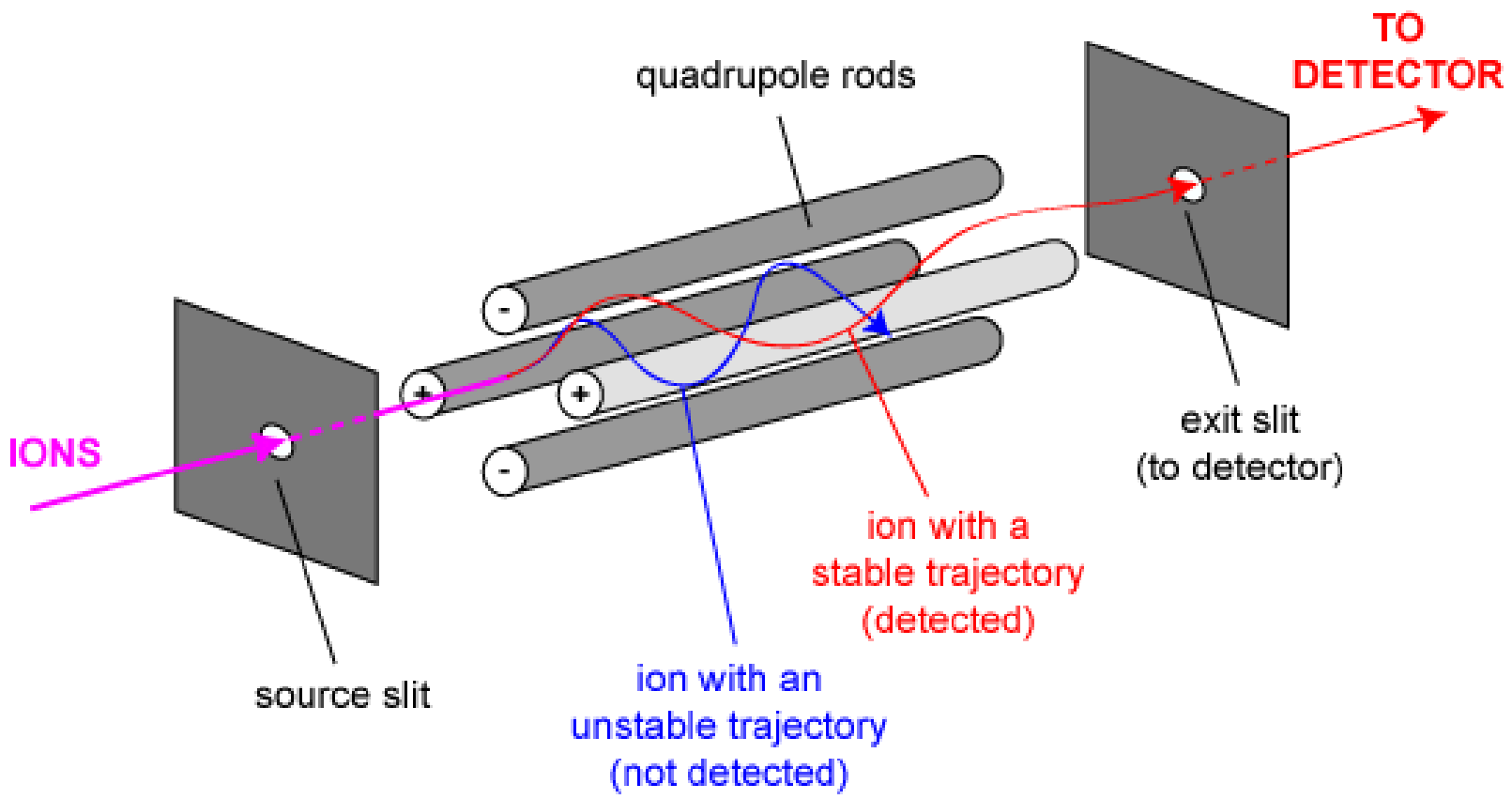
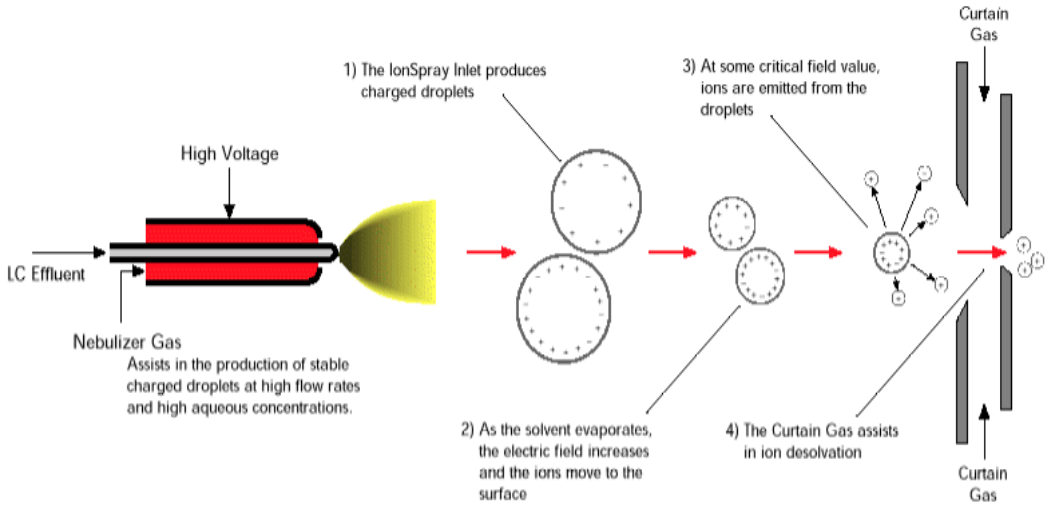
QUADRUPOLE MASS ANALYZER

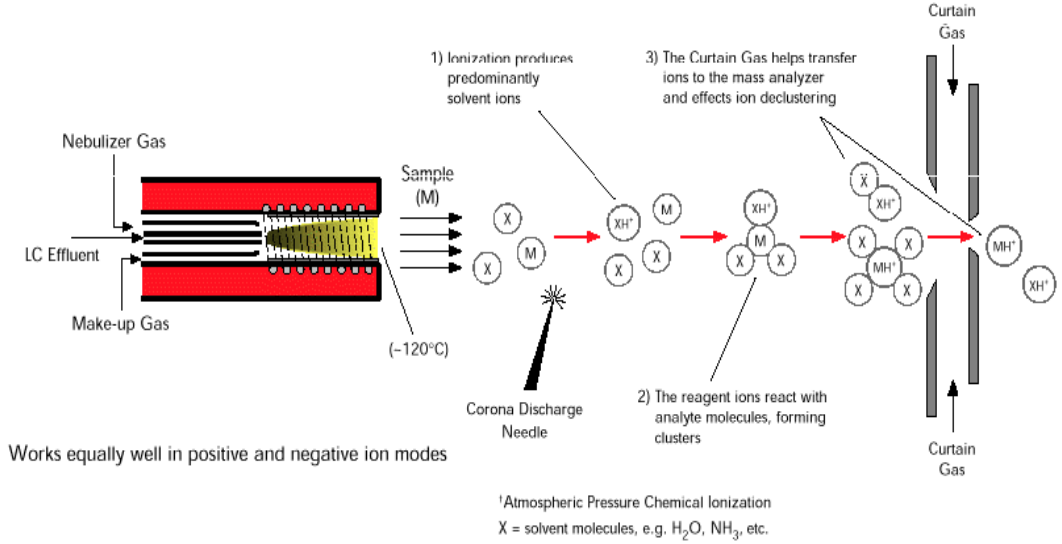
- MS measures how the trajectories of the ions respond in vacuum to various combinations of electric and magnetic fields.



Ionisation sources in LC-MS **Electron spray ionisation** (very soft) and **Atmospheric pressure chemical ionisation** (soft)

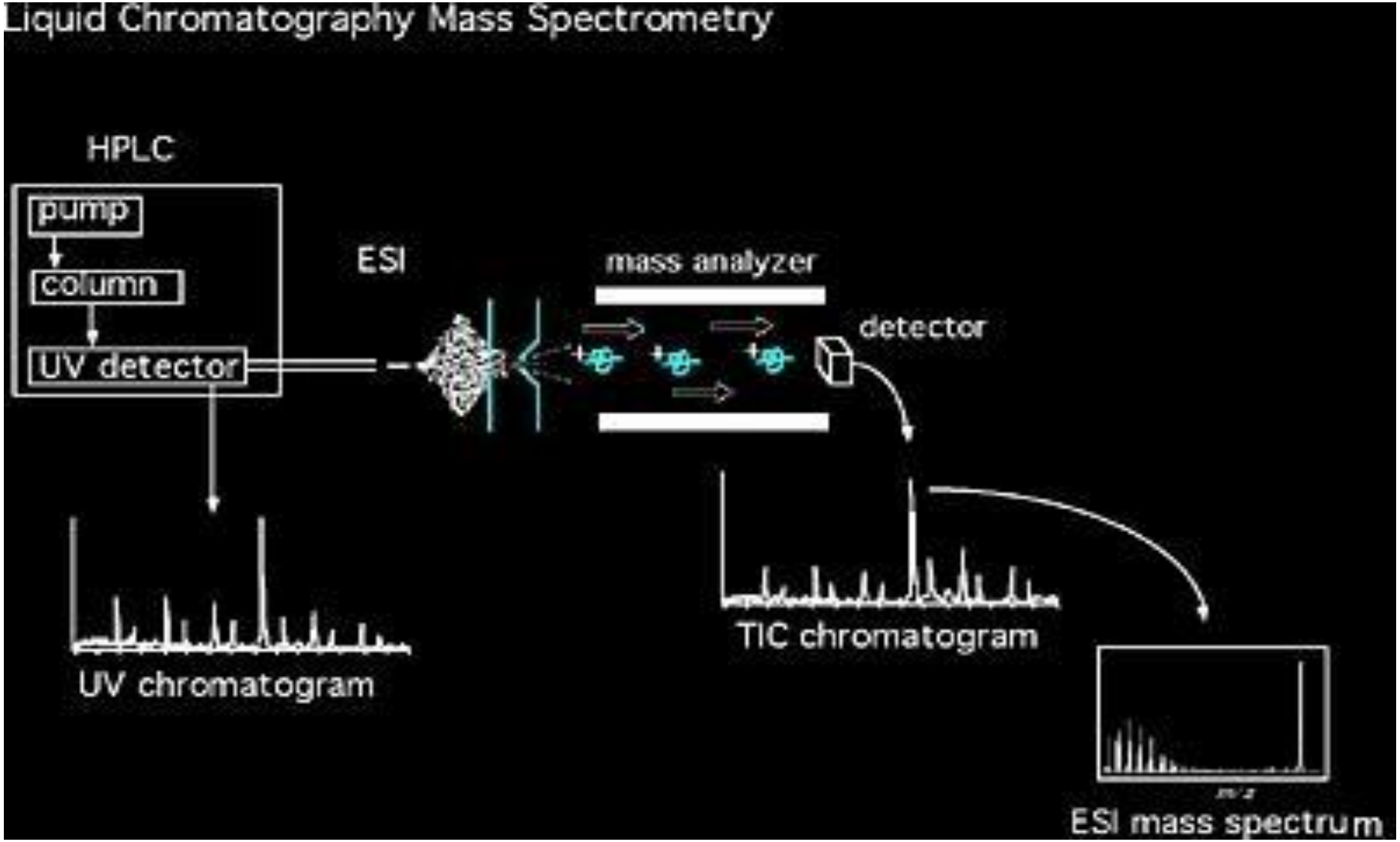


APCI

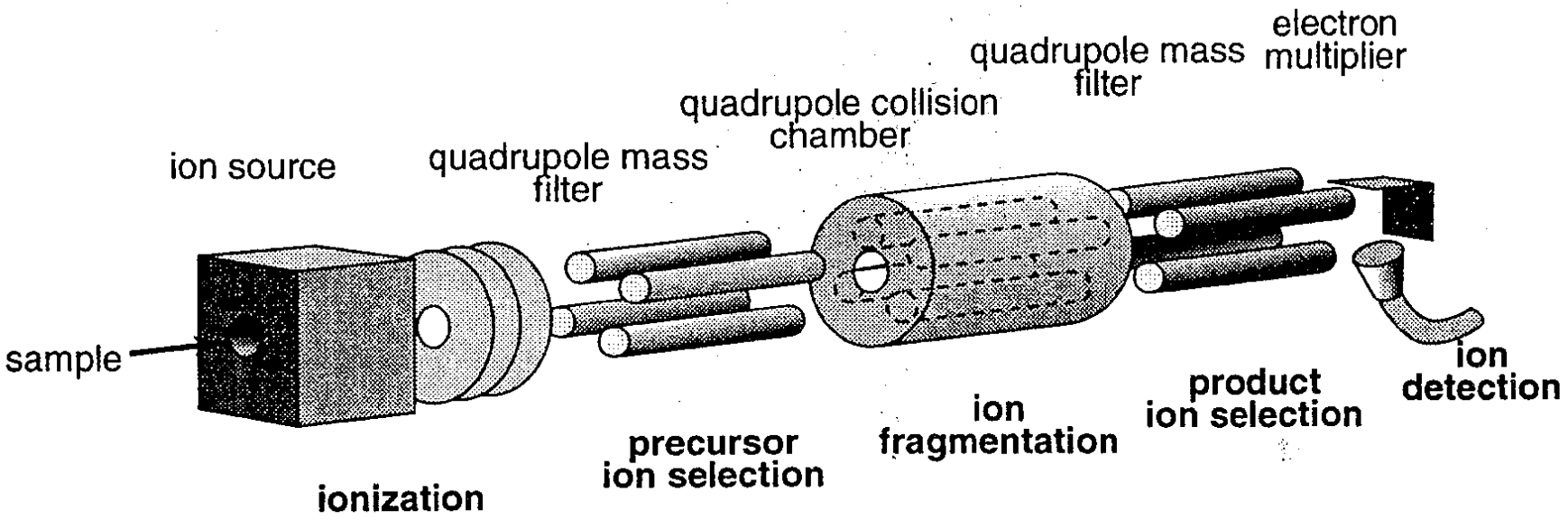


HPLC-MS vs HPLC-UV

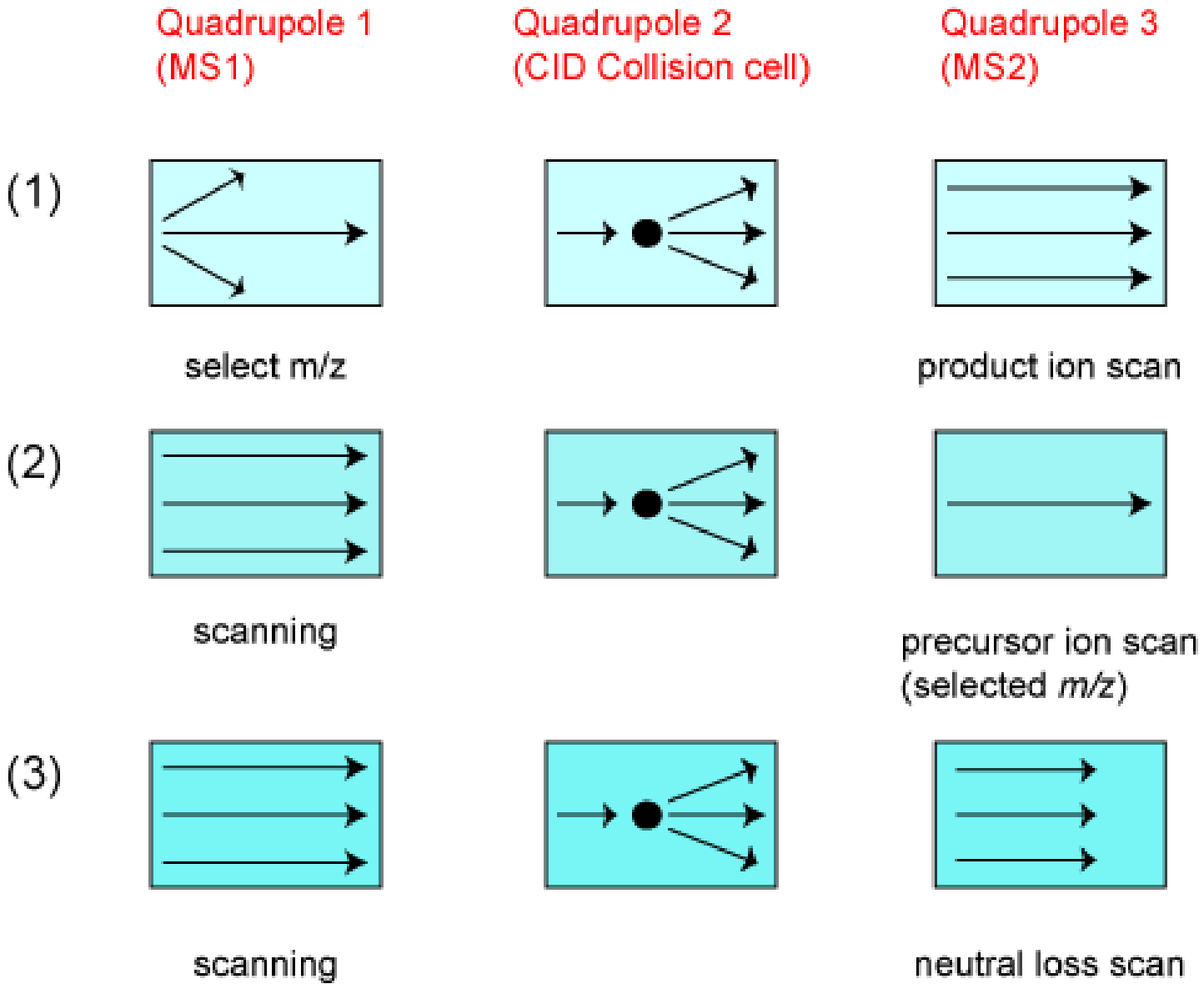
TIC = total ion current



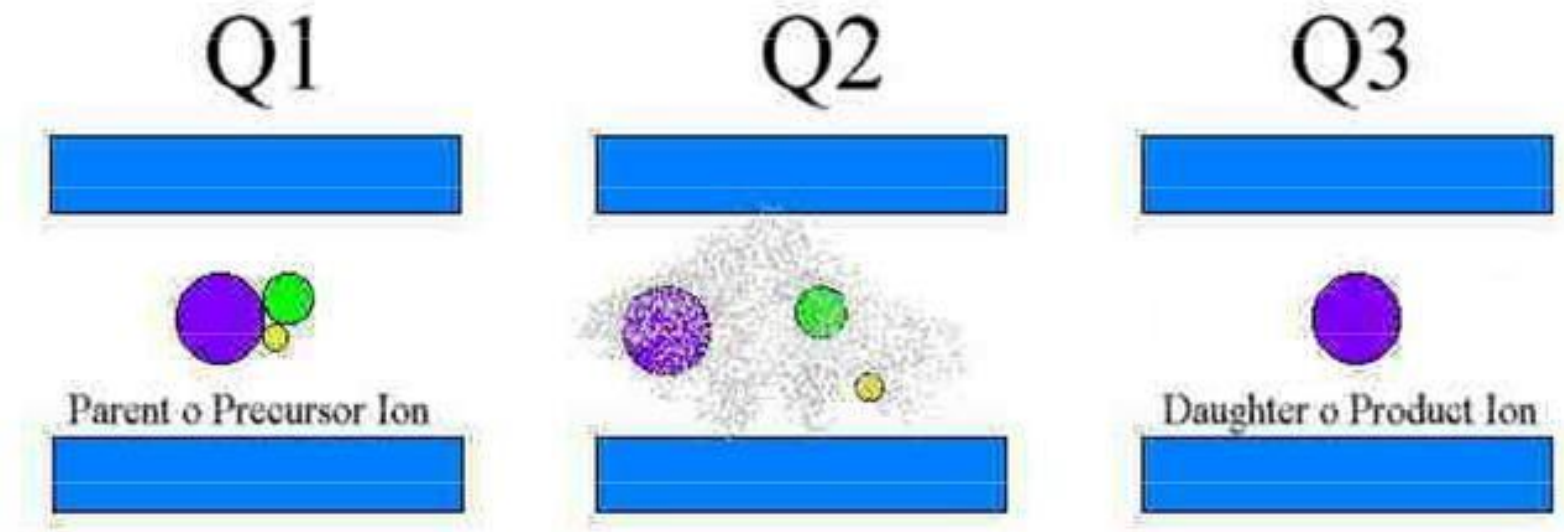
Tandem MS


ion source
quadrupole mass filter
quadrupole collision chamber
quadrupole mass filter
electron multiplier
sample
ionization
precursor ion selection
ion fragmentation
product ion selection
ion detection

Tandem MS


Quadrupole 1
(MS1)
Quadrupole 2
(CID Collision cell)
Quadrupole 3
(MS2)
(1)
select m/z
product ion scan
(2)
scanning
precursor ion scan
(selected m/z)
(3)
scanning
neutral loss scan

SELECTED REACTION MONITORING (SRM or MRM)

