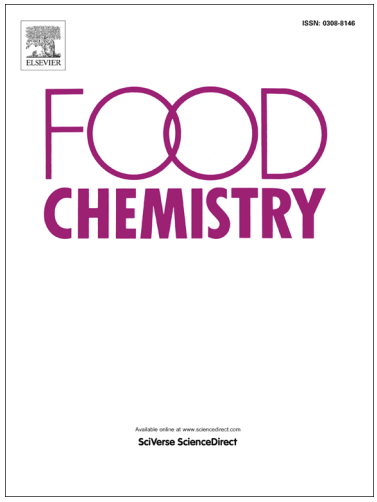

Determination of phthalate esters in distillates by ultrasound-vortex-assisted dispersive liquid-liquid micro-extraction (USVADLLME) coupled with gas chromatography/mass spectrometry

Giuseppe Montevecchi, Francesca Masino, Luca Zanasi, Andrea Antonelli

PII: S0308-8146(16)31843-X
DOI: http://dx.doi.org/10.1016/j.foodchem.2016.11.019
Reference: FOCH 20152

To appear in: *Food Chemistry*

Received Date: 28 May 2016
Revised Date: 27 October 2016
Accepted Date: 3 November 2016

Please cite this article as: Montevecchi, G., Masino, F., Zanasi, L., Antonelli, A., Determination of phthalate esters in distillates by ultrasound-vortex-assisted dispersive liquid-liquid micro-extraction (USVADLLME) coupled with gas chromatography/mass spectrometry, *Food Chemistry* (2016), doi: http://dx.doi.org/10.1016/j.foodchem.2016.11.019

Determination of phthalate esters in distillates by ultrasound-vortex-assisted dispersive liquid-liquid micro-extraction (USVADLLME) coupled with gas chromatography/mass spectrometry

Giuseppe Montevecchi[1,*], Francesca Masino[1,2], Luca Zanasi[2], Andrea Antonelli[1,2]

[1] Centro di Ricerca Interdipartimentale per il Miglioramento e la Valorizzazione delle Risorse Biologiche Agro-Alimentari BIOGEST – SITEIA, Università degli Studi di Modena e Reggio Emilia, Tecnopolo di Reggio Emilia, Piazzale Europa 1, 42124 Reggio Emilia, Italy

[2] Dipartimento di Scienze della Vita (Area Scienze Agro-Alimentari), Università degli Studi di Modena e Reggio Emilia, Via G. Amendola 2, 42122 Reggio Emilia, Italy.

* Corresponding author. Tel.: +39 0522523541; fax: +39 0522 522027.

E-mail address: giuseppe.montevecchi@unimore.it (G. Montevecchi).

Abstract

A method for the extraction of phthalate esters (PAEs) by Ultrasound-Vortex-Assisted Dispersive Liquid-Liquid Micro-Extraction (USVADLLME) approach was optimised and applied for the first time to a historical series of brandies. These contaminants are widely spread in the environment as a consequence of about half century of use in different fields of applications. The concern about these substances and the recent legal restrictions of China in distillates import need a quick and sensitive method for their quantification. The proposed method, moreover, is environmentally oriented due to the disposal of micro-quantities of solvent required. In fact, sub-ppm-limits of detection were achieved with a solvent volume as low as 160 μL.

The analysed samples were within the legal limits, except for some very ancient brandies whose contamination was probably due to a PAEs concentration effect as a consequence of long ageing and for the use of plastic pipelines no more operative.

_____

Keywords: Phthalate esters; Ultrasound-Vortex-Assisted Dispersive Liquid-Liquid Micro-Extraction; Gas chromatography-Mass spectrometry; Distillates; Brandy.

Chemical compounds studied in this article:

Dibutyl phthalate (PubChem CID: 3026)

Bis(2-ethylhexyl) phthalate (PubChem CID: 8343)

Diisononyl phthalate (PubChem CID: 590836)

Benzyl butyl phthalate (PubChem CID: 2347)

1. Introduction

Phthalate esters (alkylarylesters of 1,2-benzenedicarboxylic acid, also known as PAEs) are chemicals produced from petroleum and they represent the most common plasticisers in the world. PAEs are a family of chemicals used for over 50 years, primarily to make the plastic polymers soft and flexible, but they are used in many other materials. Because of their extensive use, PAEs are now ubiquitous in the environment and in materials in contact with food. They are process contaminants, in fact, they can migrate inside foodstuffs and the monitoring of the sources is not always easy (Staples, 2003). Moreover, PAEs contaminate surfaces of lab materials, even pure products for analysis, and can create false positives in the samples (Staples, 2003; Fankhauser-Noti and Grob, 2007). For this reason, it is necessary to check the content of PAEs in all materials used for their determination in distillates and to set up specific cleaning procedures in order to obtain phthalate-free glassware, solvents, and chemicals.

The effects on human health of PAEs are still being studied by various government agencies, including EFSA in Europe and the "Food and Drug Administration" in the United States. These substances cause great concern, mainly for risks related to human reproduction (Committee on the Health Risks of Phthalates, 2008; Owens, 2015).

On 17 February 2011, the European Commission, with Regulation 143/2011, included in Annex XIV dibutyl phthalate and bis(2-ethylhexyl) phthalate classifying them as toxic for human reproduction and identifying them as endocrine disrupters (European Union, 2011). At the beginning of 2016, 14 PAEs were included in the Candidate List for authorization (European Union, 2006), even if all of them were classified as toxic to reproduction (ECHA).

