Food & Environmental

Analysis of Endocrine Disruptors, Pharmaceuticals, and Personal Care Products in River Water

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Overview

Endocrine disrupting compounds (EDC) encompass a wide range of pollutants, including pharmaceuticals and personal care products (PPCP), pesticides, and steroids to name a few. EDC are thought to disrupt the endocrine function of mammals and fish, and as a result their biological effects are a growing concern. In order to properly assess the effects of these compounds on our environment, it is necessary to accurately monitor their presence. A method is presented for analyzing up to 100 EDC and PPCP compounds using LC-MS/MS. This method is a straight forward approach for the quantitation and identification of these compounds with excellent sensitivity and ruggedness.

Introduction

A wide range of endocrine disrupting compounds were determined in river water sampled near a water treatment plant. Compound levels upstream and downstream from the plant were quantified and compared. A combination of Solid Phase Extraction (SPE) and LC-MS/MS analysis in Multiple Reaction Monitoring (MRM) mode achieved low parts per trillion detection limits across multiple compound classes with a linear range of 3-4 orders of magnitude for all compounds.

Both positive and negative ionization modes were utilized. APCI and ESI ionization techniques were investigated using the DuoSpray™ ionization source. Electrospray ionization with polarity switching on the Turbo V™ source yielded the broadest coverage across compound classes. Two MRM transitions were monitored for each compound to achieve sensitive and specific quantitation as well as ion ratio identification. A total of 160 MRM transitions were monitored on a chromatographic time scale.

Two sets of river water samples were collected from a rural river (River 1) and an urban city river (River 2) both upstream and downstream of a sewage treatment plant in North America. The upstream and downstream samples for these two areas were then compared to determine environmental impact

Experimental

An AB SCIEX API 4000™ LC/MS/MS System equipped with a Shimadzu Prominence autosampler and binary LC pump was used. Ionization was achieved by Electrospray Ionization (ESI) and Atmospheric Pressure Chemical Ionization (APCI) using the DuoSpray™ and Turbo V™ ionization sources. All compounds were monitored using two Multiple Reaction Monitoring (MRM) transitions per compound. Each MRM transition had a dwell time of 5ms/sec. The most sensitive, first MRM transition was used for quantitation while the second MRM transition was used for qualitative identification using ion ratio determination. See Figure 3 and 4 for examples. The total cycle time for the method with polarity switching was approximately 3 seconds. Instrument conditions were as follows: CUR 20, CAD 7, GS1 75, GS2 65, IS 5000, and TEM 600. Chromatography was performed on a Phenomenex Ultracarb (20) C18 250 X 4.5 mm 5 µm reverse phase column at 30°C. The total flow rate was 600 µL/min and used a gradient starting at 95% A and held for 1 minute before ramping to 50% over 24 minutes. At a run time of 25 minutes the gradient was then ramped to 4% A over 10 minutes and held for

an additional 10 minutes. Re-equilibration time was 10 minutes for a total run time of 55 minutes. Eluent A was 0.01% formic acid in water and eluent B was 0.01% formic acid in acetonitrile.

Laboratory control samples and matrix spike samples were prepared to monitor extraction efficiency. After conditioning with 20 mL of methanol followed by 40 mL of water, 1.0 L of sample was loaded onto the cartridge at a flow rate of 25.0 mL/min. After loading, nitrogen was then pulled through the cartridge for 15 minutes to allow for sample drying. Then 5.0 mL of acetonitrile was added to the SPE bed and allowed to stand for 15 minutes. The SPE cartridges were then eluted at gravity flow into a 12 mL amber vial. Finally, water was added to the extract to a final volume of 10.0 mL. Samples were kept at 4°C ± 1°C until analysis. Figure 1 shows a schematic of the sample preparation procedure.

