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Cosmetics — Analytical methods — GC/MS method for the identification and assay of 12 phthalates in cosmetic samples ready for analytical injection

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National foreword

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A list of organizations represented on this committee can be obtained on request to its secretary.

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Cosmetics - Analytical methods - GC/MS method for the identification and assay of 12 phthalates in cosmetic samples ready for analytical injection

Cosmétiques - Méthodes analytiques - Méthode CG-SM pour l'identification et l'analyse de 12 phtalates dans des échantillons de produits cosmétiques prêts à être injectés dans un système analytique

Kosmetische Mittel - Analysenmethoden - GC/MS-Methode für die Identifizierung und die Quantifizierung von 12 Phthalaten in zur direkten Injektion geeigneten Proben kosmetischer Mittel

This European Standard was approved by CEN on 10 April 2014.

EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

CEN-CENELEC Management Centre: Avenue Marnix 17, B-1000 Brussels

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Foreword

This document (EN 16521:2014) has been prepared by Technical Committee CEN/TC 392 “Cosmetics”, the secretariat of which is held by AFNOR.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by November 2014 and conflicting national standards shall be withdrawn at the latest by November 2014.

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Introduction

Phthalates are esters of phthalic acid (Figure 1). About 80 % of all phthalates manufactured are used as “plasticizers” to make plastics flexible without sacrificing strength or durability. These compounds are present in cosmetic products like perfumes and toiletries. Some phthalates, particularly those of low molecular weight, are introduced into cosmetics as ingredients, for examples DEP and DMP are used as solvents and perfume fixatives [1-3] or DEP can be used as alcohol denaturing [2, 4]. Their presence in such products may come from their use as ingredients during the manufacturing process or may come from the migration of phthalates from packaging when plastic is used. Their presence as contaminant could also be due to the manufacturing process or raw materials used. Some analytical methods are proposed in the literature for the determination of phthalates in cosmetic products [1, 4-12].

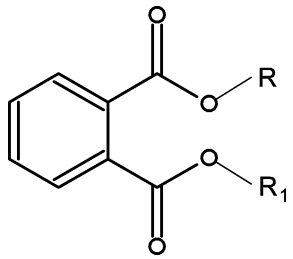


Figure 1 — Esters of phthalic acid

This standard proposes a GC/MS method for a simultaneous assay in cosmetic samples ready for analytical injection of 12 phthalates listed in Table 1. These chromatographic conditions are not suitable for the quantification of di-isononyl phthalate (DiNP) or di-isodecyl phthalate (DiDP). According to SCCP [13], the possible presence of DiNP or DiDP in cosmetics does not seem to be a problem for human health. A GC/MS method using positive chemical ionisation with ammonia as collision gas is proposed in literature for the determinations of those compounds in cosmetic products [14].

Analyses are carried out on a GC/MS system with electron impact ionization mode (Ei). The separation of phthalates is obtained on a cross-linked 5 %-phenyl/95 %-dimethylpolysiloxane capillary column 30 m × 0,25 mm (i. d.) × 0,25 µm film thickness using a temperature gradient. Phthalate quantification is performed by external calibration using an internal standard or by the standard addition. Cosmetic samples are analyzed directly or after a previous dilution in ethanol [15].

1 Scope

This European Standard describes a GC/MS method for the assay of 12 phthalates, amongst which the 8 phthalates regulated by the European cosmetic regulation 1223/2009 [16]. This method is given for the analysis of samples ready for analytical injection from cosmetic products or raw materials used in cosmetic products. Samples should be compatible with GC analysis possibly after dilution. This method does not include requirements for the preparation of samples in cosmetic matrices for which direct injection in GC is not feasible.

2 Reagents

If not otherwise specified, analytical-grade chemicals shall be used.