Based on the available information, foods are the main source of exposure to PAEs for populations (Chou and Wright, 2006; Wormuth et al., 2006). As a consequence of their lipophilicity, PAEs accumulate in all fat-containing foods, such as fresh milk and dairy products, meat, poultry, and eggs, as they are part of our daily diet (Serrano et al., 2014). Recent studies have showed that

exposure to PAEs begins in the womb and they are present even in breast milk (Latini et al., 2003; Lockett et al., 2015; Kim et al., 2015). The presence of phthalates has been well documented even in baby foods (Fierens et al., 2012).

Brandy is a distilled beverage containing a high percentage of ethanol that acts as a PAE solvent. Moreover, alcoholic beverages have a high potential PAEs contamination from earlier production process, such as transport containers, pneumatic press material, pump material, pump lines and additives (Fuller et al., 2011).

Armed with this data, the competent authority of China (AQSIQ, responsible for exports and imports) enacted a law (circular from the Ministry of Health of 22 January 2013) that made mandatory an analytical test report stating the level of three PAEs to export distillates to China (European Commission, 2015). In particular, in distillates there are restrictions as regards the content of: dibutyl phthalate (0.30 mg/kg, DBP), bis(2-ethylhexyl) phthalate (1.50 mg/kg, DEHP), diisononyl phthalate (9.00 mg/kg, DINP).

In 2014, a subsequent Chinese risk assessment stated that PAEs in spirits are safe even at higher levels in comparison with what set by AQSIQ for imports (European Commission, 2015). However, China authorities did not amend this requirement on the basis of their recent risk assessment, and other nations might follow the example of China. This would entail severe hindrances for the spirit export.

Multiple hypotheses can be done on the source of this contamination in distillates and it is crucial to deepen where the PAEs come from in order to develop a strategy for their reduction. The distillation of contaminated base wine could promote either the concentration or the abatement of PAEs in the distillates. Another suspected source of contamination is represented by the piping used to pour the semi-finished product. In addition, the technology of production makes difficult the actions that limit the content of these substances. Because of the long ageing that characterises brandy and other spirit production, as the distillates are stored in oak casks even for decades, the

effects of a possible contamination would last much more than other products. Similarly, cleaning the production line would not immediately produce positive effects.

PAE determination was carried out following several pre-treatment procedures, such as liquid–liquid extraction (LLE) (Zhu, 2006; Cai et al., 2007), solid-phase extraction (SPE) (Jara et al., 2000; Casajuana and Lacorte, 2003; Kato et al., 2003; Russo et al., 2012), solid-phase microextraction (SPME) (Prokupkovà et al., 2002; Penalver et al., 1999; Alpendurada, 2000; Polo et al., 2005; Carrillo et al., 2007; Del Carlo et al., 2008) or liquid-phase microextraction (LPME) (Psillakis and Kalogerakis, 2003; Rasmussen and Pedersen-Bjergaard, 2004), followed by chromatographic analysis. An evolution of LPME, called dispersive liquid–liquid microextraction (DLLME) was recently introduced and applied for the first time on polycyclic aromatic hydrocarbons in water samples (Rezaee et al., 2006). In comparison with LLE and SPE, DLLME is a rapid, inexpensive, simple, clean, and it assures high recovery and enrichment factors (Russo et al., 2015). Moreover, the protocol needs a minimum amount of extraction chlorinated solvent (< 200 μL) for each sample, instead of volumes in the range of 5-20 mL, as required in extractions with traditional methods. On the other hand, it provides high enrichment factors from small volumes of aqueous samples. Very recently, a protocol based on ultrasound-vortex-assisted dispersive liquid–liquid microextraction (USVADLLME) for the determination of PAEs in hydroalcoholic beverages was proposed (Cinelli et al., 2013; Russo et al., 2014). These environment friendly approaches are aimed to reduce the volume of organic solvent required and simultaneously improve the extraction efficiency without a dispersive solvent (Yan et al., 2011), and minimise the PAEs solvent carry-over with false positive cases.

Gas chromatography coupled to mass spectrometry (GC/MS) is the election technique for the PAE detection, because of its high sensitivity and the good chromatographic behaviour of phthalates (Wenzl, 2009).

The main long-term goal of the project aimed at improving the quality of production of distilled beverages by reducing PAEs content. The present work is a preliminary study focused on the definition of a robust protocol of analysis for the determination of PAEs in brandies in relation to the thresholds imposed by the Chinese law.

The screening on PAEs content was carried out on all the lots of distillates subjected to ageing in wood barrels from 1987 to 2014. As far as we know, this is a first approach to the study of phthalates in spirits.

2. Materials and methods

2.1. Sampling

Sixty samples of brandy (8 mL) were withdrawn from casks and transferred into glass tubes with ground glass stoppers. The operation was carried out by means of phthalate-free (PF) glassware. The samples were stored at 4 °C and brought to room temperature before analysis.

2.2. Chemicals

All solvents and reagents were of analytical grade. Acetone, dichloromethane, and absolute ethanol were obtained from WVR Srl (Milan, Italy). The pure standards dibutyl phthalate, bis(2-ethylhexyl) phthalate, diisononyl phthalate, and benzyl butyl phthalate, as well as carborundum, sodium chloride, and sodium hydroxide were purchased from Fluka Sigma-Aldrich® Srl (Milan, Italy). Deionised water was obtained by a Milli-Q purification system (Millipore, Milan, Italy).

2.3. Preparation of materials

2.3.1. Solvents and other chemicals PF

All the solvents used (acetone, dichloromethane, absolute ethanol, and water) were distilled to eliminate the presence of PAEs. In particular, the water was distilled in the presence of sodium hydroxide.

After the distillation, 100 mL of each solvent were concentrated up to 1 mL and it was analysed by gas chromatography-mass spectrometry (GC/MS) to verify the absence of PAEs. All distilled solvents were then stored in sealed bottles, entirely of PF glass.

Carborundum, sodium chloride, and sodium hydroxide were placed inside shallow trays in a furnace oven at 400 °C for 4 h and, after cooling, tightly sealed in PF glass bottles.

