Methods of analysis of additives and contaminants

General features:

a) levels of analytes - ultrastrace to trace (pg - mg / kg)

b) analytical procedures include variously composed sample preparation

c) analytical procedures are preferentially optimised as multicomponen / multimatrix

d) often advanced instrumental methods are used – tendency is going to sample preparation minimization followed by highly selective determination

e) in routine practice traditional (specific) methods are applied

Selected additives

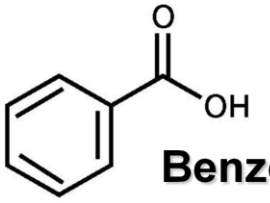
A1. Preservatives

A2. Dyes

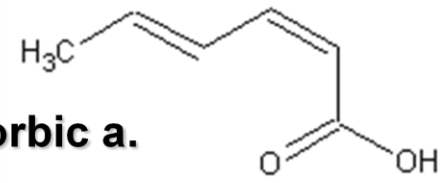
A3. Synthetic antioxidants

A4. Sweeteners

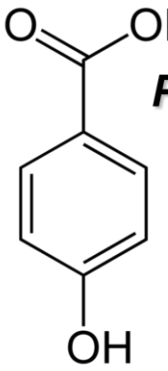
A1. Preservatives (I)



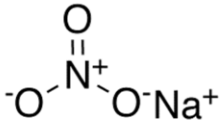
Benzoic acid and benzoates



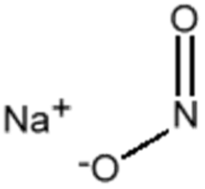
Sorbic a. and sorbates



***Para*-hydroxybenzoates (parabens)**



Sodium nitrate



Sodium nitrite

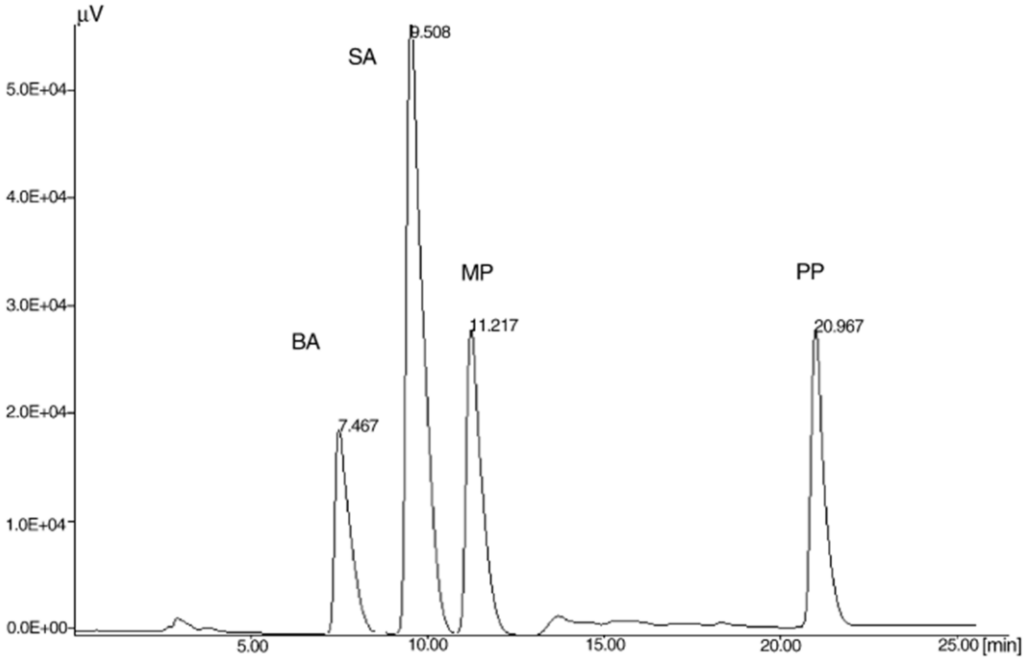
A1. Preservatives (II)

Heterogenous group of compounds

⇒ titration, SPFM (reaction) - FIA, TLC, HPLC/UV etc.

GC, CZE a ITP (organic acids)

A1. Preservatives (III)



HPLC/UV (254 nm) determination in jams and fruits:
BA – benzoic a., SA – sorbic a., MP - methylparaben, PP - propylparaben

A2. Dyes (I)

Natural (carotenoids, flavonoids, anthraquinones, betalains, pyrrole dyes)

Synthetic (azodyes, phenylmethane dyes, nitrodyes, pyrazone, xanthene, antraquinone, quinoline a indigo)

A2. Dyes (II)

Synthetic

- hydrophilic - permitted x banned (where, level, combinations)

- lipophilic - banned (packages)

Hydrophilic

Izolation – direct extraction and analysis

or conventional fiber adsorption and release

(wool, polyamide, etc.)

then *alkalic release - NH_3 solution*

or MeOH sol. of NaOH

Lipophilic

saponification, izolation from unsaponifiable part

A2. Dyes (III)

Determination:

SPFM (direct – without separation)

TLC

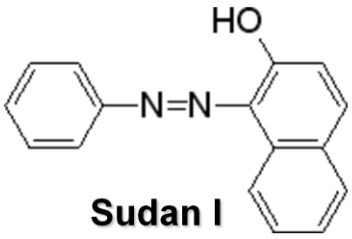
HPLC/VIS

HPLC/MS

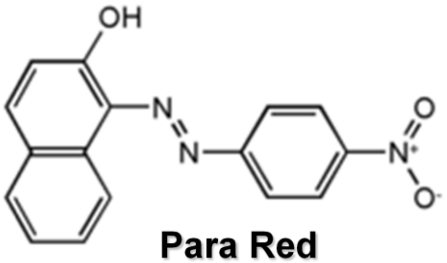
A2. Dyes (IVa)

Example: determination of banned dyes

Forbidden addition – e.g. to spices (paprika) or spicy products
- target levels: 10-100 µg/kg
Extraction with mixture 1% acetic a. : acetonitrile (5:95, v/v)



Sudan I



Para Red

(A) Sudan I
(B) Sudan II
(C) Sudan III
(D) Sudan IV
(E) Sudan Red 7B
(F) Para Red
(G) Sudan Orange G
(H) Tropaeolin 000
(I) Rhodamine B

A2. Dyes (IVb)

Method 1

***Quattro Premier Waters (TQ):* ESI - Z spray**

Quantifiction: MS^2 (MRM)

Identification: MS^2 (MRM)

X

Method 2

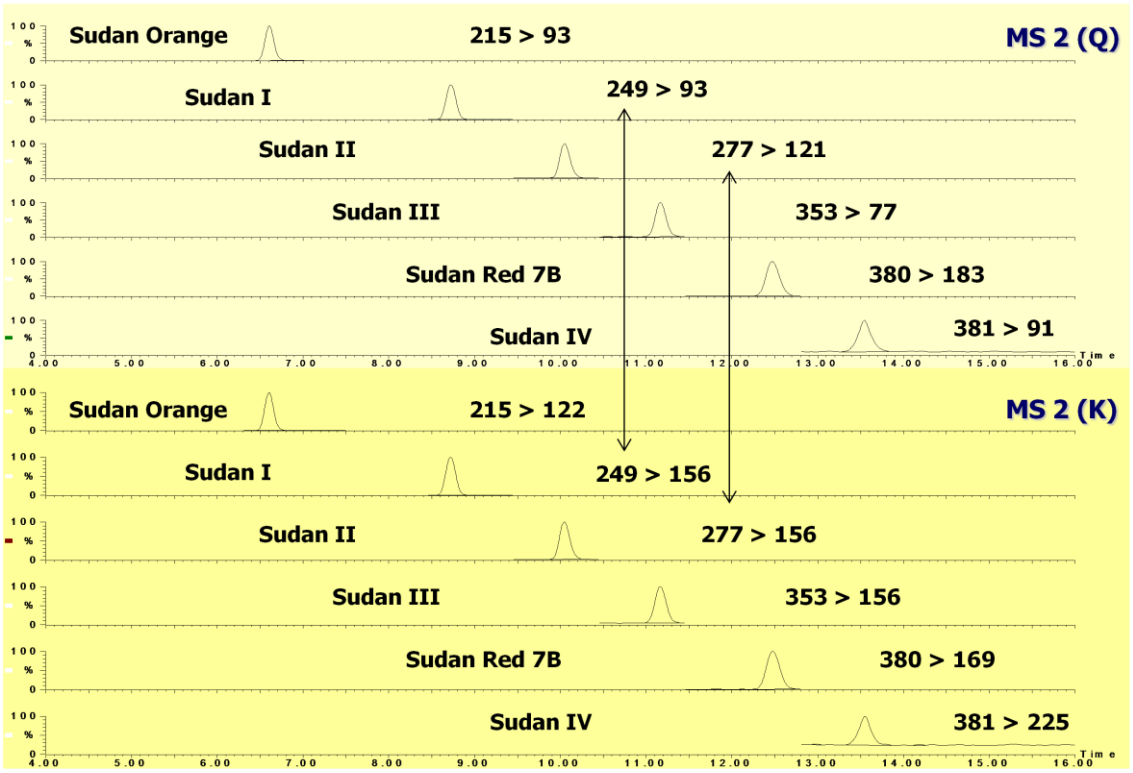
***LCQ Deca Finnigan (ITD):* APCI - direct spray**

