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Liquid chromatography at critical conditions (LCCC) — Chemical heterogeneity of polyethylene oxides

Chromatographie liquide aux conditions critiques — Hétérogénéité chimique des oxydes de polyéthylène

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Contents

Page

Foreword

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This document was prepared by Technical Committee ISO/TC 35, *Paints and varnishes*.

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

Since the first description of liquid chromatography at critical conditions (LCCC) in 1986 (see Reference [1]), the method has been continuously refined and has proved itself to be indispensable for polymer characterisation. Separation is required not only for the quantitative analysis of the individual species. It also offers the preconditions for qualitative characterisation of the fractions by means of spectroscopic and spectrometric techniques. The key factor here is the reduction of the polydispersity/chemical heterogeneity within a fraction, which represents a large problem for mass-spectrometric investigations.

The method has been described extensively in professional circles over the last two decades for different polymer systems, see References [2] to [9].

Within the framework of the Technical Committee, the extent that the method supplies consistent results for a simple, chemically heterogeneous polymer mixture was clarified as part of interlaboratory testing.

At this time, necessary experience relating to the selection of the system (interaction between the polarities separation phase/eluent/sample) was not expected of any of the participating laboratories.

The interlaboratory testing has shown that, even with a well-characterized system and with specification of all pertinent system parameters, it has to date not been possible to classify the process as a routine method in laboratories with experience in polymer analytics.

The idea presents itself of offering a validation kit (polymer mixture with the expecting separation result).

Liquid chromatography at critical conditions (LCCC) — Chemical heterogeneity of polyethylene oxides

1 Scope

This document establishes a valid method for separation of chemically heterogeneous polyethylene oxide (PEO) mixtures and for the determination the number and content of the chemically heterogeneous species in the overall sample.

The method presented in this document serves as a technical guideline and enables laboratories to learn the principle of "critical chromatography" on a validated system.

This method presented in this document with its stated system parameters is not applicable for other polymer classes, due to the diversity of the interactions between the polymer/mobile phase/stationary phase and the number of separation systems that are therefore available.

The evaluation of the interlaboratory testing has shown that many error sources relate to the technique of liquid chromatography in general. Possible error sources are described in Annex A.

Details on the evaluation of the interlaboratory testing are given in Annex B.

Elugrams of the participants (excerpts) are given in Annex C.

Investigations of the long-term stability of the test mixture are given in Annex D.

2 Normative references

There are no normative references in this document.

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

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

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

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

3.1
liquid chromatography at critical conditions
LCCC
special form of liquid chromatography of polymers at the point of adsorption, where chemically and structurally identical polymers with a certain repeat unit elute independently of the molar mass at the same retention time

Note 1 to entry: The individual monomer units do not contribute to the retention. Under these determined system parameters (defined combination of separation column/eluent mixture/temperature), a separation of polymer mixtures of the same repeat unit takes place based on chemical heterogeneity. Chemical heterogeneities can take the form of different functional groups, end groups, differences in the microstructure (e.g. copolymers and their composition) as well as topological differences (e.g. branching).

4 Principle

In the following, the method for the separation of polyethylene oxides at critical conditions of adsorption is described for the ethylene oxide repeat unit.

Three polyethylene glycols (PEG) with different molar masses (1 mg/ml to 2 mg/ml dissolved in the relevant eluent mixture (see 7.1) are measured, starting with a high proportion of the thermodynamically good solvent B (in this case acetonitrile). The eluent mixture is successively changed by increasing the proportion of component A (in this case water). This is followed by measurements in different compositions until all three standards elute independently of the molar mass at the same retention time. The determined critical solvent composition (csc) corresponds to the critical conditions.

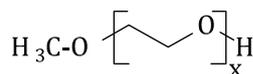Afterwards, the unknown mixtures are dissolved in this eluent mixture and measured.

From the peaks of the resulting chromatograms, the number of species with different functionalities and their relative content (taking into account the detection properties) can be determined.
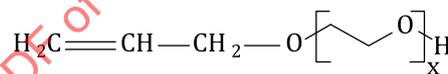
Species contained in the mixtures:

(1) Polyethylene glycol
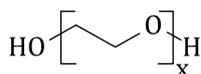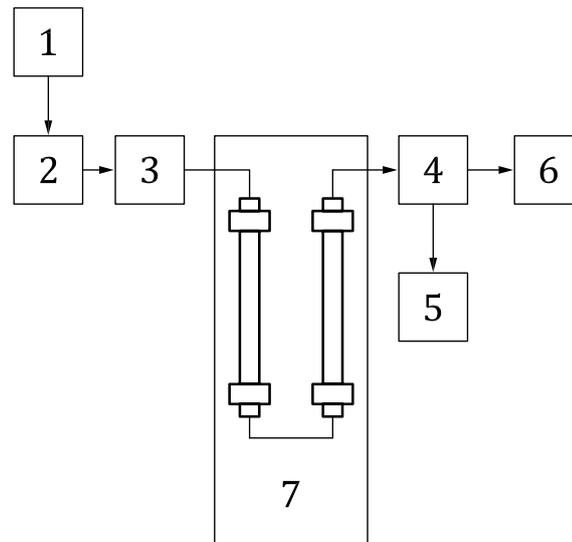
(2) Methylene polyethylene glycol

(3) Polyethylene glycol monoallyl ether

$$HO \left[\overset{O}{} \right]_x H \qquad H_3C\text{-}O \left[\overset{O}{} \right]_x H \qquad H_2C = CH - CH_2 - O \left[\overset{O}{} \right]_x H$$

5 Apparatus

5.1 General

The apparatus shall consist of the components shown in Figure 1, which are described in more detail below.

Key

1 LCCC mobile phase
2 pump, 0,1 ml/min to 2 ml/min
3 injection valve, autosampler
4 detectors: RI, ELSD, corona
5 data processing
6 waste
7 separation columns/column temperature control

Figure 1 — LCCC apparatus

All components shall come into contact with the eluent or the sample solution are resistant to them and do not exhibit any adsorption or memory effects. The individual modules should be generally connected with steel capillary tubes for polymer analytics.

5.2 Eluent supply

The eluent reservoir shall adequately protect the eluent against external influences such as the atmosphere and light, if necessary by means of a blanket of inert gas above the liquid level.

The eluent reservoir shall contain a sufficient quantity of the eluent to bring the apparatus to equilibrium and to carry out several repeat analyses.

The eluent shall be degassed either before it is introduced into the reservoir, or by use of a device fitted between the reservoir and the pump, to prevent malfunctions of the pump or the formation of bubbles in the detector. The method of degassing used (e.g. bubble trap, online purging with helium or vacuum degassing) is open to choice, but shall be stated in the test report.

For polymer mixtures that contain chromophoric groups, other detectors may be used, e.g. UV or IR detectors.

5.3 Pump

The pump ensures that the eluent flow through the separation columns is as smooth and pulse-free as possible. The flow rate shall be 0,5 ml/min to 1 ml/min, depending on the dimensions of the columns used. To fulfil these requirements, the pump shall operate at optimum efficiency at this flow rate and at the counterpressure established in the process. The variation in the flow rate of the pump used may have a variation of max. 0,1 %.

5.4 Injection system

The injection system serves to introduce into the chromatographic separation system an exactly specified amount of the sample solution (5 µl to 25 µl) in an eluent interval that is delimited as accurately as possible. This introduction may be carried out either manually or automatically.

When filling the sample loop with sample solution and subsequently introducing the sample solution into the eluent stream, the volume of liquid used shall be great enough to ensure that, even if laminar-flow effects occur, the sample loop is completely filled with the sample solution and subsequently completely flushed out.

Memory effects from the previous sample solution in the injection system shall be avoided by design or by adequate flushing.

5.5 Separation columns

The separation system consists of one or more columns connected in series, which are packed with a spherical (d < 10 µm) and porous separation material, the diameter of the pores corresponding to the size of the polymer molecules being analysed (80 Å to 300 Å for this method).

Depending on the molar mass of the samples under investigation, the pore size of the separation material may need to be adjusted accordingly.

At this point in the method, the separation material consists of a nonpolar/hydrophobic stationary phase (octadecyl-modified silica gel, reversed phase, C18). The hydrophobic properties of the material should have been further boosted by end-capping with trimethyl chlorosilane. The C-content thus obtained is ≫10 %.

To meet the objective of this document of obtaining results that agree as well as possible in different laboratories using different LC apparatus with the same sample, it is necessary to adhere to the minimum requirements specified below with regard to peak broadening (expressed in terms of a number of theoretical plates) and separation efficiency; the actually obtained values shall be stated in the test report.

Number of theoretical plates

The number of theoretical plates N shall be determined, for the apparatus used per metre of column used, from the peak width at half height. Inject up to 20 µl of a solution of the PEG 1 500 standard (min. mass concentration 1 mg/ml) on to the column, and evaluate the chromatogram obtained under the same conditions as are used for analysing polymers, using Formula (1):
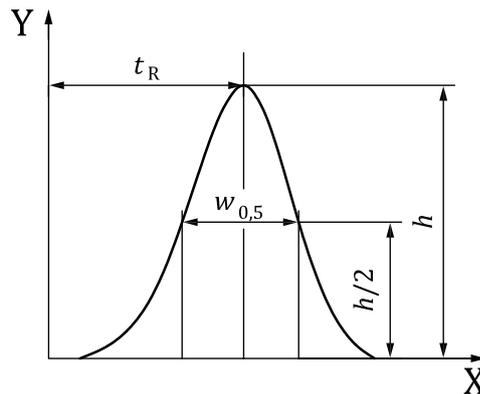
$$N = 5,54 \left(\frac{t_R}{w_{0,5}} \right)^2 \frac{100}{L} \tag{1}$$

where

t_R is the retention time at the peak maximum (the same units shall be used, e.g. min);

$w_{0,5}$ is the peak width at half height (see Figure 2);

L is the length of the column (column combination), in cm.

