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# Modified QuEChERS Method for Determination of Alfa-Endosulfan & Methyl Parathion Pesticides (OCs/OPs) in Vegetable Oils by GC-ECD/FPD

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***ABSTRACT:*** *The study was undertaken to obtain an objective and realistic overview of the analytical performance of modified QuEChERS method for analysis of specific pesticide residues using low cost adsorbent. The full in-house validation was carried out for sunflower oil by modified QuEChERS method as per SANCO guideline-2012. In this newly modified sample preparation method, easily available low cost sugarcane ash, a sugarcane industrial waste was used as an adsorbent in place of expensive Primary Secondary Amine (PSA). The sunflower oil was spiked at five different levels (0.1, 0.2, 0.4, 0.5 & 1.0 µg/g) for intra & inter assay precision studies. The intra assay percentage recoveries ranged from 80.10 – 102.68 for methyl parathion and 83.55 – 102.35 for alfa endosulfan respectively. In case of inter assay, precision values ranged from 86.35 to 102.36% for methyl parathion and from 85.05 to 99.52% for alfa-endosulfan. Percentage Relative Standard Deviation (n=7) was below 12 in inter & intra assay precision studies. Efficiency of the modified method was compared with that of original QuEChERS method by conducting parallel experiment. Pesticides were investigated at levels ≥0.05µg/g. Expanded Uncertainty was 5.65% for alfa endosulfan at 0.50 µg/g spiking level & 7.58% for methyl parathion at 1.00 µg/g spiking level. In-house validation results showed that the modified method fulfilled the requirement of SANCO guideline-2012.*

***Keywords****: Gas chromatography, In-house Method Validation, Pesticides, QuEChERS,*

## INTRODUCTION

The use of chemical pesticides in agricultural crops is becoming vital for controlling pests that greatly affect the yields, in addition to improving the quantity and quality of the products that reach the consumer. Consumer's perception of food quality has always been subject to change over time. In recent years we have observed a substantial increase in the importance placed on aspects related to pesticide residues and a growing demand for better agricultural practices, transparency and traceability in the production and marketing of conventional food. The control of pesticide residues in food for both regulatory and commercial purposes involves analysis of large numbers of samples [1 – 3].

Despite gas chromatography (GC) being the most popular technique of choice for routine pesticide residue analysis in fruits and vegetables including those with high fat content [4-8], the number of polar and non polar pesticides used in crops for increasing the yield and these pesticide residues has been analyzed by GC & other instruments [9]. Many sample preparation methods for the extraction of pesticides from vegetable oils prior to chromatographic separation have been described. Among these, the most commonly used methodology for non polar pesticides is based on gas chromatography (GC) after a comprehensive clean-up step, in most cases based on liquid–liquid partitioning extraction with different polarity solvent [10]; microwave-assisted extraction [11]; solid-phase extraction (SPE) [12]; matrix solid-phase dispersion (MSPD) [13]; solid-phase micro-extraction [14] and quick, easy, cheap, effective, rugged and safe (QuEChERS) method [15]. QuEChERS method has already received a worldwide acceptance because of the simplicity and high throughput enabling a laboratory to process significantly larger number of samples in a given time as compared to the earlier methods. Recently, the QuEChERS method has received the distinction of an AOAC official method for multiple pesticides. It has minimized the

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requirement for extensive sample cleanup, which was otherwise essential in earlier methods of analysis. The QuEChERS approach is very flexible and it serves as a template for modification depending on the analyte properties, matrix composition, equipment and analytical technique available in the lab. The template is also very rugged in that high recoveries will be achieved for many pesticides in many matrices even if different ratios and types of sample size, solvent, salts and sorbents are used in modifications. In QuEChERS procedure, Dispersive Solid Phase Extraction (d-SPE) using Primary Secondary Amine (PSA) & other sorbents as cleanup reagent is a most important step for determination of pesticide concentration in food. Even though QuEChERS meets all the requirements, there is a need of replacing the expensive PSA (adsorbent) with a cheaper material. In this direction, a successful attempt was made for the replacement of PSA with cheaply available industrial waste material i.e. fly ash of sugar cane industry.

