



# APPLICATION OF QuEChERS METHOD FOR MULTI-RESIDUE PESTICIDES DETERMINATION IN LETTUCE AND APPLE USING GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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## ABSTRACT

*This study investigated the use of the QuEChERS method for the analysis of 13 pesticides on apples and lettuces. Comparison between two methods: Citrate- (buffered and unbuffered) and acetate (buffered and unbuffered). A range of pesticides –pirimicarb, diazinon, chlorpyrifos, azoxystrobin, pendimethalin, cyprodinil, boscalid, chlorothalonil, fenhexamid were selected for this study. Triphenyl phosphate was used as the internal standard. Results obtained revealed very high percentage recoveries for the majority of the pesticides screened for with recoveries of 66.3 to 109.8% for the citrate buffered, 72.5 – 120.8% for citrate unbuffered, 53.8 – 102% for acetate buffered and 54 – 105.3% for acetate unbuffered experiments carried out using apple samples. % recoveries obtained for the lettuce samples were 32.2 to 120.5% for the citrate buffered, 33.7 – 115.0% for citrate unbuffered, 53.8 – 107.4% for acetate buffered and 54 – 105.3% for acetate unbuffered experiments. % recoveries obtained for the apple samples were 64.3 – 109.8% for the citrate buffered, 72.5 – 120.8% for the citrate unbuffered, 60.3 – 102.0% for the acetate buffered and 54 – 120.5% for acetate unbuffered. Pendimethalin and cyprodinil gave the poorest recoveries while chlorothalonil gave the best recoveries for both the different methods and samples. The organophosphorus pesticides (diazinon and chlorpyrifos) gave relatively good recoveries especially for the apple samples. Comparison of sample recoveries revealed a more favourable recovery for the apple samples compared to the lettuce samples. The limit of detection was also determined to be ~0.02µg/L (6µg/kg).*

**Keywords:** QuEChERS, Pesticide residue, apple, lettuce

## 1. INTRODUCTION

Pesticides find wide applications in agriculture to prevent moulds, pests and weeds in order to ensure good crop production worldwide. Tonnes of various pesticides are applied yearly for solely agricultural purposes and the end use is that some of the pesticides are surplus to requirement or degrade relatively slowly resulting in its detection in fruits and vegetables. Pesticide residue on fruits and vegetables has become worrisome trend more so since most of the used pesticides have been banned for use in the EU [1]. Acceptance criteria are usually provided by international organizations such as FAO [2, 3] in the form of maximum residue limits (MRLs). Monitoring pesticides residues in fruits and vegetables are particularly important as most times no heating is involved and it gives an indication of the human

exposure to the various pesticides in use. Also a good number of these fruits (e.g. apples) and vegetables (e.g. lettuce) are often eaten without prior washing increasing the risk of contamination from ingestion.

QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) has found wide spread use for the determination of pesticide residues in fruits and vegetables [1, 4-12]. It was first introduced in 2003 by Michelangelo Anastassiades and StenLehotay [13]. This method involves extraction with acetonitrile partitioned from the aqueous matrix using anhydrous magnesium sulphate (MgSO<sub>4</sub>) and sodium chloride (NaCl) followed by a dispersive solid-phase extraction clean up with MgSO<sub>4</sub> and primary secondary amine (PSA). The method has already received worldwide acceptance and, with minor modifications, has become an Official Method of the Association of Official

Agricultural Chemists (AOAC) International and the Committee of European Normalization (CEN)[14, 15]. QuEChERS follows three easy steps; 1) extraction using organic solvent and partitioning salts, 2) sample cleanup with adsorbent materials (dispersive sorbents), and 3) LC or GC analysis, or both. Dispersive cleanup sorbents can include C<sub>18</sub>, primary secondary amine (PSA), and graphitized carbon black (GCB)[13, 16]. Although QuEChERS is a known method used worldwide with various applications for different agricultural products, to the knowledge of this author there is no data on its application in Nigeria.

## 2. MATERIAL AND METHOD

Pesticide reference standards were purchased from Sigm-Aldrich (UK). Magnesium sulphate (MgSO<sub>4</sub>), sodium hydroxide (NaOH), sodium chloride (NaCl), primary secondary amine (PSA), triphenyl phosphate (TPP), sodium acetate, sodium citrate tribasic dehydrate (Na<sub>3</sub>Cit.2H<sub>2</sub>O), sodium citrate dibasic sesquihydrate (Na<sub>2</sub>HCit. 1.5H<sub>2</sub>O) was purchased from Sigma-Aldrich (UK). Analytical grade acetonitrile (ACN), acetic acid, formic acid was purchased from Fisher Scientific (UK). Apples and iceberg lettuces were purchased from the local shops and they were used for blank and fortified samples for recovery assays and as matrix-matched standards for calibration purposes.

