Time and Cost Effective Methods for Reducing Background Noise and Signal Suppression in Problem Matrices for Residue Analysis by LC-MS/MS

Joseph Kolb[1], Ariana Ramdin[1], Ryan Undeen[1], Dennis Peterson[2], Sam Ellis[2] Lisa Wanders[2]

[1]Merieux NutriSciences Corporation, Gainsville, FL 32607

[2]Thomson Instrument Company, Oceanside, CA 92054

Abstract

Several clean-up methods are compared for background reduction, analyte recovery, and cost effectiveness in order to successfully analyze a wide variety of multiclass multiresidues in difficult matrices including Chili Powder and Tobacco. The most critical aspects of reliable multiresidue analysis are the reduction of interferences from the sample matrix and analyte recovery. eXtreme|FV®, were compared to an existing ISO accredited QuEChERS method, as well as a dilute and shoot approach are analyzed in conjunction with different filtration techniques for residue analysis by LC-MS/MS for minimal number of steps, speed, reduced reagent use and reduced cost.

Experimental

In order to successfully analyze multi-residue methods on difficult matrices such as habanero flakes and tobacco, several different clean-up procedures may need to be employed. This method investigates the use of different clean-up procedures and a dilute and filter approach to successfully analyze 20 pesticide compounds facing problems from matrix effects. The cost-effectiveness of different filtering techniques was also considered.

The following difficult to analyze compounds were tested:

5-OH Thiabendazole	Clofentezine	Coumaphos	Etoxazole
Metolachlor	Phosalone	Pirimiphos-methyl	Prallethrin
Prochloraz	Pymetrozine	Pyraclostrobin	Quinoxyfen
Simazine	Spinetoram-major	Spinetoram-minor	Thiobencarb
Thiophanate-methyl	Tolyfluanid	Triazophos	Trifloxystrobin

Equipment:

Sciex API 4000 Qtrap Mass Spectrometer
Shimadzu LC-20AD Pumps
Flow Rate: 0.25 mL/min
Run Time: 20 minutes
Injection Volume: 15μL
Mobile Phases:

A: 0.1% Formic Acid and 10mM Ammonium Acetate in HPLC Water

B: 0.5% Formic Acid in Methanol

Gradient:

Time (min.)	%A	%B
	90	10
0.5	90	10
15	2	98
19	2	98
20	90	10

Column Temperature: 40˚C
Column: Waters Zorbax C18 3.5μm 3mm x 150mm
Thomson eXtreme|FV® 0.2μm PTFE (p/n 85530)*
Thomson 48 position Vial Filter Press (p/n 35015-476)
Centrifuge

Special Note: For some autosamplers it is important to adjust the needle depth of your autosampler when using Thomson filter vials to improve the reproducibility of injections

Method:

28 QuEChERS extracts were prepared and the filtration step was performed using two different approaches. Samples were evaluated for % recovery and timed. In both cases the samples need to be diluted with mobile phase prior to filtration in order to filter out precipates that are formed with the addition of aqueous solvent.

eXtreme®|FV Sample Preparation

1. Weigh 1g sample and add internal standards and standards as appropriate.
2. Dispense 10mL water and then 15mL ACN.
3. Cap and shake for 30 seconds.
4. Centrifuge for 10 minutes at 3600 rpm.
5. Transfer 400uL and filter using Thomson eXtreme 0.2 µm PTFE Filter Vial.

Traditional QuEChers Sample Preparation

1. Weigh 5g sample and add internal standards and standards as appropriate
2. Dispense 10mL water and then 15mL 1% Acetic Acid in ACN.
3. Cap and shake
4. Add Magnesium Sulfate and Sodium Acetate QuEChERS salts to tube, vortex and then shake on Genogrinder for 1 minute.
5. Centrifuge for 10 minutes at 3600 rpm
6. Decant top layer into dispersive clean-up tubes, shake and vortex for 1 min (EMR salt clean-up requires a second dispersive SPE step)
7. Centrifuge for 5 minutes at 3600 rpm
8. Dilute 1:1 with Aqueous Mobile Phase and Filter

