

Chromatography Technical Note No AS90

Fully automated QuEChERS clean-up and LC/MS-QQQ analysis of pesticides in fruits and vegetables.

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Introduction

This application note describes a fully automated method based upon the widely used QuEChERS (quick, easy, cheap, effective, rugged and safe) methodology [1-8], for the extraction and clean-up of pesticide residues in samples of fruit and vegetables.

The QuEChERS method uses acetonitrile extraction followed by the salting out of water from the sample using anhydrous magnesium sulphate, sodium chloride and buffering citrate salts to induce liquid-liquid partitioning. This initial extraction is performed offline and provides a crude extract ready for QuEChERS clean-up.

An automated dispersive solid phase extraction (DSPE) is conducted for clean-up, using a combination of primary secondary amine (PSA) to remove fatty acids and anhydrous magnesium sulphate to reduce the remaining water in the extract. After mixing and centrifugation the upper layer is ready for analysis.

Two different DSPE methods exist, the EN and AOAC, which differ in the following ways. Firstly, the buffered extraction system in the EN method uses sodium chloride, sodium citrate and disodium citrate sesquihydrate instead of sodium acetate in the AOAC extraction system. Secondly, in the DSPE step, the EN method uses 25 mg PSA per ml of extract rather than 50 mg PSA per ml of extract as stated in the AOAC method. For the purposes of the following study, samples were treated according to EN protocols. The automation of this methodology can be applied to either DSPE method, as long as suitable quantities of reagent are present in sample vials.

The determination of pesticides in fruits and vegetables is required to ensure food safety. Sample preparation is an important part of any multi residue method. The ability to automate the extraction and clean-up and to couple the extraction directly to Liquid Chromatography Mass Spectrometry – Triple Quadrupole (LC/MS-QQQ) holds the promise of dramatic improvements in laboratory productivity by streamlining the analytical process.

The presented method enables automated clean-up by DSPE immediately followed by the analysis of the pesticide extract, if required. The method shows good reproducibility, excellent recovery and limits of quantification for a wide range of pesticides with different polarities and for different matrices, ensuring the procedure is fully suitable for routine analysis.

The methodology and hardware may be utilised in two different configurations, either as a standalone MPS 3 performing the DSPE clean-up in isolation, i.e. not coupled to LC/MS-QQQ (See Figure 1) or alternatively in an MPS 3 XL Prepstation configuration, coupled directly to LC/MS-QQQ, providing the greatest levels of laboratory productivity available. (See Figure 2)

Instrumentation

Agilent 6410 Triple Quadrupole Mass Spectrometer (DMRM upgrade) fitted with Electrospray ionisation.

Agilent 1200 Series HPLC

G1312B Binary Pump SL

G1316B Thermostatted Column Compartment SL

G1379B Micro-vacuum Degasser

Gerstel MPS3 XL Prepstation fitted with injection valve.

Gerstel Solvent Filling Station

Gerstel SPE Station

Anatune CF-100 centrifuge



Figure 1. MPS 3 configured for offline QuEChERS automation.

Experimental – Clean-Up

Samples of fruit and vegetables were purchased from a local supermarket and prepared in accordance with standard protocols where possible; chopping into small pieces, when necessary, freezing and blending to achieve a thoroughly homogenous sample.

Portions of these samples (10g) were weighed into extraction tubes in preparation for EN QuEChERS extraction. Samples of apple, strawberry, lettuce, tomato, spinach and oranges were stored in the freezer until required for spiking and extraction.

For each matrix, samples (n=3) were spiked at 10 ng/g, which represents a level appropriate to the maximum residue limits (MRL's) for the matrices and compounds under investigation. Additional samples were processed unspiked to provide enough extract for the preparation of matrix matched calibration standards and blank analysis.

For the offline extraction, 10 g of sample is extracted with 10 ml of acetonitrile. Internal standard (triphenyl phosphate (TPP) 50 ng/g) is added and the sample is shaken for 1 minute. Following this shaking period the EN mixture of salts is added to buffer and remove water from the samples. The sample is then thoroughly shaken for 1 minute to mix the salts with the fruit/solvent slurry.

This mixture is then centrifuged to separate the solids from the crude acetonitrile supernatant/extract, which is ready for automated DSPE clean-up.

For the automated DSPE a 1 ml aliquot of this crude extract is added to a vial containing 25 mg of PSA sorbent and 150 mg of magnesium sulphate

(MgSO₄). This mixture is then vortexed to ensure a high degree of mixing to ensure maximum interaction between PSA and MgSO₄ materials and the crude extract. The mixture is then centrifuged at 3000 rpm for 3 minutes. For the samples of lettuce and spinach, graphitised carbon black (GCB) was also present in the clean-up vial to ensure sufficient removal of pigments and chlorophyll.

