Utilization of GCxGC-TOFMS as a Broad-Spectrum Analysis for Endocrine-Disrupting Compounds in Urban and Rural Watersheds

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1. Introduction

Endocrine-disrupting compounds (EDCs) encompass a variety of chemical classes, including drugs, pesticides, polymer additives, coatings materials, personal consumer products, industrial by-products, and pollutants. There is worldwide concern over long-term environmental exposure to EDCs leading to serious health effects, including a range of reproductive problems such as reduced fertility, male and female reproductive abnormalities, skewed male/female sex ratios, brain and behavior problems, impaired immune functions, and various cancers.

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This application note presents a robust, broad-range analysis for the detection of EDCs in impacted natural waters using comprehensive two-dimensional gas chromatography–time-of-flight mass spectrometry (GCxGC-TOFMS). GCxGC facilitates enhanced detection, chromatographic resolution, and peak capacity, while TOFMS allows the fast acquisition (up to 500 spectra per second) necessary to successfully acquire the data density needed to fully characterize low levels of targeted and untargeted compounds in complex samples. A reference standard of 108 known endocrine-disrupting compounds was prepared and analyzed. Methods for solid-phase extraction and GCxGC-TOFMS analysis were developed. GCxGC-TOFMS analysis was conducted on multiple water samples from a rural and urban Midwestern U.S. watershed. Extraction of 1.0 liter water samples was conducted using Supel-Select HLB SPE cartridges (Supelco Analytical, Sigma-Aldrich) designed to recover a wide range of analytes. Subsequent analysis was conducted by GCxGC-TOFMS, and the data was processed using ChromaTOF's reference feature. The results are reported as targeted and untargeted analytes found. This application note presents a practical, robust, sensitive, and reliable procedure for the detection of EDCs in urban and rural watersheds.

2. Results

A total of 13 duplicate one-liter water samples were analyzed from six different urban and rural locations along a Midwestern river watershed. The results from the analysis indicated 102 chemicals detected, including endocrine disruptors, personal care products, pharmaceuticals, and other industrial pollutants. The data show that out of 26 GCxGC-TOFMS analyses, 81% of the 102 chemicals detected were found at least five times.

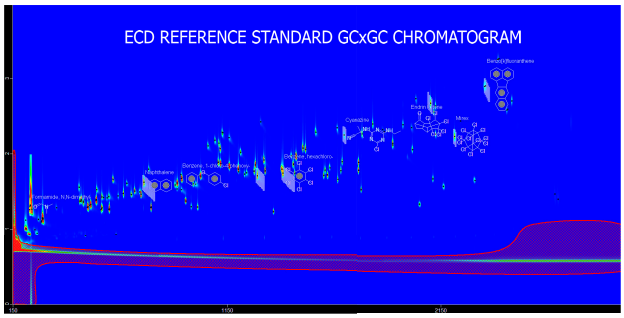

Figure 1. The two dimensional contour plot chromatogram above shows the 108 component endocrine disruptor reference standard used to develop the SPE extraction procedure and GCxGC-TOFMS method. The on-column concentration for each component is 5 nanograms.

Table 1 shows the results for 13 one-liter water samples prepared with solid-phase extraction and analyzed by GCxGC-TOFMS. A total of 102 chemical compounds were detected from six point sources in a Midwestern watershed. The compounds detected matched the reference standard with at least a 60% library match similarity. Endocrine-disrupting compounds (EDCs) encompass a variety of chemical classes, including pharmaceuticals, pesticides, polymer additives, coatings materials, personal care products, flame retardants, plasticizers, industrial by-products and miscellaneous pollutants.

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Table 1. Summarized results from duplicate analyses of 13 water samples.

