World Journal of Chemical Education, 2023, Vol. 11, No. 2, 7-12
Available online at http://pubs.sciepub.com/wjce/11/2/1
Published by Science and Education Publishing
DOI:10.12691/wjce-11-2-1

An Undergraduate Experiment: Detection of three Endocrine Disrupting Phthalates DMP, DEP and DBP in Bottled Camel Milk in the UAE

Dina Asharf, Mohammed A. Meetani*

Department of Chemistry, College of science, United Arab Emirates University, POBox 15551, Al-Ain, UAE
*Corresponding author: mmeetani@uaeu.ac.ae

Received February 20, 2023; Revised March 25, 2023; Accepted April 05, 2023

Abstract Phthalates or esters of phthalic acid are commonly known for their extensive use as plasticizers to promote the mechanical properties of industrial plastics such as malleability, strength, softness and temperature tolerance. Since phthalates are not chemically attached to the polymers, they can freely migrate from food packages and bottles into fatty food and drinks. Phthalates are proven in several toxicological studies to be of adverse impacts on human health such as respiratory disorders, cardiovascular diseases, neurological problems, birth defects, disruption of endocrine system and different types of cancer. Camel milk is uniquely rich in fats and proteins which may interfere in the analysis of phthalates; thus, a sample preparation step is needed. Therefore, liquid-liquid extraction was performed. An analytical method using gas chromatography coupled with tandem mass spectrometry (GC-MS/MS) was developed for determinations of three phthalate esters (dimethyl phthalate (DMP), diethyl phthalate (DEP), dibutyl phthalate (DBP)) in bottled camel milk samples obtained from the local markets in the UAE. Multiple reaction monitoring (MRM) mode was used for mass spectrometry detection on positive chemical ionization (PCI). Calibration curves with very good linearity were obtained for each of the three phthalates after spiking the extracted milk samples with standard concentrations of the three phthalates mixture. The analyzed milk sample was found to contain 57.6 $mg.L^{-1}$ of DBP, 0.41 $mg.L^{-1}$ of DMP and 0.25 $mg.L^{-1}$ of DEP.

Keywords: *phthalates, gas chromatography mass spectrometry, liquid extraction, camel milk, food safety*

Cite This Article: Dina Asharf, and Mohammed A. Meetani, "An Undergraduate Experiment: Detection of three Endocrine Disrupting Phthalates DMP, DEP and DBP in Bottled Camel Milk in the UAE." *World Journal of Chemical Education*, vol. 11, no. 2 (2023): 7-12. doi: 10.12691/wjce-11-2-1.

1. Introduction

Phthalates or phthalic acid esters are a group of chemical compounds excessively used as plasticizers to enhance the mechanical properties of many plastic products like their flexibility, temperature tolerance and durability [1,2]. Since phthalates are almost in every plastic product, humans are exposed to them through several pathways such as food, water, pharmaceuticals, cosmetic products, etc., but the main source of exposure for humans is food due to the use of phthalates as plasticizers at the stage of packaging and food processing [3,4,5,6,7].

The problem of phthalates is that they can easily migrate from the plastic skeleton of the food package into inside the food or the drink due to the fact that they are physically and not chemically bound to the plastic polymer [8]. Factors like storage conditions, temperature, contact time and lipid content of the food can even facilitate or accelerate this migration [8]. Phthalates were classified as endocrine disrupting chemicals which means that they can alter normal hormonal levels leading ultimately to severe health impacts [9,10]. Many studies have associated phthalates with many health issues such as cardiovascular diseases, some types of cancer, diabetes, infertility and delayed neurological development in children [11,12]. As a result, regulations on the exposure limits and the industrial use have been set on many countries around the world, for example in Japan diisononyl phthalate (DiNP) and diethyl hexyl phthalate (DEHP) are banned in kids toys industry and in food-contacting gloves [13]. Similarly, in Europe, dibutyl phthalate (DBP), and benzyl butyl phthalate (BBP) in addition to DEHP are prohibited in all plastic materials that may reach children [14].

In literature, many research studies were conducted for the evaluation of phthalates in different materials such as bottled water [15], bottled oils [16], cosmetic products [17], kids toys [18] and dairy products [19]. Different techniques were employed for the sensitive detection of phthalates such as gas chromatography mass spectrometry (GC-MS) [20,21], liquid chromatography mass spectrometry (LC-MS) [22,23] and capillary electrophoresis (CE) [24,25].

