

Original research article

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DETECTION OF MICROPLASTIC RESIDUES - DEVELOPING A METHOD FOR PHTHALATES IN HONEY

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Abstract

In this pilot study, a method for the determination of phthalates in honey was developed. The following phthalates are included: dimethyl phthalate, diethyl phthalate, di-isobutyl phthalate, di-n-butyl phthalate, bis(2-ethylhexyl) phthalate, and di-(n-octyl) phthalate. For the preparation of the samples, the method of liquid-liquid extraction with hexane with an ultrasonic bath was used. The analysis of the prepared samples was performed using gas chromatography and a mass detector. The method is reliable, sensitive, and reproducible with a detection limit of 0.28 - 1.38 µg/kg. This paper presents the results of testing samples of honey stored in glass and plastic packaging for three years in order to determine the migration of phthalates. Dimethyl phthalate was not found in the tested samples stored in plastic and glass packaging. Diethyl phthalate was not found in samples stored in glass packaging while the concentration of diethyl phthalate in samples from plastic packaging was 3.34 µg/kg. The concentrations of di-isobutyl phthalate, di-n-butyl phthalate and bis(2-ethylhexyl) phthalate, determined in samples from glass packaging were 5.32, 1.32 and 4.45 µg/kg, and in honey samples from plastic packaging 15.84, 16.01 and 14.44 µg/kg. Concentrations of di-(n-octyl) phthalate were less than the LOQ in both types of samples.

Key words: GC-MS, plastic packaging, DiBP, DBP, DEHP

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RAZVOJ ANALITIČKE METODE GASNE HROMATOGRAFIJE ZA ODREĐIVANJE FTALATA U MEDU

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Kratak sadržaj

U ovoj pilot studiji razvijena je metoda za određivanje ftalata u medu. Predmetni ftalati su: dimetil ftalat, dietil ftalat, di-isobutil ftalat, di-n-butil ftalat, bis(2-etilhexil) ftalat i di-(n-octil) ftalat. Za pripremu uzoraka je korišćena metoda tečno-tečne ekstrakcije sa hexanom uz pomoć ultrazvučnog kupatila. Analiza pripremljenih uzoraka je rađena primenom gasne hromatografije kuplovane sa masenim detektorom. Metoda je pouzdana, osetljiva i reproducibilna sa granicom detekcije od 0,28 – 1,38 µg/kg. U ovom radu prikazani su rezultati ispitivanja uzoraka meda koji je skladišten u staklenoj i plastičnoj ambalaži tokom tri godine s ciljem utvrđivanja migracije ftalata. Dimetil ftalat nije nađen u ispitanim uzorcima skladištenim u plastičnoj i staklenoj ambalaži. Dietil ftalat nije pronađen u uzorcima meda skladištenim u staklenoj ambalaži, dok je koncentracija dietil ftalata u uzorcima meda skladištenim u plastičnoj ambalaži bila 3.34 µg/kg. Koncentracija di-isobutil ftalata, di-n-butil ftalata i bis(2-etilhexil) ftalata, ispitanih u uzorcima meda skladištenim u staklenoj ambalaži je bila 5,32, 1,32 and 4,45 µg/ kg, a u uzorcima meda skladištenog u plastičnoj ambalaži 15,84, 16,01 i 14,44 µg/kg. Koncentracije di-(n-octil) ftalata su bile manje od LOQ u obe vrste uzoraka.

Ključne reči: GC-MS, plastična ambalaža, DiBP, DBP, DEHP

INTRODUCTION

Nowadays, plastic is increasingly used for food packaging due to the low cost of materials, its potential for thermal sealing, optical properties, and it is also suitable for making different shapes and sizes. Due to these properties, plastic products for food packaging and beverages have surpassed the use of materials such as glass or tinplate. Plastic packaging has many advantages - it is light, resistant, and easy to shape, i.e. it can be formed in different shapes and

sizes and thus adapted to different types of food, from solids to liquids. Plastic packaging provides good protection against damage. However, from the chemical point of view, when it comes to biodegradability, its harmful effects are the subject of a great number of research papers today. EU Regulation 1935 dating from 2004 is based on the fact that all materials or objects that come into direct or indirect contact with food must be inert enough to prevent the transfer of things to food in the quantities that are large enough to endanger human health or cause unacceptable changes in food composition or deterioration of its organoleptic properties. Plastic can be decomposed into compounds harmful to human health. These are divided into organic and inorganic compounds. The first group includes amines, phenols, and phthalates. In order to obtain softer and more flexible plastic products, so-called softeners are added during their production, and phthalates are the subject of this pilot study.

