5TH SBSE INTERNATIONAL MEETING

23 & 24 SEPTEMBRE 2019 - NOVOTEL PARIS-SUD

SBSE Technical Meeting

DETERMINATION OF 110 ENDROCRINE DISRUPTORS (EDCS) IN WATER BY USING SBSE TD – GC/MSMS UNDER THE EUROPEAN WATER FRAMEWORK DIRECTIVE

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Environment and Cleaner Production Institutes

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SUMMARY OF THE PRESENTATION

Introduction

EU Water Framework Directive /Turkish Regulation on the Management of Surface Water Quality

Twister Stir Bar Sorptive Extraction (Twister SBSE) Technique

Method

Sample preparation protocole

Analytical method by using GC/MS/MS

Validation Studies

Results

A multi-class, multi-residue method for the analysis of 110 EDCs in water

Summary

Good overview of the system

ABOUT TUBITAK

The Scientific and Technological Research Council of Turkey (TUBITAK) is the national science agency.

Marmara Research Center

- **established in 1972**
- **carries out researches on Energy, Environment, Food, Genetic Engineering and Biotechnology, Chemical Technologies, Materials, Earth and Marine Sciences through its 7 affiliated institutes.**
- **translates scientific knowledge into practical and implementable solutions for end-users in the sectors and beyond.**
- **offers original solutions to public, private and military agencies and institutions.**
- **performs its operations in "TÜBİTAK Gebze Campus" in the City of Kocaeli.**

ABOUT WATER FRAMEWORK DIRECTIVE

- In 2000 the European Community issued a directive “to establish a framework for the protection of inland surface waters, transitional waters, coastal waters and groundwater”.
- This was amended and modified by further directives in 2008 (33 priority pollutants).
- In 2013, a new Directive, 2013/39/EC, amended the Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy. Newly identified substances were added, including the setting of environmental quality standards (EQS), and EQS of some existing substances were revised (45 priority pollutants).
- The major aim of the WFD is to reach good water quality in all European waters by managing water bodies, i.e., lakes, rivers, groundwater bodies, transitional waters and coastal waters by 2027 at the latest.

TURKISH REGULATION ON THE MANAGEMENT OF SURFACE WATER QUALITY

- As a candidate for EU membership, Turkey has conducted studies on determination of specific pollutants between 2011 and 2015. In this scope, firstly, list of candidate pollutants were prepared based on the field studies, questionnaires and literature surveys.
- Different prioritization methods were then applied for candidate chemicals considering hazard characteristics, exposure levels and production/use patterns of the substances.
- In addition to prioritization scores, results of chemical monitoring studies were taken into consideration for the identification of final specific pollutants, as well. The chemicals detected at significant concentrations in surface waters were also designated as specific pollutants although their prioritization scores were lower.
- Specific pollutant list includes heavy metals, polychlorinated biphenyls, halogenated organics, endocrine disrupters, aromatic hydrocarbons and pesticides.
- Finally, 117 point sourced and 133 non-point sourced specific pollutants were designated with their national EQSs in 2016 August.

WATER SAMPLE EXTRACTION

STIR BAR SORPTIVE EXTRACTION (SBSE)

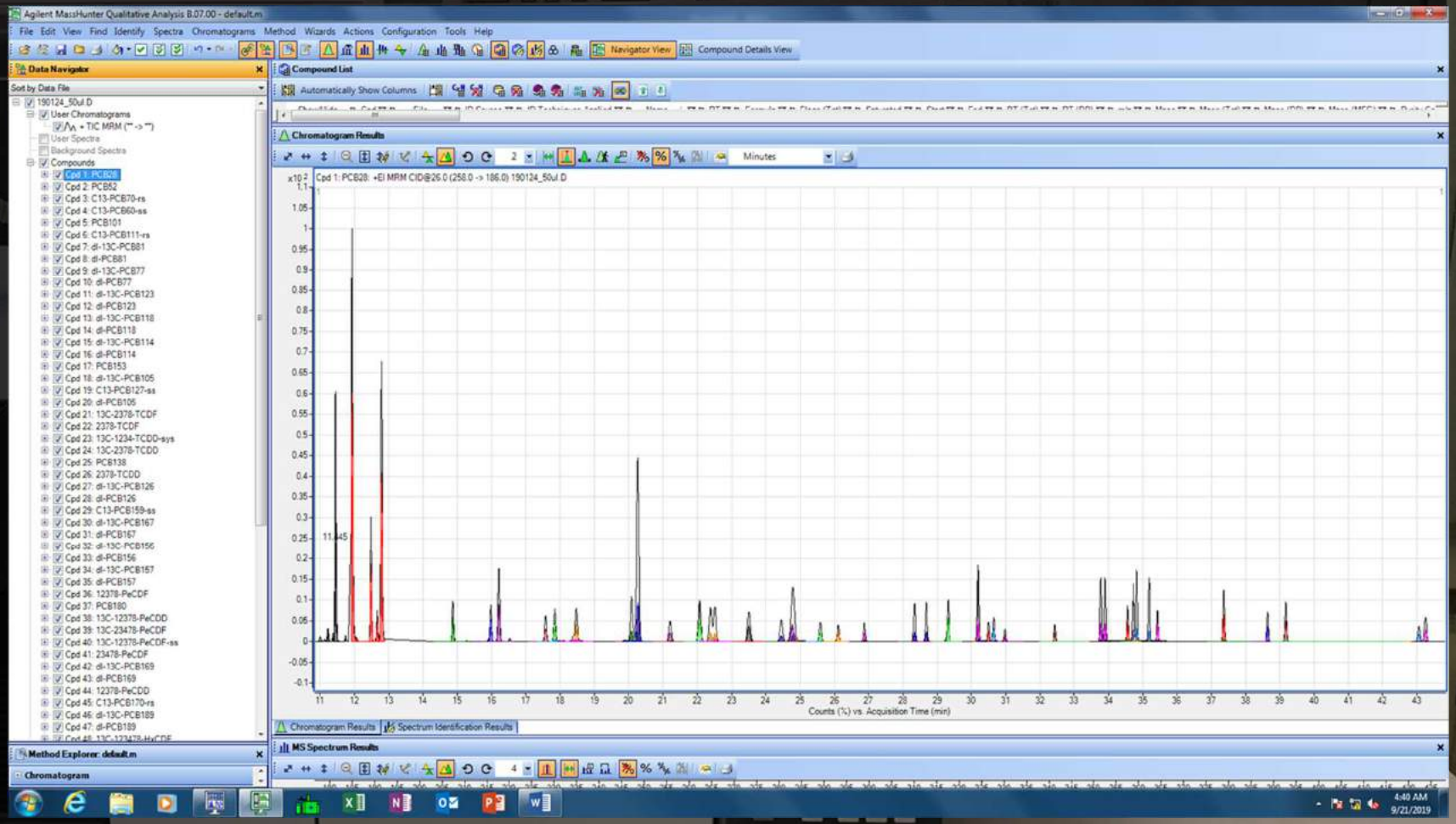
- SBSE is able to extract and preconcentrate the desired compounds from liquid matrices without using solvents.
- SBSE has been applied successfully to trace analysis of environmental samples for the determination of volatile and semi-volatile components.
- SBSE was developed in 1999 by Sandra and co-workers and commercialized under the name as *Twister*®.
- A typical SBSE unit (*Twister*®) simply containing glass, stir bar and polydimethylsiloxane (PDMS) polymeric phase.