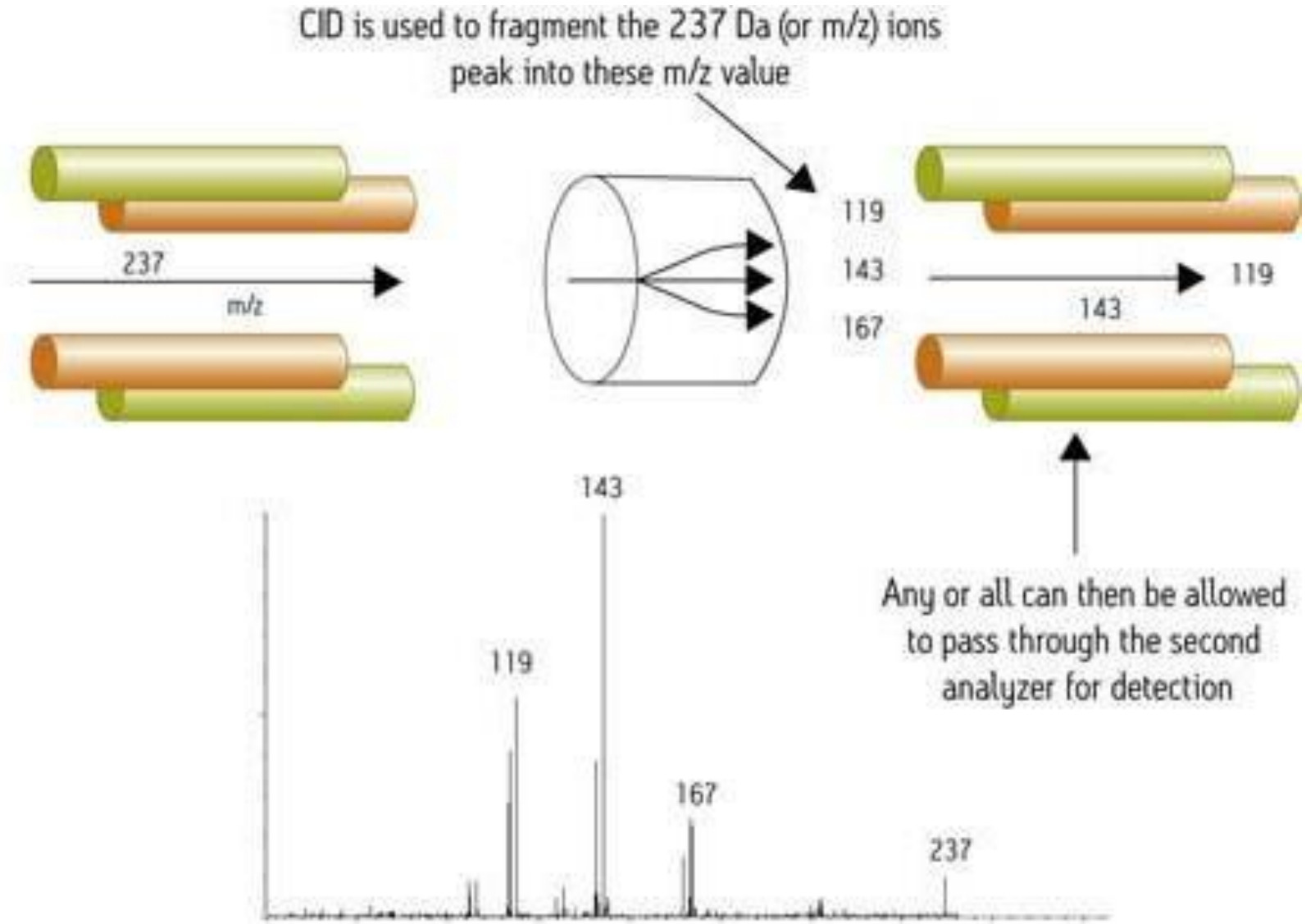


Q1 → SIM
Q2 → Collision
Q3 → SIM

This acquisition mode is used for quantitative analysis

MS-MS acts as a *detector*

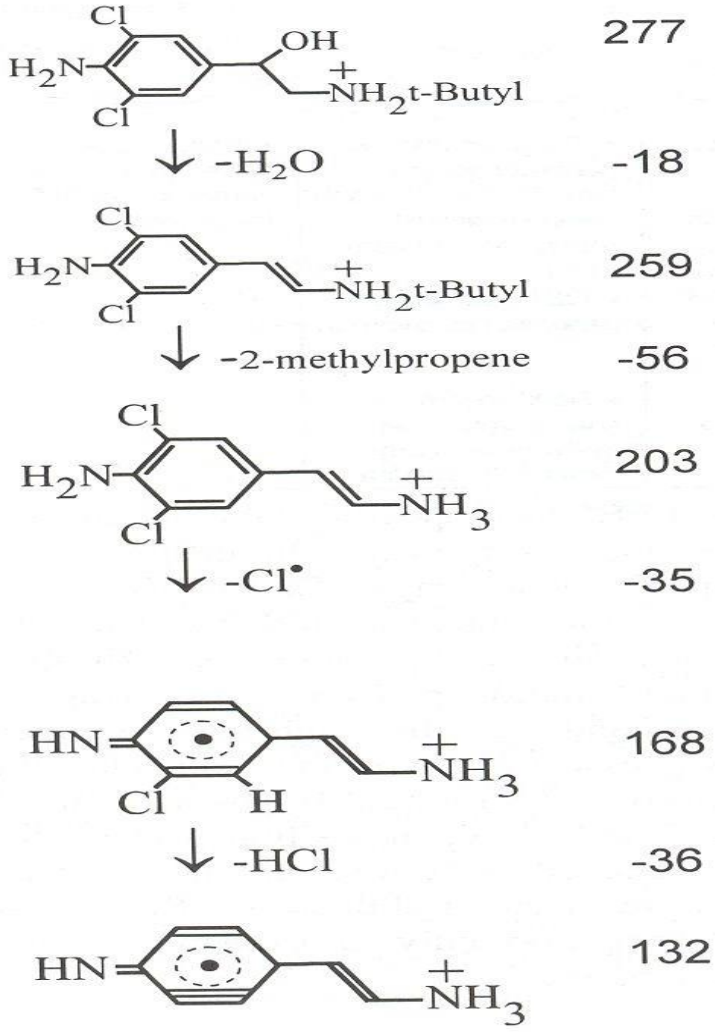
Tandem MS → SRM or PIS



SCAN MODE

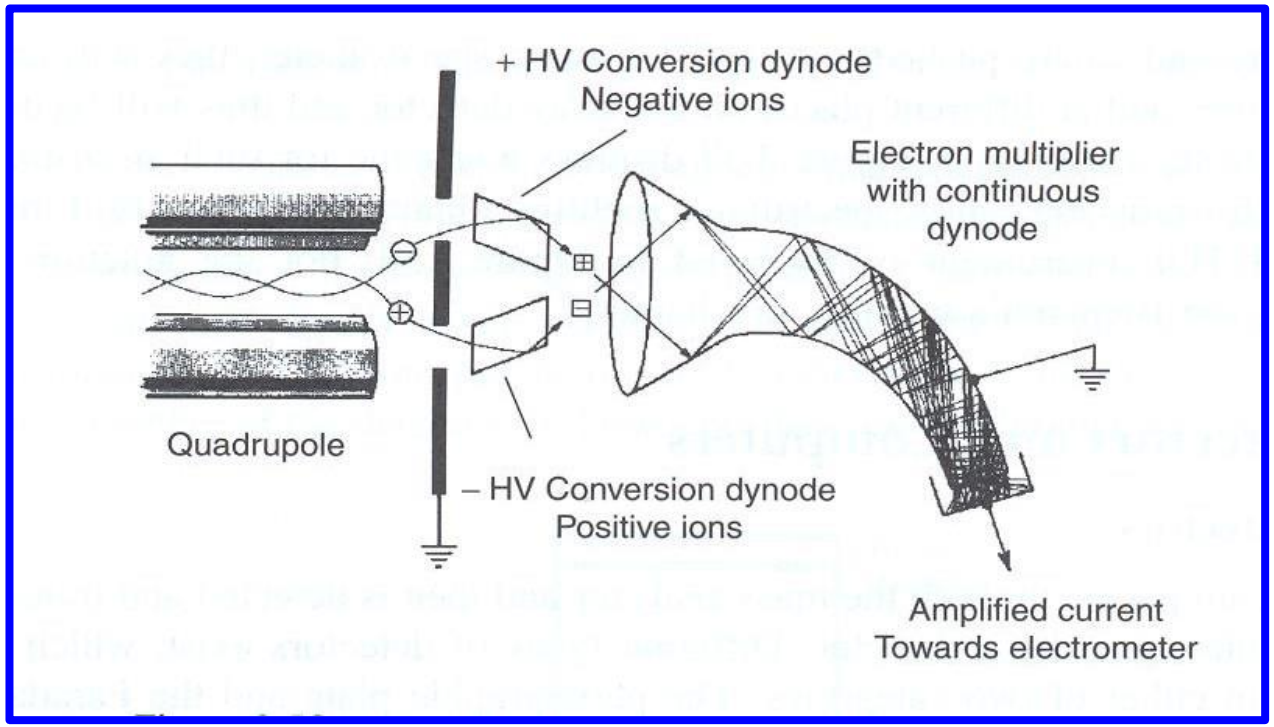
Fragmentation of Clenbuterol
(steroid-type drug that is used for veterinary purposes)

Ions are accelerated towards inert gas molecules
(i.e. N_2).
The collisions cause the ehancement of the internal energy (vibrational) until fragmentation.



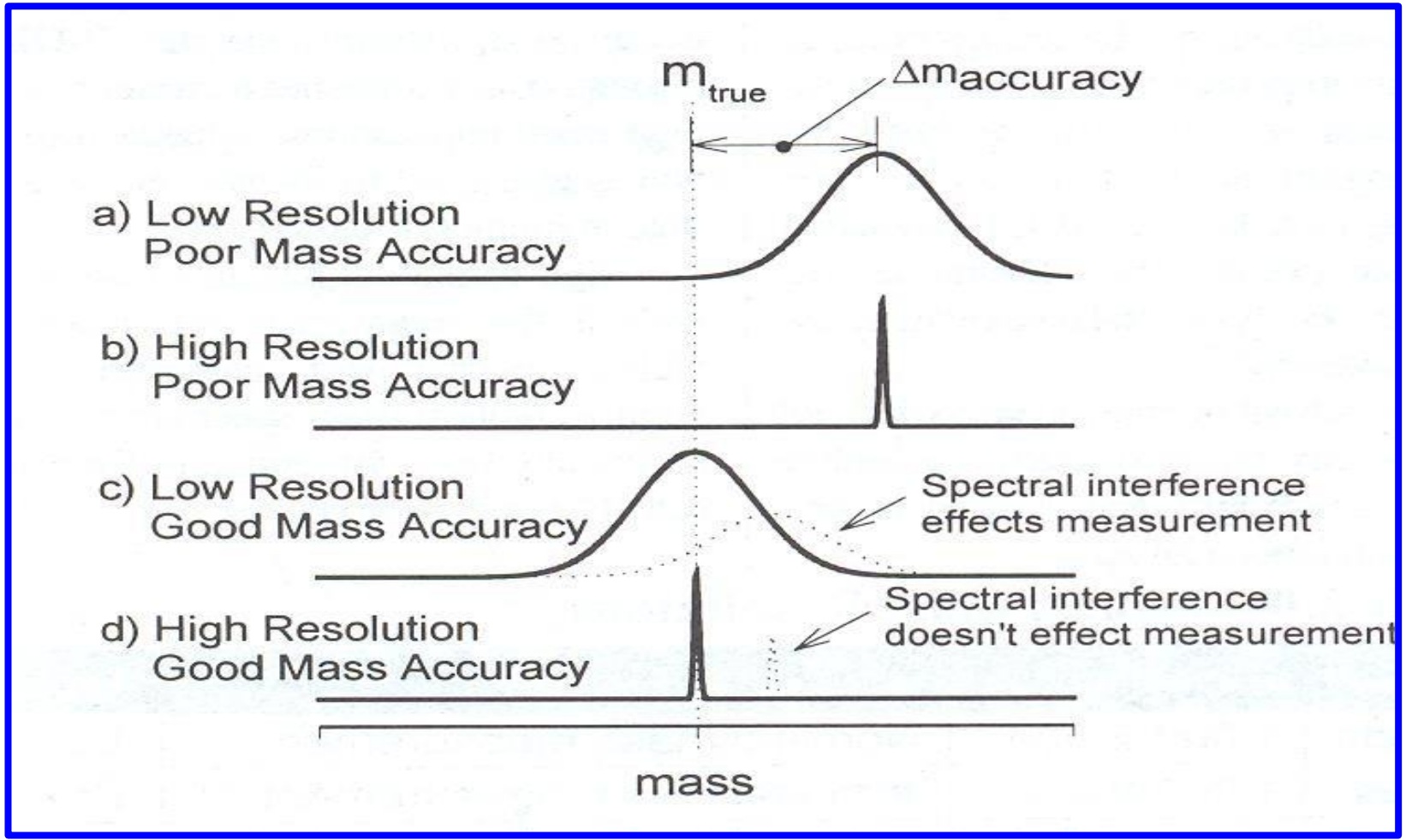
SIGNAL ACQUISITION

Electro-multiplier: ions reaches the conversion dinodes causing the electron emission; they, with a cascade effect, determine the emission of furthere electrons, obtaining a detectable current at the end of multiplier



RESOLUTION AND ACCURACY

Mass accuracy is the difference between the measured mass and the teoretical (real) mass of the analyzed compound

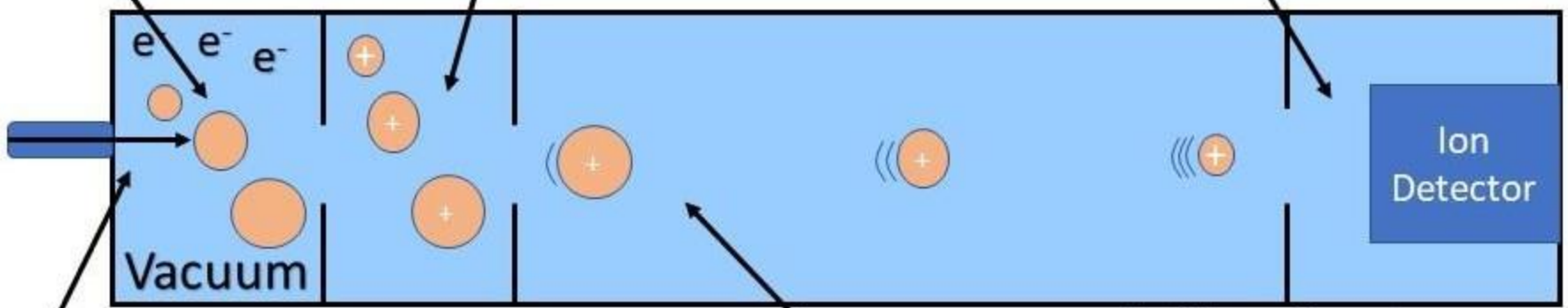


How does a Time of Flight Mass Spectrometer work?

2. Ionisation: They are then ionised into positive ions by firing electrons at them (knocking electrons from the outer energy level) This is called ***Electron Impact Ionisation***.

3. Electric Field: The positive ions are ***accelerated*** by an electric field. Lighter ions will accelerate greater than heavier ones.

5. Detector: Finally they hit the detector. Lighter ions will hit first, heavier ions later. The ions gain an electron when they hit the detector creating an electric current that can be detected.

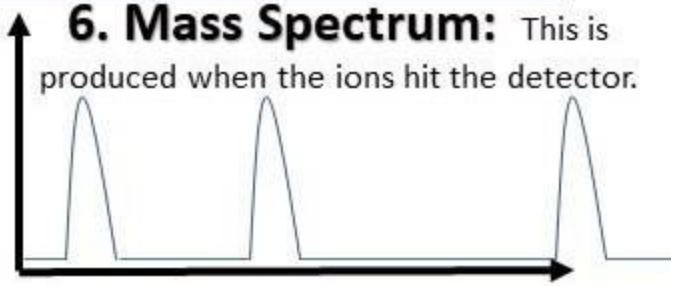


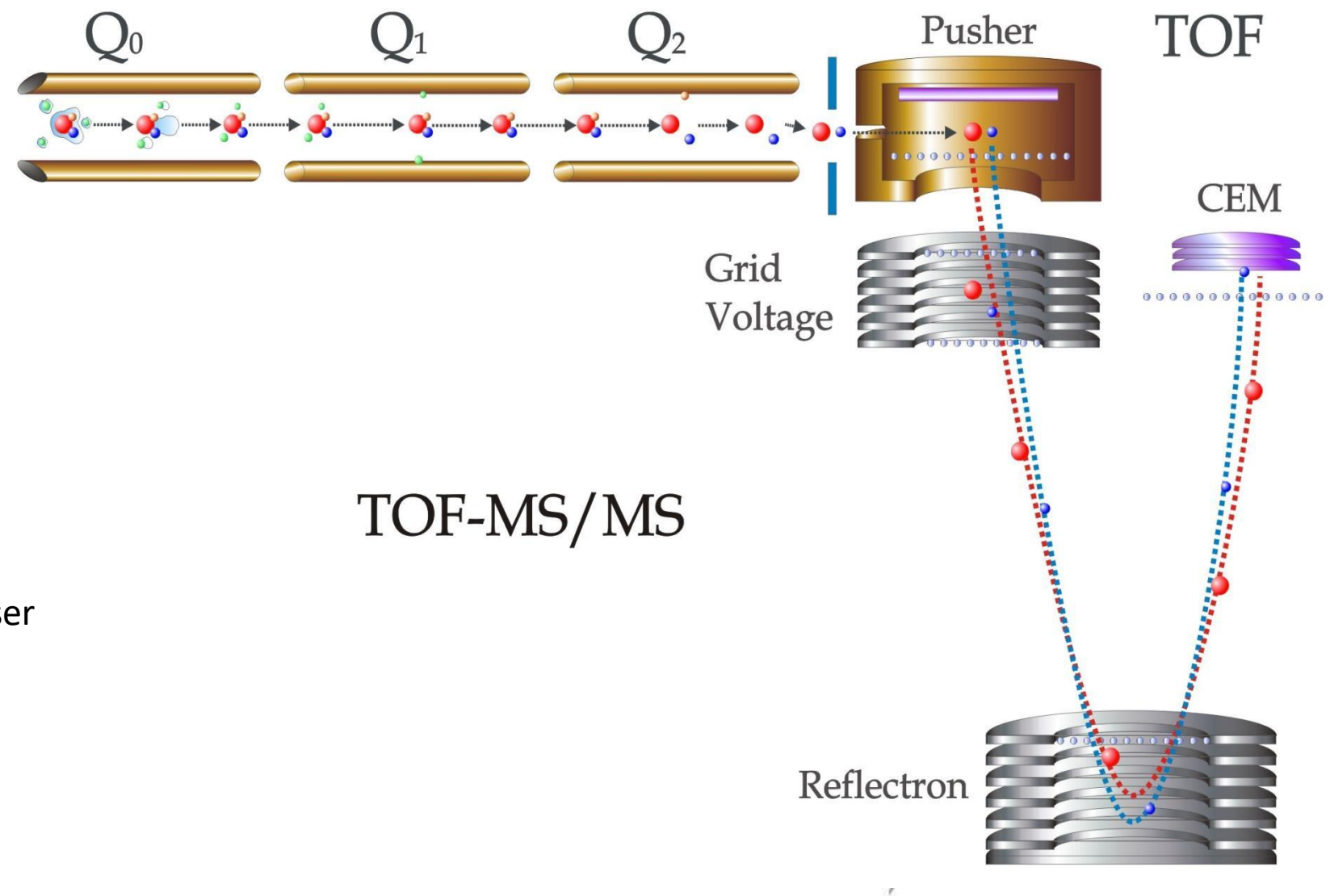
1. Vapourisation:

Samples of unknown substances (elements or compounds) are injected into a vacuum.

4. Ion Drift: They then drift with ***no*** electric field at the same speed they were travelling.

6. Mass Spectrum: This is produced when the ions hit the detector.





Time of flight analyser

UNIVERSITY OF TERAMO

Faculty of Bioscience and Technology
for Food, Agriculture and Environment

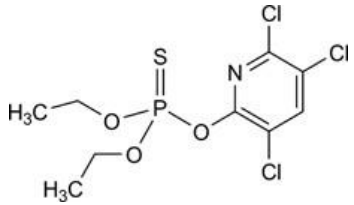
MICROEXTRACTION OF PESTICIDES FROM FOOD FOLLOWED BY UHPLC-MS/MS ANALYSIS

ANALYTICAL TARGETS

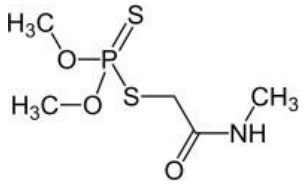
MULTIPLE CONTAMINATION

MULTICLASS ANALYSIS

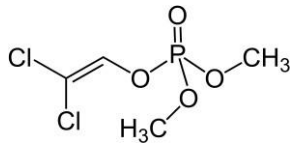
ORGANOPHOSPHATES



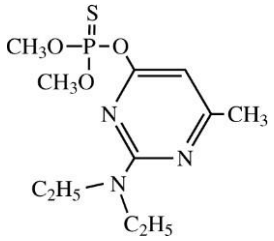
Chorpiriphos meth



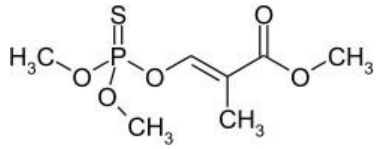
Dimethoate



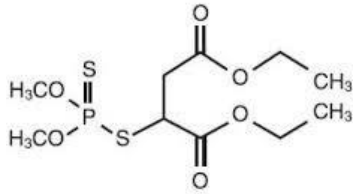
Dichlorvos



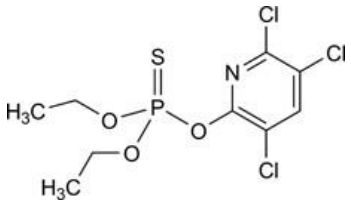
Pirimiphos Meth



Methacriphos

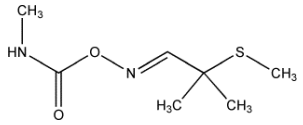


Malathion

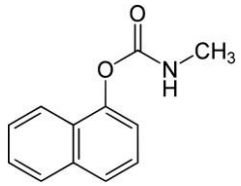


Chlorpiriphos ethyl

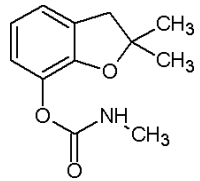
CARBAMATES



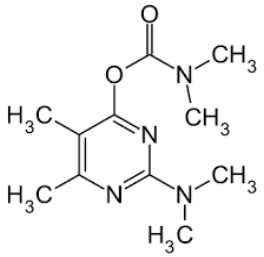
Aldicarb



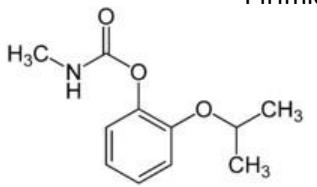
Carbaryl



Carbofuran

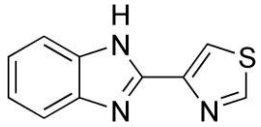


Pirimicarb

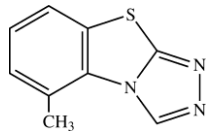


Propoxur

AZOLINE DERIV

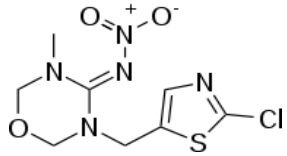


Thiabendazole

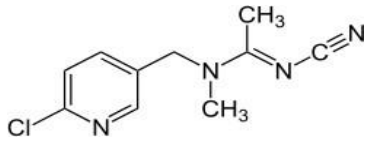


Tricyclazole

NEONICOTINOIDS



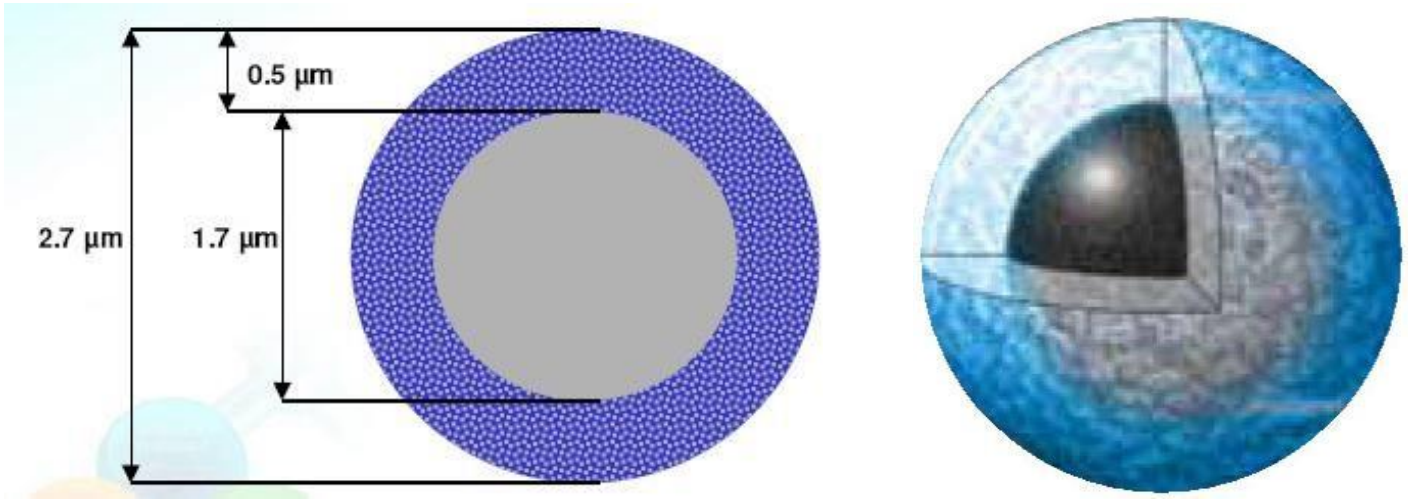
Thiametoxam

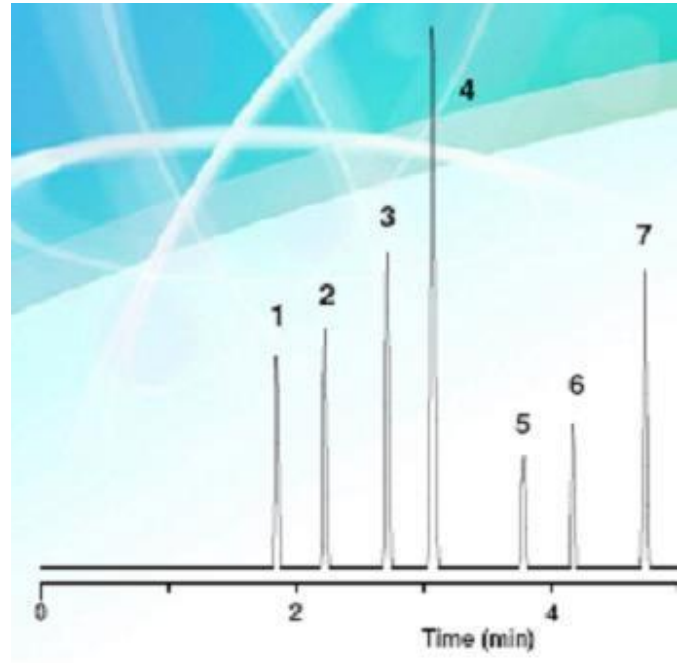


Acetamiprid

HIGH EFFICIENCY HPLC -UPLC

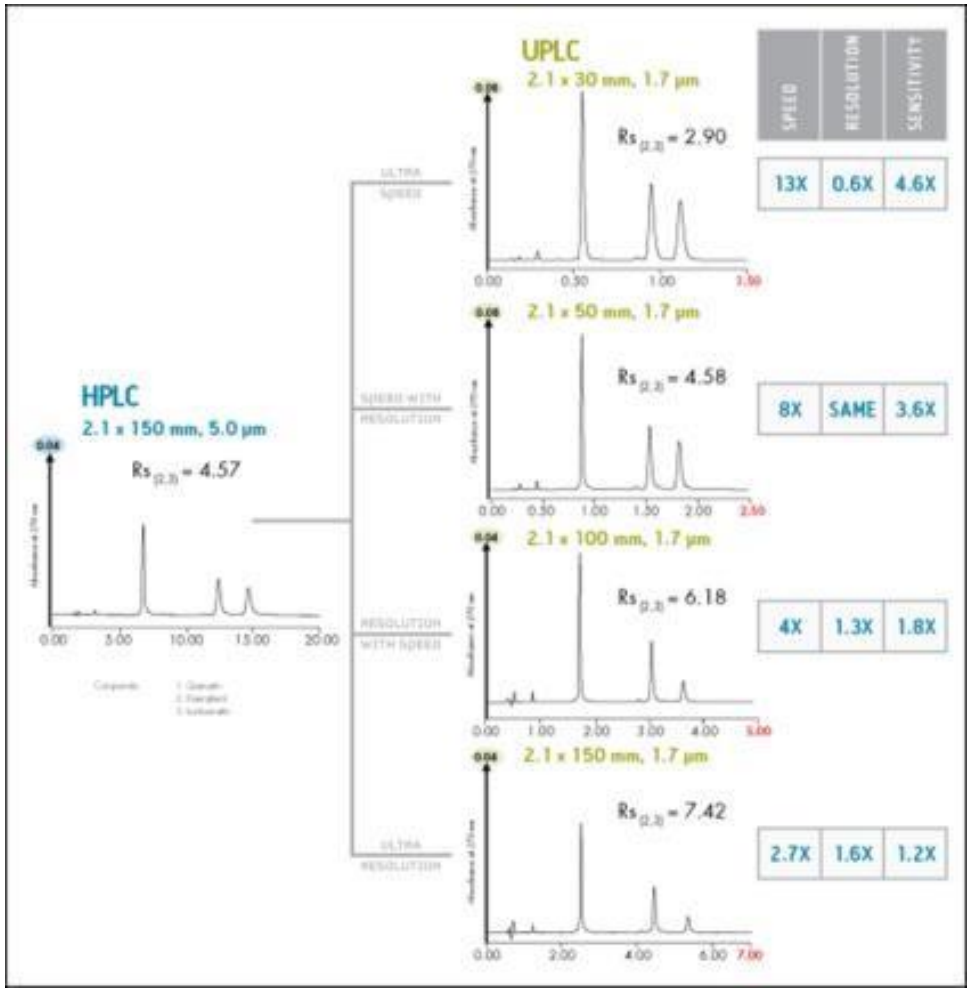
New particles feature a high-capacity, porous silica layer fused to a solid silica core