Diesters of 1,2-benzenedicarboxylic acid, better known as phthalates, are a group of man-made chemicals widely used in industry (Meeker et al, 2009). They are present primarily in plastic products, toys, medical instruments, industrial materials, food, and clothing. These compounds have the ability to disrupt the function of the endocrine system. The effects depend on the dose, duration of action, and developmental stage of the organism. The fetus, newborn, and children in puberty are the most vulnerable categories. Exposure to phthalates begins in the intrauterine period, as they freely pass through the placental barrier. It is believed that the side effects of phthalates can be manifested through neurocognitive disorders, allergies, asthma, testicular cancer, liver and kidney damage, insulin resistance and obesity, thyroid dysfunction, and respiratory system irritation. Phthalates in females can lead to anovulation, premature puberty, and changes in the duration of pregnancy (Butala et al, 2004; Yen et al, 2011; Bajkin et al, 2014). Bis(2-ethylhexyl) phthalate (DEHP), dibutyl phthalate (DBP), benzyl butyl phthalate (BBP) and di-isobutyl phthalate (DIBP) ('the four phthalates') are listed in Annex XIV to Regulation (EC) No 1907/2006 as substances that are toxic for reproduction, category 1B. Therefore, phthalate toxicity poses a significant risk to human health.

According to the "Rulebook on the Restrictions and Ban of Production, Placing on the Market and Use of Chemicals ("Official Gazette of the Republic of Serbia RS", 90/2013, 25/2015, 2/2016, 44/2017, 36/2018 and 9/2020) DBP, DEHP, BBP are prohibited for use in toys and objects intended for child care in concentrations higher than 0.1% of plasticized materials, while di- "is-nonyl" phthalate (DINP), di-„isodecyl" phthalate (DIDP), di-n-octyl phthalate (DNOP) are prohibited for use in toys and items intended for the care of children that children can put in their mouths in concentrations higher than 0.1% of plasticized materials. Given the harmful effects of phthalates on hu-

man health and the fact that they are increasingly found in plastic food packaging, there is a justified need to place clearly defined restrictions on these compounds in food packaging, and food as well. The honey whose production process does not enable contamination with phthalates, except in the case of packaging and storage in plastic packaging, is the sample whose analysis could prove the migration of phthalates in the product. Also, certain types of honey crystallize during storage- some faster and some slower. Although crystallization is a natural property of honey, a large number of consumers do not like crystallized honey, so in order to decrystallize it, they heat it, which increases the migration of phthalates. In order to prove this, it is necessary to analyse the phthalate content in honey samples. The method of liquid-liquid extraction with hexane and the analysis of the stored samples using gas chromatography-mass spectrometry (GC-MS) were chosen. The goal of this study is to develop and validate a method for the determination of phthalates in honey.

MATERIAL AND METHODS

Honey samples

For the purpose of determining the presence of phthalates in honey, ten samples of different origin were collected. Honey samples were randomly collected from two sources: honey samples that were brought to the Scientific Veterinary Institute "Novi Sad" (NIV-NS) in plastic jars and honey samples that were collected from NIV-NS's bee yard in glass jars. All the samples were in their original packaging and were transferred to the laboratory, properly labeled and stored in a dark place at room temperature for 3 years.

Reagents and materials

Standards of phthalate acid esters (PAE)" were investigated in this study, namely dimethyl phthalate (DMP; C₁₀H₁₀O₄), diethyl phthalate (DEP; C₁₂H₁₄O₄), diisobutyl phthalate (DiBP; C₁₆H₂₂O₄), dibutyl phthalate (DBP; C₁₆H₂₂O₄), bis(2-ethylhexyl) phthalate (DEHP; C₂₄H₃₈O₄), di-n-octyl-phthalate (DnOP; C₂₄H₃₄O₄)) they were purchased from dr. Ehrenstorfer GmbH (Germany). In Table 1, analytical data include Abbreviation, Retention time and Qualitative and Quantitative ion monitoring. N-Hexane was HPLC grade (Carlo Erba, Milan, Italy). The solutions of each phthalate were prepared at concentrations of 1 mg/mL. Phthalates solutions at different concentrations (0.005, 0.01, 0.1, 0.05, 0.5 µg/mL) were prepared by dilution in n-hexane. The solutions were stored in vials at -20 °C. In order to avoid cross-contamination

due to reagents, materials, and laboratory equipment, a thorough cleaning procedure was performed: the glassware was soaked and washed in acetone, dried at 140 °C for at least 4 h. All the solvents used in the analysis were tested in order to check the potential presence of PAE contamination using GC–MS analysis. Ultrapure water was produced by a Milli-Q system (Millipore, Bedford, USA).

