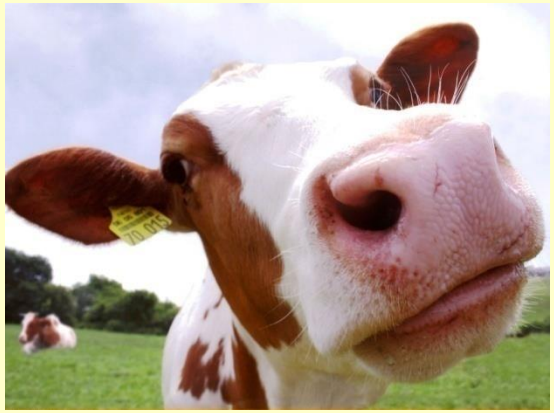
Analysis of Pesticide Residues in Food of Animal Origin using GC-MS/MS



Webinar

Björn Hardebusch, Ralf Lippold

Chemisches und Veterinärunter-suchungsamt Freiburg (Germany)

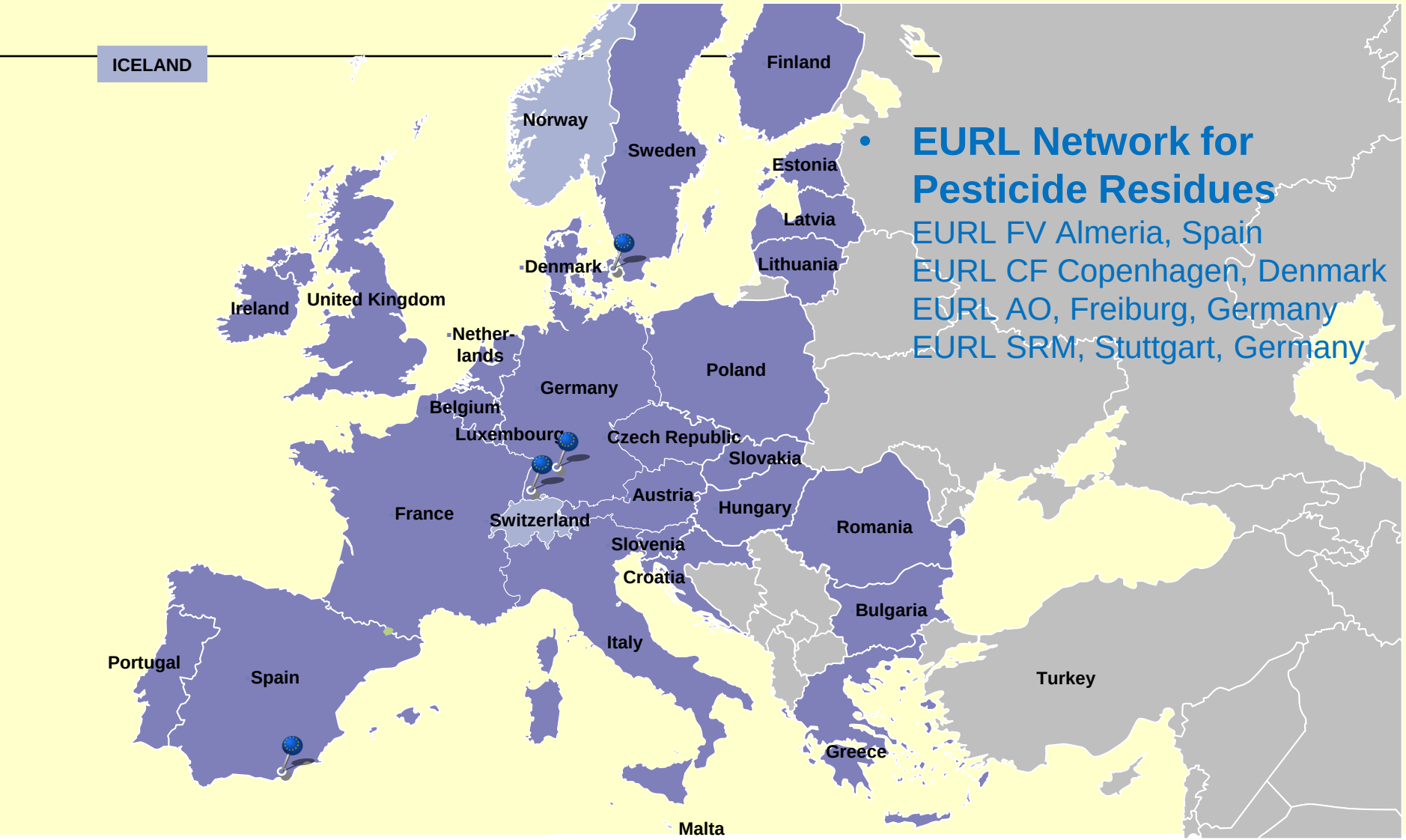
EURL AO Freiburg

CVUA Freiburg Introduction

- Investigation of more than 22000 routine samples
- Residue department responsible
  - for pesticides in food of animal origin,
  - organic contaminants in food and
  - dioxins and PCBs in food and feed

CVUA Freiburg Introduction

- Hosting of 2 European Union Reference Laboratories (EURL)
  - EURL for Dioxins and PCBs in Food and Feed and
  - EURL for Pesticides in Food of Animal Origin and Commodities with High Fat Content
- WHO/UNEP Reference Laboratories for POPs in Human Milk according Stockholm Convention



- **EURL Network for Pesticide Residues**

EURL FV Almeria, Spain
EURL CF Copenhagen, Denmark
EURL AO, Freiburg, Germany
EURL SRM, Stuttgart, Germany

EURL EUROPEAN UNION REFERENCE LABORATORY PESTICIDE RESIDUES IN FOOD OF ANIMAL ORIGIN & COMMODITIES WITH HIGH FAT CONTENT

Content

- Overview of classical methods for analysing food of animal origin
- Analysis of pesticide residues at CVUA Freiburg
- Advantages/disadvantages and alternatives
- Method development and modification on the classical approach
- Validation of honey
- Method development for liver and other matrices
- Conclusions

EN 1528 Modular System

European Standard	Description	German Standard	Modul	Description	Validated methods of CVUA Freiburg	Samples / Compounds
EN 1528-2: 1996-10 (confirmed 2001)	Extraction of fat, pesticides and PCBs and determination of fat content	§ 64 LFGB: L 00.00-34 (confirmed 1999)	E 8	Extraction of fat with hexan/acetone	PV 31 P01601	meat, fish
	Extraction of fat, pesticides and PCBs and determination of fat content	§ 64 LFGB: L 00.00-38/2 (confirmed 09/1998)	6.1	Extraction of milk	PV 31 P00402 PV 31 P00502	milk
		§ 64 LFGB: L 00.00-38/2 (confirmed 09/1998)	6.2.3	Extraction of butter	SOP 31 S00303	butter
		§ 64 LFGB: L 00.00-38/2 (confirmed 09/1998)	6.3.1 and 6.3.2	Extraction of cheese, dairy and milk powder	PV 31 P00202 PV 31 P00302 PV 31 P00602	cheese, dairy, milk powder
		§ 64 LFGB: L 00.00-38/2 (confirmed 09/1998)	6.4	Extraction of meat, fisch and products	PV 31 P00202 PV 31 P00302 PV 31 P01601	meat, fish and products
		§ 64 LFGB: L 00.00-38/2 (confirmed 09/1998)	6.5	Extraction of eggs	PV 31 P00202 PV 31 P00302 PV 31 P01601	egg
		§ 64 LFGB: L 01.00-8 (confirmed 04/1981)	-	Determination of the fat content of milk	PV 31 P00102	milk

Modular System (Meat and Fish, Egg)

- Column extraction
  - extraction in a column using n-hexane/acetone
- Soxhlet extraction
  - solvent light petroleum or diethyl ether
- Hot solvent extraction
  - Used at CVUA Freiburg
  - Solvent light petroleum or n-hexane/acetone
- Liquid-liquid partition
  - using n-hexane/acetone and sodium sulphate solution
- Centrifugation
  - Cold centrifugation using n-hexane

Extraction: Hot Soxtherm with light petroleum

clean up

European Standard	Description	German Standard	Modul	Description	Validated methods of CVUA Freiburg	Samples / Compounds
EN 1528-3: 1996-10 (confirmed 2001)	Clean-up methods	§ 64 LFGB: L 00.00-34 (confirmed 11/1999)	GPC	Gel permeation chromatography	PV 31 P00802	animal fats
		§ 64 LFGB: L 00.00-34 (confirmed 11/1999)	C 2	Adsorption chromatography on a small silica gel column	PV 31 P00902	animal fats
		§ 64 LFGB: L 00.00-37 and 38/3 (confirmed 09/1998)	11	GPC and adsorption chromatography on a small silica gel column	PV 31 P00802 PV 31 P00902 PV 31 P01002	animal fats
		§ 64 LFGB: L 00.00-12 (confirmed 08/1993)	7.4.2	Clean-up with sulfuric acid	PV 31 P01102	animal fats
EN 1528-4: 1996-10 (confirmed 2001)	Determination, confirmatory tests, Miscellaneous	§ 64 LFGB: L 00.00-38/4 (confirmed 09/1998) § 64 LFGB: L 00.00-34 (confirmed 11/1999)	4 - 5 D1 - D4	Gas chromatography with ECD, FPD, NPD, MSD	PV 31 P00802 PV 31 P00902 PV 31 P01002	organochlorine -, organophosphorus-, sulfur-containing-, nitrogen-containing-compounds, pyrethroids and other
	GC analysis of organotin compounds in fish and mussels	§ 64 LFGB: L 10.00-9 (confirmed 12/2002)	-	organotin compounds are transferred into ethyl-compounds and detected by GC-FPD and GC-MS	PV 31 P01401	fish, mussels

Detection

European Standard	Description	German Standard	Modul	Description	Validated methods of CVUA Freiburg	Samples / Compounds
EN 1528-3: 1996-10 (confirmed 2001)	Clean-up methods	§ 64 LFGB: L 00.00-34 (confirmed 11/1999)	GPC	Gel permeation chromatography	PV 31 P00802	animal fats
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EN 1528 Part 3: Clean-up

- Method A: Liquid/ Liquid partition with acetonitrile and chromatography on a florisil column
- Method B: Liquid/ Liquid partition with dimethylformamid and chromatography on a florisil column
- Method C: Column chromatography on activated florisil
- Method D: Column chromatography on partially deactivated florisil
- Method E: Column chromatography on partially deactivated aluminiumoxid
- Method F: Gel permeation chromatography (GPC)
- Method G: Gel permeation chromatography (GPC) and column chromatography on partially deactivated silica gel
- Method H: High pressure GPC (HPGPC)

