

Application Note

No. GC-10-ADI-010

GCMS QP-2010 Ultra

Determination of Organophosphorus Pesticide present in Herbal Products using QuEChERS Method

Introduction

With ever increasing use of herbal medical and global expansion of herbal medicines market, safety has become a major concern for both health authorities. From time to time, undesirable and/or undeclared substances have been present or been purported in herbal medicines or medicinal plants in many parts of the world. These substances may include pesticides, radioactive substances, microbes etc. The quality of herbal medicines has direct impact on their safety and efficacy. There are many control measures for the herbal medicines the first important step is to control the quality of medicinal plants and herbal materials. It is well known that there are many contaminants and residues that may cause harm to the consumer. For this reason there is potential global danger to health and well being of people. This risk can be reduced by ensuring that herbal medicines with harmful contaminants and residues does not reach public, by assessing the quality of medicinal plants, herbal materials and finished herbal products before they reach the market.

This application note demonstrates the analysis of herbal products for presence of residual pesticides such as Organophosphorus (OP) pesticides. These pesticides may be used as insecticides, fungicides or herbicides during growth, transportation and storage stages. A number of methods exist for the extraction and analysis of residual pesticides from different matrices. In this note we have used a simple extraction method known as "QuEChERS" (Quick, Easy Cheap, Effective, Rugged and Safe) method. This method employs Dispersive Solid Phase Extraction (dSPE) and Gas Chromatography Mass Spectrometer (GCMS).

Experimental

All the standards required for the experiment are procured from Restek Corp. USA. Organophosphorus pesticide are prepared in Toluene in the range of 10-500 ng/mL for the experiment. For the extraction of residual pesticides from herbal matrix, organic solvent is mixed with sample, gram quantity of salts and sorbents are added to partition the analytes between aqueous residue and solvent. An aliquot is removed and further mixed with different additional salts and sorbents as additional clean up step. After simple centrifugation step the supernatant is ready for GCMS analysis. **Table 1** shows the extraction procedure for herbal product. Both the standards and sample analyzed on Shimadzu GCMS QP-2010 Ultra along with Selective Ion Monitoring (SIM) mode.

Following GC conditions are used as given in **Table 2** for the analysis.

Table 1 QuEChERS method of extraction of sample.

- 1) Weigh 15 g of ground herbal sample in centrifuge tube.
- 2) Add 15 ml of 1% acetic acid in Acetonitrile and shake well till sample becomes wet.
- 3) Add 2 packets of Restek Salts containing 4g MgSO₄, 1g NaCl, 1g trisodium citrate dihydrate, 0.5g Disodium hydrogen citrate sesquihydrate. (Catalogue no 26236)
- 1) Shake by hand for 1 minute.
- 2) Centrifuge for 2 minutes at 3000 rpm and allow to settle for 5 minutes.
- 3) Transfer 1 ml of aliquot from extract to Restek dSPE tube containing 150mg MgSO₄, 25mg PSA, 25 mg C18. (Catalogue no 26216)
- 4) Shake well for 1 minute and vortex.
- 5) Centrifuge for 2 minutes at 3000 rpm and allow it to stand for 5 minutes
- 6) Take 100 uL of aliquot from above step and dilute it with Toluene to make a volume of 1ml.