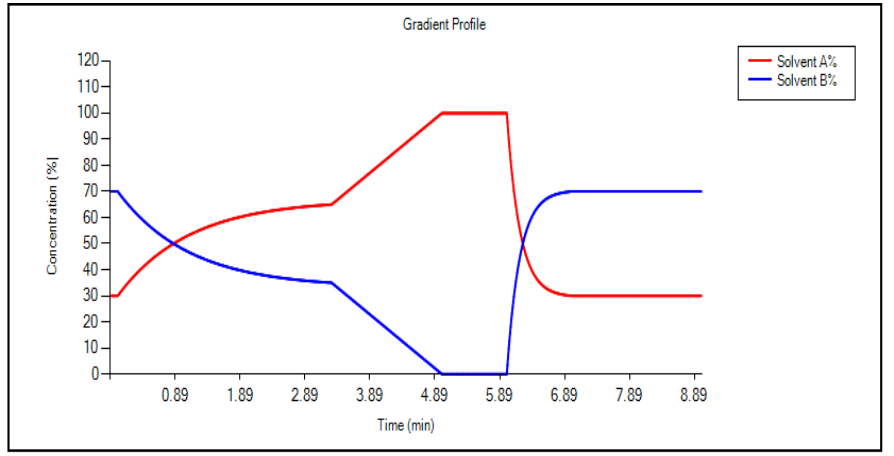
Shorter diffusion path for Fused-Core improves mass transfer and minimizes peak broadening, resulting in *efficiency similar to a sub-2 µm particle with the pressure drop of a 3 µm particle.*

UHPLC: PARTICLE SIZE



LIQUID CHROMATOGRAPHY

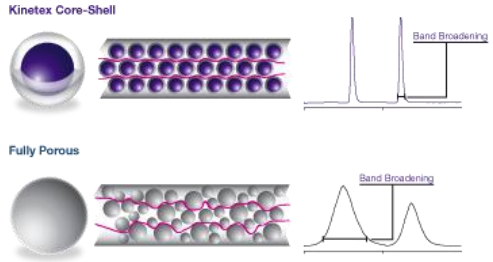
GRADIENT SCHEME



Phase A: MeOH 5mM HCOOH

Phase B: H_2O MilliQ 5mM HCOOH

- **Curved Increase of Phase A from 43% to 65% in 3,3 min**
- **Linear increase of Phase A from 65% to 100% in 1,7 min**
- **Reconditioning for 2 min**

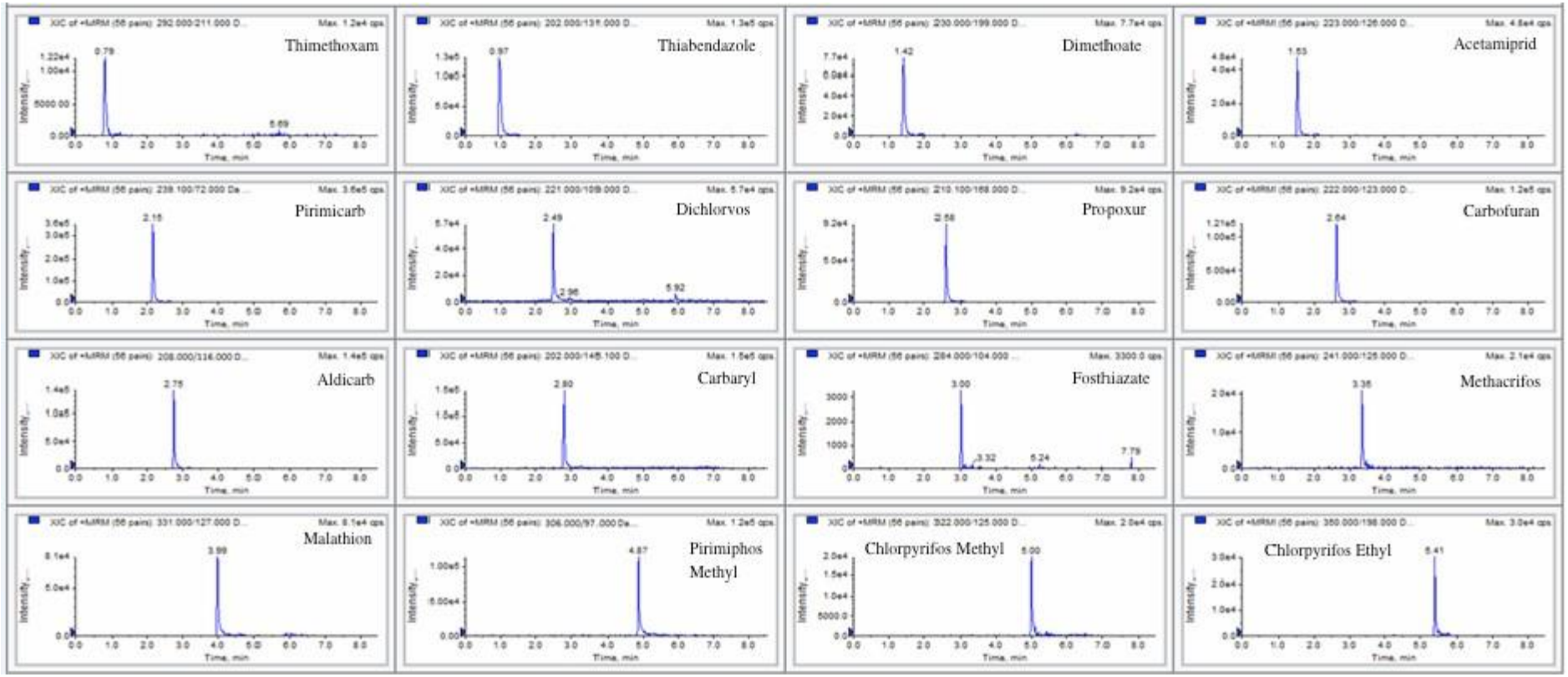


Column Phenomenex Kinetex XB-C18, 100x2,1 mm

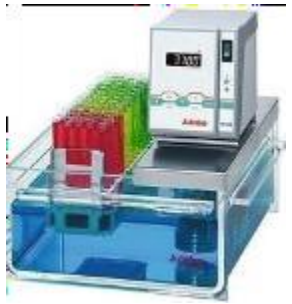
Instrumental parameters for MS/MS detection

ANALYTE	Q1 (amu)	Q3 (amu)	DP (V)	EP (V)	CE (V)	CXP (V)	t_R (min)
Thiamethoxam	292,0	211,0	60	10	17	8	0.79
		181,0			30	6	
Thiabendazole	202,0	131,0	65	8	35	9	0,97
		175,0			34	9	
Dimethoate	230,0	125,0	53	5	28	12	1,42
		199,0			13	7	
Acetamiprid	223,0	126,0	80	5	27	6	1,53
		56,0			32	5	
Pirimicarb	239,1	72,0	88	8	37	7	2,15
		182,0			22	9	
Dichlorvos	221,0	109,0	74	5	22	5	2,49
		127,0			37	8	
Propoxur	210,1	111,0	53	7	11	16	2,58
		168,0			20	8	
Carbofuran	222,0	123,0	34	4	31	15	2,64
		165,0			16	7	
Aldicarb	208,0	116,0	12	3	12	5	2,75
		89,0			23	11	
Carbaryl	202,0	145,1	14	9	12	11	2,80
		117,1			36	9	
Fosthiazate	284,0	104,0	150	10	22	10	3,00
		228,0			14	12	
Methacrifos	241,0	209,0	70	11	14	13	3,35
		125,0			19	15	
Malathion	331,0	127,0	70	9	17	8	3,99
		99,0			33	10	
Pirimiphos methyl	306,0	108,0	26	9	20	14	4,87
		67,0			29	15	
Chlorpyrifos methyl	322,0	125,0	65	4	21	9	5,00
		290,0			23	10	
Chlorpyrifos ethyl	350,0	97,0	15	9	25	5	5,41
		198,0			18	4	

XIC (extracted-ion currents) of the selected analytes



Extraction of pesticides from wheat flour

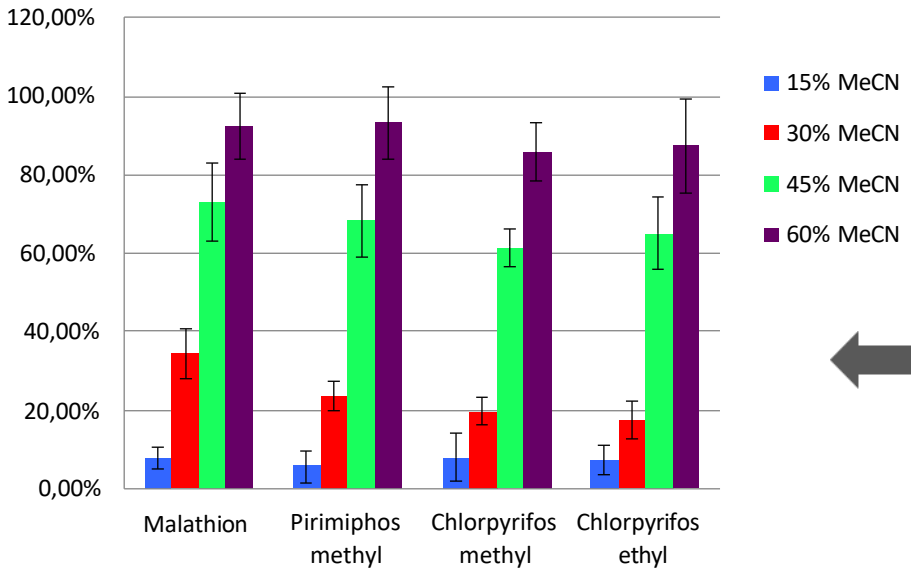


Solvent
Temperature
Ultrasounds
Centrifugation
Filtration
Stability

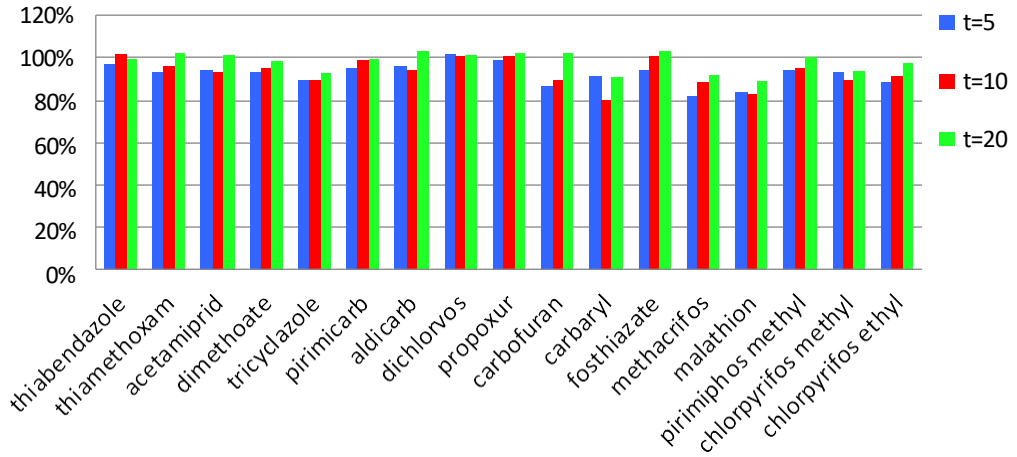
EXTRACTION OF PESTICIDES FROM WHEAT

RECOVERY

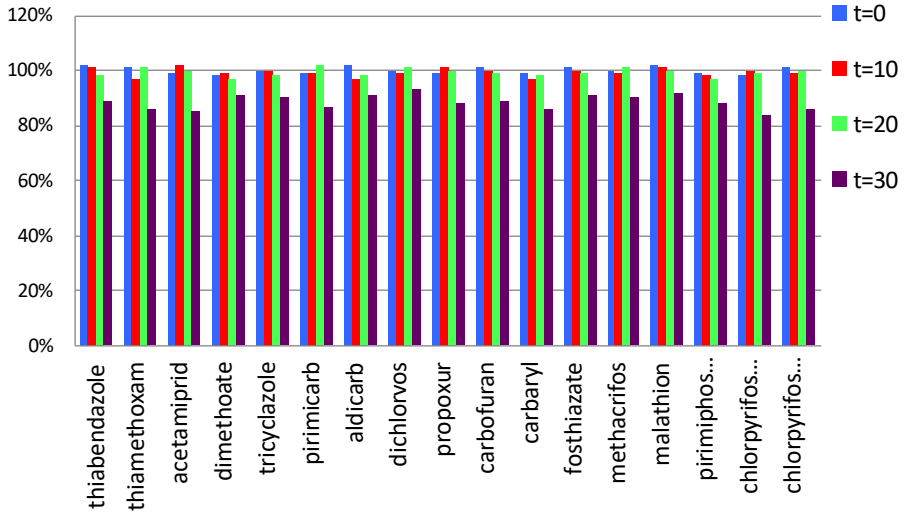
Different % AcCN



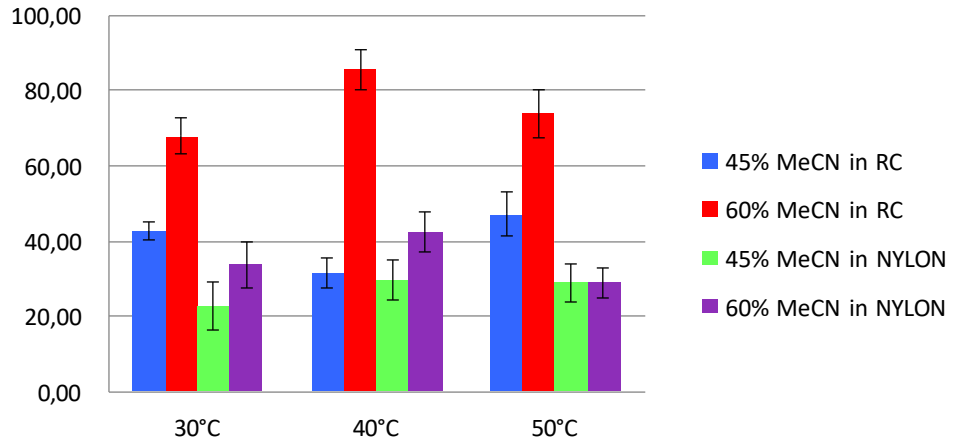
Influence of time on recovery



Stability at 40°C in std solution

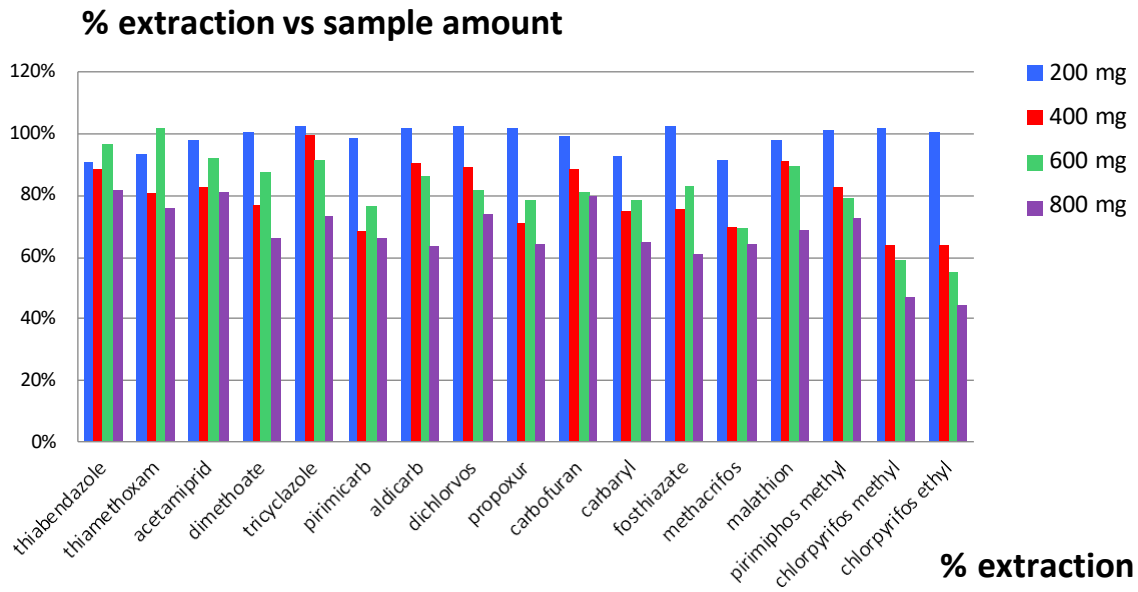


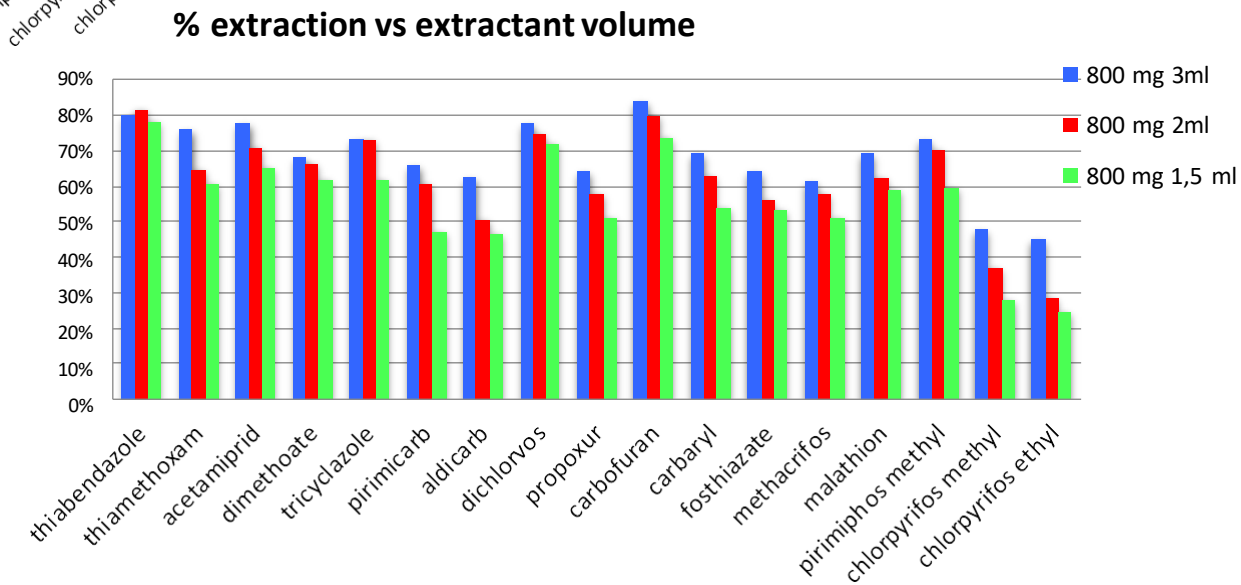
Recovery of Malathion at different T and with different filters



EXTRACTION OF PESTICIDES FROM WHEAT

ENRICHMENT

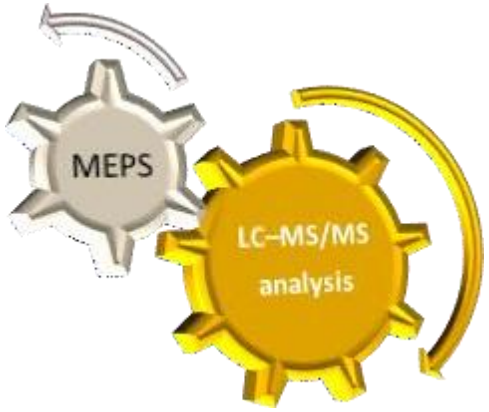




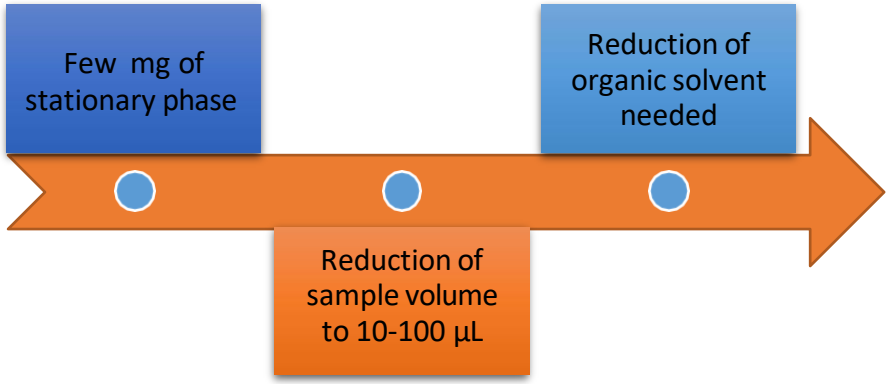
2

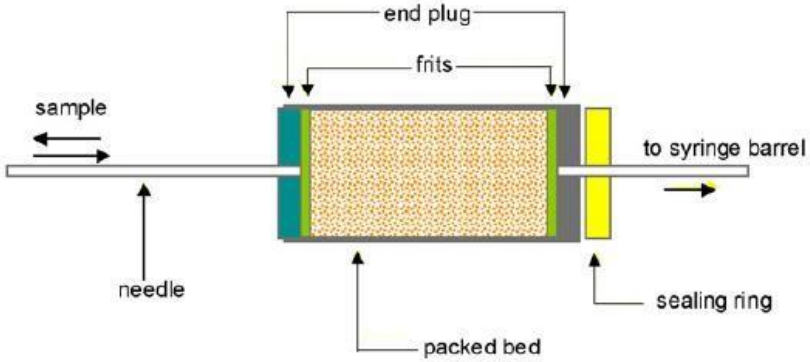
Clean-up of pesticides from wheat extract

Micro Extraction on Packed Sorbent

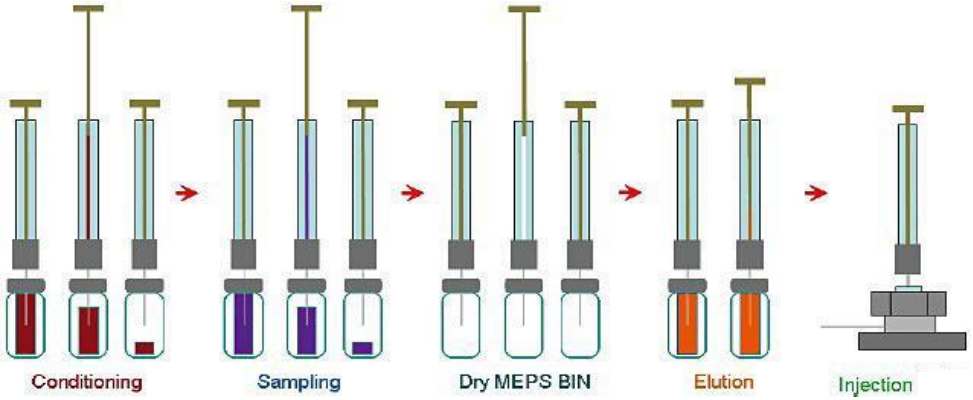


Micro Extraction on Packed Sorbent (MEPS)