Used at CVUA Freiburg

Extraction for non-polar pesticides at CVUA Freiburg

Sample preparation example

Mix 5 g (10% fat) of sample with sodium sulfate (~50 g)

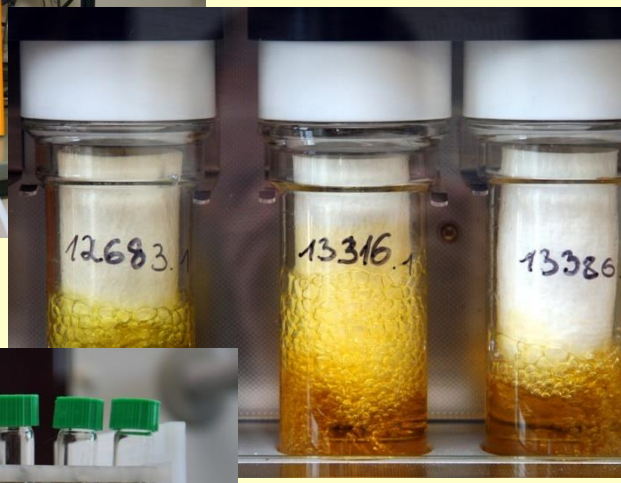
Soxtherm extraction with light petroleum

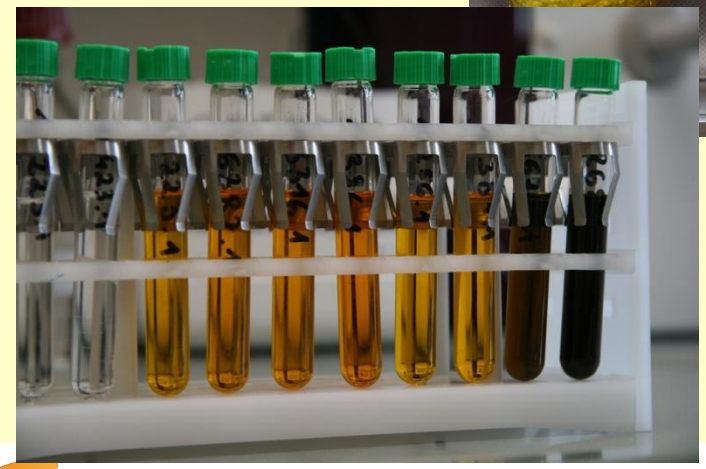
Filtrate, evaporate to dryness

Re-dissolve in ethylacetate/cyclohexane

Take an aliquot of 5 mL for clean-up (containing ~0.5 g fat)

→ clean-up





Extraction of non-polar pesticides (GC Analysis)

Sample preparation example

Mix 5 g (10% fat) of sample with sodium sulfate (~50 g)

Soxtherm Extraction with light petroleum

Filtrate, evaporate to dryness

Re-dissolve in ethylacetate/cyclohexane

Take an aliquot of 5 mL for clean-up (containing ~0.5 g fat)

→ clean-up

Clean up

Addition of internal Standards 2,4,5-TCB Mirex, Triphenylphoshat, PCB 209

GPC (Bio Beads S-X3)

small silica gel column (1.5 % water)

Fraction 2 Toluene

Fraction 3 Toluene / Acetone 95 + 5

Final volume 0.5 ml

Clean-up for GC-Analysis

Clean up

Addition of internal Standards 2,4,5-TCB Mirex, Triphenylphoshat, PCB 209

GPC (Bio Beads S-X3)

small silica gel column (1.5 % water)

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GPC (Bio Beads S-X3)

small silica gel column (1.5 % water)

Fraction 2 Toluene

Fraction 3 Toluene / Acetone 95 + 5

Final volume 0.5 ml

Quantification

Fraction 2 | **Fraction 3**

Gas chromatography 3 columns (PS 088, OV 1701, HP 5) 4 detektors (ECD, NPD, MSD, MS/MS)

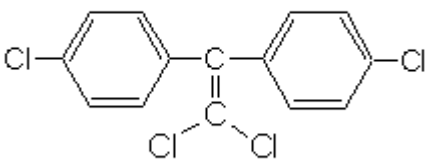
4 calibration levels, solvent/matrix calibration, internal Standards

Evaluation using ISTD

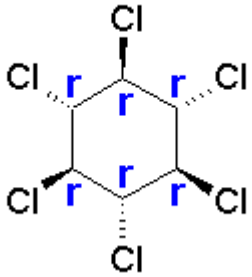
all according EN 1528

Examples for Analytes

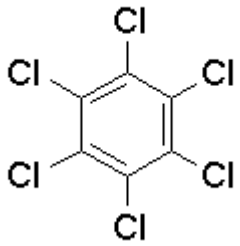
DDE, p,p-



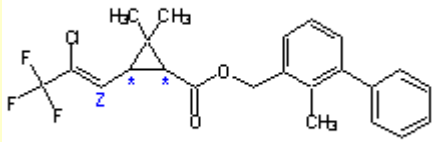
beta-HCH



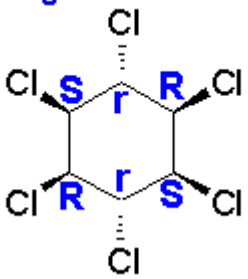
Hexachlorobenzene



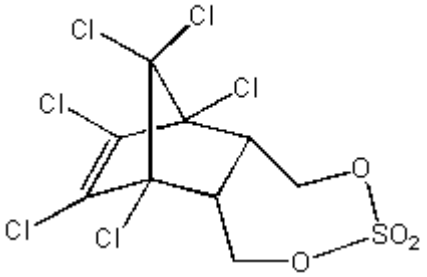
Bifenthrin



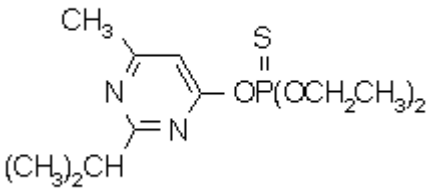
gamma-HCH



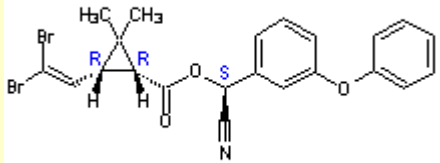
Endosulfansulfate



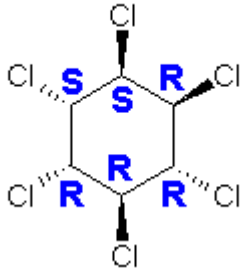
Diazinon



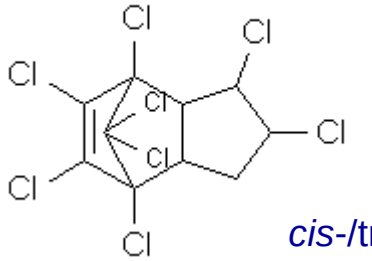
Deltamethrin



alpha-HCH

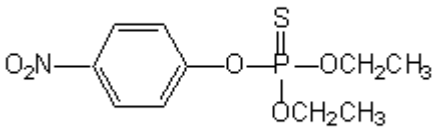


Chlordane

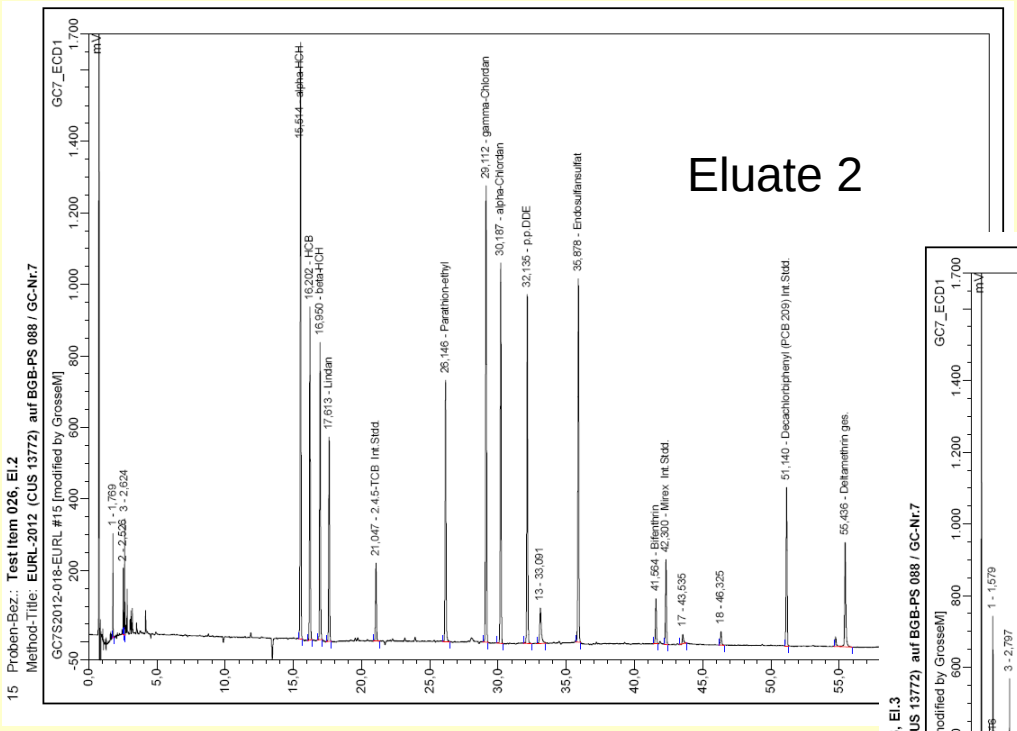


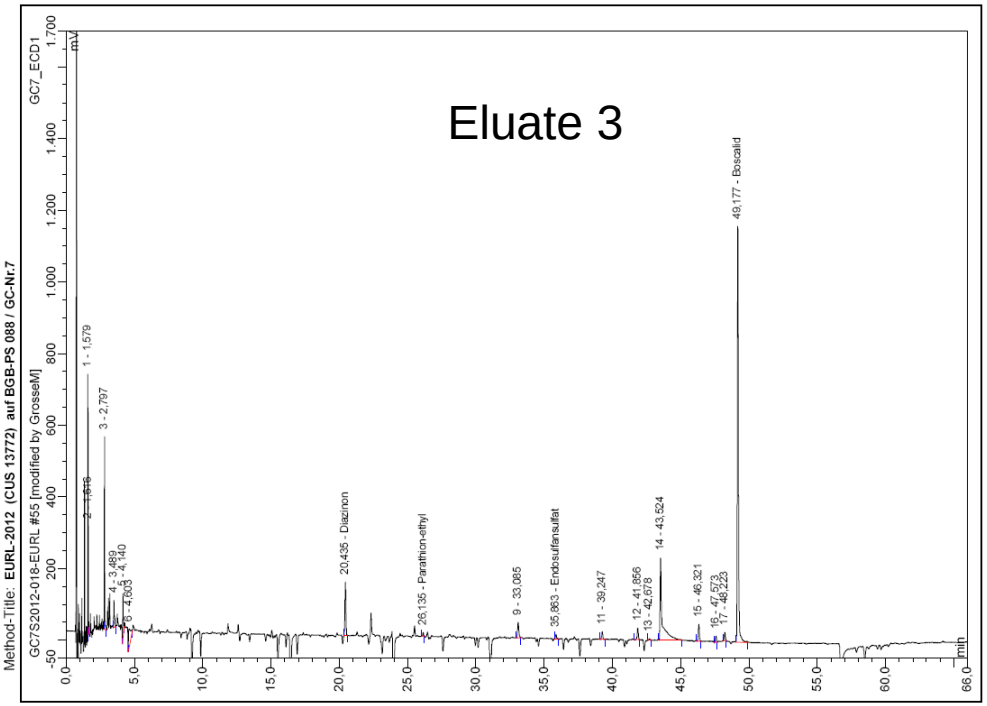
cis-/trans

Parathion



Sample chromatogram on GC-ECD (EN 1528)





