

## A Rapid Method for the Screening and Confirmation of Over 400 Pesticide Residues in Food

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### Abstract

To utilize the power of UltraPerformance Liquid Chromatography (UPLC) combined with fast MS acquisition rates, to give a rapid method for the screening of 402 pesticide residues in a single 10 minute run. A second injection, for confirmatory purposes, will meet SANCO Analytical Quality Control procedures for pesticide residue analysis (SANCO/2007/3131<sup>1</sup>).

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## Introduction

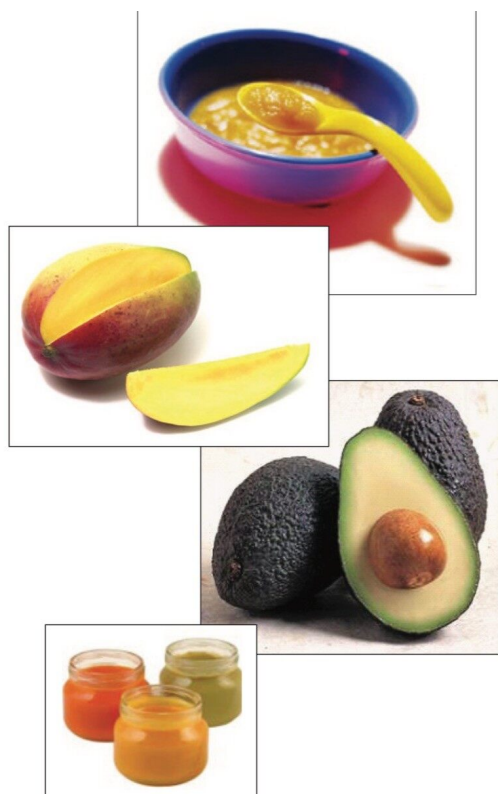
Pesticides are widely used in the production of foodstuffs to meet consumer demand for plentiful food at reasonable prices, all year round. However, continued growth in the use of pesticides, poor agricultural practices, and illegal use can pose significant risks to human health through the presence of pesticide and metabolite residues in food products. Most countries have strict regulations governing pesticides. Legislation imposes Maximum Residue Limits<sup>2</sup> (MRLs) for pesticide residues in food products requiring analytical techniques that are sensitive, selective, and robust.

Multi-residue pesticide analysis is challenging due to the low levels present, the wide variety of pesticides, and the very different chemical classes they represent. As there are currently well over 1,000 pesticides in use, laboratories are under increasing pressure to broaden the range of pesticides determined in a single analysis over a shortened run time.

The need to meet mandated detection limits, develop generic sample preparation techniques for complex matrices, and the desire to increase sample throughput are the main challenges facing food safety testing laboratories today. The use of a single multi-residue method per instrument can dramatically improve return on investment by removing the need to change method parameters. This is often the case when analyzing a wide variety of commodities with differing lists of legislated pesticides.

Advances in chromatographic separation and detection technologies have enabled analysts to increase the number of analytes determined in a single run. Tandem quadrupole mass spectrometry offers a highly specific and selective detection technique that has become the technique of choice within the laboratory.<sup>3</sup>

The following method describes a solution for the rapid analysis of pesticides in mango, avocado, and fruit-based baby food extracts that is able to exceed current worldwide legislation.



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## Experimental

Dispersive SPE, commonly referred to as “QuEChERS” , is a simple and straightforward sample preparation technique suitable for multi-residue pesticide analysis in a wide variety of food and agricultural products.<sup>4</sup> The homogenized food samples were extracted with organic solvent using Waters DisQuE dispersive sample preparation tubes. Once mixed, the pesticide residues were partitioned into the organic solvent, which was then subjected to further clean-up. The supernatant was collected, diluted, and injected onto the LC-MS/MS system as described below:

### Extraction Procedure<sup>4</sup>:

1. Add 15 g homogenized sample to a 50-mL DisQuE extraction tube containing 1.5 g sodium acetate and 6 g magnesium sulfate. Add 15 mL 1% acetic acid in acetonitrile.
2. Add any pre-extraction internal standards.
3. Shake vigorously for one minute and centrifuge > 1500 rcf for one minute.
4. Transfer 1 mL of the acetonitrile extract in to the 2-mL DisQuE extraction tube containing 50 mg PSA and

150 mg of magnesium sulphate.

5. Shake for 30 seconds and centrifuge >1500 rcf for one minute.

6. Transfer 100 µL of final extract into an autosampler vial. Add any post-extraction internal standards.  
Dilute with 900 µL water.

## Chromatographic conditions

LC system:	Waters ACQUITY UPLC System
Column:	ACQUITY UPLC BEH C <sub>18</sub> 2.1 x 100 mm, 1.7 µm
Column temp:	40 °C
Sample temp:	4 °C
Flow rate:	0.450 mL/min.
Mobile phase A:	98:2 water: methanol + 0.1% formic acid
Mobile phase B:	Methanol + 0.1% formic acid
Weak needle wash:	98:2 water: methanol + 0.1% formic acid
Strong needle wash:	Methanol + 0.1% formic acid
Total run time:	10 min
Injection volume:	20 µL, full loop injection

## Gradient:

Time(min)	Composition
0.00	95% A
0.25	95% A

Time(min)	Composition
7.75	0% A
8.50	0% A
8.51	95% A

## MS conditions

MS system:	Waters ACQUITY TQ Detector
Ionization mode:	ESI positive polarity
Capillary voltage:	1 kV
Desolvation gas:	Nitrogen, 800 L/Hr, 400 °C
Cone gas:	Nitrogen, 5 L/Hr
Source temp:	120 °C
Acquisition:	Multiple Reaction Monitoring (MRM)
Collision gas:	Argon at $3.5 \times 10^{-3}$ mBar

## Acquisition and Processing methods

The data were acquired using Waters MassLynx Software, v. 4.1. Incorporated into MassLynx, the IntelliStart technology automates optimization of MS parameters for the sample and also monitors the health of the MS system, reducing the time for operator-intensive troubleshooting and upkeep.

This data was processed using TargetLynx Application Manager. This quantification package enables automated data acquisition, processing, and reporting for quantitative data, incorporating a range of confirmatory checks that identify samples that fall outside user-specified or regulatory thresholds.

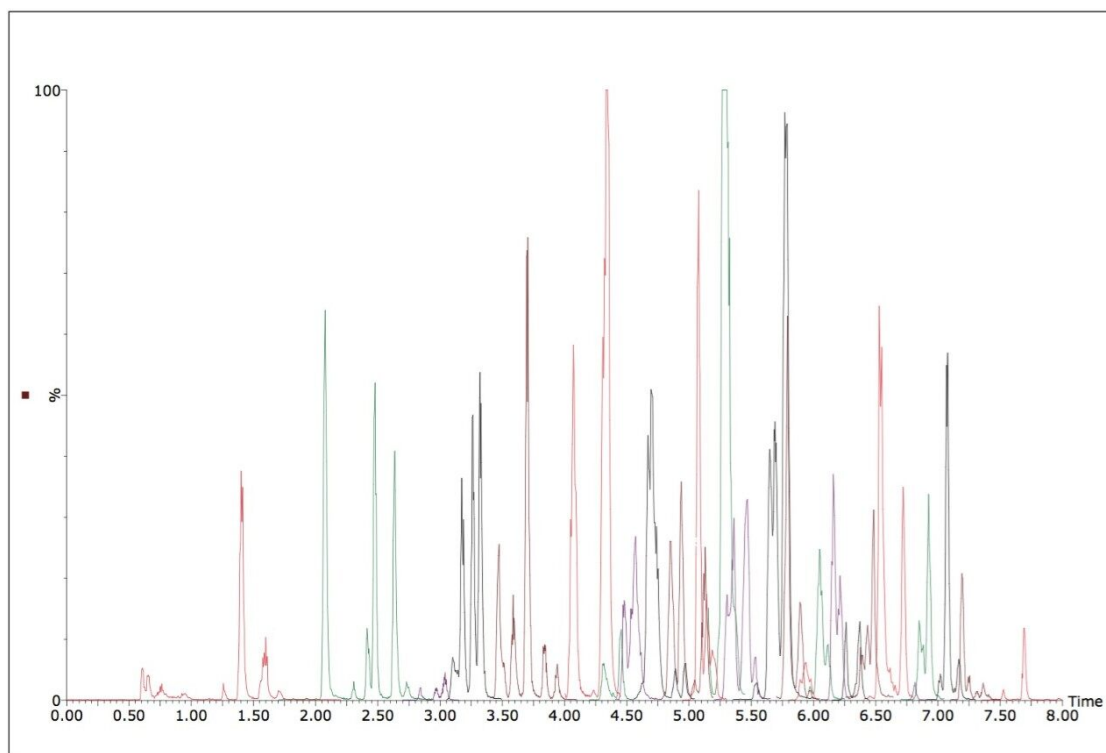
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## Results and Discussion

The analysis of 402 pesticide residues (Appendix 1) in mango, avocado, and fruit-based baby food was achieved using ACQUITY TQD: liquid chromatography combined with tandem quadrupole mass spectrometry (UPLC-MS/MS) operated in Multiple Reaction Monitoring (MRM) mode.

The rapid determination and confirmation method was achieved in two parts. Part one was a single injection with one MRM transition per pesticide, ideal for screening purposes. Part 2, where compounds of interest can then be confirmed, was achieved by two separate injections with two MRM transitions per pesticide.

Figure 1 shows all 402 pesticide residues in one 10 minute run, fully utilizing the enhanced speed and resolution of UPLC.



*Figure 1. Chromatogram showing all 402 pesticide residues in one 10 minute run in injection solvent.*

For all injections, the same UPLC conditions were used saving analytical time and costs, thus maximizing return on investment. This single setup will allow analysts with less experience to run the method as the need for changes to be made in between batches is removed.

The IntelliStart technology provides simple instrument setup and MS method development and therefore easy access even for the most inexperienced MS user.

Part 2, where compounds of interest can then be confirmed, was achieved by two separate injections with two MRM transitions per pesticide. Figures 2 and 3 show the separation of 201 pesticide residues across two run times of 10 minutes each.