2.1 Phthalates considered

Table 1 — Phthalates considered

Phthalates	CAS	Manufacturer[b]	Quality
DBP [a] (dibutyl phthalate)	84–74–2	ALDRICH	97,0 %
DEHP [a] (diethylhexyl phthalate)	117–81–7	ALDRICH	99,8 %
BBP [a] (butylbenzyl phthalate)	85–68–7	ALDRICH	97,0 %
DMEP [a] (di(2-methoxyethyl) phthalate)	117–82–8	ALDRICH	97,0 %
DnPP [a] (di-n-pentyl phthalate)	131–18–0	CIL CLUZEAU	99,0 %
DiPP [a] (diisopentyl phthalate)	605–50–5	CIL CLUZEAU	95,0 %
DPP [a] (n-pentyl isopentyl phthalate)	84777–06–0	CIL CLUZEAU	95,0 %*
DiBP [a] (diisobutyl phthalate)	84–69–5	ACROS	98,0 %
DCHP (dicyclohexyl phthalate)	84–61–7	ALDRICH	98,0 %
DEP (diethyl phthalate)	84–66–2	ACROS	98,0 %
DMP (dimethyl phthalate)	131–11–3	ACROS	98,0 %
DnOP (di-n-octyl phthalate)	117–84–0	ALDRICH	98,0 %

[a] Regulated phthalates.

[b] This is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by CEN of this product.

* Mixed isomers (DiPP, DnPP and DPP)

2.2 Ethanol

2.3 Internal standard, 4,4-Dibromodiphenyl from Fluka [1)] 97,0 % was used as internal standard (ISTD).

2.4 Internal standard stock solution (SM-ISTD), c = 1 000 µg/ml.

1) This is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by CEN of this product.

Weigh approximately 10 mg of 4,4-Dibromodiphenyl (2.3) into a 10 ml volumetric flask. Firstly, dissolve in a small amount of ethanol (2.2) and then fill to the calibration mark with ethanol. This stock solution is daily prepared.

2.5 Phthalates stock solution, c = 500 µg/ml (SM).

Weigh approximately 10 mg of each phthalate (2.1) into a 20 ml volumetric flask. Firstly, dissolve in a small amount of ethanol (2.2) and then fill to the calibration mark with ethanol. This stock solution has a shelf life of at least 2 weeks if stored in a refrigerator.

NOTE Due to the low amount of DIPP standard (sold in quantities of 10,0 mg), a stock solution of this phthalate is prepared independently from other phthalates. In this way, two intermediate stock solutions SM-1 and SM-2 at 1 000 µg/ml are prepared. SM-1 is prepared weighting approximately 10 mg of each phthalate (2.1), except DiPP, into a 10,0 ml volumetric flask, whereas SM-2 is prepared weighting approximately 5,0 mg of DiPP (2.1) into a 5,0 ml volumetric flask. The final stock solution SM is obtained mixing equal volumes of both standard solutions (c = 500 µg/ml). All these stock solutions have a shelf life of at least 2 weeks if stored in a refrigerator.

2.6 Calibration solutions (standard solutions)

1,0 ml of the phthalate stock solution SM (2.5) is transferred into a 10,0 ml volumetric flask and filled with ethanol (2.2) up to the calibration mark (c = 50 µg/ml). From this intermediate solution (S1), at least 5 calibration solutions are prepared by dilution in ethanol (2.2) after the addition of 100 µl of the internal standard stock solution (SM-ISTD). Phthalates concentrations on these calibration solutions ranges from 0,25 µg/ml to 5,0 µg/ml with an ISTD concentration fixed at 10,0 µg/ml. These calibration solutions are prepared extemporaneously and injected.

If cosmetic samples (perfume) are directly prepared in a 1,5 ml GC vial, according to 4.2, the preparation of calibration solutions have to be adapted: Calibration solutions, ranging from 0,25 µg/ml to 5,0 µg/ml, are prepared without internal standard and 10 µl of the internal standard stock solution (SM-ISTD) are added to 1,0 ml of each calibration solution directly in a 1,5 ml GC vial. These calibration solutions are prepared extemporaneously. Vials are shaken and the solution injected.

3 Apparatus and equipment

3.1 Standard laboratory equipment.

3.2 Gas chromatography/mass spectrometry apparatus.

3.3 Gas chromatography/FID (for standard purity).

3.4 Analytical separation column.

The following parameters have proved useful:

GC column, low bleeding phase: 5 %-Phenyl-95 %-dimethylpolysiloxane, 30 m × 0,25 mm (i. d.) × 0,25 µm or equivalent material.

