
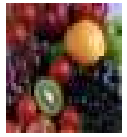
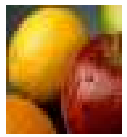


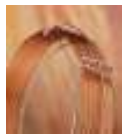

INSTITUTE OF CHEMICAL TECHNOLOGY,
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Prague, Czech Republic

TOF-MS: a Recent Challenge in GC Residue Analysis and a Strategy for Obtaining Accurate Data

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1st CRL/NRL Pesticide Residue Training Workshop, Stuttgart, Germany, 2006

Suggestions for implementation of NOVEL APPROACHES IN GC-BASED MRMs

- **SAMPLE PREP**: partition followed by dispersive SPE - **QuEChERS**
- **SAMPLE INTRODUCTION**: dirty matrix introduction injector - **DMI**
- **SEPARATION**: - low pressure chromatography - **LP-GC**
  - orthogonal chromatography - **GC×GC**

DETECTION: time-of-the-flight mass analyzer - **TOF-MS**

- QUALITY ASSURANCE: **ANALYTICAL PROTECTANTS**

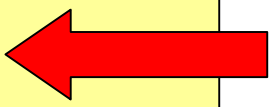
Strategies to examine food samples for occurrence of pesticide residues

TARGET ANALYSIS
➔ the list of analytes specified

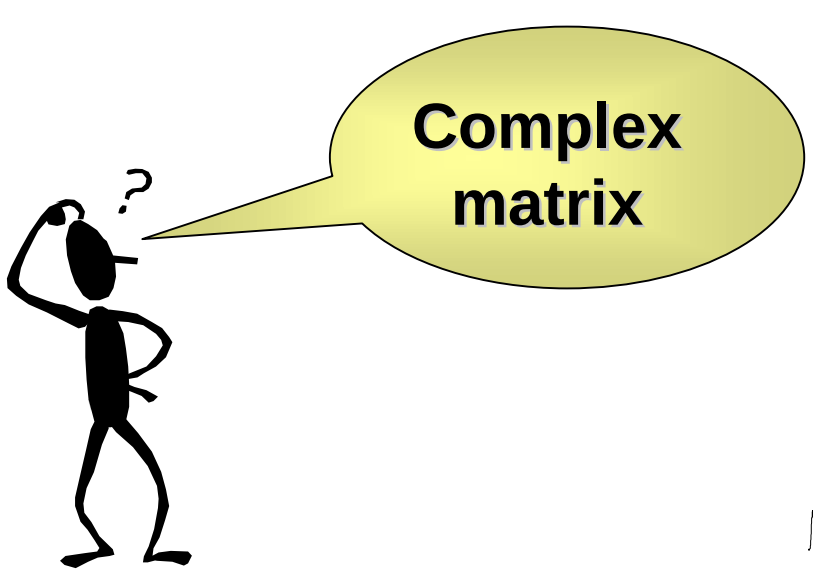
NON-TARGET SEARCH
➔ „unknowns“ to be determined

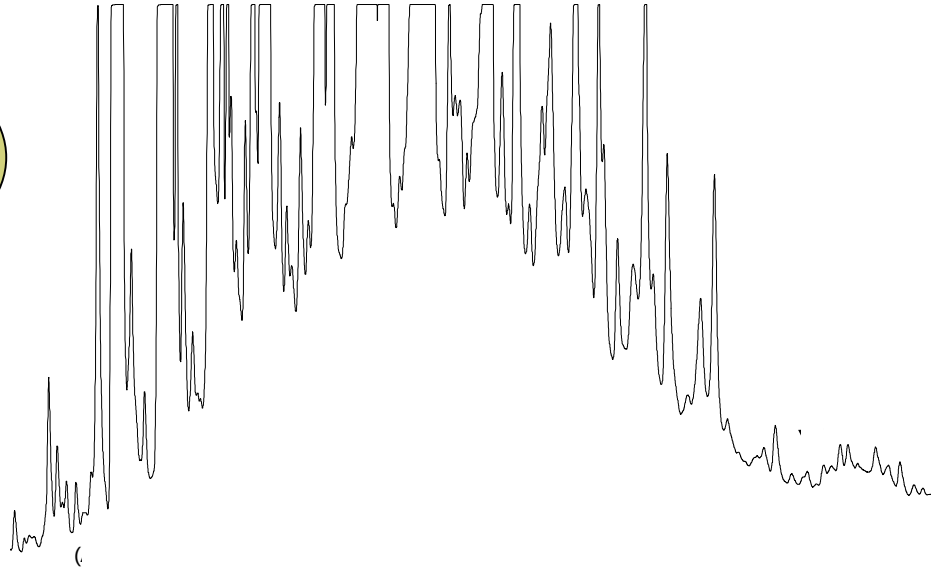
GC-MS systems conceivable for (multi)residue analysis

	Mass analyser	Mass Resolution	Acquisition rate	Detection limits
Ions separated in ***electrical*** *or* ***magnetic*** *field*	QUADRUPOLE	**0.5 amu peak width** (*R* = 2*m*, 10% valley)	**15–33 scans/s** for mass range 300 amu	**pg - fg** (SIM mode - limited by chemical noise, further improvement in MS/MS)
	ION TRAP	**1 amu peak width** (*R* = *m*, 10% valley)	**19 scans/s** for mass range 300 amu	
	SECTOR	**Up to 80,000** (10% valley)	**0.15 s/decade**	**fg (SIM mode)**
Ions separated in ***field-free tube***	**hs-TOF**	**1,400 FWHM**	**1–500 spectra/s**	**pg**
	hr-TOF	**7,000 FWHM**	**1–20 spectra/s**	**pg**



K. Maštovská, S.J.Lehotay: Practical approaches to fast gas chromatography–mass spectrometry. J. Chromatogr. A 1000 (2003) 153–180





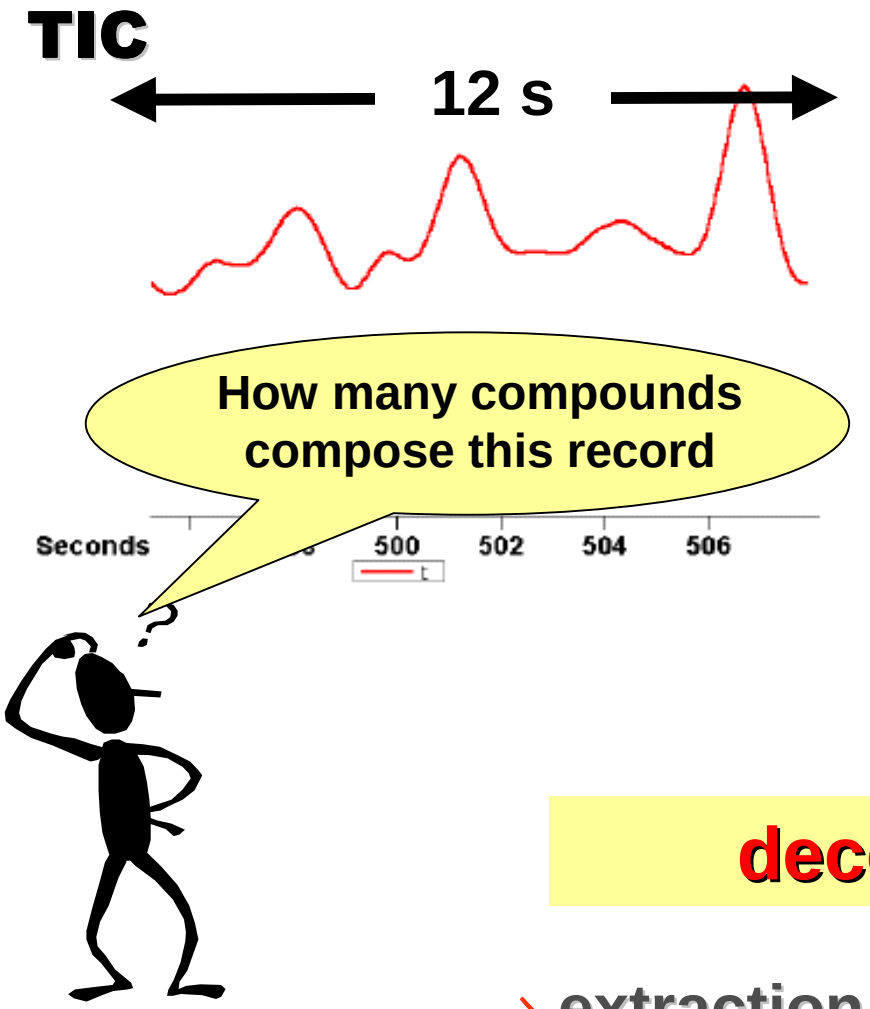
➡ **Achieve good spectral resolution !**

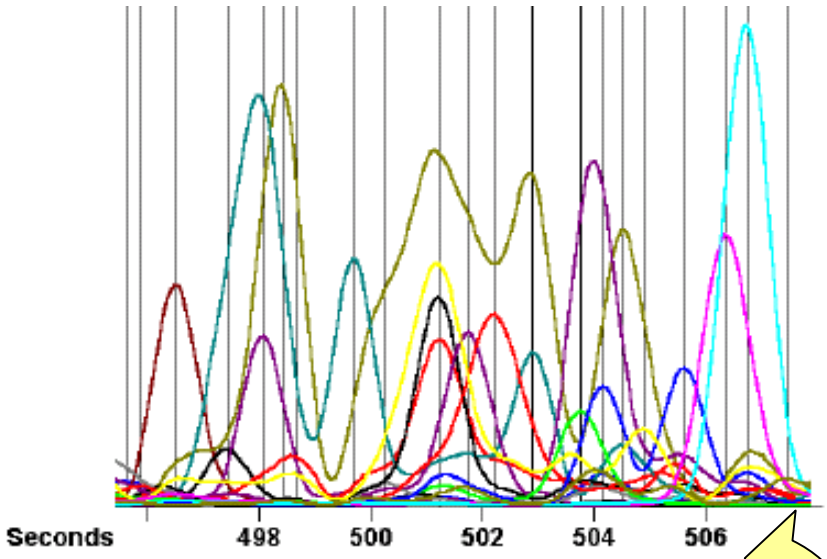
➡ **Improve chromatographic resolution !**

Non-target search:

➡ **Full spectral information needed for identification of unknown signals**

Spectral resolution of co-eluting compounds





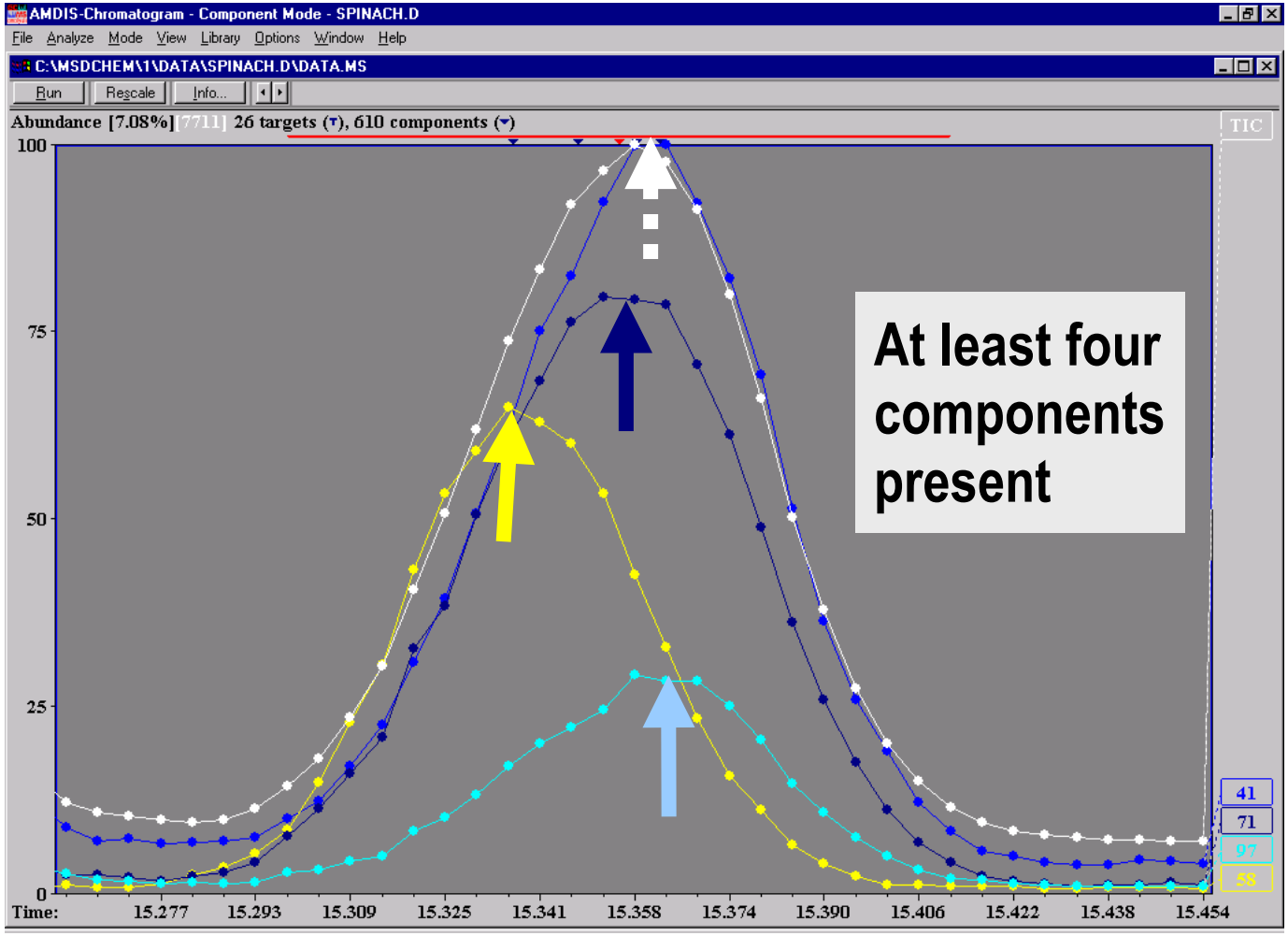
deconvolution software

→ extraction of spectral information →.....

→ library search

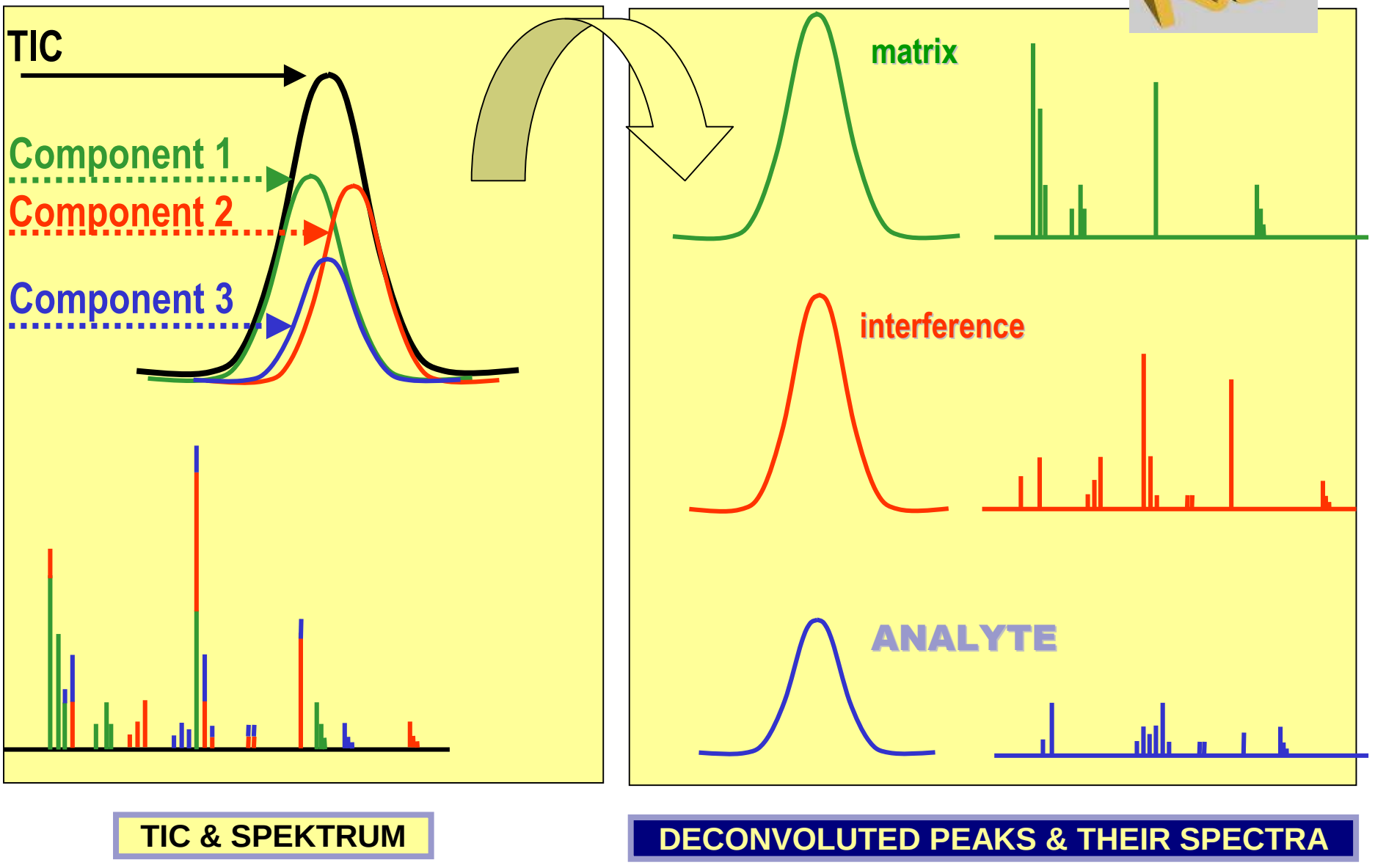
What is deconvolution...?