### 2.1 Sample Preparation

10.0 ± 0.1 g of the thoroughly comminuted samples was weighed and transferred into a 15 mL propylene centrifuge tubes (Corning UK). 100 µL of the test solutions was then added, 100 µL ACN was added for the blank experiments. The tube and contents was then vortexed for ~1 min. The pesticides were then allowed to integrate properly for 15 min and the tubes were shaken vigorously by hand for 30 secs. Pre-weighed mass of the following was then added: (a) 4 g of MgSO<sub>4</sub>& 1 g of NaCl; (b) 6 g of MgSO<sub>4</sub>& 1.5 g of sodium acetate (NaOAc); (c) 4 g MgSO<sub>4</sub>, 1 g of NaCl, 0.5 g sodium citrate dibasic sesquihydrate, 1 g of sodium citrate tribasic dehydrate. The sealed tubes were shaken vigorously for about 1 min and centrifuged for 2 mins at 3500 rpm. 1 mL of the extract (upper layer) was transferred into a dispersive solid phase clean-up sorbent tube (2 mL) containing 150 mg of MgSO<sub>4</sub> together with 50 mg PSA. The sealed tubes was then shaken vigorously and once again centrifuged at 3500 rpm for 2 mins. 0.5 mL of

the samples were transferred into 2 mL GC amber vials; 50 µL of 2 ng/µL TPP in 0.5% formic acid in ACN to all the vials. Samples were then analysed using GC-MS (ThermoQuest, UK).

### 2.2 Sample Analysis

The GC-MS system employed was a ThermoFinnigan Trace GC coupled to a ThermoFinnigan Trace MS, operating in electron ionization (EI) mode (70 eV) with selective ion monitoring (SIM). 1µL of standards and samples were injected in split/splitless mode (1.5 min splitless time). OP analyte separation was performed on a 50 m low-polarity GC column (CP-Sil 8% diphenyl-polydimethylsiloxane, film thickness 0.25 µm) with the serving as the carrier gas at a flow of 1.0 mL min<sup>-1</sup>. The injection temperature was 250°C, MS transfer line 270°C scanning from m/z 50 to 500 at 2.0 s/scan. Oven programme was set at 70°C for two minutes and ramped at 15°C/min to 180°C and held for 15 min, it was further ramped at 5°C/min to 280°C and held for 20 mins. Internal standard of triphenyl phosphate was added to all calibration standards and sample extracts.

### 2.3 Method Validation

Ten (10) pesticides were analysed in lettuce and apple matrix by the QuEChERS and GC-MS. Linearity, limit of detection were determined according to guidelines SANCO/12571/2013 [17]. Limit of detections were estimated at successive injection of dilute solutions to the lowest concentration that resulted in the S/N ratio of three. Relative standard deviation was also determined to access precision

$$\%RSD = 100 \times \frac{\sigma}{\bar{x}} \quad (1)$$

In (1),  $\sigma$  is the standard deviation of replicates,  $\bar{x}$  is the mean value of the replicates and %RSD is the relative standard deviation percentage. The accuracy was calculated as recoveries of the replicates. Values between 70% and 120% were deemed satisfactory. Recoveries were calculated:

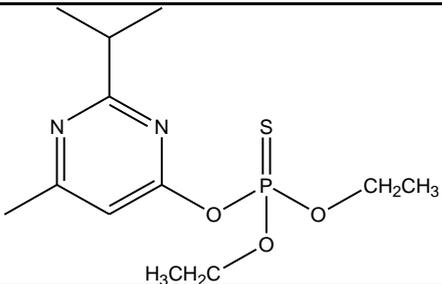
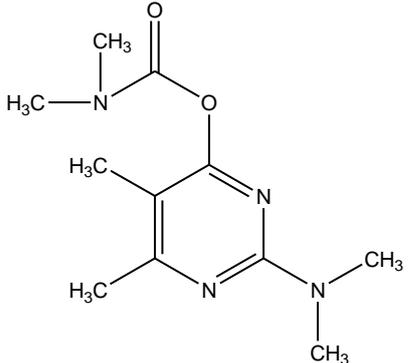
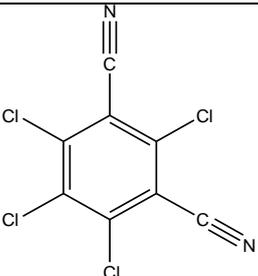
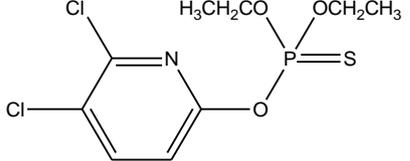
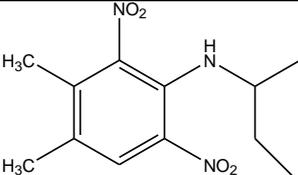
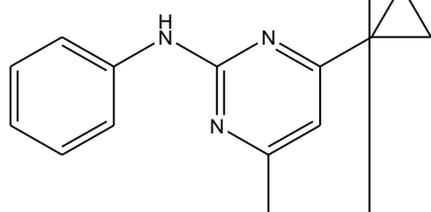
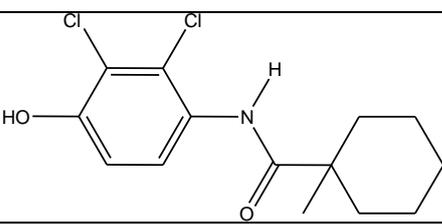
$$\%R = 100 \times \frac{X}{\mu} \quad (2)$$

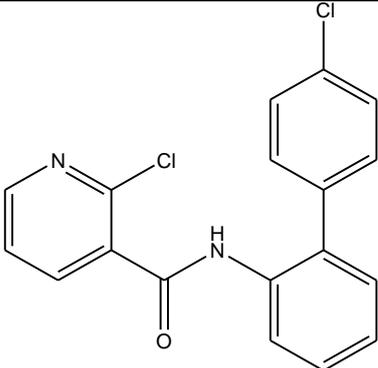
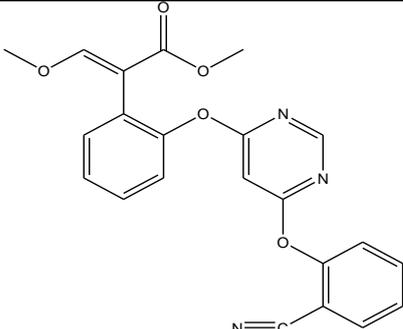
In (2), %R is the percentage recovery, X is the experimental concentration of the analyte (mg/kg),  $\mu$  is the calculated concentration of the analyte (mg/kg).

### 2.4 Statistical Analysis

Statistical analysis were done using SigmaStat® and Microsoft Excel

Table 1: List of chemicals studied

Pesticide	Chemical name	Mol. wt. (g/mol)	Chemical structure
Diazinon	O,O-diethyl O-[4-methyl-6-(propan-2-yl) pyrimidin-2-yl] phosphorothioate	304.35	
Pirimicarb	2-Dimethylamino-5,6-dimethylpyrimidin-4-yl)N,N-dimethylcarbamate	238.29	
Chlorothalonil	2,4,5,6- tetrachloroisophthalonitrile	265.91	
Chlorpyrifos	O,O-diethyl O-3,5,6-trichloropyridin-2-yl phosphorothioate	351.00	
Pendimethalin	3,4- dimethyl -2,6- dinitro-N- pentan-3-yl- aniline	281.31	
Cyprodinil	4-cyclopropyl-6-methyl-pyrimidin-2-yl) phenylamine	225.30	
Fenhexamid	N-(2,3-dichloro-4-hydroxyphenyl)-1-methyl cyclohexanecarboxamide	302.20	

Pesticide	Chemical name	Mol. wt. (g/mol)	Chemical structure
Boscalid	2-chloro-N-(4'-chlorobiphenyl-2-yl) nicotinamide	343.12	
Azoxystrobin	Methyl(2E)-2-(-2[6-(2-cyanophenoxy) pyrimidin - 4] yl] oxy]phenyl)-3-methoxy acrylate	403.39	

### 3. RESULTS AND DISCUSSION

This study utilized two different modified QuEChERS method for the determination of pesticide residue in apple and lettuce. Results obtained for the different pesticides sampled are shown in Table 2. Calibration graphs were obtained from triplicate injections of standard mixtures of the test chemicals (pesticides) containing the internal standard (TPP) for concentrations ranging from 0.1 µg/mL to 1.5 µg/mL. The levels were chosen taking into account expected environmental concentrations. The results for the intercept and slope and the various correlation coefficients ( $R^2$ ) are also presented in Table 2. A good linear correlation coefficient ( $R^2 > 0.98$ ) was observed between the peak area ratio and the standard concentration.