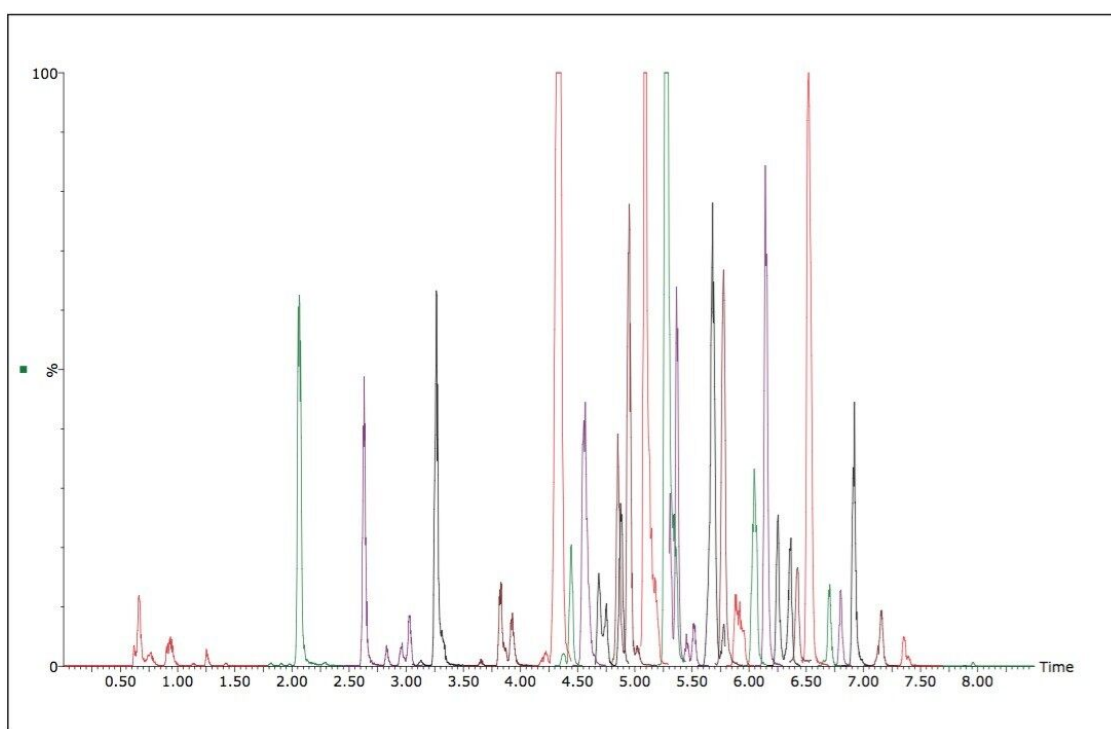
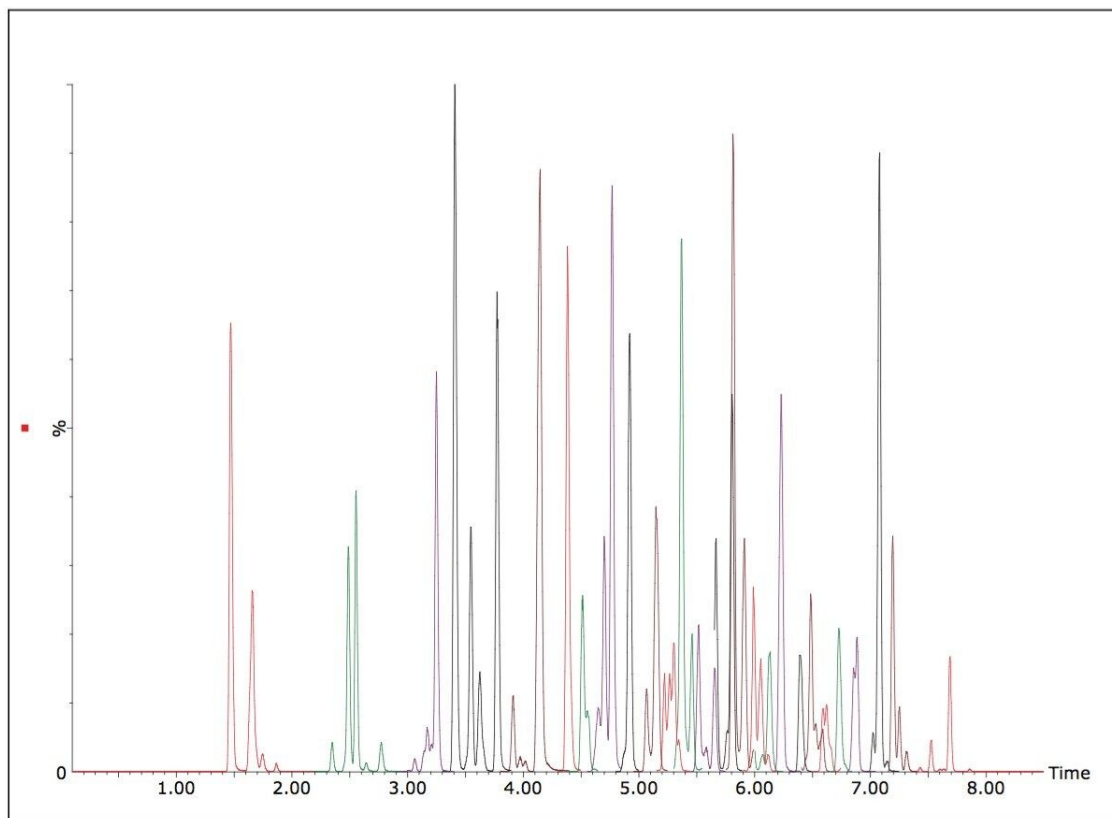


Figure 2. Chromatogram showing first 201 pesticide residues at 10  $\mu\text{g}/\text{kg}$  in injection solvent.



*Figure 3. Chromatogram showing second 201 pesticide residues at 10 µg/kg in injection solvent.*

The selectivity given using a tandem quadrupole mass spectrometer (ACQUITY TQD) shows an advantage over a single quadrupole instrument as it allows co-eluting compounds to be identified and quantified with confidence.

The enhanced speed and resolution of UPLC enabled all peaks to elute within eight minutes. Dwell times of 5 ms were used to achieve at least 12 data points across each peak for both quantification and confirmatory ions.

A calibration curve was prepared in the injection solvent (water:methanol, 90:10 v/v) and injected. Excellent linearity was achieved using a weighting factor of  $1/x$  with a high coefficient of determination achieved. This is shown in Figure 4.