4 Procedure

4.1 Standard purity

The purity of each standard and the internal standard and the respective percentages for geometrical isomers (n-pentyl isopentyl phthalate - DPP) shall be determined by GC-FID for further calculations.

4.2 Sample preparation

1,0 g of the cosmetic sample ready for analytical injection is transferred into a 10,0 ml volumetric flask and filled with ethanol (2.2) up to the calibration mark after the addition of 100 µl of the internal standard stock solution (SM-ISTD). This solution is injected. In case of excessive concentration of phthalates, an appropriate previous dilution of the sample is performed. The limit of quantification using this sample preparation was set at 5 ppm.

An alternative sample preparation consists to prepare the cosmetic sample directly in a 1,5 ml GC vial. 10 µl of the internal standard stock solution (SM-ISTD) are added to 1,0 ml of cosmetic samples (perfume). Vials are shaken and the solution injected. The limit of quantification obtained using this sample preparation was set at 0,5 ppm. Calibration solutions are prepared according to 2.6.

4.3 Gas chromatography (GC) measurement conditions

When using the apparatus (3.2) and column (3.4), the following conditions have shown to be useful:

Table 2 — GC/MS programme

Apparatus	Gas chromatography with mass selective detector (GC/MS) Autosampler		
Column	GC column, low bleeding phase: 5 %-Phenyl-95 %-dimethylpolysiloxane, 30 m × 0,25 mm × 0,25 µm (or equivalent)		
Oven programme	**Ramp**	**Temperature**	**Time**
		100 °C	0 min
	30 °C/min	200 °C	0 min
	3 °C/min	260 °C	0 min
	30 °C/min	320 °C	5 min
Injector T	300 °C		
Interface T	250 °C		
Source T	230 °C		
Injection time	30 min		
Gas / flow rate	He/1 ml/min		
Injection port	split/splitless		
Injection parameters :	1 µl/Constant pressure, Split 1/20		
Detection mode	Quadrupole		
Ionisation mode	EI (70 eV)		
Mass detection (Full/SIM)	Identification: full-scan (m/z 40 to 350) Quantification: SIM using 3 specific ions		
Internal standard	4,4-Dibromodiphenyl		
Calibration	0,25 µg/ml to 5,0 µg/ml		
Solvent used	Ethanol (injection)		

Examples of chromatogram, obtained using the GC/MS programme described in Table 2, are given in Annex A (Figure A.1).

4.4 Detection

4.4.1 General

The detection and quantitative determination can be performed by evaluating the mass traces of each phthalate. Relative Retention (RR), m/z ions used for quantification and ions ratios obtained on a standard solution for each phthalate are given in Table 3.

4.4.2 MS detection in Selected Ion Monitoring (SIM) mode

1) from 2,5 min – Ions 135 ;163 ; 194 (dwell time 50 ms/ion);

2) from 4,0 min – Ions 105 ; 149 ; 177 (dwell time 50 ms/ion);

3) from 5,0 min – Ions 104 ; 149 ; 223 (dwell time 50 ms/ion);

4) from 7,0 min – Ions 59; 104;149 ; 205; 223 (dwell time 30 ms/ion);

5) from 8,2 min – Ions 104 ; 149 ; 219, 237, 310, 312 (dwell time 25 ms/ion);

6) from 10,5 min – Ions 57; 71; 97 (dwell time 50 ms/ion);

7) from 13,0 min – Ions 91 ; 149 ; 206 (dwell time 50 ms/ion);

8) from 16,5 min – Ions 149 ; 167 ; 225; 226, 249; 279, (dwell time 25 ms/ion);

9) from 20,0 min – Ions 149 ; 167 ; 279 (dwell time 50 ms/ion).

Retention times of each group should be determined after the injection of a standard solution.

NOTE 1 A dwell time at 50 msec per ion for a SIM window including three fragments is correct. If 6 ions are present, a 25 msec per ion dwell time can be applied. At least, a minimum of 10 points of acquisition for one peak allows an acceptable quantification.