…. extraction of signals from complex mixture



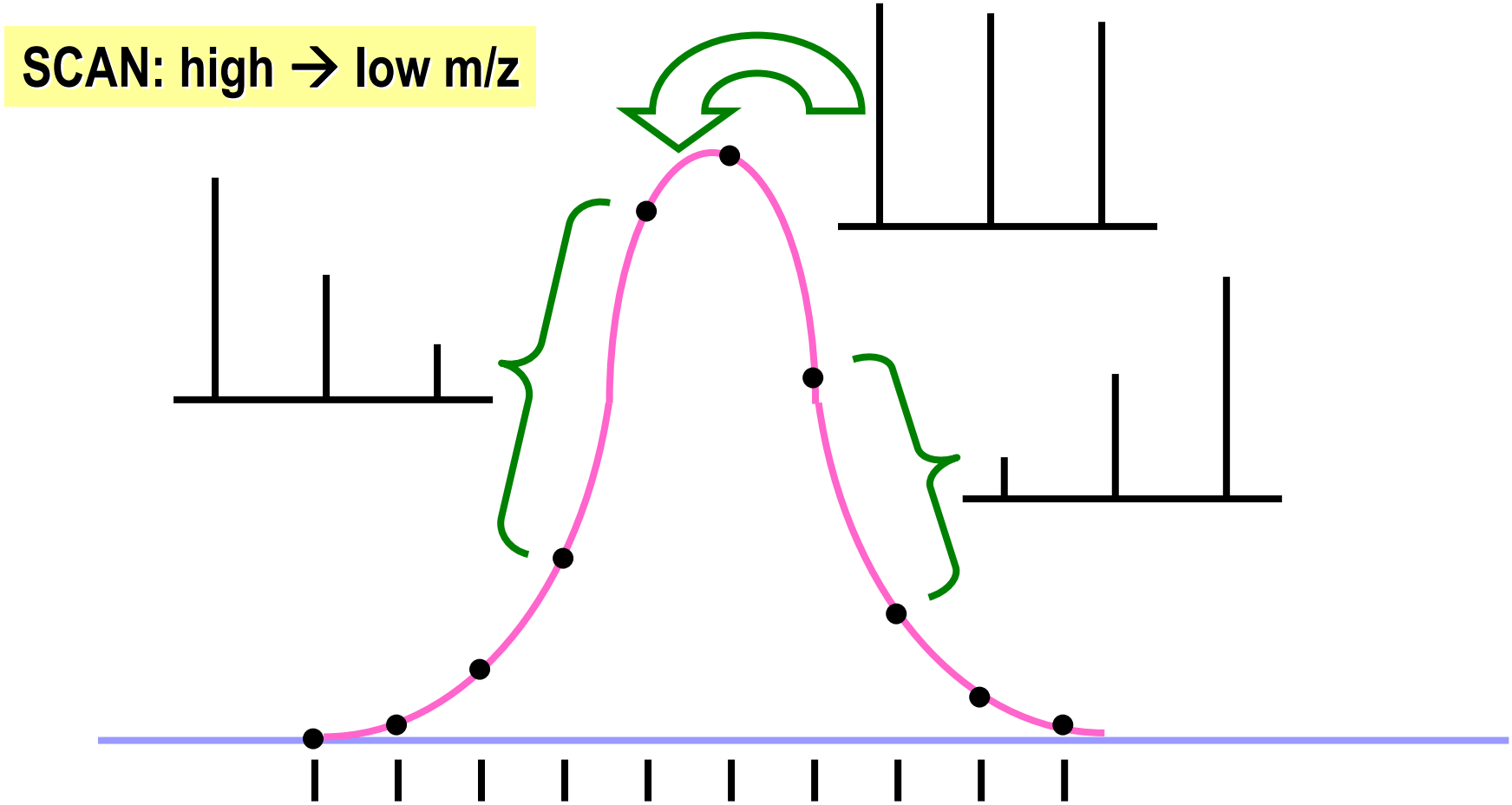
Source:: Agilent Technologies

AMDIS: Automatic Mass spectral Deconvolution Identification System

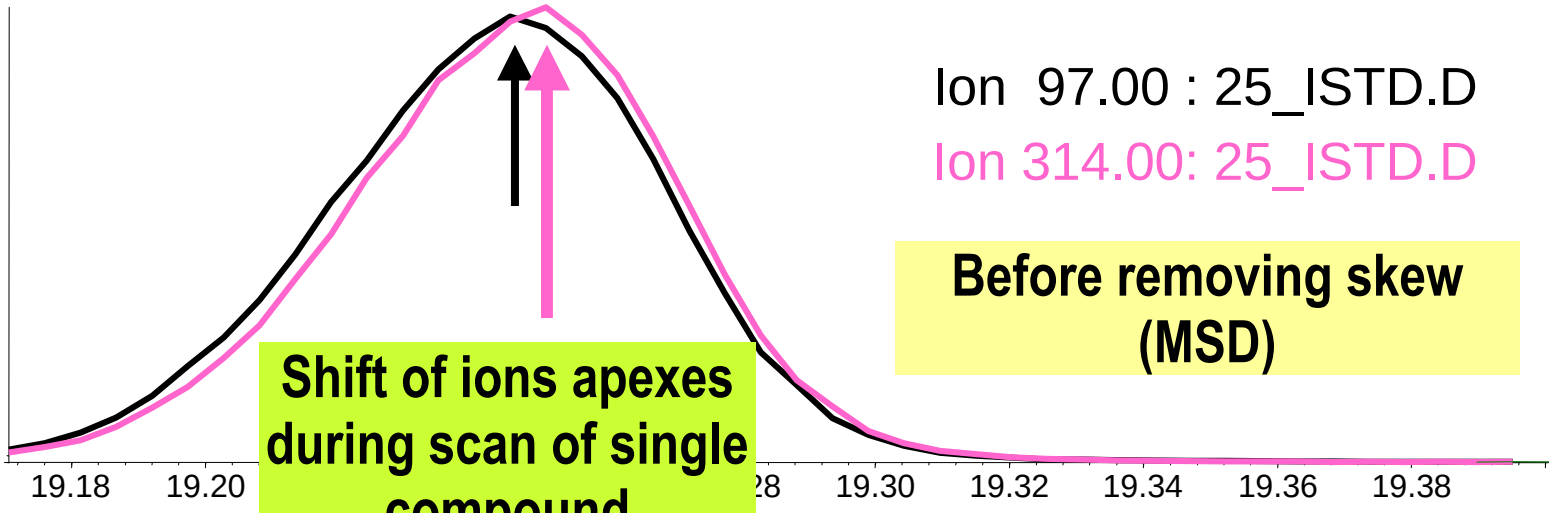


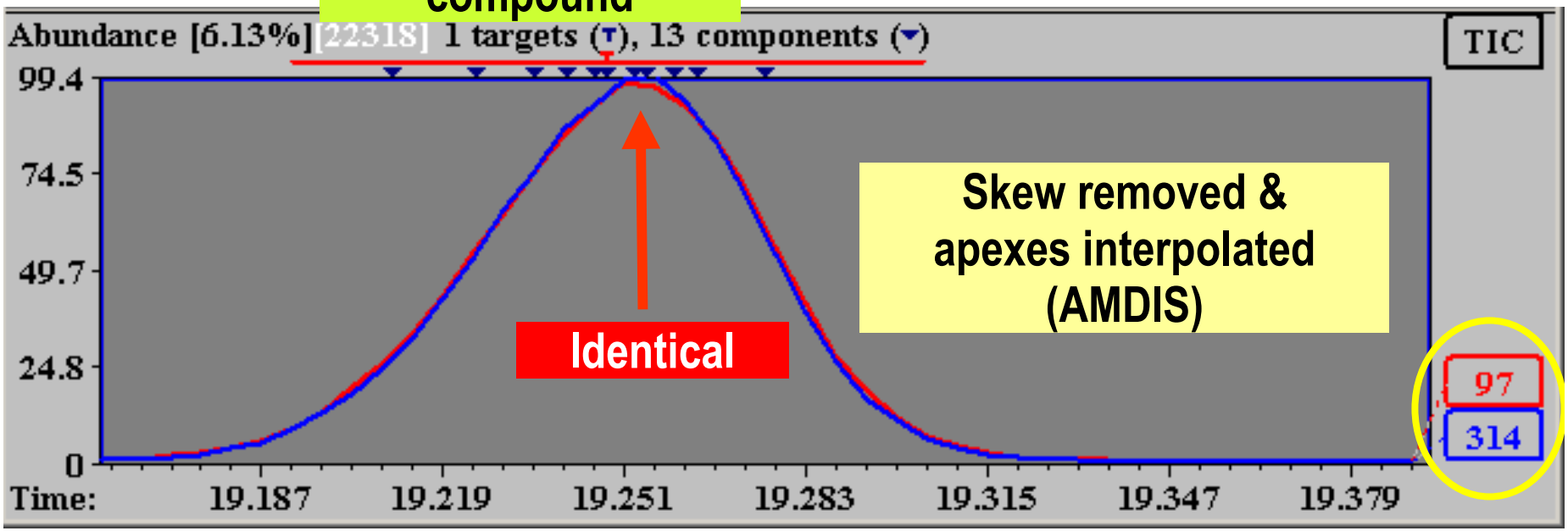
Scanning MS detector - spectral skew

Cause: changing concentration of analyte in MS source during peak elution



AMDIS: de-skew function





LIMITATION when using conventional MSD

High LODs in TIC

⇩

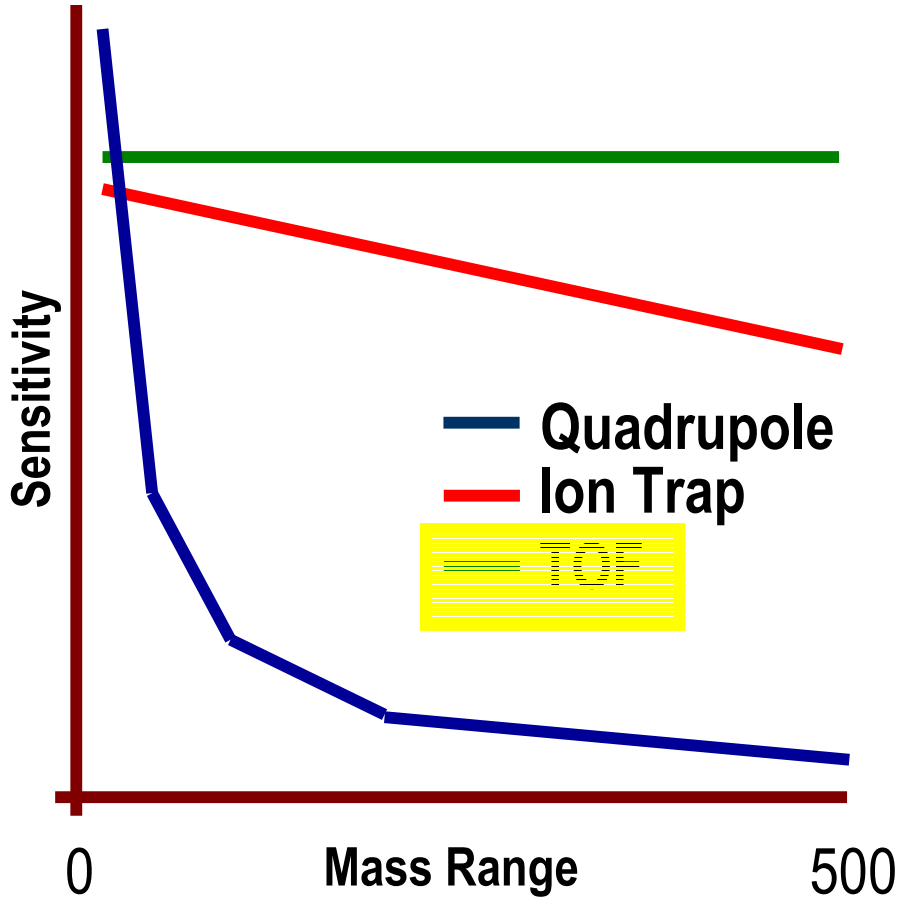
LVI-PTV

⇩

More co-extracts introduced into GC system

→ **MATRIX EFFECTS** ?

Comparison of mass analyzers sensitivity - acquisition of full spectral information



GCT,
Waters

Pegasus IV ,
Leco

HR TOF

- exact mass measurement
- conventional and fast GC

fast TOF

- fast, ultra fast GC
- GC x GC

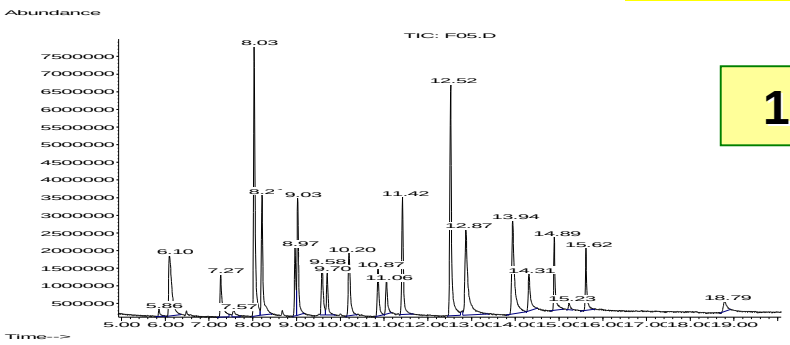
UNIQUE FEATURES OF BOTH APPROACHES:

- **Permanent acquisition of full mass spectra**
- **Absence of spectral skew** (deconvolution function)
- **High mass analyser efficiency up to 25%** (compared to 0.05% for the quadrupole) over the mass range of a 500 amu

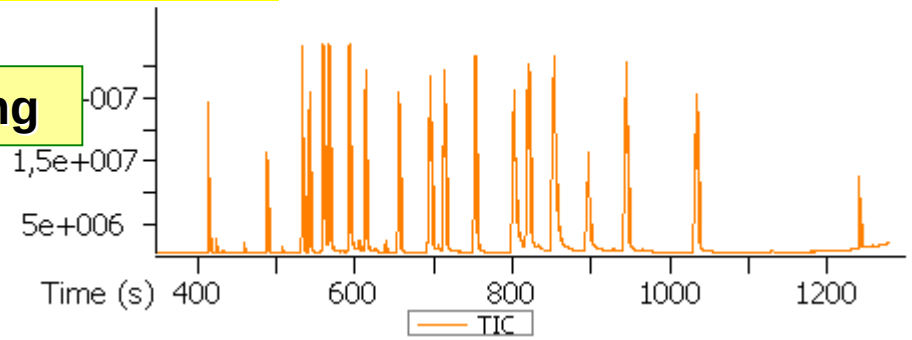
5975 MSD – full scan

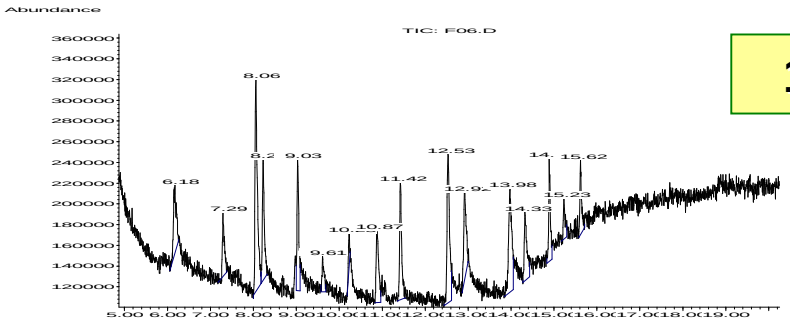
Pesticides std.

