Lores*, M.C. Casais, R. Cela

Departamento de Química Analítica, Nutrición y Bromatología, Facultad de Química, Universidad de Santiago de Compostela, E-15706 Santiago de Compostela, Spain

Received 10 October 2002; received in revised form 17 February 2003; accepted 20 February 2003

Abstract

An analytical method for the simultaneous determination of acidic and neutral pharmaceutical active compound (PhACs) residues in wastewater has been developed based on the combination of high-performance liquid chromatography (HPLC) and photochemically induced fluorimetry. The photoderivatization conditions for each particular PhAC have been assessed. Off-line optimization of the HPLC separation for both neutral and acidic compounds has been utilised and evaluated. Detection limits in the low ng/ml range have been achieved without sample pretreatment. By applying the developed analytical method combined with solid-phase extraction to real wastewater samples an enrichment factor of approximately two orders of magnitude can be obtained.
© 2003 Elsevier Science B.V. All rights reserved.

Keywords: Water analysis; Photochemically induced fluorimetric detection; Detection, LC; Derivatization, LC; Optimization; Drugs; Carbamazepine; Diazepam; Diclofenac; Ketoprofen; Profens; Naproxen

1. Introduction

Pharmaceuticals are being used in large quantities in human and veterinary medicine and many of these compounds are excreted without being entirely metabolized in the target organism, becoming a new and important contamination factor in the aquatic environment. Residues of these drugs easily reach wastewater treatment plants (WWTPs) via human urinary or faecal excretion and, although less frequently, from manufacturing sources. Since most current WWTPs were not designed to deal with this type of substance, these compounds are not totally eliminated by this treatment; consequently they can enter into sewage effluents and thus become a potential risk in the production of drinking water [1]. Nevertheless, to date, only in a few cases have pharmaceutically active compounds (PhACs) been detected at trace-levels in drinking water samples [2]. On the other hand, biodegradation, at best, leads only to a partial removal of some pharmaceutical residues [3].

The need for reliable analytical methods to determine drugs in the environment has been recently suggested [4]. These authors claim also that the knowledge about the use pattern of drugs is very

*Corresponding author. Tel.: +34-981-563-100x14386; fax: +34-981-595-012.
E-mail address: qnmlores@usc.es (M. Lores).

0021-9673/03/$ – see front matter © 2003 Elsevier Science B.V. All rights reserved.
doi:10.1016/S0021-9673(03)00392-3

limited in many European countries, with the exception of Scandinavian ones. To contribute to the improvement of this knowledge it should be useful to get not only more reliable methods, but also analytical methods that do not require expensive equipment (e.g. MS) and, consequently, could be used even in less developed areas.

Because of the large number of compounds used as constituents of medicinal products, a preselection is essential in developing adequate analytical methods [5]. In this case, the selected compounds are neutral and acidic substances: carbamazepine, 10,11-dihydrocarbamazepine, diazepam, acetylsalicylic acid, ketoprofen, naproxen, bezafibrate, diclofenac, ibuprofen and tolfenamic acid. They belong to different medicinal groups such as: lipid regulators, psychiatric drugs, anticonvulsants, analgesics and non-steroidal anti-inflammatory drugs (NSAIDs).

Up to now, many of the analytical methods reported in the literature for pharmaceutical residue analysis have been carried out by GC–MS. Nevertheless, some drawbacks can be mentioned: (i) the simultaneous determination of these two particular groups of pharmaceuticals cannot be carried out in one step due to the need of a chemical derivatization reaction for the extracted acidic compounds [6], increasing the possibility of contamination and errors; (ii) some of these compounds are thermolabile and non-volatile and may lead to degradation (e.g. carbamazepine is decomposed in the GC–MS injector forming iminostilbene as degradation product [5]).

LC–MS or LC–MS–MS are more appropriate techniques to analyze these polar and thermolabile compounds [7–12], however, when analyzing highly contaminated samples, such as sewage, a suppression of the electrospray ionization is likely to occur [5]. Besides, these instruments are still very expensive and consequently not widely distributed. On the contrary, almost every laboratory has a common HPLC system, thus our alternative proposal is to apply HPLC–photochemically induced fluorimetry (PIF) to determine the selected pharmaceuticals, which consists of an on-line post-column photoderivatization procedure: clean, fast and inexpensive. This technique has been uninterruptedly applied to the analysis of pesticides since the beginning of the 1980s [13] to the present time [14]; but, to our knowledge, this is the first application to PhACs residues' analysis.

