Influence of Matrix Effect on Selected Organochlorine Pesticide Residues in Water from the Jukskei River Catchment: Gauteng, South Africa

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DEDICATION

This study is dedicated to my parents, Mr R. C. and Mrs B. Rimayi.
ABSTRACT

One of the major problems encountered in qualitative and quantitative determination of residual pesticides by gas chromatography is the matrix effects. Matrix components have a considerable effect on the way analysis is conducted and the quality of results obtained, introducing problems such as inaccurate quantification, low analyte detectability and reporting of false positive or even false negative results. It was aimed to develop and validate a suitable method for counteracting the matrix effects so as to improve the detection and quantification of selected organochlorine pesticide residues from real water samples. The real water samples used were sampled from three points along the Jukskei River catchment area in Gauteng, South Africa for a period of 7 months from January to July 2010 so as to create a representative sample.

An automated solid phase extraction (SPE) method coupled to Gas Chromatography-Mass Spectrometry (GC-MS) method for the analysis of 20 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. Endosulphan beta, 4,4’ Dichlorodiphenydichloroethane, and Heptachlor-epoxide had the broadest linear calibration ranges of 1 ppm - 0.0156 ppm. Benzene hexachloride (BHC) delta and Lindane had the lowest statistical limits of detection of 0.018 ppm. Statistical hypothesis testing indicated that there was significant linearity in all selected organochlorine calibration curves.

Four different reversed sorbent phases, including LC18, SC18- E and Strata-X (styrene divinyl benzene) were tested for organochlorine retention efficiency. The LC-18 200 mg cartridge proved to be the most robust and effective sorbent phase as it produced better recoveries varying from 90-130% for most analytes. A breakthrough volume of 100 mL for the LC-18 200 mg cartridge was determined using an optimum matrix load curve. 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.

Four different calibration methods, namely matrix-free external standard, matrix-matched external standard, matrix-free internal standard and matrix-matched internal standard were applied to test the efficiency of computing recoveries. All calibration curves for the 20 organochlorine pesticides showed significant linearity > 0.99 when plotted on both Chemstation and Excel. The calibration methods were tested on three different matrices composed of a high sample matrix (synthetic matrix), a low sample matrix (real sample matrix) and a no sample matrix (ultrapure water).

Statistical hypothesis testing led to the decision that there are significant differences between the mean recoveries of the three water sample matrices and also that the differences in the mean recoveries of the three sample matrices are independent of the both the two calibration techniques (internal standard and external standard) and calibration types (matrix-matched and matrix-free) applied. This led to the overall conclusion that the matrix effects have an overwhelming influence on the selective determination of the selected organochlorine pesticides.
# List of Abbreviation and Symbols

**Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>GC</td>
<td>Gas Chromatography</td>
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<tr>
<td>GC-MS</td>
<td>Gas Chromatography-Mass Spectrometry</td>
</tr>
<tr>
<td>MSD</td>
<td>Mass Spectrometric Detector</td>
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<tr>
<td>SIM</td>
<td>Selective Ion Monitoring</td>
</tr>
<tr>
<td>EI</td>
<td>Electron Impact Ionisation</td>
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<tr>
<td>m/z</td>
<td>Mass to charge ratio</td>
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<tr>
<td>amu</td>
<td>Atomic mass units</td>
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<tr>
<td>HRMS</td>
<td>High Resolution Mass Spectrometry</td>
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<tr>
<td>TOF</td>
<td>Time Of Flight</td>
</tr>
<tr>
<td>GCXGC</td>
<td>Two dimensional Gas Chromatography</td>
</tr>
<tr>
<td>FT</td>
<td>Fourier Transform</td>
</tr>
<tr>
<td>POP</td>
<td>Persistent Organic Pollutant</td>
</tr>
<tr>
<td>OC</td>
<td>Organochlorine pesticide</td>
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<tr>
<td>SPE</td>
<td>Solid Phase Extraction</td>
</tr>
<tr>
<td>LLE</td>
<td>Liquid-Liquid Extraction</td>
</tr>
<tr>
<td>BHC</td>
<td>Benzene Hexachloride</td>
</tr>
<tr>
<td>DDD</td>
<td>Dichlorodiphenyldichloroethane</td>
</tr>
<tr>
<td>DDE</td>
<td>Dichlorodiphenyldichloroethylene</td>
</tr>
<tr>
<td>DDT</td>
<td>Dichlorodiphenyltrichloroethylene</td>
</tr>
<tr>
<td>PCNB</td>
<td>Pentachloronitrobenzene</td>
</tr>
<tr>
<td>PAH</td>
<td>Poly Aromatic Hydrocarbon</td>
</tr>
<tr>
<td>IUPAC</td>
<td>International Union of Pure and Applied Chemistry</td>
</tr>
<tr>
<td>%</td>
<td>Percent</td>
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</table>
°C  Degrees Celsius
min  Minute
µL  Microlitre
mL  Millilitre
mg  Milligram
mg/L  Milligram per litre
ppm  Part per million
KPa  KiloPascals
USEPA  United States Environment Protection Agency
USFDA  United States Food and Drug Agency
QC  Quality Control
SPSS  Statistical Package for the Social Sciences
ANOVA  Analysis of Variance
MANOVA  Multivariate analysis of Variance
HSD  Tukey’s Honestly Significant Difference
DF  Degrees of Freedom
Fcal  F-calculated value
Fcrit  F-critical value
Sig.  Level of significance
N  Number of variables
α  Error
STD  Standard
CI  Confidence interval
H₀  Null hypothesis
Hₐ  Alternative hypothesis
NIST  National Institute of Standards and Technology
RM  Reference Material
SRM  Standard Reference Material
CRM  Certified Reference Material
RF  Response factor
CF  Calibration Factor
IS  Internal Standard
MM  Matrix-Matched
MF  Matrix-Free
MFEXTSTD  Matrix-Free External Standard
MMEXTSTD  Matrix-Matched External Standard
MFIS  Matrix-Free internal Standard
MMIS  Matrix-Matched Internal Standard
LOD  Limit Of Detection
LOQ  Limit Of Quantification
RSD  Relative Standard Deviation
R²  Coefficient of Regression
S/N  Signal to Noise ratio
ISO  International Standardisation Organisation
RSD  Relative Standard Deviation
DCM  Dichloromethane
Mg/L  Milligram per litre
≥  Greater than or equal to
>  Greater than
≤  Less than or equal to
<  Less than
±  Plus or minus
T  Target ion
Q  Qualifier ion
C  Calibration curve
µ₁  High sample matrix mean
µ₂  Low sample matrix mean
µ₃  No sample matrix mean
µ_MM  Matrix-matched standard mean
µ_MF  Matrix-free standard mean
µ_IS  Internal standard mean
µ_EXTSTD  External standard mean
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CHAPTER 1

INTRODUCTION
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1.1 Background of the study

Generally, the sample matrix is believed to have some effects on the detection and quantification of target analytes. In analytical chemistry, matrix refers to components of a sample other than the analyte of interest and in Gas Chromatography (GC) analysis such as the analysis of residual organochlorine pesticide residues, matrix components may cause serious problems with respect to quantification and detection of analytes [1]. The aim of GC is to achieve the resolution of compounds of a given sample in the shortest possible time. GC is finding ever wider use in pesticide analysis [2]. The most used detectors in GC are the Electron Capture Detector (ECD), Nitrogen-Phosphorous Detector (NPD), Flame Photometric Detector (FPD) and most recently the Mass Spectrometer Detector (MSD) is fast becoming the detector of choice owing to its ability to effectively identify chromatographed compounds based to their mass to charge ratios (m/e) [3]. MSDs, particularly those with specific ion monitoring provide higher specificity due to their ability to give a detailed reflection on the molecular structure of a particular compound. Gas chromatography-Mass Spectrometry (GC-MS) is considered the most powerful analytical technique for quantitative analysis due to its inherent high sensitivity and selectivity. Common MSD's such as the quadrupole and the ion trap can however fail to provide unbiased confirmation about an analyte's identity [4].

