Analysis of Pesticides in Turmeric Powder by LC-MS/MS and GC-MS/MS

After Cleanup with a Novel Dual-Layer SPE Cartridge

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Introduction

Turmeric is a plant indigenous to south Asia, with a majority of its production coming from India. The rhizome of the plant is used to produce powdered turmeric, which is used in foods, cosmetics, and some medicines. It is also an essential constituent of curry, which is a mixture of spices used extensively in Indian cooking. Turmeric has also been used in traditional medicines for thousands of years, and recently has garnered attention for studies showing its potential antioxidant, anti-inflammatory, antimutagenic, antimicrobial and anticancer properties.[1]

Pesticide residue testing of turmeric and other spices is required by many countries. For example, Canada has set maximum residue limits for 42 different pesticides in turmeric root.[2] The US EPA has set tolerance limits for a variety of pesticides in root and tuberous vegetables, of which turmeric is included.[3]

Turmeric contains more than 100 different components, with two of the main constituents being curcumin and volatile oils. Curcumin gives turmeric its distinctive yellow/orange color, while the volatile oils consist primarily of terpenes. Turmeric also contains some fats; specifically, sterols and fatty acids.[4] This complex composition makes extracts produced from turmeric a challenge in the chromatographic analysis of pesticides, as residual pigments and oils can contaminate both GC-MS and LC-MS systems.

When dealing with very high background samples such as turmeric, standard QuEChERS cleanup may not offer enough capacity. For better cleanup, solid phase extraction (SPE), including dual-layer cartridges, can be used. These cartridges often contain graphitized carbon black (GCB) in the top bed and primary-secondary amine (PSA) in the bottom bed. PSA retains acidic interferences such as fatty acids. GCB removes planar molecules such as pigments and sterols. Common GCBs, however, will retain all molecules with planar structures, including some pesticide analytes such as hexachlorobenzene. To increase recoveries of these pesticides, toluene is normally added to the elution solvent. However, there are issues associated with the use of toluene. It can affect the ability of the PSA to retain fatty acids, and its presence in the final extract is problematic for HPLC analysis.[5]

In this application, a different dual-layer SPE cartridge was used in the cleanup of extracts of turmeric powder prior to pesticide analysis by GC-MS/MS and LC-MS/MS. This cartridge, the Supelclean™ Ultra 2400, was designed for the cleanup of acetonitrile extracts made from difficult matrices such as dry commodities (spices, tea, etc.) prior to pesticide residue analysis. The top bed consists of a mixture of PSA, C18 and a graphitized, spherical carbon known as Graphsphere™ 2031. This carbon was engineered to remove sufficient pigmentation while allowing for better recoveries of planar compounds, without the need for toluene in the elution solvent. The bottom layer of the cartridge contains Z-Sep, a zirconia-coated silica. Z-Sep removes oily residues and provides additional retention of some pigments. The combination of these sorbents in an SPE format offers more capacity than QuEChERS cleanup, and compared to traditional GCB/PSA dual layer cartridges, does not require the use of toluene in the elution solvent to recover planar pesticides.

Experimental

Turmeric powder was obtained from a local grocery store. Samples were spiked at 100 ng/g with the pesticides listed in **Tables 1** and **2**. Sample extracts were prepared and cleaned following the procedures in **Figure 1**. A set of 3 spiked samples and 1 unspiked (blank) were prepared and analyzed for each set of pesticides. Analysis was done by GC-MS/MS and LC-MS/MS using the conditions listed in **Tables 3** and **4** (with MS/MS transitions shown in **Tables 1** and **2**). Quantitation was performed against multi-point calibration curves prepared in unspiked turmeric extract (after cleanup). Recoveries were calculated as the average of the three spiked replicates, less anything found in the unspiked extract. No internal standards were used, thus the values reported represent absolute recoveries.