Six of the 10 studied compounds are photoreactive, after 254 nm UV irradiation, being converted into strongly fluorescent species with greater selectivity and sensitivity than the original molecule. In spite of these considerations, HPLC–PIF cannot compete in sensitivity with LC–MS, but the figures of merit reached by the presented method are good enough to determine drug residues in water samples found at concentrations up to the $\mu g\ l^{-1}$ level [2] and thus useful for defining the distributions of these potential environmental pollutants for which no maximum tolerances have so far been set.

The differences in the acid character of both groups of compounds could complicate the chromatographic separation, leading us to another aspect of this work: the utilization of a gradient simulation program (PREGA) developed by our research group [15]. This software facilitates the task of optimizing the PhACs mixture resolution. In the meantime, the robustness of the theoretical prediction made by such software has been evaluated in comparison to experimental data.

Summarizing, the concrete goals of the present work are: to assess the photoderivatization conditions for each particular PhAC; to optimize the HPLC chromatographic separation for both neutral and acidic compounds by means of PREGA evaluating the obtained results, and to apply the developed analytical method to real wastewater samples after solid-phase extraction (SPE).

2. Experimental

2.1. Reagents and solvents

Neutral pharmaceuticals: carbamazepine, 10,11-dihydrocarbamazepine and diazepam; and acidic pharmaceuticals: acetylsalicylic acid, ibuprofen, ketoprofen, naproxen, bezafibrate, diclofenac-sodium salt and tolfenamic acid were all obtained from Sigma–Aldrich (Steinheim, Germany).

LiChrosolv methanol (MeOH) and acetonitrile (MeCN) for liquid chromatography were supplied by Merck (Darmstadt, Germany). Formic acid (98%) and ethyl acetate were also purchased from Merck.

Hydrochloric acid (36%) was obtained from Prolabo (Briare, France). The water was deionized and further purified on a Milli-Q water purification system from Millipore (Bedford, MA, USA).

Stock standard solutions of each pharmaceutical were prepared by exactly weighing in both solvents: methanol and acetonitrile. It was necessary to make individual dilutions and mixtures of the analytes in the same solvents to finally prepare the injected solutions in methanol–water (1:1) and acetonitrile–water (1:1).

All stocks and diluted standard solutions were protected against light in amber vials and were stored at −18 °C and 4 °C, respectively.

2.2. Equipment

The high-performance liquid chromatograph consisted of a 600E pump (Waters, Milford, MA, USA), a diode array detector and a fluorescence detector HP Series 1100 (Hewlett-Packard, Waldbronn, Germany), connected in series. The injector is a Rheodyne, Model 7725i (Cotati, CA, USA) fitted with a 20-μl external loop.

Chromatographic separation was performed with a Nova-Pak C_{18} column, 150 mm×3.9 mm I.D., 4 μm particle size. To protect the analytical cartridge column from contamination a Waters Guard-Pak Insert Nova-Pak C_{18} was installed.

Between the column and the detector systems, a laboratory-made photoderivatization reactor [16] was inserted, which emits most of their radiation within the spectrum line of 254 nm having 40 W of total power.

Data acquisition was done by means of HP Chemstation Software (Rev. A. 06.03 [509]).

A Metrohm 654 pH meter (Herisau, Switzerland) was used for pH measurements.

2.3. HPLC analysis

The individual photochemical behaviour of each particular PhAC was investigated in isocratic mode at a flow-rate of 1 ml/min and using different mobile phases to study the solvent effect: MeOH–Milli-Q water (70:30, v/v) acidified with 0.2% formic acid and MeCN–Milli-Q water (70:30, v/v or 50:50, v/v), both containing formic acid (0.2%). The latter weaker mobile phase was used for the most polar compounds: acetylsalicylic acid, carbamazepine, 10,11-dihydrocarbamazepine and diazepam (not so polar, but having a unique selectivity behaviour in MeCN mobile phases: it elutes too near to the dead time of the column when the MeCN proportion in the mobile phase surpasses 50%). The lack of solubility in MeCN for bezafibrate, diclofenac-sodium salt and tolfenamic acid prevented the correspondent solvent study for these three compounds.