	UNTARGETED AND TARGETED ANALYTES DETECTED	COUNT # OF TIMES DETECTED	CHEMICAL TYPE
1	Phenol (CAS)	23	EDC
2	Bis(2-chloroethyl) ether	1	EDC
3	1,4-Dichlorobenzene	21	EDC
4	1,3-Dichlorobenzene	9	EDC
5	1,2-DICHLOROBENZENE	3	EDC
6	Benzylalcohol P194	19	EDC
7	2-Methylphenol	22	EDC
8	3-Methylphenol	24	EDC
9	Nitrobenzene	7	EDC
10	Isophorone * Solvent	26	EDC, INDUSTRIAL CHEMICAL
11	2-Nitrophenol	11	EDC
12	2,4-Dimethylphenol	11	EDC
13	2,4 Dichlorophenol	7	EDC
14	Benzene, 1,2,4-trichloro- (CAS)	9	EDC
15	Naphthalene	25	EDC, PAH
16	D-Glucitol, 1,4:3,6-dianhydro-2,5-di-O-methyl- * used in Personal Care Products; cosmetics	7	PCP
17	Naphthalene, 2-methyl-	20	EDC, PAH
18	Naphthalene, 1-methyl-	22	EDC, PAH
19	1,3-Isobenzofurandione (CAS) * RUBBER RETARDER, CURING AGENT	14	EDC, INDUSTRIAL CHEMICAL
20	Propofol	6	PCP, PHARMACEUTICAL
21	2,4,6-Trichlorophenol	6	EDC
22	2,4,5-Trichlorophenol	6	EDC
23	à DAMASCONE	12	FOOD, FLAVOR, FRAGRANCE
24	2-Chloronaphthalene	7	EDC, PAH
25	Phenol, 4-chloro-3,5-dimethyl- (CAS)	6	EDC
26	á-Patchoulene	7	FOOD, FLAVOR, FRAGRANCE
27	Naphthalene, 1,7-dimethyl-	7	EDC, PAH
28	1H-3a,7-Methanoazulene, 2,3,4,7,8,8a-hexahydro-3,6,8,8-tetramethyl-, [3R-(3à,3aá,7á,8aà)]-	5	EDC
29	Acenaphthylene (CAS)	14	EDC, PAH
30	Acenaphthene	21	EDC, PAH
31	BUTYL HYDROXY TOLUENE * BHT ANTIOXIDANT	23	EDC, FOOD, FLAVOR, FRAGRANCE
32	Tributyl phosphate * SOLVENT & PLASTICIZER	19	EDC, INDUSTRIAL CHEMICAL
33	Benzeneacetic acid, ethyl ester * flavor fragrance	25	EDC, FOOD, FLAVOR, FRAGRANCE
34	à-N-METHYL IONONE	6	EDC, FOOD, FLAVOR, FRAGRANCE
35	TRIPROPYLENE GLYCOL 5	21	EDC, INDUSTRIAL CHEMICAL
36	Dibenzofuran	14	EDC
37	Lilial	12	PCP, FOOD, FLAVOR, FRAGRANCE
38	1H-Benzotriazole, 4-methyl- * CORROSION INHIBITOR	4	EDC, INDUSTRIAL CHEMICAL
39	1H-Benzotriazole, 5-methyl- *RETROCURE G USED IN PREVULCANIZATION IN RUBBER MANUFATURE	4	EDC, INDUSTRIAL CHEMICAL
40	Ionol 2 * (ANTIOXIDANT- TO PREVENT GUMMING IN FUELS)	11	EDC, INDUSTRIAL CHEMICAL
41	2-tert-Butylhydroquinone * TBHQ, FOOD PRESERVATIVE ANTIOXIDANT	2	FOOD, FLAVOR, FRAGRANCE
42	Dodecanamide, N,N-bis(2-hydroxyethyl)- FOAM STABILIZER IN HOUSEHOLD DETERGENTS AND SHAMPOOS	22	PCP
43	á N METHYL IONONE	6	PCP, FOOD, FLAVOR, FRAGRANCE
44	DEET * INSECTICIDE	26	PCP, EDC, INSECTICIDE
45	Gabapentin	13	PCP, PHARMACEUTICAL
46	Fluorene	16	EDC, PAH
47	4-Chlorophenyl phenyl ether	5	EDC, FLAME RETARDANT
48	Benzothiazole, 2-(methylthio)- (CAS)	14	EDC
49	Ibuprofen	5	PCP, PHARMACEUTICAL
50	2,6-Bis(1,1-dimethylethyl)-4-(1-oxopropyl)phenol	23	EDC, UV LIGHT STABILIZER
51	Diphenylamine * ANTIOXIDANT, SCALD INHIBITIOR USED ON APPLES	2	EDC, FOOD, FLAVOR, FRAGRANCE
52	Azobenzene	9	EDC
53	Kayacure bp * used in the manufacturing of antihistamines, hypnotics, insecticides.	11	EDC, PHARMACEUTICAL, INSECTICIDE
54	N,N,N',N'-Tetraacetylethylenediamine *LIGAND FOR METAL IONS, ACRYLAMIDE POLYMERIZATION	10	EDC, INDUSTRIAL CHEMICAL
55	Clovene	18	FOOD, FLAVOR, FRAGRANCE
56	TRANS-METHYL DIHYDROJASMONATE	21	EDC, FOOD, FLAVOR, FRAGRANCE
57	Trifluralin	6	EDC, HERBICIDE
58	4-Bromophenyl phenyl ether *USED AS A (PAST) FLAME RETARDANT	4	EDC, BFR, INDUSTRIAL CHEMICAL
59	Ibuprofen-M (HO-) -H2O P329	6	PCP, PHARMACEUTICAL
60	Hexachlorobenzene	18	EDC, FUNGICIDE, POP, banned globally
61	Simazine	14	EDC, HERBICIDE, banned by EU
62	Atrazine P363	22	EDC, HERBICIDE, banned EU, US still uses
63	Myristic acid P412	24	FOOD, FLAVOR, FRAGRANCE
64	Cedryl acetate	10	PCP, FOOD, FLAVOR, FRAGRANCE
65	Ibuprofen-M (HO-) isomer-1 ME P444 * Pharmacuetical	6	PCP, PHARMACEUTICAL
66	Tris(1-chloro-2-propyl)phosphate *TCPP Flame retardant	26	EDC, INDUSTRIAL CHEMICAL
67	Phenanthrene	24	EDC, PAH
68	Anthracene	11	EDC, PAH
69	Naphthalene, 6,7-diethyl-1,2,3,4-tetrahydro-1,1,4,4-tetramethyl- (CAS)	13	EDC
70	3,5-DITERT-BUTYLBENZALDEHYDE	7	EDC
71	Caffeine (CAS)	10	PCP, FOOD, FLAVOR, PHARMA
72	Carbazole	12	EDC, INDUSTRIAL CHEMICAL
73	7-Acetyl-6-ethyl-1,1,4,4-tetramethyltetralin * synthetic musk	24	PCP, FOOD, FLAVOR, FRAGRANCE
74	Gemfibrozil * Medication to lower lipid levels	11	PCP, PHARMACEUTICAL
75	metolachlor	24	EDC, HERBICIDE
76	5H-dibenz[b,f]azepine (CAS) * Pharmaceutical - intermediate used in drug manufacturing	9	PCP, PHARMACEUTICAL
77	Benzenesulfonanilide * production of synthetic dyes, photochemicals, disinfectants, electroplating	8	EDC, INDUSTRIAL CHEMICAL
78	Sunscreen uv-15	6	PCP, PHARMACEUTICAL
79	Heptachlor epoxide	2	EDC, POP, INSECTICIDE, US limits use
80	Oxychlordane	2	EDC, POP, INSECTICIDE, banned US 1988
81	Bioallethrin	4	EDC, PESTICIDE
82	FLUORANTHENE	23	EDC, PAH