Among the investigated food samples, milk and milk products are of great importance due to their regular consumption by humans as a rich source of nutrients. Camel milk is believed to have a significant nutritional value that's even superior to the nutritional value of other types of milk [26]. Camel milk has a unique composition of proteins that makes it considered the most similar to that of the human's milk and the best alternative for babies if mother's milk is limited [26,27]. Camel milk is very rich in many minerals such as calcium potassium and iron and in a variety of vitamins such as vitamin A, vitamin C, vitamin E and vitamin B family [26,27,28,29]. Camel milk is also shown to have low percentage of the unhealthy (saturated) fats and at the same time high content of the healthy (unsaturated) fats [26,30]. Camel milk was revealed to have a promising potential to be used as in the therapy for many diseases such as diabetes [31], some types of cancer [32,33] and autism [34].

For the best of our knowledge, most of the available studies for determination of phthalates in milk were focusing on bovine milk (i.e. cows, buffaloes, goats and sheep) and breast milk, but only one study was found to investigate phthalates in samples of camel milk by liquid chromatography mass spectrometry [35].

From this point, and since camel milk is of an integral value in the United Arab Emirates and the gulf area due to its outstanding health benefits and its connection to the traditions and history of the area, the aim of this study was to investigate the levels of phthalates contamination in camel milk samples collected from the local markets in the UAE using gas chromatography coupled with mass spectrometry (GC-MS). This method could be applied as a fourth year undergraduate experiment, offering hands-on experience in gas chromatography and tandem mass spectrometry detection.

2. Experimental

2.1. Chemicals and Reagents

Analytical standards of dimethyl phthalate (DMP), diethyl phthalate (DEP) and dibutyl phthalate (DBP), in addition to, HPLC-grade solvents such as methanol, ethyl acetate, acetonitrile, and acetic acid were purchased from Sigma-Aldrich (St Louis, MO 63103 United States).

2.2. Instrumentation

Instrumental analysis was carried out on Agilent 7890 Gas chromatograph coupled with 7000E Triple Quadruple mass spectrometer equipped with an auto-sampler (Agilent, Santa Clara, California, U.S.)

2.3. Preparation of Standard Solutions

10.0 $mg.L^{-1}$ standard solution of each type of the investigated phthalates (DBP, DMP, DEP) was prepared in methanol and stored at 4°C to be used for qualitative analysis and retention time determination by gas chromatography.

A mixture of 1.0 $mg.L^{-1}$ stock solution containing the 3 phthalates was prepared in methanol and stored at 4°C. Different volumes of the prepared 1 $mg.L^{-1}$ mixture solution were added to five vials (0, 0.2, 0.4, 0.6 and 0.8 mL of milk extract was added to each vial, then methanol was added to each vial to obtain a final volume of 1 ml to apply standard addition method for calibration.

2.4. Setting GC/MS Method

GC-MS method used in the cited study was followed for setting our parameters of GC-MS analysis [36]. Table 1 summarizes GC-MS conditions.

2.5. Sample Extraction

For complex matrices like milk, extraction of analytes is a challenging step due to the presence of proteins, vitamins, lipids, and other molecules that may interfere with analysis. Camel milk in particular is believed to be more challenging due to its unique fat and protein composition. In this study liquid-liquid extraction procedures were followed using different solvents to maximize phthalates extraction and remove other contaminants. Bottled camel milk samples were purchased from a local supermarket of Al-Ain City at United Arab Emirates, and stored at 4°C until used in this study. A 15 mL sample of camel milk was mixed with different extraction mixtures as listed in Table 2. Mixture solution was allowed to vortex for 3 min to ensure well mixing and then centrifuged for 30 min at 3500 rpm. An aliquot of the supernatant was decanted out and filtered through Millex 0.45 µm PTFE syringe filters from Merck Millipore (Carrigtwohill, Ireland).