Table 1. Chemical data on the compounds investigated in this study.

Compound	Abbreviation	Retention time(min)	Quantitative ion(m/z)	Qualitative ion (m/z)
Di-methyl phthalate	DMP	7.36	163	77/194
Di-ethyl phthalate	DEP	8.44	149	177/176
Di-isobutyl phthalate	DiBP	10.78	149	104/167
Di-n-butyl phthalate	DBP	11.86	149	205/104
Bis(2-ethylhex-yl) phthalate	DEHP	20.75	149	167/206
Di-(n-octyl) phthalate	DnOP	24.89	149	279/104

Sample pretreatment

The amount of 5 g of honey and 10 mL of ultra-pure water was put into a 100 mL screw-cap glass centrifuge tube with a conical bottom and vigorously vortexed to for at least 1 min in order to form a homogeneous solution. After that, the solution was mixed with 10 mL of hexane, and submitted to extraction by shaking in a mechanical shaker for 40 min. Then, the organic phase was separated by centrifugation at 3000 rpm for 10 min and collected. The sample was once again extracted with 10 mL of hexane and the above-described procedure was repeated. The two portions of supernatant were collected and transferred to a clear conical flask and evaporated to dryness at 40 °C with a rotary evaporator. The residue was dissolved in 1.0 mL of hexane and the final solution was used for GC–MS analysis (Zhou et al, 2014). The method precision was evaluated, as described by Zhou et al. (2014). The retention times of the peaks and target ions, obtained from the standard solution of phthalates served as a base point for the phthalate's determination in samples.

GC-MS Analysis and Instrumentation

The identification of phthalates was based on a comparison of retention times of the peaks and target ions with those obtained from a standard mixture of phthalates (standards supplied by instrument manufacturer). The quantification was based on external calibration curves prepared from the standard solution of each of the examined phthalates.

The GC operating conditions are shown in Table 2. The verification of the peaks was carried out based on the retention times and target ions were compared to those of external phthalates. A solvent blank was also analyzed, phthalates were detected at the concentrations lower than LOQ.

Table 2. The GC operating conditions

Descriptions	Conditions
Instrument	Agilent 7890B/5977A MSD (Santa Clara, CA, USA)
Column	Fused silica column (30 m × 0.25 µm film of HP-5M-thickness) Agilent Technologies, Inc., (Santa Clara, CA, USA)
Temperature	Injection 280 °C MSD 280 °C Column 90 °C (1 min hold) to 210 °C at 15 °C / min (hold 2 min); then at the rate of 5 °C/min to 240 °C to hold 5 min; followed by an increase of 5 °C/min to 250 °C, and the followed by an increase of 25 °C /min to 300 °C held for 4 min.
Injection volume	2 µL

The determination was performed in splitless mode. Carrier gas was Helium, velocity: 35.698 cm/sec; pressure: 7.0 psi. The determination was made at constant flow.

RESULTS

Method Validation

Method validation and quality control were conducted following the European Commission SANTE /11813/2017 Regulation (European Commission, 2017). The method was validated in terms of the optimal linearity ($r^2 > 0.99$). Precision was evaluated by repeatability in triplicate (50.0 µg/kg, $n = 10$) and

it ranged from 0.79 – 5.72%. Recovery ranged from 88.51% to 112.23%. The obtained results are shown in Table 3.

Table 3. The average values of LOD, LOQ, precision, linearity, recovery and RSD in honey samples, spiked with 50 µg/kg (n = 20)

Compound	LOD ¹ (µg/kg)	LOQ ² (µg/kg)	Precision (%)	Linearity (r ^{2 3})	Recovery (%)	RSD ⁴ (%O)
DMP	1.38	4.68	4.26	0.9991	96.65	5.26
DEP	0.28	1.12	0.78	0.9992	112.23	10.20
DiBP	0.29	1.13	3.36	0.9991	94.62	12.62
DBP	0.89	2.76	3.49	0.9996	95.12	7.12
DEHP	0.59	2.07	3.48	0.9991	92.12	3.38
DnOP	1.22	3.89	5.72	0.9992	88.51	14.3

¹LOD—Limit of detection; ²LOQ—Limit of quantification; ³r²—Correlation coefficient; ⁴RSD—Precision in case of repeatability

Concentration of phthalates in honey

The pilot study results are presented in Table 4.

