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RESEARCH ARTICLE

Determination of Organochlorinated Pesticide Residues By Gas Chromatography - Mass Spectrometry after Elution in A Florisil Column

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Summary

The purpose of the present study was to develop an easy analytical method for determining α-hexachlorocyclohexane (α-HCH), hexachlorobenzene (HCB), heptachlor (HC), aldrin (ALD), 4,4-dichlorodiphenyldichloroethylene (4,4-DDE) and 4,4-dichlorodiphenyltrichloroethane (4,4-DDT) residues by gas chromatography with mass spectrometre (GC–MS) for routine analysis. Chicken muscles were utilized as samples in this study. A florisil packed column was used in sample preparation step and showed good performance. The average precision and accuracy ranged 8.03-24.00% and -4.36-30.67%, respectively (n=6). The average recoveries were between 95.74 and 130.67% for various spiking levels (25, 50, 100 ng/g). The average of inter- and intra-day precision was <13 and the limits of detection were ranged 7-19 ng/g, depending on different organochlorinated (OC) pesticides. In conclusion, the extraction method used in the present study was inexpensive (do not require skilled operators, high volume of solvent or costly apparatus), easy and rapid. Additionally, the validation parameters show that the proposed method in this study is sensitive, reproducible and reliable alternative to the normally used methods. Thus, it could efficiently be used in the routine and monitoring studies so that several samples can be run in parallel.

Keywords: *Organochlorinated pesticides, Residue, Florisil packed column, Gas chromatography-mass spectrometry*

Organik Klorlu Pestisid Kalıntılarının Florisil Kolonda Ayrılmasından Sonra Gaz Kromatografi - Kütle Spektrometre ile Belirlenmesi

Özet

Bu çalışmanın amacı, rutin analizler için gaz kromatografi-kütle spektrometre (GC-MS) ile α-hekzaklorosiklohekzan (α-HCH), hekzaklorobenzen (HCB), heptaklor (HC), aldrin (ALD), 4,4-diklorodifenildikloroetilen (4,4-DDE) and 4,4-diklorodifeniltrikloroetan (4,4-DDT) kalıntılarının belirlenmesi için kolay bir analitik yöntem geliştirmektir. Örnek olarak tavuk kaslarından yararlanıldı. Örnek hazırlama aşamasında, florisil dolgu kolonu kullanıldı ve iyi bir performans elde edildi. Ortalama kesinlik ve doğruluk aralığı sırasıyla %8.03-24.00 ve %-4.36-30.67, (n=6). Ortalama geri kazanım farklı düzeydeki eklenmiş doku örnekleri için (25, 50, 100 ng/g) %95.74 ve %130.67 arasındadır. Ortalama gün içi ve arası kesinlik <13 ve ölçüm limitleri farklı organik klorlu (OC) pestisitlere bağlı olarak 7-19 ng/g idi. Sonuç olarak, bu çalışmada kullanılan ekstraksiyon metodu, ucuz maliyetli (nitelikli eleman, yüksek hacimde solvent kullanımı ve maliyetli cihaz gerektirmeyen), kolay ve hızlı bir metotdu. İlaveten, validasyon parametreleri bu çalışmada önerilen metodun normalde kullanılan metodlara alternatif olarak, duyarlı, tekrarlanabilir ve güvenilir olduğunu göstermektedir. Bu yüzden bu metot rutinde ve izleme çalışmalarında birkaç örneği eş zamanlı analiz etmek için etkili olarak kullanılabilir.

Anahtar sözcükler: *Organoklorlu pestisitler, Kalıntı, Florisil dolgu kolon, Gaz kromatografi-kütle spektrometre*

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INTRODUCTION

Organochlorines (OC) is a class of pesticides that have been prohibited worldwide since the beginning of 1980s due to their toxicity, stability, high liposolubility, long biological half-life, and consequently a high degree of bioaccumulation in food chain [1,2]. Moreover, they are persistent in the environment and tend to accumulate in ecosystems. In the past extremely use of OC pesticides poses still dangerous effects such as cancer, immune systems, reduced bone mineral density, and the disruption of hormonal functions [3,4] on healty of animals, human and environment. Despite a prohibit imposed by WHO on use some of OC pesticides are still used in limited quantity in many developing countries, including India, especially for malaria control [1].

