Workshop on Non-Target Screening

Towards the harmonisation of methods for non-target screening on environmental samples

Use and benefits of Atmospheric Pressure Chemical Ionization Interfase in Gas Chromatography coupled to Tandem Mass Spectrometry in Environmental Analysis

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Workshop on Non-Target Screening
GC-MS vs LC-MS

- There is a clear trend towards **LC-MS** methods in screening analysis as **new** contaminants are more **polar**, less volatile and thermostable, thus less GC amenable
- However, **highly used** chemicals as well as other relevant contaminants are still volatile and thermostable, therefore **GC-MS** screening methods cannot be abandoned yet
- Electron ionization (**EI**) is the queen of the GC ionization techniques: robust source, standardized mass spectra, commercially available libraries but its extensive **fragmentation** is still its Achilles' heel

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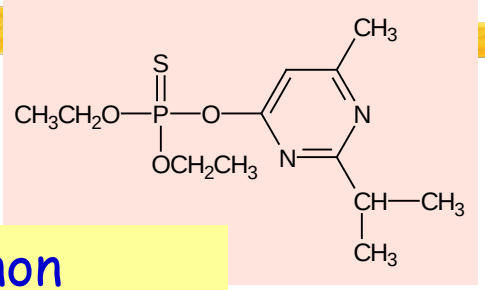
EI extensive fragmentation

- Organophosphorous insecticides

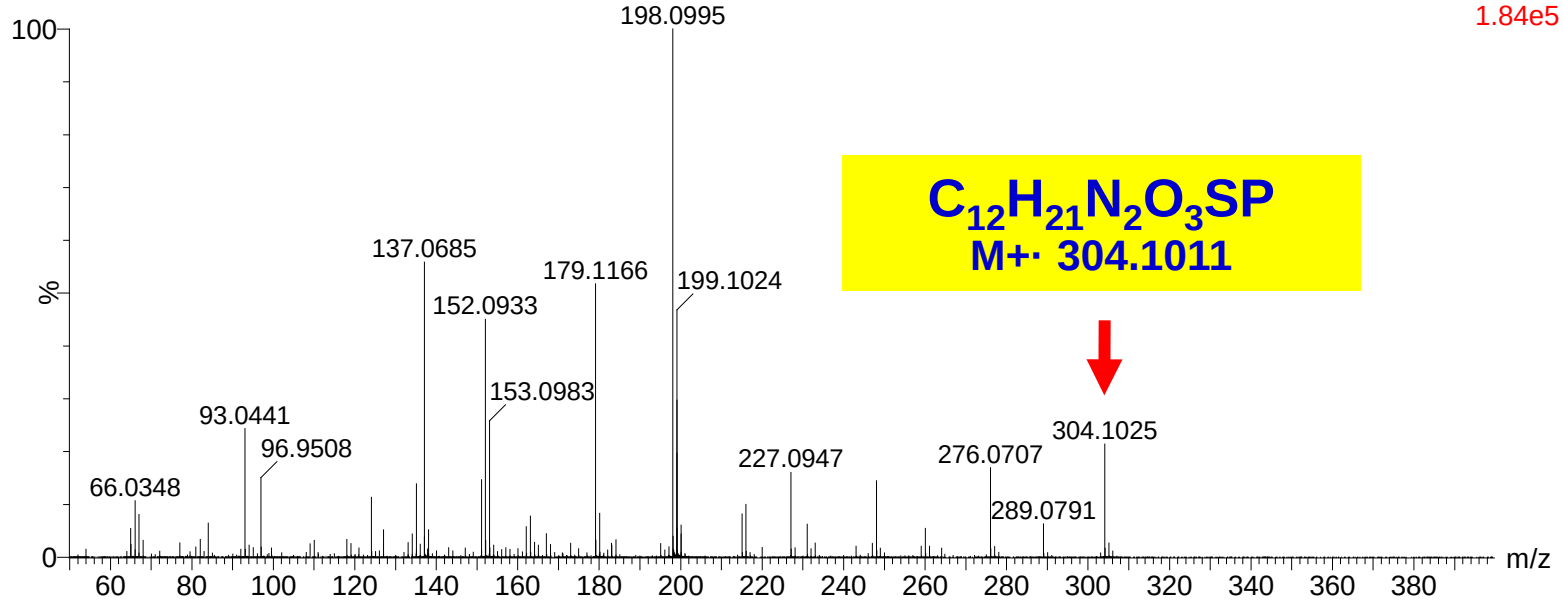


Diazinon

Mix D 1 ppm

API034 603 (11.041) Cm (599:607-627:652)

TOF MS EI+
1.84e5



$C_{12}H_{21}N_2O_3SP$
M+· 304.1011

EI extensive fragmentation

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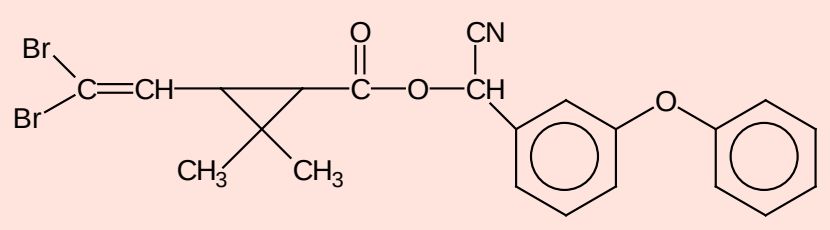
EI extensive fragmentation

- Pyrethroid insecticides

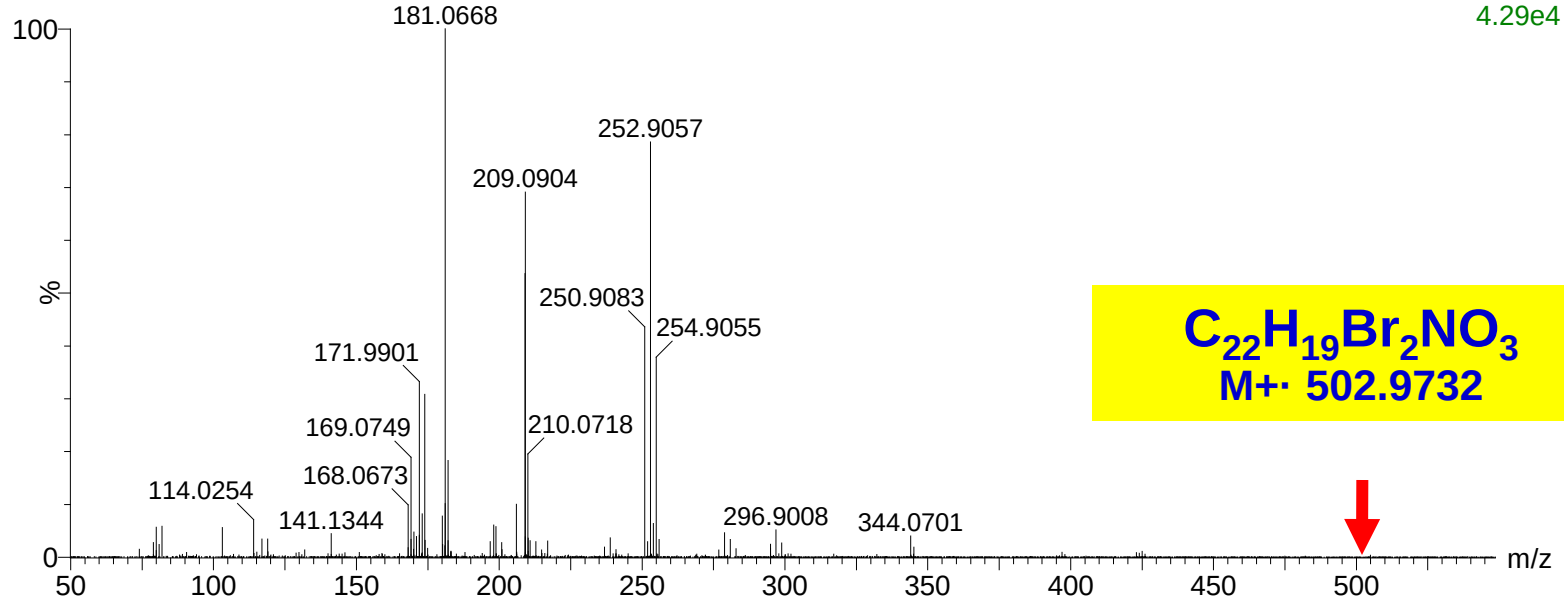


Deltamethrin

Mix D 1 ppm

API034 2062 (30.496) Cm (2058:2068-2015:2052)

TOF MS EI+
4.29e4



$C_{22}H_{19}Br_2NO_3$
M+· 502.9732

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Soft ionization in GC-MS

- **Novel soft-ionization** techniques in GC would overcome this issue, if extensive fragmentation could be considered a problem
- The **lack** of molecular ion in the EI mass spectra is a handicap during developing **tandem** mass spectrometry methods, i.e. available precursor ions only at low masses
- The **specificity** of the SRM transitions selected might be questioned when low mass fragment ions are used
- The availability of molecular ion can be used to **improve** compound discovery and identification power
- Last but not least, this source (**APGC**) allows us to couple GC to a novel Xevo tandem mass spectrometers such as **TQ-S, QTOF G2** or **Synapt**