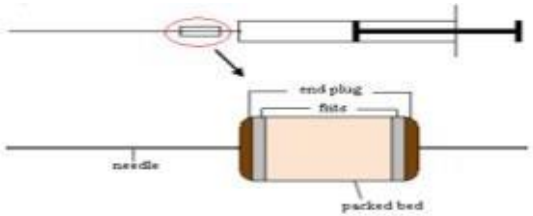


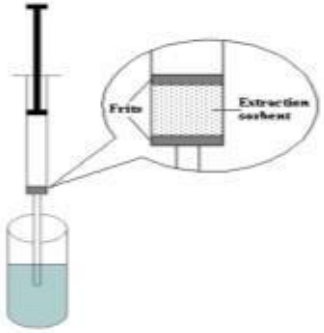
CLEAN-UP ➔ MEPS (Micro Extraction by Packed Sorbent)



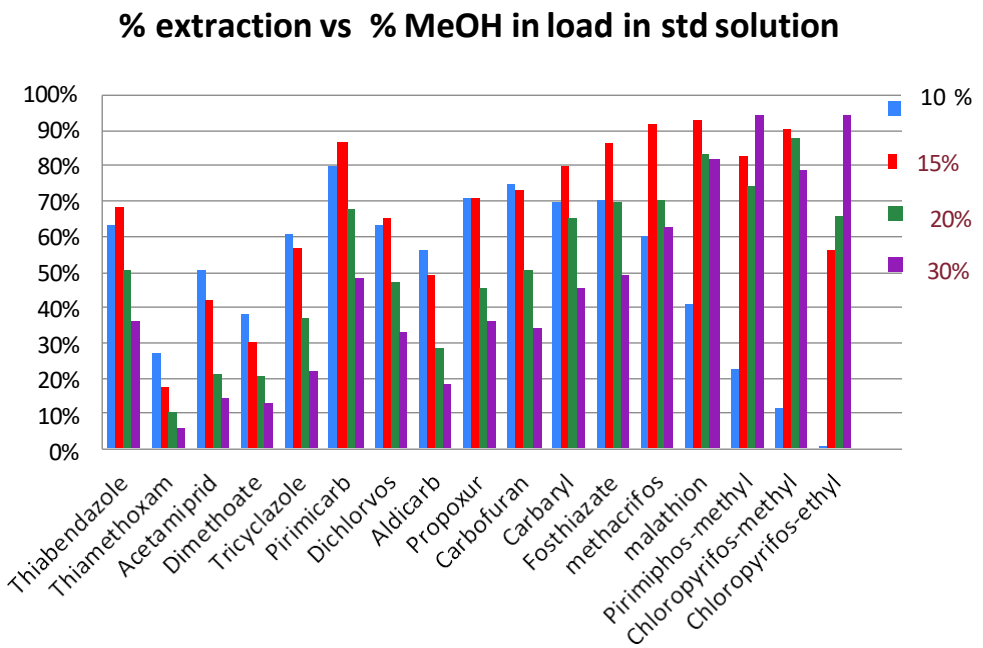
FEATURES

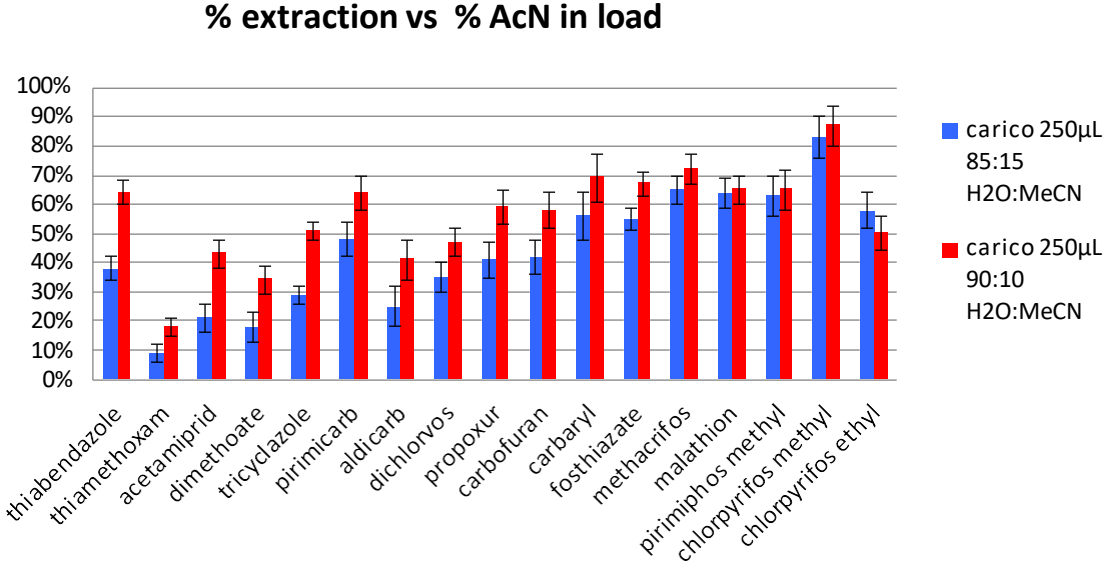
- Miniaturized SPE
- Multiple extractions :
  - ✧ Draw-eject
  - ✧ Extract-discart
- Can be used for 50 /100 samples

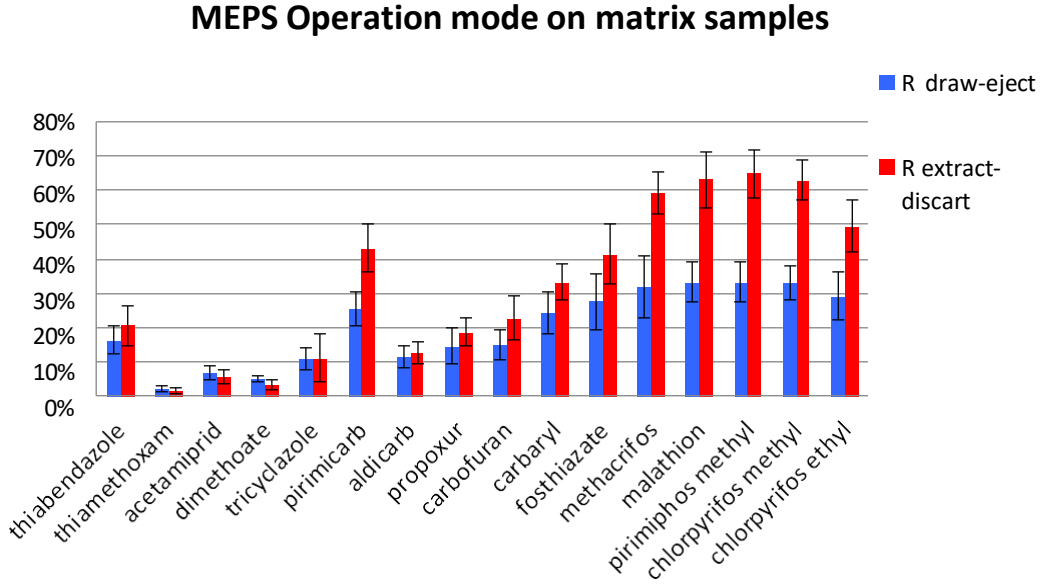


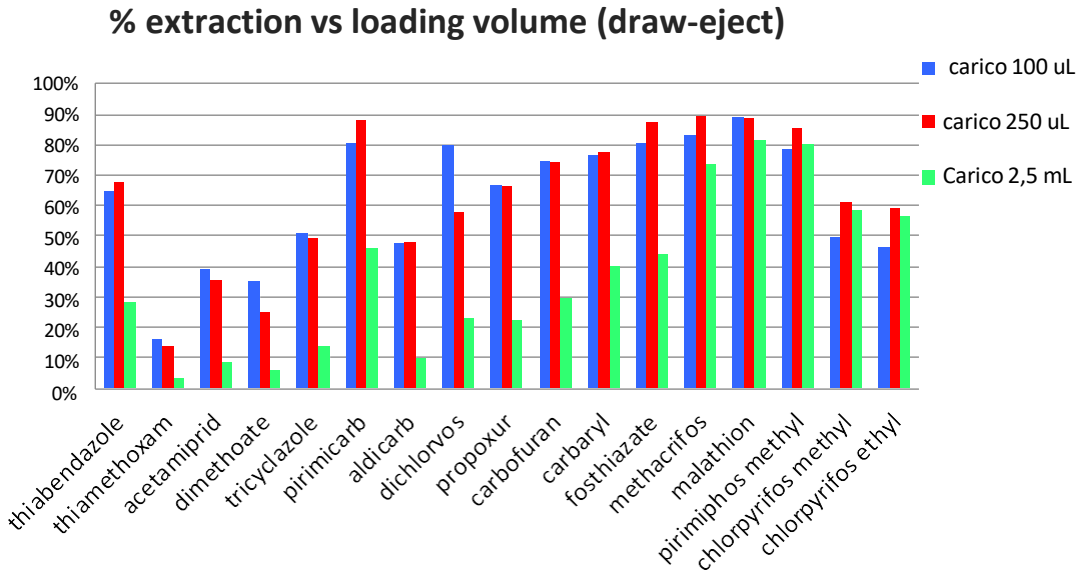


CLEAN-UP ➔ MEPS (Micro Extraction by Packed Sorbent)









SAMPLE PREPARATION PROCEDURE

1g wheat+1,5mL extraction mixture (60%MeCN+acetate buffer)

Sonication 5 min

Thermostatic bath (40°C 5 min)

Centrifugation
10000rpm
5 min 20°C

Filtration, 500µL of eluate+2,5mL acetate buffer

Centrifugation
10000rpm
5 min 4°C

MEPS C18

- Activation:100µL MeOH
- Conditioning: H_2O/MeCN 90:10
- Load: 3 mL extract-discart
- Wash: 100 µL H_2O
- Elution: 100 µL MeOH

UHPLC-MS/MS

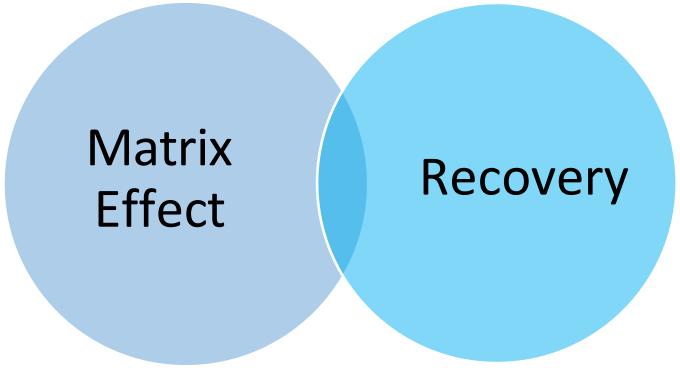
VALIDATION DATA (MRL maximum residue limit)

Analyte	Equation	r^2	Internal standard	LOD (mg/Kg)	LOQ (mg/Kg)	MLR (mg/Kg)
Thiabendazole	$y=361x+3,2x10^{-3}$	0,9992	Thiabendazole NH d_6	1.10^{-4}	3.10^{-4}	5.10^{-2}
Acetamiprid	$y=751x+3,14x10^{-3}$	0,9994	Thiabendazole NH d_6	2.10^{-4}	5.10^{-4}	5.10^{-1}
Dimethoate	$y=922x+4,46x10^{-3}$	0,9991	Thiabendazole NH d_6	1.10^{-2}	1.10^{-3}	3.10^{-2}
Tricyclazole	$y=534x+2,79x10^{-3}$	0,9996	Thiabendazole NH d_6	2.10^{-4}	5.10^{-4}	5.10^{-2}
Pirimicarb	$y=4x10^{-3}x+3,31x10^{-4}$	0,9987	Chlorpyrifos diethyl-d_{10}	3.10^{-5}	$1.\ 10^{-4}$	5.10^{-1}
Aldicarb	$y=743x+6,75x10^{-3}$	0,9983	Thiabendazole NH d_6	2.10^{-5}	5.10^{-5}	2.10^{-2}
Carbofuran	$y=907x+5,22x10^{-3}$	0,9994	Thiabendazole NH d_6	2.10^{-4}	5.10^{-4}	2.10^{-2}
Dichlorvos	$y=110x+1,16x10^{-3}$	0,9972	Thiabendazole NH d_6	7.10^{-5}	2.10^{-4}	1.10^{-2}
Propoxur	$y=912x+7,13x10^{-3}$	0,9984	Thiabendazole NH d_6	1.10^{-4}	3.10^{-4}	5.10^{-1}
Carbaryl	$y=837x+3,92x10^{-3}$	0,9992	Thiabendazole NH d_6	3.10^{-4}	1.10^{-3}	5.10^{-1}
Fosthiazate	$y=1,27x10^{-3}x+5,1x10^{-3}$	0,9997	Chlorpyrifos diethyl-d_{10}	3.10^{-4}	5.10^{-5}	2.10^{-2}
Methacrifos	$y=532x+2,29e003$	0,9998	Chlorpyrifos diethyl-d_{10}	1.10^{-5}	4.10^{-5}	5.10^{-2}
Malathion	$y=564x+5,55e003$	0,9995	Chlorpyrifos diethyl-d_{10}	2.10^{-5}	5.10^{-5}	8
Pirimiphos Methyl	$y=1,16x10^{-3}x+4,32x10^{-3}$	0,9992	Chlorpyrifos diethyl-d_{10}	2.10^{-5}	5.10^{-5}	5
Chlorpyrifos Methyl	$y=24,5x-47,2$	0,9961	Chlorpyrifos diethyl-d_{10}	7.10^{-4}	2.10^{-3}	3
Chlorpyrifos Ethyl	$y=139x-8,8$	0,9992	Chlorpyrifos diethyl-d_{10}	2.10^{-4}	5.10^{-4}	5.10^{-2}

VALIDATION DATA

Pesticide	RSD(%) intra-day			RSD(%) inter-day			Accuracy (%)		
Concentration level	½ MRL	MRL	1,5 MRL	½ MRL	MRL	1,5 MRL	½ MRL	MRL	1,5 MRL
Thiabendazole	12	4	6	14	9	9	89	91	94
Acetamiprid	8	5	4	13	8	9	97	91	94
Dimethoate	11	9	7	17	8	13	101	104	102
Tricyclazole	15	7	9	19	11	12	85	98	101
Pirimicarb	8	8	5	10	13	9	97	99	102
Aldicarb	8	6	7	15	11	10	87	90	95
Carbofuran	8	5	6	13	10	12	89	107	110
Dichlorvos	10	8	8	17	10	11	91	97	95
Propoxur	10	9	7	14	12	4	94	88	98
Carbaryl	6	2	5	12	10	8	101	103	109
Fosthiazate	7	12	10	11	14	15	100	110	98
Methacrifos	10	13	9	15	11	10	88	92	111
Malathion	11	4	9	13	10	11	103	107	111
Pirimiphos Methyl	9	12	8	13	15	11	94	87	102
Chlorpyrifos Methyl	15	13	10	20	15	12	86	88	91
Chlorpyrifos Ethyl	12	7	11	15	10	13	97	100	99

VALIDATION DATA



The recovery was calculated as the ratio of the peak area of the spiked (A) vs the area of the same sample spiked after elution of the microextraction (B).

Matrix effect was evaluated for each analyte by comparing the peak area of the quantifier ion current obtained from blank samples fortified after the extraction process (B) with the peak area of a standard at the same concentration in MeOH (C).

Analyte	Matrix effect B/C
Thiabendazole	1,00
Acetamiprid	0,98
Dimethoate	1,02
Tricyclazole	1,00
Pirimicarb	0,95
Aldicarb	0,89
Carbofuran	0,87
Dichlorvos	0,94
Propoxur	0,94
Carbaryl	0,80
Fosthiazate	0,91
Methacrifos	0,88
Malathion	0,93
Pirimiphos Methyl	0,81
Chlorpyrifos Methyl	0,78
Chlorpyrifos Ethyl	0,85

ANALYSIS ON REAL SAMPLES

ANALYTE	Flour 00 (mg/kg)	Flour 00 for pizza (mg/kg)	Flour 0 (mg/kg)	Organic flour (mg/kg)	LOQ (mg/kg)	MRL (mg/kg)
Aldicarb	0,002	0,002	< LOQ	< LOQ	$1x10^{-3}$	0,02
Chlorpyrifos methyl	< LOQ	< LOQ	0,04	< LOQ	0,03	3
Chlorpyrifos ethyl	0,003	0,003	0,002	< LOQ	0,01	0,05
Dichlorvos	0,009	0,008	0,008	< LOQ	0,005	0,01
Fosthiazate	0,006	0,006	0,006	< LOQ	$1x10^{-3}$	0,02
Malathion	0,003	0,007	0,003	< LOQ	$1x10^{-3}$	8
Methacrifos	0,007	0,005	0,007	< LOQ	$1x10^{-3}$	0,05
Pirimicarb	0,006	0,006	0,006	< LOQ	0,002	0,5
Pirimiphos methyl	0,104	0,116	0,160	< LOQ	$1x10^{-3}$	5

Determination of Pesticides in Wheat Flour Using Microextraction on Packed Sorbent Coupled to Ultra-High Performance Liquid Chromatography and Tandem Mass Spectrometry
F Di Ottavio et al… - Food Analytical Methods, 10, 1699-1708, 2017

Validation

Establishing documented evidence that provides a high degree of assurance that a specific process will consistently produce a product meeting its pre-determined specifications and quality attributes

"Validation of an analytical procedure is the process by which it is established, by laboratory studies, that the performance characteristics of the procedure meet the requirements for its intended use."

There are many reasons for the need to validate analytical procedures. Among them are **regulatory requirements, good science, and quality control requirements.**

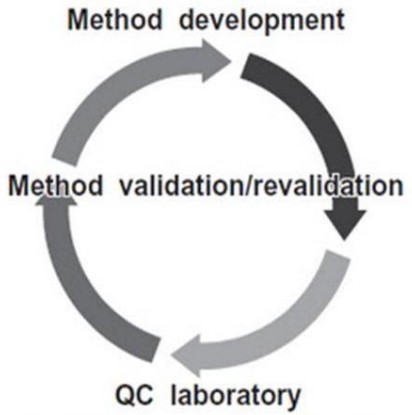


FIGURE 1 Life cycle of analytical method.

Typical validation characteristics which should be considered are:

1) **Accuracy**
2) **Precision**
3) **Specificity**
4) **Linearity**
5) **Range**
6) **Detection Limit**
7) **Quantitation Limit**
8) **Robustness/Ruggedness**
9) **Noise**
10) **Trueness**
11) **Sensitivity**

Classifications of residues (contaminants)

Directive 96/23/CE

GROUP A — Substances having anabolic effect and unauthorized substances

(1) Stilbenes, stilbene derivatives, and their salts and esters
(2) Antithyroid agents
(3) Steroids
(4) Resorcylic acid lactones including zeranol
(5) Beta-agonists
(6) Compounds included in Annex IV to Council Regulation (EEC) No 2377/90 of 26 June 199)

Classifications of residues (contaminants)

GROUP B — Veterinary drugs and contaminants

Dir. 96/23/CE

(1)**Antibacterial substances, including sulphonomides, quinolones**

(2) Other veterinary drugs

(a) Anthelmintics

(**b) Anticoccidials, including nitroimidazoles**

(c) Carbamates and pyrethroids

(d) Sedatives

(e)Non-steroidal anti-inflammatory drugs (NSAIDs)

(f)Other pharmacologically active substances

(3) Other substances and environmental contaminants

(a) Organochlorine compounds including PcBs

(b) Organophosphorus compounds

(d) Chemical elements

(d) Mycotoxins

(e) Dyes

(f) Others

DECISION 2002/657/CE

Art. 1

The Decision states the rules for the analytical methods for the official methods of analysis

Art. 3

EU member states guarantee that the official samples will be assayed with analytical methods

- **with documented instructions;**
- **following this the rules of this Decision;**
- **validated according to the Decision.**

DECISION 2002/657/CE

Art. 6

The output of an analysis will be considered non-compliant if the decision limit (CCα) is exceeded with a confirmatory method

1. If a permitted limit has been established for a substance, the decision limit is the concentration above which it can be decided with a statistical certainty of 1 – α that the permitted limit has been truly exceeded.

2. If no permitted limit has been established for a substance, the decision limit is the lowest concentration level at which a method can discriminate with a statistical certainty of 1 – α that the particular analyte is present.

For substances listed in Group A of Annex I to Directive 96/23/EC, the α error shall be 1 % or lower. For all other substances, the α error shall be 5 % or lower.

DECISION 2002/657/CE

Classification of analytical methods

Screening methods

Only those analytical techniques, for which it can be demonstrated in a documented traceable manner that they are validated and have a false compliant rate of < 5 % (β-error) at the level of interest shall be used for screening purposes in conformity with Directive 96/23/EC. In the case of a suspected non-compliant result, this result shall be confirmed by a confirmatory method.

Confirmatory methods

Confirmatory methods for organic residues or contaminants shall provide information on the chemical structure of the analyte. Consequently methods based only on chromatographic analysis without the use of spectrometric detection are not suitable on their own for use as confirmatory methods. However, if a single technique lacks sufficient specificity, the desired specificity shall be achieved by analytical procedures consisting of suitable combinations of clean-up, chromatographic separation(s) and spectrometric detection.

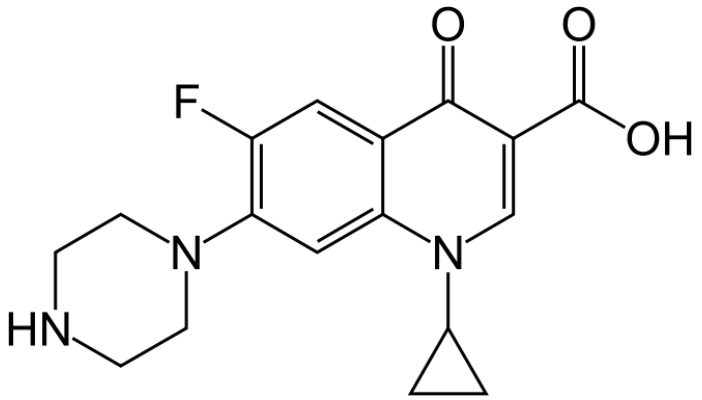
DECISION 2002/657/CE

Detection capability (CCβ)

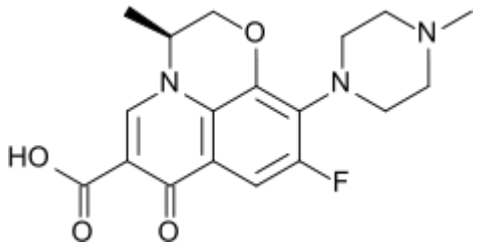
Detection capability (CCβ) means the smallest content of the substance that may be detected, identified and/or quantified in a sample with an error probability of β. In the case of substances for which no permitted limit has been established, the detection capability is the lowest concentration at which a method is able to detect truly contaminated samples with a statistical certainty of 1 – β.
In the case of substances with an established permitted limit, this means that the detection capability is the concentration at which the method is able to detect permitted limit concentrations with a statistical certainty of 1 – β.

Beta (β) error means the probability that the tested sample is truly non-compliant, even though a compliant measurement has been obtained (false compliant decision).