Exctract according to EN 1528
(Test Item of EUPT AO 07)
PS 088; GC-ECD

Advantages- and Disadvantages of Method (EN 1528)

- Method established with high experiences
- Final extracts suitable for Gas-Chromatography combined with all GC-detection systems
- For MS-Measurement only few maintenance effort necessary (1-2 times a year), few liner changes

- 6-7 different solvents are used during the analysis procedure
- High solvent volume (~500 mL per analysis), recycling at CVUA
- Time consuming steps (2-3 working days per series), esp. extraction step
- High costs for glassware und man power

➢ How to increase the efficiency (saving time and money)?

Possible alternatives?

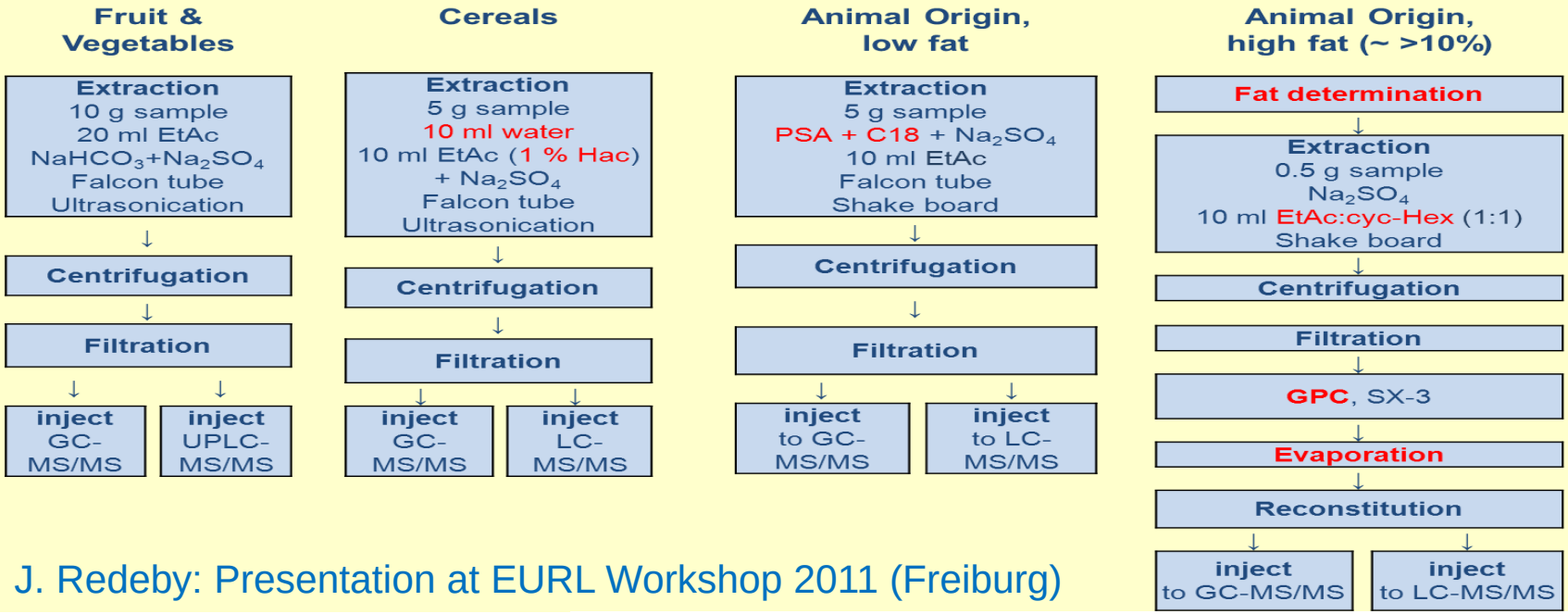
QuEChERS

SweEt

- Quick methods with fast extraction and clean-up steps
- Established for pesticides residues in fruit and vegetables
- Low solvent consumption per sample (~10 mL)
- In case of food of animal origin established for more polar pesticides detected with LC-MS/MS
- Co-extracted fat are a possible problem in GC analysis
- Low recoveries for non-polar pesticides (using QuEChERS)

SweEt Method

- Developed by Andersson and Ohlin (1989)
- Modified and miniaturized
- Simplified method published 2007
  (Anal Bioanal Chem 2007:389(6):1773-89. Epub 2007 Jul 4)



J. Redeby: Presentation at EURL Workshop 2011 (Freiburg)

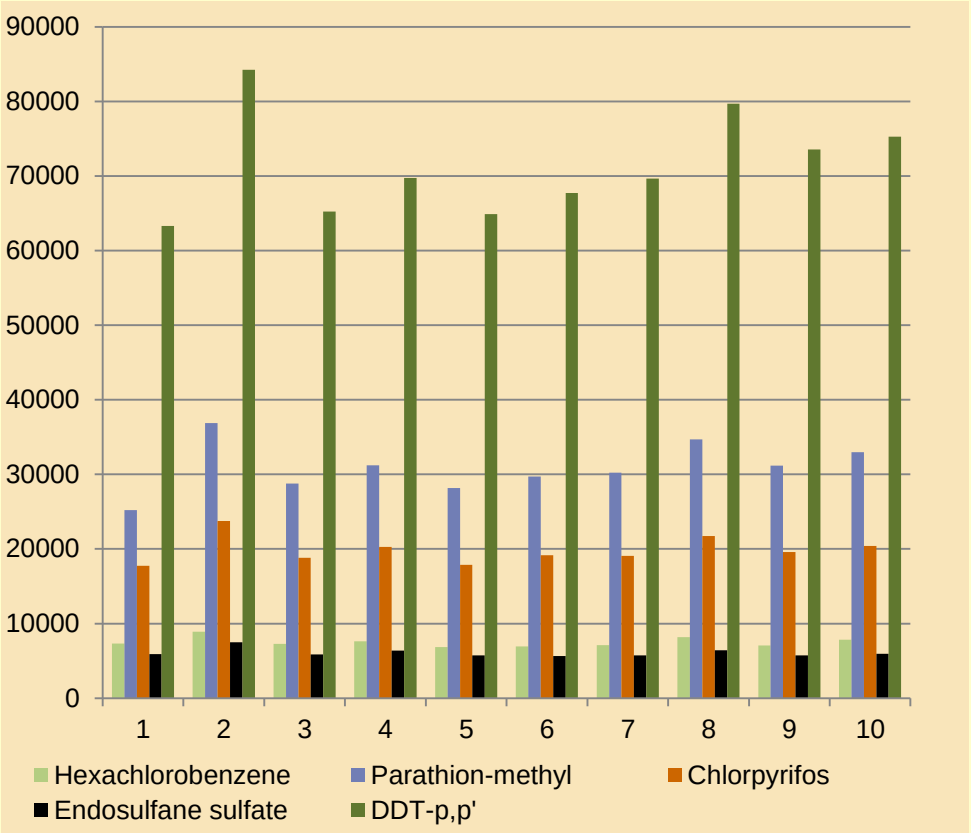
	QuEChERS	SweEt	En 1528 used at CVUA Freiburg
Sample weight	5 g	5 g	5 g
Extraction	10 mL Acetonitrile Citrate buffer $MgSO_4$	10 mL Ethylacetate Sodium sulfate PSA C_{18}	Mixing with 50 g sodium sulfate Extraction with petroleum ether for 2 h Filtration, removal of solvent Determination of fat content
	10 min shaking 6 min centrifugation (≥ 2500 g)	30 min shaking 6 min centrifugation (≥ 2500 g)	Redissolving of whole fat or aliquot (0.5 g fat) Preparing for GPC with ethylacetate/cyclohexane
Clean-up	6 mL supernatant: $MgSO_4$ PSA	Filtrate	GPC Removal of solvent Preparation of silica gel colums (conditioning)
	10 min shaking 6 min centrifugation (≥ 2500 g)		Sampling of fractions Removal of solvent 0.5 mL final volume
Detection	GC or LC detection system		

Reproducibility: GC-MSMS of QuEChERS extracts

Matrix: cream (frozen extract)

10 Injections

Injection	Hexachloro benzene	Parathion-methyl	Chlorpyrifos	Endosulfane sulfate	DDT-p,p'
1	7302	25194	17751	5904	63314
2	8908	36860	23766	7500	84235
3	7275	28771	18795	5846	65225
4	7631	31206	20292	6372	69720
5	6828	28159	17862	5730	64903
6	6938	29702	19147	5635	67724
7	7089	30243	19057	5712	69649
8	8193	34705	21717	6440	79692
9	7054	31176	19598	5717	73544
10	7852	32962	20389	5957	75251
Mean:	7507	30898	19837	6081	71326
Standard-deviation:	652	3348	1824	570	6815
CV [%]:	8,7%	10,8%	9,2%	9,4%	9,6%



No effects observable

Data obtained from test measurement on Agilent GC-MSMS

Reproducibility: GC-MSMS of QuEChERS extracts

Matrix: cream (frozen extract)

