1st NORMAN workshop on analysis of problematic compounds

How can we analyse very polar and hardly-ionisable compounds

Development and optimization strategy of LC-APPI-MS methods for the determination of halogenated compounds in environmental matrices

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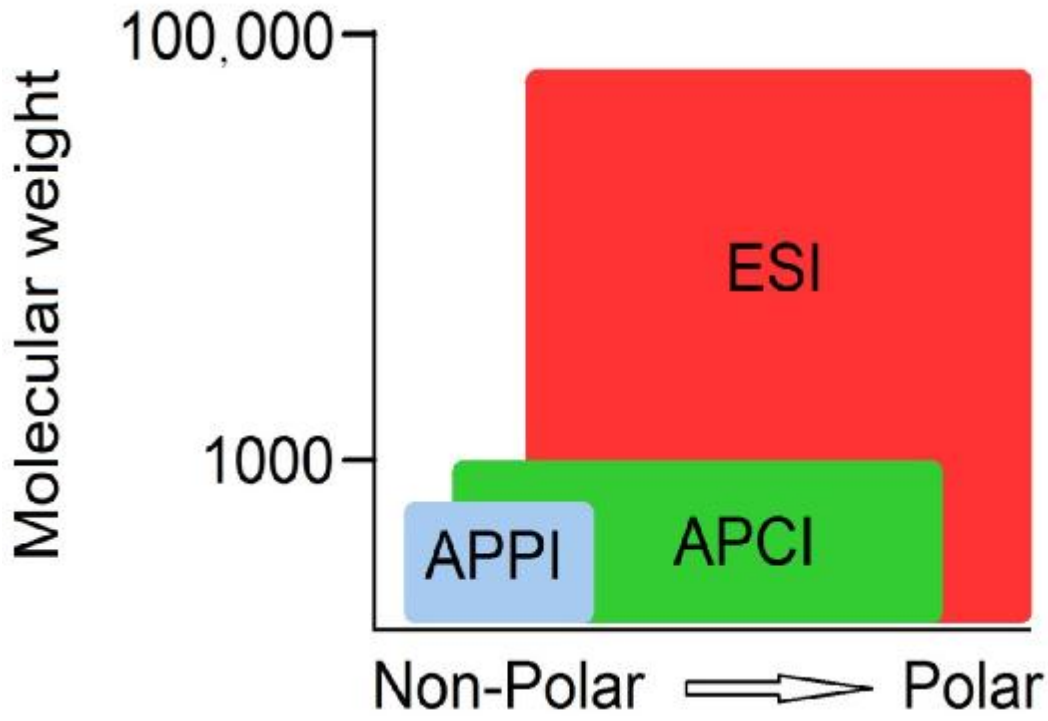
Atmospheric Pressure Photoionization (APPI)

Atmospheric pressure photoionization (APPI) is the youngest soft ionization technique. It was created in order to fill the gap of ESI and APCI sources. since it has the ability to ionize non polar molecules with relatively small molecular weights (Mr <1000).



APPI Sources for LC/MS

The first studies in which APPI was used as ionization source for LC/MS were publised in 2000 from two different groups:

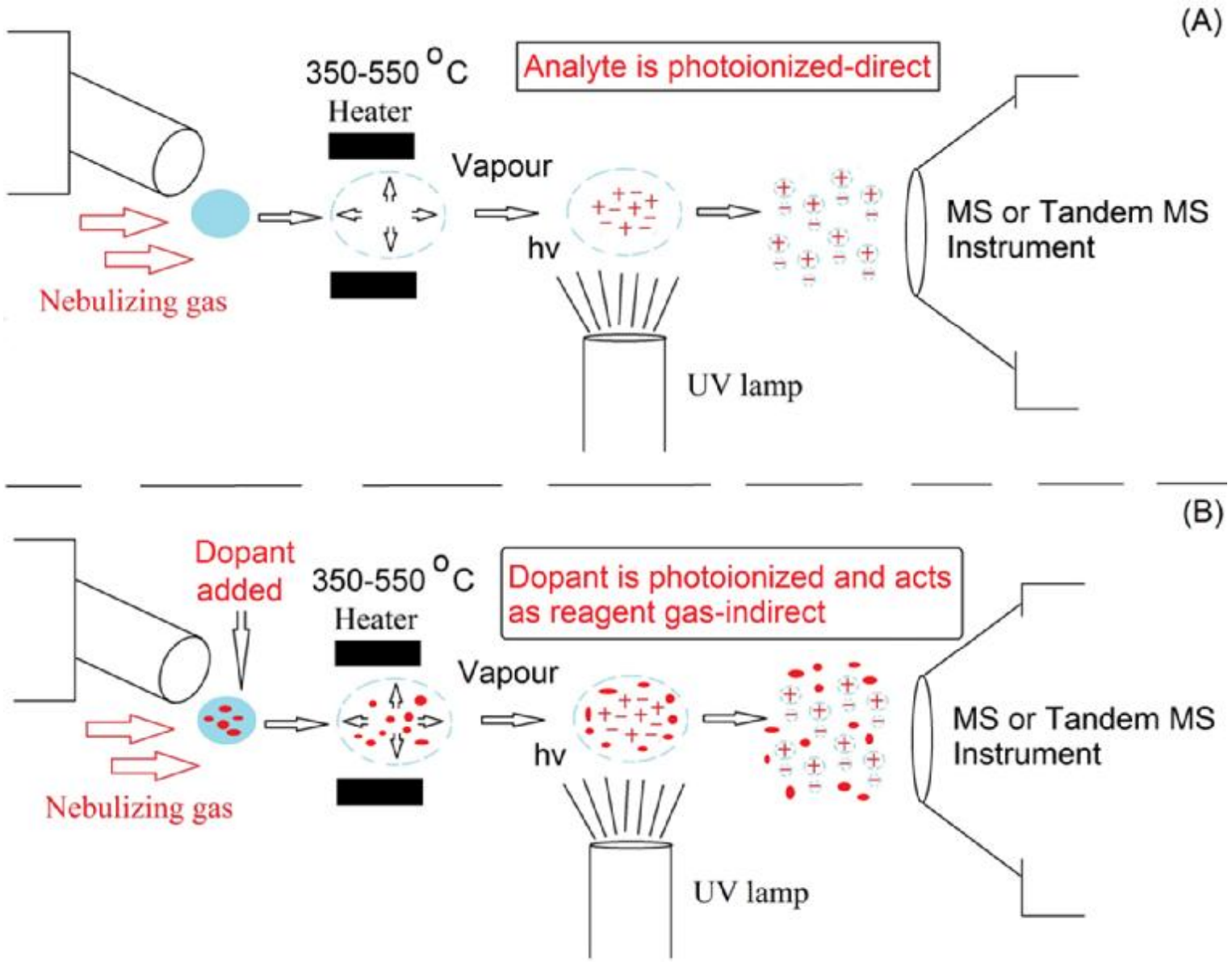
Robb's group

Source available under the trade name: PhotoSpray

Syage's group

Source available under the trade name: PhotoMate

The two sources differ in geometry.


(A)
350-550 °C
Heater
Analyte is photoionized-direct
Vapour
hv
MS or Tandem MS
Instrument
Nebulizing gas
UV lamp
(B)
Dopant
added
350-550 °C
Heater
Dopant is photoionized and acts
as reagent gas-indirect
Vapour
hv
MS or Tandem MS
Instrument
Nebulizing gas
UV lamp

Ionization mechanisms in APPI (1)

APPI is based on the interaction of a photon beam produced by a discharge lamp with the vapors formed by the nebulization of a liquid solution.

Positive ionization

The absorption of a photon (E=hv) by a molecule (M) leads to an excited molecule:

$$M + hv \rightarrow M^*$$

If $IE_M < hv$.
the molecule releases an electron:

$$M^* \rightarrow M^{\bullet +} + e^-$$

If $IE_M > hv$. may be happen:

- photodissociaton:

$$M^* \rightarrow A + B$$

- photon emission:

$$M^* \rightarrow M + hv$$

- collisional quenching:

$$M^* + C \rightarrow M + C^*$$

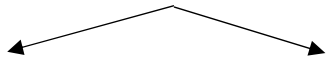
Ionization mechanisms in APPI (2)

When $IE_M > hv$, a preferentially ionized substance called **dopant (D)** can be used in order to promote the ionization of M:

$$D + hv \rightarrow D^{\bullet +}$$

Dopant: intermediate between photons and analytes.

Two mechanisms can occur for the ionization of the analyte (M) from the dopant (D):



Charge transfer.
If $EA_D > EA_M$

$$D^{\bullet +} + M \rightarrow D + M^{\bullet +}$$

Proton transfer.
if $PA_M > PA_{[D-H]\bullet}$

$$D^{\bullet +} + M \rightarrow [D - H]^{\bullet} + [M + H]^{+}$$

Ionization mechanisms in APPI (3)

Negative ionization

Three ionization mechanisms have been proposed:
- charge exchange: $M + C^- \rightarrow M^- + C$
- electron capture: $M + e^- \rightarrow M^{\bullet -}$
- proton transfer: $M + C \rightarrow [M-H]^- + [C+H]^+$

The oxygen (O_2) in the source can be ionized in $O_2^{\bullet -}$:

$$O_2 + e^- \rightarrow O_2^{\bullet -}$$

For compounds with multiple halogen atoms (X) in their molecule, a substitution reaction has been observed with electron transfer from oxygen:

$$M + O_2^{\bullet -} \rightarrow (M-X+O)^{\bullet -} + OX^{\bullet}$$

Light sources

The most common light source is a krypton lamp (Kr): photons 10.03 and 10.64eV in a ratio 4:1

Xenon lamp (Xe. 8.4eV); argon lamp (Ar. 11.7eV).

Dopants

The most common dopants are:

- toluene (IE 8.83 eV)
- acetone (IE 9.70 eV)
- anisole (IE 8.20 eV)
- chlorobenzene (IE 9.07 eV)

Dopants: IE < 9.7eV

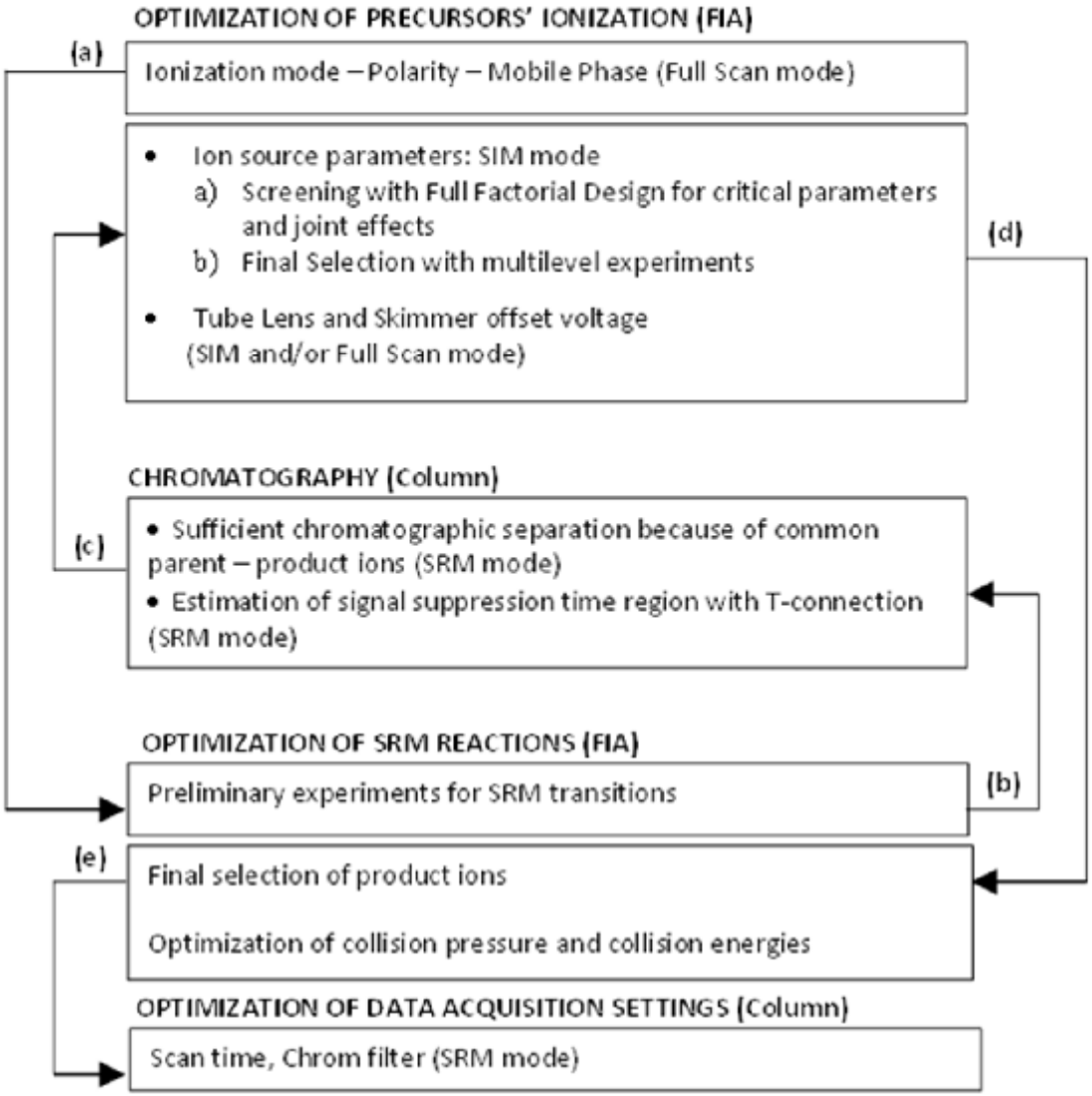
Common solvents for RP-LC: methanol, acetonitrile, water: IE > 10.84eV

