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CALIBRATION OF GC/MS METHOD FOR DETERMINATION OF PHTHALATES

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Abstract. The calibration of method for determination of phthalates by means of GC/MS was done. The obtained working range for different phthalates is as following: DBP 58.8 – 1175,3 ppb; BBP 75,2 – 1504,6 ppb; DEHP 72,7 – 1454,2 ppb; DOP 59,1 – 1182,2 ppb; DINP 484,5 – 9690,3 ppb; DIDP 573,9 – 11477,0 ppb. Dicyclohexylphtalate (DICHP) was selected as an internal standard. Hexane is used as a solvent for preparation of standard solutions of phthalates.

Keywords: phthalates; GC/MS; LOD; LOQ; calibration

Introduction

Phthalates are dialkyl- or diaryl- esters of 1,2-benzene dicarboxylic acids, also known in a practice as o-phthalic acid. The general method for their synthesis is reaction between phthalic anhydride and alcohol like methanol, ethanol until tridecyl alcohol. They are colorless liquids without taste and any smell. They also are water insoluble, but very well soluble in fats. The main application of phthalates is in polymers production, where they play role of plasticizers (Lao & Wong, 2000). Phthalates could be introduced in human organism by consuming different foods stored in polymeric packages as well as using of cosmetics, toys, building materials, etc (Pivnenko et al., 2016). They affect the reproductive system, so they leads to male and female sterility, polycystic ovaries, congenital defects of the fetus in pregnant women (Gao et al., 2017). Phthalates are one of the causes of asthma in children. This class of compounds affects the endocrine system and is the cause of obesity. Because of their toxicity, phthalates are the subject of demand and determination in polymeric goods, including polymer packaging for food products, which are now widely used in practice (Guo et al., 2010). Herein, we report the calibration as a part of introduction in laboratory practice of a GC/MS standardized method for determining the content of phthalates according to the international standards and in accordance with the conditions in the selected laboratory.[1,2)]

Experimental

Materials, methods and apparatus

Dibutyl phthalates (DBP), Benzyl butyl phthalates (BBP), Bis-(2-ethylhexyl) phthalate (DEHP), Di n-octyl phthalates (DOP), Di iso-nonyl phthalate (DINP), Di iso-decyl phthalate (DIDP), Dicyclohexyl phthalate (DICHP) and n-hexane with analytical grade were purchased by Sigma Aldrich, Germany.

Centrifuge tubes of 15 mL – ISO LAB, Glass tubes of 10mL – Valerus Ltd, Bulgaria, Syringe filters PTFE 0.45 μm, Vortex – Fisher Scientific, Ultrasonic bath – Apronecs Ltd, Bulgaria, Gas chromatograph Shimadzu QP 2010 Ultra.

Procedure for GS/MS determination

Before performing the calibration, the gas chromatograph shall be adjusted according to the manufacturer's instructions and the operating conditions specified in the measurement method.

First, a blank sample of pure solvent is injected to verify the system for the way it works and for the presence of contamination. A solution containing the individual components in the SCAN mode of the mass spectrometer is then injected and the chromatogram further used for their identification is recorded. The identification is performed by two parameters – retention time t_R (min) and the resulting mass spectrum, which is compared to a reference, located in a library of the apparatus.

From the obtained data, a SIM (select ion monitoring) method, which scans certain ions characteristic of the respective component is performed. This mode is used because of the higher sensitivity of the detector compared to the SCAN mode due to the scanning of a smaller number of ions, which reduces side effects and other types of interferences.

Chromatographic conditions

Column SLB – 5MS 29.5 m/ 0.25 mm/ 0.25 μm, Carrier gas – helium, Column oven temperature – 150.0° C, Injection temperature – 200.0 ° C, Total flow – 40.0 ml/ min, Column flow – 2 ml/ min, Injection mode – Split with split ratio – (-1.0), Ion source temperature – 200.0° C

Oven program is described in Table 1. GC/MS works in SIM mode according to the regime presented in Table 2.

Table 1. Oven program

Rate	Final temperature	Hold time
-	150.0	1.00
5.00	250.0	0.00
3.00	290.0	7.00

Table 2. Working mode of GC/MS

Start time (min)	End time (min)	m/ z
8.00	11.00	149, 223, 205
16.00	18.00	149, 91, 206
19.00	20.70	149, 249, 279, 167
20.71	31.00	149, 293, 307, 261, 279, 167, 141

Initial and working standards

The initial DICHP concentration of 10.9 mg to 20 mL in n-hexane was prepared and further it was diluted 100-fold.

