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

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.

"The research presented in this study emphasises 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."

Author Details:
John Heim, Joe Binkley, and Doug Staples
LECO Corporation
3000 Lakeview Avenue
St. Joseph, MI 49085
Phone: 800-292-6141
Fax: 269-982-8977
Email:info@leco.com
Web: www.leco.com

This research presents a robust, broad range analysis for the detection of EDC's 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 characterise low levels of targeted and untargeted compounds in complex samples. A reference standard of 108 known endocrine disrupting compounds was prepared and analysed. 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 a 152 component reference standard. The results are reported as targeted and untargeted analytes found. This research study presents a practical, robust, sensitive, and reliable method for the detection of EDCs in urban and rural watersheds.

Experimental Methods

Reference Standard Preparation

- An endocrine disruptor reference stock standard at 1ng/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. Solid Phase Extraction Procedure
- Adjust 1 liter water sample to pH 2 with 37% HCl. •Condition SPE Supel™ Select HLB, 500mg cartridge with 5mL HPLC Water/ 5%Methanol, then 5mL Acetone, followed by 5mL HPLC water.
- Load 1 Liter of water using the Supelco Visiprep Vacuum Manifold (Supelco Analytical, Sigma –Aldrich) slowly unto the SPE cartridge.
- Dry SPE tube with vacuum for approximately 15 minutes.
- Elute slowly, 3mL of acetone/5% methanol into a 20mL clean glass test tube.
- Elute slowly, 3mL of dichloromethane into the same 20mL glass test tube.
- Speedvac to dryness for approximately 2 hours.
- Reconstitute dried residue in 500µL of acetone, vortex, and pipet into auto sampler vial,
- Inject 1uL sample for GCxGC-TOFMS analysis.
  Method Development
- The solid phase extraction (SPE) procedure was developed using 1 liter of HPLC water spiked with the EDC reference standard at 50ppb.
- The GCxGC-TOFMS method was developed using the EDC reference standard injected at 5ng on-column.

Using The Reference Standard Feature

A Reference Method is built in ChromaTOF® software from a user created standard that is applied and compared to a sample. The purpose of a Reference Method is to determine the component 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 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".

Name	Type	Match	R.T. (s)	Area	UniqueMass	S/N	Library
Bisphenol A	Match	895	1724 , 2.600	740588	213	2979.2	mainlib
Ionol 2 * (ANTIOXIDANT- TO PREVENT GUMMING IN FUELS)	Out of Tolerance	918	1085 , 1.450	733755	219	5824.1	mainlib
Lindane/ EDC STD 8S rt dev 600 SIM	Not Found	600					mainlib

Table 2: An example of a peak table is shown below for the EDC Reference used for this analysis. The columns show the Compound Name, Type of Match, the Match similarity score, retention time, peak area, unique mass, signal to noise, and the library used to identify the mass spectral peak.

GCxGC-TOFMS Results

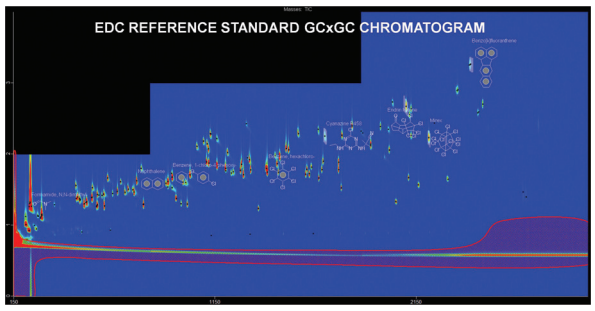

Figure 1:The two dimensional contour plot chromatogram above shows the 108 component endocrine disruptor reference standard used to develop the SPE extraction procedure and GCxGC-TOFMS method. The on-column concentration for each component is 5 nanograms.