Key

X   retention time, in minutes

Y   detection signal

t_R retention time at the peak maximum, in minutes

$w_{0,5}$ peak width at half height of the peak

h maximum peak height

$h/2$ half peak height

Figure 2 — Determination of the number of theoretical plates by the half-height method

The result is expressed as the number of theoretical plates per metre of column length. A column (column combination) should be used that achieves at least 5 000 theoretical plates per column (column combination) used.

5.6 Column temperature control

The analyses can be performed in a temperature range from 20 °C to 45 °C. The temperature of the column shall not change by more than 0,5 °C during the analysis. All analyses shall be performed at the same temperature, as the LCCC is an enthalpic process and therefore temperature-dependent[1].

5.7 Detectors

The following can be used as detectors: RID (refractive index detector), ELSD (evaporative light scattering detector) or Corona CAD (charged aerosol detector).

5.8 Eluent

All eluents that are used should have the purity "HPLC grade". The eluents should not be returned to the eluent reservoir.

5.9 Data acquisition

The signals from the detector are recorded by means of an electronic data system.

6 Sample preparation

Dissolve the samples (1 mg/ml to 2 mg/ml) in the relevant eluent. To rule out changes to the polymers in solution as a function of the time as a possible source of error, the samples should be analysed within 6 h of production of the solution.

Fill up the validation kit (mixture 2) with 2 ml of the critical solvent composition.

7 Performance of the measurements

## 7.1 Determination of the critical conditions

The critical conditions can be set up either by changing the temperature or by changing the composition of the mobile phase.

In the following procedure, keep the temperature strictly constant, and successively change the solvent composition with decreasing acetonitrile content. This can be done via separate mixing of the components or with a binary pump.

Measure the three standards with different molar masses in the relevant chosen solvent composition. At this point this this method, it is very important to make sure that the solvent in which the standard is dissolved also complies with the eluent composition.

The critical solvent composition (ACN : H_2O) is attained when all molecules of the same chemical structure (in this case PEG standards) elute at the same retention time. This means that, under these conditions, the chromatographic behaviour is no longer determined by the size of the molecule, but by chemical inhomogeneity.

To determine the critical solvent composition, plot $\lg M_p$, in g/mol, over the retention time, in min, ([Figure 3](#), left), or the retention factor k, over the content of component B in the eluent, in % ([Figure 3](#), right).

a) Logarithm of molar mass over retention time   b) Retention factor over content of component B in the eluent
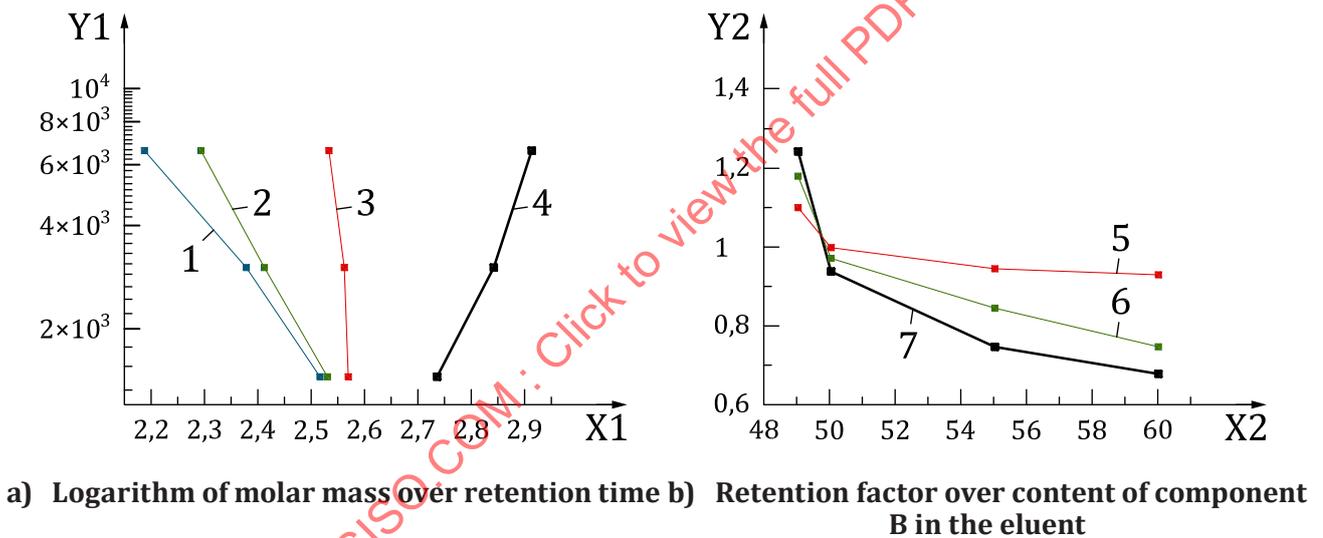
Key

X1   retention time, in minutes
Y1 $\lg M_p$, in grams per mole
X2   ACN, as volume fraction in per cent
Y2   retention factor k
1   ACN / H_2O: 60 % / 40 %
2   ACN / H_2O: 55 % / 45 %
3   ACN / H_2O: 50 % / 50 %
4   ACN / H_2O: 49 % / 51 %
5   PEG 1460
6   PEG 3020
7   PEG 6600

Figure 3 — Schematic representation of the determination of the critical solvent composition

The retention factor k is calculated using Formula (2):

$$k = \frac{(t_R - t_0)}{t_0}$$

(2)

where

t_R is the retention time of the standard in the relevant system;

t_0 is the retention time of a non-delayed substance.

7.2 Analysis of the validation kit

Mix the sample with 2 ml of the critical solvent mixture and dissolve it at room temperature under shaking.

To rule out changes to the polymers in solution as a function of the time as a possible source of error, the samples should be analysed within 6 h of production of the solution. Ensure that the temperature in the column remains constant.

The injection volume shall be matched to the set of columns used and can therefore be varied between 5 μl and 25 μl.

It is recommended to conduct a blind test (injection of the eluent/the pure sample solvent) before measuring the sample in order to identify solvent signals or peaks that are caused by the injection.

The measurement of the sample ends after complete elution of the last system peak and after the signal returns to the base line.

Conduct three repeat measurements and calculate the mean values of the retention time for the separated peaks.

Calculate the chromatographic resolution of two peaks R as a quality criterion for the separation using Formula (3):

$$R = 1{,}18 \times \frac{(t_{R2} - t_{R1})}{w_{f,hm,1} + w_{f,hm2}}$$

(3)

where

t_R is the retention time;

$w_{f,hm}$ is the full width of the peak at half maximum.

8 Test report

The test report shall contain at least the following information:

a) all details necessary for complete identification of the product tested;

b) a reference to this document (ISO/TS 23973:2020);

c) all information about the apparatus (pump, degasser, injection system, column oven, detectors, evaluation software);

d) all information about the separation columns (manufacturer, separation material, pore size type, grain size, number, dimensions, C-content);

e) the number of theoretical plates per column combination in the LC apparatus used;

f)   the column temperature;

g)   the flow rate;

h)   the critical solvent composition, in per cent, (mass fraction or volume fraction) with statement of the method of mixing (gradient pump or separate production of the mixtures);

i)   the concentration of the injected solution, in milligrams per millilitre (for samples to be weighed in by yourself);

j)   the injection volume, in microlitres;

k)   the overlay of the elugrams of the three PEGs with different molar masses;

l)   the validation kit surface percentage report, including chromatogram;

m)   a statement of the mean values of the retention time;

n)   a statement of the chromatographic resolution of the peaks R_{AB}, R_{AC} and R_{BC} according to Reference [3];

o)   the result of the test;

p)   any deviation from the method specified;

q)   any unusual features (anomalies) observed during the test;

r)   the date of the test.

Annex A
(informative)

Error sources

The evaluation of the interlaboratory testing has shown that many error sources can not only be attributed specifically to a lack of experience in terms of the chromatography of polymers, but that they relate to the technique of liquid chromatography in general.

As is well known, the resolution R depends on the separation factor α (for selectivity, measure of the separation capability of the chromatographic system), the retention factor k (for capacity, measure of the strength of the interactions) and on the number of plates N (for efficiency, measure of the band broadening of the substance zone).

If the result does not correspond to the expectations, then the following aspects should be considered:

a) Have the column dimensions and packaging material been ideally chosen?

Improvement of the resolution via the number of plates:

— column as long as possible;

— particles as small as possible;

— optimal flow rates;

— determination of the adsorption range and exclusion rate for the selected column;

— determination of the number of plates (>25 000/m);

— adjustment of the pore size to the largest molar mass to be analysed (e.g. >100 Å for molar masses above 6 000 g/mol).

b) Is the column contaminated/changed due to previous injections, is the specification no longer met?

This can alter the selectivity, and this influences the resolution more strongly than the number of plates (separation factor α).

c) Are the solvent signals and system peaks overlaying the peaks of the analyte (particularly when using RI [and UV] detectors)?

d) Have the rinsing times been complied with during the change in rinsing to new solvent compositions (reference cell rinsing!)?

e) Do the internal diameters and lengths of the capillary tubes between the column and detector meet the requirements (maximum internal diameter of 0,12 mm)?

f) Are there dead volumes (particularly in the case of detectors connected in series)?

g) Has the sample actually been analysed in the current eluent mixture?