Pegasus Leco TOF-MS

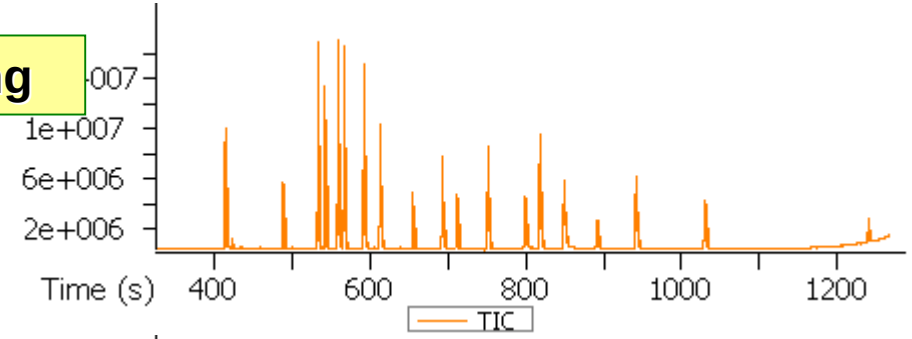


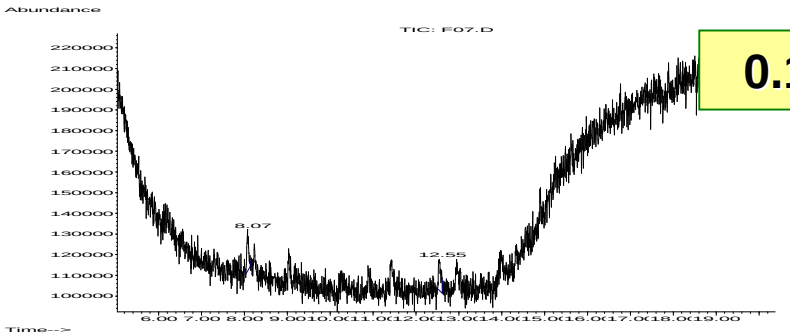
10 ng



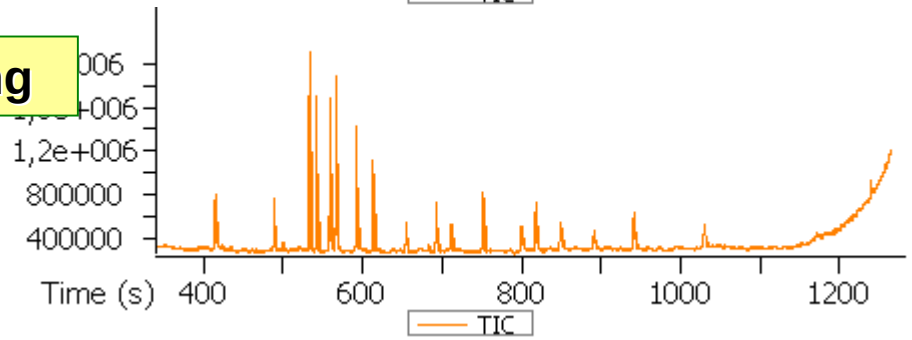


1 ng





0.1 ng



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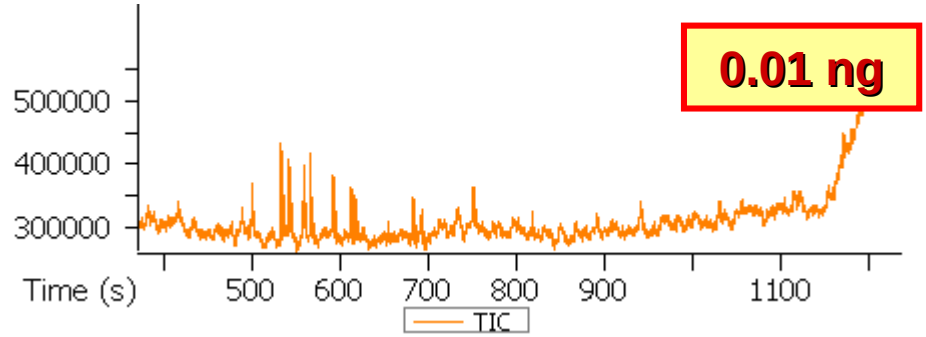


⇩

For trace levels **SIM mode** is necessary

GC-TOF MS instruments available at the market

Instrument (Manufacturer)	Mass Range (Da)	Mass Resolution	Acquisition Rate (spectra/s)	Registration of Ions	Ionisation Mode
Pegasus (Leco)	<1,000	Unit mass	1–500	ADC	EI
Tempus (Thermo)	<1,000	Unit mass	2–60	ADC	EI, CI
Kronus (Scientific Analysis Instruments)	<2,000	Unit mass	1–100	ADC	EI
GCT (Waters)	<1,500	7,000 FWHM	1–10	TDC	EI, CI, FI
GCT Premier (Waters)	<1,500	7,000 FWHM	1–20	TDC	EI, CI, FI
JMS-T100GC (JEOL)	<2,000	5,000 FWHM	1–25	ADC	EI, CI, FI

Principle of oa-TOF MS

Formation of fragment ions in the ion source (EI, CI)

⇩

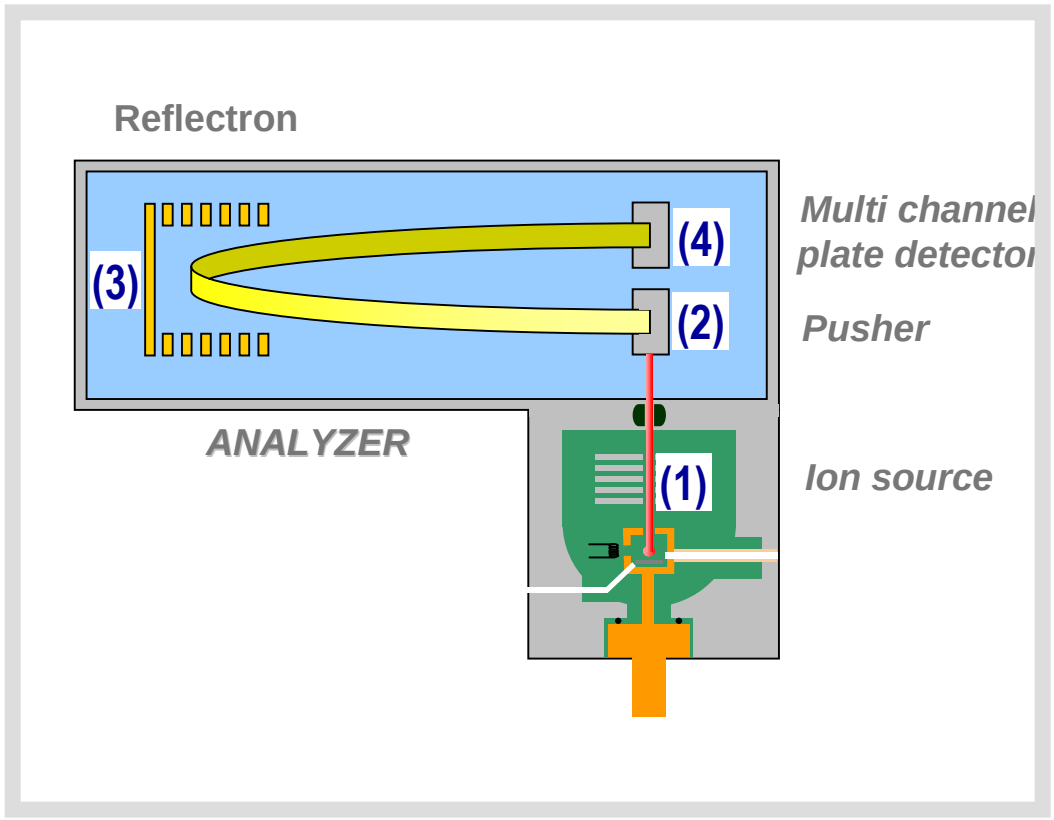
Ejection of a part of focused ion beam into a mass analyser

⇩

Energy focusing using reflectron

⇩

Detection of “ion events”

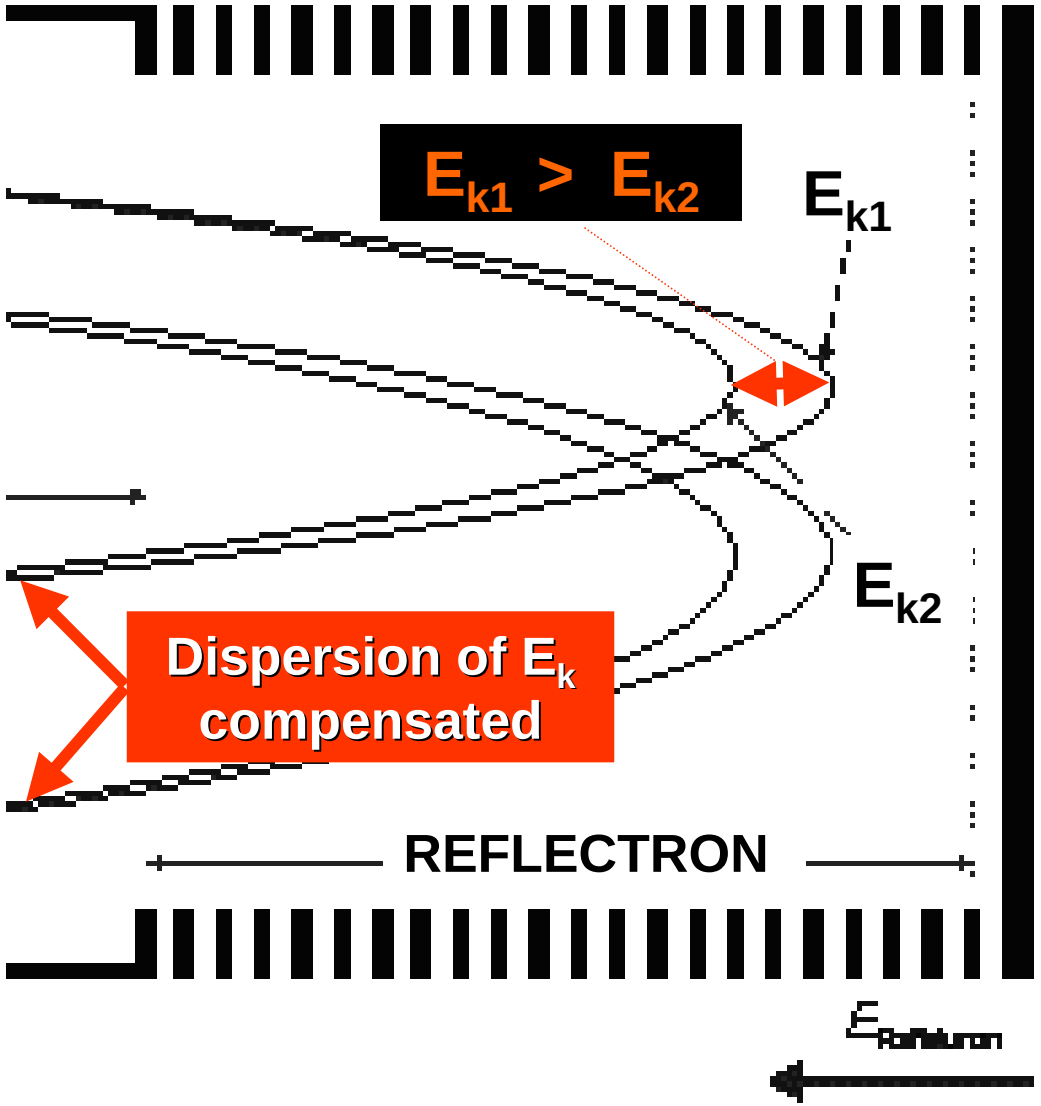


Reflectron function

$$E_k = \frac{1}{2}mv^2 = zeE$$

$$t = \sqrt{\frac{m}{z}} \cdot C$$

$$\left(C = d\sqrt{\frac{1}{2eE}} \right)$$



HR-TOF-MS

Narrow mass window setting

Example:

Matrix-matched standard of apple

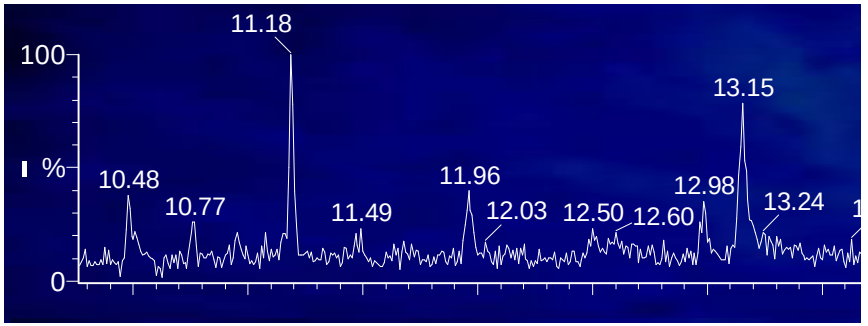
Analyte: PHOSALONE, concentration: 0.013 mg/kg

Low-resolution

S/N (PtP) = 5:1

Mass window: **1 Da**

***m/z* 182**

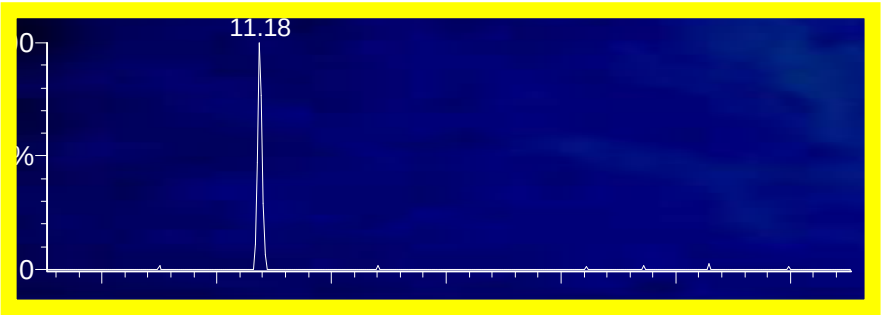


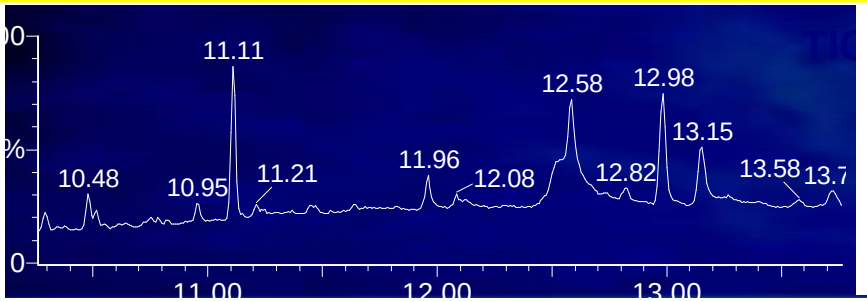
High-resolution

S/N (PtP) = 61:1

Mass window: **0.02 Da**

***m/z* 182.002**





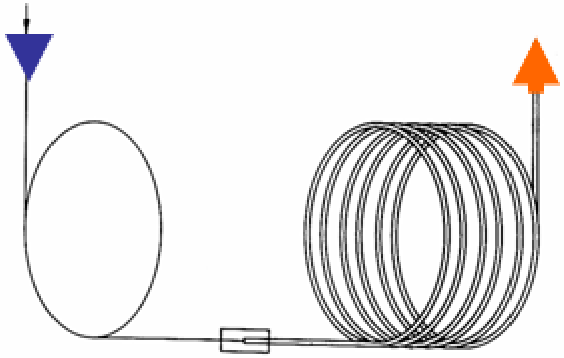
1 mg of original matrix injected

Significant elimination of background interferents from chromatogram

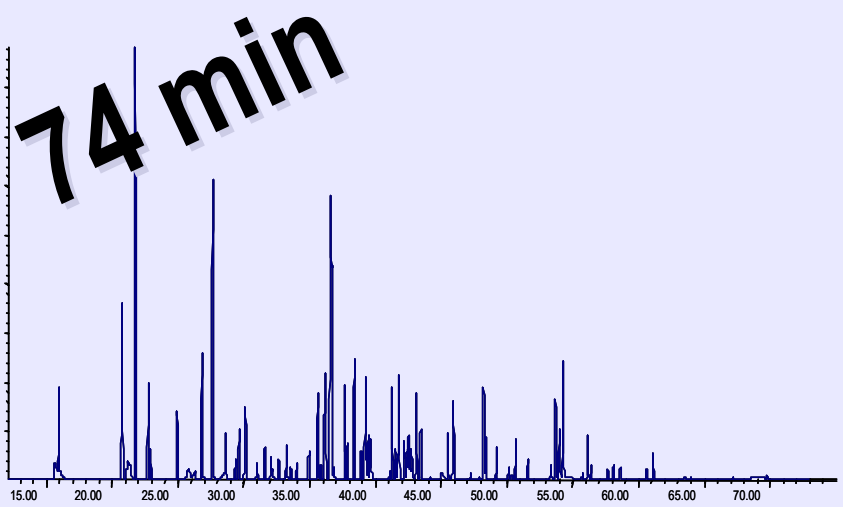
➡ **Improved detectability of analytes, low LODs**