Table 3 — List of m/z ions used for quantification and ions ratio

Compound	Indicative RR	Ion 1 (m/z)	Ion 2 (m/z)	Ion 3 (m/z)	Ion ratio 2/1 % [b]	Ion ratio 3/1 % [b]
DMP	0,43	**163**	194	135	6	6
DEP	0,52	**149**	177	105	25	10
DiBP [a]	0,78	**149**	104	223	5	2
DBP [a]	0,91	**149**	205	223	4	6
DMEP [a]	0,96	**59**	149	104	10	8
ISTD [c]	1,00	**312**	310	314	20	40
DiPP [a]	1,08	**149**	237	104	7	5
DPP [a]	1,15	**149**	237	219	5	2
DnPP [a]	1,23	**149**	237	104	6	4
BBP [a]	1,65	**149**	91	206	70	20
DCHP	2,04	**149**	167	249	30	5
DEHP [a]	2,11	**149**	167	279	50	30
DnOP	2,57	**149**	279	167	18	3

[a] Regulated Phthalates.

[b] The maximum permitted tolerances for relative ion intensities are in agreement with document [17].

[c] ISTD: 4,4 dibromodiphenyl (CAS 92–86–4).

The Relative Retention (RR) of each phthalate should be determined after the injection of a standard solution. The retention time of the ISTD (4,4 dibromodiphenyl) should be near 8,5 min. m/z ions used for quantification are bolded in the table, the 2 other m/z ions given in the table are qualifier ions used to confirm the identity of the component. Some phthalates show qualifier ions with very low intensity which leads to a weak specifity of the ion ratio (general issue for benzene derivatives such as phthalates).

Depending on the instrument, ion ratios may be determined independently from the GC/MS software according to the formula below:

$$\text{Ion ratio } (m/z_{i,j}) = [(\text{Area } (m/z_i)/\text{Area } (m/z_j)] \cdot 100$$

NOTE 2 During assay inject regularly solvent to check the absence of carry over.

5 Evaluation

5.1 Identification and quantitative determination

Phthalates are identified by comparing the retention times and the mass spectrum of the sample with those of calibration substances. The quantitative determination of the analyte is performed based on a calibration function or the standard addition. The calibration solutions are chromatographed in accordance with the conditions given in 4.2. Phthalate concentrations are calculated from the calibration by linear regression on the basis of the obtained peak areas. For DPP, the calibration curve is plotted for the major isomer. For DiPP and DnPP, the DPP isomers shall be considered.

5.2 Calculation

The phthalate content, w, with respect to the sample, is calculated using Formula (1):

$$w = \frac{c \cdot V \cdot F}{m \cdot 10\ 000} \quad (1)$$

where

w is the phthalate content, in g/100 g;

c is the phthalate concentration in the sample solution, in µg/ml, determined from the calibration function;

V is the volume of the sample solution, in ml;

F is the dilution factor, if required;

m is the initial weight of the sample, in g.

NOTE For information, validation data using this standard protocol were obtained using the analytical approach described in ISO 12787 and published in the Journal of Chromatography A, 1253 (2012) 144–153 [15]

6 Test report

The test report shall contain the following data:

a) information necessary for the identification of the sample (type, origin and designation of the sample);

b) a reference to this European Standard;

c) the date and type of sampling procedure (if known);

d) the date of receipt and date of analysis;

e) the date of test;

f) the test results and the units in which they have been expressed;

g) justification of any deviations from this official method;