2.3.2. Preparation of glassware PF

All the glassware was rinsed with PF acetone and placed in a furnace oven at 400 °C for 4 h to ensure complete elimination of PAEs. After the treatment, they were maintained closed in a cabinet to keep out dust.

2.4. Optimization of the extraction method

The extraction parameters were optimised on real samples starting from the method described by Cinelli and coll. (2013) for the determination of PAEs in the wine. However, contrary to wine, brandy samples were characterised by a high alcoholic strength: from 55 % up to 73 % ABV. For this reason, an accurate evaluation of the influence of the amount of alcohol present in the samples and volume of extracting solvent (dichloromethane) was carried out (Table 1). Accuracy was evaluated by recovery tests to study the effectiveness of the analytical method. A real sample was suitably diluted to obtain different ABV levels, then extracted with different amount of

dichloromethane and finally injected as they are and after spiking of known amounts of the three analytes (DBP 50 µL, 10 ppm; DEHP 120 µL, 100 ppm; DINP 30 µL, 100 ppm). The amount of NaCl was also evaluated.

2.5. Optimised extraction protocol

Brandy samples (1.5000 g) were accurately weighed and transferred into PF conical tubes with ground glass stoppers. Each tube was added with the internal standard (benzyl butyl phthalate), water up to 8 mL, and 1.50 g of NaCl. The sample was shaken with a vortex mixer till the complete dissolution of the salt. An aliquot of 160 µL of PF dichloromethane was quickly added to the solution in order to get the fine dispersion. The emulsion was shaken with a vortex mixer for 30 sec and the tube was placed in an ultrasonic bath for 10 min (Ultrasonic frequency 37 kHz, Elmasonic S30, Elma Schmidbauer GmbH, Singen, Germany) at room temperature. After 5 min in a water bath at 4 °C, the tube was centrifuged for 2 min at 5000 rpm to ease the separation of the organic phase. The organic phase was collected from the bottom of the tube and represented the extract ready for GC/MS analysis.

2.5.1. Evaluation of linearity, limit of detection and lower limit of quantification (sensitivity), and repeatability (precision).

Standard stock solutions (10,000 mg/L, in absolute PF ethanol) were made up for each substance. A series of increasing concentration solutions were prepared to evaluate the linearity of the instrumental response at the expected concentration range for each analyte. Limit of detection (LOD) and lower limit of quantification (LLOQ) were calculated by data obtained by study of linearity.

Precision was evaluated with a repeatability test by injecting five times the same sample.

2.6. GC-MS determination of phthalate esters

The extract (1 µL) was injected into a gas chromatograph Hewlett-Packard (HP) 6890 series instrument (Hewlett-Packard Waldbronn, Germany) with a split/splitless injection port coupled with a mass spectrometer instrument HP 5973 Mass Selective Detector (Hewlett-Packard Waldbronn, Germany), equipped with a capillary column Mega-SE52 crossbond (5% phenyl, 95% methyl polysiloxane, Mega snc, Legnano, MI, Italy) 25 m, having an internal diameter of 0.25 mm and film thickness of 0.25 µm. The oven temperature was set at 150 °C, held for 1 min and increased at 15 °C/min up to 330 °C and then held for 2 min (15 total min of analysis). The injection was performed in splitless mode at 335 °C (splitless time of 30 sec), the temperature of the transfer line was set at 330 °C. The carrier gas was ultrapure helium (constant flow rate 1 mL/min). The molecular fragmentation was obtained by electron ionization (EI). The data were obtained in full-scan mode and the mass/charge ratio (*m*/*z*) was recorded between 33 and 500 at 70 eV.

Chromatograms were acquired and processed using the software Enhanced Chem Station (G1701AA Version A.03.00, Hewlett Packard©). Peaks were identified by comparing retention times and mass spectra of pure standards and by the library NIST Databases for GC/MS (Agilent Technologies). Quantification was performed using the internal standard method. Each sample was analysed in duplicate strictly following the same procedure.

3. Results and discussion

3.1. Method optimization

The first step was the optimization of the chromatographic separation carried out on a standard solution of PAEs.

The second step was the optimization of the extraction technique using real samples, based on the method set up by Cinelli and coll. (2013). Ethanol, dichloromethane, and sodium chloride amounts were optimised. The high amount of ethanol present in the samples was carefully evaluated. In fact, ethanol is generally used as a disperser solvent, playing a critical role (co-surfactant effect) in the DLLME procedure (Cinelli et al., 2013). However, ethanol is miscible with both solvents (water and dichloromethane), thus preventing the separation of the phases.

Cinelli and coll. (2013) set an optimal range of ethanol content between 10 and 40% ABV. However, they carried out this evaluation on model solutions, while they described a strong matrix effect in the real samples, and for this reason, they adjusted the method accordingly. We did not optimise the method using model solutions and the dilution of the samples could not be avoided because of the much higher ABV content (55-73%) in brandies. With concentrations of ethanol higher than 16% ABV, there was not any separation of the phases even using as high as 160 µL of dichloromethane. This is reasonably due to a matrix effect, as well.

A recovery test on a real sample suitably diluted with water to obtain 11% and 16% ABV and spiked with PAEs was performed. This range of ABV was planned to include all the samples of brandies by a consistent dilution (1 to 5). The results obtained using 160 µL of dichloromethane (Table 2), as extracting solvent, did not show any significant differences between them. The figure for DEHP was optimal, while recoveries of DBP and DINP were around 80%.