SBSE Protocole

- Introduce Twister into water
- Stirring extraction (2 h)
- Take Twister out
- Rinse it with distillated water
- Dry it with clean wipes
- Introduce into a desorption tube
- Put the Tube into autosampler
- Thermal desorption
- Run Analysis (23.5 min)

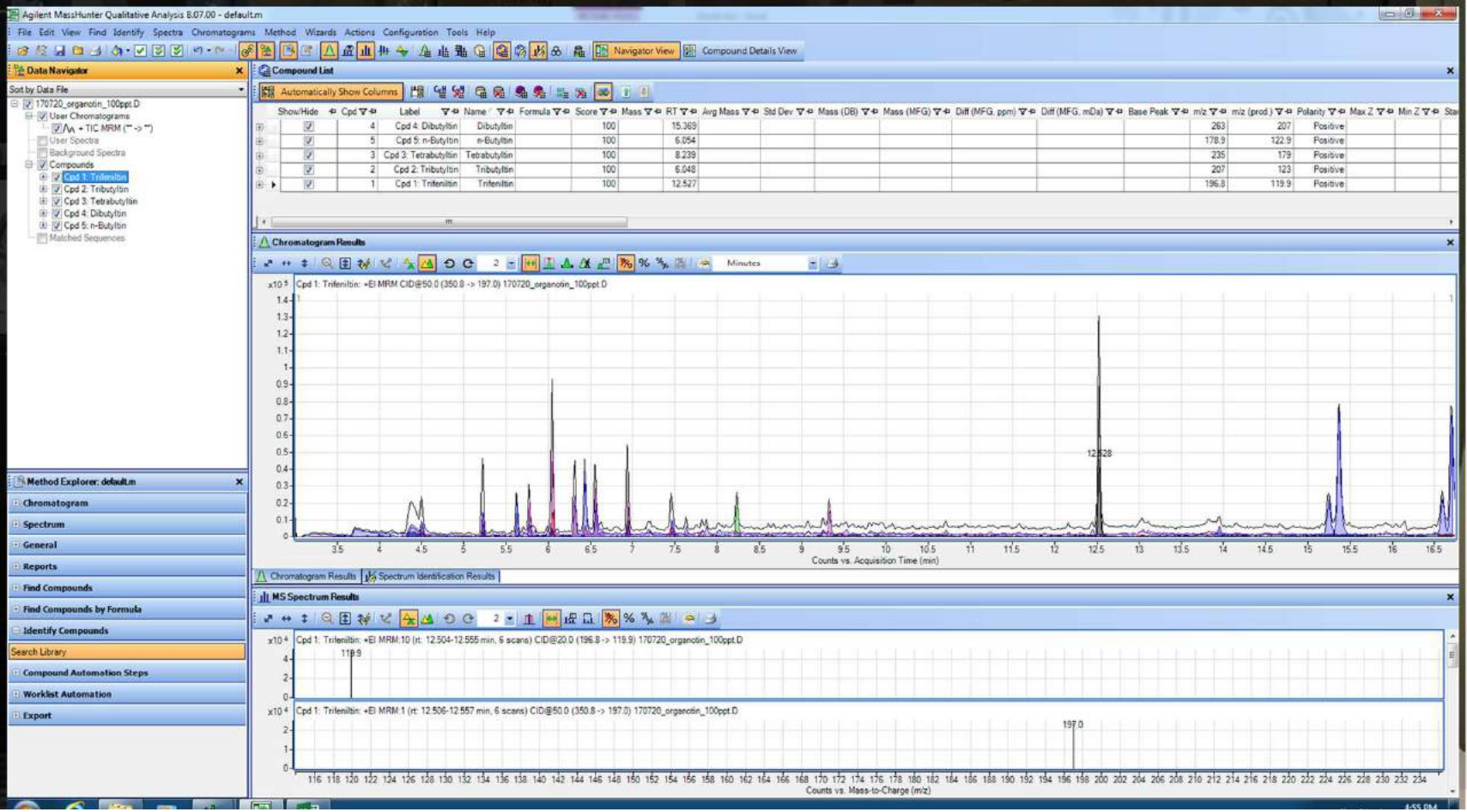
Method Development Studies with Twister

- Dioxin/Furan, dl-PCB, m-PCB analysis in single injection



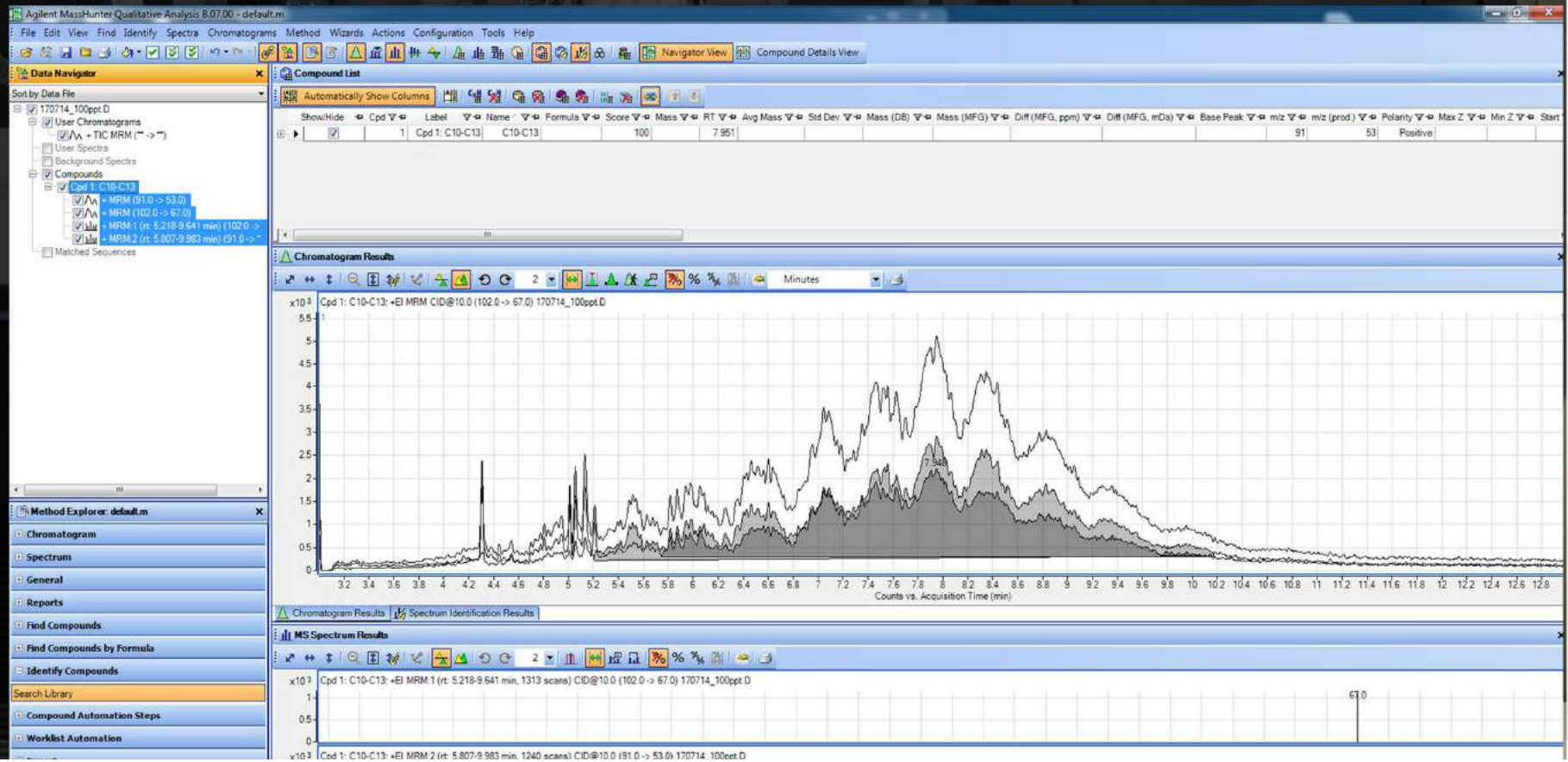
