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Textiles — Method for the detection and determination of alkylphenol ethoxylates (APEO) —

Part 2:
Method using NPLC

Textiles — Méthode de détection et de détermination des alkylphénols éthoxylés (APEO) —

Partie 2: Méthode utilisant la CLPN

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 38, *Textiles*.

A list of all parts in the ISO 18254 series can be found on the ISO website.

Any feedback or questions on this document should be directed to the user’s national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

This document specifies the normal phase liquid chromatography (NPLC) separation method for the quantitative and qualitative analysis of extractable alkylphenol ethoxylates (APEO) in textile and textile products. NPLC separation method enables alkylphenol ethoxylates to be analysed by high performance liquid chromatograph (HPLC) with Mass Spectrometer (MS), fluorescence detector (FD), charged aerosol detector (CAD) and evaporative light scattering detector (ELSD).

A study of the contribution percentage (mole fraction) of APEO congeners is presented in Annex D.

Textiles — Method for the detection and determination of alkylphenol ethoxylates (APEO) —

Part 2:
Method using NPLC

WARNING — This document calls for the use of substances/procedures that may be injurious to the health/environment if appropriate conditions are not observed. It refers only to technical suitability and does not absolve the user from legal obligations relating to health and safety/environment at any stage.

1 Scope

This document specifies the normal phase liquid chromatography (NPLC) separation method for the qualitative and quantitative analysis of extractable alkylphenol ethoxylates (APEO) in textiles and textile products.

This method provides several instrument options for the determination of alkylphenol ethoxylates (APEO) such as normal phase liquid chromatograph with mass spectrometer (NPLC/MS), normal phase liquid chromatograph with fluorescence detector (NPLC/FLD), normal phase liquid chromatograph with charged aerosol detector (NPLC/CAD) and normal phase liquid chromatograph with evaporative light scattering detector (NPLC/ELSD).

2 Normative references

There are no normative references in this document.

3 Terms and definitions

No terms and definitions are listed in this document.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at http://www.electropedia.org/

4 Principle

The test specimen is cut into small pieces, transferred to a sample vial and treated with methanol in ultrasonic water bath. The extract is filtered and collected. Subsequently, the collected extract is analysed by NPLC/MS, NPLC/FLD, NPLC/CAD or NPLC/ELSD.

5 Reagents

Unless otherwise specified, analytical grade chemicals shall be used.

5.1 Octylphenol ethoxylates (Triton®[1] X-100), (OPEO) CAS No.9002-93-1, Sigma Aldrich® Part No.T9284 (see NOTE in 5.2).

5.2 Nonylphenol ethoxylates (IGEPAL®[2] CO-630), (NPEO) CAS No.68412-54-4, Sigma Aldrich® Part No. 542334.

NOTE The mentioned brand names in 5.1 and 5.2 are given to improve the comparability of the test results amongst laboratories. Using another batch or another supplier could lead to different results.

5.3 Methanol (HPLC grade).

5.4 Acetonitrile (HPLC grade).

5.5 Acetone (HPLC grade).

5.6 Ammonium formate (HPLC grade).

5.7 Formic acid (HPLC grade).

6 Apparatus

6.1 General

Clean all glassware by rinsing with acetone (5.5) prior to use. Avoid detergents when washing labware.

6.2 Apparatus and auxiliaries for preparing the sample

6.2.1 Standard laboratory equipment.

6.2.2 Analytical balance with resolution of 0,01 g (for test specimen preparation).

6.2.3 Analytical balance with resolution of 0,001 g (for standard preparation).

6.2.4 40 ml Glass vial with screw cap (for sample pre-treatment).

6.2.5 Ultrasonic water bath (to be set up at (70 ± 5) °C).

6.2.6 Disposable syringe and membrane filter (with 0,45 µm pore size or less).

6.2.7 Glass vial (with septum cap for HPLC).

6.2.8 Solid phase extraction (SPE) cartridge for clean up (optional).

The cartridge is packed with a minimum of 200 mg of sorbent (reverse phase or silica).

6.2.9 Solid phase extraction (SPE) vacuum manifold (optional).

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[1] Triton® 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 ISO of this product.

[2] IGEPAL® 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 ISO of this product.