The pollutants are transferred to animals from either the surrounding environment or from diets. Also, the lipid content of animal influences the bioaccumulation process [5,6]. Thus, the determine of OC residues amounts is necessary in animal products. Chicken, economic and high protein and lipid content, is an important food for human life. The quality of food is directly related to contaminants levels such as, veterinary drugs, pesticide residues. Its quality control is performed to monitor residues. According to the Directive 96/23/EC, it is necessary to research OC pesticide residues in animal products [7]. Aulakh et al.[1] reported that, residues of OC pesticide are present despite complete prohibit poultry muscle and egg in India.

Until now, it is reported that many methods for determination of OC pesticide residues, such as thin-layer chromatography (TLC) and high-performance thin-layer chromatography (HPTLC) [8], and GC [9]. Because of liposoluble, low volatile pesticides except HCB, such as OC pesticides, GC-MS and electro capture detector (ECD) is obviously the prefered approach because of its high sensitivity and selectivity [10]. For gas chromatography, different clean-up procedures have been performed to the determination of OC pesticides, such as liquid-liquid extraction (LLE) [11], super critical fluid extraction (SFE) [12], accelerated solvent extraction (ASE) [13], gel permeability extraction (GPE) [14,15], microwave-assisted extraction (MAE) [10], and solid-phase extraction (SPE) [16]. As SPE, florisil, silica, alumina, C18 materials were used in clean-up step [14,16-22]. The SPE requires much lower volume in organic solvent usage is an important advantage [17].

The florisil was documented in previous studies [9,14,17,20]. So far, no clean-up methods have been reported for OC pesticides from chicken muscle by GC-MS after elution from activated florisil. The purpose of the present study was to develop an easy rapid, easy, reliable analytical method for determining some OC pesticide (α-HCH, HCB, HC, ALD, 4,4-DDE and 4,4-DDT) residues using a florisil packed-column and GC-MS for both routine analysis and screening pesticide residues in monitoring programs.

MATERIAL and METHODS

Chemicals, Reagents and Standards

In this study, all solvents (acetone, ethyl-acetate, light petroleum, *n*-hexane, methanol) were analytical grade and obtained from Merck (Darmstadt, Germany). The OC standards (α-HCH, HCB, HC, ALD, 4,4-DDE and 4,4-DDT) were obtained from Dr. Ehrenstorfer GmbH (Referans Chemical, Ankara, TURKEY). Calibration standards at concentrations of 0.01, 0.05, 0.1, 0.25, 0.5, 1 ng/µL for each substance were prepared from reference standard solution (10 ng/µL), in six replicates. Florisil (60-100 mesh) and sodium chloride were obtained from J.T. Baker (Deventer, Netherlands). Anhydrous sodium sulfate was from Merck (Darmstadt, Germany). The syring filter of 45 µm purchased from Millipore. Ultra pure water was supplied from Elga (London, UK).

Instruments

The analyses were performed in Hewlett Packard 6890 GC was connected with HP 5973 MS (HP, Agilent, Sem, Ankara, Turkey). The separation was performed on a HP-5 capillary column (HP code 19091 J-413 HP-5; 5% phenyl methyl siloxane, 30 m × 0.32 mm I.D.).

Chromatographic Condition

The column temperature program was as follows: 50°C held for 2 min; increased at a rate of 10°C/min to 150°C, and then at 3°C/min to 270°C, where it was held for 12 min. The carrier gas was helium and it was supplied in constant flow-rate of 1 mL/min at splitless mode.

Standards and Working Solutions

Concentration of stock standard solution was 10 ng/µL. Working standard solutions of all OC pesticides were prepared at six different concentrations (0.01, 0.05, 0.1, 0.25, 0.5, 1 ng/µL) in methanol. Calibration curve was prepared in the range of 0.01-1 ng/µL for all OC pesticides (α-HCH, HCB, HC, ALD, 4,4-DDE and 4,4-DDT) (n=6).

