Developing A Gas Chromatography-Flame Ionization Detector Analytical Method for the Analysis of Organochlorine Pesticides Residues

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Abstract: This paper presents the development and validation of a GC- FID method for the analysis of some organochlorine pesticides (α-BHC, γ-BHC, Heptachlor, Aldrin, Heptachlor epoxide, Dieldrin and Endrin). Validation was done through evaluation of method detection limits (MDL), linearity and recovery. The results show that, The average recoveries obtained for the studied pesticide ranged from 78.67 to 85.1% at fortification levels of 0.25, 1, 2.5 µg/ml, The calculated method detection limits (MDL) were typically in the range 0.089- 0.048 µg/ml, The correlation coefficients (r^2) were higher than 0.999 with the linear ranging from 0.1 to 5 µg/ml.

Key words: Gas chromatography · Method development · Pesticides · Validation

INTRODUCTION

Organochlorine pesticides (OCPs) are of special interest because of their high chemical stability, which results in their persistence and bioaccumulation in the environment and in animal tissues, increasing the risks to human health such as cancer and disruption of hormonal functions [1-3]. Therefore, the analytical methodology required for detection of these group of compounds should be fast, robust and simple in order to be applied after appropriate validation following quality criteria in routine laboratories [4]. Valid analytical data are essential for satisfactory monitoring and control of pesticide residues. Comprehensive overviews of validation requirements have been published [5-10] which identify many parameters by which method performance may be judged. Other authors have contributed specific statistical and computational techniques to assist with the process of method validation [11-13]. Method validation data may be needed to support accreditation or publication of the method, or to defend results generated from its use. The most common methods currently used to monitor pesticide residues derived from methods developed in the 1960s and 1970s [14,15]. However, due to the increasing cost of sensitive equipment for analysis of chlorinated pesticides (GC-MS, GC-ECD), there is a need for chemists to develop and use more cost-effective procedure to monitor pesticides in laboratories which are not equipped with GC-MS or GC-ECD [16]. Also in well equipped laboratories there is a need for a method to be an alternative to these devices in periods of routine maintenance and faults. So the main purposes of this work were to develop a method for the determination of organochlorine pesticides using gas chromatography equipped with flame ionization detector (GC-FID) and to validate this method through determination of method detection limits (MDL), linearity and recovery.

MATERIALS AND METHODS

Chemicals, Equipments and Tools: The solvents used were dichloromethane, n-hexane and acetone, (HPLC grade, Fisher Chemicals UK). Stock Pesticide standard solutions purchased as certified solutions from Accustandard. Mixture standard M-508P-A of 20 organochlorine pesticides, all are in concentration of 1000µg/ml in MtBE. Seven single standard for α-BHC, γ-BHC, Aldrin, Dieldrin, Endrin, Heptachlor, Heptaclor epoxide, all in concentration of 100µg/ml. Other necessary materials, anhydrous sodium sulfate extra pure and glass wool were purchased from Fisher chemicals.

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Optimization of Gas Chromatography Method: Analysis was carried out using Agilent gas chromatography model 7980 equipped with Flame Ionization Detector (FID). The proposed GC-FID operating condition: injector temperature, 225°C, Detector temperature 300°C, oven temperature program, initial temperature 100°C hold for 2 min., Ramp temperature 15°C/min to 160°C, then 5°C/min to 270°C and hold for 3 min. (EPA 8081a) [17], Column used for separation was *hp*-5, 30m x 0,25 mm I.D., df=0,25 µm, Carrier gas Helium purity 99.999%, Flow rate: He@ 1.2 ml/min 2µl injections of the final extract were auto-injected into the GC system. The operating condition will be optimized to obtain the best chromatogram of studied pesticides by direct injection of standard pesticides at different condition.

Validation of Optimized Method: All validation procedures were performed using extracts of free organics deionized water spiked with organochlorine pesticides (OCPs) standard solution. Method validation study depends on the determination of overall method performance parameters, These are obtained during method development following the in-house validation protocols [13]. Validation of the Gas Chromatographic method for quantitation of organochlorine pesticides will be carried out through determination of method detection limits (MDL), linearity, recovery.

