KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

COLLEGE OF SCIENCE

FACULTY OF BIOSCIENCE

LEVELS OF PHTHALATES IN SELECTED GHANAIAN ALCOHOLIC BEVERAGES

A Thesis submitted to the Department of Food Science and Technology in Partial fulfilment of the requirement for the award of the degree of Master of Science in Food Quality Management

BY

ASIGRI SALIFU

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DECLARATION

I hereby declare that the submission of this manuscript is the true findings of my own research work for the award of a MSc. in Food Quality Management and that, to the best of my knowledge, it contains no material previously published by another person nor submitted to any other university or institution for the award of degree except where due acknowledgement has been made in text. References from the work of others have been clearly stated.

Asigri Salifu	……	…….
(Student)	Signature	Date
(PG2582414)		

Certified by		
Dr. Herman Lutterodt	……	…….
(Supervisor)	Signature	Date

Certified by		
Dr. Faustina D. Wireko-Manu	…….	…….
(Head of Department)	Signature	Date

DEDICATION

This is dedicated to my family especially my lovely wife, Diana Agampim, with whose support and patience this has been realized.

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ABSTRACT

The objective of this study was to determine the presence and levels of three regulated phthalates; benzyl butyl phthalate (BBP), Di-(2-ethylhexyle) phthalate (DEHP) and Di-butyl phthalate (DBP) in locally manufactured alcoholic beverages popularly called "bitters" and packaged with polyethylene terephthalate (PET). The analysis looked at 36 samples consisting of 33 different brands of which twenty one (21) were packaged in PET bottles and fifteen (15) packaged in PET pouches/bags. Target analyte was extracted from samples into n-hexane following an optimized protocol and analyzed using gas chromatography Flame Ionization Detection (GC-FID).

The method showed good linearity in the concentration range of 1 µg/mL to 32 µg/mL with coefficient of determination (R^2) of 0.9919, 1.00 and 0.9996 for BBP, DEHP and DBP respectively. LOD and LOQ for the method detection and quantification ranged from 0.4 µg/mL to 1 µg/mL and 3 µg/mL to 4 µg/mL respectively. Recoveries for method validation purposes yielded a range of 72.24 % to 105.97% of DBP spiked at concentration of 0.1 µg/mL and 0.2 µg/mL.

At least two out of the three phthalates were detected in all samples. DPB was not detected in 5 of the samples representing 13.89 % of the samples tested. The overall mean concentration of DEHP exceeded the regulatory level by 212.67 % whilst that of DBP was as high as 1770 % above the legal limit. The highest leachable samples recorded values of 5.81 µg/mL and 6.02 µg/mL which are 287.3 % and 1906.67 % above the EU Commission regulation 10/2011 for DEHP (i.e. SML = 1.5 µg/mL) and DBP (i.e. SML = 0.3 µg/mL) respectively. The overall mean levels of BBP however complied with the regulation. Consequently, these findings indicate that the level of leaching of phthalates especially DEHP and DBP into some Ghanaian alcoholic beverages popularly called "bitters" can be quite high.

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LIST OF ABBREVIATIONS

ABS : Acrylonitrile butadiene styrene

AOAC : Association of official analytical chemists

BBP : Benzyl butyl phthalate

BHET : Bis-(hydroxyethyl) terephthalate

bw : body weight

DBP : Di-butyl Phthalate

DEHP : Di-(2-ethylhexyl) phthalate

DEP : Di-ethyle phthalate

DIBP : Di-iso- butyl phthalate

DIDP : Di- isi decyl phthalate

DIDP : Di-iso decyl phthalate

DINP : Di-isi- Nonyl phthalate

DMP : Di- methyl phthalate

DMT : Di-methyl phthalate

DNOP : Di-N-octyl phthalate

ECD : Electron capture detection

EFSA : European Food Safety Association

EG : Ethylene glycol

EPA : Environmental protection agency

EU : European Union

EUCTP : European Union China Trade Project

eV : Electron volts

FID : Flame ionization detector

GCMS : Gas chromatography mass spectrometry

FDA-Gh. : Food and Drugs Authority Ghana

GPC : Gel permeation chromatography

GSA : Ghana Standards Authority

HCWH : Health Care without Harm

HDPE : High Density Polyethylene

ICH : International Conference on Harmonization

IUPAC : International Union of pure and applied chemistry

JECFA : Joint FAO/WHO Expert Committee on Food Additives

Kg : Kilogram

Kpa : Kilo Pascal

LC-MS : Liquid chromatography mass spectrometry

LDPE : Low density polyethylene

L/L : Liquid /Liquid

LLOQ : Lower limit of quantification

LOD : Limit of detection

LOQ : Limit of quantitation

m/z : Mass to charge ratio

MS : Mass spectrometry

PAEs : Phthalate acid esters

PCI : Positive chemical ionization

PET : Polyethylene terephthalate

PP : Polypropylene

ppb : Parts per billion

ppm : parts per million

PS : Polystyrene

PVC : Polyvinyl chloride

S/N : Signal to Noise Ratio

SML : Specific migratory limit

SRM : Selected Reaction Monitoring

TDA : Tolerable Daily Allowance

TDI : Tolerable Daily Intake

UK : United Kingdom

U.S. : United States

US FDA : United States Food and Drugs Administration

USP **:** United States Pharmacopoeia

WHO **:** World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

Plastic materials are widely used within the food industry for packaging of food stuffs. It has virtually become an indispensable material within the food industry. Plastic material are made up of a number of additives usually used in production process to support effective manufacturing. These additives vary depending on stipulated regulations and the desired characteristics of the plastic. Scientific investigations point to some migration of lower molecular weight monomers of the additives from plastic packaging into food, raising questions of health and safety (Xu *et al.*, 2010; Guo *et al*., 2010: Ji *et al.*, 2013).

Phthalates are a group of chemicals used as additives (i.e. as plasticizers) to support the durability and flexibility of the plastic polymer (Staples *et al*., 1997) especially in the manufacture of PVC. They are chemically inert and have high density. They have low to medium volatility with a high solubility in organic solvents and this makes them to easily leach into the environment when the polymer material ages (Staples *et al*., 1997).

Phthalates have been banned in plastics used for food packaging because they have been shown by various studies to be toxic, and may cause reproductive and developmental defects (Tsumura *et al*., 2001; Montuori *et al*., 2008; Peterson and Jenson, 2010; EU Commission Regulation (EU) No 10/2011 of 14 January 2011, Centre for Food Safety, 2012).

Despite the ban in some countries, phthalates have been reported by various scientists to have leached from plastic material such as PET, into various food products (Hirayama *et al.*, 2001; Higuchi *et al.*, 2004; Bach *et al*., 2012).

The extent to which migration of plastic polymers occur as reported by Tehrany and Desobry, (2004) depends on the properties of the polymer and the presence of residual monomers and oligomers that are not chemically bound to the polymer matrix and are therefore free to dislodge from the polymer matrix.

The presence of oxygen at high temperature melt process of polyethylene terephthalate (PET) during the manufacturing of PET, can also promote thermally induced mechanical reactions as well as oxidation reactions (Zhang and Ward, 1995; Paci and La Mantia, 1998; Romao *et al*., 2009) thereby enhancing the migratory properties of the plastic components.

Whilst some researchers have reported on the migration of phthalates emanating from bottling lines (Higuchi *et al*., 2004) and from the resins of bottle caps (Hirayama *et al.*, 2001), others have reported migration of phthalates during storage of the product (Bach *et al*., 2012).

Liang *et al.* (2012) reported migration of phthalate plasticizers from plastic containers into soft drinks and alcoholic beverages. This, they explained to emanate from the high solubility of the phthalates in organic solvents which makes them easily released to the environment mostly with aging of the plastic. Liang *et al.* (2012) showed this when they indicated through a third party test report that phthalate acid esters (PAEs) content in a well-known domestic liquor brand was up to 260% higher than the regulated level. Cinelli *et al.* (2013) reports of increased risk of contamination of drinks with phthalates when ethanol content is high.

The Food and Drugs Administration of Taiwan reported in May 2011, a discovery of bis-(2-ethylhexyl) phthalate (DEHP) and di-iso-nonyl phthalate (DiNP) in sports drinks, fruit juices, tea beverages, fruit jams and food powders. These were illegally added as a substitute clouding agent (emulsifier) to improve the appearance of the products. The reports indicated that 965 products were found contaminated and 206 exported to 22 countries around the world (Chan and Shuang, 2012; Yang, *et al.*, 2013).

Prior to the Taiwan scandal, Sharman *et al*., (1994) carried out an investigation on DEHP in milk and cheese samples from Norway and United Kingdom (UK) and found that milk samples from Norway had higher DEHP than those from UK. This was contrary to what they observed in the case of retail cream and cheese. They then concluded that the predominant pathway for contamination of phthalates greatly affected occurrence data suggesting that occurrence data from one country cannot be extrapolated to the other.

Enneking (2006) reports of the prevalence of phthalate exposure through diet, despite the ban in the US of the use of phthalates in plastics beverage bottle manufacturing as well as food wraps, food containers, or any other plastic food packaging.

Rudel *et al*. (2011) and Schecter *et al*. (2013) have indicated that food packaging is an established contributing source of phthalate exposure. The explanations rendered in respect of the prevalence of this hazard in food include inconsistent compliance with industry claims, particularly in imported foods through recycling of the packaging material or during manufacturing (Tsumura *et al*., 2001; Montuori *et al*., 2008; Peterson and Jenson, 2010).

These and many other research findings have increased the search light of various regulatory and scientific institutions towards phthalates. The United States Food and Drugs Administration (US FDA), European Union (EU) and the Codex Alimentarius Commission have made strides towards guiding the use as well as monitoring the presence of phthalates in foods. The regulation of these hazards rests not only on the laws that govern the use of these plasticizers but also on the adequate monitoring of the recycling processes of plastics to ensure excellent separation of the different plastics to be recycled. This is in favor of the arguments that unapproved plasticizers in recycled PET for example could emanate from other plastics containing approved phthalates recycled into PET (Sax, 2010).

The less effective monitoring of the segregation of plastics prior recycling, allows bottles that have been used to package different products, with probable traces of phthalate content, such as shampoos etc. to be recycled together with those without phthalates. The resultant PET when used for food packaging may result in leaching of the toxicant (Sax, 2010). Komolprasert and Lawson (1997) have justified this by indicating the possibility of organic substances readily migrating into PET or being sorbed into the plastic polymer.

There is no doubt by far that governments and international bodies need to be proactive in food safety in respect of the global spread of phthalate tainted items. This is against the background that the taint could emanate from; deliberate illegal addition of the chemical to plastic material or direct addition to food products as it happened in Taiwan or from environmental contamination with products containing the legally used chemical (Chan and Shuang, 2012).

In Ghana, various alcoholic beverages, generally called bitters, are packaged locally into PET bottles as well as in pouches and sold in local shops and local restaurants for consumption. Recently, and still trending, plastic packaging have become a subject of safety concern by the general public. Such concerns have largely been raised on the use of plastics in packaging foods in Ghana, though it sometimes generates controversies due to the unavailability of adequate and reliable research information in this area. Agyeman and Bokpe, (2014) reports on graphic online 24th September 2014, of a researcher, Mr. Dominic Gyamfi who raised health concerns and discouraged Ghanaians from the use of plastics for packaging foods, which was not fully corroborated by the Food and Drugs Authority Ghana (FDA-Gh.), on the basis of lack of sufficient scientific evidence.

To what extent therefore, is the Ghanaian citizenry exposed to phthalates? This question remains unanswered once no one delves into this area of concern. This research piece thus looks at a section of this concern by investigating the levels of phthalates in some selected Ghanaian alcoholic beverages with

high ethanol content of the range 12-42 %, which are largely made with plant extracts and described as "bitters".

1.1 PROBLEM STATEMENT AND JUSTIFICATION

There is increase use of PET for packaging of food products in Ghana. They are used in packaging fruit juices, alcoholic liquors, palm oil, vegetable cooking oil, shito etc.

Coupled with the uncoordinated and flexible nature of regulation of the plastic manufacturing industry among key regulatory institutions such as the Environmental Protection Agency (EPA), the Food and Drugs Authority Ghana (FDA-Gh.) and the Ghana Standards Authority (GSA), alongside the background that;

- phthalates are an emerging and newly identified hazards in foods, which are present in plastics,
- there is inconsistent industry claims on the proper use of phthalates as explained by Tsumura *et al.*, (200); Montuori *et al*., (2008); Peterson and Jenson, (2010),
- there is no available data on the exposure of the Ghanaian citizenry to the prevalence of phthalates
- available literature reports on the migration of phthalates into foods such as alcoholic liquors
- phthalates are toxic to the kidney, posing fertility problems, testicular effects etc;
- the findings by Sharman *et al.* (1994) indicates that the pathway for contamination of phthalates in food greatly affect occurrence data thus, suggesting that occurrence data from one country cannot be used for another,

Ghana needs to understand the occurrence data of phthalates within its food chain.

It is often said that the lack of evidence cannot be used as evidence when no one has ever investigated. This research seeks to delve into screening alcoholic beverages generally referred as "bitters",

manufactured in Ghana and packaged in PET bottles and pouches. It shall focus on the presence of leachable chemicals such as phthalates with particular emphasis on those that have received attention from both scientific and regulatory institutions around the world owing to their toxicity namely; Bis (2-ethylehexyl) phthalate (DEHP), benzyl butyl phthalate (BBP) and Di-butyl phthalate (DBP) (Centre for Food Safety, 2012).

1.3 OBJECTIVE(S)

The research therefore seek to gather occurrence levels of bis (2-ethylehexyl) phthalate (DEHP), benzyl butyl phthalate (BBP) and di-butyl phthalate (DBP) leached into alcoholic beverages packaged with PET bottles and PET pouches, available for sale on the Ghanaian market, by using the method of Gas Chromatography Flame Ionization Detection (GC-FID).

CHAPTER TWO

2.0 LITERATURE REVIEW

During the last few years, the plastics industry has expanded, leading to the introduction of new polymers as well as the modification of old products, thus expanding the scope of hazards that it may pose.

The increase demand for plastic material in the food industry requires us to have an in-depth understanding of the leachable characteristics of the plastic polymer in various food matrices they come into contact with.

2.1 PLASTIC MANUFACTURING PROCESS

The manufacture of plastics can be made to specification depending on the intended application and desired properties. This is achieved by controlling the polymerization conditions. Plastics are generally made by a condensation polymerization reaction that allows the polymer chain to grow in a condensation reaction resulting in the formation of lower molecular weight byproducts (i.e. methanol and water). This is illustrated in the reaction in figure 2.1;

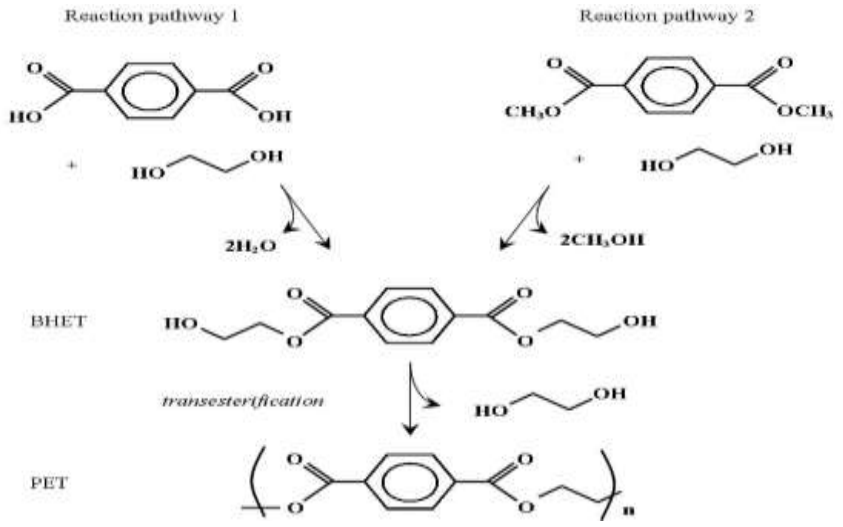


Figure 2.1: Condensation polymerization reaction for plastic production. Source: Hayden *et al*., (2013)

Ethylene glycol (EG) reacts with either (1) terephthalic acid at 240–260 °C and 300–500 kPa,or (2) dimethyl terephthalate (DMT) at 140–220 °C and 100 kPa (Kint *et al*., 1999 and Awaja, *et al*., 2005) the two reactions results in bis (hydroxyethyl) terephthalate (BHET) (Kint *et al*., 1999 and Awaja, *et al*., 2005). Two or three polymerization steps depending on the required molecular weight (Figure 2.1). Polymerization step one, as in figure 2.1 is transesterification between BHET molecules at 250–280 °C and 2–3 kPa, displacing EG, (Kint *et al*., 1999 and Awaja, *et al*., 2005). Oligomers produced are then polycondensed at 270–280°C. The synthesized raw polymer is then moulded into the required form, via extrusion, injection moulding or blow moulding.

The EPA defines two major categories of plastics; thermosets and thermoplastics (EPA, 2014). Thermosets solidify or set irreversibly when heat is applied. They are strong, durable and cannot be remolded. Thermoplastics however, are softened when exposed to heat and they assume their original condition at room temperature. These characteristics makes them ideal for food packaging.

