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Original Research

Using HPLC Method with DAD Detection for the Simultaneous Determination of 15 Drugs in Surface Water and Wastewater

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Abstract

Using HPLC method with DAD detection for the simultaneous determination of 15 pharmaceuticals from different therapeutic groups in surface water and wastewater was proposed. The determined drugs included the analgesic non-steroidal anti-inflammatory drugs (NSAIDs) paracetamol (PAR), metamizole (MTZ), aspirin (ASP), salicylic acid (SAL), ibuprofen (IBU), ketoprofen (KET), diclofenac (DIC), and naproxen (NAP); the corticosteroids dexamethasone (DEX) and prednisolone (PRE); the β-blockers carvedilol (CAR), metoprolol (MET), propranolol (PRO), and sotalol (SOT); and the anticonvulsant carbamazepine (CBM). Three solid-phase extraction (SPE) columns were examined for the pre-concentration of water samples: the Oasis HLB, NEXUS and Bond Elut ENV. The concentration level for which each method was validated in spiked water samples was 0.2 μg/L. The Oasis HLB column yielded the best recovery efficiency. Different HPLC columns were examined to achieve the best separations with the shortest possible time. The best column, C_{30} and was used for the determination of drugs from water samples.. The proposed method was applied to the analysis of water samples, mostly from rivers, and can be used for screening as a rapid and low-cost analytical tool. However, to confirm the positive findings MS techniques should be applied.

Keywords: pharmaceuticals, water samples, SPE, C_{30} column

Introduction

Pharmaceuticals in recent years have been distinguished as a group of "emerging contaminants" in environmental pollution [1]. Their presence in water samples has been caused mostly by emissions during manufacture, direct disposal of unneeded medicines, and human and animal excretion. The wastewater treatment plants (WWTPs) cannot eliminate most of these pollution and they are discharged mostly into rivers at concentrations of even μg/L [2, 3]. However, despite low concentrations found in different water samples, there is a lack of knowledge about potential effects of these pollutants on living smaller organisms in the ecosystem [4].

Low concentrations of pharmaceuticals in water samples do not allow direct injection to a chromatographic system. Therefore, some pre-concentration steps should be proposed that allows for the clean-up of samples. The most popular and effective way method of sample pre-concentration is solid phase extraction (SPE) with different commonly accessible sorbents. Liquid-liquid extraction has been of less importance to this kind of sample analysis and has not been widely used [5]. Nevertheless, the extraction of polar analytes as well as drugs with different properties (acidic, neutral, basic) using the SPE procedure is problematic in some particular cases. This is why new SPE columns

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have been proposed, mostly with polymeric sorbents that can allow us to improve recoveries for most of the compounds and allow sample extraction without pH adjustments [5, 6].

The most commonly used techniques for the determination of drugs from water samples are GC/MS or with tandem MS/MS detectors and LC/MS or LC/MS/MS [7-9]. The use of novel MS detectors allow us to increase the number of selected reaction monitoring (SRM) transitions, thus increasing confidence in the identification of analytes [9]. However, HPLC with DAD detection has also been used [10, 11]. Nevertheless, a DAD detector used without other detectors cannot give reliable identification, because DAD does not give structural information of the analytes. The most often used chromatographic columns for the determination of drugs from water samples are C_{18} [12-18]. Nevertheless, the columns with C_8 sorbent [19, 20], as well as RP-C_{16} Amide sorbents [10, 11], were also used. A literature survey reveals that C_{30} columns were not described for the determination of drugs in water samples.

The aim of this work was to develop a simple and rapid method for the simultaneous determination of 15 drugs from different therapeutic groups and their pre-concentration with the use of SPE for the best enrichment of all drugs. The proposed method could be applied for screening of water samples and for separation between negative and potential positive samples that should be confirmed in a subsequent step using MS techniques. The drugs investigated included sotalol (SOT), metamizole (MTZ), paracetamol (PAR), metoprolol (MET), aspirin (ASP), propranolol (PRO), salicylic acid (SAL), carvedilol (CAR), carbamazepine (CBM), prednisolone (PRE), dexamethasone (DEX), ketoprofen (KET), naproxen (NAP), diclofenac (DIC), and ibuprofen (IBU). The non-steroidal anti-inflammatory drugs (NSAIDs) and the β-blockers could be found in relatively high amounts (μg/L) [9, 21, 22], as well as carbamazepine in STP effluents [23]. The procedure for the pre-concentration and simultaneous determination of 15 drugs using HPLC-DAD with C_{30} column has not been described in literature, and the use of one chromatographic system to determine all drugs can save the expensive HPLC-grade chemicals. The method developed was applied for the analysis of water from different rivers and WWTP effluent and can be used for screening.