Annex B
(informative)

Evaluation of the interlaboratory testing

B.1 Participants in the interlaboratory testing

The 17 laboratories who have experience with HPLC and polymers as the substance class under investigation took part in the interlaboratory testing.

B.2 System parameters for performance of the interlaboratory testing that were made available to the participants

The samples are given in Table B.1.

Table B.1 — Samples

Designation	Type	Molar mass g/mol	Composition
S 1	PEG 1 500 Standard	1 500	
S 2	PEG 3 000 Standard	3 000	
S 3	PEG 6 000 Standard	6 000	
A 2	PEO-A	3 000	Allyl
M 2	PEO-M	5 000	Methoxy
Mi 1	PEO-X	Unknown	Unknown
Mi 2	PEO-X	Unknown	Unknown

a) Polyethylene glycol standards in the molar mass range from 1 500 g/mol to 6 000 g/mol for determination of the critical conditions of adsorption (S 1 to S 3).

b) Polyethylene oxides with different functionalities (A 2 and M 2), as well as their mixture for separation under critical conditions and for quantification of the components (Mi 1 and Mi 2).

NOTE   The sample solutions A 2, M 2 and mixture 1 needed to be manufactured by the participants (weighing, dissolution). Mixture 2 was filled up with 2 ml of the eluent and was then available for measurement.

Columns: RP 18, <300 Å, <1 µm

Column temperature: (45 ± 1) °C

Eluent: acetonitrile / water (*volume fraction* or *mass fraction*)

Flow rate: 0,5 ml/min or 1 ml/min

Detection: ELSD, Corona, RI

Injection volume: 10 µl to 20 µl

Sample concentration: around 1 ml/ml to 2 mg/ml

The samples were dissolved in the relevant eluent (mixture 2 + 2 ml eluent). To rule out changes to the polymers in solution as a function of the time as a possible source of error, the samples were analysed

within 6 h of production of the solution. It was ensured that the temperature in the column remained constant.

For the determination of the critical solvent composition, the plot M_p, in g/mol, over V_e, in ml, was chosen.

The chromatographic resolution of two peaks R was calculated from Formula (B.1):

$$R = 1{,}18 \times \frac{(t_{R2} - t_{R1})}{w_{f,hm,1} + w_{f,hm2}} \tag{B.1}$$

where

t_R is the retention time;

$w_{f,hm}$ is the full width of the peak at half maximum.

Presentation of the results:

a) exact information about the column parameters (including C-content and number of plates in the column);

b) critical solvent composition, in %, with statement of the method of mixing (gradient pump or separate production of the mixtures);

c) overlay of the chromatograms of the samples S 1 to S 3, A 2 and M 2;

d) samples Mi 1 and Mi 2 surface percentage report, incl. chromatogram; three independent weighed portions for Mi 1;

e) statement of the chromatographic resolution of the peaks in the mixtures Mi 1 and Mi 2 according to Reference [1].

B.3 Evaluation of the interlaboratory testing

All 17 participants have determined critical conditions for adsorption for PEO. The parameters are summarised in Table B.2.

The results for mixture 1 are given in Table B.3.

The results for mixture 2 are given in Table B.4.

The mean values and standard deviation for the 3 main components of mixture 2 are given in Table B.5.

Table B.2 — Compilation of the parameters of the participants

Partici-pant	Column parameters (C18)	CMC ACN/H₂O %	Detector RI	Column temperature °C	Number of plates in the column
1	Zorbax Eclipse XDB 80 Å, 5 µm, 150 × 4,6 mm 10 %	50,4/49,6 (*volume fraction*) mixed separately	ELSD	45	5 920
2	Waters, XBridge Shield 140 Å, 3,5 µm, 150 × 4,6 mm 16,7 % C	48,3/51,7 (*volume fraction*) quatro gradient pump	ELSD	80	5 120

Table B.2 *(continued)*

Partici-pant	Column parameters (C18)	CMC ACN/H₂O %	Detector RI	Column temperature °C	Number of plates in the column
3	Nucleosil 100 Å, 5 µm, 250 × 4,6 mm 18 %	53,0/47,0 (volume fraction) gradient pump	ELSD	45	5 320
4	YMC ODS A 120 Å, 5 µm, 250 × 4,6 mm 17 %	52,7/47,3 (volume fraction) gradient pump	ELSD	45	4 068
5	Nucleosil 100 Å, 5 µm, 250 × 4 mm	93,0/7,0 (volume fraction) 49,5/50,5 (volume fraction) gradient pump	ELSD	n/a	4 000
6	Nucleosil 300 Å, 5 µm, 250 × 4 mm	62,5/37,5 (volume fraction) bin. gradient pump	ELSD	n/a	1 920
7	Zorbax Eclipse XDB 80 Å, 5 µm, 150 × 4,6 mm	40,0/60,0 (volume fraction) mixed separately		25	—
8	YMC ODS A 120 Å, 5 µm, 250 × 4,6 mm 17 %	42,0/58,0 (volume fraction) gradient pump	ELSD	ambient	—
9	Nucleosil 100 Å, 5 µm, 125 × 4 mm	55,0/45,0 (volume fraction) gradient pump	ELSD	44	—
10	Zorbax Eclipse XDB C18 80 Å, 5 µm, 150 × 4,6 mm 10 %, 2×	44,4/55,6 (mass fraction) mixed separately	RI	45	4 300
11	Nucleosil (M&N) C18 120 Å, 5 µm, 250 × 4 mm 11 % C	54/46 (volume fraction) gradient pump	RI	45	330
12	Nucleosil (Knauer) C18 100 Å, 5 µm, 125 × 4 mm	56,0/44,0 (volume fraction) gradient pump	ELSD	44	1 080
13	Zorbax Eclipse XDB C18 80 Å, 5 µm, 150 × 4,6 mm 10 %, 2×	42,8/57,32 (mass fraction) mixed separately	ELSD	45,3	14 100
14	YMC ODS A 120 Å, 5 µm, 250 × 4,6 mm 17 %	50/50 (volume fraction) gradient pump	ELSD	45	9 750
15	YMC ODS A 120 Å, 5 µm, 250 × 4,6 mm 17 % C	49,8/50,2 (volume fraction) gradient pump	ELSD	45	11 375

Table B.2 *(continued)*

Partici-pant	Column parameters (C18)	CMC ACN/H$_2$O	Detector	Column temperature	Number of plates in the column
		%	RI	°C	
16	Zorbax Eclipse XDB 80 Å, 5 µm, 150 × 4,6 mm 1×	50,3/49,7 (volume fraction) gradient pump	RI	45	3 066
17	Nucleosil C8 120 Å, 5 µm, 250 × 4 mm 6,5 %	50/40 (volume fraction) gradient pump	RI	45	2 770

In the following, it is shown with the aid of examples which results should be obtained from the investigations.

Figure B.1 shows the elution curve for mixture 1 (left) and mixture 2 (right) at critical conditions for adsorption for PEO.

Mixture 1 contained PEO with methoxyl functionalisation and PEG in a quantity below 5 %.

For mixture 2, separation of 4 species was expected: (1) PEG, (2) PEO with methoxyl functionalisation, (3) PEO with allyl functionalisation, (4) unknown constituent below 2 %.

a) Elution curve for mixture 1   b) Elution curve for mixture 2

Key
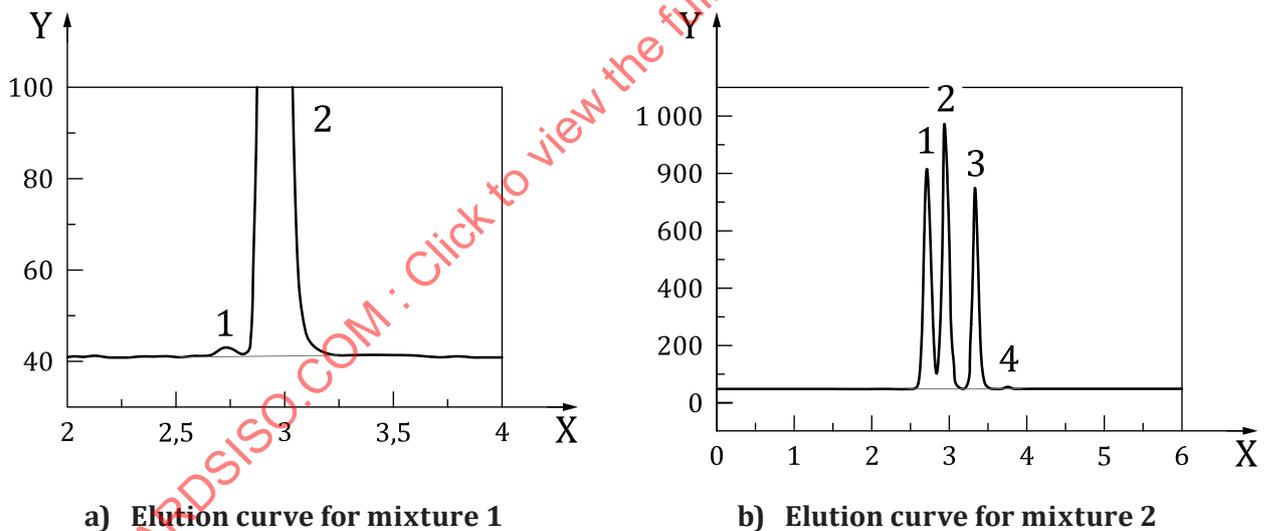
X   retention time, in minutes

Y   intensity

1   PEG

2   PEO-M

3   PEO-A

4   unknown

Figure B.1 — Elution curve for mixtures

Table B.3 — Results for mixture 1

Participant	A (PEG) % proportion of area	B (PEO-M) % proportion of area	R_{AB}	Detector
1	4,59	95,41	1,74	RI
2	0,71	99,29	1,37	ELSD
3	0,8	99,2	—	ELSD
4	0,45	99,55	1,45	ELSD
5	0,8	99,2	1,53	ELSD
6	—	100,0	—	ELSD
7	—	100,0	—	RI
8	—	100,0	—	ELSD
9	One peak with strong tailing			ELSD
10	1,58	98,42	1,23	RI
11	0	95,30	0	RI
12	0	100,0	0	ELSD
13	0,75	99,25	1,45	ELSD
14	0,20	98,60	1,72	ELSD
15	1,02	98,70	2,00	ELSD
16	0	100,00	0	RI
17	0	100,00	0	RI