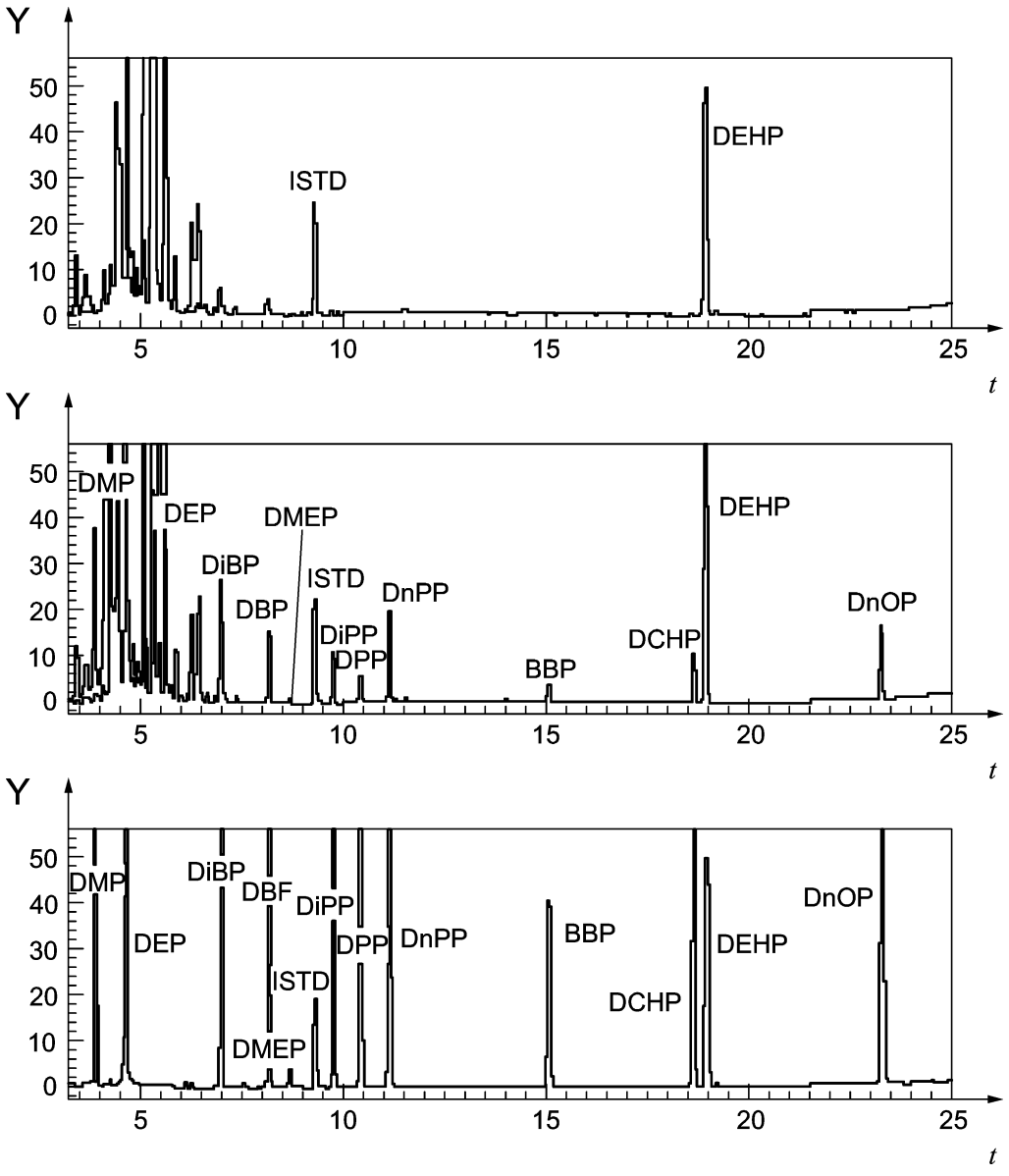
h) operations not specified in the method or regarded as optional, which might have affected the results;

i) identification of laboratory that performed the test;

j) identification, date and signature of the person responsible for the report.

Annex A
(informative)

Example of Chromatograms



Key

Higher chromatogram:	unspiked sample
Middle chromatogram:	spiked sample
Lower chromatogram:	5,0 mg/l standard solution
Y	abundance (u. a.)
t	time in min

Figure A.1 — Example of Chromatograms obtained using the GC/MS programme described in Table 2

Bibliography

[1] HUBINGER J.C., HAVERY D.C. Analysis of consumer cosmetic products for phthalate esters. *J. Cosmet. Sci.* 2006, **57** (2) pp. 127–137

[2] GÓMEZ-HENS A., AGUILAR-CABALLOS M.P. Social and economic interest in the control of phthalic acid esters. *Trends Analyt. Chem.* 2003, **22** (11) pp. 847–857