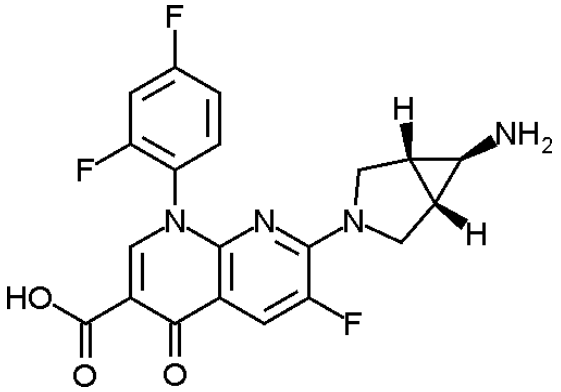
Quinolones in animal feed category B1



cyprofloxacin



levofloxacin



trovafloxacin

Quinolones: Reg. (UE) n.37/2010

Analite	Specie [a]	MLR (µg/kg)
Danofloxacin	bovine, ovine, poultry other species	200 100
Difloxacin	bovine, ovine, poultry , swine other species	400 300
Enrofloxacin	All the species	100
Flumequin	bovine, ovine, poultry, swine, fish	200 400 600
Marbofloxacin	bovine, swine	150
Oxolinic Acid	All the species	100
Sarafloxacinn	*Salmonidae*	30

[a] not for species producing eggs for human consumption

analytical procedure*

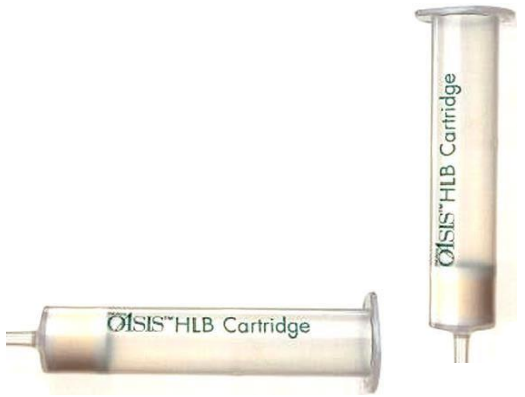
ESTRACTION: 5 g of sample + 20 ml (+20 ml) di methanol/phosphoric acid 1% (40:60, v/v);
20 ml dried under nitrogen at 50°C to evaporate methanol.

PURIFICATION: OASIS HLB (500mg/3ml) conditioned with 2 ml methanol and 2 ml water;
wash with 5 ml 1% metaphosphoric acid and 5 ml water ;
elution with 5 ml di 30% ammonia /methanol (5:95, v/v).

ENRICHMENT: solvent evapration and dilution in 0.1% formic acid

INSTRUMENTAL Analysis: HPLC-MS//MS.

- on muscle samples 100 µg/kg norfloxacin-d5 (SI) are added;
  on eggs 10 µg/kg norfloxacin-d5



* Gently provided by Dr. Annunziata IZSM Giuseppe Caporale, Teramo

HPLC-MS/MS

- HPLC Column: X-TERRA C18 100 x 2,1 mm, 3,5 µm, Waters
- Flow rate 0.2 ml/min, injection volme 10 µl
- Source API – ESI +
- Analyser Quadrupole
- MRM (Multi Reaction Monitoring) modality
- Two fragmented ion for each analyte
- Quantitative analysis on higher intensity ion
- Gradient

time (min)	acetonitrile	formic acid 0.1%
0	2	98
5	70	30
9	70	30
10	2	98
25	2	98

Instrumental Linearity

- 5 concentration levels x 3
- Levels selected according to the validation levels established for each type of sample
- Calibration curves built using analyte area/IS area *vs* concentration

fortification levels muscle

Analyte	0.5 LMR (µg/kg)	1 LMR (µg/kg)	1.5 LMR (µg/kg)
Marbofloxacin	75	150	225
Ciprofloxacin	50	100	150
danofloxacin [a]	50	100	200
Enrofloxacin	50	100	150
difloxacin [b]	150	300	400
oxolinic acid	50	100	150
flumequin [c]	200	400	600

[a] MLR danofloxacin 100-200 µg/kg

[b] MLR difloxacin 300-400 µg/kg

[c] MLR flumequin 200-400-600 µg/kg

fortification levels muscle unauthorised compounds

Analytes without MRL

Analyte	C_0 (µg/kg)	2 C_0 (µg/kg)	3 C_0 (µg/kg)
norfloxacin	10	20	30
lomefloxacin	10	20	30
sarafloxacin	10	20	30
Nalidixic acid	10	20	30

Fortified levels eggs

- Quinolones are not allowed even in traces in eggs
- fortified levels 5-10-20 µg/kg for all the analytes

Validation Plan

Validation plan	
Procedure	n.repetitions/ levels
I	6
II	6
III	6

- Verification of the normality of the data - test Shapiro Wilk test
- Verification of outliers - Grubbs test
- Variance analysis (ANOVA)
- Recoveries calculated by calibration curve in solvent
- CV%
- Calibration curves in matrices

Validation data muscle

Analyte	fortified level (µg/kg)	Recovery% (n=18)	CV (%RSD) n=18
marbofloxacin	75-150-225	97-103-99	11-9-4
norfloxacin	10-20-30	97-102-99	16-14-8
Ciprofloxacin	50-100-150	98-102-99	12-10-5
Danofloxacin	50-10-200	91-107-99	23-16-7
Lomefloxacin	10-20-30	95-105-98	13-12-7
Enrofloxacin	50-100-150	100-100-100	9-8-5
Sarafloxacin	10-20-30	98-101-99	7-8-5
Difloxacin	150-300-400	98-102-99	8-8-10
Oxolinic acid	50-100-150	99-101-96	7-9-11
nalidixic acid	10-20-30	99-101-100	11-10-7
flumequin	200-400-600	97-103-99	13-12-7

Validation data eggs

Analyte	fortified level (µg/kg)	Recovery% (n=18)	CV (%RSD) n=18
marbofloxacin	5-10-15	100-99-100	8-9-7
norfloxacin	5-10-15	101-99-100	5-4-3
ciprofloacin	5-10-15	98-102-99	9-9-5
danofloxacin	5-10-15	100-100-100	17-17-14
lomefloxacin	5-10-15	96-104-99	11-9-11
enrofloxacin	5-10-15	99-101-100	12-15-11
sarafloxacin	5-10-15	95-105-98	16-13-10
difloxacin	5-10-15	96-104-98	18-15-12
oxolinic acid	5-10-15	100-100-100	20-21-13
Nalidixic acid	5-10-15	102-98-101	17-16-16
flumequin	5-10-15	100-100-100	13-12-17

Calculation of CCα and CCβ for compounds with MLR

$$CC\alpha = MLR + 1.64\ SD_{r,MLR}$$

d where $SD_{r,MLR}$ is the intra-laboratory standad deviation at MRL

$$CC\beta = CC\alpha + 1.64\ SD_{r,CC\alpha}$$

where $SD_{r,CC\alpha}$ is the inta-laboratory standard deviation at CCα. We are assuming that SD between MLR e CCα increases linearly with concentration, (CV% is constant). Thus:

$$CC\beta = CC\alpha + 1.64\ (CV\%_{pooled} \times CC\alpha/100)$$

where $CV\%_{pooled}$ is the combination of CV% observed at MRL and CV% at 1.5 LMR

Calculation of CCα and CCβ for unauthorised compounds

$CC\alpha = C_0 + 2.33\ DS_{r,C0}$

where $DS_{r,C0}$ is the intra-lab standard deviation at the C_0 level

$CC\beta = CC\alpha + 1.64\ DS_{r,CC\alpha}$

where $DS_{r,CC\alpha}$ intra-lab standard deviation at CCα. intra-lab standard deviation We are assuming that DS between C_0 and CCα increases linearly with concentration, thus:

$CC\beta = CC\alpha + 1.64\ (CV\%_{pooled} \times CC\alpha/100)$

where $CV\%_{pooled}$ is the combination of CV% at C_0 and CV% at $2C_0$

CCα and CCβ

Analyte	Muscle		eggs	
	CCα	CCβ	CCα	CCβ
marbofloxacin	173	194	6.0	6.8
norfloxacinn	14	17	5.6	6.1
ciprofloxacin	116	132	6.0	6.9
danofloxacin	126	151	6.9	8.8
lomefloxacin	13	16	6.3	7.5
enrofloxacin	113	126	6.4	7.7
sarafloxacin	32	35	6.9	8.7
difloxacin	339	390	7.1	9.2
oxolinic acid	115	135	7.4	9.8
nalidixic acid	13	15	7.0	9.0
flumequin	234	282	6.6	8.0

Robustness

Minor changes :

- 7 potential critical factors ;
- Tests were run on 8 negative fortified samples, using Youden approach, each parameter was varied within 10%;
- Compounds were fortified at MRL or C_0 ;

Robustness – experimental design on muscle

selected parameter	Unit	High/low	Centered value	High	low
%MeOH in the etraction mixture	%	A,a	40	44	36
T of enrichment	°C	B,b	50	55	45
SPE OASIS lot	-	C,c	-	080A38157A	084038263A
pH washing SPE	pH	D,d	3.0	3.1	2.9
% ammonia in elution mixture	%	E,e	5.0	5.5	4.5
Volume of the elution mixture	ml	F,f	5.0	5.5	4.5
% of formic acid in mobile phase	%	G,g	0.10	0.11	0.09

The method was robust; CV was similar in all cases to intra-lab CV

Dioxins, dioxin-like PCBs

6.4.2017 EN Official Journal of the European Union L 92/9

COMMISSION REGULATION (EU) 2017/644

of 5 April 2017

laying down methods of sampling and analysis for the control of levels of dioxins, dioxin-like PCBs and non-dioxin-like PCBs in certain foodstuffs and repealing Regulation (EU) No 589/2014

Chemical Elements (metals)

29.3.2007 EN Official Journal of the European Union L 88/29

COMMISSION REGULATION (EC) No 333/2007

of 28 March 2007

laying down the methods of sampling and analysis for the official control of the levels of lead, cadmium, mercury, inorganic tin, 3-MCPD and benzo(a)pyrene in foodstuffs

Micotoxins

L 70/12 EN Official Journal of the European Union 9.3.2006

COMMISSION REGULATION (EC) No 401/2006

of 23 February 2006

laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs

(Text with EEA relevance)

Pesticides

ANALYTICAL QUALITY CONTROL AN METHOD VALIDATION PROCEDURES FOR PESTICIDE RESIDUES ANALYSIS IN FOOD AND FEED

Supersedes Document No. **SANTE/11945/2015. Implemented by 01/01/2018**

UNIVERSITÀ DEGLI STUDI DI TERAMO

Selective identification of conjugated polyphenol compounds in plant matrices by LC-MS/MS: a multi experiment approach

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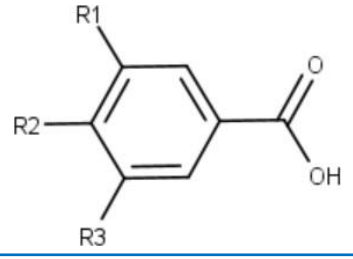
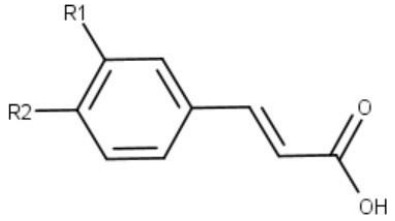
Phenolic Compounds: Classification

Phenolic Acids C_6-C_1

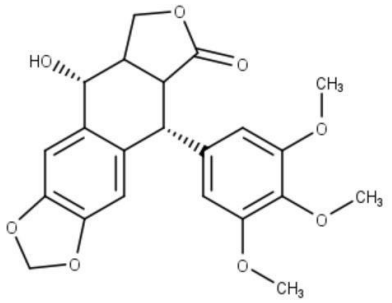
Hydroxybenzoic Acid



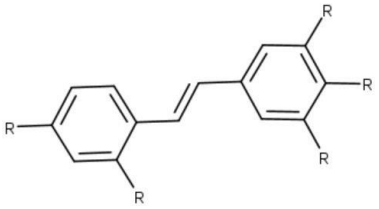
Hydroxycinnamic Acid



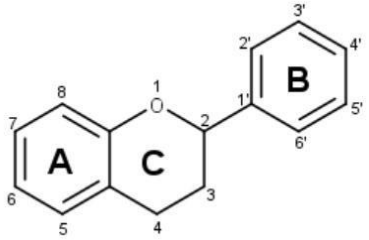
Lignans $C_{22}H_{22}O_8$



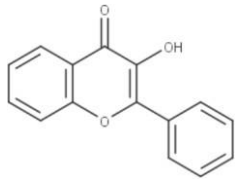
Stilbenes $C_{14}H_{12}$



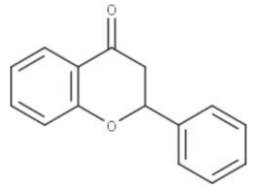
Flavonoids C_6-C_3-C_6



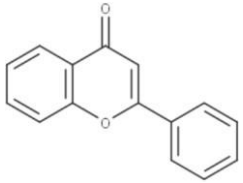
Flavonols $C_{15}H_{10}O_3$



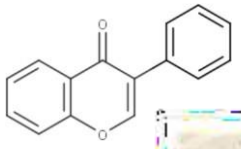
Flavanones $C_{15}H_{12}O_2$



Flavones $C_{15}H_{10}O_2$

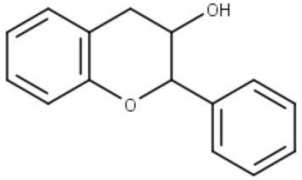


Isoflavones $C_{15}H_{10}O_2$

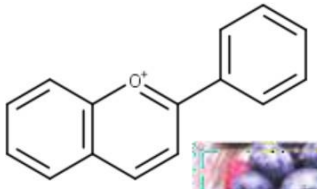


Flavan-3-ols $C_{15}H_{14}O_2$

(Catechines and Proantocianidines)

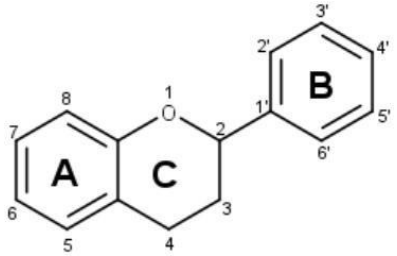


Anthocyanidinis $C_{15}H_{11}O^+$



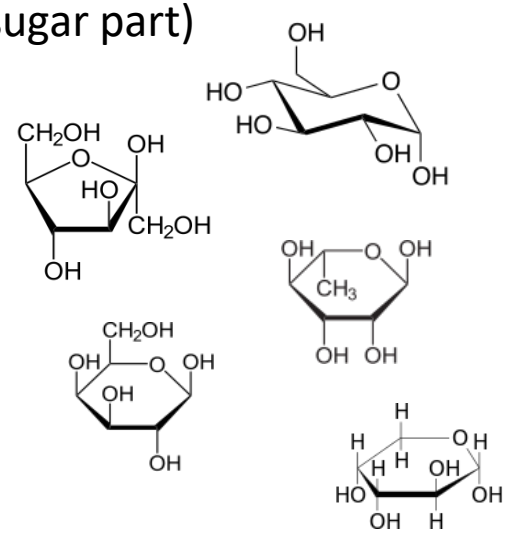
Experimental work: Glycoside derivatives

Aglycone (non sugar part)



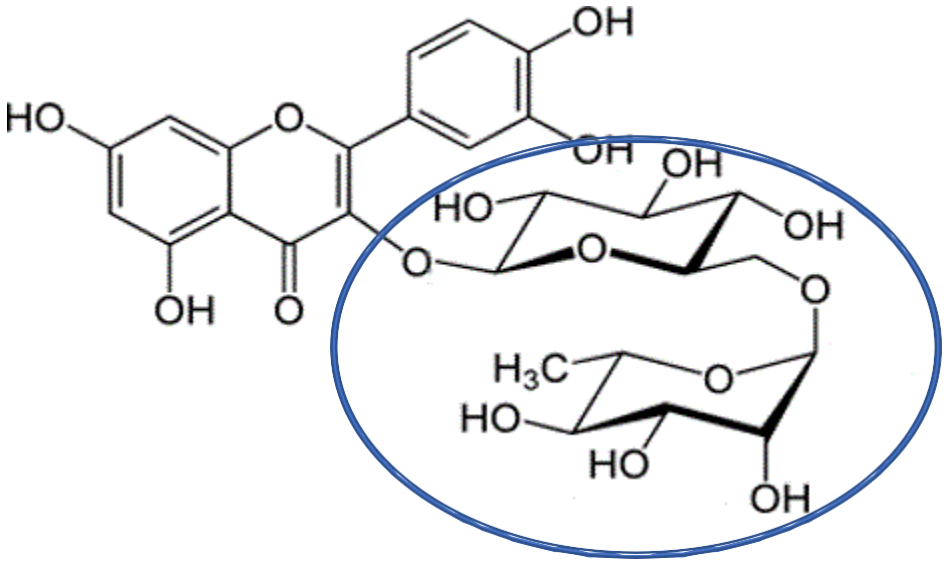
Benzene ring (A) condensed with six membered ring (C) pyran ring, which in the 2-position carriers a phenyl ring (B) as a substiuent

Glycone (sugar part)



- **glucosides** (glycone = glucose)
- **fructosides** (glycone = fructose)
- **ramnosides** (glycone = rhamnose)
- **galactosides** (glycone = galactose)
- **arabinosides** (glycone = arabinose)
- ….etc

Aglycone:
Quercetin



Glycone:
Rutinose

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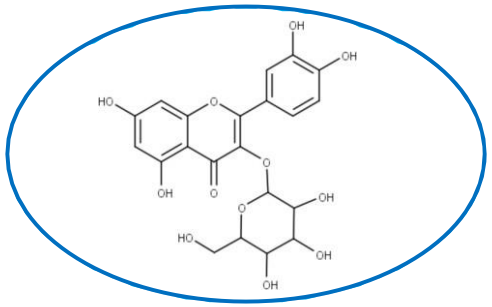
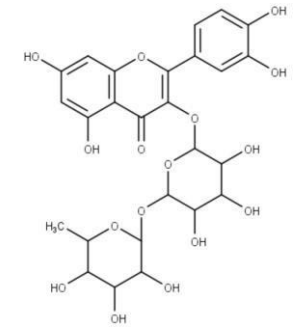
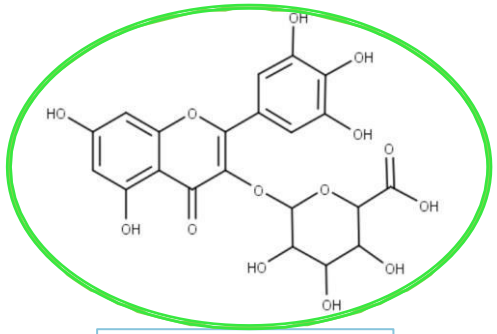
Flavonoids



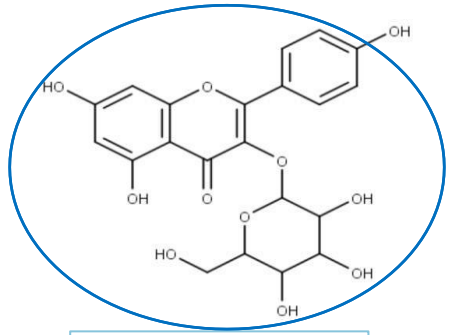
Quercetin-3-O-glucoside



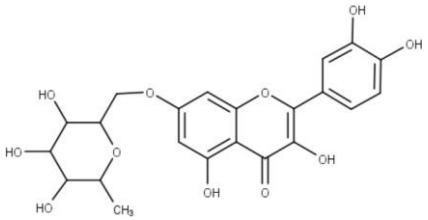
Quercetin-3-rutinoside



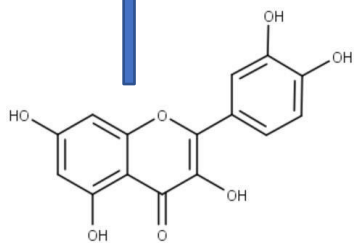
Myricetin-3-glucuronide



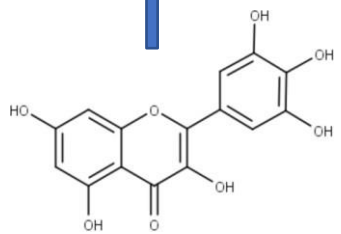
Kaempferol-3-glucoside



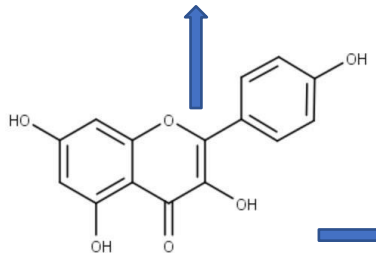
Quercetin-7-rhamnoside



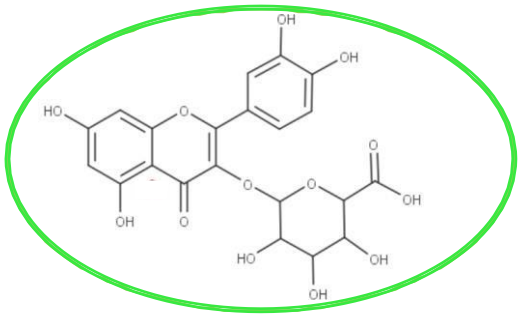
Quercetin



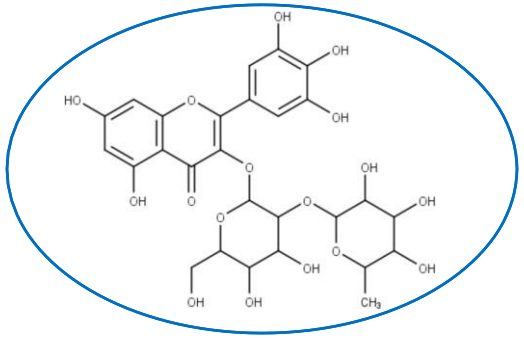
Myricetin



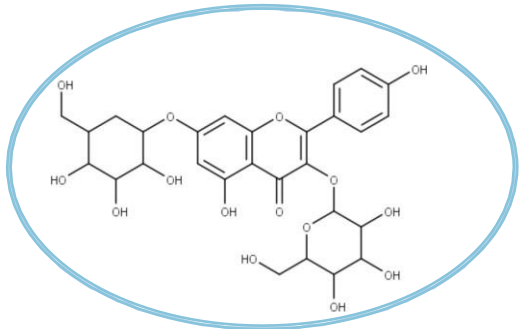
Kaempferol



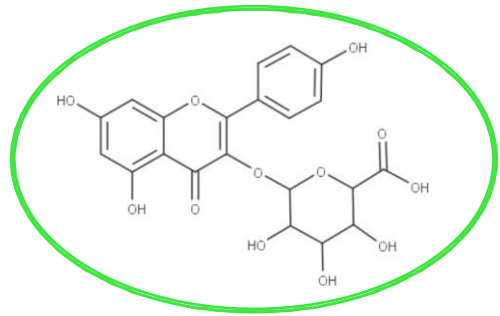
Quercetin-3-glucuronide



Myricetin-3-neohesperidoside



Kaempferol-3,7-diglucoside



Kaempferol-3-glucuronide

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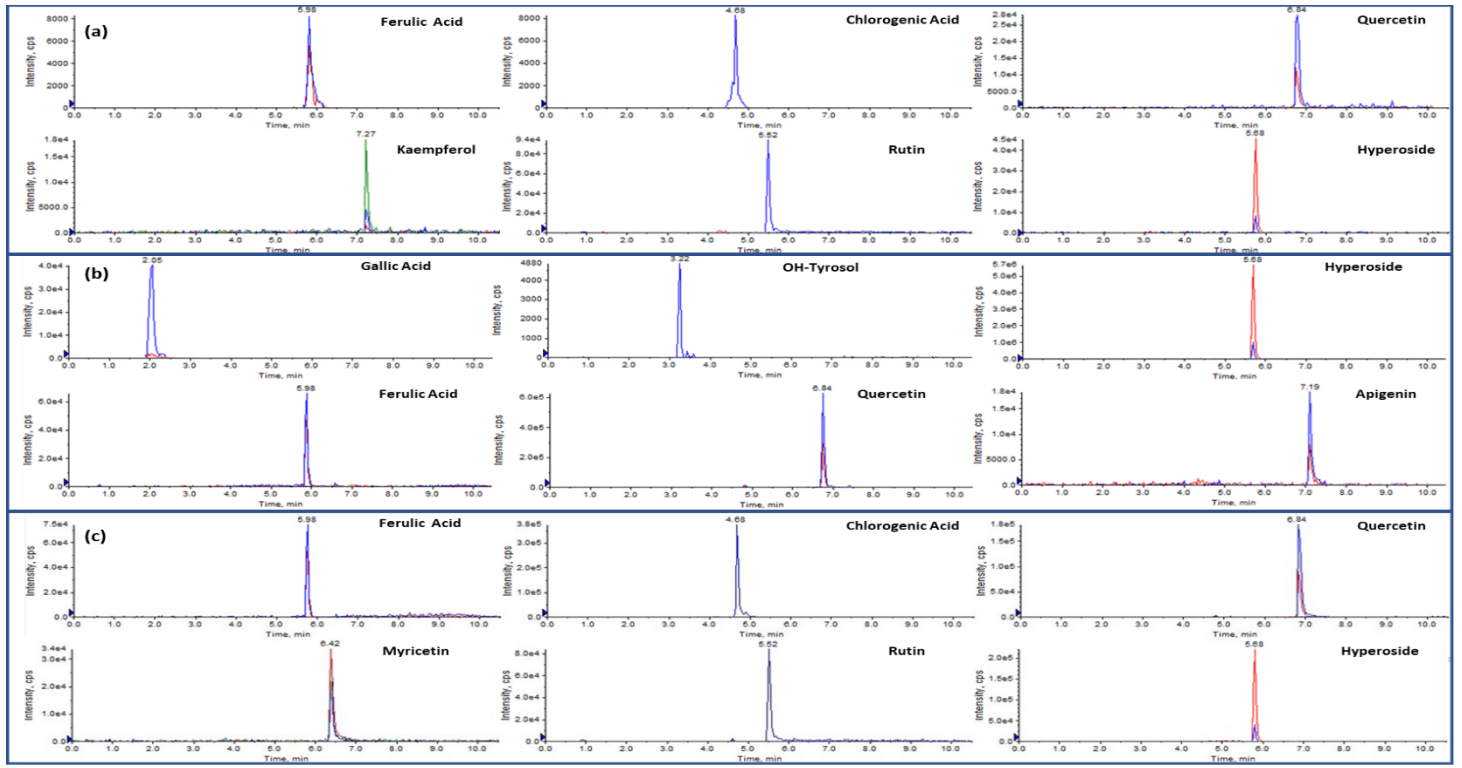
Targeted analysis: MRM



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Journal of Chromatography A

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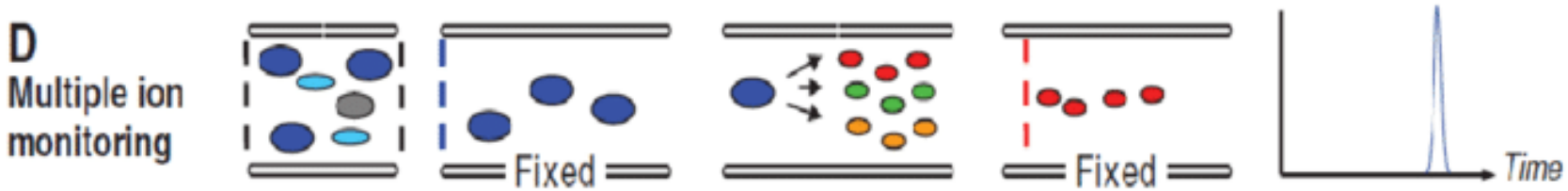
Targeted and semi-untargeted determination of phenolic compounds in plant matrices by high performance liquid chromatography-tandem mass spectrometry

Eleonora Oliva, Eduardo Viteritti, Federico Fanti, Fabiola Eugelio, Alessia Pepe, Sara Palmieri, Manuel Sergi*, Dario Compagnone

University of Teramo, Faculty of Bioscience and Technology for Food, Agriculture and Environment, Teramo, Italy

Multi-Reaction-Monitoring (MRM): quadrupole Q1 is fixed on the pseudomolecular ion and quadrupole Q3 on the fragment ion. In this mode, MS-MS is used for quantitative assays as a true detector.
In MS/MS, the yield of ions reaching the detector is lower than in single quadrupole due to the greater manipulation they undergo during transmission; however, because of the greater ion selection, the S/N ratio is undoubtedly higher and thus the sensitivity.