Mobile phases were degassed under a constant flow of helium (100 ml/min). The selected concentration for individual studies was 5 μg/ml, injecting a constant volume of 20 μl.

The wavelength for UV detection, when used, was set at 254 nm. For fluorescence detection, the excitation wavelength was fixed at 230 nm for individual as well as for mixture studies. The optimal emission wavelengths for individual detection are depicted in Table 1; while the fluorescence program used for the analysis of standard mixtures and real samples is detailed in the corresponding chromatograms. These mixtures were analyzed by means of a multisegmented gradient (Table 2) obtained using a simulation program for off-line optimization of binary gradient separations. This program called PREGA2 V 3.0 was developed by Cela et al. [17].

2.4. Solid-phase extraction procedure

A sewage water sample taken from a 100 000-inhabitant-city wastewater treatment plant was processed without and with a spike of the standard mixture to have a final concentration of 10 ng/ml. A filtered sample volume of 250 ml was brought to pH 2 with hydrochloric acid (2 *M*) and passed through a 60 mg Oasis HLB extraction cartridge (Waters) previously conditioned with 3 ml of ethyl acetate, 3 ml of methanol and 3 ml of acidified water (pH 2). The sample was loaded into the cartridge by vacuum pumping at a flow-rate of 15 ml/min. The following steps were to dry the cartridge in a nitrogen stream for 30 min and then to elute the analytes with 3 ml of ethyl acetate. The extract (0.5 ml) was evaporated to dryness in a nitrogen stream and finally redissolved in 0.250 ml of methanol plus 0.250 ml of water into a 0.50 ml conical insert. Finally, the extract was filtered through a 0.45 μm 4 mm Millex-HV filter

Table 1
Fluorescent intensity ratio between peak areas obtained with the photoreactor turned ON and OFF, using two different organic modifiers in the mobile phase: MeOH and MeCN; pharmaceutical concentration, 5 μg/ml; injection volume, 20 μl

Compound	Excitation wavelength (nm)/ emission wavelength (nm)	Solvent (%)	Ratio peak area ON/ peak area OFF
Bezafibrate	230/315	70% MeOH	∞[a]
Diclofenac	230/354	70% MeOH	29.71
Tolfenamic acid	230/354	70% MeOH	n.d.[b]
Ibuprofen	230/300	70% MeOH	1.29
		70% MeCN	1.87
Naproxen	230/360	70% MeOH	0.12
		70% MeCN	0.32
Ketoprofen	230/332	70% MeOH	∞[a]
		70% MeCN	∞[a]
Acetylsalicylic acid	230/401	70% MeOH	0.96
		50% MeCN	1.14
Carbamazepine	230/351	70% MeOH	102.02
		50% MeCN	50.83
Dihydrocarbamazepine	230/434	70% MeOH	108.96
		50% MeCN	110.15
Diazepam	230/358	70% MeOH	173.27
		50% MeCN	63.94

[a] The symbol ∞ means that the native compound is not fluorescent.
[b] n.d., not detected.

with a Durapore–poly(vinylidene difluoride) (PVDF) membrane (Millipore, Yonezama, Japan).

3. Results and discussion

3.1. Individual photochemical behaviour

To approach the first objective of this work, the photochemical behaviour of each individual PhAC was evaluated using two different solvents in the mobile phase: MeOH and MeCN, as it is described in the Experimental section. It is well known that the solvent can affect both the extension of the photoreaction and the fluorescence intensity [18].

