Method development for the influence of matrix on selected organochlorine pesticide residue analysis in surface water by GC-MS

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Abstract

An automated solid phase extraction (SPE) method coupled to GC-MS method for the analysis of selected organochlorine pesticides was developed and validated for the purposes of studying the matrix effects. The analytical method showed a significant degree of validity when tested against parameters such as linearity, repeatability and sensitivity. Four different reversed sorbent phases, including a Supelco LC18, Strata C18-E and Strata-X (styrene divinyl benzene) were tested for organochlorine extraction efficiency. The LC-18 proved to be the most robust and effective sorbent phase as it produced better recoveries varying from 90-130% for most analytes. It was then concluded that the method developed was suitable for further research towards the influence of the matrix on selective determination of the selected organochlorine pesticides.
Keywords: solid phase extraction (SPE), organochlorine pesticides, gas chromatography-mass spectrometry (GC-MS).

1 Introduction

In order to fully understand the principles behind the matrix effects in GC-MS analysis, it is of paramount importance to develop a robust and rugged validated analytical method that shows a high degree of specificity for each particular

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analyte. Validation gives an indication of the trueness of an analytical method, this will help to distinguish between the effects of the matrix on the analyte from those that may arise from random or systematic errors in the process sample handling and preparation (Quintana *et al.* [1]). The trueness of a method should always be assessed before it is applied for any routine sample analysis (Maroto *et al*. [2]). Both the amount and type of matrix can affect the perceived recovery therefore it is important to effectively isolate analytes of interest from the matrix components as even the tandem (MS/MS) mode is prone to detect co-extracted matrix components (Maroto *et al.* [2]; Poole [3]; Fenich *et al.* [4]; Ruiz-Gutierrez and Perez-Camio [5]).

Solid phase extraction (SPE) is a sample preparation technique used for extracting semivolatile and non-volatile analytes from their matrices for subsequent chromatographic analysis. It is considered one of the most powerful techniques currently available for isolating trace amounts of organic compounds such as pesticides from water and other environmental samples (Saini *et al.* [6]; Ferrer and Barcelo [7]; Gulbakan *et al.* [8]). It entails the use of SPE cartridges which are packed with silica bonded to a particular analyte adsorbing phase. SPE can be compared to other extraction techniques like liquid-liquid extraction although it is advantageous in that it provides better selectivity and extraction efficiency (recovery), eliminates problems associated with incomplete phase separation and yields quantitative extractions that are easy to perform (Supelco [9]; Nema *et al.* [10]; Marce and Borurull [11]). Selectivity is the degree to which an extraction technique can separate the analyte of interest from its matrix (Ferrer and Barcelo [7]).

The SPE method development is targeted at developing a methodology that is specific to the analytes of interest (Verpiand *et al.* [12]). Accordingly, the analytes of interest in this research are the following organochlorine compounds: Pentachlorobenzene, BHC-alpha, Hexachlorobenzene, BHC-beta, Lindane (BHC gamma), Pentachloronitrobenzene, BHC-delta, Heptachlor, Aldrin, Heptachlor-epoxide, Chlordane trans (gamma), Endosulfan (I) alpha, Chlordane cis (alpha), Dieldrin, 4,4-DDE, Endrin, Endosulfan (II) beta, 4,4-DDD, Endosulfan sulphate, 4,4 DDT and Mirex.

2 Materials and methods

Grade A volumetric flasks and pipettes, funnels, spatula and Pasteur pipettes were used for reagent preparation. Methanol, Dichloromethane, Toluene, Acetone, Hexane, SPE cartridges, collection vials, 2ml vials, caps, inserts, test tubes and nitrogen gas were also used in the SPE method development. A Mettler Loledo AX105 Delta Range® analytical balance was used to weigh the standards to four decimal places.

3 Quality control

All volumetric flasks and pipettes were calibrated before use. Analytical balances were calibrated annually and verified daily using standard reference

masses. Grade A volumetric glassware and analytical (pesticide) grade reagents were also used for the entire analysis with a purity >99%. All cartridge testing for SPE method development was done in at least duplicate analysis. Deionised ultrapure water was sourced from a Millipore Milli-Q system. The water was passed through an organic compound scavenger resin bed before passing to the Milli-Q system. The certified pesticide neat standards had a purity of at least 98.5% (obtained from Dr Ehrenstorfer and Chemservice) and 100mg/l stock solution and subsequent cocktails were prepared in toluene and stored at ≤-18°C. Spiking solutions were prepared in acetone. Temperatures for the laboratory atmosphere and freezers were monitored daily.

4 GCMS configuration

An Agilent Technologies 6890 GC coupled to an Agilent Technologies 5975 Quadrupole Mass Selective Detector was used for analysis using a 30 m x 0.25mm x 0.25µm DB-5MS column with stationary phase 5% phenyl and 95% dimethylpolysiloxane. The mobile phase of choice used was 99.999% helium gas supplied by Airliquide South Africa.