The standards used are in ppb concentrations presented in Table 3.

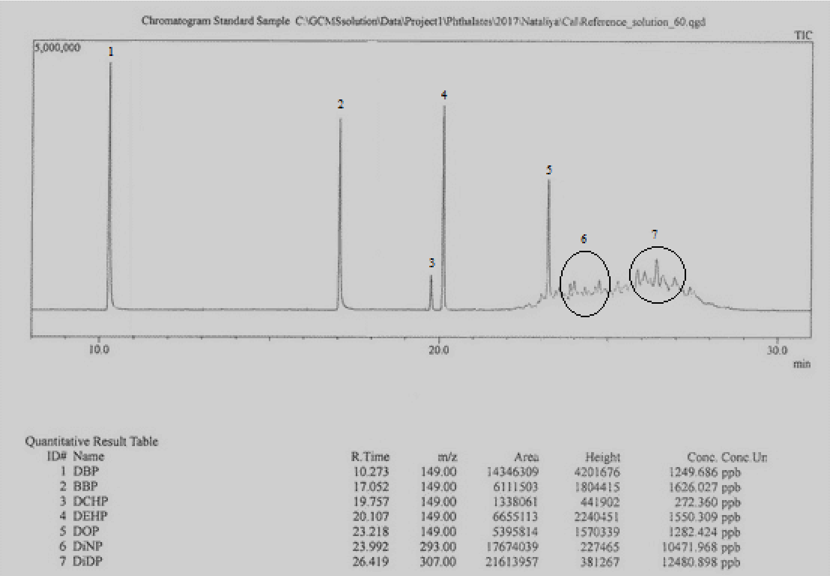
Table 3. Concentration in ppb of used standard solutions of determined phthalates

DBP	BBP	DEHP	DOP	DINP	DIDP
58,7640	75,2290	72,7080	59,1085	484,5150	573,8500
117,5280	150,4580	145,4160	118,2170	969,0300	1147,7000
293,8200	376,1450	363,5400	295,5425	2422,5750	2869,2500
587,6400	752,2900	727,0800	591,0850	4845,1500	5738,5000
881,4600	1128,4350	1090,6200	886,6272	7267,7250	8607,7500
1175,2800	1504,5800	1454,1600	1182,1700	9690,3000	11477,0000

1 mL of all prepared standards are mixed with 50 µL internal standard of DICHP and they are further injected to the GS/MS apparatus.

Results and discussion

The characteristic ions for monitored phthalates were determined at the beginning of analysis using t_R of chromatogram as well as their characteristic ions (Fig. 1). They all are presented in Table 4.



Chromatogram Standard Sample C:\GCMSsolution\Data\Project1\Phthalates\2017\Nataliya\Cal\Reference_solution_60.qgd

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Quantitative Result Table
  ID# Name                          R.Time     m/z       Area     Height        Conc. Conc.Un
    1 DBP                           10.273   149.00   14346309   4201676     1249.686 ppb
    2 BBP                           17.052   149.00    6111503   1804415     1626.027 ppb
    3 DCHP                          19.757   149.00    1338061    441902      272.360 ppb
    4 DEHP                          20.107   149.00    6655113   2240451     1550.309 ppb
    5 DOP                           23.218   149.00    5395814   1570339     1282.424 ppb
    6 DiNP                          23.992   293.00   17674039    227465    10471.968 ppb
    7 DiDP                          26.419   307.00   21613957    381267    12480.898 ppb
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Figure 1. Chromatogram of target phthalates. Compounds 6 and 7 are mixture of isomers. They are manually integrated and the zone of their presents is determined by means of characteristic peaks of all isomers

Table 4. t_R and m/z ration of characteristic target ions for monitored phthalates

Name	t_R, min	characteristic ion, m/z	Q1, m/z	Q_2, m/z
DBP	10.26	149	205	233
BBP	17.04	149	91	206
DEHP	19.73	149	167	249
DEHP	20.08	149	167	279
DOP	23.19	149	261	279
DINP	23.96	293	149	167
DIDP	26.39	307	149	141

Each of the prepared calibration solutions is injected 10 times in the SIM mode of the mass spectrometer. A blank sample of pure solvent is injected between different concentration levels in order to confirm the absence of system contamination. Each of the components is identified by both parameters retention times t_R and m/z/ of characteristic ions (Table 2). The obtained data are used for standard curve drawing. It shows the relations of the average of the ratio between areas of each phthalate and the internal standard as a function of the respective concentrations of phtalates. As a results of obtained data, standard curves, presented on Figs. 2 – 7, are drawn.