Results and Discussion

Background

Prior to cleanup, the extract appeared orange-brown in color with a yellow oily residue (**Figure 2**). After cleanup for both LC and GC, the extracts appeared substantially lighter and clearer. **Figures 3** and **4** show

(continued on next page)

Table 1. Pesticides Studied in Turmeric Powder by GC-MS/MS Analysis

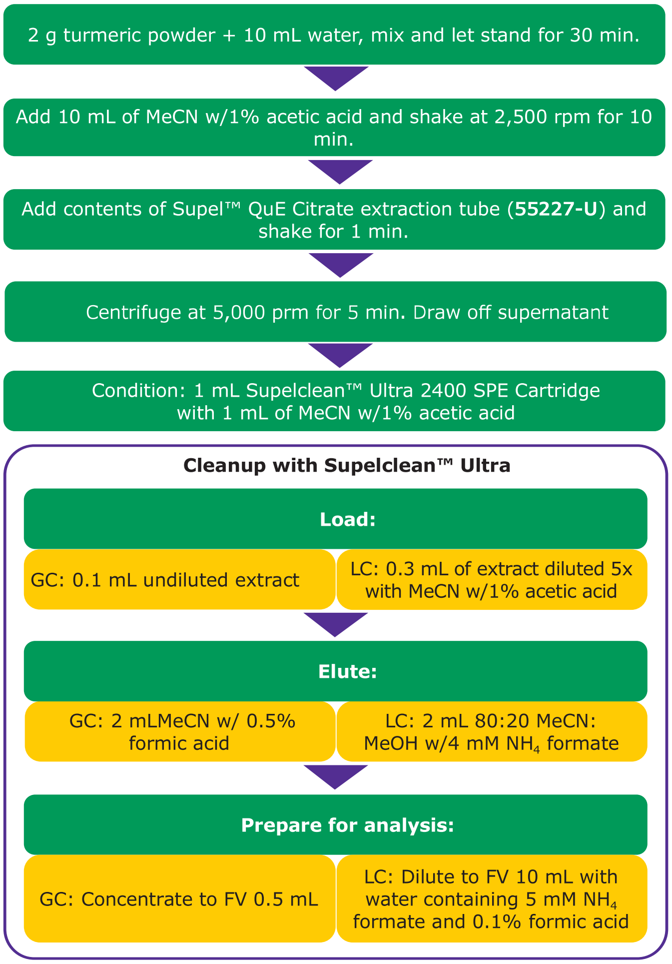
	MRM 1	CE	MRM 2	CE
Alachlor	188/160	10	188/130	40
Aldrin	263/193	35	263/191	35
γ-BHC	183/147	15	181/145	5
Azinphos-methyl	160/77	15	132/77	15
Chloropyrifos	314/286	5	314/258	15
Chloropyrifos-methyl	286/93	20	288/93	20
Cypermthrins	165/91	10	163/91	10
4,4'-DDT	235/199	15	235/165	25
Diazinon	199/135	15	137/84	10
Dichlorvos	185/93	25	145/109	25
Dimethoate	125/79	20	93/63	10
Disulfoton	88/60	5	88/59	15
Endosulfan β	241/206	15	241/170	30
Endosulfan-α	241/206	15	241/170	30
Ethion	231/129	20	121/65	10
Fenitrothion	277/125	20	277/109	20
Heptachlor	274/239	15	272/237	15
Hexachlorobenzene	284/249	20	284/214	35
Iprodione I	314/56	35	187/124	25
Iprodione II	316/56	35	187/124	25
Malathion	173/99	15	158/125	5
Metalaxyl	234/174	10	234/146	20
Methoxychlor	227/169	30	227/141	30
Mevinphos	192/127	25	192/109	25
Parathion-methyl	233/109	10	124/47	10
Permethrins	183/168	10	183/165	10
Phenthoate	274/125	15	274/121	10
Phorate	260/75	5	231/129	25
Phosalone	182/102	15	182/75	30
Pirimiphos-methyl	290/151	20	290/125	25
Profenophos	339/269	15	339/188	15
Quintozene	295/237	20	237/143	30
Vinclozolin	212/145	30	187/124	20

Table 2. Pesticides Studied in Turmeric Powder by LC-MS/MS Analysis

	MRM	Frag (V)	CE (V)	Cell Acc (V)
Acephate	184/143	70	0	5
Acetamiprid	223.1/126	80	27	2
Boscalid (Nicobifen)	343/307.1	145	16	6
Carbendazim (Azole)	192.1/160.1	105	16	2
Chlorbufam	224/172.02	120	5	3
Cycluron	199.2/72	120	20	2
Diflubenzuron	311/158	80	8	2
Fenoxanil	329.08/189	80	30	3
Fosthiazate	284/61	90	60	2
Methabenzthiazuron	222.1/165.1	90	12	2
Methamidophos	142/125	85	10	2
Methomyl	163.1/106	50	4	2
Monocrotophos (Azodrin)	224.1/193	65	0	5
Nitralin	346.11/304	100	10	3
Oxamyl	237.1/72	60	12	2
Pirimicarb	239.15/72.1	100	20	2
Procymidon	301/284*	70	8	2
Propaquizafop	444.12/100.1	125	16	2
Tetraconazole	372/159	130	36	2
Uniconazole-P	292.1/125	135	40	2

