Development of a New Analytical Method for Determining Pesticide Residues by Gas Chromatography-High Resolution Mass Spectrometry using the Zebron™ ZB-5MS*PLUS*™ GC Column

Javier Arrebola, PhD[1], Roberto Romero, PhD[1], Antonia Garrido, PhD[1], Irene Dominguez, PhD[1], Francisco Egea, PhD[1], Sean Orlowicz[2], Richard Jack, PhD[2], Ramkumar Dhandapani, PhD[2]

[1]Department of Chemistry and Physics, University of Almeria, Carr. Sacramento, s/n, 04120 La Cañada, Almeria, Spain, [2]Phenomenex, Inc., 411 Madrid Ave., Torrance, CA 90501, USA

Introduction

Food from plant origin, fresh or processed ones, may contain pesticide residues due to agricultural practices. Food safety control is highly relevant for the protection of consumers and therefore, pesticide residues are regulated by governments and professional associations. Maximum residue limits (MRLs) establish the maximum allowed amount of pesticides in fruits and vegetables to be consumed. Although MS/MS based methods are popular for pesticide analysis, GC-HRMS adds a new dimension of authenticity with accurate mass measurement.

HRMS instruments present high capability for unequivocal identification of compounds thanks to the measurement of accurate mass. The combination of gas chromatography with HRMS is a very powerful tool together with nominal spectral libraries for screening purposes. Here we show the capabilities of the Zebron ZB-5MS*PLUS* columns in separating residual pesticides by coupling GC with HRMS. The ZB-5MS*PLUS* stationary phase provides a deactivated silica surface and a 5 % phenyl-arylene selectivity that provides the best peak shape for challenging pesticide compounds. Such methodology has a higher impact on users than a traditional one based on low resolution mass spectrometry.

Materials and Methods

Sample Preparation

Fruit and vegetable samples were chopped and homogenized according to the method established in Directive 2002/63/EC. Samples were processed following a citrated buffered roQ™ QuEChERS protocol (KS0-8909).

A 10 g portion of sample was weighed in a 50 mL polypropylene centrifuge tube. 10 mL of Acetonitrile was added to the sample, and was vortexed for 2 min. Afterwards, 4 g of Magnesium Sulfate, 1 g of Sodium Chloride, 1 g of Trisodium Citrate Dehydrate, and 0.5 g of Sodium Hydrogencitrate Sequihydrate were added. The sample was vortexed for 2 min. The tube was centrifuged for 6 min at 5000 rpm (4136 x g).

Then, a clean-up step was carried out. 5 mL of the extract was transferred to a 15 mL centrifuge tube containing 750 mg of Magnesium Sulfate and 125 mg of Primary/Secondary Amine. The sample was again vortexed for 1 min. The tube was then centrifuged for 5 min at 3700 rpm (3601 x g). Finally, a 1 mL aliquot of the clean extract was evaporated with a Nitrogen stream to dryness and re-dissolved with 950 µL of Ethyl Acetate and 50 µL of Propoxur-d7.

GC-HRMS Conditions

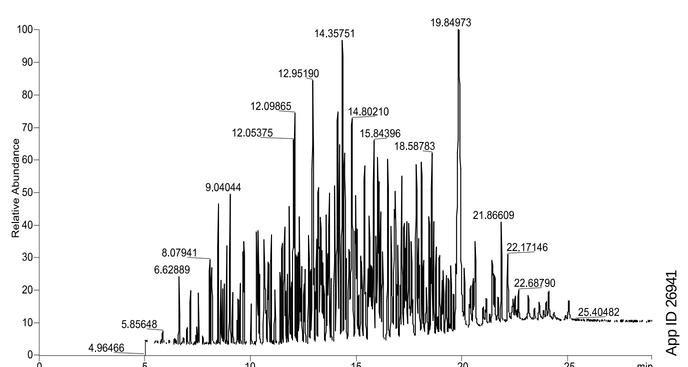
Column: Zebron ZB-5MS*PLUS*
Dimension: 30 meter x 0.25 mm x 0.25 µm
Part No.: 7HG-G030-11
Injection: Splitless @ 280 °C, 1 µL
Carrier Gas: Helium @ 1 mL/min (Constant Flow)
Oven Program:

Ramp(°C/min)	Temp (°C)	Time(min)
-	50	1.0
20	170	0.0
10	310	8.0

Detector: GC-MS
Detector Temperature: 250 °C

Results

Figure 1. Profile of 268 Pesticides on a Zebron ZB-5MS*PLUS* GC Column.



Results

Figure 2. Calibration Curve Obtained for the Pesticide Atrazine. Working Range Between 0.001 and 0.250 mg/kg.

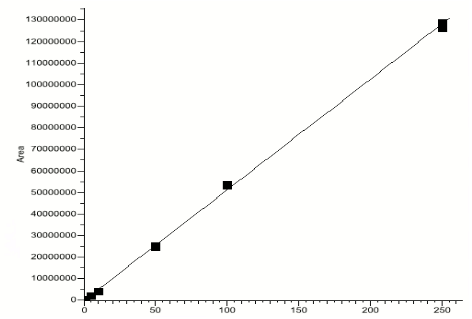
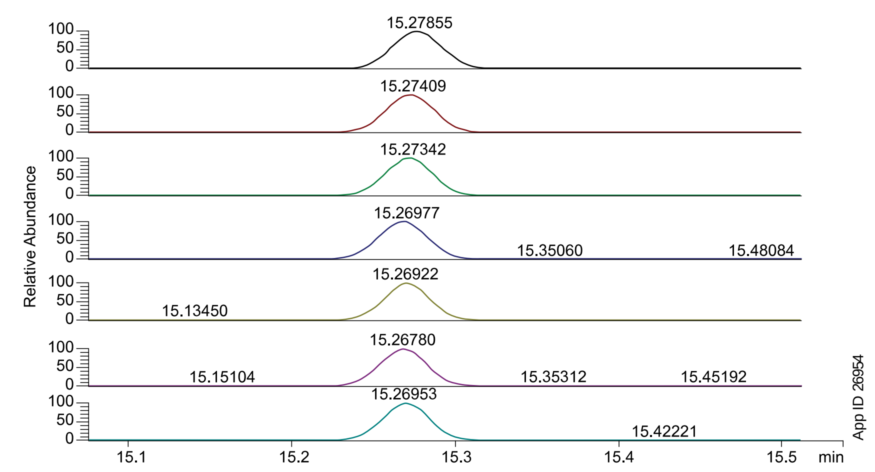


Table 1. Calibration Curve R2 Values for Pesticides.