Table 1. GC-MS conditions

GC conditions	
Column	Agilent J&W GC, DB-5MS UI capillary column, 30.00 m x 0.25 mm x 0.25 µm
Inlet temperature	280°C
Injection volume	2 µL
Carrier gas flow	Helium at 1.0 mL/min
Injection mode	Splitless, Pulse injection at 30 psi for 0.6 min
Oven program	50°C for 2 min, to 280°C at 30°C /min, to 310°C at 15°C /min and hold for 3.33 min, for a total run time of 15 min
Solvent	Methanol

Mass spectrometer conditions	
Ion source	Chemical ionization (Positive mode)
CI reagent gas	Methane
Filament Delay	4 min
Source Temperature	220°C
Transfer Line Temperature	280°C
MS 1 Quad Temperature	150°C
MS 2 Quad Temperature	150°C

Table 2. Composition of extraction mixtures

	Extraction A	Extraction B	Extraction C	Extraction D
Volume of camel milk sample (mL)	15	15	15	15
Acetonitrile (mL)	15	15	-	10
Ethyl acetate (mL)	15	-	-	10
Methanol (mL)	-	15	15	10
Acetic acid (mL)	0.6	0.6	0.6	0.6
Tert-butyl methyl ether (mL)	-	-	15	-

3. Results and Discussion

For performing qualitative and quantitative analysis of the three investigated phthalates (DMP, DBP, DEP) in milk samples, GC-MS analysis was conducted for the three types of phthalates at the optimized conditions to obtain the retention time for each compound and the mass spectrum with the characteristic product ions. Three different modes of GC-MS were applied: full scan mode, product ion scan mode and multiple reaction monitoring (MRM) mode. Full scan mode was used first for qualitative detection to test whether the investigated phthalates will be detected or not under the conditions set for analysis as described in experimental section. In this mode, mass range of 50 to 500 Da was scanned since the molar mass of the three phthalates under study lie within this range, and then the exact mass of the analyte was extracted from the obtained total ion chromatogram.

Product ion scan mode was operated afterwards to optimize collision energies. In product ion scan mode, product ions of each analyte precursor ion were scanned in the range of m/z 50 to 500 Da using collision energies of 5, 10, 15 and 20 eV. Table 3 summarizes the data obtained from product ion scan mode which were essential to optimize parameters for MRM analysis.

Multiple reaction mentoring (MRM) mode of analysis was applied for maximum sensitivity and selectivity analysis for each phthalate. Under the applied conditions of GC-MS, DMP was found to appear at retention time of 7.743 min while DEP and DBP at 8.316 and 9.678, respectively. From these results, it was observed that retention time increases as the alkyl group elongates and molar mass increases. This trend agrees with the increase in boiling point for the three phthalates (DMP, DEP and DBP) which is 283°C, 296°C, and 340°C, respectively. Figure 1 shows the results of GC-MS analysis obtained on MRM mode for 10 mg.L^{-1} concentration of DBP as an example. High intensity peaks up to 10^5 were obtained.

Table 3. MRM parameters as optimized from product ion scan analysis

Phthalate	Retention time (minutes)	M/Z of the product ions	Collision energy
DMP	7.743	77	20
		91	15
		133	20
		151	15
		163	15
DBP	9.678	57	20
		149	20
		205	5
DEP	8.316	149	15
		177	10