Quantitative analysis was performed in the selected ion monitoring mode (SIM) using one target ion (in bold) and the qualifying ion (see Table 3). The data collected for the apple and lettuce samples showed that some analytes had different recoveries. The change in cleanup process for the lettuce samples from citrate buffered and unbuffered to acetate buffered and unbuffered revealed an increase in the % recovery for the pesticides diazinon and pirimicarb from 35.3 % and 35% for citrate buffered and unbuffered to 103.0 % to 107.4% respectively. These observed differences may be attributable mostly to the cleanup process. A matrix based standard was

prepared to avoid possible ionisation enhancement or suppression. The pesticides pendimethalin and cyprodinil gave the poorest recoveries for all the method studied with a range of recovery of 55.0 to 72.5% for pendimethalin and 53.8 - 83.6% for cyprodinil. A look at their chemical structure revealed a similar amino group which could be responsible for their similar behaviour. The pesticide chlorothalonil was observed to have the best recoveries for the various methods (citrate and acetate - buffered and unbuffered) and the different samples (apple and lettuce). Values obtained ranged from 93.6 to 113.0%. Lehotay et al; [18] reported good recoveries for chlorothalonil using similar methods although the sample extracted was peas. The organophosphorus pesticides (diazinon and chlorpyrifos) revealed relatively good recoveries for most the methods tested (both buffered and unbuffered). However the recoveries for diazinon for citrate test (buffered and unbuffered) were found to be very poor with values of 35 - 35.3%. Unfortunately, it is difficult to attribute these values to the method as the recoveries for the apples samples were high (109 - 114%). Comparison of the recoveries from the two different samples (apples and lettuce) revealed a major bias for the apples samples with relatively higher recoveries were obtained for most of the apple samples compared to the lettuce samples. The detection limit (determined on the basis of a signal - to - noise ratio (S/N) of 3)

was also determined. In the absence of certified reference material, the method was validated by measuring the percentage of recovery after the addition of known amounts of standards to apple and lettuce samples. In all the cases the relative standard deviation (RSD) values obtained for the apple and lettuce samples were less than 12% which are acceptable values for these types of complex samples. The limit of detection was found to be 0.003µg/L with a true value of 0.006 mg/kg. Differences in recoveries for the various extraction and cleanup methods were found to be statistically significant in all the pesticides samples based on a one way analysis of variance (ANOVA) at a 95% confidence interval.

Table 2: Level of pesticides recovered from the Apple and lettuce samples using different QuEChERS modified methods.

Table 3: Retention times for the studied pesticides and their target (in bold) and qualifying ions

Pesticide	Retention time	Precursor ion (m/z)
Diazinon	15.42	304, 169
Pirimicarb	18.43	238, 166
Chlorothalonil	36.37	265.91, 266, 268
Chlorpyrifos	21.51	350.9, 199, 197, 314
Pendimethalin	23.55	281, 252, 162
Cyprodinil	23.64	225, 77
Fenhexamid	29.77	302, 97, 55
Boscalid	33.90	343, 140
Azoxystrobin	45.10	388, 344
TPP (internal standard)	30.89	326, 215, 170

Monitoring the levels of these pesticides in locally available apple and lettuce revealed concentrations below the limit of detection and quantification of the GC-MS used.

Table 2: % Recoveries of selected pesticides for the different methods

Pesticide	Line equation	R <sup>2</sup>	Apple				Lettuce			
			Citrate buffered	Citrate un-buffered	Acetate buffered	Acetate un-buffered	Citrate buffered	Citrate un-buffered	Acetate buffered	Acetate un-buffered
Diazinon	Y=-0.0018 +4.2178x	0.9934	109.8	114.5	97.9	94.2	35.3	35.0	103.0	107.4
Pirimicarb	Y= -0.8816 +19.0393x	0.9957	89.2	110.9	71.9	72.5	32.2	33.7	102.0	106.2
Chlorothalonil	Y = -0.3195 +0.5530x	1.0000	102.7	112.2	93.6	102.3	102.7	113.0	93.6	102.3
Chlorpyrifos	Y = -0.0400+ 0.7316x	0.9969	83.2	98.2	72.2	69.7	83.2	98.2	72.2	69.7
Pendimethalin	Y = -0.0101 +1.2088x	0.9968	64.3	72.5	60.3	55.0	64.3	72.5	60.3	55.0
Cyprodinil	Y= -1.9434 +27.2743x	0.9975	66.2	83.6	53.8	54.0	66.2	83.6	53.8	54.0
Fenhexamid	Y= -9.3878 +156.378x	0.9984	107.0	97.0	93.0	98.0	65.0	64.0	71.0	109.0
Boscalid	Y = -0.1778 +1.5154x	0.9664	100.7	98.1	102.0	99.3	102.0	99.3	100.7	98.1
Azoxystrobin	Y = -0.1563 +1.0181x	0.9983	100.6	120.8	100.7	105.3	120.5	115.0	98.9	85.9

