Determination of Phthalate Esters in Soft Drinks by Gas Chromatography and Mass Spectrometry

O. E. Odumade, L. O. Ekebafe, D. K. Obodo

Department of Chemistry, University of Lagos, Lagos, Nigeria
Corresponding author E-mail: lekebafe@unilag.edu.ng

ABSTRACT

This study investigates the presence of phthalate esters in bottled soft drinks and evaluates the associated health risks and regulatory considerations. Extraction was done using 15 mL of dichloromethane (DCM). A gas chromatograph equipped with a split/splitless injector and an auto-sampler coupled with a triple quadrupole mass spectrometer was employed for the analysis. Analysis of various brands revealed significant concentrations of di(2-ethylhexyl) phthalate (DEHP) and dibutyl phthalate (DBP);DEHP had the highest concentration in Tem (36.17µg/mL) and the lowest in Sup(22.44µg/mL), while DBP ranged from 12.97µg/mL in Mira to 22.87µg/mL in Tem. DEHP also demonstrated the highest mean concentration of 28.20 ± 5.75 µg/mL. Known for its adverse effects on liver and testes. These findings underscore the potential health hazards associated with phthalate exposure, particularly to vulnerable populations. Regulatory comparisons highlight the absence of universally accepted maximum contaminant levels for phthalates in beverages, emphasizing the need for standardized guidelines to safeguard consumer health. Furthermore, discussions encompass the influence of storage conditions on phthalate leaching, with higher temperatures exacerbating contamination levels. Recommendations include consumer education on proper storage practices, industry adoption of phthalate-free packaging, and global harmonization of regulatory frameworks.

Keywords: Phthalate esters, Soft drinks, Gas Chromatography, Mass spectrometry

INTRODUCTION

Numerous chemicals could enter our food at different stages of the food manufacturing process, either intentionally or unintentionally. These include environmental contaminants, food additives, and compounds derived from objects that come into touch with food, such as processing and packaging equipment. Maintaining food safety regulations and protecting public health depend on determining exposure to these compounds[1,2]. Phthalates are one of these compounds that enter our meals through a variety of routes and call for careful monitoring.

Plasticizers have been essential in a variety of industries, including consumer goods and automotive applications. In the beginning, camphor was employed to plasticize celluloid; however, tricresyl phosphate eventually took its place in the manufacturing of PVC (poly vinyl chloride).

The most common class of plasticizers since their introduction in the 1920s are phthalic acid esters[3]. Phthalate chemicals have caused public concern since the 1980s because of their possible negative health consequences and migration problems. Among the most often used compounds in the world are phthalates, of which di(2-ethylhexyl) phthalate (DEHP) is the most common.

The production of DEHP ranged from 2.2 to 8.8 billion pounds per year worldwide in 1994, with Western Europe producing more than one billion pounds in 2006 (European Chemicals Bureau (ECB), 2008)[4]. From 1.2 billion pounds in 1997 to 750 million pounds in 2007, the EU's output of DEHP drastically decreased due to health concerns (European Chemicals Agency (ECHA) DEHP, 2009)[5].

The use of non-phthalate plasticisers has increased as a result of governmental restrictions and environmental awareness. Numerous industries, including medical devices, construction materials, toys for kids, food packaging, personal care items, and apparel, employ phthalate chemicals extensively[6-8]. Because they are dangerous, phthalates' migration from food packaging materials into foodstuffs has drawn a lot of public attention.

Since Nigerians consume a lot of beverages in plastic bottles, it is essential to comprehend the existence and possible leaching of phthalates in order to protect the public's health. Consumers may be at risk for health problems if phthalates, which are frequently present in PVC products, seep into beverages because of weak connections. In Nigeria, ensuring the safety of food and drinks in plastic bottles helps to ensure food security and safety.