6.3 Chromatographic equipment

6.3.1 **High performance liquid chromatograph with mass spectrometer** (LC/MS), or

6.3.2 **High performance liquid chromatograph with fluorescence detector** (LC/FLD), or

6.3.3 **High performance liquid chromatograph with charged aerosol detector** (LC/CAD), or

6.3.4 **High performance liquid chromatograph with evaporative light scattering detector** (LC/ELSD).

6.3.4.1 **C18 short column for separation of OPEO and NPEO**.

Dimension 4,6 mm × 50 mm, Particle size 1,7 µm, equivalent is available.

6.3.4.2 Normal phase column for the separation of each APEO congener by the number of ethoxylate group.

Hydrogen bond adsorption column, dimension 4,6 mm × 150 mm, particle size 3 µm, equivalent is available.

7 Procedure

7.1 Standard preparation

The OPEO and NPEO are weighed accurately with analytical balance (6.2.3) and dissolved with methanol (5.3) containing 1 000 mg/l for stock solution. The reservation condition of solution is suggested to be in dark and less than 4 °C.

7.2 Mobile phase for HPLC

7.2.1 Methanol with 0,1 % formic acid and 0,01 % ammonium formate

Dissolve 0,1 g of ammonium formate (5.6) in ml of methanol (5.3), subsequently add 1 ml of formic acid (5.7).

7.2.2 Acetonitrile with 0,1 % formic acid

Add 1 ml of formic acid (5.7) in 1 000 ml of acetonitrile (5.4).

7.3 Sample preparation

The test specimen is cut into pieces with 5 mm × 5 mm and pieces are mixed homogeneously. The specimen is weighed 1 g to the nearest 0,01 g with analytical balance (6.2.2) and put into the 40 ml glass vial (6.2.4) for extraction.

7.4 Sample extraction

20 ml of methanol (5.3) are added to sample vial (see 7.3) and then ultrasonic extraction is performed in ultrasonic water bath (6.2.5) at 70 °C for 60 min. Afterwards the extract is cooled down to room temperature, 1 ml approximately 2 ml of extract is filtered into HPLC sample vial (6.2.7) using disposable syringe equipped with membrane filter (6.2.6). The HPLC sample vial is closed with cap immediately for further analysis.

7.5 Sample analysis

Qualitative and quantitative analysis of APEO is performed using LC/MS, LC/FLD, LC/CAD or LC/ELSD.

Congeners with 2 to 16 ethoxylate groups shall be used for quantification.

If the result is interfered by matrix, additional clean up procedure could be applied using SPE cartridge and the examples of SPE procedure are given in Annex C.

Guidelines for suitable chromatographic conditions and examples of chromatograms are given in Annex A and Annex B.

8 Calculation and calibration

8.1 Calculation of contribution percentage of each APEO congener in standard solution

It is necessary to confirm the composition ratio of APEO congeners (from APEO 2 to APEO 16) because the composition of congeners in standard mixture may be varied depending on batch or brand of standard. Use APEO reagents (5.1 and 5.2) to calculate the composition of each congener in standard mixture. Obtain a chromatogram of each peak and identify all congeners according to Annex A. Calculate the contribution percentage, R_i, of each congener according to Formula (1).

$$R_i = \frac{A_{is}}{A_t} \times 100 \tag{1}$$

where

R_i is the contribution percentage of each APEO congener in standard solution, in %;

A_{is} is the area response of each APEO congener in standard solution;

A_t is the total sum of APEO area responses (from APEO 2 to APEO 16) in standard solution.

8.2 Calculation of concentration of each APEO congener in standard solution

$$C_{is} = \frac{R_i}{100} \times C_s \tag{2}$$

where

C_{is} is the concentration of each APEO congener in standard solution, in mg/l;

R_i is the concentration percentage of each APEO congener in standard solution and calculated according to Formula (1);

C_s is the concentration of APEO standard solution, in mg/l.

