APPLICATION NOTE

Liquid Chromatography

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Safeguarding Food from Pesticides by UHPLC After Extraction with the QuEChERS Method

Introduction

The detection of pesticides in food matrices, such as fruit and vegetables, requires an ever increasing use of new technologies. Chromatographic techniques, both gas (GC) and liquid (LC), offer a suitable means of addressing the analytical requirements. They enable screening to be carried out rapidly both in gaseous and liquid phases – on samples prepared using the "QuEChERS" (Quick, Easy, Cheap, Effective, Rugged, Safe) system, a fast and effective multi-residue extraction method.

Employing UHPLC (Ultra High Performance Liquid Chromatography) with a PDA (Photo Diode Array) detector offers considerable advantages over standard LC analysis. Indeed, it provides a very fast means of monitoring phytodrugs which are permitted in concentrations of up to 10 µg/Kg. The use of a PDA detector, together with the development of a library of spectra, enables each substance to be analyzed and identified correctly.

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Figure 1. Flexar FX-10 UHPLC.

Table 1. HPLC instrumental conditions.

UHPLC System	PerkinElmer Flexar FX-10
Column	C18, 100 mm x 3 mm ID, 1.8 µm
Injection Volume	20 µL
Mobile phase	A) o-phosphoric acid 0.1% v/v B) Acetonitrile
Gradient	Equilibrium 3' 75% A – 25% B
Total analysis time: 16 minutes	Step 1: 2 min 60% A – 40% B curve -2 Step 2: 3 min 50% A – 50% B curve 2 Step 3: 2 min 40% A – 60% B curve 2 Step 4: 3 min 25% A – 75% B curve 3 Step 5: 2 min 15% A – 85% B curve 2 Step 6: 2 min 5% A – 95% B curve 2 Step 7: 2 min 5% A – 95% B
Back pressure	From 7000 psi (Step 1) to 4500 psi (Step 7)
Flow rate	0.65 mL/min for all steps
Wavelength	210 nm
Column temperature	40 °C

Experimental phase

The study also includes the extraction of the samples using a standardized approach known as QuEChERS, which is an official, AOAC-compliant method for determining pesticide residues in samples of fruit and vegetables (Multi-residual pesticides) as per the European standard EN-15662.

Extraction procedure using the QuEChERS method

Place 15 g of homogenized sample in a 50 mL extraction tube containing 1.5 g of sodium acetate and 6 g of magnesium sulphate.

- Add 15 mL of an acetonitrile solution containing 1% glacial acetic acid. Add the internal standard, if required.
- Agitate vigorously for at least 1 minute and centrifuge for 1 minute at > 1,500 g.
- Transfer 1 mL of the supernatant (equivalent therefore to 1 g of sample) into a 2 mL extraction tube containing 50 mg of PSA and 150 mg of magnesium sulphate. Agitate vigorously for at least 30 seconds and then centrifuge for 1 minute at > 1,500 g.
- Take the clear supernatant and place in a vial for injection into the UHPLC system, or dilute it first, if necessary, with an appropriate solvent.

The analysis is carried out using a UHPLC system with Photo Diode Array detection.

Preparation of standards

Nine mixtures of different pesticides were used, starting with individual solutions of each in acetone at a concentration of 200 ppm. From each of these mixtures, diluting as required in 1:1 acetonitrile:water, 6 standard solutions were prepared at concentrations of 10, 40, 100, 400, 2,000 and 4,000 ppb. Chromatograms for each of the mixtures are shown in Figure 3.

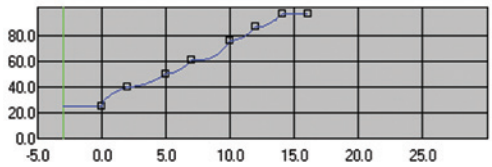

Figure 2. Pump gradient profile (solvent B).