Table 2 Analytical Conditions

Gas Chromatograph : Shimadzu GCMS QP-2010 Ultra

Oven Temp. Program

Oven temp.	°C/min	Next °C	Hold Min
Initial		110	0
Ramp rate 1	5	280	2

Injector Temp 250°C Split less 2 mins

Carrier Gas Helium

Detector Quadrapole Mass Spectrometer

Acquisition Mode SIM

Column OP Pesticides (Restek) 30m x0.25mm x 0.25µm

Inj. Volume 1 uL

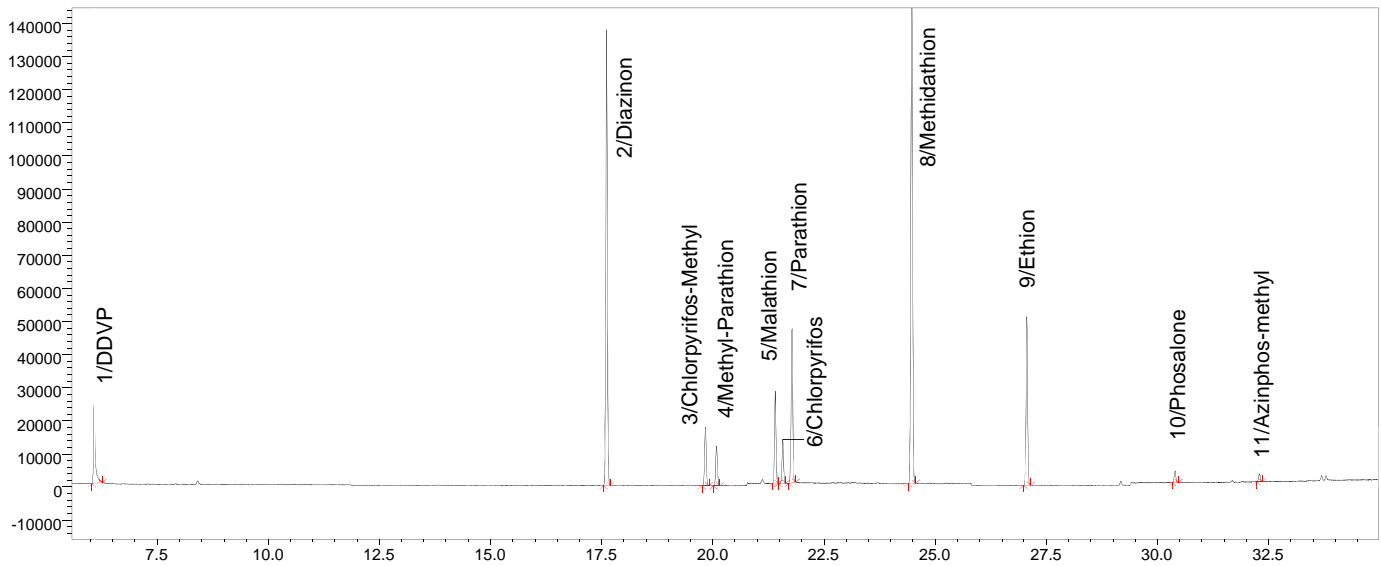


Fig 1 TIC of Organophosphorus Pesticide at 100 ng/mL Standard

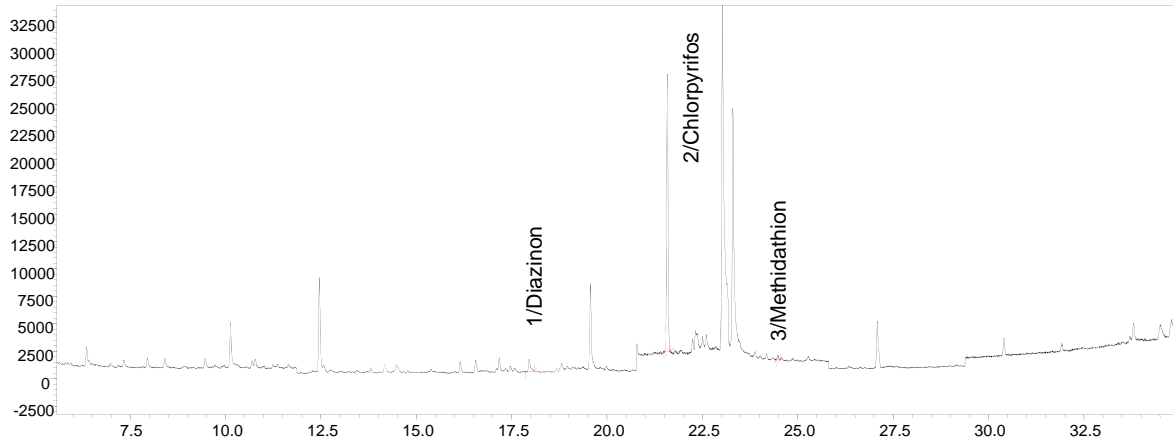


Fig 2 TIC of Herbal Powder sample

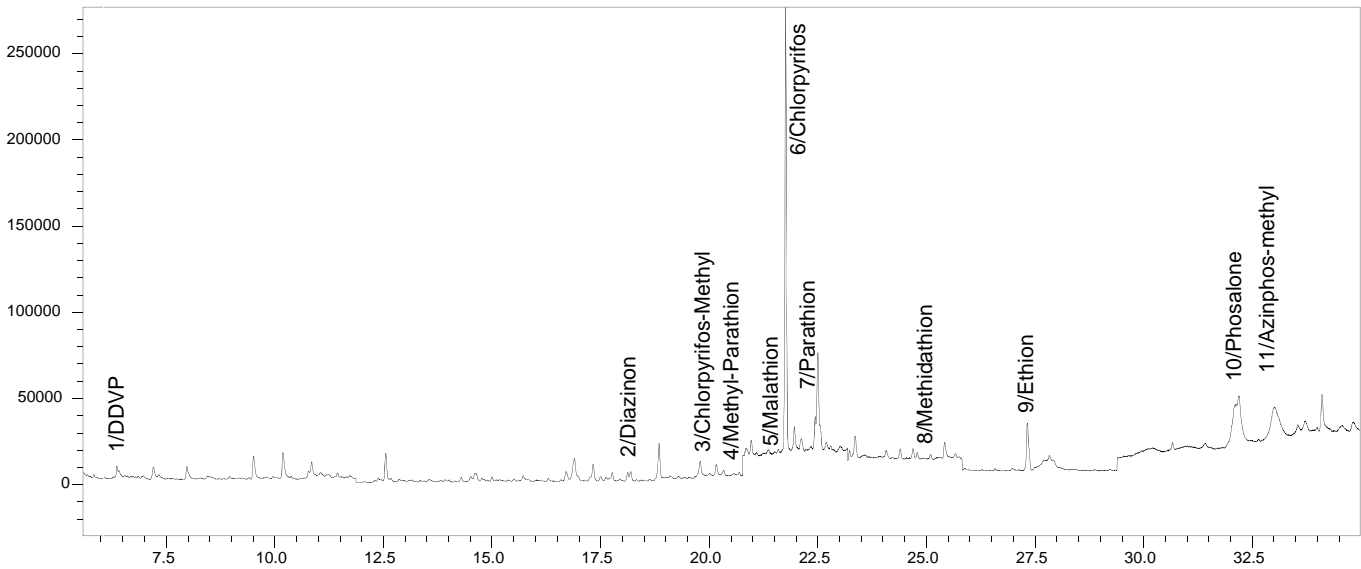


Fig 3 TIC of ORG-118 Spiked

Table 3 List of Organophosphorus Pesticides displaying %RSD and Regression Coefficient values

ID	Compound Name	Retention Time (min)	%RSD (n=6)			R ² Value	%Recovery
			250 ng/mL	100 ng/mL	50 ng/mL		
1	DDVP	6.085	4.61	3.62	3.68	0.999	105.57
2	Diazinon	17.66	1.84	2.21	3.82	0.999	80.14
3	Chlorpyrifos-Methyl	19.84	1.46	1.79	3.92	0.999	100.70
4	Methyl-Parathion	20.1	1.04	1.23	1.99	0.997	107.15
5	Malathion	21.44	1.14	1.9	3.02	0.998	111.39
6	Chlorpyrifos	21.585	1.48	1.97	2.38	0.999	92.02
7	Parathion	21.83	1.00	1.19	3.17	0.996	99.09
8	Methidathion	24.54	1.57	2.13	4.47	0.998	106.47
9	Ethion	27.1	0.74	1.28	3.8	0.998	105.36
10	Phosalone	31.68	1.29	3.95	4.06	0.999	93.98
11	Azinphos-methyl	32.315	2.34	0.85	4.96	0.992	100.06

Table 4 Quantitation of Unknown OPs present in Herbal Powder

ID	Compound Name	Retention Time (min)	Target Ions	Conc. (ng/mL)
1	DDVP	6.085	109	ND
2	Diazinon	17.66	137	9.63
3	Chlorpyrifos-Methyl	19.84	125	ND
4	Methyl-Parathion	19.995	109	ND
5	Malathion	21.44	93	ND
6	Chlorpyrifos	21.585	97	168.64
7	Parathion	21.83	109	ND
