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APPLICATION OF GAS CROMATOGRAPHY TIME-OF-FLIGHT HRMS MASS SPECTROMETRY TO FOOD ANALYSIS

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During the last years the introduction of liquid chromatography high resolution mass spectrometry (LC-HRMS) has become popular in pesticide residues laboratories. The advantages in getting exact mass of the analytes have been evaluated giving an important and new solution to common problems of these analyses. But, till now practically all applications have been focused on LC. Recently new GC-HRMS are intending to cover a similar position for typical GC pesticide residues. Some questions remain to clarify if the established workflows in LC can be applicable for GC-HRMS considering of the differences between ionization systems as ESI or EI.

This work reports the development and evaluation of a rapid automated screening method for determining pesticide residues in food using gas chromatography time-of-flight mass spectrometry (GC−TOF-MS) based on the use of an accurate-mass database. The database (including 87 GC amenable pesticides) created include, ions obtained under electron impact ionization at 70 eV and retention times of each pesticide under retention time looking of pesticides at constant flow.

This customized database was associated to commercially available software which extracted all the potential compounds of interest from the GC−TOF MS raw data of each sample and matched them against the database to search for targeted compounds in the sample.

The automatic identification with the developed workflow has been tested in tomato, orange, potato and onion extract spiked at 10, 20, 50 and 100 μg/ml level, most pesticides present in the sample were identified correctly, the identification criteria were a retention time window of 0.5 min, and a mass error tolerance of 10 ppm for at least two fragments for each pesticide.

GC MASS SPECTROMETRIC CONDITIONS

Agilent 7200 Q-TOF GC/MS

Injection volume: 1μL

Mode: Splitless MM Inlet

Inyector temperature: 280ºC

2 columns: HP5MSI (350ºC) 15mx250μmx0.25μm

Full Scan EI Mode

Constant Flow
Column 1 = 1.225ml/min
Column 2= 1.425ml/min

OVEN PROGRAM

Rate(ºC/min)	Value(ºC)	Hold Time(min)	Run Time(min)
	60	1	1
40	120	0	2.5
5	310	0	40.5

Post Run: 310ºC

Post Run Time: 2 min

BACKFLUSH CONFIGURATION

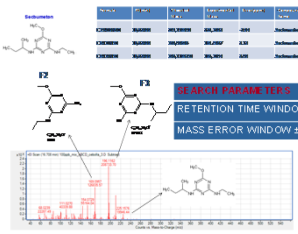
Ion source temperature: 280ºC

Transfer line temperature: 280ºC

ACURRATE MASS DATABASE

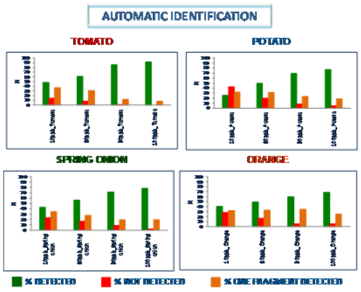
87 PESTICIDES
2 FRAGMENTS AT LEAST FOR EACH PESTICIDE
RETENTION TIMES OF EACH PESTICIDE

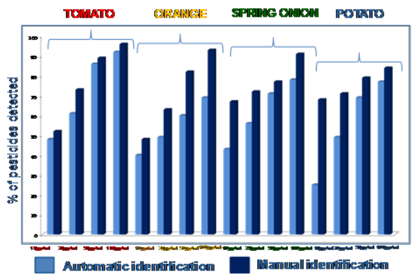
### Formula	Retention Time	Mass	Compound name	Description
# Formula	RT	Mass	Cpd	Comments
C17H16Br2O3	28,12347	425,9466196	Bromopropylate	
C13H9Br2O	28,12347	338,9020151	Bromopropylate F1	
C7H6BrO	28,12347	184,9602024	Bromopropylate F2	
C6H4Br	28,12347	154,9496377	Bromopropylate F3	
C13H12BrCl2N3O	27,92085	374,95408	Bromuconazole	
C10H8BrCl2O	27,92085	292,9135579	Bromuconazole F1	
C13H12Cl2N3O	27,92085	296,0357424	Bromuconazole F2	
C10H9Cl2O	27,92085	215,0030453	Bromuconazole F3	
C7H3Cl2O	27,92085	172,9560951	Bromuconazole F4	
C8H7Cl2	27,92085	172,9924806	Bromuconazole F5	
C13H24N4O3S	24,01706	316,1569113	Bupirimate	
C11H18N3O3S	24,01706	272,1068871	Bupirimate F1	
C11H18N3O	24,01706	208,1449872	Bupirimate F2	
C5H4N2O	24,01706	108,0323628	Bupirimate F3	
C16H23N3OS	23,84666	305,1561831	Buprofezin	
C9H9N2S	23,84666	177,048644	Buprofezin F1	
C7H5NO	23,84666	119,0371138	Buprofezin F2	
C7H8N	23,84666	106,0656743	Buprofezin F3	
C7H11N2OS	23,84666	171,0592087	Buprofezin F4	
C8H16N2S	23,84666	172,1034192	Buprofezin F5	
Continue...	....	....	....	