a comparison between extracts at the same level of dilution with and without cleanup. The LC extract (in 80% aqueous) was almost devoid of color, with very little cloudiness. The extract for GC analysis was a pale yellow color, with substantially less oily residue. Full scan GC-MS analyses of GC extracts are shown in

Figure 1. Extraction and Cleanup Procedure Used for Turmeric Powder, GC and LC



2 g turmeric powder + 10 mL water, mix and let stand for 30 min.

Add 10 mL of MeCN w/1% acetic acid and shake at 2,500 rpm for 10 min.

Add contents of Supel™ QuE Citrate extraction tube (**55227-U**) and shake for 1 min.

Centrifuge at 5,000 prm for 5 min. Draw off supernatant

Condition: 1 mL Supelclean™ Ultra 2400 SPE Cartridge with 1 mL of MeCN w/1% acetic acid

Cleanup with Supelclean™ Ultra

Load:

GC: 0.1 mL undiluted extract

LC: 0.3 mL of extract diluted 5x with MeCN w/1% acetic acid

Elute:

GC: 2 mLMeCN w/ 0.5% formic acid

LC: 2 mL 80:20 MeCN: MeOH w/4 mM NH_4 formate

Prepare for analysis:

GC: Concentrate to FV 0.5 mL

LC: Dilute to FV 10 mL with water containing 5 mM NH_4 formate and 0.1% formic acid

Table 3. GC-MS/MS Run Conditions for the Analysis of Pesticides in Turmeric

column:	SLB®-5ms, 30 m × 0.25 mm ID, 0.25 µm (**28471-U**)
oven:	50°C (2 min), 8°C/min to 320°C (5 min)
inj. temp.:	250°C
carrier gas:	helium, 1.4 mL/min, constant
detector:	MRM (see **table 1**)
MSD interface:	320°C
injection :	1µL, splitless (splitter open at 0.75 min)
liner:	4 mm I.D., split/splitless type, single taper wool packed FocusLiner™ design (**2879901-U**)

Table 4. LC-MS/MS Run Conditions for the Analysis of Pesticides in Turmeric

column:	Ascentis® Express C18, 10 cm × 2.1 mm ID, 2 µm (**50813-U**)
mobile phase:	[A] 5 mM ammonium formate, 0.1% formic acid in water; [B] 5 mM ammonium formate, 0.1% formic acid in methanol
gradient:	95% A, 5% B held for 1 min; to 50% A in 3 min; to 100% B in 8 min; held for 1 min;, to 95% A in 1.5 min; held at 95% A for 1.5 min
flow rate:	0.4 mL/min
detector:	MRM (see **table 2**)
injection:	5 µL

Figure 2. Undiluted Acetonitrile Extract of Turmeric Powder Before Cleanup

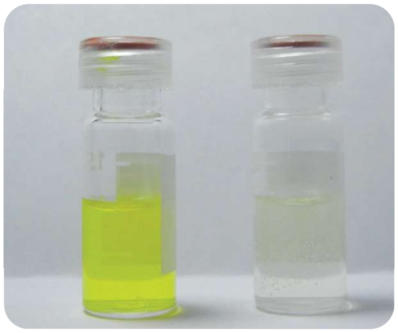


Figure 3. Turmeric Extracts at the Same Dilution (167X total); Without Cleanup, and After Cleanup for LC/MS/MS Analysis

Figure 4. Turmeric Extracts at the Same Dilution (5x); Without Cleanup, and After Cleanup for GC/MS/MS Analysis

Figure 5 as total ion chromatograms (TICs). The peak pattern is similar between the two, with the main peaks consisting primarily of terpenes. These compounds are easily volatilized in the GC inlet, and do not pose issues with system contamination, however they can interfere with mass spectral detection, requiring the use of MS/MS for selectivity. The overall amplitude of the peaks was less after cleanup, as is shown by a 21% reduction in the peak area sums for each in **Figure 5**.