Total runtime for the analysis was 31.87 minutes with initial temperature of 70°C and hold time of 2 minutes. Ramp 1 was 25°C/ min to 150°C, with no hold time. Ramp 2 was 3°C/min to 200°C, with no hold time and ramp 3 was 8°C/min to 280°C with no hold time. A constant pressure of 129.9 KPa was maintained with an average velocity of 50 cm/second. Data was analysed using chemstation software from Agilent Technologies. A 1 µl volume of sample was injected using an ALS autosampler.

5 Peak identification

To identify the peaks of interest, 10mg/l (ppm) neat standards were injected to determine the retention time for each analyte. To increase the specificity of the analytical method, particularly in the presence of a matrix, Selective Ion Monitoring (SIM) mode was configured into the GCMS. An average of 4 major ion fragments from each analyte were selected for use in identification of the compounds, using criteria of a balance between the highest mass and abundance, since each compound has a specific ion spectrum.

All the peaks from the 1mg/l cocktail mix having been identified, calibration standards were then made up by serial dilution for validating the GCMS instrument method, using the calibration levels: 1mg/l, 0.5mg/l, 0.25mg/l, 0.125mg/l, 0.0625mg/l, 0.0313mg/l, 0.0156mg/l and 0.0078 mg/l. The 1mg/l cocktail was also used to test SPE cartridges for efficiency of extraction and determination of validation criteria for the SPE method.

6 Solid phase extraction

The solid phase extraction was performed using an automated instrument, the Gilson GX-271 liquid handling instrument. Sample preparation is the most

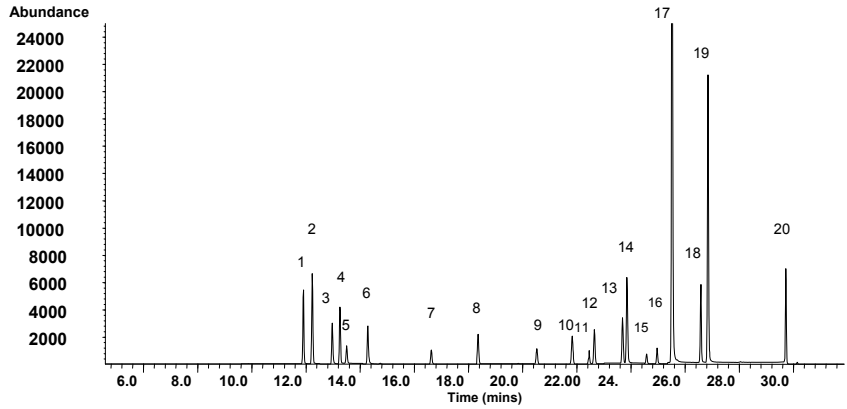

Figure 1: SIM chromatogram of organochlorine cocktail.

tedious and time consuming step and a possible source of errors (Huck and Bonn [13]; He *et al.* [14]). Automated solid phase extraction was employed as it is more rapid, precise and accurate compared to the conventional manual SPE extraction (Parker *et al.* [15]; Rossi and Zhang [16]).

6.1 SPE cartridges used

6.1.1 Strata-X (500mg)

The Strata-X is a reversed phase bed cartridge suitable for the extraction of polar and non polar analytes with hydrocarbon and aromatic groups which form a surface modified styrene divinyl benzene group. This gives it an advantage over other sorbents in that it is deconditioning resistant and has better selectivity for polar and non polar compounds (Countryman *et al.* [17]). The styrene divinyl structure also has the advantage of selective interaction with aromatic rings as those in DDT through formation of specific π-π interactions (Ferrer and Barcelo [7]; Marce and Borurull [11]). Reversed phase cartridges are frequently used in environmental chemistry to extract organic substances from aqueous samples such as water (Saini *et al.* [6]). The cartridge contained a bed mass of 500mg and is suitable for the analysis of Organochlorine compounds and the functional group is displayed below in figure 2.

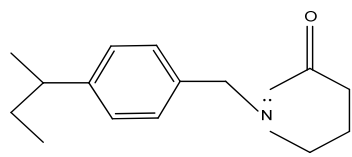

Figure 2: Functional group of Strata-X.

6.1.2 LC-18 (Supelco) (200mg)

The LC-18 (Supelco) cartridge consists of an octadecyl bonded endcapped silica reversed phase bed. It is suitable for non polar to moderately polar compounds such as the organochlorine compounds under study. The hydrophilic silanol groups at the surface of the raw silica packing have been chemically modified with hydrophobic alkyl or aryl functional groups by reaction with the corresponding silates (Supelco [9]). The functional group is displayed below in figure 3.