Table 4. Concentration of phthalates in honey and blank (µg/kg)

Compound	Blank (hexan + water)	3 years stored honey in glass jar ¹	3 years stored honey in plastic jar ²
DMP	<LOQ	<LOQ	<LOQ
DEP	<LOQ	<LOQ	3.34
DiBP	<LOQ	5.32	15.84
DBP	<LOQ	1.32	16.01
DEHP	<LOQ	4.45	14.44
DnOP	<LOQ	< LOQ	< LOQ

¹ Average concentration of 5 measurements of 3 years stored honey in glass jar;

² Average concentration of 5 measurements of 3 years stored honey in plastic jar

DISCUSSION

Various analytical methods, liquid and gas chromatographic techniques with different detectors have been used to determine phthalates over the years. (Glaser et, 1981; Prokúpková et al, 2002; Batlle & Nerín, 2004; Li et al, 2004; Xu et al, 2007; Farajzadeh et al, 2012; Yan et al, 2012), LC–UV (Jen and Liu, 2006; Ling et al, 2007; Li et al, 2008; Zhao et al, 2008; Kamarei et al, 2011), GC–MS (Penalver et al, 2000; Feng et al 2005; Serôdio & Nogueira, 2006; Sørensen, 2006; Shen et al, 2007; Liu et al, 2008; Regueiro et al, 2008; Cacho et al, 2012; Jiao et al, 2012; Sun et al, 2012; Huang et al, 2012; Yan et al, 2012; Kong et al, 2012) and LC–MS/MS (López-Jiménez et al, 2005). GC–MS detection methods for the identification and quantification of phthalates are most commonly used for routine analyses due to their relatively high sensitivity and selectivity. In addition to the development of methods for quantifying phthalates, a significant step in this process is the development of a method for sample preparation. Over the years, different techniques and matrices have required different methods of sample preparation and optimization, in order to obtain a fast and reliable method of preparation. Besides SPE preparation (Harris et al, 1997; Liu et al, 2008; Li et al, 2008; Kamarei et al, 2011; Yan et al, 2012), single-drop microextraction (Batlle and Nerín, 2004), solid phase microextraction (Penalver et al, 2000; Prokúpková et al, 2002; Li et al, 2004; Feng et al, 2005), liquid- liquid extraction were also used (LLE) (Zhou et al, 2014).

In this study, performance development methods included verification of linearity, limit of detection (LOD) and limit of quantification (LOQ), precision, recovery using a Guardiennes for pesticides residues (SANTE/2019). The calibration curves were obtained by measuring standard solutions injected in five level of concentration (0.005; 0.01; 0.05; 0.1; 0.5 µg/mL). Figure 1. shows chromatograms of solvent blank (water and hexane) and standard solution, which means that the applied parameters of the method will enable phthalates separation. The linearity of the method was determined by calibration in five calibration levels and good linearity characterized by a coefficient of linearity was obtained for all phthalates of interest > 0.999 ($r^2 > 0.999$). LOQ and LOD were determined by injecting five consecutive samples of the first calibration point (0.005 ug / mL) with an acceptable accuracy of $\pm 20\%$. The accuracy was assessed on the basis of the data obtained by injecting standard solutions in five replicates in two calibration levels. It ranged between 0.78 – 5.72. Recovery was determined by spiking a sample of honey into two concentration levels and two mean values obtained are shown as a result. Recovery values range between 88.51 - 112.23% which is in line with the Sante guidelines. The result of the recovery, which corresponds to the recommendation for residual

determinations, also shows us that the applied phthalate extraction method is satisfactory. During the development of the method, no calibration method was used through the matrix, but a blank sample was taken that underwent the same preparation procedure as the honey sample and contained water and hexane. The obtained results on the presence of phthalates in the blank are shown in Table 4. Namely, the values of phthalates in the blank are quantified, but below the LOQ values with a low level of confidence and therefore are not shown. However, it is important to point out that the solvent blank must work due to the wide distribution of phthalates. Notardonato et al (2020) got linearity > 0.999 , but recovery was in the range from 69.3 to 98.8%. Zhou et al (2014) also got good linearity and recovery was between 82.9 – 110.9%.

