Method validation for phthalate analysis from water

Irina Dumitraşcu

Environmental Health Center, Cluj-Napoca, Romania; Babeş-Bolyai University, Faculty of Environmental Sciences and Engineering, Cluj-Napoca, Romania. Corresponding author: I. Dumitraşcu, irina.dumitrascu@ehc.ro

Abstract. The goal of method validation is to provide objective evidence that the evaluated method will show acceptable reproducibility and accuracy so as to be applicable. The objective of this paper is to present a validation method for quantitative phthalates analysis from water by solid phase extraction (SPE) and determination by gas chromatography in combination with mass spectrometry detector (GC–MS) in electronic ionization mode (EI) with selected-ion monitoring (SIM) acquisition method. Every method, even if it is standardized, has to be validated in the laboratory because method performance varies with the instrument used, environmental condition and of course the analyst. Performance parameters of the method like linearity, precision, detection limit and uncertainty are evaluated for each compound. This paper proves that the method is hold into the legal estimation or estimation required by the laboratory management where they are no legally regulations.
Key Words: phthalates, validation, Horwitz.

Introduction. Phthalates are esters of phthalic acid based on the structure in Figure 1. Due to man's activities they are present in the environment in quite large quantities, since they are a group of chemicals which have been used for about the last 50 years as plastifying agents, mainly to make polyvinyl chloride (PVC) supple and flexible. However, not all the phthalates are used for this, some are used to stop nail varnish flaking, to make perfumes last longer, or to make tool handles stronger and more resistant. Others reinforce or increase the effect of adhesives, paint pigments, caulking and many other materials. They can be found in many industrial sectors: paint, petrochemical, packing, cosmetics, etc. and in view of this widespread use, phthalates have been the subject of intensive research concerning effects on health and the environment (Sablayrolles et al 2005).
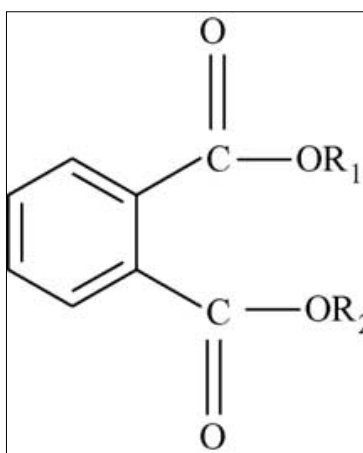

Figure 1. General formula for phthalates
($R_1 = R_2$ or $R_1 \neq R_2$).

Phthalates are easily released and migrate into foods, beverages and drinking water from the packaging or bottling materials or manufacturing processes. This process accelerates as plastic products age and break down. With respect to their endocrine disrupting potential, phthalates such as benzyl butyl phthalate (BBP), di-butyl phthalate (DBP) and di-isobutyl phthalate (DIBP) have been found to elicit estrogenic responses in *in vitro* assays. It is possible that phthalates are a contributory factor to endocrine-mediated adverse effects observed in wildlife and humans over the past few decades (Amiridou & Voutsa 2011).

Method validation is the process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Results from method validation can be used to judge the quality, reliability and consistency of analytical results; it is an integral part of any good analytical practice. Analytical methods need to be validated or revalidated before their introduction into routine use; whenever the conditions change for which the method has been validated (e.g., an instrument with different characteristics or samples with a different matrix), and whenever the method is changed and the change is outside the original scope of the method (Ludwig Huber 2007).

The purpose of this study is to describe a validation method of phthalates from water. The study is based on the idea that the validation must be made through a comparison of experimental and theoretical results, each set including its level of uncertainty. Usually, experimental results are presented with their uncertainties, which correspond to imperfections in the measurement apparatus. On the other hand, this is rarely the case for theoretical results, which are simply those generated by the simulation. However, data obtained and used for the simulation are themselves associated with an uncertainty which propagates through to the results of the computation. We can come to more appropriate conclusions on the effective validation of a theoretical result if we compare it with the experimental result outcome along with their respective uncertainties (Aude et al 2000).