Results

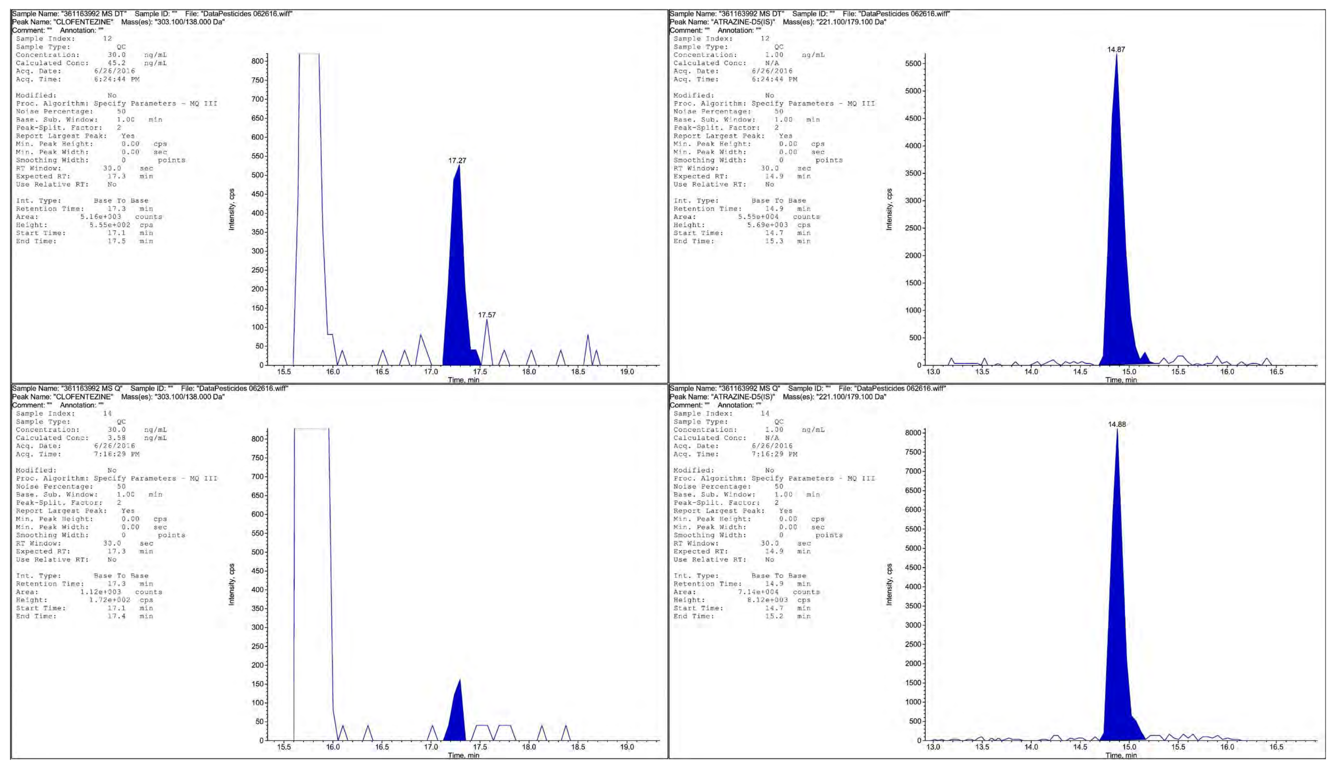
Data Comparison Table of 20 Analyte Recoveries from different extracts/matrices spiked at 30ppb. Habanero Flakes and Tobacco showed less matrix effects and increased reproducibility using the dilute and filter method and compared to the QuEChers and filter method.

Analyte	Habanero Flakes QuEChERS +PSA %Recovery	Habanero Flakes QuEChERS + EMR %Recovery	Habanero Flakes Dilute and Filter %Recovery	Tobacco QuEChERS +PSA %Recovery	Tobacco Dilute and Filter %Recovery
5-OH Thiabendazole	30.9	41.8	75.6	35.5	59.3
Clofentezine	11.9	206	151	232	82.2
Coumaphos	15.3	107	87.9	129	135
Etoxazole	65	80.8	92.4	447	189
Metolachlor	32.8	110	150	117	174
Phosalone	54.7	121	86.3	135	111
Pirimiphos-methyl	192	409	262	267	264
Prallethrin	128	351	321	232	28.0
Prochloraz	75.8	186	130	146	140
Pymetrozine	136	129	328	449	319
Pyraclostrobin	28.1	35.6	77.6	98.7	103
Quinoxyfen	51.6	132	83.1	39.1	91.0
Simazine	73.6	117	186	112	97.9
Spinetoram-major	49.6	160	104	120	124
Spinetoram-minor	46.9	114	92.7	119	146
Thiobencarb	28.5	69.6	78.1	71.5	83.5
Thiophanate-methyl	18.5	105	94.7	314	128
Tolyfluanid	14.4	71.3	54.9	101	115
Triazophos	15.3	8.94	34.8	27.4	29.4
Trifloxystrobin	40.8	137	108	75.7	106

Data

For the pesticides we compared the traditional QuEChERS method and cleaned up with PSA and syringe & filter to simply dilute and shoot with the eXtreme PTFE Thomson Vial for Chili Powder and Tobacco. Diluting the samples gives better or comparable sensitivity with several difficult analytes in which we have been experiencing matrix suppression. Here are some of the analytes where the dilute and shoot method counteracted matrix suppression: 5-Hydroxythiabendazole, Clofentezine, Coumaphos, Etoxazole, Metolachlor, Phosalone, Pirimiphos-methyl, Prallethrin, Prochloraz, Pymetrozine, Pyraclostrobin, Quinoxyfen, Simazine, Spinetoram, Thiobencarb, Thiophanate-methyl, Tolyfluanid, Triazophos, and Trifloxystrobin. The dilution extraction helped us to include these analytes in our screen despite the heavy matrix effect we saw in QuEChers extraction.

Note: Several high recoveries (>200%) caused by matrix suppression of internal standard or matrix enhancement of analyte.



Conclusion

Using a dilute and filter approach on difficult matrices allows several analytes to be included in multiresidue pesticide screens that would have otherwise been excluded due to matrix suppression; or worse yet, yielded a false negative result if only the traditional QuEChERS approach was employed. The dilute and filter method may not be useful for all analytes where sensitivity is a concern, however in several cases the dilute and filter method actually improved sensitivity of the analyte as matrix suppression was minimized without having to use more expensive clean-up techniques. Moreover, this approach is an extremely cost effective way to ensure problem analytes on difficult matrices can be included in a screen. The Thomson filter vials are also a tremendous time and money saver when replacing traditional syringe filtration techniques.

Time Savings of Filter Vials

The first approach was a traditional QuEChers method including filtration using a syringe, 0.2µm PTFE filter, and needle. The time taken to assemble the syringes and filter, as well as the time to mix the extract and mobile phase prior to placing in the syringe was included in the timing. The entire process took 64 minutes and 52 seconds.

With the second approach, the extract and mobile phase were placed into the bottom of a Thomson eXtreme Filter Vial together, the 0.2µm PTFE filter and cap was placed on top of the vials, and all the samples were pressed simultaneously using the Thomson Multi-Use Press. The entire process took 12 minutes and 51 seconds. Giving a time savings of 52 minutes!

Total Time Sample Filtration for 48 Samples