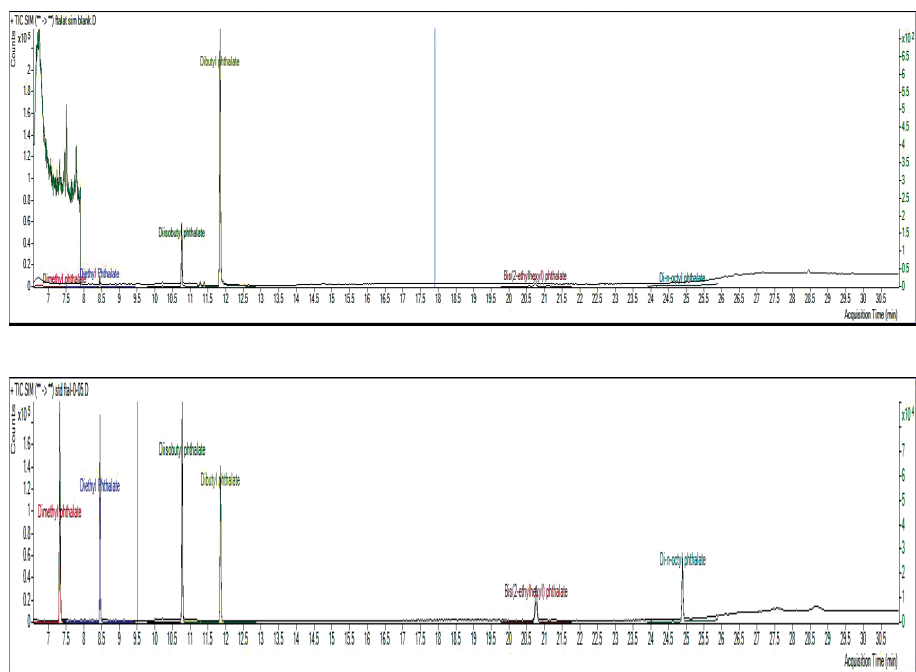


Figure 1. SIM mode blank chromatographs (hexane and water) (above) and phthalate standard with a concentration of 0.05 µg/g (below).

Notardonato et al (2020) also used gas chromatograph (GC) coupled with ion trap mass spectrometry (GC-MS) with selected ion monitoring (SIM) mode for phthalates determination same like Goodman W. (2009) who made an application note for determination of phthalates using GC-MS. If we look the condition parameters, we can see that we all had used similar parameters. We all used silica column with same diameters (30 m x 0.25 mm x 0.25 µm),

while the start temperature was not exactly the same, but it was similar (between 90 and 100 °C). However, the response for each compound was linear with r^2 greater than 0.999 for all phthalates compounds and for all authors.

In this study we analyzed and found phthalates in honey. Some other authors also reported phthalates contamination of honey (Notardonato et al, 2020; Zhou et al, 2014; Lo Turco et al, 2016). The concentration that we registered was much lower than that found by other authors who analyzed more samples (Notardonato et al., 2020; Lo Turco et al, 2016).

DMP and DnOP were not detected in these ten honey samples, while other analyzed phthalates were present. The maximum contents of PAEs were within 16.01 µg/kg for DBP; 15.84 µg/kg for DiBP; 14.44 µg/kg for DEHP; 3.34 µg/kg for DEP.

These examinations show that the samples in glass bottle had lower level of phthalates. Notardonato et al. (2020) analyzed 47 nectar honey samples and they found DMP in only one sample while DEP were found in 5 samples in the range from 25.4 to 374 µg/kg; DIPB were detected in six samples ranging between 28.7 and 553.1 µg/kg, DBP was in the range from 11.5 to 996.8 µg/kg; DEHP was detected in 37 samples in the range from 4.9 to 502.8 µg/kg and DnOP in range from 5.1 to 888.2 µg/kg in 25 samples. Our examination shows a lower level of contamination in honey than in other studies (Lo Turco et al, 2016; Notardonato et al., 2020;).

As some plastic can migrate from food through different materials, the EU Commission has defined the presence and the levels of small amounts of additives in food. Particularly, according to the EU Regulation No. 10/2011 and 2005/2018, the safety limit defined by each specific migration limits (SMLs) in food, for DMP, DEP, DiBP, and DnOP is 60 mg/kg, and 0.3, 0.05, and 1.5 mg/kg for DBP, BP-A, and DEHP, respectively. The limit of 60 mg/kg requires consideration: this high value means that the additive is permitted to be used in the polymer production for food packaging and there are no restrictions. The LOQs that we had were lower than the SML set by the EU Commission: this means that the method investigated is sensitive enough to analyze the threshold limits of the different compounds in the collected honey samples.