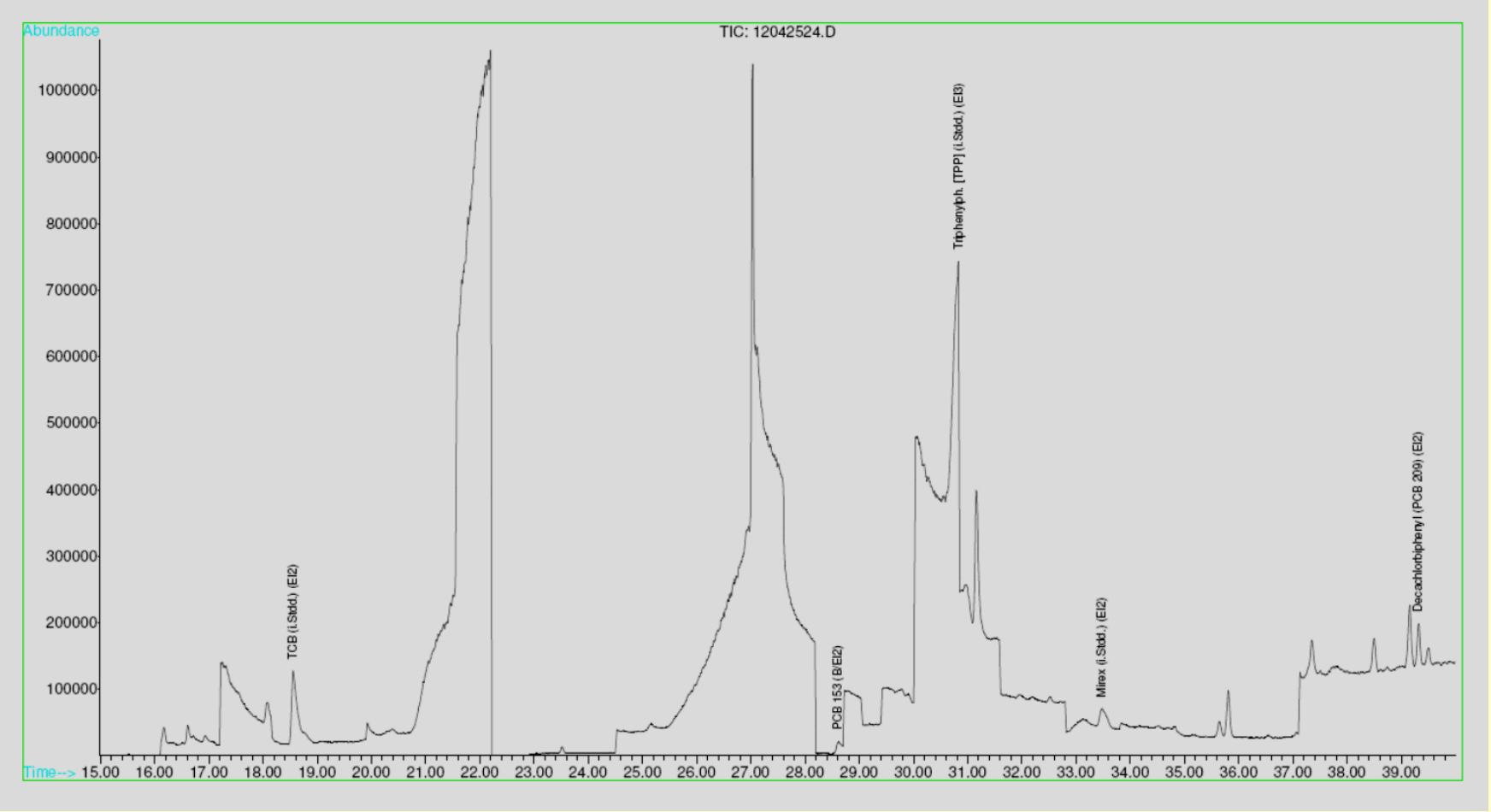
10 Injections

Injection	Deltamethrin	Fenvalerate I	Fenvalerate II
1	595	14625	9101
2	534	14129	7450
3	208	7461	3736
4	250	7432	4113
5	191	5426	3479
6	192	5564	2858
7	156	4994	3588
8	127	5500	2107
9	138	4596	1566
10	132	4283	1382
Mean	252	7401	3938
standard-deviation	169	3826	2496
cv [%]	67.2%	51.7%	63.4%

Loss of sensitivity – no reproducibility with „dirty“ extracts.

Data obtained from test measurements on Agilent GC-MSMS

GC-MSD: extracted matrix of animal origin



Modification of existing method for pesticide residues

- Modification of the extraction procedure
- Ethylacetate like in SweEt method approach, first
- Mixture of ethylacetate and cyclohexane used for extraction
  - Combination with existing EN 1528 – clean-up approach
  - The extract should be ready to use for clean-up
  - No evaporation step after extraction
- GPC cleanup
- Optional silica gel cleanup for difficult matrices
- Starting with matrix honey

Modification of existing method for pesticide residues

5 g sample (honey)

addition of Internal Standards

Wait 10 min

+ 5 ml water
+ citrate buffer salts (as in QuEChERS)

Shake 10 sec

+ 10 ml ethylacetate/ cyclohexane (1:1)

Shake 10 min

Modification of existing method for pesticide residues

+ 10 g Na_2SO_4
+ 0,2 g PSA

Shake 10 min

Centrifuge
(2500 g)

Take 5 ml extract

Proceed with
GPC

Modification of existing method for pesticide residues

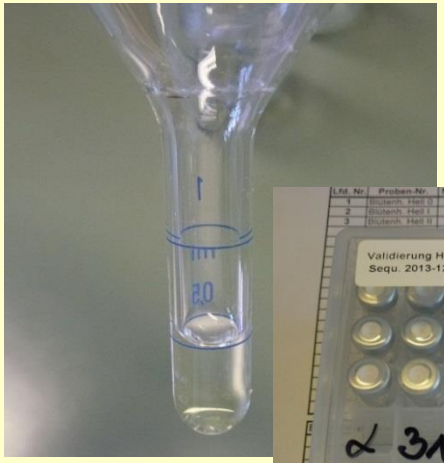
Evaporate solvent

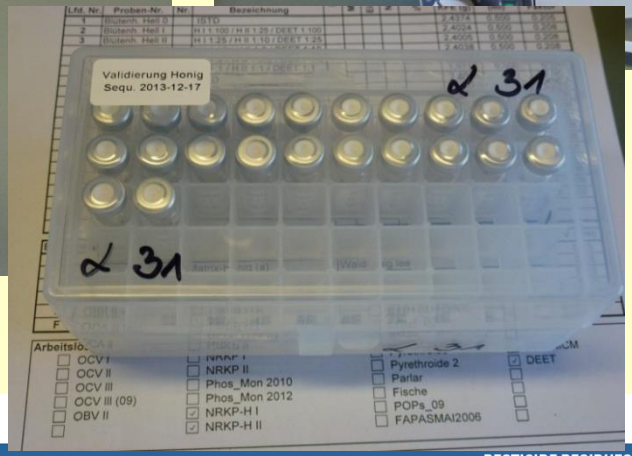
Optional Cleanup Step (difficult matrices)
- Re-dissolve in isooctane
- silica gel column

Re-dissolve in cyclohexane

Final volume 0.5 mL

GC-MS/MS-Analysis





Analysis of samples

- Use of matrix matched calibrations
  (using extracted honey expected to be free of pesticide residues)
- Analysis performed using the Agilent 7890A with 7000B QQQ
  (Pesticide Analyzer)

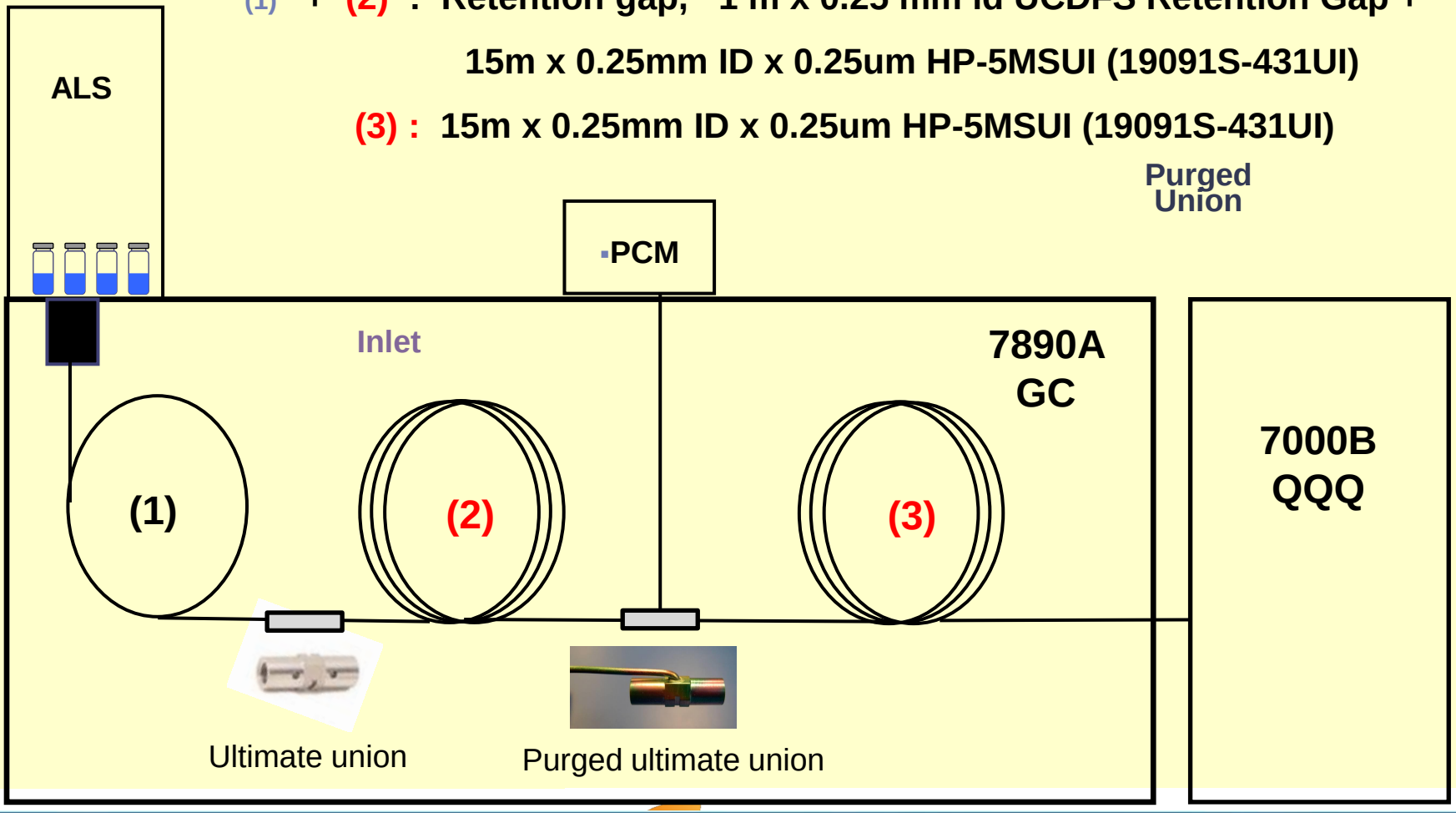
- Validation requirements*:
  - Recovery 70-120 %
  - Coefficient of variation (CV) < 20 %
  - Blank < 30% LOQ
  - 5 replicates per level