The clean supernatant is then filtered using the SPE Station to 0.45 µm through PTFE filters, ensuring that the extract is suitable for rapid resolution LC/MS-QQQ.

In the case of online QuEChERS automation, the resulting filtrate is injected into an LC/MS-QQQ in a “just in time” fashion ensuring that analysis is always completed as soon as extracts are available.



Figure 2. MPS 3 XL Prepstation configured for online QuEChERS.

Experimental – Mass Spectrometry

Using a solution of approximately 200 compounds in acetonitrile, the QQQ dependant parameters necessary for any multi residue method were determined and optimised, using the MassHunter Software Add-On Optimizer.

Optimised fragmentor voltages and collision energies were selected for each compound for both a quantifier and qualifier transition where available. All other instrumental parameters were set and optimised using the Autotune function. Once the QQQ transitions were identified, retention time data was gathered for each compound and the associated quantifier and qualifier ion transitions. Following this a dynamic multiple reaction monitoring method was generated.

Generic electrospray chamber conditions were chosen due the wide range of compounds under investigation. Ionisation was conducted in the positive mode, with the following conditions; Gas temperature – 250 °C, Drying gas flow 12 L/min, nebuliser pressure – 35 psi and capillary voltage – 4000 V.

Experimental – HPLC

Separation of the compounds under investigation was conducted on a Zorbax Eclipse Plus RR C18 column (100 x 2.1 mm ID 1.8 µm). Separation was achieved using 5 mM ammonium formate and 0.01 % formic acid (A) with methanol and 5 mM ammonium formate and 0.01 % formic acid (B) using the following gradient conditions at 0.3 ml/min, 0 min 10 % B, 1 min 10 % B, 18 min 100 % B, 20 min 100 % B, 20.1 min 10 % B with a total cycle time of 25 minutes. Injection volume was 5 µl and was stacked using an injector program to ensure the highest on column concentrations whilst limiting solvent strength to achieve good peak shape and separation See Figure 3.

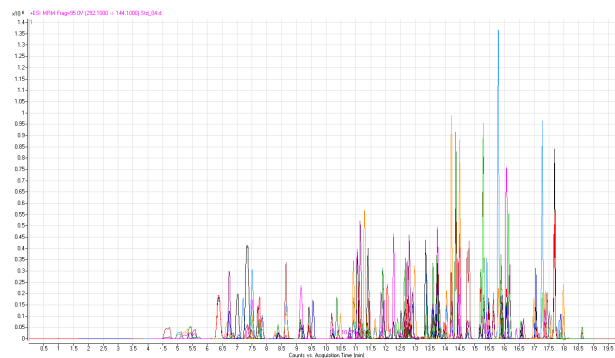


Figure 3. – A solvent based standard. 10ng/ml

Results

From the 200 pesticides analysed, 24 were selected for data comparison purposes. This selection was made based upon the availability of recently published recovery data, for manual clean-up using QuEChERS.

Linear calibrations (See Figure 4) for all compounds, in each matrix were achieved in all cases (See Appendix 1). Matrix blanks were also analysed during the analysis to provide evidence for any analyte contribution arising from the sample itself; all instrument blanks for all matrices for the compounds under investigation showed no positives determinations. (See Figure 5).

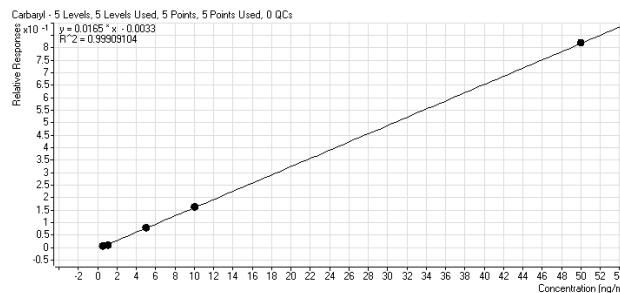


Figure 4. Example calibration curve 0.5 – 50 ng/g

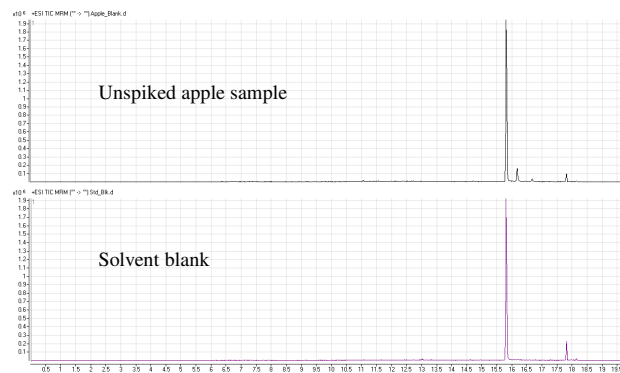


Figure 5. Solvent blank vs. Unspiked apple sample TICs

The use of TPP as an internal standard at 50 ng/g allowed the generation of calibration curves by plotting the relative responses of analytes (peak area of analytes/peak area of TPP) against the relative concentration of the analyte (concentration of analyte/concentration of TPP). Successful determination of the 1 ng/g calibration standard in all matrices shows that the quantification



limits (LOQ) are lower than the required MRL's.