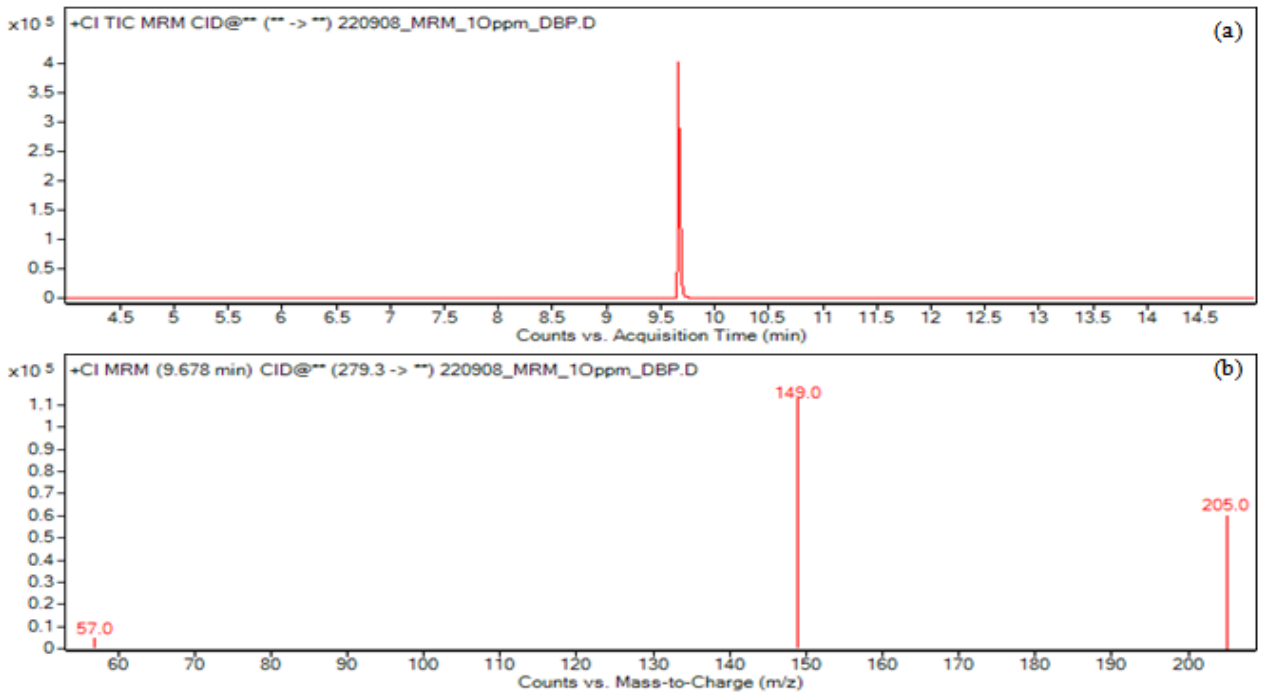

Figure 1. (a) Total ion chromatogram of 10 ppm DBP standard solution obtained on MRM mode, (b) positive chemical ionization mass spectrum of DBP (MWt = 278.34 g/mol)

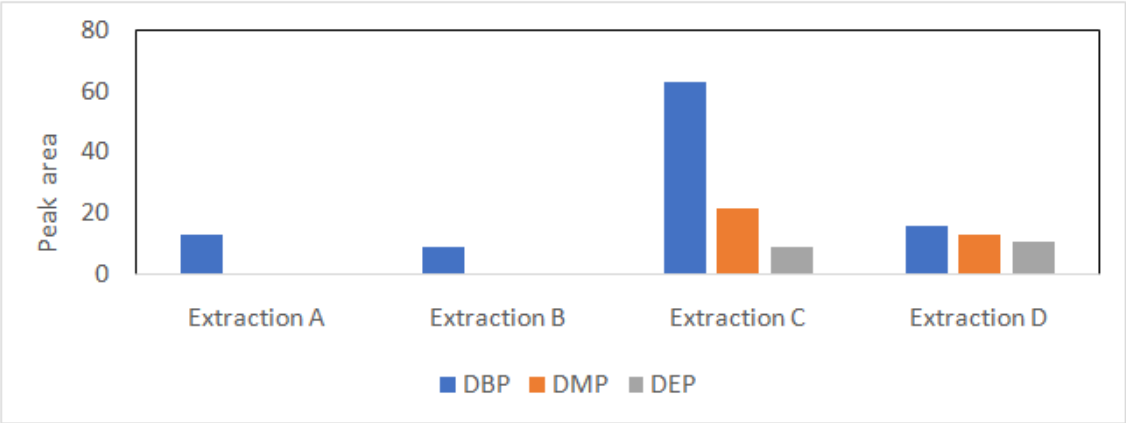

Figure 2. Peak area of each investigated phthalate obtained by different extraction methods

All the previous analyses were conducted in methanol solution to find retention time and test efficiency of the applied method. For quantitative analysis, we used standard addition method for calibration and elimination of matrix effect, where we spike the extracted camel milk with different standard volumes of the three phthalates under study. In order to do so, extraction steps were required first to remove other compounds that may interfere with analysis. Liquid-liquid extraction was applied, and different solvents were investigated for best extraction efficiency as described in experimental section. Results shown in Figure 2 show the measured peak area of each phthalate for each extraction procedure. Extraction C has shown to give the highest peak areas for the three phthalates. Therefore it was selected for extraction in all analysis procedures. The highest efficiency of this extraction method can be attributed to the presence of a polar solvent which is methanol that increases the polarity of the solution leading to minimal co-extraction of lipids and fats, at the same time the presence of tert-butyl methyl ether which is the least polar solvent among all used solvents that may have helped in maximizing the extraction of phthalates due to their non-polar nature.