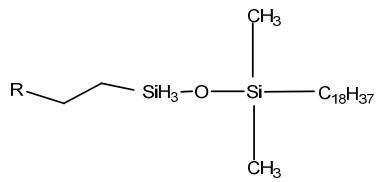

Figure 3: Functional group of LC-18 (Supelco).

6.1.3 Strata C18-E (200mg) and Strata C18- E (500mg)

The Strata C18-E (S C18-E) cartridge used was a reversed phase absorbent with a hydrocarbon and aromatic functional group. Its retention mechanism is through hydrophobic interactions, hydrogen bonding and aromatic interactions (Pavlovic *et al.* [18]; Li *et al.* [19]; Fontanels *et al.* [20]). A 200mg and a 500mg sorbent bed mass were used for the method development, particularly to test the effect of increasing the sorbent bed mass on analyte retention. The two functional groups of the Strata C18- E cartridge sorbent bed shown below in figure 4;

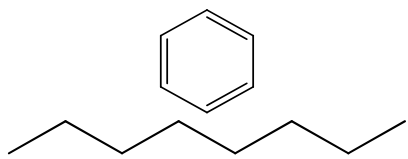

Figure 4: Functional groups of Strata C18- E.

6.2 SPE test procedure

The automated SPE method development was designed to configure the best procedure to use for the extraction of the organochlorine compounds under study. The test procedure considered the following parameters;

1) Sorbent (solid phase) choice
2) Sorbent treatment
3) Sorbent mass
4) Sample pre-treatment
5) Sample volume and flow mechanism
6) Solvent choice

The Gilson GX-271 liquid handling instrument is capable of performing the following cartridge treatment steps:

1) Conditioning cartridges: this is done to activate the sorbent bed
2) Loading cartridges: this is done to introduce the analytes to the sorbent bed where they are adsorbed.
3) Drying cartridges: this is done to remove any water remaining on the sorbent bed and prevent the introduction of water to the elute.
4) Eluting cartridges: this is the process of desorbing the adsorbed analytes by adding a polar solvent that washes the analytes from the sorbent bed (Poole *et al.* [21]).

For effective mass transfer of analytes onto the sorbent a consistent flow rate applied at low pressure was used (Nema *et al.* [10]). The Gilson GX-271 liquid handling instrument utilises positive pressure elution which makes it increasingly easy to control flow rates (Gilson [22]). Extensive cleanup of extracts may result in the partial loss of some compounds, hence this method development was aimed at retaining as much analyte as possible within the final extract (Hajslova *et al.* [23]).

7 Results and discussion

7.1 GCMS instrument method validation

Method validation is essential as it confirms that an analytical method is effective in measuring the parameters it is intended to measure. Successful validation of the GC-MS method developed confirmed that the methods, procedures and protocols applied in the analysis were reliable and accurate and as a result valid conclusions were postulated.

7.2 Validation parameters

For the purposes of method validation the parameters tested were linearity, working range, repeatability, limits of detection, limits of quantitation and analysis of variance (ANOVA) as shown in Table 1.

The table below shows the calibration range in which acceptable accuracy, linearity and precision can be obtained.

Table 1 also displays the linear regression for the calibration curves, repeatability, Limits of Detection (LOD) and Limits of Quantification (LOQ). However, from the table above DDT showed no significant linearity and therefore a quadratic fit was used. BHC-delta and Lindane (BHC gamma) showed the lowest LOD's with DDE 4, 4', and Endosulfan beta showing the broadest linear ranges of 1mg/l to 0.0156 mg/l.

Hypothesis testing was done using the analysis of variance (ANOVA) F-test with the null hypothesis H_0= there is no significant linearity in the selected organochlorines at a 95% confidence level.

Table 1: Validation parameters for selected organochlorine compounds (n = 11).

Compound Name	Linear Range(mg/l)	R^2	Repeatability (RSD%)	LOD (mg/l)	LOQ (mg/l)
Aldrin	1-0.0313	0.9990	6.45	0.038	0.125
BHC-alpha	1-0.125	0.9984	9.63	0.062	0.205
BHC-beta	1-0.0313	0.9985	3.92	0.060	0.201
BHC-delta	0.5-0.0313	0.9980	4.59	0.018	0.060
Chlordane cis (alpha)	1-0.0313	0.9995	3.22	0.048	0.161
Chlordane, trans (gamma)	1-0.125	0.9993	3.23	0.049	0.162
DDD 4,4'	1-0.125	0.9982	5.93	0.083	0.277
DDE 4,4'	1-0.0156	0.9993	4.31	0.068	0.228
DDT 4,4'	Non linear	Non linear	5.25	0.078	0.261
Dieldrin	0.5-0.0313	0.9999	3.75	0.025	0.082
Endosulfan alpha	1-0.0313	0.9986	4.23	0.047	0.155
Endosulfan beta	1-0.0156	0.9990	2.65	0.019	0.063
Endosulfan SO4	1-0.25	0.9988	7.17	0.027	0.091
Endrin	0.5-0.125	0.9994	5.81	0.021	0.071
Heptachlor	0.5-0.125	0.9980	5.17	0.037	0.123
Heptachlor-epoxide	0.5-0.0156	0.9991	4.39	0.029	0.095
Hexachloro-benzene	0.5-0.0625	0.9983	2.75	0.023	0.077
Lindane	0.5-0.0313	0.9982	4.20	0.018	0.059
Mirex	1-0.125	0.9995	4.41	0.056	0.187