To the best of our knowledge, no application of the modified QuEChERS method using low cost adsorbent to the analysis of pesticides in vegetable oil samples has been published. Therefore, the aim of this study was to validate the efficient, sensitive and interference-free method in combination with Gas chromatography. For this purpose, Modified QuEChERS method was evaluated in terms of cleanliness of the oil sample extracts, efficiency of the extraction (recoveries), analytical performance, matrix effects and sensitivity (limits of detection) for analyses of pesticides (alfa endosulfan & methyl parathion) in vegetable oil samples collected from local markets in Mysore city, Karnataka-India. There is no screening data available for alfa endosulfan & methyl parathion in vegetable oils from Mysore region. Thus we undertook a systematic study of monitoring the alfa endosulfan & methyl parathion residues level in vegetable oils.

## EXPERIMENTAL SECTION

### Oil Sample

Different vegetable oils (each 5) were collected from local vendors of Mysore city, Karnataka state, India. The sample was stored at room temperature (25±2) for analysis.

### Reagents

All analytical grade reagents were procured unless otherwise stated. Alfa endosulfan & methyl parathion Pesticide reference standards were purchased from Sigma-Aldrich and Laborchemikallen, GmbH respectively. Acetonitrile, n-hexane, acetone, toluene & anhydrous sodium sulfate were purchased from Merck (India). Magnesium sulfate ($MgSO_4$) and Primary Secondary Amine (PSA) were procured from Agilent Technology (U.S). Sodium bi carbonate was obtained from s.d. Chem Pvt Ltd (India).

### Sugarcane Ash

Sugarcane ash was obtained from Kisansahkari sugar mill, Sampurnanagar, Kheeri (U.P, India). The material was pulverized and sieved using 20 -200 mesh. Glass column (45 cm x 2cm) was plugged with cotton, over which 15 g of sugarcane ash was packed. The prepared column was eluted with 200 ml acetone & n-hexane (50:50) and dried first at room temperature and later in an electric oven at 110°C for 2 h.

### Carbon (%) in Sugarcane Ash

One step pyrolysis method was followed to determine the activated carbon in sugarcane ash [16]. For this, the test samples were divided into three portions; the first part was mixed with 10% phosphoric acid (100 g sample + 100 mL of $H_3PO_4$, wt/v) and the second part was mixed with 10% potassium hydroxide (100 g sample + 100 mL of KOH, w/v) and the third part was used as control without any addition. Both the control and treated samples were pyrolyzed at 400°C for 1 h in muffle furnace. After activation, the mixture was removed from the furnace and allowed to cool at room temperature. The pyrolysed carbons were leached with 2% HCl (v/v) for 2 h and washed several times with de-ionized hot water until a neutral pH was achieved. Later the carbon paste was dried in hot air oven at 110°C for 24 h. The activated carbon yield was calculated by applying the formula [17].

$$X\,(\%) = (M/Mo) \times 100$$

Where X is activated carbon yield (%), M is the activated carbon mass (g) and Mo is the raw sample mass (g).

### Stock & Working Solutions

The stock solutions of alfa-endosulfan & methyl parathion were prepared in n-hexane & toluene (50:50). Other working standard solutions of 0.01, 0.025, 0.05, 0.1, 0.25, 0.5, 1.0, 2.5 and 5.0 µg/ml concentrations were prepared by serial dilution with n-hexane. The standard solutions were stored in a refrigerator at 4 -5 °C.

**Apparatus**

*GC Instrument*

Gas Chromatograph-Hewlett Packard HP 6980 series (USA) equipped with split/split less auto-injector model HP 7683B series was used for the analysis. The non-polar stationary phase was a fused silica capillary column (25 m x 0.20 mm, 0.33 µm film) by Hewlett Packard (USA), which were equivalent to HP-50 (50% phenyl polysiloxane). For the control of instrument and data analysis, 'Chemstation' software (rev.b.02.01) was used. Parallel Gas Chromatograph-Shimadzu 2010 (Shimadzu, Kyoto, Japan) equipped with split/split less auto-injector model AOC-20i was also used for the analysis. The non-polar stationary phase used was a fused silica capillary column (30 m x 0.25 mm, 0.25 µm film thickness) by Supelco, USA which were equivalent to DB-1 & DB-5 (1% phenyl polysiloxane & 5% phenyl polysiloxane). For the control of instrument and data analysis, 'GC Solution' software (2.1) was used.