The analysis of the obtained results in these preliminary experiments (Table 1), shows four different trends in the photochemically induced fluorescence of the studied compounds: (i) pharmaceuticals with native fluorescence, acetylsalicylic acid and ibuprofen, which remain fluorescent after photoreaction (the ratio peak area photoreactor ON/OFF is near unity); (ii) substances that present a strong increase in their fluorescence response after UV light application: bezafibrate, carbamazepine, dihydrocarbamazepine, diazepam, diclofenac and ketoprofen; (iii) the strong native fluorescence of naproxen

Table 2
Suggested identity of some fluorescent photoproducts formed in the post-column photoderivatization process (based on the literature)

Compound	Suggested photoproduct	Refs.
Diclofenac	Carbazole-1-acetic acid	[20,21]
Naproxen	Naphthalene, 2-methoxy-6-(1-methoxyethyl)	[22]
Ketoprofen	Bencenemethanol, 3-(ethyl-α-phenyl)	[22]
Diazepam	*o*-Aminobenzophenone derivative	[23]
Bezafibrate	Phenol derivative	[24]
Carbamazepine	Cyclobutyl dimer	[25]

decreases after being photoirradiated; (iv) finally, tolfenamic acid which is not fluorescent either before or after photoreaction and thus cannot be determined by fluorescence detection.

The photoreaction extent is mostly independent of the solvent nature for most of the studied compounds, except for carbamazepine, ketoprofen and diazepam, for which the fluorescence response is clearly higher in MeOH. Another factor that leads to the choice of methanol as working solvent was the shorter life of PTFE photoreactors when using acetonitrile [19].

The photochemistry of most of the investigated PhACs has been deeply studied from different points of view, mainly photoreactivity, potential phototoxicity and photodegradation, but some photoderivatization publications can also be found [20–25]. Based on the literature, the identity of some photoproducts formed in the post-column photoderivatization reaction can be suggested as it is depicted in Table 2.

3.2. Analytical performance

Once methanol was chosen as the optimum solvent for the photoreaction of PhACs, the next step was to optimize the chromatographic separation of the mixture. Software developed in our research group named PREGA (Programmed Elutions by Genetic Algorithms) [15,17] facilitates this task, giving theoretical programmed elutions with binary gradients, from which the user can select the best one. The best gradient prepared by PREGA to solve this particular mixture of PhACs is detailed in Table 3.

One of the advantages of PREGA is the possibility to choose between different methods to build a fit for proposed empirical simulation models, instead of using theoretical equations [26] that assume a linear relationship between the retention factor and the organic modifier percentage. Chromatography practice shows that a multitude of compounds do not follow strictly such behaviour [27], and in these cases, the retention times will not be predicted correctly. Table 4 shows the excellent agreement between experimental and theoretical retention data obtained with PREGA (relative error is between 0.2 and 2.1%), changing the simulation algorithm when needed; the experimental chromatogram obtained for the standard mixture solution is also shown in Fig. 1.

Table 3
Off-line optimized multisegment gradient by means of PREGA2 V 3.0[a]

Modifier (%)	Step time (min)	Cumulative time (min)
40	0.0	0.0
44	14.0	14.0
48	7.7	21.7
52	0.6	22.3
56	0.0	22.3
60	0.1	22.4
64	0.0	22.4
68	7.2	29.6
72	0.0	29.6
76	0.0	29.6
80	10.4	40.0

[a] All individual steps were programmed with a Waters gradient curve 11 (equivalent to a vertical change).

The wavelength program set in the fluorescence detector to acquire analytical data after the post-column photochemical derivatization is depicted in Fig. 2. The excitation wavelength has been fixed at 230 nm during the whole analysis, because all of the pharmaceuticals studied absorbed light in this spectrum region. But, each PhAC has been detected at its maximum emission wavelength, except when the peaks elute so close that the wavelength change disturbs the baseline. In this case, a compromise value near the maxima affected has been selected. Fig. 2 shows a fluorescence chromatogram of the standard mixture obtained in the optimized conditions, where the baseline drift observed in the UV chromatogram (Fig. 1) is not appreciable, improving the integration accuracy. It should be pointed out that tolfenamic acid (peak 3 in figures) is not detectable by this method (no reaction to fluorescent products) and ibuprofen (peak 4) has too high detection limits (low sensitivity). Acetylsalicylic acid (peak 7) can be detected without or with photoderivatization, but the sensitivity is equivalent in both cases. Fluorescence response for naproxen (peak 5) decreases after the photochemical reaction, however, even after the reaction it has the best detection and quantification limits. Determination of the six compounds left is clearly better or only possible after the photochemical derivatization.