CONCLUSION

In this study, a fast and reliable method for the determination of six phthalates from honey was developed. Using liquid-liquid extraction, satisfactory recovery values were obtained and the analysis was performed on GC-MS. This paper can be considered as a pilot study for the determination of phthalates in honey and honey products. During the analysis, phthalates were found even

in blank solvent, in concentrations lower than LOQ, and their presence even in the blank indicates the need to expand the study. During the test, phthalates were found in all tested honey samples. Given the findings, in the future, we will focus on potential sources of phthalate contamination in the process of production of honey and honey products and will continue more extensive testing of these contaminants not only in honey but also in other foods.

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Author's Contribution

B.K. drafting the manuscript and have made substantial contributions to basic idea; B.K. and J.P. carried out the GC-MS analysis and have been involved in drafting the manuscript; J.V. carried out sample collection and sample preparation and performed the statistical analysis; J.V. and R.R. have been involved in drafting the manuscript; B.Đ. have been involved in data collection; J.V. and R.R. revised the manuscript critically.

Competing interest

The authors declare that they have no competing interests.

REFERENCES

1. Bajkin I., Bjelica A., Ičin T., Dobrić V., Kovačev-Zavišić B., Medić-Stojanoska M. 2014. Effects of phthalic acid esters on fetal health. *Medicinski preglod*, 67, 5-6, 172-175. doi: 10.2298/mpns1406172b.
2. Batlle R. and Nerín C. 2004. Application of single-drop microextraction to the determination of dialkyl phthalate esters in food simulants. *Journal of Chromatography A*, 1045, 1-2, 29-35. doi: 10.1016/j.chroma.2004.06.001.
3. Butala J.H., David R.M., Gans G., McKee R.H., Guo T.L., Peachee V.L., White Jr K.L. 2004. Phthalate treatment does not influence levels of IgE or Th2 cytokines in B6C3F1 mice. *Toxicology*, 201, 1-3, 77-85. doi: 10.1016/j.tox.2004.04.004.

4. Cacho J.I., Campillo N., Viñas P., Hernández-Córdoba M. 2012. Stir bar sorptive extraction coupled to gas chromatography–mass spectrometry for the determination of bisphenols in canned beverages and filling liquids of canned vegetables. *Journal of chromatography A*, 1247, 146-153. doi: 10.1016/j.chroma.2012.05.064.
5. COMMISSION REGULATION (EU) No. 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food
6. COMMISSION REGULATION (EU) No. 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC
7. COMMISSION REGULATION (EU) No. 2005/2018 of 17 December 2018 amending Annex XVII to Regulation (EC) No 1907/2006 of the European Parliament and of the Council concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) as regards bis(2-ethylhexyl) phthalate (DEHP), dibutyl phthalate (DBP), benzyl butyl phthalate (BBP) and diisobutyl phthalate (DIBP)
8. European Commission, 2017. SANTE/11813/2017. Guidance Document on Analytical Quality Control and Method Validation Procedures for Pesticide Residues and Analysis in Food and Feed. Implemented by 01/01/2018.
9. Farajzadeh M.A., Djozan D., Mogaddam M.R.A., Norouzi J. 2012. Determination of phthalate esters in cow milk samples using dispersive liquid-liquid microextraction coupled with gas chromatography followed by flame ionization and mass spectrometric detection. *Journal of separation science*, 35, 5-6, 742-749. doi: 10.1002/jssc.201100853.
10. Feng Y.L., Zhu J., Sensenstein R. 2005. Development of a headspace solid-phase microextraction method combined with gas chromatography mass spectrometry for the determination of phthalate esters in cow milk. *Analytica Chimica Acta*, 538, 1-2, 41-48. doi: 10.1016/j.aca.2005.02.020.
11. Glaser J. A., Foerst D.L., McKee G.D., Quave S.A., Budde W.L. 1981. Trace analyses for wastewaters. *Environmental Science & Technology*, 15, 12, 1426-1435. doi: 10.1021/es00094a002.
12. Goodman W. 2009. Application note: GC/MS Analysis of Phthalates in Children's Products. PerkinElmer, Inc.
13. Harris C.A., Henttu P., Parker M.G., Sumpter J.P. 1997. The estrogenic activity of phthalate esters in vitro. *Environmental health perspectives*, 105, 8, 802-811. doi: 10.1289/ehp.97105802.