Understanding and addressing potential sources of contamination like phthalates is essential for maintaining public trust in food and beverage products. The presence of phthalates in drinks can negatively impact brand image, potentially resulting in financial setbacks and product withdrawals. This research aims to highlight and reduce such risks, supporting healthier business operations and encouraging long-term sustainability.

The outcomes of this study can also support the development of informed policies aimed at minimizing phthalate exposure and protecting public well-being. Using scientific findings to shape regulations can lead to improved oversight of plastic packaging materials and ensure they meet health and safety guidelines. By analyzing the levels of different phthalates in soft drinks packaged in plastic bottles, the study adds to the broader conversation about chemical contaminants in consumables.

MATERIALS AND METHODS

Materials: HPLC grade Dichloromethane was purchased from Fisher Scientific (Loughborough, UK). Anhydrous sodium sulfate of analytical reagent grade was purchased from MP Biomedicals, LLC (Strasbourg, France).

Six different brands of frequently consumed soft drinks designated as; Mira, Pei, Sup, Spi, Ola and Tem were selected for this study. The soft drink samples were geographically randomly collected in Dec. 2023 from popular stores in the

University of Lagos but were of different batches for each brand. All the samples were packed in PET plastic bottles with High Density polyethylene (HDPE) plastic caps and were stored *in the dark at 4°C in their original seal containers until the analysis was made (less than 48 hours).*

Extraction Procedure:

100 mL of the samples was measured into the separating funnel and was extracted serially with three different portions of 15 mL of dichloromethane (DCM). The three extracts were added together in another separating funnel and further extraction was done twice with 5 mL 0.1M sodium carbonate to get rid of free fatty acids and some aqueous entraps in the organic constituent that had been extracted with DCM. The extracts were dehydrated over an anhydrous sodium sulfate filter paper and the solvent was left to evaporate with time when exposed to air in a fume cupboard. The residue was re-dissolved in 2 mL DCM before it was cleaned up in silica gel packed column.

A gas chromatograph equipped with a split/splitless injector and an auto-sampler (Varian CP-3800, Varian Inc., Middleburg, The Netherlands) coupled with a triple quadrupole mass spectrometer (Varian 320, Varian Inc., Walnut Creek, USA) was employed for the analysis. A 30 m VF-5MS capillary column (5% phenyl - 95% methyl polysiloxane) with a 0.25 µm film thickness and 0.25 mm internal diameter (CP8944, J and W Agilent, Folsom, California, USA) was used for compound separation. Helium carrier gas at 99.999% purity was used at a constant rate of 1 ml min^{-1}. Injections of 1 µl were made in splitless mode using an injector temperature set at 275°C. The oven temperature was initially programmed at 40°C for 3.5 min then was increased by a rate of 5°C min^{-1} until reaching 250°C and then held for 10 min. 33 Finally, the temperature was increased at a rate of 2°C min^{-1} reaching 280°C and then held for 5 min. Electron ionization of 70 eV was employed and the quadrupoles were scanned from 50- 550 amu. at a rate of 0.5 scans sec^{-1} . The transfer line, ion source and manifold of the mass spectrometer were set at 250, 200 and 40°C respectively. The data were acquired in full scan mode and recorded on a workstation (Varian 6.9) equipped with a National Institute of Standards and Technology (NIST, US Department of Commerce) mass spectral library. The target PAEs compounds were identified in the samples based on retention time matching to the standard, the presence of two to four qualifier ions and their correct ratios in accordance to the mass spectra characteristic features obtained using the standards.

Microsoft Excel was used to calculate the mean, standard deviation, and coefficient of variation of the different Phthalate concentrations from the GC-MS analysis.

The phthalates esters concentrations in the soft drinks brands were determined, and the results are presented in the table below:

RESULT AND DISCUSSION

Table 1: Concentration of phthalates esters in bottled samples

BRAND	DBP (µg/mL)	DEHP (µg/mL)
Mira	12.97	27.01
Pei	ND	ND
Sup	16.37	22.44
Spite	14.78	27.18
Ola	ND	ND
Tem	22.87	36.17

*ND – Not detected.