Workshop on Non-Target Screening
APGC interface. Instruments

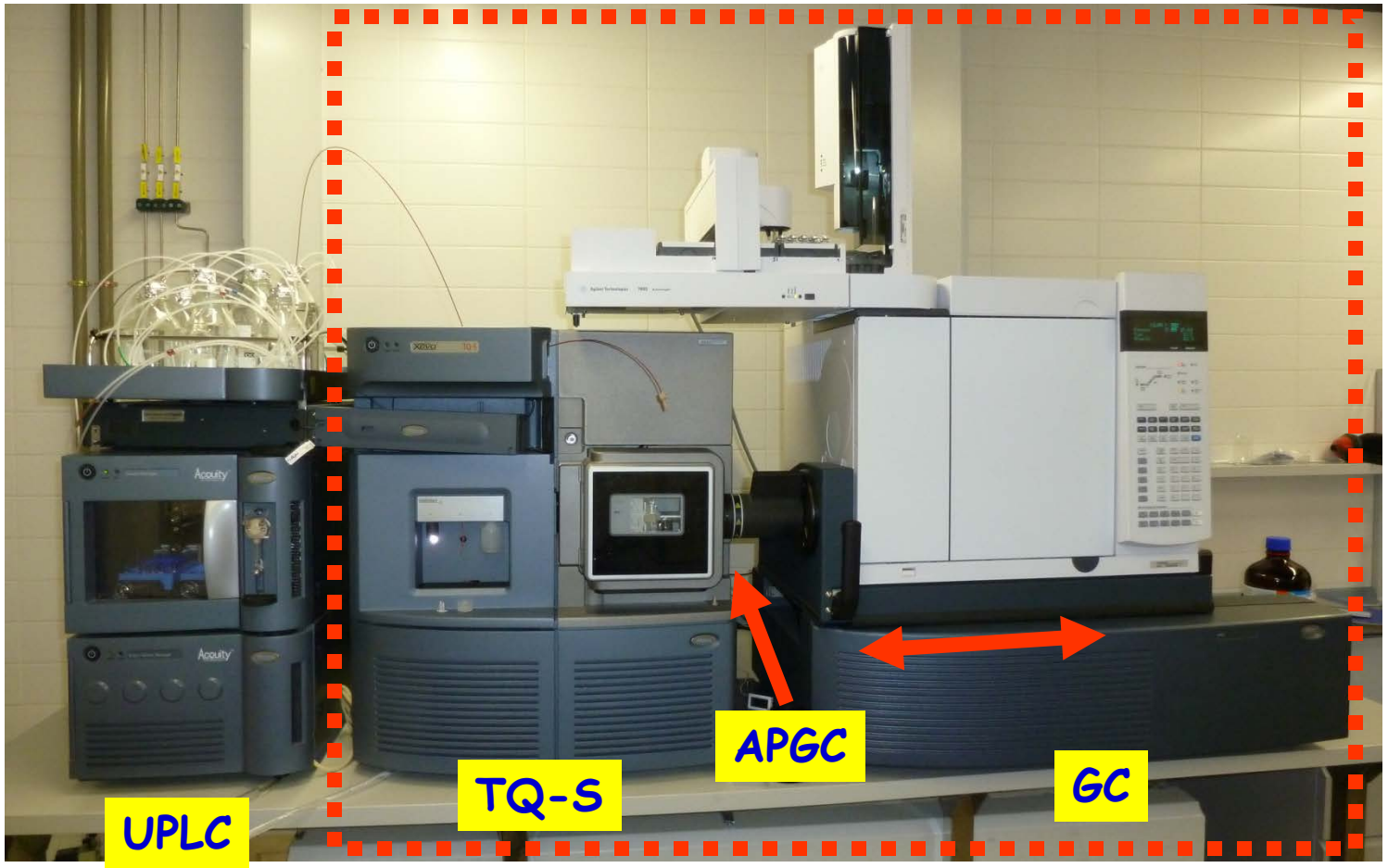
UPLC TQ-S APGC GC

TARGET APPLICATIONS

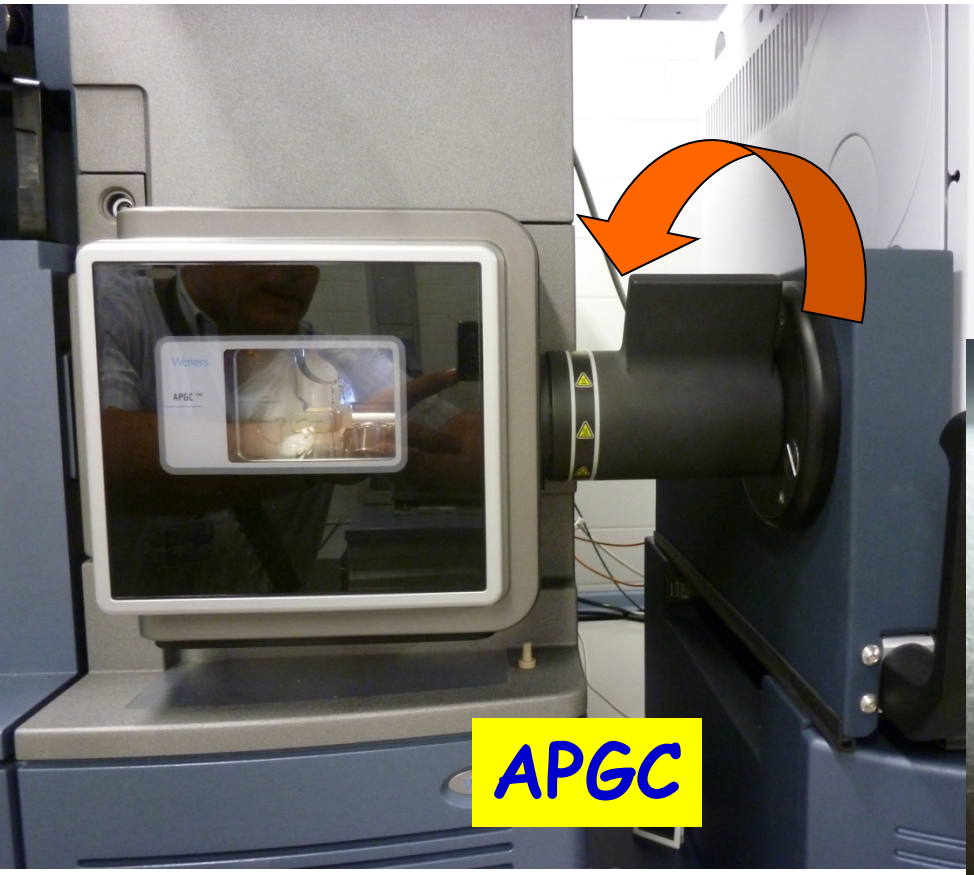
UPLC G2 QTOF APGC GC

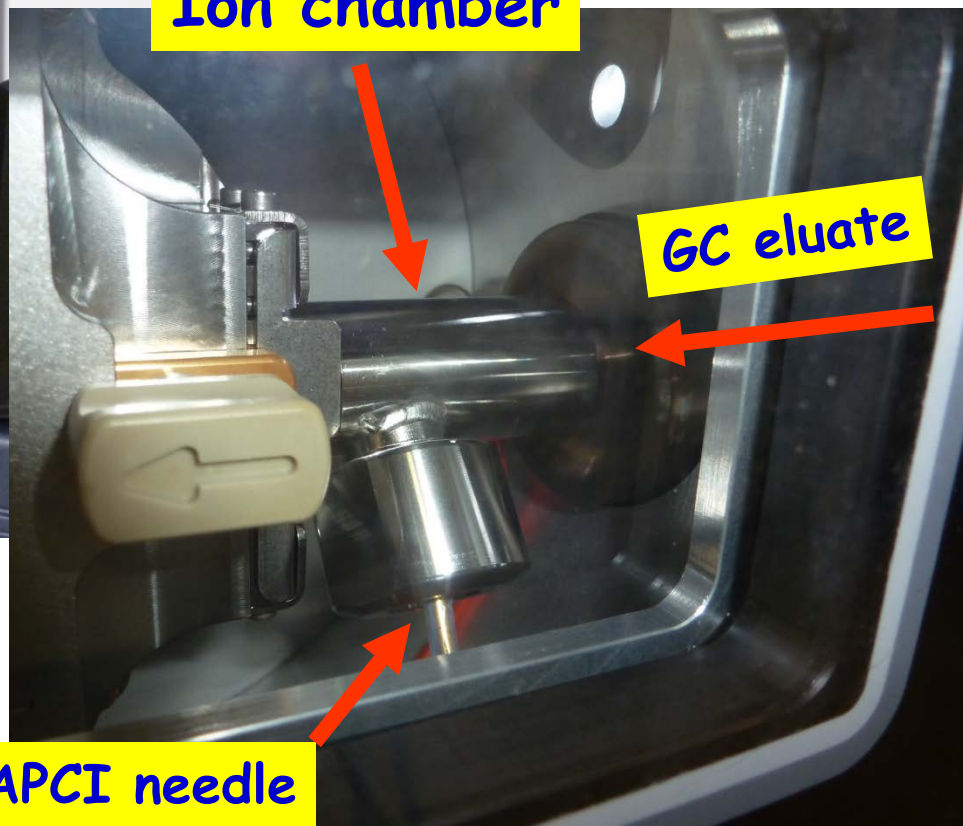
SCREENING

Workshop on Non-Target Screening
GC-APGC-MS/MS



Workshop on Non-Target Screening
APGC source





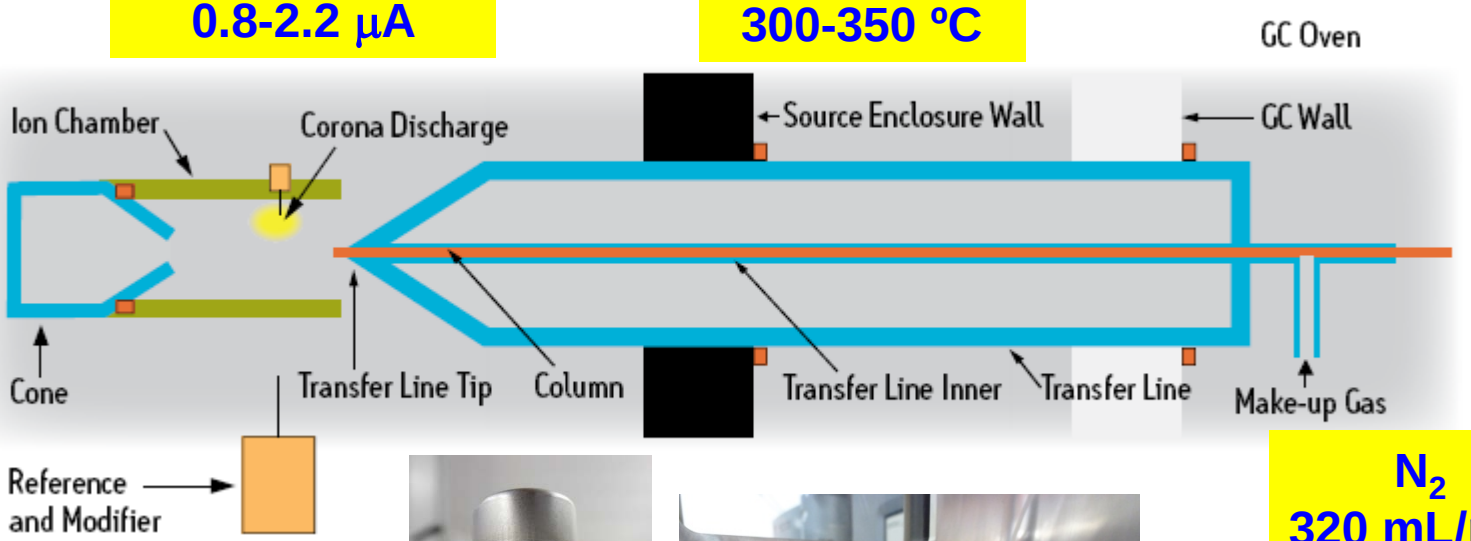
Workshop on Non-Target Screening
APGC source

- "APCI-like" soft ionization for GC eluate

Cone
30-50 V

Corona current
0.8-2.2 μA

Transfer line
300-350 °C

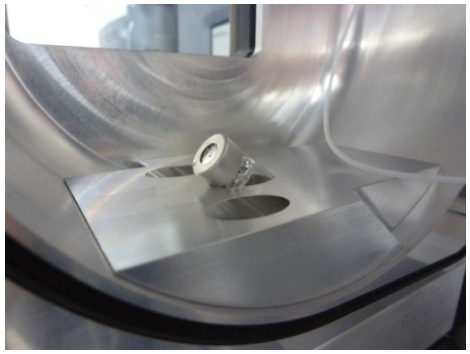


GC eluate

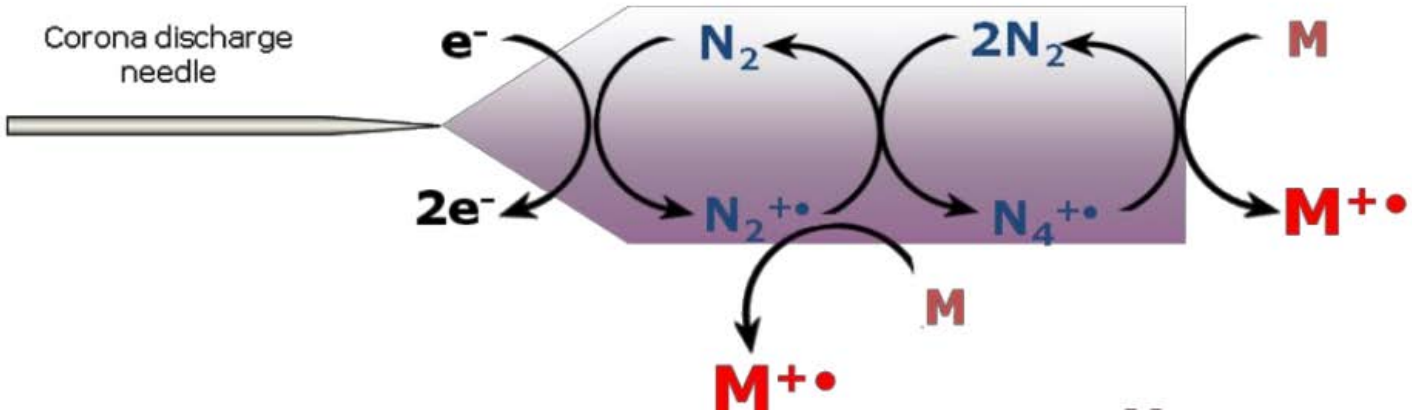
N_2
320 mL/min

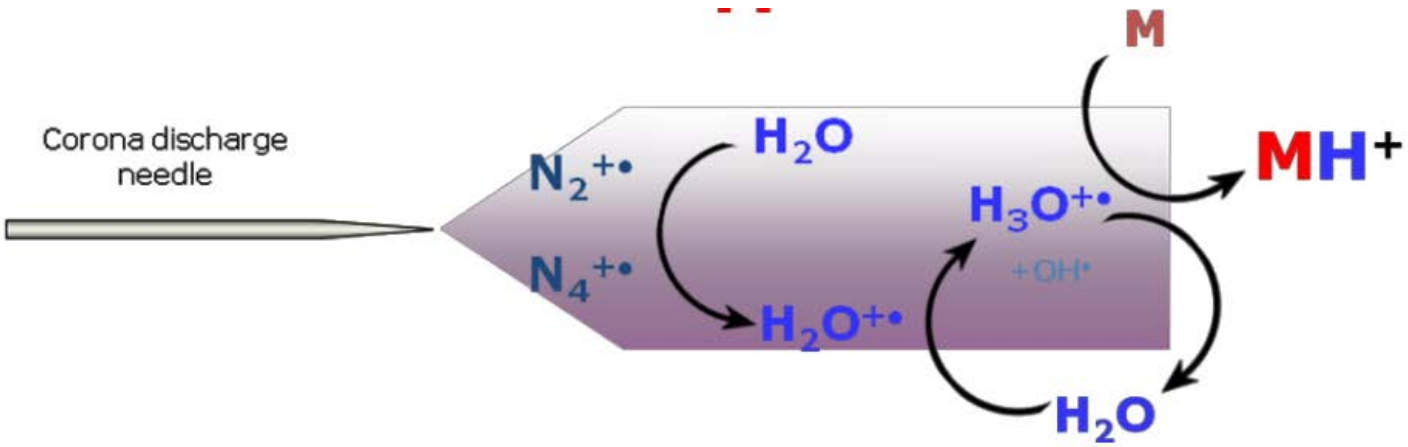
Cone gas
150-200 L/hr

Modifiers



Workshop on Non-Target Screening
APGC source – Ionization modes





Modifiers (H_2O, MeOH)

