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# RESULTS OF COLLABORATIVE INTERLABORATORY STUDY TO ESTIMATE THE PERFORMANCE CHARACTERISTICS OF THE GAS CHROMATOGRAPHIC METHOD FOR DETERMINATION OF DI(2-ETHYLHEXYL)PHTHALATE IN WATER

J. PETRE[a]*, M. MITRITA[a], I. CRUCERU[b], L. CRUCERU[a], V. IANCU[a]

[a]*National Research and Development Institute of Industrial Ecology, 90-92 Panduri Road, Bucharest, Romania*
*E-mail*: *ecoind@incdecoind.ro*
[b]*Faculty of Chemistry, University of Bucharest, Bucharest, Romania*

**Abstract**. Di(2-ethylhexyl)phthalate (DEHP) is widely spread in the environment arising from anthropogenic sources rather than from natural ones. DEHP is a highly lipophilic, moderately persistent and presents a high degree of accumulation in a variety of aquatic intervertebrates, fish and amphibians. Due to its carcinogenic character DEHP was included in the list of priority dangerous substances (Directive No 76/464/CEE transposed into HG 351/2005). In order to ensure the ecological security by controlling the concentrations of this pollutant in water it was developed a gas chromatographic method after extraction in hexane at pH = 3. The estimation of the performance characteristics of the method was performed by a collaborative interlaboratory study. Each participant laboratory has analysed a set of five water samples with di(2-ethylhexyl)phthalate in different levels of concentration. The results obtained after the statistic processing of the experimental data showed that the standard deviations of repeatability and reproducibility are lying in the normal domains for a chromatographic method. Consequently, the proposed method can be used by any environmental laboratory which performs the control of the level of pollution with this priority dangerous substance.

*Keywords*: collaborative study, di(2-ethylhexyl)phthalate, analysis, gas-chromatography, water.

## AIMS AND BACKGROUND

The continuous control of the pollution level from different categories of water and the comparison of the obtained concentration values with the maximum accepted limits involve the use of analytical methods with a high level of repeatability and reproducibility.

In accordance with the European Directives concerning environmental pollution, the discharges of dangerous priority substances were normed also in our country according to HG 351/2005 (Ref. 1). For di(2-ethylhexyl)phthalate the maximum accepted concentration in natural water sources was established at 0.33 μg /l.

* For correspondence.

The method developed in the laboratory for the analysis of di(2-ethylhexyl)phthalate in water consists in the extraction of the sample with hexane at pH 3.5, followed by gas-chromatographic analyses of the organic extract. The chromatographic separation of the compound is performed on a low polar capillary column and the detection is done with a flame ionisation detector (FID).

In order to test the analytical performances of this new method developed, was initiated an interlaboratory collaborative scheme. In this way it was possible to observe whether the method has deficiencies and also to make proposals for modification/improvement of the analytical technique.

## EXPERIMENTAL

The testing scheme applied in this study ( Fig. 1) is in accordance with the requirements of the Guide ISO/IEC 43/1 (Ref. 2) and of the standard ISO 5725:1994, parts 2 and 6 (Ref. 3).

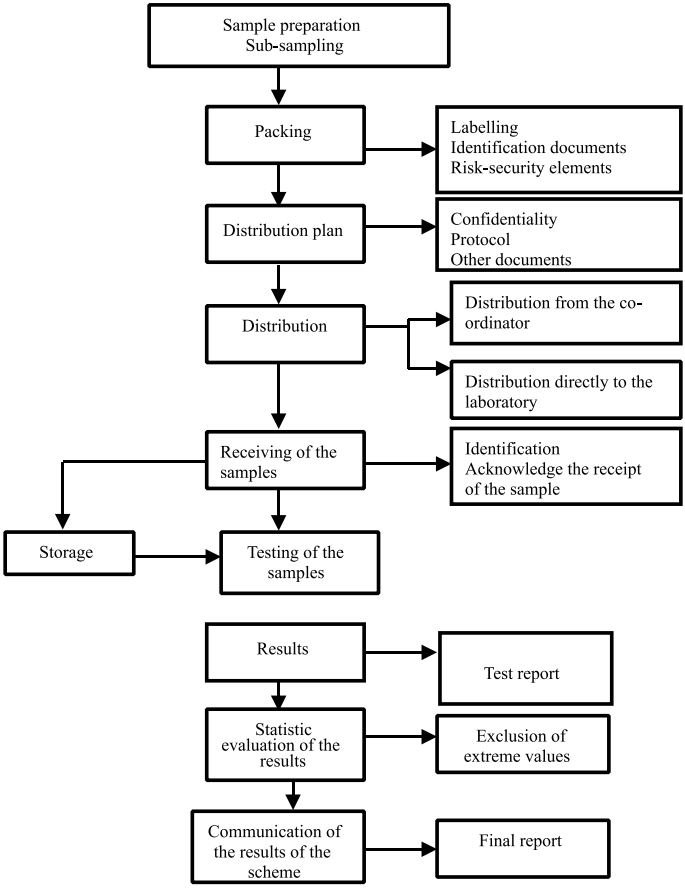


**Fig. 1**. Activities during the interlaboratory study

The samples to be tested were divided into sub-samples and sent by the co-ordinator of the scheme simultaneously to all the laboratories participant in the scheme. The samples were put in glass containers tightly closed and kept in cooling boxes at 4°C after which they were distributed.

