THE STUDY OF PHTHALATES MIGRATION IN WINE PRODUCTS BY GC-MS METHOD¶

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Abstract. A number of studies have shown phthalates' potential impact on human health due to their carcinogenic and endocrine-disrupting effects. More than 2000 analyses for determination of phthalates' rests in alcoholic beverages were done in the laboratory of National Center for Quality Testing of the Alcoholic Beverages (Republic of Moldova) using modern method of analysis like GC-MS.

Keywords: phthalates, wine, gas-chromatography, mass-spectrometry, migration.

1. Introduction

Today, in modern, industrialized society people can hardly imagine life without home appliances, communication systems, a convenient plastic packaging, fragrance and cosmetics. Most of these and many other chemical products have their properties such as strength, ductility, durability, incombustibility, etc., owing to a number of synthetic organic chemicals. Phthalates are among the members of this series. Phthalates (esters of phthalic acid) are included in the compositions of almost all types of plastics, rubber, paints and varnishes, giving them elasticity and strength [1]. Almost 90% of produced phthalates are used as plasticizers. Phthalates act as solvents and flavor fixatives especially in perfumes and cosmetics.

Humans always are surrounded by materials containing phthalates, such as linoleum, insulation of wires, pipes, plastic housings of domestic appliances, toys, varnishes and paints.

It is supposed that phthalates accumulate in the human body, which negatively affects its hormones, liver and kidneys may also become the causes of allergies, asthma and cancer, neurodevelopmental disorders and abnormalities in the development of children. Molecules of phthalates are not structural elements of the polymer chains and therefore easily stand out in the environment, getting into the human body through food, skin or by inhalation.

In a number of investigated wine-products released by vendors the presence of phthalates was detected. Particular attention was given to the dibutylphthalate.

2. Experimental

2.1. Material and methods

Measuring the concentration of phthalates in wine and base-wine relied on its elimination by chloroform extraction, chromatographic separation on a capillary column, identify the retention time and mass spectrum, and quantify with the characteristic ions m/z . Measuring the concentration of phthalates in alcoholic beverages such as vodka, brandy, cognac alcohol, rectified ethyl alcohol was based on chromatographic separation of the sample on a capillary column using Aldrin with a purity above 99.3% and supplied by SUPELCO as an internal standard, the identification was made by retention times and mass spectrum, quantification of characteristic ions m/z for phthalates and for Aldrin. Tab.1.

Table 1

Measured m/z ratios characteristic for phthalates and IS

Compound	Abbreviation	m/z
Dimethylphthalate	DMP	163, 164, 194
Diethylphthalate	DEP	149, 177
Dibutylphthalate	DBP	149
Bis(2-ethylhexyl)phthalate	DEHP	149, 167, 279
Dioctylphthalate	DOP	149, 279
Didecylphthalate	DDP	149, 307
Aldrin (IS)		250, 261, 263, 265, 291, 293, 298

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The background solution (synthetic wine) was used to prepare the calibration solutions. It consisted of aqueous solution of 15% ethanol and tartaric acid (6g/dm^3) (tartaric acid, supplied by Fluka, puriss. p.a. for ion chromatography) and carried to the pH to 3.5 with 5M sodium hydroxide. Synthetic wine was used for calibration standard solutions with concentrations of phthalates: 0 - 1,00 mg/dm^3 (DMP - 99.6%, DEP - 99.6%, DBP - 99.8%, DEHP - 99.7%, DNOP - 99.7%, DNDP - 99.8% Pestanal from Sigma-Aldrich). For the extraction of phthalates, 100 ml of sample (calibration solution) was placed in a separatoring funnel of 250 cm^3 with addition of 10 cm^3 of chloroform (Chloroform, LGC Promochem, for HPLC). Extraction was implemented in 10 min with continuous shaking. After separating the organic layer the bottom layer of chloroform was drained through a paper filter with anhydrous sodium sulfate (sodium sulfate anhydrous, Stanchem, Spain). Collected 10 ml of the chloroform extract was transferred into a gas chromatography vial, from which was selected 1,0 µl of extract by microsyringe directly for analysis using gas chromatography with mass-spectrometer.