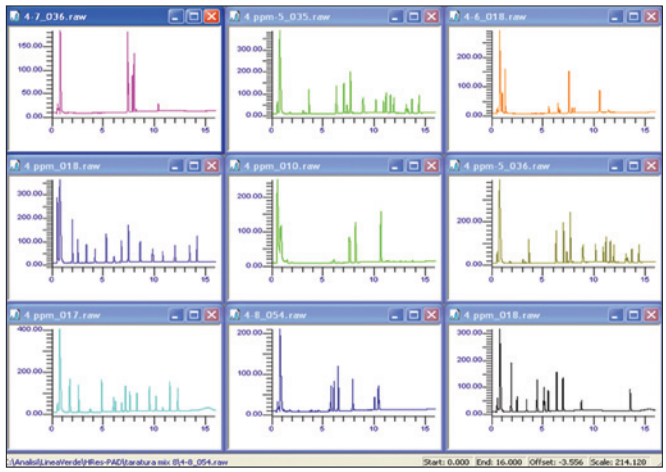

Figure 3. Pesticides standard solutions.

The calibration was linear over the range 10-4,000 ppb with a correlation value r^2 in the range 0.999-0.9999 for all components (see Appendix 1), and Figure 4 illustrates an example calibration curve for a component chosen at random (highlighted in blue).

Table 2. **List of compounds analyzed.**

Cyflutryn	Chiorpropham	Chlorpyrifos-Methyl	Fenbuconazol	Sethoxydim
Cymoxanil	Acrinathrin	Hexaconazole	Pencycuron	Iprovalicarb
Deltamethrin	Triadimefon	Cycloxidim	Thiacloprid	Methiocarb
Dicloran	Fludioxonil	Cyromazine	Pyraclostrobin	Difenoconazole
Indoxacarb	Azoxystrobin	Napropamide Fenarimol	Folpet	Metribuzin
Iprodion	Boscalid	Fenhexamid	Captan	Phomix
Metalaxyl	Diflubenzuron	Fenpyroximate	Hexythiazox	Fenamidone
Primicatb	Bitertanol	Tebuconazol	Phosalone	Pyriproxyfen
Tolcoflos Methyl	Buprofezin	Imidacloprid	Dimethomorph isomer mixture E+2 isom	Fenazaquin
Chlorothalonil	Cypermethrin isomer mixture	Etridiazole	Clomazone beta	Dithianon
Cyproconazole	Diclofuanid	Flufenoxuron	Chlorthal-dimethyl	Famoxadone
Cypronidil	Fluazifop-Buthyl	Oxyfluorfen	Aclonifen	Oxamyl
Lambda- Cythalothrin	Azinphos-Methyl	Phenamifos	Endusulfan sulphate	Carbosulfan
Benalaxyl	Azinphos-Ethyl	Propachlor	Fenvalerate	Cyazofamid
Pyrimethanil	Pirimphos-Ethy	Propaquizafop	Etofenprox	Propham
Thiram	Prochloraz	Pymetrozine	Phenmedipharm	Benfuracarb
Malathion	Fluvalinate isomer mixture	Quizalofop-p-ethyl	Lenacil	Teflubenzuron
Linuron	Fenoxaprop	Tetraconazole	Sethoxydim	

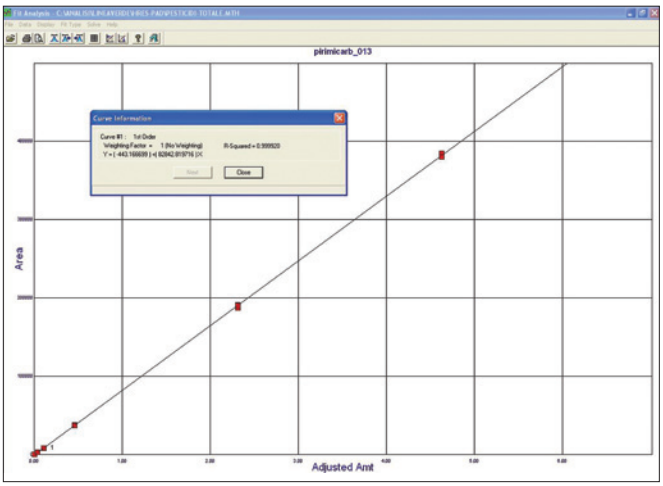

Figure 4. Calibration curve.