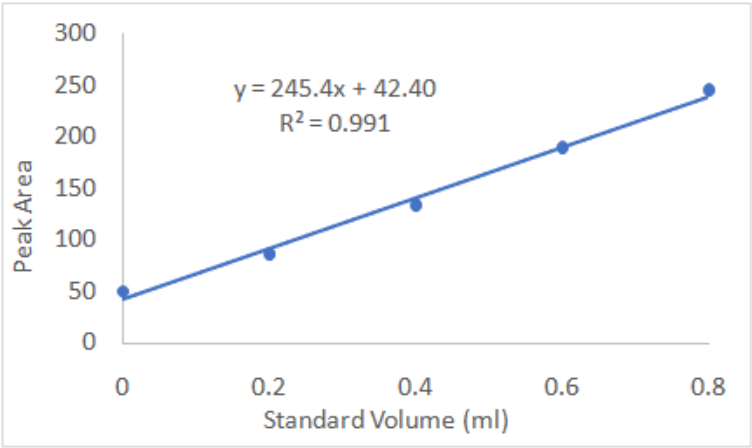

Figure 3. Standard addition calibration curve for DBP

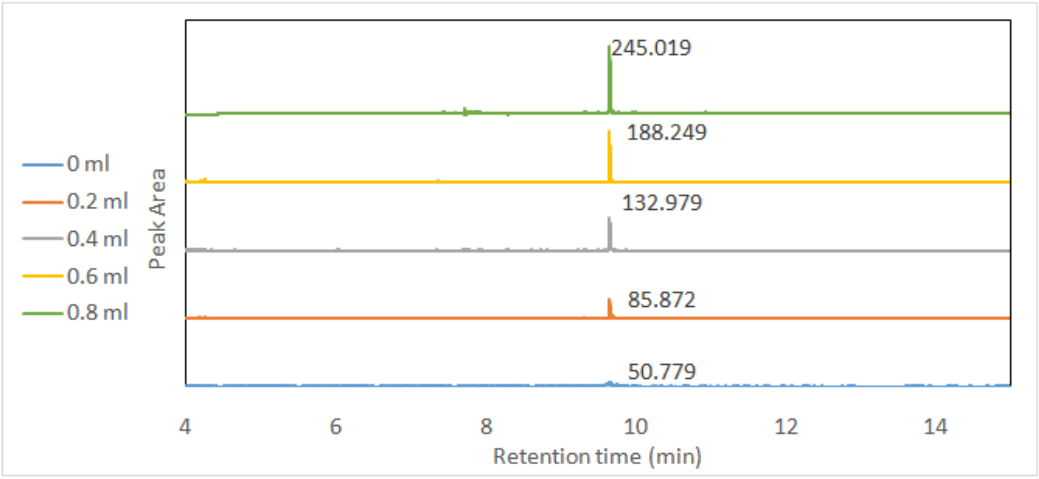

Figure 4. Peak area obtained for each spiked standard volume of DBP

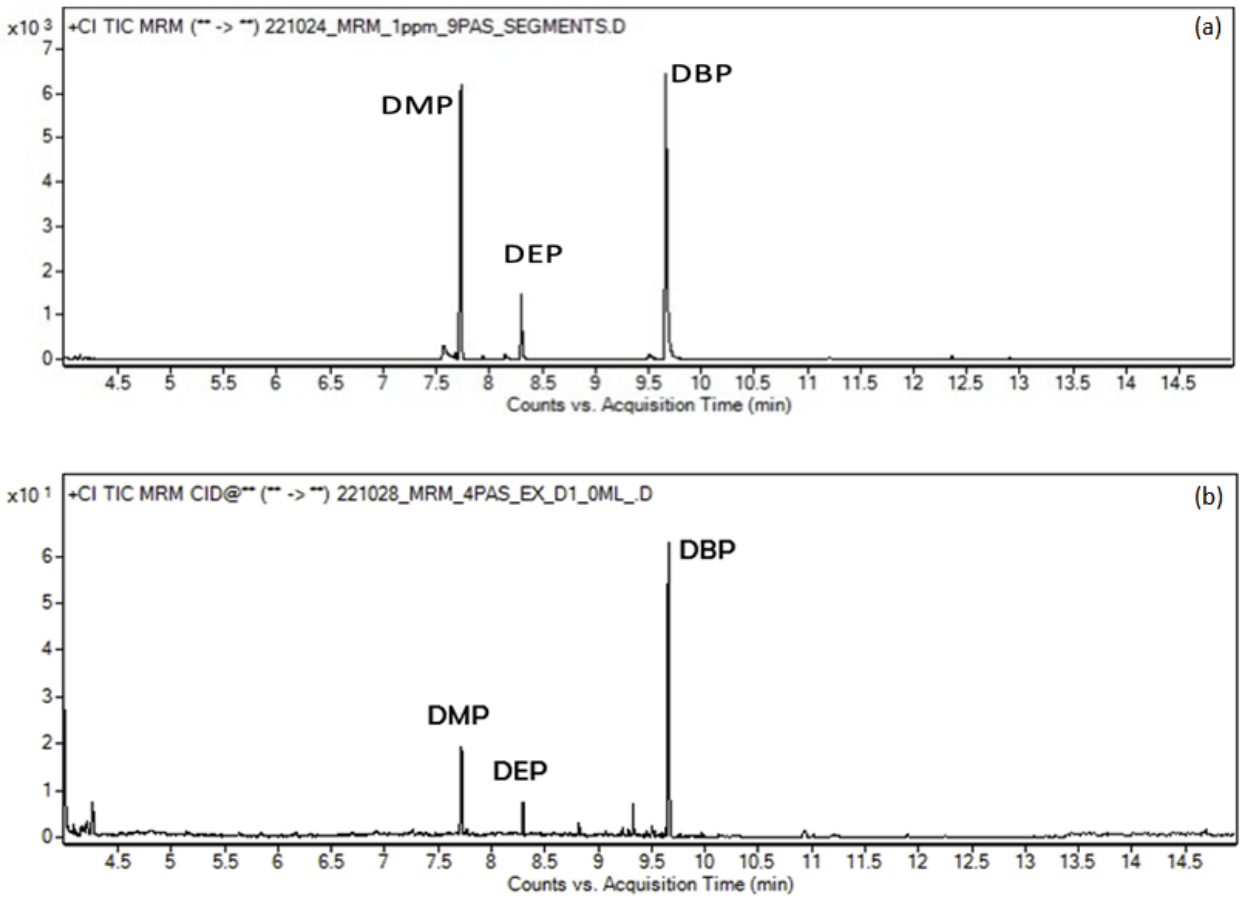

Figure 5. (a) reference GC chromatogram of 1ppm mixture of the three phthalates, (b) Obtained GC chromatogram for the analyzed camel milk sample

Calibration curves were generated by the standard addition method where milk extract was spiked by different volumes of 1 ppm of the three phthalates mixture (0, 0.2, 0.4, 0.6 and 0.8 ml). Standard addition Calibration curve for DBP is shown in Figure 3 with regression coefficient of 0.9917 which indicates good linearity.

Figure 4 summarizes the peak areas of DBP at each standard volume as an example for calibration process.

Figure 5 shows the obtained results of the sample compared to the reference total ion chromatogram of 1 ppm mixture of the three phthalates in methanol solution. DMP, DEP and DBP were all detected in the camel milk sample. Using the following standard addition calibration equation:

$$C_x = \frac{bC_s}{mV_x},$$

where: C_x = sample concentration, V_x = Sample volume, C_s = concentration of the standard, m = the slope and b = y-intercept of the calibration curve, concentration of each phthalate was calculated and DBP was found to be present in 57.6 ppb, while the concentrations of DMP and DEP were 0.41 and 0.25 ppb, respectively. The European Union has