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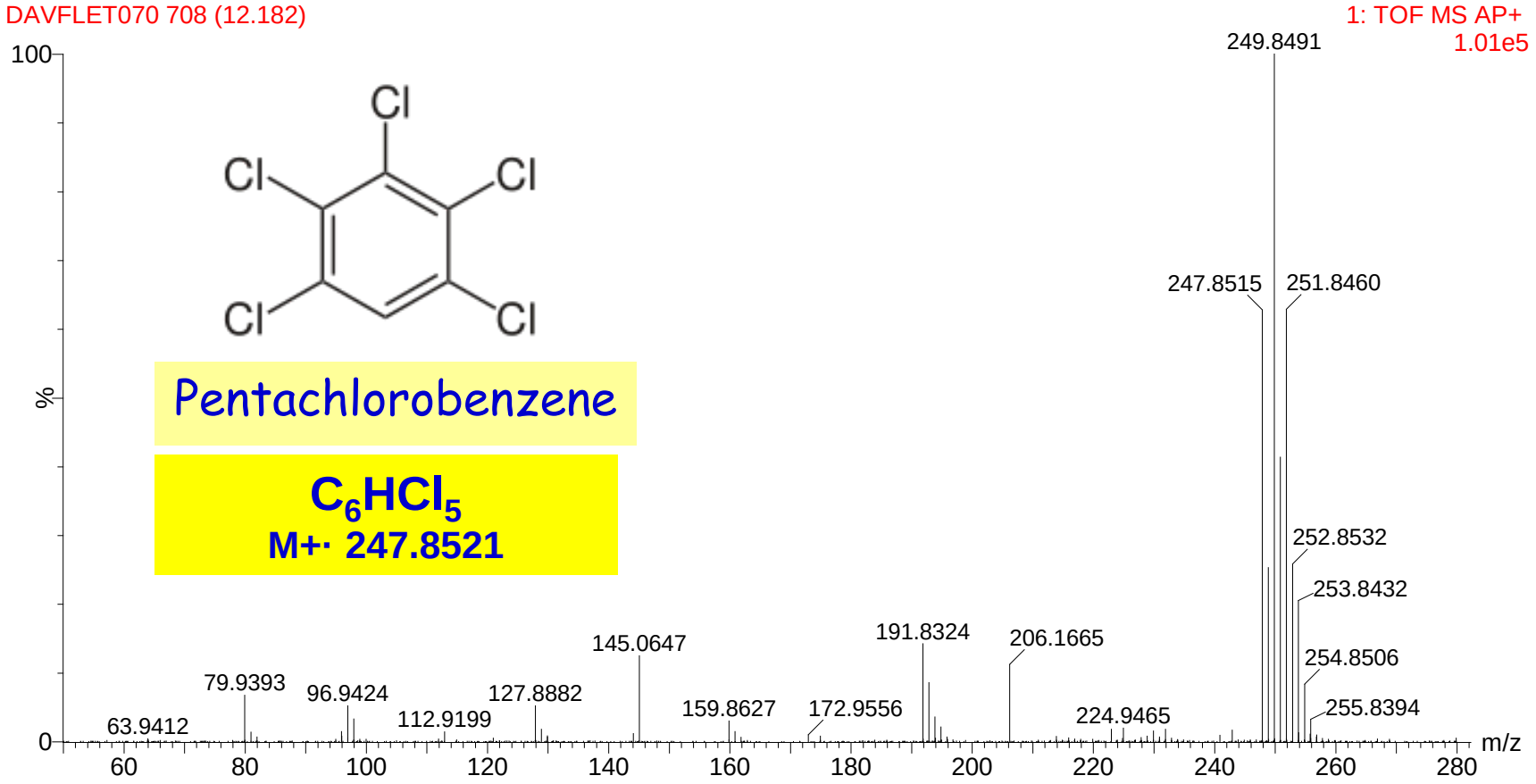
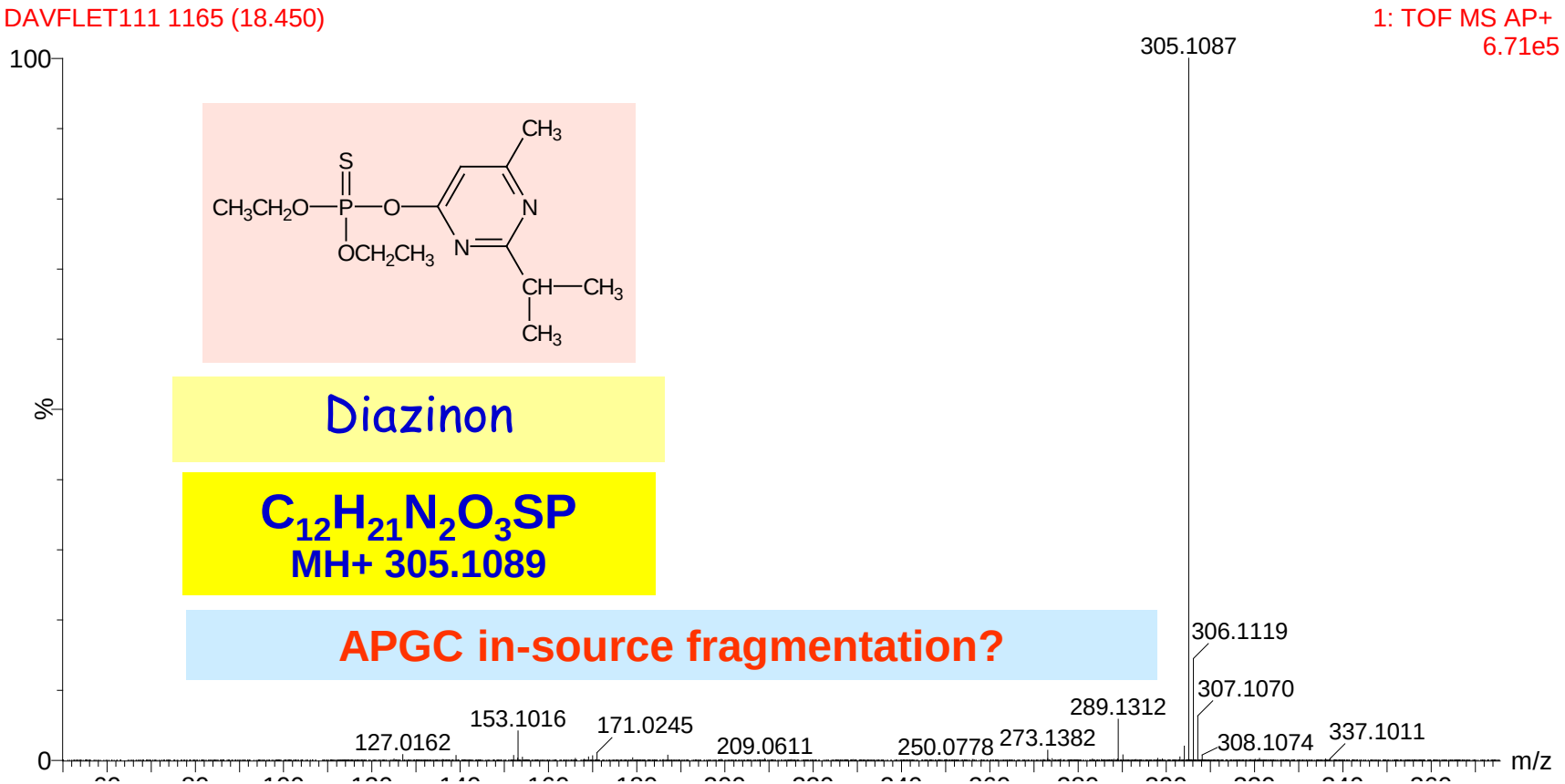
APGC source – Ionization modes

- $N_2^{+\cdot} + M \rightarrow \mathbf{M^{+\cdot}} + N_2$



Workshop on Non-Target Screening
APGC source – Ionization modes

- $H_3O^+ + M \rightarrow \mathbf{MH^+} + H_2O^+$



Workshop on Non-Target Screening
APGC interface. Ionization behaviour

>100 GC-amenable pesticides

Research Article

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Potential of atmospheric pressure chemical ionization source in GC-QTOF MS for pesticide residue analysis

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The potential applications of a new atmospheric pressure source for GC-MS analysis have been investigated in this work. A list of around 100 GC-amenable pesticides, which includes organochlorine, organophosphorus and organonitrogenated compounds, has been used to evaluate their behavior in the new source. Favoring the major formation of the molecular ion in the source has been the main goal due to the wide-scope screening possibilities that this fact brings in comparison with the traditional, highly fragmented electron ionization spectra. Thus, the addition of water as modifier has been tested as a way to promote the generation of protonated molecules. Pesticides investigated have been classified into six groups according to their ionization/fragmentation behavior. Four of them are characterized by the abundant formation of the protonated molecule in the atmospheric pressure source, mostly being the base peak of the spectrum. These results show that wide-scope screening could be easily performed with this source by investigating the presence of the protonated molecule ion, MH+. The developed procedure has been applied to pesticide screening in different food samples (nectarine, orange and spinach) and it has allowed the presence of several pesticides to be confirmed such as chlorpyriphos ethyl, deltamethrin and endosulfan sulfate. The availability of a quadrupole time-of-flight instrument made it feasible to perform additional MS/MS experiments for both standards and samples to go further in the confirmation of the identity of the detected compounds. Results shown in this paper have been obtained using a prototype source which exhibits promising features that could be applied to other analytical problems apart from those illustrated in this work.

Keywords: atmospheric pressure chemical ionization; GC; quadrupole time-of-flight mass spectrometry; pesticides; screening

Introduction

The increasing use of high-resolution full spectrum acquisition techniques in the last decade, such as time-of-flight mass spectrometry (TOF MS), has allowed a huge amount of chemical information on sample composition to be obtained, widening the number of analytes that can be investigated in a single experiment. This analyzer provides the selectivity and sensitivity required for efficient, wide-scope screening, as it combines high full-spectral sensitivity with high mass resolution. The useful and accurate mass data obtained can be processed in both target and/or non-target way, which gives the instrument an interesting versatility depending on the aim of the analysis and allows the user to look at an analytical problem from a different point of view.[1–5] Additionally, the full spectrum dataset remains and offers the analyst the possibility of performing a retrospective analysis, i.e. to make a careful examination of old raw data looking for the presence of any compound that becomes interesting later. All these characteristics make this technique ideal to perform screening analysis that provides greater analytical information and allows the user to efficiently discriminate samples with no detectable residues from those with contaminants or residues at a relevant level. In the pesticide residue analysis field, there is a clear trend toward liquid chromatography coupled to mass spectrometry (LC-MS) as new pesticides are more polar, less volatile and thermostable, and consequently, less GC amenable. However, high usage pesticides (in Europe or in developing countries) are still volatile and thermostable, therefore GC-MS methods cannot be abandoned yet.