Materials and Methods

Chemicals and Reagents

Metamizole monohydrate was bought from Riedel-de Haën (Seelze, Germany) and paracetamol was purchased from Fluka BioChimika (Darmstadt, Germany). Diclofenac sodium, ibuprofen, aspirin, salicylic acid, carbamazepine, naproxen, ketoprofen, sotalol hydrochloride, metoprolol tartrate, propranolol hydrochloride, carvedilol, prednisolone, and dexamethasone were all bought from Sigma-Aldrich (Milwaukee, WI). HPLC grade acetonitrile, water, methanol, trifluoroacetic acid (TFA), and formic acid were bought from Merck (Darmstadt, Germany), and analytical grade methanol was purchased from POCH (Gliwice, Poland).

The stock solution of carvedilol, ibuprofen, and aspirin was prepared by dissolving 10 mg of the standard in 10 mL of analytical-grade methanol. Stock solutions of the remaining pharmaceuticals were prepared by dissolving 10 mg of the standard in 10 mL of a mixture of distilled water/methanol (50/50, *v/v*). Stock solutions, except aspirin, was stable for at least three months at -18ºC. The stock solution of aspirin were stable for two weeks. Working solutions were prepared daily by mixing the appropriate volume of each stock solution with a mixture of distilled water/methanol (90/10, *v/v*) and were stable for three months at 4ºC.

Instrumentation

The HPLC system included a quaternary gradient pump L-2130 (LaChrom Elite, Merck Hitachi), a L-2455 diode array detector (LaChrom Ultra, Merck Hitachi), analytical columns: LiChroCart Purospher®Star C_{18e} (250 mm x 3 mm, 5 μm particle size) (Merck), TSK-GEL ODS (150 mm x 4.6 mm, 5 μm) (Tosoh Bioscience), Chromolith® RP-18e (100 mm x 4.6 mm, monolithic) (Merck), Develosil® RPAQUEOUS-AR-5 C30 (250 mm x 4.6 mm, 5.8 μm) (Nomura Co.), and LiChrosorb RP-8 (250 mm x 4 mm, 7 μm) (Merck). For sample injections, a Rheodyne injector 7725i with 20 μL sample loop was used. The data was collected using EZChrom Elite software. The solid phase extraction (SPE) was performed using J.T. Baker spe-12G (Deventer, Netherlands).

The SPE Procedure

Three different SPE columns with polymeric sorbents were used for the sample extraction procedure for water samples. These columns included a NEXUS column (6 mL, 200 mg, Varian), a Bond Elut ENV column (6 mL, 500 mg, Varian), and an Oasis HLB column (6 mL, 500 mg, Waters). The extraction procedure was as follows: conditioning (except for non-conditioned NEXUS column) with 6 mL of methanol and 6 mL of distilled water at pH 7, at a flow rate of 1 mL/min. One-litre spiked distilled water with all 15 pharmaceuticals (0.2 μg each) was passed through the columns at a flow rate of approximately 6 mL/min. Then, each column was dried for 10 minutes. The analytes were eluted with 5 mL of methanol (at a flow rate of 1 mL/min), evaporated to dryness under a nitrogen stream, and reconstituted in 1 mL of methanol/distilled water (10/90, *v/v*). 20 μL of the obtained extracts were injected into the HPLC system. The proposed procedure was then examined on 1 L tap water samples spiked with all 15 drugs (0.2 μg each).

Water Samples

Water samples were collected from different locations in Poland and one from the Czech Republic, mostly from mainstream rivers. All samples were stored at 4ºC until analyzed.

Seven surface water samples were collected from the Wisła River from different cities: Skoczów, Kraków, Kazimierz, Warszawa, Bydgoszcz, and two from Toruń: before the Old City (Toruń 1) and after the Old City (Toruń 2). The remaining water samples were collected from different rivers: the Vltava (Prague), the Odra (Wrocław), the Brda (Bydgoszcz), the Warta (Zawiercie and Czestochowa), the Krzywa (Bielsko-Biała), the Kłodnica (Gliwice), the Potok Toszecki (Toszek), the Mała Panew (Zawadzkie), and the Troja (Nowa Cerekwia). One sample was collected from the wastewater treatment plant (WWTP) effluent from Bielsko-Biała. The samples were collected from September 2009 to December 2009 and processed using SPE procedure. Collected extracts were analyzed using the HPLC method.

