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Development and validation of modified QuEChERS based GC-MS/MS method for determination of residual pesticides in herbal tea

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Abstract

A simple, rapid analytical method for the quantitative determination of pesticide residues in herbal tea was established by employing GC-MS/MS. Herbal tea samples were extracted via modified QuEChERS. Samples were analysed for the residues of certain common pesticides such as Dicofol (as DCBP), Ethion and Quinalphos by GC-MS/MS. The limits of detection and quantification range of pesticides were 5µg/kg and 10µg/kg respectively. The developed method was validated for various parameters such as Specificity, linearity, Precision, Recovery, Repeatability and Ruggedness by employing GC-MS/MS.A wide linear range of 10-200 µg/kg was observed with r^2 values ≥ 0.99. Pesticides free herbal tea samples were spiked at 10, 25 and 50 µg/kg fortification levels. Better recoveries between 80% to 120% were obtained with the acceptable relative standard deviation (RSD) i.e. <20%. The proposed rapid monitoring technique efficiently screens multiple pesticides in herbal tea indicating the high selectivity of the modified method.

Keywords: Herbal tea, Pesticide residues, QuEChERS.

1. Introduction

Herbal tea, a non-caffeinated drink made from plants, herbs, or spices has been used throughout history for its potential medicinal benefit. Dried plant material found in herbal tea poses a significant challenge to the analytical chemist to detect trace levels of pesticide residues **[1]**. Pesticides were used in tea farming to control insects, mites, leaf-eating beetles, and caterpillars. Certain pesticides such as Dicofol, Ethion and Quinalphos are commonly used for the control of pests and diseases in tea **[2]**. The public has become upset by the pesticides commonly used, as many tea pests have developed the resistance against them. Therefore, determination of pesticide residues in tea is an important contribution to a safer tea. Determination of pesticide residues is very important, especially for prevention in the field of public health safety but it is a big challenge due to the complex matrix of herbs, consisting of polyphenol compounds, pigments, sugars and alkaloids **[3]**.

Dicofol is a miticide used for foliar application to a variety of food/feed crops **[4].** Dicofol (Figure 1) is unstable in solution and its major degradation product is dichlorobenzophenones (DCBP) which is toxic. Ethion and Quinalphos, (Figure 1) are an inhibitor of acetylcholinesterase and accepted as broad-spectrum organophosphate pesticide **[5],** widely used in tea plantation and therefore a big concern among the regulation agencies.