Sample Preparation

Homogenized chicken muscles were utilized as samples in this study. Ten grams sample was transferred to a 250 mL centrifuge tube, and then 50 mL light petroleum-acetone (1/1, v/v) was added. The mixture

was homogenized for 2 min at 9500 rpm and 50 mL sodium chloride solution (5%) was added. This mixture was centrifuged for 10 min at 1600 rpm. The organic phase transferred to a column of anhydrous sodium sulfate (10 g in a glass tube, 200×20 mm I.D.). The eluate was collected with 2×25 mL portions of light petroleum into a 250 mL Erlenmeyer flask. The eluate was evaporated by a rotary evaporator (bath temperature of 40°C with reduced pressure). Florisil was activated at 130°C for at least 48 h. A little cotton wool was put into a glass column (300×20 mm I.D.) Then, 2.5 g activated florisil and anhydrous sodium sulfate (1 cm) were added to the glass column, respectively. The column was conditioned with 15 mL *n*-hexane (3×5 mL) and not allowed to run dry. After dissolving extract in 1 mL *n*-hexane was transferred to this glass column. The eluate was collected with 30 mL *n*-hexane/ethyl-acetate (v/v, 25/5), (6×5 mL). The extract was mixed together and 1 mL isooctane was added to extract as protective.It was evaporated by an evaporatory (reduced pressure and bath temperature was 50°C). Residue was dissolved with 1 mL of *n*-hexane and filtered through a syringe filter of 0.45 µm. The extract (1 µL injection volume) was injected to GC-MS.

Validation

Linearity, precision, accuracy, limit of detection (LOD), limit of quantitation (LOQ), and recovery parameters were determined for validation of method for OC pesticides. Six levels (0.01-1 ng/µL) calibration series with three analyses at each concentration level were determined for linearity and calibration curve was calculated automatically.

Samples for recovery experiments were spiked with the target pesticides and let for 60 min before performing the extraction. The recoveries were calculated as the percentage using extraction process after spiking from 25 to 100 ng/g with three different levels of OCs (n=6). Precision and relative standard errors (accuracy) were determined on spiked pesticide-free chicken samples at three concentrations by calibration curve prepared (n=6). The inter- and intra-day reproducibility was determined at 0.01, 0.1, and 1 µg/mL concentrations. All samples were freshly prepared from standard solutions (1 ng/µL). Considering a signal-to-noise ratio of 3 and 10 for LOD and LOQ, respectively.

RESULTS

Calibration graphs were established through the range of 0.01-1 ng/µL with correlation of coefficients from 0.996 to 0.999 for all analytes. The correlation of coefficients (R^2), LODs and LOQs are shown in *Table 1*. Retention times of OC pesticides were 17.21, 17.50, 21.82, 23.50, 29.06 and 33.42 min for α-HCH, HCB, HC, ALD, 4,4-DDE and 4,4-DDT, respectively. Intra-assay variations were determined by measuring six replicates (n=6) of three standard samples used for calibration curves. The average of inter- and intra-day precision was <13.

Chromatograms of blank chicken muscle sample, OC mix standards (25 ng/mL) and spike sample 50 ng/g on a HP-5 capillary column are shown, respectively (*Fig. 1, Fig. 2* and *Fig. 3*).

The recoveries of spiked samples, precision and accuracy are shown in *Table 2*. The average precision and accuracy ranged 8.03.00-24.00% and -4.36-30.67%, respectively (n=6). The average recoveries were between 95.74 and 130.67% for various spiking levels (25, 50, 100 ng/g).

Table 1. *Limit of detections (LOD, ng/g), Limit of quantitations (LOQ, ng/g) and Correlation cofficients (R^2) in 0.01-1 ng/µL standard solution range with different levels*

Tablo 1. *Farklı düzeydeki (0.01-1 ng/µL) standart solüsyon aralığında Tespit limiti (LOD, ng/g), Hesaplama limiti (LOQ, ng/g) ve Korelasyon katsayıları (R^2)*

Pesticide	LOD (ng/g)	LOQ (ng/g)	Correlation (R^2)
α- HCH	19	57	0.997
HCB	7	21	0.999
HC	12	36	0.998
ALD	8	24	0.999
4,4-DDE	10	30	0.998
4,4-DDT	9	18	0.996