Chromatographic Separations

Different chromatographic columns were examined to obtain the best chromatographic system for determining 15 drugs. The mobile phase used for all columns consisted of: 0.05% TFA in water or 0.1% formic acid (solvent A), methanol (B), and acetonitrile (C). For the optimization of separations and analysis time, 0.1% formic acid was used in exchange for 0.05% TFA. All separations were achieved at ambient temperature (c.a. 22ºC) using HPLC equipment with a DAD detector. The column eluent was analyzed at the characteristic detection wavelength for each drug in the absorbance range 200-450 nm.

Results and Discussion

Drugs Determination

Different chromatographic columns (LiChrosorb C_8, Purospher Star C_{18}, TSK-GEL ODS, Chromolith C_{18}, and Develosil C_{30}) were examined to achieve the best separations in the shortest possible analysis time. The gradient elution programme was comprised of three solvents: 0.05% TFA in water (A), methanol (B), and acetonitrile (C), which were used on each column. The C_8 column did not give satisfactory separations, even with the modifications of gradient. Three C_{18} columns from different manufacturers gave quite good separations with different analysis times; however, the best separations (as well as greater sensitivity) were achieved on the C_{30} column from Nomura Co. This column gave the best separations for all 15 drugs with analysis time under 30 minutes for gradient with 0.05% TFA. Nevertheless, the use of 0.1% formic acid as solvent A allowed us to shorten analysis time to 25 minutes with better separations. This gradient elution was finally used for the determination of drugs in water samples (Table 1, Fig. 1). The retention times, standard deviations, and analytical wavelengths are shown in Table 2.

Table 1. The best gradient elution programme: A – 0.1% formic acid in water, B – methanol, C – acetonitrile.

Time [min]	Solvent			Flow rate [mL/min]
	A [%]	B [%]	C [%]	
0.0	89	10	1	1.0
10.0	50	30	20	1.0
15.0	50	30	20	1.0
17.0	30	20	50	1.0
25.0	5	5	90	1.0
30.0	89	10	1	1.0

Recovery

The SPE columns chosen for the recovery efficiencies testing investigated pharmaceuticals were as follows: NEXUS, Bond Elut ENV, and Oasis HLB. All chosen columns have the polymeric sorbents that seemed to be the

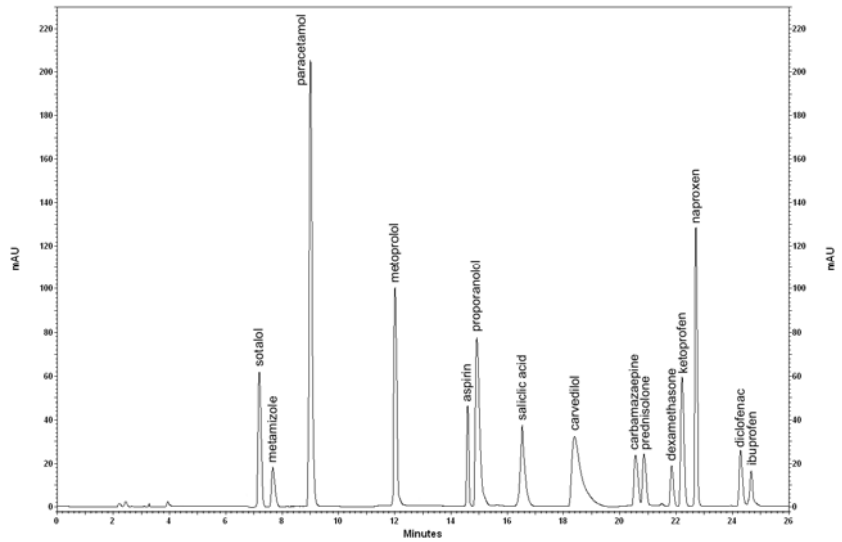

Fig. 1. The chromatogram of standards mixture containing 3 µg/mL for all drugs performed on the DAD detector.

Table 2. Wavelengths, retention times, standard deviation, and coefficient of variation (n=6).