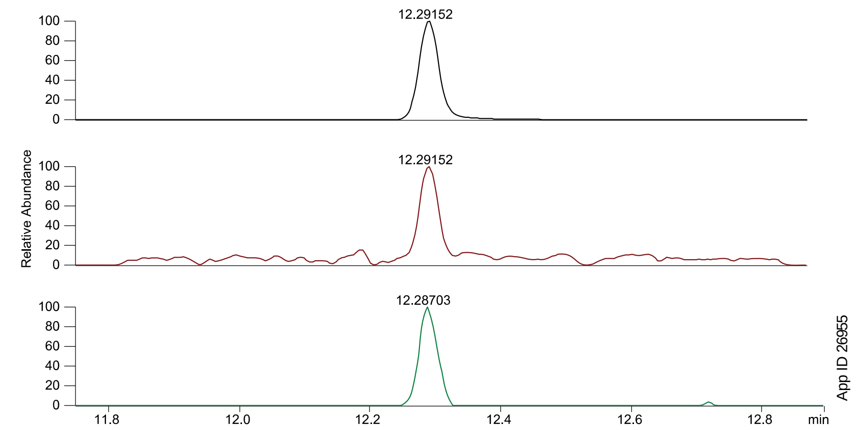
Compound Name	R^2	Compound Name	R^2
1,4-Dimetilnaftaleno	0.998942219	Disulfoton	0.99793204
2-phenylphenol	0.998386807	Ditalimfos	0.998949092
3,5-Dichloroaniline (3,5-Dichlorobenzenamine)	0.998630422	Edifenphos	0.998653756
4,4-Dibromobenzophenone	0.998386517	Endosulfan ether	0.998638926
4,4-Diclorobenzofenon	0.998228884	Endosulfan sulfate	0.999399315
4-Chloro-3-methylphenol	0.996688092	Endrin	0.998706712
Aclonifen	0.99353018	Endrin ketone	0.999094107
Acrinathrin	0.998209792	EPTC (Eptam)	0.999078096
Alachlor	0.999028669	Esfenvalerate	0.998456522
Aldrin/Aldrin-r	0.998418941	Ethion	0.991168804
Anthraquinone	0.998542205	Ethoprophos	0.999075798
Atrazine	0.999238283	Ethoxyquin	0.99138868
Azoxystrobin	0.99829505	Etridiazole	0.999002176
Benalaxyl	0.998976646	Etrimfos	0.999232783
Benfluralin	0.995444223	Famfur (Fonofos)	0.999047473
Benfuresate	0.998892031	Fenamiphos	0.998265698
Benodalil	0.998558157	Fenamiphos sulfone	0.99780723
Benoxacor	0.99725214	Fenamiphos sulfoxide	0.999045189
Benzene, hexachloro	0.999296242	Fenarimol	0.998445175
Benzyl benzoate	0.997957247	Fenazaquin	0.995167663
Bifenazato	0.97333699	Fenbuconazol	0.998503846
Biphenyl	0.998079986	Fenchlorphos/Ronnel	0.99932361
Bifenox	0.999503797	Fenhexamid	0.991202336
Bifenthrin	0.998892937	Fenitrothion	0.999177411
Bitertanol	0.996683478	Fenobucarb	0.999194468
Boscalid	0.998999274	Fenoxaprop-P-ethyl	0.998502038
Bromacil	0.9988172	Fenoxycarb	0.998782417
Bromocyclen	0.998985819	Fenpropathrin	0.998961115
Bromophos-ethyl	0.999045465	Fenpropimorph	0.999120732
Bromophos-methyl	0.999417057	Fenson	0.997326316
Bromopropylate	0.99904998	Fenthion	0.999217523
Bupirimate	0.998402377	Fenvalerate	0.998299334
Buprofezin	0.998783956	Fipronil	0.99877209
Butafenacil	0.995907777	Fipronil sulfone	0.998210819
Butilate	0.997556098	Flucythrinate	0.998086264
Butralin	0.99961669	Fluchloralin	0.994865125
Cadusafos	0.998957049	Fludioxonil	0.99906409
Carbophenothion	0.998919631	Flumetralin	0.999314275
Carbophenothion methyl	0.987212444	Flumioxazin	0.993295923
Cinidon-ethyl	0.995907777	Fluotrimazole	0.998048853
CIS 1,2,3,6-Tetra-hydrophthalimide	0.995423132	Fluquinconazol	0.99941145
Clodinafop-propargyl	0.993635698	Fluvalinate (Tau)	0.994672664
Chlorfenapyr	0.999057548	Fonofos	0.999106042
Chlorfenprop methyl	0.998813165	Formothion	0.999287337
Chlorfenson /Ovex	0.999135823	Furalaxyl	0.99916366
Chlorfenvinphos	0.999093123	Halfenprox	0.990197591
Chlormephos	0.998383875	Heptacloro-epoxido-A-endo (cis)	0.998631709
Chlorpropham	0.998562723	Heptacloro-epoxido-B-exo (trans)	0.99819743
Clortion	0.975372542	Heptachlor	0.999479566
Chlozolinate	0.998486175	Heptenophos	0.998630245
Crimidine	0.998782904	Hexachlorocychlohexane-alfa	0.999450172
Cyanofenphos	0.998919631	Hexaconazole	0.999224733
Cyanophos	0.998731622	Hexazinone	0.998034805
Cycloate	0.999135817	Indoxacarb	0.997968095
Cyflufenamid	0.999161703	Iodofenphos	0.999332257
Cyfluthrin	0.998741963	Iprodione	0.997695069
Cypermethrin	0.997370526	Isobenzan	0.998425394
Cyproconazole	0.998974836	Isocarbophos	0.99880613
Cyprodinil	0.999115342	Isodrin	0.998901603
Chinomethionate	0.998256561	Isofenphos	0.99858637
Chlordane (CIS)	0.998506236	Isofenphos-methyl	0.998971183
Chlorflurenol-methyl	0.999313753	Isomethiozin	0.999269117
Chloropropylate	0.997820634	Isopropalin	0.999300247
Chlorothalonil	0.999210031	Isoprothiolane	0.998929638
Chlorpyrifos – ethyl	0.998568648	Kresoxim-methyl	0.998635139
Chlorpyrifos – methyl	0.999224693	Lambda Cyhalothrin	0.999275047
DCPA (Clortal dimetil)	0.998981624	Lenacile	0.998788016
DDD-o,p (Mitotane)	0.998890375	Leptophos	0.998874746
Deltamethrin	0.997991024	Lindane-gamma	0.999450172
Diazinone	0.998518326	Malathion	0.996880777
Diclofop-methyl	0.998777327	Mefenpyr-diethyl	0.997472144
Dicofol, 4,4	0.998832171	Mepanipyrim	0.998854165
Dichlobenil (Benzonitrile, 2,6-dichloro-)	0.99907646	Metalaxyl	0.998927861
Dichlofenthion	0.998759718	Metazachlor	0.999315932
Dichloran (Botran)	0.99916187	Methamidophos	0.991286302
Dichlorvos	0.998739233	Methidation	0.998985109
Dieldrin	0.998706712	Methoxychlor	0.995563934
Difenoconazole	0.998548285	Metolachlor	0.998984445
Diflufenican	0.998751621	Mevinphos	0.998805054
Dimethomoph	0.996333623	Mirex	0.999450884
Diniconazole	0.999205997	Myclobutanil	0.998623477
Diphenylamine	0.998483731		