	UNTARGETED AND TARGETED ANALYTES DETECTED	COUNT # OF TIMES DETECTED	CHEMICAL TYPE
83	Lauryl acrylate * for manufacturing polymers used in hairstyling	6	PCP
84	Naproxen * Pharmaceutical	1	PCP, PHARMACEUTICAL
85	Triclosan * antibacterial, antifungal agent used in toothpaste	7	PCP, PHARMACEUTICAL
86	Pyrene	23	EDC, PAH
87	Butyl citrate * used as a plasticizer, antifoam agent	5	EDC, INDUSTRIAL CHEMICAL
88	Bisphenol A * Used in making plastics	13	EDC, INDUSTRIAL CHEMICAL
89	Nitrofen	2	EDC, PESTICIDE, banned in EU and US
90	Endrin P1112	2	EDC, INSECTICIDE, banned 2004
91	TCPP _ Tris(1,3-dichloroisopropyl)phosphate * (PBDE) flame retardant REPLACEMENT	16	EDC, INDUSTRIAL CHEMICAL
92	2H-1-Benzopyran-2-one, 7-(diethylamino)-4-methyl- * (Optical bleach in textile industry)	4	EDC, INDUSTRIAL CHEMICAL
93	Hexazinone P513	1	EDC, HERBICIDE
94	Bifenthrin	5	EDC, INSECTICIDE, class C carcinogen
95	Benzo[a]anthracene	4	EDC, PAH
96	Chrysene (CAS)	8	EDC, PAH
97	Methoxychlor	2	EDC, INSECTICIDE U.S. BANNED 2003
98	Mirex	2	EDC, INSECTICIDE U.S. BANNED 1976
99	Benzo[b or k]fluoranthene	15	EDC, PAH
100	Benzo[a]pyrene (CAS)	3	EDC, PAH
101	Fenvalerate isomer-1 P1241	1	EDC, INSECTICIDE
102	Fenvalerate isomer-2 P1242	2	EDC, INSECTICIDE