n= number of replicates.

The decision rule used: If F-calculated > F-critical = reject H_0, led to the decision that there was significant linearity for all Organochlorine compounds.

8 Results of real sample and blank analysis

The matrix is a burden on pesticide residue analysis (Poole [3]). Unfortunately, it is impossible to completely eliminate the matrix from a real sample matrix in order to isolate the analyte of interest. Dedicated SPE application techniques have been developed to give extracts with comparatively low matrix burden but several problems still arise in GC-MS pesticide residue analysis of the matrix based extracts (Poole [3]). One set of the four test cartridges was loaded with

10ml spiked real samples (s) and another set with 10 ml blank deionised water samples (b) using the following developed optimised conditions.

1) Condition 6ml methanol with flow rate 6ml/min.
2) Load 10ml sample with flow rate 1.5ml/min.
3) Dry using nitrogen gas for 2 minutes with flow rate 6ml/min.
4) Elute with 6ml DCM with rate 1.5ml/min.

The results below indicate that the LC-18 cartridge produced the best real sample recoveries which were acceptable for most analytes as they were in the target range of 100±30% recovery.

Figure 5 also shows that the real sample matrix samples seemed to exhibit matrix induced enhanced chromatographic effect as all but one of the analytes produced recoveries greater than 100%.

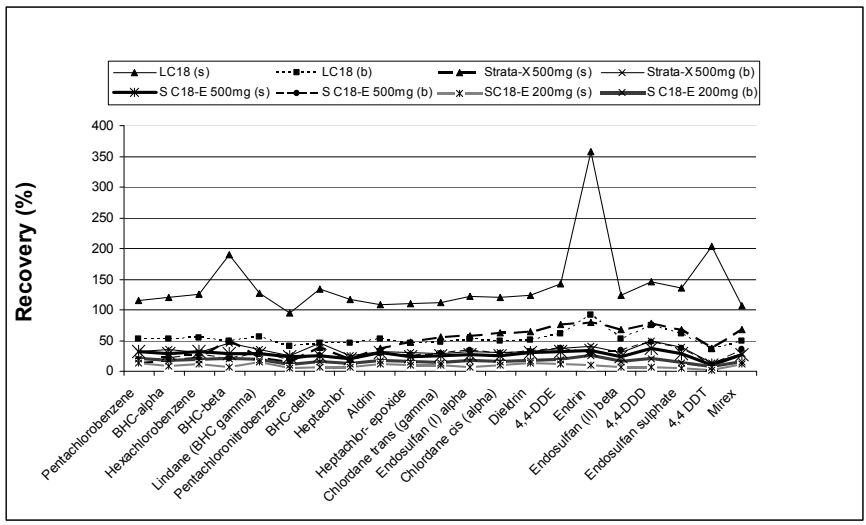

Figure 5: Recovery of spiked real sample and blank water.

Other cartridges produced recoveries of less than 100% for both the real sample and blank determinations. This indicates that there was a matrix induced diminished chromatographic response. It is not unusual to obtain recoveries as high as >200% in pesticide residue analysis in the presence of a real sample matrix as many labs worldwide have documented such cases (Hajslova *et al.* [23]).

9 Conclusions

The method developed for the analysis of the selected organochlorine pesticides showed a significant degree of validity in terms of trueness. Most validation criteria such as repeatability, linearity, sensitivity and ANOVA were met. The

LC-18 cartridge proved to be the most robust for the analysis of the selected organochlorine pesticides as it produced better recoveries overall, even when significant changes were made to the sample preparation procedure. The method development has clearly shown that the matrix does have an effect on the quantitation and detection of analytes although the effects of the matrix were not the focal point of this paper. Automation of SPE has proved to be an important aspect of SPE analysis and is equally as beneficial as the use of a GC automatic sample injector in terms of drastically increasing precision and accuracy and also substantially reducing the chances of human error. However it should be noted that the issue of defining acceptable recoveries remains a controversial and subjective issue where matrix based extractions are involved.

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