Regarding the huge amount of full spectrum with accurate mass data generated by TOF instruments, choosing the right strategy to get the maximum information from the data is one of the major keys to success. The way to proceed is mainly driven by the kind of mass spectrum delivered by the system. Normally, in LC-TOF MS analysis, the ionization occurs at atmospheric pressure by electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI), atmospheric pressure photo ionization[3,6,7] or ambient ionization, e.g. direct analysis in real time or desorption ionization.[8,9] These types of ionization processes promote the formation of protonated or deprotonated molecules with very little fragmentation, yielding typically the $[M+H]^+$ or $[M-H]^-$ ions as the base peak of the spectrum. Recent screening applications have been developed using LC-(ESI)TOF MS technique.[5,10,11] The predictable presence of the (de)protonated molecule in LC-TOF data has allowed an automatic and rapid 'post-target' searching[11,12] for many LC amenable compounds (including pesticides, antibiotics, veterinary drugs and banned dyes, among others, as well as several metabolites) by extracting chromatograms, with narrow-mass windows, at the exact mass

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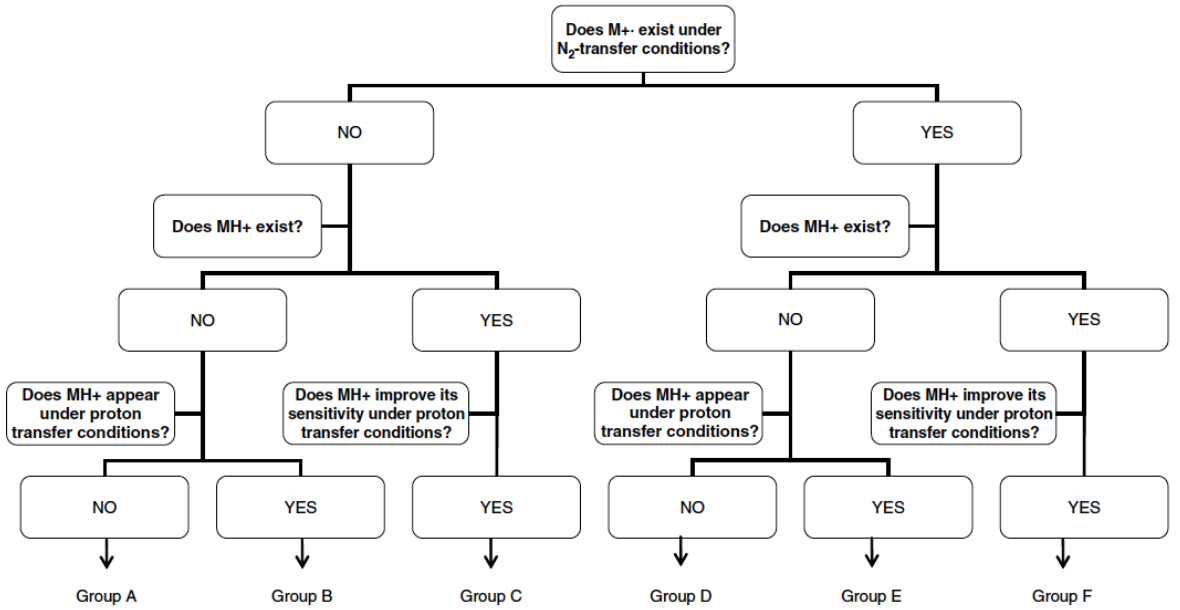


Figure 4. Scheme of the different behaviors of pesticides when using the APCI source.

Workshop on Non-Target Screening
APGC interface. Ionization behaviour

Atmospheric pressure chemical ionization source in GC-QTOF MS

Journal of MASS SPECTROMETRY

Table 1. Groups of pesticides according to its ionization/fragmentation behavior in APCI source

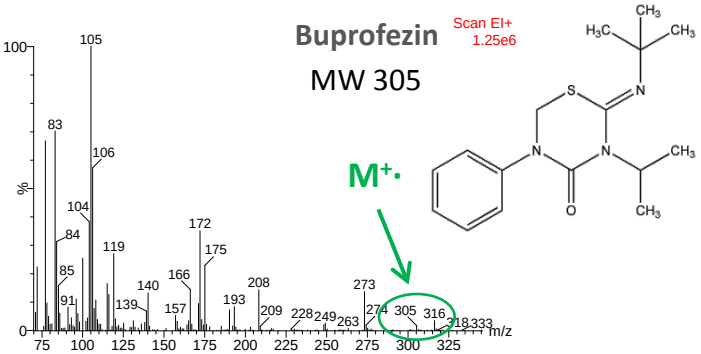
Group A	Group B	Group C	Group D	Group E	Group F	
α-HCH	Malathion	Methamidophos	Pentachlorobenzene	Isodrin	Tecnazene	Quinalphos
γ-HCH	Phosmet	Dichlorvos	HCB	Dieldrin	Diphenylamine	Procymidone
β-HCH	Terbufos	Ethoprophos	*p,p′*-DDE	Endosulfan sulfate	Trifluralin	Profenofos
δ-HCH	Disulfoton	Dimethoate	Trans-chlordane	Mevinphos	Simazine	Myclobutanil
p,p′-DDT	Methidathion	Fonofos	*p,p′*-DDD	Methacrifos	Atrazine	Bupirimate
Mirex	Azinphos methyl	Tolclofos methyl	Heptachlor	Heptenophos	Terbuthylazine	Endrin
		Fenchlorphos	Fluvalinate	Chlorpropham	Propyzamide	Oxadixyl
		Chlorfenvinphos		Phorate	Pyrimethanil	Ethion
		α-Endosulfan		Dichlofluanid	Diazinon	Triazophos
		Tetrachlorvinphos		Tolylfluanid	Chlorthalonil	Fosalone
		Imazalil			Etrimfos	Lambda-Cyhalothrin I
		β-Endosulfan			Endosulfan ether	Fenarimol
		Propiconazole I			Pirimicarb	Pyrazophos
		Tebuconazole			Fosfamidon	Pyridaben
		Pyriproxyfen			Metribuzin	Coumaphos
		Iprodione			Parathion methyl	Cypermethrin
		Metoxychlor			Metalaxil	Deltamethrin
					Fenitrothion	Azoxystrobin
					Pirimiphos methyl	Quintozene
					Aldrin	Chlorpyrifos methyl
					Fenthion	Alachlor
					Chlorpyrifos ethyl	Buprofezin
					Parathion ethyl	Bifenthrin
					Pirimiphos ethyl	Permethrin I
					Cyprodinil	Fenvalerate
					Heptachlor epox A	Esfenvalerate
					Chlozolinate	

N_2

$M^{+\cdot}$

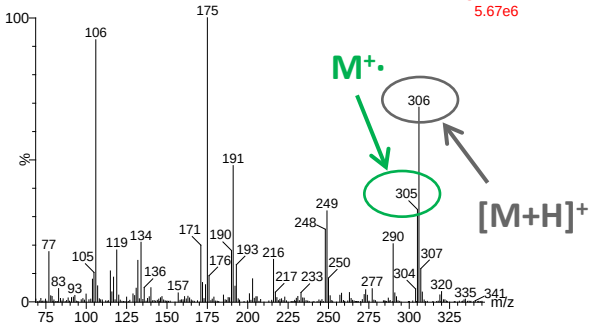
Workshop on Non-Target Screening
APGC interface. Ionization behaviour

EI



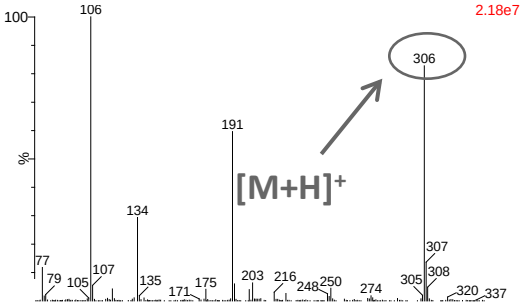
APCI

Charge-transfer conditions



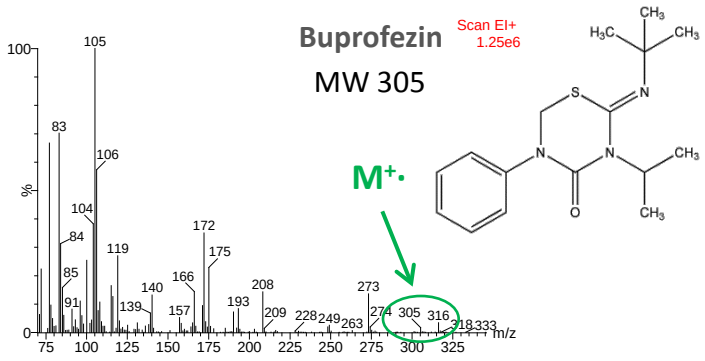
APCI

Proton-transfer conditions



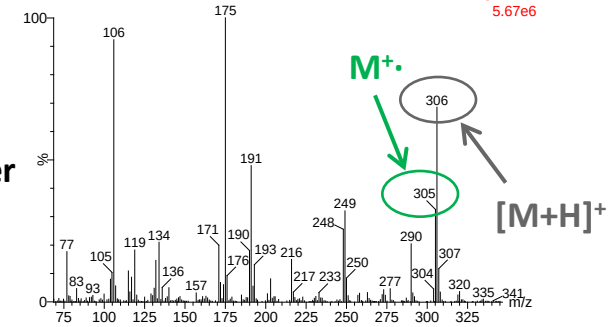
Workshop on Non-Target Screening
APGC interface. Ionization behaviour