Drug	Wavelength [nm]	Retention time [min]	Standard deviation [min]	Coefficient of variation [%]
Aspirin	229	14.625	0.019	0.11
Carbamazepine	215	20.564	0.034	0.20
Carvedilol	227	18.524	0.117	0.68
Dexamethasone	241	21.834	0.038	0.22
Diclofenac	275	24.258	0.052	0.30
Ibuprofen	225	24.666	0.043	0.25
Ketoprofen	254	22.181	0.035	0.20
Metamizole	259	7.693	0.024	0.14
Metoprolol	227	12.098	0.070	0.41
Naproxen	231	22.672	0.045	0.26
Paracetamol	241	9.017	0.075	0.44
Prednisolone	241	20.853	0.037	0.22
Propranolol	227	15.009	0.131	0.76
Salicylic acid	241	16.513	0.036	0.21
Sotalol	227	7.129	0.038	0.22

most suitable for sample pre-treatment and pre-concentration when dealing with pharmaceuticals of different properties (acidic, neutral, basic) [24]. Moreover, the use of polymeric sorbents without the necessity of pH adjustments provides some great advantages over the other sorbents and procedures [6].

The results for recovery efficiency are presented in Table 3. The spiking level for each pharmaceutical was 0.2 μg/L. The recoveries obtained were mostly on the same level (within the standard deviations), both in distilled and tap water for examined pharmaceuticals, respectively. The recovery efficiencies were over 90% for most of the drugs on each column, besides carvedilol and paracetamol for the NEXUS column (under 50%), paracetamol for ENV (under 50%), and metamizole for Oasis HLB (under 60%). The recoveries of over 50% for paracetamol and metamizole were found only in one publication [25]. The highest recovery efficiencies in tap water samples were achieved for the Oasis HLB column over 90% for most of the drugs, except for metamizole and paracetamol. However, satisfactory recovery efficiencies, most over 80%, were achieved on the other two NEXUS column (except for carvedilol, paracetamol, and salicylic acid), and ENV column (except for paracetamol and salicylic acid).

Linearity, Limit of Detection (LOD) and Limit of Quantification (LOQ)

Standard curves for examined pharmaceuticals were determined using linear regression:

$$y = ax + b$$

...where y is the peak area, a is the slope, x is the respective concentration, and b is the intercept. The parameters of the calibration curves for all pharmaceuticals are presented in Table 4. The limit of detection (LOD) and limit of quantification (LOQ) were determined using the parameters of standard curves and then recalculated including the appropriate recovery level of each drug in one-litre tap water. LOD and LOQ values were determined by the addition of standards after SPE procedure. The LOD values were determined as:

$$LOD=3.3s/a$$

...where s is the standard deviation of intercept (S_b) and a is the slope. The LOQ values were calculated as LOQ=3LOD. Low LOD and LOQ values were achieved using the C_{30} chromatographic column and DAD detector. For most of the examined drugs, LOD values were under 0.07 μg/L. For aspirin and carvedilol the LOD values were over 0.10 μg/L.

Application to Surface and Wastewater Samples

The HPLC method with DAD detection and SPE as a pre-concentration was applied to the simultaneous determination of 15 drugs in water samples. The drugs in real water samples were identified by comparison of the retention of standard solutions and absorption spectra, and by the addition of detected analyte to the extract, in order to check the

Table 3. Recoveries (n=6) for all pharmaceuticals in 1 L of spiked (0.2 μg/L) distilled and tap waters.