[3] FDA April 19, 2001 Updated March 31, 2005 and February 7, 2008 “Phthalates and Cosmetic Products“

[4] GODLY E.W., MORTLOCK A.E. The determination of Di-n-alkyl phthalates in cosmetic preparations by gas-liquid chromatography. *Analyst (Lond.)*. 1973, **98** (168) pp. 493–501

[5] HUBINGER J.C. A survey of phthalate esters in consumer cosmetic products. *J. Cosmet. Sci.* 2010, **61** (6) pp. 457–465

[6] SHEN H.Y., JIANG H.L., MAO H.L. et al. Simultaneous determination of seven phthalates and four parabens in cosmetic products using HPLC-DAD and GC-MS methods. *J. Sep. Sci.* 2007, **30** (1) pp. 48–54

[7] KOO H.J., LEE B.M. Estimated exposure to phthalates in cosmetics and risk assessment. *J. Toxicol. Environ. Health*. 2004, **67** pp. 1901–1914

[8] CHEN H., WANG C., WANG X. et al. Determination of phthalate esters in cosmetics by gas chromatography with flame ionization detection and mass spectroscopic detection. *Int. J. Cosmet. Sci.* 2005, **27** pp. 205–210

[9] DE ORSI D., GAGLIARDI L., PORRÀ R. et al. A environmentally friendly reversed-phase liquid chromatography method for phthalates determination in nail cosmetics. *Anal. Chim. Acta*. 2006, **555** (2) pp. 238–241

[10] SU R., ZHAO X., LI Z. et al. Poly(methacrylic acid-co-ethylene glycol dimethacrylate) monolith microextraction coupled with high performance liquid chromatography for the determination of phthalate esters in cosmetics. *Anal. Chim. Acta*. 2010, **676** pp. 103–108

[11] KAMAREI F., EBRAHIMZADEH H. et al. Optimization of ultrasound-assisted emulsification microextraction with solidification of floating organic droplet followed by high performance liquid chromatography for the analysis of phthalate esters in cosmetic and environmental water samples. *Microchem. J.* 2011, **99** (1) pp. 26–33

[12] MAHUGO-SANTANA C. et al. Application of new approaches to liquid-phase microextraction for the determination of emerging pollutants. *Trends Analyt. Chem.* 2011, **30** (5) pp. 731–748

[13] SCCP/1016/06, Opinion on phthalates in cosmetic products. Scientific Committee on Consumer Products

[14] GEORGE C., PREST H. 2002. “Determination of phthalate esters by positive chemical ionization MS with retention-time locked GC”, LCGC North America 20 (2), www.chromatographyonline.com (accessed September 2003)

[15] GIMENO P., MAGGIO A.F., BOUSQUET C. et al. Analytical method for the identification and assay of 12 phthalates in cosmetic products: Application of the ISO 12787 international standard “Cosmetics-

Analytical methods-Validation criteria for analytical results using chromatographic techniques. *J. Chromatogr. A*. 2012, **1253** pp. 144–153

[16] Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products, OJ L 342, 22.12.2009, p. 59–209, Annex II (entrance 675, 677, 678, 1151 and 1152) and Article 15, with Regulation (EC) 1272/2008, Annex VI and Regulation (EC) 790/2009, Annexes II & V

[17] 2002/657/EC: Commission Decision of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results (notified under document number C (2002) 3044), OJ L 221, 17.8.2002, p. 8–36

[18] European Pharmacopoeia 7.0; 5.3 “Statistical analysis of results of biological assays and tests”

[19] GARRIDO FRENICH A., MARTÍNEZ VIDAL J.L., FERNÁNDEZ MORENO J.L. et al. Compensation for matrix effects in gas chromatography –tandem mass spectrometry using a single point standard addition. *J. Chromatogr. A*. 2009, **1216** pp. 4798–4808

[20] ANTLER M., JANE MAXWELL E. et al. Online Standard additions Calibration of Transient Signals for Inductively Coupled Plasma Mass Spectrometry. *Anal. Chem.* 2007, **79** pp. 688–694

[21] J. C. Miller and J. N. Miller “Statistics for Analytical Chemistry”, Halsted Press, New York, 117-120 (2nd edition)

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