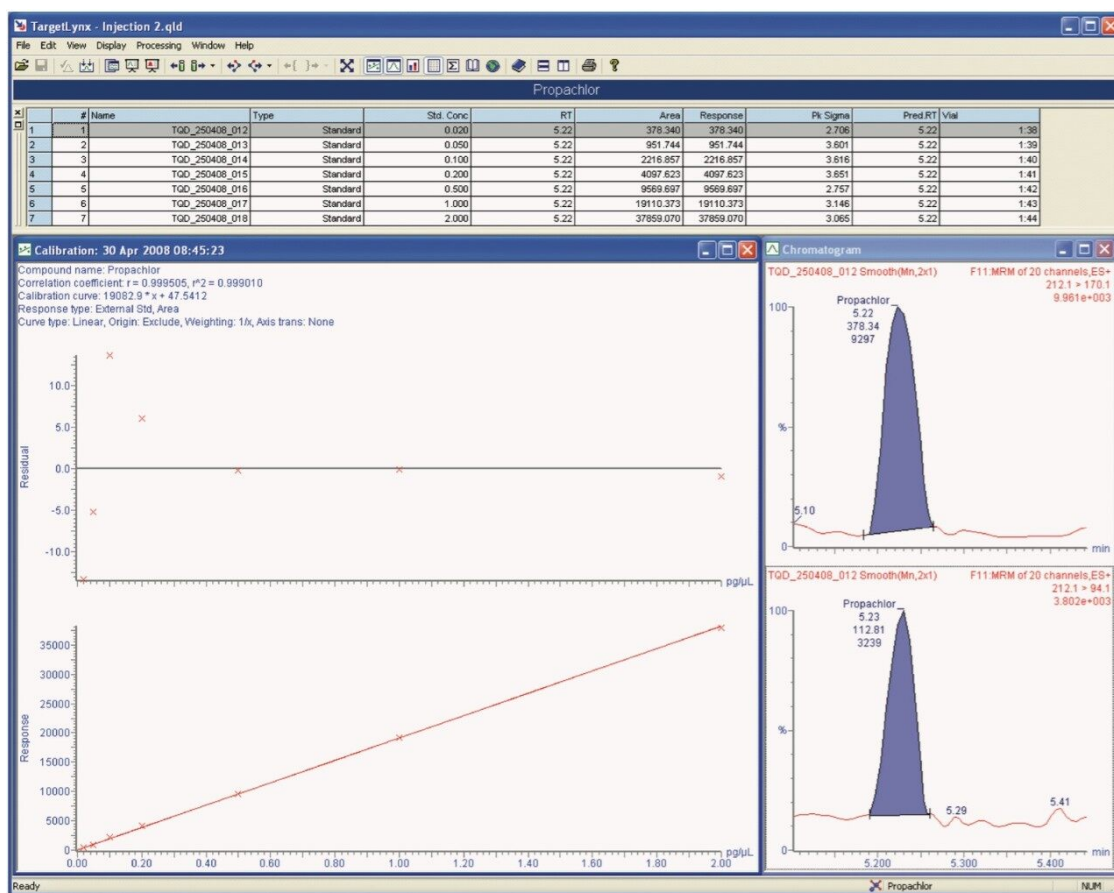
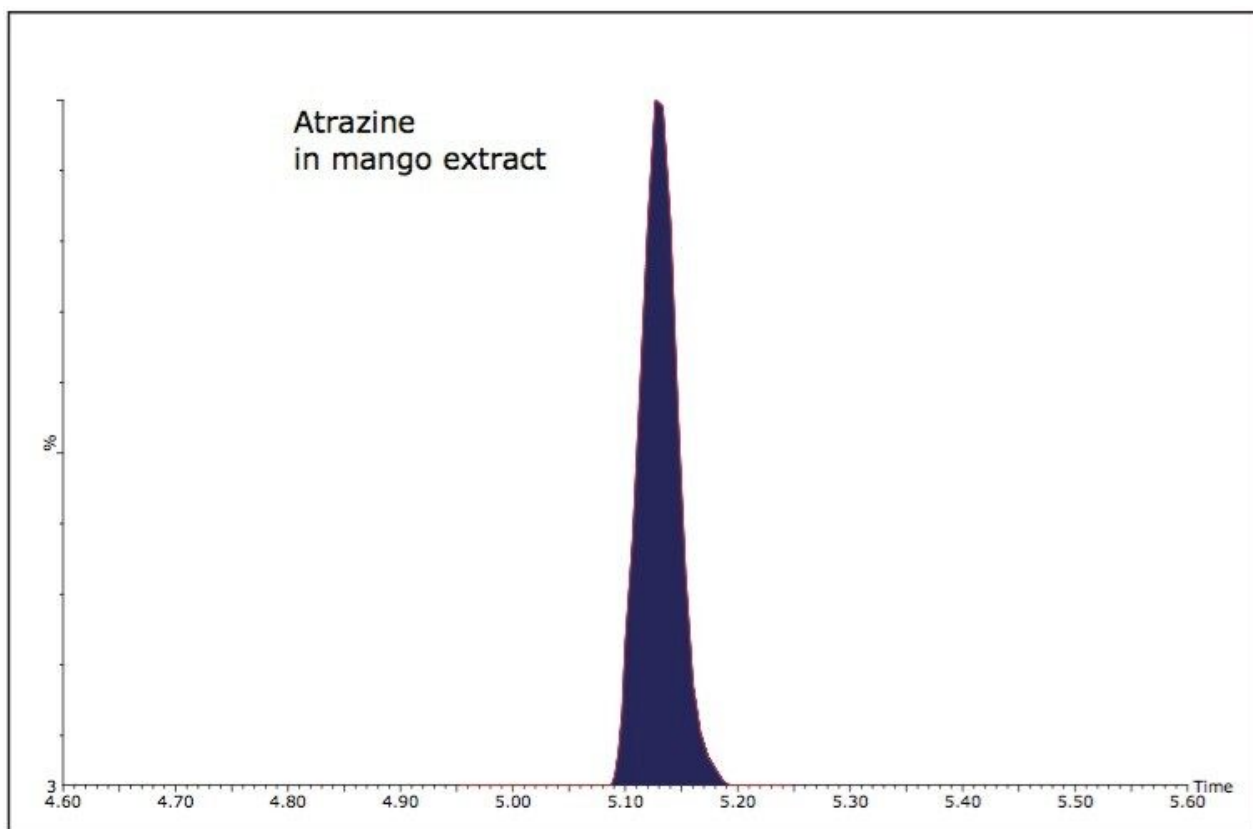
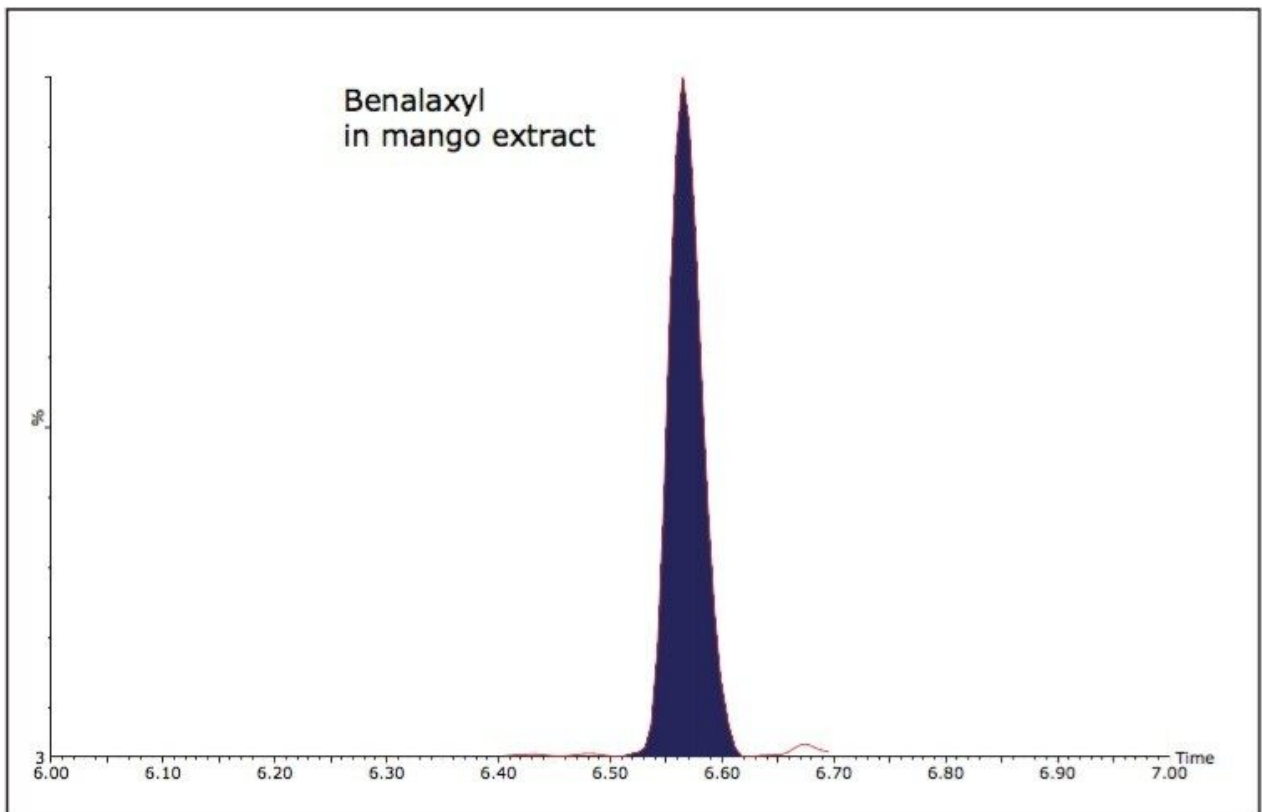
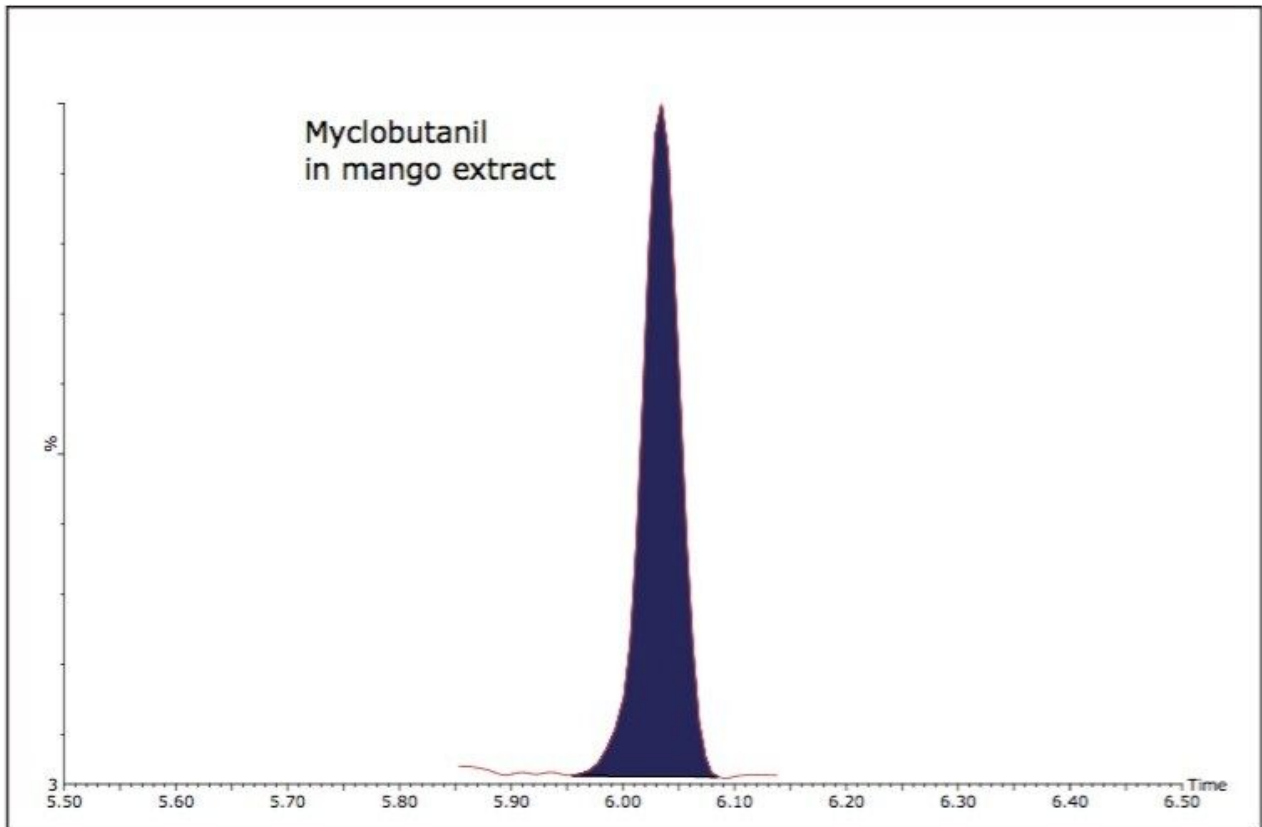
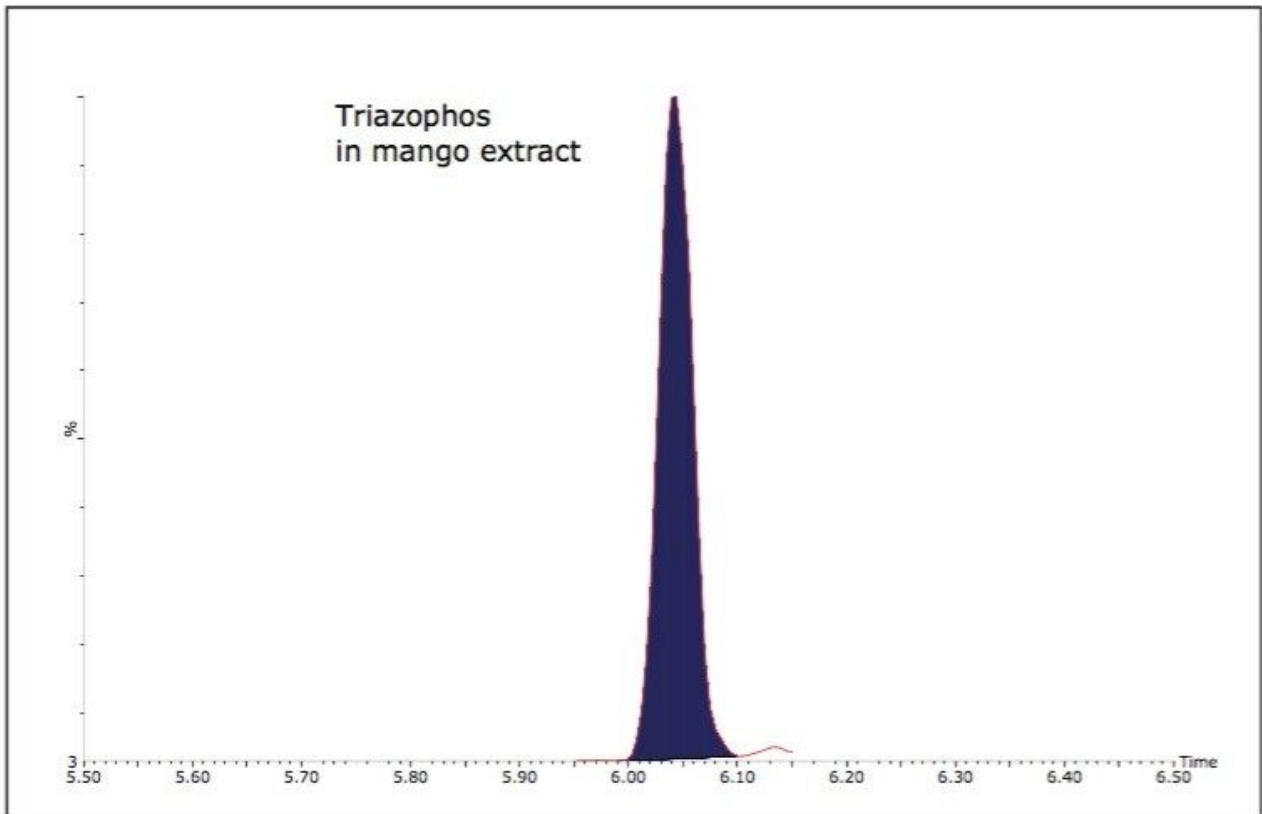


Figure 4. TargetLynx view showing a solvent standard calibration curve over a linear range from 0.02-2 pg/μL. The highlighted chromatogram is at 0.02 pg/μL.