3. Experimental

An endocrine disruptor reference stock standard at 1 ng/µL was prepared in acetone with EPA Method standards purchased from Restek Corp. The commercial standards purchased were Method 8270 Megamix, Method 527 pesticide mix # 1, Method 551.1 pesticide/herbicide mix, plus Bisphenol A. The solid-phase extraction (SPE) procedure was developed using one liter of HPLC water spiked with the EDC reference standard at a concentration of 50 pg/µL. The GCxGC-TOFMS method was developed using the EDC reference standard injected at 5 ng on-column.

Solid Phase Extraction Procedure

One-liter water samples were adjusted to pH 2 with 37% HCl. SPE Supel™ Select HLB 500 mg cartridges were conditioned with 5 mL HPLC Water / 5% Methanol, then 5 mL Acetone, followed by 5mL HPLC water. Samples were loaded onto the Supelco Visiprep Vacuum Manifold (Supelco Analytical, Sigma–Aldrich) and were drawn slowly through the SPE cartridge. The SPE tube was then dried with vacuum for approximately 15 minutes. The cartridge was eluted slowly with 3 mL of acetone/5% methanol followed by 3 mL of dichloromethane into a 20 mL clean glass test tube. Extracted samples were placed in a Speedvac for approximately 2 hours until completely dry. The dried sample residue was reconstituted in 500 µL of acetone, vortexed, and pipetted into an autosampler vial. The GCxGC-TOFMS analysis followed with a 1 µL injection.

GCxGC-TOFMS Analysis Parameters

The Agilent 7890 Gas Chromatograph was equipped with a LECO dual-stage, quad jet thermal modulator and a GERSTEL MPS2 auto-sampler. The GC Primary Column used was a 30 m x 0.25 mm id. x 0.25 µm film thickness Rxi-5Sil MS (Restek Corp.). The GC Secondary Column used was a 1.0 m x 0.18 mm id. x 0.18 µm film thickness Rxi-17Sil MS (Restek Corp.). The carrier gas used was Helium set @1.5 mL/min. A 1 µL injection was made in splitless mode with an inlet temperature of 250°C. A LECO Pegasus® 4D mass spectrometer was used. The TOFMS method parameters used a mass range of 35–800 m/z. The acquisition rate was set at 200 spectra/s with an ion source temperature of 230°C.

Using the ChromaTOF® REFERENCE Feature

A Reference Method is built in ChromaTOF® software based on user-defined retention time and spectral information that are applied and compared to a sample. The purpose of a Reference Method is to determine differences between a sample and a reference standard within user-defined limits of retention time, peak area, and spectral match. In this study, untargeted components found in the water extracts were added to the original 108 component reference standard. The final Reference used in data analysis contained 152 compounds. The Reference feature is applied as part of the data processing method. The processed sample peak table displays each compound from the reference in the Type column as either a "Match", found but "Out of Tolerance" by percent, or "Not Found".