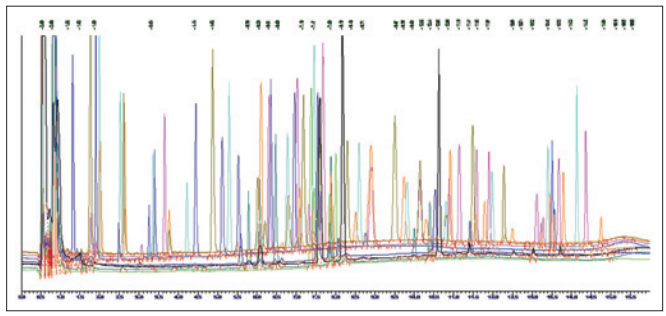

Figure 5. Overlay of all 90 pesticides.

UV spectrum identification

This method involves the identification of the individual components using 3 different libraries obtained at different concentrations:

High Concentration: 4,000 ppb

Medium Concentration: 400 ppb

Low Concentration: 40 ppb

Thus, it is always possible to optimize the identification as a function of the concentration of the sample.

An example identification based on the spectrum obtained (Figure 6) using the appropriate library is described below.

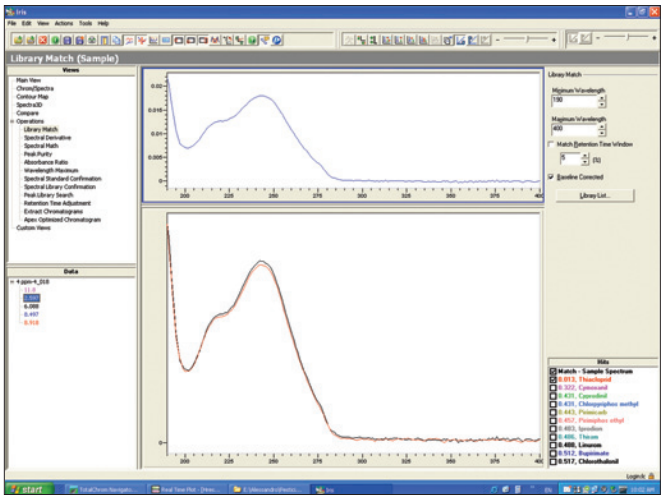

Figure 6. Identification using spectral library.

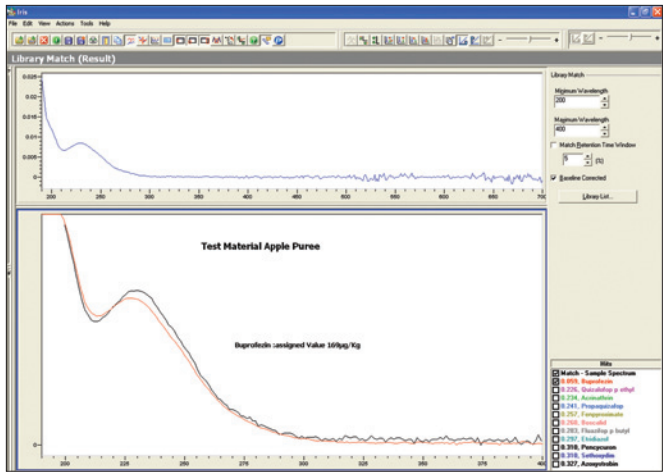

Figure 8. Spectral profile identification of apple puree test material.