Table 1 presents the concentration of two phthalate esters, DBP (Di-n-butyl phthalate) and DEHP (Di-2-ethylhexyl phthalate), in various bottled samples of different brands. The concentrations are measured in micrograms per milliliter (µg/mL). Additionally, the table indicates when a phthalate ester was not detected in a particular sample, denoted as "ND" (Not Detected). DBP and DEHP are specific types of phthalate esters commonly used as plasticizers in various consumer products, including food and beverage packaging materials. Table 1 lists different brands of bottled samples, including Mira, Pei, Sup, Spite, Ola, and Tem.

For each brand, the concentration of DBP and DEHP is provided. These concentrations represent the amount of each phthalate ester detected in the bottled sample per milliliter of the liquid content.:Mira: Contains 12.97 µg/mL of DBP and 27.01 µg/mL of DEHP; Pei: DBP and DEHP were not detected in this sample; Sup: Contains 16.37 µg/mL of DBP and 22.44 µg/mL of DEHP; Spi: Contains 14.78 µg/mL of DBP and 27.18 µg/mL of DEHP; Ola: DBP and DEHP were not detected in this sample; Tem: Contains 22.87 µg/mL of DBP and 36.17 µg/mL of DEHP. ND (Not Detected): This designation indicates that the specific phthalate ester was not found in detectable amounts in the respective sample.

Overall, the data suggest that phthalate esters, particularly DBP and DEHP, are present in varying concentrations in bottled samples of different brands. The presence or absence of these phthalate esters can have implications for consumer health and regulatory considerations related to food and beverage safety.

Table 2: Mean, Standard deviation and coefficient of variation of phthalates esters

Phthalates	DBP (µg/mL)	DEHP (µg/mL)
Mean	16.75	28.20
Standard deviation	4.31	5.75
Coefficient of variation %	25.73	20.39

Table 1 summarizes the concentration of phthalates in the bottled samples range was between 12.97 - 27.01 µg/mL for Mira, Pei shows no detection, 16.37 – 22.44 µg/mL for Sup, 14.78 – 27.18 µg/mL for Spi, Ola shows no detection and 22.87 – 36.17 µg/mL for Tem.

Lowest DBP concentration was detected in Mira (12.97µg/mL) and highest in Tem (22.87 µg/mL). Maximum concentration of DEHP was detected in Tem (36.17 µg/mL) and minimum in Sup (22.44 µg/mL)