8.3 Calculation of concentration of each APEO congener in sample

$$X_i = \frac{A_i \times C_{is} \times V \times DF}{A_{is} \times M} \tag{3}$$

where

X_i	is the concentration of each APEO congener in sample, in mg/kg;
C_{is}	is the concentration of each APEO congener in standard solution and calculated according to Formula (2) in mg/l;
A_i	is the area response of each APEO congener in sample extract solution;
A_{is}	is the area response of each APEO congener in standard solution;
V	is the final volume of extract solution, in ml;
M	is the mass of test specimen, in g;
DF	is the dilution factor.

8.4 Calculation of total concentration of all APEO congener in sample

Calculate the total concentration, X_t, of all APEO congener using Formula (4):

$$X_t = \sum X_i \tag{4}$$

where

X_t	is the total sum of concentration of each APEO congener in sample, in mg/kg;
X_i	is the concentration of each APEO congener in sample and calculated according to Formula (3), in mg/kg.

8.5 Calibration curve

Prepare a calibration curve including the concentration range of each APEO congener (from APEO 2 to APEO 16) to be determined. Calibration curves are prepared with at least three calibration points.

NOTE The concentration range of calibration curve is subject to change upon the need of each laboratory and equipment used.

For quantification, the calibration curve shall have a correlation coefficient greater than 0,995 (R^2 greater than 0,990).

9 Test report

The test report shall include the following information:

a) a reference to this document, i.e. ISO 18254-2:2019;

b) identification of sample and the date of analysis;

c) detection method and quantification method and name of all the standards used (NPEO and OPEO);

d) the result as total sum of each congener (from EO 2 to EO 16) of APEO (NPEO and OPEO are reported individually);

e) any deviation from the procedure specified and all circumstances that may have influenced the results.

Annex A
(informative)

Examples of chromatographic condition

A.1 Preliminary remark

As the instrumental equipment of the laboratories may vary, no generally applicable instructions can be provided for chromatographic analyses. The parameters given in this annex have been successfully tested and used.

A.2 Chromatographic conditions

A.2.1 Normal phase liquid chromatographic condition

Mobile phase 1: Acetonitrile with 0,1 % formic acid

Mobile phase 2: Methanol with 0,1 % formic acid and 0,01 % ammonium formate

Stationary phase: ZORBAX Eclipse plus C18®[3] (1,8 µm); (50 × 4,6) mm plus SHARC 1 (3,0 µm); (150 × 4,6) mm

Flow rate: 0,6 ml/min (flow gradient, see below)

Injection volume: 5 µl (MS), 50 µl (FLD, CAD, ELSD)

Gradient condition (1) for the separation of each APEO congener

Gradient:	Time [min]	Mobile phase 1 [%]	Flow [ml/min]
	0	98	0,6
	1	98	0,6
	15	51	0,6
	18	51	0,6
	19	20	0,6
	25	20	0,6

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[3] This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

Gradient condition (2) for the confirmation of OPEO and NPEO

Gradient:	Time [min]	Mobile phase 1 [%]	Flow [ml/min]
	0	60	0,6
	6	60	0,6
	10	20	0,6
	15	20	0,6

A.2.2 Detector condition

A.2.2.1 Mass spectrometer (MS)

Ionization mode: API-ES

Polarity: Positive

Acquisition mode: SIM/SCAN mode

Mass range: 150 amu approximately 1 500 amu

Nebulizer gas: Nitrogen

Sheath gas flow rate: 40 psi

Aux gas flow rate: 10 (arbitrary units)

Spray voltage: 4 kV

Capillary temperature: 300 °C

See Table A.1.

Table A.1 — Masses for the quantification of APEO in LC/MS

NPEO	m/z[M + NH_4]	OPEO	m/z[M + NH_4]
NPEO 2	326	OPEO 2	312
NPEO 3	370	OPEO 3	356
NPEO 4	414	OPEO 4	400
NPEO 5	458	OPEO 5	444
NPEO 6	502	OPEO 6	488
NPEO 7	546	OPEO 7	532
NPEO 8	590	OPEO 8	576
NPEO 9	634	OPEO 9	620
NPEO10	678	OPEO10	664
NPEO11	722	OPEO11	708
NPEO12	766	OPEO12	752
NPEO13	810	OPEO13	796

Table A.1 *(continued)*

NPEO	m/z[M + NH_4]	OPEO	m/z[M + NH_4]
NPEO14	854	OPEO14	840
NPEO15	898	OPEO15	884
NPEO16	942	OPEO16	928

A.2.2.2 Tandem mass spectrometer (MS/MS)

Ionization mode: API-ES

Polarity: Positive

Acquisition mode: SRM mode

Mass range: 150 amu approximately 1 500 amu

Nebulizer gas: Nitrogen (flow: 50 l/hr)

Nebulizer gas temperature: 300 °C

Capillary voltage: 3 000 V

Collision gas: Argon

Collision gas pressure: 1,0 mTorr

See Table A.2.