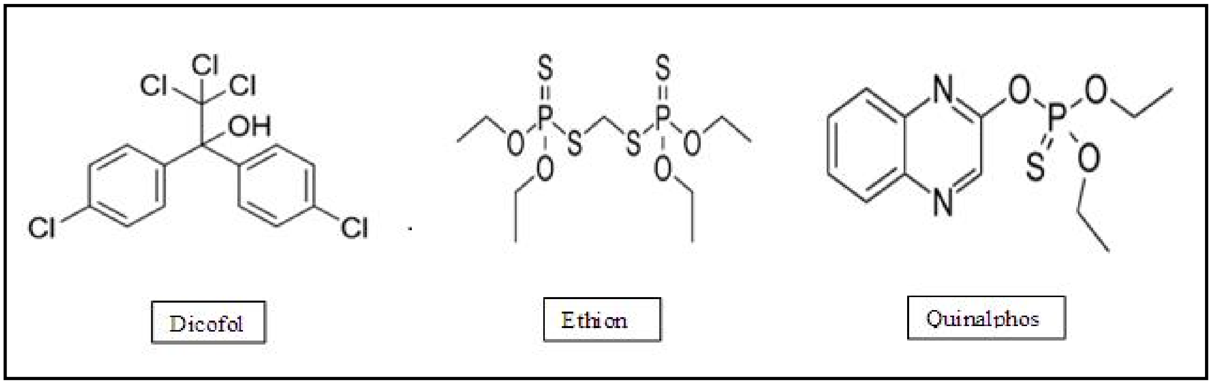

Figure 1: Chemical structures of Dicofol, Ethion and Quinalphos

The analysis of pesticide residues in tea and herbal products follows the regulations of the European Directorate General for Health and Consumer Affairs (SANCO) for “Method Validation and Quality Control Procedures for Pesticide Residue Analysis in Food and Feed”**[6]**. MRLs for tea were established under regulation No. 396 in 2005, and commodity and pesticide specific MRLs were laid down in Annex II of Regulation 149 in 2008 (OJEU, 1990-2014) **[7]**. The default values of MRLs set by the EU in 2008 for pesticides Dicofol, Ethion and Quinalphos in tea are 20mg/kg, 3mg/kg and 0.05mg/kg respectively. These MRLs are much lower than other guidelines which will help the country to limit the presence of undesirable substances in tea.

Also, larger number of analytical methods have been published for the determination of pesticide residues in tea. Some methods include the solvent extraction combined to many clean-up techniques, such as solid phase extraction (SPE) or gel permeation chromatography (GPC). The principle of SPE involves the partitioning between a liquid (sample matrix or solvent with analytes) and a solid (sorbent) phase. Solid Phase extraction is one of the excellent methods for pesticide analysis but QuEChERS method has important advantages over these extraction methods using dispersive SPE over classical SPE clean-up. It enables yielding high recovery rates for wide range of analytes including no SPE manifold, no Vacuum/Pressure, no conditioning, no flow control and drying-out, no elution step needed, less sorbent needed, faster and cheaper. QuEChERS approach is also in accordance with so-called green chemistry due to low solvent consumption and absence of chlorinated solvents and a very small waste generation **[8]**. QuEChERS (Quick, easy, cheap, effective, rugged, and safe) is a sample preparation methodology for multi-residue pesticide analysis, which was first published by ANASTASSIADES and co-workers (2003) **[9]**.

Sample preparation is an essential step, especially for botanical matrices, which are often complex and contain interfering matrix compounds that results in ion suppression, co-elution, and instrument contamination **[10]**.The aim of this paper is to optimize modified QuEChERS extraction method followed by simultaneous determination of pesticide residues in herbal tea using GC-MS/MS **[11]**.Nowadays, there is a growing demand for high-throughput multiresidue methods (MRMs), which should be easy to perform, rapid and of low cost, require a minimum volumes of solvents, provide a high selectivity without complicated clean-up solutions, and allow analysing broad range of analytes **[12]**.

In this modified QuEChERS method, ethyl acetate was used for solvent extraction in primary step followed by addition of salt mixture which leads to partitioning. Aliquot was added to sorbents and centrifugation was done to get the dispersive solid phase extraction (DSPE). Gas Chromatography with Mass Spectrometer was used to analyze the residual pesticides as it provides better results in various food matrices and drinking water.

2. Materials and Methods

2.1. Chemicals and reagents

Reference standards were purchased from Sigma Aldrich. Ethyl acetate (HPLC grade) and deionized water (Millipore-Advantage A10) was used for the preparation of standard solutions and samples. Magnesium sulfate heptahydrate (Agilent), sodium chloride (Merck), Sodium citrate dibasic sesquihydrate (Sigma-Aldrich) and Sodium citrate tribasic dihydrate (Sigma-Aldrich) were used in the present work. Carbon 18 and Graphitized Carbon Black (GCB) were purchased from Agilent.

2.2. Preparation of standard solutions

Diluent: Ethyl acetate is an organic compound, colorless and volatile liquid with a moderately sweet smell and is widely used as an extracting solvent. Although it is not miscible with water, it is miscible with many organic solvents. Ethyl acetate was selected as the standard and sample diluents because of its ability to dissolve a wide range of pesticides and is an ideal solvent for GC-MS/MS analysis of pesticides in herbal tea.

2.3. Preparation of stock standard solution

Standard stock solution of 1000 mg/ kg concentration for Dicofol, Ethion & Quinalphos were prepared by weighing accurately 10 mg of the respective standard in 10 mL of volumetric flask. The volume was made upto the mark using diluent.