EI



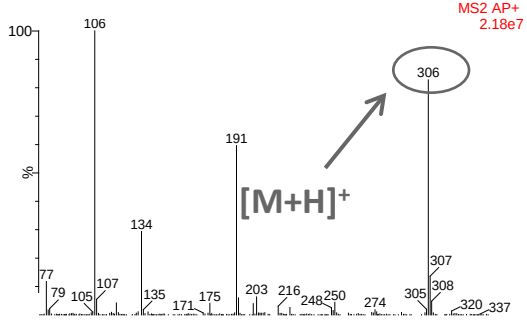
APCI

Charge-transfer conditions

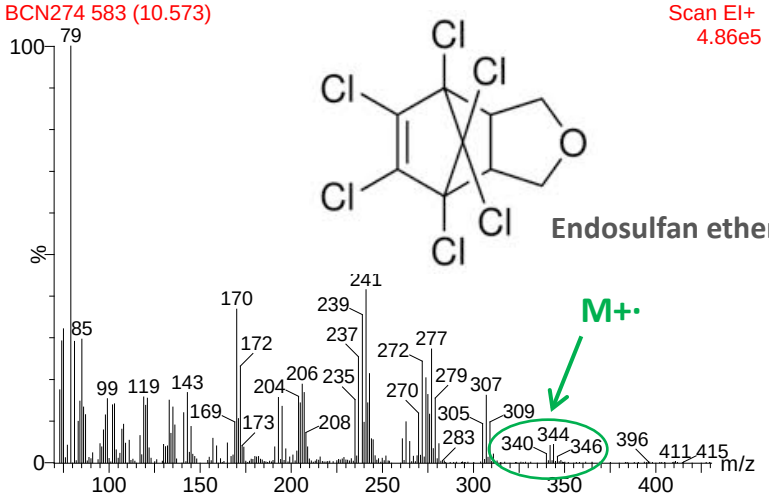


APCI

Proton-transfer conditions

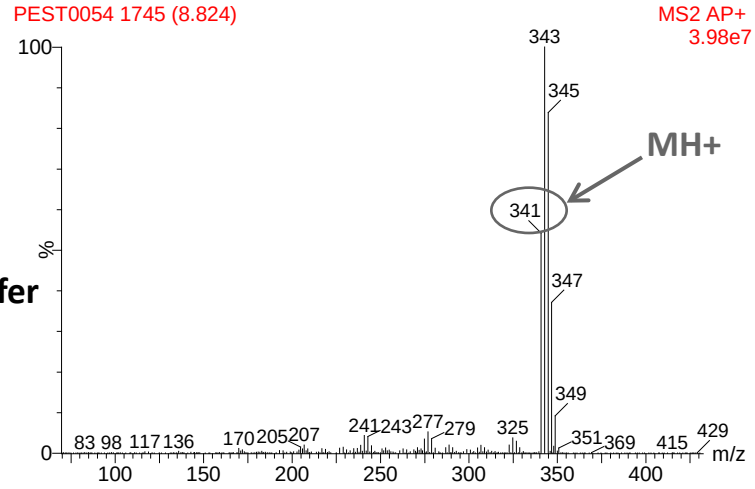


EI



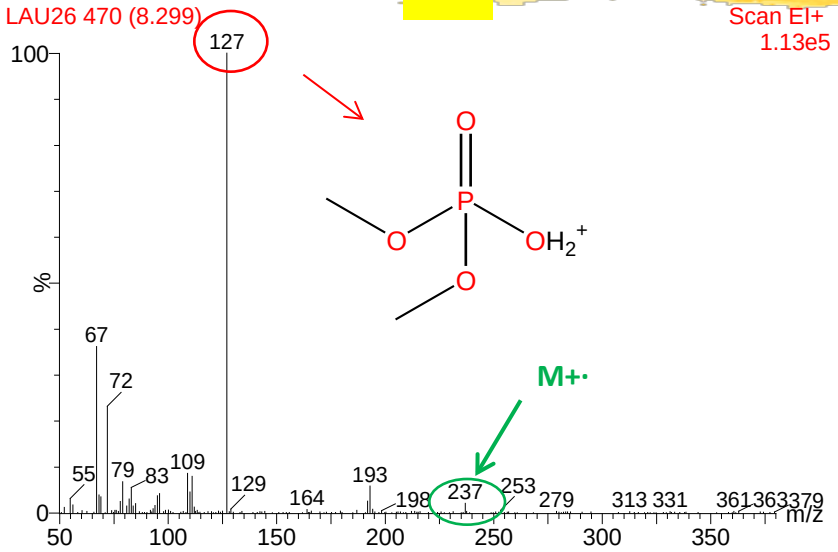
APCI

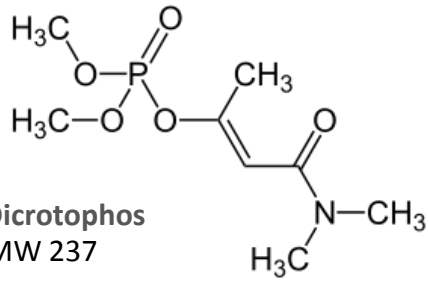
Proton-transfer conditions



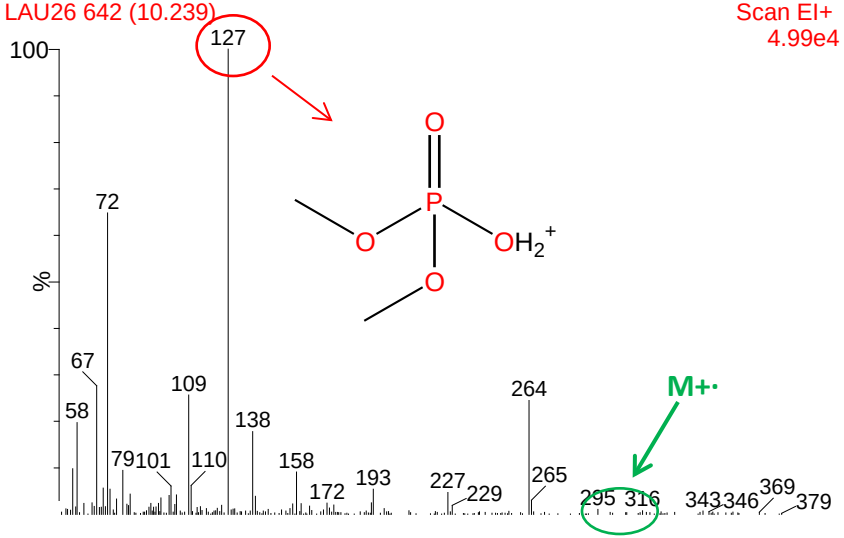
Workshop on Non-Target Screening
APGC interface. Ionization behaviour

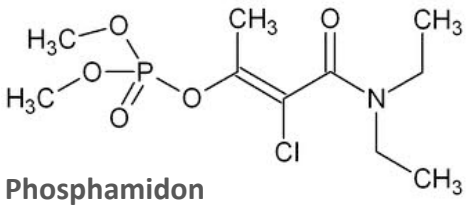
EI





Dicrotophos
MW 237

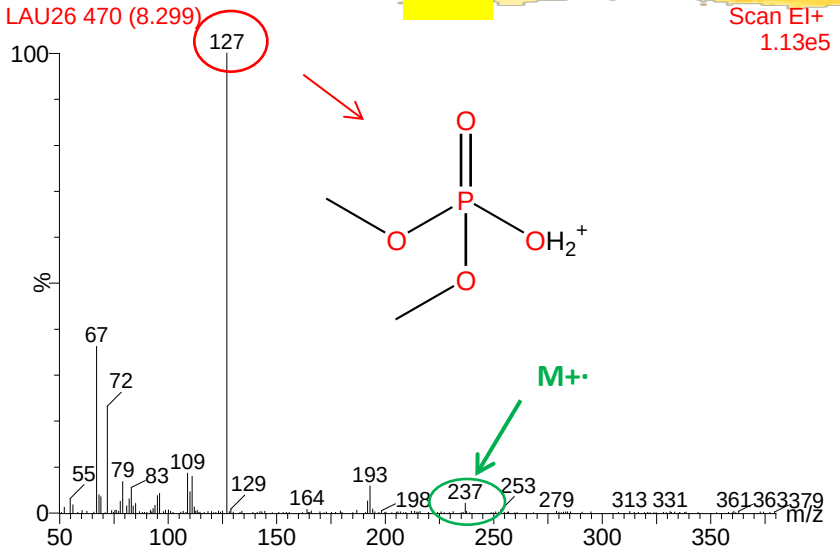


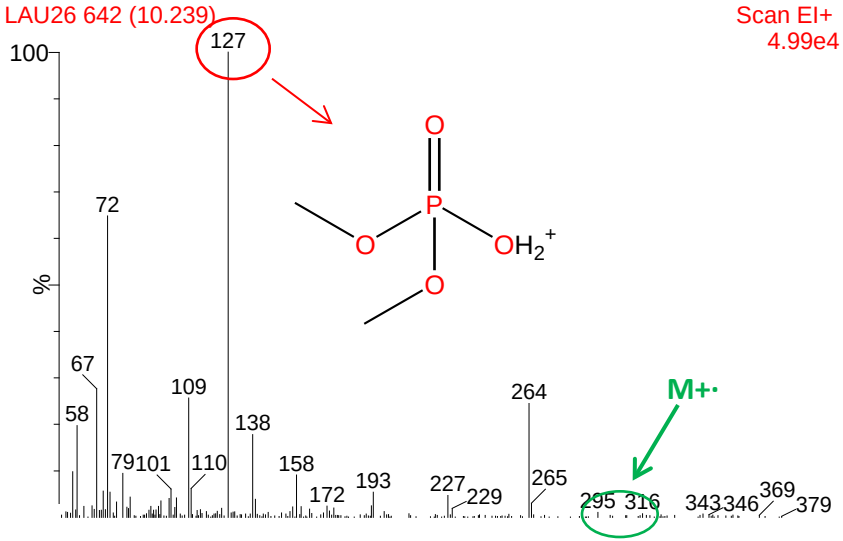


Phosphamidon
MW 299

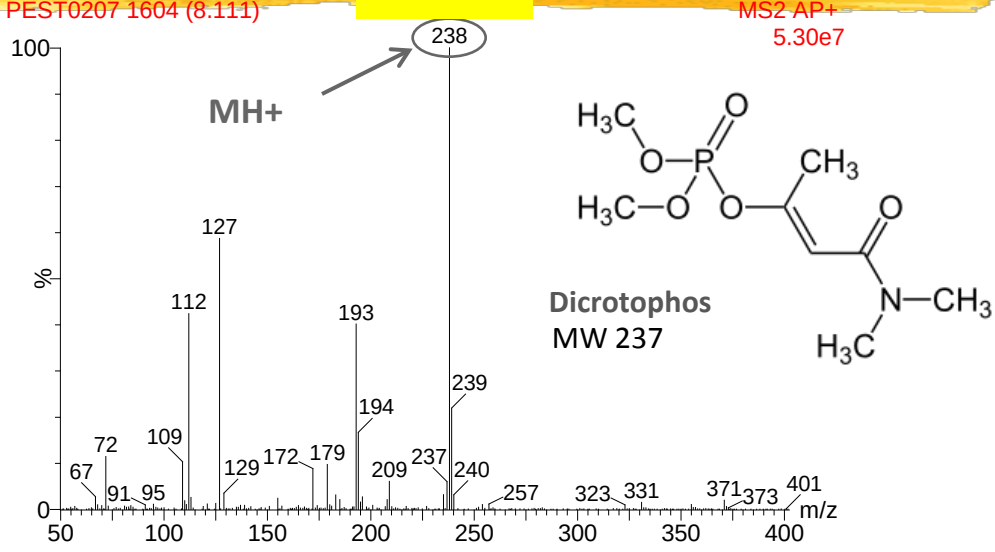
Workshop on Non-Target Screening
APGC interface. Ionization behaviour

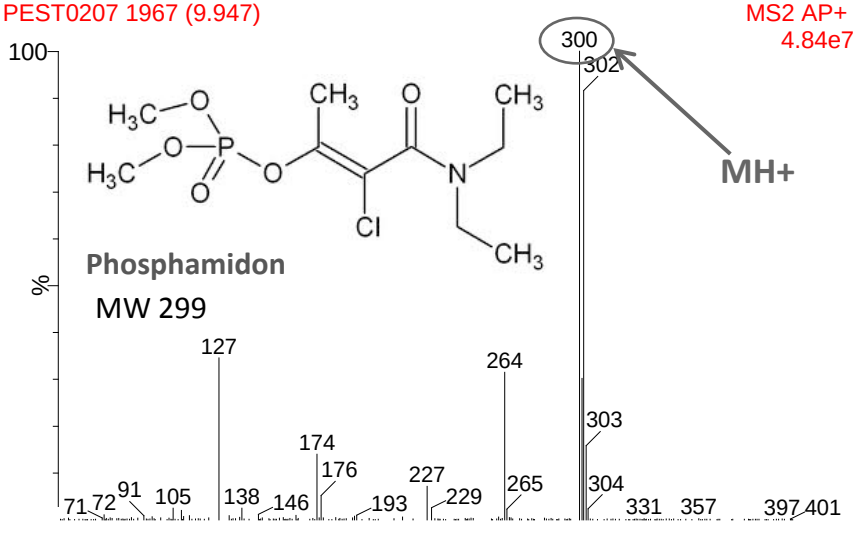
EI





APGC





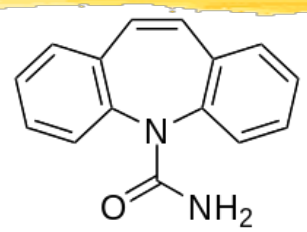
Workshop on Non-Target Screening
Suspect screening of GC-compounds

- Difficult to ensure a **unique** ionization mechanism for a great number of contaminants tested
- Run the samples **twice**, first in dry-source conditions, second adding water as modifier
- Search for accurate-mass measured **$M^{+\cdot}$** and/or **[M+H]+** in charge-transfer data. Only **[M+H]+** under proton-transfer conditions
- **Confirmation of the identity** could be **simultaneously** performed in the same run thanks to **MS^E** acquisition mode.