Applications of APPI

APPI has been tested and applied in the analysis of several compounds in various type of samples:

- clinical (i.e. testosterone. cortisone)
- drugs (i.e. cyclosporine. morphine)
- environmental samples (i.e. PAHs)
- lipids (i.e. trielaidin)
- natural compounds (i.e. aflatoxins)
- pesticides (i.e. metolachlor)
- synthetic organics (i.e. PBDEs)
- petroleum derivatives (i.e. sulfur compounds)

Optimization strategy of ESI and APCI

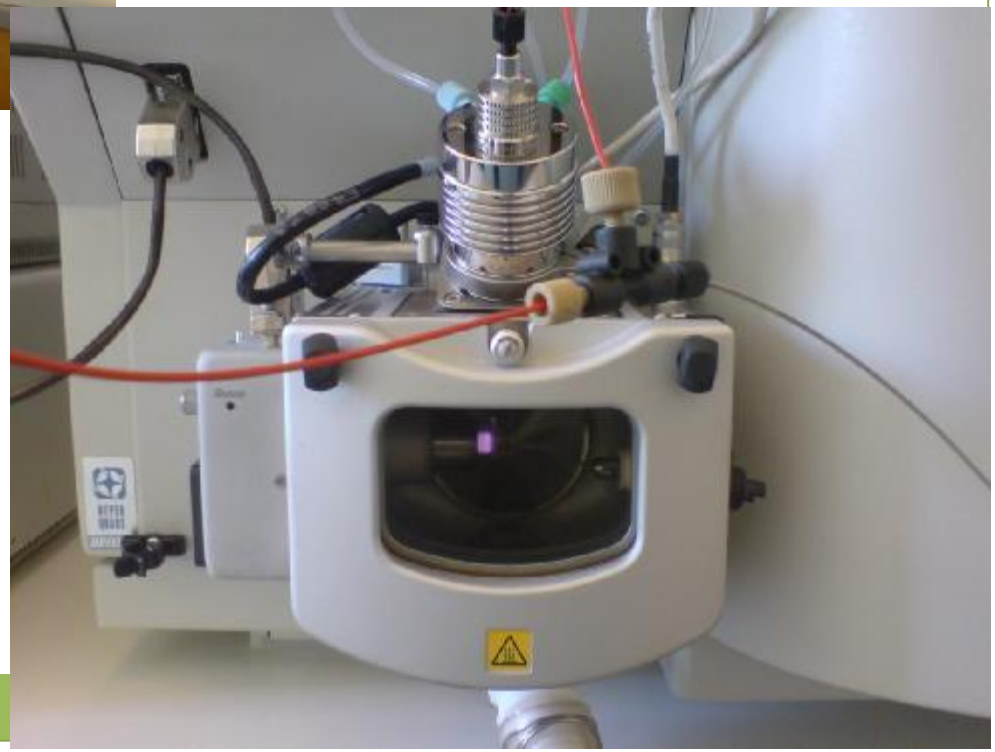


Maragou et al.. J. Am. Soc. Mass Spectrom. 2011. 22: 1826-1838

Thermo LC-APPI-MS/MS system - PhotoMate source

Optimization

- Mobile phase/column
- Dopant
- Source parameters:
  - Position
  - Gases
  - Skimmer offset
  - Vaporizer temperature
  - Capillary temperature



Objectives

→ Study of the most important ionization parameters

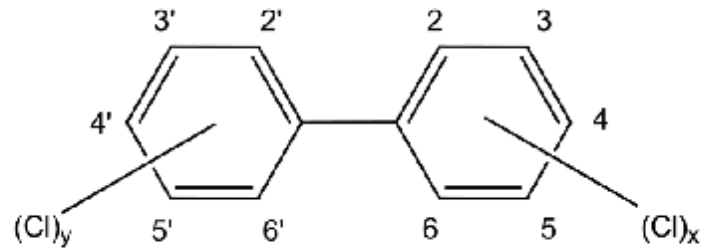
→ Development of an optimization strategy for LC-APPI-MS methods using experimental designs in order to understand the behavior of molecules ionized by APPI as well as the joint effects of ionization parameters.

→ Development of new applications for the technique LC-MS/MS using the APPI source.

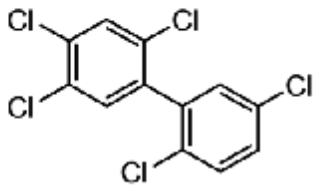
For these reasons three categories of halogenated compounds were studied:

- **Polychlorinated Biphenyls (PCBs)**
- **Polychlorinated Naphthalenes (PCNs)**
- **Polybrominated Diphenyl Ethers (PBDEs)**

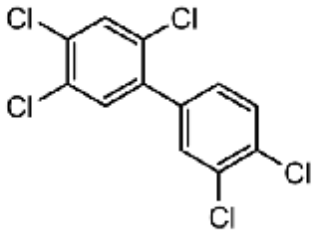
Polychlorinated Biphenyls (PCBs)



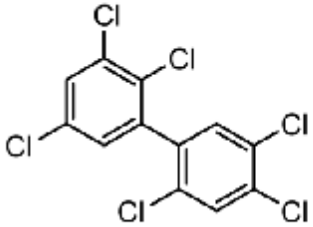
PCBs' basic molecular structure and the conventional numbering of the substituent positions, where x+y=n.



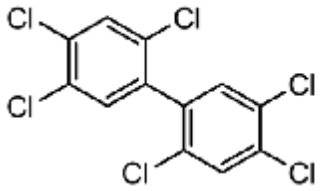
PCB 101
(2,2',4,5,5'-pentachlorobiphenyl)
Mr = 324



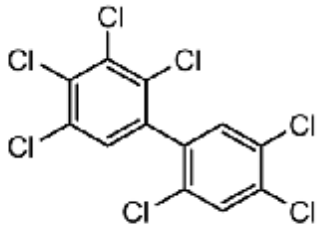
PCB 118
(2,3',4,4',5-pentachlorobiphenyl)
Mr = 324



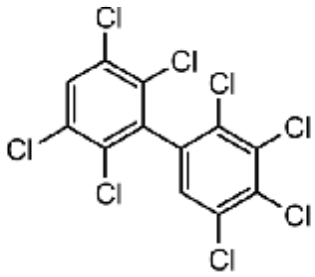
PCB 138
(2,2',3,4,4',5'-hexachlorobiphenyl)
Mr = 358



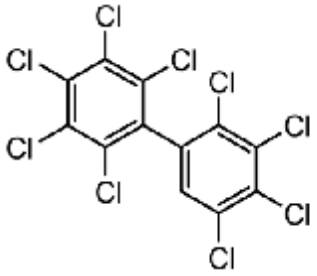
PCB 153
(2,2',4,4',5,5'-hexachlorobiphenyl)
Mr = 358



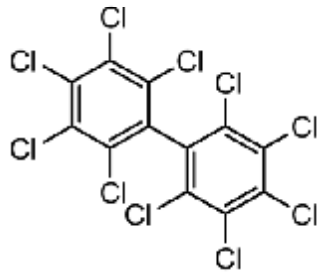
PCB 180
(2,2',3,4,4',5,5'-heptachlorobiphenyl)
Mr = 392



PCB 199
(2,2',3,3',4,5,5',6'-octachlorobiphenyl)
Mr = 426



PCB 206
(2,2',3,3',4,4',5,5',6-nonachlorobiphenyl)
Mr = 460



PCB 209
(decachlorobiphenyl)
Mr = 494

Strategy for the development and optimization of a method for the determination of PCBs by LC-APPI-MS/MS (1)

Infusion of standard solution (10 µg/mL) for each PCB. Record full scan MS spectra.

Selection of ionization source and polarity: **APPI in negative ionization**.

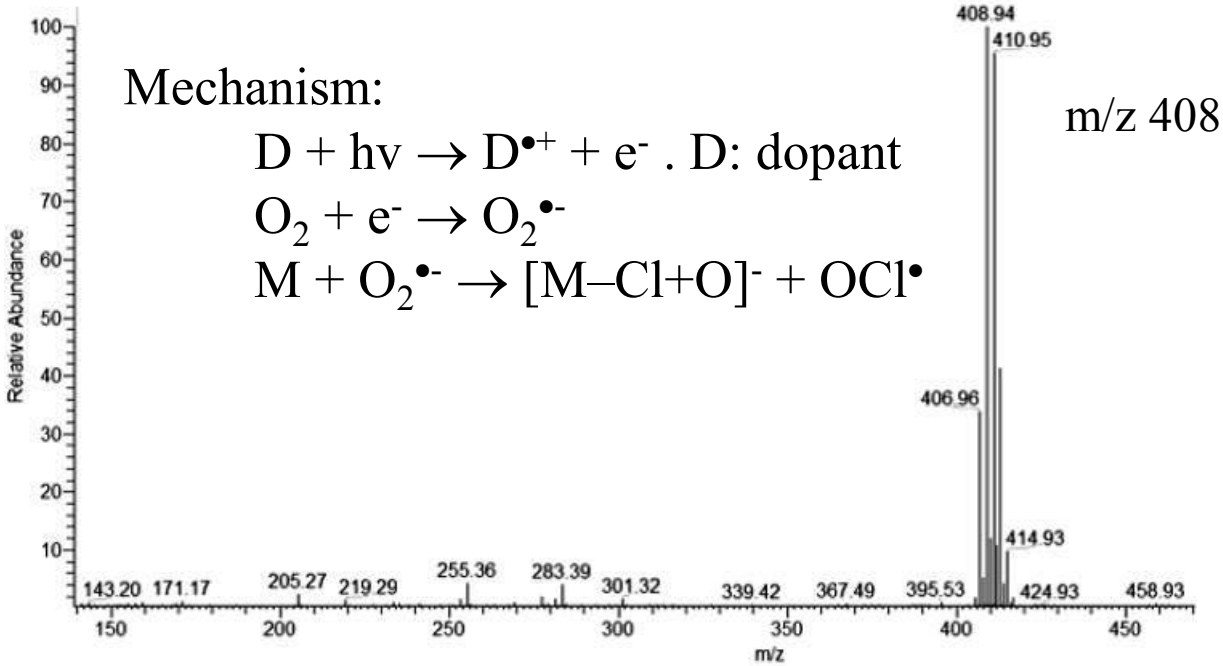
Intense peak appeared at m/z attributed to **[M-Cl+O]$^-$**.

Mechanism:

$D + hv \rightarrow D^{\bullet +} + e^-$. D: dopant

$O_2 + e^- \rightarrow O_2^{\bullet -}$

$M + O_2^{\bullet -} \rightarrow [M–Cl+O]^- + OCl^{\bullet}$



PCB 199

Strategy for the development and optimization of a method for the determination of PCBs by LC-APPI-MS/MS (2)

Congener	Observed SRMs (negative ionization)	
	Q_1 (precursor ion)	Q_3 (four most intense product ions)
PCB 101	$[M - Cl + O]^-$ (307)	$[M - Cl_2 + O]^-$ (271, 269), Cl^- (37, 35)
PCB 118	$[M - Cl + O]^-$ (307)	$[M - Cl_2 + O]^-$ (271, 269), $[M - Cl_3 + O]^-$ (233, 235)
PCB 138	$[M - Cl + O]^-$ (341)	$[M - Cl_2 + O]^-$ (305, 303), $[M - Cl_3 + O]^-$ (269, 267)
PCB 153	$[M - Cl + O]^-$ (341)	$[M - Cl_2 + O]^-$ (305, 303), $[M - Cl_3 + O]^-$ (269, 267)
PCB 180	$[M - Cl + O]^-$ (375)	$[M - Cl_2 + O]^-$ (339, 337), $[M - Cl_3 + O]^-$ (303, 301)
PCB 199	$[M - Cl + O]^-$ (409)	$[M - Cl_2 + O]^-$ (373), $[M - Cl_3 + O]^-$ (337, 339), Cl^- (35)
PCB 206	$[M - Cl + O]^-$ (445)	$[M - Cl_2 + O]^-$ (409), $[M - Cl_3 + O]^-$ (375, 373), Cl^- (37)
PCB 209	$[M - Cl + O]^-$ (479)	$[M - Cl_2 + O]^-$ (444), $[M - Cl_3 + O]^-$ (405, 407, 409)

Strategy for the development and optimization of a method for the determination of PCBs by LC-APPI-MS/MS (3)

Univariate optimization of the most important APPI parameters was conducted by loop injections of standard solutions for each PCB (1μg/mL, 5 μL).