2.4. Preparation of working standard solutions

Series of working standard solutions of Dicofol, Ethion & Quinalphos in concentration range of 10-200µg/kg were prepared using respective standard stock solution.

2.5. Extraction procedure

Herbal tea sample was collected from the market and ground into powder for homogenization. Prior to ethyl acetate extraction of the target compounds from tea matrix, 1 g of the sample was weighed into 50 mL capacity PTFE centrifuge tube and soaked in 10 mL of deionized water for 30 minutes to improve the extraction efficiency. Sample was extracted with 10ml of ethyl acetate and a salt mixture of 4g anhydrous $MgSO_4$, 1g NaCl, 1g sodium citrate dibasic sesquihydrate and 0.5g of sodium citrate tribasic dihydrate after 10 minutes of vigorous shaking using multitube vortexer followed by centrifugation at 8000rpm for 10 minutes. Further dispersive clean-up was given to 5ml of supernatant organic layer with 900 mg of anhydrous $MgSO_4$, 300 mg of C18 and 45 mg of GCB in another 15ml capacity PTFE centrifuge tube followed by vortex for 2 minutes and centrifugation at 10000rpm for 5 minutes. Solution was passed through 0.45µm membrane filter prior to injection on GC-MS/MS.

2.6. Instrumentation and conditions

Analysis was carried out on GC-MS/MS-TQ8040 system (Shimadzu) equipped with mass analyzer. The capillary column DB-5MS (60mm x 250µm x 2.5mm) was used for separation with the acquisition mode of MRM. The operating conditions of GC-MS/MS are as mentioned in **Table 1**.

Table-1: GC-MS/MS-TQ8040 Operating Conditions

Carrier gas	Helium
Carrier gas flow rate	Constant flow, 1ml/min
Column oven: Start Ramp 1 Ramp 2 Ramp 3	Temperature Programme: 70 °C, for 1 min 20 °C/min to 150 °C, 2 min 5 °C/min to 250 °C, 10 min 10 °C/min to 300 °C, 0 min
Ion source temperature	230 °C
Injector temperature	250 °C
Injection volume	1µl
Total run time	42 minutes

3. Results

Method validation

The method validation was done by evaluating Specificity, linearity, Precision, Limit of Detection (LOD), Limit of Quantification (LOQ), Recovery, Repeatability and Ruggedness.

3.1. Specificity

The specificity criterion tries to demonstrate that the result of the method is not affected by the presence of interferences **[13]**. The sample was spiked with the standard and was chromatographed to examine interference, if any.

3.2. Linearity

The linearity of an analytical method is its ability within a definite range to obtain results directly proportional to the concentrations (quantities) of an analyte in the sample. Matrix match linearity was constructed at five point concentration over a range of 10 to 200µg/kg. Matrix-matched linearity was obtained by preparing working standard solutions of 10, 25, 50, 100 and 200µg/kg. Calibration curves were calculated by linear least squares regression using peak areas.

The correlation coefficient (r^2) value was found to be higher than 0.99 and the calibration curves were linear within the range. The linearity values are shown in **Table 2** and respective linearity graphs are shown in **Figures 2, 3 & 4**.

Table 2: Linearity data for Dicofol; Ethion and Quinalphos

S.No.	Concentration (µg/kg)	Area			Rf		
		Dicofol	Ethion	Quinalphos	Dicofol	Ethion	Quinalphos
1	10	40151	128801	45607	0.000249	0.000078	4560.7
2	25	98024	320879	114554	0.000255	0.000078	4582.2
3	50	187445	624922	219429	0.000267	0.000080	4388.6
4	100	379483	1335249	450176	0.000264	0.000075	4501.8
5	200	763818	2807945	923585	0.000262	0.000071	4617.9