The test was carried out on the same samples using different amounts of dichloromethane, which allowed the maximum extraction of the analytes. As already reported by Cinelli and coll. (2013), volumes of 40, 80, and 120 µL of dichloromethane did not allow the separation of the phases on real samples, while a volume of 160 µL did it for both concentrations of ethanol (11% and 16% ABV). The results obtained using 200 µL of dichloromethane were reported in Table 2. In both cases (11% and 16% ABV) the recoveries decreased in comparison with what found for 160 µL of dichloromethane and with higher relative standard deviations.

Sodium chloride was added to help the migration of the analytes into the organic phase and the separation of the phases (salting out effect). Cinelli and coll. (2013) found that a concentration of sodium chloride below 25 g L^{-1} reduced PAE solubility in the organic phase, whereas there were not differences above that value. On the basis of this data, the concentration of sodium chloride was increased in order to enhance the separation of the organic phase in real samples. However, ethanol concentration reduced the solubility of sodium chloride in the samples. In fact, 16% ABV limited to 1.50 g the dissolution of sodium chloride in the sample.

Vortex and ultrasonication times were not modified (Cinelli et al., 2013), and these steps were carried out at room temperature. As Cinelli and coll. observed, some samples could not be well separated and it was necessary to carry out a further centrifugation at 2000 rpm for 2 min at low temperature. To have a consistent protocol, all the samples were subjected to centrifugation. As centrifugation tends to heat the samples, test tubes were kept for 5 min in a water bath at 4 ºC in order to improve the separation of the phases.

Finally, accurately weighed 1.5000 g of each sample was diluted up to 8 mL to obtain an ABV between 12.5 and 16.0 % depending on the different alcoholic strength of the brandies.

3.2. Method evaluation

The concentration ranges of the analytes used for the calibration are showed in Table 3, along with LOD and LLOQ. For each substance, the concentration range was selected around the specific limits imposed by the Chinese law and we did not explore the sensitivity of the method for lower concentrations. The limits were satisfactory for DBP and DEHP, while DINP presented higher values (LLOQ = 1.00 mg/kg). However, the legal limit for DINP is much higher (9.00 mg/kg).

Linearity was verified in the considered concentration range by coefficients of determination (R^2) of the calibration curves for the three PAEs. All R^2 were higher than 0.98 (Table 3) and the background was particularly low (b coefficients). Precision was evaluated by the repeatability test

by injecting five times the same sample (Table 3). Relative standard deviation (RSD) was contained within 5% for all PAEs.

A comparison of the data of linearity, precision, and accuracy from different methods is showed in table 4. Data about LOD and LLOQ were not comparable with what showed by other authors and they did not appear in the table, but their figures were consistent for the aim of this work. For this reason, we did not explore for further enhancement of sensitivity.

3.3. Real samples

The data of the PAE concentrations in the real samples are showed in table 5. DBP had the highest concentrations in the samples with ageing higher than 15 years, exceeding the threshold (0.30 mg/kg) in 7 samples. In the samples from 1999 onwards it did not exceed the concentration of 0.10 mg/kg, with a few exceptions only. Similar considerations can be made for DINP, which was found only in the samples with ageing higher than 20 years, although it did not exceed in any case the threshold of 9.00 mg/kg. It is remarkable that DINP was no longer detected after 1994. The DEHP differed from the others compounds because it was less subjected to an accumulation in the most aged samples. This substance was present in concentrations above the threshold 1.50 mg/kg for two samples only. In particular, in brandy dated 1989/01/05 the concentration was very high (4.18 mg/kg), while in brandy dated 2001/11/23 it was a little higher the critical level (1.62 mg/kg). Only one sample (1989/01/05) showed both concentrations of DBP and DEHP higher than the legal limit.

The analysis of PAE concentrations over the years (Figure 1) showed that the younger is the brandy the lower is its PAE content. In fact, DBP decreased from 0.27 mg/kg to 0.05 mg/kg, while DEHP from 0.55 mg/kg to 0.30 mg/kg. However, the data for DEHP related to 1987-1990 and 2001-2005 were both characterised by the presence of the two figures above the critical threshold and they can

be considered as outliers. Finally, DINP showed an increase between 1987-1990 and 2001-2005; then these substances completely disappeared.

This fact is a direct consequence of ageing. In fact, distillates undergo a slow concentration process by water and ethanol evaporation through the staves. As a consequence, PAEs could tend to increase their concentration. PAEs content was, in fact, lower than the legal limits in all samples from 2002 onwards. This is a very delicate aspect to be dealt with and it is related with the most valuable products (longest ageing). The importance of the quality of the raw materials (wines) destined at long ageing production of brandies (over 20 years) and the grapes for wine production have therefore a crucial role. This aspect is less important for 3- and 5-years aged brandies, as the effect of concentration is lower, obviously.

The maximum concentrations for DBP and DEHP found in the literature for wine samples are: 0.244 ppm and 0.276 ppm (Del Carlo et al., 2008); 0.312 ppm and 0.026 ppm (Cinelli et al., 2013); 0.557 ppm and 0.007 ppm (Guo et al., 2012), respectively. Based on the literature data, DBP should be considered the most critical compound because its concentrations were very close to the Chinese law limits (0.30 mg/kg) already in the starting wine. In addition, it is necessary to study the behaviour of PAEs during distillation in order to establish if these molecules are able to follow ethanol during distillation, thus concentrating during this process.

It was not verified whether the barrels were responsible for the release of PAEs in the brandies during ageing, although it seems unlikely. However, in a few cases an abnormal accumulation of DEHP and DINP was registered. DINP behaviour was thoroughly considered. Between 1994 and 1997, the company production was stopped for unknown reasons, and since then this PAE was no longer detected in the samples subjected to ageing. All this suggests that the source of contamination could have been removed at that time, even unintentionally.