Workshop on Non-Target Screening
Suspect screening of GC-compounds

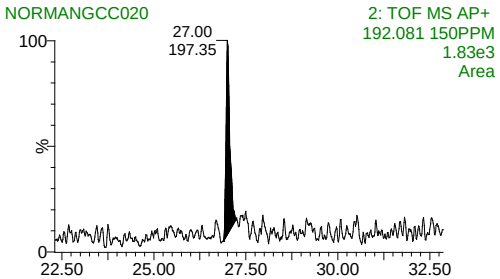
Carbamazepine

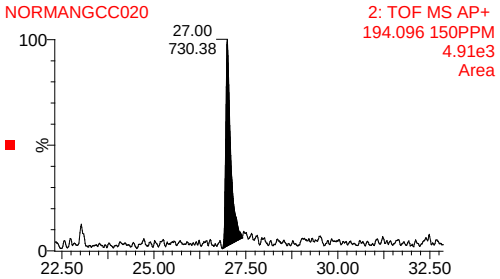
$C_{15}H_{12}N_2O$
MH+ 237.1028

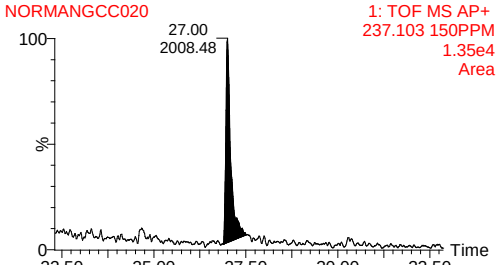


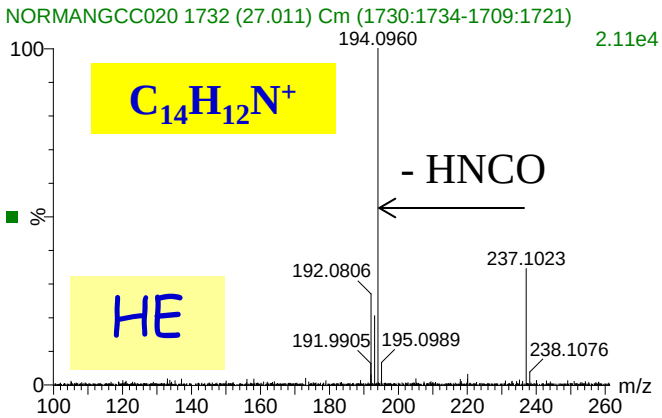
Massbank

UPLC-ESI-QTOF MS^E



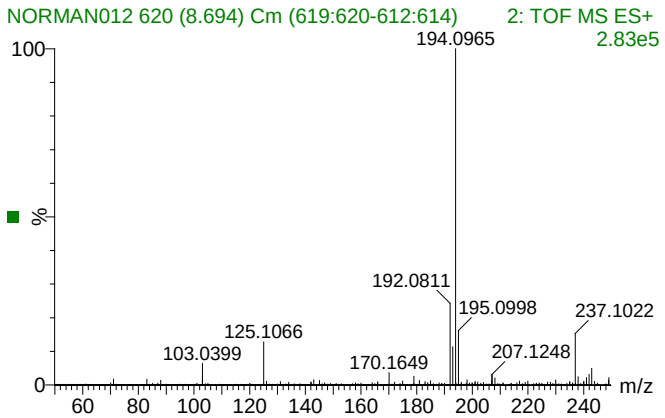


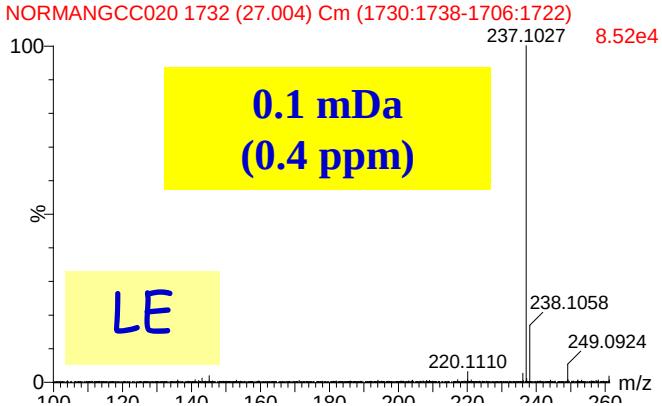




$C_{14}H_{12}N^+$

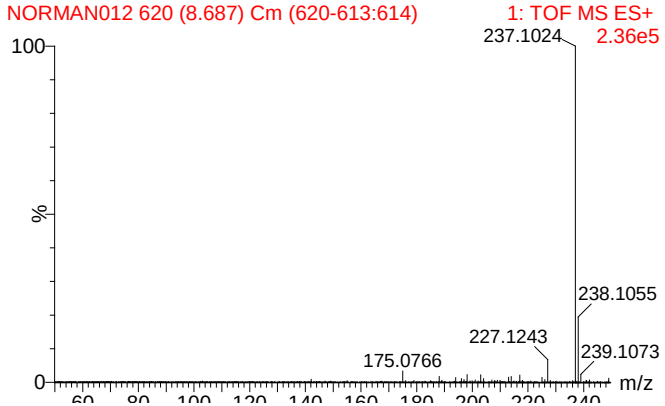
HE





0.1 mDa
(0.4 ppm)

LE



Workshop on Non-Target Screening
Non-Target Screening of GC-compounds

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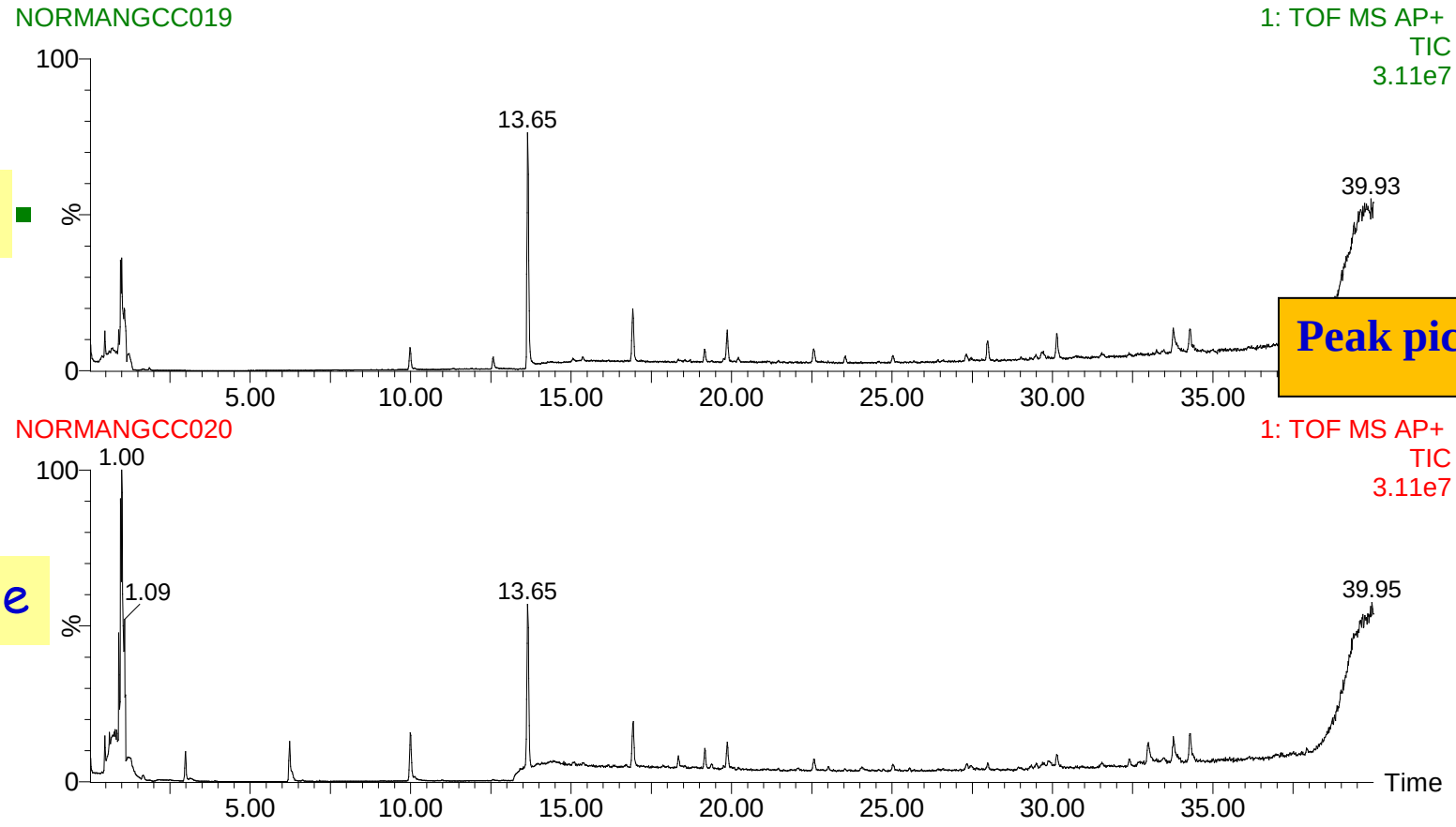
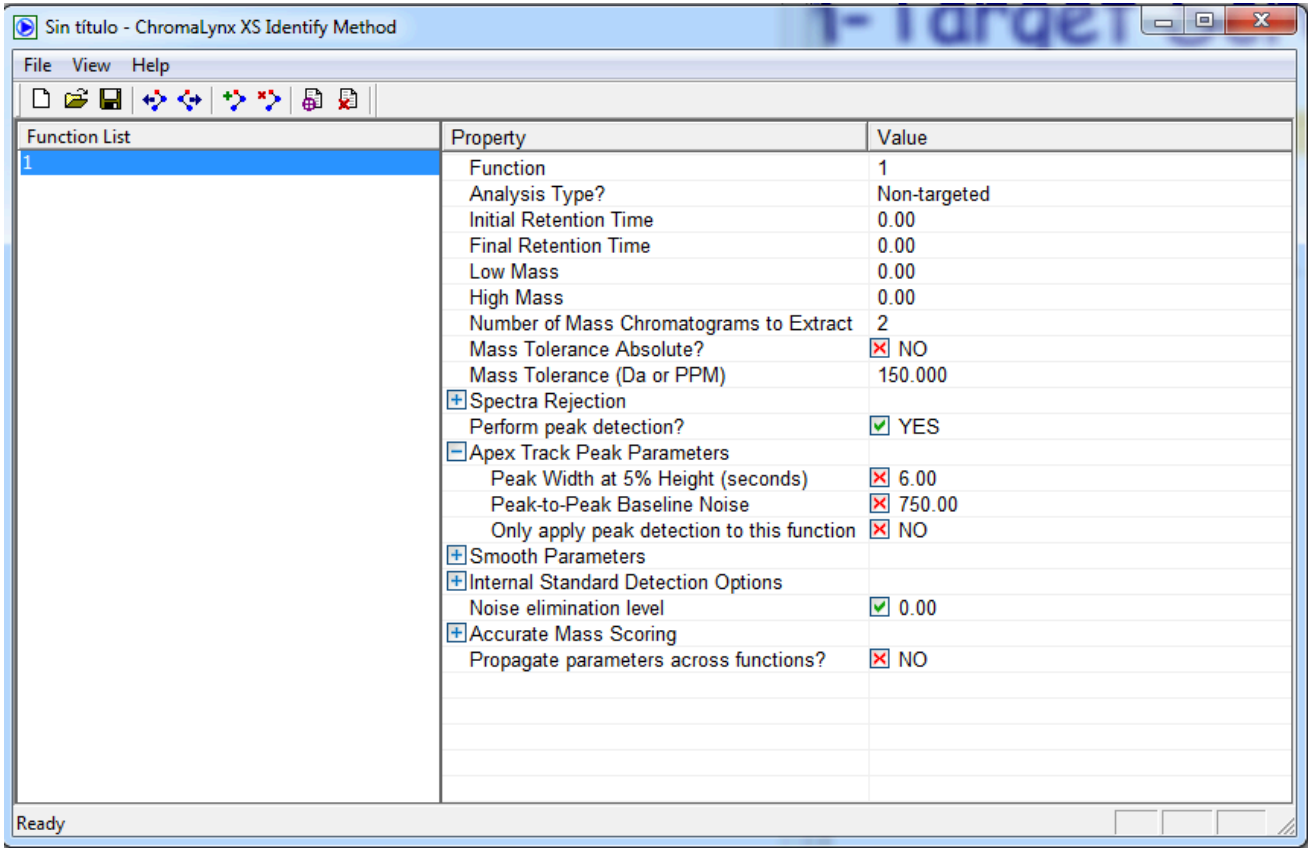
Sample