Four concentration levels injected in triplicate

Table 4
Relative errors between simulated and experimental retention data for the 10 PhAC mixture, detailing the selected simulation algorithm for each particular compound

Compound	Theoretical retention time (min)	Experimental retention time[a] (min)	Repeatability of experimental retention time[b]	Modelization algorithm	Relative error (%)
Acetylsalicylic acid	7.82	7.79	0.05	Least-squares fit	0.4
Carbamazepine	12.25	12.38	0.09	Least-squares fit	1.0
Dihydrocarbamazepine	14.48	14.55	0.10	Least-squares fit	0.5
Diazepam	25.26	25.51	0.12	Least-squares fit	1.0
Ketoprofen	26.93	26.54	0.23	Least-squares fit	1.5
Naproxen	28.62	28.68	0.06	Cubic spline	0.2
Bezafibrate	30.01	29.81	0.05	Cubic spline	0.7
Diclofenac	33.94	33.38	0.06	Cubic spline	1.7
Ibuprofen	35.04	34.33	0.06	Cubic spline	2.1
Tolfenamic acid	38.58	38.20	0.04	Cubic spline	1.0

[a] Mean of five injections.
[b] Standard deviation of five injections.

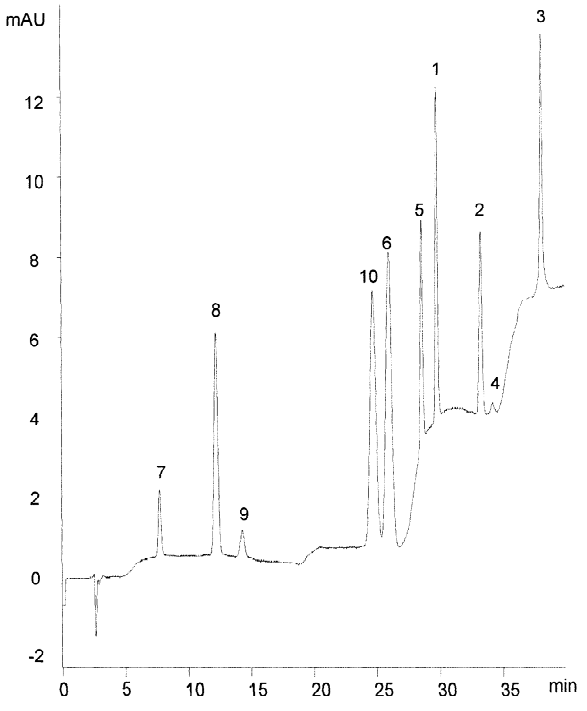

Fig. 1. Real chromatogram of a 5 μg/ml standard mixture solution obtained by off-line optimization with PREGA, without post-column UV irradiation (detection at λ=254 nm). 7, Acetylsalicylic acid; 8, carbamazepine; 9, dihydrocarbamazepine; 10, diazepam; 6, ketoprofen; 5, naproxen; 1, bezafibrate; 2, diclofenac; 4, ibuprofen; 3, tolfenamic acid.

were used to build the calibration curves, by means of an external standard method based on peak areas. Table 5 displays quality parameters of the analytical procedure for each compound: concentration range, regression equations, correlation coefficients (always higher than 0.99), limits of detection (from 2 to 120 ng/ml), limits of quantification (from 7 to 390 ng/ml), repeatability (intra-day precision) and reproducibility (inter-day precision). The utilization of solid-phase extraction to process real samples [28] will give an enrichment factor of one to two orders of magnitude depending on the nature of the sample matrix (83-fold in the experimental conditions selected for this work).