set Specific Migration Limits (SMLs) which are defined as the maximum allowed concentration of a certain substance migrated or released from a food contact material (FCM) into food [37]. For phthalates, the European Union SMLs are 0.3 mg/kg for DBP, 30.0 mg/kg for benzyl butyl phthalate (BBP) and 1.5 mg/kg fs for DEHP [38]. Other types of phthalates yet don't have an individual SML. So, comparing the sample content of phthalates with these limits shows that concentrations of phthalates in the analyzed milk sample are far below the allowed limits.

4. Conclusion

In this study, GC-MS/MS analytical method was applied for determination of three types of phthalates in camel milk samples obtained from the local market in the UAE. Liquid-liquid extraction was used as a concentration and isolation step of the phthalates using a mixture of (methanol: tert-butyl methyl ether: acetic acid) at a ratio of (49:49:2) %, respectively. Standard addition calibration method has been utilized to determine the levels of the three phthalates in the camel milk samples. They were found in very low concentrations of 57.6 $mg.L^{-1}$ of DBP, 0.41 $mg.L^{-1}$ of DMP and 0.25 $mg.L^{-1}$ of DEP.

Acknowledgements

This study was only made possible by the financial support of the United Arab Emirates university research office (UPAR fund # 12S090).

References

[1] Bradley EL, Burden RA, Leon I, et al. (2013): Determination of phthalate diesters in foods. *Food Addit Contam*, 30, 722-734.