Each laboratory had to test 5 representative samples corresponding to the following concentration range: 0.025-0.095 mg di(2-ethylhexyl)phthalate/l, with two duplicates for each level of concentration.

The results were collected by the coordinator laboratory and evaluated using the statistical calculation model specific for the precision experiments.

The next steps were followed:

➤ A critical examination of the results given by the participants at the scheme in order to identify and treat adequately the extreme results and other irregularities by applying the graphical method (the Mandel test *h* and *k*) and also the numerical calculation method (the Cochran test) (Ref. 4).

For the Mandel test of interlaboratory coherence (*h)* the following formula was used:

$$h_{ij} = \frac{\bar{y}_{ij} - \bar{\bar{y}}_j}{\sqrt{\frac{1}{(p_j - 1)} \sum_{i=1}^{p_j} (\bar{y}_{ij} - \bar{\bar{y}}_j)^2}} \tag{1}$$

where $\bar{y}_{ij}$ is the mean value in the cell; $\bar{\bar{y}}_j$ – general mean of the tests ($\hat{m}_j$).

For the Mandel test of intralaboratory coherence (*k*) the following formula was used:

$$\sqrt{\frac{\sum S_{ij}^{\ 2}}{p_j}} \text{ for each level} \tag{2}$$

$$k_{ij} = \frac{S_{ij}\sqrt{p_j}}{\sqrt{\sum S_{ij}^{\ 2}}} \text{ at every concentration level.} \tag{3}$$

The Cochran numerical test, as a measure of the variability inside the laboratory, was calculated with the following formula:

$$C = \frac{s_{\max}^{\ 2}}{\sum_{i=1}^{p} s_i^{\ 2}} \tag{4}$$

where $s_{\max}$ is the highest value of the standard deviation from the set.

➤ Determination of the precision values and of the mean values for each concentration level by calculation of:

– standard deviation of repeatability ($s_r^{\ 2}$)

– interlaboratory standard deviation ($s_L^{\ 2}$)

– standard deviation of reproducibility ($s_{Rj}$)

– general mean ($\hat{m}_j$).

The relation between $s_r$, $s_R$ and $\hat{m}_j$ may be represented graphically and can be included in one of the following situations:

- $s_r = b\hat{m}_j$ – a straight line passing through the origin;
- $s_r = a + b\hat{m}_j$ – a straight line with positive ordinate at the origin;
- $\lg s_r = c + d\lg \hat{m}_j$ – an exponential relation.

## RESULTS AND DISCUSSION

The results reported by the laboratories which participated in the scheme are presented in Table 1.

**Table 1**. Results obtained in the interlaboratory study for di(2-ethylhexyl)phthalate

| Laboratory | Concentration (mg/l) | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| 1 | 0.025 | 0.033 | 0.057 | 0.074 | 0.096 |
| | 0.027 | 0.035 | 0.055 | 0.076 | 0.095 |
| 2 | 0.024 | 0.034 | 0.056 | 0.076 | 0.098 |
| | 0.022 | 0.034 | 0.053 | 0.074 | 0.096 |
| 3 | 0.026 | 0.036 | 0.055 | 0.075 | 0.094 |
| | 0.024 | 0.035 | 0.052 | 0.076 | 0.096 |
| 4 | 0.023 | 0.033 | 0.055 | 0.073 | 0.092 |
| | 0.025 | 0.036 | 0.057 | 0.075 | 0.094 |
| 5 | 0.024 | 0.036 | 0.054 | 0.075 | 0.095 |
| | 0.025 | 0.037 | 0.055 | 0.075 | 0.094 |

Using both statistical techniques – graphical method (the Mandel test) and the numerical calculation method (the Cochran test) there have not been found outliers or extreme values in the results reported by the participants at the interlaboratory comparison scheme. The results are presented in Figs 2 and 3 and Table 2 .