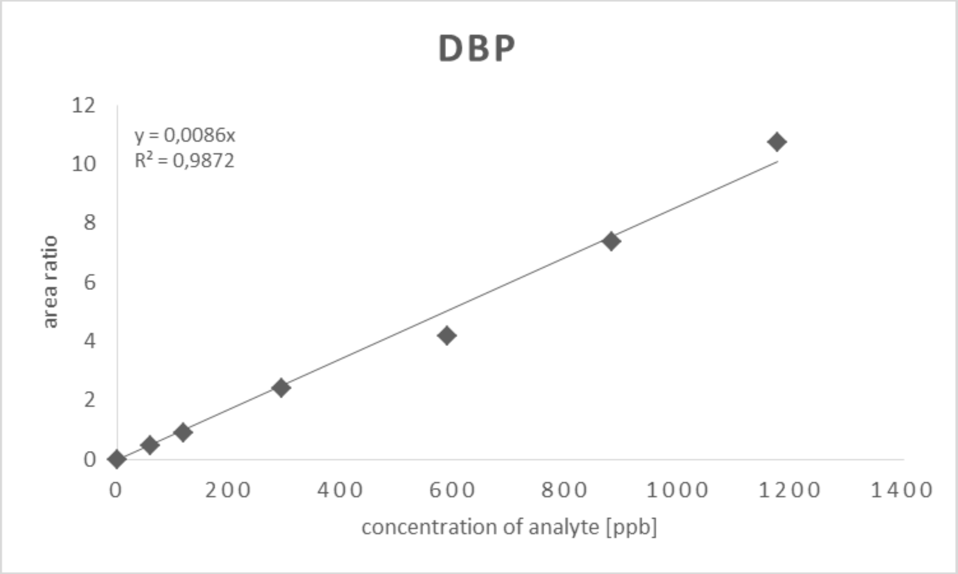

Figure 2. Standard curve for dibutyl phthalates

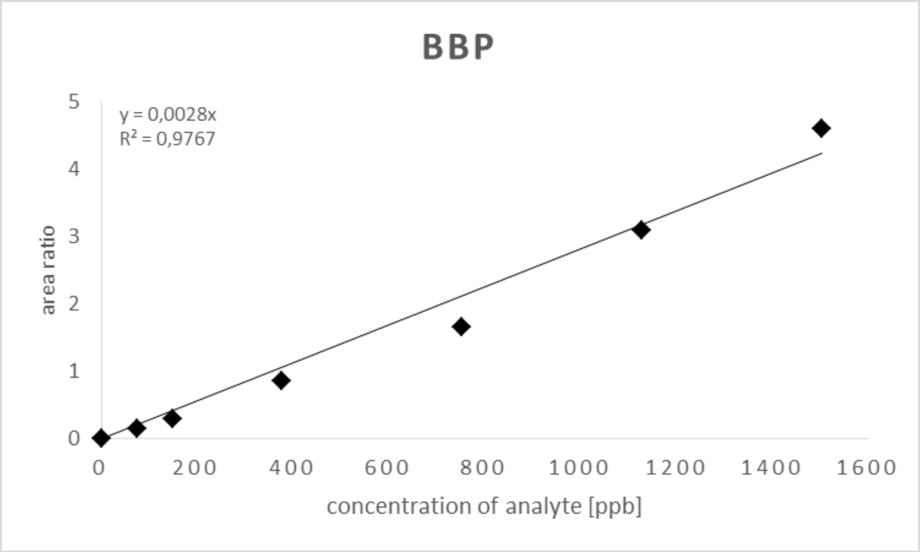

Figure 3. Standard curve for benzyl butyl phthalates

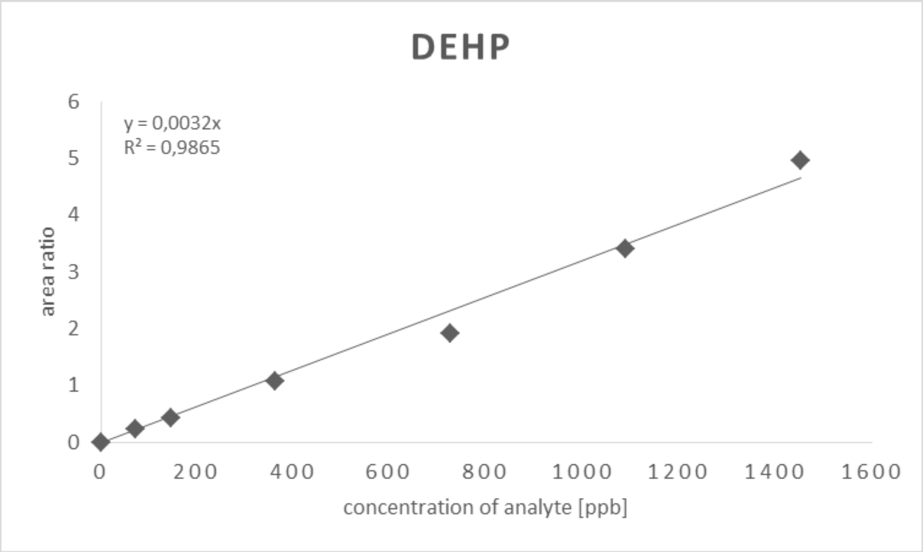

Figure 4. Standard curve for bis-(2-ethylhexyl) phthalate

All obtained data show liner response of detector for the chosen concentration interval of phthalates. A measure of linearity is the correlation coefficient “R”. As its square is closer to one, the higher the degree of linearity. The correlation coefficients for aim phthalates are summarized in Table 5.

Table 5. Correlation coefficients R^2 of target phthalates

name of aim phthalate	R^2	LOQ [ppb]	S [ppb]	LOD [ppb]
DBP	0,9872	58,7640	5,8764	17,6292
BBP	0,9767	75,2290	7,5229	22,5687
DEHP	0,9865	72,7080	7,2708	21,8124
DOP	0,9797	59,1085	5,9109	17,7327
DiNP	0,9791	484,5150	48,4515	145,3545
DiDP	0,9781	573,8500	57,385	172,1550