Evaluation and validation of analytical methods and laboratory procedures are therefore of paramount importance, prominent means being the use of adequate (preferably certified) reference materials and participation in interlaboratory proficiency tests. Quality demands made upon the infrastructure, equipment, operating procedures, personnel and organisation of the laboratory are to be deduced from the quality requirements that the produced chemical information should meet. A formal recognition of this type of quality can be achieved through accreditation or certification, based on international quality standards and guidelines, as issued by ISO, OECD and CEN (Van Zoonen et al 1999).

Hereinafter are presented results for method validation of phthalates from water analyzed according to SR EN ISO 18856-2006.

Material and Method

Reagents and materials*.* Phthalate esters were supplied from Cambridge Isotope Laboratories and Supelco. Ethyl acetate, methanol, isooctane and anhydrous sodium sulfate were supplied from Merck. Solid phase extraction (SPE) cartridges were purchased from Thermo Scientific.

Analytical determination*.* Water samples are poured through preconditioned SPE cartridges with ethyl acetate and methanol. The analytes were eluted with ethyl acetate and transferred to a sample vial.

The analysis was performed using a gas chromatograph (Shimadzu GC-2010) coupled with a mass spectrometer (Shimadzu QP 2010) and an autosampler (AOC 20i, Shimadzu Corporation). Compounds were separated on a TraceGold TG–5MS 5% diphenyl–95% dimethyl polysiloxane capillary column (30m length, 0.25mm i.d., 0.25mm film thickness) from Thermo Scientific.

The compounds were separated using the following oven program: the column temperature was initially set at $80^{o}C$ for 2 min, then increased at a rate of $17^{o}C/min$ up to

320°C which was maintained for 5 min. Helium carrier gas (99.9999% purity) was maintained at a constant rate of 1.2 mL/min. The temperature at the injector was 150°C. The ion source and transfer line temperature was set at 280°C and at 320°C, respectively. Mass spectra were obtained using electron impact ionization (70 eV).

The identification of target compounds was based on the relative retention time, the presence of target ions and their relative abundance. Three ions were chosen to be monitored by mass spectrometer detector with selected-ion monitoring (MS-SIM) mode according to the mass spectra characteristic features obtained in the full-scan mode and by comparison with the NIST05 library reference spectral bank (Table 1). To evaluate the mass spectral fragmentation pattern of each compound, a standard solution of each compound was analyzed by capillary GC–MS in the full-scan mode, for which the target (base peaks) and qualifier ions were chosen to attain the best response in the SIM mode acquisition. (Serodio & Nogueira 2006).

Table 1

SIM ions, retention time for the analyzed phthalates by GC–MS(EI-SIM)

Compound	SIM ions	Retention time (min)
Dibutyl phthalate (DBP)	149/73/147	11.797
Di-iso-butyl phthalate (DiBP)	149/57/41	11.225
Benzyl butyl phthalate (BBP)	149/91/65	14.014
Di(2-ethylhexyl) phthalate (DEHP)	149/167/57	14.938

Results and Discussion

Linearity and calibration curve. The instrumental calibration was performed with standard mixtures ranging from 0.18 to 0.90 µg L^{-1} for the four phthalates, using the corresponding target ion abundances. The linearity was checked for each of the phthalates. According to ISO 8466-1990 a calibration curve is linear within the chosen range if the correlation coefficient (R) is equal or greater than 0.997 and the PG value for F homogeneity test is smaller than 5.35.

To F homogeneity test checks whether there are significant differences in the concentration range ends. Chromatographic area measurements were performed using 10 replicates for the minimum level of concentration and 10 replicates for the maximum concentration (n = 10). Then they were calculated variances (s2) values obtained for the two levels of concentration. Results are shown in Table 2 and were compared with the table value function $F_{9, 9; 0.99}$ = 5.35 (Tanase et al 2007).

Table 2

Linearity values

Compound	PG Homogeneity	PG Linearity	R
Dibutyl phthalate (DBP)	4.72	3.50	0.998
Di-iso-butyl phthalate (DiBP)	4.60	0.75	0.999
Benzyl butyl phthalate (BzBP)	2.95	0.46	0.998
Di(2-ethylhexyl) phthalate (DEHP)	4.73	0.13	0.998

Calibration curve and the equations for each phthalate ester are displayed in Figures 2 and 3.

