Application of QuEChERS Pesticide Multiresidue Method in Traditional Saudi Medicine and Analysis by Gas Chromatography Mass Spectrometry

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***Abstract*—The Quick Easy Cheap Effective Rugged and Safe multiresidue method (QuEChERS) has been validated for the extraction of 9 organochlorine pesticides (OCPs) in different matrices of traditional Saudi medicine plants (TSMP). The nine OCPs were hexachlorohexane isomers (HCH, including α-BHC, β-BHC, γ-BHC,), heptachlor, heptachlor-epoxide, and DDT derivatives (o,p-DDE, p,p-DDE, o,p-DDT, p,p-DDT). The method employed a rapid, simple and cost effective procedure. The spiking levels for the recovery experiments were 0.1, 0.5 and 2.0 mg kg-1. Mean recoveries mostly ranged from 82.0% to 104.0% (93.0% on average), while the linearity for 9 OCPs, in the working standard solutions of six concentration levels between 1 and 100 ng/ml, varied from 0.989 to 0.999. Precision, expressed as relative standard deviation (RSD) were generally below 10% (4.72% on average). Based on these results, the methodology was shown to be robust and highly efficient and thus, suitable for monitoring MRL compliance in a wide range of commodity. The contamination status of 9 OCPs on different TSMPs fenugreek, ginger cloves and cinnamon marketed in Riyadh, Saudi Arabia. Among a total of 48 samples which were also tested using a previously validated method, 31.0% (15 samples) contained at least one of the 9 pesticides. We conclude that QuEChERS is a valid method suitable for investigate of OCPs residues in TSMPs.**

***Index Terms*—Organochlorine pesticides, QuEChERS, medicinal plant, Saudi Arabia.**

I. Introduction

Saudi Arabia is one of the richest biodiversity areas in the Arabian Peninsula and comprises very important genetic resources of crop and medicinal plants. In addition to its large number of endemic species, the components of the flora are the admixture of the elements of Asia, Africa and the Mediterranean region [1]. According to Collenette [2], the greatest species diversity has been observed in Asir and Hijaz, the western mountainous area of the Kingdom, which borders the Red Sea. And it is due to a greater rainfall and range of altitude from sea level to 9300 ft at Jabal Sawdah, near Abha. A total of 2250 species in 142 families are represented in the flora of the Kingdom of Saudi Arabia [2]. Of these, there are 242 endemic and 600 rare and endangered species in the wild; thus an action plan should be taken for their conservation and sustainable development. According to Al-Yahya [3], the Arabian peninsula is the birth place of herbal drugs, and the use of folk medicine has existed there since time immemorial. According to Mossa [4], the Kingdom of Saudi Arabia is gifted with a wide range of flora, consisting of a large number of medicinal herbs, shrubs and trees. It has been estimated that the flora of Saudi Arabia have a great medicinal species diversity expected to be more than 1200 (over 50%) out of its 2250 species. Some sporadic publications on the folk medicines of Saudi Arabia [3]-[5] are available.

Indigenous knowledge (IK) of uses of plants of Saudi Arabia for the cure of many ailments is ancient and still available among the tribal and local people and medicinal healers (Hakim). These IK and traditional experiences are disappearing day by day with development and modernization. There is, therefore, an urgent need to document these vast stores of knowledge through ethnobotanical surveys throughout the Kingdom before their disappearance from the community.

Organochlorine pesticides (OCPs) were widely used in agriculture starting in the early 1950s, and most OCPs are resistant to photochemical, biological and chemical degradation for a long period of time [6]. They persist in various media and some can be transported over long distances to regions where they have never been used. The use of most OCPs is now prohibited in China, but their low biodegradability means that they can still be detected in the environment [7]. Because many herbs such as American ginseng are plant materials, they carry a risk of contamination from agricultural chemicals [8]. Therefore, residuals of environmentally persistent pesticides may be carried by the plants and soil to varying degrees. To date, a maximum residue level has not been set for American ginseng [9], and only a few analytical methods for determining pesticide residues in herbs have been reported [10], [11]. In addition, the method of preparing sample solutions is troublesome because of the complex chemical composition and certain sample matrices. As a result, techniques that combine a short analysis time, sufficient selectivity and adequate sensitivity have become necessary.

The Quick Easy Cheap Effective Rugged and Safe multiresidue method (QuEChERS) is a novel sample preparation methodology for pesticide multiresidue analysis that was developed between 2000 and 2002 and first reported in 2003 [12]. Although a very new method, it has already been widely accepted by the international community of pesticide residue analysis, and many publications already

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deal with this method in its original form or variations [10], [13]-[15]. Additional advantages of the method include the high sample throughput and the low amounts of solvent, glassware, and bench space required [16].