With what solvent ?

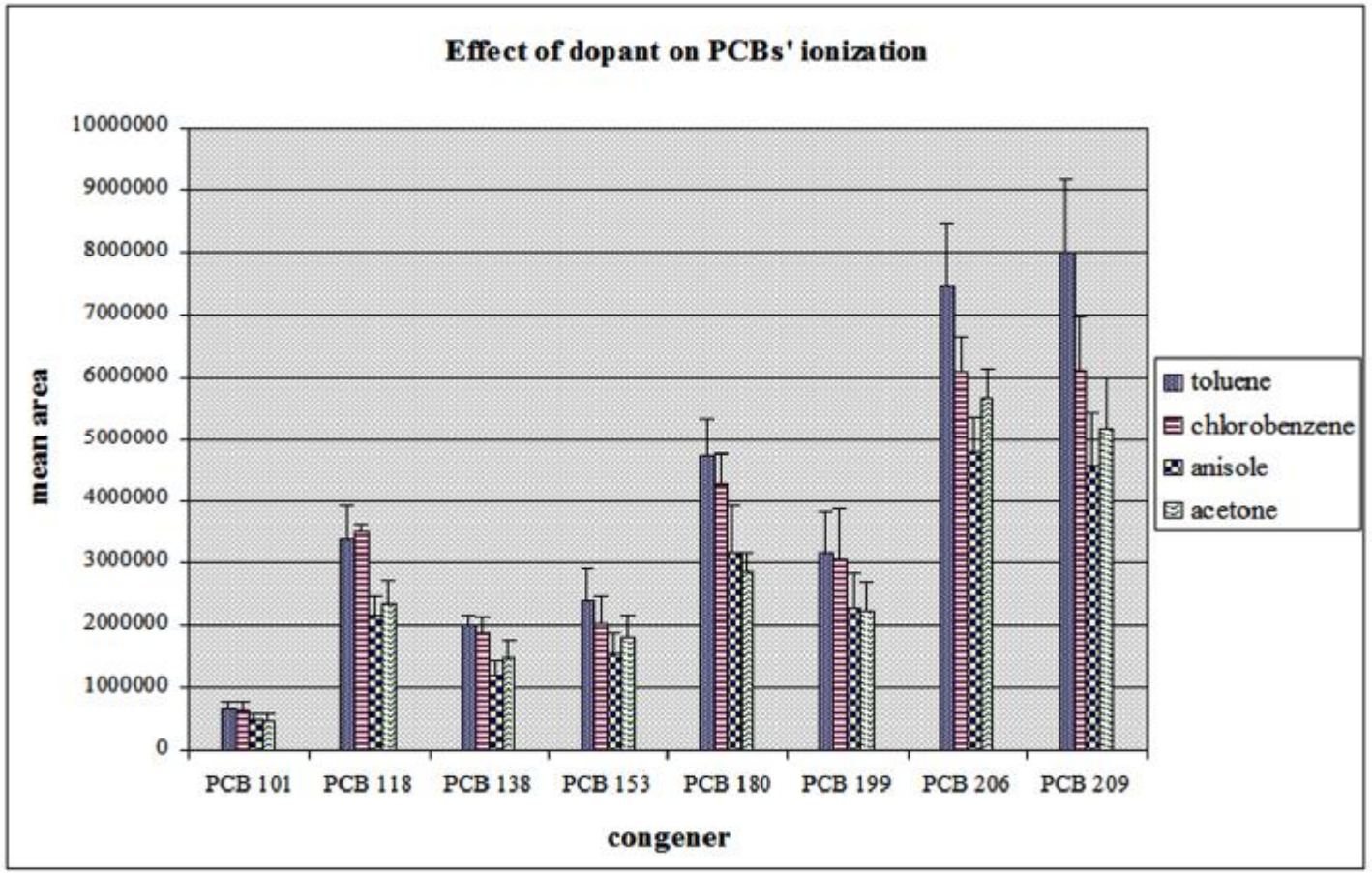
Before this step. preliminary experiments were conducted in order to determine the basic parameters of LC (column and mobile phase). All analytes were eluted when mobile phase was **100% methanol**.

So, methanol will be used as solvent during the optimization process.

Strategy for the development and optimization of a method for the determination of PCBs by LC-APPI-MS/MS (4)

Dopant

(n=5 injections, full scan MS)

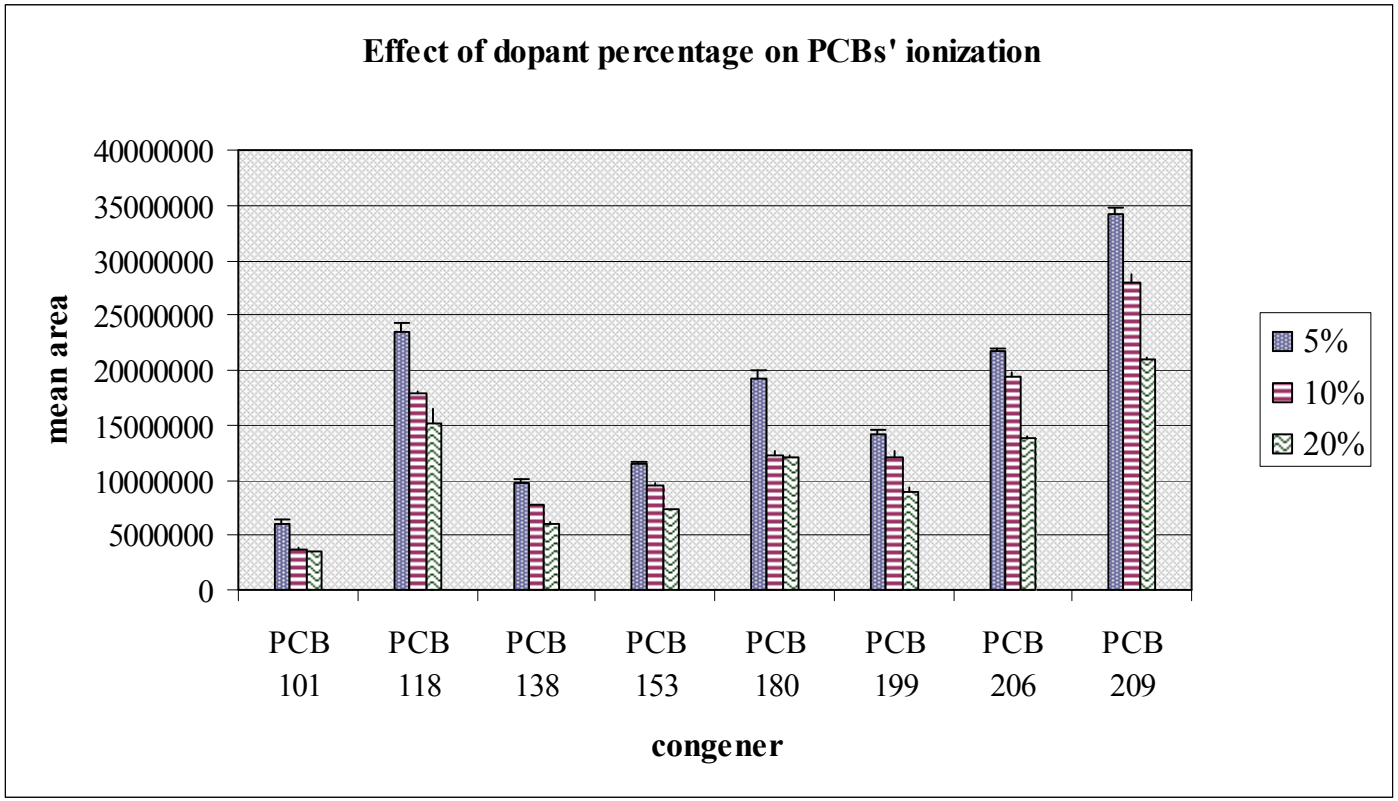


Final selection: **toluene**

Strategy for the development and optimization of a method for the determination of PCBs by LC-APPI-MS/MS (5)

For the rest parameters of the univariate optimization, loop injections (n=4) were conducted recording the selected SRM for each PCB.

Dopant flow

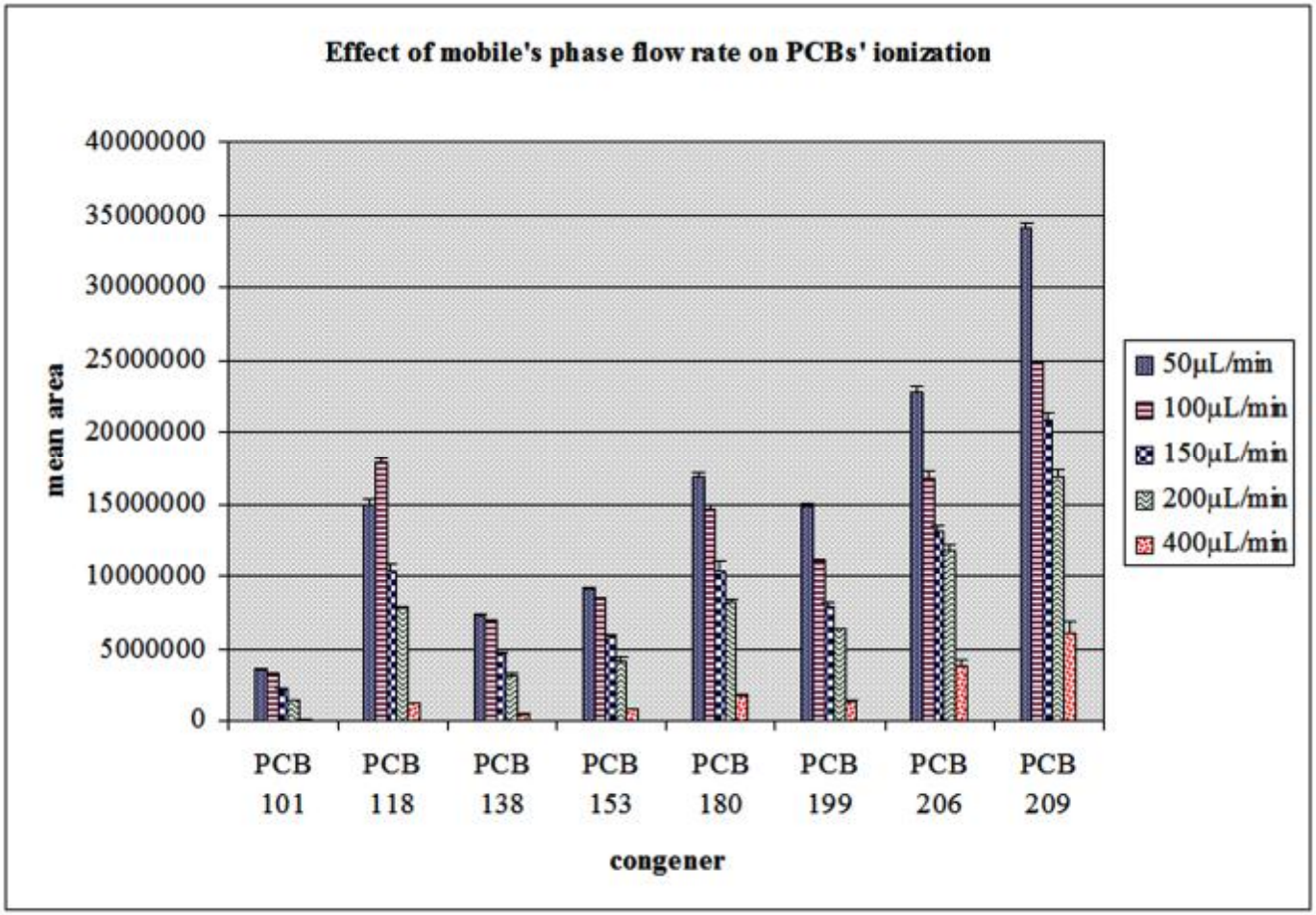


final selection: 5%

Strategy for the development and optimization of a method for the determination of PCBs by LC-APPI-MS/MS (6)