2.2. Instruments

SHIMADZU GCMS-QP-2010S (IS) with a COMBI PAL autosampler (CTC ANALYTICS, Zwingen, Switzerland) equipped with fused silica column RESTEK-Rtx-5MS (30m/0.25mm/0.25µm 5% diphenyl / 95% dimethylpolisiloxane phase) was used to perform injections and gas chromatographic analyses in an automated way.

2.3. Gas chromatography–mass-spectrometry

The oven program started at an initial temperature of 160°C for 1 min. Temperature was then increased at a rate of 10°C/min to 200°C, maintained for 1 min, then increased at a rate of 20°C/min to 320°C and maintained for 10 min. The carrier gas was helium at 1.0 ml/min (99.9990%), split 5. Ionisation was performed by electron impact (EI). The temperatures used were 320°C for the injector, 320°C for the transfer line, and 200°C for the ion source. The compounds were quantified in selected ion monitoring (SIM) mode[3]. The analyte to internal standard peak area ratio was used as analytical signal for constructing the calibration graphs.

Duration of gas chromatography-mass spectrometric analysis for phthalates constituted 30 minutes.

For the analysis of strong alcoholic beverages calibration solutions of phthalates were prepared on the basis of 50% water-alcohol mixture with the addition of a solution of aldrin as internal standard (IS).

3. Results and discussion

The studies conducted in the laboratory of National Center for Quality Testing of the Alcoholic Beverages (Republic of Moldova) included more than 2000 samples of the bottled wine, base-wine and strong alcoholic beverages for the presence of phthalates.

For determination of optimal conditions of extraction there were done a number of studies described below.

There was made comparative analysis (investigation) of extraction grade obtained with different organic solvents, such as chloroform, diethyl ether, hexane, carbon tetrachloride, benzene and isoamyl alcohol. Conditions: ratio wine/ organic solvent = 100mL/10mL; extraction time = 10min. The results are shown in fig.1. and fig. 4. (average between four extractions).

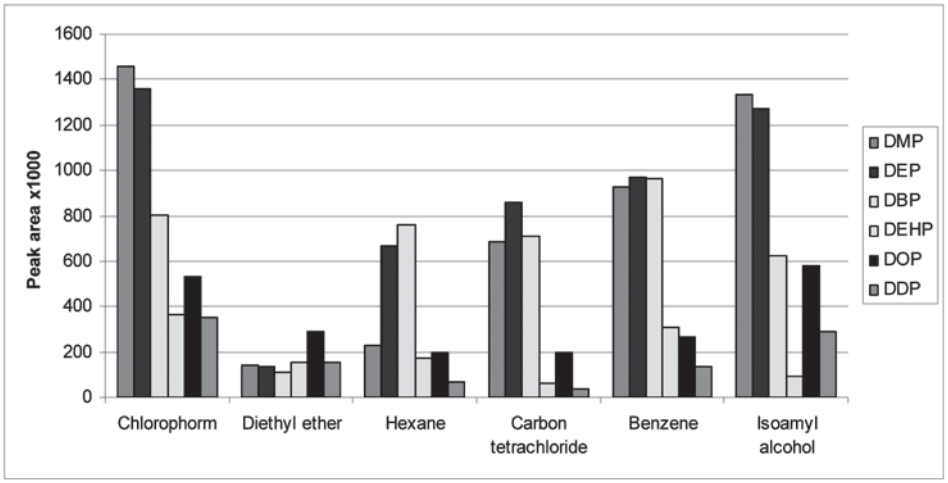

Fig. 1. Signals intensity of phtalates in various extractants

In fig.1 there are shown signal intensities which correspond to extraction of phthalates from the same model contaminated solution by various solvents. RSD% (relative standard deviation) was calculated from the results of four parallel extractions, what is shown in fig. 2 with prices for 1L of solvent grade *p.a.* or similar in Euro.