Fast run - low pressure GC/MS

Example: **analysis of 100 pesticides in baby food (buffered QUeCHERS extract)**

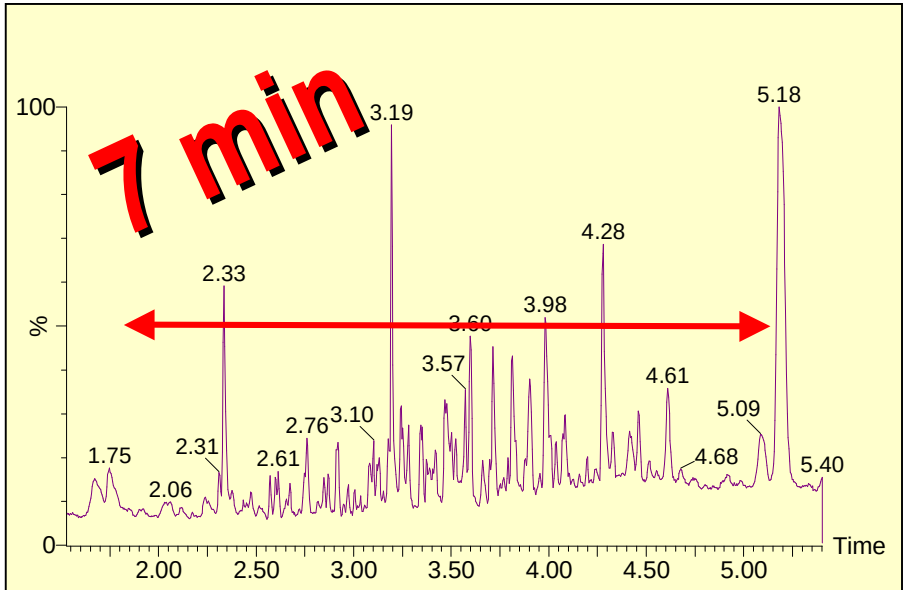


Conventional GC–MS (SIM)



Dichlorvos t_R = 17.6 min
Azoxystrobin t_R = 72.8 min

LP-GC-HR-TOF MS

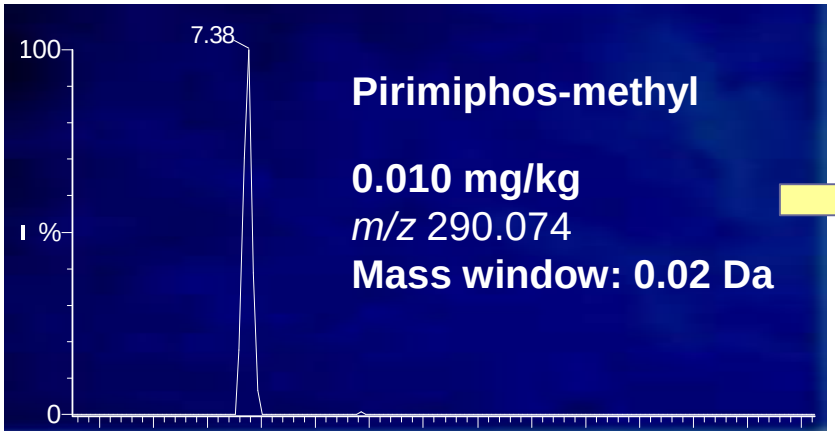


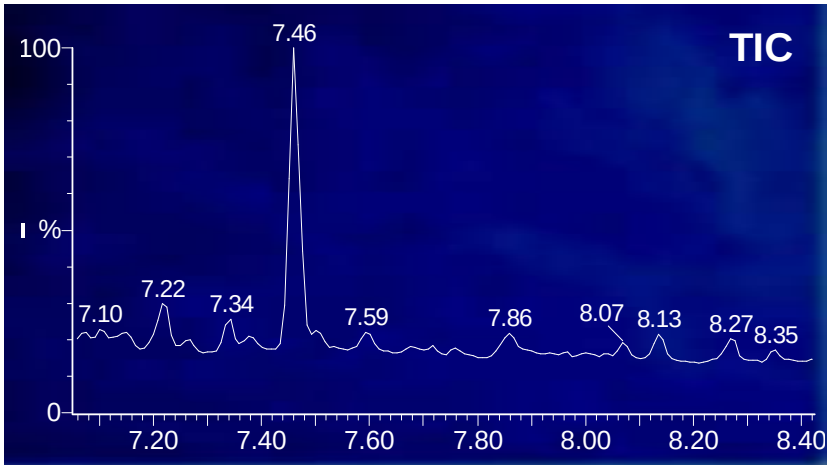
Dichlorvos t_R = 1.7 min
Azoxystrobin t_R = 5.1 min

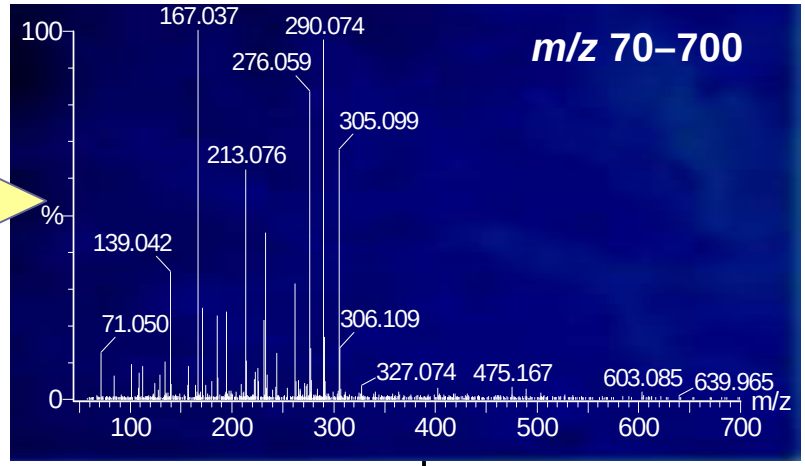
1. *Čajka T., Hajšlová J.: J. Chromatogr. A 1058, 251–261 (2004).*

Acquisition of full mass spectra

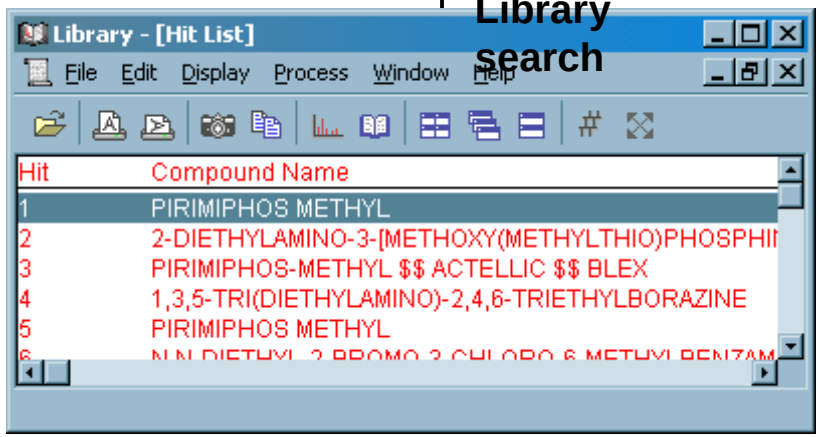
➡ **Full capabilities of library reference spectra search - identification / confirmation even at very low concentrations**





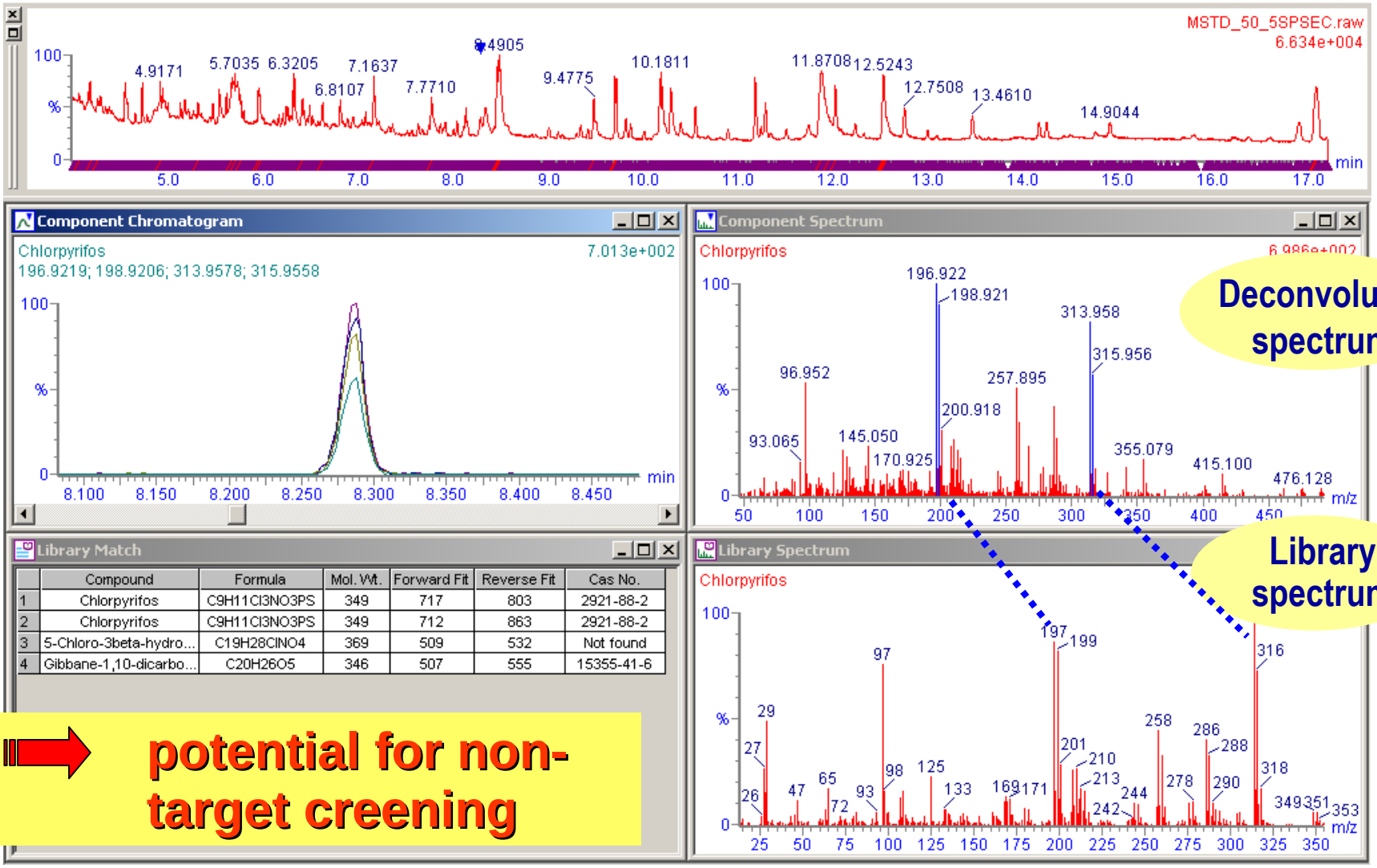


Library search

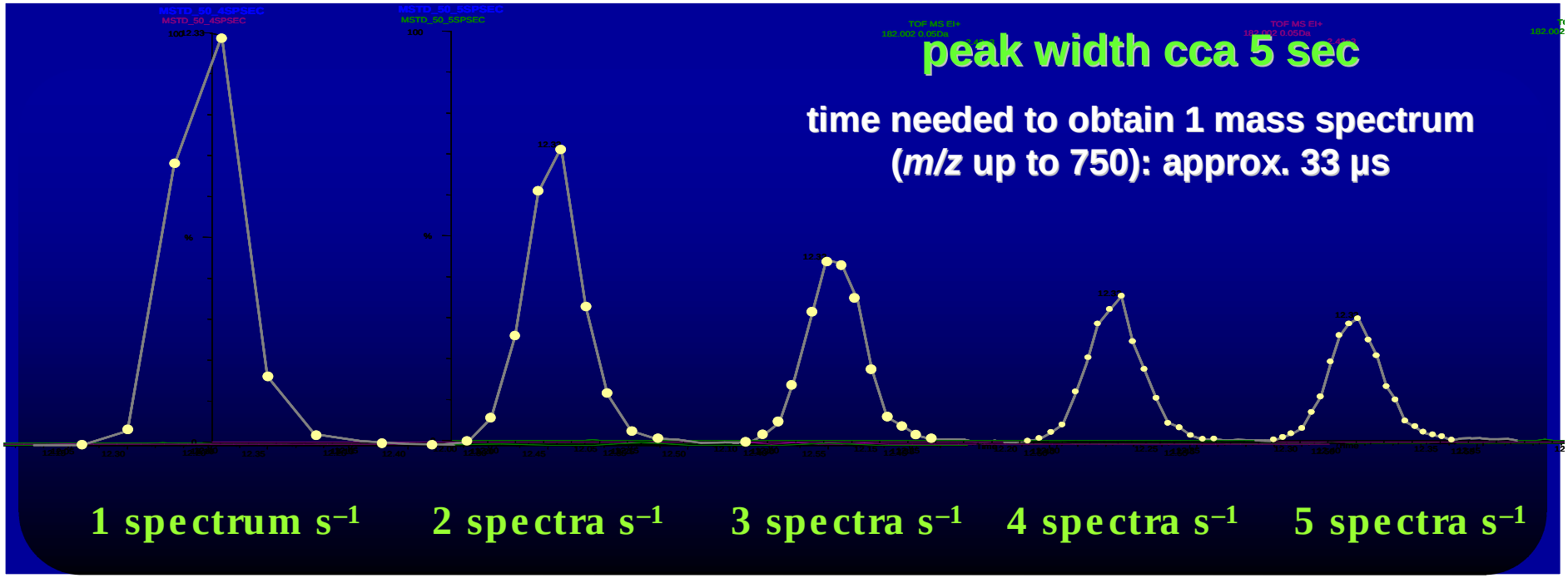


Exact mass deconvolution, library search

0.05 mg/kg CHLORPYRIFOS in peach, *acquisition rate 5 spectra/sec*



Changing acquisition speed



1 point = sum (average) of 33,000 primary spectra/s

Good repeatability of signal even at lowest speed i.e. 1 spectrum s⁻¹
(contrary to scanning instruments)

- ↑ acq. speed ⇒
- ↑ points/peak ⇒
- ↑ LOD

Pesticide residues in apples: LOQs (µg/kg)

endosulfan-SO4	1.1	o,p-DDD	2.8	endrin	5.3	methamidophos	9.6
penconazole	1.3	p,p-DDT	3.0	chlorpyrifos-methyl	5.4	mevinphos	9.8
vinclozolin	1.4	procymidone	3.1	cypermethrin I	5.4	acephate	10
chlorothalonil	1.7	cyprodinyl	3.5	cypermethrin II	5.4	fenthion	10
tetraconazole	2.0	ethion	3.5	cypermethrin III	5.4	folpet	11
triadimefon	2.0	endosulfan-beta	3.6	methidathion	5.5	cypermethrin IV	11
kresoxim-methyl	2.0	etrimfos	3.7	phosmet	5.5	ometoate	12
pyridaben	2.0	fenitrothion	4.0	triazophos	5.7	phosphamidone I	12
beta-cyfluthrin I	2.0	beta-cyfluthrin II	4.0	diazinon	5.8	phosphamidone II	12
tetradifon	2.1	endosulfan-alfa	4.1	azinfos-Me	5.8	carbaryl	12
tolylfluanid	2.2	chlorpyrifos	4.3	deltamethrin	5.9	captan	13
p,p-DDE	2.3	fenarimol	4.3	tebuconazol	6.1	iprodione	13
bifenthrin	2.3	dichlofluanid	4.4	azinfos-Et	6.1	chlorfenvinphos II	15
HCHγ (lindane)	2.4	dichlorvos	4.5	phosalone	6.4	hexythiazox	15
p,p-DDD	2.4	bromopropylate	4.5	malathion	7.0	imazalil	15
HCHα	2.5	pirimiphos-methyl	4.8	heptenophos	7.2	fenoxycarb	15
heptachlor	2.5	difenylamin	4.9	monocrotophos	7.2	propham	24
aldrin	2.5	parathion	5.0	trifloxystrobin	7.2	metalaxyl	30
o,p-DDT	2.6	triazamate	5.0	cyhalothrin-λ	7.4	chlorfenvinphos I	30
HCHβ	2.7	dieldrin	5.0	methacrifos	7.5	chlorpropham	32
HCHδ	2.7	permethrin I	5.0	dimethoate	7.8	difenoconazole-I	32
HCB	2.8	permethrin II	5.0	bupirimate	7.8	difenoconazole-II	32
quinalphos	2.8	parathion-methyl	5.2	pirimicarb	9.1	fenvalerate I	48
o,p-DDE	2.8	tolclofos-methyl	5.2	thiabendazole	9.3	fenvalerate II	48