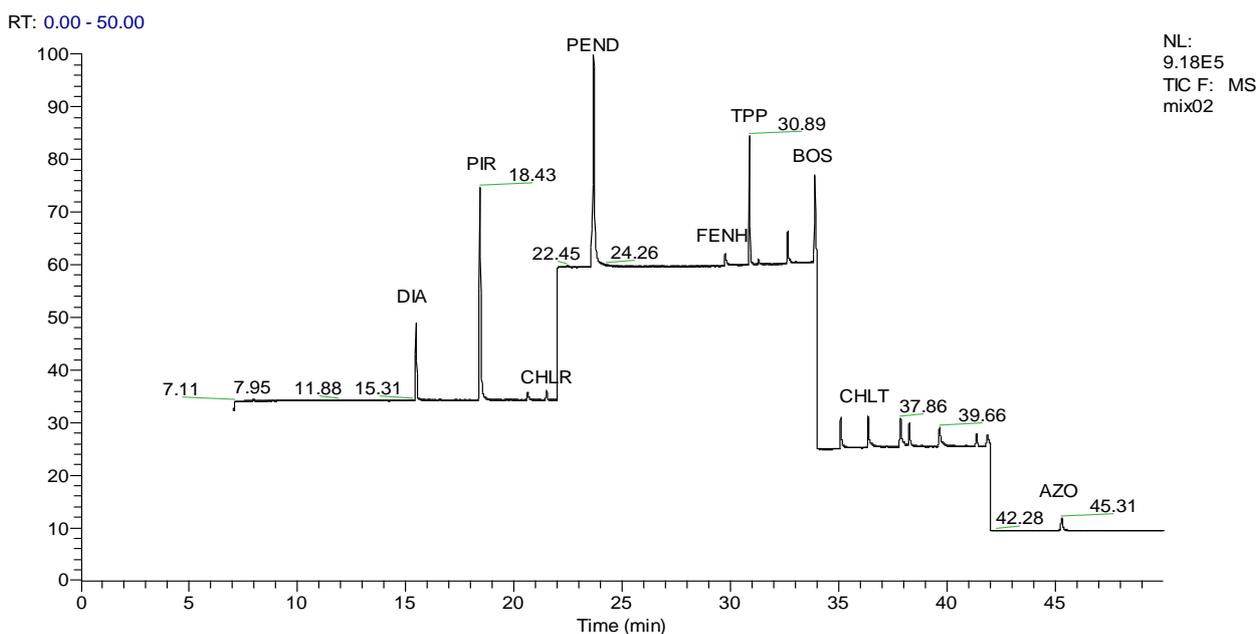


Figure 1: Chromatogram of the separated pesticide DIA- (Diazinon), PIR - (Pirimicarb), CHLR - (Chlorpyrifos), PEND - (Pendimethalin), TPP - (Triphenyl phosphite - internal standard), BOS - (Boscalid), CHLT (Chlorothalonil), AZO (Azoxystrobin).

#### 4. CONCLUSION

The use of QuEChERS as an analytical tool for the assessment of pesticide residues on fruit and vegetable was carried out in this study. QuEChERS acetonitrile extraction and salting procedures with SPE cartridge clean-up was used to develop a sensitive assay for a range of pesticides (pyrethroids, organophosphorus, carbamates etc.) in apple and lettuce samples using GC-MS analysis. Recoveries were in the range of 54% to 107% with RSD less than 10.9. It is proved to be the optimal method for extraction multi-class pesticides from complex matrices. The SPE clean-up provided high efficiency of clean-up with low matrix effects. This method is sensitive and accurate in routine multi-residue analysis in herbs, and has been proven to be useful in the determination of pesticide residues in apples and lettuce samples. It also provides a theoretical basis for detection of pesticide residues in Nigeria in future.

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