8	Methidathion	24.54	145	ND
9	Ethion	27.1	97	ND
10	Phosalone	31.68	182	ND
11	Azinphos-methyl	32.315	77	ND

Linearity of the OP standards is studied at different levels. The calibration curve is plotted by using the data obtained by injecting 1 μ L of standards at 10-500 ng/mL. All the pesticides of interest have excellent linearity with R² values. (Refer **Table 3**). The recovery study was carried out by spiking 0.25 μ g/mL of standard in the sample. Fig 3 shows the TIC of sample spiked with 0.25 μ g/ml of standard. The spiked sample treatment for extraction is as shown in the **Table1**. The recovery data of the spiked sample is shown in **Table 3**.

The unknown OPs are quantified based on the external standard method. The detected OPs that are present in the sample are listed in **Table 4**.

□ Conclusion

Unknown Organophosphorus pesticides present in Herbal Powder are extracted using QuChERS method. This method is found to be simple, fast and helps the purification of sample and enrichment of residual pesticide in herbal product. The impurities and mainly the matrix effect did not interfere with quantitation process of target ions.

□ Reference

1. M.Anastassiades,S.J.Lehotay,D.Stajnbaher,F.J.Schenck Fast and Easy Multi residue Method employing Acetonitrile Extraction/ Partition and Dispersive Solidphase Extraction for determination of Pesticide Residue in produce, J AOAC Int. Mar-Apr 86(2):412-431
2. S.J.Lehotay, Interlaboratory Validation of QuChERS Method to analyze Pesticide residue in fruits and Vegetables Proceedings AOAC Annual Meeting, St. Louis MO USA (2004)
3. <http://www.restek.com/Technical-Resources/Technical-Library/Foods>