Table B.4 — Results for mixture 2

Partici-pant	A (PEG) % proportion of area	B (PEO-M) % proportion of area	C (PEO-A) % proportion of area	Unknown % proportion of area	R_{AB}	R_{AC}	Detector
1	36,3	34,67	27,34	1,68	1,31	2,73	RI
2	35,31	37,17	27,19	0,41	1,07	3,24	ELSD
3	36,5	37,9	25,1	0,4	1,63	4,29	ELSD
4	34,03	38,56	26,88	0,53	1,39	2,52	ELSD
5	36,1	37,9	25,7	0,3	1,23	3,44	ELSD
6	—	51,35	48,65	—	—	0	ELSD
7		54,7	45,3	—	—	0	RI
8	7,31	70,37	21,96	—	—	0	ELSD
9	One peak, suggestion of two components				0	—	ELSD
10	35,66	38,23	24,34	1,77	1,06	3,41	RI
11 (FIT)	Peak with shoulder			0	0	0	RI
12	One peak, tailing			0	0	0	ELSD
13	37,76	39,65	22,6	0	1,66	5,07	ELSD
14	36,7	37,87	24,7	0,47	1,44	4,45	ELSD
15	36,45	36,1	26,6	0,58	1,7	5,0	ELSD
16	36,5	38,01	24,3	1,18	0,81	2,12	RI
17	40,5	31,5	28,0	1,09	1,09	1,74	RI

Of 17 participants, 8 were unable to identify the present PEG (marked in <u>Figure B.2</u> at the top with a box). For 9 participants a mean value of 1,2 % with a standard deviation of 1,3 was calculated.
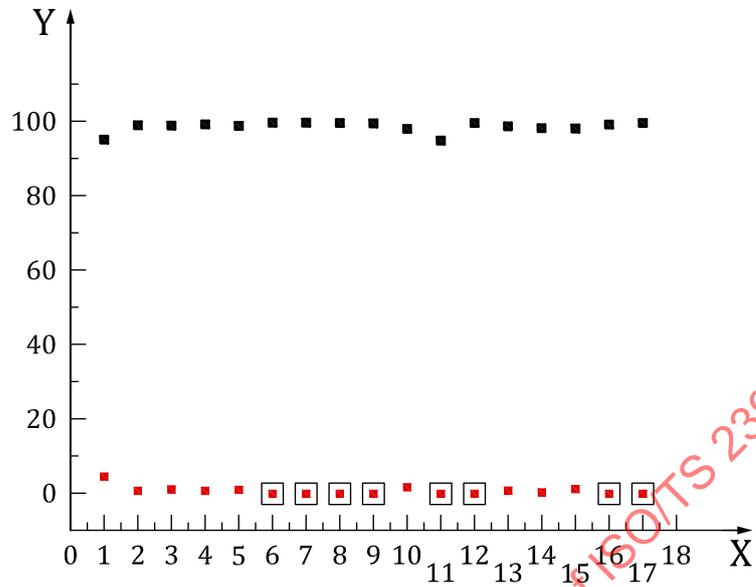
a) Results in % for the detected components in mixture1
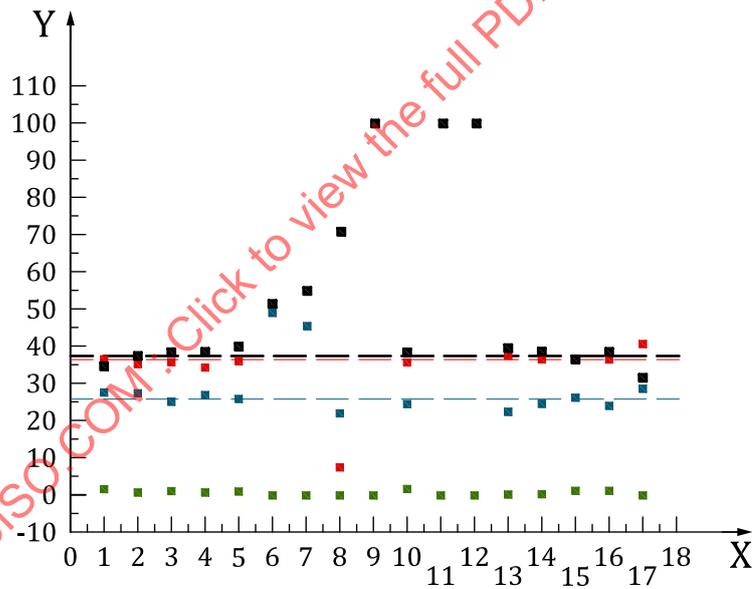
b) Results in % for the detected components in mixture 2

Key

X   participant

Y   proportion, as a percentage

■   PEG

■   PEO-M

■   PEO-A

■   unknown

_ _   PEG mean value

▬ ▬   PEO-M mean value

_ _   PEO-A mean value

**Figure B.2 — Results in % for the detected components
(evaluation for 11 of 17 participants, without participants 6, 7, 8, 9, 11 and 12)**

Table B.5 — Mean values and standard deviation for the 3 main components of mixture 2

	Mean value %	Standard deviation
PEG	36,5	1,6
PEO-M	37,2	2,4
PEO-A	25,7	1,7

The best separation was achieved by participants 13, 14 and 15 (hatched area in Figure B.3). But sufficient separation was also recorded for numbers of plates >5 000/column (participants 1, 2 and 3).
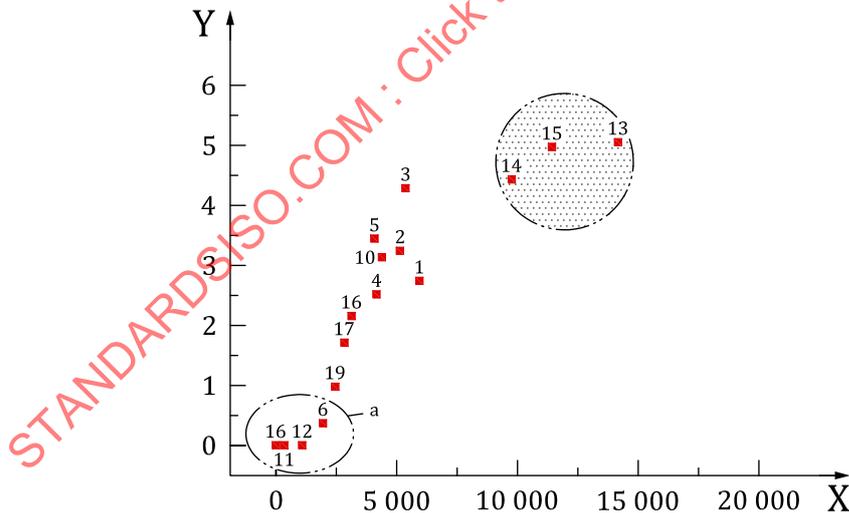
Key

X   number of plates/column

Y $R_{\text{PEG/PEO-A}}$

a   Column no longer complies with the given specification, capillary dimensions, column dimensions, no critical conditions.

Figure B.3 — Dependency of the resolution $R_{\text{PEG/PEO-A}}$ on the number of plates in relation to the column length used

Annex C
(informative)

Elugrams of the participants (excerpts)

