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QuEChERS Method for Determination of Some Chlorinated Hydrocarbon and Synthetic Pyrethroid Residues in Sheep Meat by Gas Chromatography-Electron Capture Detector

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A rapid, simple and efficient multiresidue method was developed and optimized for the identification and quantification of organochlorine pesticides (OCP) and synthetic pyrethroids (SPs) in sheep meat samples. The method consists of a modified Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) sample preparation method. Samples were extracted with acetonitrile, and the extracts were cleaned up by dispersive solid phase extraction with primary secondary amine (PSA) sorbent and anhydrous magnesium sulphate. Determination and quantification of OCP residues was carried out using a GC-ECD. Mean recoveries were found in the ranges 70-110 % and 84-99 % for the investigated OCPs and SPs, respectively, with the RSD was less than 20%. This method was found more efficient and reliable enabling more number of samples to be analysed in less time. Moreover lipid removal was achieved to a large extent to get desired result.

Keywords: QuEChERS, Multiresidue analysis, Sheep meat, Chlorinated hydrocarbons, Synthetic pyrethroids, GC-ECD.

Sheep meat is contaminated with pesticides in feed, water and during exposure of animals to pest control activity directly or indirectly. Biological activity of pesticides is not only restricted to the target organisms but also extends to non targets animals (Singh and Dhaliwal, 1993). In India for crop protection, the usage of organochlorines, particularly, cyclodienes are banned but restricted use of these chemicals is allowed in the case of public health programme, especially malaria control programme. Pyrethroids have been used widely throughout the world for insect control in agriculture and to combat human and animal parasites (Mastovska *et al.,* 2006). Pesticides used for cattle disease control can affect public health and international trading of food products, if maximum residue levels (MRL) are above those stipulated by FAO and WHO. Chlorinated hydrocarbons have low acute toxicity but possess greater potentials for causing chronic toxicity due to liphophilic nature. The OCP residues in poultry egg samples varied from BDL to 0.031 µg/g (Suganthy *et al.,* 2009). Pesticide residues accumulating in the tissues of animals lead to bio-accumulation in different levels and ultimately to biomagnification. Government of India and Directorate of Plant Protection and Quarantine in the year 2008, banned diclofenac, a veterinary drug due to hazards biocidal nature to folken birds.

The most commonly reported methods for the analysis of pesticide residues in meat and animal tissues are based on an extraction step with ethyl acetate (Muhammad *et al.,* 2010), acetone (Barbini *et al.*, 2007) and hexane-acetonitrile (Darko and Acquaah, 2007) followed by partition and clean-up in order to remove the fatty interference, followed by a gas chromatographic determination with electron capture or mass spectrometry detection (GC-ECD, GC-MS).

Unlike many earlier pesticide residue analysis methods developed for traditional chromatographic detection systems (e.g. Ultra Violet/vis absorbance, fluorescence, element-selective detectors), the QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) approach takes advantage of the wide analytical scope and high degree of selectivity and sensitivity provided by gas and liquid chromatography (GC and LC) coupled. GC, GC-MS and Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) have become the main analytical tools in most pesticide monitoring laboratories to meet world standards. Thus the streamlined features, practical benefits and excellent results provided by the QuEChERS sample preparation approach lead to the great popularity of QuEChERS concept (Anastassiades *et al.,* 2003, Lehotay *et al.,* 2010). The QuEChERS has been applied with success on several food matrices such as fruits and vegetables (Anastassiades *et al.,* 2003,) and low-fatty (2-20%) food matrices, such as milk, egg, and avocado (Lehotay *et al.*, 2005).

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In fat samples, conventional methods for the analysis of pesticide residues usually involve laborious and time-consuming clean-up steps. Moreover, analytical problems associated with lipids extraction when using gas chromatographic system, especially electron capture detector, due to matrix interferences, may cause signal suppression or enhancement. In multi-residue analysis, the sample preparation process cannot be selective to remove chemical compounds of the matrix if they have similar properties as the analytes. Modifications of QuEChERS method can be made by adjusting solvents, salt volumes, and clean-up sorbents. The aim of the study was to develop a simple and efficient multiresidue analysis in sheep meat. In order to shorten the analytical procedure during extraction and cleanup.

Materials and Methods

Chemicals

Acetone, acetonitrile, and hexane of HPLC grade, anhydrous magnesium sulphate, sodium chloride of analytical grade all from M/s. Merck (Mumbai, India), primary secondary amine (PSA) from M/s. Varian, India.

Preparation of standards

Pesticide standards were obtained from Accustandard; Inc, (New Haven, CT06513, USA). Each insecticide was dissolved in hexane to make a 1000 µg/g stock standard solution. Intermediate and working standard solutions were prepared from stock solution by serial dilution technique. Five working standard solutions ranging from 0.01, 0.05, 0.1, 0.5, and 1 µg/g were prepared from standard mixture for a linearity check. Spiking solutions were used for fortifying the sample and also for the calculation after appropriate dilution. All the solutions were protected against light with aluminium foil and stored at -4°C.