**Sample Preparation**

For analysis, about 1.00 ± 0.03 g homogenized vegetable oil sub-samples was weighed and transferred to a 50 mL centrifuge tube and 10 mL of acetonitrile added to each tube. The contents of the tubes were homogenized using high speed Ultra turrax t18 basic homogenizer for 5-6 min at 14000-15000 rpm. An aliquot of 6.0 mL acetonitrile extract (upper layer) was transferred to the 15 mL dispersive-SPE tubes containing 0.10 ± 0.001 g (PSA) and 2.00 ± 0.01 g anhydrous $MgSO_4$ and also tubes with 0.010 g (sugarcane ash) sorbent and 2.00 ± 0.01 g anhydrous $MgSO_4$ for cleanup (dispersive solid phase extraction, DSPE). The tubes were tightly capped and shaken vigorously for 1 min and later centrifuged at 5,000 rpm for 2 min. Two mL of the supernatant acetonitrile extract was transferred to a clean dry test tube and completely evaporated using turbo-vap nitrogen concentrator, with the water bath maintained with temperature at 50°C and nitrogen flow rate at 15 psi. The residue was reconstituted in 1 mL n-hexane : toluene (50:50) and analyzed by Gas Chromatograph (GC) with Electron Capture Detector (ECD) and Flame Photometry Detector (FPD). In case of ECD, GC separation was conducted at following conditions : $N_2$ gas flow, 0.79 mL/min; Make up, 30 mL/min; inlet temperature, 280°C; injection volume, 1 µl; Spilt ratio, 1:10; Detector temperature, 300°C; initial oven temperature, 170°C, held for 5 min, then a 1.5°C/min ramp to 220°C, held for 10 min followed by a 4°C/min ramp to 280°C (held for 7 min). In case of FPD, GC separation was conducted at following conditions: $N_2$ gas flow, 5.0 mL/min; inlet temperature, 200°C; injection volume, 2 µl; Spilt ratio, 1:10; Detector temperature, 250°C; Hydrogen gas flow: 60 mL/min; Air Flow: 70mL/min; initial oven temperature, 80°C, held for 10 min, then a 120°C/min ramp to 200°C, held for 6 min followed by a 20°C/min ramp to 250°C (held for 10 min).

**Method validation as per SANCO guideline 2012**

As per SANCO guideline-2012, the method was tested to assess for mean recovery, sensitivity (as a measure of trueness), precision and limit of quantification (LOQ). This effectively means that spiked experiments, to check the accuracy of the method should be undertaken minimum of 7 replicates is required to check the precision and sensitivity of the method. The LOQ is defined as the lowest validated spike level meeting the method performance acceptability criteria (mean percentage recoveries) for each representative commodity in the range 70-120%, with an RSD (d" 20%). Other approaches to demonstrate that the analytical method complies with performance criteria may be used, provided that they achieve the same level and quality of information [18].

**RESULT & DISCUSSION**

**Method Validation**

Optimized QuEChERS method using sugarcane ash (containing 35% carbon) as a cleanup adsorbent was validated for sunflower oil samples according to the SANCO guidelines-2012 by GC-ECD/FPD for alfa-endosulfan & methyl parathion pesticides. The validation of the analytical method was performed using the following parameters: linearity, selectivity accuracy, precision, system suitability, limit of detection, limit of quantification and repeatability.

*Linearity*

A linear regression analysis was carried out by plotting the chromatographic response (chromatogram area) for each pesticide (y-axis) versus the final concentrations of pesticides (x-axis). We constructed a calibration curve for the five point concentration of mixed analytes. Calibration curve of pesticides plotted in range 0.10 – 0.75µg/mL for Alfa Endosulfan, and 0.20 – 2.0 µg/mL for methyl parathion. The regression equations were y = 726230x + 8310.5 for alfa endosulfan & y = 740992x +23440 for methyl parathion. Correlation coefficients ($r^2$) were

found higher than 0.996 for alfa endosulfan & methyl parathion.