Table 1. Compound list including MRM transitions (positive polarity)

		Quantifier		Qualifier				Quantifier		Qualifier	
Compound	**Type**	**Q1**	**Q3**	**Q1**	**Q3**	**Compound**	**Type**	**Q1**	**Q3**	**Q1**	**Q3**
Acetaminophen	Analgesic	152	110	152	65	*Estradiol*	Estrogen	255	159		
Ketoprofen	Analgesic	255	105	255	77	*Ethinylestradiol*	Estrogen	271	133		
Codeine	Analgesic	300	215	300	165	*17α-Hydroxy-progesterone*	Estrogen	331	97		
Hydrocodone	Analgesic	300	199	300	171	*Progesterone*	Estrogen	315	109	315	97.
Androstenedione	Androgen	287	97	287	97	*Equilin*	Estrogen replacement	269	211	269	157
Testosterone	Androgen	289.5	97	289	109	*Diethylstilbestrol*	Estrogen replacement	269	135	269	107
Dilantin	Anti-convulsant	253	182			*TCEP*	Flame retardant	285	223	285	239
Meprobamate	Anti-anxiety	219	158	219	115	*Simazine*	Herbicide	202	132	202	124
Sulfadiazine	Antibiotic	251	92	251	65	*Isoproturon*	Herbicide	207	72		
Sulfamethoxazole	Antibiotic	254	92	254	108	*Chlorotoluron*	Herbicide	213	72	213	140
Sulfathiazole	Antibiotic	256	156	256	92	*Atrazine*	Herbicide	216	174	216	68
Sulfamerazine	Antibiotic	265	92	265	108	*Chloridazon*	Herbicide	222	104	222	92
Sulfamethizole	Antibiotic	271	156	271	92	*Propazine*	Herbicide	230	146	230	188
Sulfamethazine	Antibiotic	279	92	279	124	*Diuron*	Herbicide	233	72	233	46
Sulfachlorop-yridazine	Antibiotic	285	92	285	65	*Hexazinone*	Herbicide	253	171	253	85
Trimethoprim	Antibiotic	291	230	291	123	*Bromacil*	Herbicide	261	205	261	188
Sulfadimethoxine	Antibiotic	311	156	311	92	*Metazachlor*	Herbicide	278	134	278	210
Ciprofloxacin	Antibiotic	332	288			*Metolachlor*	Herbicide	284	252	284	175
Penicillin G	Antibiotic	335	176	335	217	*DEET*	Insect repellant	192	119		
Amoxicillin	Antibiotic	366	114	366	208	*Bezafibrate*	Lipid regulator	362	139	362	121
Lincomycin	Antibiotic	407	126	407	359	*Diazepam*	Muscle-relaxant	285	154	285	193
Doxycycline	Antibiotic	445	428	445	339	*Norethisterone*	Ovulation Inhibitor	299	109	299	91
Tetracycline	Antibiotic	445	410	445	154	*Theophylline*	Stimulant	181	124	181	96
Oxytetracycline	Antibiotic	461	426	461	443	*Theobromine*	Stimulant	181	138	181	110
Chlortetracycline	Antibiotic	479	462	479	154	*Caffeine*	Stimulant	195	138	195	110

Compound	Type	Q1	Q3	Q1	Q3	Compound	Type	Q1	Q3	Q1	Q3
Virginiamycin	Antibiotic	526	109	526	67	*Oxybenzone*	Sunscreen	229	151	229	105
Monensin	Antibiotic	694	461	694	479	*Sildenafil*	Virility regulator	475	100	475	283
Erythromycin	Antibiotic	735	158	735	576	*Vardenafil*	Virility regulator	490	72	490	114
Roxithromycin	Antibiotic	838	679	838	158	*Salicylic Acid*	Skin care, acne	139	61	139	79
Tylosin	Antibiotic	917	174	917	772	*Cotinine*	Nicotine metabolite	177	80	177	98
Meclocycline Sulfosalinicyclate	Antibiotic	477	460			*4-Aminoantipyrine*	Aminopyrine metabolite	204	56		
Sulfadimethoxine	Antibiotic	311	156			*Ketorolac*	Anti-inflammatory	256	105	256	77
Sulfachloro-Pyridazine	Antibiotic	285	156			*Fenoprop*	Herbicide	269	181	269	85
Norifloxacin	Antibiotic	320	276			*Meclofenamic acid*	Anti-inflammatory	296	278	296	243
Enroflofacin	Antibiotic	360	316			*Piroxicam*		332	95	332	121
Fluoxetine	Antidepressant	310	148			*Nifedipine*	Dihydropyridine calcium channel blocker	347	315		
Carbamazepine	Anti-seizure	237	194	237	193	*Indomethacin*	Anti-inflammatory	358	139	358	75
Pentoxifylline	Blood viscosity reducing agent	279	181	279	138	*Diatrizoate*	Radiocontrasting agent	615	361		

Table 1 (continued). Compound list including MRM transitions (negative polarity)