Method Development Studies with Twister

- Dioxin/Furan, dl-PCB, m-PCB analysis in single injection
- **Organotins analysis by derivatization (***sodium* tetraethylborate (*NaBEt*(4))



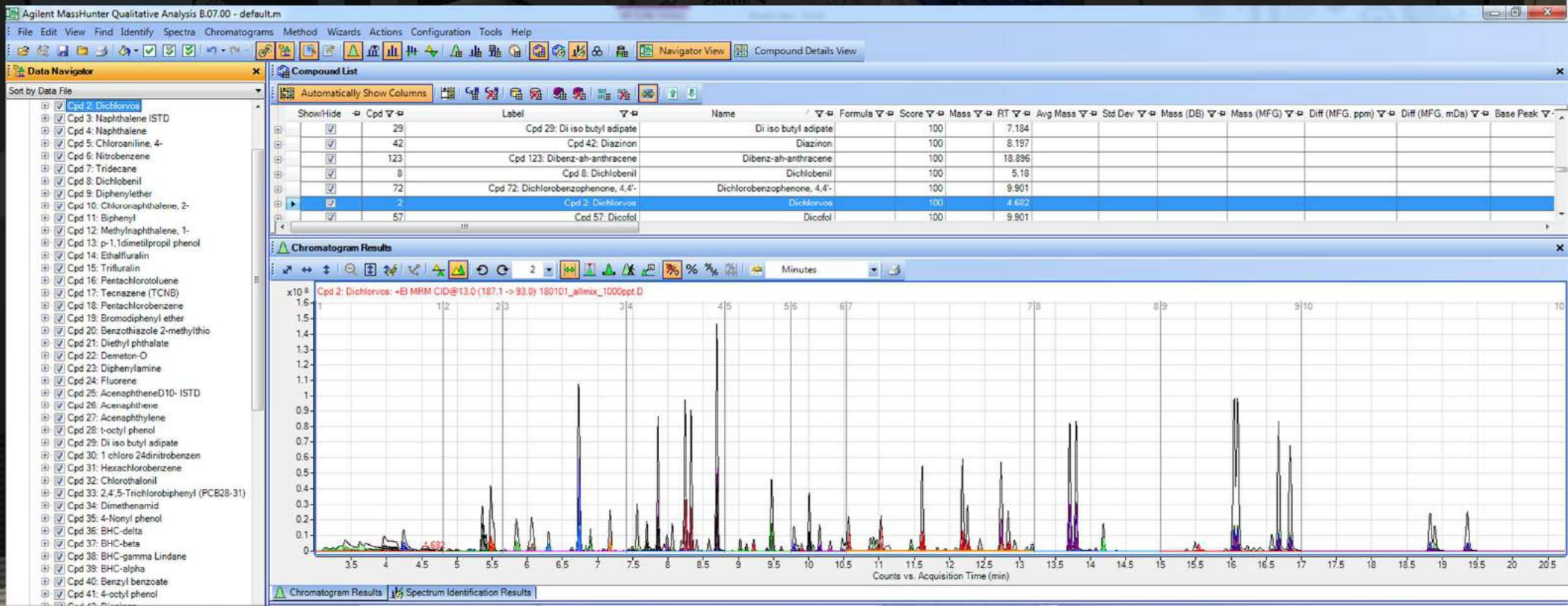
Method Development Studies with Twister

- Dioxin/Furan, dl-PCB, m-PCB analysis in single injection
- Organotins analysis by derivatization (*sodium* tetraethylborate (*NaBEt*(4))
- **Short Chain Chlorinated alkanes C10-13**