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Targeted analysis: UHPLC MS/MS parameters

ID	t_R (min)	Q_1 (amu)	DP (V)	EP (V)	Q_3 (amu)	CE (V)	CXP (V)	ID	t_R (min)	Q_1 (amu)	DP (V)	EP (V)	Q_3 (amu)	CE (V)	CXP (V)
Gallic Acid	2.05	168.9	-70.00	-10.50	124.9	-21.00	-9.00	Isoquercetin	5.69	463.0	-100.00	-9.00	300.9	-33.00	-11.00
					96.9	-26.00	-6.00						270.9	-58.00	-10.00
OH-Tyrosol	3.22	152.9	-75.00	-7.00	122.9	-22.00	-8.00	Ferulic Acid	5.96	192.8	-63.00	-11.00	134.0	-21.00	-8.00
					104.6	-30.00	-7.00						177.8	-18.00	-6.00
Protocatechuic Acid	3.95	152.8	-64.00	-12.00	108.90	-23.00	-6.00	Hesperidin	6.01	609.1	-130.00	-4.50	300.9	-38.00	-11.00
					53.0	-34.00	-4.00						286.2	-60.00	-9.00
EGC	4.53	305.0	-98.00	-6.50	136.9	-36.00	-10.00	Rosmarinic Acid	6.20	359.0	-78.00	-4.50	160.8	-24.00	-7.00
					166.9	-28.50	-6.50						197.0	-24.50	-6.50
3/4-OH-Benzoic Acid	4.60	136.9	-12.00	-9.00	89.0	-15.50	-6.00	Oleuropein	6.20	539.2	-55.00	-10.00	275.0	-32.00	-9.00
					66.0	-30.00	-10.00						377.1	-24.00	-9.00
Tyrosol	4.61	137.0	-65.00	-9.00	119.0	-21.00	-8.50	o-Coumaric Acid	6.31	163.0	-50.00	-5.00	119.0	-19.00	-8.00
					106.9	-23.00	-5.50						117.0	-33.00	-7.00
Chlorogenic Acid	4.64	353.1	-60.00	-7.00	190.9	-25.50	-6.50	Sinapic Acid	6.35	222.9	-75.00	-11.00	164.0	-23.00	-6.00
					160.9	-35.00	-6.00						208.0	-22.00	-7.00
Epicatechin	4.73	288.9	-85.00	-8.00	245.0	-24.00	-9.00	Myricetin	6.42	316.9	-115.00	-5.00	150.9	-34.00	-6.00
					108.9	-38.50	-8.00						136.9	-36.00	-9.50
Caffeic Acid	5.05	179.0	-60.00	-10.00	135.0	-22.00	-8.00	Luteolin	6.79	284.9	-100.00	-8.00	150.9	-35.00	-11.00
					107.0	-32.00	-10.00						199.0	-35.00	-6.00
Vanillic Acid	5.06	167.1	-49.00	-11.00	107.9	-28.50	-5.00	Quercetin	6.84	300.9	-94.00	-10.00	151.0	-30.00	-6.00
					151.9	-19.50	-6.50						178.8	-26.00	-6.00
Catechin	5.10	289.0	-95.00	-5.00	245.0	-25.00	-8.00	Trans-Cinnamic Acid	6.92	147.2	-50.00	-8.00	102.9	-16.00	-4.00
					108.9	-32.50	-9.00						77.2	-30.00	-6.00
EGCG	5.19	457.2	-10.00	-7.00	168.8	-20.00	-17.00	Naringenin	6.95	271.0	-112.00	-5.00	150.9	-25.00	-7.00
					124.9	-40.00	-17.00						118.8	-37.00	-8.00
Siringic Acid	5.21	196.9	-57.00	-11.00	181.9	-19.00	-6.00	Isoxhanthoumol	7.06	353.1	-114.00	-10.00	233.1	-26.00	-8.00
					120.9	-22.00	-8.00						189.1	-35.00	-9.00
Orientin	5.35	447.1	-105.00	-11.00	356.9	-29.00	-11.00	Apigenin	7.19	268.9	-110.00	-4.00	116.9	-50.00	-9.00
					297.0	-43.00	-11.00						151.0	-34.00	-5.00
Rutin	5.52	609.2	-100.00	-6.00	300.9	-45.00	-11.00	Diosmetin	7.27	299.1	-90.00	-6.00	255.8	-40.00	-9.00
					254.7	-70.00	-9.00						150.9	-40.00	-10.00
p-Coumaric Acid	5.67	162.8	-60.00	-5.00	119.0	-20.00	-9.00	Kaempferol	7.27	285.0	-105.00	-7.00	228.8	-40.00	-9.00
					116.7	-42.00	-9.00						159.0	-42.00	-6.00
Hyperoside	5.68	462.9	-110.00	-5.00	300.9	-48.00	-10.00	Xhanthoumol	9.48	353.0	-120.00	-8.00	164.9	-42.00	-9.00
					299.900	-40.00	-11.00						203.0	-45.00	-8.00

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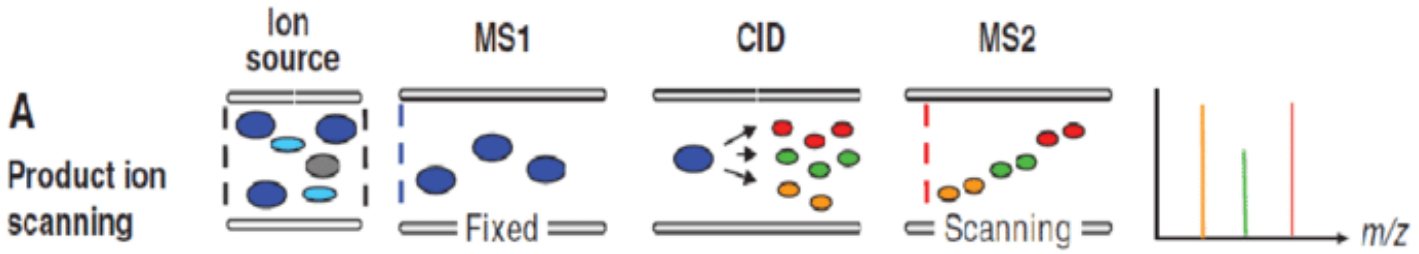
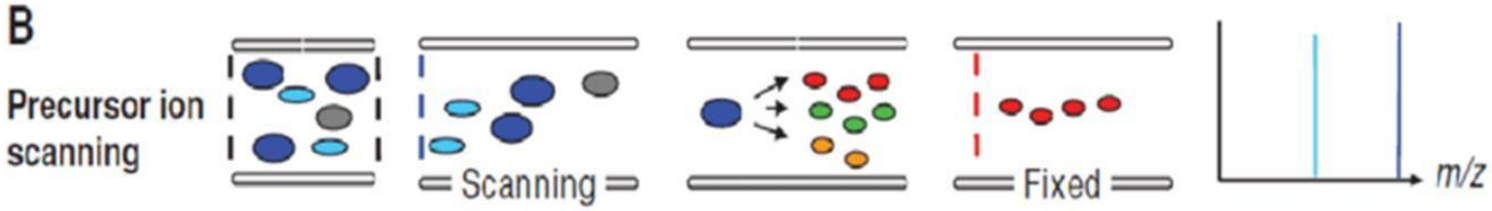
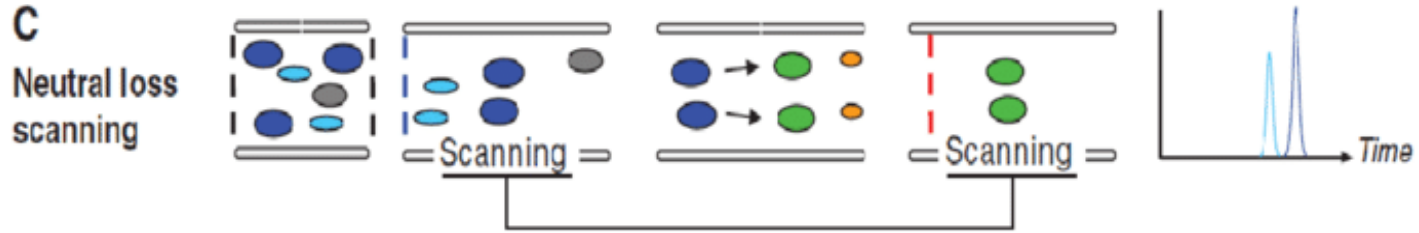
Product Ion Scan: selected by the first mass analyzer (quadrupole or sector system) being appropriately fragmented in the collision cell and the fragments thus generated (called "daughter ion," but nowadays called "product ion") being characterized by a second mass analyzer. (Q1 fixed, Q2 collision cell, Q3 scanning).



Precursor Ion Scan: reverses the previous (Q1 scan on precursor ions, Q3 fixed on the common fragment), exploring which precursor ions produce by cleavage a particular and specific fragment. *Used for the identification of homologues and metabolites.*



Neutral Loss Scan: allows exploration with the first quadrupole (Q1 in scanning) of those precursor ions that in cleavage can produce ionic fragments with different masses due to loss of the same neutral fragment. *Used for the identification of homologues and metabolites.*



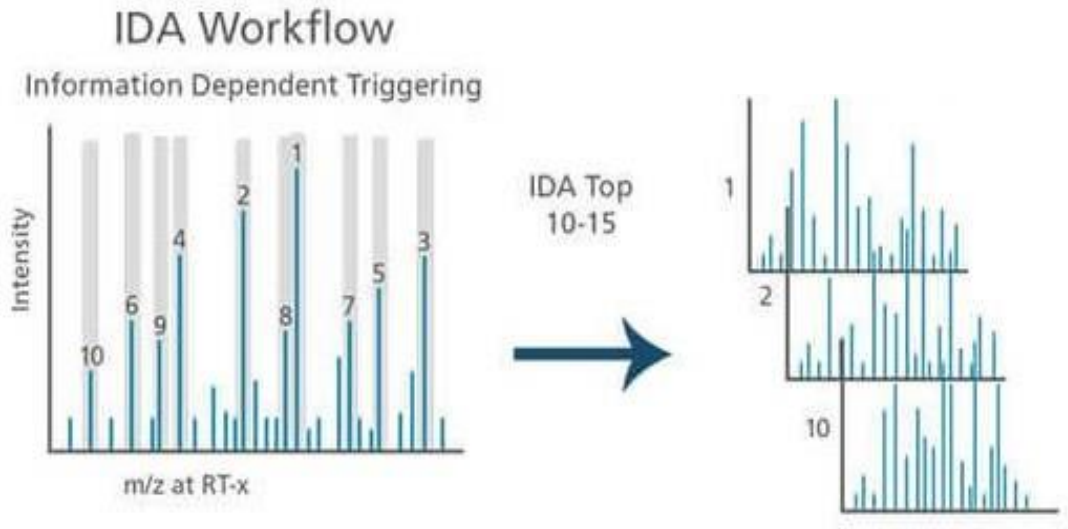
MS³ : The isolated ion is further fragmented into the linear ionization trap and the resulting product ions are scanned out of the trap. All the resulting product ions are transmitted to the linear ionization trap (lit), where only one product ion will be isolated.

Enhanced Product Ion Scan: is the standard method for performing MS/MS on the QTRAP instrument. It is actually a hybrid QqQ/LIT scan because it combines the capabiliti of the QqQ with the LIT to achieve a performance level not possible with either analyzer alone. This provides *extremely high sensitivity*, high quality MS/MS data because the fragment ions are captured and analyzed in the LIT.

IDA Information Dependent Acquisition (IDA): is an acquisition method that analyzes data during acquisition. IDA can be used to change the experimental conditions depending on the results of the analysis. These real-time changes are controlled by criteria set in the acquisition method, including:

- Ion intensity and charge state.
- Inclusion and exclusion lists.
- Isotope pattern.

Being able to optimize data acquisition settings while the data is being acquired enables to keep both the sample and working time on an instrument.



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Targeted and Semi-untargeted strategies

- MRM
- PREC, NL, MS^3
- NL-IDA-EPI

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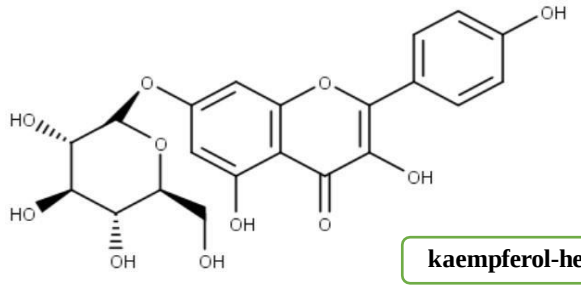
Semi-untargeted mode

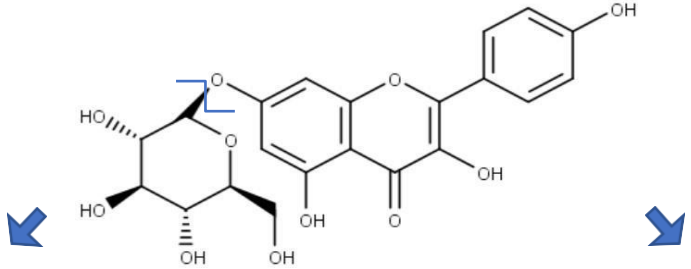
Name:
Luteolin-hexoside/Kaempferol-hexoside

Molecular Weight:
448.4 g/mol

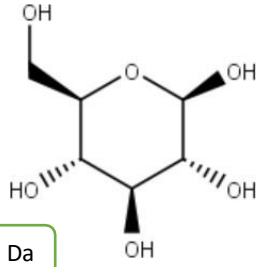
T_R:
4,79



kaempferol-hexoside



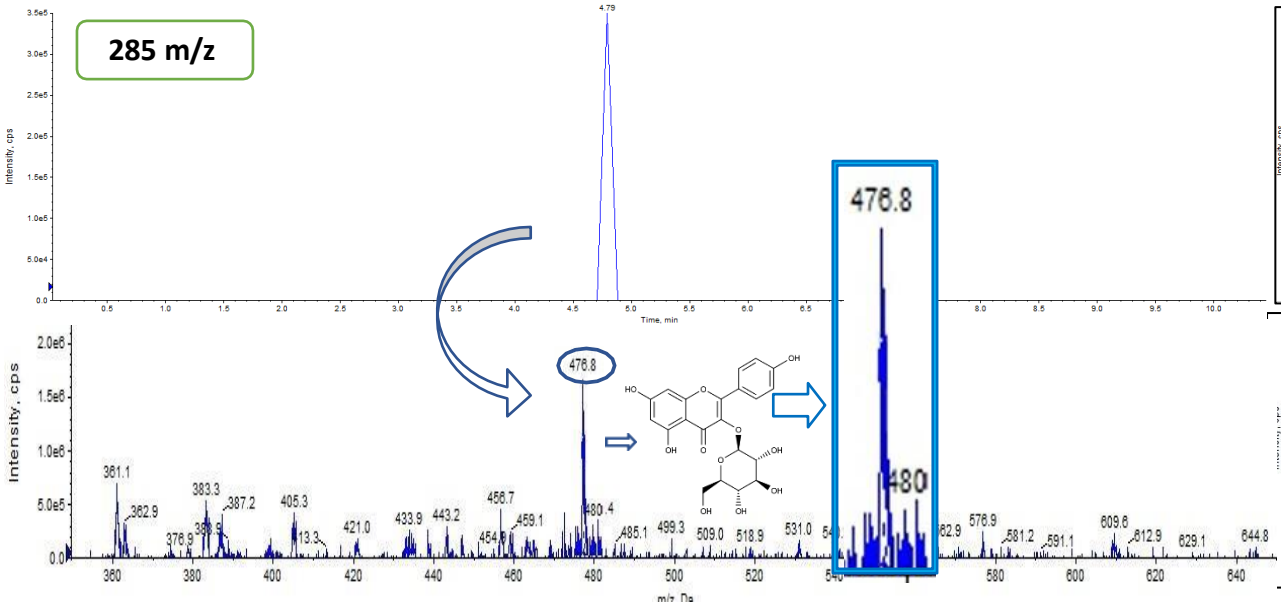
Hexoside 180,157 g/mol



MW-H_2O=162 Da

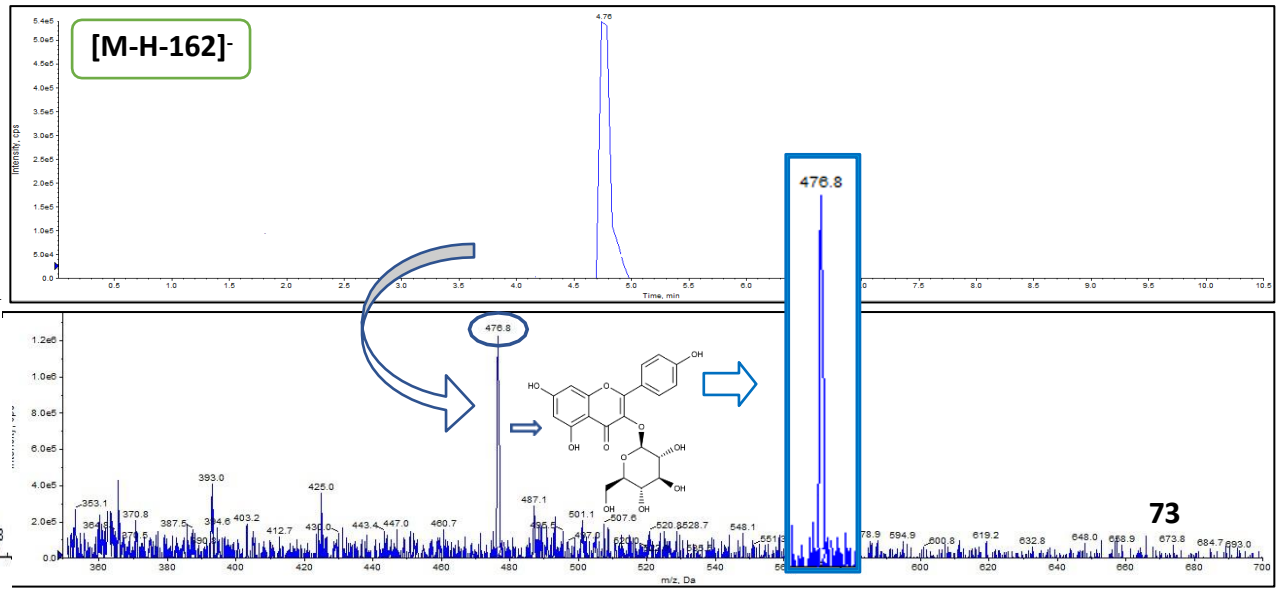
Precursor ion scan

285 m/z

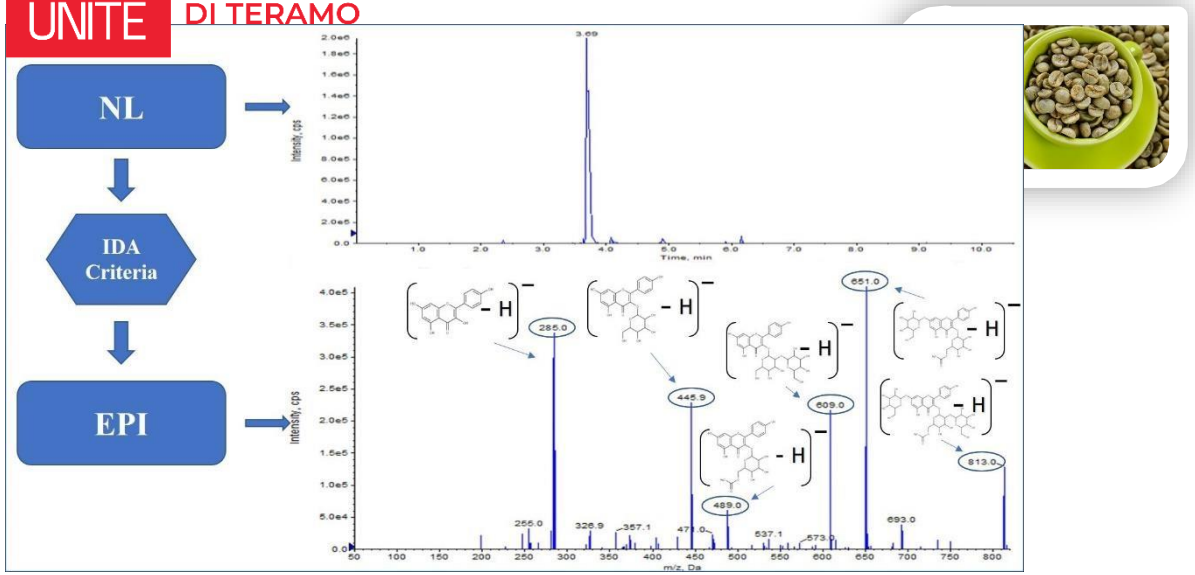


Neutral loss scan

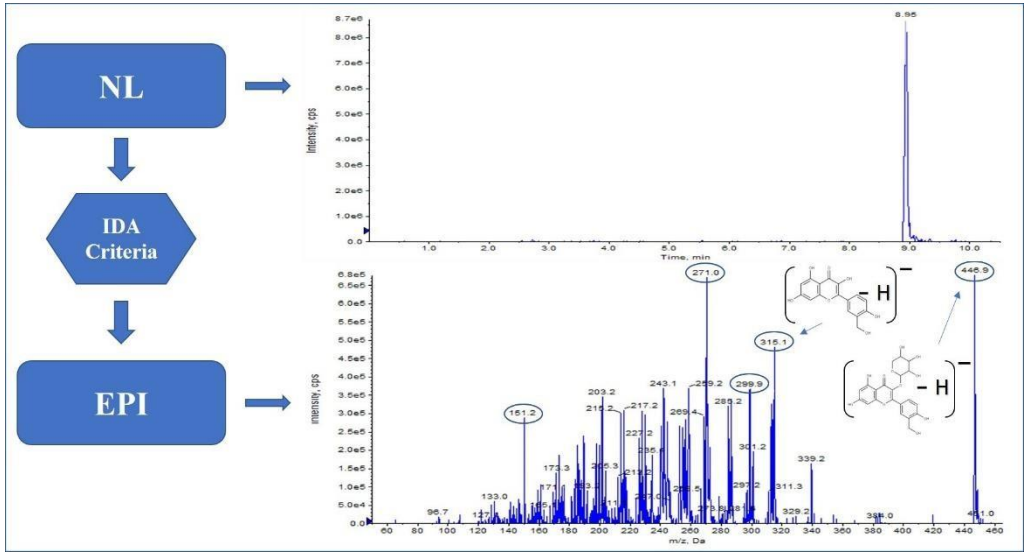
[M-H-162]-



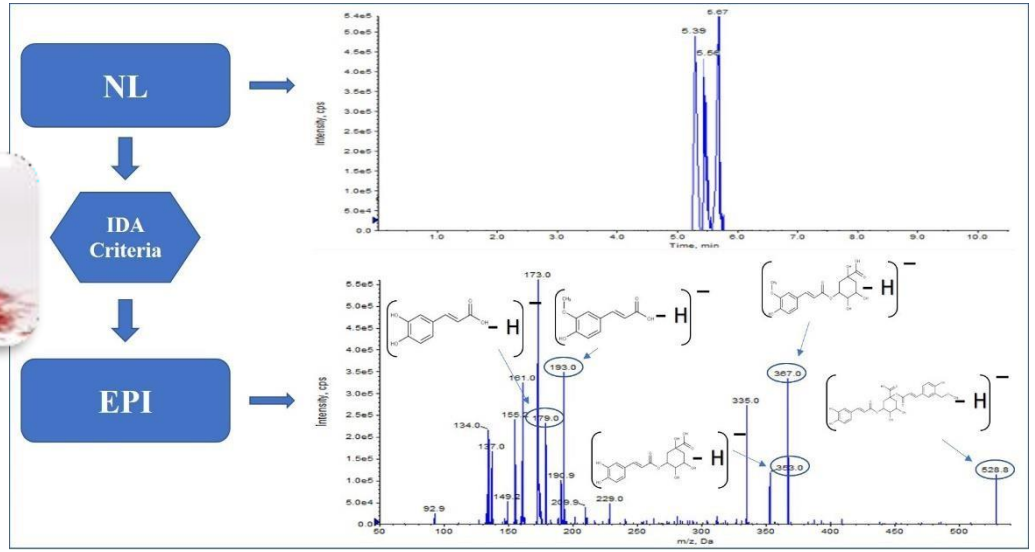
Semi-untargeted: NL-IDA-EPI Results



Feruloyl-5-caffeoylquinic acid fragmentation pattern in green coffee



Isorhamnetin-xyloside pattern in hop



Kaempferol-glucosyl-(1->2)-(6"-acetylgalactoside)-hexoside fragmentation pattern in Saffron