*Document SANCO 12751/2013

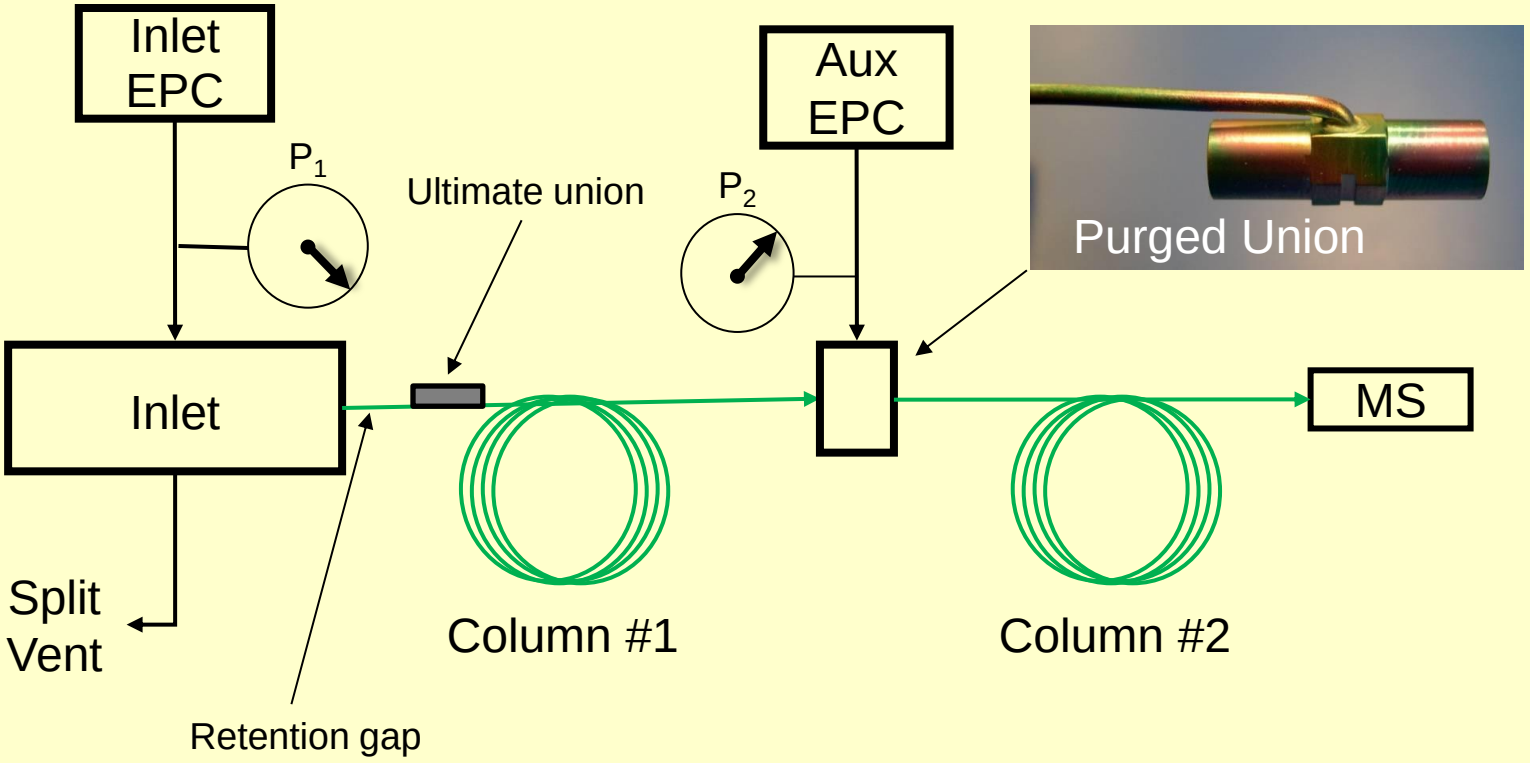
GC-MS/MS Pesticide Analyzer 40 minute CF Method + 1 m Pre-Column

(1) + (2) : Retention gap, 1 m x 0.25 mm id UCDFS Retention Gap + 15m x 0.25mm ID x 0.25um HP-5MSUI (19091S-431UI)

(3) : 15m x 0.25mm ID x 0.25um HP-5MSUI (19091S-431UI)

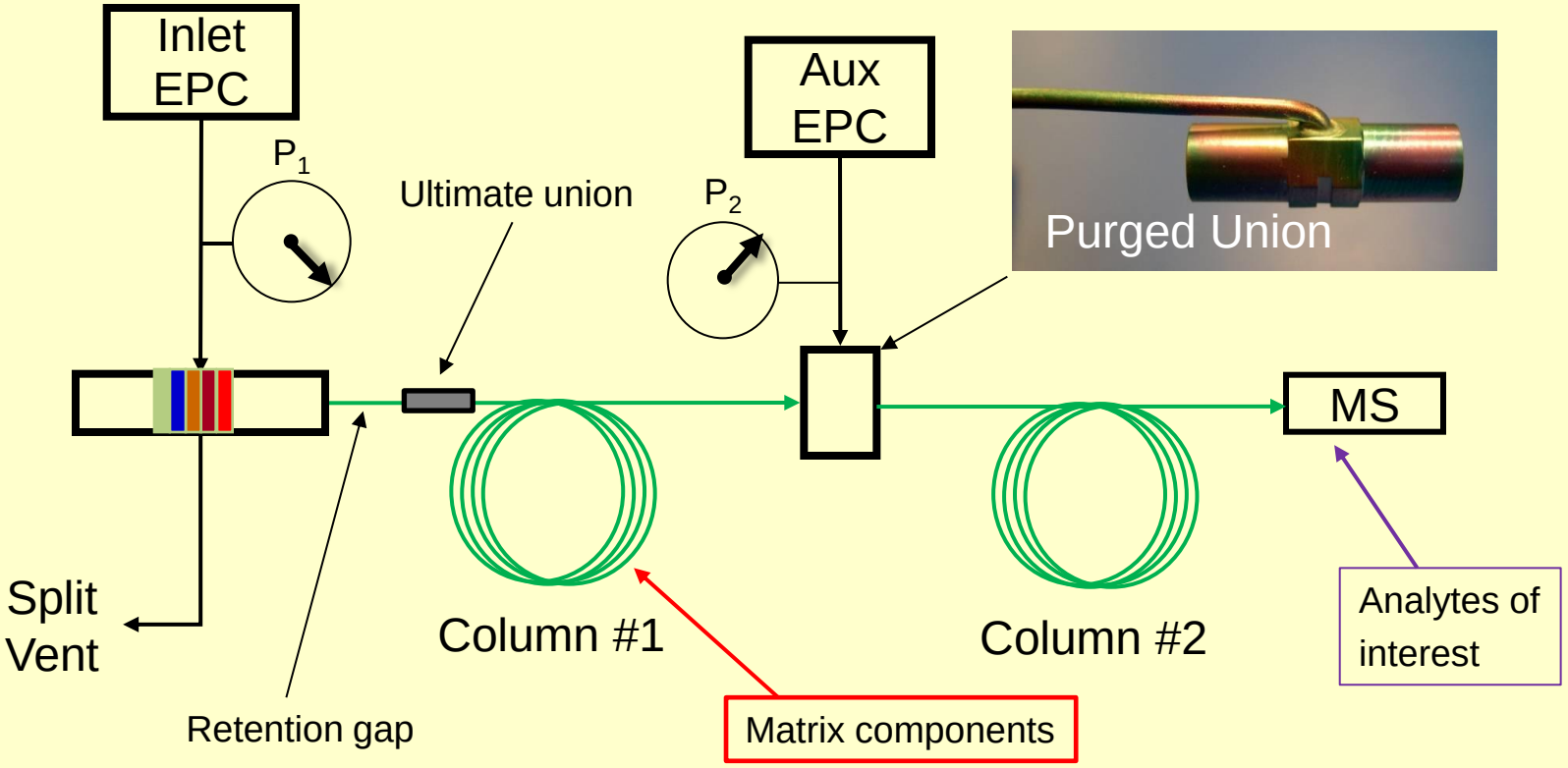


Mid-column, post run back flush



During the run, $P_1 > P_2$, Carrier gas flows towards MS

Mid-column, post run back flush



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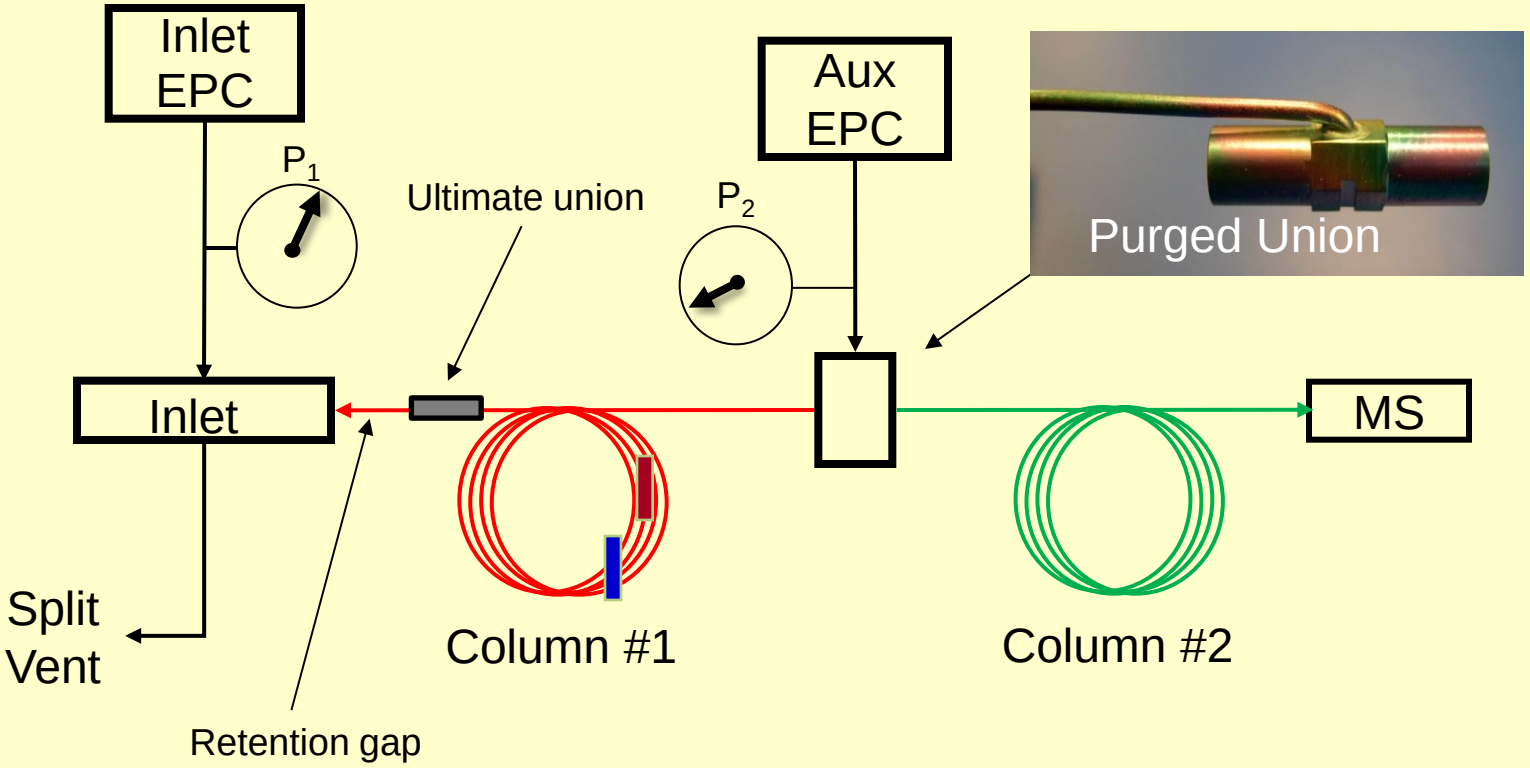
Mid-column, post run back flush