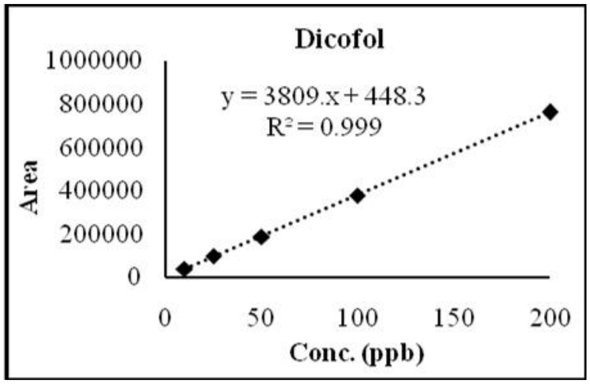

Figure 2: Linearity graph of Dicofol

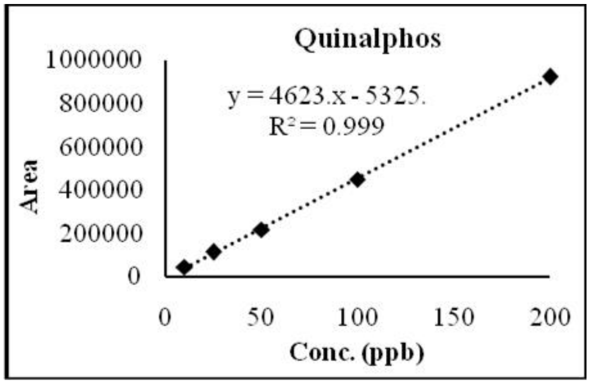

Figure 3: Linearity graph of Quinalphos

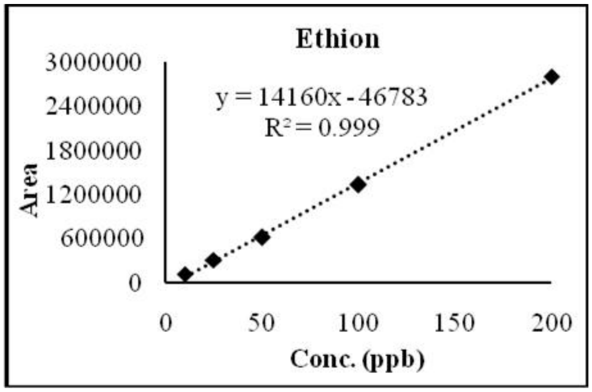

Figure 4: Linearity graph of Ethion

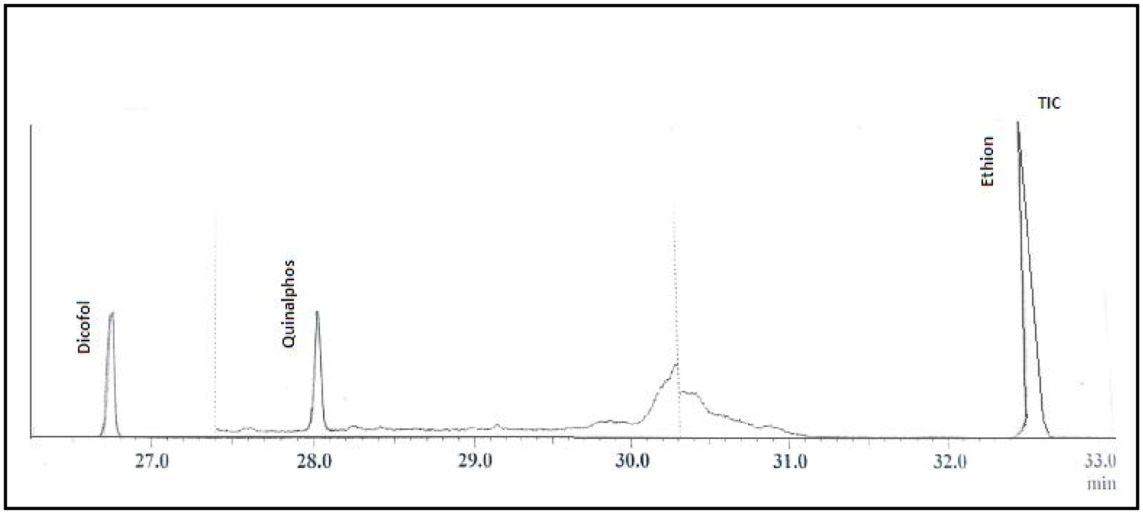

Figure 5: Typical chromatograms of Dicofol, Quinalphos and Ethion

3.3. Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ were calculated by instrumental and statistical methods. For the instrumental method, LOD is determined as the lowest amount to detect and LOQ is the lowest amount to quantify, by the detector. The LOD and LOQ were determined based on Linearity. For LOD a value of signal-to-noise ratio equal to or greater than three ($S/N \geq 3$) was chosen. For LOQ a value of signal-to-noise ratio equal to or greater than ten ($S/N \geq 10$) was chosen. The Limit of detection and quantification for Dicofol, Ethion and Quinalphos are 5µg/kg and 10µg/kg respectively.

3.4. System Precision/System Suitability

It is the evaluation of the components of an analytical system to show that the performance of a system meets the standards required by a method **[14]**. System Precision was estimated from the analysis of samples in six replicates at LOD and LOQ level. The acceptance criterion was within 20% relative standard deviation (RSD).The precision data is as shown in **Table 3.**

Table 3: Precision data at 5µg/kg (LOD) & at 10µg/kg (LOQ)

S. No.	5µg/kg (LOD)			10µg/kg (LOQ)		
	Dicofol	**Ethion**	**Quinalphos**	**Dicofol**	**Ethion**	**Quinalphos**
1	24752	69217	25466	49886	99167	40963
2	25390	68888	25417	49351	95058	39191
3	25857	64649	25544	50628	94638	39649
4	27632	63544	25402	48715	87969	37446
5	25028	57641	22464	45787	86096	38097
