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Extraction and quantification of phthalates in plastic coca-cola soft drinks using high performance liquid chromatography (HPLC)

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ABSTRACT

Benzene-1,2-dicarboxylic acid esters, also known as phthalates, are commonly used in the productions of plastics such as polyvinylchloride (PVC) plastics, non-polymers and adhesives. Phthalates are ubiquitous compounds which are difficult to measure in drinks and foods containing trace level. Phthalates have been associated with developmental alteration and endocrine disruption. This study was aimed at identifying and quantifying concentrations of four different phthalates namely; Diethyl phthalate (DEP), Bis-(2-ethylhexyl) phthalate (DEHP), Dimethyl phthalate (DMP), Dibutyl phthalate (DBP), and diphenyl phthalate (DPhP), in plastic Coca-Cola soft drink purchased from five different strata or locations of University of Ibadan, Ibadan, Nigeria. Liquid-liquid extraction with dichloromethane was used for the extraction of the phthalates, followed by cleaned up in a silica gel column. This was followed by high performance liquid chromatographic analysis (HPLC) for quantification. The linear range of the HPLC calibration curve was 0 mg/L to 10 mg/L with a mean correlation coefficient (R^2) of 0.998 and the percentage recovery was between 56 and 106%. This result indicated that DEHP had the highest concentration of 11.52±3.31 µg/mL. Heat and longer storage of plastic soft drinks increased the content of leached phthalates in the drinks; therefore consumers of plastic packaged drinks should desist from frequent intake to avoid chronic toxicity of phthalate esters.

Capsule Summary: Due to an increase in endocrine related diseases and cancer, phthalates were assessed for in plastic cokes commonly consumed in University of Ibadan, Nigeria using HPLC, and findings showed that they contained high level of DEP and DEHP.

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INTRODUCTION

There has been a growing interest and concern for the study of the impact of man-made chemicals on wildlife, humans, and the environment and these studies have suggested that some synthetic and naturally occurring substances in the environment may affect the normal functioning of the endocrine system (Adewuyi et al., 2012). One of the major classes of environmental substances is the endocrine disrupting chemicals (EDCs), which interfere with the function of the endocrine system. In recent years, these endocrine disrupting chemicals have become one of the most important topics of research in the field of environmental sciences (Olowu et al., 2010). Health agencies and scientists are deeply concerned about the exposure of human to EDCs, which are widely distributed in the environment (Carlsen et al., 1992). EDCs are refers to as exogenous substances that cause adverse effect in organisms or its progeny, consequent to changes in endocrine function. They are ubiquitous in the environment as a result of their large number of uses in industrial, residential and agricultural applications (Olowu et al., 2010). One of the most important classes of EDCs are esters of phthalic acid (1,2- benzenedicarboxylic acid) or phthalates, which are ubiquitous in the environment. Phthalates have been found in sediments, natural water, soil and aquatic organisms (Giam et al., 1984; Staples et al., 1997), and also in drinking water (Capen, 1992).

Global phthalate production has increased from very low levels at the end of World War II to approximately 5 million metric tons/year, in which additives in flexible polyvinylchloride (PVC) account for 80–90% in the estimated global production of phthalates (Bornehag et al., 2004). Di (2-ethylhexyl) phthalate (DEHP) is widely used to soften polyvinylchloride (PVC) plastics and non-polymers (Rusyn et al., 2012). The lower molecular weight phthalates, such as diethyl phthalate (DEP), dibutyl phthalate (DBP), and dimethyl phthalate (DMP) are widely used for adhesives, paints, ink, varnishes, personal care products (e.g perfumes, lotions, cosmetics) (Gevao et al., 2013).