Quantifiction: MS^2 (SRM)

Identification: MS^3 (SRM)

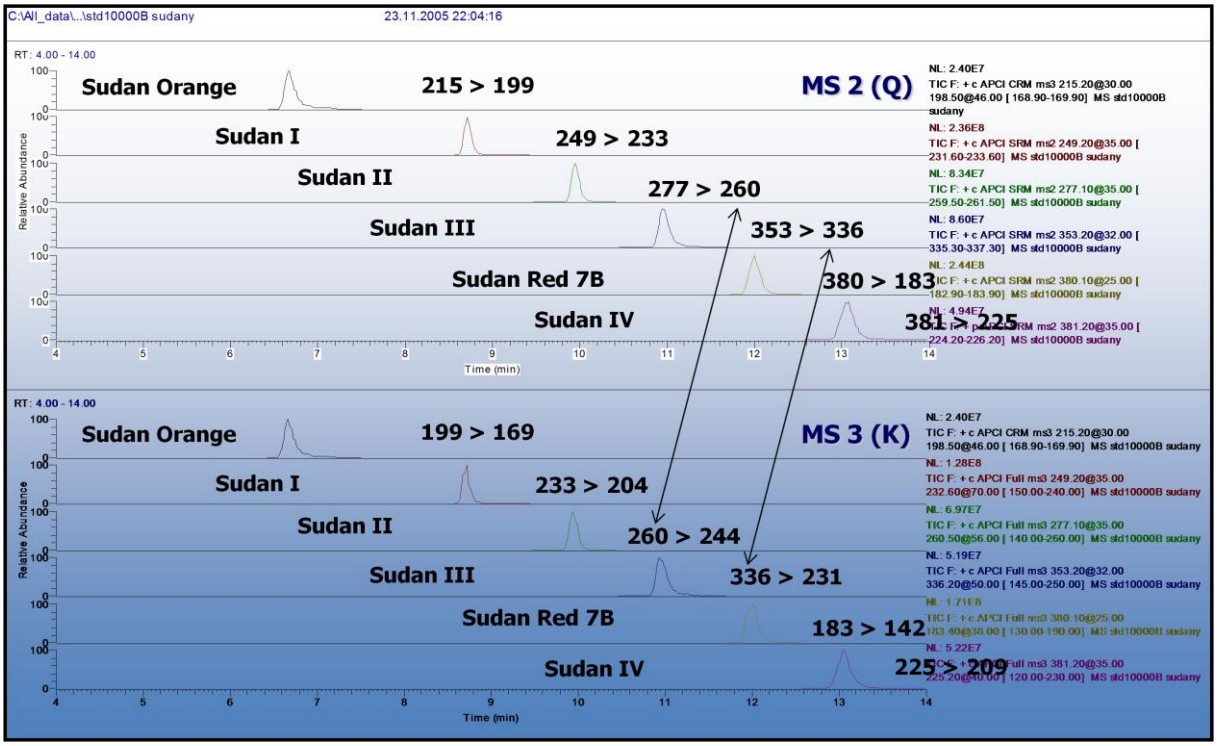
A2. Dyes (IVc)

Record of azodyes analysis – MS^2 in space (tandem quads)

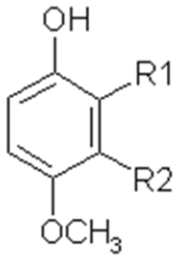


A2. Dyes (IVd)

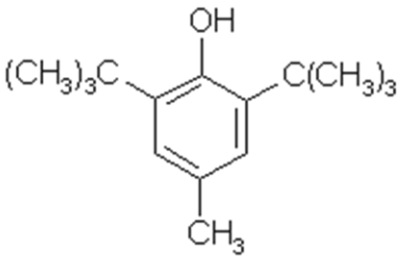
Record of azodyes analysis – MS^2 a MS^3 in time (3D ion trap)



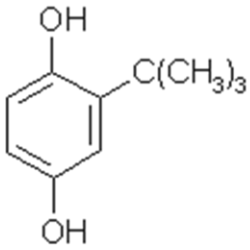
A3. Synthetic antioxidants (I)



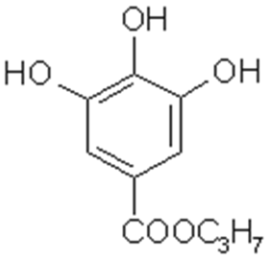
BHA



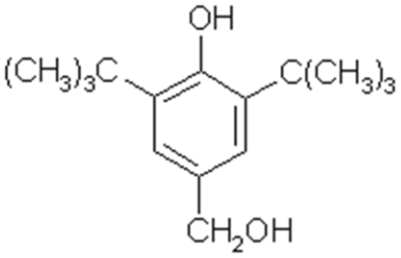
BHT



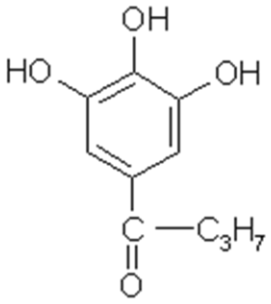
TBHQ



PG



4-Hydroxymethyl-
2,6-ditertiary butylphenol



THBP
(2,4,5-Trihydroxy-
butyrophenone)

A3. Synthetic antioxidants (II)

Non-polar phase - emulgation, co-isolation with lipids, then separation or direct isolation by alcohols

Samples - lard, oil, various fats

Determination:

SPFM - UV (232, 241, 252, 300 nm etc.)

- VIS after reaction (400, 520 nm etc. according to agent

580 nm - $FeSO_4$)

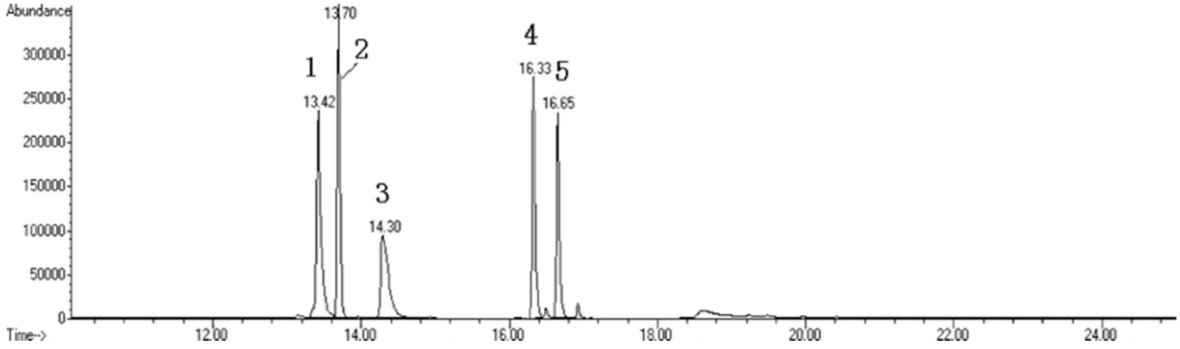
TLC - agents: AgNO3 + NH3, phosphomolybdenic a. + NH3