During post run back flush $P_1 < P_2$, Carrier flow reversed in column

The Most Comprehensive and Flexible MRM database

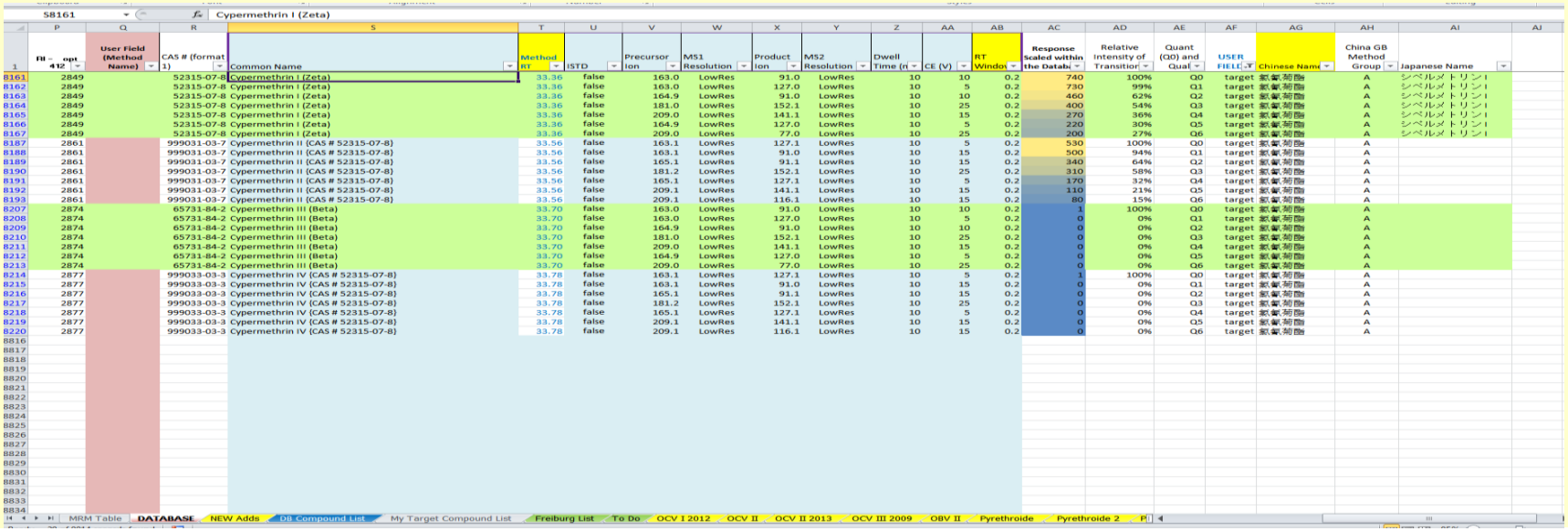
- **1000+ Pesticides & Environmental Pollutants**

Compound Classification	Total Number
Pesticides (fungicides, herbicides, insecticides, rodenticides and others)	675
Breakdown Products	42
Deuterated Compounds	6
Polybrominated Diphenyl Ether (PBDE)	4
Polybrominated Biphenyl (PBB)	1
Polychlorinated Biphenyl (PCB)	209
Polycyclic Aromatic Hydrocarbon (PAH)	26
Phthalates	17
Additional Semi-volatile Pollutants	94

- **Average of 8 MRM transitions for each compound (Total > 8500 transitions)**
  - -- provides alternatives to **avoid matrix interferences**
  - -- includes relative intensities for all transitions

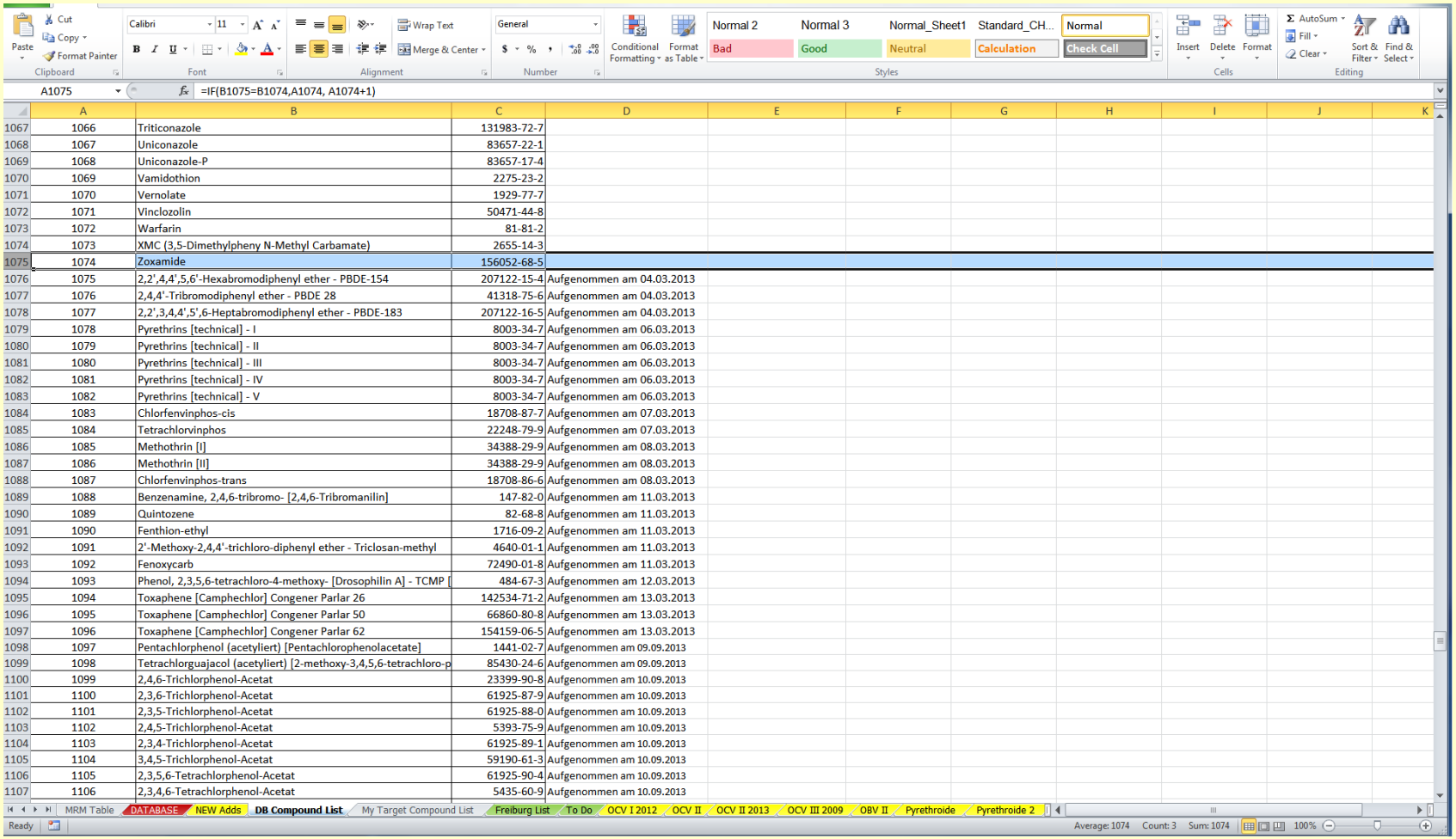
Pesticide Analyser with MRM database

- Database containing > 1000 pesticides and environmental pollutants
- Specific mass transitions and intensities especially for Agilent GC-MSMS
- Selection of the best transitions, no further tuning necessary
- Inclusion of other missing analytes possible
  (they have to be tuned of course!)