The 402 pesticide mix was spiked into the three matrices and the extracts analyzed. Figures 5, 6, and 7 show pesticides at 10 μg/kg, equivalent to the lowest worldwide (EU) legislation, in mango, avocado, and fruit-based baby food extracts respectively.







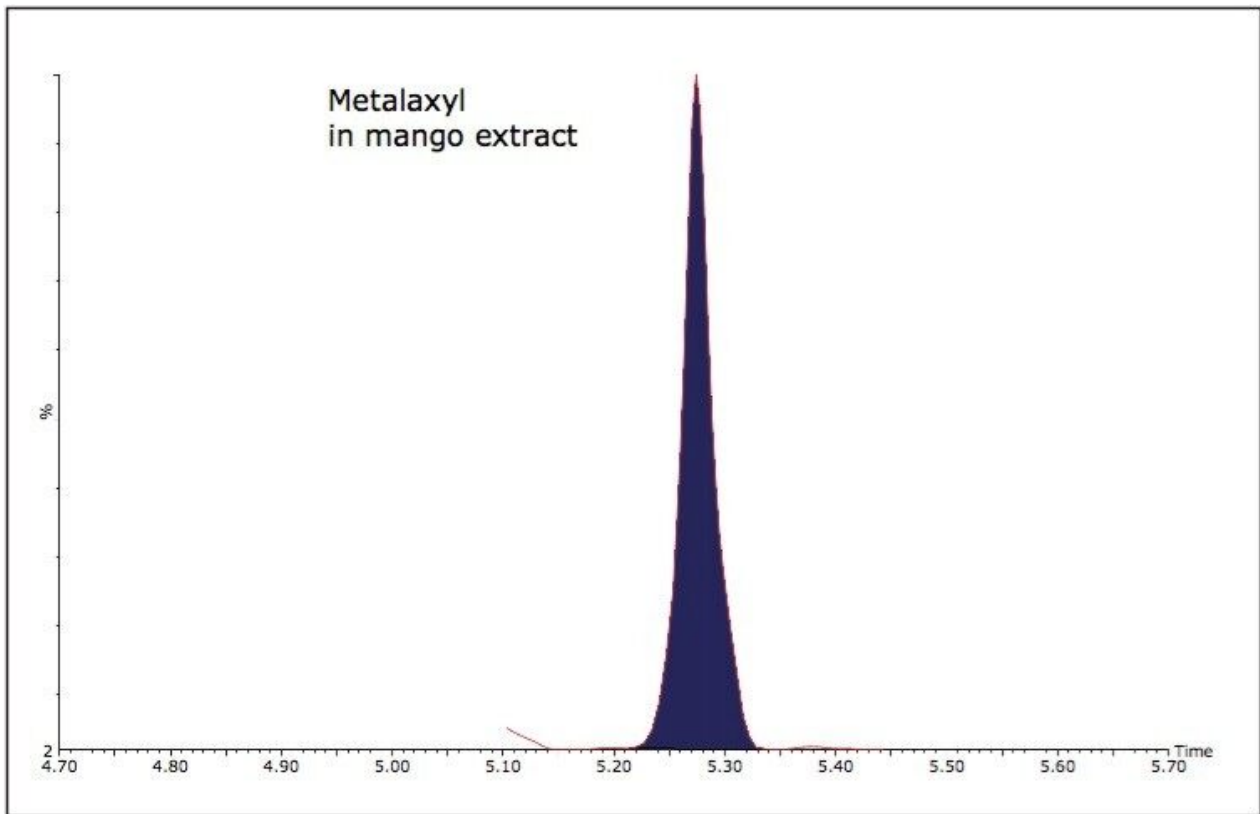
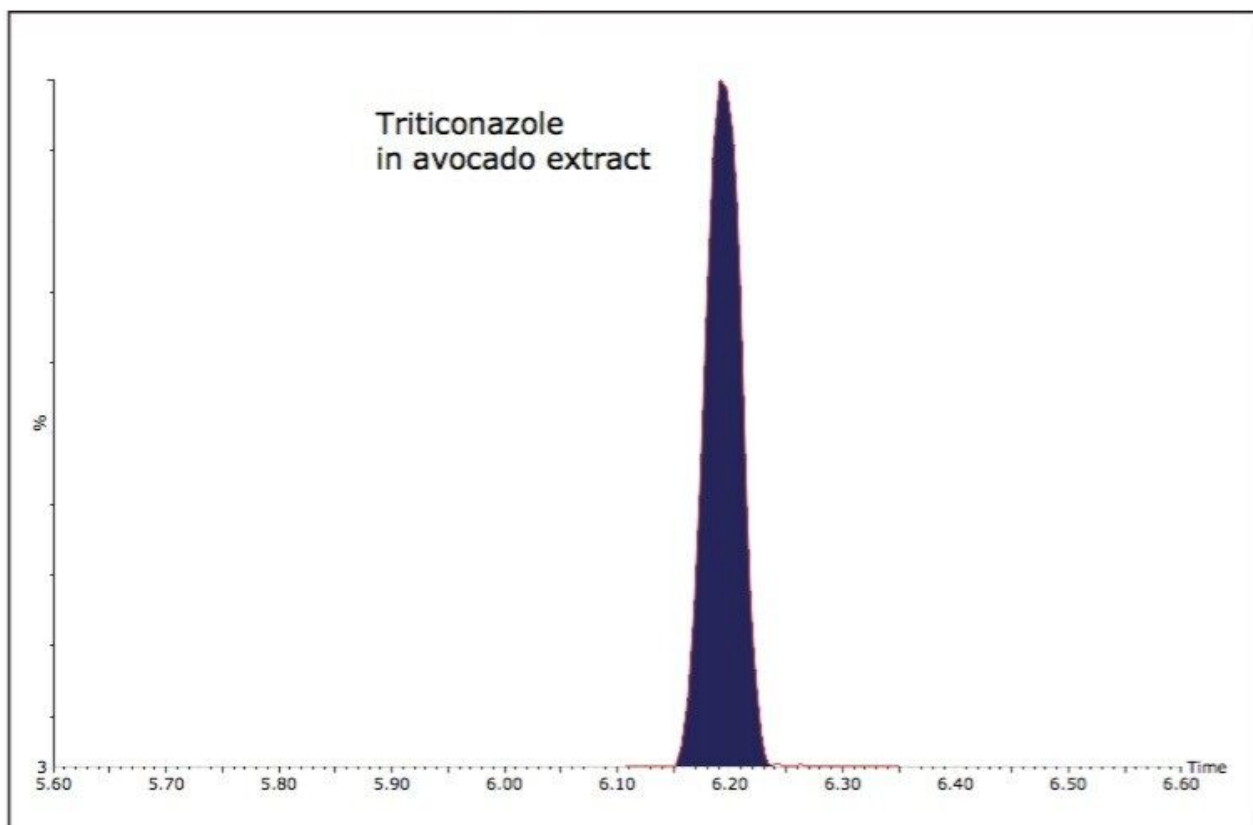
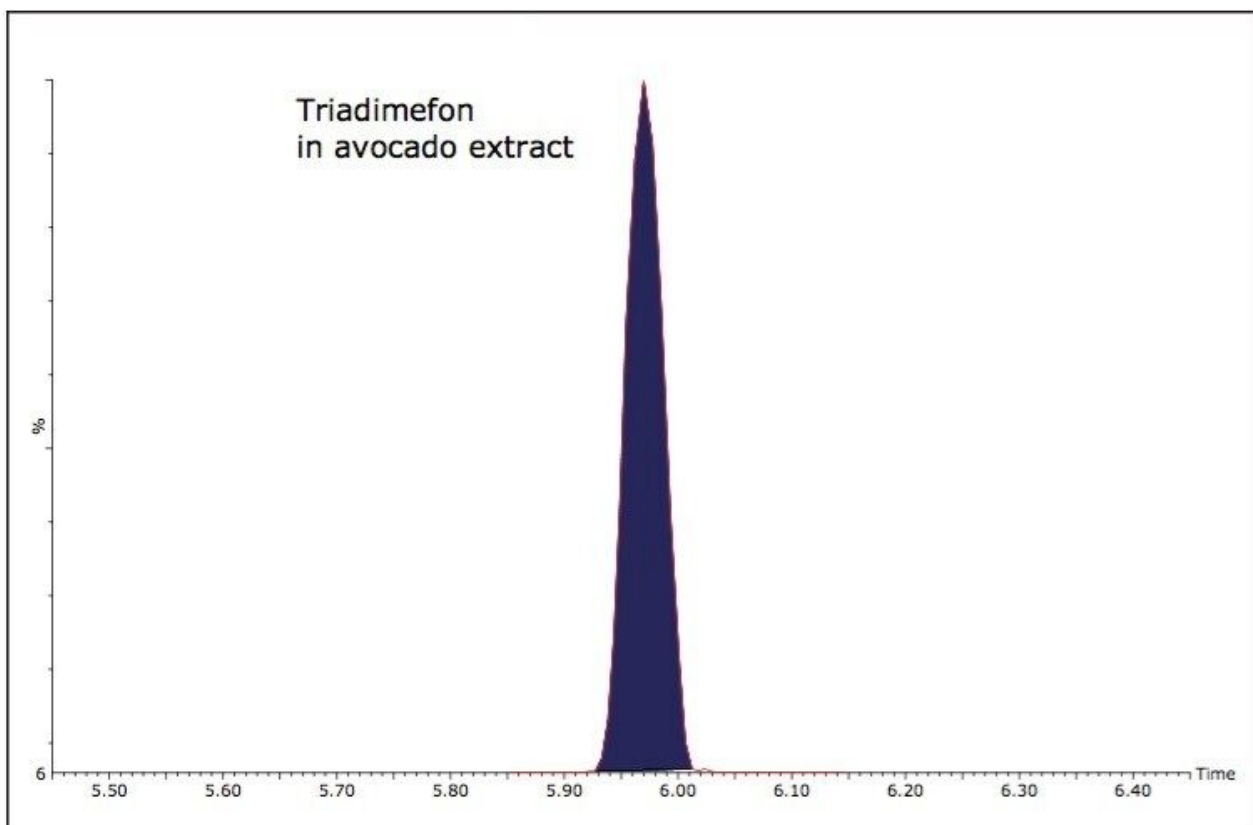
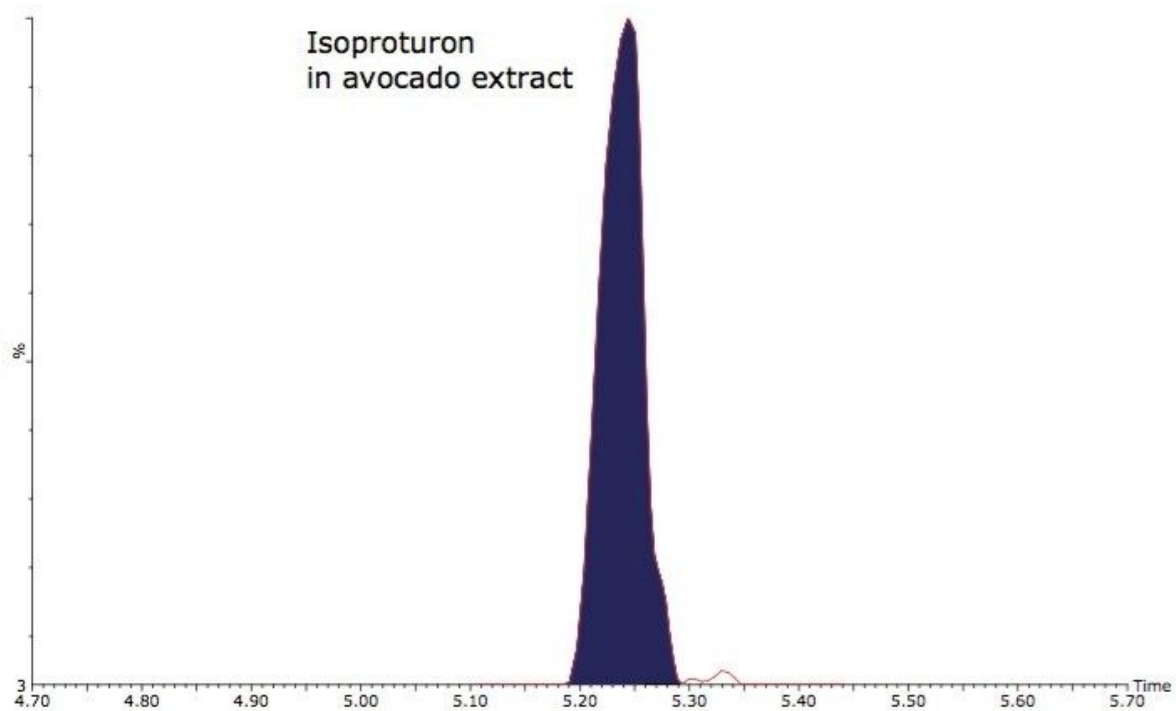