Figures C.1 to C.17 show the elugrams of the participants 1 to 17 of the interlaboratory test.
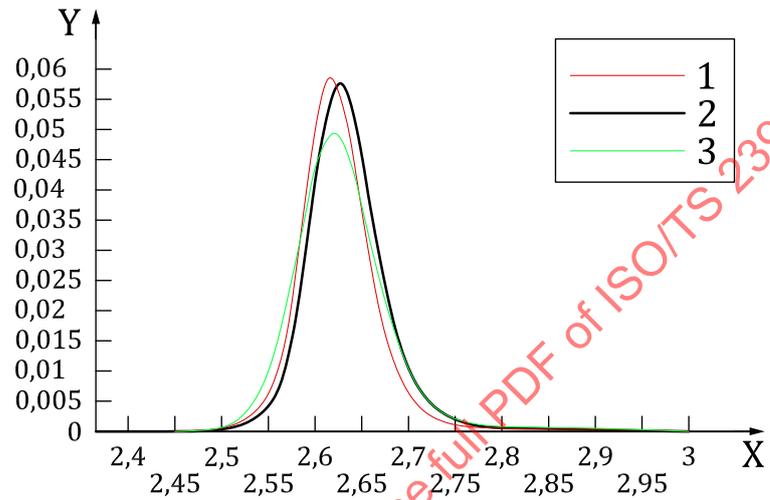
a) S 1 to S 3
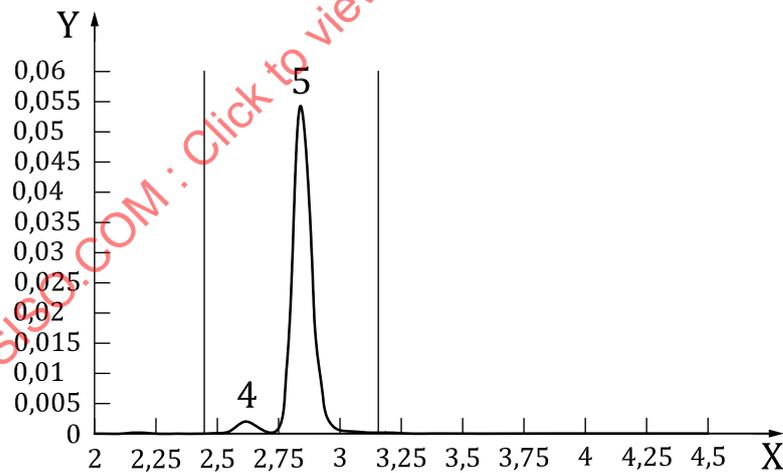
b) Mixture 1
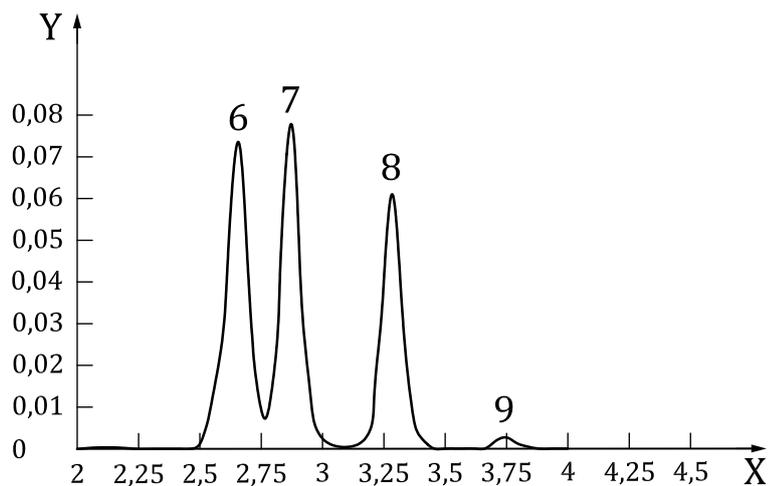
c)   Mixture 2

Key

X   elution volume, in millilitres

Y   detection signal, in volts

1   S1 PEG 1500

2   S2 PEG 3000

3   S3 PEG 6000

4   2,61 ml, PEG

5   2,84 ml, PEO-M

6   2,65 ml, PEG

7   2,86 ml, PEO-M

8   3,27 ml, PEO-A

9   3,74 ml, unknown

Column: C18 – 80 Å, 5 µm, 150 × 4,6 mm 2 ×

Number of plates: 19 700 per m / 5 ,900 column

Figure C.1 — Participant 1

a) S 1 to S 3

b) Mixture 1

c) Mixture 2

Key
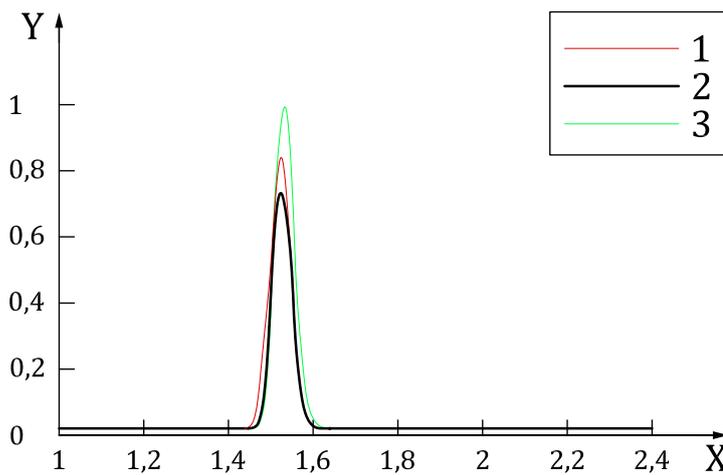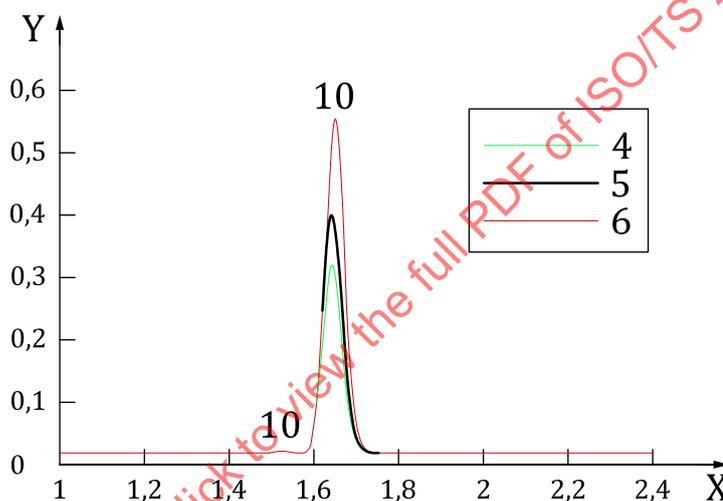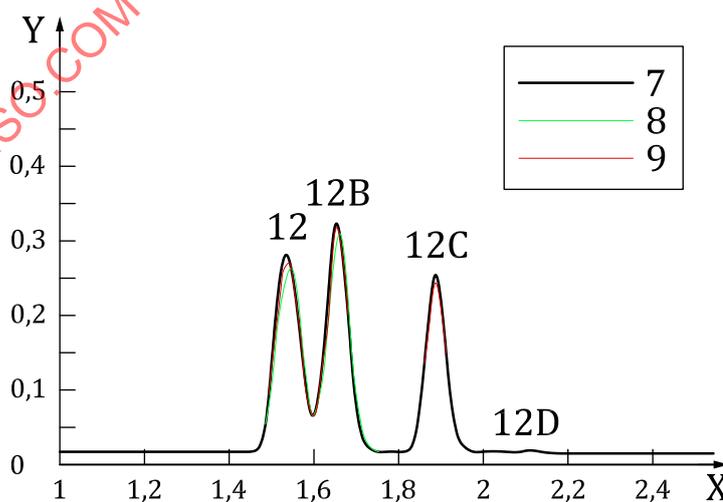
X   elution volume, in millilitres

Y   detection signal, in millivolts

1   S1 PEG 1500 Standard ELSD

19

2 S3 PEG 6000 Standard ELSD

3 S2 PEG 3000 Standard ELSD

4 mixture 1 a ELSD

5 mixture 1 b ELSD

6 mixture 1 c ELSD

7 mixture 2 PEO-Xc ELSD

8 mixture 2 PEO-Xb ELSD

9 mixture 2 PEO-X ELSD

10, 12, 12B, 12C, 12D peaks

Column: C18 – 140 Å, 3,5 μm, 150 × 4,6 mm

Number of plates: 34 000 per m / 5 100 column

Figure C.2 — Participant 2

a) S 1 to S 3

b) Mixture 1

c) Mixture 2

Key
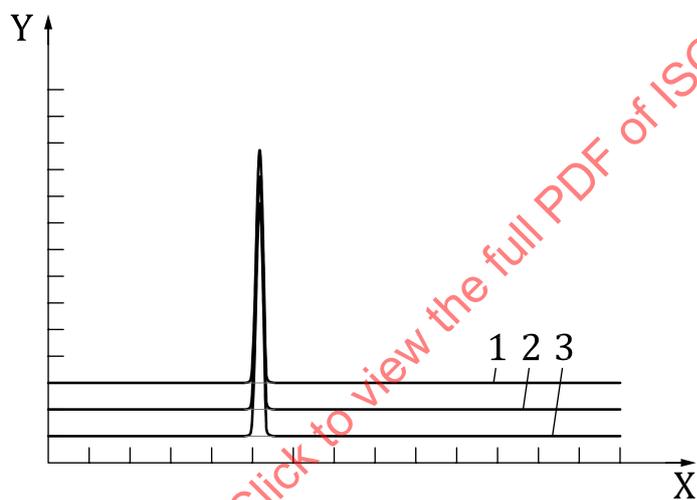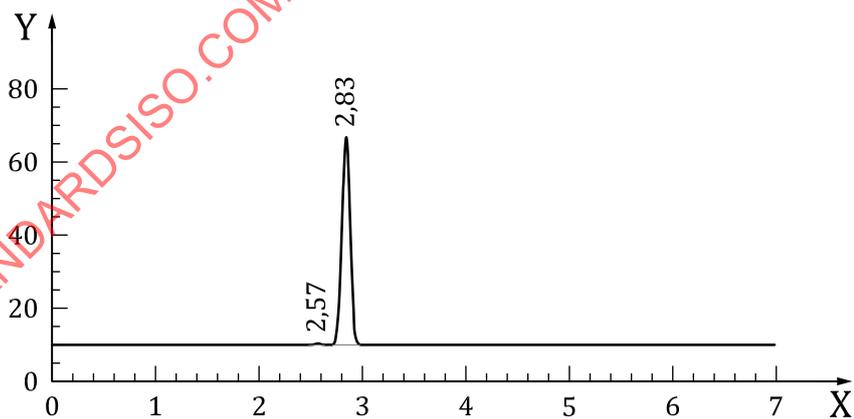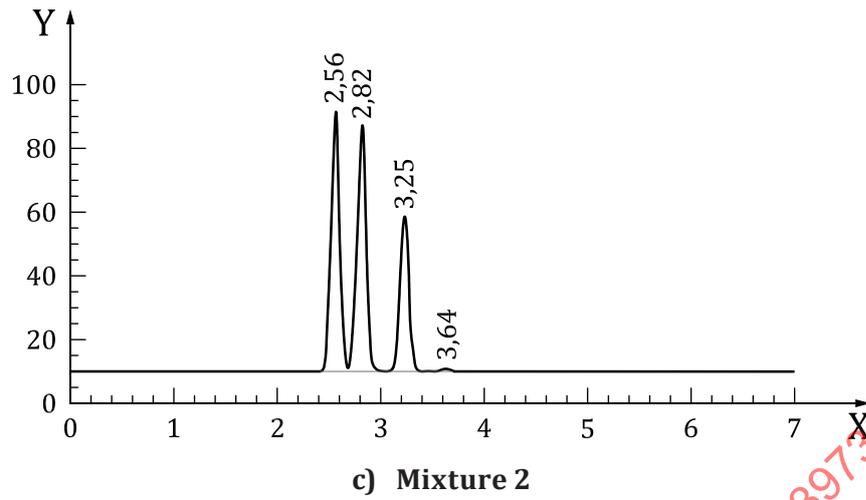
X   retention time, in minutes