Workshop on Non-Target Screening
Non-Target Screening of GC-compounds

Chromalynx XS Non-targeted

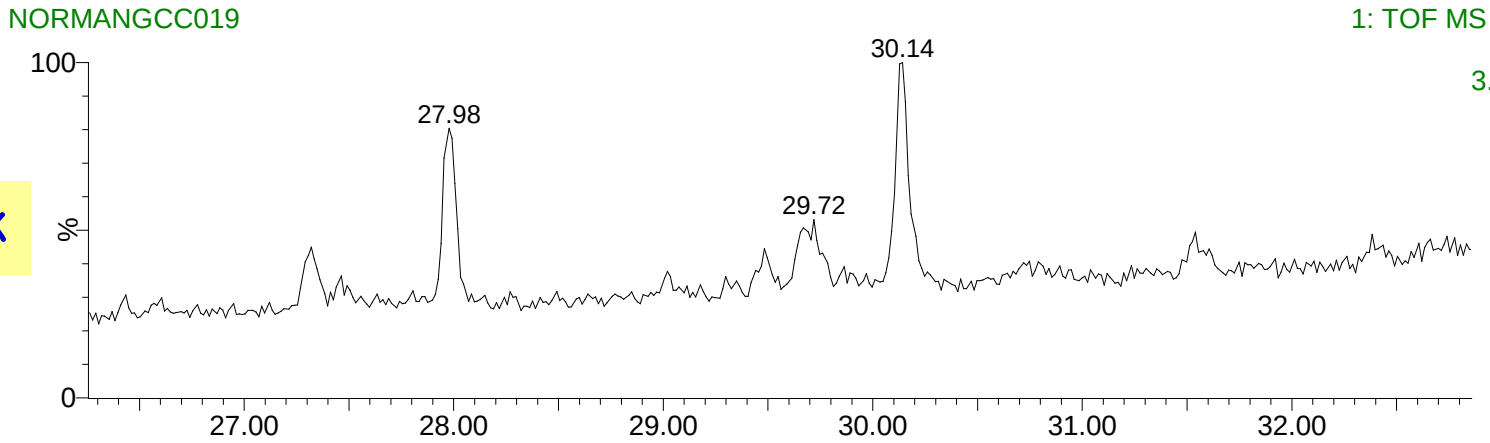


Workshop on Non-Target Screening
Non-Target Screening of GC-compounds

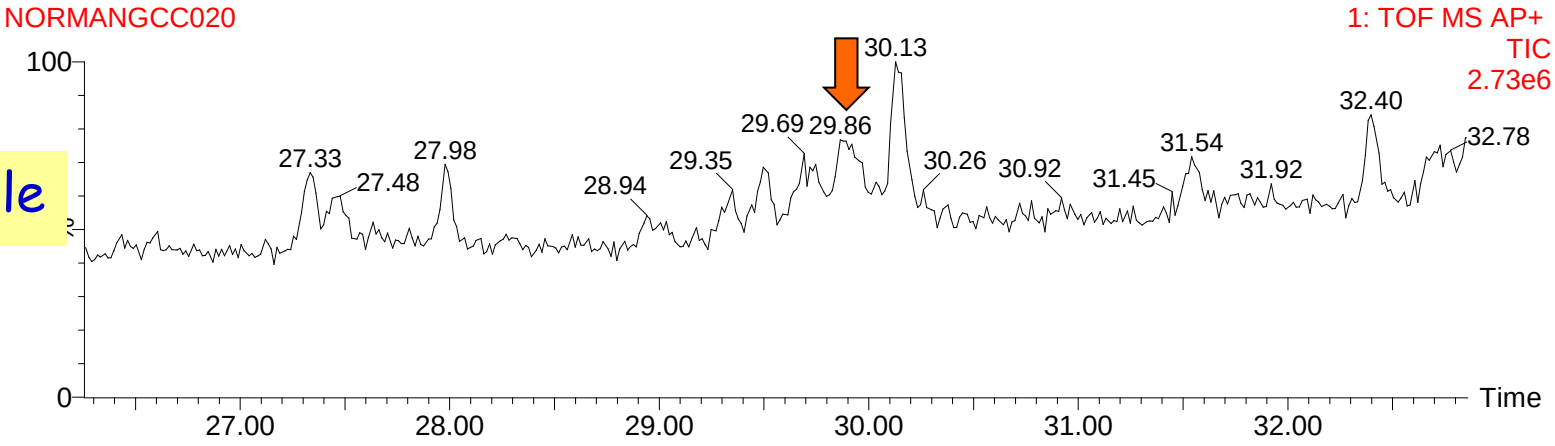
Chromalynx XS Non-targeted

m/z 331.2849
RT 29.89 min

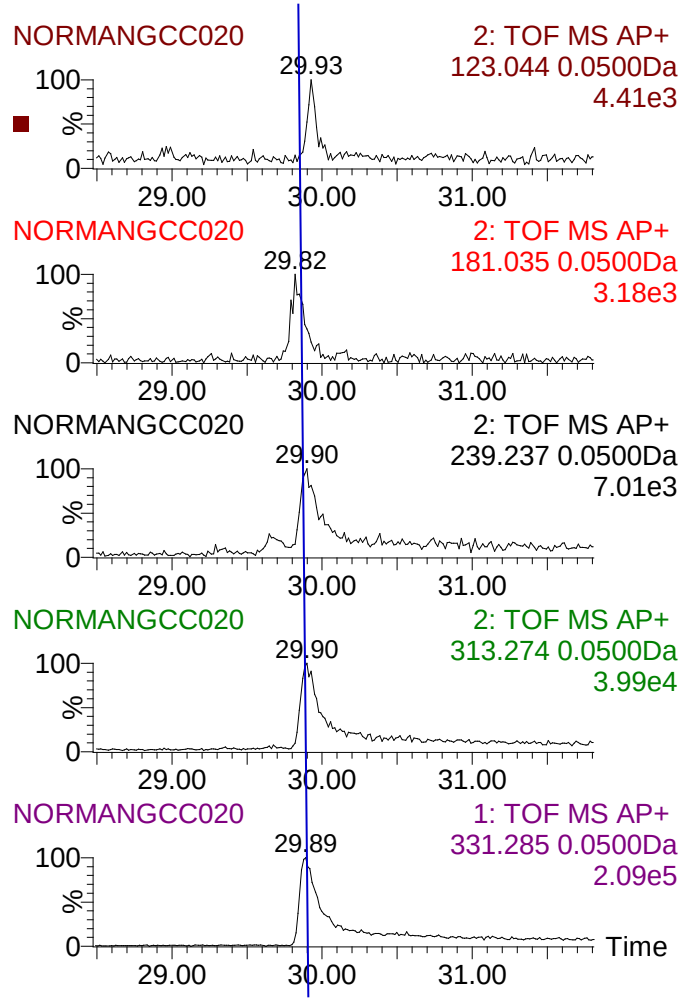
Blank

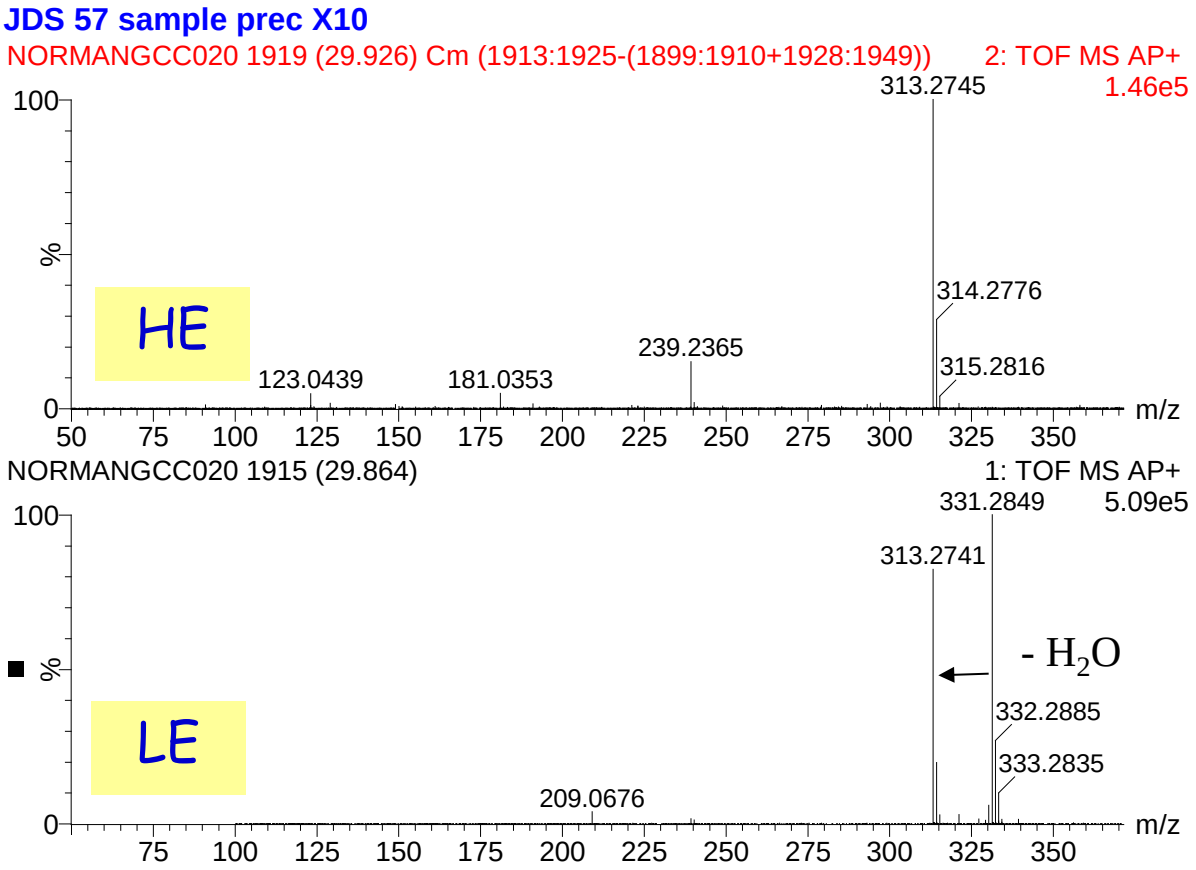


Sample



Workshop on Non-Target Screening
Non-Target Screening of GC-compounds





Workshop on Non-Target Screening
Non-Target Screening of GC-compounds

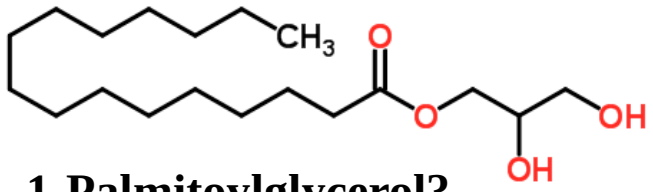
Elemental Composition

File Edit View Process Help

Multiple Mass Analysis: 2 mass(es) processed
Tolerance = 3.0 mDa / DBE: min = -1.5, max = 50.0
Element prediction: On (Carbon range ± 5)
Number of isotope peaks used for i-FIT = 3
Monoisotopic Mass, Even Electron Ions
2771 formula(e) evaluated with 4 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-100 H: 0-200 N: 0-5 O: 0-5 P: 0-1 S: 0-6

Mass	RA	Calc. Mass	mDa	PPM	DBE	Formula	i-FIT	i-FIT Norm	Fit Conf %	C	H	N	O	P	S
313.2741	82.31	313.2743	-0.2	-0.6	1.5	C19 H37 O3	545.8	n/a	n/a	19	37		3		
331.2849	100.00	331.2848	0.1	0.3	0.5	C19 H39 O4	658.5	0.687	50.32	19	39		4		
		331.2862	-1.3	-3.9	5.5	C20 H35 N4	658.9	1.051	34.94	20	35	4			
		331.2878	-2.9	-8.8	0.5	C18 H40 N2 O P	659.8	1.914	14.74	18	40	2	1	1	

JDS 57 sample prec X10
NORMANGCC020 1915 (29.864)
1: TOF MS AP+
5.09e+005



1-Palmitoylglycerol?