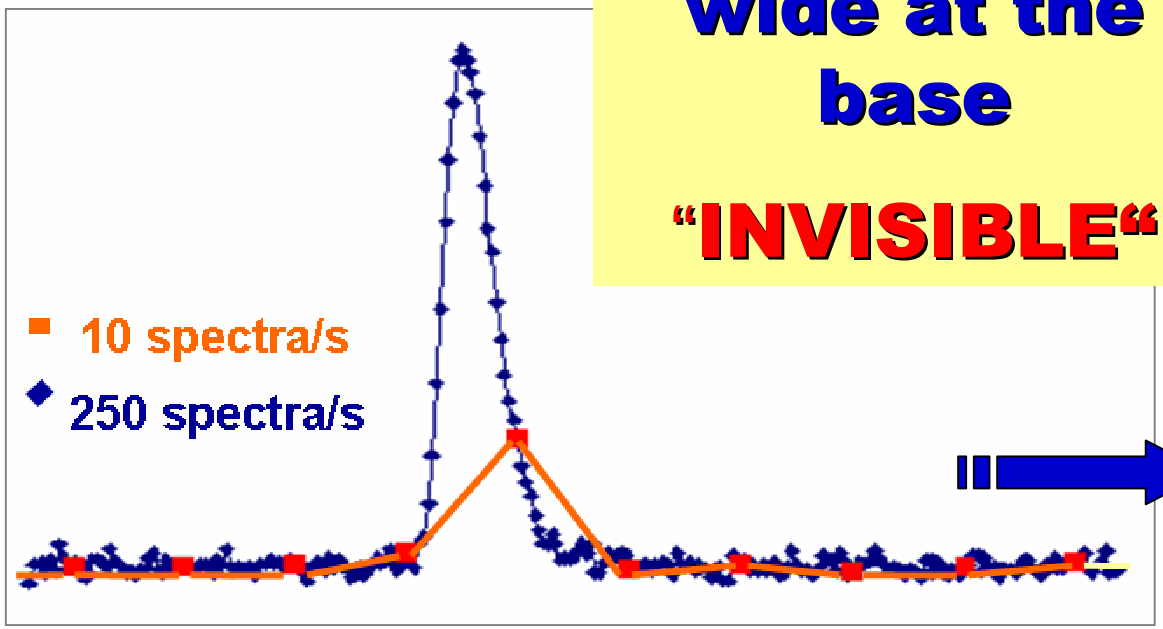
LOQs expressed as Lowest Calibration Levels (LCL), RSD 8 – 12%

Limitations of hr-TOF MS

- Detection / identification / quantification of very narrow peaks difficult – not enough data points per peak

Peak 150 ms wide at the base

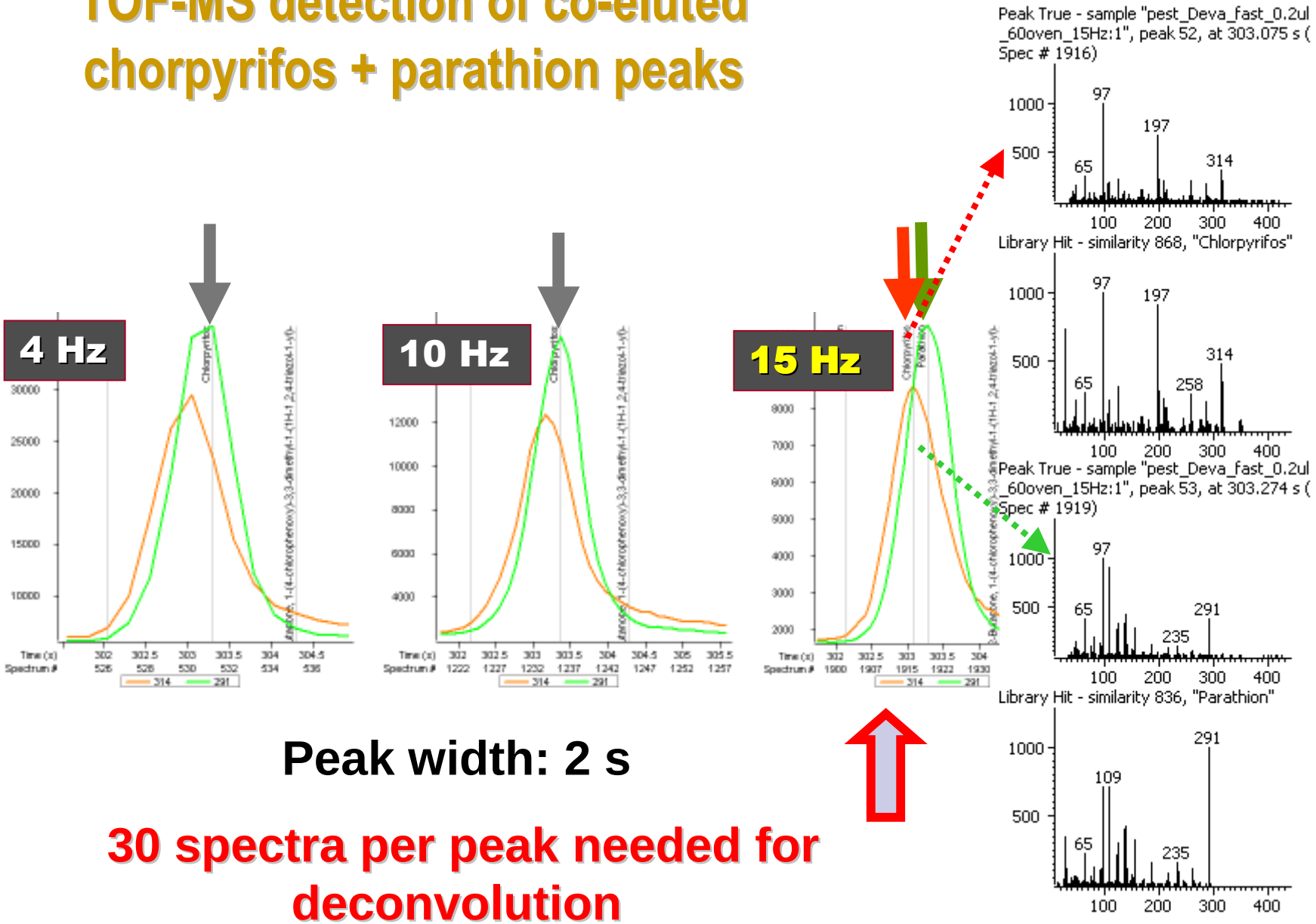
"INVISIBLE"



Fast TOF

Example:

TOF-MS detection of co-eluted chorpyrifos + parathion peaks



Peak width: 2 s

30 spectra per peak needed for deconvolution

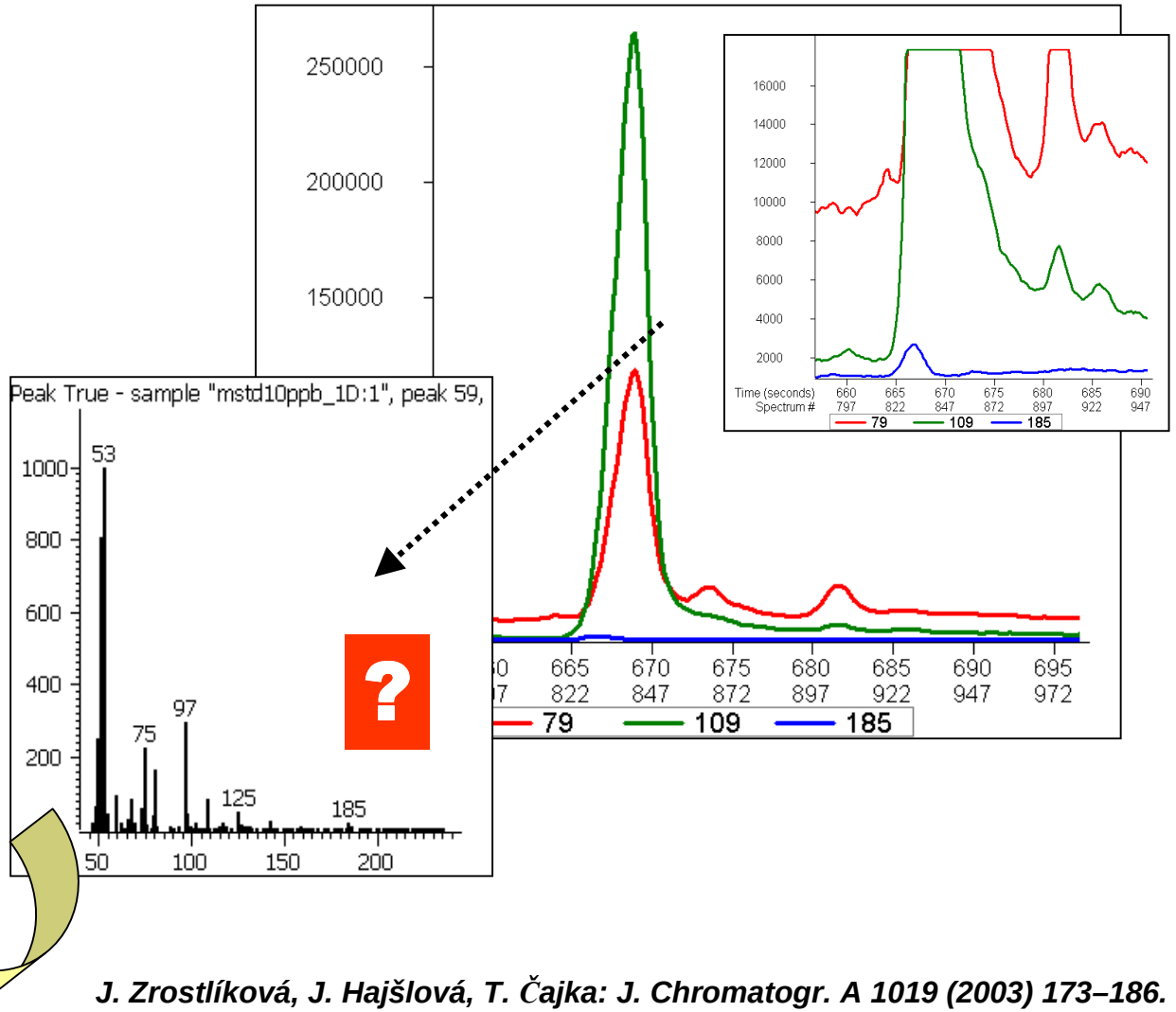
Example:
Separation of target analyte (dichlorvos. 0.01 mg/kg) from co-extract

1D-GC

INTERFERENCE:

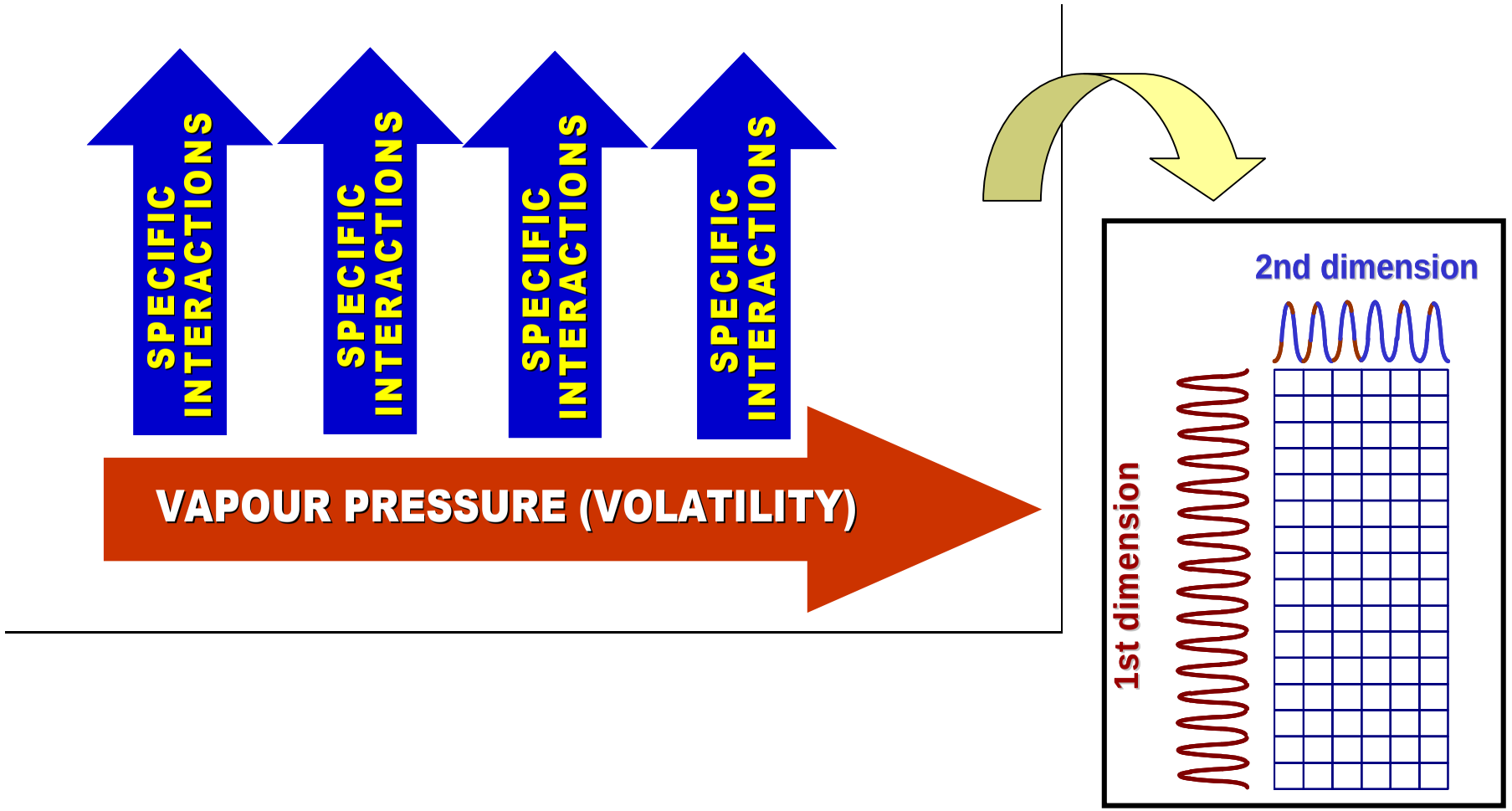
- ***m/z* 109** (quantification)
- ***m/z* 79** (identification)

no spectral match with NIST library



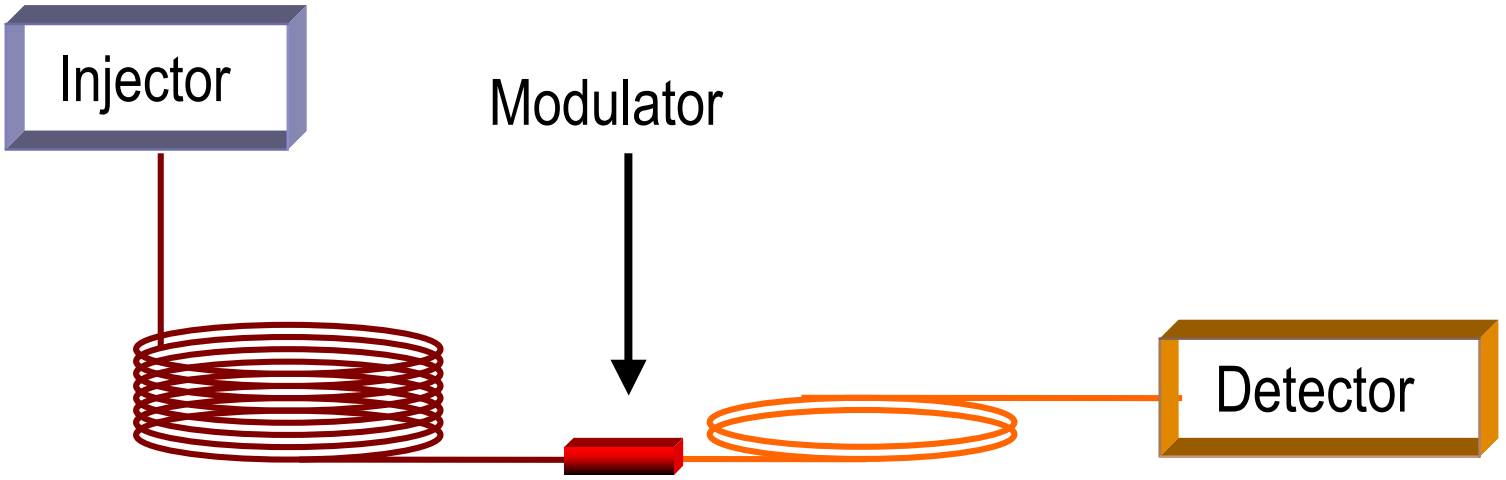
J. Zrostlíková, J. Hajšlová, T. Čajka: J. Chromatogr. A 1019 (2003) 173–186.