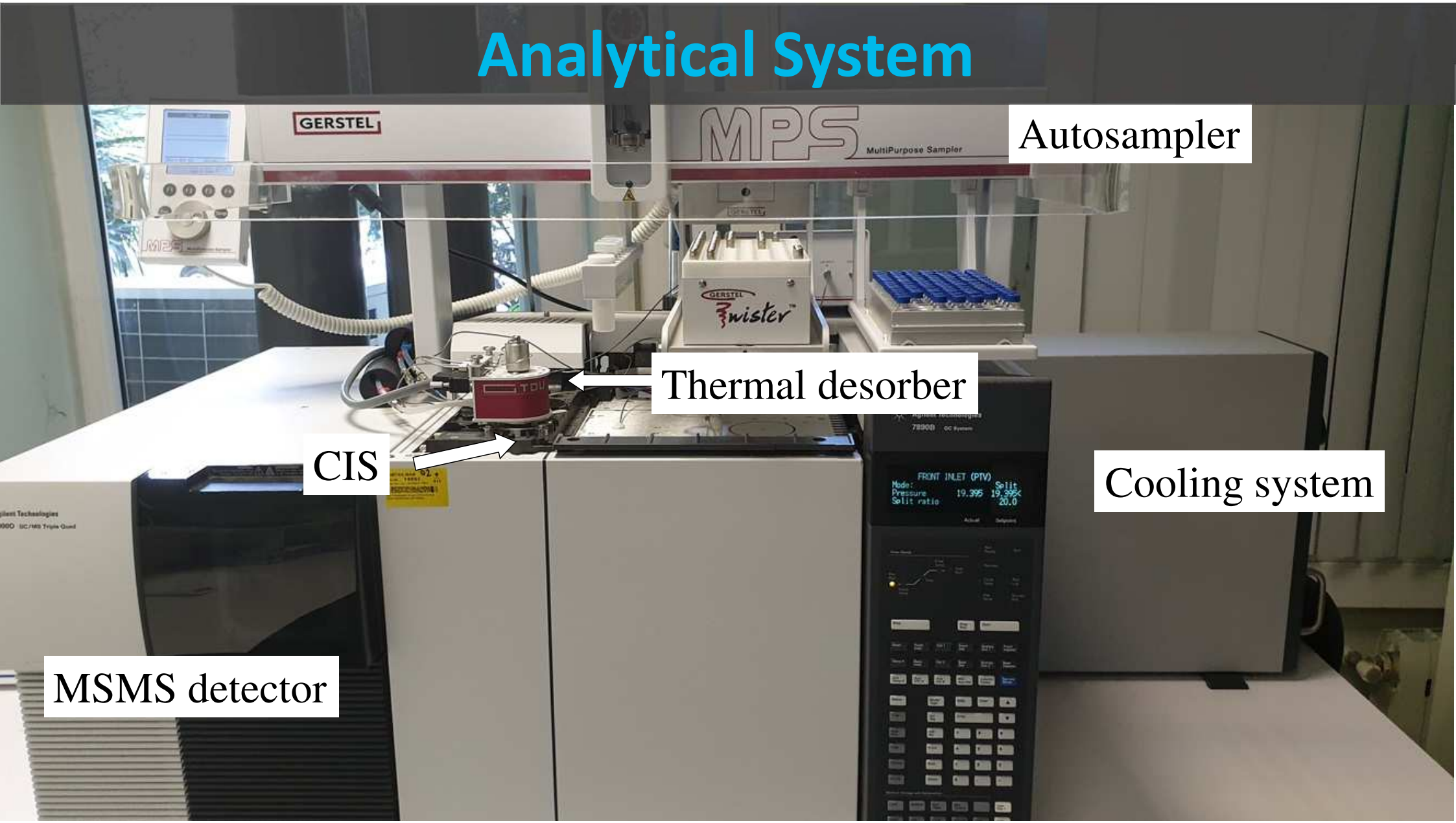


Method Development Studies with Twister

- Dioxin/Furan, dl-PCB, m-PCB analysis in single injection
- Organotins analysis by derivatization (*sodium* tetraethylborate (*NaBEt*(4))
- Short Chain Chlorinated alkanes C10-13
- **Multi-residue method for the analysis of 110 EDCs**



Analytical System



GC/MSMS METHOD PARAMETERS

	Rate (°C/min)	Value (°C)	Hold Time (min)	Run Time (min)
Initial Temp	50	50	1	1
Ramp I	40	170	0	4
Ramp II	10	315	5	23.5
Column HP 5 Ultra Inert 30 m x 250 µm x 0.25 µm				

Flow (mL/min)	1.7
Pressure (psi)	19.4
Average Velocity (cm/sec)	34.5
Holdup Time	1.4494
Total Flow (mL/min)	38.7
Split Ratio	20:1

Dynamic Multiple Reaction Monitoring (dMRM) as an acquisition tool in MSMS system enable us to make Easier Method maintenance and Better performance also efficiently quantitate/screening more than 100 targets in a single run. Using dMRM within retention-time window is easily scheduled than MRM even retention time shifts.