		Quantifier		Qualifier				Quantifier		Qualifier	
Compound	Type	Q1	Q3	Q1	Q3	Compound	Type	Q1	Q3	Q1	Q3
Acetylsalicylic acid	Analgesic	179	137	179	93	*Estrone*	Estrogen	269			
Ibuprofen	Analgesic	205	161	205	159	*Estradiol*	Estrogen	271			
Naproxen	Analgesic	229	183	229	155	*Estriol*	Estrogen	287			
Warfarin	Anti-coagulant	307	161	307	250	*Ethinylestradiol*	Estrogen	295			
Diclofenac	Anti-arthritic	294	250	294	214	*Tetrabromo-bisphenol A*	Flame retardant	443	103	443	239
Carbadox	Antibiotic	261	122			*2,4-D*	Herbicide	219	161	219	125
Triclosan (Irgasan)	Antibiotic	287	35			*Clofibric acid*	Metabolite of lipid regulator	213	127	213	85
Chloramphenicol	Antibiotic	321	257	321	152	*Iopromide*	X-ray contrast agent	790	127		
Gemfibrozil	Anti-cholesterol	249	121			*2,4-Dichloro-benzoic acid*		189	101	189	145

Results and Discussion

Quantitative optimization in Analyst® Software was utilized to streamline method development for this large list of compounds. The final method contains the analytes and MRM transitions listed in Table 1.

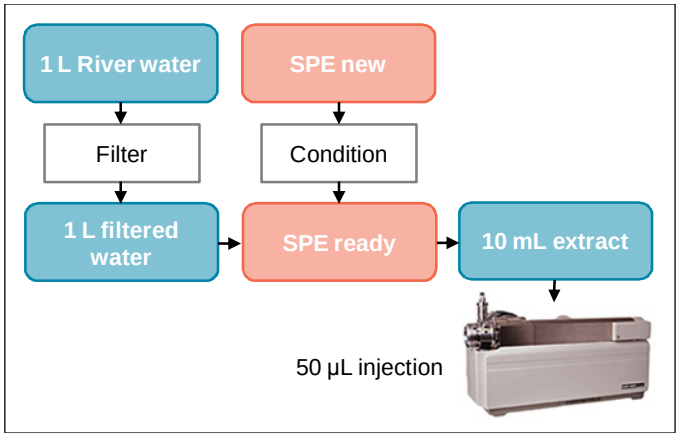

Figure 1. Sample preparation procedure for solid phase extraction

A calibration curve was prepared in water/acetonitrile (1/1) at the following concentrations, 0.2, 0.4, 1.6, 3.1, 6.3, 25, and 100 ng/mL. Linearity was achieved for all monitored compounds. Examples of linearity are shown in Figure 4.

Samples were collected and extracted using the procedure described above. To monitor the extraction efficiency of the sample preparation a laboratory control sample (LCS) was prepared. This sample consisted of tap water being free of all target compounds. This water was then spiked with all of the target analytes. The final concentration of all analytes in the LCS was 20 ng/L.

Recoveries in the LCS ranged from 30 to 115% across all compounds. Based on these results, it was determined that the sample preparation procedure used is adequate for a full screen of the compounds reported. For future work, once the final sample list is determined, surrogate compounds will be selected for each compound class to closely monitor the sample preparation procedure. If possible, a deuterated surrogate will be chosen for each compound class and will only be used to monitor sample preparation efficiency and not instrument variability. It has been shown in previous work that an internal standard, used to monitor instrument variability, may introduce more error in the quantitation results of this large list of compounds.

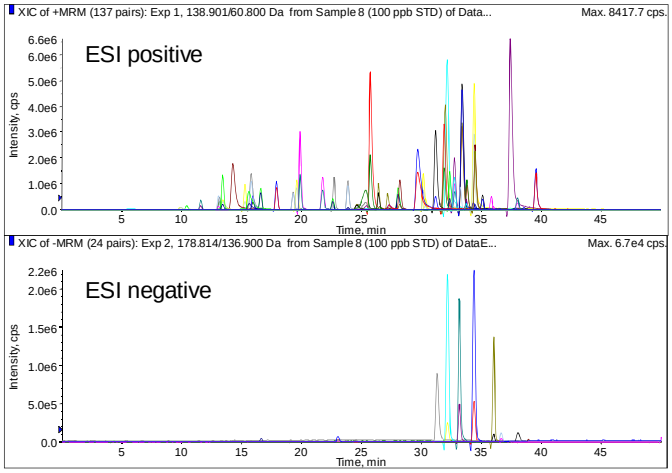

Figure 2. Polarity switching is utilized to encompass a large list of analytes – 100 ng/mL standard injection