Mobile phase flow: final selection 100μL/min

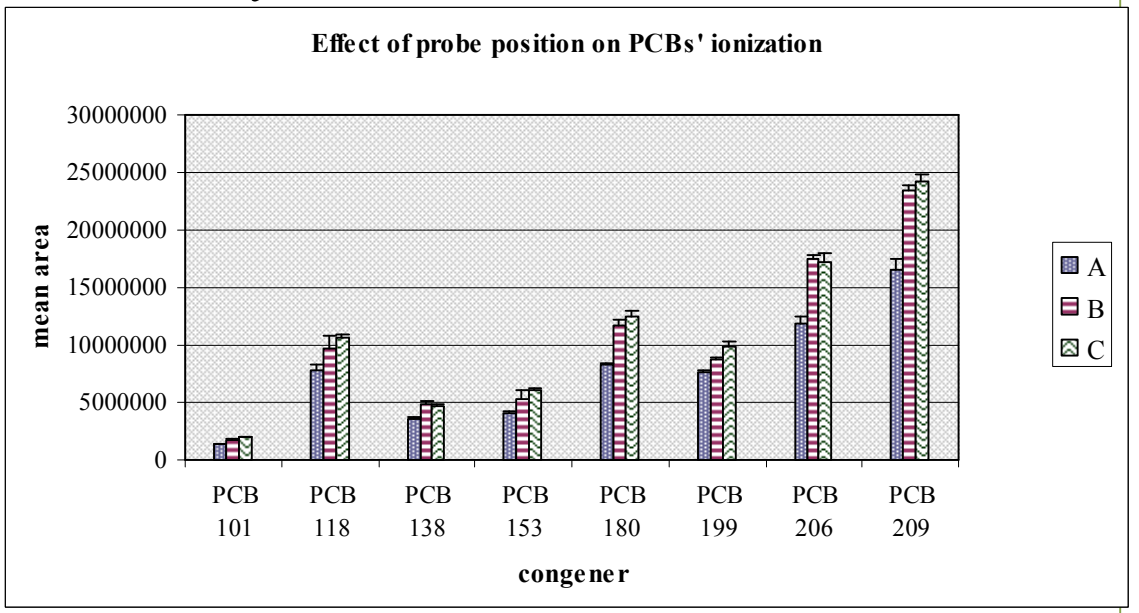
- Compromise between ionization. peak shape and analysis time.



Strategy for the development and optimization of a method for the determination of PCBs by LC-APPI-MS/MS (7)

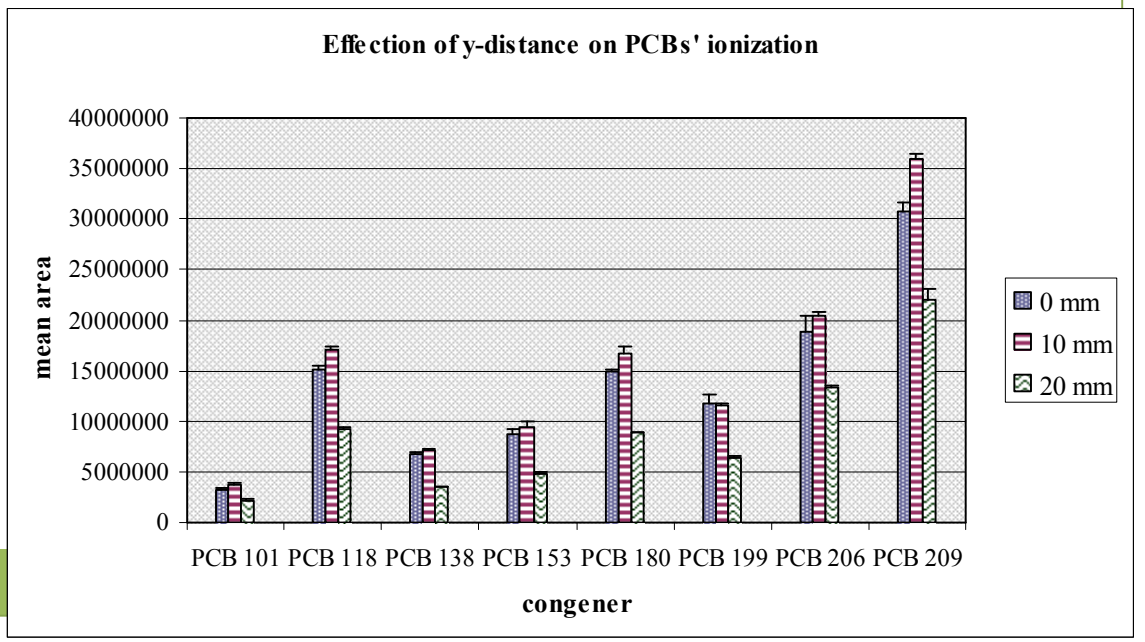
Probe position

final selection: position C



Y-distance

initial selection: 0a.u.

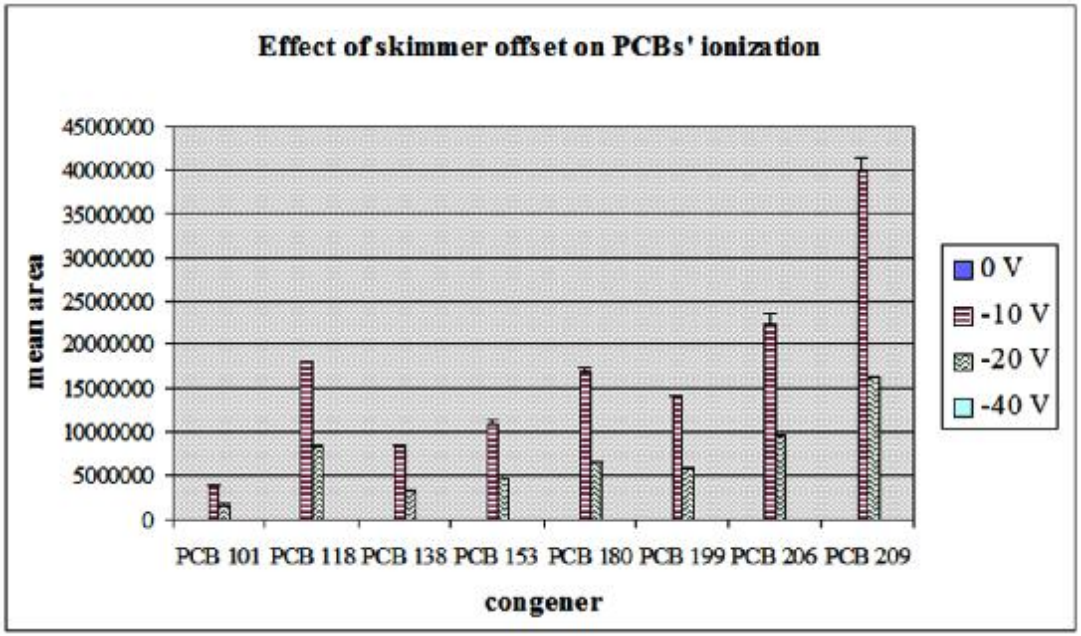


Strategy for the development and optimization of a method for the determination of PCBs by LC-APPI-MS/MS (8)

Skimmer offset

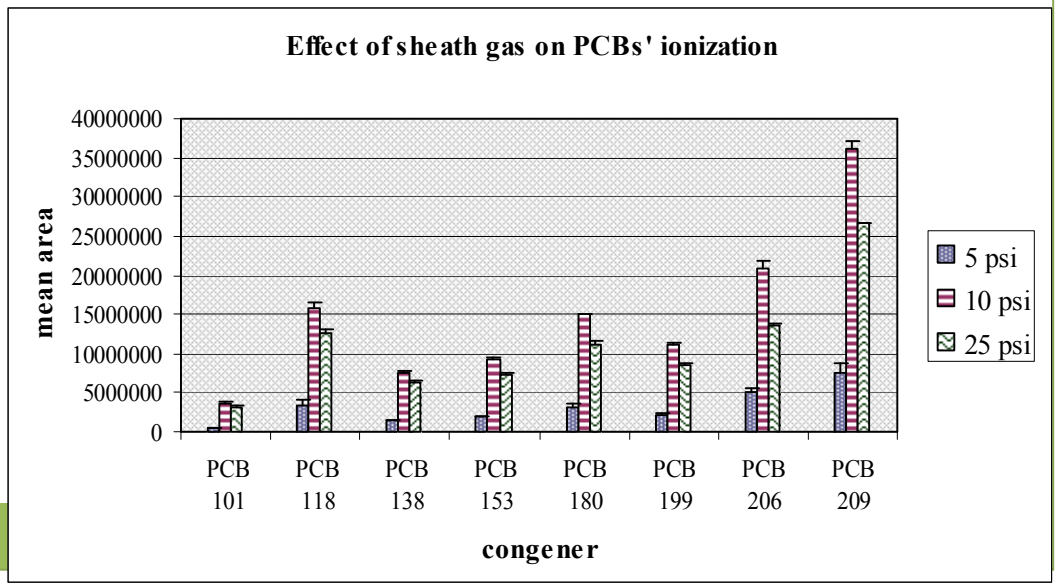
final selection: -10V

A small voltage at skimmer was necessary for desolvation and the increase of the signal.



Sheath gas

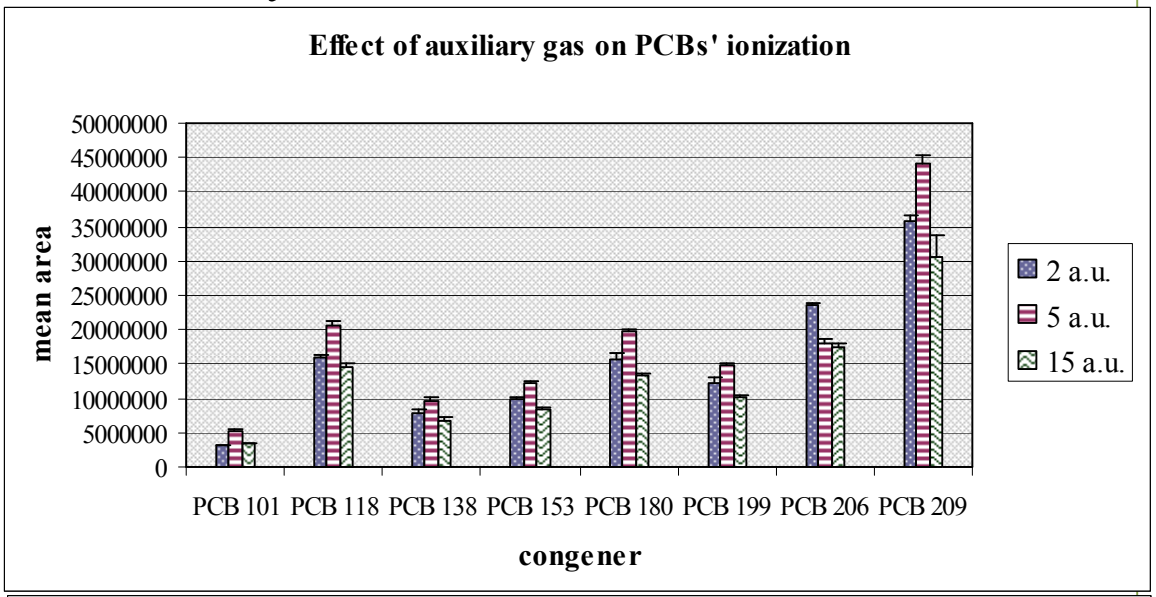
initial selection: 10psi



Strategy for the development and optimization of a method for the determination of PCBs by LC-APPI-MS/MS (9)

Auxiliary gas

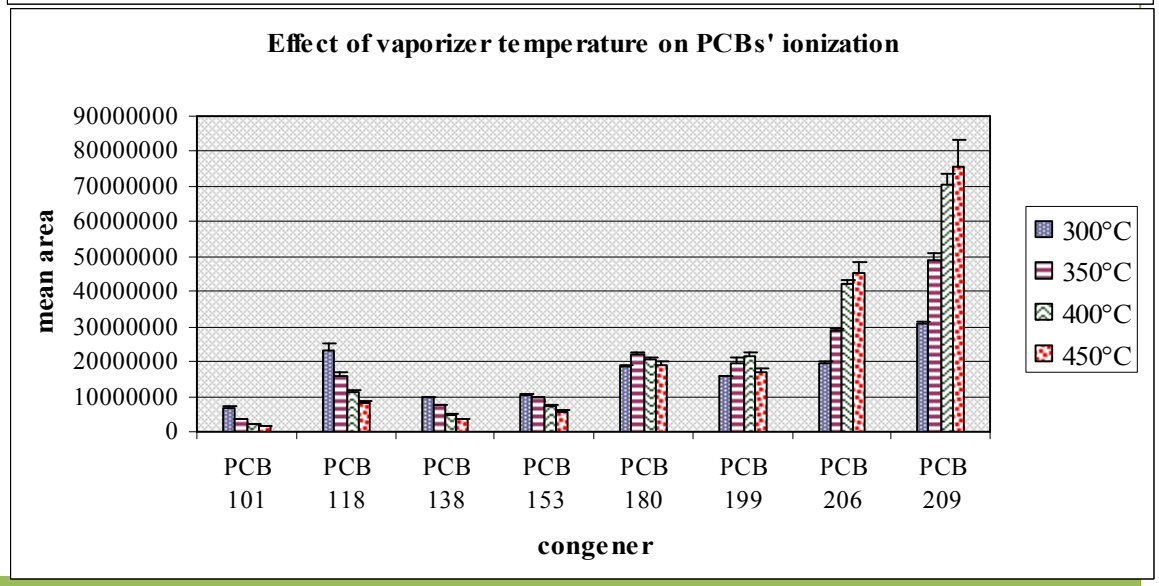
initial selection: 5a.u.