Y   response

1   S3 PEG 6000 Standard ELSD

2   S2 PEG 3000 Standard ELSD

3   S1 PEG 1500 Standard ELSD

Column: C18 – 100 Å, 5 μm, 250 × 4,6 mm / ELSD

Number of plates: 21 280 per m / 5 320 column

Figure C.3 — Participant 3

a) S 1 to S 3

b) Mixture 1

c) Mixture 2

Key
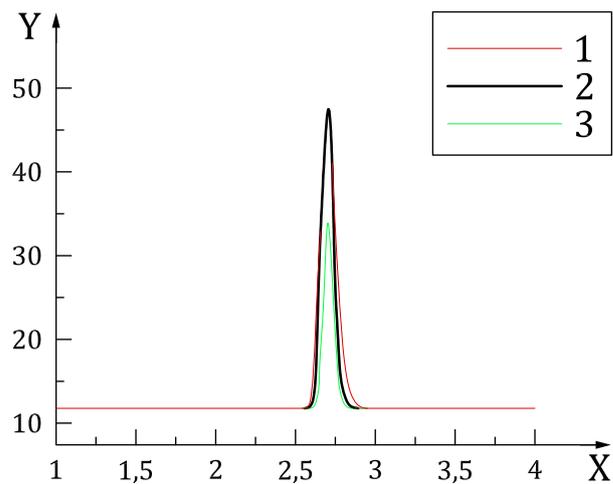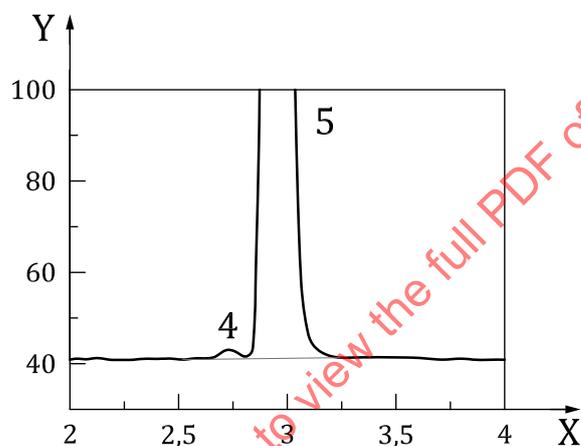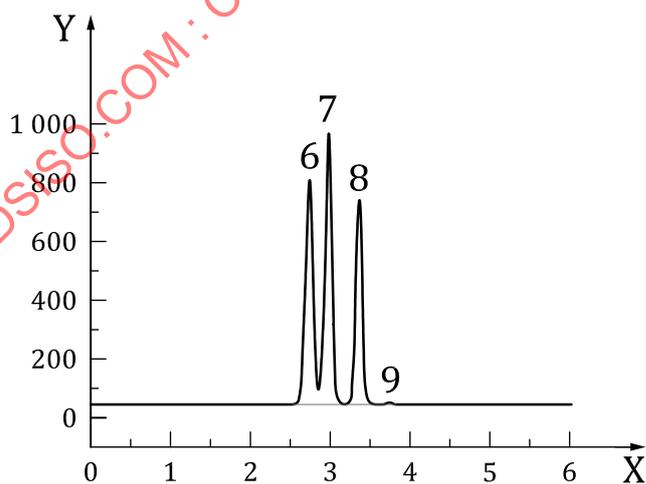
X   retention time, in minutes

Y   intensity

1   PEG6690

2   PEG3020

3   PEG1460

4 to 9 peaks
Column: C18 – 120 Å, 5 μm, 250 × 4,6 mm / ELSD
Number of plates: 16 300 per m / 4 070 column

Figure C.4 — Participant 4
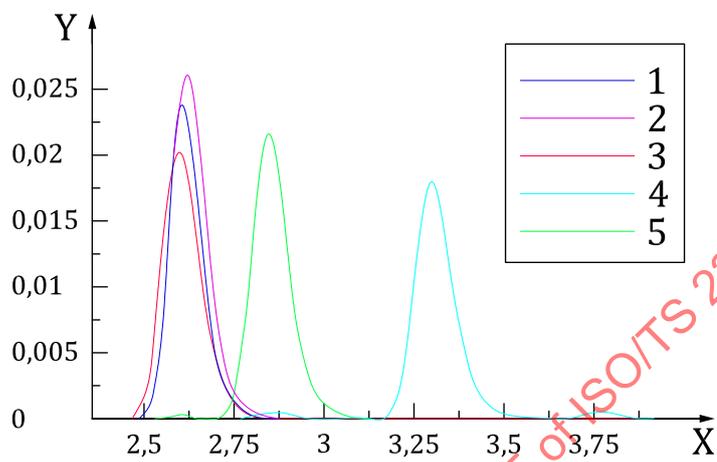
a) S 1 to S 3

b) Mixture 1

c) Mixture 2

Key
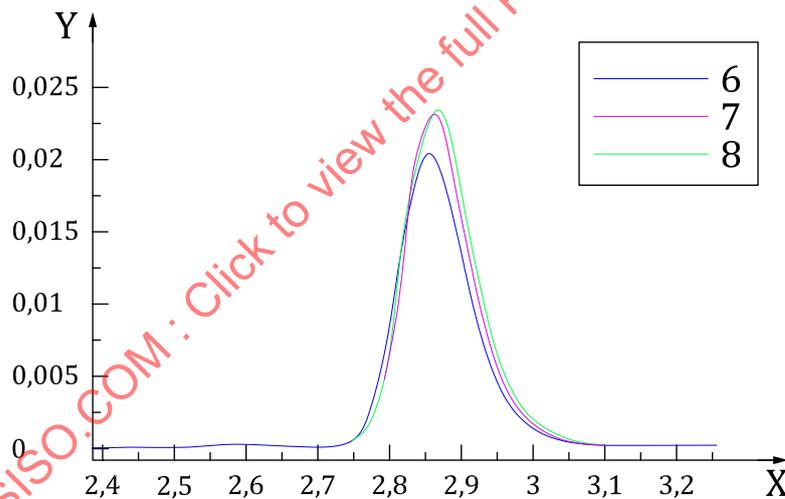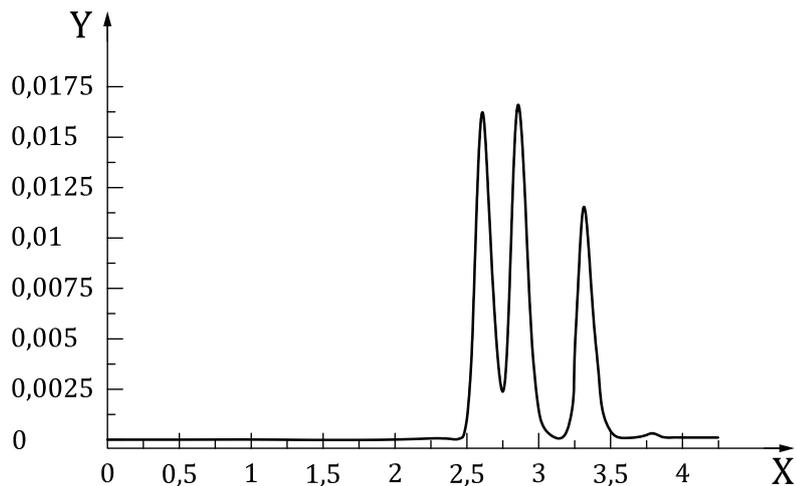
X   elution volume, in millilitres
Y   detection signal, in volts
1   03d S1
2   03d S2
3   03d S3
4   03d A2
5   03d M2
6   03dE1 Mi1
7   03dE2 Mi1
8   03dE3 Mi1

Column: C18 – 100 Å, 5 μm, 250 × 4 mm / ELSD
Number of plates: 16 000 per m / 4 000 column

