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Development and Validation of Gas Chromatography - Mass Spectrometry Method for Determination of Aldrin and Dieldrin in Serum

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ABSTRACT

Aim: Exposure to many banned pesticides continues all over the world and in our country due to the long half-lives of pesticides. Therefore, the purpose of the study was to obtain a rapid, simple, and convenient method for the simultaneous determination of aldrin and dieldrin.

Material and Methods: Method improving and validation assessment are the most important elements for establishing reference techniques and reliable analysis results. In order to analyze analytes, validation of the analytical method is carried out by checking the parameters of specificity, recovery, precision, linearity, the limit of detection (LOD), and the limit of quantification (LOQ).

Results: In our study, an easily applicable, so rapid, effective, and safe GC-MS method was created for the determination of aldrin and dieldrin in serum. In the developed method, 4,4'-Dichlorobenzophenone (4,4-DBP) was used as the internal standard. Aldrin and dieldrin were analyzed in serum in a very short time of 4 minutes. Linear correlation coefficients ($R2 \geq 0.99$) of the linear calibration curves between a range of 1-250 ng/mL of analytes in serum were established. The limit of detection for aldrin and dieldrin were 0.28 and 0.29 ng/mL, respectively. More than 80% recovery of aldrin and dieldrin were obtained at high concentrations, and consistent relative standard deviation (RSD < 6.03%) values were found for both pesticides.

Conclusion: This new method can reliably and quickly be used in routine analysis. The study showed that exposure to aldrin and dieldrin can be detected and monitored in such a short time as four minutes.

Keywords: Aldrin; dieldrin; serum; validation; GC-MS.

Serumda Aldrin ve Dieldrin Tayini için Gaz Kromatografisi - Kütle Spektrometresi Yönteminin Geliştirilmesi ve Doğrulanması

ÖZ

Amaç: Tüm dünyada ve ülkemizde yasaklanmış pestisitlere maruziyet, pestisitlerin uzun yarılanma ömürleri nedeniyle devam etmektedir. Bu nedenle bu çalışmanın amacı, serumda aldrin ve dieldrin maruziyetinin eş zamanlı tayini için hızlı, basit ve kullanışlı bir yöntem elde etmektir.

Gereç ve Yöntemler: Metot geliştirme ve doğrulama değerlendirmeleri; referans teknikleri oluşturmak ve güvenilir analiz sonuçları elde etmek için en önemli unsurlardır. Analitleri analiz etmek için, analitik yöntemin doğrulanması; özgüllük, geri kazanım, kesinlik, doğrusallık, saptama sınırı ve ölçüm sınırı parametrelerin kontrol edilmesiyle gerçekleştirilir.

Bulgular: Çalışmamızda; serumda aldrin ve dieldrin tayini için kolaylıkla uygulanabilir, çok hızlı, etkili ve güvenli bir GC-MS yöntemi oluşturuldu. Geliştirilen yöntemde internal standart olarak 4,4'-Diklorobenzofenon (4,4-DBP) kullanıldı. Aldrin ve dieldrin, serumda 4 dakika gibi çok kısa bir sürede analiz edildi. Serumda 1-250 ng / mL analit aralığında doğrusal kalibrasyon eğrilerinin doğrusal korelasyon katsayıları ($R^2 \geq 0,99$) saptandı. Aldrin ve dieldrin için tespit limiti sırasıyla 0,28 ve 0,29 ng / mL idi. Yüksek konsantrasyonlarda aldrin ve dieldrinin % 80'den fazla geri kazanımı elde edildi ve her iki pestisit için tutarlı bağıl standart sapma (RSD < % 6,03) değerleri bulundu.

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Sonuç: Bu yeni yöntem; rutin analizlerde güvenilir ve hızlı bir şekilde kullanılabilir. Çalışma, aldrin ve dieldrin maruziyetinin dört dakika gibi kısa bir sürede tespit edilebildiğini ve izlenebildiğini gösterdi.
Anahtar Kelimeler: Aldrin; dieldrin; serum; doğrulama; GC-MS.