4. Conclusions

The study is of great importance as it appears to be the first one to have analysed PAEs contamination in a historical series in the brandy production. The results showed that only in the longest aged samples PAEs concentrations exceeded the limits of the Chinese law. This would suggest that the content of PAEs in the samples depends on the quality of the wines and on the effect of concentration that occurs during ageing. The strict selection of the raw material remains a crucial requirement. However, the behaviour of PAEs during distillation is still unknown and deserves deeper investigations.

The ultrasound-vortex-assisted dispersive liquid-liquid micro-extraction technique showed innovative and a suitable routine practice also due to the reduction of solvent use and disposal and operator health safeguard.

Acknowledgments

The research was supported by a financial contribution from Emilia Romagna region *"Interventi a favore della ricerca industriale delle imprese operanti nelle filiere maggiormente coinvolte dagli eventi sismici del maggio 2012"*

References

Alpendurada, M. F., J. (2000). Solid-phase microextraction: a promising technique for sample preparation in environmental analysis. *Journal of Chromatography A*, *889*, 3-14.

Cai, Y., Cai, Y. E., Shi, Y., Liu, J., Mou, S., & Lu, Y. (2007). A liquid–liquid extraction technique for phthalate esters with water-soluble organic solvents by adding inorganic salts. *Microchimica acta*, *157(1-2)*, 73-79.

Carrillo, J. D., Salazar, C., Moreta, C., & Tena, M. T. (2007). Determination of phthalates in wine by headspace solid-phase microextraction followed by gas chromatography–mass spectrometry: fibre comparison and selection. *Journal of Chromatography A*, *1164(1)*, 248-261.

Casajuana, N., & Lacorte, S. (2003). Presence and release of phthalic esters and other endocrine disrupting compounds in drinking water. *Chromatographia*, *57(9-10)*, 649-655.

Chou, K., & Wright, R. O. (2006). Phthalates in food and medical devices. *Journal of Medical Toxicology*, *2(3)*, 126-135.

Cinelli, G., Avino, P., Notardonato, I., Centola, A., & Russo, M. V. (2013). Rapid analysis of six phthalate esters in wine by ultrasound-vortex-assisted dispersive liquid–liquid micro-extraction coupled with gas chromatography-flame ionization detector or gas chromatography–ion trap mass spectrometry. *Analytica Chimica Acta*, *769*, 72-78.

Committee on the Health Risks of Phthalates (2008). Phthalates and Cumulative Risk Assessment The Task Ahead. National Academies Press, Washington, D.C., U.S.A.

Del Carlo, M., Pepe, A., Sacchetti, G., Compagnone, D., Mastrocola, D., & Cichelli, A. (2008). Determination of phthalate esters in wine using solid-phase extraction and gas chromatography–mass spectrometry. *Food Chemistry*, *111(3)*, 771-777.

ECHA. European Chemical Agency. Candidate List of substances of very high concern for Authorisation. http://echa.europa.eu/web/guest/candidate-list-table/ Accessed 25.05.16.

European Union (2006). Regulation EC no. 1907/2006, REACH of 18 December 2006, concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) and establishing a European Chemicals Agency. 30.12.2006 Official Journal of the European Union L 396/1.

European Union (2011). Regulation EC no. 143/2011 of 17 February 2011, amending Annex XIV to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH). 18.2.2011 Official Journal of the European Union L 44/2.

European Commission (2015). CHINA – Sanitary and Phytosanitary Issues – Unjustified barrier for alcoholic beverages due to phthalate (plasticisers) levels. (2015). http://madb.europa.eu/madb/sps_barriers_details.htm?barrier_id=155505/ Accessed 25.05.16.

Fankhauser-Noti, A., & Grob, K. (2007). Blank problems in trace analysis of diethylhexyl and dibutyl phthalate: investigation of the sources, tips and tricks. *Analytica chimica acta*, *582(2)*, 353-360.

Fierens, T., Servaes, K., Van Holderbeke, M., Geerts, L., De Henauw, S., Sioen, I., & Vanermen, G. (2012). Analysis of phthalates in food products and packaging materials sold on the Belgian market. *Food and Chemical Toxicology*, *50(7)*, 2575-2583.

Fuller, N. J., Lee, S. H., & Buglass, A. J. (2011). Residues in Alcoholic Beverages. In A. J. Buglass (Ed.), *Handbook of alcoholic beverages: technical, analytical and nutritional aspects* (pp. 1076-1092). Hoboken, NJ, U.S.A.: John Wiley & Sons.

Guo, Y., Zhang, Z., Liu, L., Li, Y., Ren, N., & Kannan, K. (2012). Occurrence and profiles of phthalates in foodstuffs from China and their implications for human exposure. *Journal of agricultural and food chemistry*, *60(27)*, 6913-6919.

Jara, S., Lysebo, C., Greibrokk, T., & Lundanes, E. (2000). Determination of phthalates in water samples using polystyrene solid-phase extraction and liquid chromatography quantification. *Analytica Chimica Acta*, *407(1)*, 165-171.

Kato, K., Shoda, S., Takahashi, M., Doi, N., Yoshimura, Y., & Nakazawa, H. (2003). Determination of three phthalate metabolites in human urine using on-line solid-phase extraction–liquid chromatography–tandem mass spectrometry. *Journal of Chromatography B*, *788(2)*, 407-411.

Kim, S., Lee, J., Park, J., Kim, H. J., Cho, G., Kim, G. H., Cho G., Kim G.H., Eun S.H., Lee J.J., Choi G., Suh E., Choi S., Kim S., Kim Y.D., Kim S.K., Kim S.Y., Kim S., Eom S., Moon H.B., Kim S., Choi, K. (2015). Concentrations of phthalate metabolites in breast milk in Korea: Estimating exposure to phthalates and potential risks among breast-fed infants. *Science of the Total Environment*, *508*, 13-19.