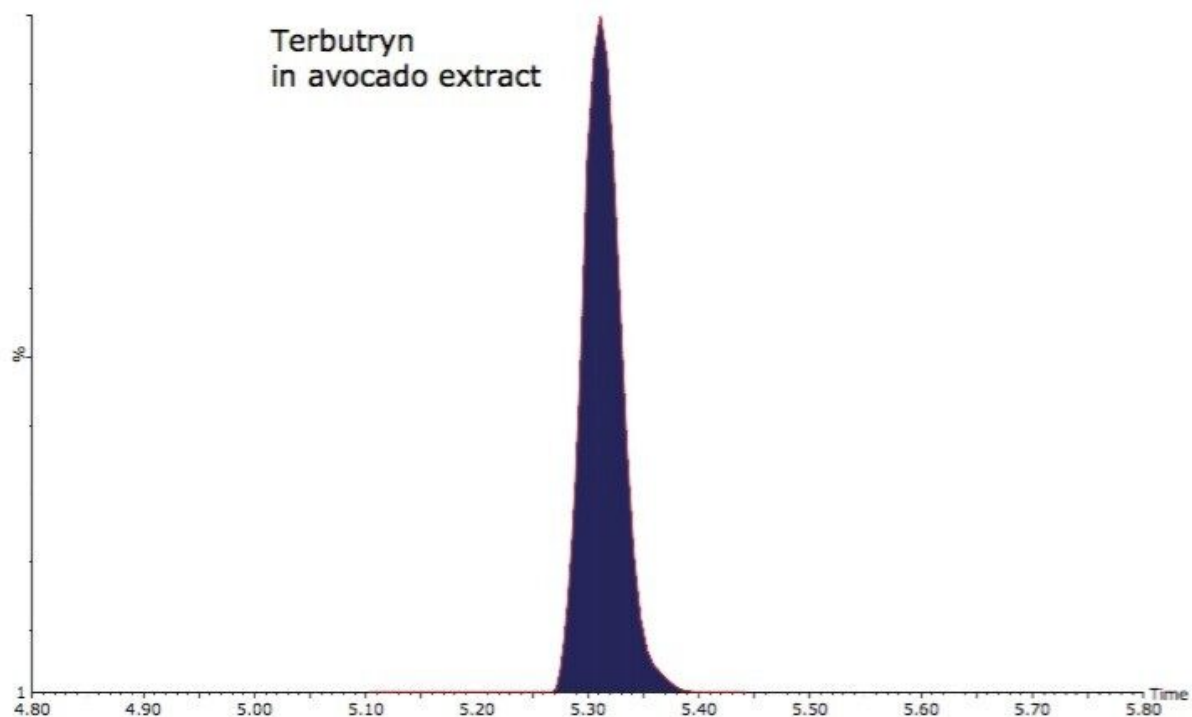
Figure 5. Five pesticides in mango extract at 10  $\mu\text{g/kg}$ .