Vaporizer temperature

initial selection: 300°C

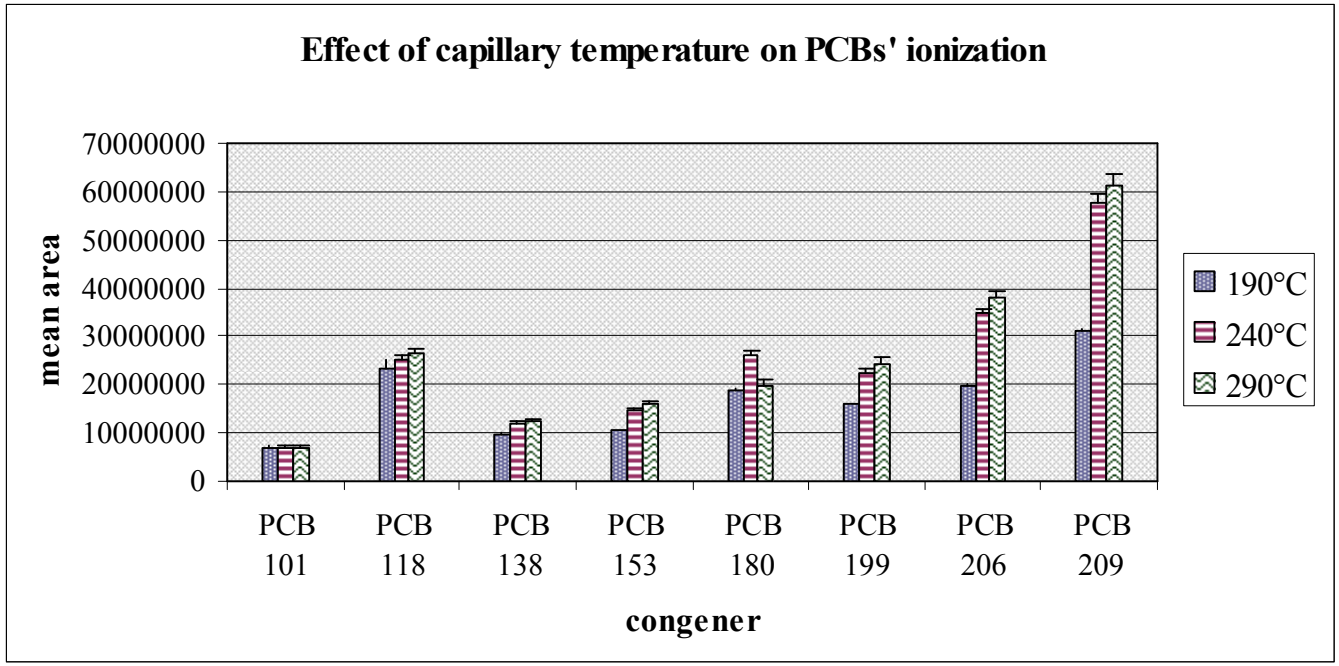
(preference on less sensitive PCBs)



Strategy for the development and optimization of a method for the determination of PCBs by LC-APPI-MS/MS

Capillary temperature

Initial selection: 190^{o}C



Strategy for the development and optimization of a method for the determination of PCBs by LC-APPI-MS/MS

Screening full factorial experimental design. Peak area was the response variable. Statgraphics Centurion XV Software was used.

Set a low and a high value for each parameter:

Levels	Y-distance (a.u.)	Sheath gas (psi)	Auxiliary gas (a.u.)	Vaporizer temperature (°C)	Capillary temperature (°C)
-1	0	8	2	300	200
0	5	14	5	375	250
+1	10	20	8	450	300

Conducted by loop injections of standard solutions of each PCB (1μg/mL, 5 μL) at each combination.

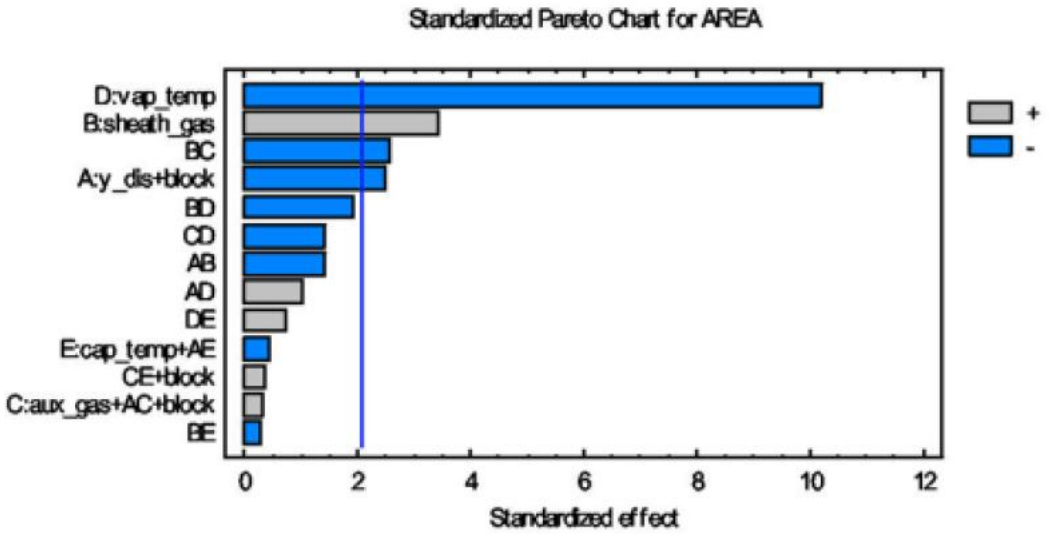
Strategy for the development and optimization of a method for the determination of PCBs by LC-APPI-MS/MS (12)

Pareto charts:

- Important parameters: **vaporizer temperature, auxiliary gas, sheath gas**.
- Less important parameters take their final value:

  y-distance: 0a.u.

  capillary temperature: 200°C



PCB 101

Strategy for the development and optimization of a method for the determination of PCBs by LC-APPI-MS/MS

For the critical parameters (sheath gas. auxiliary gas and vaporizer temperature). a **surface response design (CCD)** of experiments was planned in order to locate their optimal values. The response value was again the peak area.

Conducted by loop injections of standard solutions of each PCB (1μg/mL, 5 μL) at each combination.

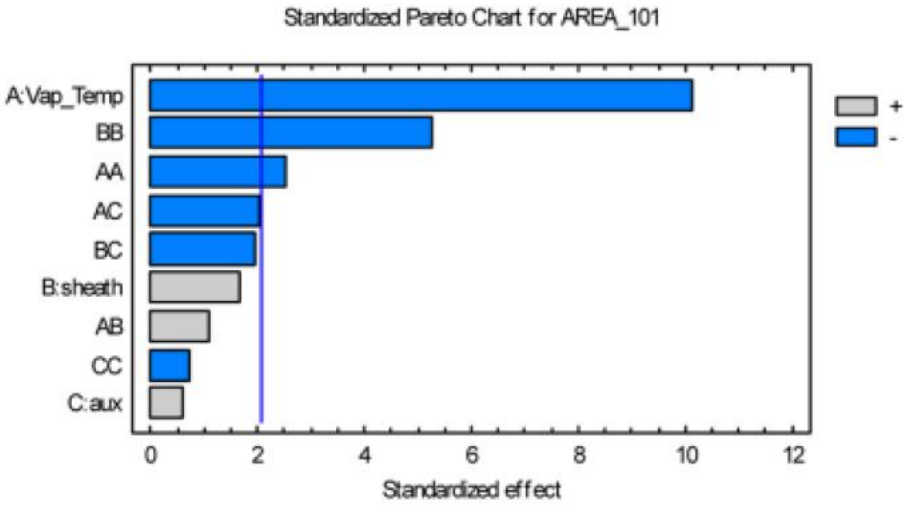
Set a low and a high value for each parameter:

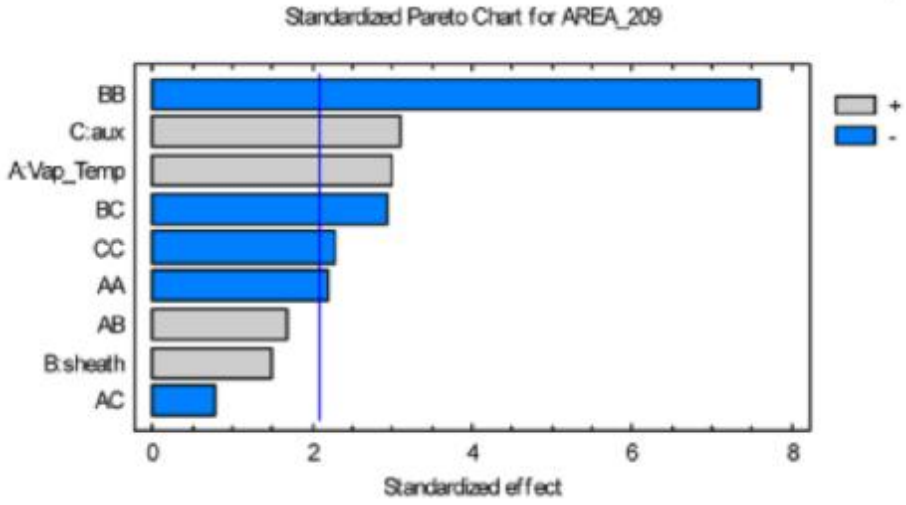
Levels	Sheath gas (psi)	Auxiliary gas (a.u.)	Vaporizer temperature (^{o}C)
-a	3	0	232
-1	10	2	280
0	20	5	350
1	30	8	420
+a	37	10	468

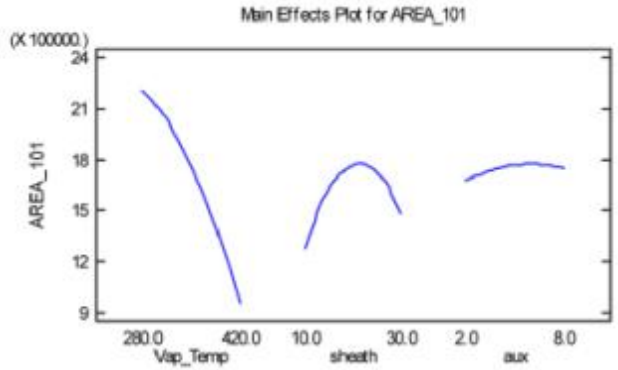
Strategy for the development and optimization of a method for the determination of PCBs by LC-APPI-MS/MS (14)

Pareto Charts

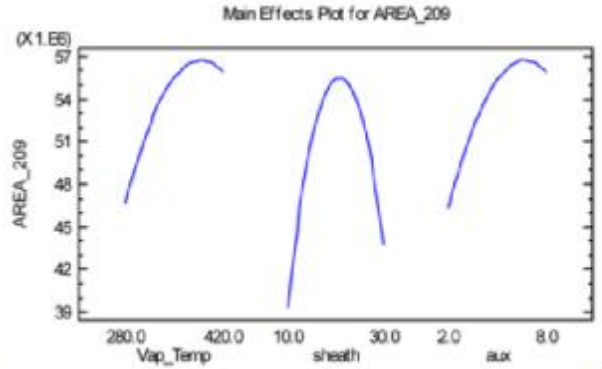
- more important parameter: **vaporizer temperature**
- less important parameter: auxiliary gas