Latini, G., De Felice, C., Presta, G., Del Vecchio, A., Paris, I., Ruggieri, F., & Mazzeo, P. (2003). In utero exposure to di-(2-ethylhexyl) phthalate and duration of human pregnancy. *Environmental health perspectives*, *111(14)*, 1783-1785.

Leng, G., Chen, W., Zhang, M., Huang, F., & Cao, Q. (2014). Determination of phthalate esters in liquor samples by vortex□assisted surfactant□enhanced□emulsification liquid–liquid microextraction followed by GC–MS. *Journal of Separation Science*, *37(6)*, 684-690.

Lockett, G. A., Huoman, J., & Holloway, J. W. (2015). Does allergy begin in utero? *Pediatric Allergy and Immunology*, *26(5)*, 394-402.

Owens M. (Ed.) (2015). Phthalates: Health Effects, Detection and Exposure Prevention. Hauppauge, N.Y., U.S.A.: Nova Science Publishers.

Penalver, A., Pocurull, E., Borrull, F., & Marce, R. M. (1999). Trends in solid-phase microextraction for determining organic pollutants in environmental samples. *TrAC Trends in Analytical Chemistry*, *18(8)*, 557-568.

Polo, M., Llompart, M., Garcia-Jares, C., & Cela, R. (2005). Multivariate optimization of a solid-phase microextraction method for the analysis of phthalate esters in environmental waters. *Journal of Chromatography A*, *1072(1)*, 63-72.

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.

Psillakis, E., & Kalogerakis, N. (2003). Developments in liquid-phase microextraction. *TrAC Trends in Analytical Chemistry*, *22(9)*, 565-574.

Rasmussen, K. E., & Pedersen-Bjergaard, S. (2004). Developments in hollow fibre-based, liquid-phase microextraction. *TrAC Trends in Analytical Chemistry*, *23(1)*, 1-10.

Rezaee, M., Assadi, Y., Hosseini, M. R. M., Aghaee, E., Ahmadi, F., & Berijani, S. (2006). Determination of organic compounds in water using dispersive liquid–liquid microextraction. *Journal of Chromatography A*, *1116(1)*, 1-9.

Russo, M. V., Notardonato, I., Cinelli, G., & Avino, P. (2012). Evaluation of an analytical method for determining phthalate esters in wine samples by solid-phase extraction and gas chromatography coupled with ion-trap mass spectrometer detector. *Analytical and bioanalytical chemistry*, *402(3)*, 1373-1381.

Russo, M. V., Notardonato, I., Avino, P., & Cinelli, G. (2014). Fast determination of phthalate ester residues in soft drinks and light alcoholic beverages by ultrasound/vortex assisted dispersive liquid–liquid microextraction followed by gas chromatography-ion trap mass spectrometry. *RSC Advances*, *4(103)*, 59655-59663.

Russo, M. V., Avino, P., Perugini, L., & Notardonato, I. (2015). Extraction and GC-MS analysis of phthalate esters in food matrices: a review. *RSC Advances*, *5(46)*, 37023-37043.

Serrano, S. E., Braun, J., Trasande, L., Dills, R., & Sathyanarayana, S. (2014). Phthalates and diet: a review of the food monitoring and epidemiology data. *Environ Health*, *13(1)*, 43.

Staples, C. A. (Ed.) (2003). Phthalate esters. Springer Science & Business Media, part Q, vol. 3. Berlin, Germany: Springer.

Wenzl T. (2009). Methods for the determination of phthalates in food Outcome of a survey conducted among European food control laboratories. European Commission Joint Research Centre Institute for Reference Materials and Measurements, European Commission.

Wormuth, M., Scheringer, M., Vollenweider, M., & Hungerbühler, K. (2006). What are the sources of exposure to eight frequently used phthalic acid esters in Europeans? *Risk Analysis*, *26(3)*, 803-824.

Yan, H., Cheng, X., & Liu, B. (2011). Simultaneous determination of six phthalate esters in bottled milks using ultrasound-assisted dispersive liquid–liquid microextraction coupled with gas chromatography. *Journal of Chromatography B*, *879(25)*, 2507-2512.

Zhu, J., Phillips, S. P., Feng, Y. L., & Yang, X. (2006). Phthalate esters in human milk: concentration variations over a 6-month postpartum time. *Environmental science & technology*, *40(17)*, 5276-5281.

Figure captions

Fig. 1 Mean concentration of the three PAEs over the years. DBP = dibutyl phthalate; DEHP = bis(2-ethylhexyl) phthalate; DINP = diisononyl phthalate.