14. Huang G., Li H.F., Zhang B.T., Ma Y., Lin, J.M. 2012. Vortex solvent bar microextraction for phthalate esters from aqueous matrices. *Talanta*, 100, 64-70.
15. Jen J.F. and Liu T.C. 2006. Determination of phthalate esters from food-contacted materials by on-line microdialysis and liquid chromatography. *Journal of Chromatography A*, 1130, 1, 28-33. doi: 10.1016/j.chroma.2006.06.029.
16. Jiao Y., Fu S., Ding L., Gong Q., Zhu S., Wang L., Li H. 2012. Determination of trace leaching phthalate esters in water by magnetic solid phase extraction based on magnetic multi-walled carbon nanotubes followed by GC-MS/MS. *Analytical methods*, 4, 9, 2729-2734.
17. Kamarei F., Ebrahimzadeh H., Yamini Y. 2011. Optimization of ultrasound-assisted emulsification microextraction with solidification of floating organic droplet followed by high performance liquid chromatography for the analysis of phthalate esters in cosmetic and environmental water samples. *Microchemical Journal*, 99, 1, 26-33. doi: 10.1016/j.microc.2011.03.006.
18. Kong S., Ji Y., Liu L., Chen L., Zhao X., Wang J., Bai Z., Sun Z. 2012. Diversities of phthalate esters in suburban agricultural soils and wasteland soil appeared with urbanization in China. *Environmental Pollution*, 170, 161-168. doi: 10.1016/j.envpol.2012.06.017.
19. Li X., Zeng Z., Chen Y., Xu, Y. 2004. Determination of phthalate acid esters plasticizers in plastic by ultrasonic solvent extraction combined with solid-phase microextraction using calix [4] arene fiber. *Talanta*, 63, 4, 1013-1019. doi: 10.1016/j.talanta.2004.01.006.
20. Li J., Cai Y., Shi Y., Mou S., Jiang G. 2008. Analysis of phthalates via HPLC-UV in environmental water samples after concentration by solid-phase extraction using ionic liquid mixed hemimicelles. *Talanta*, 74, 4, 498-504. doi: 10.1016/j.talanta.2007.06.008.
21. Ling W., Jiang G.B., Cai Y.Q., Bin H.E., Wang Y.W., Shen D.Z. 2007. Cloud point extraction coupled with HPLC-UV for the determination of phthalate esters in environmental water samples. *Journal of Environmental Sciences*, 19, 7, 874-878. doi: 10.1016/S1001-0742(07)60145-4.
22. Liu H.C., Den W., Chan S.F., Kin K.T. 2008. Analysis of trace contamination of phthalate esters in ultrapure water using a modified solid-phase extraction procedure and automated thermal desorption-gas chromatography/mass spectrometry. *Journal of Chromatography A*, 1188, 2, 286-294. doi: 10.1016/j.chroma.2008.02.078.
23. Lo Turco V., Di Bella G., Potortì A.G., Tropea A., Casale E.K., Fedè M.R., Dugo G. 2016. Determination of plasticisers and BPA in Sicilian and Calabrian nectar honeys by selected ion monitoring GC/