[2] Chen M, Chen J, Tang C, et al. (2008): The internal exposure of Taiwanese to phthalate- An evidence of intensive use of plastic materials. *Environ Int*, 34, 79-85.

[3] Cao XL. 2010. Phthalate esters in foods: sources, occurrence, and analytical methods. *Compr Rev Food Sci*. 9: 21-43.

[4] Silva, M. J., Samandar, E., Preau, J. J. L., Reidy, J. A., Needham, L. L., and Calafat, A. M. (2005). Automated solid-phase extraction and quantitative analysis of 14 phthalate metabolites in human serum using isotope dilution- high-performance liquid chromatography-tandem mass spectrometry. *J. Anal. Toxicol*. 29, 819-824.

[5] Mankidy, R., Wiseman, S., Ma, H., and Giesy, J. P. (2013). Biological impact of phthalates. *Toxicol. Lett.* 217, 50-58.

[6] T. Wenzl, European Commission, Methods for the determination of phthalates in food, 2009.

[7] Miclean, Mirela & Cadar, Oana & Roman, Cecilia. (2012). Determination of phthalates in bottled milk using headspace solid-phase microextraction coupled with gas chromatography-mass spectrometry. *Studia Universitatis Babes-Bolyai Chemia*. 57. 127-133.

[8] Fasano, E., F. Bono-Blay, T. Cirillo, P. Montuori, and S. Lacorte. 2012. Migration of phthalates, alkylphenols, bisphenol A anddi(2-ethylhexyl)adipate from food packaging. *Food Contr*. 27: 132-138.

[9] Schug, T.T.; Johnson, A.F.; Birnbaum, L.S.; Colborn, T.; Guillette, L.J., Jr.; Crews, D.P.; Collins, T.; Soto, A.M.; Vom Saal, F.S.; McLachlan, J.A.; et al. Minireview: Endocrine disruptors: Past lessons and future directions. *Mol. Endocrinol*. 2016, 30, 833-847.

[10] Grindler, N.M.; Vanderlinden, L.; Karthikraj, R.; Kannan, K.; Teal, S.; Polotsky, A.J.; Powell, T.L.; Yang, I.V.; Jansson, T. Exposure to phthalate, an endocrine disrupting chemical, alters the first trimester placental methylome and transcriptome in women. *Sci. Rep.* 2018, 8, 1-9.

[11] Wang Y, Qian H. Phthalates and Their Impacts on Human Health. Healthcare (Basel). 2021 May 18; 9(5): 603.

[12] Eales J, Bethel A, Galloway T, Hopkinson P, Morrissey K, Short RE, Garside R. Human health impacts of exposure to phthalate plasticizers: An overview of reviews. *Environ Int.* 2022 Jan; 158:106903.

[13] Mutsuga, M.; Wakui, C.; Kawamura, Y.; Maitani, T. Isolation and identification of some unknown substances in disposable nitrile-butadiene rubber gloves used for food handling. *Food Addit. Contam.* 2002, 19, 1097-1103.

[14] EUR-Lex. EU Phthalates Directive 2005/84/EC. 14 December 2005. Available online: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32005L0084.

[15] Salazar-Beltrán D, Hinojosa-Reyes L, Ruiz-Ruiz E, Hernández-Ramírez A, Luis Guzmán-Mar J. Determination of phthalates in bottled water by automated on-line solid phase extraction coupled to liquid chromatography with uv detection. *Talanta.* 2017 Jun 1; 168: 291-297.

[16] Luo Q, Liu ZH, Yin H, Dang Z, Wu PX, Zhu NW, Lin Z, Liu Y. Global review of phthalates in edible oil: An emerging and nonnegligible exposure source to human. *Sci Total Environ*. 2020 Feb 20; 704: 135369.

[17] Chen H, Wang C, Wang X, Hao N, Liu J. Determination of phthalate esters in cosmetics by gas chromatography with flame ionization detection and mass spectrometric detection. *Int J Cosmet Sci.* 2005 Aug; 27(4): 205-10.

[18] Ashworth MJ, Chappell A, Ashmore E, Fowles J. Analysis and Assessment of Exposure to Selected Phthalates Found in Children's Toys in Christchurch, New Zealand. *Int J Environ Res Public Health.* 2018 Jan 25; 15(2): 200.

[19] Lin, Jialu & Chen, Wanxin & Zhu, Hangcui & Wang, Chengjun. (2015). Determination of free and total phthalates in commercial whole milk products in different packaging materials by gas chromatography-mass spectrometry. *Journal of dairy science*. 98.