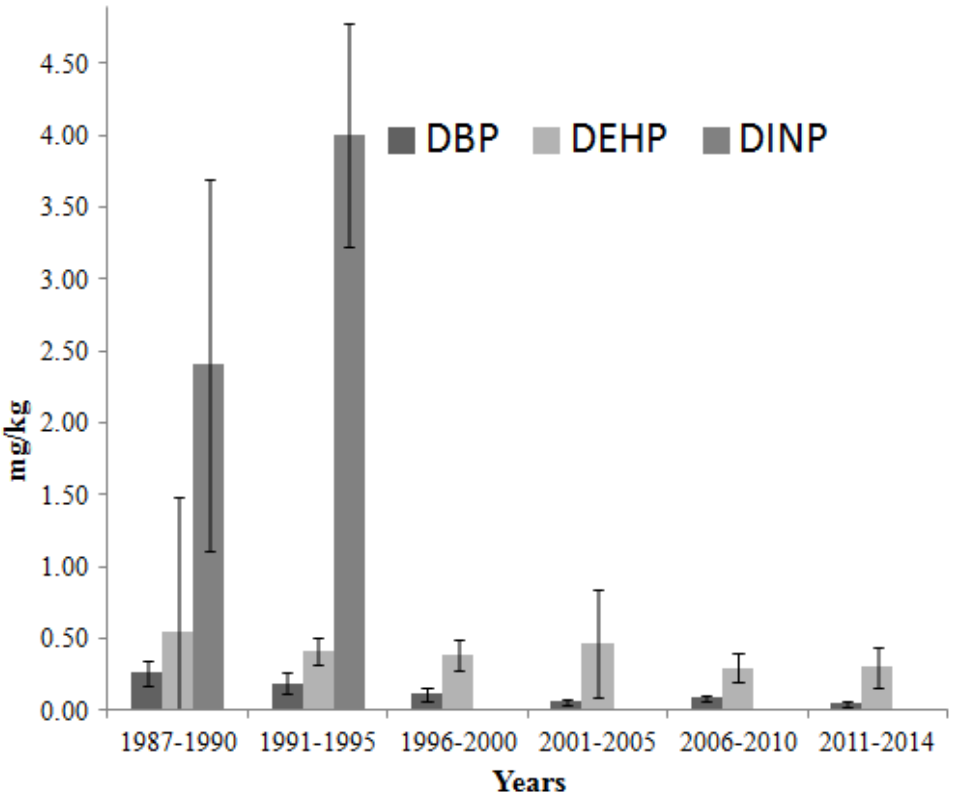

Fig. 1

Table 1

Amount of ethanol (% ABV), sodium chloride (g), and dichloromethane (µL) tested for optimization of PAEs micro-extraction on real samples.

Ethanol (% ABV)	11	16	26	40	
Sodium chloride (g)	0.25	0.50	1.00	1.50	2.00
Dichloromethane (µL)	40	80	120	160	200

Table 2

Recovery evaluation on a real sample properly diluted (11% and 16% ABV) spiked with DBP 50 µL (10 ppm), DEHP 120 µL (100 ppm), and DINP 30 µL (100 ppm). The extraction was carried out with 160 µL and 200 µL of dichloromethane.

RSD = relative standard deviation. DBP = dibutyl phthalate; DEHP = bis(2-ethylhexyl) phthalate; DINP = diisononyl phthalate.

	Recovery (% ± RSD)			
	CH_2Cl_2 160 µL		**CH_2Cl_2 200 µL**	
	11% ABV	**16% ABV**	**11% ABV**	**16% ABV**
DBP	78.67 ± 3.75	79.14 ± 3.89	75.26 ± 6.63	73.38 ± 5.25
DEHP	99.29 ± 4.97	100.84 ± 5.36	93.47 ± 5.84	91.33 ± 7.79
DINP	81.63 ± 5.21	82.06 ± 4.70	77.95 ± 6.11	80.92 ± 4.38

Table 3

Main parameters of method validation for standard compounds. Evaluation of linearity (R^2), limit of detection (LOD) and lower limit of quantification (LLOQ), and repeatability. RSD = relative standard deviation. DBP = dibutyl phthalate; DEHP = bis(2-ethylhexyl) phthalate; DINP = diisononyl phthalate.

	Range for calibration (mg/kg)	Straight line equations	Linearity (R^2)	LOD (mg/kg)	LLOQ (mg/kg)	Repeatability (RSD, %; n = 5)
DBP	0.01-0.45	y = 6.40x - 0.05	0.9997	0.003	0.011	3.82
DEHP	0.01-6.80	y = 2.13x - 0.04	0.9840	0.040	0.130	4.62
DINP	0.30-9.28	y = 0.76x - 0.65	0.9940	0.300	1.000	4.35

Table 4

Comparison between the proposed method and the other protocols reported in the literature for PAEs extraction and determination on alcoholic beverages. A = DBP; B = DEHP.

Method	Matrix	Coefficient of determination (R^2)	Repeatability (RSD)	Recovery (%)	Ref.
HS-SPME-GC-MS (CW-DVB fiber)	Wine	0.996-0.999[A] 0.980-0.982[B]	2-5[A] 8-24[B]	100-122[A] 72-122[B]	Carrillo et al. (2007)
HS-SPME-GC-MS (PDMS-DVB fiber)	Wine	0.990-0.998[A] 0.995-0.999[B]	4-6[A] 9-20[B]	77-130[A] 70-105[B]	Carrillo et al. (2007)
SPE-GC-MS	Wine	0.995[A] 0.997[B]	16[A] 16[B]	65-109[A] 69-90[B]	Del Carlo et al. (2008)
SPE-GC-MS	Wine	0.9993[A] 0.9995[B]	1.0[A] 0.9[B]	90-102[A] 28-78[B]	Russo et al. (2012)
USVADLLME-GC-MS	Alcoholic (10-40% ABV)	0.9822[A] 0.9938[B]	5.8[A] 7.9[B]	92.8-95.4[A] 85.0-91.5[B]	Cinelli et al. (2013)
USVADLLME-GC-MS	Alcoholic (<6% ABV) Soft drinks	0.9801[A] 0.9959[B]	3.6[A] 3.9[B]	96.7-98.7[A] 94.2-95.6[B]	Russo et al. (2014)
VSLLME-GC-MS	Chinese liquor	0.9948[A] 0.9938[B]	6.8-8.3[A] 7.5-10.4[B]	90.5-93.7[A] 87.6-90.2[B]	Leng et al. (2014)
USVADLLME-GC-MS	Brandy	0.9997[A] 0.9840[B]	3.8[A] 4.6[B]	78.7-79.1[A] 99.3-100.8[B]	This work

Table 5

Concentration of phthalates esters expressed as the mean values of two replicates in mg/kg (± standard deviation) in real samples.

n.d.: non detected. s.d.: standard deviation.

In bold, data exceeding the legal limit of the Chinese law: DBP (dibutyl phthalate) = 0.30 mg/kg; DEHP [bis(2-ethylhexyl) phthalate] = 1.50 mg/kg; DINP (diisononyl phthalate) = 9.00 mg/kg.