Pesticide Recovery and Reproducibility

The average recoveries obtained from spiked turmeric samples (n=3) are presented in **Table 5**. Of the 51 pesticides spiked, all except hexachlorobenzene had recovery of greater than 70%. Hexachlorobenzene, a pesticide with a planar structure, was recovered at 67% after cleanup. It should be noted that this was without using toluene in the elution solvent, as is necessary to obtain good recoveries from dual-layer cartridges containing graphitized carbon black.[5] Although not shown here, higher recovery of hexachlorobenzene has been obtained by loading more turmeric extract (300 µL) on the Supelclean™ Ultra 2400 cartridge. This indicates that the presence of more matrix displaced the hexachlorobenzene, thus reducing its retention on the carbon. However the higher sample loading produced an extract with more color, a sign that the cleanup capacity of the cartridge had been reached or exceeded for this matrix.

Reproducibility, calculated as %RSD for the sets of spiked replicates, was less than 20% for 44 of the 51 pesticides. As is indicated in **Figure 6**, many compounds had RSD values of less than 10%. Pesticides with RSD values greater than 20% were attributed to those showing low response in the MS/MS method.

Table 5. Pesticide Recoveries and % RSD Values (***n***=3) for Spiked Replicates; Turmeric Spiked at 100 ng/g

Pesticide	Avg. Recovery	RSD	Analysis
Alachlor	99%	23%	GC-MS/MS
Aldrin	85%	10%	GC-MS/MS
Azinphos-methyl	89%	11%	GC-MS/MS
γ-BHC	83%	8%	GC-MS/MS
Chloropyrifos	96%	12%	GC-MS/MS
Chloropyrifos-Methyl	113%	6%	GC-MS/MS
Cypermthrin (isomer 1)	99%	15%	GC-MS/MS
4,4'-DDT	95%	8%	GC-MS/MS
Diazinon	92%	14%	GC-MS/MS
Dichlorvos	78%	31%	GC-MS/MS
DIsulfoton	86%	7%	GC-MS/MS
Endosulfan β	86%	35%	GC-MS/MS
Endosulfan-α	92%	23%	GC-MS/MS
Ethion	97%	7%	GC-MS/MS
Fenitrothion	63%	5%	GC-MS/MS
Heptachlor	81%	7%	GC-MS/MS
Hexachlorobenzene	67%	9%	GC-MS/MS
Iprodione (isomer 1)	103%	5%	GC-MS/MS
Malathion	90%	10%	GC-MS/MS
Metalaxyl	86%	21%	GC-MS/MS
Methoxychlor	78%	12%	GC-MS/MS
Mevinphos	73%	7%	GC-MS/MS
Parathion-Methyl	88%	8%	GC-MS/MS
Permethrin (isomer 1)	104%	24%	GC-MS/MS
Phenthoate	89%	7%	GC-MS/MS
Phorate	82%	10%	GC-MS/MS
Phosalone	90%	7%	GC-MS/MS
Pirimiphos-methyl	74%	3%	GC-MS/MS
Profenophos	88%	7%	GC-MS/MS
Quintozene	75%	8%	GC-MS/MS
Vinclozolin	90%	6%	GC-MS/MS
Acephate	89%	6%	LC-MS/MS
Acetamiprid	102%	4%	LC-MS/MS
Boscalid (Nicobifen)	86%	7%	LC-MS/MS
Carbendazim (Azole)	106%	7%	LC-MS/MS
Chlorbufam	92%	18%	LC-MS/MS
Cycluron	103%	5%	LC-MS/MS
Diflubenzuron	101%	5%	LC-MS/MS
Fenoxanil	91%	10%	LC-MS/MS
Fosthiazate	95%	4%	LC-MS/MS
Methabenzthiazuron	96%	4%	LC-MS/MS
Methamidophos	85%	5%	LC-MS/MS
Methomyl	106%	6%	LC-MS/MS
Monocrotophos (Azodrin)	97%	3%	LC-MS/MS
Nitralin	124%	55%	LC-MS/MS
Oxamyl	104%	3%	LC-MS/MS
Pirimicarb	97%	3%	LC-MS/MS
Procymidon	91%	13%	LC-MS/MS
Propaquizafop	97%	1%	LC-MS/MS
Tetraconazole	98%	2%	LC-MS/MS
Uniconazole-P	103%	19%	LC-MS/MS

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Figure 5. GC-MS Scan Analyses of Turmeric Extracts Before and After Cleanup with Supelclean™ Ultra 2400 cartirgde Shown with same Y-scale. Sum of area counts for all peaks is indicated with each.