PET production is more economical and energy saving than the glass production. Its light weight makes it easier for merchants and consumers to handle. Energy is saved during transport of PET, especially in long distance haulage, reasons for which plastics have gained popularity in the food packaging industry. Despite these positive attributes of PET bottles, in recent years, their use as non-returnable beverage containers has contributed to increasing volumes of waste in the environment. An attempt by certain economies to manage this, have resulted in the recycling of these PET bottles for reuse. Further, the discovery of certain hazardous chemical in food matrices (such phthalates/ phthalate esters, bis-phenol A, antimony etc.) packed with PET material has raised lots of eyebrows regarding the use of PET for food packaging (Sax, 2010).

It is worth noting that the hazardous chemicals, phthalates, are not used in manufacture of PET nor are they used as substrate or precursors in the manufacture of PET. Yet, there are several citations that point to phthalates being recovered from the contents of PET bottles and the matrices in which they house, pointing to leaching of these contaminants from the bottle walls (Sax, 2010).

Polyethylene terephthalate (PET) is actually chemically different from phthalates. Whereas phthalates are monoesters of lower molecular weight made from orthophthalic acid, PET are polyesters of high molecular weight made from terephthalic acid (Sax, 2010).

These Phthalates are plasticizers usually used to soften other types of plastics (but not in PET). They are generally classed as additives in the manufacture of plastics.

2.1.1 Additives in Plastic Manufacturing

Different additives are used in plastic manufacture depending on the desired characteristics of the final product. These either improve the performance and aging properties of the plastic materials or improve processing properties for the shaping process (i.e. injection moulding, extrusion, blow moulding, vacuum moulding etc.). It is important to mention also that some additives are used to reduce the price of the plastic compound rather than to improve the properties of the plastics (Sax, 2010).

PVC is one of the cheapest plastics on the market with most additives added. Phthalates are still the most popular and cheap additives added to PVC (Vest, *et al.*, 2003). Plastic additives can be classed as

- Functional additives (stabilizers, antistatic agents, flame retardants, plasticizers, lubricants, slip agents, curing agents, foaming agents, biocides, etc.)
- Colorants
- Fillers (mica, talc, kaolin, clay, calcium carbonate, barium sulphate)
- Reinforcements (e.g. glass fibres, carbon fibres) (Vest *et al.*, 2003).

These are not usually chemically bound to the polymer and are therefore not part of the polymer chain. This makes them vulnerable to dislodging from the surface of the polymer, to be present in the environment housed by the polymer. The manufacturing process as well as the process of adding the additives to plastics could result in the formation of chemical substances through degradation, as well as use of the plastic material (i.e. aging during use), suggesting that the chemistry of plastics, the environmental and health impacts can be difficult to predict.

PET is supposed to comply with international food contact regulations especially regarding the use, levels and presence of additives.

2.1.2 Phthalates a Plastic Additive

Phthalates are di-alkyl or alkyl aryl esters of 1, 2-benzenedicarboxylic acid (USEPA, 2007). The length and isomeric structure of the chain largely influences its chemical characteristics.

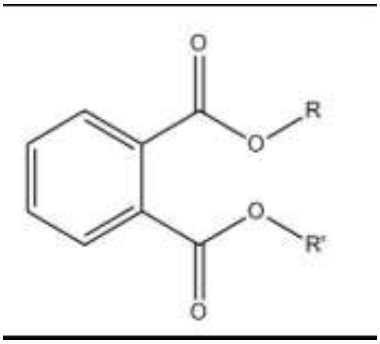


Figure 2.2: General structure of phthalate. Source: USEPA, (2007)

Chemical structure of phthalate; phthalic acid di-alkyl ester; if R, R'=alkyl groups, phthalic acid monoesters; if R=alkyl, R'=H

Illustrated in Table 2.1 is an overview of some regulated phthalates and their corresponding formulas, abbreviations, molar masses and CAS numbers.

Table 2.1: Regulated Phthalates and their Chemical Descriptions

PHTHALATE	ABBREVIATION	MOLAR MASS	CAS NO.
Di-(2-ethylhexy) phthalate	DEHP $C_{24}H_{28}O_4$	390.6	117-81-7
Di-butyl phthalate	DBP $C_{16}H_{22}O_4$	278.4	84-74-2
Di-iso-nonyl phthalate	DINP $C_{26}H_{42}O_4$	418.6	28553-12-0
Di-iso-decyl phthalate	DIDP $C_{28}H_{46}O_4$	446.7	26761-40-0
Benzyl butyl phthalate	BBP $C_{19}H_{20}O_4$	312	85-68-7
Di-n-Octyl phthalate	DNOP $C_{24}H_{38}O_4$	390	117-84-0

Source: Tienpont, (2004)

As additives, they are selected to soften plastics and to impact on the durability, stability and strong performance on the material. Ideally, because they bind into the material in which they are added, they should not evaporate, diffuse or migrate out of the product (Vest *et al.*, 2003).

Phthalates are also used as solvents and other additives in a wide range of consumer products such as mosquito insect repellents, personal care products (nail polish, skin moisturizers, perfumes, air fresheners, etc.). Though they are not supposed to be used in plastics for food packaging such as PET, researchers have found phthalates in food (Wormuth *et. al.*, 2006; Schecter *et al.*, 2013).

In the U.S.A, a survey of 72 foods purchased from a supermarket indicated detection of phthalates in all classes of the foods such as pork, dairy products, vegetable oils and grains sampled, with DEHP mostly

detected in majority of the food categories (despite small sample size) (Schecter *et al.*, 2013). Wormuth *et al.* (2006) has found this to be consistent with concentrations observed in European foods viewed in a scenario based model. In the midst of these, the American Plastics Council states that:

Phthalates are not to be used in plastic beverage bottles, nor are they to be used in plastic food containers, food wraps, or any other type of plastic food packaging sold in the United States (Enneking, 2006).

The lack of parallelism in this directive with the observations made has been explained by Petersen and Jensen (2010); Tsumura *et al.*, (2001); Montuori *et al.*, (2008) to include inconsistent compliance with industry claims, particularly in imported foods, the introduction of phthalates from recycled content or during manufacturing, the inappropriate use of packaging materials (non-food packages used for food), and use of PVC and other phthalate-containing plastics in food processing and handling.

The variety of food contact materials that defines the specific sources of phthalate contamination in food has been difficult. The large number of manufacturers of foods and food packaging materials have also been a contributing factor to the challenge. Surveys of phthalates in foods and food contact materials have further been limited by the cost of laboratory measurements as well as the technical challenge of direct measurement of phthalate dieters, which are prone to laboratory contamination.

Owing to the glass-like transparency properties combined with adequate gas barrier properties to support the retention of carbonation, plastics has become the choice especially for beverages. PET bottles exhibits a high toughness to weight property ratio, which enables a lighter weight to secure large volumes of substances without breaking (Welle, 2011).

There are different grades of plastics depending on the basis of classification. They differ mainly in molecular weight, optical appearance, and intrinsic viscosity etc.

2.2 PLASTIC CLASSIFICATION

In order to ensure the identification and proper disposal or recycling of plastics, the Society of the Plastics Industry (SPI) established a classification system to help consumers make informed choices. This classification system is based on the chemical makeup of the plastic. Manufacturers are supposed to follow a coding system and place a number usually at the bottom of the bottle. For example polyethylene terephthalate (PET) or PETE**,** are usually marked with an SPI code of "1" at the bottom of PET bottles, whilst High density polyethylene products are marked with SPI code "2". Table 2.1 illustrates a summary of the various classifications of plastics and their characteristics;

Table 2**.2:** Classification of plastics and their common properties

Plastic Type	General Properties	Common Household Uses
 Polyethylene Terephthalate	Good gas & moisture barrier properties High heat resistance Clear Hard Tough Microwave transparency Solvent resistant	Mineral Water, fizzy drink and beer bottles Pre-prepared food trays and roasting bags Boil in the bag food pouches Soft drink and water bottles Fibre for clothing and carpets Strapping Some shampoo and mouthwash bottles
 High Density Polyethylene	Excellent moisture barrier Properties, Excellent chemical resistance, Hard to semi-flexible and strong Soft waxy surface, Permeable to gas, HDPE films crinkle to the touch, Pigmented bottles stress resistant	Detergent, bleach and fabric Conditioner bottles Snack food boxes and cereal box liners Milk and non-carbonated drinks bottles Toys, buckets, rigid pipes, crates, plant pots Plastic wood, garden furniture Wheeled refuse bins, compost containers

Source: https://www.ryedale.gov.uk/attachments/article/690/Different_plastic_polymer_types.pdf

Table 2.**2 Continued:** Classification of plastics and their common properties

Plastic Type	General Properties	Common Household Uses
 Polyvinyl Chloride	Excellent transparency Hard, rigid (flexible when plasticized) Good chemical resistance, Long term stability, Good weathering ability Stable electrical properties, Low gas permeability	Credit cards Carpet backing and other floor covering Window and door frame guttering Pipes and fittings, wire and cable sheathing Synthetic leather products
 Low Density Polyethylene	Tough and flexible Waxy surface Soft-scratches easily Good transparency Low melting point Stable electrical properties Good moisture barrier properties	Films, fertilizer bags, refuse sacks Packaging films, bubble wrap Flexible bottles Irrigation pipes Thick shopping bags (clothes and produce) Wire and cable applications Some bottle tops Most bottle tops
 Polypropylene	Excellent chemical resistance High melting point Hard, but flexible Waxy surface Translucent Strong	Ketchup and syrup bottles Yoghurt and some margarine containers Potato crisp bags, biscuit wrappers Crates, plant pots, drinking straws Hinged lunch boxes, refrigerated containers Fabric/ carpet fibres, heavy duty bags/tarpaulin
 Polystyrene	Clear to opaque Glassy surface Rigid or formed Hard Brittle High clarity Affected by fats and solvents	Yoghurt containers, egg boxes Fast food trays Video cases Vending cups and disposable cutlery Seed trays Coat hangers Low cost brittle toys

Source : https://www.ryedale.gov.uk/attachments/article/690/Different_plastic_polymer_types.pdf

Table 2.**2 Continued:** Classification of plastics and their common properties

Plastic Type	General Properties	Common Household Uses
	There are other polymers that have a wide range of uses, particularly in engineering sectors. They are identified with the number 7 and OTHER (Or a triangle with numbers from 7 to 19).	Nylon (PA) Acrylonitrile butadiene styrene (ABS) Polycarbonate (PC) Layered or multi-material mix polymers

Source : https://www.ryedale.gov.uk/attachments/article/690/Different_plastic_polymer_types.pdf

2.3 RECYCLING OF PLASTIC MATERIALS: A SOURCE OF PHTHALATE CONTAMINATION

Recycling defines a process whereby an original material is reused. It is generally known that PET is the most widely recycled plastic in the world. The advantage lies in its ability to be recycled many time and to be used for different end products. An unrecycled PET is termed as virgin PET. Virgin PET usually meets high level of hygienic and safety standards. This level of standard is seldom achieved in the case of recycled PET especially within the jurisdiction of developing countries despite the presence of regulatory enforcers.

Plastic classification amongst other things facilitate the sorting and appropriate recycling of the polymer. Despite this, some complications are usually encountered which may result in the presence of unwanted additives or chemicals in the final products. There are two main recycling procedures; mechanical and feedstock recycling. Thermoplastics, such as PET bottles are usually recycled by the mechanical process. In mechanical recycling, the plastic is simply washed, cut into small pieces and used as raw material for new products relevant for the type of plastics and additives in question (Hansen *et al.*, 2013).

The process becomes challenging when the materials are very dirty and quite difficult to remove by simply washing (for example; when food or oil are attached to the surface of the plastics polymer). Different plastics are usually mixed (PE, PVC, ABS, PET etc.), these materials contain different additives or blowing agents which may now be illegal to use. Recycling these may lead to the introduction of these

unapproved plastic materials into the final products. Nerin *et al*. (2000) have indicated the observation of higher concentrations of phthalates in recycled PET compared to virgin PET.

Further, degradation of the polymer caused by aging can pose a challenge to the recycling of thermoplastics. This makes the degraded plastic monomer incapable of linking with neibouring molecules (Brandrup *et al*., 1996) hence supporting migration into the content it houses.

2.4 CHEMICAL MIGRATION FROM PASTICS

When chemical substances move from a plastic polymer to the surface and sometimes into the medium in which the plastic houses, migration is the term use to describe that phenomenon. Some of these chemical substances may be toxic to humans, such as phthalates, bisphenol "A", antimony etc.

The migratory properties of chemical substances depend on their size. Faster migrators are mostly monomers and residual solvents. Smaller molecules migrate faster because of lower boiling points. It is estimated that, most additives used in plastic have molecular weights ranging between 200-2000 g/mol (Hansen *et al*., 2013).

Migration to the contact media also depend on the exposure temperature as well as the characteristics of the contact media, i.e. Gas, liquid or solid. The contact media and the migratory chemical both have a synergistic interaction regarding the rate of migration. The amount of chemical migrated into the medium is also dependent on the contact time.

Migration generally is expressed mathematically in a law known as Fick's law as;

$M = C_0 \times t^{0.5} \times K \times EXP\ (-E/RT)$

Where M: Migration, C_0: Concentration of the migrant in the polymer, t: Time, K: Constant, T: Temperature, E: Activation energy, R: Gas constant (Hansen *et al*., 2013).

A number of reports have described phthalate esters migrating from flexible plastic polymers into food, this particularly includes oils and fatty foods because of the lipophilicity of the phthalate esters (Xu *et al*., 2010; Guo *et al*., 2010; Fan *et al*., 2012; Ji *et al*., 2013). The chemical characteristics of phthalate plasticizers makes them very soluble in alcohol and as such they can be transferred into liquor during production, transportation, and storage (Liang, *et al*., 2012).

The basic principles in respect of migration can be summarized as follows;

- Organic molecules like gases and solvents that have low boiling points and high vapor pressure have faster migratory rate.
- Molecules which have a low solubility in the plastic will migrate faster than molecules with a high solubility in the plastic;
- Chemical substance with low tendency to migrate have molecular weights greater than 600 g/mol.
- The rate of migration is proportional with temperature variations;
- For a plastic material, migration is higher at the amorphous regions of a semi-crystalline plastic material. This is because of better space between the plastic polymers in the amorphous region.
- An increased Migration rate is observed in a contact medium if the solubility of the migrating substances is high in the contact medium (e.g. phthalate plasticizers to vegetable oils and alcohols),
- When concentration of the migrating substance decreases with time, the migration rate decreases.

Dawei *et al*. (2014) have indicated in their research into 36 liquor samples from different locations in China, the detection of phthalates at levels 86.1 %, 55.6 %, 97.2 %, 86.1 % and 91.7 % for DMP, DEP, DBP, DIBP and DEHP respectively. This prompted them to recommend that these phthalates should be monitored in alcoholic liquors.

Migration limits have been set by various nationals and international bodies to guide the regulation of such leachable chemicals. The European Union for instance sets migration limits based on the conventional assumptions that;

- 1 kg of food is consumed by 60 kg person per day
- the food is packaged in a cubic container of 6 dm^3 surface area releasing the substance

(EU Commission Regulation 10/2011).

A suitable packaging material is therefore defined by its migration limits (ML). This spells out the maximum amount of leachable constituents of a packaging material allowed per unit area. Per the Commission Regulation 10/2011 on plastic materials and articles intended to come into contact with food, food matrice must not habor packaging constituents (migrated from packing) in an amount larger than 10 mg/dm^2 or 60 mg/kg of food or food simulant.

The regulation also defines the specific migration limit (SML), as the highest amount of substance permitted to migrate from the packaging into the food. SML equals 1.5 mg/kg for DEHP and 0.3 mg/kg for DBP for example (EU Commission Regulation 10/2011). Migratory limits have been a subject of concern because of the health implication of such migrated toxicants into food.

2.5 HEALTH IMPLICATIONS OF EXPOSURE TO PHTHALATES

Several literature have pointed to the toxic effects of phthalates to the human body. Various scientist have viewed the toxic effect of phthalates from diverse dimensions.

Phthalates have been implicated to have an anti-androgenic effects regarding reproduction and human development (Reed, 2010). They have been demonstrated to reduce sperm count, cause histological changes in the testis and affect fertility in males. Concerns have also been raised on phthalates causing fetal death, reduction in weights of new born and malformations of the fetus (Heudorf, 2007). Di(2-

ethylhexyl) phthalate (DEHP), di-butyl phthalate (DBP) and butyl benzyl phthalate (BBP) are the main phthalates that have received major attention by the scientific and regulatory institutions because of toxic effects on reproduction and development in experimental animals (Centre for Food Safety, 2012). Humans exposure to phthalates is usually via food, the air, water including other sources such as cosmetics or pharmaceutical products (JRC, 2009). Reed (2010) in his research has been baffled at the effects of phthalates, considering that they do not bio accumulate. Phthalates such as DEHP, BBP DBP, di-isononyl phthalate (DINP), and di-isodecyl phthalate (DIDP) have low acute toxicity and they do not accumulate because they are metabolized and excreted quickly (JRC, 2003 and JRC, 2008).

Both the US and the European Union have recorded wide spread reports on the exposure of phthalates, where in majority of the populations, the route of exposure of DEHP and DINP has been through diet. DEHP has been found to contaminate food directly as it is used in food packaging (Rodgers *et al.*, 2014) In 2009, Huang and his colleagues in a small Taiwanese study on humans showed that phthalates affected female babies which sometimes resulted in abnormal sexual behavior. This, they indicated resulted from the passing from mother to fetus absorbed phthalates through the placenta.