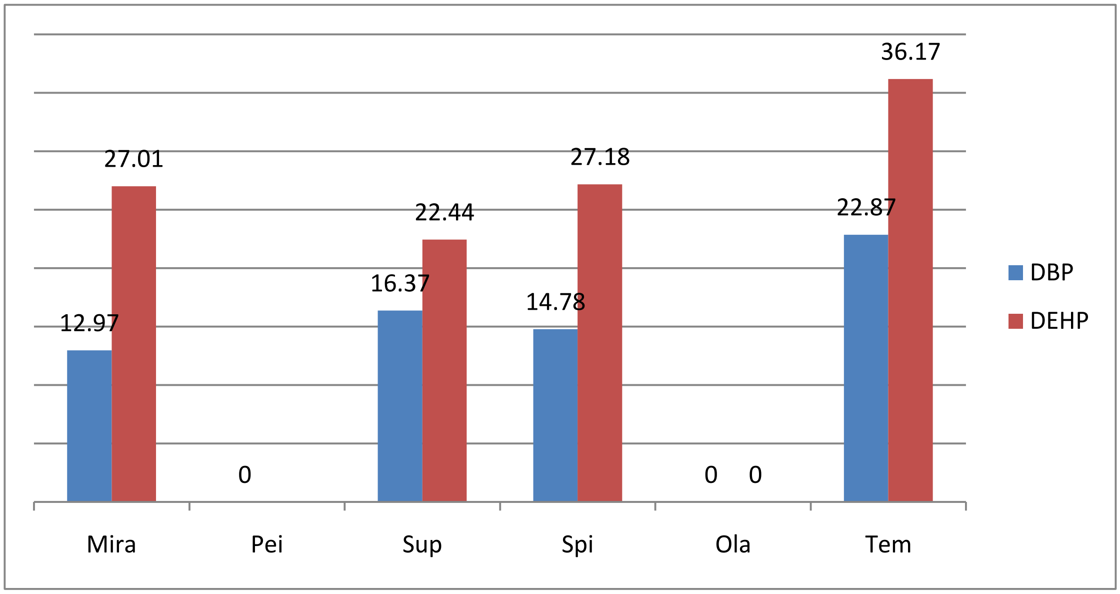

Figure 1: concentration of phthalates esters.

DEHP Bis(2-ethylhexyl) phthalate had the highest concentration of 36.17 µg/mL in Tem and the lowest concentration of 22.44 µg/mL in Sup. DBP (Di-butylphthalate) had the lowest concentration in Mira 12.97µg/mL and highest concentration in Tem 22.87 µg/mL

Pei and Ola showed no sign of DBP and DEHP, which could be as a result of the high increase in the pH value. The mean of the overall concentrations of phthalates (µg/mL) are presented in Table 2. DEHP Bis(2-ethylhexyl) phthalate had the highest mean concentration of 28.2 ± 5.75 µg/mL.

The main organs affected by DEHP toxicity are the liver and testes. DEHP has been shown to cause liver damage by altering lipid and carbohydrate metabolism, often accompanied by visible structural changes. One of the most notable effects is the increase in the number of peroxisomes in liver cells, as previously reported by the World Health Organization in 1996[7]. Based on the data presented in Table 2, DEHP had the highest average concentration among the phthalates studied, followed by DBP. This result aligns with earlier studies[8-10], which reported DEHP as a frequently detected phthalate in plastic materials.

Regulatory bodies like the United States Environmental Protection Agency, the European Union, and the World Health Organization have set the maximum allowed concentration of DEHP in bottled water at 6 and 8 micrograms per liter respectively. These values were used as reference points in this study since there are no established safety limits for other phthalates in beverages at either national or international levels. In this research, both DEHP and DBP concentrations were found to be lower than these recommended thresholds.

DEHP and DBP appear to be frequent contaminants in bottled water across various regions, with most reported concentrations falling between 0 and 1 microgram per liter. The likelihood of these chemicals leaching into drinks increases with temperature. Storage conditions have a major impact on the extent of leaching, with the highest levels observed in bottles kept outside, followed by those stored at room temperature and then at 4 degrees Celsius. These findings are consistent with earlier research, which demonstrated that phthalate leaching is triggered by hydrolysis, with higher temperatures accelerating the process.

Bottles exposed to warmer environments are more prone to releasing higher amounts of phthalates, supporting the evidence that heat encourages leaching. This explains why bottles stored outdoors tend to show the highest concentrations of these substances. An increase in DEHP levels in PET bottled water left in direct sunlight at 34 degrees Celsius for 17 hours was observed in another study[6]. Similarly, a study reported that temperature, duration of storage, and sunlight exposure significantly influenced the release of DEHP and DnBP from juice containers, especially those holding acidic beverages[9-12]. Another study also concluded that lower temperatures, such as 4 degrees Celsius, are less likely to support phthalate migration in acidic drinks[8]. These findings emphasize that higher temperatures promote the hydrolysis process that leads to greater phthalate leaching.