Instrumental parameters

A GC-2010 (Shimadzu, Japan), equipped with ECD with ^{63}Ni radio isotope 370 MBQ (10mci) as source of detector in gas chromatography. Capillary fussed silica column (J&W, scientific, USA), DB-5, 30m X 0.25 mm id X 0.25 µm film thickness. Carrier gas: nitrogen 2.0 ml/min (constant flow mode), make up gas-nitrogen; Injector temperature 250°C and Detector temperature 300°C.

The GC column oven was initially set at a temperature of 160° C for 1 min, increased @ 3°C / min to 200°C, held for 2 min and increased @ 4°C / min to 220°C held for 4 min finally increased @ 5°C / min to 250°C held for 15 min. Split injection at a volume of 1 µl by Shimadzu AOC 20i auto injector and AOC 20s auto sampler.

Sample preparation

Meat samples were collected from the local market. The meat sample (1 kg) was blended with high volume blade homogeniser and used for the recovery study.

Recovery studies

A representative (10 g) homogenised meat sample was mixed with a known amount of OCPs and SPs mixture in a 50ml polypropylene centre fuge tube. In controlled sample, same amount of hexane was added. Both tubes were kept for 30 minutes to allow the pesticide standard reference materials to get imbibed in to the ground meat.

Extraction and cleanup

The extraction of pesticide residue from meat sample was followed by QuEChERS method with modification (Anastassiades *et.al.* 2003). An aliquot of 10 g. of homogenised sample was weighed in to a 50 ml of polypropylene centrifuge tube and 20 ml of acetonitrile was added and hand shaken immediately for 2 minutes. Four grams of anhydrous magnesium sulphate and 1 g. of sodium chloride were further added and shaken immediately for 30 seconds with the closed screw cap. The mixture was then centrifuged at about 10000 rpm for 10 minutes using a centrifuge (Plasto Craft®) to separate the sediments and water from acetonitrile. Next the 4 ml (equivalent to the 2 g.of sample) of supernatant of acetonitrile layer obtained after salting out was loaded in to a 15 ml centrifuge tube containing 100 mg of primary secondary amine and 600 mg of magnesium sulphate and shaken immediately followed by the centrifugation at about 5000 rpm at 5 minutes . After centrifugation, 2 ml of supernatant was taken out in to the turbovap tube and concentrated to dryness by using the Turbovap evaporator (Caliper Life Sciences, Russelsheim, Germany) at 40°C with a gentle stream of nitrogen and the residues were redissolved in 1 ml of hexane and subsequently filtered through membrane filter paper to remove the excess of colouring materials and transferred in to GC vials. The concentration of sample represented by the test solution was 1g/ml. The detection and quantification was carried out by GC-ECD.

Results and Discussion

In the present work, the QuEChERS method was followed to extract the pesticide residues from the sheep meat. This approach can significantly reduce the analysis time and solvent consumption. Anastassiades *et al.,* (2003) reported a QuEChERS method of pesticide extraction by using acetonitrile as a vortex mixture, the cleanup procedure was performed by dispersive solid phase extraction using the primary secondary amine and finally the extract was injected in to GC-ECD system. Electron capture detector was found to be fairly sensitive to all selected pesticides under standardised GC-ECD parameters. Blank as well as control sample did not show any peak that could be attributed to the

Table 1. Average recovery and relative standard deviation of chlorinated hydrocarbon from sheep meat samples by modified QuEChERS method

Chlorinated hydrocarbon	Retention time (min)	Fortification level (µg / g)	*Recovery (%)	SD	RSD (%)
Alpha HCH	6.72	0.01	75.48	4.09	5.41
Gamma HCH	7.92	0.01	91.93	3.64	3.95
Beta HCH	8.38	0.01	99.17	5.51	5.55
Delta HCH	9.40	0.01	79.07	5.29	6.69
Heptachlor	10.57	0.01	114.77	4.55	3.97
Aldrin	12.10	0.01	71.87	1.58	2.20
Heptachlor epoxide	14.16	0.01	106.73	4.52	4.24
Alpha endosulphan	16.09	0.01	103.27	8.21	7.95
p,p, -DDE	17.67	0.01	92.20	5.65	6.12
Dieldrin	17.91	0.01	76.23	5.75	7.54
Endrin	18.91	0.01	82.57	3.95	4.78
Beta endosulphan	19.70	0.01	93.20	8.17	8.76
p,p, -DDD	20.50	0.01	95.30	8.85	9.29
Endrin aldehyde	20.80	0.01	108.73	4.35	4.00
Endosulphan sulphate	22.20	0.01	86.50	5.51	6.37
p,p, -DDT	22.50	0.01	90.50	8.15	9.01
Methoxychlor	26.82	0.01	104.53	4.23	4.05

* Means of three replication, SD- standard deviation, RSD-Relative standard deviation

studies of pesticides. All the pesticides gave well resolved peaks. However, permethrin, beta-cyfluthrin, fenvalerate and deltamethrin gave two peaks due to separation of isomers and the mean of their two peakes was used to calculation. The linearity range of multiresidue pesticides was worked out by plotting the response against a standard and ranged from 0.01 to 1 µg / g.