	Recoveries (SD) [%]					
	Distilled water			Tap water		
	ENV	NEXUS	HLB	ENV	NEXUS	HLB
Aspirin	83.2 (6.5)	72.4 (8.2)	88.2 (6.7)	77.4 (6.6)	70.7 (6.3)	85.8 (7.0)
Carbamazepine	95.5 (6.3)	97.7 (9.8)	104.5 (8.7)	95.0 (6.8)	99.8 (5.7)	97.9 (5.6)
Carvedilol	90.4 (5.5)	45.6 (5.3)	89.9 (7.1)	87.7 (6.4)	40.3 (8.8)	91.7 (8.3)
Dexamethasone	103.7 (6.7)	95.7 (8.7)	97.6 (8.9)	97.7 (9.7)	98.1 (9.3)	102.5 (9.4)
Diclofenac	105.1 (6.4)	104.6 (9.0)	100.0 (8.3)	98.5 (6.1)	96.0 (7.5)	100.1 (8.8)
Ibuprofen	101.6 (5.7)	102.1 (5.7)	96.2 (6.3)	91.4 (8.2)	96.9 (8.4)	97.9 (7.9)
Ketoprofen	100.5 (7.7)	104.0 (8.3)	102.3 (7.1)	99.6 (5.7)	97.0 (10.6)	98.0 (10.3)
Metamizole	81.3 (6.1)	88.2 (7.9)	58.9 (7.4)	79.0 (9.2)	82.3 (5.7)	64.0 (7.9)
Metoprolol	88.6 (8.0)	98.8 (6.6)	103.0 (7.3)	90.8 (9.7)	94.2 (10.7)	99.6 (9.6)
Naproxen	98.3 (8.7)	99.5 (5.4)	98.1 (6.4)	99.3 (8.8)	98.2 (5.5)	95.9 (7.6)
Paracetamol	38.0 (3.2)	37.3 (1.6)	69.0 (5.7)	34.1 (4.5)	38.2 (2.1)	65.9 (7.0)
Prednisolone	100.8 (4.2)	100.5 (8.5)	103.0 (7.3)	95.5 (9.5)	105.5 (9.6)	97.1 (8.5)
Propranolol	87.4 (9.1)	94.2 (11.6)	97.7 (9.5)	90.6 (12.5)	96.9 (11.5)	96.3 (6.2)
Salicylic acid	79.8 (8.3)	61.5 (6.3)	88.0 (9.0)	76.3 (9.3)	68.1 (4.2)	89.5 (9.1)
Sotalol	99.4 (10.5)	88.6 (10.1)	96.3 (5.3)	96.8 (11.0)	84.7 (7.6)	101.1 (6.8)

Table 4. Parameters of calibration curves, linearity ranges, and LOD and LOQ values.

Drug	Linear range [μg/mL]	Slope (a)	S_a	Intercept (b)	S_b	S_{xy}	R^2 (n=6)	LOD [μg/L]	LOQ [μg/L]
Aspirin	0.550-10	50,704	544	-13,311	2,535	4,607	0.9995	0.183	0.548
Carbamazepine	0.090-10	191,483	411	5,801	1,714	3,597	0.9999	0.029	0.086
Carvedilol	0.320-10	392,615	2,570	-128,711	11,794	21,114	0.9998	0.105	0.315
Dexamethasone	0.300-10	233,389	173	1,237	706	1,549	0.9999	0.010	0.030
Ibuprofen	0.190-10	299,442	4,800	13,816	5,532	6,435	0.9994	0.063	0.188
Ketoprofen	0.015-10	501,775	805	3,454	679	1,409	0.9999	0.004	0.013
Diclofenac	0.040-10	146,517	1,099	-1,523	516	925	0.9997	0.012	0.036
Metamizole	0.050-10	172,721	605	-879	556	1,068	0.9999	0.016	0.047
Metoprolol	0.110-10	186,658	478	-5,332	2,003	4,094	0.9999	0.035	0.106
Naproxen	0.025-10	1,042,352	2,471	24,412	2,083	4,324	0.9999	0.007	0.021
Paracetamol	0.020-10	818,137	294	-2,619	1,225	2,620	0.9999	0.007	0.020
Prednisolone	0.125-10	119,237	355	3,977	1,486	3,039	0.9999	0.041	0.122
Propranolol	0.110-10	504,707	1277	-34,493	5351	10,939	0.9999	0.034	0.103
Salicylic acid	0.030-10	213,189	138	1,559	576	1,225	0.9999	0.010	0.029
Sotalol	0.055-10	166,050	206	-5,886	866	1771	0.9999	0.017	0.051

Table 5. Concentrations and standard deviations (μg/L) of pharmaceuticals in different surface water samples (n=3).