- MS. Food Additives & Contaminants: Part A, 33, 11, 1693-1699. doi: 10.1080/19440049.2016.1239030.
24. López-Jiménez F.J., Rubio S., Pérez-Bendito D. 2005. Determination of phthalate esters in sewage by hemimicelles-based solid-phase extraction and liquid chromatography-mass spectrometry. *Analytica chimica acta*, 551, 1-2, 142-149.
 25. Meeker J.D., Sathyanarayana S., Swan S.H. 2009. Phthalates and other additives in plastics: human exposure and associated health outcomes. *Philosophical transactions of the royal society B: biological sciences*, 364, 1526, 2097-2113. doi: 10.1098/rstb.2008.0268.
 26. Notardonato I., Passarella S., Ianiri G., Di Fiore C., Vincenzo Russo M., Avino P., 2020. Analytical Method Development and Chemometric Approach for Evidencing Presence of Plasticizer Residues in Nectar Honey Samples. *Int. J. Environ. Res. Public Health* 2020, 17, 1692;
 27. Penalver A., Pocurull E., Borrull F., Marce R.M. 2000. Determination of phthalate esters in water samples by solid-phase microextraction and gas chromatography with mass spectrometric detection. *Journal of Chromatography A*, 872, 1-2, 191-201. doi: 10.1016/s0021-9673(99)01284-4.
 28. Prokůpková G., Holadová K., Poustka J., Hajšlová J. 2002. Development of a solid-phase microextraction method for the determination of phthalic acid esters in water. *Analytica Chimica Acta*, 457, 2, 211-223. doi: 10.1016/s003-2670(02)00020-x.
 29. Regueiro J., Llompart M., Garcia-Jares C., Garcia-Monteagudo J.C., Cela R. 2008. Ultrasound-assisted emulsification-microextraction of emergent contaminants and pesticides in environmental waters. *Journal of Chromatography A*, 1190, 1-2, 27-38. doi: 10.1016/j.chroma.2008.02.091.
 30. REGULATION (EC) No 1935/2004 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 27 October 2004 on materials and articles intended to come into contact with food and repealing Directives 80/590/EEC and 89/109/EEC
 31. Rodil R., and Moeder, M. 2008. Stir bar sorptive extraction coupled to thermodesorption – gas chromatography – mass spectrometry for the determination of insect repelling substances in water samples. *Journal of Chromatography A*, 1178, 1-2, 9-16. doi: 10.1016/j.chroma.2007.11.051.
 32. Serôdio P., and Nogueira J. M. F. 2006. Considerations on ultra-trace analysis of phthalates in drinking water. *Water research*, 40, 13, 2572-2582. doi: 10.1016/j.waters.2006.05.002.
 33. Shen H.Y., Jiang H.L., Mao H.L., Pan G., Zhou L., Cao Y.F. 2007. Simultaneous determination of seven phthalates and four parabens in cosmetic products using HPLC-DAD and GC-MS methods. *Journal of separation*

- science, 30, 1, 48-54. doi: 10.1002/jssc.200600215.
34. Sørensen L. K. 2006. Determination of phthalates in milk and milk products by liquid chromatography/tandem mass spectrometry. *Rapid Communications in Mass Spectrometry: An International Journal Devoted to the Rapid Dissemination of Up-to-the-Minute Research in Mass Spectrometry*, 20, 7, 1135-1143. doi: 10.1002/rcm.2425.
 35. Sun H., Yang Y., Li H., Zhang J., Sun N. 2012. Development of multiresidue analysis for twenty phthalate esters in edible vegetable oils by microwave-assisted extraction–gel permeation chromatography–solid phase extraction–gas chromatography–tandem mass spectrometry. *Journal of agricultural and food chemistry*, 60, 22, 5532-5539. doi:10.1021/jf3009603.
 36. Xu J., Liang P., Zhang T. 2007. Dynamic liquid-phase microextraction of three phthalate esters from water samples and determination by gas chromatography. *Analytica chimica acta*, 597, 1, 1-5. doi: 10.1016/j.aca.2007.06.047.
 37. Yan H., Cheng X., Yan K. 2012. Rapid screening of five phthalate esters from beverages by ultrasound-assisted surfactant-enhanced emulsification microextraction coupled with gas chromatography. *Analyst*, 137, 20, 4860-4866. doi: 10.1039/c2an36046b.
 38. Yan H., Cheng X., Yang G. 2012. Dummy molecularly imprinted solid-phase extraction for selective determination of five phthalate esters in plastic bottled functional beverages. *Journal of agricultural and food chemistry*, 60, 22, 5524-5531. doi: 10.1021/jf300660m.
 39. Yen T.H., Lin-Tan D.T., Lin J.L. 2011. Food safety involving ingestion of foods and beverages prepared with phthalate-plasticizer-containing clouding agents. *Journal of the Formosan Medical Association*, 110, 11, 671-684. doi: 10.1016/j.jfma.2011.09.002.
 40. Zhao R.S., Wang X., Yuan J.P., Lin J.M. 2008. Investigation of feasibility of bamboo charcoal as solid-phase extraction adsorbent for the enrichment and determination of four phthalate esters in environmental water samples. *Journal of Chromatography A*, 1183, 1-2, 15-20. doi: 10.1016/j.chroma.2008.01.021.
 41. Zhou J., Qi Y., Wu H., Diao Q., Tian F., Li Y. 2014. Simultaneous determination of trace migration of phthalate esters in honey and royal jelly by GC–MS. *Journal of separation science*, 37, 6, 650-657. doi:10.1002/jssc.201300778.

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