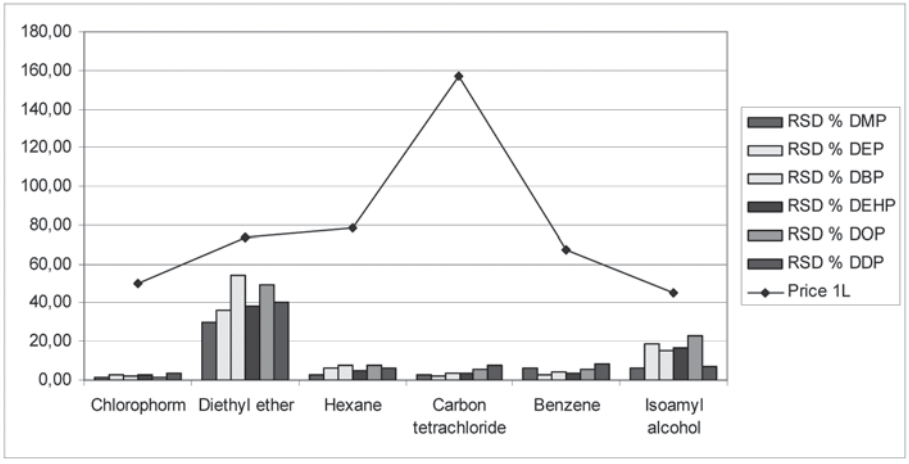

Fig. 2. Relative standard deviation of four extractions. Prices of organic solvents for 1L/€*

** - Prices of organic solvents according to the catalogue (SIGMA-ALDRICH 2011) for 1L grade p.a. or similar*

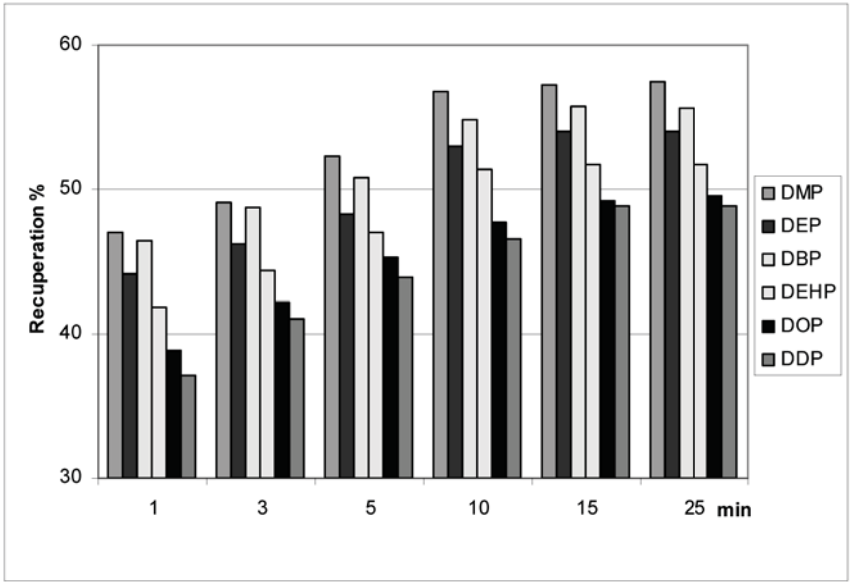

Fig. 3. Determination of optimum phthalates extraction time from wine with chloroform

For the purpose of improvement of analytical characteristics the comparative analysis of two technics of injection has been carried out: method of direct injection of liquid samples into the capillary column and the solid phase microextraction (SPME) – method was recommended by Carrillo J. D. at al. [2]. The results are shown in tab.1.