TDU Parameters

Initial	
Initial Temp (°C)	40
Delay Time (min)	0.50
Initial Time (min)	0.50

Ramp I	
Rate (°C/min)	720
End Temp (°C)	300
Hold Time (min)	5
Transfer Temp (°C)	310
Desorption mode	Split

CIS Parameters

Initial	
Initial Temp (°C)	-20
Equlibr. Time (min)	0.10
Initial Time (min)	1

Ramp I	
Rate (°C/sec)	12
End Temp (°C)	310
Hold Time (min)	5

	Compound name	Precursor ion	Product ion	Retention time (min)	A
1	Nitrobenzene	123	77	3,119	
2	Tridecane	71,2	43,1	3,547	
3	2,6 dimethyl phenol				
4	Naphthalene				
5	Chloroaniline, 4-				
6	Dichlorvos				
7	4chloro 3methylphenol				
8	1,2,4,5 tetrachlorobenzene				
9	Dichlobenil				
10	Biphenyl				
11	p-1,1 dimethylpropylphenol				
12	Chloronaphthalene, 2-				
13	Diphenylether				
14	Methylnapthalene, 1-				
15	2,6 ditersiyer butylphenol				
16	2amino4chlorophenol				
17	Demeton-O				
18	Acenaphthylene				
19	Terbutyl 4methoxyphenol				
20	Acenaphthene				
21	Pentachlorobenzene				
22	Diethyl phthalate				
23	2,4,6 tri tert butylphenol				
24	t-octyl phenol				
25	Fluorene				
26	Tecnazene (TCNB)				
27	Pentachlorotoluene				
28	Benzothiazole-2-methylthio				
29	1chloro 24dinitrobenzen				
30	Diphenyl amine				
31	Ethalfluralin				
32	Trifluralin				
33	Di isobutyl adipate				
34	Bromodiphenyl ether				
35	Thiometon				
36	Dimethoate				
37	BHC-alpha				
38	Hexachlorobenzene				
39	Nonyl Phenol Branched				
40	4-Octyl phenol				
41	Benzyl benzoate				
42	BHC-beta				
43	BHC-gamma (Lindane)				
44	Diazinon				
45	Demeton-S				
46	Phenanthrene				
47	Anthracene				
48	Tefluthrin, cis-				
49	Chlorothalonil				
50	BHC-delta				
51	4-Nonyl phenol	220	107	8,526	
52	Musk Xylene	282	265	8,72	
53	Dimethenamide	230	154,1	8,778	
100	Dieldrin	277	241	11,481	
101	Nitrofen	282,9	253	11,607	
102	Chlorfenapyr	327,8	246,8	11,652	
148	PBDE-183	723,8	563,8	19,603	

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Triple Quadrupole MS Method Editor

Method
- Acquisition
- Chromatograms
- Timed Events
- Instrument Curves
- Tune File Parameters
- Compound Browser
- Update RT

Tune File

atunes.eiex — Browse...

Source Parameters

Ion Source: EI
Source Temperature (°C): 300
Electron Energy Mode: Use Tune Setting
Electron Energy (eV): 70

Detector Setting

Use Gain Factor: 20
Use Delta EMV
Calculated EMV: 1409.8
EM Saver — Limit
Data Saved

Run Time

Run time (min): 1
Solvent Delay (min): 3

Filtering

Automatically Subtract Baseline
* Advanced MRM/SIM filtering
* The feature is instrument dependent

Scan Type: dMRM
(use this to revert to non-dMRM method)

Compound Table

Show: All

	Enable	Compound Name	CAS#	ISTD	Precursor Ion	MS1 Resolution	Product Ion	MS2 Resolution	RT (min)	Left RT Delta (min)	Right RT Delta (min)	Average Dwell (min)	CE (eV)
1	☑	Nitrobenzene		☐	123	Wide	77	Wide	3.12	.1	.5	30.1	12
2	☑	Nitrobenzene		☐	107	Wide	77	Wide	3.12	.1	.5	30.1	7
3	☑	Nitrobenzene		☐	77	Wide	51	Wide	3.12	.1	.5	30.1	16
4	☑	Nitrobenzene		☐	77	Wide	50	Wide	3.12	.1	.5	30.1	40
5	☑	Tridecane		☐	71.2	Wide	43.1	Wide	3.55	.5	.5	21.9	5
6	☑	Tridecane		☐	71.2	Wide	41.1	Wide	3.55	.5	.5	21.9	5
7	☑	Decamethyl Penta Siloxane		☐	355	Wide	267	Wide	3.91	.5	.5	17.2	11
8	☑	Decamethyl Penta Siloxane		☐	267	Wide	251	Wide	3.91	.5	.5	17.2	19
9	☑	Decamethyl Penta Siloxane		☐	267	Wide	179	Wide	3.91	.5	.5	17.2	45
10	☑	2,6 dimethyl phenol		☐	107.1	Wide	77.1	Wide	4.03	.5	.5	16.3	15
11	☑	2,6 dimethyl phenol		☐	107.1	Wide	51.1	Wide	4.03	.5	.5	16.3	25
12	☑	Naphthalene		☐	128.1	Wide	102.1	Wide	4.38	.5	.5	13.5	24
13	☑	Naphthalene		☐	128.1	Wide	78.1	Wide	4.38	.5	.5	13.5	20
14	☑	Naphthalene		☐	127.1	Wide	77.1	Wide	4.38	.5	.5	13.5	15
15	☑	Chloroaniline, 4-		☐	127	Wide	100	Wide	4.49	.5	.5	12	10
16	☑	Chloroaniline, 4-		☐	127	Wide	92	Wide	4.49	.5	.5	12	15
17	☑	Chloroaniline, 4-		☐	127	Wide	65	Wide	4.49	.5	.5	12	20
18	☑	Chloroaniline, 4-		☐	92	Wide	65	Wide	4.49	.5	.5	12	10
19	☑	Dichlorvos		☐	187.1	Wide	93	Wide	4.63	.5	.5	10.8	13
20	☑	Dichlorvos		☐	184.9	Wide	93	Wide	4.63	.5	.5	10.8	12
21	☑	Dichlorvos		☐	109	Wide	79	Wide	4.63	.5	.5	10.8	5
22	☑	4chloro 3methylphenol		☐	142	Wide	107	Wide	4.83	.5	.5	9.6	15
23	☑	4chloro 3methylphenol		☐	107	Wide	77	Wide	4.83	.5	.5	9.6	15
24	☑	1,2,4,5 tetrachlorobenzene		☐	216	Wide	180.9	Wide	5.09	.5	.5	7.9	15
25	☑	1,2,4,5 tetrachlorobenzene		☐	214	Wide	178.9	Wide	5.09	.5	.5	7.9	15
26	☑	Dichlobenil		☐	171	Wide	136.1	Wide	5.17	.5	.5	7.2	10
27	☑	Dichlobenil		☐	171	Wide	100.1	Wide	5.17	.5	.5	7.2	20
28	☑	Biphenyl		☐	154.1	Wide	153.1	Wide	5.33	.5	.5	6.6	15
29	☑	Biphenyl		☐	153.1	Wide	152.1	Wide	5.33	.5	.5	6.6	15