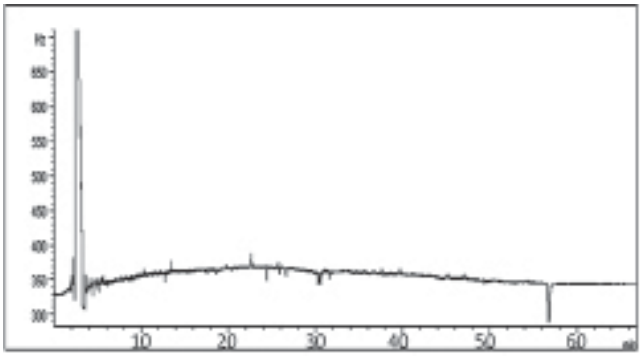

Fig 1. Chromatogram of blank sample on HP-5 capillary column

Şekil 1. Kör örneklerin HP-5 kapillar kolondaki kromatogramı

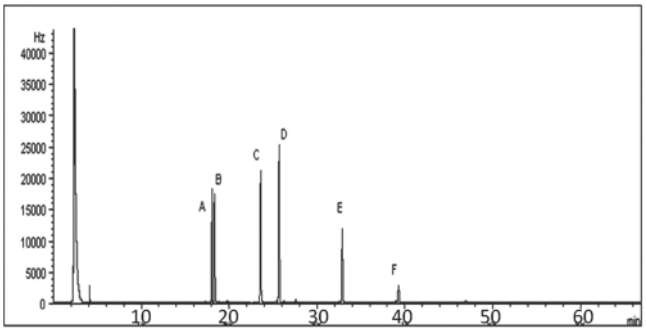

Fig 2. Chromatogram of OC pesticide mix standard (25 ng/mL) on HP-5 capillary column: A= α-HCH, B=HCB, C=HC, D=ALD, E=4,4-DDE, F=4,4-DDT

Şekil 2. Organik klorlu pestisit standartlarının (25 ng/mL) HP-5 ka-pillar kolondaki kromatogramı: A=α-HCH, B=HCB, C=HC, D=ALD, E=4,4-DDE, F=4,4-DDT

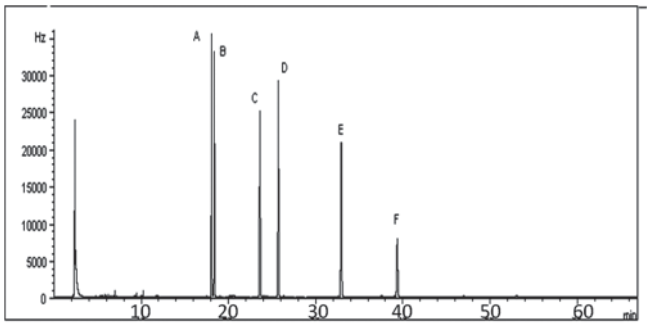

Fig 3. Chromatogram of spiked sample (50 ng/g) on a HP-5 capillary column: A=α-HCH, B=HCB, C=HC, D=ALD, E=4,4-DDE, F=4,4-DDT

Şekil 3. Standart madde eklenmiş (50 ng/g) örneğin HP-5 kapillar kolondaki geri kazanım kromatogramı: A= α-HCH, B=HCB, C=HC, D=ALD, E=4,4-DDE, F=4,4-DDT

Table 2. *The recoveries of spiked samples (n=6), precision and accuracy (%) in three levels*
Tablo 2. *Üç düzeyde standard madde eklenmiş örneklerin (n=6) % geri kazanım, kesinlik ve doğruluk oranları*

Pesticides	Spiked Level (ng/g)	Recovery (%)	Precision (%)	Relative Error (Accuracy, %)
α-HCH	25	96.68	26.62	-3.32
	50	113.24	25.74	13.24
	100	87.40	19.64	-12.60
	average	99.10	24.00	-0.90
HCB	25	103.88	15.25	3.88
	50	97.94	13.36	-2.06
	100	85.41	7.448	-14.59
	average	95.74	12.01	-4.36
HC	25	134.08	12.05	34.08
	50	137.94	10.37	37.94
	100	120.00	6.48	20.00
	average	130.67	9.63	30.67
ALD	25	105.32	10.47	5.32
	50	106.58	13.06	6.59
	100	92.86	5.00	-7.14
	average	101.58	9.51	1.58
4,4-DDE	25	98.04	10.24	-1.96
	50	101.82	8.22	1.82
	100	90.63	5.64	-9.37
	average	96.83	8.03	-3.17
4,4-DDT	25	108.12	18.10	8.12
	50	125.32	16.20	25.32
	100	107.00	14.30	7.00
	average	113.48	16.20	13.48

DISCUSSION

Theoretically, the nature of the preferred sorbent/ solvent combination is determined primarily by the polarity of the target analytes and the nature of the sample matrix and an appropriate solvent or solvent mixure should be use for the eluation of target analyte from matrix components [22]. As meat is a very complex

matrix in extraction and clean-up steps is recommended to decrease the presence of interferents in eluate and also to avoid the deterioration of the chromatographic column [2,13].