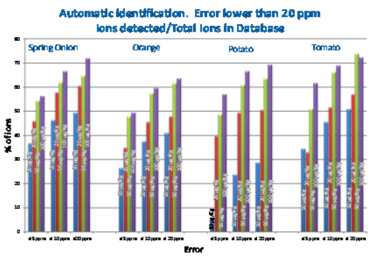


SEARCH PARAMETERS
RETENTION TIME WINDOW ± 0,5 min
MASS ERROR WINDOW ± 10 ppm

AUTOMATIC IDENTIFICATION VS MANUAL IDENTIFICATION

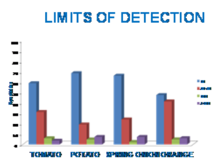






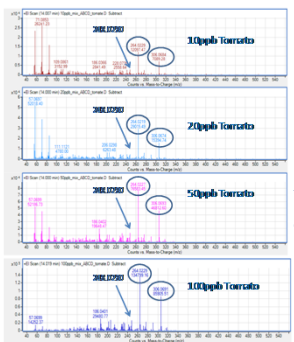
VALIDATION STUDY

LIMITS OF DETECTION



Compound	Spring Onion			Orange		
	R2	LOD(ppb)	Rep. RSD(%) (n=3)100ppb	R2	LOD(ppb)	Rep. RSD(%) (n=3)100ppb
Pirimicarb	0.99629	10	2,9	0.98896	10	4,7
Pirimiphos-Methyl	0.99617	10	1,4	0.99220	10	2,5
Procymidone	0.99214	10	1,4	0.99093	10	0,6
Prometon	0.99328	10	1,3	0.99110	10	2,4
Prometryn	0.99165	10	1,9	0.99136	10	1,4
Prothiophos	0.99827	10	1,4	0.99164	10	0,6
Pyriproxyfen	0.99094	10	1,4	0.99806	10	0,9
Quinoxyfen	0.99070	10	8,8	0.99625	10	0,9
Secbumeton	0.99157	10	0,1	0.99095	10	1,7
Tebufenpyrad	0.99236	10	0,8	0.99033	10	3,6
Tecnazene	0.99051	10	3,5	0.99213	10	0,5
Terbumeton	0.99417	10	0,1	0.99961	10	1,4
Terbutryn	0.99351	10	1,2	0.99230	10	1,7
Continue...	...	...	...	...	...	...

Linearity, Repeatibility and Limits of Detection in spring onion and orange matrices

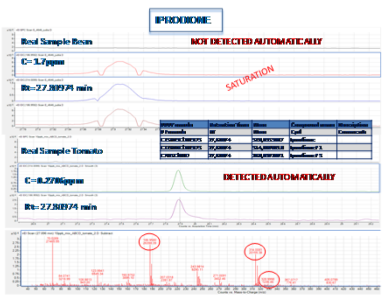


TRIFLURALIN

	10ppb	20ppb	50ppb	100ppb
Absolute Abundances				
264.0232	*12097.47*	*29015.49*	*76583.45*	*134799.16*
306.0702	7089.28	15394.74	46812.60	85905.51
248.0280	2802.65	4210.54	13306	24920.55
Relative Abundances to base peak 264.0232				
306.0702	59%	53%	61%	64%
248.0280	23%	15%	17%	18%