3.3. Application

The developed HPLC–PIF method was applied to a real sewage water sample previously submitted to the solid-phase extraction procedure described in the Experimental. The sample was first injected with no spiking, two of the investigated pharmaceuticals being found: acetylsalicylic acid and naproxen. Another aliquot of the sewage water sample was spiked at the low ng/ml level with the target compounds before the extraction step. The solid-phase extraction process used has been tested for the acidic compounds [28] giving recoveries from 90% to 115% for all of them. The extract was injected in triplicate and the obtained results are shown in Table

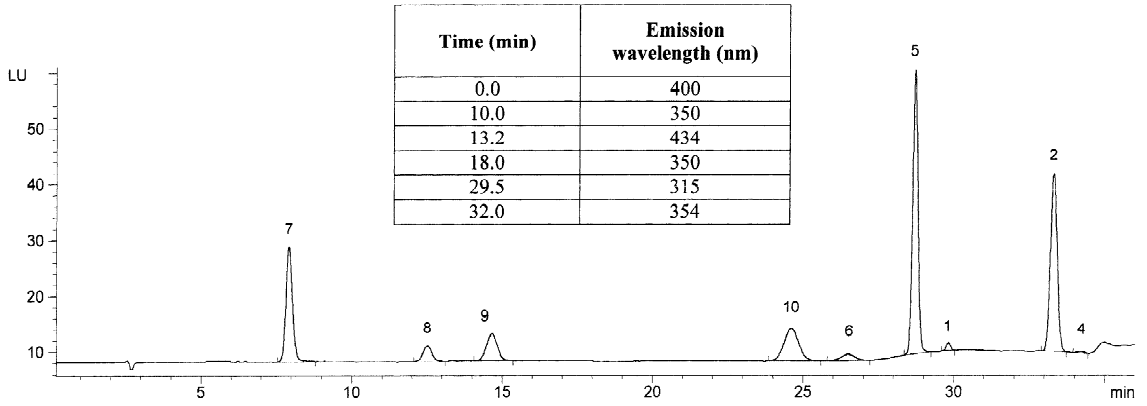

Fig. 2. Photochemically induced fluorescence (PIF) chromatogram of a 700 ng/ml standard mixture obtained in the optimized conditions with post-column UV irradiation. The fluorescence wavelength program is included. 7, Acetylsalicylic acid; 8, carbamazepine; 9, dihydrocarbamazepine; 10, diazepam; 6, ketoprofen; 5, naproxen; 1, bezafibrate; 2, diclofenac; 4, ibuprofen.

6, the naproxen concentration being corrected for that found in the blank (4.3±1.4 ng/ml). Fig. 3 shows the chromatograms of the original and the spiked sewage water sample.

Acetylsalicylic acid behaves in an unexpected way: the concentration found in the sewage water without addition (26.3±1.0 ng/ml) is higher than with addition (16.0±0.6 ng/ml). Two factors can affect this result: the high adsorption tendency of acetylsalicylic to every kind of solid surface and the easy interconvertibility between the pharmaceutical and its main metabolite: salicylic acid [29]. To avoid the first drawback, all glassware and syringes were always carefully cleaned. Thus, probably the second factor is responsible for the strange results in real samples, taking into account the linear behaviour of

Table 5
Figures of merit of the analytical procedure (without solid-phase extraction) for the selected pharmaceuticals

Compound (concentration range)	Regression equation	R^2	LOD (ng/ml)[a]	LOQ (ng/ml)[b]	Repeatability (RSD) ($n=6$)	Reproducibility (RSD) ($n=6$)
Acetylsalicylic acid (27–2069 ng/ml)	$y = 0.430x + 0.542$	0.9984	3	9	1.9	2.7
Carbamazepine (353–2259 ng/ml)	$y = 0.059x - 3.988$	0.9928	30	100	3.1	3.4
Dihydrocarbamazepine (129–2279 ng/ml)	$y = 0.138x - 2.654$	0.9999	13	45	1.9	1.9
Diazepam (128–2258 ng/ml)	$y = 0.196x - 1.678$	0.9972	18	61	5.2	5.2
Ketoprofen (447–2265 ng/ml)	$y = 0.043x + 0.280$	0.9980	66	219	1.8	1.8
Naproxen (26–2196 ng/ml)	$y = 0.839x - 33.715$	0.9970	2	7	3.3	3.8
Ibuprofen (701–5380 ng/ml)	$y = 0.012x + 0.554$	0.9998	120	390	8.7	8.2
Bezafibrate (341–5274 ng/ml)	$y = 0.022x + 1.462$	0.9995	42	139	3.7	4.4
Diclofenac (27–2244 ng/ml)	$y = 0.422x + 12.368$	0.9957	3	9	8.8	8.3

[a] The limit of detection was determined applying a signal-to-noise ratio of 3.
[b] The limit of quantitation was determined applying a signal-to-noise ratio of 10.