	ASP	CAR	CBM	DEX	IBU	KET	DIC	MET	MTZ	NAP	PAR	PRE	PRO	SAL	SOT
WWTP B.-B.	-	-	-	-	-	0.052 (0.006)	0.128 (0.023)	0.234 (0.042)	0.152 (0.048)	0.082 (0.011)	-	-	< LOQ	0.227 (0.056)	-
Wisła Skoczów	-	-	-	-	-	-	0.074 (0.015)	-	-	-	< LOQ	-	-	-	-
Wisła Kraków	-	-	-	-	-	-	-	-	-	0.249 (0.041)	-	-	-	-	-
Wisła Kazimierz	-	-	-	-	-	-	-	-	-	0.162 (0.041)	-	-	-	0.243 (0.019)	-
Wisła Warszawa	0.368 (0.032)	-	-	-	-	-	-	-	-	0.079 (0.011)	-	-	-	0.113 (0.018)	-
Wisła Bydgoszcz	-	-	-	-	-	0.044 (0.005)	0.094 (0.016)	-	-	0.161 (0.016)	-	-	-	0.057 (0.008)	-
Wisła Toruń 1	-	-	-	-	-	-	0.064 (0.009)	-	-	0.133 (0.017)	-	-	-	0.159 (0.035)	-
Wisła Toruń 2	< LOQ	-	-	-	-	-	0.097 (0.009)	-	-	0.149 (0.030)	0.043 (0.006)	-	-	-	-
Vltava Prague	0.307 (0.058)	-	0.112 (0.022)	-	-	0.116 (0.018)	0.104 (0.015)	-	-	0.157 (0.030)	0.050 (0.007)	-	< LOQ	0.475 (0.059)	-
Odra Wrocław	0.733 (0.146)	-	-	-	-	0.258 (0.024)	0.429 (0.038)	-	0.902 (0.194)	0.128 (0.018)	0.021 (0.002)	-	-	0.205 (0.026)	-
Warta Zawiercie	-	-	-	-	-	-	-	-	-	0.095 (0.005)	-	-	< LOQ	0.125 (0.019)	-
Warta Częstoch.	-	-	-	-	-	-	0.277 (0.046)	-	-	0.143 (0.016)	0.073 (0.008)	-	-	-	-
Brda Bydgoszcz	-	-	-	-	-	-	0.042 (0.007)	-	-	0.143 (0.022)	-	-	< LOQ	-	-
Kłodnica Gliwice	-	-	-	-	-	-	0.057 (0.008)	-	-	0.753 (0.074)	-	-	-	-	-
Krzywa B.-B.	-	-	-	-	-	-	0.174 (0.033)	-	-	0.040 (0.010)	-	-	-	-	-
Mała Panew Zawadzke	-	-	-	-	-	-	-	-	-	0.088 (0.014)	-	-	-	-	-
Troja Nowa Cerekwia	-	-	-	-	-	-	0.186 (0.024)	-	-	0.187 (0.035)	-	-	-	0.154 (0.038)	-
Potok Toszecki Toszek	-	-	-	-	-	-	0.305 (0.037)	-	-	-	-	-	-	-	-

retention time and peak shape. Naproxen and diclofenac were present in almost every tested water sample at concentrations mostly under 0.31 μg/L (Table 5). Higher concentrations were found in the Odra from Wrocław (for diclofenac) and in the Kłodnica from Gliwice (for naproxen). Salicylic acid was found in nine water samples (concentrations ranged from 0.057-0.475 μg/L), paracetamol in five samples (concentrations ranged from 0.021-0.073 μg/L), and ketoprofen in four samples (concentrations ranged from 0.044-0.258 μg/L). Other drugs were found in single water samples, beside carvedilol, dexamethasone, ibuprofen, prednisolone, and sotalol, which have not been found in any of the water samples. Eight drugs were found in the Vltava river from Prague and seven in WWTP effluent from Bielsko-Biała and the Odra from Wrocław. In the Vltava river most drugs were present at concentrations over 0.1 μg/L and two (aspirin and salicylic acid) over 0.3 μg/L. Relatively high concentrations were found in the Odra from Wrocław for aspirin and metamizole – over 0.7 μg/L. The high concentrations of some drugs in those rivers could be caused by the presence of the WWTPs in the nearest locations. In the remaining water samples the presence of two