Expanded Peak Capacity and Resolution of GCxGC

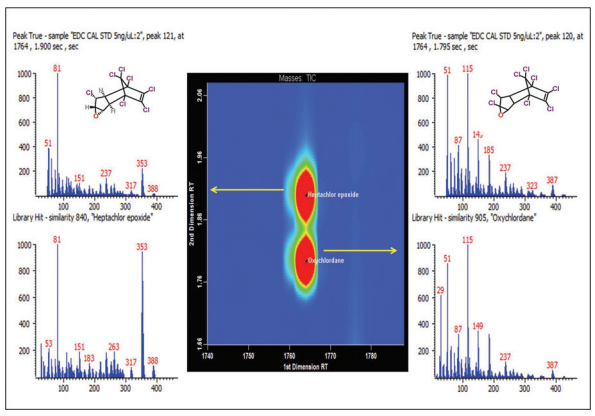

Figure 2: The example above shows the 2D Contour Plot for 2 peaks that are resolved in the second dimension by 105 milliseconds yet coeluted completely in the 1st dimension separation at 1764s. The expanded peak capacity and advantage of a two-dimensional separation is illustrated by this example of two chemically similar pesticides that would be coeluted and difficult to characterise by one dimensional chromatography. Notice the high mass spectral match scores by the NIST 08 library searches of 84%and 90.5% for both compounds.

Conclusions

In conclusion, comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry was utilised 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 optimised GCxGC method was developed using a conventional non-polar and mid-polarity column set. The GCxGC method utilised variable modulation which aids in optimisation of the available peak capacity and chromatographic resolution of the first and second dimension separation. A TOFMS method was created which offers continuous full range non-skewed mass spectral information, True Signal Deconvolution®, and fast acquisition rates ideal for the characterisation of EDCs and other contaminants in water.

Thirteen solid phase extractions were conducted on 1 liter aqueous samples obtained from 6 different rural and urban point sources along a Midwestern watershed. GCxGC-TOFMS analysis was followed by data processing that utilised the "Reference" feature in ChromaTOF software. The final reference developed to detect EDCs contained 152 compounds which was applied as part of the data processing method. Results of this research detected 102 chemicals in aqueous extractions from 6 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