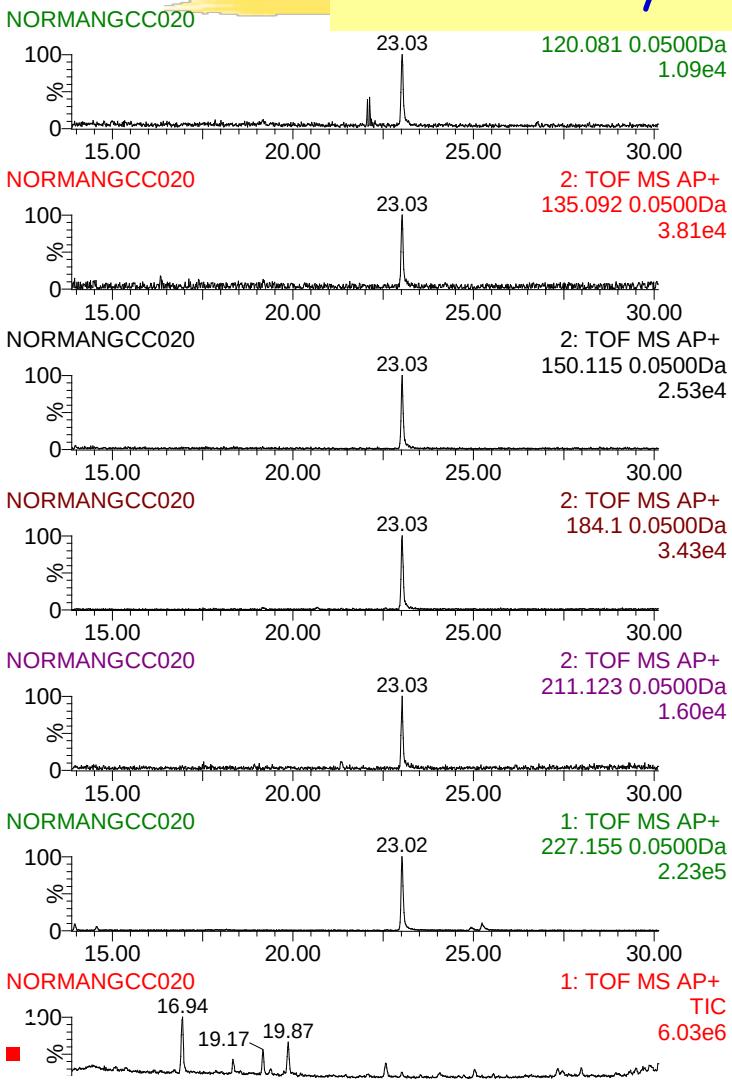
$C_{19}H_{39}O_4^+$

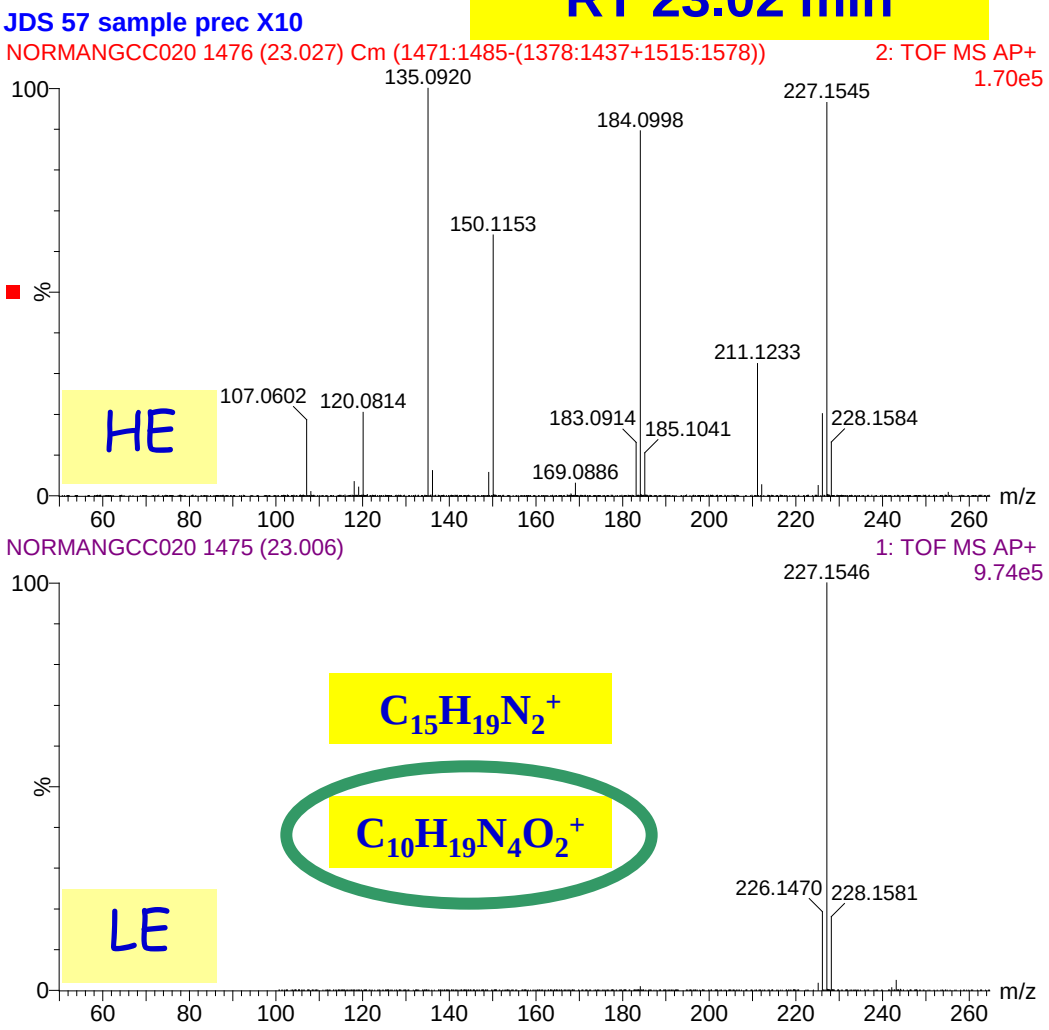
For Help, press F1

Workshop on Non-Target Screening
Non-Target Screening of GC-compounds

Chromalynx XS Non-targeted

m/z 227.1546
RT 23.02 min



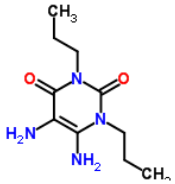
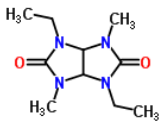
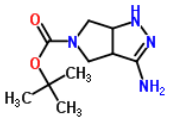
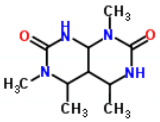
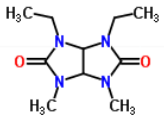


Workshop on Non-Target Screening
Non-Target Screening of GC-compounds

100 hits returned from a total of 199.
Search terms: **C10H18N4O2**
(Found by molecular formula)

Grid | Tile | Table | Names/Structures | Names

1 2 3 4 5

ID	Structure	Molecular Formula	Molecular Weight	# of Data Sources	# of References	# of PubMed	# of RSC
4274252		$C_{10}H_{18}N_4O_2$	226.2755	27	29	0	0
2113217 - 0/2 defined		$C_{10}H_{18}N_4O_2$	226.2755	18	19	0	0
22524993 - 0/2 defined		$C_{10}H_{18}N_4O_2$	226.27552	16	20	0	0
504662 - 0/4 defined		$C_{10}H_{18}N_4O_2$	226.2755	14	16	0	0
617707 - 0/2 defined		$C_{10}H_{18}N_4O_2$	226.2755	14	15	0	0

Workshop on Non-Target Screening GC-APGC-MS/MS

Conclusions

- APGC is a valuable ionization source for coupling GC to novel tandem mass spectrometers (Xevo TQ-S, Xevo G2 Q-Tof, Synapt G2-S HDMS or Xevo TQD)
- APGC allows a "universal" soft-ionization for GC-amenable compounds
- APGC allows selecting molecular ion/protonated molecule as precursor ion rendering more selective and sensitive SRM transitions than EI
- GC-APGC-QTOF allows suspect creening and confirmation of GC-amenable contaminants based on expected $M^{+\cdot}$/MH^+ and MS^E acquisitions, like UPLC-ESI-QTOF
- GC-APGC-QTOF shows potential for non-target screening and elucidation of GC-amenable contaminants

Thank you for your attention

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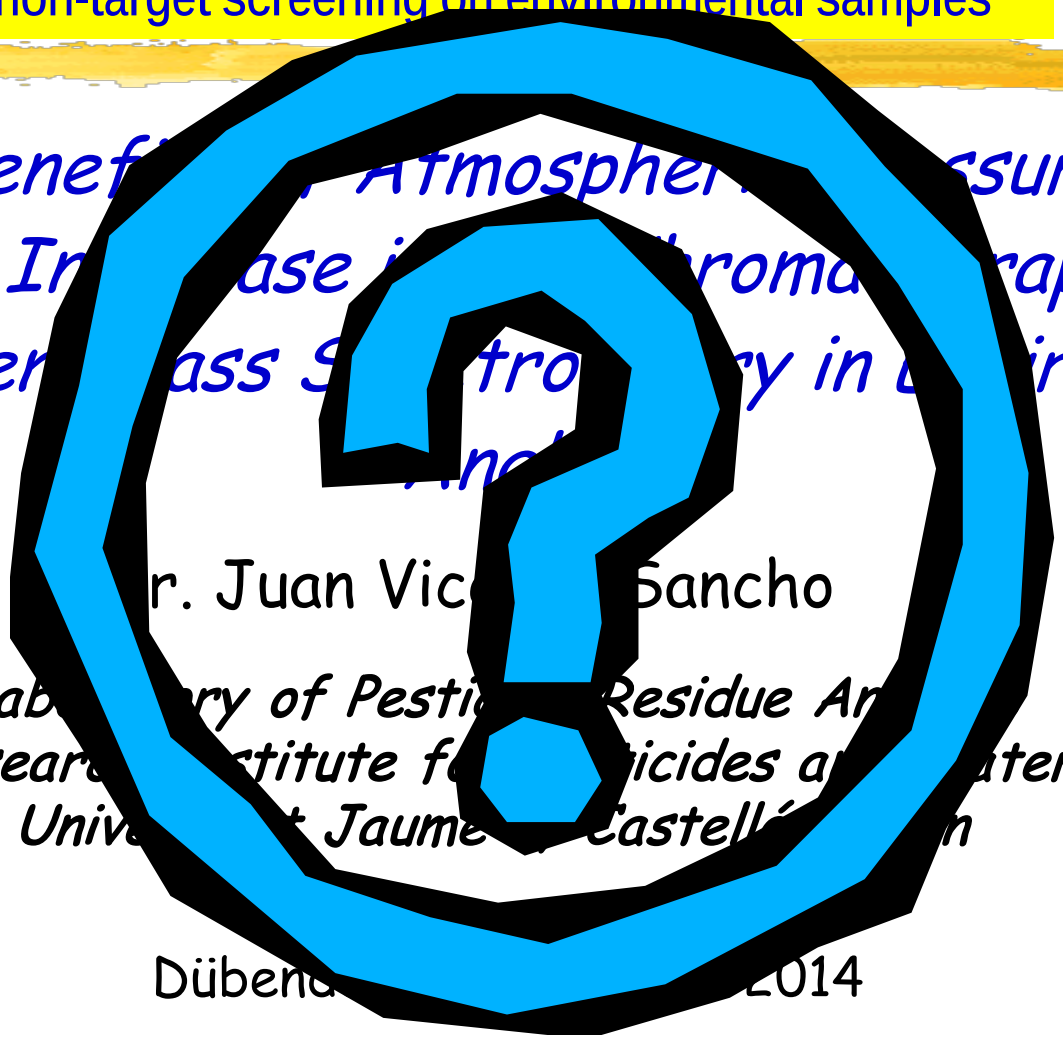
Workshop on Non-Target Screening

Towards the harmonisation of methods for non-target screening on environmental samples

Use and benef[illegible] Atmospher[illegible]ssure Chemical Ionization In[illegible]ase i[illegible]roma[illegible]raphy coupled to Tander[illegible]ass S[illegible]tro[illegible]ry in [illegible]ironmental [illegible]



Dr. Juan Vic[illegible] Sancho

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Univ[illegible]t Jaume [illegible]Castellón[illegible]n

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