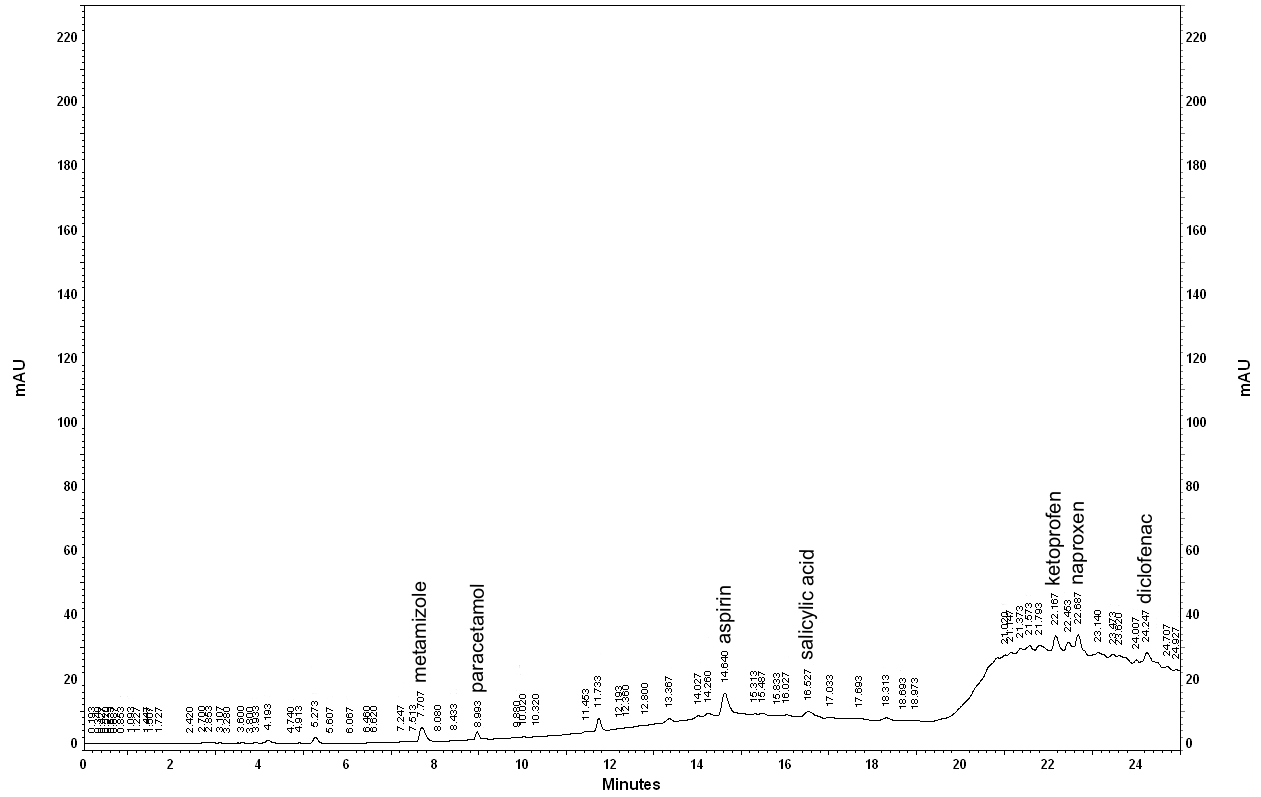

Fig. 2. The chromatogram of the extract from Odra River (Wrocław) after SPE procedure.

to five drugs was confirmed, beside some smaller rivers or locations without any WWTPs in the nearest area where only single drugs were found (Potok Toszecki from Toszek, Mała Panew from Zawadzkie, and Wisła from Kraków). The concentrations of all determined pharmaceuticals ranged from 0.021-0.902 μg/L, but in most of the samples drugs were found in low ng/L levels and only in a few cases were higher than 0.3 μg/L. Nevertheless, in order to confirm the positive findings, MS techniques, which allow us to have reliable identification, should be applied. The chromatogram of sample extract from the Odra river (Wrocław) is presented in Fig. 2.

Conclusions

A rapid and simple method has been developed for the simultaneous determination of 15 pharmaceuticals using HPLC method with DAD detection. The C_{30} chromatographic column allowed us to achieve good separation for examined drugs in 25 minutes. The other advantage of the C_{30} column were lower LOD and LOQ values, which made possible the screening of selected drugs in water samples.

The SPE columns tested revealed good recovery efficiency (over 80% for most of the drugs) and can be used as a pre-treatment and pre-concentration step. However, the best polymeric column, from those examined, was the Oasis HLB column with the highest recovery efficiency for selected drugs. All columns were used for the real water samples.

The analysis of many rivers, mostly from different locations in Poland, showed that many of the drugs selected were present in those waters. Nevertheless, the concentrations were at mostly low, several tens ng/L, levels.

In conclusion, the HPLC method with C_{30} column and DAD detector can be used for screening and detecting selected drugs in water samples, and can be a useful tool for rapid and low-cost monitoring of water pollution in rivers and WWTPs effluents. The method can also be used in laboratories that do not possess expensive LC-MS/MS equipment. DAD does not give structural information for the reliable identification of the analytes; therefore, positive findings should be confirmed by additional analysis using MS to avoid reporting false positives.

Acknowledgements

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