The introduction of pesticides into the environment is considered a risk for human health due to the toxicity of the pesticides [1]. Semi-volatile persistent organic pollutants have been detected in natural waters from a variety of different regions. These include organochlorine pesticides such as Dichlorodiphenyltrichloroethane (DDT) and Benzene hexachlorides (BHC). Organochlorines have very low solubilities in water, are fat soluble and are resistant to metabolism [5]. Multiple determination of pesticides nowadays is applied to separate groups of semi-polar and thermally unstable contaminants with a wide range of physico-chemical properties within the same run in a relatively short period of time [6]. Multi-residue analysis has been identified as a cost effective and labour saving method for determination of a wide range of analytes within
a single run, but obtaining optimum recoveries for all analytes is practically impossible [7]. The analysis of pesticides in water samples involves isolation of the analytes from the sample matrix, removing the bulk co-extracts from the crude extract, identification and quantification of the pesticides.

Most analytical methods are based on liquid partitioning with organic solvents such as dichloromethane. Over the years new extraction techniques such as solid phase extraction have been developed to overcome the drawbacks caused by high amounts of glassware and toxic solvents in the classical liquid extraction methods [2]. Despite the increasing success of GC-MS, reports of matrix susceptibility have shown the limitations of this powerful analytical technique. Matrix components, which are unavoidably present in analysed samples, may be responsible for adverse effects impairing different stages of the GC-MS determinative step. The matrix effects therefore have to be investigated during the early development of any GC analytical method [8].

It is well known that matrix effects can seriously degrade the accuracy of GC-MS analysis results. The matrix effects can be defined as the effect of co-eluting residual matrix components on the ionisation of the target analytes [9]. They result in either signal suppression or enhancement. The matrix effect strongly depends on the nature of the analyte and on the properties of the co-eluting compounds, as some of the co-eluting compounds elute as chromatographic peaks and cause ionisation efficiency change only in a limited retention time range. The specific mechanism of the matrix effects is still not fully resolved [7].

Methods of taking the matrix effect into account have been studied but they do not necessarily reduce its influence [10]. Accounting for the matrix effects in principle can lead to corrected results, but for methods which undergo stronger ionisation suppression, its efficiency is limited [11]. Furthermore, since the nature and amount of these co-eluting compounds are usually variable between samples, the matrix effects can be highly variable and difficult to predict, making it difficult to compensate for them in practice [12]. Whilst different techniques can be applied to compensate for the matrix
effects and produce quantitatively accurate results, the loss in method sensitivity that is accompanied by signal suppression and the variability in method sensitivity that occurs between samples cannot be eliminated [7].

There are several commercially available robotic laboratory automation systems which serve to minimise and manage the matrix interferences by performing purification and extraction protocols. Matrix effects are however complex and system specific as each sample presents different management challenges and each analytical method is affected differently by the matrix components [13]. The most obvious way to reduce the matrix effects is to reduce the amount of matrix components entering the chromatographic system. An alternative strategy to reduce matrix effects is their compensation using appropriate calibration methods such as matrix-matched standards, standard addition method and the use of isotopically labelled calibration standards. Another alternative method is the use of analyte protectants which block the active sites in the injector [2].

For a solution, the concept of the matrix includes not only the compounds available naturally, but also those that may be present or added to the solution as part of the preparation because their nature and concentration may influence the intensity of the analyte signal. Two main types of matrices are a real sample matrix and a synthetic matrix [14].

A real sample matrix consists of a matrix and analytes that have combined with each other in nature and hence are naturally incorporated into the matrix of interest. They are also known as co-extracted matrix components. A real sample matrix is mainly made up of humic substances which are the most stable fraction of organic matter in soil and water and can persist for thousands of years [15]. Synthetic matrices are also called fortified matrices and consist of a matrix and analytes that have been combined together in a laboratory type production process rather than a natural setting [16]. Both types of matrices are useful and provide information that is critical to a successful
accreditation and certification program, although there is a strong debate about which type of matrix provides the best information about the analytical test [16].