Table A.2 — Masses for the quantification of APEO in LC/MS/MS

NPEO	m/z (Q1)	m/z (Q3)	Collision energy (eV)	OPEO	m/z (Q1)	m/z (Q3)	Collision energy (eV)
NPEO 2	326	183	12	OPEO 2	312	183	12
NPEO 3	370	227	12	OPEO 3	356	227	13
NPEO 4	414	271	15	OPEO 4	400	271	14
NPEO 5	458	315	16	OPEO 5	444	315	17
NPEO 6	502	359	18	OPEO 6	488	359	18
NPEO 7	546	291	23	OPEO 7	532	515	16
NPEO 8	590	291	23	OPEO 8	576	560	22
NPEO 9	634	291	26	OPEO 9	620	277	24
NPEO 10	678	291	30	OPEO 10	664	277	26
NPEO 11	722	291	30	OPEO 11	708	277	31
NPEO 12	766	291	30	OPEO 12	752	277	30
NPEO 13	810	291	32	OPEO 13	796	277	32
NPEO 14	854	291	34	OPEO 14	840	277	34
NPEO 15	898	291	34	OPEO 15	884	277	32
NPEO 16	942	291	35	OPEO 16	928	277	38

A.2.2.3 Fluorescence detector (FLD)

Wavelength: 277 nm for excitation and 314 nm for emission

A.2.2.4 Charged aerosol detector (CAD)

Full scale range of corona: 100 pA

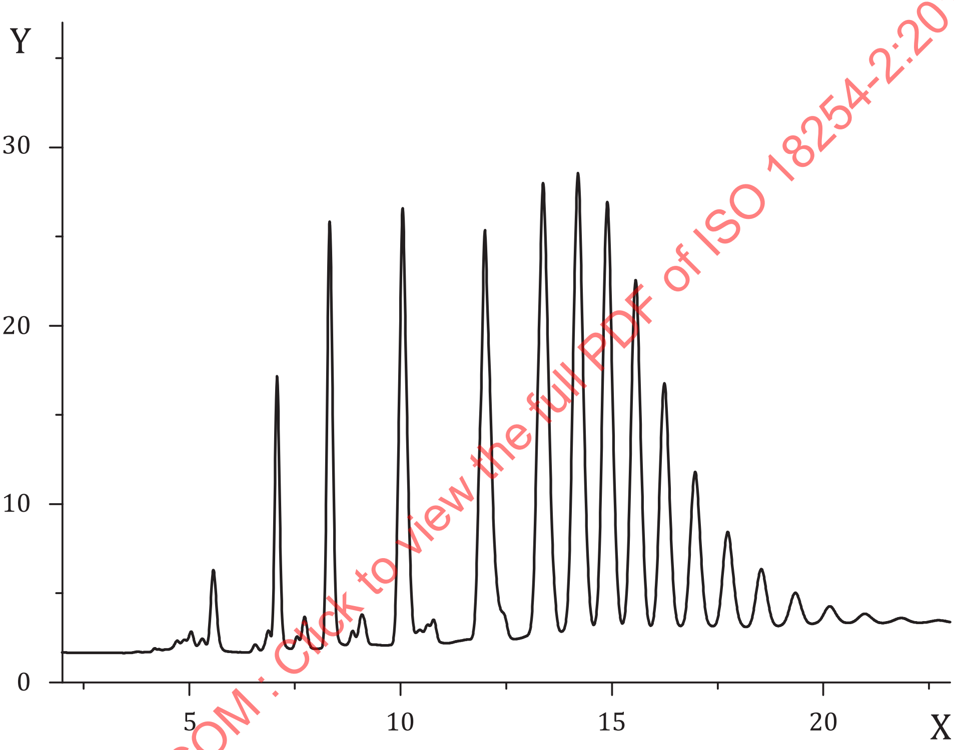
Nebulizer gas temperature: 30 °C

A.2.2.5 Evaporative light scattering detector (ELSD)

Gas flow: Nitrogen (flow: 1,5 l/min)

Nebulizer gas temperature: 50 °C

See Figures A.1 to A.5.