It is noted that PG < F for all the phthalates esters and R > 0.997, proved that the dispersions are uniform and therefore correct concentration range was chosen.
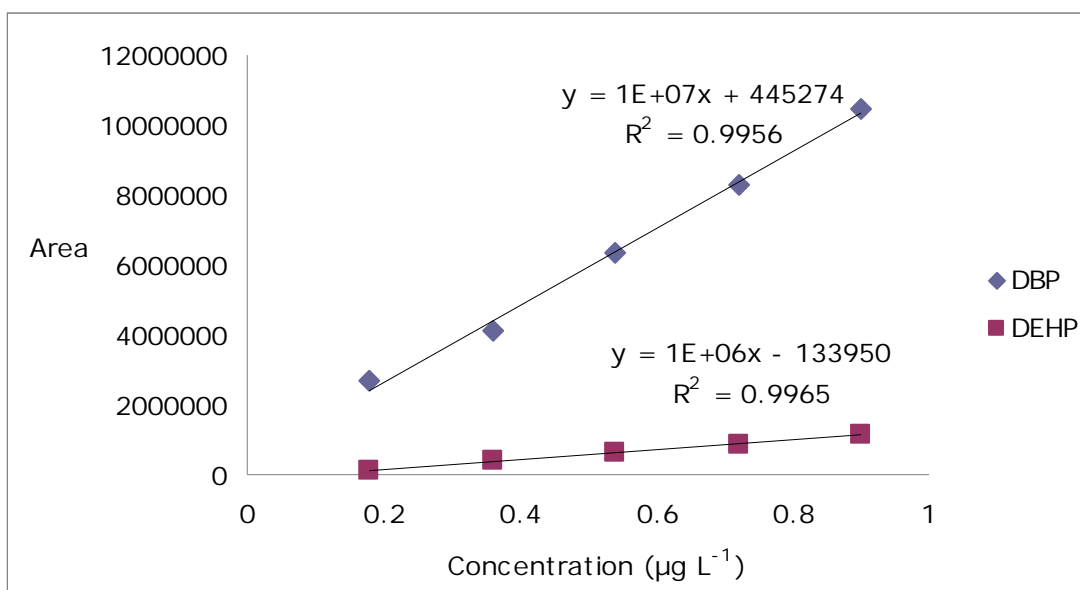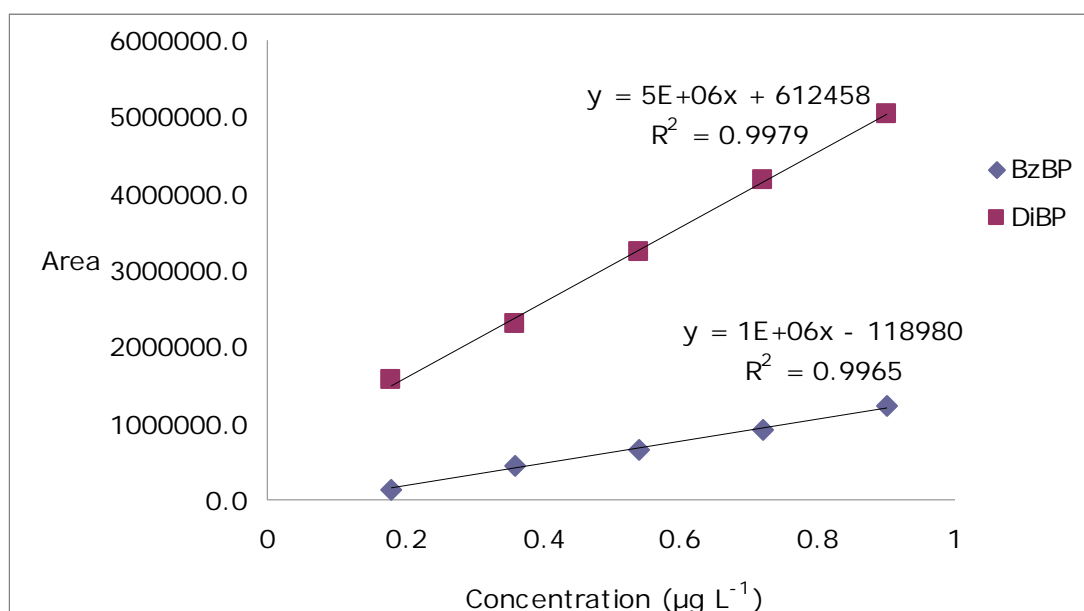
Figure 2. Calibration curve for DBP and DEHP.