Table 1

Comparative analysis of injection methods - direct injection and SPME

Methods	Liquid injection	SPME
Sample preparation	Extraction: chloroform/ wine sample 10ml/100ml	Solid Phase Micro Extraction with CW-DVB fiber.
RSD %	2,18±0.51	3,11±0.72
The cost of presampling of one sample	~1.65€*	~9.55€*

*- According to the catalogue prices (SIGMA-ALDRICH)

In order to optimize the extraction process of phtalates during presampling were investigated some dependencies:

a) Effect of pH on the level of recovery was established. Samples of synthetic wine with different values of pH (3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0) were contaminated by DBP. The level of recuperation for a solution with pH = 7.0 was taken as 100%. The results are expressed in fig. 4.

b) Similarly, the influence of sugar content on the extractability of DBP was investigated with Synthetic wine (2.1). Sugar concentration in the samples was formed using concentrated must (C (DBP) <0.01mg/dm^3). DBP was added to the obtained model solutions with concentrations of sugars: 0, 30, 50, 100 and 150 g/dm^3. Chloroform extracts of these samples were analyzed. The results are expressed in fig. 5.

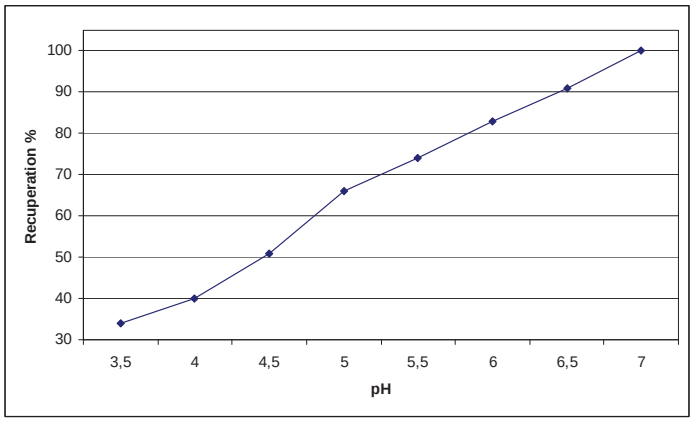

Fig. 4. Dependence: Recuperation level % =f(pH)

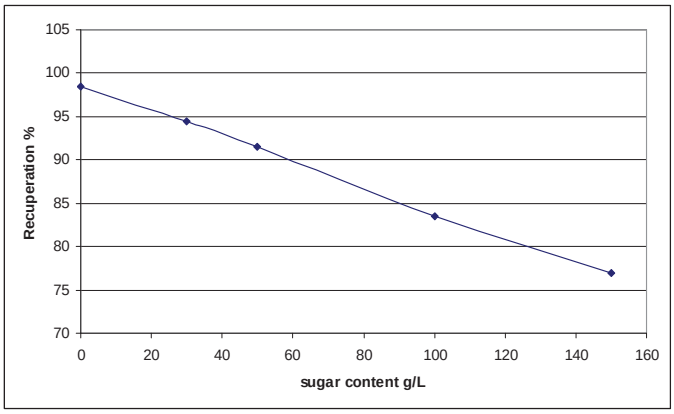

Fig. 5. Dependence: Recuperation level % =f(sugar content g/L)

c) Effect of alcohol content on the extractability of DBP was also investigated using synthetic wine (2.1). Alcohol content in the samples was formed by ethyl alcohol (C (DBP) <0.01mg/dm^3). DBP was added to the obtained model solutions with concentrations of alcohol: 6, 9, 12, 15, 18 and 21% v/v. Chloroform extracts of these samples were tested. As it follows from the results of investigation alcohol content doesn't influence significantly on the level of recovery. (Fig. 6).