Date	DBP		DEHP		DINP	
	mg/kg	± s.d.	mg/kg	± s.d.	mg/kg	± s.d.
1987/11/23	**0.40**	0.01	0.41	0.05	3.01	0.32
1987/12/16	0.25	0.03	0.26	0.04	1.08	0.24
1988/01/04	**0.33**	0.09	0.26	0.01	1.42	0.09
1988/11/02	**0.35**	0.03	0.42	0.01	2.86	0.18
1988/11/30	0.22	0.03	0.21	0.01	2.13	0.28
1988/12/13	**0.32**	0.03	0.34	0.01	1.81	0.03
1989/01/05	**0.30**	0.06	**4.18**	0.69	< 1.00	
1989/01/05	**0.43**	0.03	0.24	0.03	1.93	0.21
1989/10/25a	**0.33**	0.04	0.39	0.03	3.01	0.15
1989/10/25b	0.26	0.01	0.35	0.03	2.89	0.04
1989/11/08	0.26	0.01	0.30	0.00	1.98	0.21
1989/11/22	0.17	0.03	0.31	0.05	3.15	0.79
1989/12/13a	0.20	0.03	0.19	0.01	2.03	0.31
1989/12/13b	0.18	0.00	0.22	0.02	1.69	0.06
1990/10/16	0.15	0.02	0.56	0.08	6.68	1.18
1990/10/25	0.15	0.02	0.24	0.01	2.49	0.01
1990/11/06	0.21	0.01	0.39	0.02	1.68	0.11
1991/10/08	0.29	0.00	0.37	0.07	4.70	0.73
1991/10/17	0.21	0.06	0.30	0.01	3.57	0.05
1992/10/15	0.14	0.00	0.52	0.02	4.61	0.14
1994/11/03	0.13	0.03	0.45	0.06	3.12	0.45
1997/10/27	0.10	0.01	0.38	0.08	n.d.	
1998/10/27	0.20	0.02	0.28	0.05	n.d.	
1998/11/18	0.14	0.01	0.47	0.01	n.d.	
1999/10/21	0.07	0.02	0.30	0.05	n.d.	
1999/11/02	0.05	0.01	0.26	0.01	n.d.	
2000/10/18	0.16	0.03	0.47	0.07	n.d.	
2000/10/26	0.08	0.01	0.56	0.04	n.d.	
2000/12/14	0.08	0.00	0.34	0.03	n.d.	
2001/10/17	0.10	0.02	0.66	0.07	n.d.	
2001/11/14a	0.07	0.00	0.32	0.01	n.d.	
2001/11/14b	0.07	0.02	0.42	0.05	n.d.	
2001/11/23	0.04	0.00	**1.62**	0.25	n.d.	
2002/10/17	0.07	0.02	0.28	0.00	n.d.	
2002/10/30	0.06	0.00	0.43	0.05	n.d.	
2003/10/14	0.03	0.00	0.22	0.03	n.d.	
2003/10/23	0.08	0.02	0.20	0.00	n.d.	
2003/12/12	0.03	0.00	0.66	0.01	n.d.	

2004/10/15	0.06	0.00	0.31	0.02	n.d.
2004/10/25a	0.07	0.01	0.34	0.06	n.d.
2004/10/25b	0.06	0.01	0.36	0.01	n.d.
2005/10/13	0.06	0.01	0.30	0.05	n.d.
2006/10/16	0.09	0.00	0.13	0.02	n.d.
2007/09/25	0.13	0.03	0.22	0.03	n.d.
2008/09/29	0.10	0.00	0.25	0.02	n.d.
2008/10/03	0.09	0.04	0.30	0.03	n.d.
2008/10/07	0.10	0.04	0.53	0.19	n.d.
2008/10/24	0.08	0.02	0.21	0.00	n.d.
2009/09/30	0.09	0.01	0.26	0.05	n.d.
2009/10/06	0.10	0.01	0.47	0.01	n.d.
2009/10/15	0.07	0.01	0.35	0.02	n.d.
2009/10/19	0.07	0.00	0.27	0.03	n.d.
2010/09/29	0.06	0.01	0.35	0.00	n.d.
2010/10/11	0.08	0.01	0.29	0.00	n.d.
2010/10/15	0.10	0.01	0.34	0.02	n.d.
2010/11/11	0.06	0.00	0.31	0.02	n.d.
2010/12/14	0.06	0.02	0.19	0.01	n.d.
2011/09/22	0.05	0.02	0.25	0.03	n.d.
2011/09/28	0.05	0.00	0.28	0.04	n.d.
2011/10/03	0.04	0.00	0.29	0.02	n.d.
2011/10/07	0.05	0.00	0.29	0.00	n.d.
2012/10/12	0.04	0.01	0.27	0.02	n.d.
2013/10/10	0.04	0.00	0.14	0.02	n.d.
2013/11/14	0.10	0.00	0.64	0.05	n.d.
2014/09/30	0.03	0.00	0.30	0.01	n.d.
2014/10/22	0.03	0.00	0.24	0.04	n.d.

Determination of phthalate esters in distillates by ultrasound-vortex-assisted dispersive liquid-liquid micro-extraction (USVADLLME) coupled with gas chromatography/mass spectrometry

Giuseppe Montevecchi, Francesca Masino, Luca Zanasi, Andrea Antonelli

Highlights: > Phthalate esters (PAEs) are toxic ubiquitous process contaminants > China imposed concentration limits on PAEs for imported distillates > A liquid-liquid micro-extraction method for determination in distillates was set up > A complete historical series (27 years) of brandies for the first time was analysed > In the longest aged samples, concentrations exceeded the limits of the Chinese law.