Figure 3. Calibration curve for DiBP and BzBP.

Accuracy. Practical assessment of trueness relies on comparison of mean results from a method with known values, that is, trueness is assessed against a reference value (i.e. true value or conventional true value) (The Fitness for Purpose of Analytical Methods 1998). Accuracy can be evaluated at three levels: repeatability, intermediate precision and reproducibility. In this study only repeatability was demonstrate, which is a measure of the variability of the measurements made by the same method on identical samples of the same laboratory and in a short time.

Chromatographic area measurements were performed using 10 replicates for the minimum level of concentration, 10 replicates for medium level of concentration and 10 replicates for the maximum concentration.

Repeatability is usually expressed by the percentage relative standard deviation of repeatability (RSD%) and the values obtained are shown in Table 3 (Tanase et al 2007).

Table 3

RSD values

Compound	Concentration ($\mu g\ L^{-1}$)	RSD (%)	RSD by Horwitz
Dibutyl phthalate (DBP)	0.18	3.86	12.5
	0.54	1.63	10.5
	0.90	1.35	9.8
Di-iso-butyl phthalate (DiBP)	0.18	5.64	12.5
	0.54	1.77	10.5
	0.90	1.39	9.8
Benzyl butyl phthalate (BzBP)	0.18	4.43	12.5
	0.54	1.23	10.5
	0.90	1.25	9.8
Di(2-ethylhexyl) phthalate (DEHP)	0.18	3.24	12.5
	0.54	1.38	10.5
	0.90	1.41	9.8

According to Horwitz equation, we calculated relative standard deviation for this level of concentration (Tanase et al 2007). As it can be seen, the value obtained for RSD% is within the specified limits.

Limit of detection. Where measurements are made at low analyte or property levels, e.g. in trace analysis, it is important to know what is the lowest concentration of the analyte or property value that can be confidently detected by the method (The Fitness for Purpose of Analytical Methods 1998).

Chromatographic area measurements were performed using 6 replicates of blank. Standard deviation was calculated for the results. Limit of detection (LOD) is 10 times the standard deviation for blanks values (ISO 5725/1994). Results are shown in Table 4.

Table 4

LOD values

Compound	Standard deviation	LOD ($\mu g\ L^{-1}$)
Dibutyl phthalate (DBP)	0.00082	0.00818
Di-iso-butyl phthalate (DiBP)	0.00016	0.00162
Benzyl butyl phthalate (BzBP)	0.00022	0.00219
Di(2-ethylhexyl) phthalate (DEHP)	0.00163	0.01630

Uncertainty. Uncertainty is a parameter associated with the result of a measurement, that characterizes the dispersion of the values that could reasonably be attributed to the concentration of the analyte (EURACHEM/CITAC Guide CG 4/2012).

In estimating the overall uncertainty, it is necessary to take each source of uncertainty and treat it separately to obtain the contribution from that source (Figure 4). Each of the separate contributions to uncertainty is referred to as an uncertainty component (EURACHEM/CITAC Guide CG 4/2012).

Having identified the uncertainty sources the next step is to quantify the uncertainty arising from these sources. This can be done by evaluating the uncertainty arising from each individual source and then combining them. Following the estimation of individual components of uncertainty and expressing them as standard uncertainties, the next stage is to calculate the combined standard uncertainty (EURACHEM/CITAC Guide CG 4/2012). Results are shown in Table 5.