COMPREHENSIVE CHROMATOGRAPHY:
dramatic increase of chromatographic resolution



Orthogonal GC (GC×GC) set-up

➔ two columns with differing selectivity connected via modulator



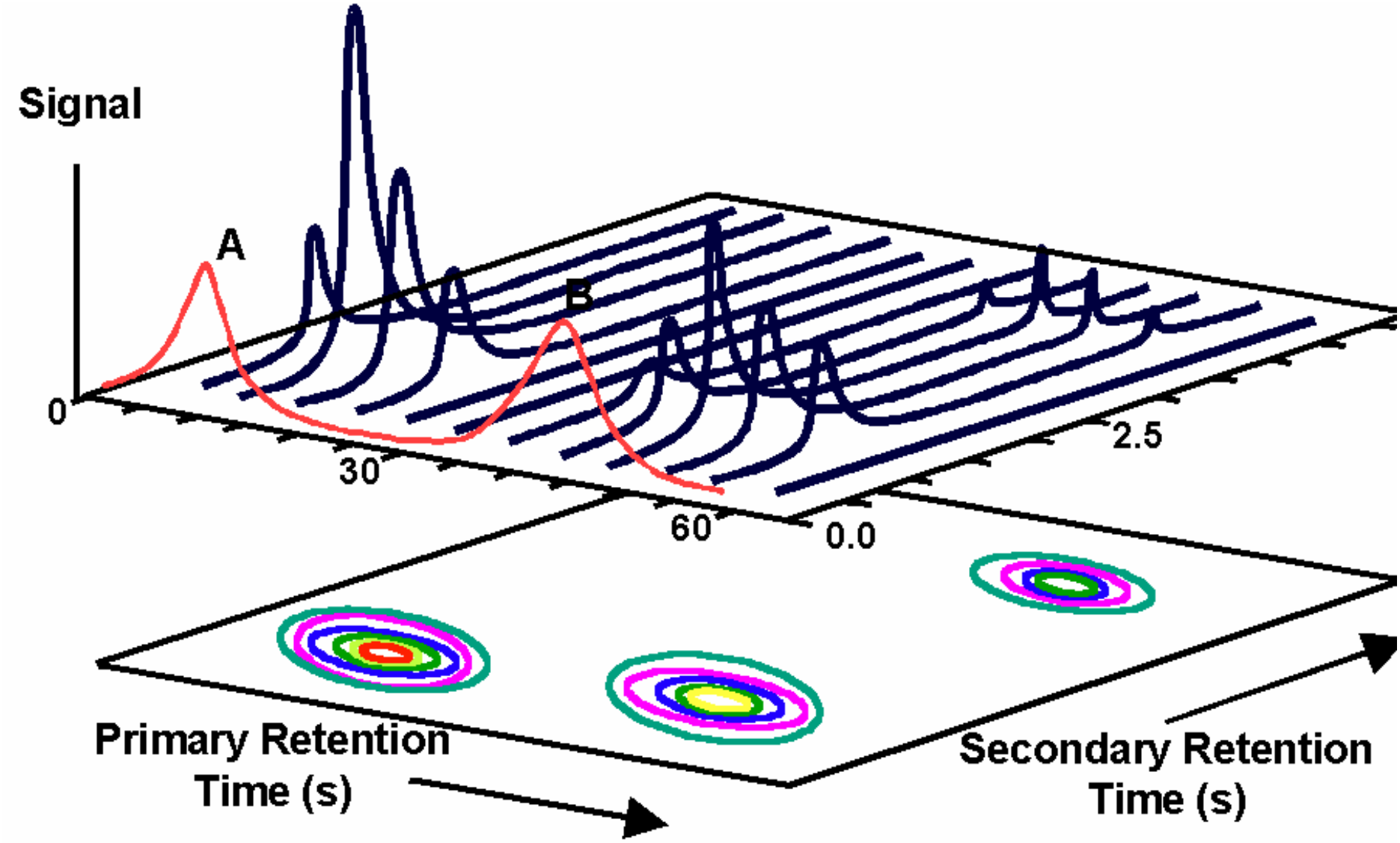
1st dimension

Typicaly narrow bore non polar column (30 m × 0,25 mm I.D. × 0,25 µm film)

2nd dimension

Typically microbore polar column (1 m × 0,1 mm I.D. × 0,1 µm film)

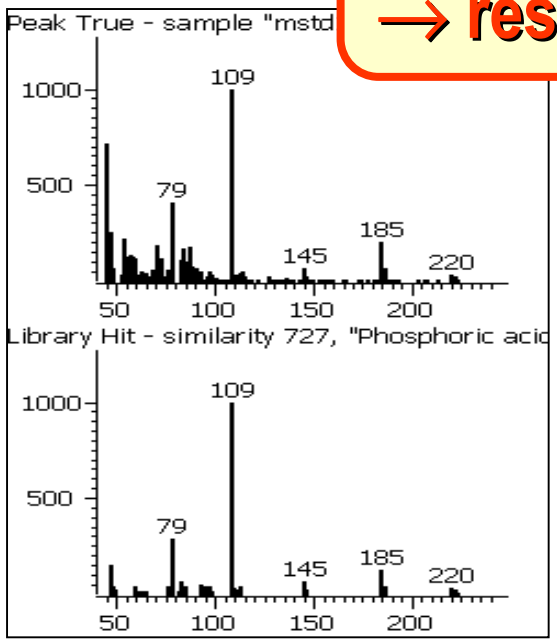
Two - dimensional chromatogram



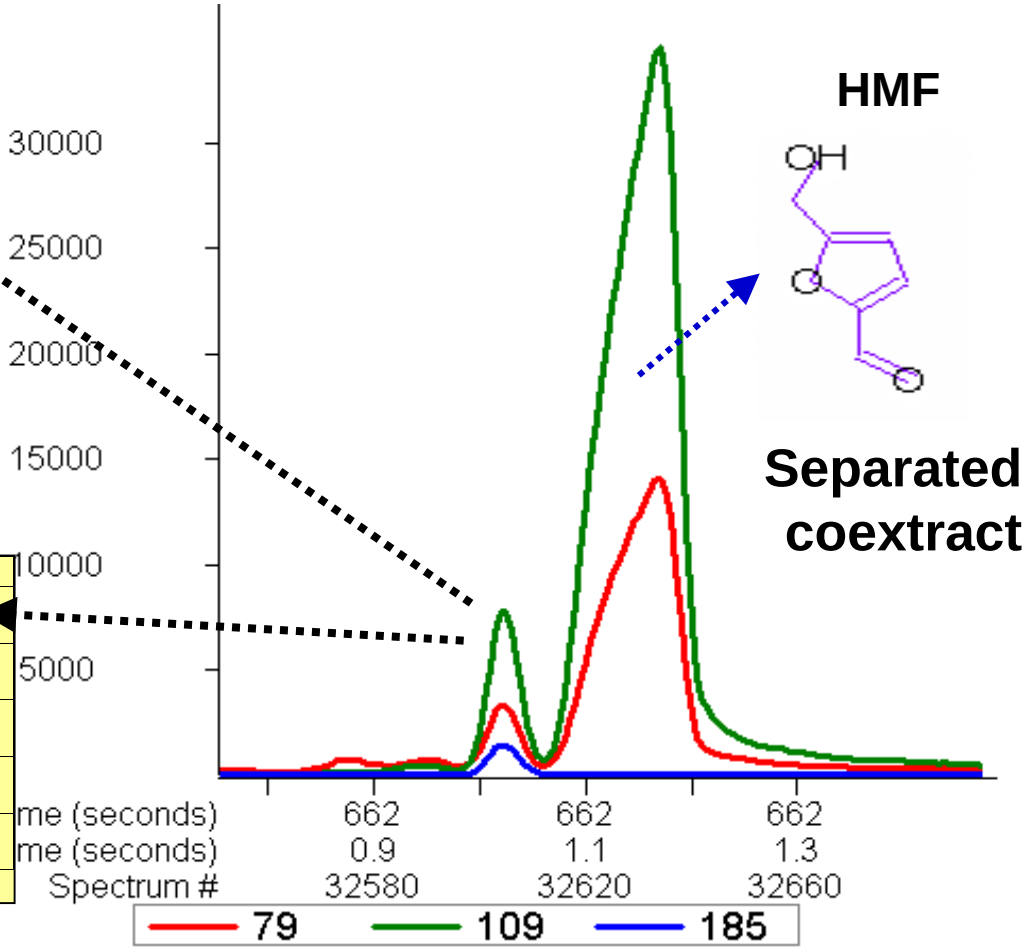
Example:
Separation of target analyte (dichlorvos. 0.01 mg/kg) from co-extract

GC×GC

Dichlorvos reliably identified and quantified
→ result of increased GC peak capacity



Hit	Name	Reverse	CAS
1	**Phosphoric acid, 2,2-dichlorovinyl dimethyl ester**	**940**	62-73-7
2	Phosphoric acid, 2,2-dichlorovinyl dimethyl ester	729	62-73-7
3	Phosphoric acid, 2,2-dichlorovinyl dimethyl ester	707	62-73-7
4	Phosphoric acid, 2,2-dichlorovinyl dimethyl ester	704	62-73-7
5	Phosphoric acid, 2,2-dichlorovinyl dimethyl ester	700	62-73-7

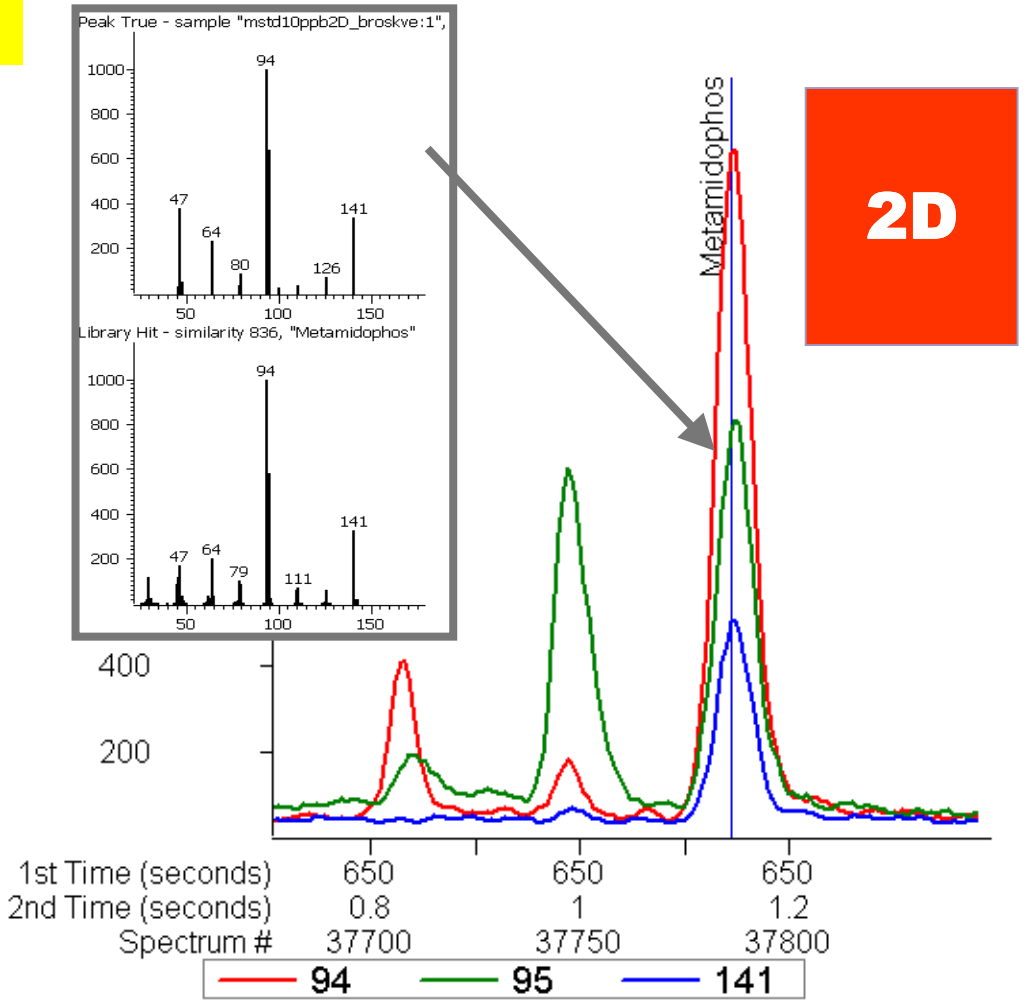


Difficult pesticides

METHAMIDOPHOS
in GPC purified peach extract (10 pg injected)

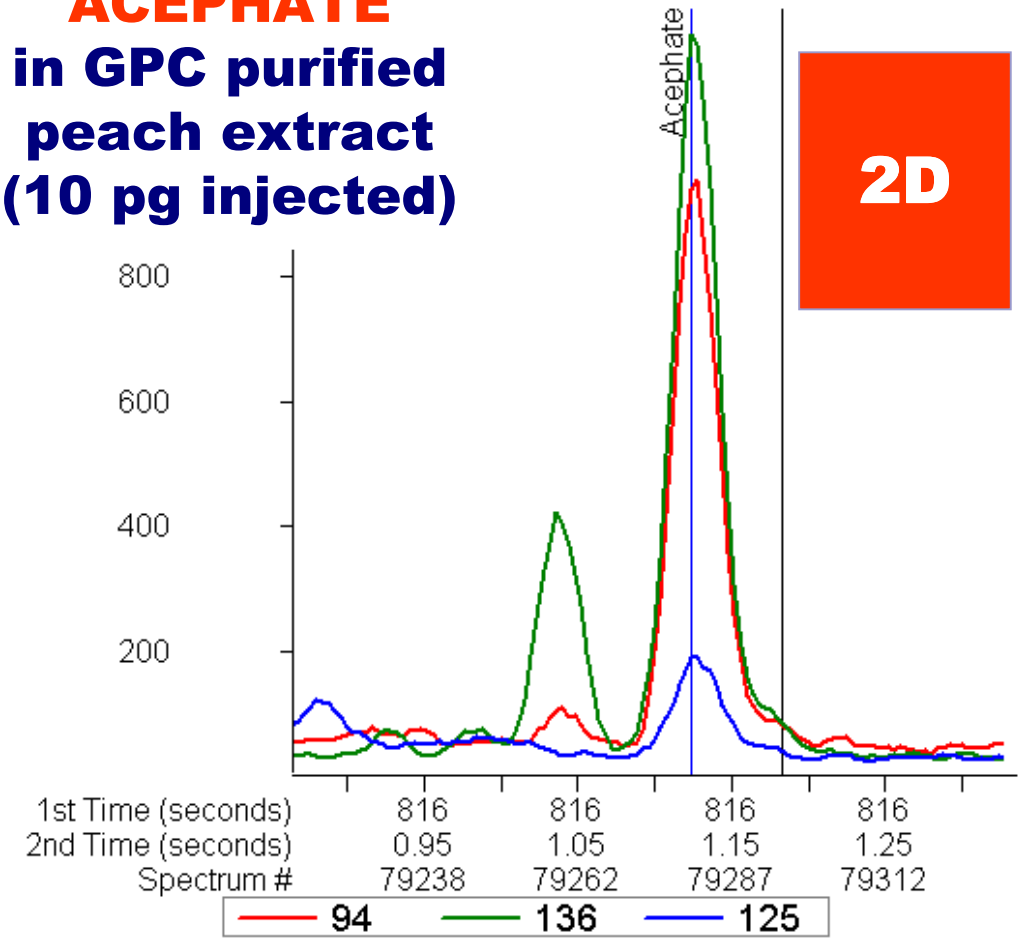
1D:
spectral match 611 (only)

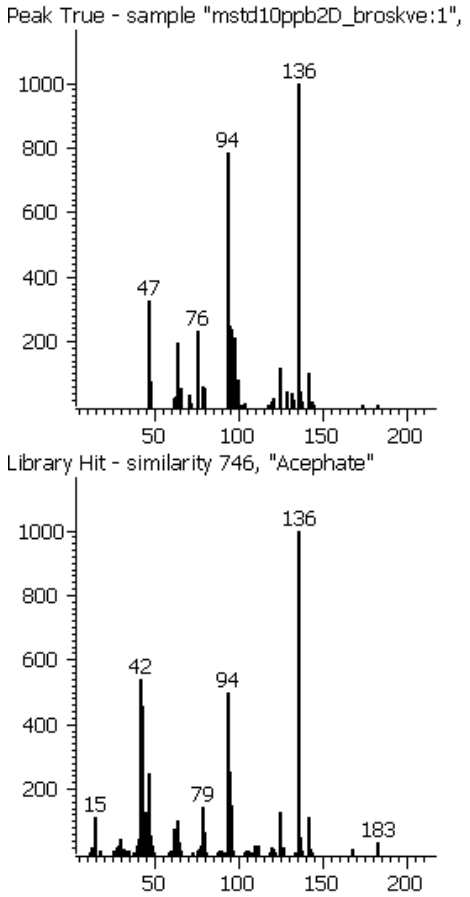
2D:
very good spectral match



Hit	Name	Reverse	Similarity	CAS
1	**Metamidophos**	**841**	**836**	10265-92-6
2	Phosphoramidothioic acid, O,S-dimethyl ester (mainlib)	829	823	0-00-0
3	Phosphoramidothioic acid, O,S-dimethyl ester	829	823	10265-92-6
4	1H-Pyrrole, 2,3-dimethyl-	818	656	600-28-2