### *Selectivity*

Percentage relative standard deviations (%RSD) of retention time (seven replicate injections) ranged from 0.042 to 0.258% meaning good selectivity for alfa endosulfan & methyl parathion. Extracted control oil sample was injected to evaluate the method for selectivity. There was no interferences at particular retention time of pesticides in control oil sample (Fig. 1).

### *Preliminary Recovery (%) Experiment*

Analytical recoveries were calculated using modified method in sunflower oil with values ranging from 83.18 to 106.35% for alfa endosulfan and from 80.20 to 102.65 for methyl parathion, Repeatability studies the showed the relative standard deviation (RSD) values ranging from 5.25 to 9.58% for methyl parathion and from 7.25 to 9.95% for alfa endosulfan. The methodology was successfully applied to full in house validation as per SANCO guidline-2012. Validation data is presented in table 1 & 2.

### *Inter & Intra Assay Precision*

Precision of analytical method was evaluated by calculating relative standard deviation (RSD) or coefficient of variation (CV) of a set of data. Precision of GC-ECD/FPD method was checked to assess the reproducibility of instrument response to target analyte. In order to assess the analytical method precision, measurements were done under conditions of repeatability. To determine the precision of inter and intra assay of this methodology, samples were spiked at 0.10, 0.20, 0.40, 0.50 and 1.00 µg/g levels by

**Table 1**
**Percentage recovery studies of methyl parathion at 0.1, 0.2, 0.4, 0.5 & 1 µg/g spiking levels using Primary Secondary Amine & sugarcane ash as clean-up sorbent in sunflower oil**

| *Type of Study* | *Adsorbent* | *Spiking Level* | | | | |
|---|---|---|---|---|---|---|
| | | *0.10 µg/g* | *0.20 µg/g* | *0.40 µg/g* | *0.50 µg/g* | *1.00 µg/g* |
| Preliminary | Primary Secondary | 81.15* | 90.15 | 90.65 | 99.59 | 100.65 |
| recovery (%) | Amine | ±9.15** | ±8.85 | ±7.95 | ±7.85 | ±6.85 |
| Study | Sugarcane ash | 80.20 | 91.22 | 93.65 | 98.35 | 102.65 |
| | | ±9.95 | ±9.88 | ±8.15 | ±8.69 | ±7.25 |
| Intra-assay | Primary Secondary | 81.98 | 90.58 | 95.58 | 99.65 | 99.85 |
| recovery (%) | Amine | ±8.55 | ±8.12 | ±7.25 | ±7.99 | ±6.89 |
| study | Sugarcane ash | 80.10 | 91.58 | 94.05 | 95.05 | 102.68 |
| | | ±8.96 | ±9.68 | ±8.89 | ±8.63 | ±7.55 |
| Inter-assay | Primary Secondary | 80.21 | 92.65 | 96.65 | 90.25 | 106.14 |
| recovery (%) | Amine | ±10.25 | ±9.65 | ±9.88 | ±8.87 | ±7.58 |
| Study | Sugarcane ash | 86.35 | 90.36 | 97.65 | 97.36 | 102.36 |
| | | ±9.65 | ±9.88 | ±9.06 | ±8.25 | ±7.23 |

**Table 2**
**Percentage recovery studies of alfa-endosulfan at 0.1, 0.2, 0.4, 0.5 & 1 µg/g spiking levels using Primary Secondary Amine & sugarcane ash as clean-up sorbent in sunflower oil**