For some methods there are legal estimation of uncertainty, but for that method they are not available. Laboratory management required estimation less than 30% of the analyte concentration.
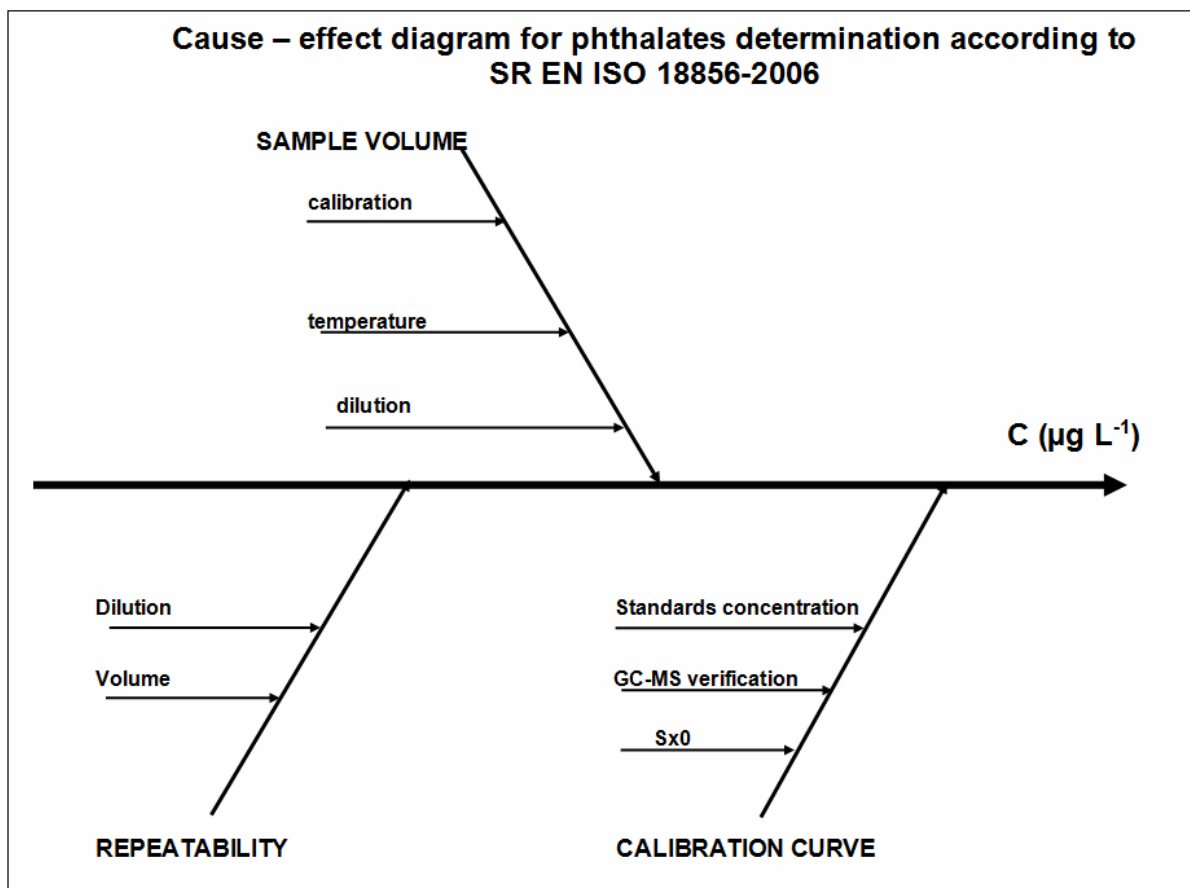
Figure 4. Source of uncertainty.

Uncertainty values

Compound	Uncertainty of the method (%)
Dibutyl phthalate (DBP)	9.02
Di-iso-butyl phthalate (DiBP)	6.81
Benzyl butyl phthalate (BzBP)	8.19
Di(2-ethylhexyl) phthalate (DEHP)	8.23

Conclusions. The method proved to be suitable for its intended purpose because it is linear, accurate and precise:

- the method proved to be linear on concentration range between 0.18-0.90 µg L^{-1} by conducting verification tests: dispersion homogeneity and linearity;

- the method is precise, which results in proving the repeatability;

- the method is accurate, as demonstrated by calculating the bias on calibration range.

The method meets the requirements for which it is intended to use.

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Author:
Irina Dumitraşcu, Environmental Health Center, 58 Busuiocului Street, 400240, Cluj-Napoca, Romania; Babeş-Bolyai University, Faculty of Environmental Sciences and Engineering, 30 Fântânele Street, 400294, Cluj-Napoca, Romania, e-mail: irina.dumitrascu@ehc.ro