Ormond *et al*. (2009) also indicated in their study that the development of hypospadias, a reproductive birth defect was more prone (two to three times more likely) in women who were exposed to phthalates in the workplaces.

Phthalates exposure was also found to correlate with premature breast development in Taiwanese young girls (Chou, *et al*., 2009). Similarly, higher phthalates levels in urine of adult males was found to be correlated with increased waist circumference and insulin resistance (Stahlhut, *et al*., 2007).

Phthalate esters are usually classed as endocrine disruptors or environmental hormones (Heudorf, *et al*., 2007; Kambia, *et al*., 2001; Gomez-Hens and Aguilarcm, 2003). Various researchers have reported the reduction of sperm production, sperm mobility and male fertility resulting from long term exposure to phthalate esters (Swan, *et al*., 2005; Huang, *et al*., 2007). Reports have also implicated phthalates to be responsible for interfering with endocrine system of fetuses as well as affecting the gender of children (Swan, *et al*., 2005; Huang, *et al*., 2007).

These and many other research findings point to the need for the scientific community especially the regulatory bodies (both national and international) to put in place measures aimed at ensuring that exposure levels of phthalates are minimal.

2.6 REGULATIONS ON PHTHALATES

Various nations regulate residues of phthalates in food products. Most of the regulatory policies in respect of phthalates are at the infantry stage, both internationally and at the national level (Center for Food Safety, 2012).

The World Health Organization (WHO) stated a tolerable daily intake (TDI) of 0.025 mg/Kg body weight (bw) for DEHP in drinking water. In 2005, EFSA also evaluated five phthalates for their use in food contact surfaces as follows; TDIs of 0.05 mg/kg bw for DEHP, 0.5 mg/Kg bw for BBP, 0.01 mg/Kg bw for DBP, and 0.15 mg/Kg bw for DINP and DIDP respectively were allocated (EFSA-Q, 2003; EFSA-Q-192, 2003; EFSA-Q-194, 2003 and EFSA-Q-194, 2003).

There is currently no codex standard for phthalates such as DEHP in foods. Though it has generally been stated that phthalates cannot be added to food, some action limits have been set by the Centre for Food Safety (CFS) as follows; 1.5 mg/Kg for DEHP, 9 $mgKg^{-1}$ for DINP/DIDP (for sum of the two), 30 mg/Kg

for BBP, 0.05 mg/Kg for DIMP, 0.3 mg/Kg for DBP in food resulting from the DEHP-tinted clouding agent incident that originated in Taiwan in May 2011.

An evaluation in 1988 by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) lead to a recommendation that human exposure to DEHP in food should be reduced to the lowest attainable magnitude.

The European Union through the EU laws have set limits to the use of phthalates in plastics that come into contact with food narrowing down to specific maximum amounts that could possibly migrate into food, described as specific migratory limits (i.e. SML) including other specific requirements on selected phthalate compounds (EU Commission Regulation, 10/2011). The EU regulation sets specific Maximum Limits for regulated phthalates as in table 2.3. These limits guide the levels of leaching allowed in a packaging material for the particular toxicant indicated.

There has not been any acceptable levels of phthalates in wines and spirits. Currently, at least until the beginning of the ban in the EU Commission Regulation 10/2011 of 14 January, 2011. Special tolerance for imports were defined unilaterally by EU member states. The necessity of monitoring the presence of the most toxic phthalates (BBP, DBP, and DEHP) in wines, spirits and alcoholic beverages and materials that come into contact with phthalates within member countries has been stressed.

U.S. data on levels of DEHP indicate < 1 ppm though in some processed and /or fatty foods, the levels may be higher.

Table 2.3: Specific Maximum Limits (SML) for some selected phthalates from selected standards

Risk Assessment EFSA			Risk Management (European Commission)	
phthalate	**TDI mg/kg bw/day**	**Remarks**	**SML mg/kg food simulant**	**Restrictions of use**
DEHP	0.05	Exposure in range of TDA	1.5	Very restricted use
BBP	0.50	Exposure well below TDI	30	Restricted use
DBP	0.01	Exposure in range of TDA	0.3	Very restricted use
DINP	0.15	Exposure well below TDI	9	Restricted use
DIDP	0.15	Exposure well below TDI	9	Restricted use

Source: Schafer, (2013).

Besides the SML, the EU China Trade project also stipulated maximum permitted daily amounts of the toxicants for 60 kg person known as the tolerable daily intake (Schafer,2013)

The TDI refers to the daily amount of a chemical that has been assessed safe for human consumption usually for one year.

Table 2.4: Tolerable Daily Intake (TDI) of a 60 kg person

Phthalates	**TDI mg/kg bw per day**	**Limit of ingestion for 60 kg person**	**Limit in food**
DEHP	0.05	3mg	Take into account the
BBP	0.50	30mg	consumption of the
DBP	0.01	0.6mg	food and relevant
DINP	0.15	9mg	factors
DIDP	0.15	9mg	

Source: Schafer, (2013).

2.7 ANALYSIS OF PHTHALATES

A number of techniques have been used for the analysis of phthalate esters in food matrices. The most widely used method has been gas chromatography and liquid chromatography alongside various detectors such as mass spectrometry (MS), flame ionization detectors (FID) etc. (Yao *et al*., 2008; Yan *et al*. 2010; Cheng, and Yan 2012; Chen *et al.*, 2012; Yan *et. al*., 2012; Jiao *et al*., 2012; Wolfgang *et al.*, 2012; Dong *et al.*, 2013).

The analytical procedure is usually preceded by an extraction and purification procedure to obtain the pure analyte.

The methods of extraction and cleanup as well as the choice of instrumentation pays much emphasis on avoiding contamination in the laboratory. Contamination could occur at all steps such as from glass ware, solvents, materials for columns in silica or alumina or even the laboratory air could contain phthalates that would contaminate your analyte (Lopez-Avila *et al.*, 1990).

To avoid this, some proposals have been made by some researchers to;

- heat thoroughly the cleaned glassware to 400^{o}C and deactivate the glass surface with appropriate solvent.
- use regular blanks for controlling contamination. Blank values are defined as "a reading or result originating from the matrix, reagent and any residual bias in the measurement device or process, which contributes to the value obtained. Blank values are hardly constant, suggesting the need to control them. Whiles some laboratories would include a blank in each sequence run, others include additional blanks at the end of the sequence. Others may run a blank with each food sample for the quantity in the analytical procedure"
- if possible, reduce the cleanup process as far as possible
- use new and annealed glassware for phthalate analysis if possible

The challenges of contamination has been experienced by Vikelsøe *et al.*, (2002) after they still found DBP in significant amounts on their glass ware even after all efforts put at avoiding contamination. They latter resorted to the use of new and annealed glassware for all phthalate work.

2.7.1 Extraction

The extraction procedure for phthalates into a medium for analysis largely depends on the state of the medium in which the hazard is originally suspected to be present. For most liquid food matrices, the methods used have largely been Liquid-liquid extraction procedures with variations in the liquid extractant and modifications of the process reagents to maximize extraction output. Most European laboratories do not apply any clean-up procedures prior to analysis especially for liquid-liquid extraction in media such as water, soft drinks and alcoholic beverages (Wenzl, 2009).

Phthalates in non-fatty liquid samples are extracted with the use of non-polar organic solvents, and frequently measured without any additional clean-up (Tsumura *et al*., 2001). This especially occurs for soft drinks, and alcoholic beverages where most laboratories within the EU apply liquid-liquid (L/L) extraction procedures for the isolation of phthalates from the matrix as reported by Wenzl, (2009) in a survey conducted among food control laboratories in the EU. The extraction solvents mostly used in that survey included chloroform, n-hexane, n-heptane, or isooctane.

The Guangzhou Inspection and Quarantine Technology Centre method makes use of dichloromethane extraction solvent via a soxhlet extraction procedure (Huang *et al*., 2011).The extracted analyte is usually subjected to analysis by various techniques depending on several factors pertaining to the choice of the researcher. Clean-up procedure is usually not advised, as it increases risk of contamination, except in cases where large amounts of co-extractants are recovered alongside the extraction protocol (Heise and Litz, 2004).

Techniques usually applied include, liquid-liquid partitioning and gel permeation chromatography (GPC) with S-X30 biobeads, in the column (Heise and Litz, 2004).

2.7.2 Techniques for Measuring Phthalates

A clean extracted analyte is subjected to measurement techniques for the realization of research deliverables. Though different methods have been used by the scientific community to measure phthalates in various media, gas chromatography with mass spectrometric detection has been the major techniques for the measurement of phthalates. Alternatively, the use of Gas chromatography with flame ionization detection (GC-FID), or electron capture detection (GC-ECD) has also been used, but are less frequently used (Heise and Litz, 2004).

The principle of flame ionization detector (FID) relies on the combustion of organic compounds in a hydrogen flame and detecting ions formed during the process. A metal collector with a high voltage detects and identifies the ions. The concentration of the sample hydrocarbon as in the gas determines the rate of ionization and thus the current across the collector. FID is usually stated as carbon pictograms per sec (pg C/Sec). The detector responds linearly irrespective of the compound structure, to the mass of carbon flowing through it (Matthew, 2016). The equation below is an illustration of the combustion sequence for a typical hydrocarbon.

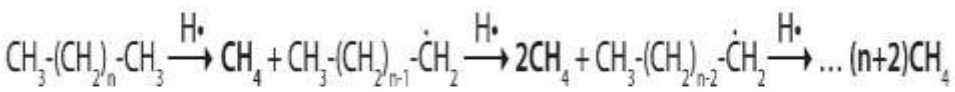


Figure 2.3: Combustion sequence of a typical hydrocarbon. Source: (Matthew, 2016)

Despite that FID remains the most straight forward detection method, an extensive clean-up of samples is required for phthalate analysis so as to avoid interference from matrix compounds. This is especially

critical when trace levels are being measured (Ostrovsky *et al.*, 2011). GC-FID has been adopted in some literature (Rastogi, 1998). The determination of phthalates by using GC-FID has been explored by Ostroysky *et al.* (2011) where the use of alkaline hydrolysis to phthalic acid at temperature of 80^{o} C for 20 hours was put to test. Selective removal of interferences by lipophilic agents using n-hexane at pH 1 achieved a reduction in interference of the detector system.

The method has been indicated by Huang *et al.* (2011) as suitable for analysis of phthalates without isomerides and that it gives rise to high Limits of detections compared to other methods.

The US EPA methods 606 and 8060 describes the use of ECD for phthalate analysis even though ECD responds towards halogenated compounds. It is however relatively sensitive for phthalates compared to FID.

Some laboratories use Positive chemical ionization (PCI) as an alternative to electron ionization (EI). PCI by applying both methane and ammonia as reagent gas which produces significantly different mass spectra, containing more abundant peaks of the molecular ions of the individual phthalate, supporting a better identification of the chromatographic peaks as well as differentiation of different phthalates (George and Prest, 2001). The procedure is helpful in the analysis of complex mixtures of various isomeric forms of phthalates (George and Prest, 2001).

Various analysers have been put to use, comprising quadrupole analyzers, triple quadrupole analyzers, ion traps and magnetic sector instruments. The choice of benchtop quadruple system has basically to do with stability, robustness, reduced cost and linear dynamic range.

The use of high performance liquid chromatography electrospray ionization tandem mass spectrometry (HPLC-ESI-MS-MS) has been utilized by Swan (2011), where acetonitrile extraction was used for the separation phthalates from milk samples.

In a survey among European food control laboratories on analytical methods applied for the determination of phthalates in food, a number of laboratories who used the GCMS method of analysis reported to have operated the mass spectrometer in scan mode, covering a mass-to-charge range of 50 to 350 or even higher. At 70 eV, electron ionization, phthalates generates major fragment ion of mass-to charge ratio of 149 resulting from the protonated form of the phthalic acid anhydride. This is with the exception of DMP as reported (Wenzl, 2009). The phthalic ion anhydride is usually the ion for quantification of the analyte content. The most abundant quantitation ion for DMP is m/z 163. Below is the catalogue of the various quantitation and qualifier ions for the regulated phthalates.

Table 2.5: Quantitation/qualifier ions for some regulated phthalates

Compound	m/z Quantitation Ions	Qualifier Ion
DEHP	149,167, **279**	167
BBP	91, 149, **206**	91
DNOP	149, 167, 261, **279**	279
DINP	149, 167, **293**	293
DIDP	149, 167, **307**	307
DBP	149, 167, 205, **223**	205

Suggested quantitative ions are bolded. Source: Chan and Shuang, (2012)

A reaction equation for the generation of the phthalate Qualifier ion has been illustrate as in figure 2.4.

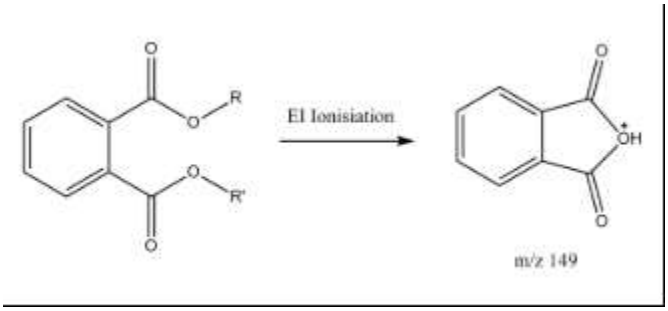


Figure 2.4: Phthalates and their major fragmentation in EI ionization
Source: Kathryn, (2016)

The qualifier ion is usually the most abundant ion in the mass spectrum of the analyte when the mass spectrum is operated in the scan mode. It is sometimes referred to as the "quantitation" ion though not used in the quantification of the peak (Kathryn, 2016).

Liquid chromatography such as HPLC has also been used by some researchers to measure the levels of phthalates in analyst. Whiles some researchers use this in combination with UV detection (HPLC-DAD), others use it in combination with tandem quadrouple mass spectrometry (HPLC MS/MS) in selected reaction monitoring mode (SRM) (Wenzl, 2009).

Various standard methods have been developed for use in analyzing the levels of phthalates, recommended for specific food matrices. These methods tend to specify optimum conditions for the achievement of best results.

The Guangzhou inspection and Quarantine Technology Centre makes use of gas chromatography mass spectrometry for quantification by isotopic dilution.

Analysis by Gas chromatography-mass spectrometry has been reported to offer higher sensitivity for the measurement of phthalates compared to liquid chromatography-mass spectrometry. LC-MS however offers some advantages including better selectivity in respect of molecular weight information in isomeric mixtures. It also facilitates faster analysis and is more reliable in the quantification of isomeric mixtures (Dawei *et al*., 2014).

Dawei *et al.* (2014) have used high resolution mass spectrometry to analyze phthalates in liquors with limits of detection and quantification ranging between 0.1 and 1microgram per litre and 0.4 and 2 micogram per litre respectively.

2.7.3 Method Validation

The quality of analytical procedure is usually appraised on the basis of its suitability for the intended purpose, recovery, standardization requirements, sensitivity, analyte stability, ease of analysis etc. Key to every experimental procedure is to establish the suitability of that procedure. Performance matrices mostly used include;

- Accuracy: which shows the closeness of the average analytical result to the actual
- Precision; describes how close replicate results are to each other and it usually includes repeatability (i.e. same instrument and operator), intermediate precision (i.e. same lab but different operators and over extended time frame) and reproducibility (different instruments and operators)
- Specificity describes the discriminatory ability of the test method from potential interferences with the test analyte.
- Limit of detection (LOD); points to the lowest amount of target analyte capable of being detected within the limits of statistical validity.
- Limit of Quantification (LOQ) refers to the minimum Amount of target analyte that can be identified and quantified in the actual sample, viewed within statistical rules.
- Linearity and range makes references to the shape of the response curve of the target analyte obtained from the concentration/amount range over which target analyte has been quantified.

2.7.3.1 Limit of Detection (LOD) and Limit of Quantification (LOQ)

Alternatively, LOD/LOQ could be used to establish the suitability of a method for an analyte quantitation. Whilst the LOD defines a statistically valid lowest amount of the target analyte capable of being detected in a standard that is free of matrix, the LOQ defines the statistically valid lowest amount of target analyte that can be identified and quantified in real samples (David and Armbruster, 2008). In terms of signal to noise ratio, the LOD is the concentration of analyte which induce signal (S) that is three times higher than the background noise level (N), S/N=3.

The Limit of Quantification, LOD, the smallest concentration of analyte which induce signal (S) that is 10 times higher than the background noise (N), S/N=10.