| *Type of Study* | *Adsorbent* | *Spiking Level* | | | | |
|---|---|---|---|---|---|---|
| | | *0.10 µg/g* | *0.20 µg/g* | *0.40 µg/g* | *0.50 µg/g* | *1.00 µg/g* |
| Preliminary | Primary Secondary | 85.10* | 88.22 | 98.55 | 104.19 | 108.22 |
| Recovery (%) | Amine | ±8.98** | ±11.85 | ±8.95 | ±6.95 | ±6.25 |
| Study | Sugarcane ash | 83.18 | 81.82 | 97.12 | 99.15 | 106.35 |
| | | ±9.58 | ±8.66 | ±7.15 | ±6.89 | ±5.25 |
| Intra-assay | Primary Secondary | 82.28 | 83.95 | 98.88 | 105.05 | 98.25 |
| recovery (%) | Amine | ±9.25 | ±9.59 | ±8.25 | ±6.19 | ±6.09 |
| Study | Sugarcane ash | 83.55 | 86.58 | 99.05 | 102.35 | 96.88 |
| | | ±9.98 | ±10.52 | ±7.19 | ±7.63 | ±6.15 |
| Inter-assay | Primary Secondary | 83.60 | 82.75 | 93.25 | 100.36 | 104.14 |
| recovery (%) | Amine | ±12.25 | ±8.69 | ±8.78 | ±6.27 | ±5.20 |
| Study | Sugarcane ash | 85.05 | 84.66 | 90.44 | 95.18 | 99.52 |
| | | ±10.35 | ±10.22 | ±9.82 | ±7.25 | ±6.03 |

*% Recovery & **% RSD (n=7)