dMRM Statistics

Total MRMs	440
Number of MRMGroups	226
Minimum Concurrent MRMs	2
Maximum Concurrent MRMs	64
Minimum Dwell Time (ms)	2.3
Maximum Dwell Time (ms)	98.88
Minimum Cycle Time (ms) (hardware limit)	85.09

Parameters

Cycles Per Second (data points for a 1sec peak): 5
Cycle Time (ms): 200
Min Dwell Time (ms): 2

Overwrite Delta RT
Left RT Delta (min): .5
Right RT Delta (min): .5
Overwrite

Plot Type: Concurrent MRMs — Select Transition On Click

VALIDATION STUDIES

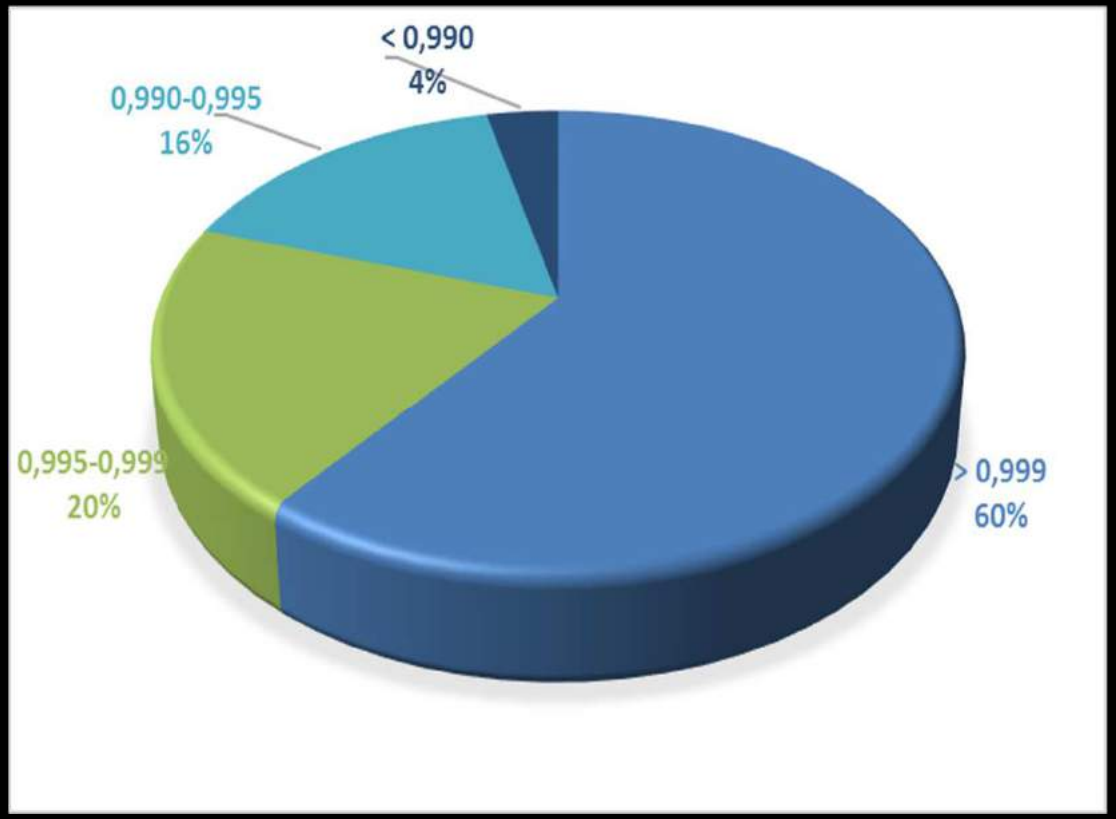
- *J. Guerra, Pharm. Tech. March 1986*
- *G. Maldener, Chromatographia, July 1989*

The method fully validated according to ISO 17025.

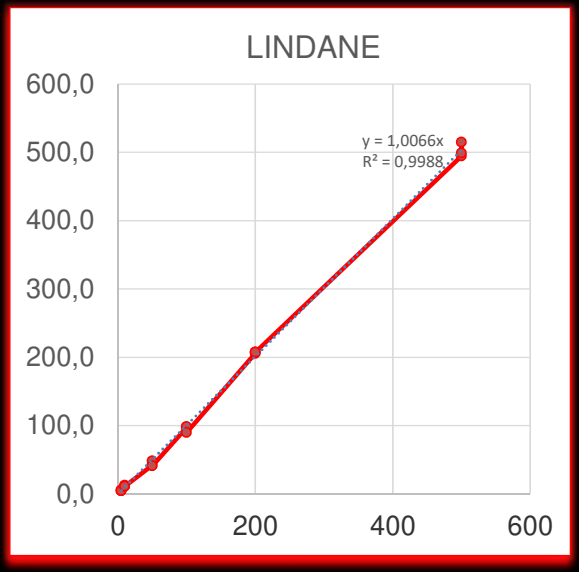
VALIDATION STUDIES

- Linearity
- *Selectivity*
- Detection limit (LOD) and Quantitation limit (LOQ)
- Repeatability
- Reproducability
- Matrix Effects