Table 3. EDC's & Pollutants Detected

	UNTARGETED AND TARGETED ANALYTES DETECTED	1ST DIMENSION RETENTION TIME (s)	COUNT # OF TIMES DETECTED	CHEMICAL TYPE
1	Phenol (CAS)	354	23	EDC
2	Bis(2-chloroethyl) ether	364	1	EDC
3	1,4-Dichlorobenzene	392	21	EDC
4	1,3-Dichlorobenzene	394	9	EDC
5	1,2-DICHLOROBENZENE	418	3	EDC
6	Benzylalcohol P194	420	19	EDC
7	2-Methylphenol	440	22	EDC
8	3-Methylphenol	458	24	EDC
9	Nitrobenzene	480	7	EDC
10	Isophorone * Solvent	532	26	EDC, INDUSTRIAL CHEMICAL
11	2-Nitrophenol	548	11	EDC
12	2,4-Dimethylphenol	566	11	EDC
13	2,4 Dichlorophenol	600	7	EDC
14	Benzene, 1,2,4-trichloro- (CAS)	608	9	EDC
15	Naphthalene	623	25	EDC, PAH
16	D-Glucitol, 1,4:3,6-dianhydro-2,5-di-O-methyl- * used in Personal Care Products; cosmetics	731	7	PCP
17	Naphthalene, 2-methyl-	770	20	EDC, PAH
18	Naphthalene, 1-methyl-	791	22	EDC, PAH
19	1,3-Isobenzofurandione (CAS) * RUBBER RETARDER, CURING AGENT	794	14	EDC, INDUSTRIAL CHEMICAL
20	Propofol	842	6	PCP, PHARMACEUTICAL
21	2,4,6-Trichlorophenol	842	6	EDC
22	2,4,5-Trichlorophenol	845	6	EDC
23	à DAMASCONE	863	12	FOOD, FLAVOR, FRAGRANCE
24	2-Chloronaphthalene	878	7	EDC, PAH
25	Phenol, 4-chloro-3,5-dimethyl- (CAS)	884	6	EDC
26	á-Patchoulene	887	7	FOOD, FLAVOR, FRAGRANCE
27	Naphthalene, 1,7-dimethyl-	911	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)]-	929	5	EDC
29	Acenaphthylene (CAS)	971	14	EDC, PAH
30	Acenaphthene	1010	21	EDC, PAH
31	BUTYL HYDROXY TOLUENE * BHT ANTIOXIDANT	1025	23	EDC, FOOD, FLAVOR, FRAGRANCE
32	Tributyl phosphate * SOLVENT & PLASTICIZER	1031	19	EDC, INDUSTRIAL CHEMICAL
33	Benzeneacetic acid, ethyl ester * flavor fragrance	1037	25	EDC, FOOD, FLAVOR, FRAGRANCE
34	à-N-METHYL IONONE	1043	6	EDC, FOOD, FLAVOR, FRAGRANCE
35	TRIPROPYLENE GLYCOL 5	1046	21	EDC, INDUSTRIAL CHEMICAL
36	Dibenzofuran	1052	14	EDC
37	Lilial	1055	12	PCP, FOOD, FLAVOR, FRAGRANCE
38	1H-Benzotriazole, 4-methyl- * CORROSION INHIBITOR	1070	4	EDC, INDUSTRIAL CHEMICAL
39	1H-Benzotriazole, 5-methyl- *RETROCURE G USED IN PREVULCANIZATION IN RUBBER MANUFATURE	1076	4	EDC, INDUSTRIAL CHEMICAL
40	Ionol 2 * (ANTIOXIDANT- TO PREVENT GUMMING IN FUELS)	1085	11	EDC, INDUSTRIAL CHEMICAL
41	2-tert-Butylhydroquinone * TBHQ, FOOD PRESERVATIVE ANTIOXIDANT	1088	2	FOOD, FLAVOR, FRAGRANCE
42	Dodecanamide, N,N-bis(2-hydroxyethyl)- •FOAM STABILIZER IN HOUSEHOLD DETERGENTS AND SHAMPOOS	1091	22	PCP
43	á N METHYL IONONE	1109	6	PCP, FOOD, FLAVOR, FRAGRANCE
44	DEET * INSECTICIDE	1112	26	PCP, EDC, INSECTICIDE
45	Gabapentin	1127	13	PCP, PHARMACEUTICAL
46	Fluorene	1130	16	EDC, PAH
47	4-Chlorophenyl phenyl ether	1139	5	EDC, FLAME RETARDANT
48	Benzothiazole, 2-(methylthio)- (CAS)	1151	14	EDC
49	Ibuprofen	1160	5	PCP, PHARMACEUTICAL
50	2,6-Bis(1,1-dimethylethyl)-4-(1-oxopropyl)phenol	1166	23	EDC, UV LIGHT STABILIZER
51	Diphenylamine * ANTIOXIDANT, SCALD INHIBITIOR USED ON APPLES	1169	2	EDC, FOOD, FLAVOR, FRAGRANCE
52	Azobenzene	1175	9	EDC
53	Kayacure bp * used in the manufacturing of antihistamines, hypnotics, insecticides.	1178	11	EDC, PHARMACEUTICAL, INSECTICIDE
54	N,N,N',N'-Tetraacetylethylenediamine *LIGAND FOR METAL IONS, ACRYLAMIDE POLYMERIZATION	1178	10	EDC, INDUSTRIAL CHEMICAL
55	Clovene	1181	18	FOOD, FLAVOR, FRAGRANCE
56	TRANS-METHYL DIHYDROJASMONATE	1196	21	EDC, FOOD, FLAVOR, FRAGRANCE
57	Trifluralin	1214	6	EDC, HERBICIDE
58	4-Bromophenyl phenyl ether *USED AS A (PAST) FLAME RETARDANT	1250	4	EDC, BFR, INDUSTRIAL CHEMICAL
59	Ibuprofen-M (HO-) -H2O P329	1253	6	PCP, PHARMACEUTICAL
60	Hexachlorobenzene	1259	18	EDC, FUNGICIDE, POP, banned globally
61	Simazine	1298	14	EDC, HERBICIDE, banned by EU
62	Atrazine P363	1307	22	EDC, HERBICIDE, banned EU, US still uses
63	Myristic acid P412	1313	24	FOOD, FLAVOR, FRAGRANCE
64	Cedryl acetate	1331	10	PCP, FOOD, FLAVOR, FRAGRANCE
65	Ibuprofen-M (HO-) isomer-1 ME P444 * Pharmacuetical	1334	6	PCP, PHARMACEUTICAL
66	Tris(1-chloro-2-propyl)phosphate *TCPP Flame retardant	1349	26	EDC, INDUSTRIAL CHEMICAL
67	Phenanthrene	1352	24	EDC, PAH
68	Anthracene	1358	11	EDC, PAH
69	Naphthalene, 6,7-diethyl-1,2,3,4-tetrahydro-1,1,4,4-tetramethyl- (CAS)	1364	13	EDC
70	3,5-DITERT-BUTYLBENZALDEHYDE	1373	7	EDC
71	Caffeine (CAS)	1406	10	PCP, FOOD, FLAVOR, PHARMA
72	Carbazole	1415	12	EDC, INDUSTRIAL CHEMICAL
73	7-Acetyl-6-ethyl-1,1,4,4-tetramethyltetralin * synthetic musk	1415	24	PCP, FOOD, FLAVOR, FRAGRANCE
74	Gemfibrozil * Medication to lower lipid levels	1490	11	PCP, PHARMACEUTICAL
75	metolachlor	1529	24	EDC, HERBICIDE
76	5H-dibenz[b,f]azepine (CAS) * Pharmaceutical - intermediate used in drug manufacturing	1550	9	PCP, PHARMACEUTICAL
77	Benzenesulfonanilide * production of synthetic dyes, photochemicals, disinfectants, electroplating	1589	8	EDC, INDUSTRIAL CHEMICAL
78	Sunscreen uv-15	1592	6	PCP, PHARMACEUTICAL
79	Heptachlor epoxide	1612	2	EDC, POP, INSECTICIDE, US limits use