6	24019	54574	22658	48386	84034	36519
Average	25446.3	63085.5	24491.8	48792.2	91160.3	38644.2
STDEV	1236.4	5933.1	1497.7	1678.2	5966.7	1608.5
%RSD	4.9	9.4	6.1	3.4	6.5	4.2

3.5. Accuracy/Recovery

Accuracy of method was measured in terms of recovery. The recovery of an analyte is the extraction efficiency of an analytical process, reported as a percentage of the known amount of an analyte carried through the sample extraction and processing steps of the method **[15]**. Recovery was calculated by analyzing extracted spiked samples at high and low concentration in relationship with the curve calibration, compared with the control samples. The recovery was evaluated at three concentration levels (sample concentrations were 10, 25 and 50µg/kg) of spiked samples with 6 replicates at each level. The relative standard deviations (RSDs) for all spiked levels were lower than 20%. The recovery data is as shown in **Table 4**.

Table 4: Data for Accuracy &Recovery studies of Dicofol, Ethion and Quinalphos in herbal tea

Pesticides	Fortification Level (µg/Kg)	Mean Recovery (µg/Kg)	% Recovery ± SD
Dicofol (as DCBP)	10	10.55	106.0 ± 3.3
	25	27.16	108.5 ± 1.6
	50	44.26	88.3 ± 1.8
Ethion	10	10.76	109.0 ± 4.4
	25	29.25	117.1 ± 1.5
	50	48.95	98.5 ± 1.8
Quinalphos	10	10.36	103.7 ± 3.1
	25	27.20	107.1 ± 2.1
	50	42.81	86.6 ± 1.5

3.6. Repeatability/Reproducibility

To study repeatability of the method samples were analysed at three concentration levels (sample concentrations were 10, 25 and 50µg/kg) and injected in six replicates. The acceptance criterion was within 20% relative standard deviation (RSD). The repeatability data is as shown in **Table 5.**

Table 5: Data for Repeatability and Ruggedness of Dicofol, Ethion and Quinalphos in herbal tea