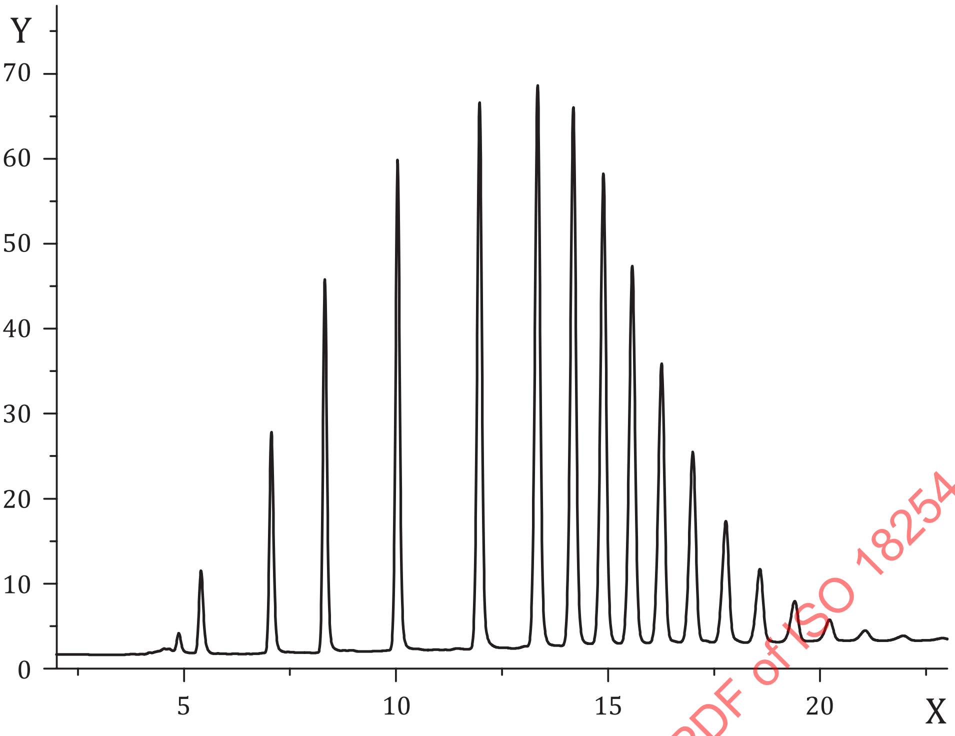


Key

X retention time, min

Y abundance

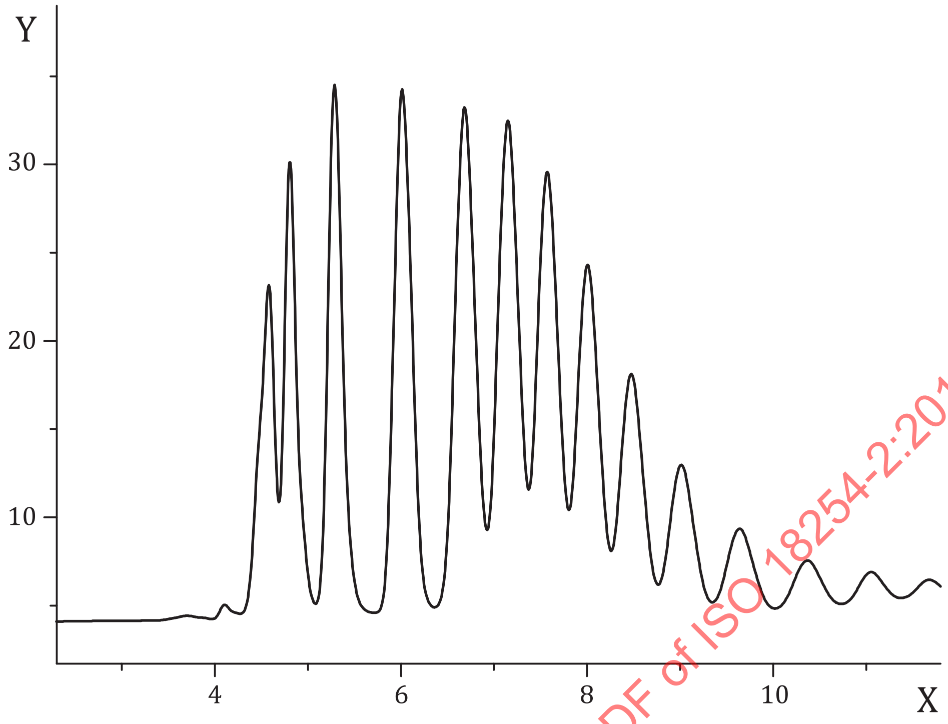
Figure A.1 — FLD chromatogram of NPEO using gradient condition (1)