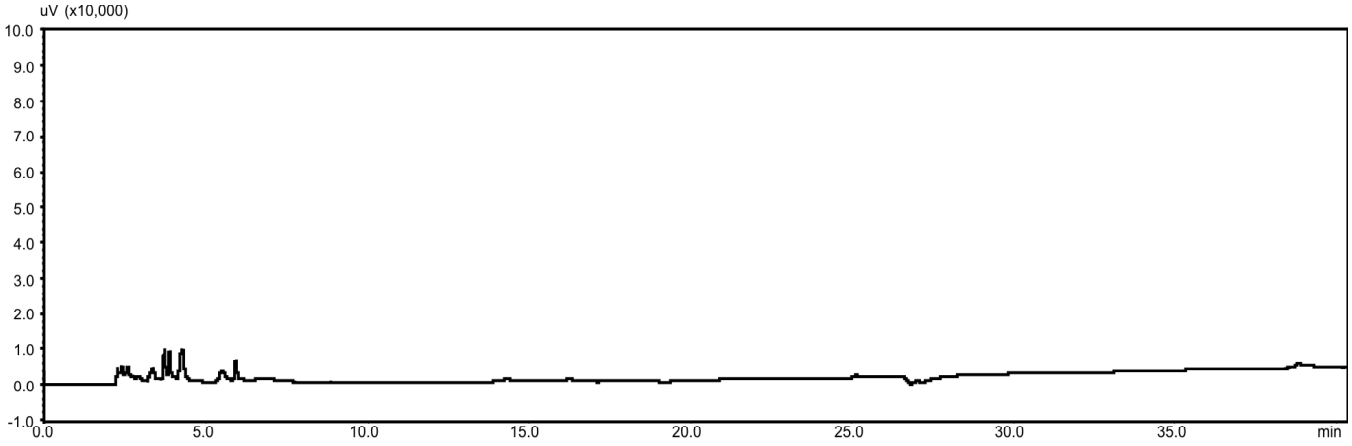


**Figure 1: Chromatogram of control sunflower oil**

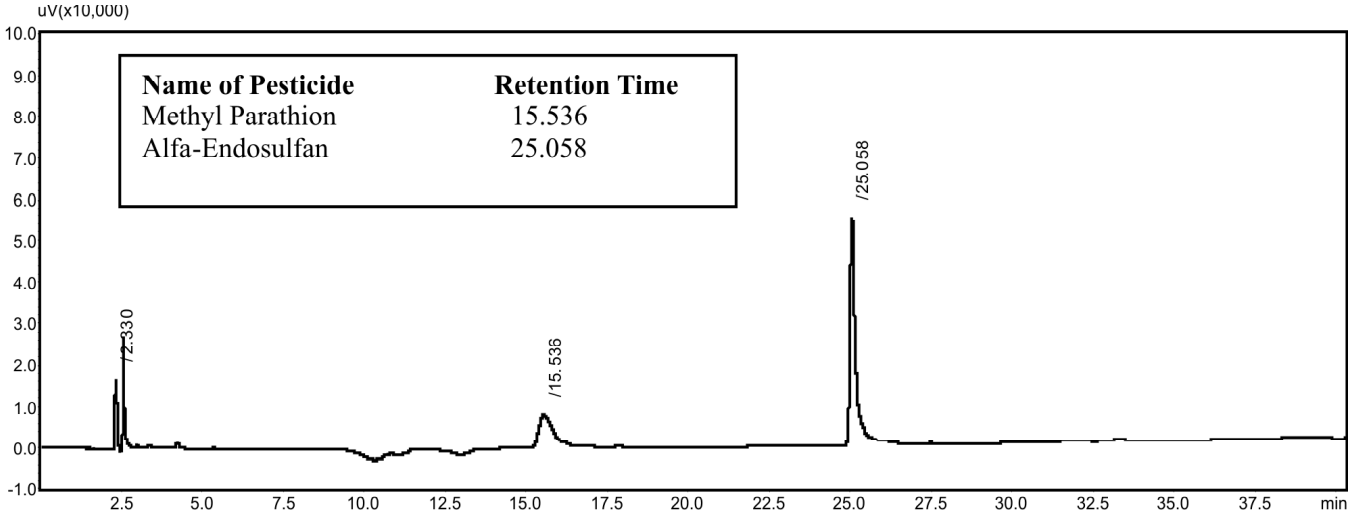


**Figure 2: Standard chromatogram of 0.4 µg/mL for alfa endosulfan & methyl parathion**

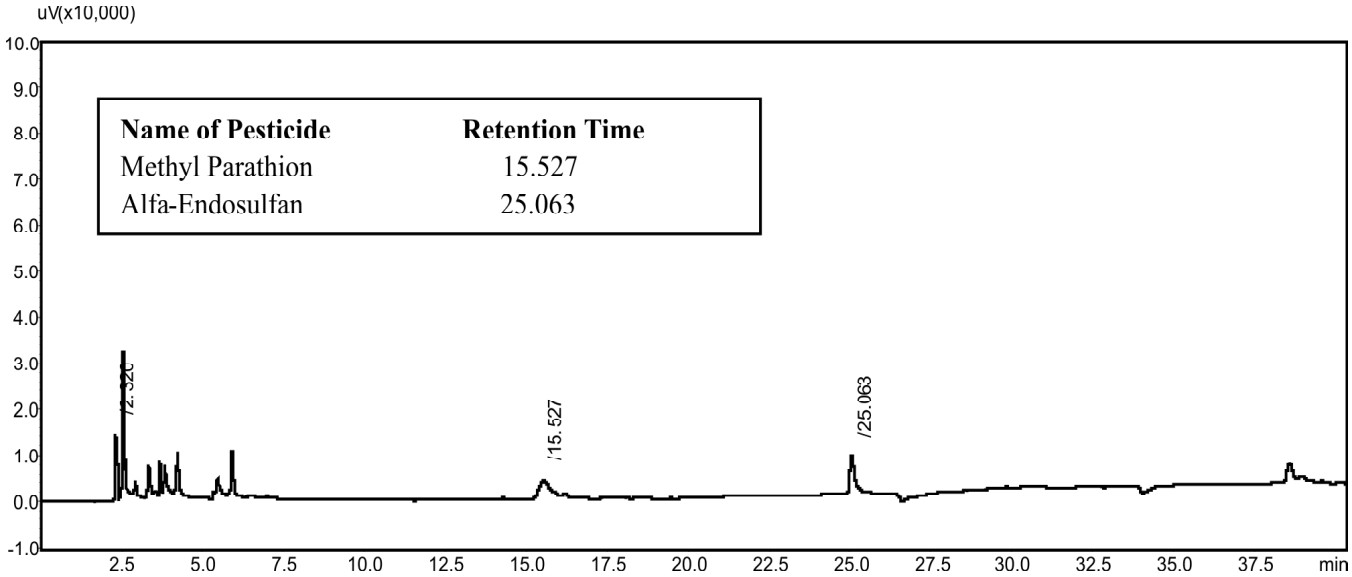


**Figure 3: Chromatogram of spiked sunflower oil at 0.5 µg/g with alfa endosulfan & methyl parathion**

three analysts in sunflower oil for alfa endosulfan & methyl parathion. The analytical recoveries (%) were used to evaluate the trueness of the method. The repeatability was calculated as within-day RSD (%) of analyte concentration and intermediate precision was evaluated as RSD (%) of analyte concentration, obtained in consecutive days by three analysts. Table 1 & 2 showed the analytical intra & inter assay precision & percentage RSD values.