ACEPHATE
in GPC purified peach extract (10 pg injected)



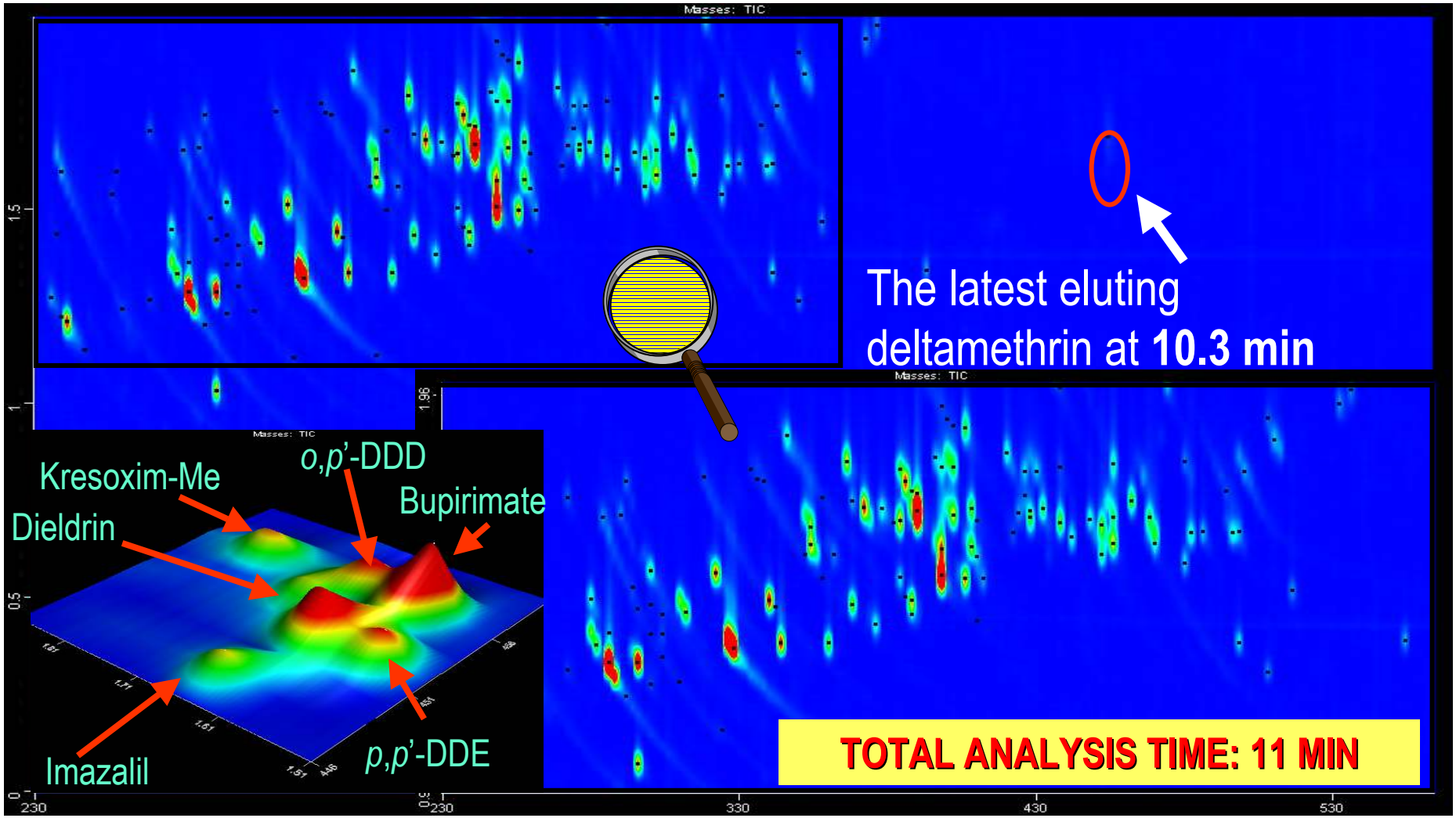


Hit	Name	Reverse	Similarity	CAS
1	**Acephate**	810	746	30560-19-1
2	Acephate	821	738	30560-19-1
3	Acephate (mainlib)	804	734	0-00-0
4	Acephate	804	734	30560-19-1
5	Disulfide, ethyl 1-methylethyl	697	573	53966-36-2

RESULTS:

Analysis of 91 pesticides by high speed GCxGC/TOF-MS

LODs < 0.01 mg/kg, RSD 5 – 24% at 0.02 mg/kg level)



LODs (ug/kg)
in 2D →lower
by factor
2 - 50

PROBLEM:

late eluting broad peaks of pyrethroids

→6 - 7 segments /peak

→ higher LODs

pesticide	m/z	1D			2D		
		noise	S/N	LOD	noise	S/N	LOD
methamidophos	94	400	43.7	6	60	28	2
dichlorvos	109	3000	24	10	50	261	0.2
acephate	136	400	25	10	40	30	2
dimethoate	125	1000	62	4	60	40	1.3
lindane	181	1000	16.4	15	30	66	0.8
carbaryl	144	1200	37.2	6.7	50	78	0.6
methiocarb	168	600	35.2	7.1	40	34	1.5
heptachlor	272	210	29.5	9	20	45	1.5
pirimiphos-Me	290	300	24.8	10	40	22.5	2.2
chlorpyrifos	197	200	47	5.3	40	30	1.7
procymidone	96	3000	17	15	80	71.25	0.7
thiabendazole	202	300	43.3	2.3			
captan	149	600	6	30	40	7.5	7
endosulfan I	241	200	13.5	18	30	10.6	5
endosulfan II	241	200	10.2	24	30	5	10
endosulfan-SO_4	272	150	19	13	30	6	7.5
phosalone	182	400	10.7	23	40	8	6.3
propargite	173	250	13	19	30	7.3	7
permethrin I*	183	400	6.7	37	30	8.3	30
permethrin II*	183	400	10.7	23	30	25	10
deltamethrin**	181	300	3.5	150	20	5	100

** estimated from the analysis of peach matrix-matched standard at 50 mg/ml for both techniques*
*** estimated from the analysis of peach matrix-matched standard at 100 mg/ml for both techniques*

GCxGC/HS-TOF-MS:
A powerful tool for non-target analysis

Example

- Tomato sample from the EU Proficiency Test containing a wide range of pesticide residues
- The aim - testing the ability to detect pesticides in complex matrix

No information about target analytes provided in advance

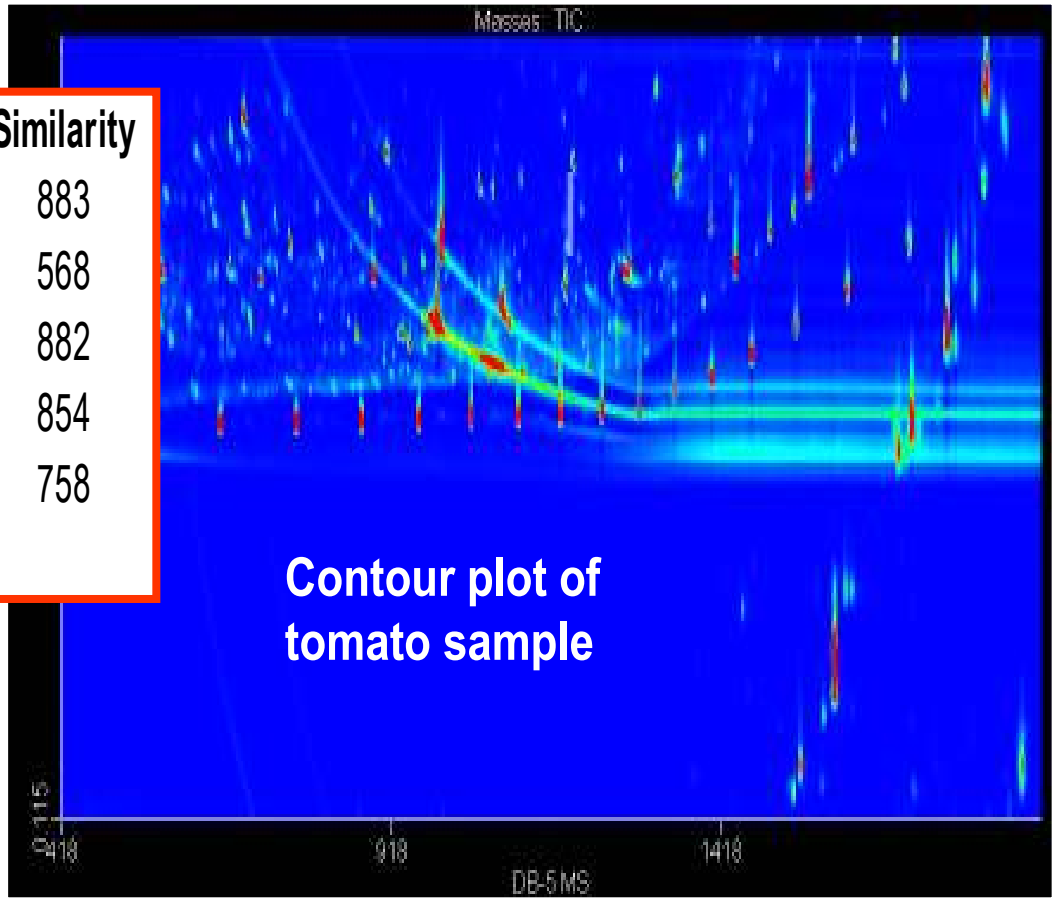
RESULTS OF NON-TARGET SCREENING

IDENTIFIED PESTICIDES (NIST library) – deconvoluted:

Pesticide	Similarity	Pesticide	Similarity
Dimethoate	895	Endosulphan I	883
Diazinon	929	Imazalil	568
Chlorothalonil	855	Endosulphan II	882
Metalaxyl	837	Bromopropylate	854
Procymidone	808	Azoxystrobin	758
Thiabendazole	834		

Oxydemethon-Me
imidacloprid
Acrinatrin

These pesticides were not present in the used library version nor in standard



WHAT ARE THE CHALLENGES OFFERED BY TOF MS?

hsTOF

- Unit resolution
- High sensitivity
- Very fast data acquisition

hrTOF

- High mass accuracy
- Very high sensitivity
- Fast data acquisition

▶ **Full spectral information available even at low levels**

- UNKNOWNS IDENTIFICATION / ON-LINE CONFIRMATION

- RELIABLE, UNBIASED QUANTIFICATION

Hajšlová J., Čajka T.: In: Food Toxicant Analysis, Y. Picó (editor), Elsevier, Oxford, UK (*in press*), ISBN: 0-444-52843-1

Main requirements and expectations in analysis of pesticide residues

Requirements

- **Low limits of detection**
- **GOOD ACCURACY**
- **High degree of confirmation**
- **Robustness**

QUALITY CONTROL PROCEDURES FOR PESTICIDE RESIDUES ANALYSIS

Document N° SANCO/10232/2006

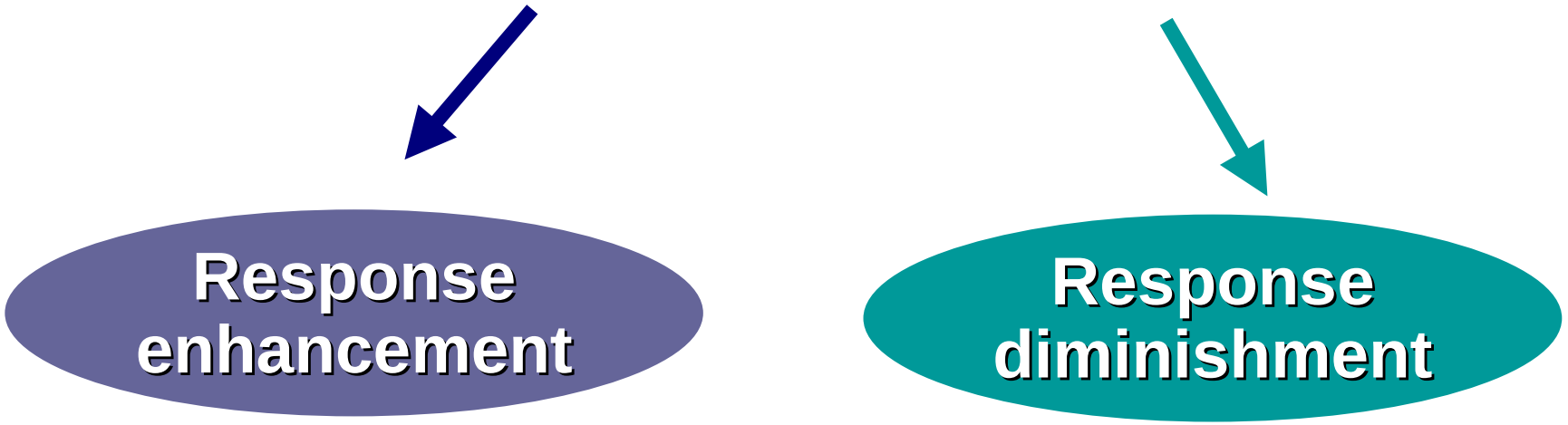
24/March/2006

ADDITIONAL FEATURES REQUIRED

- Short analysis time
- Broad MRM scope
- Affordable cost

MATRIX EFFECTS:
trueness issue both in LC and GC

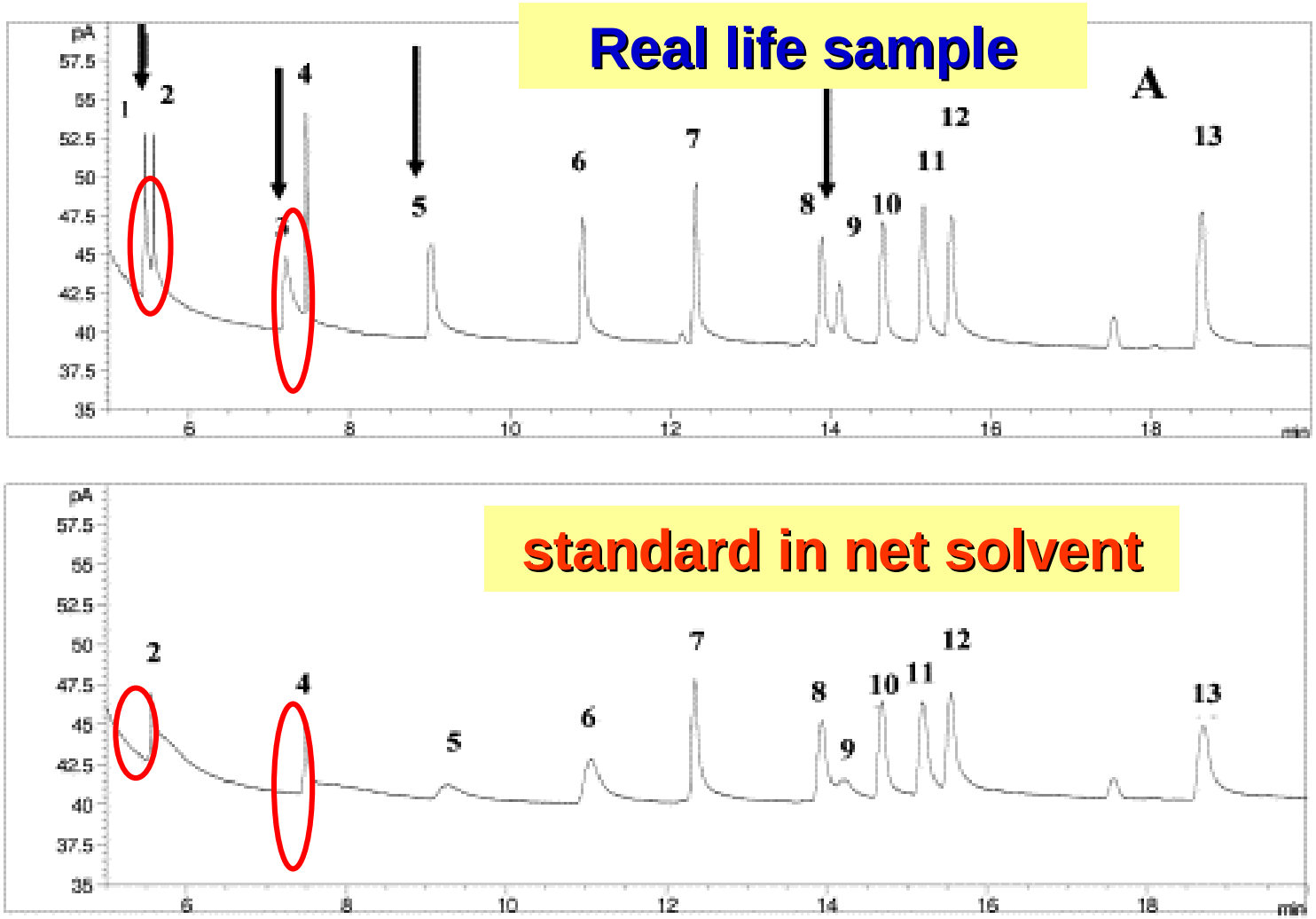
Change of a chromatographic **peak intensity** and **shape** of affected compounds when they are injected in the presence of a complex matrix