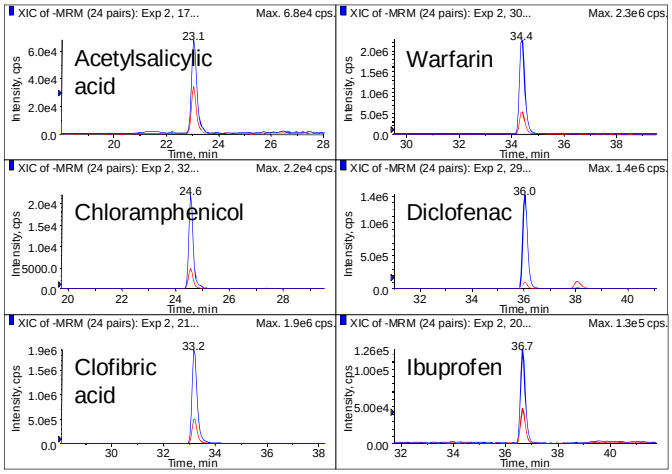

Figure 3. Overlay of two MRM transitions used for six selected analytes. The most sensitive transition in blue for each analyte is used for quantitation. The area ratio of the second MRM in red is used for identification

Table 2. Lower Limits of Quantitation (LLOQ) of selected analytes

Analyte	LLOQ (ng/L) ppt	Analyte	LLOQ (ng/L) ppt
DEET	11.6	*Propazine*	0.46
Ketoprofen	3.3	*Progesterone*	3.9
Sulfadiazine	13.0	*Trimethoprim*	6.4
Fluoxetine	280	*Androstenedione*	4.7
2,4-D	2.3	*Erythromycin*	14.0

Result of both River 1 and River 2 showed detection of several compound classed. As expected, a significantly larger number of compound classes were detected in the urban river (River 2). Lower limit of quantitation (LLOQ) was determined to be the level at which a peak is detected with a signal to noise of at least 10:1. This level was theoretically determined using the standards and assuming linearity down to zero concentration. Table 2 shows a selected list of compounds and their LLOQ. All compounds had LLOQ in the sub part per billion (ppb) range.

Detection of each analyte was identified using the area ratio of two MRM's collected. For River 2, Erythromycin, Ketorolac, and Meprobamate along with 20 other compounds were detected in either the upstream and downstream samples. Ion ratios on the samples were compared to the ion ratios measure on the standards for compound identification. See Figure 5. Final results of River 1 and River 2 are shown in Table 3.

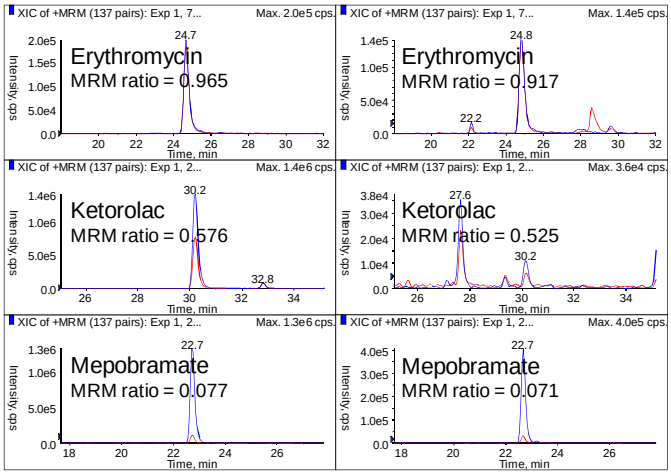

Figure 4. Measured ion ratios of three select analytes (Erythromycin, Ketorolac, and Meprobamate) in the standard and the upstream and downstream sample of river 2, respectively. Despite low level detection like that seen for Ketorolac in the River 2 sample, the ion ratios of the two MRM transitions still confirm with the standard. MRM ratio calculation was done automatically using the Analyst® Reporter software