Table 6
Levels of six pharmaceutical active compounds in a spiked (10 ng/ml) sewage water sample

Compounds	Concentration (ng/ml)[a] mean value ($n=3$)
Carbamazepine	12.1±1.1
Dihydrocarbamazepine	9.8±0.2
Diazepam	13.0±0.8
Ketoprofen	9.4±0.6
Naproxen	9.4±0.9
Diclofenac	8.6±0.9

[a] The confidence interval for the concentration was calculated following the international organization for standardization (ISO) normative.

the standard itself (Table 5). For the remaining compounds, the good agreement between added and found quantities confirms the applicability of the proposed analytical method. Moreover, another aliquot of the same extract was processed in parallel by GC–MS to determine neutral compounds confirming the data obtained by means of the HPLC–PIF method.

4. Conclusions

The developed analytical method, reliable and inexpensive, enables the simultaneous determination of some acidic and neutral pharmaceutical active compounds in wastewaters: carbamazepine, dihydrocarbamazepine, diazepam, ketoprofen, naproxen, bezafibrate and diclofenac. Determination of acetylsalicylic acid is problematic in real wastewater samples; ibuprofen has high detection limits and tolfenamic acid cannot be determined by fluorescence detection.

Acknowledgements

Financial support from the Spanish DGICYT (project REN 2000-0984HIP) is acknowledged. C.G.

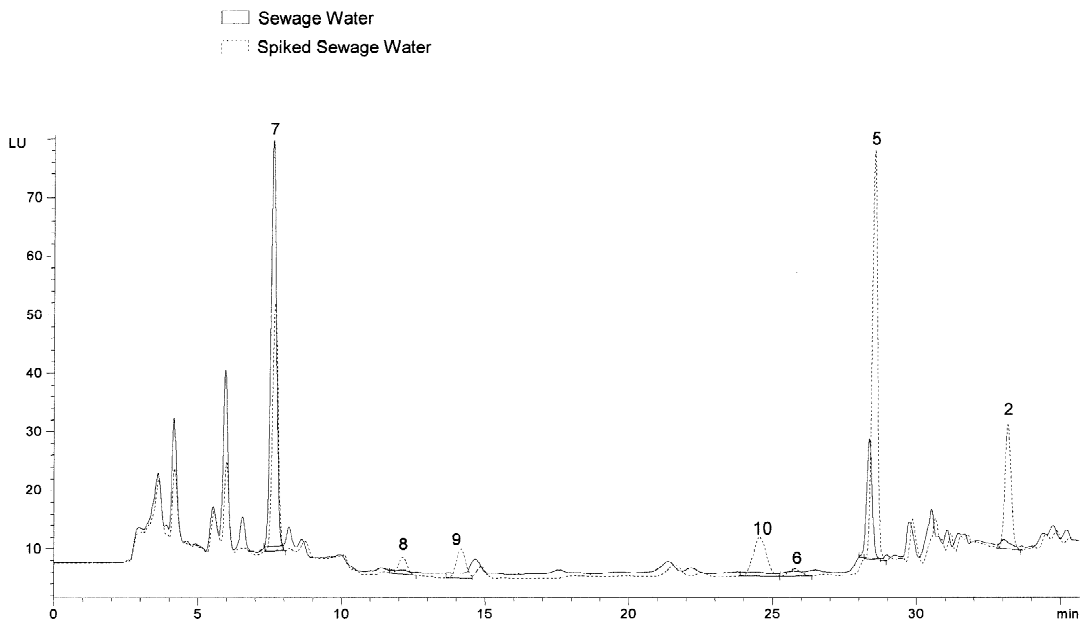

Fig. 3. Photochemically induced fluorescence (PIF) chromatogram of a spiked (10 ng/ml) real sewage water sample. The fluorescence wavelength program is the same as depicted in Fig. 2. 7, Acetylsalicylic acid; 8, carbamazepine; 9, dihydrocarbamazepine; 10, diazepam; 6, ketoprofen; 5, naproxen; 1, bezafibrate; 2, diclofenac; 4, ibuprofen.

is indebted to the Xunta de Galicia for a predoctoral grant.