The LOD is calculated as a multiple of the peak-to peak noise (i.e. as 2 or 3 x noise) whilst the LOQ as a multiple of the LOD (i.e. 10-20 x LOD). The noise is determined using a clean standard analytical system. Various guidelines exist for LOD and LOQ method validation procedures (Shrivastava and Gupta, 2011). It is either determined based on; Visual evaluation, signal to noise or based on standard deviation of the response and slope. Tables 2.6 and 2.7 are extracts comparing the different guidelines available for the calculation of LOD and LOQ;

Table 2.6: Comparison of different guidelines for "detection limit" parameter of analytical method validation

Guidelines	ICH	US FDA	AOAC	USP	IUPAC
Definition	Lowest amount of analyte in the sample, which can be detected but not necessarily quantified under stated experimental conditions	Explicitly not described	Lowest content that can be measured with reasonable statistical certainty	lowest amount of analyte in the sample, which can be detected but necessarily quantifies under stated experimental conditions	Smallest amount of analyte in the sample that can be reliably distinguished from zero
method	1. By visual evaluation 2. Based on s/n ratio Applicable to procedure, which exhibits baseline noise Low conc. Of analyte is compared with blank 3. Based on SD of response and slope LOD= 3.3 σ/s s-slope of calibration curve σ -SD of response; Can be obtained by standard deviation of blank response Residual standard deviation of the regression line Standard deviation of the y-intercept of the regression line S_{yx} i.e. standard error of the estimate	Not described	Based on more than 20 blank readings	For non-instrumental: analysis of sample with known concentration of analyte and by establishing minimum conc. at which analyte can be reliably detected. For instrumental: process for non-instrumental process can be adopted. Detection limit should be sufficiently low for analysis of samples with known concentration of analyte above and below the required detection limit	Not specified
Expression /calculation	If based on visual examination or S/N ratio relevant chromatogram is to be presented If by calculation/extrapolation estimate is validated by analysis of suitable no. of samples known to be near or prepared at detection limit	Not specified	the mean value of the matrix blank readings (n≥20) plus three standard deviations of the mean, expressed in analyte concentration	Not specified	Not specified
Acceptance criteria	**S/N** ratio> 2-3, not specified in other cases	Not specified	Not specified	Not specified	Not specified

ICH-International Conference on Standardization, **US FDA**-United States Food and Drugs Administration, **AOAC**- Association of Analytical Chemists, **USP**-United States Pharmacopoeia, **IUPAC**- International Union of Pure and Applied Chemistry (Shrivastava and Gupta, 2011)

Table 2.7: Comparison of different guidelines for 'quantitation limit' parameter of analytical method validation

Guidelines	ICH	US FDA	AOAC	USP	IUPAC
Definition	Lowest amount of analyte in the sample, which can be quantitatively determined with suitable precision and accuracy	The lowest amount of analyte that can be quantitatively determined with suitable precision and accuracy also called LLOQ (lower limit of quantification)	The limit of quantitation is the lowest amount of analyte in a sample, which can be quantitatively determined with precision and accuracy appropriate to analyte and matrix considered	Lowest amount of analyte in a sample, which can be quantitatively determined with suitable precision and accuracy	Not defined
Method	1. By visual evaluation 2. Based on S/N ratio Applicable to procedure, which exhibits base line noise Low conc. of analyte is compared with blank 3. Based on S.D. of response and slope LOQ=10 σ /s s-slope of calibration curve σ- S.D. of response; can be obtained by Standard deviation of blank response Residual standard deviation of the regression line Standard deviation of the y-intercept of the regression line $S_{y/x}$ i.e. standard error of estimate	Preparation of standard curve and lowest conc. on the calibration curve should be accepted as LLOQ if it satisfies following condition. Response at LLOQ = 5 x Response by blank Analyte peak should be identifiable discrete and reproducible with precision of 20% and accuracy of 80%-120%	Not specified	1. By visual evaluation 2. Based on S/N ratio Applicable to procedure, which exhibits baseline noise. Low con. Of analyte is compared with blank 3. Based on S.D. of response and slope LOQ = 10 σ /s s-slope of calibration curve σ- S.D. of response; can be obtained by Standard deviation of blank response Residual standard deviation of the regression line Standard deviation of the y-intercept of the regression line $S_{y/x}$ i.e. standard error of estimate	Not recommended; only recommends expressing uncertainty of measurement as function of concentration

ICH-International Conference on Standardization, **US FDA**-United States Food and Drugs Administration, **AOAC**- Association of Analytical Chemists, **USP**-United States Pharmacopoeia, **IUPAC**- International Union of Pure and Applied Chemistry (Shrivastava and Gupta, 2011).

Table 2.7 Continued: Comparison of different guidelines for 'quantitation limit' parameter of analytical method validation

Guidelines	ICH	US FDA	AOAC	USP	IUPAC
Recommendation	Limit should be validated by the analysis of suitable no. of samples known to be near or prepared at the quantitation limit	Not specified	Not specified	Not specified	Not specified
Expression /calculation	Limit of quantitation and method used for determining should be presented. Expressed as analyte conc.	Not specified	Mean value of the matrix blank reading plus 10 standard deviations of the mean, expressed in analyte conc.	Expressed as analyte conc. (% or ppm)	Not specified
Acceptance criteria	Not specified	Not specified	Not specified	Not specified	Not specified

ICH-International Conference on Standardization, **US FDA**-United States Food and Drugs Administration, **AOAC**- Association of Analytical Chemists, **USP**-United States Pharmacopoeia, **IUPAC**- International Union of Pure and Applied Chemistry (Shrivastava and Gupta, 2011)

2.7.3.2 Recovery

Recovery is usually estimated by a spiking experiment where a known amount of the reference standard is added to the test sample and subjected to the same procedure for analysis in order to recover it. Recovery is one of the criteria used to validate the method used for analysis. Choosing it as a method of validation depends on the procedure for the analysis. It is usually specified for most individual analytes within the ranges 80 % to 110 % (Wenzl, 2009).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 SAMPLING

A total of 36 samples (made up 33 different brands of 750 mL x 20 PET bottles, 200 mL x 1 PET bottle and 50 mL x 15 PET plastic pouches) were obtained from the Ashaiman Market, Tema whole- sale points located at Community one and Community two.

Bottled samples were labelled S_1 to S_{21} and pouch samples were labelled S_{p1} to S_{p15}. Samples were stored at room temperature (21-28^0 C) until the day of the experiment.

It is important to state that no particular inference should be made from the presence or absence of any particular brand in this survey. The samples only represent current products at the market at the time of the sampling.

3.2 CHEMICALS

Benzyl butyl phthalate (BBP), reference standard; Dibutyl phthalate (DBP), reference standard; Di(2-ethylhexyl) phthalate (DEHP), reference standard; Di-n-Octyl phthalate (DNOP), reference standard; Di-isononyl phthalate (DINP) reference standards all obtained from Accustandard USA, n-hexane obtained from Park scientific limited Northampton, Methanol obtained from sigma-Aldrich.

3.3 APPARATUS

Glass dropper, Eppendorf centrifuge 5804, water bathe, glass test tubes, GC-FID, Vortex mixer, volumetric flasks of different volumes, 25ml glass measuring cylinder, micropipettes of different sizes.

Note: all apparatus used for testing were made of glass not plastics, and was rinsed with n-hexane and dried before use.

3.4 STANDARD (STD) SOLUTION PREPARATION

Volumes of 10 µl, 40 µl, 80 µl, 160 µl and 320 µl each of 100 µg/ml standard solutions of BBP (STD 1), DBP (STD 2), DEHP (STD 3) were taken and made up with methanol to 1ml, to obtain the corresponding concentrations of standard working solutions 1 µg/ml, 4 µg/ml, 8 µg/ml, 16 µg/ml and 32 µg/ml each for GC injection.

3.5 EXTRACTION

Following a method optimization of the China regulation GB/T 21911-2008 for the determination of phthalates in foods as described by Liang *et al. (*2012), 5ml each of the samples was transferred into separate glass test tubes using separate glass pipettes and evaporated on a water bathe (temperature 65^{o}C) for about 4 hours to obtain about a 2-3 ml of the samples in each glass test tube. Samples were allowed to cool to room temperature (21-28^{0}C) after which 2 ml of n-hexane was added, vortexed for 2 minutes and finally centrifuged at 4000 rpm for 5 minutes. The supernatant was transferred into glass vails for GC injection.

3.5.1 Spike Recovery Extraction

Samples S_3 and Sp_8 were spiked with 50 µl and 100 µl of standard 10 µg/ml DBP to obtain four spiked solutions, 2 of S_3 (concentrations 0.1 µg/ml and 0.2 µg/ml) and 2 of Sp8 (concentrations 0.1 µg/ml and

0.2 µg/ml). The resultant solutions were then taken through the extraction protocol as in section **3.5** to obtain the analyte for GC injection.

3.5.2 Identification and Quantification

Samples were loaded on an auto sampler set to dispense One milli litre (1ml) of the extractant into the GC-FID set-up in accordance to the set operation conditions in **Table** 8.

The FID was operated in the splitless mode.

Table 3.1: GC-FID Operating conditions

Instrument	Shimadza GC-2010plus with split/ splitless injector, FI detector AOC 20i auto injector, AOC 20s auto sampler
GC column:	Varian Factor Four capillary column VF 5MS: 30 m x 0.25 mm ID X 0.25 µm
Temperature Program	Start temperature 80° C for 1 min, 10° C per min to 280° C, 10 min at 280° C
Carrier gas	N_2, 1 ml/min
Injector	280° C, injection volume 1 µl
Detector	Ionisation, 280° C

The standard solutions were run first and used to set the conditions for automatic identification and auto-detection of baselines of relevant sample peaks for the target analyte.

3.5.3 Solvent Blanks

Solvent blank made of n-hexane was run periodically (after very five runs) through the GC-FID to monitor for potential contamination.

3.5.4 Data Analysis

Stats Graphics Centurion Version 15, 2015 was used for all data analysis. Data basically presented as means plus or minus standard deviations as well as percentages, in order to support analysis.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

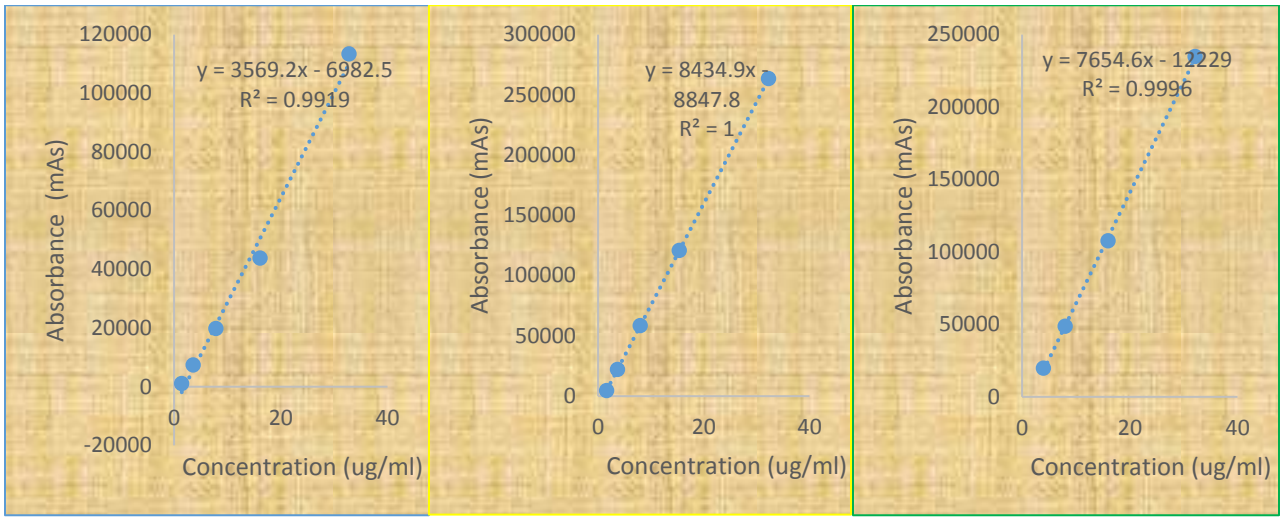
The project set forth to investigate in some popular Ghanaian alcoholic beverages the presence or otherwise and the levels of three most reported and toxic phthalates, namely BBP, DEHP and DBP (Cinelli *et al.*, 2013) using Gas chromatography flame ionization detector (GC/FID).

4.1 RETENTION TIMES

The closest peaks of interest in the samples chromatograms were identified by using the respective retention times of the reference standard chromatograms, in an automated set-up system. The GC retention times of phthalates were stable throughout the study with maximum coefficient of variation of 0.01.

The sequence of the mean retention times of the standards are consistent with the molecular weights (MW) of the compounds as based on the principle of chromatography, thus, the early elution of smaller molecular weight compounds (MW(DBP) = 278.4 g/mol, MW(BBP) = 312 g/mol and MW(DEHP) = 390.6 g/mol).

4.2 LINEARITY



BBP **DEHP** **DBP**

Figure 4.1: Calibration curves of reference standards BBP, DEHP and DBP

Standard calibration curves of all three reference standards as in figure 4.1 above show excellent linearity with coefficients of determination (R^2) of 0.9919 (BBP), 1.000 (DEHP) and 0.9996 (DBP). In other words the regression line well approximates the real data points. The calibration curve for DEHP perfectly fit the data. These were achieved within the concentration ranges of 1µg/mL to 32 µg/mL of standard solutions. Whilst Dawei *et al.* (2015) obtained a good linearity with $R^2 > 0.995$ in concentration range of 0.001-0.1 µg/ mL and 0.1-0.5 µg/ mL Mario *et al.* (2011) obtained this with $R^2 \geq 0.9992$ within concentration range of 0.01 -10 µg/mL, both using different detector instrumentation approach, high resolution mass spectroscopy and ion trap mass spectrometer detector respectively.

4.3 DETECTION AND QUANTITATION

Limits of Detections (LOD) of 0.4 µg/mL, 1.0 µg/mL, 1.0 µg/mL and Limit of Quantification (LOQ) of 3 µg/mL, 3 µg/mL, 4 µg/mL for BBP, DEHP and DBP respectively were calculated at signal to noise ratio of 3 and 10. Statistically valid detected and quantifiable data obtained from the samples are thus indicated in table 4.1;

Table 4.1: Analysis of mean concentrations with respect to LOD, LOQ and sample package type

Phthalate	**Sample Package type**	**Samples [mean] > LOQ (by package type)/** µg/Ml	**Samples [mean]> LOQ (Overall)/** µg/mL	**Percentage of [samples]> LOD (%)**	**Percentage of [samples]> LOQ (%)**
BBP	Pouch	5.03 ± 0.44	5.03 ± 0.55	100	30.56
	Bottle	5.70 ± 0.71			
DEHP	Pouch	5.81	4.69 ± 0.97	100	8.33
	Bottle	4.13 ± 0.71			
DBP	Pouch	5.61 ± 2.43	5.61 ± 2.43	86.11	8.33
	Bottle	-			

[samples] = concentration of samples in µg/mL, [mean] = mean concentration of samples in µg/mL. Sample mean overall represents the mean of the phthalates in both pouch and bottle

The percentage frequecy of samples concentration above the Limit of Detection (LOD) was calculated as 100%, 100% and 86.11% for BBP, DEHP and DBP respectively. Thus, BBP and DEHP were detected in all samples whilst DBP was detected in 86.11% (31 samples) of the samples. This results show that to a large extent, the three investigated pthalates are present in plastic packaged alcholic liqours in Ghana, with highest mean levels of leaching recorded for DBP (5.61 µg/mL ± 2.43 µg/mL).

The percentage frequency of measured cocnentrations of samples above the Limit of Quantification (LOQ) was calculated as 30.56%, 8.33% and 8.33% for BBP, DEHP and DBP respectively. Of the 36 samples, DBP was not detected in 13.89% (5 samples) of the samples.

These observations in Ghanaian alcoholic liquor are similar to the findings of Dawei *et al.* (2015) and Chatonnet *et al.* (2014) where they both reported the presence of DBP and DEHP in significant qunatities sometimes above the Specific Migratory Limits (SML) (as spelled out in the European Regulation 10/2011), in alcohols and wines in France respctively. Chatonnet *et al.* (2014) has indicted that BBP, DEHP and DBP are most frequently detected phthalates in the French wines sampled for their study. Yingying *et al.* (2013) also found 63 % (19: 30) of samples of white spirits in China having levels of DBP exceeding the SML.

Reasons for this finding in Ghanaian alcoholic beverages could be attributed to one or more of either of the following as articulated by Petersen and Jensen (2010), Tsumura *et al.* (2001) and Montuori *et al.* (2008). First, is the incosistent industry claims on the use of plastic additives as one possible reason. Second, is the practice of the plastic industry in Ghana to recycle plastics for reuse which could be the source of contamination, emmanating from improper seperation of various types of plastics during recycling. This is possible because of the legal presence of these additives in non-food packaging plastics materials that are usually collected together with food packaging plastics. Further more, one would not rule out the possibility of contamination emmanating from the caps of bottles made up of High Density Polyethylene (HDPE) during transport of the products. To obtain an entirely complaint packaging would require the use of virgin PET for production of plastic packaging materials meant for food packaging or strict adherencce to the rules of separation based on plastic classification, as well as as ensureing the use of compliant plastic material in bottle lines during production.

4.4 RECOVERY

A spike recovery test carried out at concentrations of 0.1 µg/mL and 0.2 µg/mL yielded recoveries within the ranges of 72.24 % to 105.97%. The mean recovery is calculated as 86.79%. The mean recovery value is within acceptable values for validation procedures which is usually within 80-120% (Kocourek, 2012). Mario *et al.* (2011) obtained recoveries of 73 % - 71 % and 96 %- 99 % for red wines spiked at 0.02 and 0.05 µg/ mL of PAE respectively. This was achieved using a solid –phase extraction with an ion trap mass spectrometry. In a similar research in light alcoholic beverages using dispersive liquid –liquid micro-extraction with ion trap mass spectrometry, Mario *et al.* (2014) recorded recoveries of 95.6 % to 99.4 %. The methods used in these separate researches are more specific and delivering lower detection and quantification limits compared to the method used in this research. Despite, the achieved recoveries obtained have been observed to be within acceptable validation limits as stipulated by Kocourek, (2012) above.