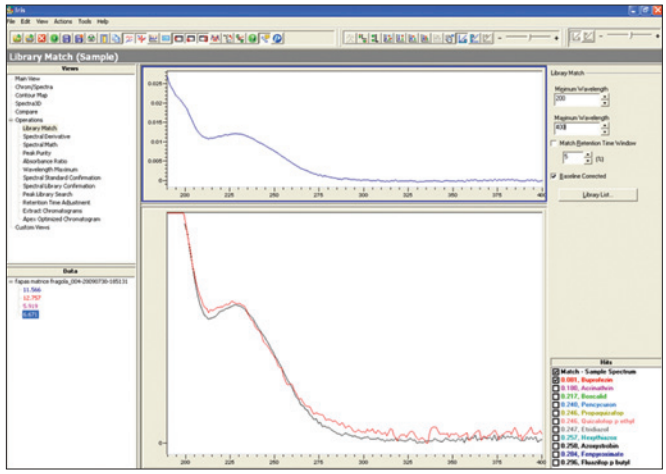

Figure 9. Spectral profile identification.

Analysis of a certified "Strawberry" sample

To demonstrate the effectiveness of the method, a certified sample of strawberries was analyzed (FAPAS® Ref. Number T1986 Strawberry Purée) containing Buprofezin. The substance was identified correctly as shown below (150 µg/kg).

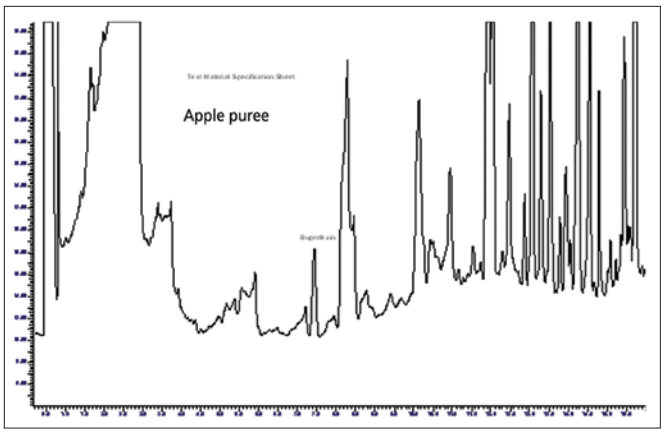

Figure 7. Chromatogram of apple puree test material.

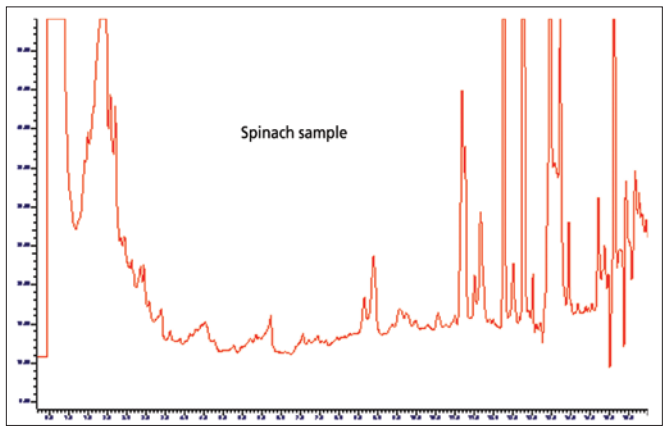

Figure 10. Chromatogram of spinach sample.

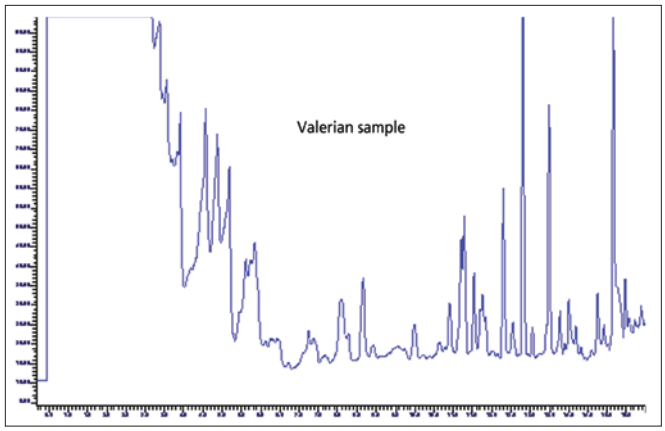

Figure 11. Chromatogram of valerian sample.