Results

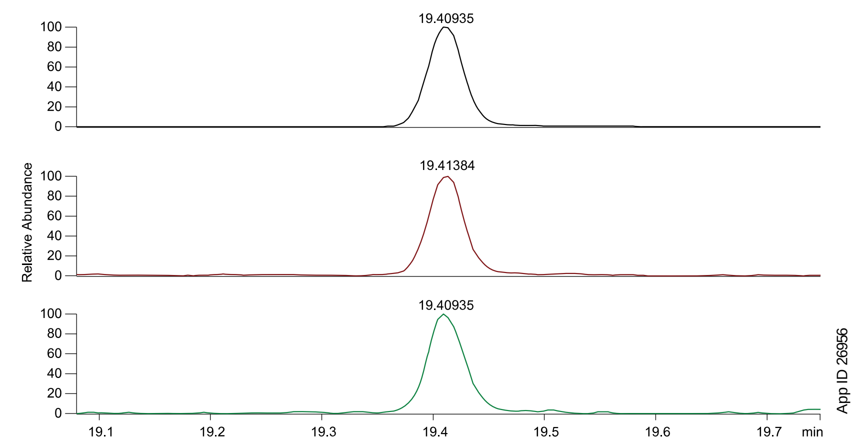
Figures 3. Chromatographic Peaks obtained Monitoring the Quantification Ion of the Pesticide Bromophos-Ethyl.