GC – directly or derivatization (methylderivatives), FID, MS

HPLC – reverse phase, UV 280 nm, RID, ECD

And… electroanalytical and electromigration methods

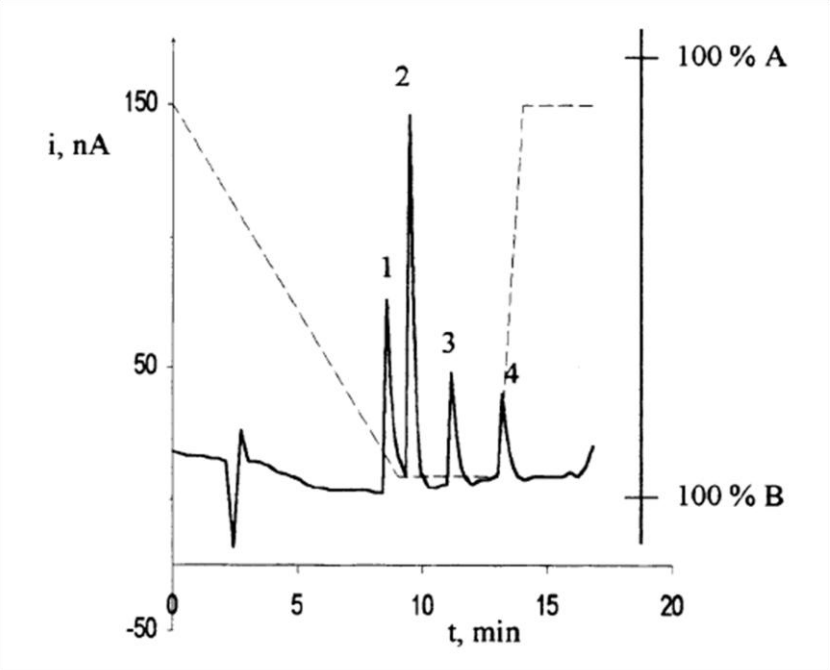
A3. Synthetic antioxidants (III)



GC-MS analysis (TIC record standard mixtures of 5 antioxidants - 5 mg/l

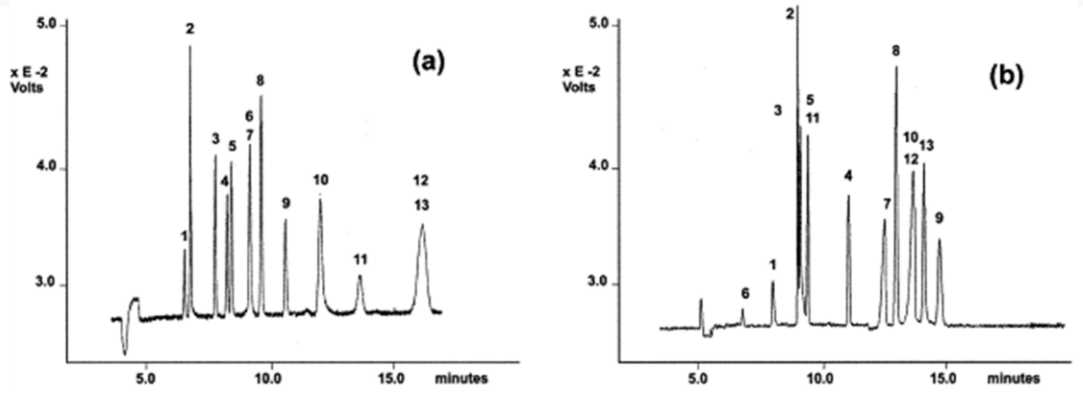
(1) BHA; (2) BHT; (3) TBHQ; (4) ethoxyquine; (5) Ionox-100

A3. Synthetic antioxidants (IV)

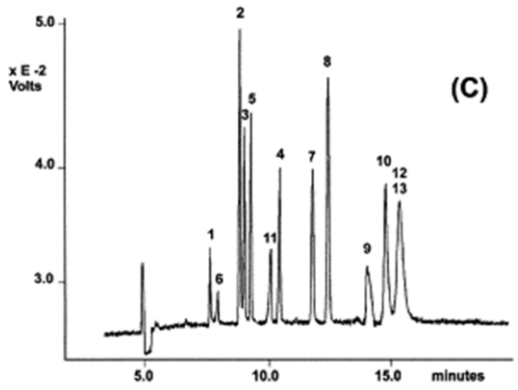


Chromatogram of HPLC(C18)-ECD analysis - mixture (2 mg/ml): PG (1), BHA (3), BHT (4) and 5 mg/ml TBHQ (2), amperometric detection.

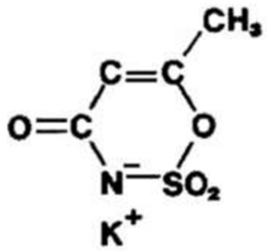
A3. Synthetic antioxidants (V)



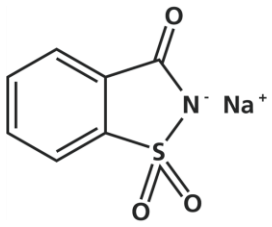
MECC separation using various micellar agents, detection - UV 214 nm, drinks:
aspartame (1), propylgalate (2), methylparaben (3), sorbic a. (4), ethylparaben (5), *terc*-butylhydro-quinone (6), benzoic a. (7), saccharin (8), acesulfame-K (9), octylgalate (10), butyl-hydroxyanisole (11), butylhydroxytoluene (12), dodecylgalate (13)
(a) - pufr 50 m*M* sodium dodecyl sulphate (SDS), 20 m*M* borát - pH 9.5
(b) pufr 50 m*M* sodium cholate (SC) 20 m*M* borate pH 9.5
(c) pufr 50 m*M* sodium deoxycholate (SDC), 20 m*M* borate - pH 9.5



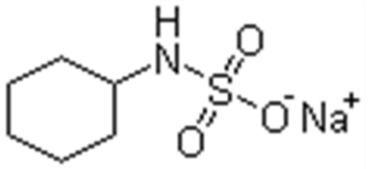
A4. Sweeteners (I)



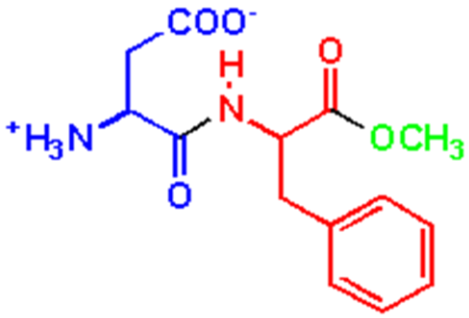
Acesulfame K



Saccharin

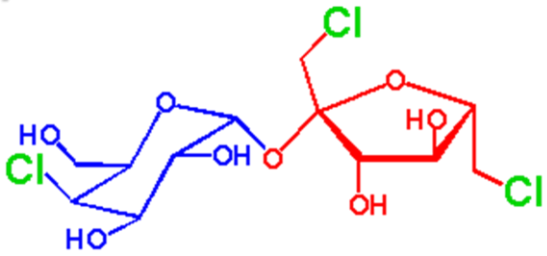


Cyclamate



Aspartyl-phenylalanine methyl ester

Aspartame



1,6-dichloro-1,6-dideoxy-
beta-D-fructofuranosyl-
4-chloro-4-deoxy-
alpha-D-galactopyranoside

Sucralose

A4. Sweeteners (II)

Polar extraction – according to solubility - dilution, clarification, filtration, deffating, SPE …

Determination: HPLC/UV or VIS (derivatization)

→ RP(C18)

MECC

A4. Sweeteners (III)

Example of HPLC/UV analysis

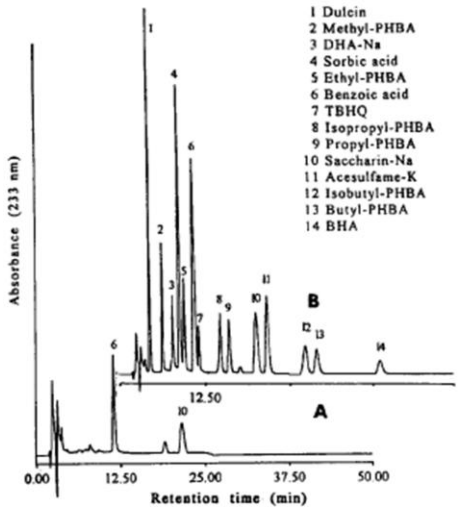


Figure 6
Chromatogram of additives in sugared fruit extracted by a Sep-Pak C_{18} cartridge. Mobile phase, acetonitrile/50 mM aqueous α-hydroxyisobutyric acid solution (pH 4.5) (2.2 : 3.4, v/v) containing 2.5 mM HTA with a flow rate of 1.0 mL/min. Detection was at 233 nm. A: sugared fruit only; B: sugared fruit spiked with additives at a concentration of 25 mg/g each.