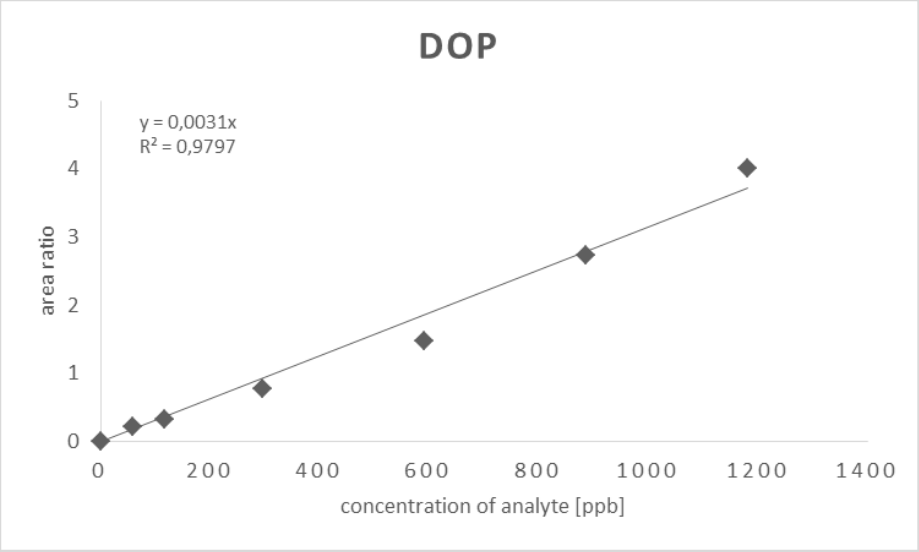

Figure 5. Standard curve for di n-octyl phthalates

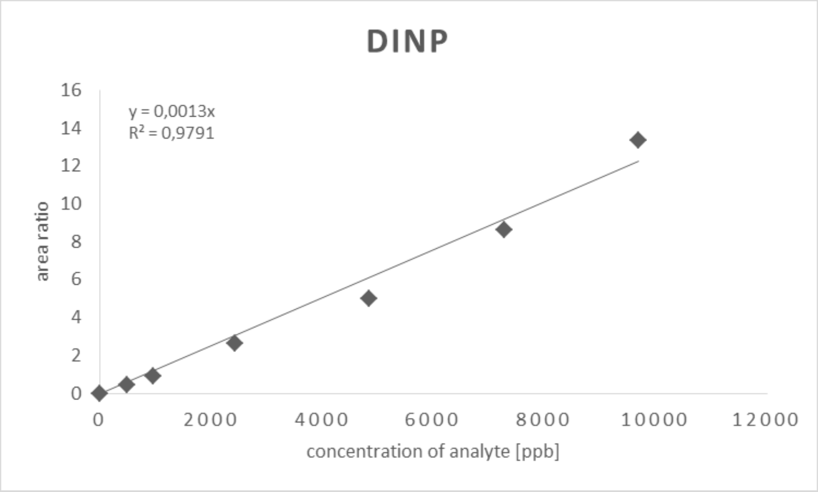

Figure 6. Standard curve for di iso-nonyl phthalate

Limit of quantification (LOQ)

By definition the limit of quantification (LOQ) is the smallest amount of the determinable component, which can be calculated with a certain probability [7]. If a technical instrument with a defined calibration band is used, the LOQ coincides with the first point in that range. For the specific case the values are presented in Table 3.

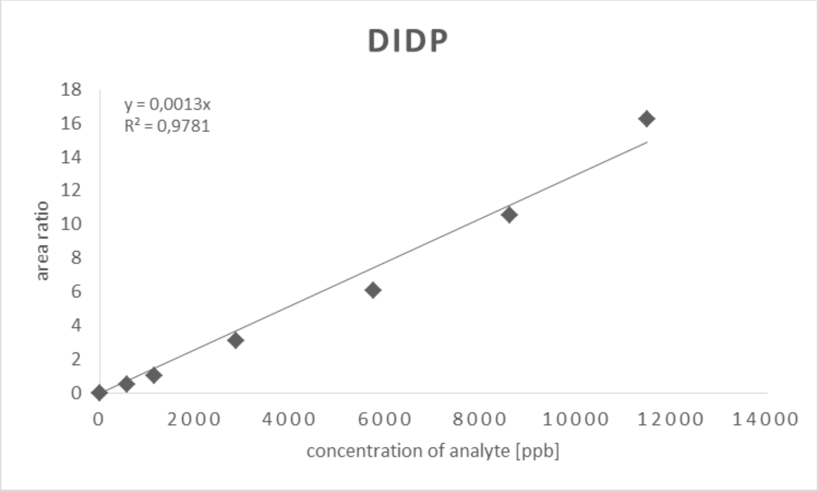

Figure 7. Standard curve for di iso-decyl phthalate

Moreover, LOQ can be calculated by means of Eq. (1):

$$LOQ = 10S_0 \tag{1}$$

where, S_0 is a standard deviation SD obtained after a test blank sample with a dimension equal to that of the measured quantity. By means of mathematical equation presented above the S_0 values can be easily calculated and for the target compounds they are summarized in Table 3.

Limit of detection (LOD)

The detection limit (LOD) represents the smallest amount of the detectable component that can be detected with a certain probability.[3] It can be calculated by means of Eq. (2).

$$LOD = 3S_0 \tag{2}$$

The calculated values for LOD of target phthalates is presented in Table 3.

Conclusion

The calibration of GC/MS system Shimadzu QP 2010 Ultra was successfully done for five phthalates - Dibutyl phthalates (DBP), Benzyl butyl phthalates (BBP), Bis-(2-ethylhexyl) phthalate (DEHP), Di n-octyl phthalates (DOP), Di iso-nonyl phthalate (DINP), Di iso-decyl phthalate (DIDP) using Dicyclohexyl phthalate (DICHP) as an internal standard. The linear range for further work, LOQ and LOD values were also determined succesfully.

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NOTES

1. http://www.oiv.int/public/medias/2693/oiv-ma-bs-33-en.pdf
2. http://www.oiv.int/public/medias/2589/oiv-ma-as323-10.pdf
3. http://www.bds-bg.org/bg/bg/standard/?natstandard_document_id=46634

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