Table 3

	UNTARGETED AND TARGETED ANALYTES DETECTED	1ST DIMENSION RETENTION TIME (s)	COUNT # OF TIMES DETECTED	CHEMICAL TYPE
80	Oxychlordane	1613	2	EDC, POP, INSECTICIDE, banned US 1988
81	Bioallethrin	1625	4	EDC, PESTICIDE
82	FLUORANTHENE	1628	23	EDC, PAH
83	Lauryl acrylate * for manufacturing polymers used in hairstyling	1640	6	PCP
84	Naproxen * Pharmaceutical	1655	1	PCP, PHARMACEUTICAL
85	Triclosan * antibacterial, antifungal agent used in toothpaste	1661	7	PCP, PHARMACEUTICAL
86	Pyrene	1679	23	EDC, PAH
87	Butyl citrate * used as a plasticizer, antifoam agent	1721	5	EDC, INDUSTRIAL CHEMICAL
88	Bisphenol A * Used in making plastics	1721	13	EDC, INDUSTRIAL CHEMICAL
89	Nitrofen	1769	2	EDC, PESTICIDE, banned in EU and US
90	Endrin P1112	1772	2	EDC, INSECTICIDE, banned 2004
91	TCPP _ Tris(1,3-dichloroisopropyl)phosphate * (PBDE) flame retardant REPLACEMENT	1838	16	EDC, INDUSTRIAL CHEMICAL
92	2H-1-Benzopyran-2-one, 7-(diethylamino)-4-methyl- * (Optical bleach in textile industry)	1854	4	EDC, INDUSTRIAL CHEMICAL
93	Hexazinone P513	1882	1	EDC, HERBICIDE
94	Bifenthrin	1958	5	EDC, INSECTICIDE, class C carcinogen
95	Benzo[a]anthracene	1962	4	EDC, PAH
96	Chrysene (CAS)	1970	8	EDC, PAH
97	Methoxychlor	1974	2	EDC, INSECTICIDE U.S. BANNED 2003
98	Mirex	2062	2	EDC, INSECTICIDE U.S. BANNED 1976
99	Benzo[b or k]fluoranthene	2198	15	EDC, PAH
100	Benzo[a]pyrene (CAS)	2262	3	EDC, PAH
101	Fenvalerate isomer-1 P1241	2302	1	EDC, INSECTICIDE
102	Fenvalerate isomer-2 P1242	2322	2	EDC, INSECTICIDE

Table 3: The results for thirteen 1 liter water samples prepared with solid phase extraction and analysed by GCxGC-TOFMS are shown below. 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 (EDC's) encompass a variety of chemical classes, including pharmaceuticals, pesticides, polymer additives, coatings materials, personal consumer products, flame retardants, plasticisers, industrial by-products and miscellaneous pollutants. This data shows that out of 26 GCxGC-TOFMS analyses, 81% of the 102 chemicals detected were found at least 5 times.

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The research presented in this study emphasises 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 illustrates 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 characterise a broad range of chemical contaminants in a Midwestern watershed.