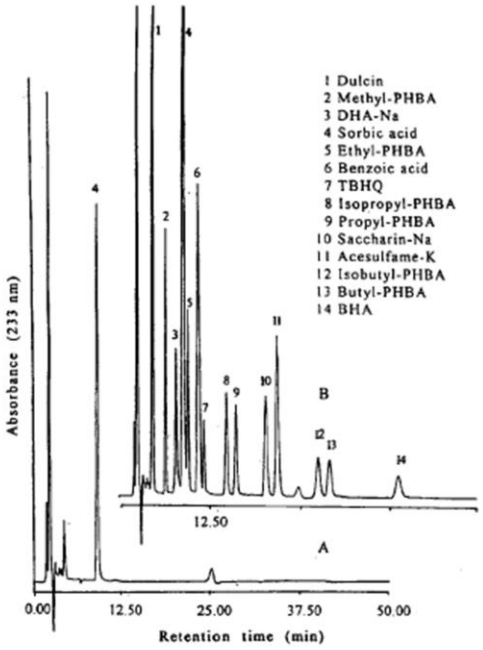


Figure 7
Chromatogram of additives in dried roast beef extracted by a Sep-Pak C_{18} cartridge. Mobile phase, acetonitrile/50 mM aqueous α-hydroxyisobutyric acid solution (pH 4.5) (2.2 : 3.4, v/v) containing 2.5 mM HTA with a flow rate of 1.0 mL/min. Detection was at 233 nm. A dried roast beef only; B: dried roast beef spiked with additives at a concentration of 25 mg/g each.

Selected contaminants

B1. Pesticides

B2. Organic industrial contaminants

B3. Metals

B4. Pharmaceutics

B5. Migrants (from packages)

B1. Pesticides (Ia)

Intentionally applied compounds for extermination of pests

- various weeds and animal

Terms: rezidue – amount remaining after protection period

„incurred residues“ - componds incorporated to matrix

- i.e. characterization of long term presence in matrix

perzistent pesticides (rezidues) – remain tens of years

multi(rezidue) analysis – investigation of tens to hundreds compounds within a method

Occurence: primary in treated crops

secondary various (all) parts of environment

Legislation: MRL, directives…

B1. Pesticides (Ib)

Classification according to purpose of application:

herbicides, fungicides, insecticides...

Classification according to mechanism of action:

contact X systemic

Analytical classification: polarity, volatility, stability,

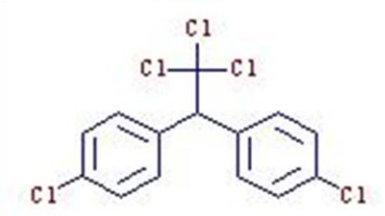
forms of occurence

multi or sigle rezidue compound

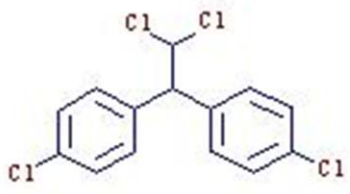
- possible isolation:

plant matrix ...X... animal matrix

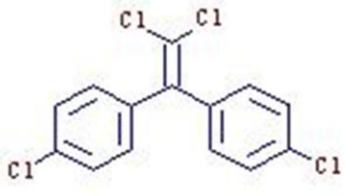
B1. Pesticides (Ic)



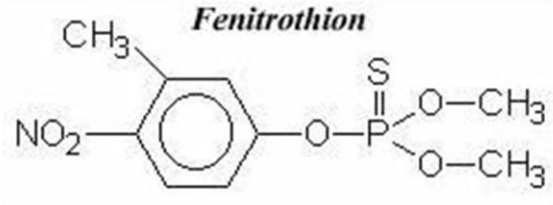
p, p´-DDT (4,4'-DDT)



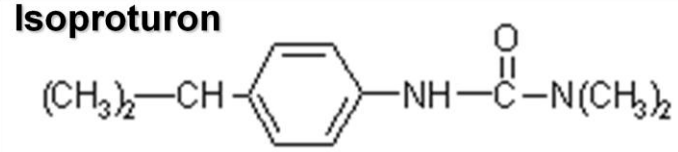
p,p´-DDE

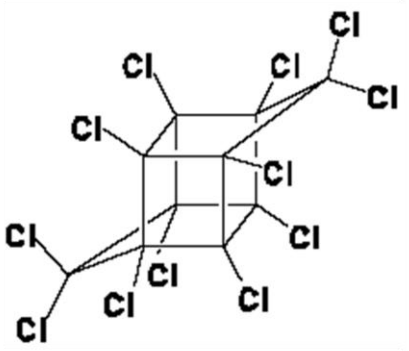


p,p´-DDD



Isoproturon





MIREX

B1. Pesticides (II)

Pesticides analysis:

target ...X... non-target

Target: list of compounds (standards available),
expected levels

Non-target: screening, monitoring (knowns, exceptionally unknowns)

B1. Pesticides (III)

Applied methods:

Multirezidue / multimatrix:

→ amenable for gas or liquid chromatography

Specific (single rezidue):

investigation of difficultly determinable compounds -

- special sample preparation

include degradation products or metabolites

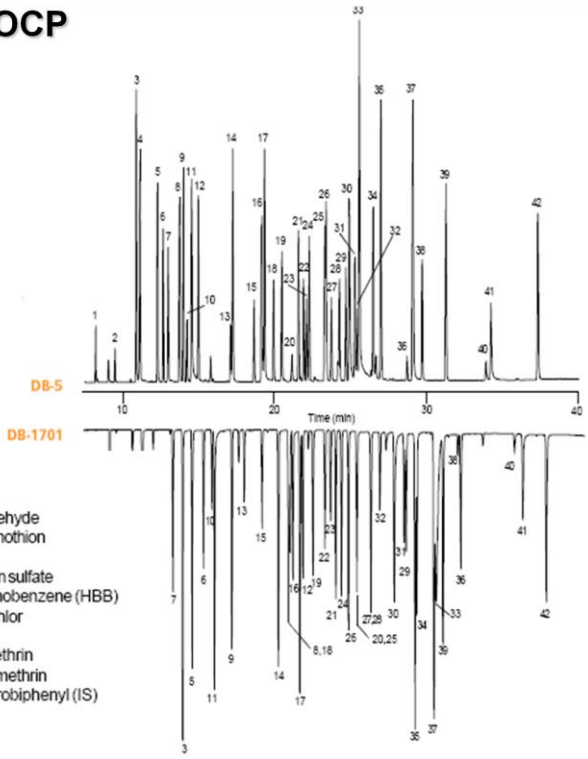
Common methods: **GC, HPLC – conventional detectors or MS**

Other methods: **CZE, ITP, MECC, CEC**

B1. Pesticides (IVa)

GC/2xECD - parallel separation of OCP

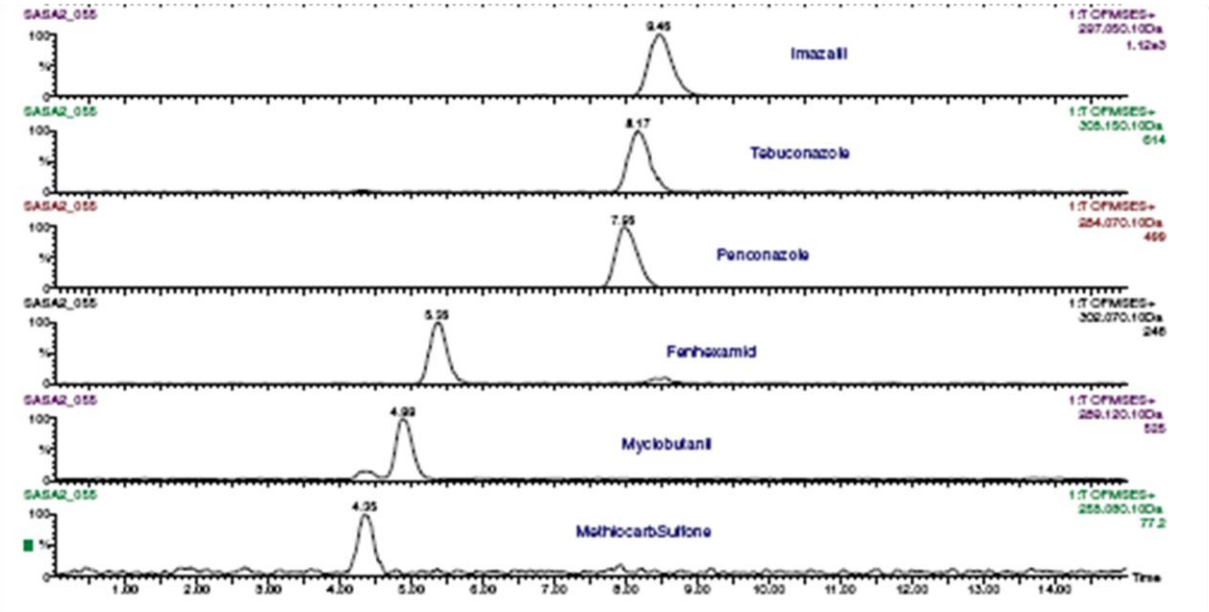
Columns:	**DB-5**
	30 m x 0.32 mm I.D., 0.25 µm
	DB-1701P
	30 m x 0.32 mm I.D., 0.21 µm
Guard Column:	**10 m x 0.53 mm I.D.**
	3-way union
J&W P/N:	**009-5007**
Carrier:	Helium at 29.2 cm/sec, measured at 150°C
Oven:	60°C for 0.5 min
	60-140°C at 20°/min
	140-280°C at 11°/min
	280°C for 23 min
Injector:	Splitless, 200°C
	2.0 µL, 20-200 pg/µL
Detector:	ECD, 325°
	Nitrogen makeup gas at 30 mL/min