Extraction of Spiked Blanks: Extraction of spikes was carriedout using Liquid/liquid extraction (LLE). LLE is recognized as an popular method for screening tests of unknown pesticides not only because of its simplicity, robustness, minimal operator training, efficiency and a wealth of available analytical data, but also because of its wide acceptance in many standard methods [18, 19]. However, this age-old technique requires large-volumes of high-purity solvents, which are hazardous, yields small sample-to-solvent volume ratios and is time-consuming. Although automation has overcome some of its shortcomings, multistage operation and problems of emulsions formation counteract this capability [20]. About 1 litre of water sample was extracted by shaking in 2 litre separatory funnel with 60 ml of dichloromethane for 1minute. The organic layer is separated through a glass funnel containing 30 grams of anhydrous sodium sulphate (dried at 550°C for 6h). The extraction procedure is repeated two more times with 50 ml of dichloromethane each time. The extract is evaporated using a rotary evaporator and the solvent is exchanged into n-hexane

and adjust the final extract volume to 1 ml using a gentle stream of clean nitrogen. The extract is ready now for GC analysis [21].

RESULTS AND DISCUSSION

Optimization of GC- FID Method: The proposed method was not efficient enough to completely separate all pesticides (20) peaks contained in the used standard solution. The method was modified many times till complete separations of analytes were obtained. The best conditions were as follow: injector temperature, 250°C, Detector temperature 300°C, oven temperature program, initial temperature 80°C hold for 1 min, Ramp temperature 30°C/min to 175°C hold for 4 min, then 3°C/min to 225°C and hold for 6 min. Fig. 1 shows how a slight change in temperature program leads to complete separation of two overlapped peaks. Fig. 2 represents the a typical GC-FID full chromatogram abtained using the modified method, it shows the complete separation of all compounds (20) within 27 miniutes. As shown in the chromatograms, peak shapes for the pesticides were good.

Method Validation: Validation is performed to verify that the method is fit for purpose, which means that the desired commodities and analytes are evaluated to achieve acceptable recoveries, reproducibilities and detection limits [16] Validation of a methodology can involve evaluating many parameters, In this paper the method of organochlorine pesticides determination using gas chromatography equipped with flame ionization detector (GC-FID) was validated through evaluation of method detection limits (MDL), Linearities and Recoveries for seven out of twenty pesticides contained in the mix standard solution these pesticides are α-BHC, γ-BHC, Heptachlor, Aldrin, Heptachlor epoxide, Dieldrin and Endrin.