Table 3: Retention time of phthalate esters

BRAND	DBP	DEHP
Mira	7.264	9.713
Pei	ND	ND
Sup	7.230	9.679
Spi	7.264	9.685
Ola	ND	ND

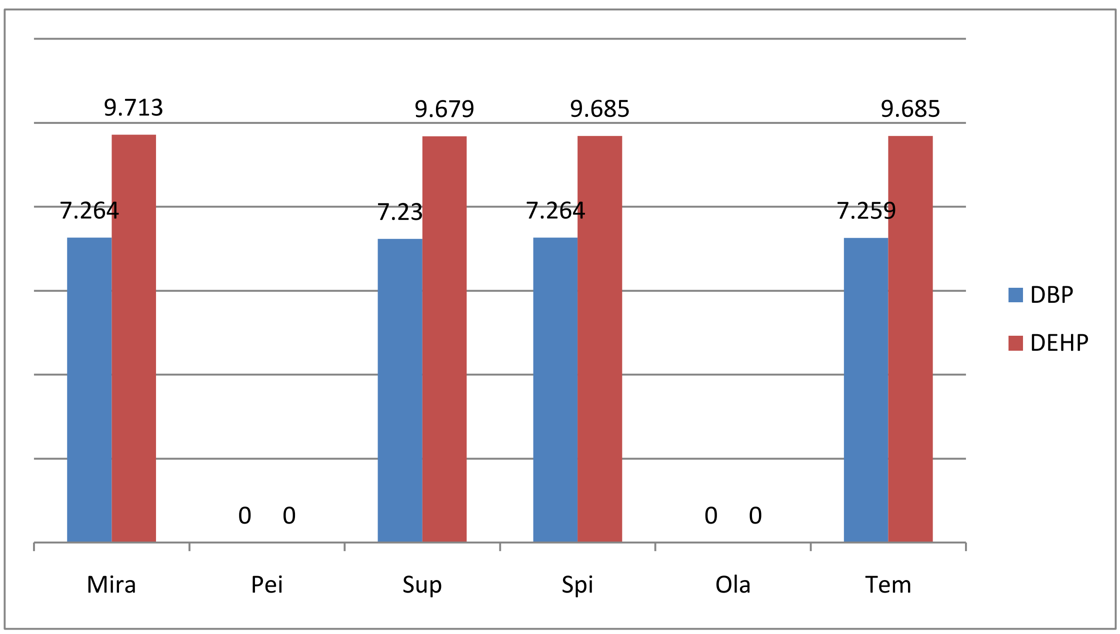

Figure 2: Retention time of determined phthalates esters.

The retention time of each of the studied phthalate esters in each sample are: Mira: DBP – 7.264, DEHP – 9.713; Pei: DBP – ND, DEHP - ND; Sup: DBP – 7.230, DEHP-9.679; Spi: DBP

– 7.264, DEHP - 9.685; Ola: DBP – ND,DEHP-ND; Tem: DBP - 7.259, DEHP - 9.685. The obtained retention times implied that the phthalate esters eluted according to their molecular mass.

CONCLUSION

This study reveals a widespread presence of phthalate esters, especially DEHP and DBP, in soft drinks sold in plastic bottles, raising serious concerns about potential health impacts. DEHP was the most dominant compound found, with levels surpassing the limits allowed in drinking water. The lack of clear global standards for phthalate levels in beverages highlights the need for uniform safety regulations to protect consumers. It also shows that storage conditions play a key role, with warmer temperatures increasing the release of these chemicals, emphasizing the importance of educating consumers on appropriate storage.

Considering the harmful health effects linked to phthalates, such as liver toxicity and hormonal disruptions, it is essential to reduce human exposure. The study suggests that manufacturers switch to phthalate free packaging, enforce strict quality checks, and align international regulations to ensure consistent safety practices. It also calls for more long term studies to examine how continuous exposure to phthalates affects health over time. There is also an urgent need to innovate eco-friendly packaging solutions that reduce phthalate use while maintaining product safety. Ultimately, a comprehensive strategy combining policy, industrial change, consumer education, and further research is necessary to minimize exposure and protect public health.

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