Key

X	retention time, min
Y	abundance

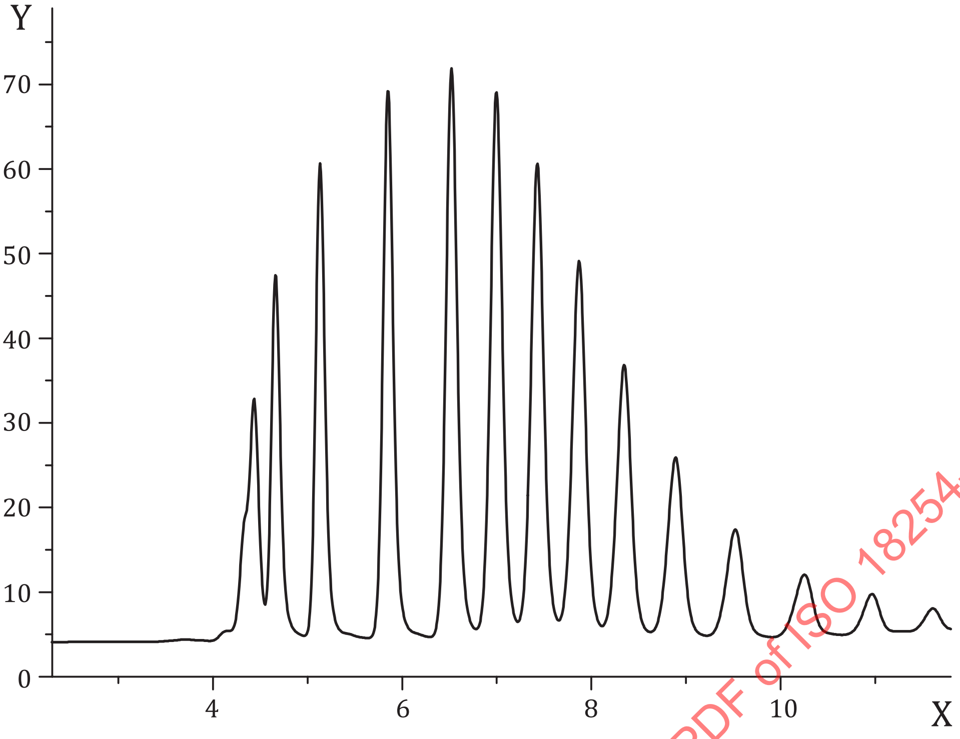
Figure A.2 — FLD chromatogram of OPEO using gradient condition (1)



Key

X	retention time, min
Y	abundance

Figure A.3 — FLD chromatogram of NPEO using gradient condition (2)