Plasticizers, which are also known as phthalates, make plastics flexible and durable. A plasticizer may reduce the melting point, viscosity, lower the glass transition temperature (Tg), or lower the elastic modulus of a product. On the basis of data from several studies on phthalate concentrations in the general population, it is obvious that profiles of phthalates and phthalate esters varied significantly among different geographic area. It was found that the ubiquitous human exposure to phthalates occurred in both developing and industrialized countries (Guo et al., 2011). It appears that adolescents and adults had significantly lower levels of monobutyl phthalate (MBP), monobenzyl phthalate (MBzP), and mono (2-ethylhexyl) phthalates (MEHP), but had significantly higher concentrations of monoethyl phthalate (MEP) in comparison with children. Females had remarkably higher concentrations of MEP, MBzP, and reproductive toxicant MBP than did males, but similar MEHP levels. As they are not chemically bound to the polymers or products, they are released from the products by evaporation and abrasion (Gevao et al., 2013).

In Nigeria, a lot of people patronize and/or consume beverages packaged in plastic bottles a lot; coke is one of the mostly consumed. Plastic bottles used in coke packing is a polyvinylchloride material (PVC) and thus, is expected to be incorporated with phthalates in its production and which could subsequently leach into the drink due the weak bond connecting it with the plastic. The level of different kinds of phthalates present in the plastic bottled coke was therefore investigated and compared with that of glass bottled coke to ascertain level of phthalates present.

MATERIAL AND METHODS

Site description

The site investigated were consumer outlets in the campus of the University of Ibadan, Ibadan, Nigeria, where dwellers buy varieties of daily need such as food, toiletries and confectionaries. As expected, there are designated places (densely populated areas) across the campus where these outlets are located amongst which are Halls of Residence, Staff quarters, Student Union Building, Recreational areas and University gate. The figure below is a map showing the majors roads, streets and locations on the university campus.

Sampling

The sampling area was the campus of University of Ibadan, Nigeria. It was geographically divided into five strata namely; Indepedence/Awolowo Hall (Indy/Awo), Student Union Building/Bello Hall (SUB/Bello), Faculty of Agriculture/Veterinary Medicine (F.A/V.M), Abadina/spices (Aba/Spices) and University's main gate (Gate). About 10 plastic coke samples were taken from each stratum, a composite of these samples from each stratum was made, followed by making triplicate of the composites and transferred into a pre-cleaned and baked glass bottles.

Sample preservation and preparation

Coke is a carbonated soft drink and was preserved with carbon (IV) oxide in the course of production which results in strong effervescence of the sample when shaken, which affect the separation of the extraction mixture and therefore hinder extraction efficiency.

As a result, the collected and prepared samples were left uncovered and placed in a fume cupboard for about 48 hours to rid of the carbonation and enhance extraction of the sample. They are tightly covered after the loss of CO_2 and stored in the refrigerator at 4^oC prior to extraction.

Standards and reagents

Analytical grade solvents were obtained and double distilled prior to their application. Solvents include dichloromethane (DCM), hexane, acetone and acetonitrile, ultra-pure distilled water was also obtained to prepare Na_2CO_3 (sodium carbonate). Standards namely; Diethyl phathalate (DEP), Bis(2-ethylhexyl) phthalate (DEHP), Dimethyl phathalate (DMP), Dibutyl phthalate (DBP), and diphenyl phthalate (DPhP) and internal standard (benzyl benzoate) of high purity (>98%) were purchased from Sigma Aldrich (from St. Louis, MO, U.S.A) and activated silica gel was purchased from Merck (Darmstadt, Germany).

Extraction procedure

Fig. 1: Map of University of Ibadan showing major roads, locations and sampling points.

100 mL of the coke sample 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 extract was dehydrated over an anhydrous Na_2SO_4 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.

Clean up procedure

A glass chromatographic column of 50 mL was packed with 5 g of silica gel. The column was tapped to settle the silica gel for efficient column packing. Then, about 1 g of Na_2SO_4 was added to the top of the silica gel and the column was pre-eluted with 10 mL of n-hexane at a flow rate of about 2 mL/min. The re-dissolved extract was then quantitatively transferred into the column and few mL of n-hexane was used to complete the extract transfer down the column. About 15 mL of n-hexane was used to elute the column for non-polar constituent and the fraction was discarded. 20 mL of acetone was then used to elute the phthalate esters prior to exposure to sodium sulphate layer and the eluate was concentrated to 1 mL while opened in a fume cupboard. 2 mL of acetonitrile was added to the concentrate prior to HPLC analysis.