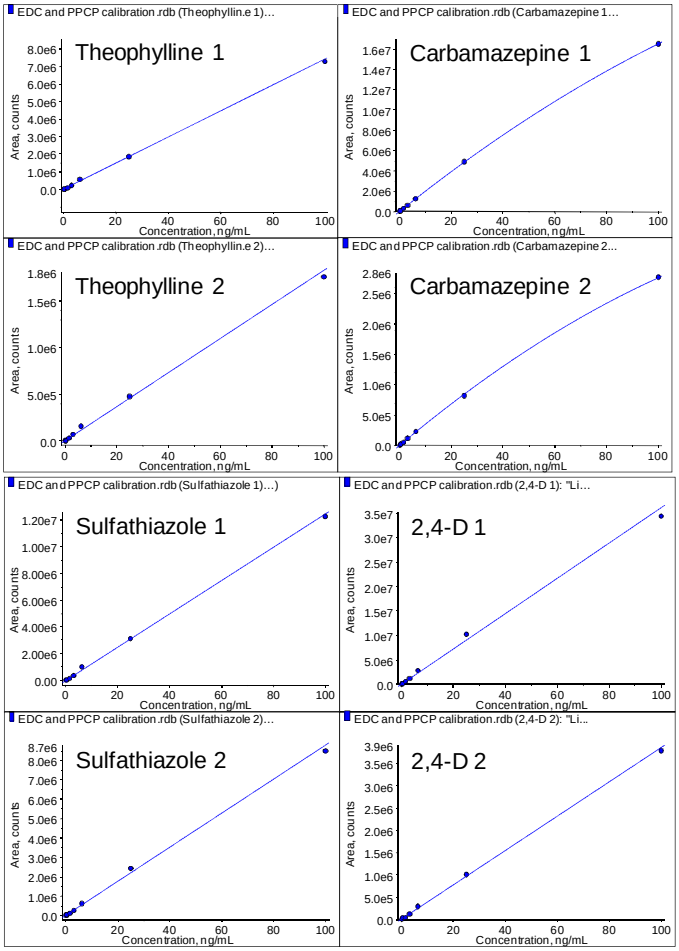

Figure 5. Example calibrations for selected analytes

Table 3. Eight EDC and PPCP compounds were detected in the samples of river 1. Despite the rural nature of this location, low level of these widely used herbicides and pharmaceuticals are still detected. As expected a larger list of compounds were detected in the river 2 samples because of it urban origin. In total 23 EDC and PPCP compounds were founds at low to mid part per trillion (ppt) levels. These results show that it is possible to scan for a functionally diverse set of compounds in one analysis and achieve high sensitivity and accurate quantitation

Analytes in River 1	Concentration (ng/L) upstream	Concentration (ng/L) downstream	Analytes in River 2	Concentration (ng/L) upstream	Concentration (ng/L) downstream
Erythromycin	3.08	53.5	*Oxybenzone*	ND	6.25
Carbamazepine	65.5	152	*Bromacil*	ND	7.40
2,4-D	ND	9.35	*Diazepam*	ND	0.388
DEET	1.49	7.67	*Warfarin*	ND	0.930
Sulfamethoxazole	13.2	13.3	*Triclosan (Irgasan)*	5.90	31.4
Caffeine	41.0	23.5	*Codeine*	17.1	77.5
Ciprofloxacin	3.81	ND	*Diuron*	1.38	4.35
Cotinine	2.05	ND	*Trimethoprim*	58.5	123
			Lincomycin	1.53	3.02
			Carbamazepine	870	1305
			DEET	24.0	29.9
			Ketorolac	2.49	3.06
			Meprobramate	85.5	97.5
			Atrazine	1.08	0.88
			Sulfamethoxazole	95.5	74.5
			Pentoxifylline	6.60	3.39
			Caffeine	57.0	13.5
			Cotinine	14.4	ND
			Simazine	1.01	ND
ND not detected			*Norethisterone*	1.15	ND
Increases by more than 2x			*Erythromycin*	135	ND
Within ± 2x			*Tylosone Tartrate*	4.28	ND
Decreases by more than 2x			*2,4-D*	3.24	ND

Summary

LC-MS/MS analysis has been shown to be a highly feasible approach for the monitoring of a large set of endocrine disrupting compounds spanning multiple categories and chemical classes. MRM mode allows for the determination of these compounds in river water matrix with low detection limits and high selectivity.

Additional compound identification was achieved by the simultaneous monitoring of a second MRM transition and calculation of the corresponding ion ratio, which was done automatically by Analyst Reporter™ software. Electrospray ionization with polarity switching was found to be the most suitable approach.

References

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