Pesticides	Fortification Level (µg/kg)	Repeatability		Ruggedness	
		Mean Concentration recovered (µg/kg)	% Recovery ± SD	Mean Concentration recovered (µg/kg)	% Recovery ± SD
Dicofol (as DCBP)	10	9.88	97.8 ± 2.3	11.29	112.0 ± 1.7
	25	23.71	93.6 ± 1.6	25.94	102.9 ± 1.6
	50	49.81	97.6 ± 2.7	53.94	107.2 ± 1.7
Ethion	10	10.64	106.6 ± 1.1	11.20	111.9 ± 5.0
	25	25.48	100.5 ± 2.0	27.78	110.3 ± 1.7
	50	51.72	102.1 ± 2.2	57.84	115.1 ± 1.9
Quinalphos	10	9.20	90.9 ± 3.3	10.83	107.7 ± 3.1
	25	22.21	87.0 ± 5.1	23.72	94.5 ± 1.7
	50	46.70	92.5 ± 1.2	48.67	97.2 ± 2.4

3.7. Ruggedness

Ruggedness of the method was evaluated by performing the sample analysis in six replicates at three different levels using different analyst on different days. The % RSD values of less than 20% indicate that the method adopted is rugged. The data of Ruggedness is shown in **Table 5.**

4. Discussion

Evaluation of the QuEChERS method

The QuEChERS method requires sample moisture content of about 80% or above for maximal pesticide extraction (ANASTASSIADES et al., 2003) **[16]**. Therefore, water was added to dried tea powder before extraction to hydrate the sample. Due to the high complexity of the tea matrix, many complex chemical substances are extracted at the same time, causing interference and seriously affecting the detection limits. To ensure the accuracy and the precision of experimental data, at the time of each analysis, a solvent blank and a recovery was added. In this extraction method, $MgSO_4$ and NaCl salts were used for better partitioning the layers and upper layer had given significant volume which was explored for high recoveries. C18 was used to eliminate the fatty compounds and other polar interferences. The solvent blank showed no signal interference. From the results obtained, the QuEChERS extraction method can be considered acceptable for residue analysis in herbal tea samples. According to the results, all three pesticides showed recovery results ranging from 80% to 120%.

Matrix effect

The matrix effects were assessed by comparing the slopes of matrix-matched calibration curves to solvent calibration curves and given in percentage. Ion suppression and enhancement are the two causes of the matrix effect. The ME is evaluated by the following equation:

$$\text{ME (\%)} = \frac{\text{Slope of matrix-matched curve} \quad \text{x } 100}{\text{Slope of Solvent}}$$

When the ME% is within -20 and +20 it is considered as low matrix effect and if ME% is within -50 and +50 it is considered as strong matrix effect **[17]**. However, the matrix effects on all analysed pesticides were within ±20%. This modified QuEChERS method eliminated most of the pigments and with that it minimize the matrix effect. Herbal teas give a high matrix effect because of the existence of many pigments. In the dispersive clean-up step, GCB was used to eliminate chlorophylls and some of the polyphenols and other pigments. GCB can absorb some planar pesticides when used at higher concentrations. In this investigation, the use of GCB gave equal or better recoveries for most pesticides. The higher recoveries were obtained by the reduction of the matrix effect, which was a result of the application of GCB.

5. Conclusion

The QuEChERS, a fast analytical sample preparation method has been developed and validated for the residual content of Dicofol, Ethion & Quinalphos and analysed by GC-MS/MS. Modified QuEChERS allowed reduced sample preparation, high recoveries for a wide range of chemically diverse pesticide compounds. The TQ8040 GC-MS/MS delivered high sensitivity and matrix selectivity, which delivered low chemical matrix background with well-defined pesticide peaks that were safe and easy to integrate, thus eliminating the need for time-consuming manual baseline corrections.

Quantitative calibrations were performed in a standard matrix and showed excellent linearity and precision. In the present research work, the extraction efficiency, recovery percentage was found to be good and influence of matrices was also found to be minimum by employing C18+GCB absorbent in combination during dispersive clean-up step. The validation data showed that this method has good accuracy and sensitivity and could be applied for the determination of multi-residue pesticides in herbal tea samples. The method well suits for the intended purpose.

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