4.5 COMPLIANCE OF MEASURED CONCENTRATIONS TO EU SET SML (EU REGULATION 10/2011)

All valid quantifiable values (i.e. based on LOQ) in this experiment for DEHP and DBP exceeded the Specific Migratory Limit (SML) (DEHP; SML = 1.5 mg/kg, DBP; SML = 0.3 mg/kg as per the EU Regulation 10/2011). **An alcoholic beverage with a phthalate content above the SML as imposed by the regulation on materials in contact with food (EU Regulation 10/2011) would mean it had been in contact with non-compliant material and thus renders the packaging material and the product non-compliant**. Based on the above premise, all affected products that fall within this ambit as indicated in Table 6.1 of appendix III (i.e. values in green) are non-compliant. The contamination could emanate

from the packaging or from the production process as associated with plastic materials within the production lines amongs others.

Though the highest leached amount was recorded for BBP (8.72 µg/mL ± 0.62 µg/mL), it complied with the regulation (i.e. SML (BBP) = 30 mg/kg) . No spsecific reason can be attributed to this owing to the fact that several factors may affect leaching properties of additives within a plastic polymer including; the size of the additive, temperature conditions, presence of micoorgamisms as well as the extend of branching of the additive molecule.

All the valid quantifiable amounts for DBP were observed in only the pouch packaged liquor (3 samples). Pouch packages are more prone to leaching as they are thinner and more succeptile to environemtnal conditions such us temperatrue and handling conditions. Permeability of the pouch polymer structure to the liquid content is comparativley higher as rubbery polymers have larger polymer gaps and thus higher diffusion rates.

Whilst the overall sample mean concentration of BBP is less than the SML (5.03 µg/mL ± 0.55 µg/mL < SML 30 mg/kg) making it compliant to the regulation, that of DEHP (4.69 µg/mL ± 0.97 µg/mL) and DBP (5.61 µg/mL ± 2.43 µg/mL) are higher than the permitted levels leachable (i.e. < 1.5 mg/kg and < 0.3 mg/kg for DEHP and DBP respectively) into food matrix as per EU regulation 10/2011, which therefore makes the products non-compliant.

The overall mean concentration of DEHP as indicated exceeds the regulatory level by 212.67 % whilst that of DBP exceeds the regulatory levels by as much as 1770%. Highest leached concentrations of DEHP and DBP of 5.81 µg/mL and 6.02 µg/mL, are 287.3 % and 1906.67% above the EU set regulatory limits respectively. The finding are quite higher when compared to those of Liang *et al.* (2012), as their findings

of levels of PAEs in a Chinese local liquor recorded 260% higher than regulatory levels. Differences in levels of phthalates occurrence based on geographical locations are expected, of which Shaman *et al.* (1994) indicated that occurrence data from one country cannot be used for another. This situation is as a result of the different compliance levels by the industry players as well as the difference in stringency of regulatory controls by various nations.

In short, while argueing from the perspective of valid quantifiable amounts measured in this experiment, each of the samples have recorded at least one non-compliance in respect of levels of one or more of the phthalates under study, suggesting that a critical look at the production process, the use of plastic contact surfaces for alcoholic beverage production and the packaging materials is imperative. Indeed, the results point to the fact that the levels of leaching of phthalates into some of the alcoholic beverages can be quite high (when compaired to the SML which defines the acceptable limits) and thus deserves keen attention and monitoring by both the scientific and regulatory communitites as well as the manufacturers of both the packaging plastic materials and the alcoholic liqours.

This call is further hightened when we recall findings of Cinelli *et al.* (2013), reporting the increased risk of contamination by phthalates when food matrix has high ethanol content as well as the listing of BBP, DEHP and DBP as the most toxic phthalates in wines, spirits and soft drinks (Mario *et al.*, 2014).

The implication of the results may even be viewed to be more complex when analyzed from the perspective of aggregate effect of the substances especially their common effect on specific organs of the human body. This indeed creates formidable barriers in our day to day regulatory control once the absence of an aggregate view of toxic substances is unavailable to direct research of common effect and from

multiple sources. As explained by HCWH, (2002), what may appear to be a tolerable level of exposure to a single compound can actually contribute to an unsafe aggregate (when viewed from similar compounds with additive toxic effects) since the tolerable daily intake are ordinarily determined by assessing the toxicity of a single compound.

The implication of this observation is that as low as a milli litre of the non-compliant alcoholic bitters consumed is enough to exceed the tolerable limits of say a 60 kg person, stated as 3 mg and 0.6 mg for DEHP and DBP respectively as stipulated in the EU China trade project agreement (see Table 2.1). Interestingly 1 mL is far too small a volume to consume, compared to the volume of the so called "tot" or say a 50 mL pouch package.

A further worrying concern may set forth when we review literature with indication pointing to a much wider source of phthalate contamination within the environment. This would hype our suspicion of phthalates exposure in the Ghanaian populace which may be beyond alcoholic beverages, and may include other food products packaged with plastics. Paganetto *et al.* (2000), Sathyanarayana *et al.* (2008) and many others have pointed to human exposures to phthalates being caused by the introduction of fatty foods kept in plastic tools or through skin with the use of body creams, oils and lotions. Sadighi *et al.* (2015) have even detected DBP in toothbrushes and quantified DEHP at 1.15 ppm as well, though the experimental sample size was small. These practices involving packaging and use of products that make us susceptible to phthalate exposure are common with the Ghanaian community. Recalling the common saying that "Little drops of water makes a mighty ocean" should increase our zeal at uncovering fully the occurrence levels of phthalates within our community, a first step to planning for our safety as Ghanaians.

CHAPTER FIVE

5.0 RECOMMENDATIONS AND CONCLUSION

This research has showed that BBP, DEHP and DBP leach into the sampled Ghanaian alcoholic beverages with levels of DEHP and DBP exceeding the acceptable levels allowed in the EU regulation 10/2011. The overall mean concentration of BBP was 5.03 µg/mL ± 0.55 µg/mL and this fell well below the acceptable level as per the EU regulation 10/2011. Whilst the mean concentration of DEHP exceeded the regulatory level by 212.67%, that of DBP exceeded by 1770%. The exposure of Ghanaian to Phthalates (especially DEHP and DBP) through alcoholic bitters can therefore be said to be significant and should not be neglected. The need for further research in this area to unravel the true source of the phthalate contamination is evident.

This research piece, though challenged by the small sample size, may just be a tip of the iceberg waiting to unleash its effects or already doing so but remain unnoticed.

The public and the trader need to be enlightened on these emerging hazards by being encouraged to buy food from reliable suppliers. They ought to pay particular attention to the suitability of the package to holding hot, fatty or acidic foods. Traders need to ensure that food packaging complies with relevant regulations. Above the vigilance on the part of the public and the trader, the regulatory and standard body of Ghana need to apt their game in ensuring that the scope of regulatory activities pays keen attention to quality and conforming characteristics of the production process as well as the food packaging materials.

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APPENDICES

APPENDIX I

1.0 FORMULA FOR CALCULATING CONCENTRATIONS OF THE STANDARD SOLUTIONS FOR GC-FID RUNS

$C_1 V_1 = C_2 V_2$

Where C_1 =Initial concentration, C_2 = final concentration, V_1 = initial Volume, V_2 = final volume

2.0 FORMULA FOR CALCULATING LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTIFICATION (LOQ)

$$LOQ = 10 * \frac{\text{Standard Deviation of Response}}{\text{Slope of calibration curve}}$$

$$LOD = 3* \frac{\text{Standard Deviation of Response}}{\text{Slope of calibration curve}}$$

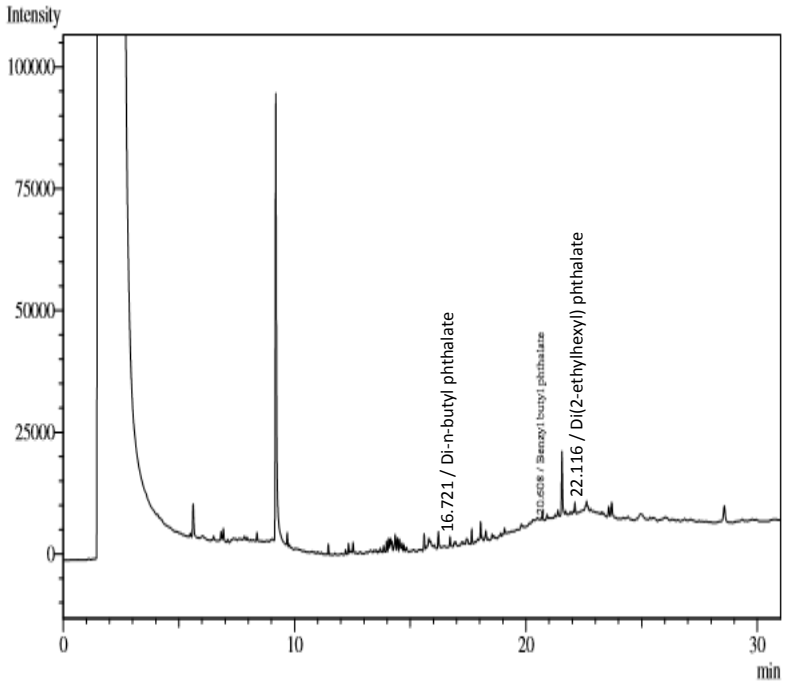
3.0 RECOVERY CALCULATED WITH THE FORMULA

$$\textbf{Recovery} = \frac{\text{Mean Value}}{\text{Added amount}} *100$$

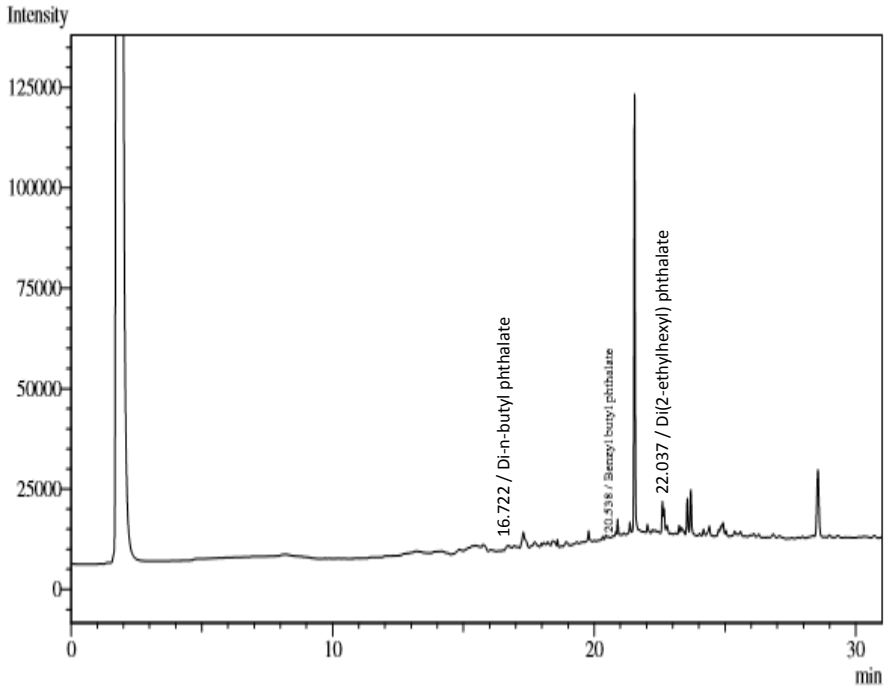
APPENDIX II

1.0 CHROMATOGRAMS OF REFERENCE STANDARDS, SAMPLES AND BLANKS

1.1 SAMPLES

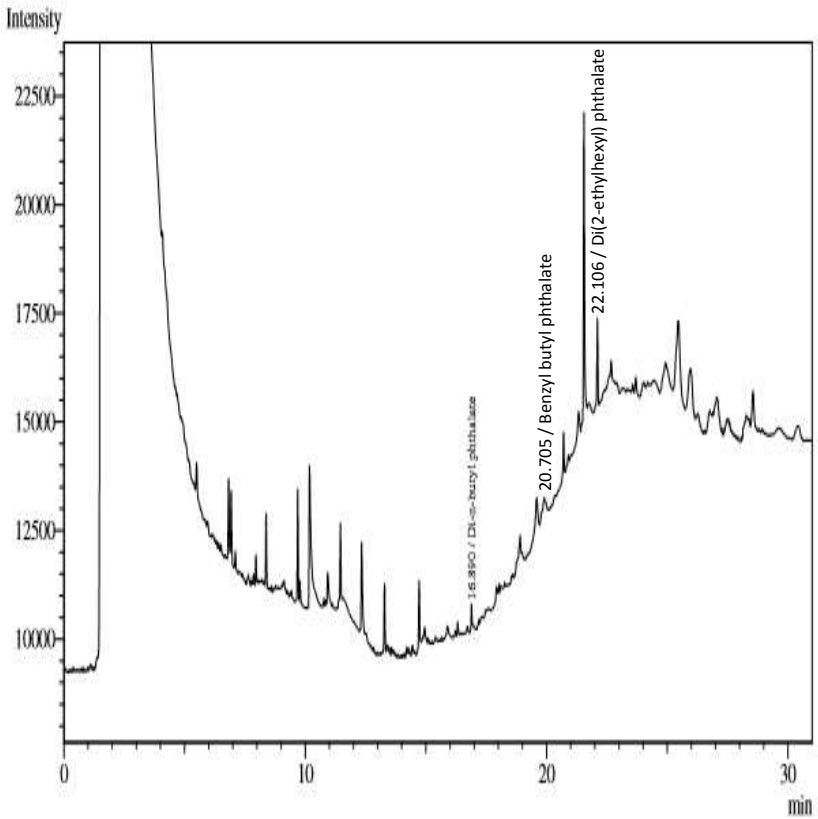


S_{P1}

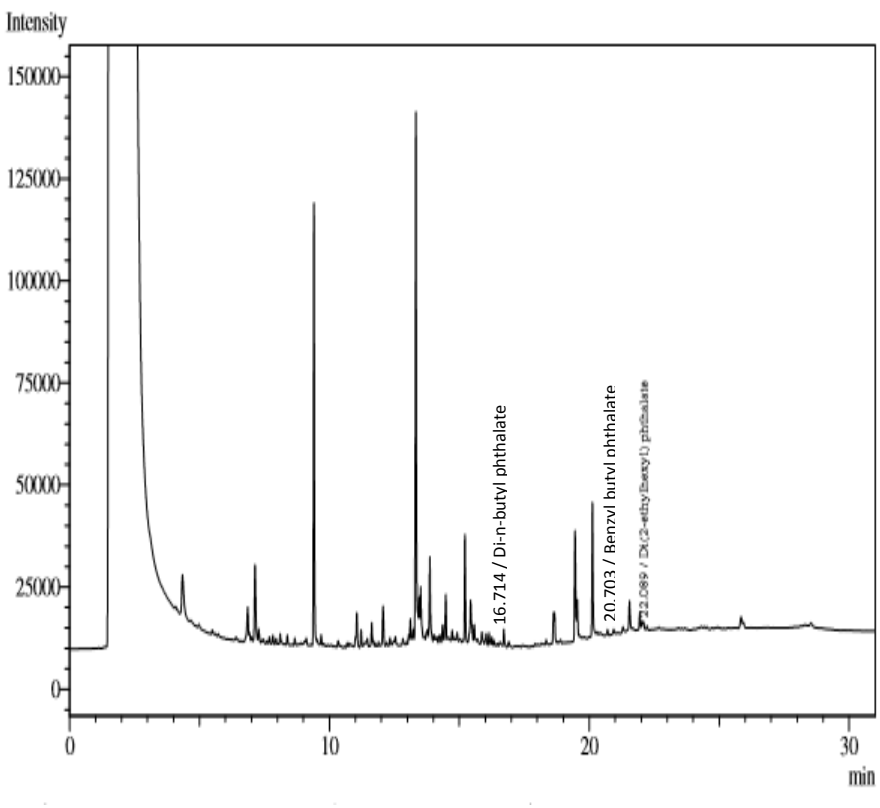


S_{P2}

Figure 6.1 Continued: Chromatograms of samples showing peaks for BBP, DEHP and DBP. (Sp_1-Sp_{15} = pouch samples, S_1-S_{21} = Bottled samples)