In this study, we employed a modified QuEChERS method, in combination with gas chromatography (GC), for the analysis of the contamination status of 14 OCPs in 60 samples, taken from six different areas (including Canada, America, Hebei Province, Shangdong Province, Liaoning Province and Beijing City in China).

II. Experimental

A. Reagents and Standards

All OCP standards α-HHC, β-HHC, γ-HHC, heptachlor, heptachlor-epoxide, o,p-DDE, p,p-DDE, o,p-DDT and p,p-DDT, (purity 98%) were obtained from Sigma, Germany. A stock solution of each pesticide was prepared by dissolving 10 mg with 50 ml of n-hexane in volumetric flask. The concentration of the stock solution was 1000 ng/ml for all pesticides. All the solvents used, including acetonitrile, petroleum ether (60–90˚C) and methanol, were analytical grade. Graphitized carbon black (GCB), primary secondary amine (PSA), sodium acetate and anhydrous MgSO4 were also manufactured by Supelco Co.

B. Sampling

A total of 48 samples of four different medicinal plants (fenugreek, ginger, cloves and cinnamon) were collected from markets Riyadh, Saudi Arabia. All samples were milled (particle size about 2 mm) and stored at -18˚C prior to analyses for OCPs.

C. Extraction and Clean-up

Extraction and clean-up were carried out according to a modified version of the QuEChERS method. Two grams of sample flour were weighed into the 50-ml centrifuge tubes. The main extraction involved the addition of 20 ml of acetic acid–water–acetonitrile (1:5:94, v/v). The tube was closed and shaken vigorously by hand for 1 min. To induce phase separation and pesticide partitioning, a buffer–salt mixture (consisting of 0.5 g sodium acetate and 3 g anhydrous MgSO4) was added to the suspension. The tube was closed, shaken vigorously by hand for 1 min, and centrifuged for 5 min at 650 g. For clean-up, 1.5 ml of the upper organic phase was transferred into a 2-ml centrifugation tube already containing 25 mg PSA, 50 mg GCB and 150 mg anhydrous MgSO4. The tube was closed, shaken vigorously by hand for 1 min, and centrifuged at 5000 rpm for 5 min. An aliquot of the upper organic phase (about 1 ml) was transferred into a vial for GC/MS analyses.

D. GC Analyses

An Agilent 7890N gas chromatograph (Agilent, Ltd, USA) equipped with 63Ni electron capture detection (ECD) system and 7683B Series injector was used. Nitro-gen of purity greater than 99.99% was used as carrier gas. For separation, a fused-silica capillary column DB-5 (30 m × 0.25 mm i.d., film thickness 0.25 μm), Agilent) was employed. The column temperature was maintained at 80 ˚C for 1 min, and then ramped at 10 ˚C/min up to 250 ˚C and kept for 20 min. The total run time was 20 min at a constant flow rate of 1.5 ml/min. The injector temperature was 250 ˚C and the detector temperature was 260 ˚C. The sample injection volume was 1μl.

III. Results and Discussion

Standard calibration curves were obtained by plotting analyte concentrations against the peak area. The linearity for 9 OCPs, in the working standard solutions of seven concentration levels between 1 and 100 ng/ml, varied from 0.989 to 0.999. The calibration curves were sufficient for the detection of 9 OCPs in American ginseng samples. Precision and limit of detection (LOD) are shown in Table I. The accuracy and precision of the method were sufficiently high, spiked within the range of 0.1–1 ng/g. The recovery values oscillated between 86% (*α*-BHC), 82% (*β*-BHC), 87.3% (*γ*-BHC), 96.1% (heptachlor), 102% (heptachlor-epoxide), 97.8% (*o*, *p*-DDE) and 104% (*p*, *p*-DDE), 95.5% (*o*, *p*-DDT) and 97.2% (*p*, *p*-DDT) at three levels added (0.1, 0.5 and 2 ng/g, respectively), and relative standard deviations (RSD) were generally below 8.0%. The LODs were between 0.15 and 0.35 ng/g, based on a signal-to-noise ratio of 3:1.

TABLE I: Precision, LOD, Linearity Ranges and Recovery % of Various Tested Pesticides.

Pesticide	Precision (RSD %)	LOD (ng/ml)	Linear range (ng/ml)	Rec. %
α-HCH	4.83	0.05	1 - 100	86
β-HCH	6.52	0.05	1 - 100	82
γ-HCH	4.55	0.05	1 - 500	87.1
Heptachlor	3.26	0.02	1 - 100	96.1
Heptachlor-epoxide	3.56	0.02	1 - 100	102
O, *P*-DDE	5.63	0.01	1 - 100	97.8
P, *P*-DDE	5.48	0.01	1 - 100	104
O, *P*-DDT	4.52	0.01	1 - 100	95.5
P, *P*-DDT	5.12	0.01	1 - 100	97.2