Figure C.5 — Participant 5
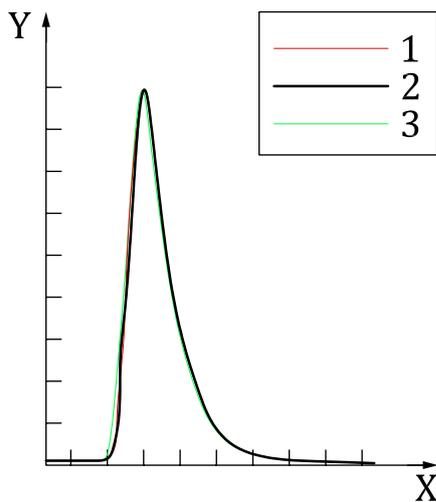
a) S 1 to S 3

b) Mixture 1

c) Mixture 2

Key
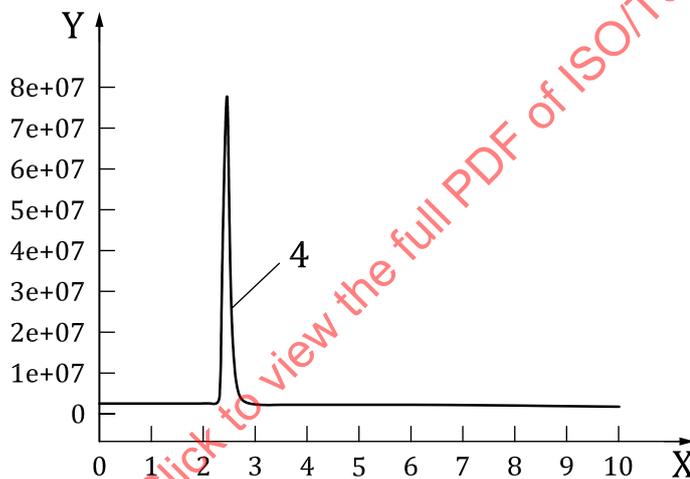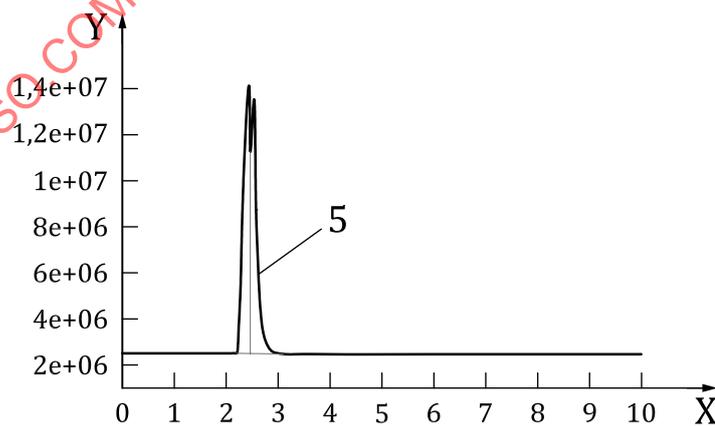
X   retention time, in minutes

Y   detector signal, in millivolts

1   Agilent 35900E Interface ELSD, S 1

2   Agilent 35900E Interface ELSD, S 2

3   Agilent 35900E Interface ELSD, S 3

4   Agilent 35900E Interface ELSD, mixture 1

5   Agilent 35900E Interface ELSD, mixture 2

Column: C18 – 300 Å, 5 μm, 250 × 4 mm

Number of plates: 7 680 per m / 1 920 column

Figure C.6 — Participant 6
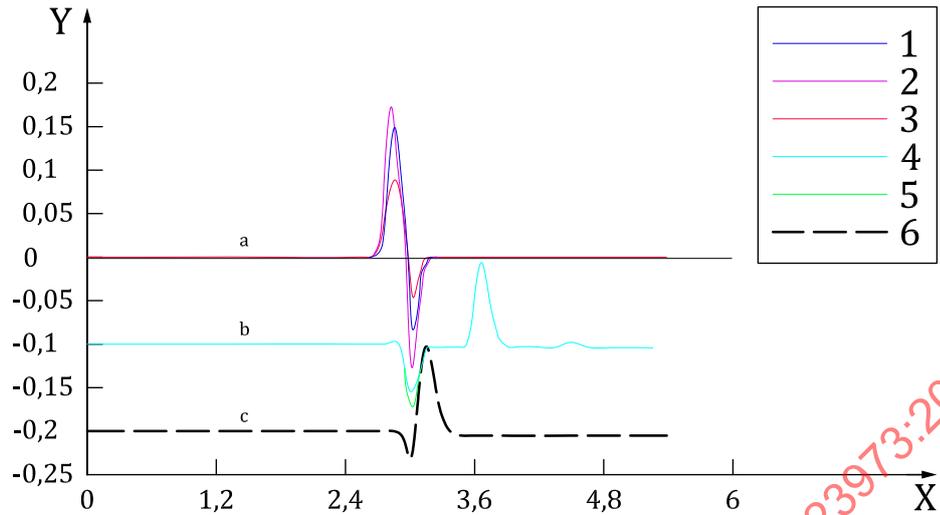
a) S 1 to S 3
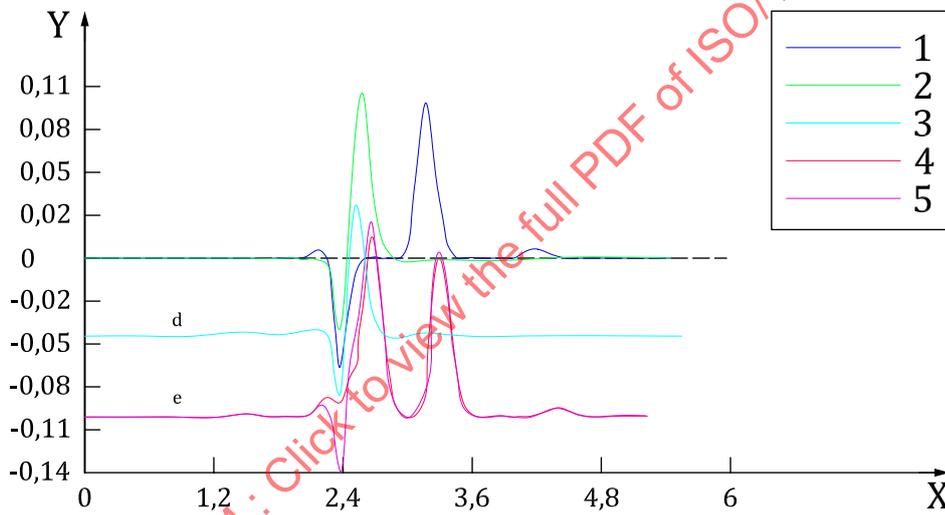
b) Mixture 1 (middle) and mixture 2 (bottom)

Key

X	retention time, in minutes	6	PEG 77 %
Y	response	a	PEG 1460, 3020, 6690.
1	PEG 73 %	b	PEO-A.
2	PEG 74 %	c	PEO-M.
3	PEG 75 %	d	Mixture 1.
4	PEG 76 %	e	Mixture 2.
5	PEG 76 %		

Column: C18 – 80 Å, 5 µm, 150 × 4,6 mm

Number of plates: not known

Figure C.7 — Participant 7
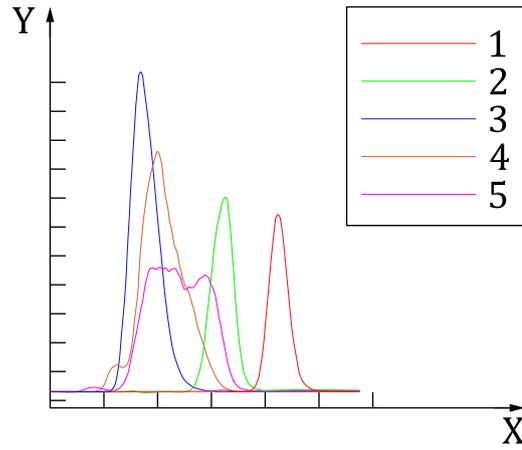
a) S 1 to S 3
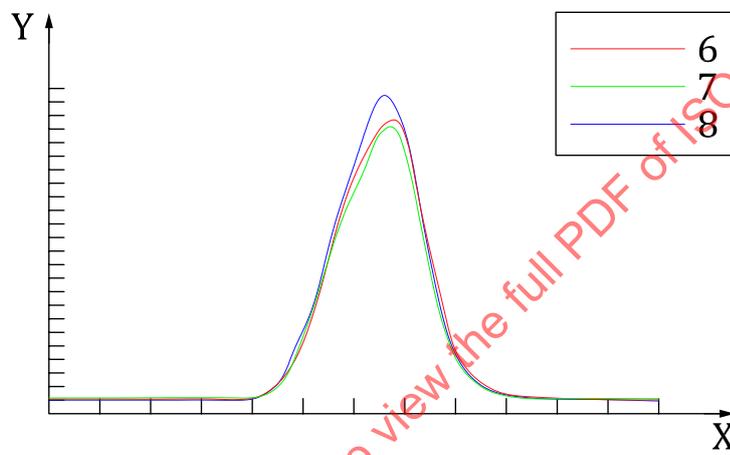
b) Mixture 1