PCB 101



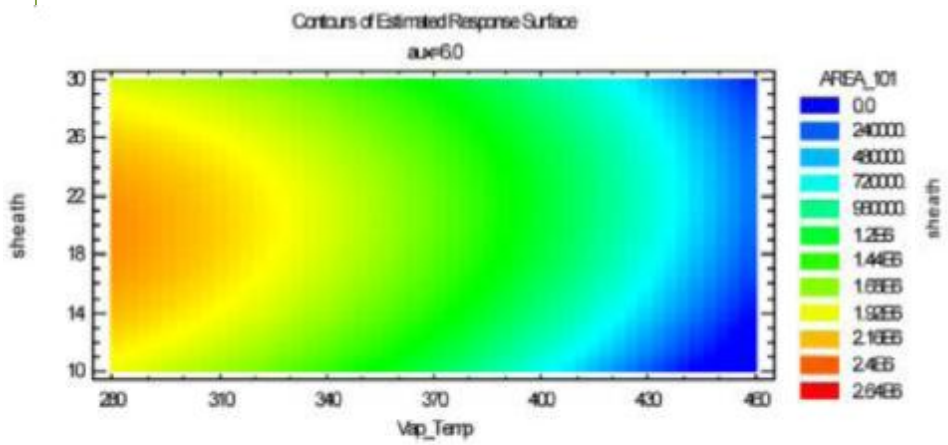
PCB 209

Strategy for the development and optimization of a method for the determination of PCBs by LC-APPI-MS/MS

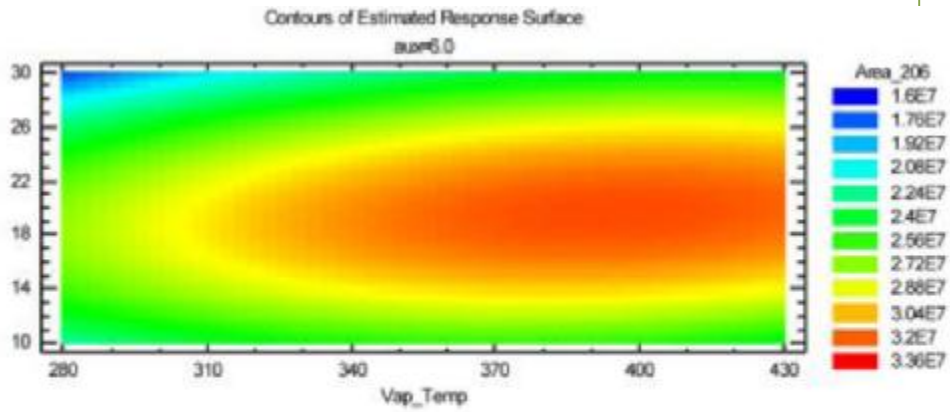
Surface response plots for each congener were created keeping the auxiliary gas constant.

The optimum value for sheath gas was approximately the same for all PCBs.

What about vaporizer temperature?



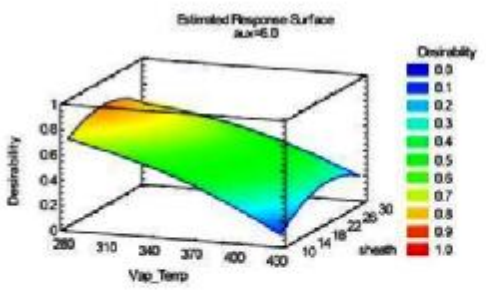
PCB 101



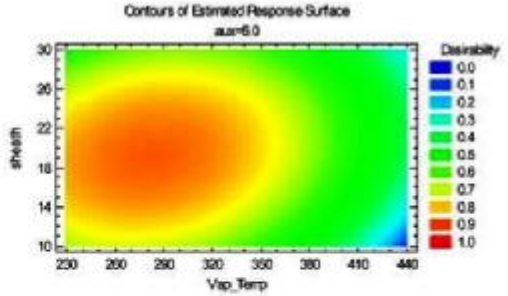
PCB 206

Strategy for the development and optimization of a method for the determination of PCBs by LC-APPI-MS/MS

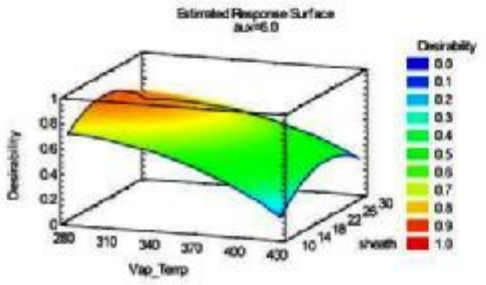
Desirability plots for the **less sensitive compound, PCB 101,** were constructed with a representative PCB congener of each group.



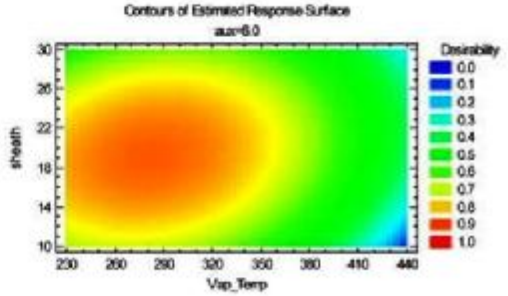
PCB 101 – PCB 138



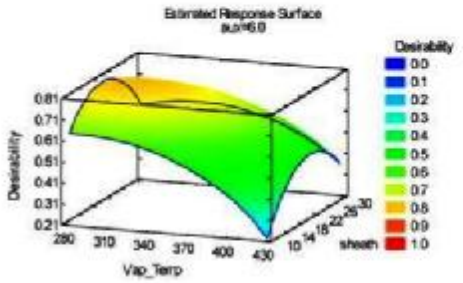
PCB 101 – PCB 138



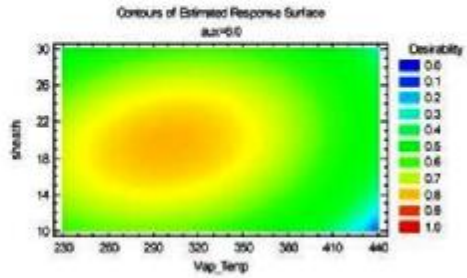
PCB 101 – PCB 180



PCB 101 – PCB 180



PCB 101 - PCB 209



PCB 101 - PCB 209

Final values for the parameters:

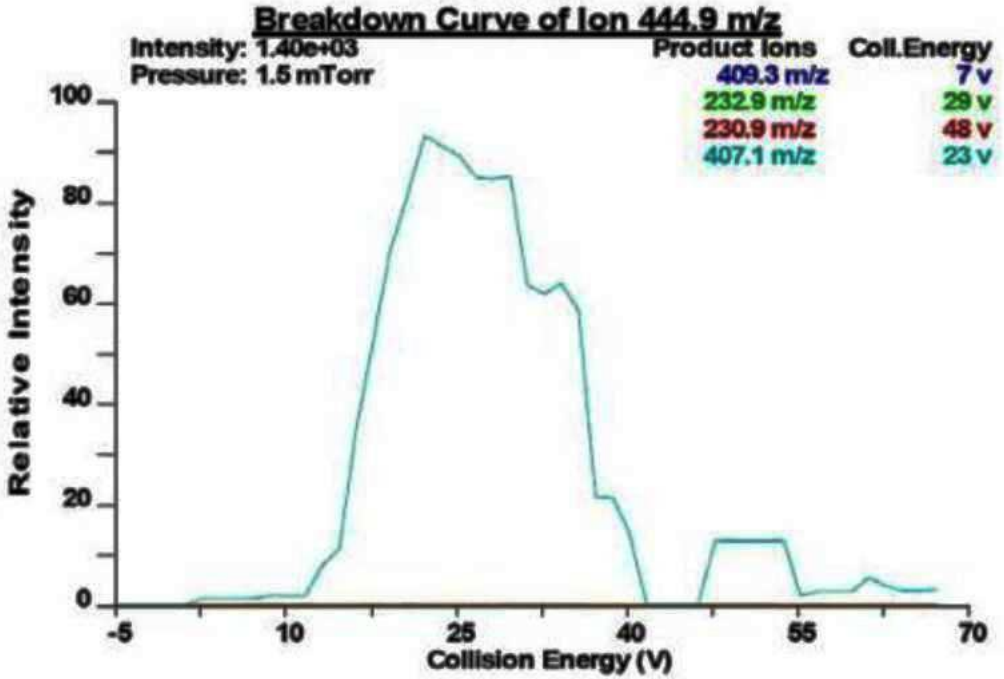
vaporizer temperature: 280ºC
sheath gas: 20 psi
auxiliary gas: 6 a.u.

Strategy for the development and optimization of a method for the determination of PCBs by LC-APPI-MS/MS

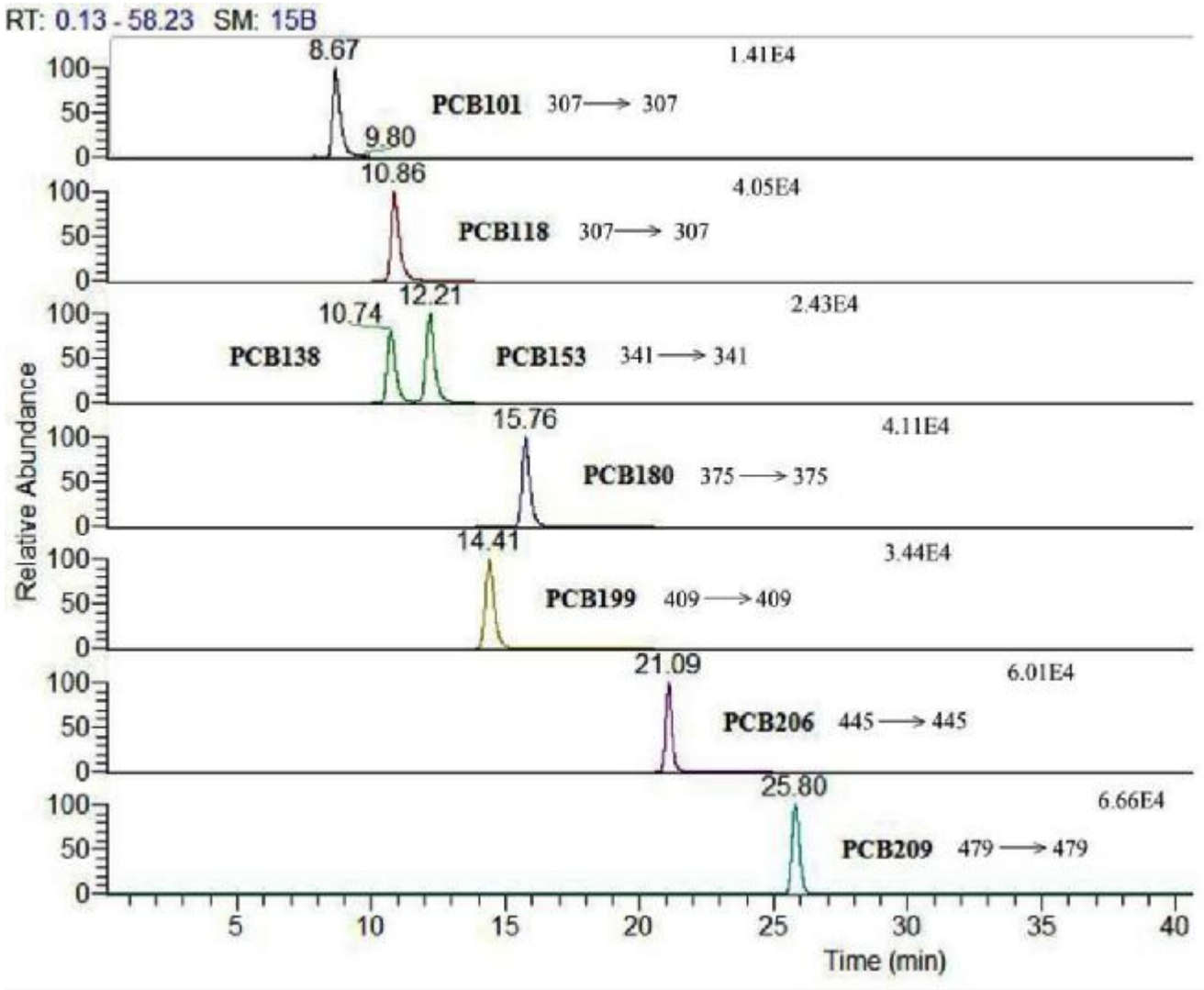
Experiments with loop injections were realized to confirm the selected precursor ion of each congener and its product ions under the final conditions.