1.2 Justification

In any analytical research, the matrix effect is always present in real samples and its reduction is one of the most challenging aspects of the method development process. The importance of this research is to investigate and understand the matrix effect phenomenon on GC-MS analysis of selected residual organochlorine pesticides with the aim of understanding and manipulating the mechanism of this phenomenon so as to improve quantification and detection of analytes.

1.3 Background of problem

In GC-MS analysis of residual pesticides, there is a propensity of the analytical system to produce inaccurate quantification, low analyte detectability and high background noise when all instrumental parameters indicate otherwise. Many researchers and laboratories also particularly noticed the trend of a systematic occurrence of reproducibly low recoveries even when all GC-MS parameters were optimally standardized, indicating that there is a factor within the sample that is adversely affecting the results (matrix factor) [16]. This research was mainly focused on organochlorine pesticides, testing different kinds of matrices and calibration techniques on their detection and quantification and also exploring the use of matrix-matched standards to address these highlighted problems.

1.4 Aims and Objectives

The study aimed to produce data of sufficient quality to be able to meet the following research goals:
• To highlight and evaluate the role of the matrix effect in GC-MS pesticide residue analysis.
• To develop and establish a suitable method to handle the matrix effects in pesticide residue analysis with GC-MS.
• To determine the validity and significance of the matrix effects.
• To determine the significance of the matrix type.
• To investigate the use of matrix-matched standards versus matrix-free standards.
• To investigate the use of the internal standard and external standard calibration methods.

All research data collected underwent rigorous quality assurance review, which assessed, among other things, accuracy, precision, bias, representativeness, completeness, and comparability.

1.5 Precision, Accuracy, and Bias

Precision is the degree of agreement between replicate analyses of a sample under identical conditions and is a measure of the random error associated with the analysis, usually expressed as Relative Percent Difference (RPD) or Relative Standard Deviation (RSD). Accuracy is the measure of the difference between an analytical result and the true value, usually expressed as a percentage. The accuracy of a result is affected by both systematic errors (bias) and random errors (imprecision). Bias is the systematic or persistent distortion of a measurement process that causes errors in one direction. The precision, accuracy, and bias for the data collected were evaluated by one or more of the following quality control (QC) procedures:

• Analysis of various laboratory QC samples such as method blanks, matrix spikes, certified reference materials, duplicates/triplicates and positive and negative controls.
• Collection and analysis of field replicate samples. Field replicate results should exhibit a relative percent difference less than 150% in order for the evaluation of the spatial and aerial chemical concentrations to be meaningful.

1.6 Representativeness

Representativeness expresses the degree to which sample data accurately and precisely represents a characteristic of a population, parameter variations at the sampling point, or an environmental condition. Laboratory representativeness was achieved by proper preservation and storage of samples along with appropriate subsampling and preparation for analysis.

1.7 Comparability

Comparability is a qualitative parameter expressing the confidence with which one data set can be compared with another. This goal was achieved through using standard techniques to collect and analyze representative samples, along with standardized data validation and reporting procedures.

1.8 Scope

The scope of this research was limited to:
• 20 organochlorine pesticides which was sufficiently manageable by the researcher.
• GC-MS analysis so as to ensure more robust results on the matrix effects, by eliminating inter-instrument uncertainty.
• Water sample analysis so as to obtain a more uniform matrix and also to eliminate inter-sample uncertainty when computing and comparing analytical results.
1.9 Hypotheses

- There is no significant difference between real sample matrix and matrix-free analysis of residual organochlorine pesticides by GC-MS.
- There is no significant difference between real sample matrix and synthetic sample matrix analysis of residual organochlorine pesticides by GC-MS.
- There is no significant difference between the use of matrix-free standards and matrix-matched standards for calibration.
- There is no significant difference between external standard calibration and internal standard calibration.

1.10 Research Plan

This research is an experimental design and involved analytical method development, collection of water samples from three points along the Jukskei River catchment area in South Africa for laboratory analysis. The data obtained was statistically evaluated and validated by analysis of variance (ANOVA) using Statistical Package for the Social Science (SPSS).
References