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Semi-untargeted: NL-IDA-EPI Results

Green coffee

t_R	m/z	NL (Da)	Main fragments				Compound identification
3.34	352,9	$[M-H-162]^-$ or $[M-H-191]^-$	135	161	179	191	Caffeoylquinic acid (I)
3.76	352,9	$[M-H-162]^-$ or $[M-H-191]^-$	135	161	179	191	Caffeoylquinic acid (II)
3.77	451,0	$[M-H-162]^-$	109	125	245	289	Catechin-hexoside
3.88	352,9	$[M-H-162]^-$ or $[M-H-191]^-$	135	161	179	191	Caffeoylquinic acid (III)
4.37	366,9	$[M-H-162]^-$ or $[M-H-176]^-$	134	135	179	193	Feruloylquinic acid
4.58	337,0	$[M-H-146]^-$ or $[M-H-162]^-$	117	119	161	191	p-Coumaroylquinic acid
4.90	514,9	$[M-H-162]^-$ or $[M-H-191]^-$	161	179	191	353	Dicaffeoylquinic acid (I)
5.13	514,9	$[M-H-162]^-$ or $[M-H-191]^-$	161	179	191	353	Dicaffeoylquinic acid (II)
5.39	529,0	$[M-H-162]^-$ or $[M-H-176]^-$or $[M-H-191]^-$	179	193	353	367	Feruloyl-5-caffeoylquinic acid (I)
5.56	529,0	$[M-H-162]^-$ or $[M-H-176]^-$or $[M-H-191]^-$	179	193	353	367	Feruloyl-5-caffeoylquinic acid (II)
5.67	529,0	$[M-H-162]^-$ or $[M-H-176]^-$or $[M-H-191]^-$	179	193	353	367	Feruloyl-5-caffeoylquinic acid (III)
5.90	542,9	$[M-H-162]^-$ or $[M-H-176]^-$	134	179	193	367	Diferuloylquinic acid (I)
6.13	542,9	$[M-H-162]^-$ or $[M-H-176]^-$	134	179	193	367	Diferuloylquinic acid (II)
6.30	542,9	$[M-H-162]^-$ or $[M-H-176]^-$	134	179	193	367	Diferuloylquinic acid (III)

Saffron

t_R	m/z	NL (Da)	Main fragments				Compound identification
3.27	787,0	$[M-H-146]^-$ or $[M-H-162]^-$ or $[M-H-308]^-$	317	463	479	625	Myricetin-rutinoside-hexoside
3.38	771,0	$[M-H-162]^-$	255	285	446	609	Kaempferol-sophoroside-hexoside
3.47	755,0	$[M-H-146]^-$ or $[M-H-162]^-$ or $[M-H-308]^-$	255	285	446	593	Kaempferol-rutinoside-hexoside
3.69	813,0	$[M-H-162]^-$ or $[M-H-308]^-$	446	489	609	651	Kaempferol-glucosyl-(6''-acetylgalactoside)-hexoside
3.77	314,6	$[M-H-162]^-$ or $[M-H-191]^-$	53	109	135	153	Protocatechuic acid-hexoside
3.77	609,0	$[M-H-162]^-$	159	255	285	446	Kaempferol-sophoroside
4.12	651,0	$[M-H-146]^-$ or $[M-H-162]^-$ or $[M-H-308]^-$	151	179	447	489	Quercetin-O-(6''-acetyl-galactoside)-O-rhamnoside
4.21	448,5	$[M-H-162]^-$	213	231	259	287	Cyanidin-hexoside
4.21	624,8	$[M-H-162]^-$	151	179	301	463	Quercetin-sophoroside
4.29	639,0	$[M-H-162]^-$	151	271	315	477	Isorhamnetin-sophoroside
4.41	609,0	$[M-H-146]^-$ or $[M-H-162]^-$ or $[M-H-308]^-$	151	179	271	301	Quercetin-rutinoside
4.64	623,0	$[M-H-146]^-$ or $[M-H-162]^-$ or $[M-H-308]^-$	151	271	300	315	Isorhamnetin-rutinoside
4.89	651,0	$[M-H-162]^-$	255	285	446	489	Kaempferol-(6''-acetyl-glucoside)-glucoside

Hop

t_R	m/z	NL (Da)	Main fragments				Compound identification
3.34	352,9	$[M-H-162]^-$ or $[M-H-191]^-$	135	161	179		
3.76	352,9	$[M-H-162]^-$ or $[M-H-191]^-$	135	161	179		
3.88	352,9	$[M-H-162]^-$ or $[M-H-191]^-$	135	161	179		
4.60	609,0	$[M-H-162]^-$ or $[M-H-308]^-$	151	179	271		
4.77	592,8	$[M-H-162]^-$ or $[M-H-308]^-$	133	159	229		
5.31	463,0	$[M-H-162]^-$	151	179	271		
7.16	396,8	$[M-H-162]^-$	173	207	281		
7.44	396,8	$[M-H-162]^-$	173	207	281		
7.65	396,8	$[M-H-162]^-$	173	207	281		
8.49	433,2	$[M-H-132]^-$	151	179	271		
8.95	446,9	$[M-H-132]^-$	151	271	300		
9.84	311,0	$[M-H-162]^-$ or $[M-H-191]^-$	103	135	179	249	Caffeoyltartaric acid

molecules

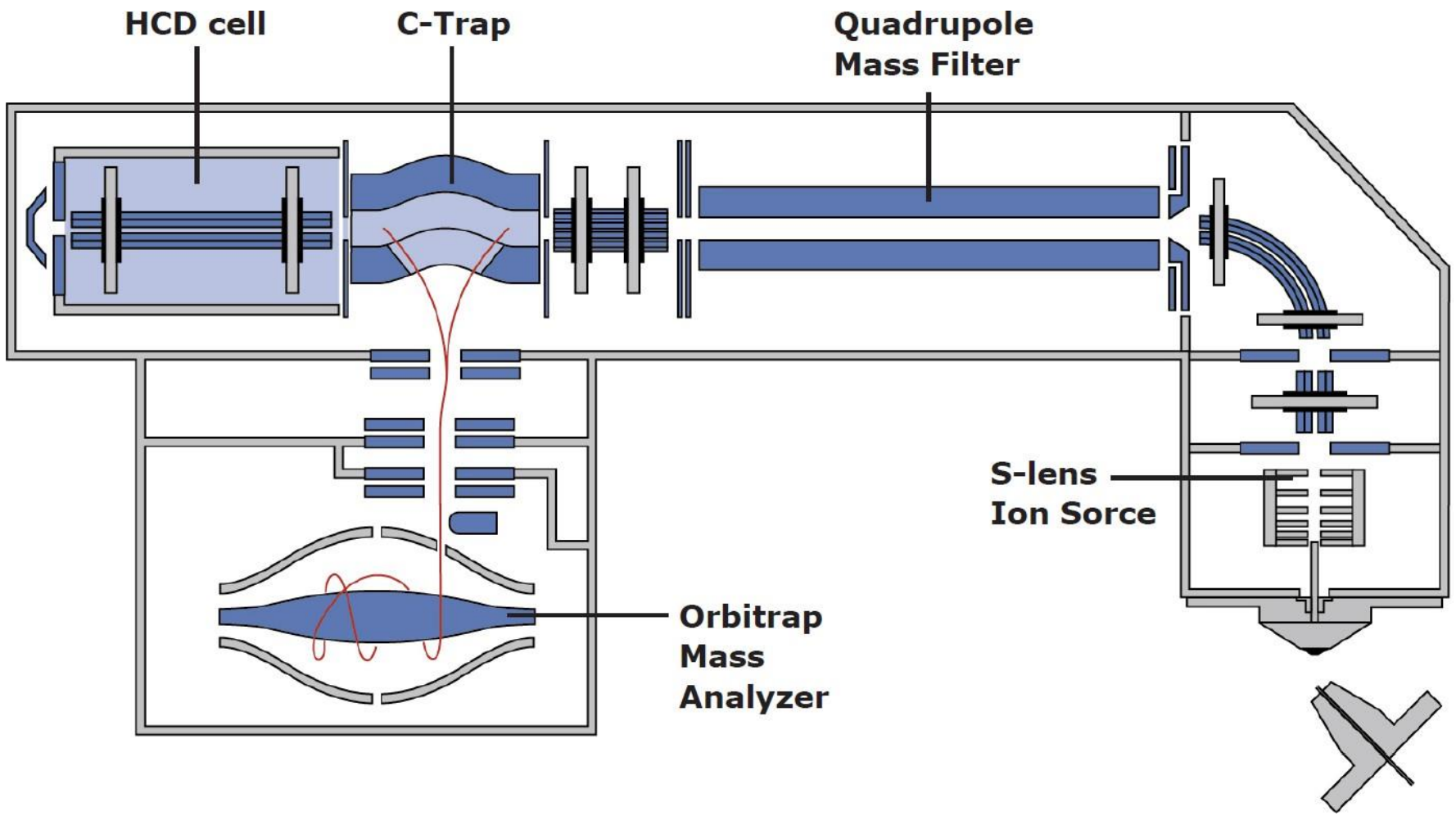
MDPI

Article

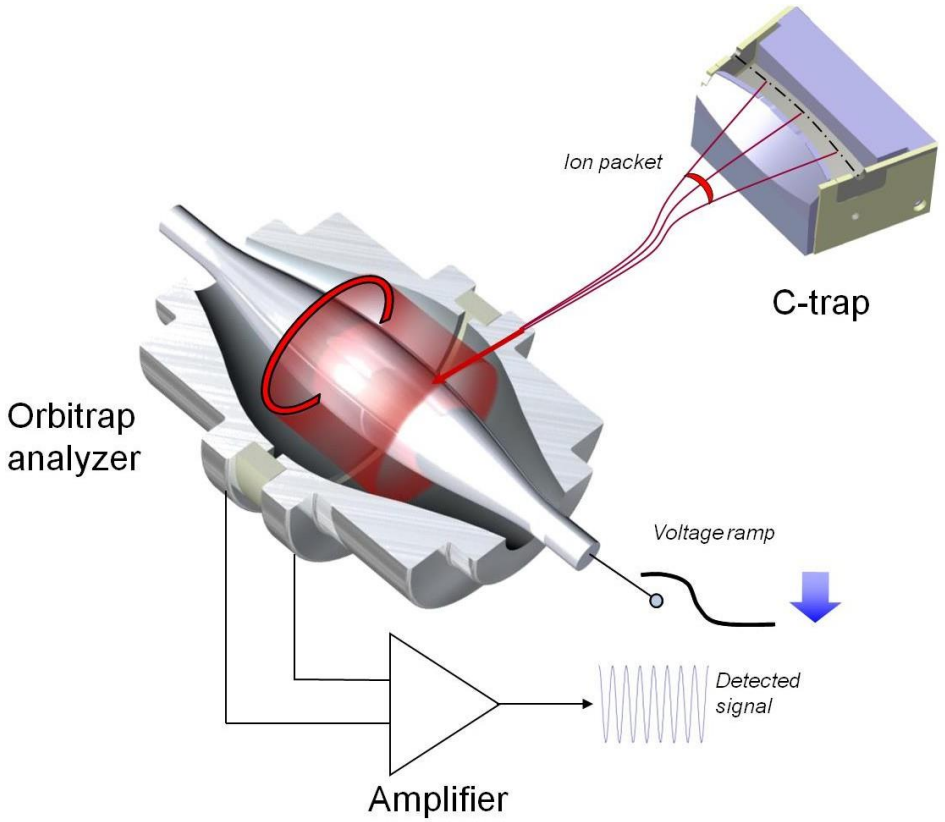
Predictive Multi Experiment Approach for the Determination of Conjugated Phenolic Compounds in Vegetal Matrices by Means of LC-MS/MS

Eleonora Oliva, Federico Fanti *, Sara Palmieri, Eduardo Viteritti, Fabiola Eugelio, Alessia Pepe, Dario Compagnone and Manuel Sergi

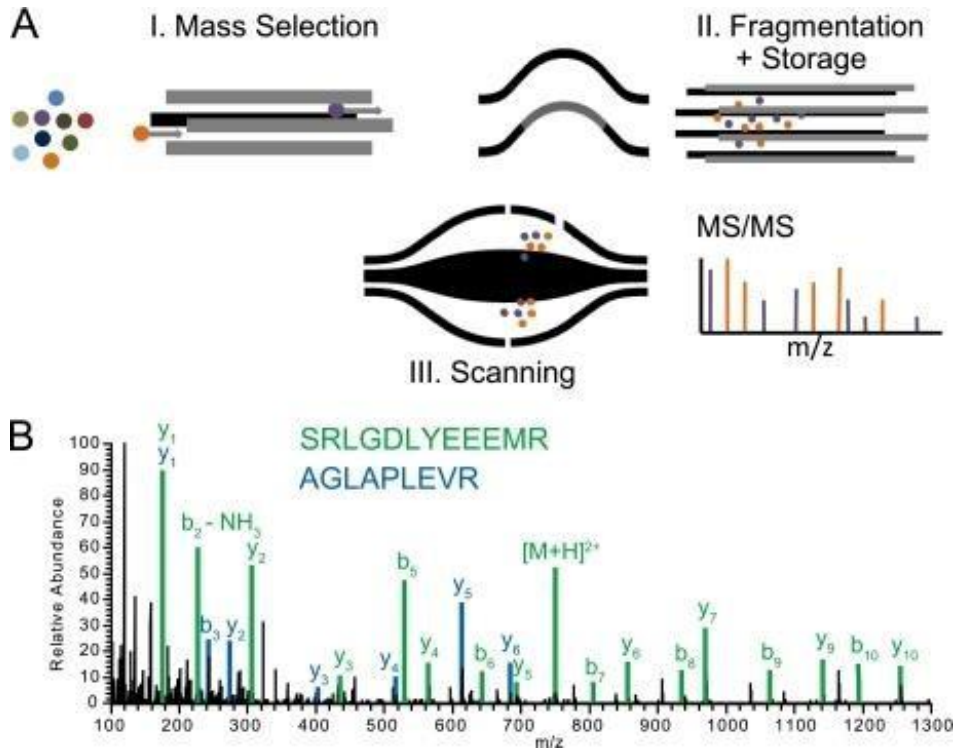
ORBITRAP MASS ANALYZER (untargeted analysis)

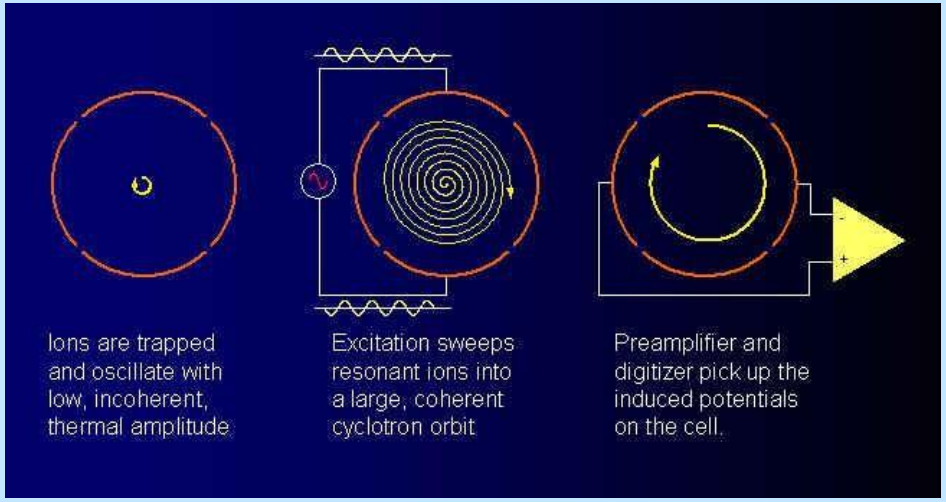


ORBITRAP MASS ANALYZER



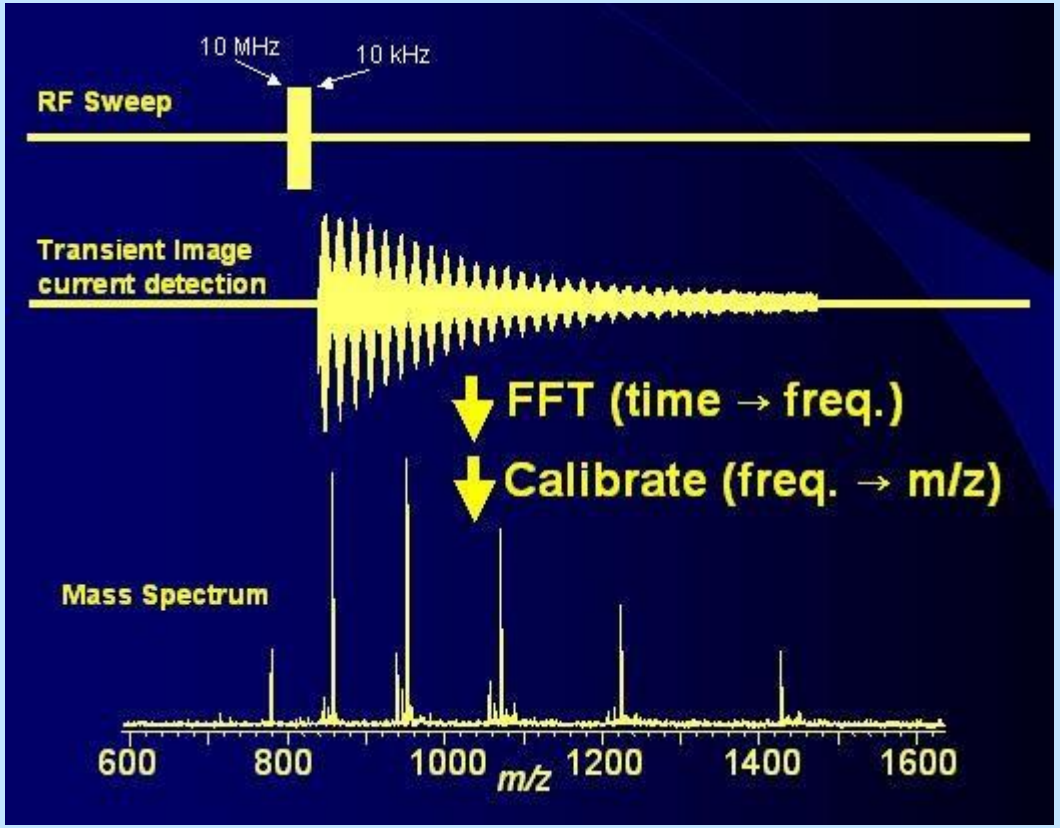
high resolution MS detector





Fourier transform FT-MS
Or FT-ICR-MS (ion ciclotron resonance

Magnetic fiels is sweeped to get all the ions trapped



Untargeted analysis of pesticides

combine high resolution and accuracy mass spectrometry (fourier transform FT-MS) techniques combined with new generation chemical software for detecting and identifying contaminants in food commodities

- **untarget approach in routine analysis of contaminants and metabolites in food**
- **Increase number and classes of compounds to be searched**
- **Satisfy the requests of EU about food safety**

Stone fruits commodity ***(DOCUMENT SANTE 11945/2015)***

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Untargeted approach

- **Extraction (non-specific wide range of polarity)**

)

- **Acquisition of HS mass spectra**

- **Data mining using specific softwares**

- Interpretration of the spectra and identification of the molecules combining parameters as mass accuracy, isotopic clusters, fragmented ions using specific databases .

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Sample treatment

comparing extractions and potentially interfering compounds

1) **MeOH dilution and injection**

2) **MeCN dilution and injection**

3) **QuEChERS (UNIEN15662) no PSA.**

4) **QuEChERS (UNIEN15662) + PSA**

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UHPLC-HRAMS analysis

- ✓ **Chromatography: Accucore aQ column (100 mm x 2.1 mm)**
  mobile phase A) 5 mM ammonium formiate in water
  B) mM ammonium formiate 5 in Methanol
- ✓ **Ion Source: ESI positive ion mode**
- ✓ **mass spectrometer:Q-Exactive (Orbitrap)**

"exclusion list" **containing 70 compounds in a mass range 100 -700 Da .**

UHPLC-HRAMS analysis

Acquisition of 4 spectra for each sample and "sample blank"

- **Acquisition and storage of the Data for identification of parental ions**

N°3 run acquisition in FullScan-All Ion Fragmentation

FullScan: Parental Ions resolution 140000 FWHM (3° decimal after MW) -range m/z 110-950

AIF: resolution 35000 FWHM -range m/z 63- 700 Da.

- **Structural analysis of the peaks over a fixed threshold**

N°1 run acquisition Data Dependent Scan (ddMS^2)

FullScan: Parental Ions resolution Ion 70000 FWHM -range m/z 110-950

ddMS^2: fragments resolution 17500 FWHM (mass accuracy ≤ 2 ppm).

Possibility to make retrospective analysis !