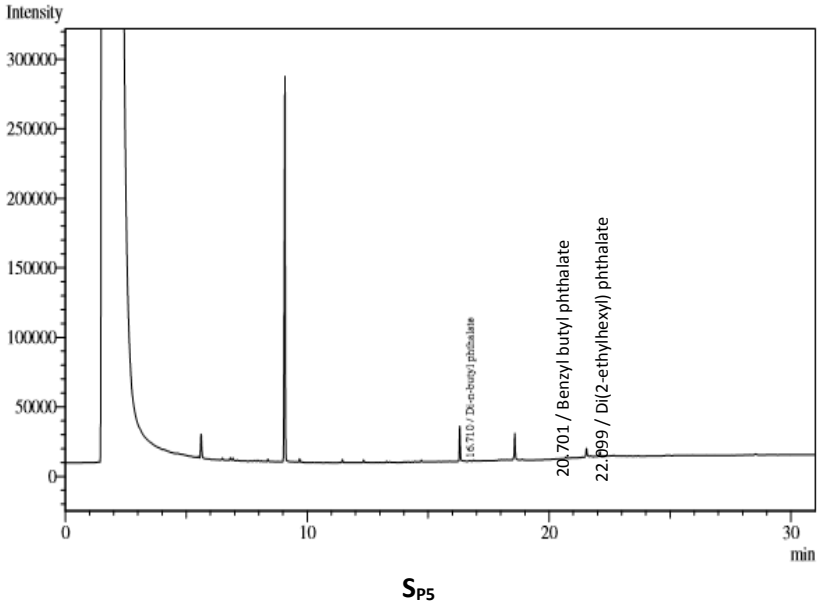


S_{P3}

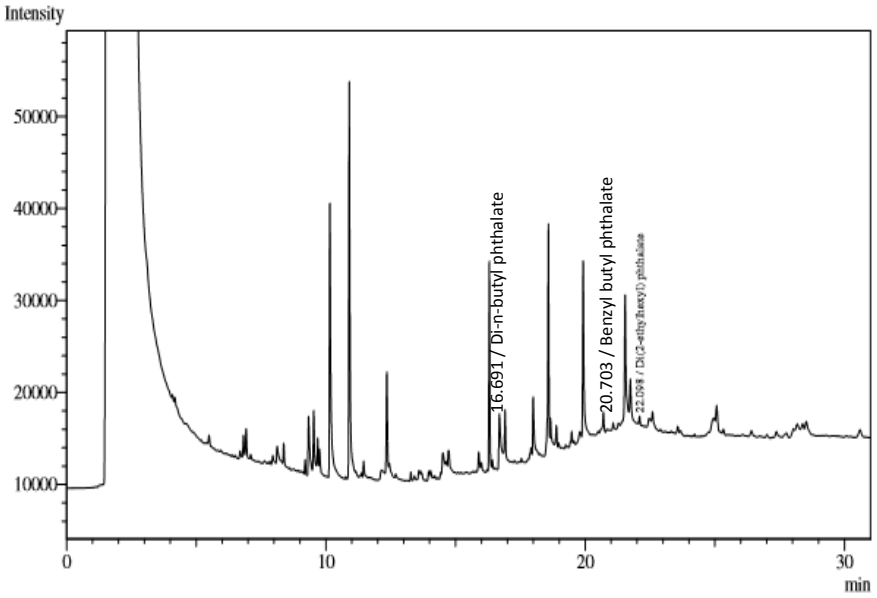


S_{P4}

Figure 6.1 Continued: Chromatograms of samples showing peaks for BBP, DEHP and DBP. (Sp_1-Sp_{15} = pouch samples, S_1-S_{21} = Bottled samples)

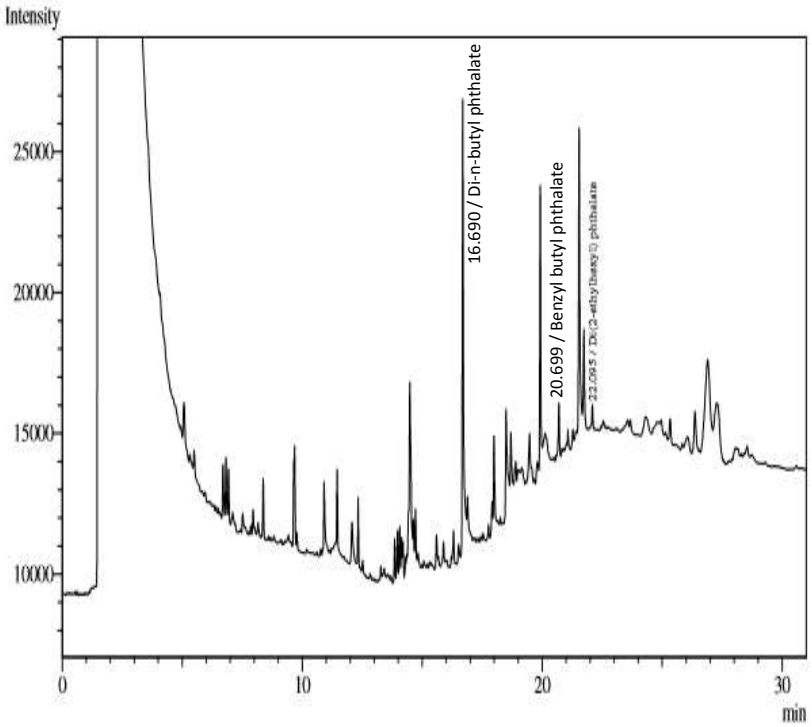


S_{P5}

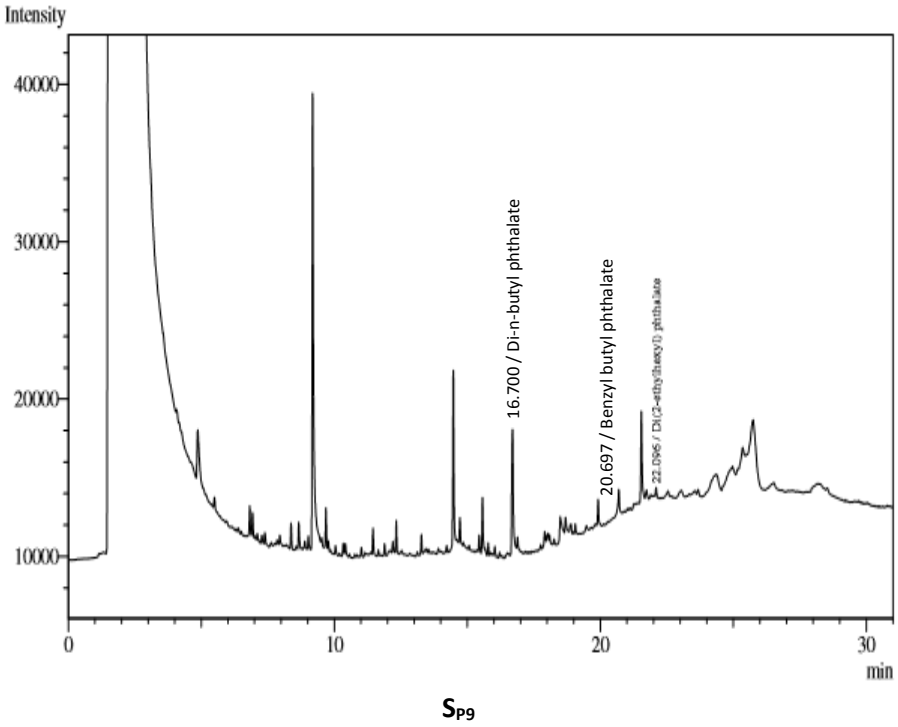


S_{P6}

Figure 6.1 Continued: Chromatograms of samples showing peaks for BBP, DEHP and DBP. (Sp_1-Sp_{15} = pouch samples, S_1-S_{21} = Bottled samples)

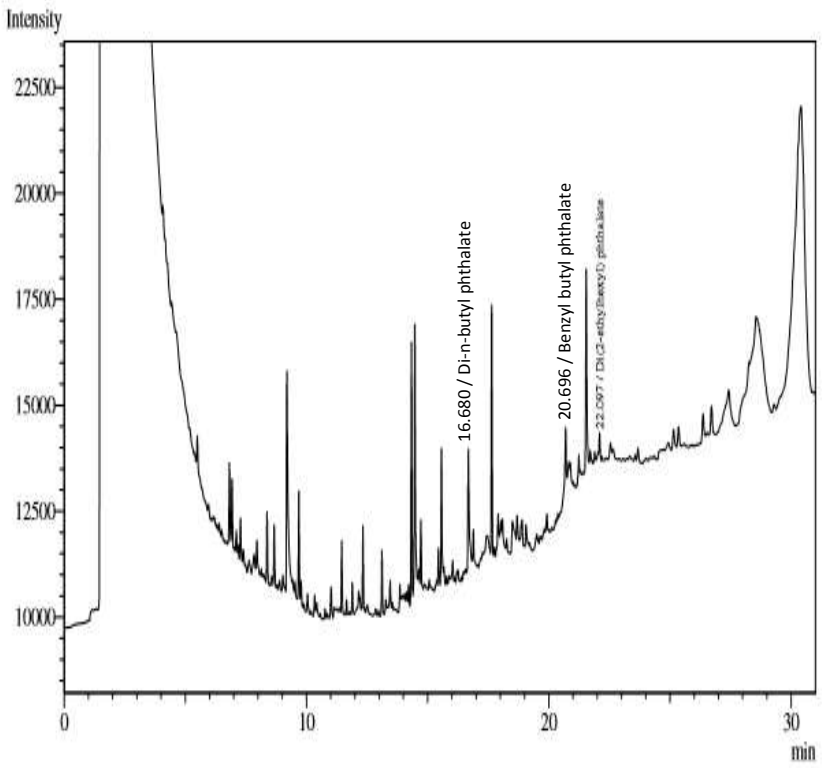


S_{P7}

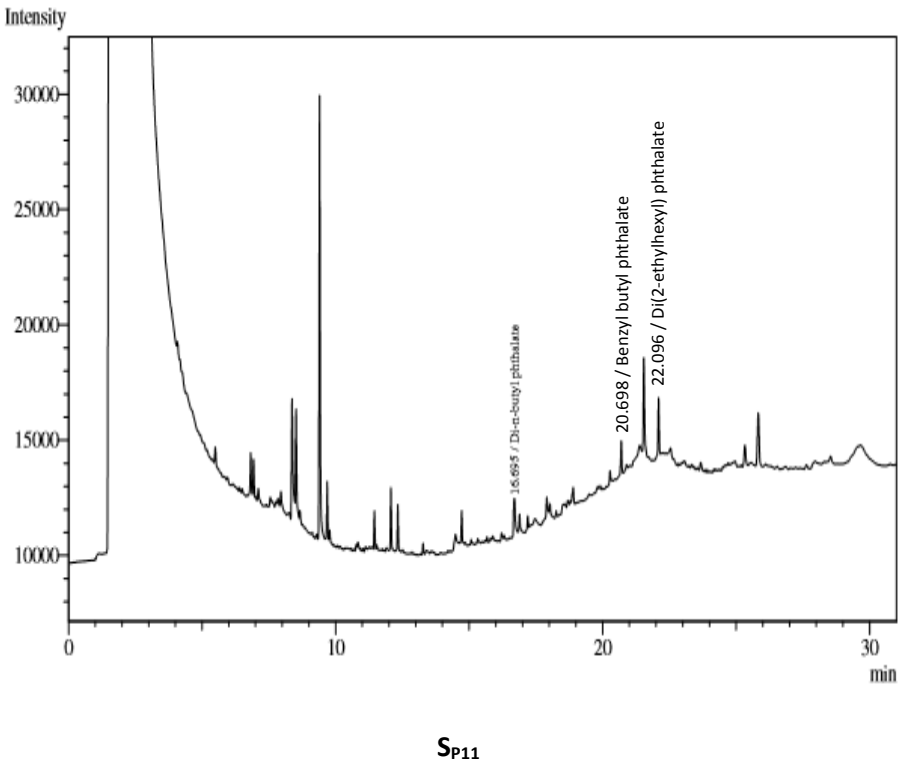


S_{P9}

Figure 6.1 Continued: Chromatograms of samples showing peaks for BBP, DEHP and DBP. (Sp_1-Sp_{15} = pouch samples, S_1-S_{21} = Bottled samples)

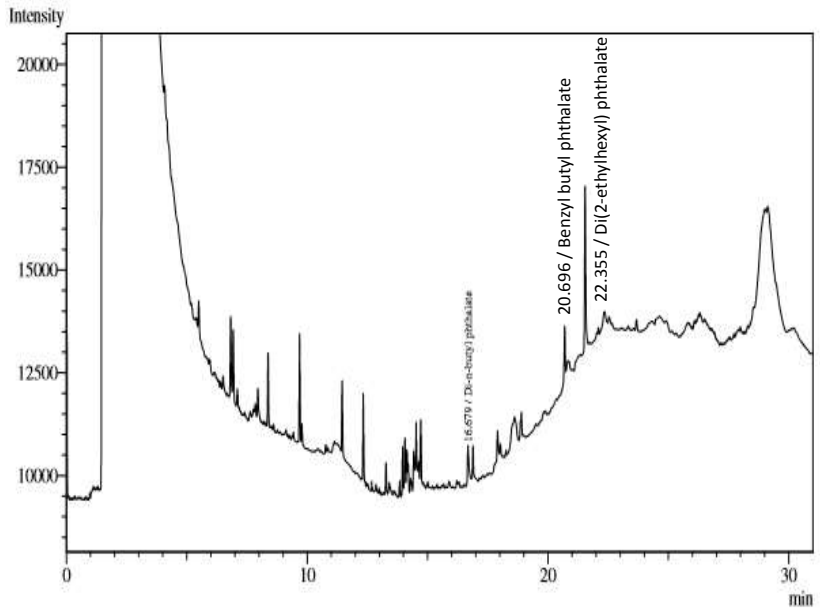


S_{P10}

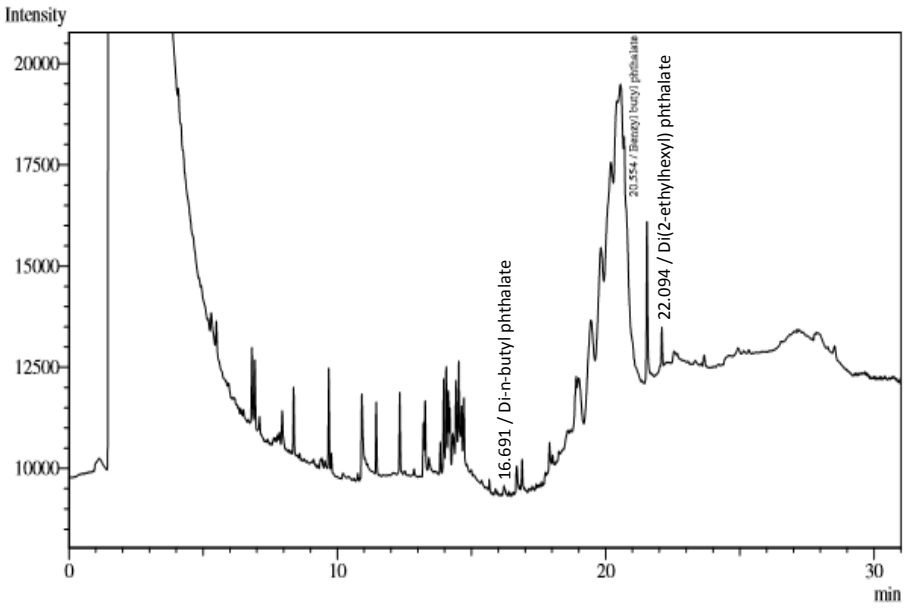


S_{P11}

Figure 6.1 Continued: Chromatograms of samples showing peaks for BBP, DEHP and DBP. (Sp_1-Sp_{15} = pouch samples, S_1-S_{21} = Bottled samples)

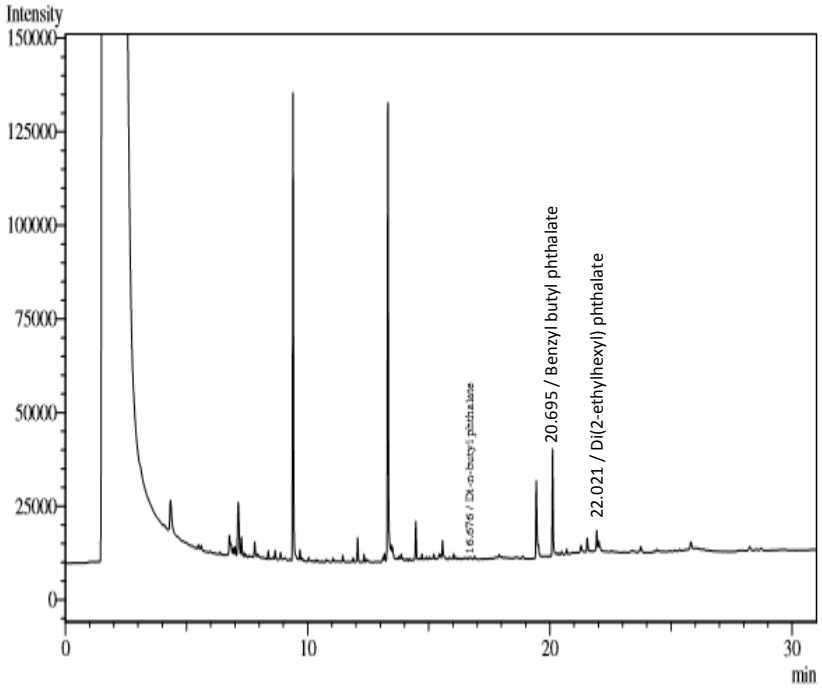


S_{P12}

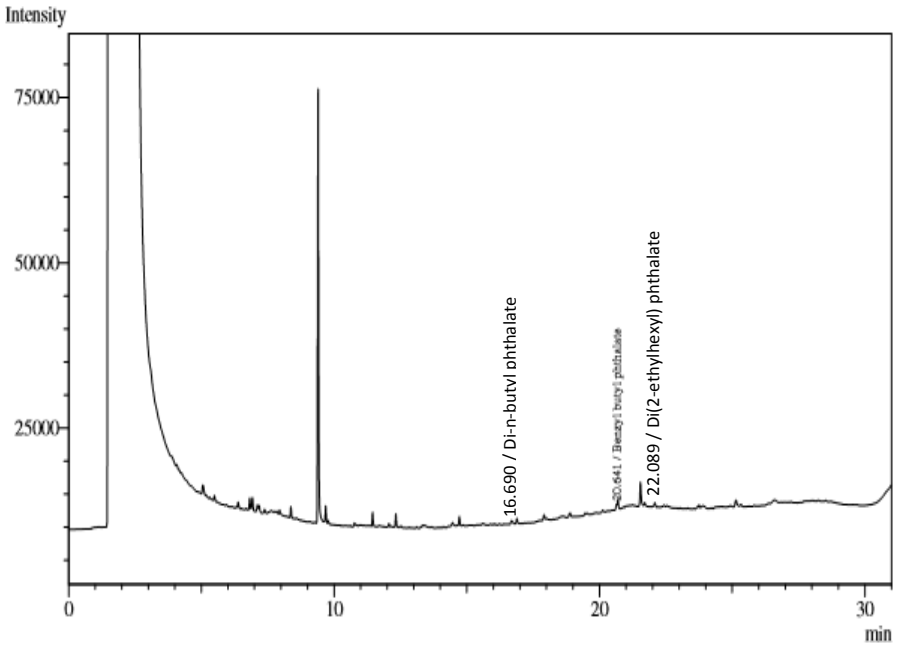


S_{P13}

Figure 6.1 Continued: Chromatograms of samples showing peaks for BBP, DEHP and DBP. (Sp_1-Sp_{15} = pouch samples, S_1-S_{21} = Bottled samples)