Calibration and Linearity: Calibration was carried out using 6 concentration levels (0.1, 0.25, 0.5, 1, 2, 5) µg/ml of organochlorine pesticides mix standard solution containing the seven analytes. Linearity was evaluated by calculation of a six-point linear plot with three replicates, based on linear regression and squared correlation coefficient, r^2, which is >0.9990. The studied seven pesticides had a linear range from 0.1 to 5µg/mL. Calibration curve for each analytes are presented in Fig. 3 and the results are summarized in Table 1.
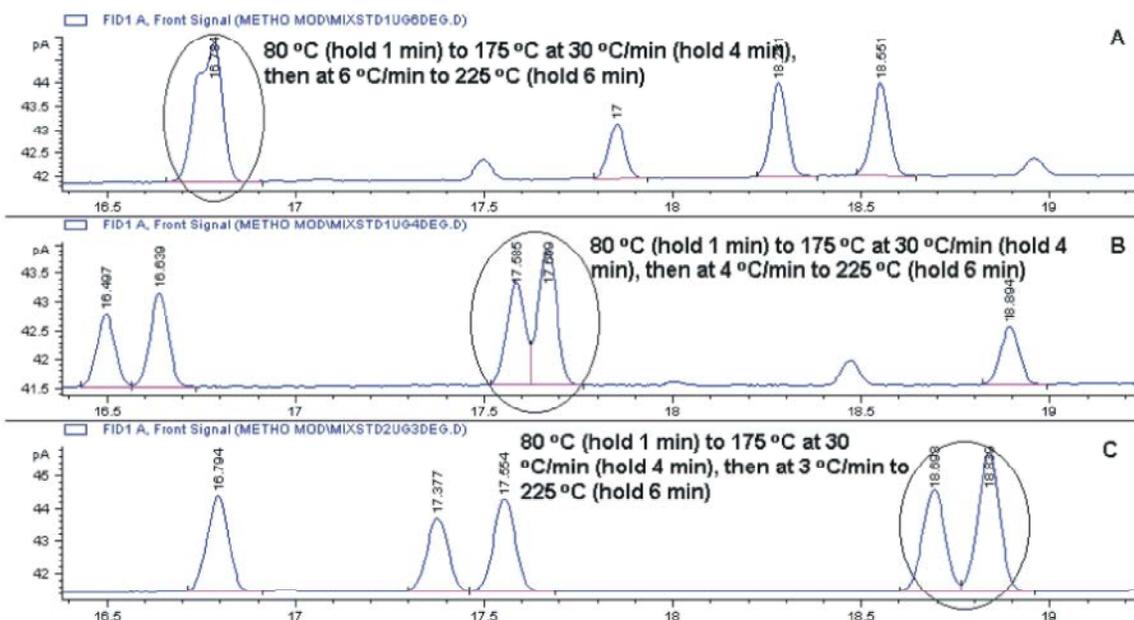
Fig. 1: Two overlapped peaks were separated by different heating rates, chromatogram A, ramp 6°C/min, chromatogram B, ramp 4°C/min, chromatogram C, ramp 3°C/min
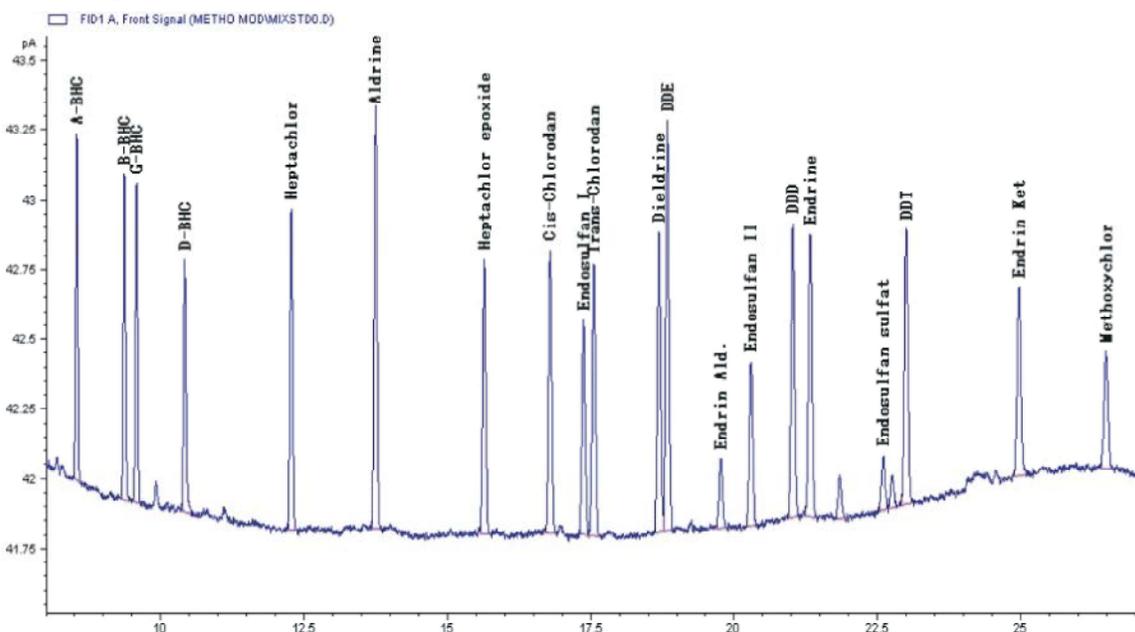

Fig. 2: Typical GC-FID chromatogram represent complete separation of 20 organochlorine pesticides in a mixture standard (1µg/ml) using the modified method

Method Detection Limits (MDL): Method Detection Limits (MDL) were calculated as three times the standard deviation of seven blanks spiked with pesticides mix standard (7 compounds) at low level concentration (0.2µg/l). Detection Limit were calculated according to IUPAC Recommendation [22, 23]. Values obtained are shown in Table 1. All of the extraction and clean-up procedures were applied to these seven spiked samples. The MDL values obtained are below the first calibration level.