The **fragmentation of PCB199, PCB206 and PCB209 was not efficient** under flow conditions

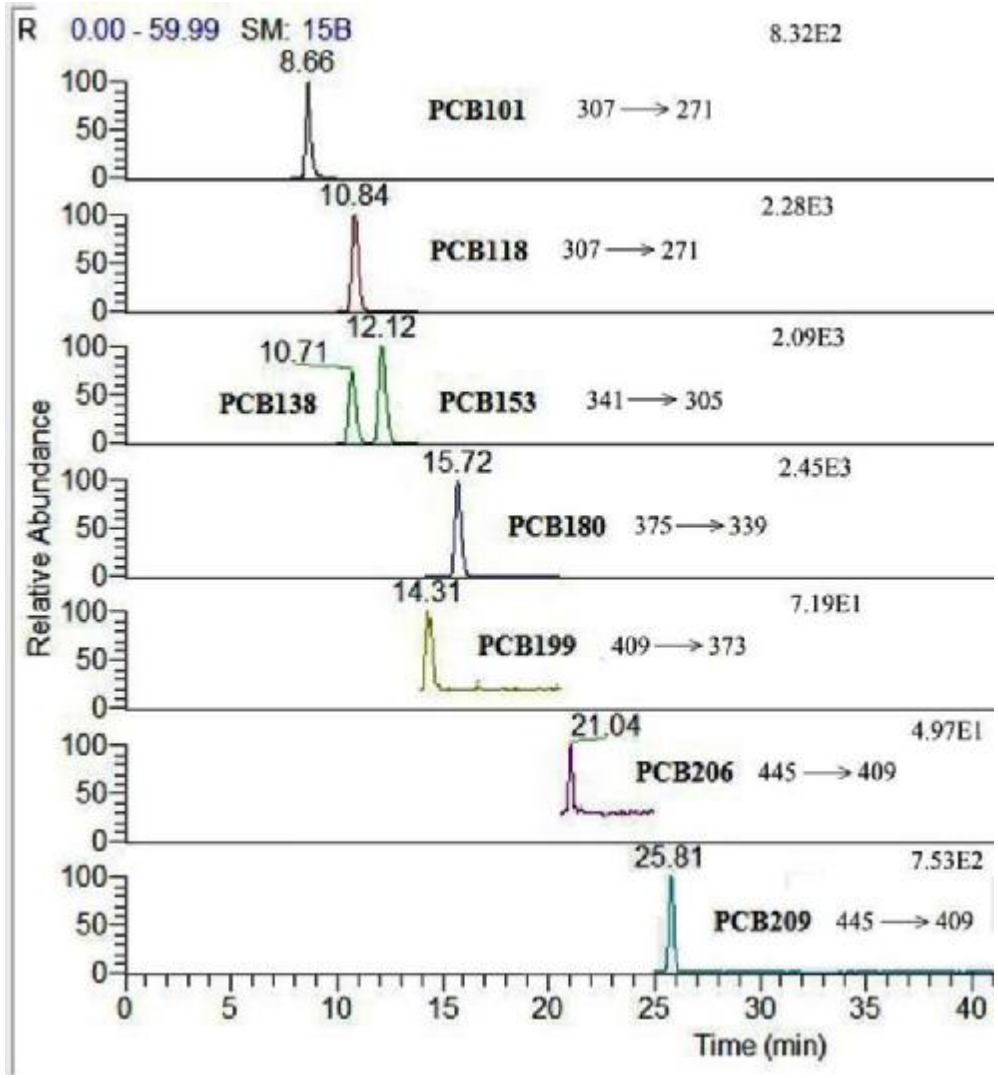
To overcome this problem. the use of **pseudo-SRM technique** was performed.



PCB206



Pseudo-SRM chromatogram of multi-congener standard solution 100 µg/L



SRM chromatogram of multi-congener standard solution 100 µg/L

Mobile phases / Column

Methanol-water gave higher signals for all PCBs.

Acetonitrile-water gave better separations of isomeric PCBs.

Column Waters XSELECT HSS T3 (100 x 2.1 mm. 2.5 µm) was selected (better peak shapes).

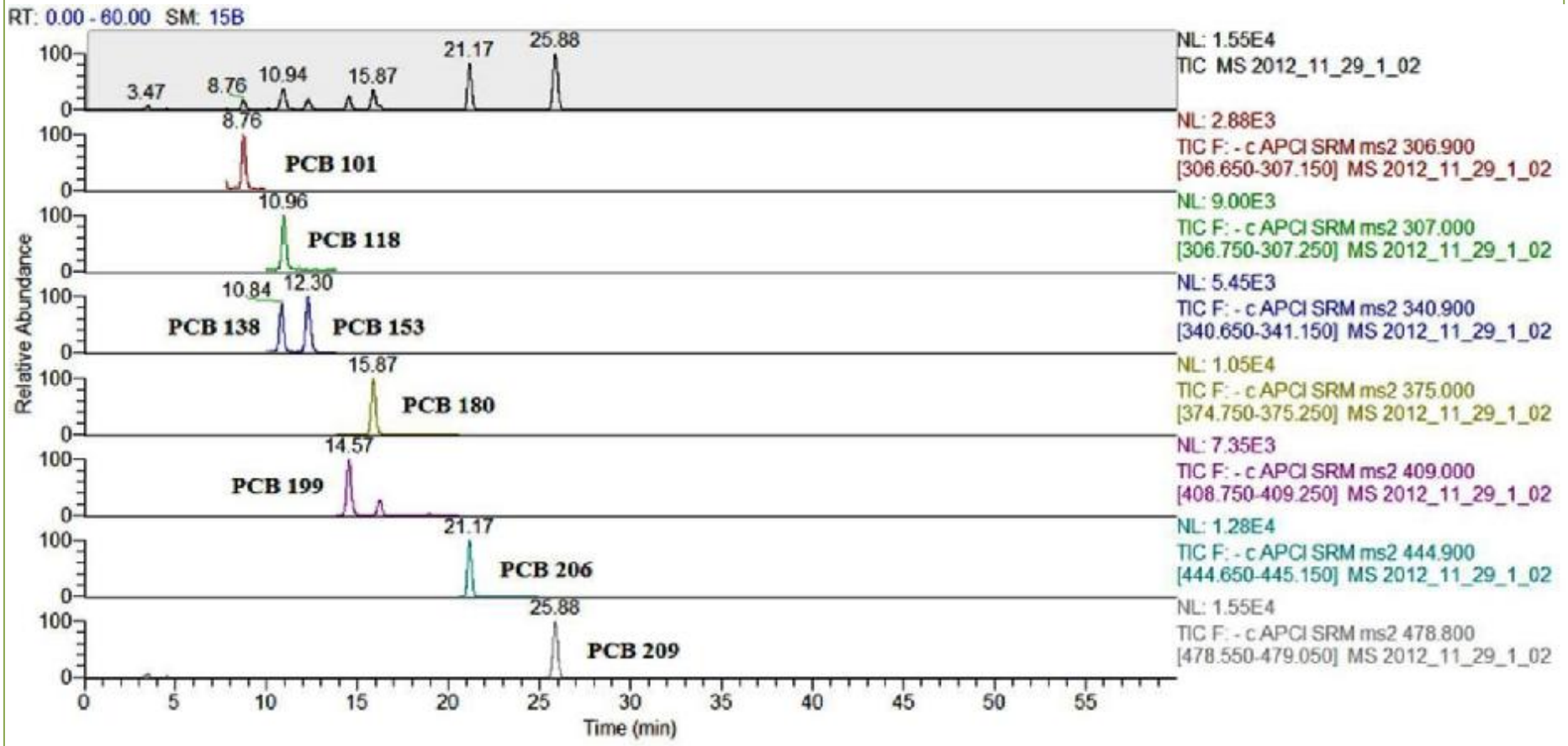
Column Temperature: 25°C

Injection volume: 5µL

Time (min)	Flow (mL/min)	Water (%)	Methanol (%)
0	0.100	8	92
3	0.100	0	100
30	0.100	0	100
31	0.100	8	92
60	0.100	8	92

Method validation for the determination of PCBs by LC-APPI-MS/MS

Congener	LoD (µg/L)	LoQ (µg/L)	Precision (n=6. %RSD)		Trueness (mean recovery ± SD. n=6)		Overall recovery %	Correlation coefficient (R^2)	Regression coefficients with standard errors of the calibration equations	
			0.01 µg/L	0.1 µg/L	0.01 µg/L	0.1 µg/L			$(b \pm s_b)\times10^5$	$(a \pm s_a)\times10^5$
PCB 101	0.0030	0.0092	3.5	8.8	76.8 ± 5.7	110 ± 10	73	0.992	6.73 ± 0.34	0.057 ± 0.035
PCB 118	0.0030	0.0092	6.2	5.2	80.7 ± 9.3	109.7 ± 6.1	86	0.994	26.5 ± 1.2	0.18 ± 0.12
PCB 138	0.0024	0.0072	4.9	5.7	72.4 ± 4.9	112.8 ± 6.7	84	0.991	13.31 ± 0.75	0.079 ± 0.077
PCB 153	0.0022	0.0067	5.2	8.1	75.6 ± 6.4	108.8 ± 9.2	83	0.995	17.62 ± 0.75	0.086 ± 0.077
PCB 180	0.0031	0.0094	7.8	5.7	70.0 ± 9.4	110.7 ± 6.6	90	0.993	32.0 ± 1.6	0.16 ± 0.16
PCB 199	0.0016	0.0048	7.2	2.4	70.5 ± 7.6	110.2 ± 2.7	96	0.994	28.4 ± 1.3	0.10 ± 0.13
PCB 206	0.0023	0.0071	8.3	6.0	68.1 ± 9.0	109.7 ± 6.8	90	0.994	36.4 ± 1.7	0.14 ± 0.17
PCB 209	0.0024	0.0072	8.6	9.7	68.2 ± 10.6	113 ± 11	84	0.991	46.0 ± 2.7	0.26 ± 0.27

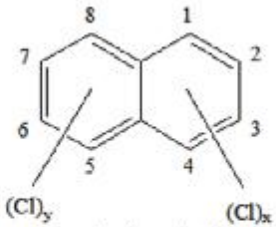


Pseudo SRM chromatogram of spiked wastewater sample (0.05 μg/L)

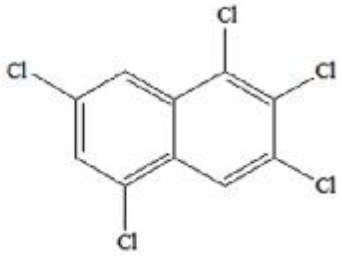
For more information:

Athanasios I. Moukas, Nikolaos S. Thomaidis, Antonios C. Calokerinos, 2014. Determination of polychlorinated biphenyls by liquid chromatography–atmospheric pressure photoionization–mass spectrometry. Journal of Mass Spectrometry 49: 1096 - 1107.

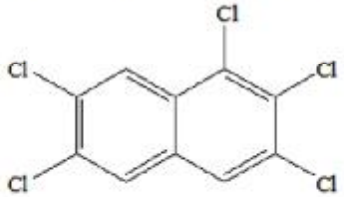
Polychlorinated Naphthalenes (PCNs)



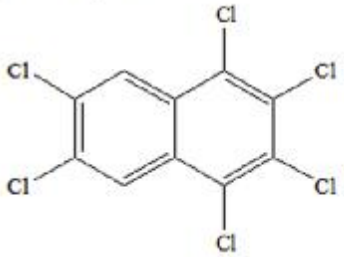
PCNs' basic molecular structure and the conventional numbering of the substituent positions, where x+y=n.