*Inter Assay precision*

Analytical recoveries were acceptable in sunflower oil for both pesticides. Percentage recovery values ranged from 86.35 to 102.36 for methyl parathion, percentage recoveries obtained from 85.05 to 99.52 for alfa endosulfan. RSD (%) values were calculated from 7.23 to 9.65 for methyl parathion and from 6.03 to 10.35 for alfa endosulfan.

*Intra Assay precision*

The intra assay percentage recovery was in the range of 80.10 – 102.68 for methyl parathion and 83.55 – 102.35 for alfa endosulfan respectively. RSD (%) values were ranged 7.55 – 8.96 for methyl parathion & from 6.15 to 9.98 for alfa endosulfan. In both cases intra & inter assay precision, RSD was less than 12% for representative commodities i.e. sunflower oil. As per SANCO guideline-2012, the recoveries should be in the range of 70 to 120% & RSD should be less than 20%. Hence, the modified method full-filled the requirement of SANCO guideline-2012.

***System Suitability***

The RSD values were calculated on the basis of seven replicates at 0.5µg/mL level, it was 0.051% & 0.043% for alfa endosulfan & methyl parathion respectively when injected individually. In case of mixed standard (0.5µg/mL), it was found 0.029%, and 0.031% for alfa endosulfan & methyl parathion respectively. These results of RSD showed good repeatability for both pesticides.

***Limit of detection, limit of quantification and measurement uncertainty***

The LOD was defined as the lowest concentration of pesticides that could be differentiated as signal with a signal-to-noise ratio (*S/N*) greater than 3. The limit of quantification (LOQ) was determined experimentally by spiked blank sunflower oil extracts with both the pesticides with a signal-to-noise ratio (*S/N*) greater than 10. LOD of alfa endosulfan & methyl parathion was 0.02µg/mL. LOQ was calculated 0.05µg/g for alfa endosulfan & methyl parathion. Measurement uncertainty (MU) was accessed according to ISO/TS 21748:2004 (International Organization for Standardization, 2004) [19] and EURACHEM guide (EURACHEM, 2000) [20]. Uncertainty of measurement was estimated using data obtained from in house method validation & participating laboratories. Expanded Uncertainty was calculated 5.65% for alfa endosulfan at 0.50 µg/g spiking level & 7.58% for methyl parathion at 1.00 µg/g spiking level.

Validation study fulfilled the requirement of SANCO guideline-2012. Data obtained from sugarcane ash as a cleanup reagent was compared with data of PSA in validation study, there was no significant difference (Table 1-2). To check the performance of modified method, z-scores were also compared with different participating laboratories by conducting PT programme. The sunflower oil was spiked at 0.5 µg/g for alfa endosulfan & 1 µg/g for methyl parathion and sent to 21 different laboratories in India. The participating laboratories followed their own sample preparation technique for analysis of sunflower oil. It was found that the performance of 19 laboratories including coordinating laboratory was found in acceptable range (z-score within ±2). Data obtained from in-house validation is also very similar to those reported by other authors for the Matrix Solid Phase Dispersion (MSPD) extraction of pesticides from food matrixes including oils [21-26].

**Analysis of Real Oil Samples**

The methodology was applied for analysis of different oil samples (each 5 only) which were purchased from different vendors of Mysore city for determination of alfa endosulfan & methyl parathion. Methyl parathion & alfa-endosulfan residues were detected below detection limit in sunflower, mustard, groundnut, coconut and soya bean oils.

**CONCLUSION**

The modified & optimized QuEChERS method using sugarcane ash as a cleanup reagent was found suitable for determination of the two pesticides (alfa-endosulfan & methyl parathion) in vegetable oils, demonstrating the great versatility of QuEChERS method that may be used for other pesticide residue analysis also in matrices with high fat content by Gas Chromatography. As laboratories demand faster, more rugged, and high-throughput sample preparation methods compatible with modern instrumentation, QuEChERS applications should

continue to grow. The easily customizable steps of QuEChERS provide an essential path towards future pesticide analysis applications.

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