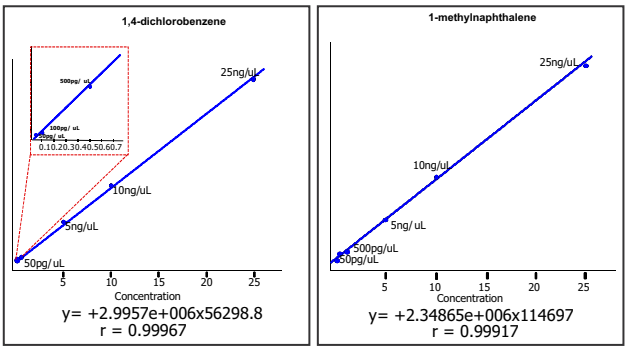
An example of a peak table result from the EDC Reference used for this analysis is shown below in Table 2. The columns show the Compound Name, Type of Match, the Match similarity score, 1st and 2nd dimension retention times, peak area, unique mass, signal-to-noise, and the library used to identify the mass spectral peak.

Table 2. Example of peak table result after processing against a reference.

Name	Type	Match	R.T.(s)	Area	Unique Mass	S/N	Libraray
Bisphenol A	Match	895	1724, 2.600	740588	213	2979.1	mainlib
Ionol 2 * (ANTIOXIDANT-TO PREVENT GUMMING IN FUELS)	Out of Tolerance	918	1085, 1.450	733755	219	5824.1	mainlib
Lindance/EDC STD 8s rt dev 600 SIM	Not Found	600					mainlib

Calibration Linearity and Targeted Quantitation

Six point calibration curves for 1,4-dichlorobenzene and 1-methylnaphthalene are shown below in Figure 2. Calibration linearity for both components above show calculated Pearson's r values greater than 0.999. Targeted analysis was utilized in the data processing method with calibration curves for components contained within the endocrine disruptor standard. The peak table below shows the quantitative results calculated for two selected analytes detected in a one-liter water sample.



Name	Concentration	Conc. Units	R.T. (s)	Similarity	Area	Height	Unique Mass	S/N	Library
1,4-Dichlorobenzene	0.08	ng	396 , 1.345	941	169194	12113	146	364.46	Wiley9
Naphthalene, 1-methyl-(CAS)	0.06	ng	791 , 1.830	732	7533.1	605.24	141	70.566	Wiley9

Figure 2. Calibration curves and quantitative results for 1,4-dichlorobenzene and 1-methyl-naphthalene.

4. Conclusions

In conclusion, comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry was utilized in this research to detect endocrine disruptors, personal care products, as well as other pollutants in samples obtained from urban and rural point sources along a Midwestern watershed. A solid-phase extraction method was developed using a hydrophilic modified, styrene-based polymer for a broad range of compounds from aqueous samples. An optimized GCxGC method was developed using a conventional non-polar and mid-polarity column set. The GCxGC method utilized variable modulation which aids in optimization of the increased peak capacity and chromatographic resolution of the first- and second-dimension separation. A TOFMS method was created which utilizes continuous full range non-skewed mass spectral information, True Signal Deconvolution®, and fast acquisition rates ideal for the characterization of EDCs and other contaminants in water. Thirteen solid-phase extractions were conducted on one liter aqueous samples obtained from six different rural and urban point sources along a Midwestern watershed. GCxGC-TOFMS analysis was followed by data processing that utilized the "Reference" feature in the ChromaTOF software. The final reference method containing 152 EDCs was applied as part of the data processing method. Results of this research detected 102 chemicals in aqueous extractions from six different point sources along the watershed. The detected compounds matched the reference standard with at least a 60% library match similarity. Furthermore, results show that 81% of the 102 chemicals detected were found at least five times. The research presented in this study emphasizes the need for instrumentation that will detect and identify sources of long- term environmental exposure to EDCs that can lead to ecological destruction and serious health effects. The application of GCxGC-TOFMS for this work presents an excellent instrumental tool for the detection of targeted and untargeted pollutants in impacted natural waterways. The data presented illustrate the advantages and benefits of GCxGC-TOFMS to provide a robust analysis as well as a data mining strategy using the "Reference" feature of ChromaTOF software to characterize a broad range of chemical contaminants in natural waterways.