Instrumental analysis

High performance liquid chromatography was carried out with Agilent technologies 1200 gradient series HPLC system available at the Central Science Laboratory in Obafemi

Table 1: Retention time of phthalate esters

Phthalate Esters	Retention time (min)
DMP	1.44
DEP	1.68
DPhP	2.04
Internal Standard (benzyl benzoate)	2.91
DEHP	5.56

Awolowo University, Ile-Ife, Nigeria. The instrument was equipped with an online vacuum degasser, a quaternary pumping system, a rheodyha injector, an auto sampler, a variable wavelength diode array UV detector and a computer system with HP chemstation software. Chromatographic separation was achieved using 100 mm×4.6 mm, 3.5 µm Agilent C_{18} analytical column with particle with particle of 5 µm. The analytical column was housed in a thermostated column compartment at ambient room temperature. The detection of phthalate was done at 254 nm wavelength. Chromatographic separation was performed under isocratic elution condition using acetonitrile, water and methanol (35:35:30) as mobile. Under these condition separation lasted for about ten minutes with flow rate of 1 mL/min. A 20 µL was used as the injection volume and phthalates were detected at 228 nm. Identification of phthalate ester was based on its retention time and quantification was by combination of internal standardization and response factor. Individual phthalate esters in the standard mixture and sample were identified according to their retention times.

Standards preparation

A 200 µg/mL of five different phthalate standards were prepared separately in 10 mL standard flasks and a mixture of all the standard was made from the stocks at 1:1:1:1:1:1 for DEP, DEHP, DPhP, DBP, DMP respectively. 200 µg/mL of internal standard (benzyl benzoate) was also prepared in a 10 mL standard flask as stock and a 5 µg/mL working standard is prepared from it. Equal volume of the internal was spiked with equal volume standards and samples accordingly.

Quality assurance

The sampling, sample preservation and preparation, sample extraction and sample clean-up were all carefully done to ascertain good quality assurance. Recovery study on the phthalate esters was also done to ascertain the efficiency of the extraction and the instrumental analytical procedure due to the non-availability of standard reference material (SRM).

Recovery study

Recovery study was done to assess the efficiency of the methodology. The study was carried out by spiking equivalent amount of the sample with 100 µg/mL of the phthalate standard mixture and internal standard followed by extraction and HPLC analysis as described earlier (Adewuyi et al., 2012).

Quantification of phthalate ester in bottle coke samples

Five-point calibration curves were prepared for each phthalate ester. Phthalates were identified in sample extracts by comparing HPLC retention times with standards and quantified using the standard calibration curve of each of the phthalate ester. Sample extracts were diluted as necessary to confirm that the concentration is in the linear range of the calibration curve. Phthalate ester concentration in each sample are recorded while those with without define peaks or response are recorded as not detected (ND).

Concentration of each phthalate ester in the extract was determined from the expression below:

Response Factor (RF) =

$$\frac{\text{Peak Area of Phthalate Esters}}{\text{Peak Area of Internal Standard}} = \frac{\text{Conc.of phthalate ester in the extract}}{\text{Conc.of Internal Standard}}$$

RESULTS AND DISCUSSION

This study assessed the amount of DEP, DMP, DPhP and DEHP in various samples of Coke. Even though the samples used are from the same manufacturer, different production dates, time of production, batch numbers and expiry are the properties that differentiate them. The retention time of each of the studied phthalate esters are: DMP - 1.44, DEP – 1.68, DPhP – 2.04, DEHP – 5.56 while that of internal standard (benzyl benzoate) was 2.91 as shown in Table 1. The obtained retention times implied that the phthalate esters eluted from the chromatographic column according to their molecular mass (MM) and as well similar to the previous works done on determination of phthalate using HPLC (Adewuyi et al., 2012 and Ogunfowokan et al., 2006).

HPLC calibration curves obtained for DEP, DPhP, DMP and DEHP were found to be linear over the concentration range of 0 µg/mL to 10 µg/mL. All correlation coefficients were better than 0.994 with the phthalates having the following correlation coefficients (r^2) from the calibration curves: DMP – 0.9973, DEP – 0.9987, DEHP – 0.9987 and DPhP – 0.9985. This revealed a high level of linearity between the concentration of phthalate in the sample and the instrument's response.