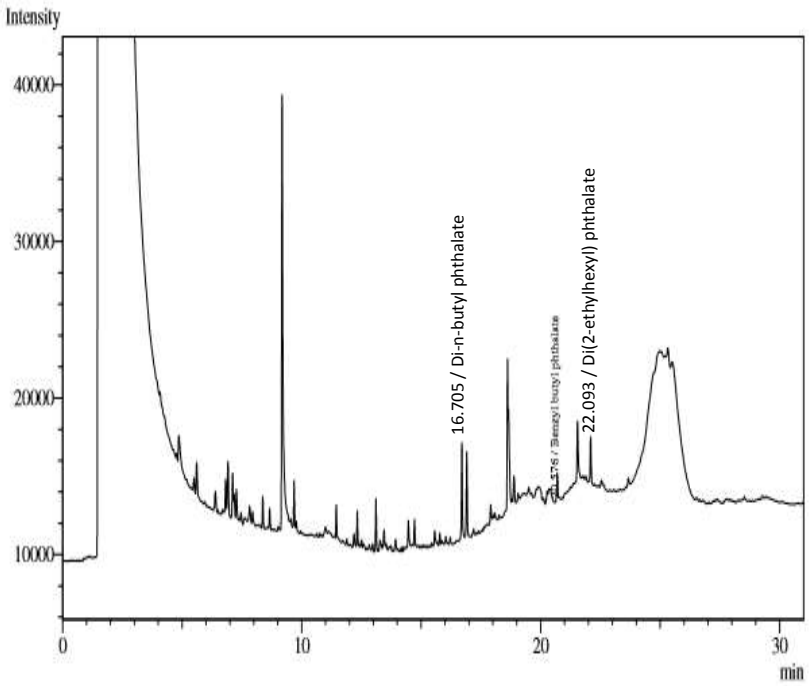


S_{P14}

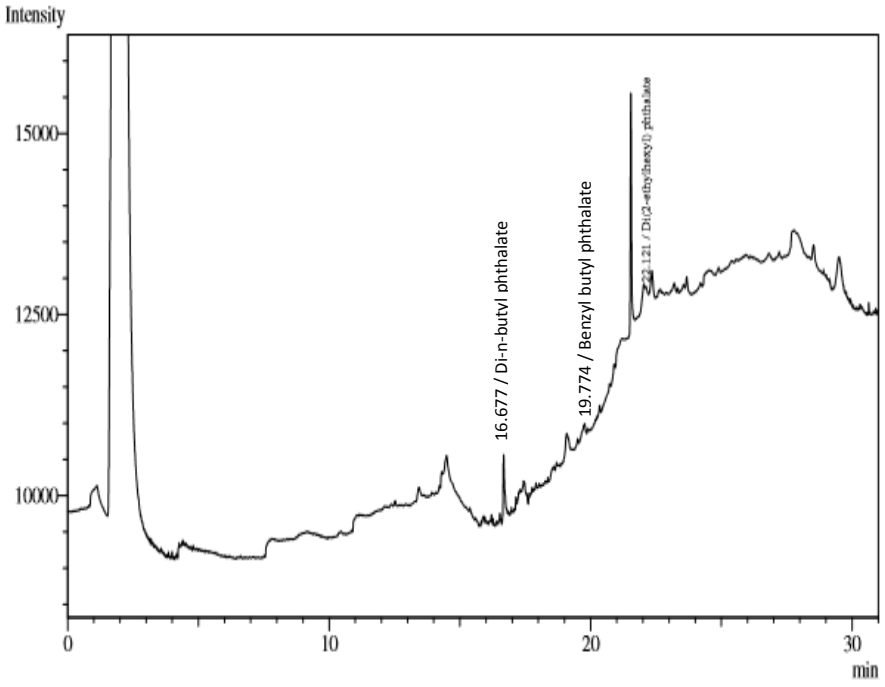


S_{P15}

Figure 6.1 Continued: Chromatograms of samples showing peaks for BBP, DEHP and DBP. (Sp_1-Sp_{15} = pouch samples, S_1-S_{21} = Bottled samples)

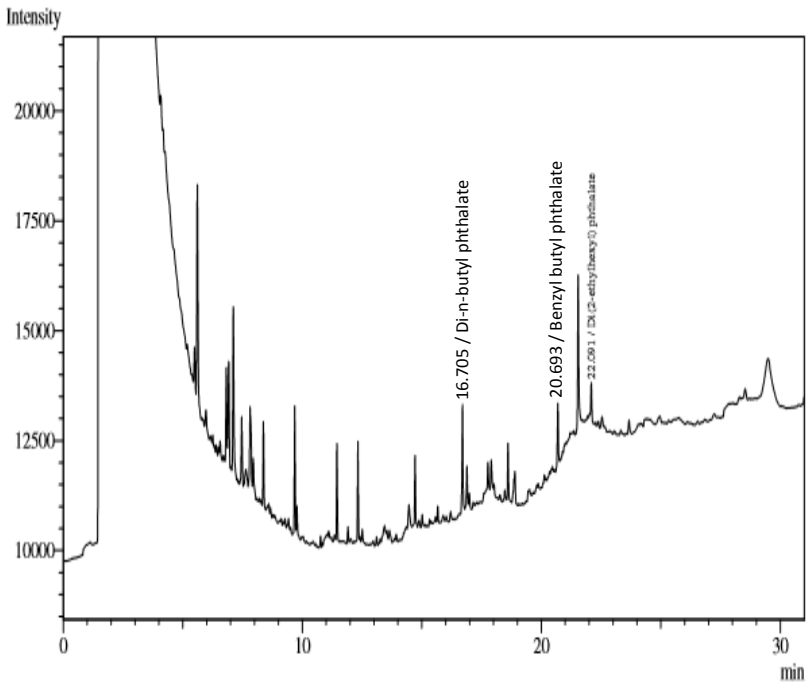


S_4

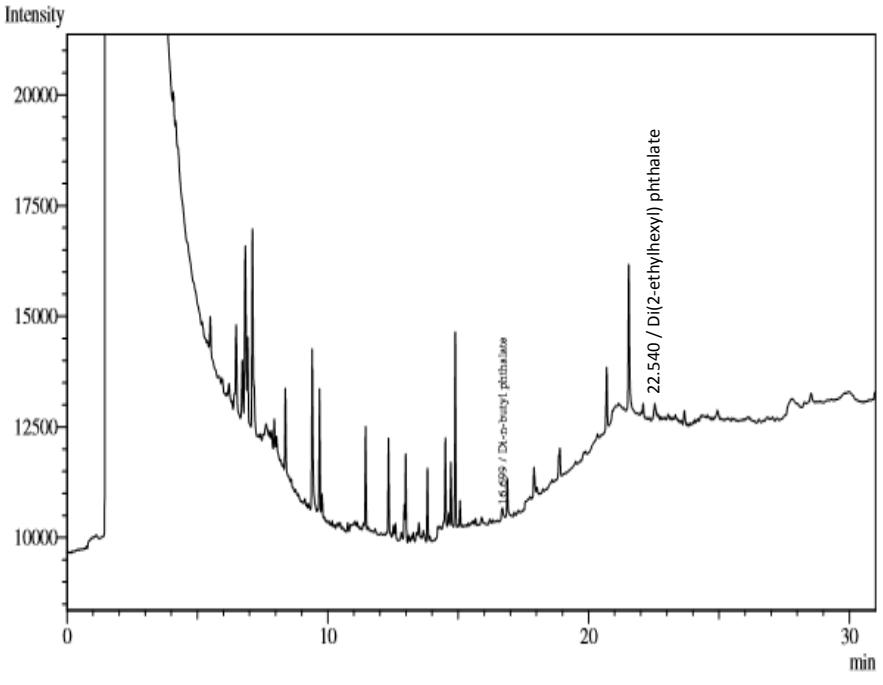


S_5

Figure 6.1 Continued: Chromatograms of samples showing peaks for BBP, DEHP and DBP. (Sp_1-Sp_{15} = pouch samples, S_1-S_{21} = Bottled samples)

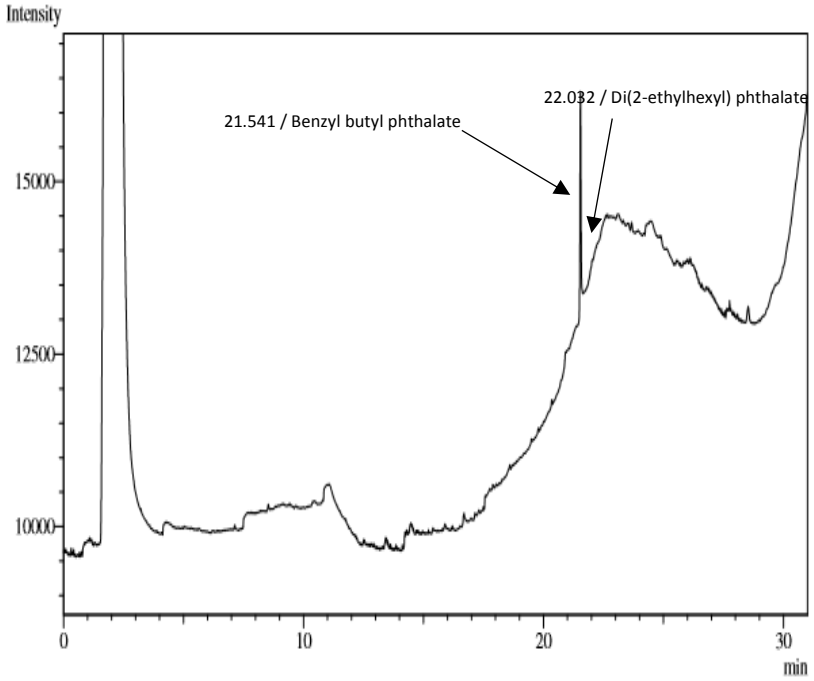


S_7

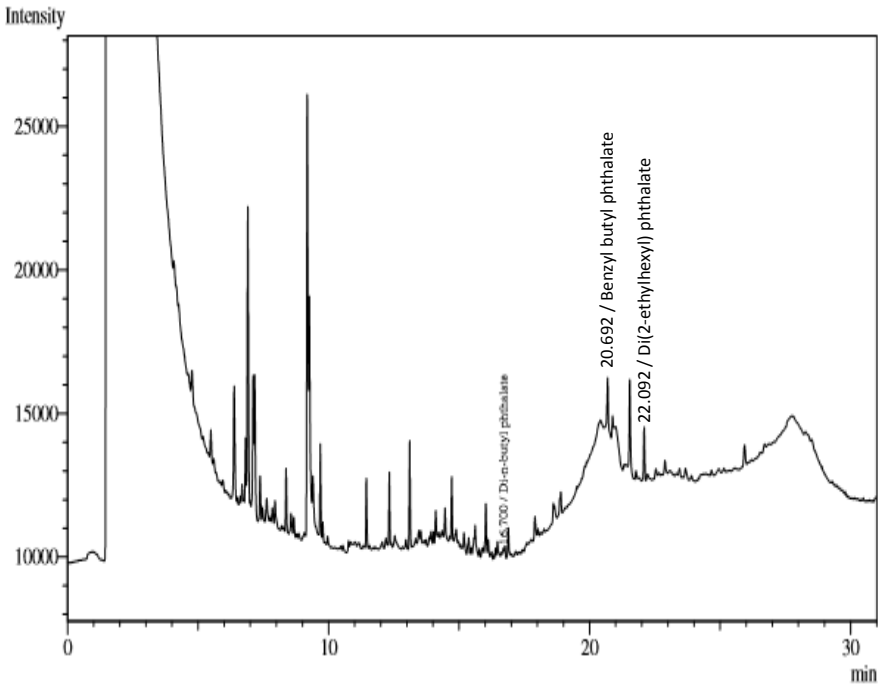


S_8

Figure 6.1 Continued: Chromatograms of samples showing peaks for BBP, DEHP and DBP. (Sp_1-Sp_{15} = pouch samples, S_1-S_{21} = Bottled samples)

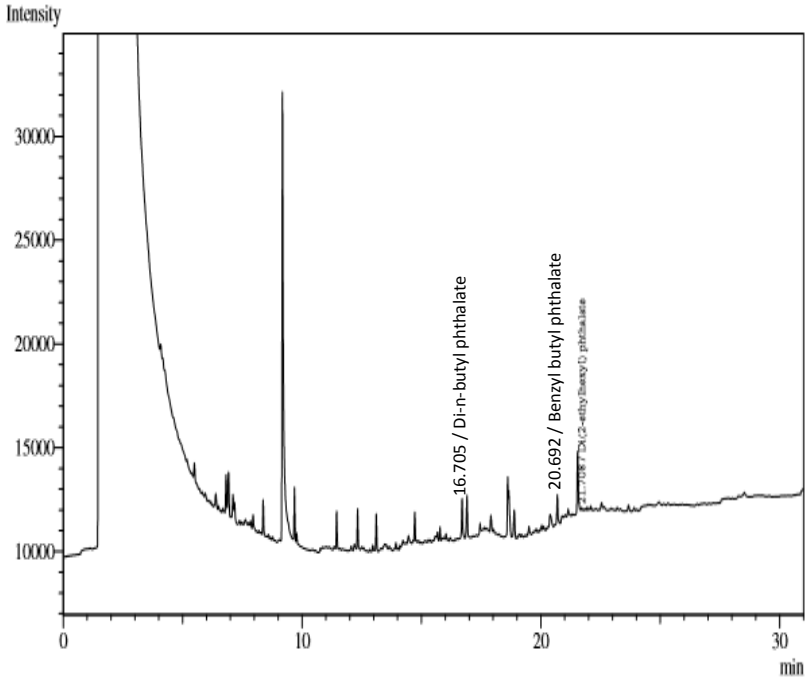


S_9

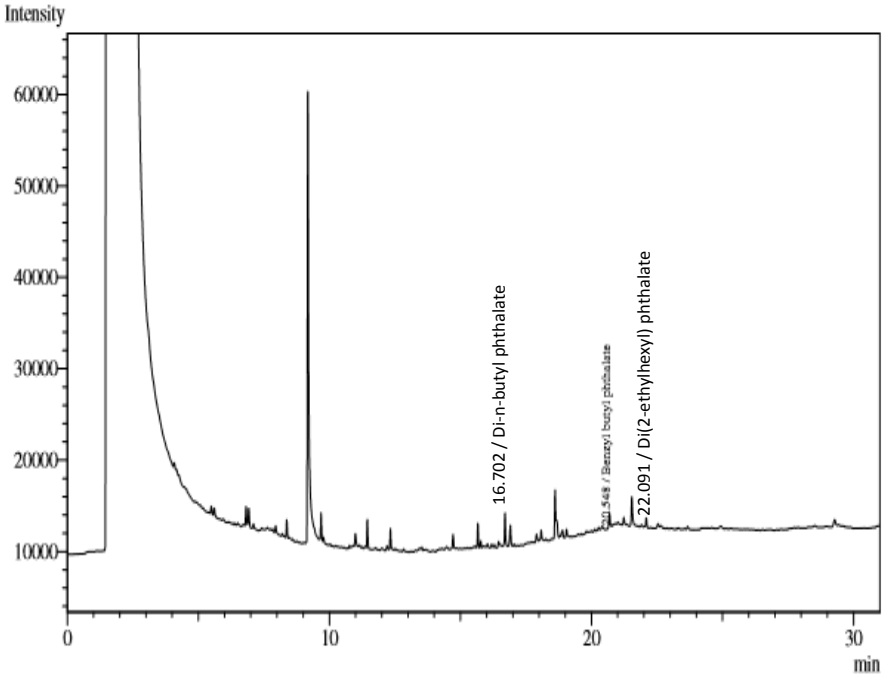


S_{10}

Figure 6.1 Continued: Chromatograms of samples showing peaks for BBP, DEHP and DBP. (Sp_1-Sp_{15} = pouch samples, S_1-S_{21} = Bottled samples)