The relative abundance is constant at different concentrations

ANALYSIS OF REAL SAMPLES



REAL SAMPLES

Muestra	GC-TOF automatico	GCQQQMS	GC-TOF manual
Pepper	Boscalid (0.25mg/Kg)	Boscalid(0.23mg/Kg)	
Tomato_1	Iprodione (0.20mg/Kg)	Iprodione(0.27mg/Kg)	
	Pyriproxifen (0.054mg/Kg)	Pyriproxifen (0.062mg/Kg)	
Tomato_2	**Bifenthrin 1F(0,12**mg/Kg**)**	**Bifenthrin (0.13mg/Kg)**	**Ion 166.099 error 140mg/Kg**
Melon_1	Bupirimate (0.15mg/Kg)	Bupirimate(0.13mg/Kg)	
Tomato_3	Buprofezin(0.49mg/Kg)	Buprofezin(0.16mg/Kg)	
	Metalaxyl(0.060mg/Kg)	Metalaxyl(0.068mg/Kg)	
Zucchini	Pirimicarb(0.33mg/Kg)	Pirimicarb(0.24mg/Kg)	
	Bupirimate(0.11mg/Kg)	Bupirimate(0.048mg/Kg)	
	Penconazole(0.091mg/Kg)	Penconazole(0.074mg/Kg)	
Brussels sprouts	Boscalid(0.21mg/Kg)	Boscalid(0.12mg/Kg)	
Tomato_4	**Bifenthrin 1F (0.10mg/Kg)**	**Bifenthrin (0.125mg/Kg)**	**Ion 166.099 error 108mg/Kg**
Bean_1*	**Iprodione 1F(0.830mg/Kg)**	**Iprodione(1.02mg/Kg)**	**Saturation***
Bean_2	Azoxystrobin ND	Azoxystrobin(0.337mg/Kg)	**Coelute with column peak**
	Iprodione(0.36mg/Kg)	Iprodione(0.25mg/Kg)	
Bean_3	Azoxystrobin ND	Azoxystrobin(0.11mg/Kg)	**Coelute with column peak**
	Iprodione(0.19mg/Kg)	Iprodione(0.078mg/Kg)	
Melon_2	Azoxystrobin ND	Azoxystrobin(0.16mg/Kg)	**Coelute with column peak**
	Bupirimate(0.11mg/Kg)	Bupirimate(0.08mg/Kg)	
Bean_4*	Iprodione ND(1.82mg/Kg)	**Iprodione(1.70mg/Kg)**	**Saturation***
Bean_5*	Iprodione ND(1.27mg/Kg)	**Iprodione(1.70mg/Kg)**	**Saturation***
Grape	Iprodione(0.15mg/Kg)	Iprodione(0.11mg/Kg)	
	Myclobutanil 0.041mg/Kg	Myclobutanil(0.041mg/Kg)	

*These samples were injecting diluted 1:2 to obtain a correct identification and quantification and the mass spetrum show at least 2 fragments ions.

CONCLUSIONS

The development of an accurate mass database in GC-TOF-MS has been performed, in a first step the pesticides were analyzed under EI at 70eV and with retention time locked. Their mass spectrum were investigated, each experimental ion has been assigned to a part of the molecule in order to calculate the theoretical exact mass of each fragment. In most of cases the molecular ion was not present in the mass spectrum.

The accurate mass data base created includes at least two fragments for each pesticide and its retention time.

The accurate mass data base was tested for automatic identification in four matrices: tomato, spring onion, orange and potato in four different concentrations. In all cases the error was lower than ±20ppm and in around a 65% of the detected ions the error was lower than ± 5ppm.

The automatic identification was compared with manual identification and differences arise especially at low concentrations.

15 real samples were processed for the automatic identification with the developed data base, results were compared with those obtained by GC-QQQ-MS, from a total of 22 pesticides presents in the real samples 14 were correctly identified automatically, and three pesticides were not identified correctly by GCQTOF because the high concentration detected saturate the detector.

This work is a first approach to the accurate mass database for automatic identification in GC-TOF-MS. It can be considered as a starting point for a large database and for an improved methodology for automatic identification.