The mean of the overall concentrations of phthalate (µg/mL) are presented in Table 2. DEHP (bis-(2-ethylhexyl) phthalate) had the highest mean concentration of 11.52±3.31 µg/mL. The estimated average daily adult dose from the consumption of commodities highly likely to be contaminated (such as milk, cheese, margarine) is about 200 µg (DHS, 1985). The primary target organs for DEHP toxicity are liver and testes, DEHP can cause functional hepatic damage, as reflected by morphological changes, alterations in energy linked enzyme activity, and changes in lipid and carbohydrate metabolism. The most striking effect is proliferation of hepatic peroxisomes (WHO, 1996). DPhP (diphenyl phthalate) was detected in only two samples, as a

Table 2: Mean, standard deviation and coefficient of variation of phthalate esters

Phthalates	DMP	DEP	DPhP	DEHP
Mean	3.71	1.25	ND	11.52
Standard Deviation	1.76	0.75	ND	3.31
Coefficient of Variation %	47.42	59.94	ND	28.75

*ND – Not detected.

Table 3: Concentration of phthalates esters in bottled coke samples

SAMPLE	DMP (μg/mL)	DEHP(μg/mL)	DEP (μg/mL)	DPhP (μg/mL)
P Indy/Awo 1	10.22	15.23	0.85	ND
P Indy/Awo 2	8.64	10.98	1.16	ND
P Indy/Awo 3	3.59	18.67	1.23	ND
P Gate 1	3.73	8.34	0.43	0.37
P Gate 2	2.75	7.91	ND	ND
P Gate 3	1.68	9.63	ND	ND
P Aba/Spices 1	2.97	12.06	1.21	ND
P Aba/Spices 2	4.11	10.51	2.86	ND
P Aba/Spices 3	2.12	12.32	0.93	ND
P SUB/Bello 1	1.32	7.43	ND	ND
P SUB/Bello 2	3.96	8.36	0.16	ND
P SUB/Bello 3	4.08	11.21	ND	ND
P F.A/V.M 1	5.18	16.69	1.22	0.63
P F.A/V.M 2	4.86	10.01	2.14	ND
P F.A/V.M 3	7.74	13.52	1.56	ND

Table 4: Percentage recovery of phthalate esters in bottled coke sample

Phthalate Esters	Retention Time (min)	Percentage Recovery (%)
DMP	1.44	106
DEP	1.68	98
DPhP	2.04	56
DEHP	5.56	110

result DPhP can be said not to be part of materials used as plasticizers for plastic coke bottles or rather to have very slow leaching rate over time. The order of decreasing of the mean concentration of the phthalates as represented in Table 2 is DEHP > DMP > DEP > DPhP. The high concentration of DEHP is not unusual as it is a commonly detected phthalate in most plastic materials previously studied (Adewuyi and Olowu, 2012).

The recovery studies of the phthalate esters (PEs) for DMP, DEP, DPhP and DEHP are 106, 98, 56 and 110 % respectively. The percentage recoveries obtained by spiking the samples with standard are within and comparable with the percentage recoveries obtained in related previous study: 50 – 105 % (Vitali, 1997) and 23.78 – 85.5 % (Fatoki and Ogunfowokan, 1993). Invariably, the percentage recoveries are good and therefore validate the results obtained for this study.

CONCLUSIONS

Results obtained from this study indicated that phthalates esters can be efficiently extracted from coke sample via liquid-liquid extraction and it also revealed that high level of phthalates esters can be obtained from plastic bottled coke via leaching, precisely DEHP and DMP. It is therefore recommended that consumers of plastic packaged drinks should desist from frequent intake to avoid chronic toxicity of phthalate esters and that Standard Organization of Nigeria (SON), in collaboration with National Agency for Food and Drug Administration and Control (NAFDAC), should formulate the minimum standards for phthalates in drinking water, as well as in plastic containers.

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