[20] Yang J, Li Y, Wang Y, Ruan J, Zhang J, Sun C: Recent advances in analysis of phthalate esters in foods. *TrAC – Trend Anal Chem* 2015, 72: 10-26.

[21] Sanchis Y, Yusa` V, Coscolla` C: Analytical strategies for organic food packaging contaminants. *J Chromatogr A* 2017, 1490: 22-46.

[22] Ho Y-M, Tsoi Y-K, Leung KS-Y: Ionic-liquid-based dispersive liquid–liquid microextraction for high-throughput multiple food contaminant screening. *J Sep Sci* 2013, 36: 3791-3798.

[23] Kataoka H, Ise M, Narimatsu S: Automated on-line in-tube solid-phase microextraction coupled with high performance liquid chromatography for the analysis of bisphenol A, alkylphenols, and phthalate esters in foods contacted with plastics. *J Sep Sci* 2002, 25: 77-85.

[24] Yue M-E, Lin Q, Li Q, Xu J, Jiang T-F: Determination of PAEs by integrative coupling method of headspace in-tube microextraction and reverse-flow micellar electrokinetic capillary chromatography. *Food Anal Method* 2017, 10: 3565-3571.

[25] Sun J, He H, Liu S: Determination of phthalic acid esters in Chinese white spirit using dispersive liquid–liquid microextraction coupled with sweeping b-cyclodextrin-modified micellar electrokinetic chromatography. *J Sep Sci* 2014, 37:1679-1686.

[26] Ho TM, Zou Z, Bansal N. Camel milk: A review of its nutritional value, heat stability, and potential food products. *Food Res Int.* 2022 Mar; 153: 110870.

[27] Zibaee S, Hosseini SM, Yousefi M, Taghipour A, Kiani MA, Noras MR. Nutritional and Therapeutic Characteristics of Camel Milk in Children: A Systematic Review. *Electron Physician.* 2015 Nov 20; 7(7): 1523-8.

[28] Soliman, G. Z. A. (2005). Comparison of chemical and mineral content of milk from human, cow, buffalo, camel and goat in egypt. *The Egyptian Journal of Hospital Medicine*, 21, 116-130.

[29] Farah, Z., Rettenmaier, R., & Atkins, D. (1992). Vitamin content of camel milk. *International Journal for Vitamin and Nutrition Research*, 62(1), 30-33.

[30] Dreiucker, J., & Vetter, W. (2011). Fatty acids patterns in camel, moose, cow and human milk as determined with GC/MS after silver ion solid phase extraction. *Food Chemistry*, 126(2), 762-771.

[31] Mohamad, R. H., Zekry, Z. K., Al-Mehdar, H. A., Salama, O., El-Shaieb, S. E., El- Basmy, A. A., ... Sharawy, S. M. (2009). Camel milk as an adjuvant therapy for the treatment of type 1 diabetes: Verification of a traditional ethnomedical practice. *Journal of Medicinal Food*, 12(2), 461-465.

[32] Korashy, H. M., Z. H. Maayah, A. R. Abd-Allah, A. O. S. El-Kadi and A. A. Alheider. 2012. Camel milk triggers apoptotic signaling pathways in human hepatoma HepG2 and breast cancer MCF7 cell lines through transcriptional mechanism. *J. Biomed. Biotechnol.* 2012: 1-9.

[33] Magjeed, N. A. 2005. Corrective effect of milk camel on some cancer biomarkers in blood of rats intoxicated with Aflatoxin B1. *J. Saudi Chem.* Soc. 9(2): 253-263.

[34] Shabo Y. Yagil R. Etiology of autism and camel milk as therapy. *Journal of Endocrine Genetics*, 2005; 4 (2): 67-70.

[35] Hammouri, Mahmoud & Barakat, Salem & Bella, S.Y.. (2016). Evaluation of phthalates in Jordan diary products by HPLC/MS-MS. *Rasayan Journal of Chemistry*. 9. 160-168.

[36] Haibo Wang and Kurt Thaxton Varian: Determination of Phthalate Compounds in Plastic Toys Using the Varian 225-MS Ion Trap Mass Spectrometer. Application note 02100 www.agilent.com/chem.

[37] Commission Regulation (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food, Official Journal of the European Union.

[38] http://echa.europa.eu/documents/10162/c6781e1e-1128-45c2-bf48-8890876fa719.