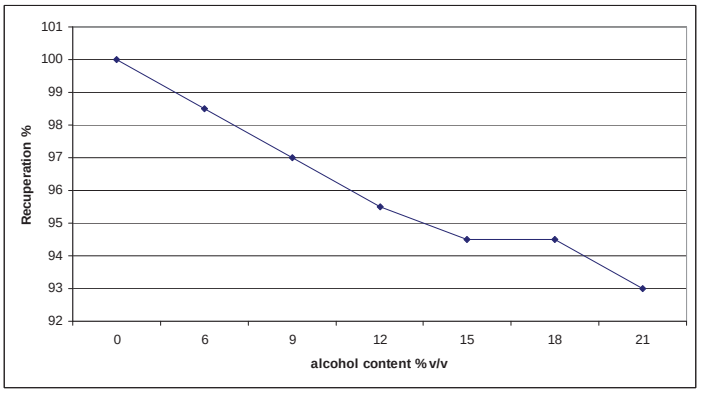

Fig. 6. Effect of alcohol content on the recuperation level of DBP

To establish the sources of DBP pollution in wines there were studied samples of sulfitated and concentrated must: <0.01-0.15ppm of DBP was detected. The lowest concentration level of DBP was characteristic for sulfitated

must, then concentrated - 0.05-0.15ppm. The results of investigations of 15 grapes samples were negative or represent traces. In addition, at the five wineries was investigated water used in wine production. It was found that concentration of DBP in natural water is lower than LOQ, while content in flushing water is 0.04-0.05ppm, and 0.09-0.15 ppm of DBP in softened water.

Therefore contamination with phthalates has mainly a technogenic character, and it is the result of contact with polymeric materials. In the sequel, we studied samples of different materials, which were in contact with wine production during the winemaking process and storage, such as paints, varnishes, primers, pipes, rubber seals. All these tests were conducted according the Directive 2007/19/EG. Also was investigated migration of DBP to a model solution - 15% aqueous ethanol solution, acidified with tartaric acid. Migration of phthalates from materials, which are in contact with wine, is a continuous process that can continue throughout the period of production or storage. The rate of migration was determined basing on these investigations. Studies have been conducted on materials submitted by Moldovan winemakers and distributors. In addition to fresh paint (intended for contact with food) were analyzed paints, which were in contact with wine during a certain period of time. Fresh (liquid) paint was applied to the flask's inner surface, dried on air in 2-3 days, and then a model solution was placed into the flask. Content of DBP was determined in the model solution, which was in contact with the dry polymer within 1 day. Ratio of polymer and model was 1:100. Migration took place at the room temperature (20-22°C).

36 different samples of cork stoppers (for wine and brandy), 6 samples of polymer stoppers for sealing wines and more than 20 samples of caps, seals, dispensers bottle and other polymer elements, which can contact with bottled alcoholic beverages have been studied as a potential source of contamination. All the samples were crushed to accelerate the potential migration of phthalates in the model solution, in which further was determined the content of phthalates. In some cases, migration of phthalates was determined from the surface of the products. As the model solutions were used water-alcohol solution and acidic water-alcohol mixtures simulating wine. As a result, it should be noted that DBP was detected in trace amounts only in four of the investigated samples. In these cases, the observed DBP was on the surface of cork, what is probably due to a violation of the conditions of capping material storage. Quantities of phthalates sufficient for essential change of them in beverages were found in no one of the studied samples capping materials.

4. Conclusions

In the context of studies conducted in the laboratory of National Center for Quality Testing of the Alcoholic Beverages (Republic of Moldova) were included more than 2000 samples of the bottled wine, base-wine and some types of strong alcoholic beverages for the presence of most widespread and toxic phthalate – dibutylphthalate. Results display presences DBP in 85% of studied samples of wines, i.e. a content of DBP more than LOQ (0.01mg/dm^3). Samples of sulfitated and concentrated must, natural and softened water and grapes samples were studied to establish the sources of DBP pollution in wines. Has been determined that contamination of phthalates has a technogenic character, and it is the result of contact with polymeric materials. Optimum conditions of extraction DBP from liquid samples were obtained. Also has been established, that significant influence on extractability is performed by pH value and sugars content value, the alcohol contents in synthetic wine has not displayed significant effect. In addition migration DBP from polymeric materials has been learnt. In the nearest future we plan to research plugs and other materials used in winemaking process on presence of DBP and its migration.

Acknowledgments

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