References

[1] C.G. Daughton, A.T. Ternes, Environ. Health Perspect. 107 (1999) 907.
[2] T. Heberer, Toxicol. Lett. 131 (2002) 5.
[3] C. Zwiener, T. Glauner, F.H. Frimmel, J. High Resolut. Chromatogr. 23 (2000) 474.
[4] S.E. Jorgensen, B. Halling-Sorensen, Chemosphere 40 (2000) 691.
[5] T.A. Ternes, Trends Anal. Chem. 20 (2001) 419.
[6] S. Öllers, H.P. Singer, P. Fässler, S.R. Müller, J. Chromatogr. A 911 (2001) 225.
[7] R. Hirsch, T.A. Ternes, K. Haberer, A. Mehlich, F. Ballwanz, K.-L. Kartz, J. Chromatogr. A 815 (1998) 213.
[8] W. Ahrer, E. Scherwenk, W. Buchberger, J. Chromatogr. A 910 (2001) 69.
[9] M. Farré, I. Ferrer, A. Ginebreda, M. Figueras, L. Olivilla, L.L. Tirapu, M. Vilanova, D. Barceló, J. Chromatogr. A 938 (2001) 187.
[10] X.-S. Miao, B.G. Koenig, C.D. Metcalfe, J. Chromatogr. A 952 (2002) 139.
[11] T. Ternes, M. Bonerz, T. Schmidt, J. Chromatogr. A 938 (2001) 175.
[12] T. Heberer, J. Hydrol. 266 (2002) 175.
[13] C.E. Werkhoven-Goewie, W.M. Boon, A.J.J. Praat, R.W. Frei, U.A.Th. Brinkman, C.J. Little, Chromatographia 16 (1982) 53.
[14] A. Muñoz de la Peña, M.C. Mahedero, A.J. Bautista-Sánchez, J. Chromatogr. A 950 (2002) 287.
[15] J.A. Martínez-Pontevedra, New Developments in the Optimization of Chromatographic Separations by means of Genetic Algorithms, University of Santiago de Compostela, 2002, Ph.D. Thesis.
[16] M. Lores, C.M. García, R. Cela, J. Chromatogr. A 724 (1996) 55.
[17] R. Cela, J.A. Martínez, Quim. Anal. 18 (1999) 29.
[18] J.W. Birks, Chemiluminescence and Photochemical Reaction Detection in Chromatography, VCH, New York, 1989, Chapters 1 and 6.
[19] C. De Ruiter, J.F. Bohle, G.J. De Jong, U.A.Th. Brinkman, R.W. Frei, Anal. Chem. 60 (1988) 666.
[20] T. Poiger, H.-R. Buser, M.D. Müller, Environ. Toxicol. Chem. 20 (2001) 256.
[21] S. Encinas, F. Boscá, M.A. Miranda, Photochem. Photobiol. 68 (1998) 640.
[22] F. Boscá, M.L. Marín, M.A. Miranda, Photochem. Photobiol. 74 (2001) 637.
[23] P.J.G. Cornelissen, G.M.J. Beijersbergen van Henegouwen, Neth. Pharm. Weekbl. Sci. Ed. 2 (1980) 547.
[24] N. Canudas, F. Vargas, M.A. Miranda, Drug Res. 46 (1996) 694.
[25] J.K. Robson, D. Sharples, J. Pharm. Pharmacol. 36 (1984) 843.
[26] L.R. Snyder, J.W. Dolan, J.R. Gant, J. Chromatogr. 165 (1979) 3.
[27] M.M. Hsieh, J.G. Dorsey, J. Chromatogr. 631 (1993) 63.
[28] I. Rodríguez, J.B. Quintana, J. Carpinteiro, A.M. Carro, R.A. Lorenzo, R. Cela, J. Chromatogr. A 985 (2003) 265.
[29] T.T. Ternes, M. Stumpf, B. Schuppert, K. Haberer, Wasser 90 (1998) 295.