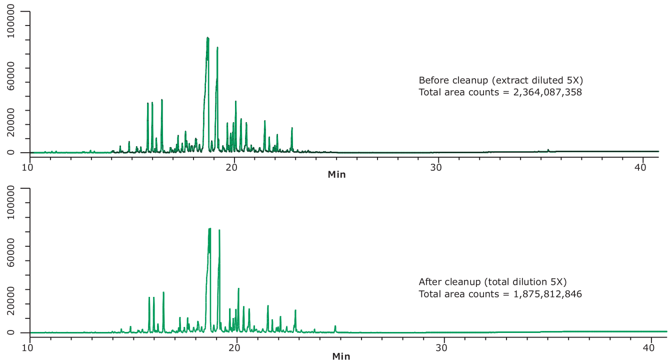
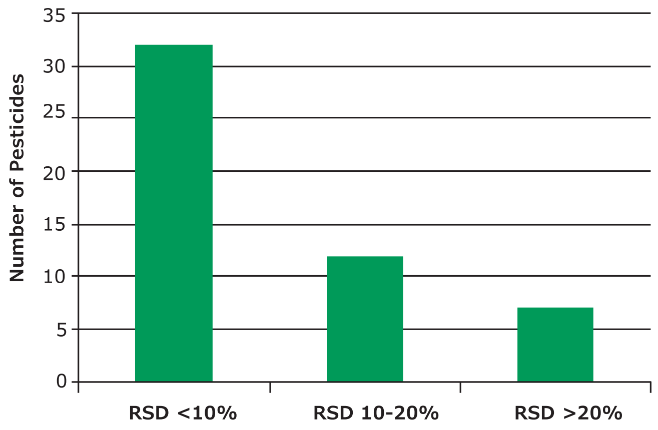


Figure 6. Number of Pesticides with Average Recoveries Within Indicated Percent Relative Standard Deviation (%RSD) Ranges After Cleanup with Supelclean™ Ultra 2400

(Recoveries from turmeric powder spiked at 100 ng/g.)



Conclusion

A new cleanup method has been developed using the Supelclean™ Ultra 2400 dual-layer SPE cartridge. The selection of sorbents in this cartridge allows for cleanup of acetonitrile extracts of very difficult samples such as spices and other dry commodities. The Graphsphere™ 2031 carbon used in the upper layer removes/reduces pigmentation while still allowing for recovery of planar pesticides without the use of toluene in the elution solvent. Z-Sep sorbent in the bottom layer of the cartridge removes oils and some pigments, as was indicated in the cleanup of turmeric extracts for both GC and HPLC analysis. Suitable recoveries for a wide range of pesticides of different polarities and classes were obtained from turmeric extract, and minimal background interference was noted. In this work, a 1 mL Supelclean™ Ultra 2400 cartridge was used. A larger 3 mL version of the cartridge is also available which can accommodate a higher sample loading.

Acknowledgements

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Featured Products

Description	Cat. No.
Supelclean™ Ultra 2400 SPE Cartridges	
1 mL, pk of 108	**52779-U**
3 mL, pk of 54	**54281-U**
Supel™ QuE QuEChERS Products	
Citrate Extraction Tube, 12 mL, pk of 50	**55227-U**
Empty Centrifuge Tube, 50 mL, pk of 50	**55248-U**
Columns	
SLB®-5ms Capillary GC Column, 30 m × 0.25 mm I.D., 0.25 µm	**28471-U**
Ascentis® Express C18 HPLC Column, 10 cm × 2.1 mm I.D., 2 µm particle size	**50813-U**
Accessories	
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QuEChERS Shaker and Rack Starter Kit, Schuko plug, AC input 230 V	**55438-U**
Visiprep™ DL 12-port Solid Phase Extraction Manifold	**57044**
Disposable valve liners, PTFE, 100 ea.	**57059**

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Description	Cat. No.
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Accessories	
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