Recovery: A typical organochlorine pesticides mix standard was analyzed by the method under validation (GC-FID) both in its original state and after the addition
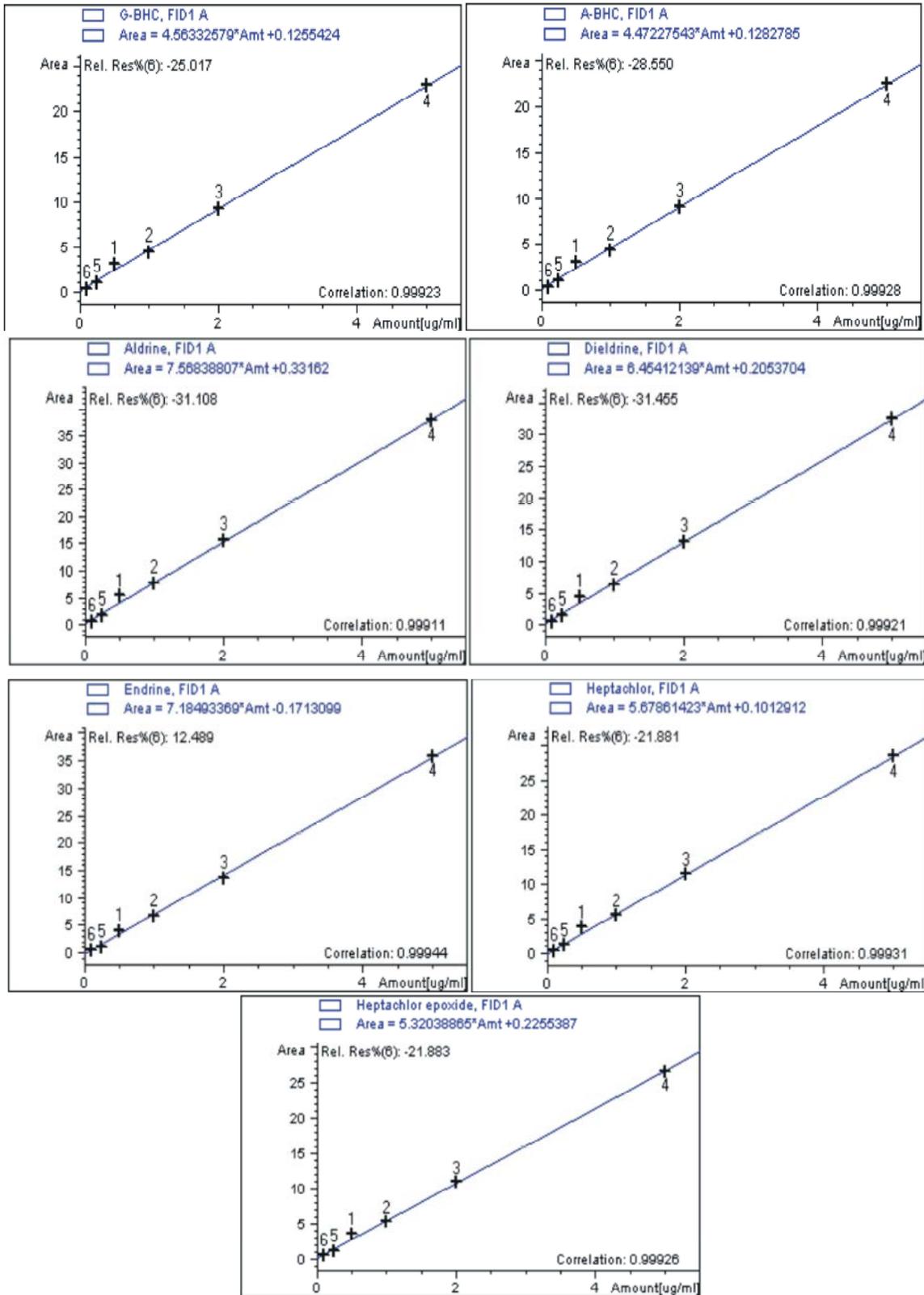
Fig. 3: Six level calibration curves for seven organochlorine pesticides using GC-FID method