1. Etridiazole
2. Chloroneb
3. Propachlor
4. Tetrachloro-m-xylene (IS)
5. Trifluralin
6. α-BHC
7. Hexachlorobenzene
8. β-BHC
9. γ-BHC
10. Pentachloronitrobenzene
11. *p,p'*-Dichlorobiphenyl
12. δ-BHC
13. Heptachlor
14. Alachlor
15. Aldrin
16. Chlorpyrifos
17. DCPA
18. Isodrin
19. Heptachlor epoxide
20. Captan
21. γ-Chlordane
22. *o,p'*-DDE
23. Endosulfan I
24. α-Chlordane
25. Dieldrin
26. *p,p'*-DDE
27. *o,p'*-DDD
28. Endrin
29. Endosulfan II
30. Chlorobenzilate
31. *p,p'*-DDD
32. *o,p'*-DDT
33. Endrin aldehyde
34. Carbophenothion
35. *p,p'*-DDT
36. Endosulfan sulfate
37. Hexabromobenzene (HBB)
38. Methoxychlor
39. Mirex
40. *cis*-Permethrin
41. *trans*-Permethrin
42. Decachlorobiphenyl (IS)

B1. Pesticides (IVb)

LC-TOFMS: Ion chromatograms of pesticides



B2. Organic industrial contaminants (I)

Polychlorinated compounds (PCB, PCDD/F)

Polybrominated compounds (PBB, PBDE)

Polycyclic aromatic hydrocarbons (PAU, NPAU)

Perfluorinated compounds (PFC - PFAS, telomeric alcohols)

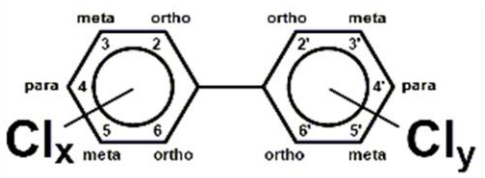
Strategy of analysis

- similar to pesticides

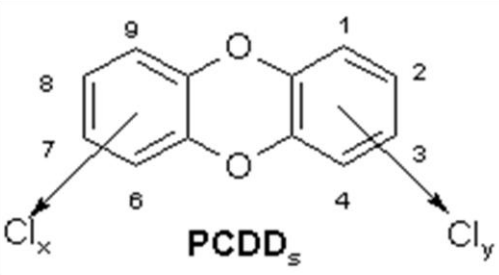
- typically groups of related compounds

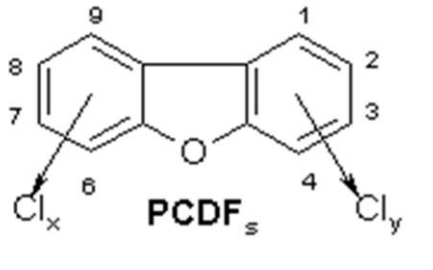
→ simpler method optimization

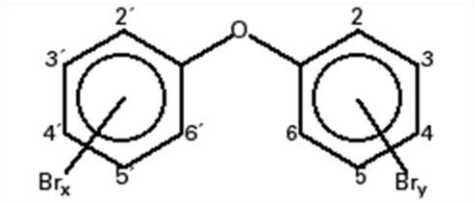
B2. Organic industrial contaminants (IIa)



PCB - polychlorinated bifenyls







PBDE - polybrominated difenylethers

B2. Organic industrial contaminants (IIb)

PCB, PCDD/F, PBB, PBDE

- sample preparation: isolation of non-polar part: LSE, LLE

separation - cleanup: LLE, GPC, SPE, LSC

concetration: Rotavap, N_2, solvent exchange

-special preseparation: dialysis (lipids separation),

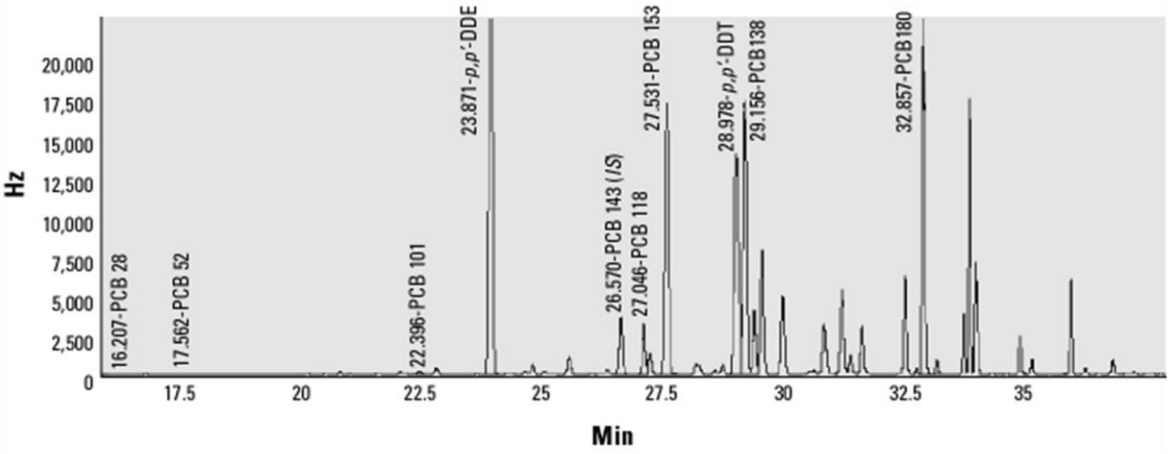
pyrenyl or carbon columns - longer

retention of planar congeners, PCDD/F

- instrumental determination: primary GC/ECD or MS (EI a NCI)

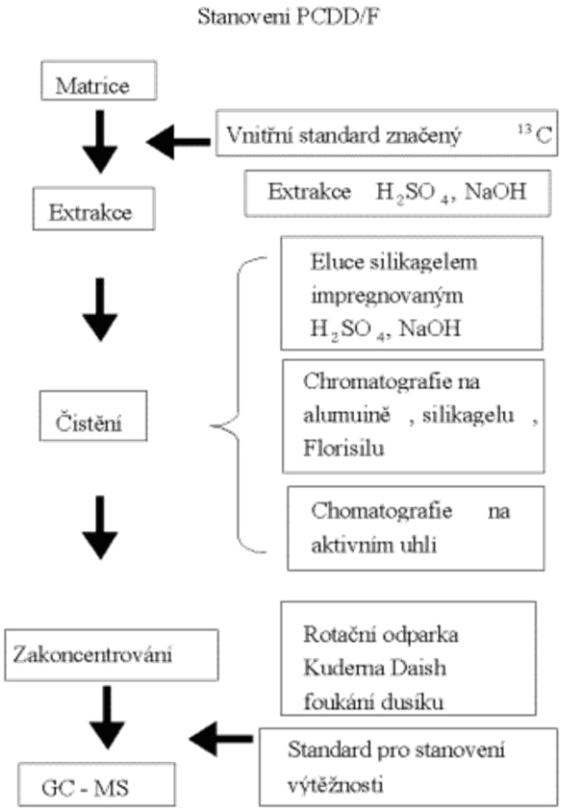
or HPLC/MS

B2. Organic industrial contaminants (IIc)



Typical chromatogram of GC/ECD analysis of indicator PCBs

B2. Organic industrial contaminants (IId)