INTRODUCTION

Aldrin and dieldrin are broad-spectrum insecticides, chemicals produced in the laboratory, and not naturally found in the environment. Developed as alternatives to DDT in the 1940s, aldrin, and dieldrin were widely used from the 1950s to the early 1970s. EPA has done many studies on aldrin and dieldrin, and found important results (1). One of these studies was showed that 0.1 ppm dieldrin exposure caused liver tumors. The other study was showed measurable amounts of dieldrin in composite food samples such as dairy products. Another study in humans was determined that 99.5% of human adipose tissue contains traces of dieldrin. Based on this information and carcinogenicity data, the EPA issued suspension notices for the use of aldrin and dieldrin in October 1974. But these insecticides were widely used around the world for 20 years until their use was banned by the EPA.

Even if these insecticides are banned, their exposure continues and humans' exposure to aldrin and dieldrin occurs through inhalation, ingestion, and skin absorption (2). Acute poisoning due to aldrin and dieldrin causes motor convulsions, coordination disorders, dizziness, and gastrointestinal disturbances. Many studies documenting deaths and acute intoxications from aldrin and dieldrin intake are evidence of their fairly common use (1,3-5).

Due to their widespread use, aldrin and dieldrin generate chronic exposure, and these insecticides have proven to be more toxic to humans than their target insects. The low solubility of these chlorinated compounds in water, and their lipophilic nature make them highly toxic. In addition, only 7.8% and 7.7% of these substances are excreted from the body by urine after human's exposure to aldrin and dieldrin. This rate of excretion is very low, indicating that most of these pesticides accumulate in the body (6,7).

Aldrin is metabolized into dieldrin, one of the most persistent of all pesticides, both in the body and nature (Figure 1).

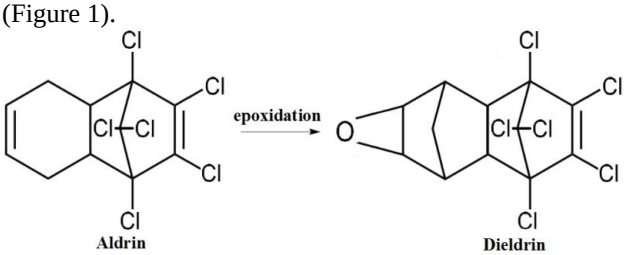

Figure 1. Metabolism of aldrin to dieldrin

For this reason, in the primary exposure environment, high aldrin concentration is first detected in plants, and then dieldrin concentration increases (8). It has been shown that this metabolism depends on many factors such as enzymes, hormones, pH, carbon and nitrogen sources, and the amount of light (9).

The metabolite dieldrin is the primary marker of aldrin exposure in humans. It has been determined that dieldrin biotransformation of aldrin occurs in the liver, lungs, and skin (2,10). Aldrin epoxidation occurs by the activity of mono-oxygenases linked to cytochrome P-450 forms (9,11,12). Although aldrin and dieldrin are no longer used, their exposure continues in many parts of the world. In order to prevent or reduce the negative effects of aldrin and dieldrin residues in humans, these pesticides should be analyzed in serum and their current status should be monitored.

Various analytical methods have been developed for the analysis of these pesticides in human samples (13-16). Due to the similar properties of these pesticides such as lipophilic and stability, some steps in the applied analytical methods are almost the same (17,18). Generally, analytical methods include sample preparation and analysis steps.

Analytical method validation is an essential requirement for evaluating the items we want to analyze (19). Method validation is also the effort of providing the data which is necessary to verify that an analytical testing system is suitable and can provide reliable analytical data. Each method verification review that is created is specific to the specified product (20).

In light of all these, the aim of this study was to optimize and validate a simple, fast and effective method for the analysis of even low concentrations for aldrin and dieldrin in human serum samples.

MATERIAL AND METHODS

Materials and Chemicals

Aldrin and dieldrin reference standards were of analytical grade and purchased from Fluka (Steinheim, Germany). 4,4′-Dichlorobenzophenone (4,4-DBP) was used as an internal standard and obtained from Sıgma-Aldrıch (Steinheim, Germany). All used solvents, which had high purity grade, were sulphuric acid, methanol, and diethyl ether. They were purchased from Sigma Aldrich (St. Louis, USA). 2-isopropanol and n-hexane were from Merck (Darmstadt, Germany). Cartridges for solid-phase extraction were Sep-Pak Silica Classic Cartridges (Wat051900).