EURL EUROPEAN UNION REFERENCE LABORATORY PESTICIDE RESIDUES IN FOOD OF ANIMAL ORIGIN & COMMODITIES WITH HIGH FAT CONTENT

MRM database additions of EURL AO



	A	B	C	D
1067	1066	Triticonazole	131983-72-7	
1068	1067	Uniconazole	83657-22-1	
1069	1068	Uniconazole-P	83657-17-4	
1070	1069	Vamidothion	2275-23-2	
1071	1070	Vernolate	1929-77-7	
1072	1071	Vinclozolin	50471-44-8	
1073	1072	Warfarin	81-81-2	
1074	1073	XMC (3,5-Dimethylpheny N-Methyl Carbamate)	2655-14-3	
1075	1074	Zoxamide	156052-68-5	
1076	1075	2,2',4,4',5,6'-Hexabromodiphenyl ether - PBDE-154	207122-15-4	Aufgenommen am 04.03.2013
1077	1076	2,4,4'-Tribromodiphenyl ether - PBDE 28	41318-75-6	Aufgenommen am 04.03.2013
1078	1077	2,2',3,4,4',5',6-Heptabromodiphenyl ether - PBDE-183	207122-16-5	Aufgenommen am 04.03.2013
1079	1078	Pyrethrins [technical] - I	8003-34-7	Aufgenommen am 06.03.2013
1080	1079	Pyrethrins [technical] - II	8003-34-7	Aufgenommen am 06.03.2013
1081	1080	Pyrethrins [technical] - III	8003-34-7	Aufgenommen am 06.03.2013
1082	1081	Pyrethrins [technical] - IV	8003-34-7	Aufgenommen am 06.03.2013
1083	1082	Pyrethrins [technical] - V	8003-34-7	Aufgenommen am 06.03.2013
1084	1083	Chlorfenvinphos-cis	18708-87-7	Aufgenommen am 07.03.2013
1085	1084	Tetrachlorvinphos	22248-79-9	Aufgenommen am 07.03.2013
1086	1085	Methothrin [I]	34388-29-9	Aufgenommen am 08.03.2013
1087	1086	Methothrin [II]	34388-29-9	Aufgenommen am 08.03.2013
1088	1087	Chlorfenvinphos-trans	18708-86-6	Aufgenommen am 08.03.2013
1089	1088	Benzenamine, 2,4,6-tribromo- [2,4,6-Tribromanilin]	147-82-0	Aufgenommen am 11.03.2013
1090	1089	Quintozene	82-68-8	Aufgenommen am 11.03.2013
1091	1090	Fenthion-ethyl	1716-09-2	Aufgenommen am 11.03.2013
1092	1091	2'-Methoxy-2,4,4'-trichloro-diphenyl ether - Triclosan-methyl	4640-01-1	Aufgenommen am 11.03.2013
1093	1092	Fenoxycarb	72490-01-8	Aufgenommen am 11.03.2013
1094	1093	Phenol, 2,3,5,6-tetrachloro-4-methoxy- [Drosophilin A] - TCMP [	484-67-3	Aufgenommen am 12.03.2013
1095	1094	Toxaphene [Camphechlor] Congener Parlar 26	142534-71-2	Aufgenommen am 13.03.2013
1096	1095	Toxaphene [Camphechlor] Congener Parlar 50	66860-80-8	Aufgenommen am 13.03.2013
1097	1096	Toxaphene [Camphechlor] Congener Parlar 62	154159-06-5	Aufgenommen am 13.03.2013
1098	1097	Pentachlorphenol (acetyliert) [Pentachlorophenolacetate]	1441-02-7	Aufgenommen am 09.09.2013
1099	1098	Tetrachlorguajacol (acetyliert) [2-methoxy-3,4,5,6-tetrachloro-p	85430-24-6	Aufgenommen am 09.09.2013
1100	1099	2,4,6-Trichlorphenol-Acetat	23399-90-8	Aufgenommen am 10.09.2013
1101	1100	2,3,6-Trichlorphenol-Acetat	61925-87-9	Aufgenommen am 10.09.2013
1102	1101	2,3,5-Trichlorphenol-Acetat	61925-88-0	Aufgenommen am 10.09.2013
1103	1102	2,4,5-Trichlorphenol-Acetat	5393-75-9	Aufgenommen am 10.09.2013
1104	1103	2,3,4-Trichlorphenol-Acetat	61925-89-1	Aufgenommen am 10.09.2013
1105	1104	3,4,5-Trichlorphenol-Acetat	59190-61-3	Aufgenommen am 10.09.2013
1106	1105	2,3,5,6-Tetrachlorphenol-Acetat	61925-90-4	Aufgenommen am 10.09.2013
1107	1106	2,3,4,6-Tetrachlorphenol-Acetat	5435-60-9	Aufgenommen am 10.09.2013

Mass Hunter Software

EURL EUROPEAN UNION REFERENCE LABORATORY

PESTICIDE RESIDUES IN FOOD OF ANIMAL ORIGIN & COMMODITIES WITH HIGH FAT CONTENT

Honey Results 1

	Level I			Level II			Level III			Level IV			Level V		
	Rec.	Stddev.	cv [%]	Rec.	Stddev.	cv [%]	Rec.	Stddev.	cv [%]	Rec.	Stddev.	cv [%]	Rec.	Stddev.	cv [%]
Hexachlorbenzene HCB	164.1	0.08	6.1	118.5	0.16	6.6	84.4	0.17	5.0	80.3	0.34	5.2	84.6	1.11	6.4
Lindan gamma-HCH	124.4	0.08	8.0	113.1	0.29	12.2	106.8	0.38	8.6	101.7	0.43	5.2	104.9	1.62	7.5
HCH, alpha-	138,4	0.05	4.5	114.7	0.15	6.6	95.8	0.19	4.8	92.3	0.43	5.6	100.5	2.01	9.7
HCH, beta-	133.2	0.12	11.0	120.3	0.27	10.9	110.5	0.46	10.1	108.9	0.97	10.8	105.0	3.01	13.9
DDE, pp-	74.0	0.14	11.1	93.2	0.53	13.7	95.4	0.83	10.6	92.9	0.68	4.4	97.6	2.47	6.1
DDT, op-	110.2	0.08	17.7	106.5	0.18	16.8	101.1	0.18	8.9	99.8	0.18	4.4	100.6	0.35	3.4
DDT, pp-	118.0	0.08	17.0	105.2	0.15	14.1	101.8	0.15	6.9	96.9	0.18	4.6	98.1	0.39	3.9
DDD, pp-	81.9	0.02	3.6	88.9	0.33	11.2	92.5	0.45	5.9	102.7	0.49	2.9	99.6	1.60	2.0
Chlorbenzilate	81.7	0.01	6.9	87.0	0.08	10.8	93.0	0.10	5.2	102.8	0.07	1.6	99.0	0.72	3.5
Vinclozolin	41.9	0.14	83.6	83.2	0.22	16.2	97.1	0.22	5.4	101.0	0.35	4.2	101.7	3.19	7.6
Coumaphos	119.9	0.24	24.4	84.7	0.56	20.1	81.2	0.72	10.7	92.2	1.00	6.6	91.9	5.8	7.7
Malathion	54.2	0.02	20.0	83.0	0.11	16.3	94.2	0.18	9.4	104.9	0.29	6.7	104.8	1.06	4.9

Honey Results 2

	Level I			Level II			Level III			Level IV			Level V		
	Rec.	Stddev.	cv [%]	Rec.	Stddev.	cv [%]	Rec.	Stddev.	cv [%]	Rec.	Stddev.	cv [%]	Rec.	Stddev.	cv [%]
Chinomethionate	4.6	0.05	244.9	76.8	0.32	25.3	113.9	0.41	8.8	103.5	0.54	6.4	114.6	2.11	4.5
Cyfluthrin	86.6	0.72	10.2	97.6	2.69	13.4	91.8	4.15	11.0	101.3	8.52	10.2	103.5	7.23	3.4
lamda-Cyhalothrin	53.0	0.45	10.3	62.5	1.70	13.2	59.0	3.11	12.8	61.5	3.29	6.5	67.7	3.34	2.4
Deltamethrin	77.8	0.25	7.9	97.6	1.28	12.7	100.2	3.06	14.8	104.6	2.05	4.8	113.8	2.81	2.4
Cymiazol	69.0	0.03	20.7	75.4	0.01	16.4	77.2	0.03	19.0	95.6	0.27	6.8	96.3	2.72	13.7
Brompropylate	74.3	0.04	13.9	88.3	0.16	10.7	102.2	0.42	10.0	106.2	0.47	5.4	101.9	3.26	7.8
Fenvalerate	89.0	0.38	10.3	96.4	1.58	15.9	93.9	2.86	14.8	101.1	4.71	11.3	105.9	2.69	2.5
DEET	66.1	0.14	102.7	101.6	0.07	8.3	98.7	0.09	4.6	107.9	0.35	7.9	109.5	2.30	10.2
Chlordimeform	56.9	0.10	82.0	96.3	0.36	44.9	70.7	0.71	49.0	114.9	1.57	33.2	114.1	9.20	39.2
Fenoxycarb	59.9	0.15	31.1	85.5	0.37	13.1	94.9	0.59	7.5	102.0	0.74	4.4	101.5	6.90	8.3
Phosalon	91.1	0.09	22.7	88.2	0.2	13.7	93.4	0.51	13.2	105.5	0.55	6.4	106.1	1.40	3.2
Cypermethrin	97.1	0.76	9.5	103.1	2.90	13.7	96.0	4.19	10.6	103.4	7.93	9.3	105.2	4.86	2.2

Honey Limit of Quantification (LOQ)

Substance	LOQ (µg/kg)	EU-MRL (µg/kg)
Hexachlorbenzene HCB	2.1	10
Lindan gamma-HCH	2.1	10
HCH, alpha-	2.1	10
HCH, beta-	2.1	10
DDE, pp-	1.6	
DDT, op-	0.4	
DDT, pp-	0.4	
DDD, pp-	0.8	
DDT, Summe		50
Chlorbenzilat	0.2	10
Vinclozolin	1.6	10
Coumaphos	8.2	100
Malathion	0.8	20

Honey Limit of Quantification (LOQ)

Substance	LOQ (µg/kg)	EU-MRL (µg/kg)
Chinomethionat	4.1	10
Cyfluthrin	8.2	10
Cypermethrin	8.2	50
lamda-Cyhalothrin*	8.2	50
Deltamethrin	4.1	30
Cymiazol	0.8	10
Brompropylat	0.4	10
Fenvalerat	4.1	10
DEET	0.8	10
Chlordimeform	failed	10
Fenoxycarb	3.3	10
Phosalon	1.6	10

Method for analysing pesticide residues in liver

5 g sample

+ Internal Standards
- wait 10 min

+ 5 ml water
+ citrate buffer salts

- Shake 10 sec

+ 10 ml ethylacetate/ cyclohexane (1+1/v+v)

- Shake 10 min

Method for analysing pesticide residues in liver

+ 10 g Na_2SO_4
+ 0,2 g PSA

+ 10 g Na_2SO_4
+ 0,2 g PSA
+ 0,5 g zirconium salt

- Shake 10 min

Centrifuge (2500 g)