Conclusion

This project involved the analysis of over 90 pesticides which are commonly used in treating many species of fruit and vegetables. They were not determined by GC due to incomplete separation and low sensitivity.

The chromatographic analysis obtained with UHPLC enables the pesticides under investigation to be characterized with high precision identification and good sensitivity over the range 40 µg-4,000 kg in less than 16 minutes. In addition the use of the QuEChERS extraction system facilitates very rapid sample preparation. Given that a real sample will be unlikely to contain all 90 pesticides, it is not the primary aim of this method to achieve a baseline separation of every single one. The objective of using this method is to provide a rapid and reliable method of analysis which combines UHPLC with fast sample preparation. Furthermore, identification via a library of UV spectra created under the same UHPLC analytical conditions ensures that the individual compounds will be identified accurately.

Appendix 1

Pesticide	Linear regression coefficient (r^2)
Pirimicarb	0.999920
Clopyralid	0.999705
Imazamox	0.999616
Chloridazon	0.999908
Imidacloprid	0.99988
Cymoxanil	0.999779
Thiram	0.999832
Pyrimethanil	0.999775
Thiacloprid	0.999885
Lenacil	0.999699
Metribuzin	0.995980
Cyprodinil	0.999512
Benfuracarb	0.998758
Carbosulfan	0.999448
Bupirimate	0.999270
Metalaxyl	0.999944
Prochloraz	0.997694
Propaclor	0.999848
Propham	0.999334
Clomazone	0.999958
Buprofezin	0.999455
Dichloran	0.999696
Cyproconazol	0.999116
Dimethomorph	0.998258

Pesticide	Linear regression coefficient (r^2)
Methiocarb	0.999662
Phenmedipham	0.998339
Linurom	0.999913
Fludioxonil	0.999184
Cyazofamid	0.999129
Fenazaquin	0.999951
Azinphos methyl	0.998249
Captan	0.999921
Fenamiphos	0.999767
Iprovalicarb	0.999892
Azoxystrobin	0.999666
Triadimefon	0.999645
Fenhexamid	0.999473
Tebuconazol	0.999608
Boscalid	0.997999
Tetraconazole	0.999703
Sethoxydim	0.999757
Napropamid	0.999802
Chlorpropham	0.999489
Fenamidone	0.999874
Hexaconazole	0.999090
Dithianon	0.995886
Fenbuconazol	0.999163
Diflubenzuron	0.999441

Appendix 1 continued

Pesticide	Linear regression coefficient (r^2)
Iprodion	0.999230
Chlorothalonil	0.999785
Fenoxaprop-p-ethyl	0.999658
Bitertanol	0.998290
Malathion	0.998745
Folpet	0.999858
Azinphos ethyl	0.998583
Etridiazol	0.996639
Aclonifen	0.998505
Dichlofluanid	0.999348
Benalaxyl	0.999051
Difenconazol	0.999642
Chlorthal dimethyl	0.999152
Famoxadone	0.999825
Pyraclostrobin	0.997821
Chlorpyriphos methyl	0.999293
Toclofos methyl	0.998704
Pencycuron	0.999220
Pirimiphos ethyl	0.998005
Endosulfan sulfate	0.998667

Pesticide	Linear regression coefficient (r^2)
Phosalone	0.998413
Teflubenzuron	0.999864
Cycloxidim	0.999844
Indoxacarb	0.991326
Quizalofop-p-ethyl	0.997600
Propaquizafop	0.996202
Pyriproxyfen	0.998287
Pyriproxyfen	0.998287
Oxyfluorfen	0.997626
Fluazifop-p-butyl	0.997439
Flufenoxuron	0.998968
Hexythiazox	0.993939
Fenpyroximate	0.997778
Cyfluthrin	0.999320
Lambda cyhalothrin	0.993716
Deltamethrin	0.999384
Fenvalerate	0.999085
Acrinathrin	0.999826
Fluvalinate	0.996263
Etofenprox	0.996979
Phoxim	0.998382

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