The recovery and reproducibility of this method was evaluated by spiking the matrices with an appropriate amount of multi-component standard to give a concentration of 10 ng/g. These samples were quantified against the matrix matched calibration of 0.5 to 50 ng/g to ensure any suppression or enhancement effects due to the presence of matrix impurities was catered for. The analysis was performed in triplicate (n=3), the data is shown in Table 1. It can be seen in the results for the selected 24 pesticides, that acceptable recoveries (74 to 120 %) are generated with an average of 100 %

Table 2 shows the precision data for the analysis; the % RSD ranged from 0.2 to 19.3 with an average of 6.5, which is fully acceptable for quantitative analysis at a spike of 10 ng/g.

The resulting chromatograms from the analysis still showed sharp well resolved peaks as can be seen in figure 6.

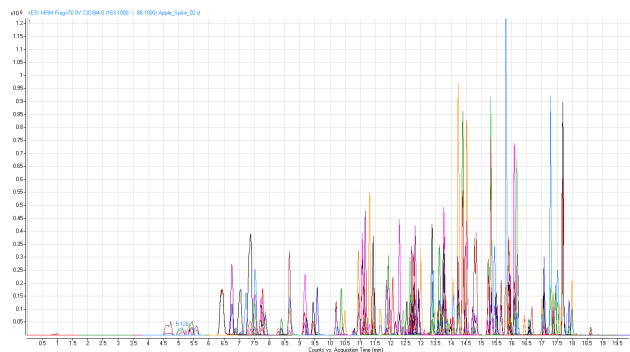


Figure 6. Apple sample spiked at 10ng/g

Conclusions

The recovery and reproducibility data based upon the matrices studied at the levels spiked show that the system is fully suitable for automating the QuEChERS DSPE clean-up. Automating this process ensures the entire procedure is fast, easy, and offers time and labour savings, while ensuring consistency. The hardware can process 50 samples in roughly 11 hours. The samples are analysed in a "real-time" fashion if an online configuration is employed, ensuring accurate results by minimising any possible sample analyte degradation time. The cycle time of the clean-up is shorter than the analytical run time providing the highest throughput, ensuring the LC-QQQ is never waiting for a sample, maximising your LC-QQQ investment. The system has a capacity of 98 samples ensuring even the highest throughput scenarios are catered for.

If operated in a standalone configuration the system can provide extracts for a non dedicated LC-QQQ system with a sample preparation time of approximately 13.5 minutes.

The system is suitable for online use when combined with a GC-QQQ. Certain selected pesticides are more amenable to GC rather than LC and further work will be completed at Anatune to assess the performance of the system when coupled to GC

References

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Pesticide	Sample Recovery (%)						Previous Data
	Apple	Strawberry	Spinach	Lettuce	Tomato	Orange	
Acetamiprid	118	101	103	94	106	96	103
Aminocarb	109	98	95	90	105	96	102
Azoxystrobin	103	111	92	98	109	99	98
Carbaryl	113	102	100	90	107	101	93
Carbendazim	109	100	98	86	107	98	98,90
Clothianidin	113	100	102	94	107	99	103
Dimethomorph	114	101	88	89	105	82	99
Fenhexamid	74	75	82	86	87	93	93
Fenobucarb	114	105	104	94	112	99	120
Fenoxycarb	113	103	102	97	110	104	98
Flufenoxuron	105	109	87	83	103	99	111
Flutolanil	120	109	103	99	108	102	99
Imidacloprid	114	101	93	93	104	94	96,105
Kresoxim-methyl	111	105	101	97	112	108	102,103
Lufenuron	101	94	89	74	102	91	100
Methoxifenozyd	118	106	105	100	111	93	104
Monolinuron	112	100	97	89	105	89	101
Propoxur	113	105	102	93	109	94	100
Pyriproxyfen	117	109	102	97	110	103	102
Siduron	118	105	75	92	105	100	101
Spinosyn A	116	101	81	90	105	99	97
Spirodiclofen	106	99	93	90	101	103	101
Thiabendazole	104	92	87	78	102	97	102,90
Thiacloprid	116	102	107	99	111	101	98

Table 1. Recovery data for selected pesticides in all matrices investigated.