Isoproturon  
in avocado extract



Terbutryn  
in avocado extract





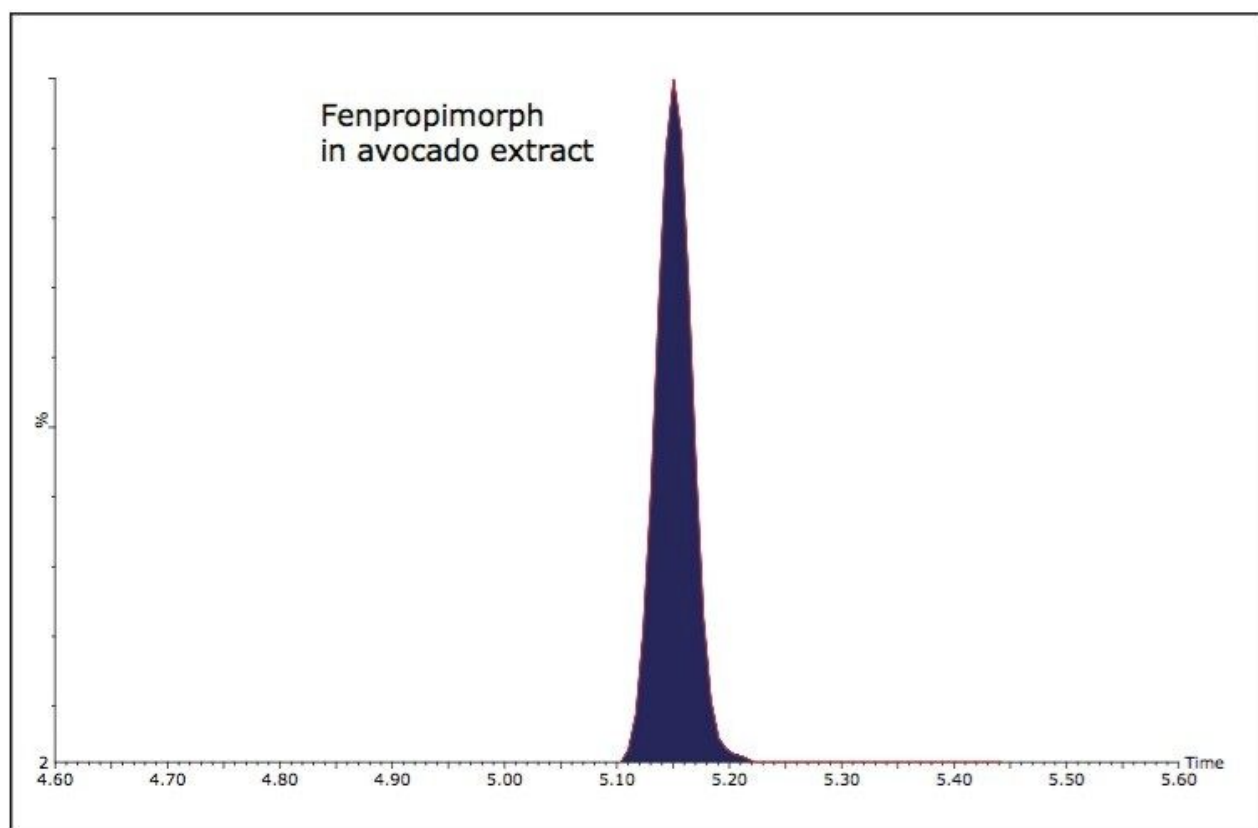
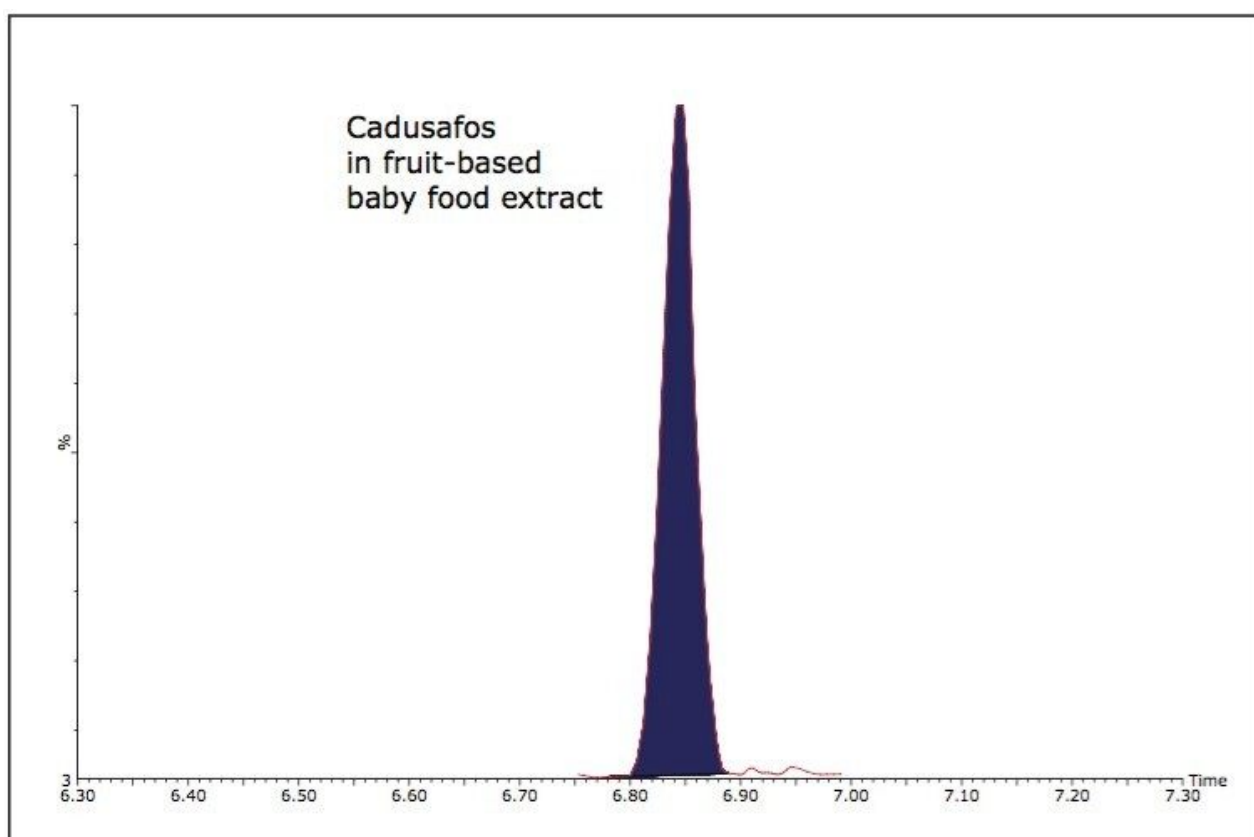
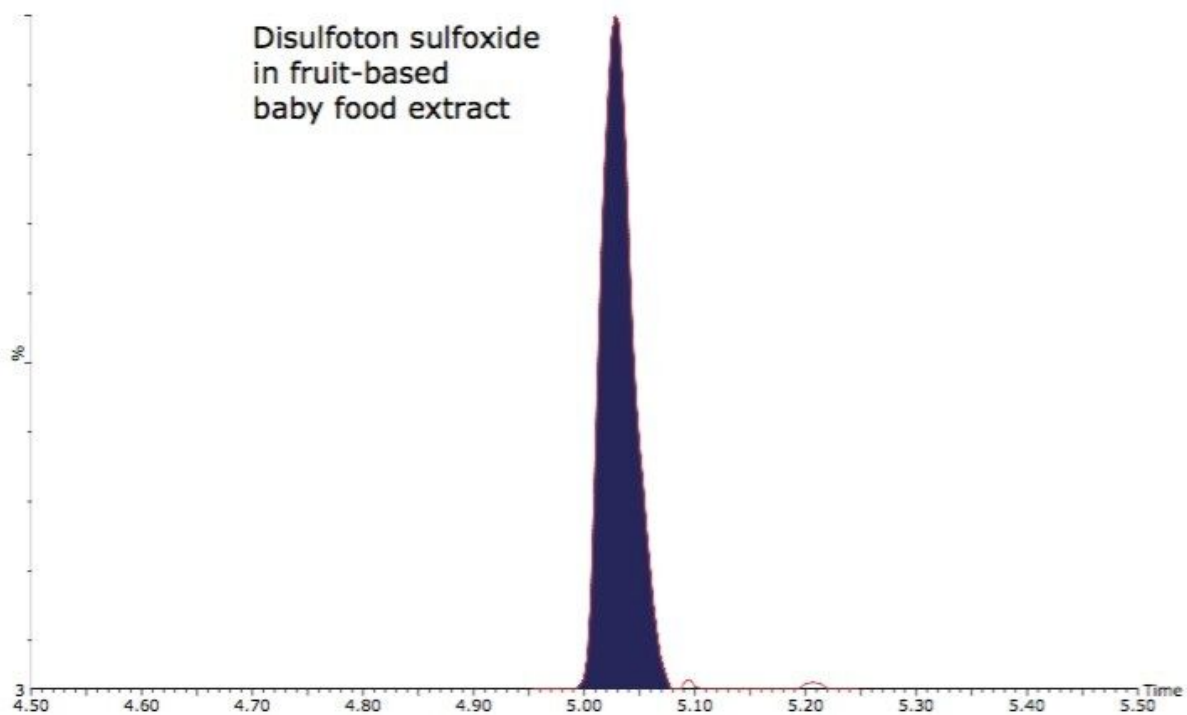
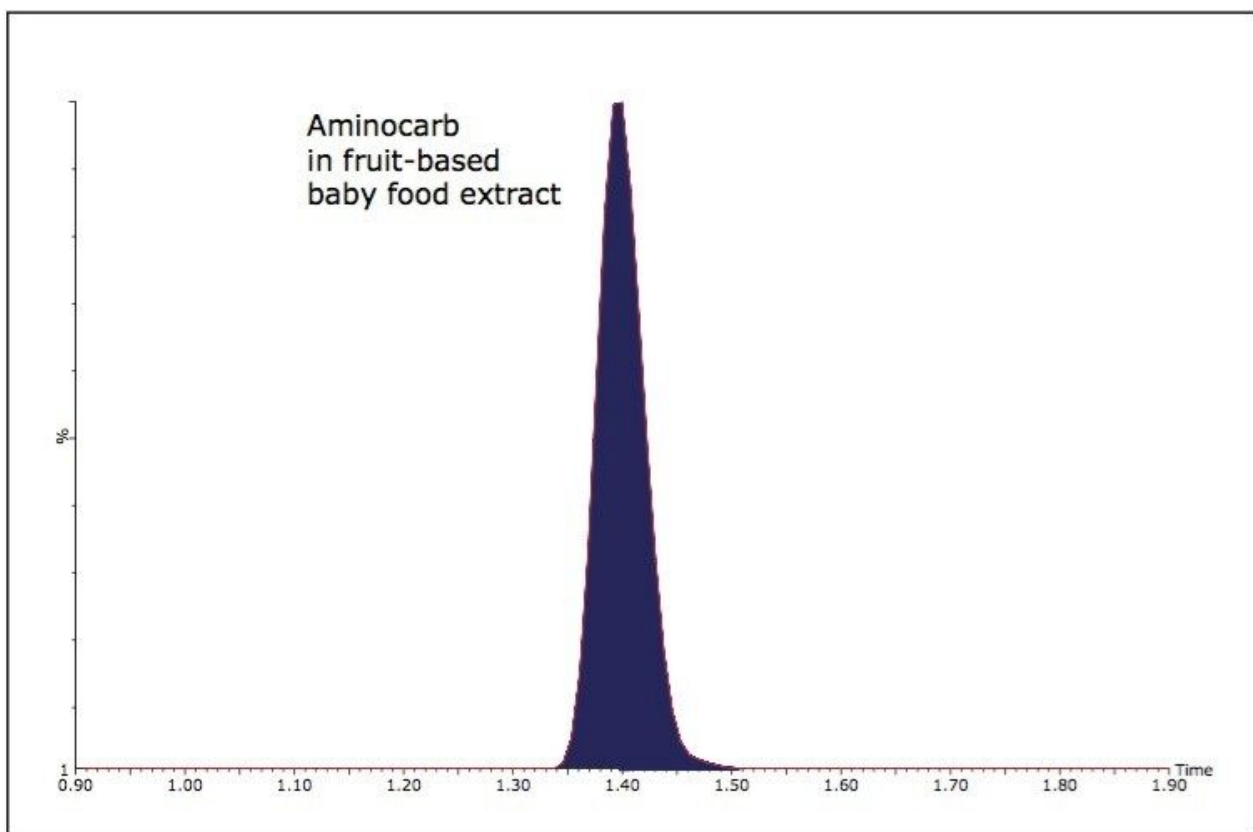


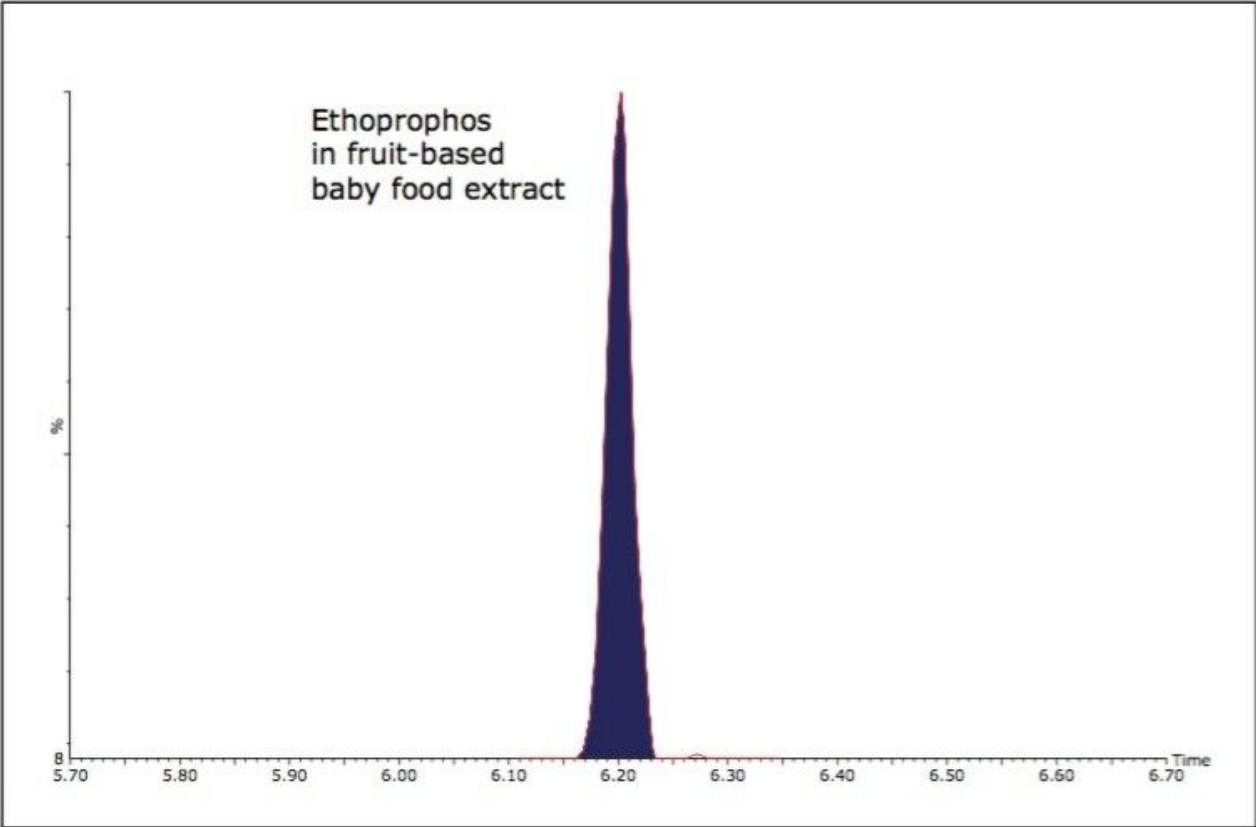
Figure 6. Five pesticides in avocado extract at 10  $\mu\text{g/kg}$ .