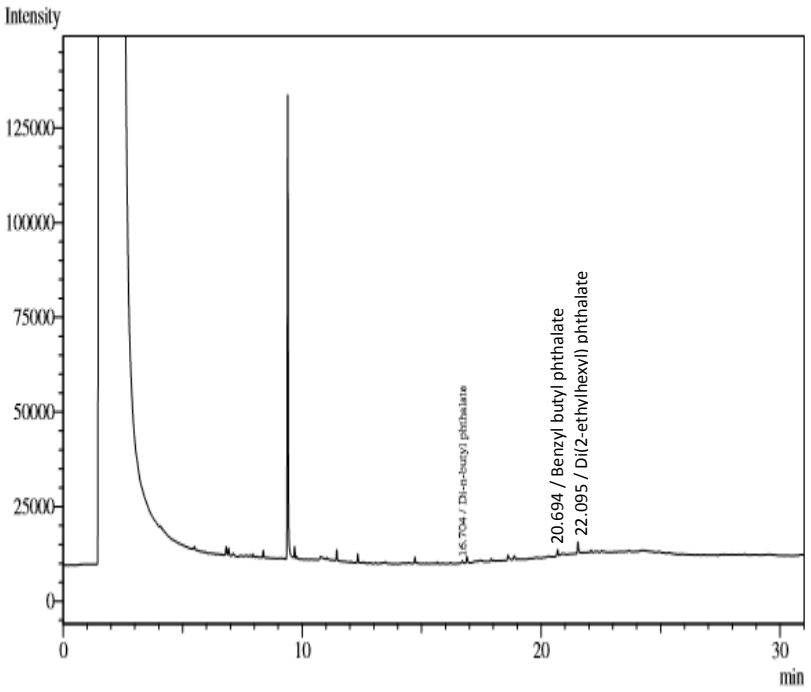


S_{12}

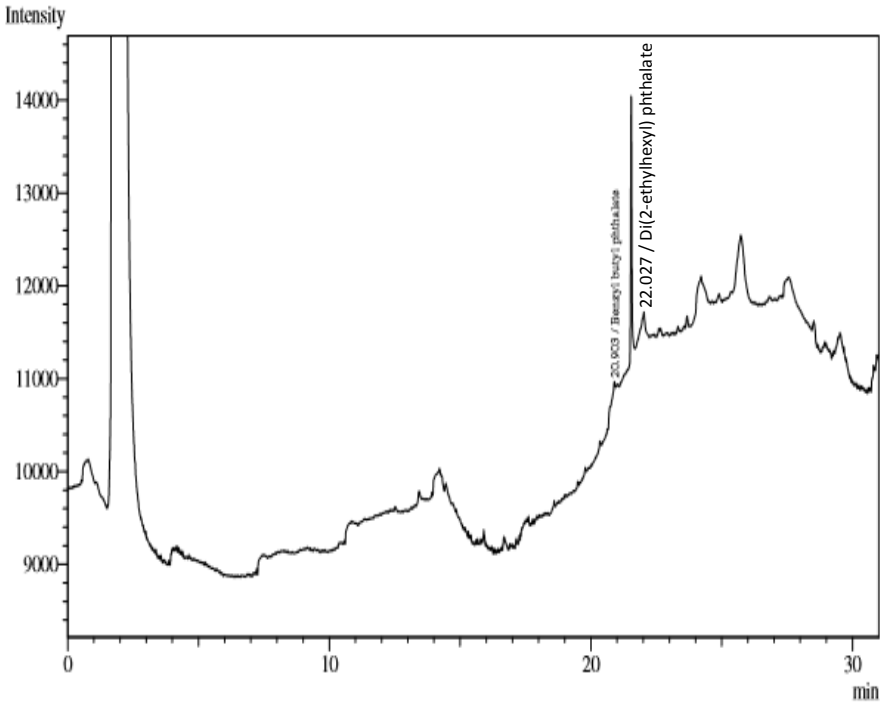


S_{13}

Figure 6.1 Continued: Chromatograms of samples showing peaks for BBP, DEHP and DBP. (Sp_1-Sp_{15} = pouch samples, S_1-S_{21} = Bottled samples)



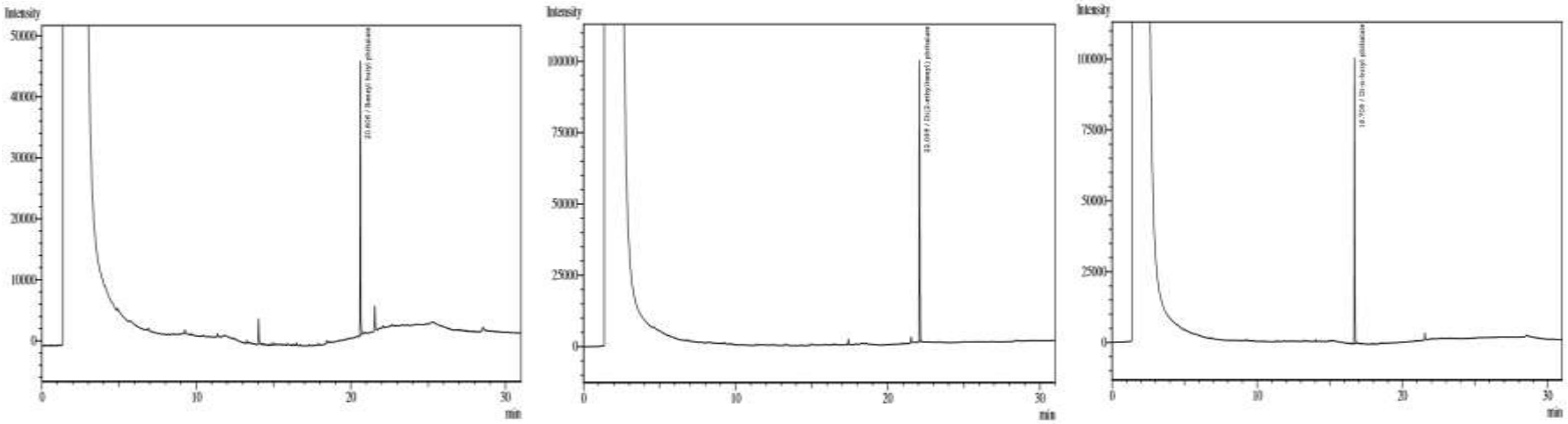
S_{19}



S_{20}

Figure 6.1 Continued: Chromatograms of samples showing peaks for BBP, DEHP and DBP. (Sp_1-Sp_{15} = pouch samples, S_1-S_{21} = Bottled samples)

1.2 REFERENCE STANDARDS



Benzyl butyl phthalate standard (32.822 µg/ml) Di(2-ethylhexyl) phthalate standard (32.291 µg/ml) Di-n-butyl phthalate standard(32.150 µg/ml)

Figure 6.2: Chromatograms of reference standards at 32 µg/ml concentrations

1.3 BLANK

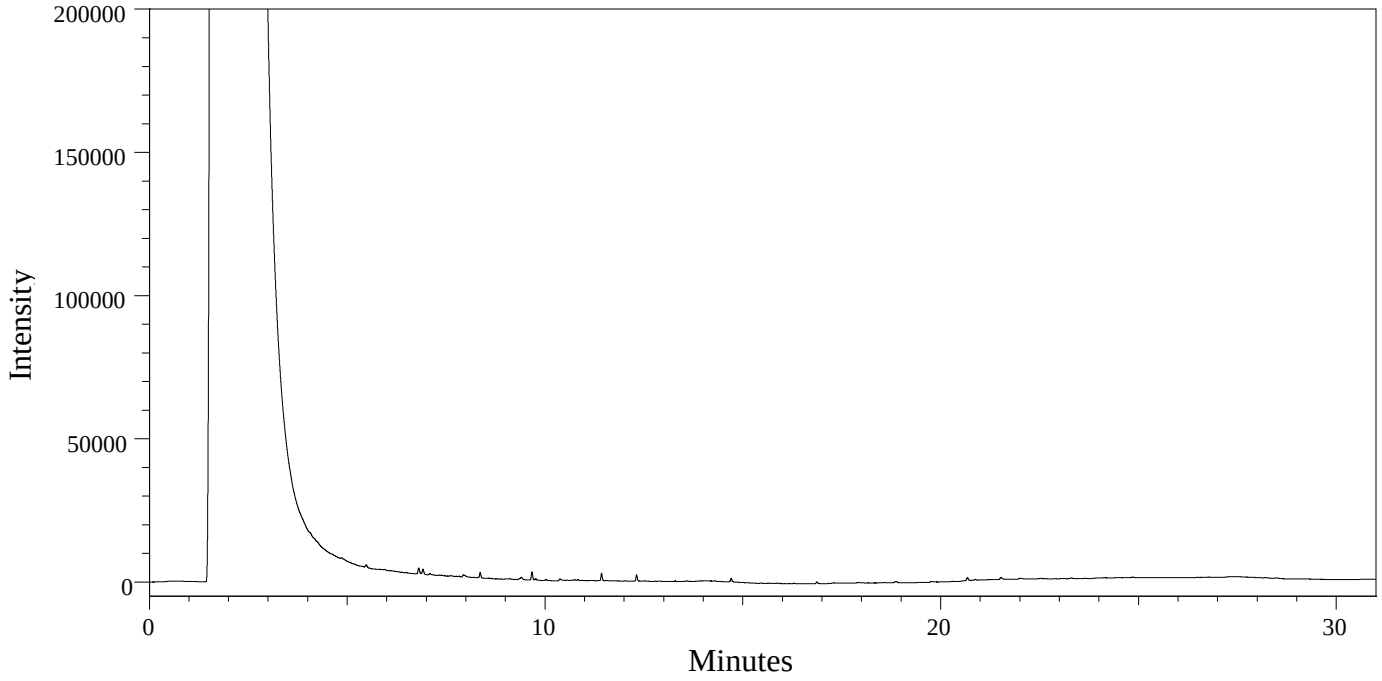


Figure 6.3: Chromatogram of Blank (Hexane solution)

APPENDIX III

TABLE OF RESULTS

Table 6.1: Raw data of the concentrations of BBP, DEHP and DBP as obtained from chromatograms of samples

		BBP				DEHP				DBP			
SAMPLE NO.	% ALC.	REP1	REP2	AVR	±SD	REP1	REP2	AVR	±SD	REP1	REP2	AVR	±SD
S_{P1}	40	5.95	4.81	5.38	0.81	4.03	1.18	2.60	2.01	2.54	1.83	2.18	0.50
S_{P2}	43	2.07	2.44	2.25	0.26	1.96	1.30	1.63	0.47	3.74	3.23	3.49	0.36
S_{P3}	42	3.13	3.17	3.15	0.03	3.06	1.90	2.48	0.82	1.86	1.90	1.88	0.02
S_{P4}	42	8.28	9.16	8.72	0.62	2.21	2.39	2.30	0.12	3.30	3.82	3.56	0.37
S_{P5}	42	2.57	3.44	3.01	0.61	4.77	6.85	5.81	1.47	1.82	1.80	1.81	0.02
S_{P6}	22	4.48	2.57	3.53	1.35	1.78	1.42	1.60	0.25	5.94	5.22	5.58	0.51
S_{P7}	45	3.35	1.64	2.50	1.21	1.41	1.19	1.30	0.15	9.84	2.57	6.02	5.14
S_{P8}	40	3.10	2.92	3.01	0.13	1.06	1.17	1.11	0.08	2.13	2.38	2.26	0.18
S_{P9}	42	4.54	3.81	4.18	0.52	1.38	1.19	1.29	0.14	6.38	4.06	5.22	1.64
S_{P10}	40	5.04	4.85	4.95	0.14	1.35	1.98	1.67	0.45	3.53	1.99	2.76	1.09
S_{P11}	30	3.46	4.22	3.84	0.54	1.90	1.81	1.86	0.06	2.69	3.04	2.87	0.24
S_{P12}	40	2.87	2.68	2.77	0.13	1.83	1.27	1.55	0.40	2.25	1.81	2.03	0.31
S_{P13}	40	3.21	4.97	4.09	1.25	1.41	2.29	1.85	0.62	2.09	1.86	1.97	0.17
S_{P14}	40	2.43	2.36	2.40	0.05	4.00	1.27	2.63	1.93	2.56	1.94	2.25	0.44
S_{P15}	42	2.08	2.13	2.11	0.03	1.35	1.45	1.40	0.07	2.02	2.91	2.46	0.63
S_1	42	3.02	2.78	2.90	0.17	2.04	3.12	2.58	0.76	ND	ND	**ND**	ND
S_2	18	2.63	2.78	2.71	0.11	1.18	1.28	1.23	0.07	2.12	1.92	2.02	0.14
S_3	40.5	3.24	3.56	3.40	0.22	2.12	2.44	2.28	0.23	2.01	1.97	1.99	0.03
S_4	42	1.42	2.36	1.89	0.67	3.38	1.18	2.28	1.55	4.08	3.40	3.74	0.48

REP1 = Replicate 1, **REP2** = Replicate 2, **AVR** = Average, **SD** = Standard Deviation, **ND** = not detected, **S_1-S_{21}** = samples in bottles, **Sp_1- Sp_{15}** = samples in pouches, mean concentrations above the LOQ, mean concentrations above LOD but below LOQ, mean concentration above LOQ but one of whose replicate values is below LOQ

Table 6.1 Continued: Raw data of the concentrations of BBP, DEHP and DBP as obtained from chromatograms of samples

		BBP				DEHP				DBP			
SAMPLE NO.	**% ALC.**	**REP1**	**REP2**	**AVR**	**±SD**	**REP1**	**REP2**	**AVR**	**±SD**	**REP1**	**REP2**	**AVR**	**±SD**
S_5	40	1.56	2.65	2.10	0.77	1.30	1.32	1.31	0.02	2.21	1.88	2.05	0.23
S_6	40	2.56	2.48	2.52	0.05	1.43	1.17	1.30	0.18	1.82	1.93	1.88	0.08
S_7	25	2.51	2.45	2.48	0.04	1.36	1.19	1.28	0.13	2.71	1.88	2.29	0.59
S_8	40	2.51	2.28	2.39	0.16	1.31	1.18	1.24	0.09	1.81	1.82	1.82	0.01
S_9	30	4.19	2.06	3.12	1.50	1.18	1.18	1.18	0.00	ND	ND	**ND**	ND
S_{10}	35	8.63	7.83	8.23	0.57	3.69	4.85	4.27	0.82	1.8	1.98	1.90	0.13
S_{11}	35	4.52	6.43	5.48	1.35	3.56	4.42	3.99	0.61	2.46	2.89	2.68	0.30
S_{12}	42	2.57	3.08	2.83	0.36	1.19	1.21	1.20	0.01	2.39	2.32	2.35	0.05
S_{13}	40	1.69	2.61	2.15	0.65	1.49	1.18	1.33	0.21	3.06	4.56	3.81	1.06
S_{14}	40	2.05	3.41	2.73	0.96	3.56	2.05	2.81	1.07	4.64	3.45	4.05	0.84
S_{15}	40	1.24	1.56	1.40	0.23	1.45	1.74	1.60	0.21	1.27	1.82	1.55	0.39
S_{16}	35	1.88	2.8	2.34	0.65	1.26	1.2	1.23	0.04	1.99	1.91	1.95	0.06
S_{17}	12	2.31	2.56	2.44	0.18	1.35	1.31	1.33	0.03	1.83	1.68	1.75	0.11
S_{18}	42	1.23	1.31	1.27	0.06	2.48	1.96	2.22	0.37	ND	ND	**ND**	ND
S_{19}	42	2.64	2.73	2.69	0.06	1.41	1.18	1.29	0.16	1.98	1.88	1.93	0.07
S_{20}	40	1.38	1.28	1.33	0.07	1.66	1.45	1.56	0.15	ND	ND	**ND**	ND
S_{21}	40	3.94	3.86	3.90	0.06	2.05	1.98	2.02	0.05	ND	ND	**ND**	ND

REP1 = Replicate 1, **REP2** = Replicate 2, **AVR** = Average, **SD** = Standard Deviation, **ND** = not detected, **S_1-S_{21}** = samples in bottles, **Sp_1-Sp_{15}** = samples in pouches, mean concentrations above the LOQ, mean concentrations above LOD but below LOQ, mean concentration above LOQ but one of whose replicate values is below LOQ

Table 6.2: Spike recovery data for DBP

	Di-n-butyl Phthalate (DBP)			
Recovery Calculation	**Sample = S_{P8}**	**Sample = S_3**	**Sample = S_{P8}**	**Sample = S_3**
Sample Conc. (ppb)	2.26	1.99	2.26	1.99
Concentration Spiked (ppb)	0.1	0.1	0.2	0.2
Total Concentration Injected (ppb)	2.36	2.09	2.46	2.19
Recovered from GC Analysis (ppb)	2.50	1.84	1.77	1.77
% Recovery	105.97	88.13	72.24	80.81

Key: Conc. = concentration, ppb = parts per billion