Compound	RSD %					
	Apple	Strawberry	Spinach	Lettuce	Tomato	Orange
Acetamiprid	10.7	13.9	2.2	1.1	6.6	3.5
Aminocarb	9.5	10.1	3.1	0.2	2.9	3.2
Azoxystrobin	12.7	13.9	7.5	12.0	10.3	3.3
Carbaryl	4.8	9.7	1.2	3.5	4.3	8.3
Carbendazim	8.6	9.4	3.4	1.0	2.9	7.3
Clothianidin	9.3	15.9	2.7	9.1	8.0	14.6
Dimethomorph	8.3	7.4	2.6	0.9	7.2	6.6
Fenhexamid	10.4	16.8	1.1	2.3	3.4	4.2
Fenobucarb	10.2	7.9	4.4	6.6	7.1	3.9
Fenoxycarb	8.9	8.4	1.6	3.6	3.1	1.9
Flufenoxuron	7.8	8.7	1.9	19.3	0.8	2.1
Flutolanil	9.6	9.3	3.9	1.7	7.9	7.5
Imidacloprid	18.2	11.1	3.8	6.5	6.5	8.9
Kresoxim-methyl	11.7	9.8	6.7	6.0	1.5	4.6
Lufenuron	12.8	11.4	5.8	19.3	6.0	6.4
Methoxifenozyd	9.2	9.0	2.9	3.5	4.1	10.4
Monolinuron	12.1	10.6	2.7	5.9	6.7	0.9
Propoxur	10.1	3.7	4.6	1.2	5.3	4.6
Pyriproxyfen	7.5	7.0	1.4	2.6	0.6	6.9
Siduron	17.2	7.6	14.7	6.1	2.4	8.2
Spinosyn A	11.6	9.3	8.0	4.5	4.0	3.5
Spirodiclofen	9.0	8.1	2.7	2.5	5.6	1.3
Thiabendazole	9.1	7.5	3.6	2.3	2.2	2.6
Thiacloprid	10.3	8.4	1.6	1.7	0.8	2.3

Table 2. Relative standard deviation data for selected pesticides in all matrices investigated.



Appendix 1.

Compound	Correlation co-efficients					
	Apple	Strawberry	Spinach	Lettuce	Tomato	Orange
Acetamiprid	0.9993	0.9960	0.9986	0.9996	0.9984	0.9941
Aminocarb	0.9985	0.9961	0.9969	0.9993	0.9979	0.9942
Azoxystrobin	0.9988	0.9894	0.9952	0.9986	0.9739	0.9844
Carbaryl	0.9991	0.9987	0.9962	0.9987	0.9969	0.9993
Carbendazim	0.9991	0.9983	0.9994	0.9994	0.9990	0.9944
Clothianidin	0.9975	0.9983	0.9960	0.9977	0.9953	0.9914
Dimethomorph	0.9990	0.9927	0.9954	0.9968	0.9970	0.9765
Fenhexamid	0.9997	0.9924	0.9981	0.9991	0.9948	0.9962
Fenobucarb	0.9999	0.9984	0.9986	0.9978	0.9972	0.9898
Fenoxycarb	0.9995	0.9979	0.9989	0.9998	0.9984	0.9966
Flufenoxuron	0.9981	0.9948	0.9971	0.9993	0.9972	0.9967
Flutolanil	0.9986	0.9995	0.9992	0.9991	0.9997	0.9894
Imidacloprid	0.9955	0.9929	0.9924	0.9985	0.9989	0.9950
Kresoxim-methyl	0.9991	0.9980	0.9988	0.9993	0.9996	0.9968
Lufenuron	0.9959	0.9854	0.9986	0.9958	0.9976	0.9887
Methoxifenozyd	0.9990	0.9992	0.9995	0.9999	0.9993	0.9748
Monolinuron	0.9995	0.9966	0.9957	0.9996	0.9984	0.9939
Propoxur	0.9994	0.9978	0.9987	0.9993	0.9992	0.9947
Pyriproxyfen	0.9987	0.9994	0.9999	0.9999	0.9999	0.9976
Siduron	0.9972	0.9979	0.9987	0.9986	0.9991	0.9966
Spinosyn A	0.9988	0.9973	0.9949	0.9989	0.9990	0.9965
Spirodiclofen	0.9989	0.9945	0.9945	0.9988	0.9978	0.9961
Thiabendazole	0.9991	0.9937	0.9972	0.9993	0.9962	0.9933
Thiacloprid	0.9987	0.9979	0.9997	0.9987	0.9996	0.9967

Appendix showing all correlation co-efficients in all matrices for all compounds.