Disulfoton sulfoxide  
in fruit-based  
baby food extract







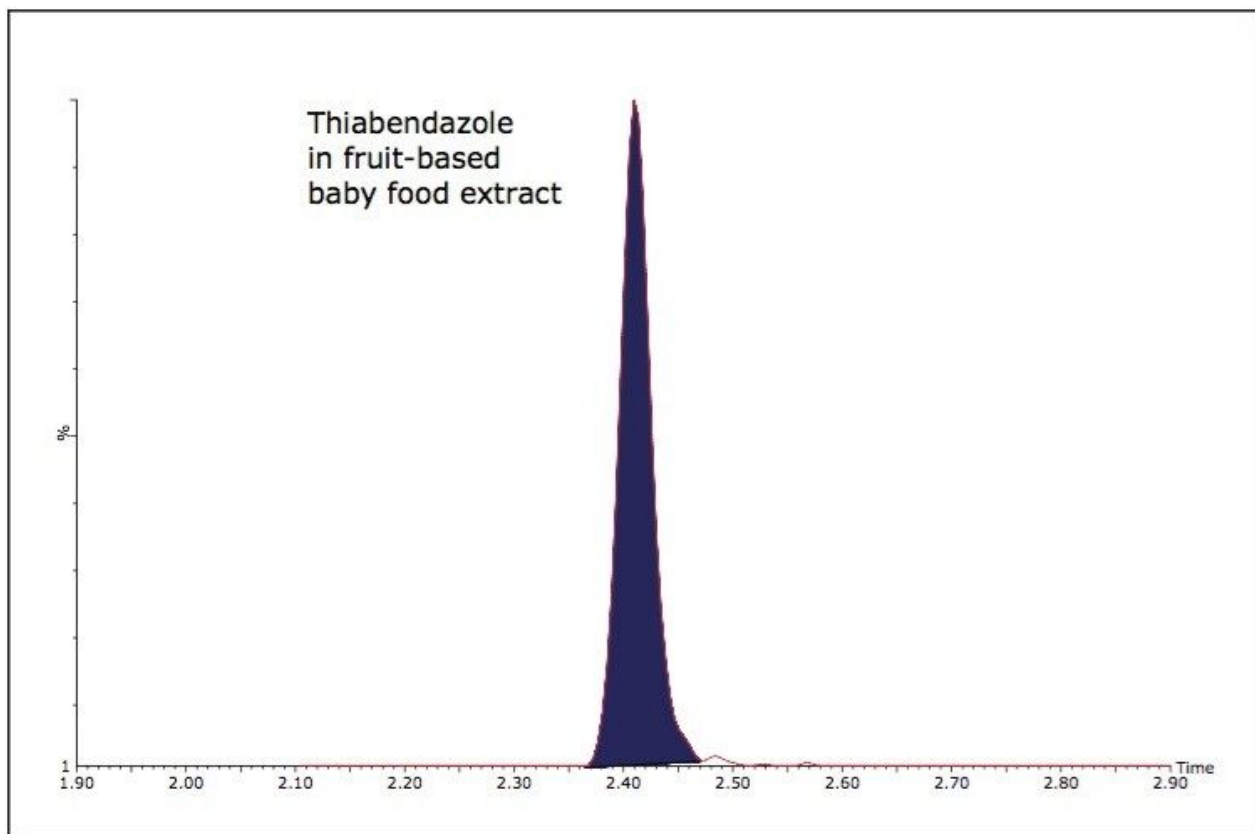
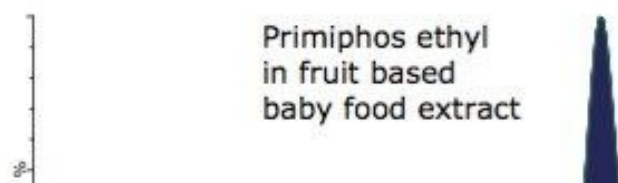
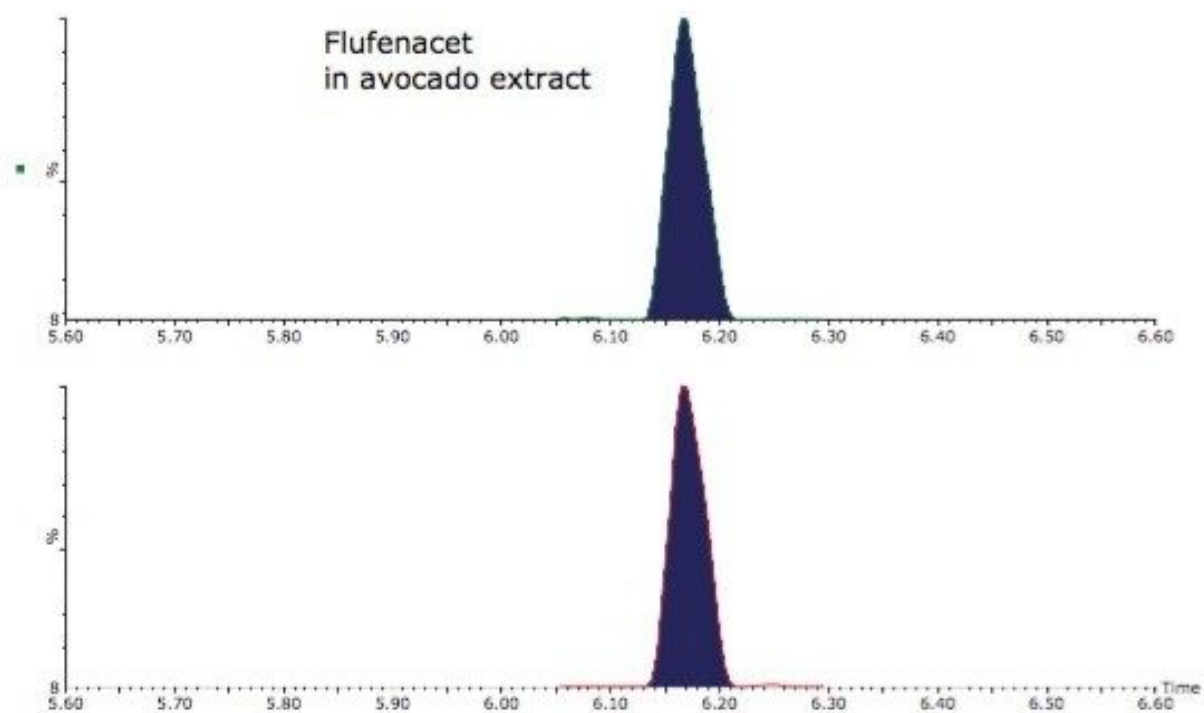
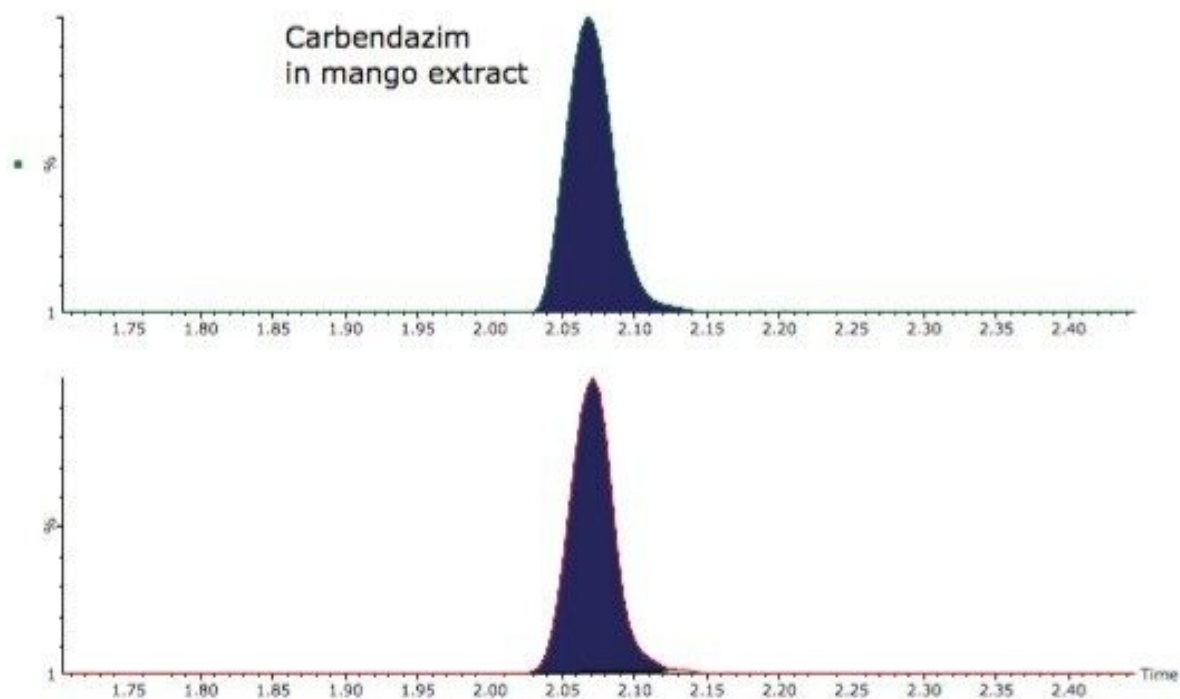


Figure 7. Five pesticides in fruit-based baby food extract at 10 µg/kg.