Table 1: GC-FID Method Detection Limits (MDL) and squared correlation coefficient (r^2) and linear range for studied organochlorine pesticides

Pesticides	Linear range µg/ml	r^2	MDL (µg/ml)
α-BHC	0.1 – 5	0.99928	0.089
γ-BHC	0.1 – 5	0.99923	0.061
Heptachlor	0.1 – 5	0.99931	0.059
Aldrin	0.1 – 5	0.99911	0.051
Heptachlor epoxide	0.1 – 5	0.99926	0.055
Dieldrin	0.1 – 5	0.99921	0.048
Endrin	0.1 – 5	0.99944	0.057

Table 2: Mean recovery and relative standard deviations (RSD) of seven organochlorine pesticides in eight spikes at three levels (0.25, 1, 2.5 µg/ml)

Pesticide	0.25 µg/ml		1 µg/ml		2.5 µg/ml	
	Mean recovery (%)	R.S.D. (%)	Mean recovery (%)	R.S.D. (%)	Mean recovery (%)	R.S.D. (%)
α -BHC	81.34	3.71	85.07	2.25	84.33	3.87
γ -BHC	80.43	2.86	84.4	3.70	82.92	3.68
Heptachlor	78.67	2.71	80.96	1.95	82.22	2.52
Aldrin	81.22	3.78	82.68	3.56	83.29	2.13
Heptachlor epoxide	82.28	3.54	81.45	4.34	84.68	2.30
Dieldrin	81.63	3.7	83.38	3.69	83.51	3.82
Endrin	82.01	2.84	83.05	3.57	85.1	1.83

(spiking) of a known mass of the analyte to deionized free organic water. The difference between the two results as a proportion of the mass added is called the recovery [24]. The spiking was performed at three fortification levels (0.25, 1 and 2.5µg/l). These three levels also represented the values at low, middle and high parts of the linear range. The recovery of each pesticide at each fortification level was evaluated. Eight liters of free organic deionized water were spiked with pesticides at each fortification level at the same day. Mean recoveries data and relative standard deviations (RSD.) obtained are given in Table 2. The high relevance of such analytical data led to the harmonization of quality control criteria with the support of the European Commission with a view to ensuring reliable and comparable results. Mean recoveries within 70–110% are considered satisfactory [4, 25]. From the recovery study presented in Table 2, The recoveries obtained ranged from 78.67 to 85.1% the results are comparable to or better than those provided by existing methods for the determination of these compounds in water samples.

CONCLUSION

The method for analysis of organochlorine pesticides using gas chromatography equipped with flame ionization detector (GC-FID) was successfully modified for separation of twenty organochlorine pesticides in a run of about 27 minutes. In this research paper seven pesticides (α-BHC, γ-BHC, Heptachlor, Aldrin, Heptachlor epoxide,

Dieldrin and Endrin) were successfully validated at three fortification levels (0.25, 1, 2.5 µg/ml) The recoveries obtained ranged from 78.67 to 85.1%. The calibration curves were linear in the range (0.1-5 µg/ml) the evaluation was based on the squared correlation coefficient, r^2, which was >0.9990. The method detection limits (MDL) were typically in the range (0.089-0.048 µg/ml) for the pesticides included in the study. Finally, The results obtained indicate that the methodology developed could be a successful alternative for laboratories to measure organochlorine pesticides.

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