Take 5 ml extract

GPC-cleanup

Method for analysing pesticide residues in liver

Evaporate solvent

↓

Cleanup Step
(difficult matrices)
Re-dissolve in
isooctane
silica gel column

↓

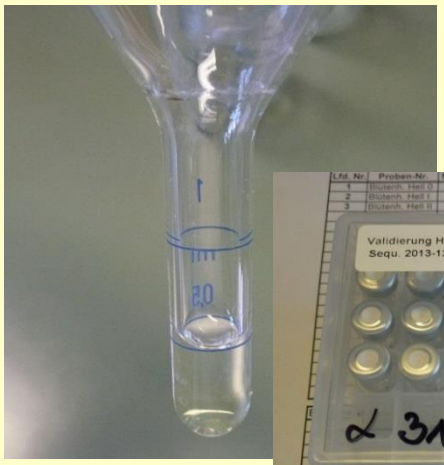
Re-dissolve in
cyclohexane

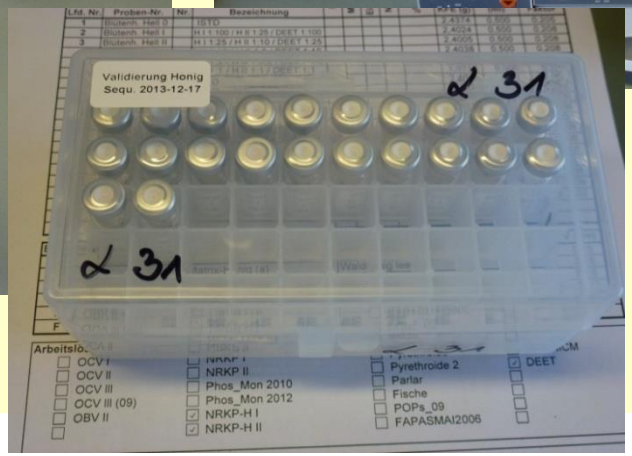
↓

Final volume 0.5 mL

↓

GC-MS/MS-Analysis





Method for analysing pesticide residues in liver final modification

- Zirconium salt was used during the extraction procedure resulting in cleaner extracts (visual control)
- As final modification the zirconium salt was layered over the silica gel
- With this modification the results for a selection of 70 pesticides and contaminants were very good
- The method has to be validated according to SANCO/12571/2013 (matrix matched calibration)

Residues in liver (final modification)

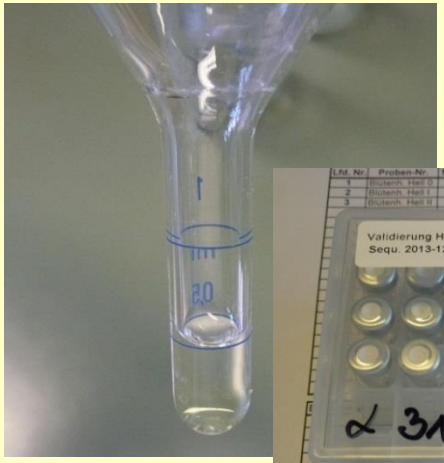
Evaporate solvent

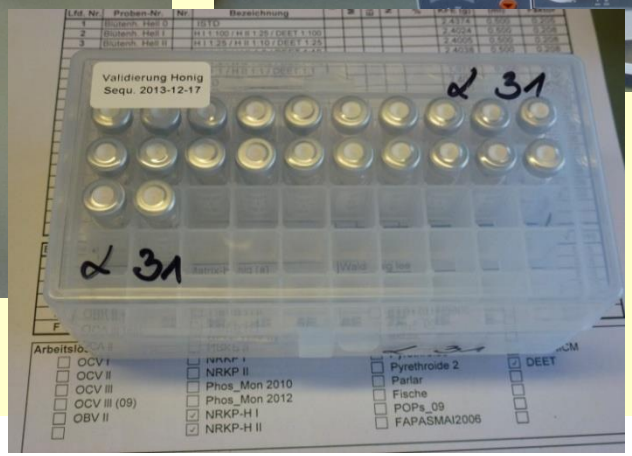
Cleanup Step
(difficult matrices)
- Re-dissolve in isooctane
- silica gel column with zirconium salt layer

Re-dissolve in cyclohexane

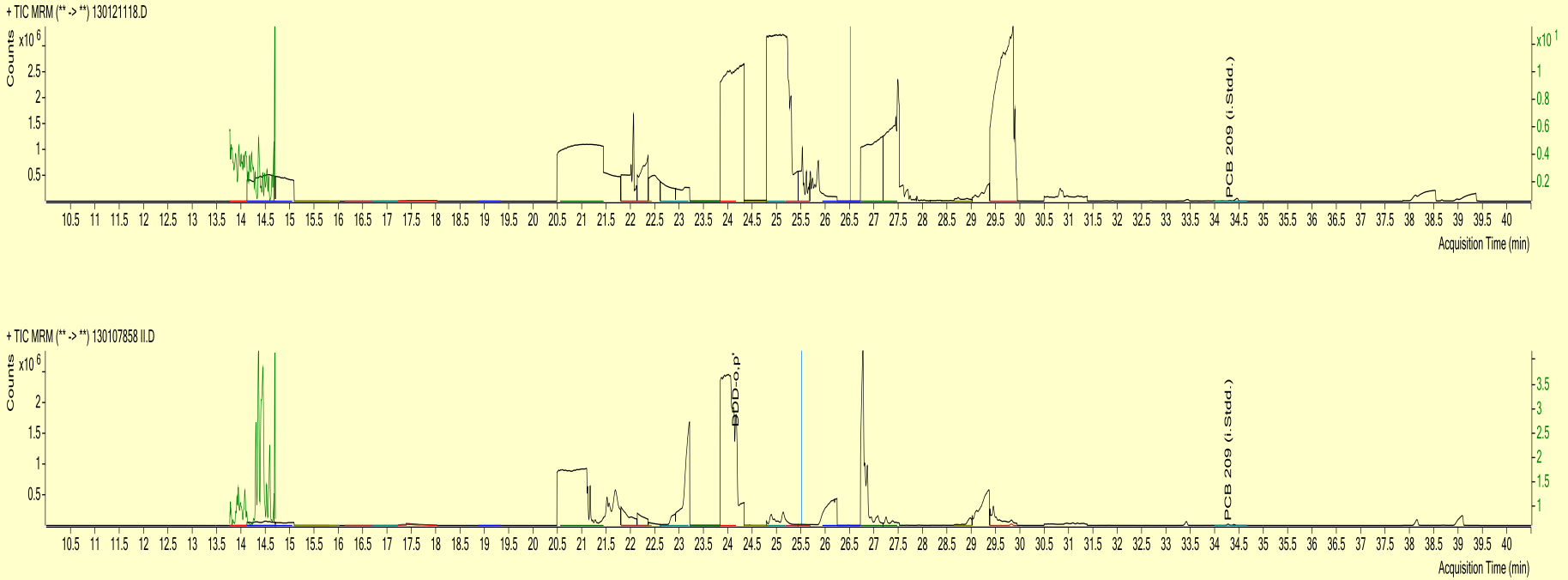
Final volume 0.5 mL

GC-MS/MS-Analysis



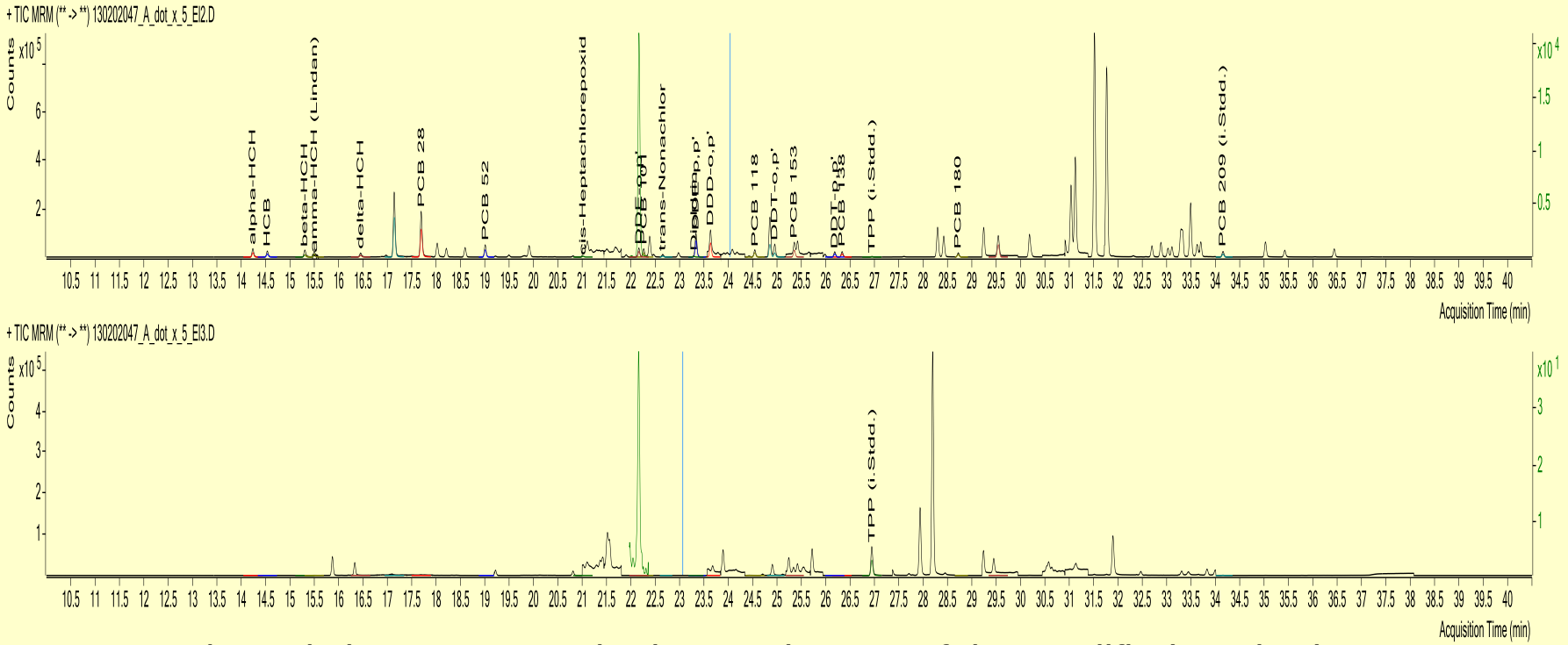


TIC (MRM; extracts of liver according EN 1528)



- Non sufficient cleanup
- matrix effects caused by co-extractives from liver matrix
- Evaluation of single substances possible
- Non applicable for multi component analysis caused by shifting retention times

TIC (MRM; extracts of liver with modifications)



- TIC (MRM) demonstrates the better cleanup of the modified method
- Results against standards in extracted lard matrix
  - Recovery rates for majority of analytes (> 80%) between 70-120%
  - matrix effects occur for the rest of analytes

➢ Validationexperiments to be evaluated against standards in liver extracts

Summary

- A method was established and validated for the matrix honey derived from SweEt extraction and modules of EN 1528
- The limits of quantification on a GC-MS/MS-system are comparable to the original EN 1528 based method
- For difficult matrices (e.g. liver) additional cleanup steps can be used (e.g. mini silica gel column, zirconium salt)
- The scope of the method will be enlarged to other matrices
  - Meat
  - Egg
  - Milk
- For quantifying the use of matrix matched calibration is mandatory

Thank you for your attention!

GC-Liner after the validation study