The advantage of using ACQUITY TQD is that ion ratio confirmation is also possible. This is used to confirm the identity of any pesticide that was presumptive positive from the screening method. Within the EU, ion ratio confirmation is important for pesticide analysis as documented in SANCO/2007/3131<sup>1</sup>.

In Part 2, the confirmatory runs, all 402 pesticides were chromatographed with both primary (for quantitation) and secondary (for confirmation) MRM transitions present. Figure 8 shows three more compounds in the three matrices with both MRM transitions.



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*at 10 µg/kg in matrix. The ion ratios for carbendazim, primiphos ethyl, and flufenacet are 0.16, 0.18 and 0.61 respectively.*

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## Conclusion

A rapid multi-residue method was developed for the screening of over 400 pesticides in one 10 minute run with one MRM transition per pesticide. For confirmation, two 10 minute runs were required with two MRM transitions per pesticide. The analysis of pesticides in mango, avocado and fruit-based baby food extracts was able to exceed current worldwide legislated limits.

Improved efficiency and increased sample throughput were realized through the combination of powerful UPLC and fast MS acquisition technologies. The Waters ACQUITY TQD as shown in Figure 9 offers:

- Enhanced chromatographic resolution and short analysis times
- Incorporation of confirmatory MRM traces
- Compliance with legislative regulations such as SANCO
- IntelliStart technology is designed to reduce the burden of complicated operation, training new users, time-intensive troubleshooting, and upkeep
- The small footprint of the ACQUITY TQD will give any laboratory an advantage as it removes the need for larger instrumentation.





*Figure 9. ACQUITY TQD.*

The benefits of this Waters UPLC-MS/MS solution for a revenue conscious laboratory can be realized through increased efficiency through analytical time savings and decreased need for sample retesting, resulting in increased lab productivity. Cost savings can be made by lowering the use of lab consumables with the environmental impact of solvent usage also being reduced.

The sensitivity achieved for a large number of pesticide residues in real food matrices indicates this UPLC-MS/MS method is an ideal basis for the rapid analysis of pesticides in a wide range of food samples.

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## References

1. Website: [http://ec.europa.eu/food/plant/protection/resources/qualcontrol\\_en.pdf](http://ec.europa.eu/food/plant/protection/resources/qualcontrol_en.pdf)
2. Commission of the European Communities EC 396/2005, OJ 2005; L70:1.
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4. Lehotay, J. AOAC Int. 90(2) 2007, 485-520.

## Acknowledgements

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## Appendix: List of the 402 pesticides analyzed

3,4,5-Trimethacarb”	Dibrom	Indoxacarb	Propanil
Acephate	Dichlofluanid	Iodosulfuron methyl	Propaquizafop
Acetamiprid	Dichlorvos	Iprobenphos	Propazine
Acibenzolar-S-methyl	Diclobutrazol	Iprovalicarb	Propetamphos
Acitidone	Dicrotophos	Isazophos	Propham
Aldicarb	Diethofencarb	Isocarbamide	Propiconazole
Aldicarb sulfone	Difenoconazole	Isocarbofos	Propoxur
Aldicarb sulfoxide	Difenoхuron	Isofenphos	Propyzamide
Ametryn	Diflubenzuron	Isomethiozin	Prosulfocarb
Amidosulfuron	Dimefuron	Isonoruron	Prosulfuron
Aminocarb	Dimepiperate	Isoprocab	Pymetrozine
Amitrole	Dimethachlor	Isopropalin	Pyracarbolid
Anilazine	Dimethametryn	Isoproturon	Pyraclostrobin
Anilofos	Dimethenamid	Isoxaben	Pyrazophos
Asulam	Dimethirimol	Kresoxim-methyl	Pyrazosulfuron-ethyl

Atraton	Dimethoate	Lenacil	Pyridaben
Atrazine	Dimethomorph	Linuron	Pyridafol
Atrazine-desethyl	Dimetilan	Malaoxon	Pyridaphenthion
Atrazine-desisopropyl	Dimoxystrobin	Malathion	Pyridate
Azaconazole	Diniconazole	Mecarbam	Pyrifenox
Azamethiphos	Dioxacarb	Mefenacet	Pyrimethanil
Azinphos-ethyl	Diphenamid	Mepanipirim	Pyriproxifen
Azinphos-methyl	Diphenylamine	Mephosfolan	Pyroquilon
Aziprotryne	Disulfoton	Mepronil	Quinalphos
Azobenzene	Disulfoton-sulfone	Mesosulfuron-methyl	Quinmerac
Azoxystrobin	Disulfoton-sulfoxide	Mesotrione	Quinoxifen
Benalaxyl	Ditalimfos	Metalaxyl	Quizalofop-ethyl
Benazolin	Dithiopyr	Metamitron	Quizalofop-methyl
Bendiocarb	Diuron	Metazachlor	Rabenzazol
Benfuracarb	DMST	Metconazole	Rotenone
Benfuresate	Dodemorph	Methabenzthiazuron	Sebuthylazin
Bensulfuron methyl	Edifenphos	Methacrifos	Sebuthylazin-desethyl
Bensulide	Epoxiconazole	Methamidophos	Secbumeton

Bentazone	EPTC	Methfuroxam	Sethoxydim
Benzoximate	Esprocarb	Methidathion	Siduron
Benzthiazuron	Ethidimuron	Methiocarb	Simazine
Bifenazate	Ethiofencarb	Methiocarb sulfone	Simeconazole
Bitertanol	Ethiofencarb sulfone	Methiocarb sulfoxide	Simetryn
Boscalid	Ethiofencarbsulfoxide	Methomyl	Spinosad A
Bromacil	Ethirimol	Methoprotryne	Spinosad D
Bromuconazole	Ethofumesate	Methoxyfenozide	Spiromesifen
Bupirimate	Ethoprophos	Metobromuron	Spiroxamine
Buprofezin	Ethoxyquin	Metolachlor	Sulcotrione
Butocarboxim	Ethoxysulfuron	Metolcarb	Sulfallate
Butocarboxim sulfoxide	Etofenprox	Metosulam	Sulfaquinoxaline
Butoxycarboxim	Famphur	Metoxuron	Sulfometuron-methyl
Buturon	Fenamidone	Metrafenone	Sulfosulfuron
Butylate	Fenamiphos	Metribuzin	Sulfotep
Cadusafos	Fenamiphos sulphone	Metsulfuronmethyl	Tebuconazole
Carbaryl	Fenamiphos sulphoxide	Mevinphos	Tebufenozide
Carbendazim	Fenarimol	Molinate	Tebufenpyrad

Carbetamide	Fenazaquin	Monocrotophos	Tebupirimfos
Carbofuran	Fenazox	Monolinuron	Tebutam
Carbofuran-3-hydroxy	Fenbuconazole	Monuron	Tebuthiuron
Carbofuran-3-keto	Fenfuram	Myclobutanil	Temephos
Carbosulfan	Fenhexamid	Napropamide	Tepraloxydim
Carboxin	Fenobucarb	Naptalam	Terbufos
Carfentrazone-ethyl	Fenoxycarb	Neburon	Terbufos-sulfone
Chlorbromuron	Fenpiclonil	Nicosulfuron	Terbufos-sulfoxide
Chlorfenvinphos	Fenpropathrin	Nicotine	Terbumeton
Chlorfluazuron	Fenpropidin	Nitenpyram	Terbumeton-desethyl
Chloridazon	Fenpropimorph	Nitralin	Terbutylazine
Chloroxuron	Fenpyroximat	Nuarimol	Terbutylazine-2-hydroxy
Chlorpropham	Fensulfothion	Ofurace	Terbutylazine-desethyl
Chlorpyrifos	Fenthion	Omethoate	Terbutryn
Chlorpyrifos-methyl	Fenthion-sulfone	Orbencarb	Tetrachlorvinphos
Chlorsulfuron	Fenthion-sulfoxide	Oryzalin	Tetraconazole
Chlorthiophos	Fenuron	Oxamyl	Thiabendazole

Chlortoluron	Flamprop-isopropyl	Oxasulfuron	Thiacloprid
Cinidon-ethyl	Flamprop-methyl	Oxycarboxin	Thiamethoxam
Cinosulfuron	Fluazafop-P-butyl	Oxydemeton-methyl	Thiazafluron
Clethodim	Fluazifop	Paclobutrazol	Thidiazuron
Clodinafop-propargyl	Flucycloxuron	Paraoxon-methyl	Thifensulfuron methyl
Clomazone	Flufenacet	Parathion	Thiodicarb
Clopyralid	Flufenoxuron	Pebulat	Thiofanox
Cloquintocet-mexyl	Fluomethuron	Penconazole	Thiofanox-sulfone
Clothianidin	Fluoxastrobin	Pencycuron	Thiophanate
Coumaphos	Fluroxypyr	Pendimethalin	Thiophanate-methyl
Cruformate	Fluroxypyr-meptyl	Phenmedipham	Tolylfluanid
Cyanazine	Flurtamone	Phenthoate	Topramezone
Cyanofenphos	Flusilazole	Phorate	Tralkoxidym
Cyazofamid	Flutolanil	Phorate sulfone	Triadimefon
Cycloate	Flutriafol	Phorate sulfoxide	Triadimenol
Cycloxydim	Fonofos	Phosalone	Triallate
Cycluron	Foramsulfuron	Phosphamidon	Triasulfuron
Cyflufenamid	Formetanate	Phoxim	Triazophos
Cymoxanil	Fosthiazate	Picloram	Triazoxid

Cyproconazole	Fuberidazole	Picolinafen	Trichlorfon
Cyprodinil	Furathiocarb	Picoxystrobin	Tricyclazole
Cyromazine	Halosulfuron methyl	Piperonyl butoxide	Trietazine
Daminozide	Haloxyfop	Piperophos	Trifloxystrobin
Demeton O	Haloxyfop-2-ethoxyethyl	Pirimicarb	Trifloxysulfuron
Demeton S	Haloxyfop-methyl	Pirimiphos-ethyl	Triflumizole
Demeton-S-methyl	Heptenophos	Pirimiphos-methyl	Triflumuron
Demeton-S-methyl-sulfon	Hexaconazole	Procloraz	Triflusulfuron-methyl
Desmedipham	Hexazinone	Profenofos	Triticonazole
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