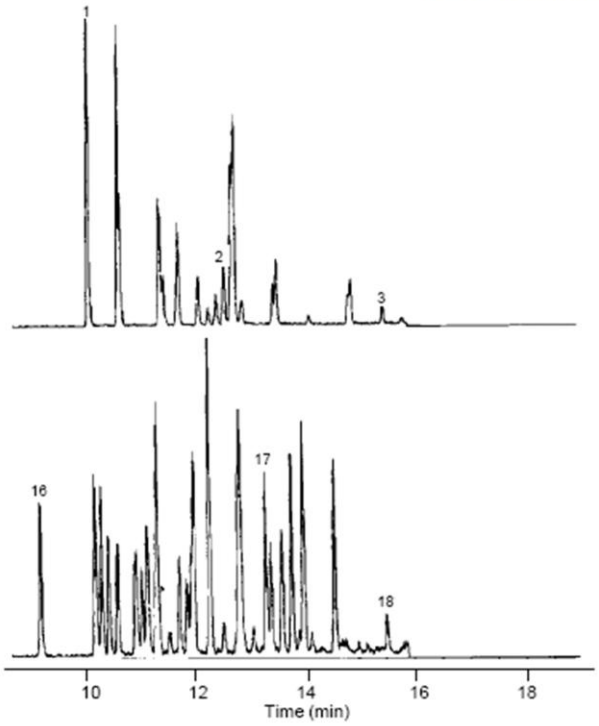
B2. Organic industrial contaminants (IIe)

Dioxins and Furans

Column: **DB-Dioxin**
60 m x 0.25 mm I.D., 0.15 µm
J&W P/N: **122-2461**
Carrier: Helium at 34.3 cm/sec, measured at 250°C
Oven: 180°C for 1 min
180-270°C at 2.5°/min
270°C for 40 min

1. 1,3,6,8-TCDD
2. 2,3,7,8-TCDD
3. 1,2,8,9-TCDD
4. 1,2,4,6,8-/1,2,4,7,9-PeCDD
5. 1,2,3,7,8 + unknown-PeCDD
6. 1,2,4,8,9-PeCDD
7. 1,2,4,6,7,9-/1,2,4,6,8,9-HeCDD
8. 1,2,3,4,7,8-HeCDD
9. 1,2,3,4,6,9-HeCDD
10. 1,2,3,6,7,8-HeCDD
11. 1,2,3,7,8,9-HeCDD
12. 1,2,3,4,6,7-HeCDD
13. 1,2,3,4,6,7,9-HpCDD
14. 1,2,3,4,6,7,8-HpCDD
15. OcCDD
16. 1,3,6,8-TCDF
17. 2,3,7,8-TCDF
18. 1,2,8,9-TCDF
19. 1,3,4,6,8-PeCDF
20. 1,2,3,4,8-PeCDF
21. 1,2,3,7,8-PeCDF
22. 1,2,3,4,6-PeCDF
23. 2,3,4,7,8-PeCDF
24. 1,2,3,8,9-PeCDF
25. 1,3,4,6,7,9-HeCDF
26. 1,2,3,4,7,8/1,2,4,6,8,9-HeCDF
27. 1,2,3,6,7,8-HeCDF
28. 2,3,4,6,7,8-HeCDF
29. 1,2,3,7,8,9-HeCDF
30. 1,2,3,4,8,9-HeCDF
31. 1,2,3,4,6,7,8-HpCDF
32. 1,2,3,4,7,8,9-HpCDF
33. OcCDF

Tetra Isomers



B2. Organic industrial contaminants (IIf)

Chromatograms of PBDE analysis using GC-TOFMS

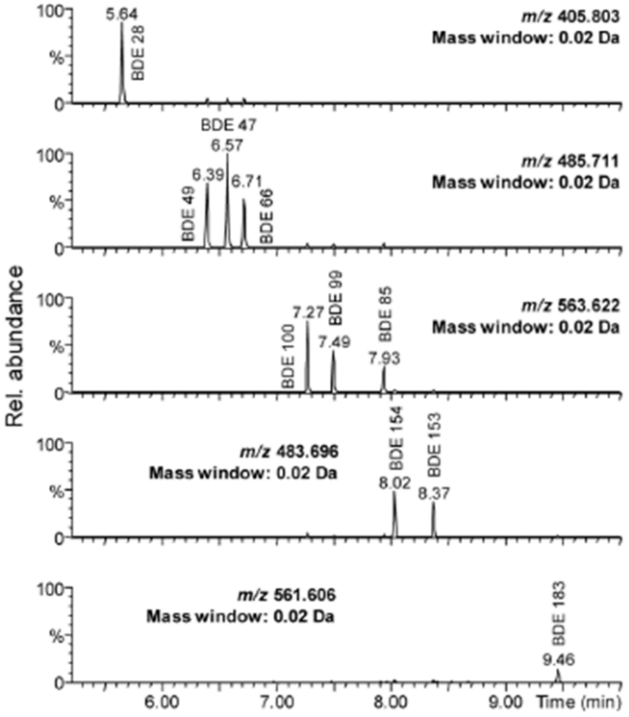
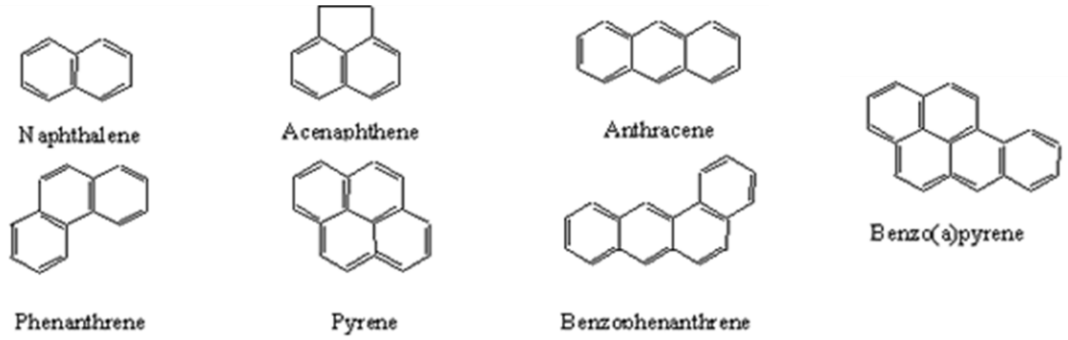


Figure 1. GC–TOF MS chromatograms of PBDE standard solution in EI mode (10 pg of each analyte injected). The target ions were extracted using a 0.02 Da mass window.

B2. Organic industrial contaminants (IIIa)

PAH - polycyclic aromatic hydrocarbons



16 PAH – US EPA:
naphthalene, acenaphthylene, acenaphthene, fluorene, fenanthrene, antracene, fluoranthene, pyrene, benzo(a)antracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, indeno(123cd)pyrene, dibenzo(ah)anthracene, benzo(ghi)perylene

B2. Organic industrial contaminants (IIIb)

PAH, NPAH

- sample preparation: isolation of non-polar part

or saponification (MeOH solution of NaOH)

separation - cleanup: LLE, GPC, SPE, LSC

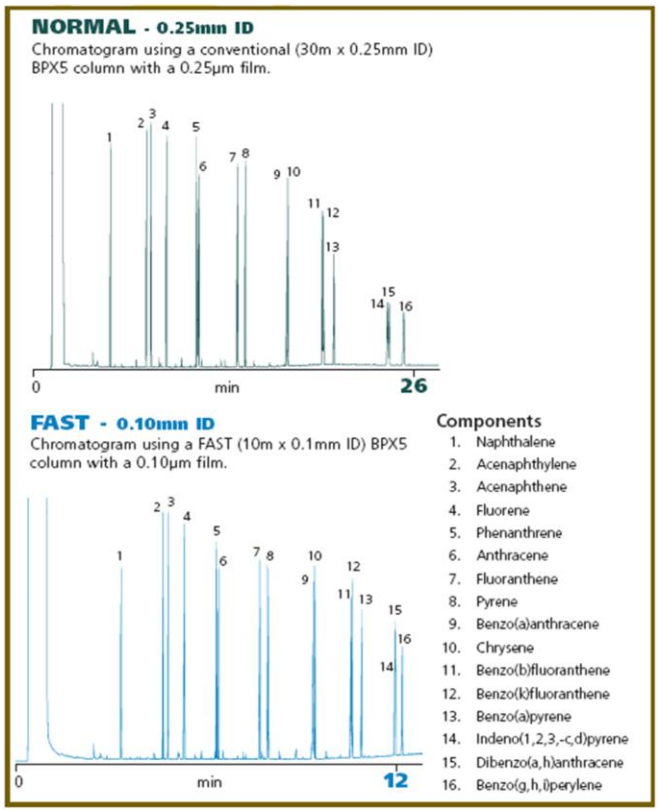
concentration: Rotavap, N_2, solvent exchange

- special conditions: light (UV) protection (photolability)