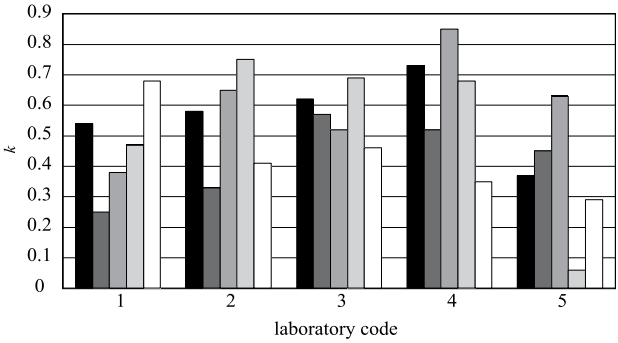


**Fig. 2**. Results of the Mandel test ($k$) for di(2-ethylhexyl)phthalate

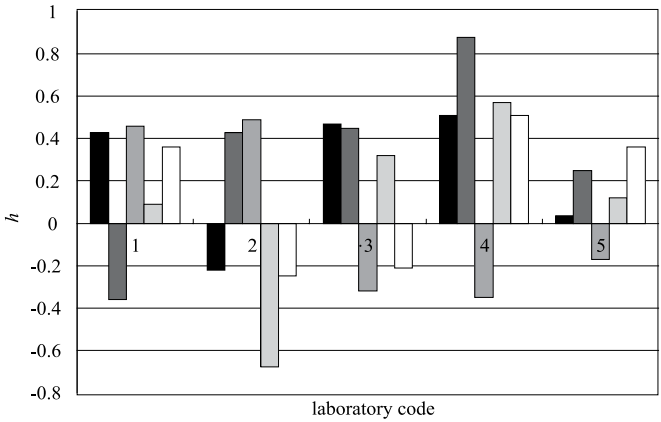


**Fig. 3**. Results of the Mandel test (*h*) for di(2-ethylhexyl)phthalate

**Table 2**. Results of the Cochran numerical test for di(2-ethylhexyl)phthalate

| Level | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| *C*, calculated | 0.1176 | 0.0316 | 0.1666 | 0.0322 | 0.1428 |
| *p*=5, *n*=2 , significance level 1%, *C*=0.928; *p*=5, *n*=2 , significance level 5% *C*=0.841 | | | | | |

The experimental study offered to the participant laboratories also useful information about their analytical performance, respectively the repeatability of the intralaboratory determinations (with the Mandel test *k*) and the reproducibility of the results in case of interlaboratory determinations (with the Mandel test *h*).

Using the intralaboratory coherence test, *k*, it can be noticed that all laboratories presented relatively small and constant variations for most of the concentration levels (Fig. 2).

Concerning parameter *h*, all laboratories presented positive and negative results, with relatively small variations for laboratory number 5 but with higher level of variation for laboratories numbers 3 and 4 (Fig. 3 ).

Also, in the case of the numerical Cochran test (test of the variability inside the laboratory) there have not been observed outliers or extreme results for none of the concentration levels. The values calculated for parameter '*C*' were compared with the critical values of the Cochran test for *p*=5, *n*=2 and significance levels of 1% (0.928) and 5% (0.841). The values obtained for this parameter are situated in the range of 0.031-0.14.

Because all the values obtained by the participant laboratories were in the range of the accepted values, some other statistical parameters such as *m*, $s_r$, $s_R$, were calculated and are presented in Table 3.

**Table 3**. Calculated values for $m$, $s_r$ and $s_R$ of the method of analysis for di(2-ethylhexyl)phthalate

| Level | P (participant laboratory) | $m$ (the mean value) (mg/l) | $s_r$ (standard deviation of repeatability) (mg/l) | $s_R$ (standard deviation of reproducibility) (mg/l) |
|---|---|---|---|---|
| 1 | 5 | 0.0245 | 0.00153 | 0.00175 |
| 2 | 5 | 0.0350 | 0.00151 | 0.00176 |
| 3 | 5 | 0.0549 | 0.00162 | 0.00179 |
| 4 | 5 | 0.0749 | 0.00164 | 0.00168 |
| 5 | 5 | 0.0950 | 0.00156 | 0.00172 |

For the tested concentrations, the calculated values for the standard deviation of repeatability ($s_r$) remained relatively constant related to the increase of the general mean value: 0.0015-0.0016 mg/l. The same behaviour can be observed also for the standard deviation of reproducibility: 0.0016-0.0017 mg/l. In both cases the values of the standard deviation are situated in the normal limits for a chromatographic method.

## CONCLUSIONS

Using both statistical techniques – graphical (the Mandel test) and the numerical calculation method (the Cochran test) were not observed outliers or extreme values in the results reported by the laboratories which participated to the interlaboratory comparison scheme concerning the determination method for di(2-ethylhexyl)phthalate in water.

Concerning the dependence of precision on the general mean value, it was observed that on the tested concentrations both standard deviations ($s_r$ and $s_R$) remained relatively constant related with the increase of the general mean value.

The method performance parameters (repeatability and reproducibility) are situated in the accepted limits for a chromatographic method, so that the methods elaborated can be used by different environmental laboratories for the determination of di(2-ethylhexyl)phthalate from water samples.

## REFERENCES


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