The OC compounds, nonpolar, accumulate in the lipid portion of tissues, and the lipid yield in extract might cause discrepancies in the results. Because lipids with a high polarity such as phospholipid are not likely to be easily extracted into organic solvent, it is necessary to determine the efficiency of the lipid extraction from biological samples [13,23]. Animal products having a high lipid content are homogenized with acetone-*n*-hexane or diethyl ether-light petroleum in presence of anhydrous sodium sulphate was commonly used procedure for extraction of OC pesticides [13,15,18]. Saito et al.[13] reported that, acetone produced better lipid extraction efficiencies than all the other solvents. Hexane, an apolar solvent, is used to remove less polar matrix components, such as the lipids [22]. Ethyl acetate has moderately nonpolar and polar properties and thus serves as o good solvent for extraction. It is more polar than traditional extraction solvents of fats, such as hexane, but it is not as polar as acetonitrile or acetone which are completely miscible with water [24]. Garrido Frenich et al.[25] reported that the best results were found with the ethyl acetate solvent for almost compounds in extraction of pesticides from animal tissues. In proposed method in this study, extraction was performed with light petroleum-acetone (1/1, v/v) solvent in a glass column anhydrous sodium sulphate and *n*-hexane/ethyl acetate (25/5, v/v) was used in clean-up step. Used solvents in the present study, showed good performance. Obtained results were consistent with previous reports [13,15,18,22,25].

Florisil, on which these polar analytes are retained very strongly, has been usually used for the clean-up of apolar pesticides and PCBs [22,26] in fatty matrix due to its potential to retain lipids. Also, it retains the high polar materials [19]. The florisil column is capable of effecting isolation and clean-up of pesticide residues from food samples [9,10,27]. Doong and Lee [16] reported that florisil SPE cartridge is appropriate for the clean-up and can provide a reliable quantification of OC pesticides due to effectively eliminate matrix components in different kinds of foods (fish, shelfish, meat, cereals) having different fat contents. Garrido Frenich et al.[25] reported that, the use of a co-column was also desirable to enhance sample clean-up: Florisil and alumina-N cartridges were studied. The use of alumina-N cartridges provided, in general, very poor recoveries (lower than 50%) whereas with the florisil cartridges were much better, therefore this was the clean-up sorbent chosen in the clean-up of liver chicken samples. Armishaw et al.[14] reported that, florisil column was simple and proven technique in clean-up step. Carro et al.[21] reported that pesticides α-BHC, β-BHC and HC presented higher recoveries when florisil was used as adsorbent in aquaculture samples. In the present study, florisil was chosen as adsorbent in clean-up process. The florisil has been used to reduce the carryover of fat material into the *n*-hexane/ethyl acetate eluate. Sample was very good cleaned from fats. Obtained results were consistent with previous reports [14,16].

It is reported that the recovery of HCB is usually low in different matrices [9,21,28]. The result of this study was in agreement with previous reported. In our study, the average recovery of HCB was found lower than others. The lower in recovery of HCB may be related to high volatile and lipophilic properties of its. However, the highest average recovery was obtained for HC and this finding was inconsistent with the result reported by Nardelli et al.[29] reported that, the recoveries are satisfactory for all compounds apart for HEPO (not greater than 76%); this low value can be ascribed to the acidic treatment that probably converts such epoxide into the correspondent diol or sulphate, which are much more soluble in the water phase. In the present study, obtained average recoveries (95.74-130.67%) were consistent with previous reports [9,14,29]. Obtained results were satisfactory and the use of eluting solvents they may be improve the corresponding recoveries significantly. The recovery values were not correlated with the increasing spiking levels in the present study however, the good recoveries were determined all pesticides at 50 ng/g spiked level.

In the present study, a new and simultaneous method has been developed and validated in chicken samples by GC-MS after elution from a florisil column for several OC pesticides. The main advantage of the developed method is that extraction and clean up are performed in less time with a low volume of solvent. Additionally, the proposed method is sensitive, reproducible and reliable alternative to the normally used methods, moreover it was inexpensive (do not require skilled operators, high volume of solvent or costly apparatus), easy and rapid. Thus, it could efficiently be used in the routine and monitoring studies so that several samples can be run in parallel. In future studies, developed method can be tried for analysis not only other OC pesticides and their major metabolites but also in different matrices.

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