- instrumental determination: GC/MS, HPLC/UV or FLD

alternatively LC-MS (APPI)

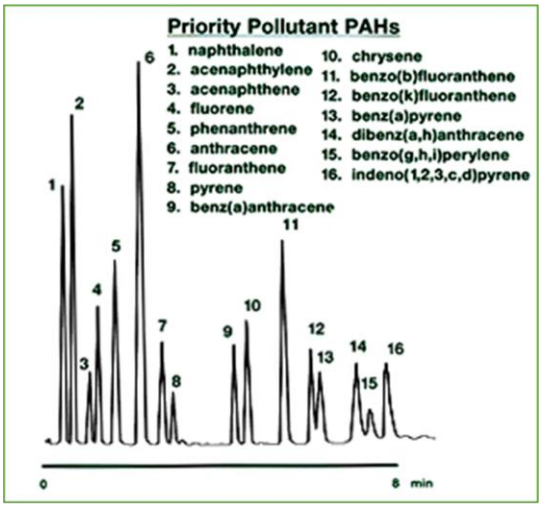
B2. Organic industrial contaminants (IIIc)



GC

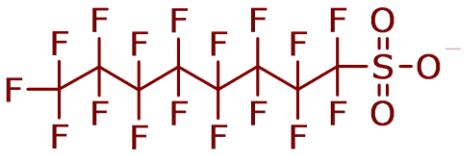
...X...

HPLC



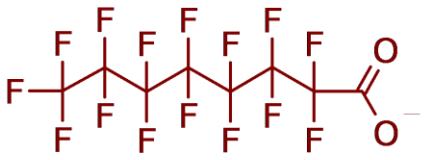
B2. Organic industrial contaminants (IVa)

Perfluorinated compounds (PFC)



PFOS

(perfluorooctanesulfonate)



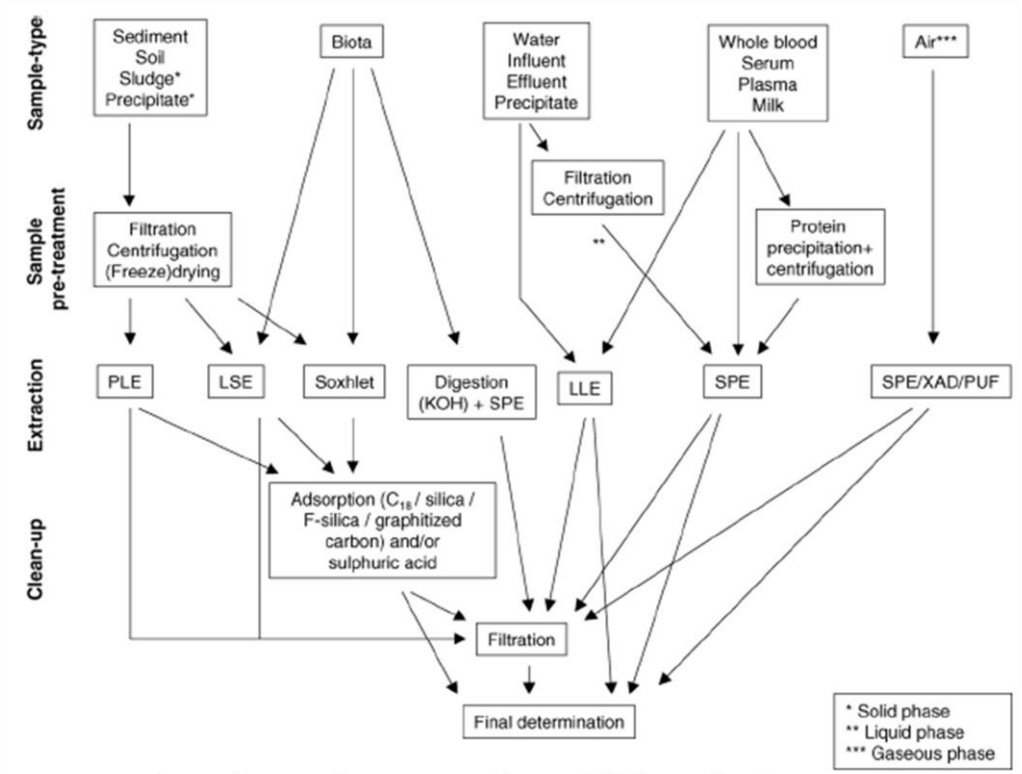
PFOA

(perfluorooctanoic acid)

- NH_2 **PFOSA**

(perfluorooctanesulfonamide)

B2. Organic industrial contaminants (IVb)



Various strategies of sample preparation – PFC analysis

B2. Organic industrial contaminants (IVc)

Methods of PFC determination

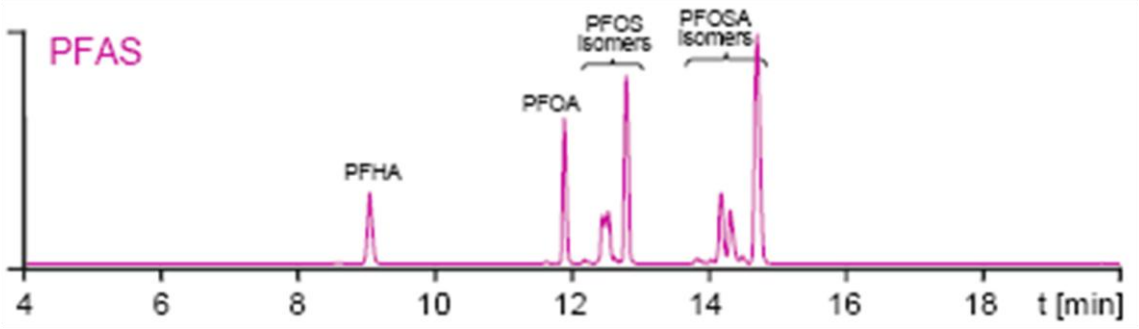
A. GC/MS – some of them – no need of derivatization

B. HPLC/MS – simpler sample preparation

- fast optimization and determination

B2. Organic industrial contaminants (IVd)

LC-TOF/MS determination of PFC (ESI-)



B3. Metals (I)

Toxic (at low amount) - Pb, Hg, Cd - accumulation

- Ba, Sr, Be, Sb, Cr(IV)

Toxic (at high amount) - Sn, As

Esential, but toxic at high amount - Cu, Zn, Fe

And others ... Al, Se, Ni ...

Analytical procedures

a) sample preparation: removal – matrix decomposition → mineralization

possibly chelation (dialcylcarbonates ...)

b) determination: total content ...X... forms of occurence – speciation

B3. Metals (II)

Determination:

HPLC/UV

voltamperometry - polarography

AAS, AES, ICP-MS

enzyme methods

GC – volatile forms

B4. Pharmaceuticals (I)

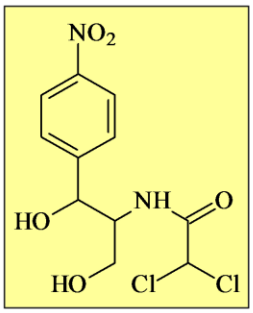
Veterinary - permitted ...X... banned:

sulfonamides, chloramfenicol

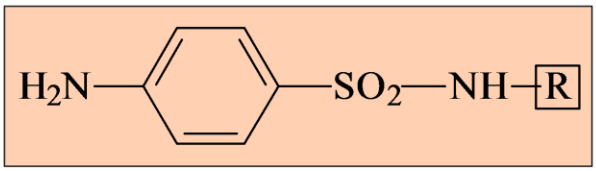
Environmental contaminants – municipal waste

Occurence: honey, meat, fish products (farms)

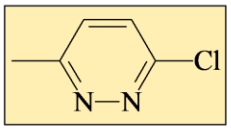
B4. Pharmaceuticals (II)



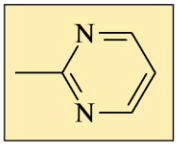
CHLORAMFENICOL



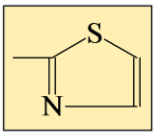
SULFONAMIDES



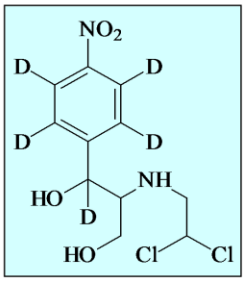
SULFACHLORPYRIDAZINE



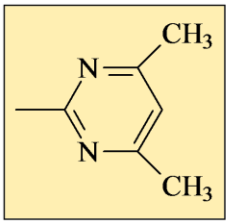
SULFADIAZINE



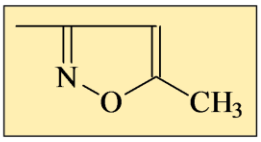
SULFATHIAZOLE



D5 - CHLORAMFENICOL



SULFADIMIDINE



SULFAMETHOXAZOLE

B4. Pharmaceuticals (III)