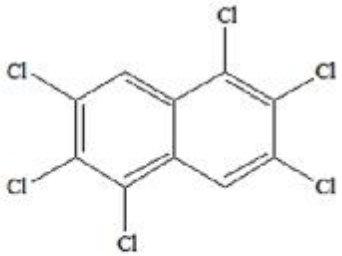
PCN 52
(1,2,3,5,7-pentachloronaphthalene)
Mr = 298



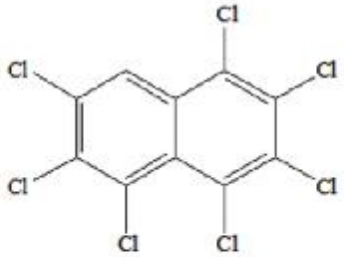
PCN 54
(1,2,3,6,7-pentachloronaphthalene)
Mr = 298



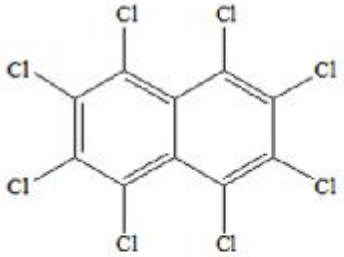
PCN 66
(1,2,3,4,6,7-hexachloronaphthalene)
Mr = 332



PCN 67
(1,2,3,5,6,7-hexachloronaphthalene)
Mr = 332



PCN 73
(1,2,3,4,5,6,7-heptachloronaphthalene)
Mr = 366

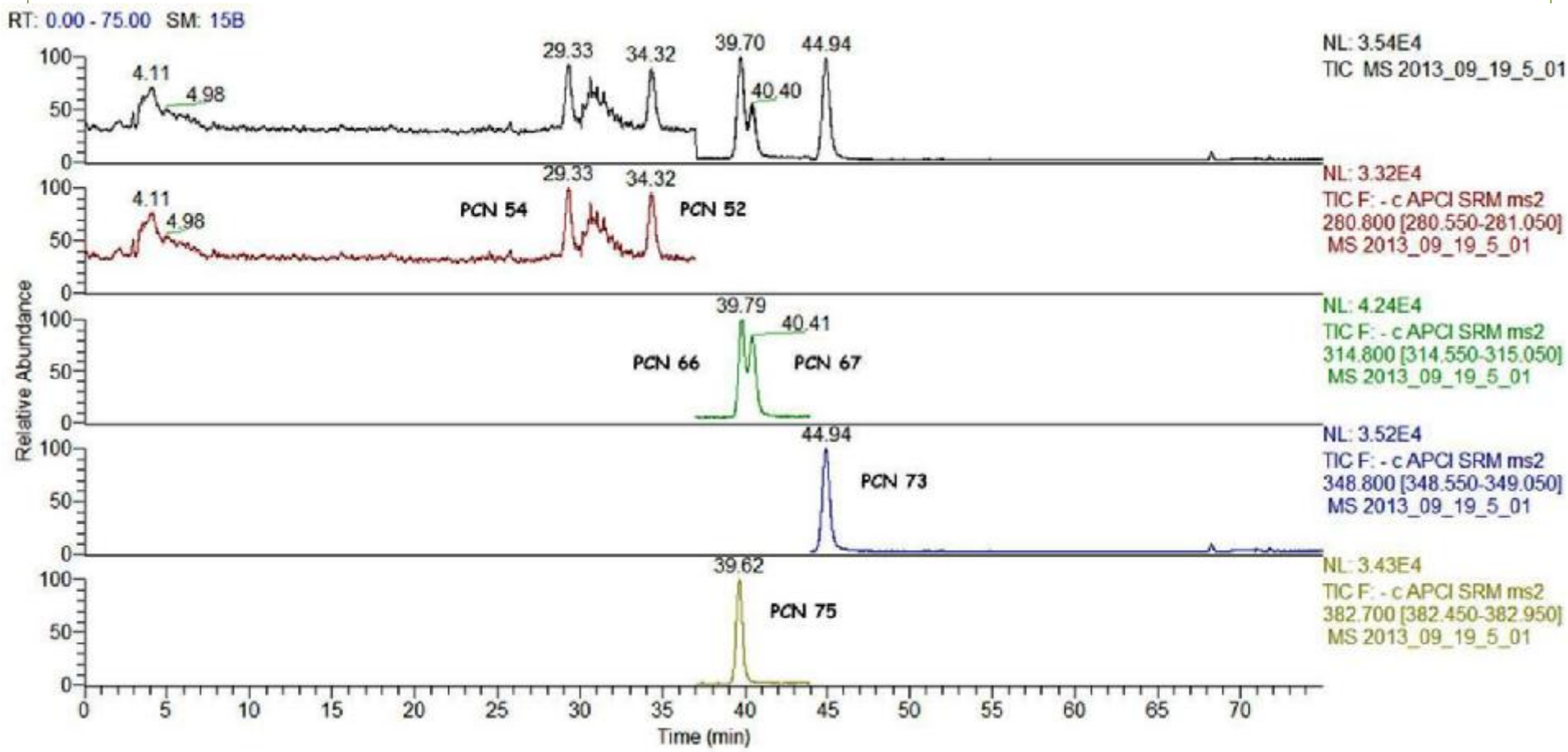


PCN 75
(octachloronaphthalene)
Mr = 400

Six compounds were selected as analytes and the same strategy as for PCBs was applied.

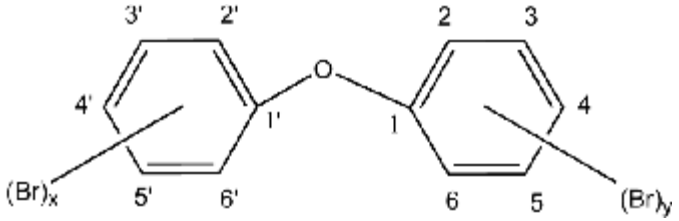
Method validation for the determination of PCNs by LC-APPI-MS/MS

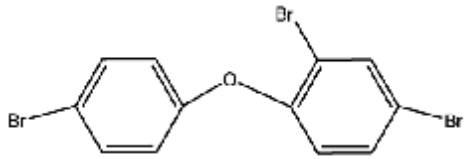
Congener	LoD (µg/L)	LoQ (µg/L)	Precision (n=6. %RSD)		Trueness (mean recovery ± SD. n=6)		Overall recovery %	Correlation coefficient (R^2)	Regression coefficients with standard errors of the calibration equations	
			0.05 µg/L	0.5 µg/L	0.05 µg/L	0.5 µg/L			$(b \pm s_b)\times10^5$	$(a \pm s_a)\times10^5$
PCN 52	0.015	0.045	11	17	82.7 ± 8.9	77 ± 11	97	0.977	69.0 ± 5.3	-2.7 ± 3.0
PCN 54	0.021	0.063	15	12	82 ± 11	79.6 ± 9.0	99	0.982	64.6 ± 4.3	-2.1 ± 2.4
PCN 66	0.011	0.032	9.5	16	67.5 ± 6.4	81 ± 11	99	0.984	123.2 ± 8.0	-5.3 ± 4.5
PCN 67	0.012	0.035	10	15	69.3 ± 7.1	82 ± 12	98	0.986	115.0 ± 6.9	-3.9 ± 3.9
PCN 73	0.009	0.029	8.4	13	68.6 ± 5.7	82.1 ± 9.4	97	0.984	127.4 ± 7.9	-5.6 ± 4.4
PCN 75	0.010	0.030	8.4	14	70.6 ± 5.9	82 ± 10	97	0.986	103.7 ± 6.2	-4.1 ± 3.5



Pseudo SRM chromatogram of spiked surface water (0.1 µg/L)

Polybrominated Diphenyl Ethers (PBDEs)

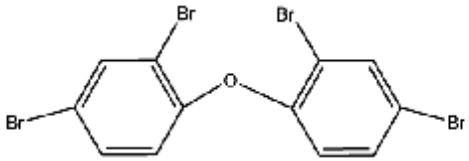




BDE 28

(2,2',4-tribromodiphenyl ether)

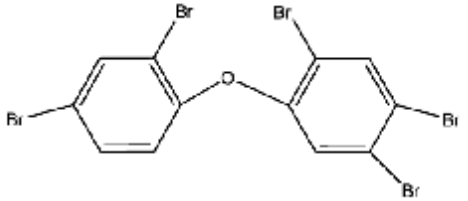
Mr = 404



BDE 47

(2,2',4,4'-tetrabromodiphenyl ether)

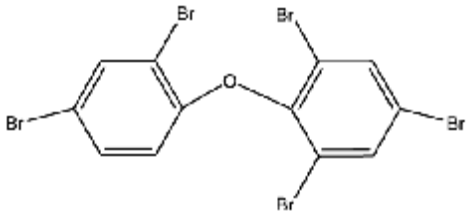
Mr = 482



BDE 99

(2,2',4,4',5-pentabromodiphenyl ether)

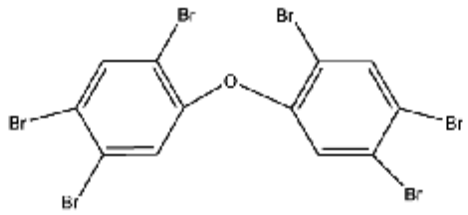
Mr = 560



BDE 100

(2,2',4,4',6-pentabromodiphenyl ether)

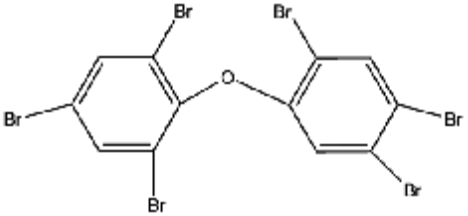
Mr = 560



BDE 153

(2,2',4,4',5,5'-hexabromodiphenyl ether)

Mr = 638



BDE 154

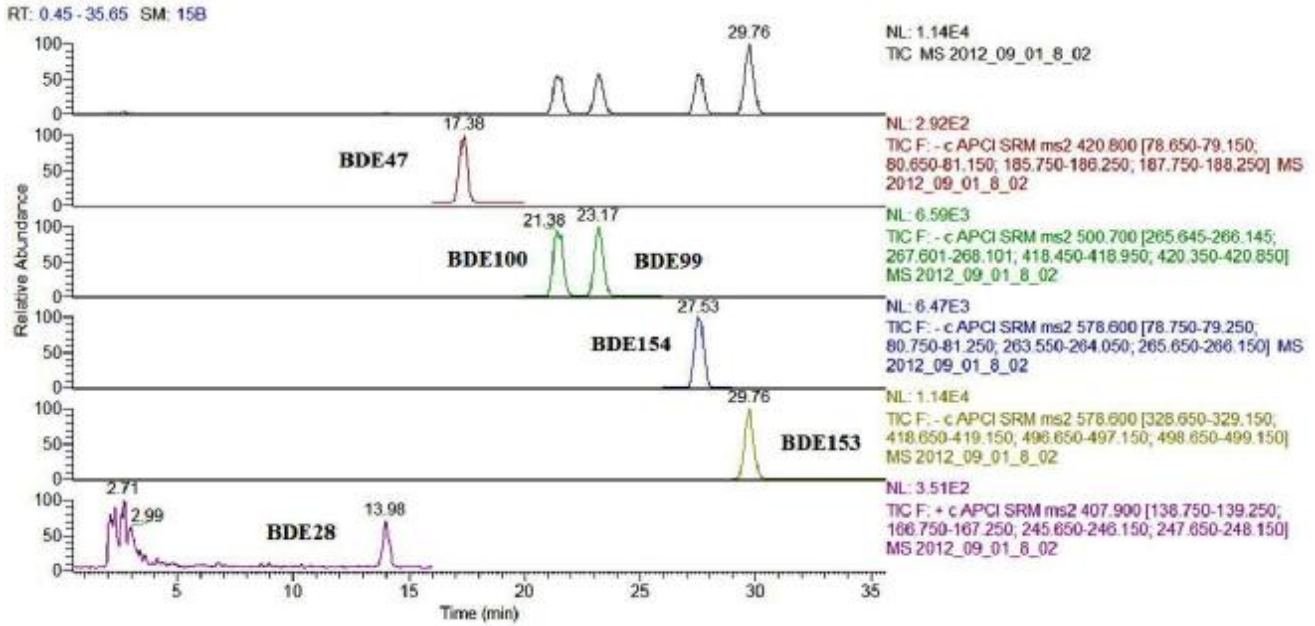
(2,2',4,4',5,6'-hexabromodiphenyl ether)

Mr = 638

Six compounds were selected as analytes and the same strategy as for PCBs was applied.

Method validation for the determination of PBDEs by LC-APPI-MS/MS

Pseudo-SRM chromatogram of a spiked surface water (0.25 µg/L)



Congener	LoD (µg/L)	LoQ (µg/L)	Precision (n=6, %RSD)	Trueness (mean recovery ± SD, n=6)
			0.25 µg/L	0.25 µg/L
BDE 28	0.024	0.073	16	95 ± 14
BDE 47	0.0019	0.0062	12	82.6 ± 9.5
BDE 99	0.0051	0.017	1.6	81.1 ± 1.3
BDE 100	0.0034	0.011	5.1	83.6 ± 4.2
BDE 153	0.0012	0.0041	2.5	80.5 ± 2.0
BDE 154	0.0036	0.011	5.0	82.4 ± 4.1

Conclusions

- LC-APPI-MS/MS technique can be used for the determination of polyhalogenated polyaromatic compounds in traces, especially for those with more than four halogen atoms.
- Dopant, mobile phase composition, the skimmer offset and the vaporizer temperature are the more critical instrumental parameters.

THANK YOU FOR YOUR ATTENTION