RESULTS OF LINEARITY AND WORKING RANGE

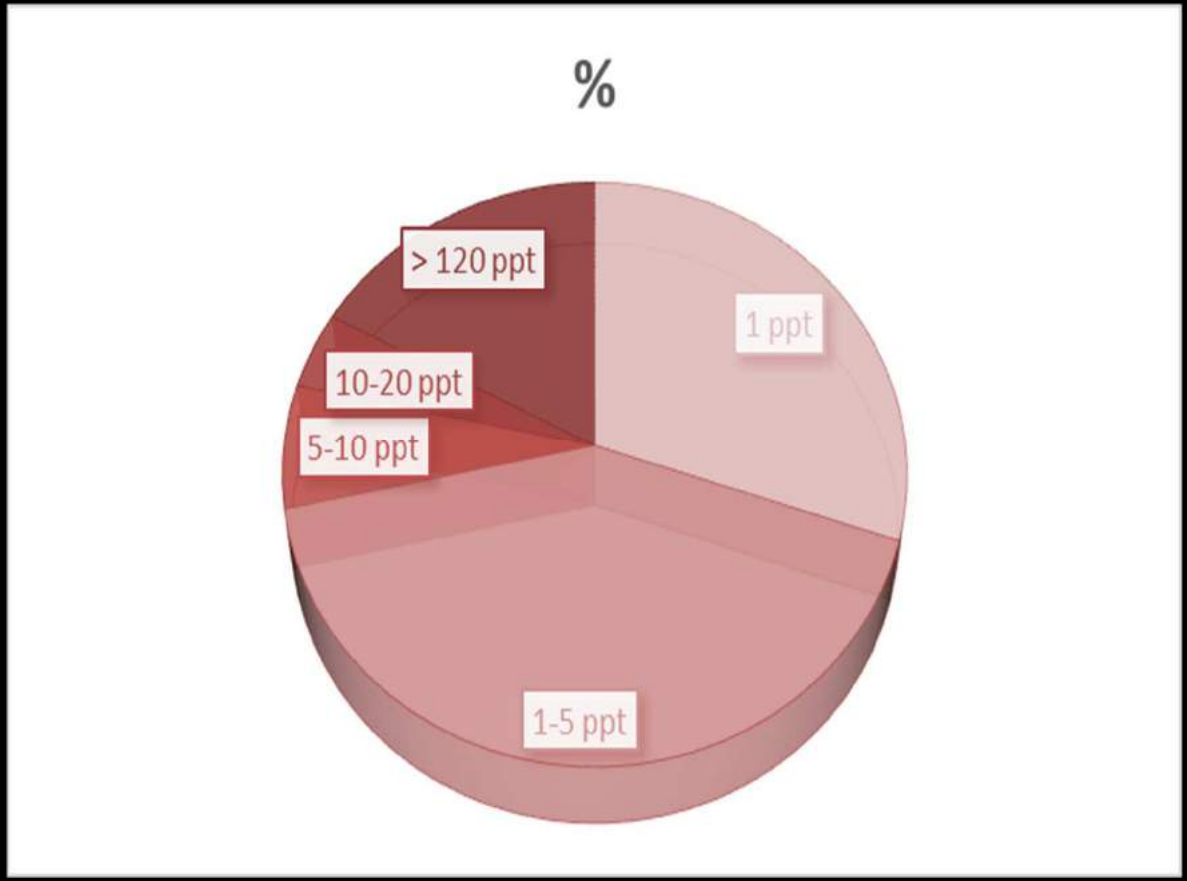


CORRELATION	% PERCENT
> 0,999	70%
0,995-0,999	23%
0,990-0,995	19%
< 0,990	4%

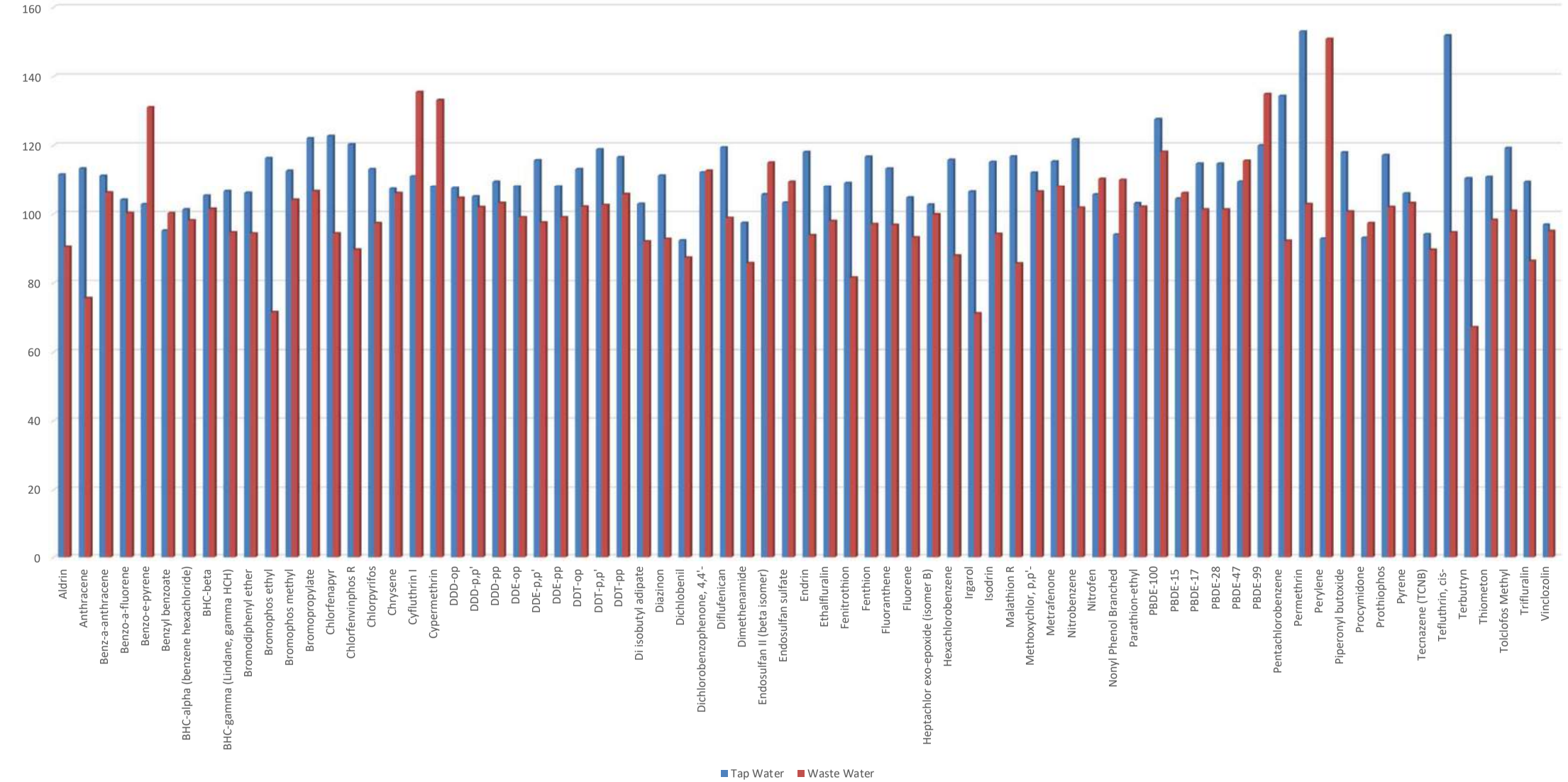


Working range;
1- 2-5-10-20-50-100 ppt

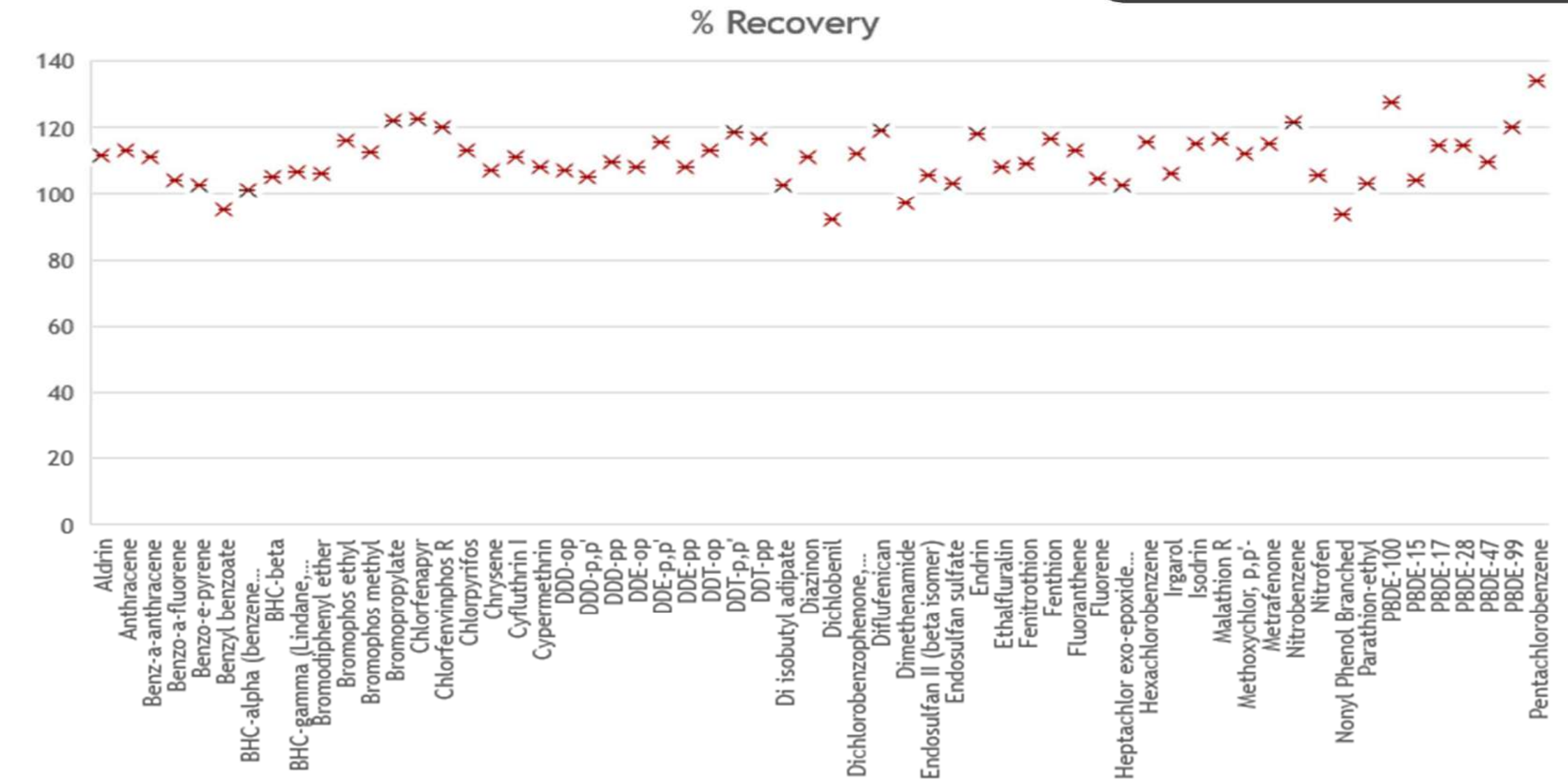
STUDY OF QUANTITATION LIMIT (LOQ)



MATRIX EFFECT



RESULTS OF TRUENESS



VALIDATION STUDY COMPARISON OF CHEMICAL GROUPS

RESULTS

- Aim of this work was meet the requirements of EWFD chemical pollutants list analysis in routine laboratories to accomplish with SBSE GC/MSMS technique.
- Twister® was used for analytes enrichment from 100 mL water samples.
- Compounds were thermally desorbed and separated by GC/MSMS
- The analysis method was comprehensively validated
- The method includes various endocrine disruptor compounds included in the WFD, such as polychlorinated biphenyls, polycyclic aromatics hydrocarbons, polybrominated diphenyl ethers, phenols, phthalates and pesticides etc.
- The method is also applicable to the analysis of similar contaminants that are not in this list.
- The SBSE which is quick, easy, solventlessly, lab friendly is taken totally two hours.
- The quantification limits (LOQs) obtained ranged from 0.12 to 50 ppt
- Precision (in terms of RSD) was lower than 40%, recoveries ranged between 40 and 120%, and determination coefficients were higher than 0.990 for all analytes.

MERCI DE VOTRE ATTENTION

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