Sample preparation:

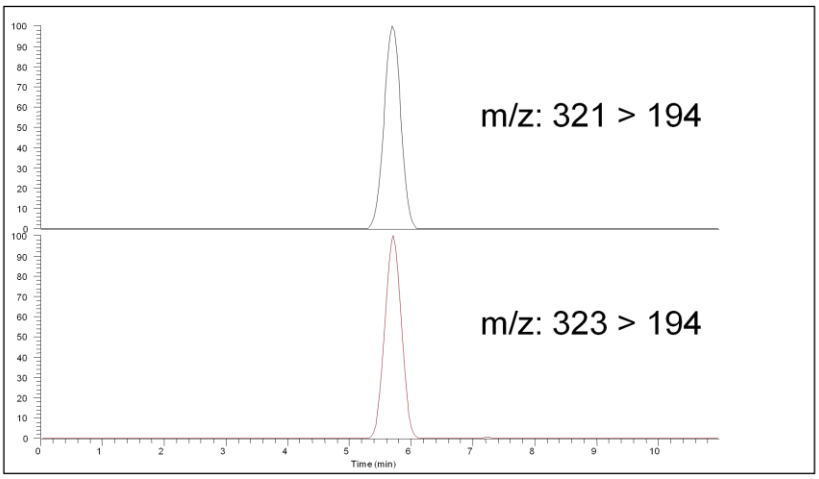
* **Direct analysis:** (ultra)filtrated extracts
* **SPE:** concetration and cleanup of samples (possibly immunosorbents)

Instrumental determination:

* **HPLC-UV:** robust, less sensitive
* **HPLC-FLD:** medium robust, highly selective and sensitive (derivatization)
* **LC-MS:** (highly robust, highly selective and sensitive)

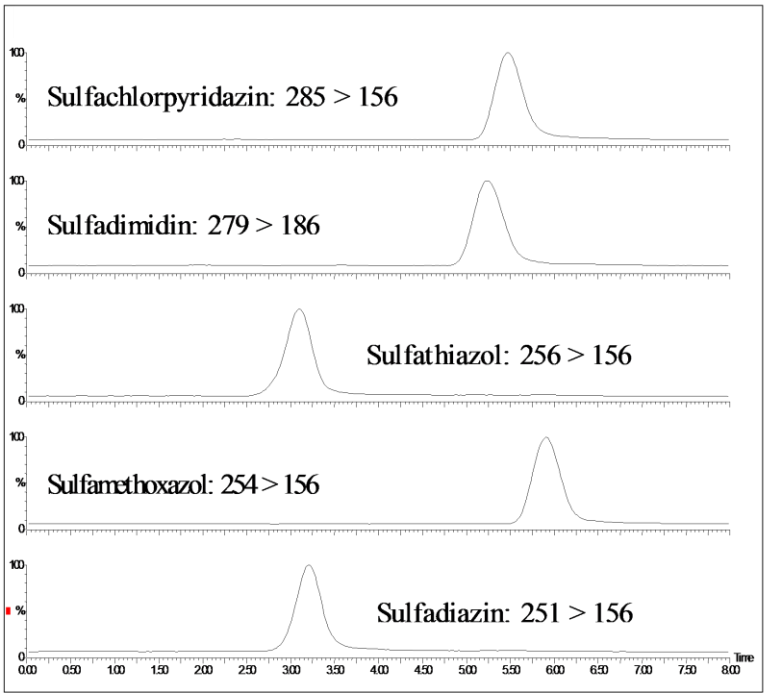
B4. Pharmaceuticals (IV)

HPLC-MS/MS chloramfenicol analysis



B4. Pharmaceuticals (V)

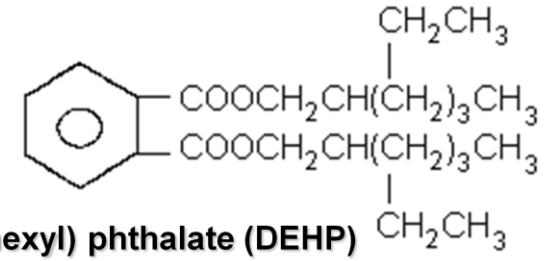
HPLC-MS/MS sulfonamides analysis



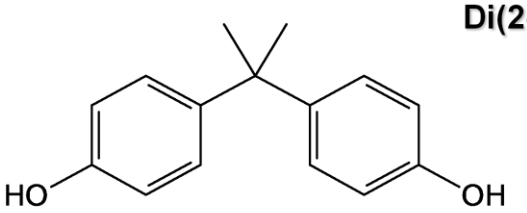
B5. Migrants (from packages) (I)

Various compounds:

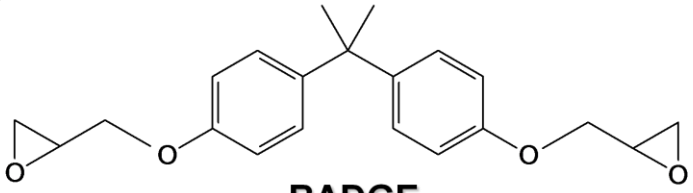
phthalates, bisphenols...



Di(2-ethylhexyl) phthalate (DEHP)



Bisphenol A



BADGE

B5. Migrants (from packages) (II)

Occurence: packed food - bottles, cans ...

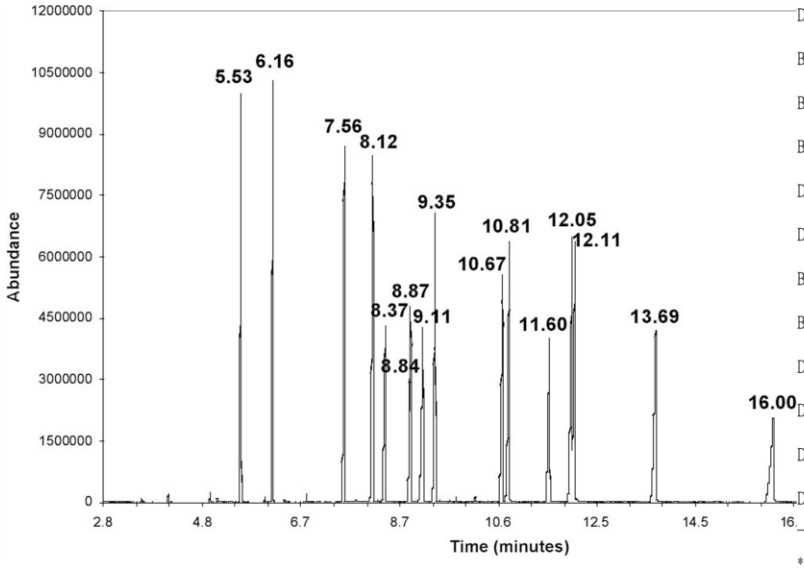
Mostly lipophilic, moderately polar:

extraction – organic solvents, SPME

Analysis: GC (derivatization), HPLC

B5. Migrants (from packages) (III)

GC-MS phthalates analysis (standard)



Name	CAS No.	Retention Time (min.)
Dimethyl phthalate (DMP)	131-11-3	5.53
Diethyl phthalate (DEP)	84-66-2	6.16
Diisobutyl phthalate (DIBP)	84-69-5	7.56
Di-n-butyl phthalate (DnBP)	84-72-0	8.12
Bis(2-methoxyethyl) phthalate (BMEP)	117-82-8	8.37
Bis(4-methyl-2-pentyl) phthalate (BMPP)	146-50-9	8.84, 8.87*
Bis(2-ethoxyethyl) phthalate (BEEP)	605-54-9	9.11
Diamyl phthalate (DAP)	131-18-0	9.35
Dihexyl phthalate (DHP)	84-75-3	10.67
Butyl benzyl phthalate (BBP)	85-68-7	10.81
Bis(2-n-butoxyethyl) phthalate (BBEP)	117-83-9	11.60
Dicyclohexyl phthalate (DCP)	84-61-7	12.05
Di(2-ethylhexyl) phthalate (DEHP)	117-81-7	12.11
Di-n-octyl phthalate (DOP)	117-84-0	13.69
Dinonyl phthalate	84-76-4	16.00

* Two isomers

B5. Migrants (from packages) (IV)

HPLC-FLD bisphenols analysis (standard)