Sample Preparation

First, stock solutions of each pesticide analyte were prepared in hexane at a concentration of 100 µg / ml. Multiple pesticide concentrations were then prepared from these primary stock solutions by dilution and mixing them. A primary standard solution was prepared at a concentration of 100 µg / ml in the internal standard. From this main stock, 200 ng / mL intermediate stock solution was prepared and used by diluting it in the analysis.

Steps of serum preparation, the serum samples were extracted as described in previous studies (21-23). Methanol and ethyl ether/hexane mixture was added to the serum. The organic phase, which was obtained by centrifugation, was evaporated. 0.5 mL of concentrated sulfuric acid was added to the residue and then extracted with hexane. The organic phase was dried again. The residue was redissolved in hexane and purified in solid-phase extraction with a silica Sep-Pak cartridge. In the last step, extracted samples were fortified with the internal standard of 4,4-DBP and analyzed by using GC / MS.

Chromatographic conditions

The analysis of all these samples which were transferred into the vials was carried out by gas chromatography (GC-Agilent 7890B) coupled to a mass spectrometer (MS-

Agilent 5977A). Separation of pesticides was carried out with a column Agilent J&W DB-5 (30 m, 0.25 mm internal diameter, 0.25 µm film thickness).

By using this device and column, 2 µL of sample volume was injected in the mode of splitless pulsed, and with a purge flow of 60 mL/min, the injector temperature was 250 °C. The temperature program which was used for the analysis is as follows: 100 °C/min, 50 °C/min to 300 °C (hold 1 min). The carrier gas which was used was helium with a constant flow at 2 mL/min. The temperature of the interface between the GC and the mass spectrometer was maintained constant at 290 °C. The ions were separated by a quadrupole filter according to their mass/charge ratio (m/z). The detection was performed by ion selection mode (SIM: Selected Ion Monitoring).

Validation

Calibration curves were constructed on the peak area ratio of analyte/internal standard versus concentrations by using linear regression. The method was evaluated by the obtained parameters of linearity, the limit of detection (LOD), sensitivity, repeatability, and accuracy. Two different concentrations of aldrin and dieldrin (7.5 and 100 ng / mL) were analyzed to determine the accuracy of the test. The limit of quantification (LOQ) was determined as the lowest concentration level that can be verified with acceptable values for recovery and precision.

RESULTS

In the SIM mode of GC-MS, the molecular ion of m/z 66 for aldrin, m/z 79 for dieldrin, and m/z 250 for internal standard were the quantification ions. The details about the GC-MS method with single ion monitoring (SIM) and settings, which were used throughout this study, are given in Table 1.

Table 1. Optimized GC-MS acquision method parameters of each pesticide

	Retention time	Fragmentation ions (m/z)[a]
Aldrin	3.528	66*, 263, 91
Dieldrin	3.923	79*, 81, 263
4,4-DBP (IS)	3.597	111, 139, 250*

Abbreviation: m/z, mass/charge; 4,4-DBP, 4,4′-dichlorobenzophenone; IS, internal standard.

[a] SIM ions; ions marked with an asterisk were quantification ions

The chromatogram of aldrin, internal standard and dieldrin in SIM mode of the generated method is shown in Figure 2.

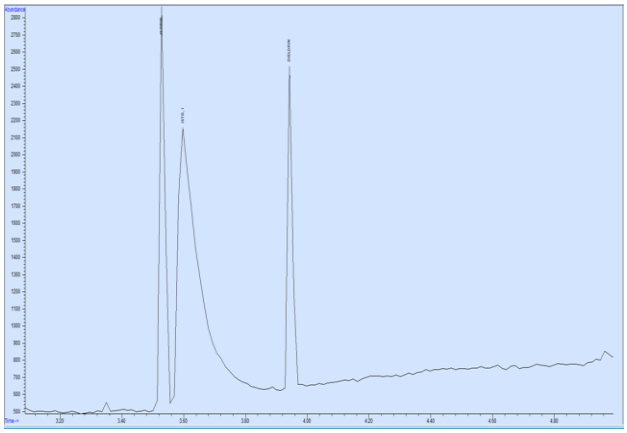

Figure 2. The chromatogram of aldrin (100 ng/mL), internal standard (50 ng/mL), and dieldrin (100 ng/mL)