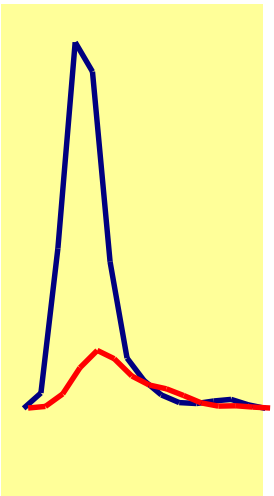


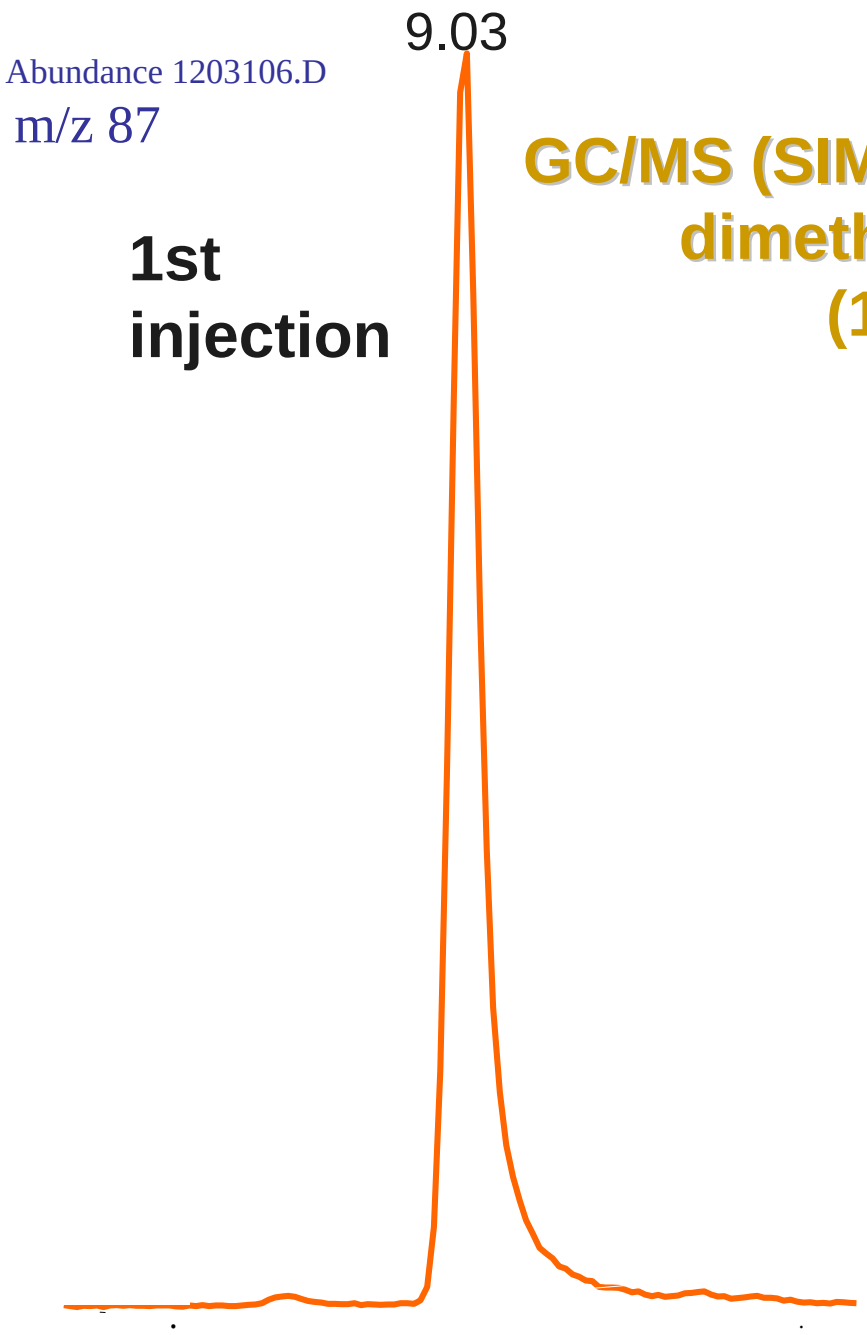
J. Hajšlová and J. Zrostlíková, J. Chromatogr. A 1000 (2003) 181

Example:

GC/MS analysis (splitless injjection) of pesticide residues (0.02 mg/kg) in apple extract (A) and net solvent (B)





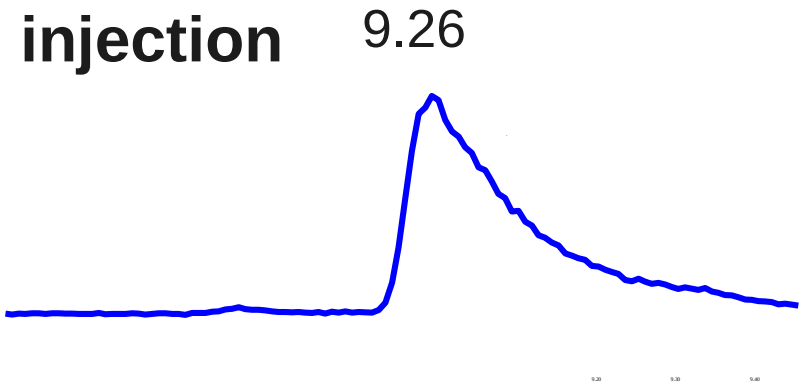


1st injection

Example:

GC/MS (SIM, m/z 87) splitless injection 5 pg dimethoate in crude plant extract (1.5 µL, 2.5g matrix /ml)

50th injection



Elimination *vs.* compensation for matrix effects

➔ **ELIMINATION**

- No active sites in GC system
- No matrix compounds

Impossible in practice

➔ **COMPENSATION**

- Standard addition
- **Matrix-matched standards**
- Isotopically labeled internal standards

Impractical for routine

- **Analyte protectants**

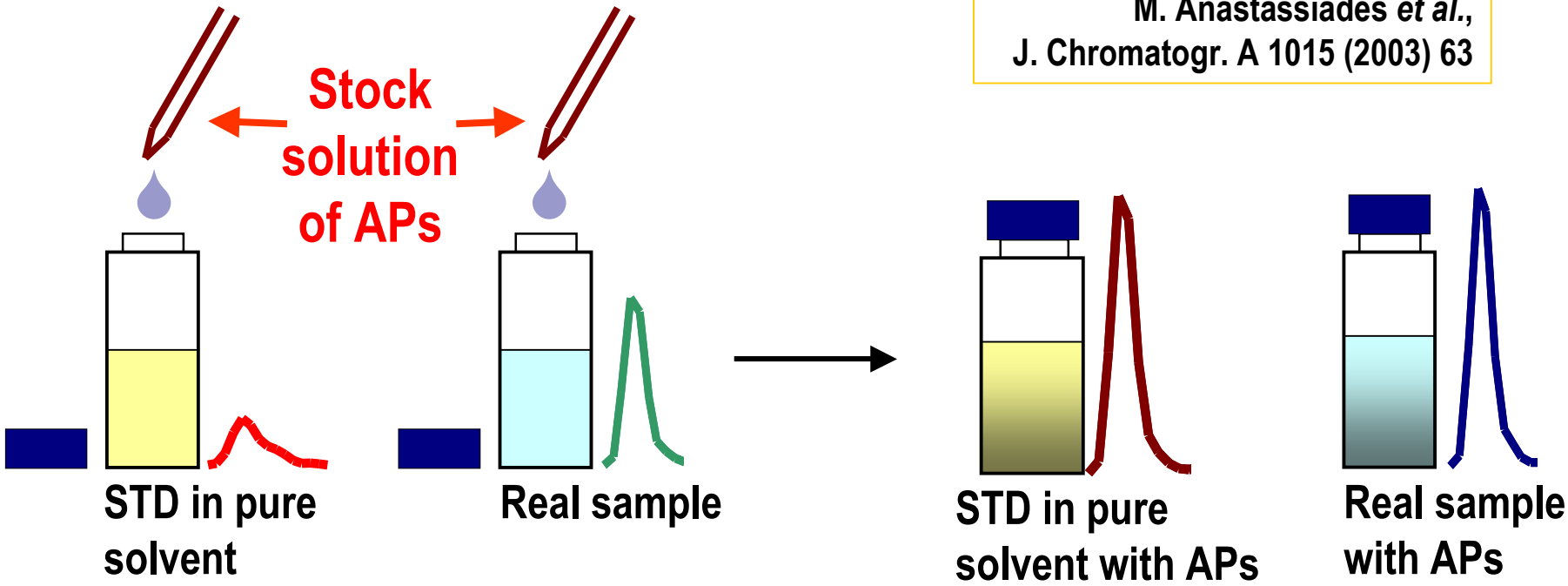
CHALLENGE!

ANALYTE PROTECTANTS

GENERAL CONCEPT: Replacement of laborious matrix-matched standards by **addition of compounds simulating (mimicking) matrix to standard in pure solvent**

Compounds strongly interacting with active sites in the GC system

M. Anastassiades *et al.*, J. Chromatogr. A 1015 (2003) 63



USE OF ANALYTE PROTECTANS

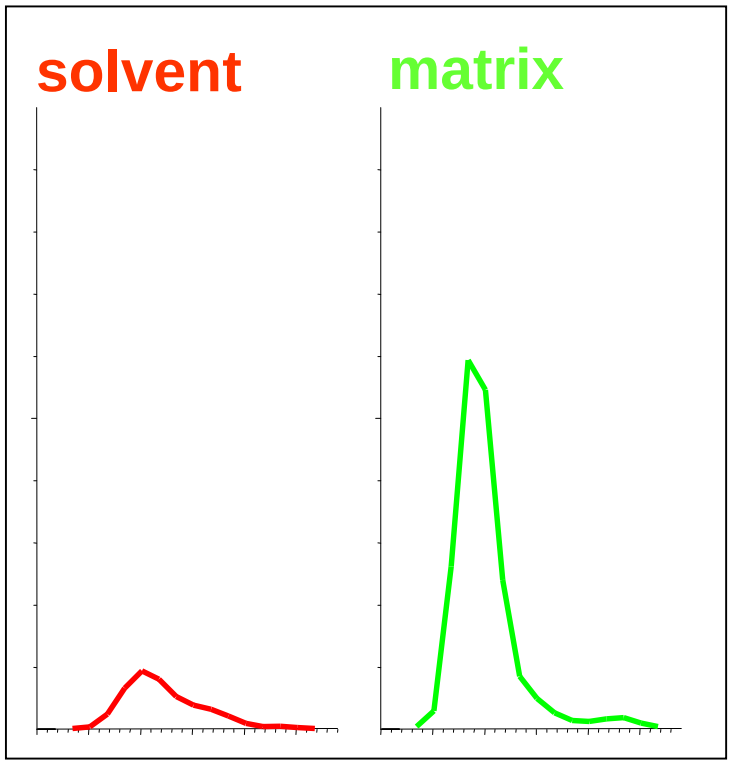
→ obtaining more accurate data

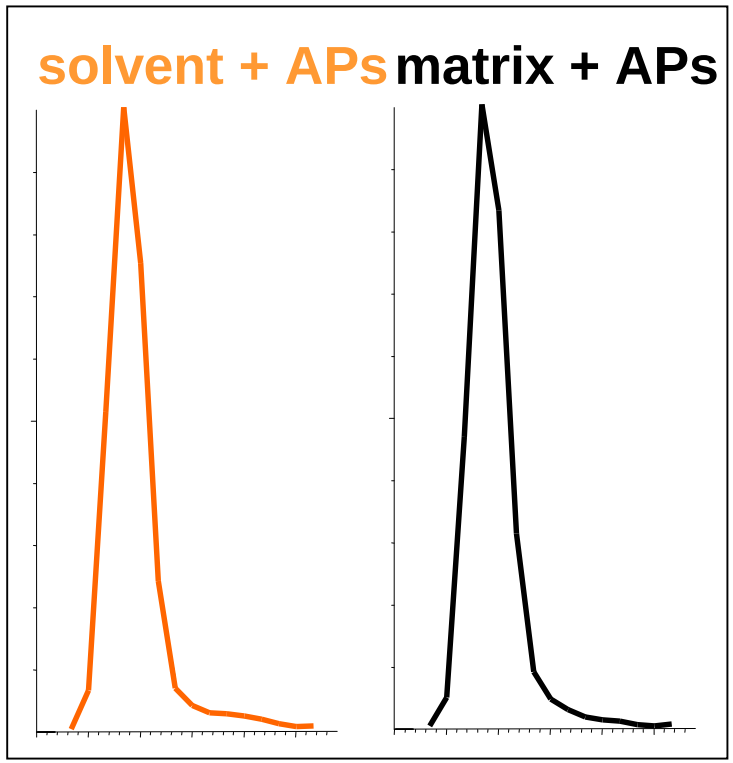
PHOSALON

0.005 mg/kg, apple

~~**0.022 mg/kg**~~

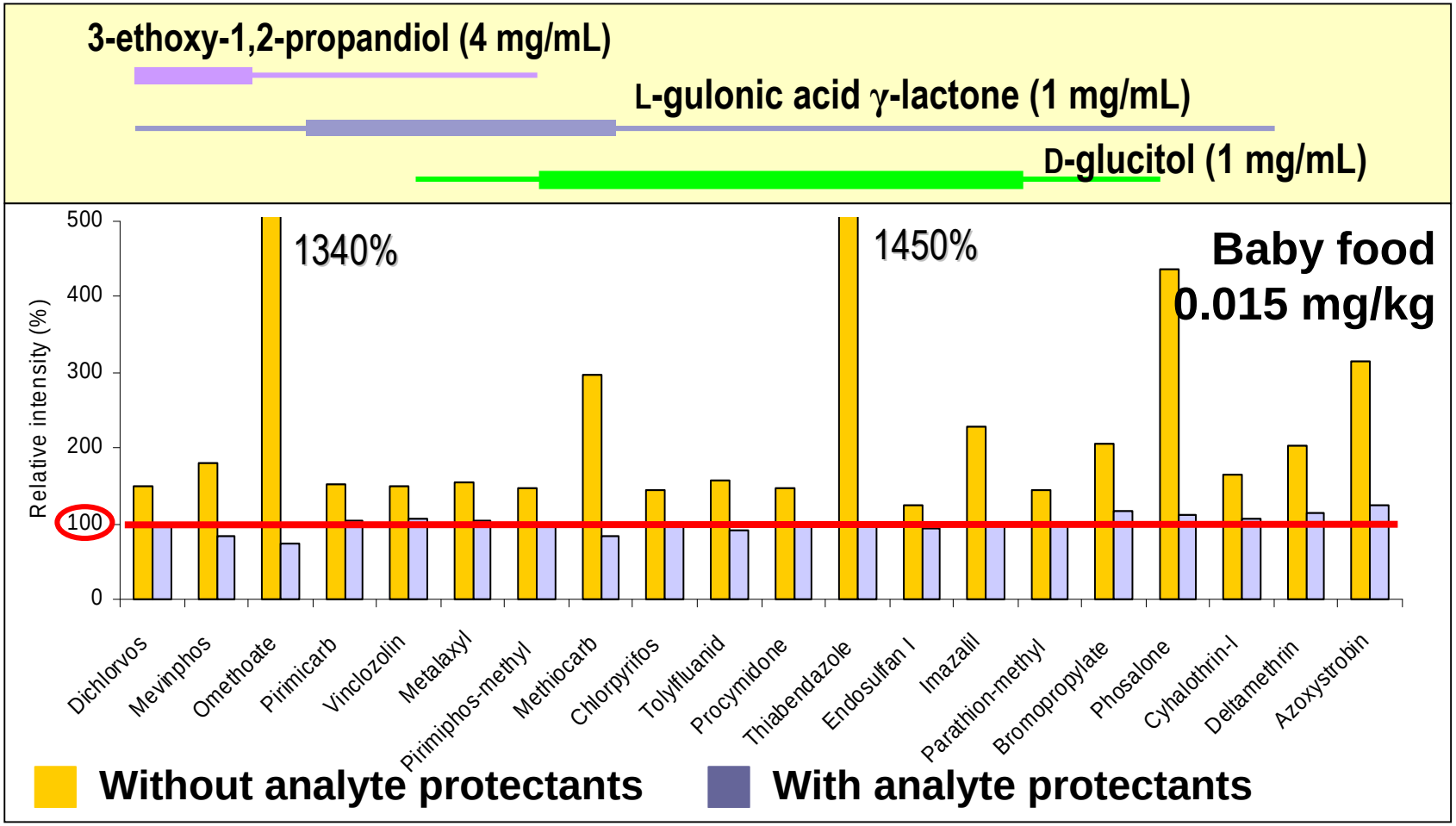
0.0045 mg/kg





Comparison of Generated Data

Accurate data over a broad analyte spectrum !



Enjoy moving ahead…..