The methodology was applied to the analysis of medicinal plant samples collected from different markets in Riyadh, Saudi Arabia during 2012. In the present study, nine out of 48 samples contained at least one of the 9 pesticides, as shown in Table II. None of the 48 samples contained all 9 tested pesticides. None of the hexachlorohexane isomers (*α*-BHC, *β*-BHC, *γ*-BHC), heptachlor, heptachlor-epoxide, and DDT derivatives (*o*, *p*-DDE, *p*, *p*-DDE, *o*, *p*-DDT, *p*, *p*-DDT) were detected at over 26 ng/g in one sample. Results indicated that ginger showed that the highest mean concentration of the 9 tested OCPs were 13.60 (0-22.6) ng/g and 30.21 (0-25.75) ng/g for ΣHCHs and ΣDDTs, respectively. The ΣHCHs are 6.64 (0-9.62) ng/g, 4.41 (0-4.42) ng/g and 3.17 (0-3.45) ng/g, in cinnamon, fenugreek and cloves, respectively, while ΣDDTs are 17.35 (0-13.68) ng/g, 15.5 (0-10.42) ng/g and 14.89 (0-18.47) ng/g, in cloves, cinnamon and fenugreek, respectively. Frequency (positive samples %) of 9 tested OCPs were 17 % (8 samples), 25 % (12 samples), 6 % (3 samples), 7 % (3 samples), 9 % (4 samples), 27 % (13

samples), 31 % (15 samples), 7 % (3 samples), and 8 % (4 samples), of analysed samples, for *α*-BHC, *β*-BHC, *γ*-BHC, heptachlor, heptachlor-epoxide, *o*, *p*-DDE, *p*, *p*-DDE, *o*, *p*-DDT and *p*, *p*-DDT, respectively.

TABLE II: ORGANOCHLORINE PESTICIDES CONTAMINATION OF FENUGREEK, GINGER, CLOVES AND CINNAMON COLLECTED FROM SAUDI MARKETS

Pesticide	Fenugreek	Ginger	Cloves	Cinnamon	Frequency %
α-HCH	1.52 (0-2.55)	3.56 (0-7.23)	0.78 (0-1.64)	2.32 (0-5.64)	17
β-HCH	0.78 (0-1.52)	2.15 (0-4.85)	1.45 (0-3.45)	3.46 (0-9.62)	25
γ-HCH	2.11 (0-4.42)	7.89 (0-22.60)	0.94 (0-2.97)	0.85 (0-2.18)	6
ΣHCHs	4.41	13.60	3.17	6.64	-
Heptachlor	1.35 (0-3.23)	2.25 (0-6.94)	1.59 (0-2.28)	2.38 (0-6.42)	7
Heptachlor-epoxide	0.52 (0-2.65)	1.36 (0-3.84)	2.35 (0-4.46)	3.66 (0-6.85)	9
O, *P*-DDE	5.12 (0-12.38)	14.56 (0-25.75)	7.26 (0-13.68)	4.12 (0-10.42)	27
P, *P*-DDE	6.45 (0-18.47)	8.94 (0-23.21)	4.35 (0-11.44)	3.95 (0-7.35)	31
O, *P*-DDT	0.56 (0-1.96)	2.15 (0-6.44)	1.25 (0-2.75)	0.54 (0-1.23)	7
P, *P*-DDT	0.89 (0-2.34)	0.95 (0-2.73)	0.55 (0-2.25)	0.85 (0-1.56)	8
ΣDDTs	14.89	30.21	17.35	15.5	-

Although the pesticide was detected, the level was lower than the maximum allowable residual limits (MRLs) established by the China Pharmacopoeia Committee (total BHC 200 ng/g, heptachlor and heptachlor-epoxide 100 ng/g and total DDT 200 ng/g, for different medicinal herbs, 2010).

We expect that more attention will be paid to monitoring the presence of pesticide residues to guarantee high quality of medicinal herbs in Saudi Arabia. Our results suggest that contamination of medicinal herbs with OCPs is widespread. The MRLs for OCPs commonly found in tested Saudi old medicinal herbs and other herbs should be established as quickly as possible. The apparent current explosion of interest in commercial utilization of Saudi medicinal herbs should be accompanied by accurate quality control. Prompt analytical methods would be very useful for setting up realistic MRLs and other regulatory guidelines in the management of pesticide residues in herb products.

IV. CONCLUSION

The proposed QuEChERS procedure was successfully applied to the sample preparation of 9 OCPs from medicinal herbs. This method showed satisfactory validation parameters, such as accuracy, precision and lower limits of detection. For all of pesticides studied, the sensitivity of the method was good enough to ensure reliable determination at levels lower than the respective MRLs. The proposed method was applied for the determination of pesticide levels in 48 samples collected from different markets. OCPs were found at different levels in the samples investigated. The present study provides significant data on OCP contamination in Saudi herbs and helps in risk assessment of consumer exposure to the expected OCP levels.

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