Selection and optimization of extraction and cleanup procedure are very important in multiresidue analysis. The extraction of pesticide residue depends upon the polarity of the pesticides as well as the type of pesticide matrix. Owing to the wide range of polarity and solubility exhibited by the compounds investigated, acetonitrile was selected as one of the solvents for extraction of pesticides because of its effectiveness of polar and non polar pesticides from the diverse range of matrices. The clean up step was modified by the addition of 100mg PSA to the remaining fats. The added PSA effectively removed many polar matrix components, such as organic acids, certain polar pigments, and sugars, from the food extracts. The co-extractives in the extract, because of insufficient cleanup of sample cause rapid deterioration of gas chromatographic system especially electron capture detector, thereby precluding reliable results. In the QuEChERS method originally used for pesticide extraction, 10 ml of acetonitrile was added to 10 g. of sample followed by addition of magnesium sulphate and salting out step to remove water and PSA to bind with organic acids polar colouring material or glucosides. The sample matrix was less disturbed when using primary secondary amines which minimised procedure with GC-ECD.

Table 1 and 2 show recoveries and relative standard deviation (RSD) values obtained for spiked samples at spiking level. These values evidenced that the method achieved acceptable recoveries for all pesticides (70-120%), with RSD lower than 15%. Most pesticides gave 70-120% acceptable recoveries with associated RSD<15%, therefore developed method may be used as a quantitative method for this analytes.

More lipophilic pesticides (alpha HCH, delta HCH, aldrin, and dieldrin) gave recoveries of 70-80 % (Table 1) and reasonably consistent (RSD<10%). Lower recoveries were unavoidable in some pesticides for the satisfactory chromatographic performance, as well to remove co-extractive fats. Consistent recoveries and accurate quantitation of most lipophilic residues will require either the use of an internal standard matched closely to the lipophilic analytes, the use of an extracted matrix-matched calibration or to correct the results for the recovery factor.

Table 2. Average recovery and relative standard deviation of synthetic pyrethroids from sheep meat samples by modified QuEChERS method

Synthetic pyrethroid	Retention time (min)	Fortification level (µg/g)	*Recovery (%)	SD	RSD (%)
Fenpropathrin	26.37	0.05	76.28	2.89	3.79
Lambdacyhalothrin	30.04	0.05	78.93	3.50	5.23
Permethrin I	34.00	0.05	91.34	2.24	2.46
Permethrin II	34.05	0.05	95.32	2.84	2.97
Beta cyfluthrin I	36.03	0.05	81.65	2.84	3.47
Beta cyfluthrin II	36.06	0.05	82.50	3.91	4.73
Alpha cypermethrin	35.76	0.05	74.40	4.15	5.58
Fenvalerate I	39.98	0.05	85.53	5.00	5.85
Fenvalerate II	36.20	0.05	100.77	4.65	4.62
Deltamethrin I	44.08	0.05	110.23	9.28	8.42
Deltamethrin II	44.08	0.05	77.73	6.24	8.03

* Means of three replication, SD- standard deviation, RSD-Relative standard deviation

Among synthetic pyrethroids, alpha cypermethrin (74.40%) recorded the lowest recovery value of 74.40 % and deltamthrin I recorded the highest value of 110.23 %.Fenpropathrin (76.28), lambda cyhalothrin (78.93%),permethrin I (91.34%), permethrin II (95.32%), beta cyfluthrin I (81.65%), beta cyfluthrin II (82.50%), fenvalerate I (85.53%), fenvalerate II (100.77%), and deltamethrin II (77.73%) showed acceptable range of recovery values. Analytical problems associated with the presence of lipids were not observed in spiked samples after the analysis of routine sequences: Retention time drifted about 0.1% and response drifted lower than 30% at spike level.

Detector was found quite sensitive not only for organochlorine but also synthetic pyrethriods. The limit of detection (LOD) of the method was worked out based on the signal to noise ratio of 1:3 and limit of quantification (LOQ) based on 1:10 ratio. ECD was found to be fairly sensitive for all the 24

pesticides under standardised GC parameters all the pesticides gave well resolved peaks. The QuEChERS method takes the advantage of several features of acetonitrile to provide a rather selective isolation of pesticide residue over a wide polarity range.

The proposed method is sensitive, quick, easy, and allows the multiresidue determination of 24 representative pesticides including OCPs and SPs pesticides in fat samples, with recoveries ranging from 70% to 120% for most of them with the optimized conditions. The proposed method shows good sensitivity and recovery and allows for rapid analysis. This method requires only small volumes of solvents per sample and needs no special equipments. Thereby, this method could be used for routine analysis in laboratories for national and community monitoring programs of different families of pesticides in animal fat with equipment that allows quantitation and confirmation in one analysis.

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