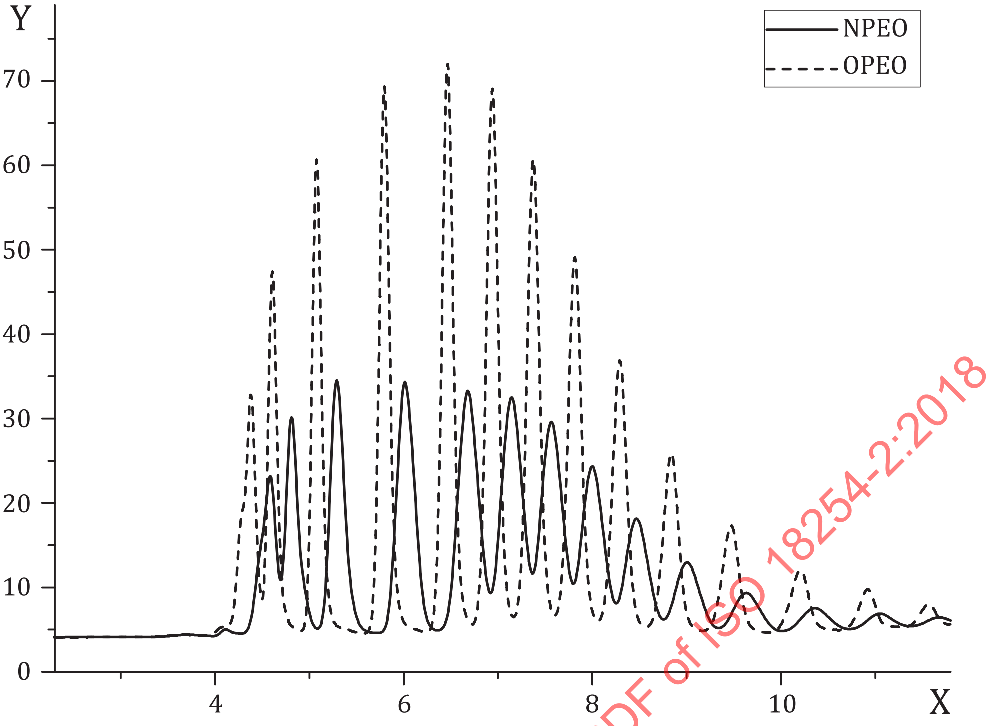


Key

X retention time, min

Y abundance

Figure A.4 — FLD chromatogram of OPEO using gradient condition (2)



Key

X retention time, min

Y abundance

Figure A.5 — Overlay of FLD chromatograms of OPEO and NPEO using gradient condition (2)

Annex B
(informative)

Examples of solid phase extraction (SPE) procedure for clean up

B.1 General

In general, sample extract is analysed without clean up procedure, however if the result is interfered by the matrix in the sample extract, the following clean up procedure could be applied.

The following procedures are described for commercially available 6 ml SPE cartridge packed with 500 mg of sorbent based on silica and on co-polymer of divinylbenzene – vinyl pyrrolidinone. Similar sorbent material is applicable and the manufacture's guidance for other material of the SPE cartridge shall be preferred.

B.2 Procedure for silica sorbent

After the procedure of sample extraction, take 10 ml of sample extract and concentrate to dry with vacuum concentration equipment and then dissolve the residue with 3 ml of hexane – ethyl acetate (1:1) solution.

Condition the sorbent of SPE cartridge with 5 ml of hexane – acetone (4:1) solution, followed by 5 ml of hexane – ethyl acetate (1:1) solution.

Apply the dissolved sample residue solution thoroughly into SPE cartridge with controlling flowing rate about 1 ml/min.

Wash the SPE cartridge with 5 ml of hexane – acetone (4:1) solution and then drain the solution to dry using SPE vacuum manifold for 10 min.

Elute with 5 ml of methanol and collect the eluate for further analysis.

B.3 Procedure for divinylbenzene — Vinyl pyrrolidinone co-polymer sorbent

After the procedure of sample extraction, take 10 ml of sample extract and concentrate to dry with vacuum concentration equipment and then dissolve the residue with 3 ml of methanol – water (3:2) solution.

Condition the sorbent of SPE cartridge with 5 ml of methanol – dichloromethane (1:4) solution, followed by 5 ml of methanol and then 5 ml of water.

Apply the dissolved sample residue solution thoroughly into SPE cartridge with controlling flowing rate about 1 ml/min and then drain the solution to dry using SPE vacuum manifold until the residual water is removed.

Elute with 5 ml of methanol – dichloromethane (1:4) solution and collect the eluate.

Concentrate the eluate using gentle flow of nitrogen and then dissolve the residue with 2 ml of methanol for further analysis.