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statistical analysis and databases

- **Thermo Xcalibur™ 3.1 Software**
- **Compound Discoverer 2.0**
- **Aggregated Computational Toxicology Resource (ACToR)**

EPA Toxcast; FDA UNII - NLM; FooDB; Pesticide Common Names; PubChem, Chemistry World from the Royal Society of Chemistry

R. Judson et all. Appl. Pharmacol., 233 (2008) 7-13.

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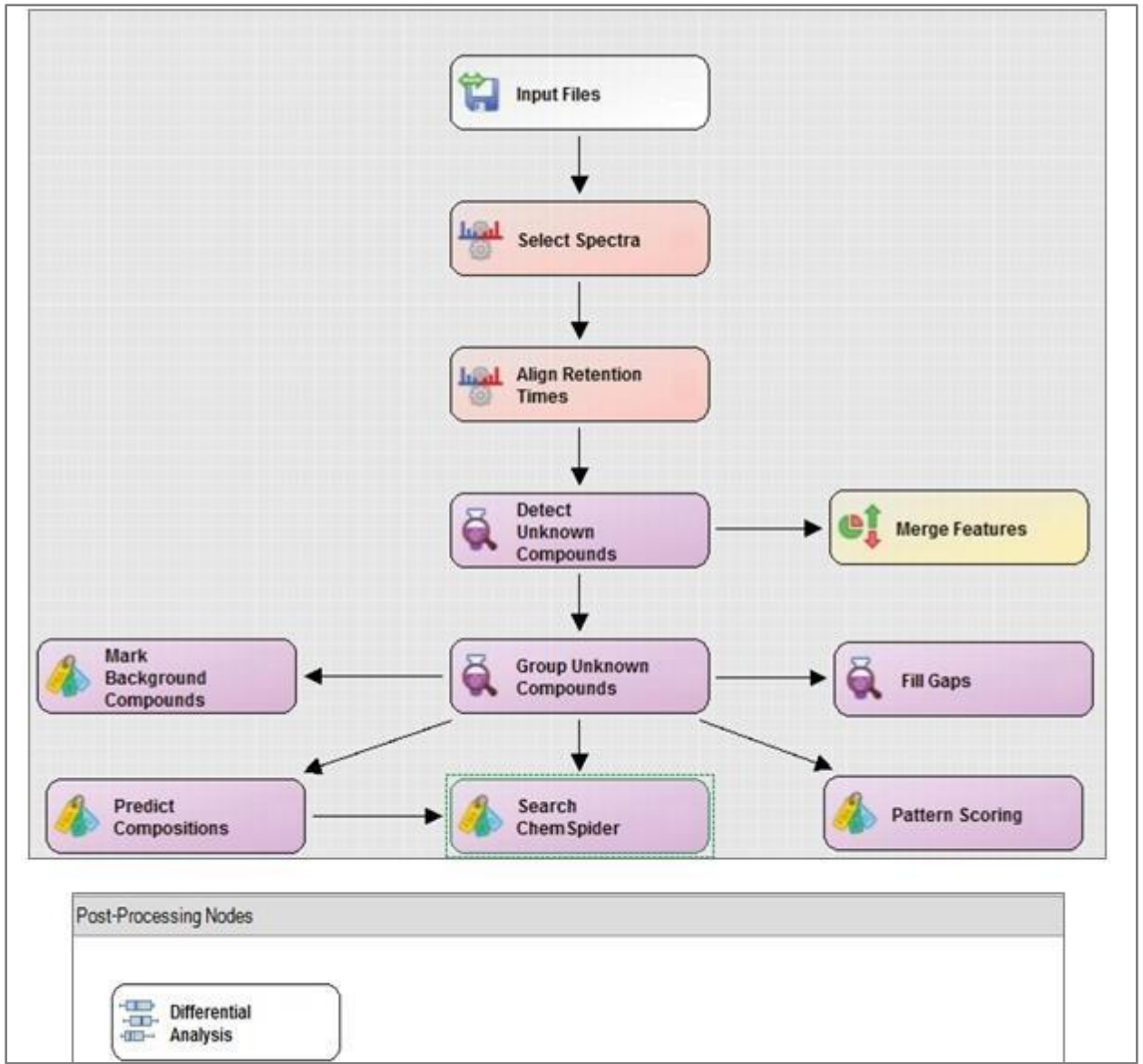
data treatment

Compoud discoverer Workflow



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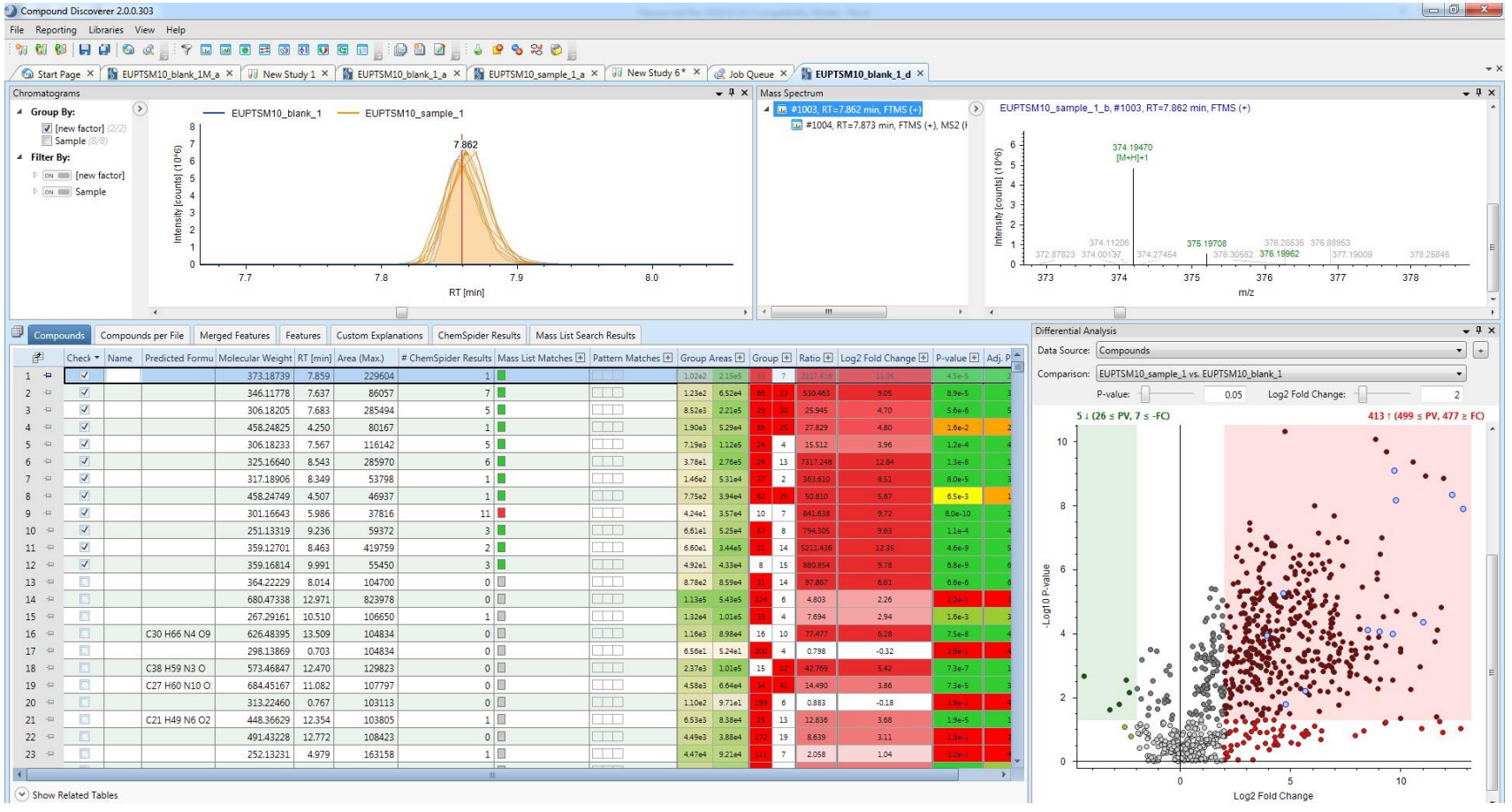
differential analysis

Unknown sample/Blank sample



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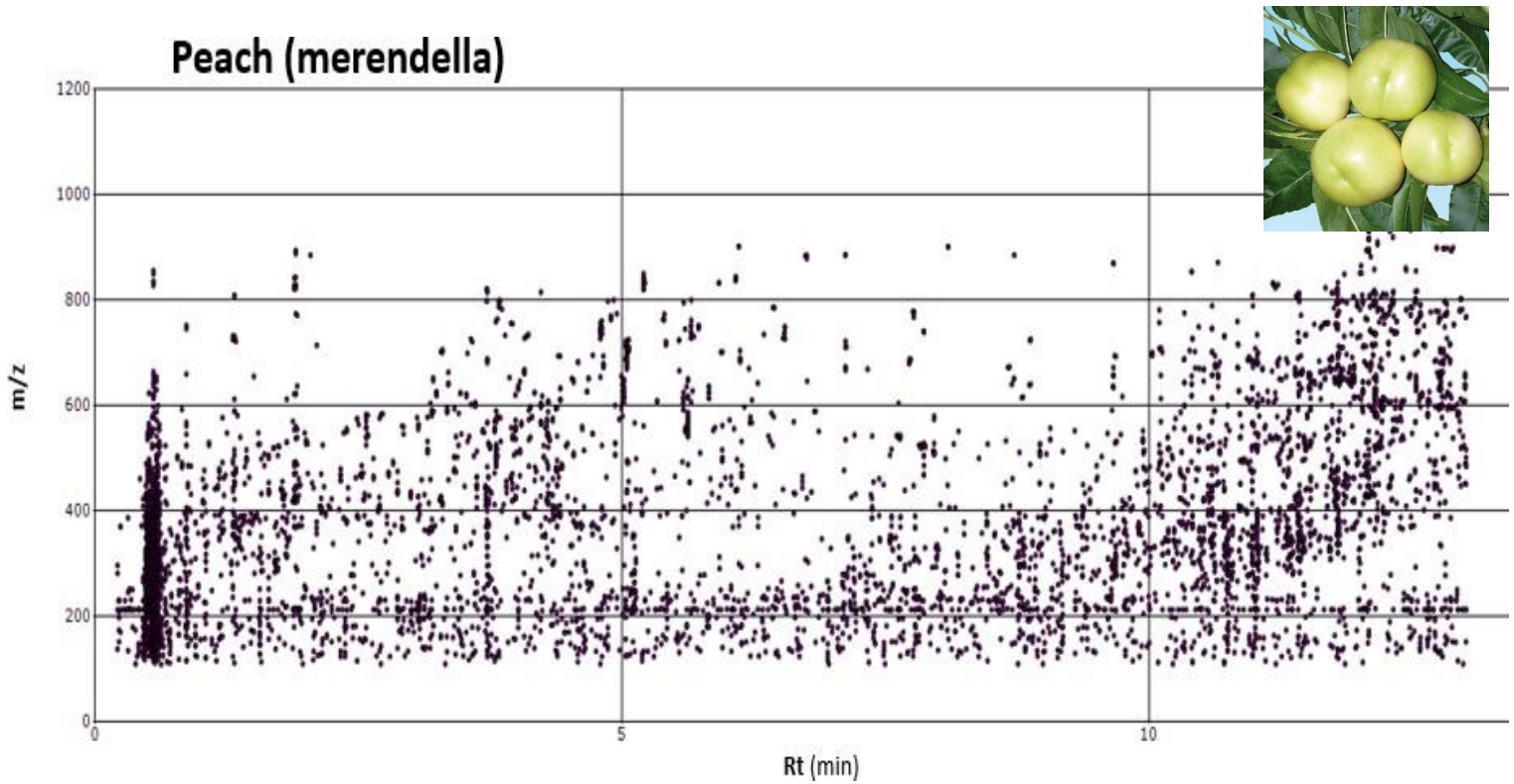

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Extraction protocol number molecules detected

Matrix	Molecular components			
	MeOH	ACN	ACN-QuEChERS without clean-up	ACN-QuEChERS with clean-up
Plum (White)	3716	3324	3904	3107
Plum (Red)	3729	3972	5409	4587
Apricot (Reale)	4050	5919	6235	4581
Apricot (Goldrich)	4562	6304	6639	4874
Peach (nettarine)	4993	5517	6383	5035
Peach (merendella)	6712	8660	9732	7287

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«Dilute-and-shoot» Acetonitrile

Compound	Molecular Formula	Molecular Weight	m/z	CSID	MeOH	ACN	ACN-QuEChERS without clean-up	ACN-QuEChERS with clean-up
Fenamiphos (*)	$C_{13}H_{22}NO_3PS$	303.357	304.11308	28827	RD	RD	RD	RD
Fenamiphos sulfone (*)	$C_{13}H_{22}NO_5PS$	335.356	336.10290	33142	RD	RN	RD	RN
Fenamiphos sulfoxide (*)	$C_{13}H_{22}NO_4PS$	319.357	320.10800	33141	RD	RD	RD	NR
Fosthiazate (*)	$C_9H_{18}NO_3PS_2$	283.348	284.04385	82856	RD	RD	RD	RD
Propamocarb (*)	$C_9H_{20}N_2O_2$	188.267	189.15975	30114	RD	RD	RD	RD
Malathion (*)	$C_{10}H_{19}O_6PS_2$	338.358	331.04334	3864	RD	RD	RD	RD
Thiamethoxam (*)	$C_8H_{10}ClN_5O_3S$	291.715	292.02656	96828	RD	RD	RD	RD
Haloxyfop-P (*)	$C_{15}H_{11}ClF_3NO_4$	361.700	362.03980	395627	RD	RD	RD	RD
Methiocarb (*)	$C_{11}H_{15}NO_2S$	225.307	226.08963	15417	RD	RD	RD	RD
Methiocarb-sulfone (*)	$C_{11}H_{15}NO_4S$	257.306	258.07950	15729	RD	RD	RN	RN
Methiocarb-sulfoxide (*)	$C_{11}H_{15}NO_3S$	241.307	242.08450	16568	RD	RD	RD	NR
Myclobutanil alcohol metabolite (**)	*$C_{15}H_{17}ClN_4O$*	*305.116*	*305.11637*	*164596*	*RD*	*RD*	*RD*	*NR*
Chlorpyrifos (*)	$C_9H_{11}Cl_3NO_3PS$	350.586	349.93356	2629	RD	RD	RD	RD
Acephate (*)	$C_4H_{10}NO_3PS$	183.166	184.01918	1905	RD	RD	RD	RD
Cymoxanil (*)	$C_7H_{10}N_4O_3$	198.179	199.08290	4514714	RD	RD	RD	NR

RD Detected and quantified

RN detected and not quantified

NR non detected and non quantified

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Validation approach

Data compared Versus already validated method

55 compounds detected and identified by the software using the operating conditions selected

No false positives, false negatives < 5%

By comparison with targeted methods

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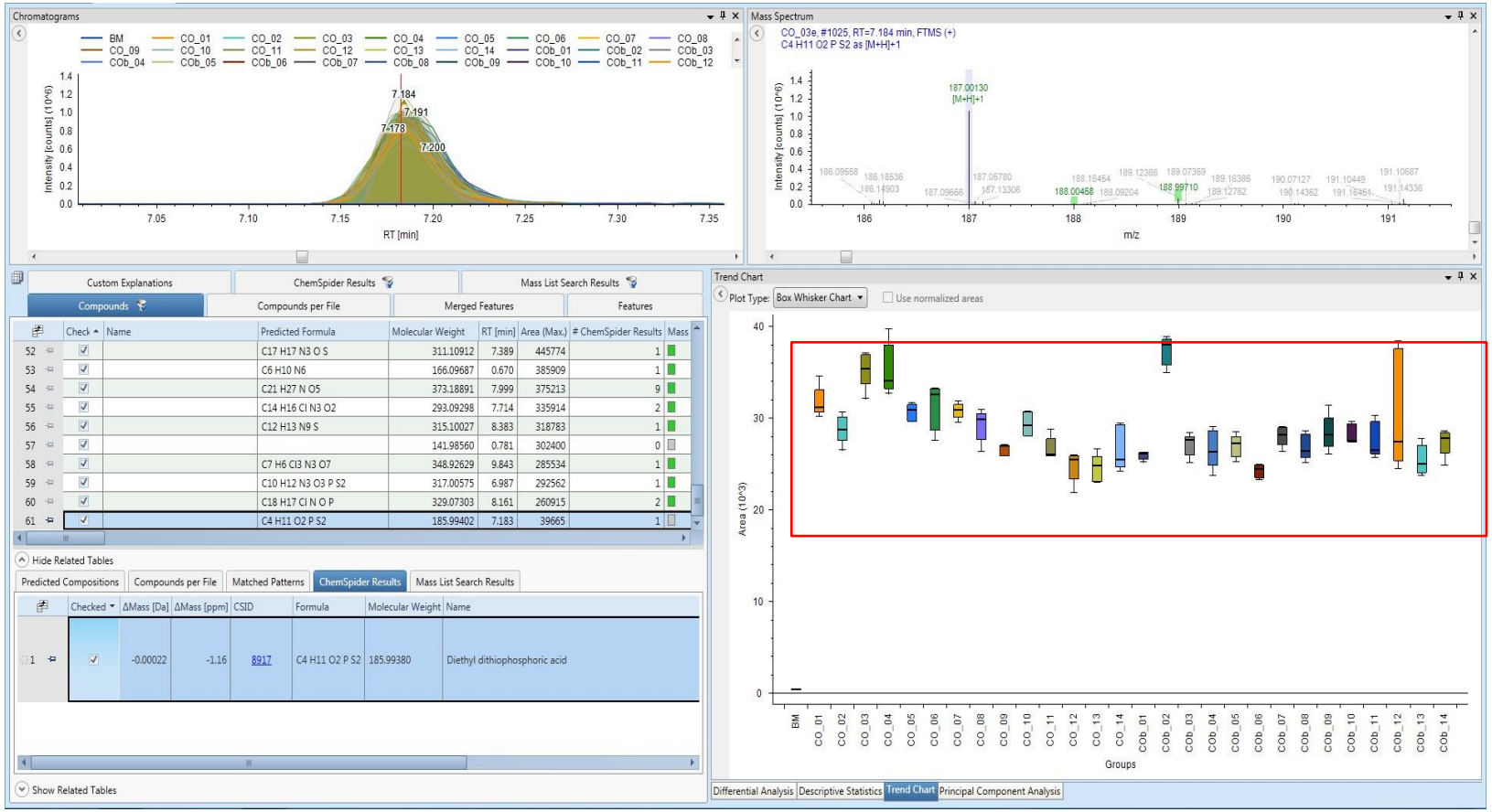
Identification of degradation products



Identification of Diethyl dithiophosphoric acid ($C_4H_{11}O_2PS_2$) 3 chromatographic runs acquisitions in FullScan-AIF

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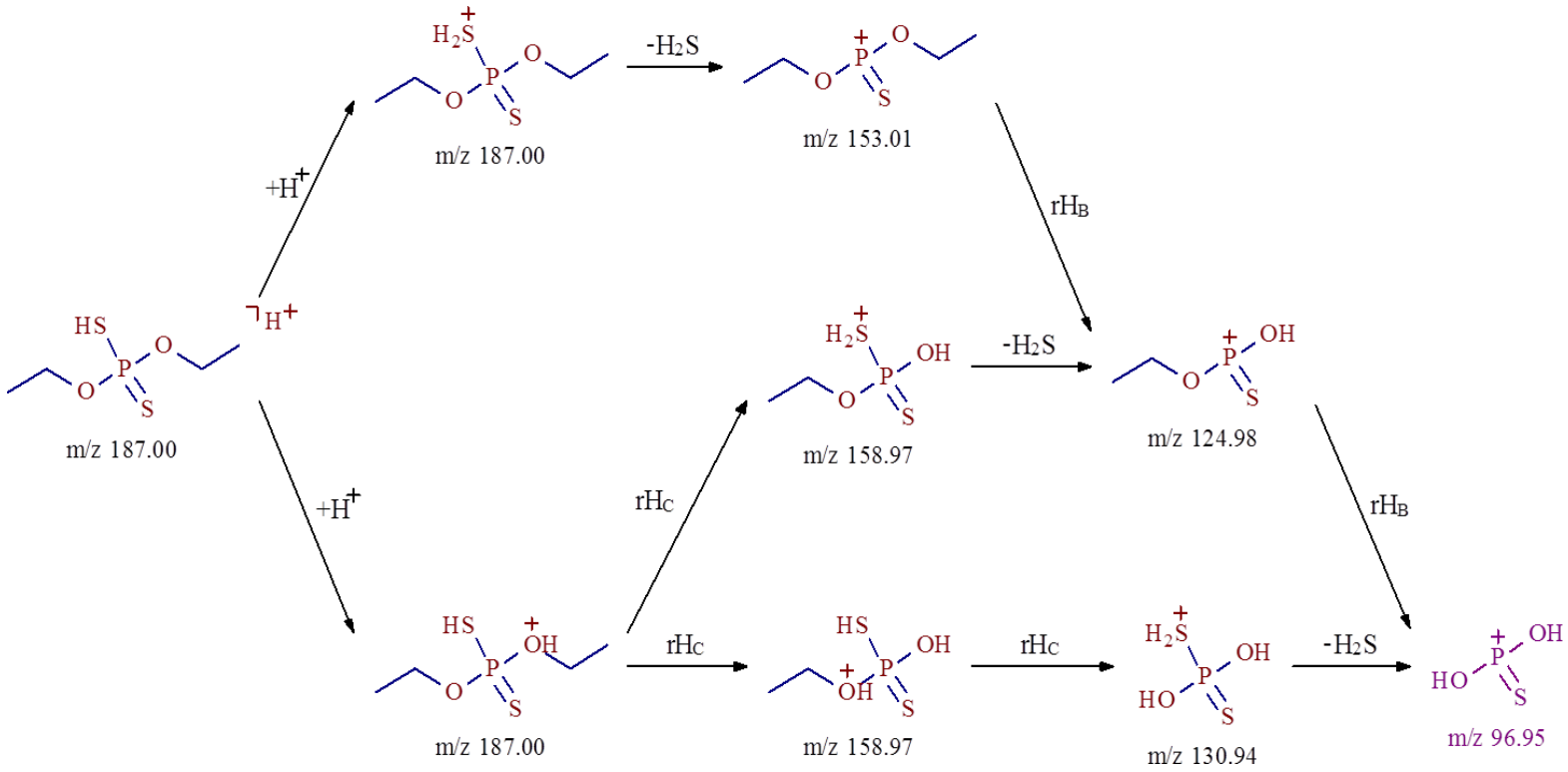
Identification of degradation products



Diethyl dithiophosphoric acid ($C_4H_{11}O_2PS_2$) fragmentation mechanism simulated by Mass Frontier 7.0

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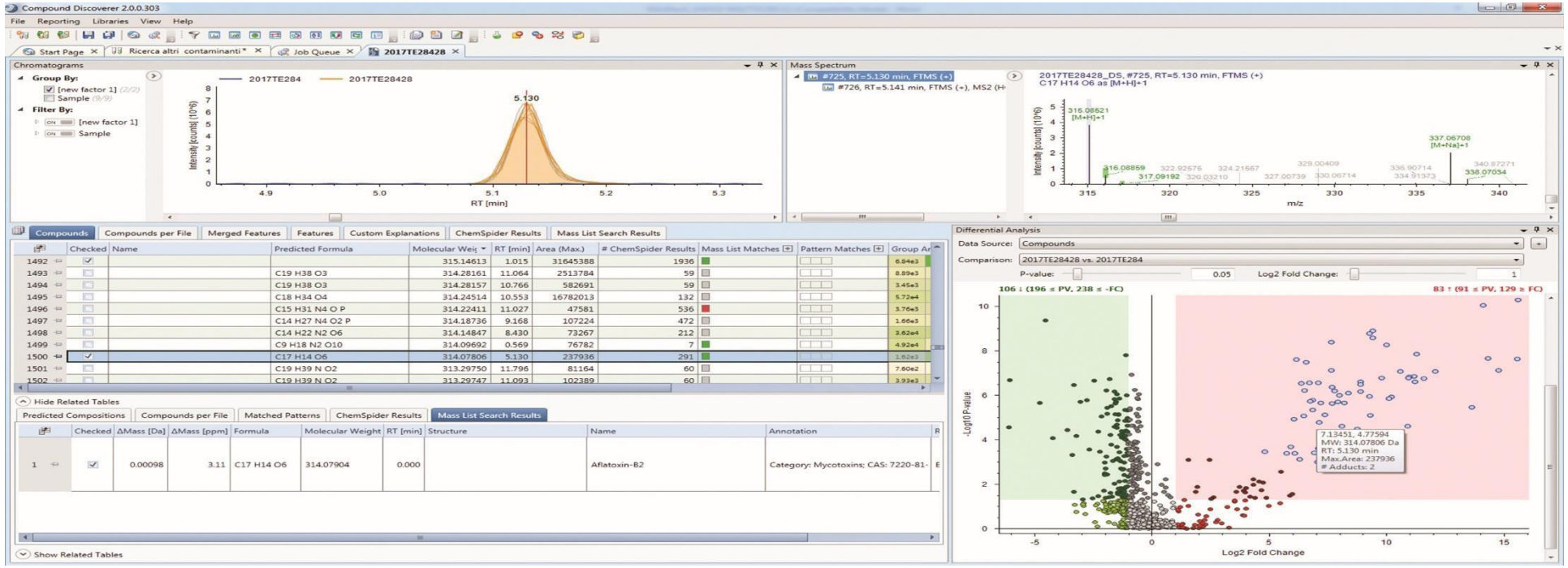
Identification of other xenobiotics !



Identification of Aflatoxyn B2 through Compound Discoverer 2.0