The analytical performance of the method has been examined by looking at its linearity, regression coefficient (R2), recovery, relative standard deviation (RSD), the limit of detection (LOD), and limit of quantification (LOQ). Seven standards were used to calibrate aldrin, dieldrin, and the calibration curves between 1 and 250 ng / mL were generated. The results from the quantitative determination of aldrin and dieldrin were expressed in Table 2.

Table 2. Validation parameters of gas chromatography-mass spectroscopy method.

	Linearity (ng/mL)	R^2	Recovery (%) (7.5-150 ng/mL)	RSD %	LOD (ng/mL)	LOQ (ng/mL)
Aldrin	1.0-250.0	0.9920	62.67-81.90	3,46	0.28	0.95
Dieldrin	1.0-250.0	0.9958	70.67-88.10	6,03	0.29	0.98

Abbreviation: R^2, regression coefficient; RSD, relative standard deviation; LOD, the limit of detection; LOQ, limit of quantification

DISCUSSION

Determination of optimal injector, furnace temperature, and flow rate for the analysis of aldrin, dieldrin, and 4,4-DPB was performed by using standard pesticide mixtures and full scan mode. For base peak ions (m/z), each pesticide was run in full scan mode, and ions were obtained from the mass spectrum. The appropriate m / z selection for each pesticide, which is to be monitored, was carefully made to ensure the specificity and sensitivity of the analysis. The pesticides from the chromatogram were determined by comparing the retention time and 3 target ions (Table 1).

The retention time of the study is very important as the retention time is the key parameter in chromatographic methods to separate and quantify the compounds of interest from the complex sample. With the developed method, the retention times of aldrin and dieldrin in serum are less than 4 minutes, which makes our study faster and more specific than many other studies (24-26).

The calibration functions were linear within Table 2's concentration range which was considered for each pesticide. The R^2 values for the calibration curves indicated excellent linearity and were found to be above 0.99. LOD and LOQ were defined as the concentration of a sample resulting in a peak signal-to-noise ratio. LOD was calculated by taking three times of the noise level, and the LOQ was calculated by taking ten times of the noise level. Calculated LOQ concentrations were sufficient to determine acute and chronic exposure to both aldrin and dieldrin in serum (21, 27, 28).

A recovery study was carried out for low and high concentrations of these compounds to evaluate the efficiency of the method in the study. The recoveries of aldrin and dieldrin at levels of 7.5 and 150 ng / mL were inspected, and the range of their recovery was found between 62.67% to 88.10%. These recoveries are in the acceptable range for multiple residue pesticide analysis and can be used in the routine analysis (29). The precision of the method was also evaluated and expressed as relative standard deviation (RSD%). The RSD values obtained from our method (RSD < 6.03%) were found much lower than the 15% acceptance limit set by the FDA (30).

Validity results of the presented analytical method prove its suitability for biomonitoring of aldrin and dieldrin. It

can be said that the determined validation parameters are the reproducibility of the method, can be successfully applied for the simultaneous determination of aldrin and dieldrin in blood serum samples, and can be used in monitoring human exposure.

CONCLUSION

Since dieldrin is converted in the body after aldrin exposure, it is very important to simultaneously analyze aldrin and dieldrin in serum. Thought this way, both acute and chronic exposure can be detected. This study demonstrates that GC-MS is a powerful analytical technique for the simultaneous analysis of aldrin and dieldrin in serum. Furthermore, our results are important for the advancement of GC-MS methods and the use of the validated method is the basis for monitoring exposure to these substances in serum. The validated method is so fast, specific and reproducible that it can be used to determine the exposure of these pesticides at ppb (ng / mL) level and easily used in routine analysis.

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