Figures 4. Orange Sample Containing 0.016 mg/kg of Pyrimethanil.

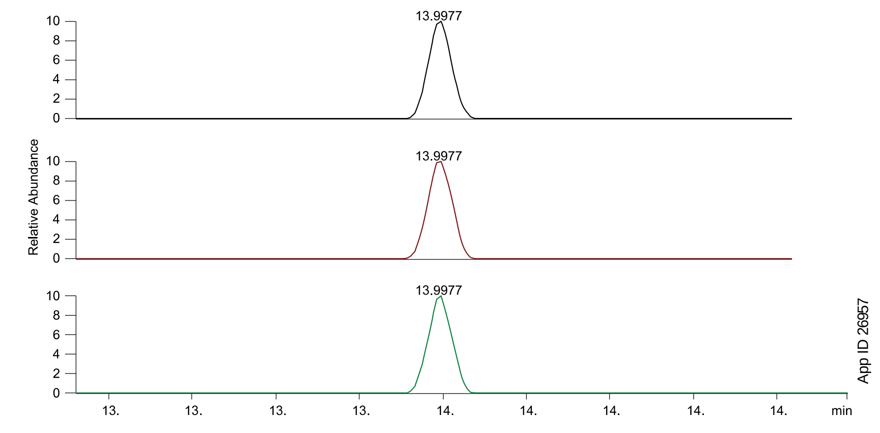


Figures 5. Lemon Sample Containing 0.023 mg/kg of Pyriproxifen.



Results

Figures 6. Banana Sample Containing 0.043 mg/kg of Chorpyrifos-Ethyl.



Discussion

Pesticide analysis is extremely challenging due to the number of pesticide compounds that one might be looking for. 268 pesticides were included in the developed method. All the compounds were monitored with three characteristic ions that provide adequate selectivity for their determination. High resolution applied for obtaining such ions ensure a drastic diminution of interferences and elimination of matrix effect. It reduces significantly the risk of false positive or negative results. All the compounds were registered in the software for automatic detection of the compounds in target mode. The highest intensity ion is used for quantification of the compounds by monitoring the extracted ion chromatogram. Chromatographic peaks were quantified by peak area relative to the one of the internal standards. The other two ions were used for confirming the results. Mass accuracy resulted very useful for increasing reliability of the results. In **Figure 1**, a total ion chromatogram obtained with the proposed experimental conditions is shown for an apple extract spiked at a concentration level of 0.500 mg/kg.

Calibration was performed using three replicates of each calibration point. The working range for every compound was calculated and considered a linear calibration because it is the most typical calibration function selected in routine laboratories. **Figure 2** shows an example of the linearity with the results of the pesticide Atrazine. All the proposed working ranges generated a R^2 higher or equal to 0.99 and adequate recoveries for all the studied concentrations (**Table 1**). An example of the chromatographic peaks obtained while monitoring the quantification ion of one of the studied pesticides for the calibration curve can be seen in **Figure 3**. This shows the stability of the retention time for the pesticide. The recovery and precision of the method have been calculated at 0.01 and 0.1 mg/kg (n=5). Recoveries were considered as acceptable when they were between 70 and 120 %. Precision was expressed as relative standard deviation (%RSD).

Over 200 real samples were analyzed to evaluate the robustness of the chromatographic method and stationary phase on the Zebron ZB-5MS*PLUS* GC column. Different fruits and vegetables were tested. A selection of matrices were used here as an example including oranges, lemons and bananas, to detect trace levels of pesticides in them (**Figures 4-6**).

Conclusion

The study here demonstrates detection and recovery of 268 multi pesticide compounds from various fruit matrices. In addition, the method is linear over a wide range to accommodate trace level detection of pesticides from real samples.

The Zebron ZB-5MS*PLUS* GC column not only gave adequate separation and inertness, but also provided reproducible retention with multiple injections of real samples and proving the robustness of the stationary phase..

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