c) **Mixture 2**

Key
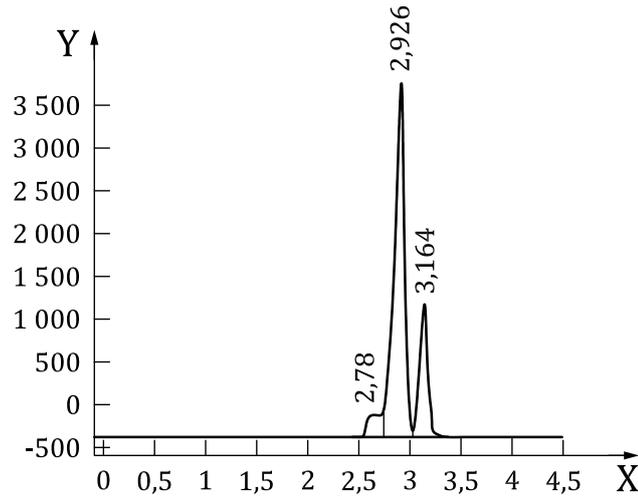
X   retention time, in minutes

Y   detector signal, in millivolts

1   sample A2 PEO-A

2   sample MO PEO-M

3   sample PEG 1460

4   sample PEG 3020

5   sample PEG 6690

6   sample M1-A PEO-X

7   sample M1-B PEO-X

8   sample M1-C PEO-X

Column: C18 – 120 Å, 5 μm, 250 × 4,6 mm

Number of plates: Not known

Figure C.8 — Participant 8

a) 1 S to 3 S

b) Mixture 1

c) Mixture 2

Key
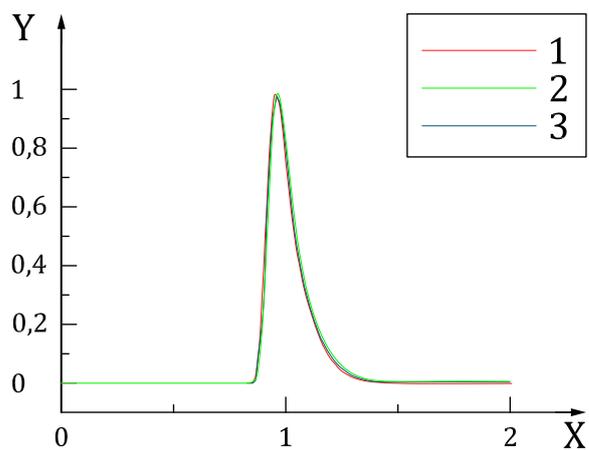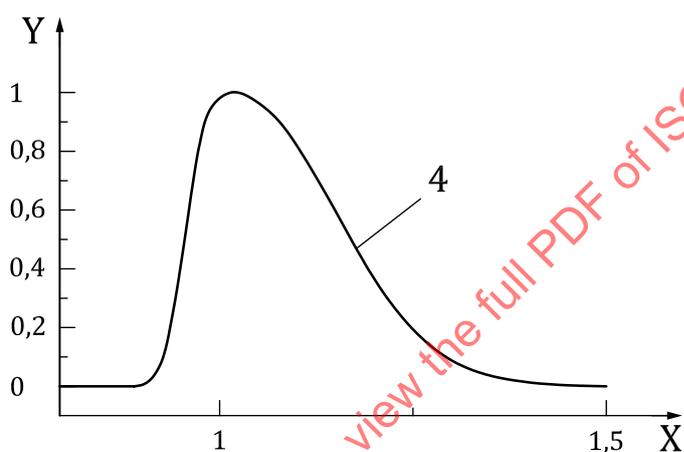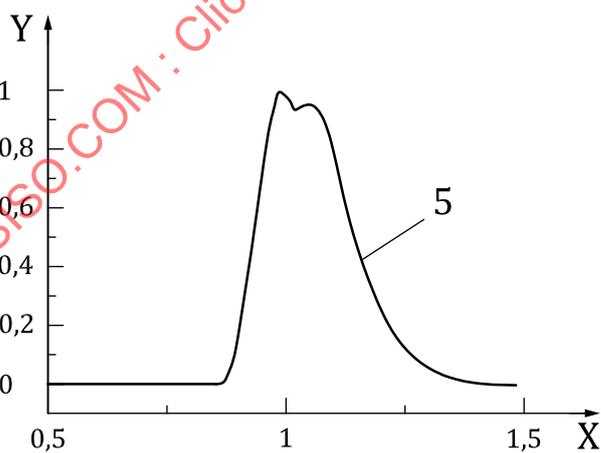
X   elution volume, in millilitres

Y   normalized ELSD signal

1   PEG 1450-55-45 ACN-H20

2   PEG 3000-55-45 CAN-H20

3   PEG 6650-55-45 CAN-H20

4   Mi1 PEO X (3) 55-45

5   Mi2 PEO X (2) 55-45

Column: C18 – 100 Å, 5 µm, 125 × 4 mm

Number of plates: not known

Figure C.9 — Participant 9
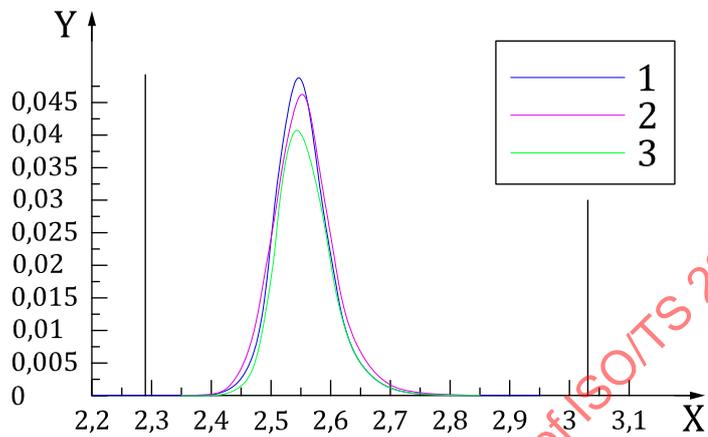
a) 1 S to 3 S
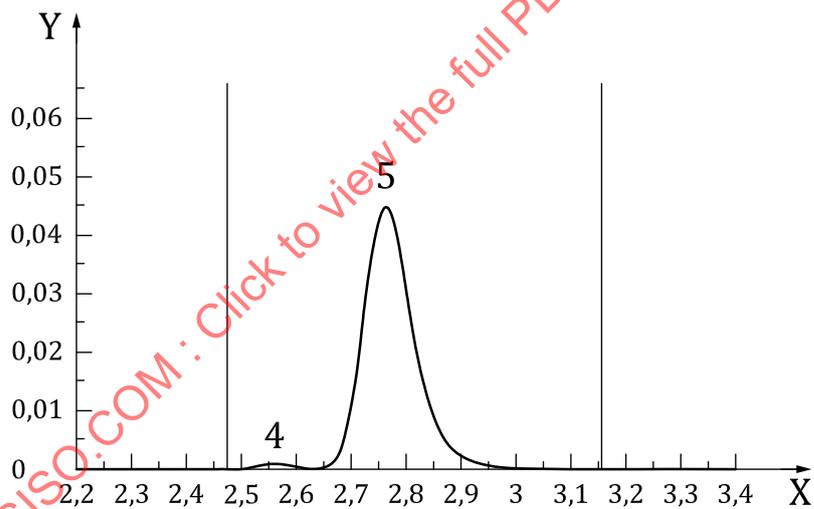
b) Mixture 1
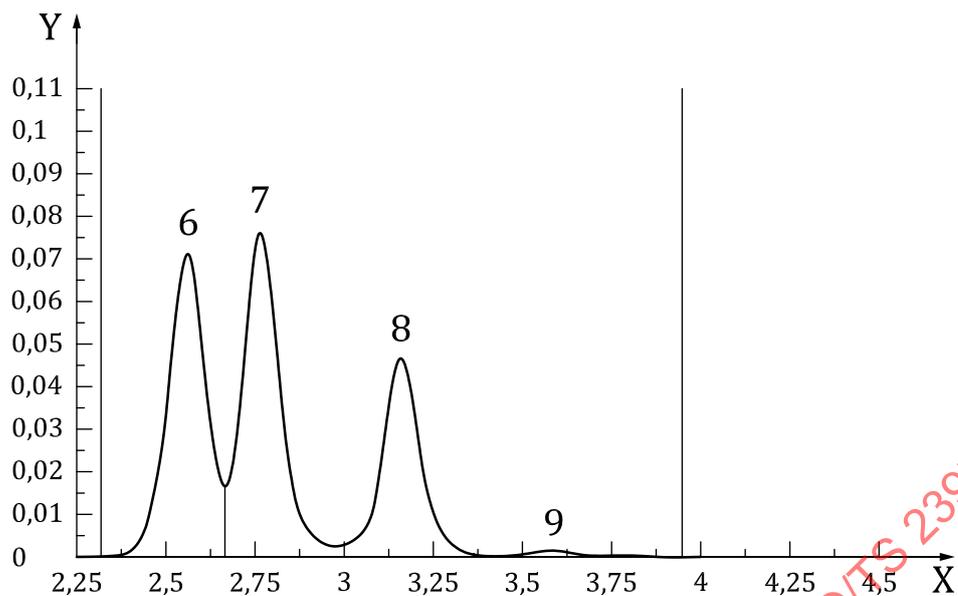
c) Mixture 2

Key

X   elution volume, in millilitres

Y   detection signal, in volts

1   S1 PEG 1500

2   S2 PEG 3000

3   S3 PEG 6000

4   2,56 ml, PEG

5   2,76 ml, PEO-M

6   2,55 ml, PEG

7   2,75 ml, PEO-M

8   3,15 ml, PEO-A

9   3,59 ml, unknown

Column: C18 – 80 Å, 5 μm, 150 × 4,6 mm 2×

Number of plates: 16 000 per m / 4 000 column

Figure C.10 — Participant 10
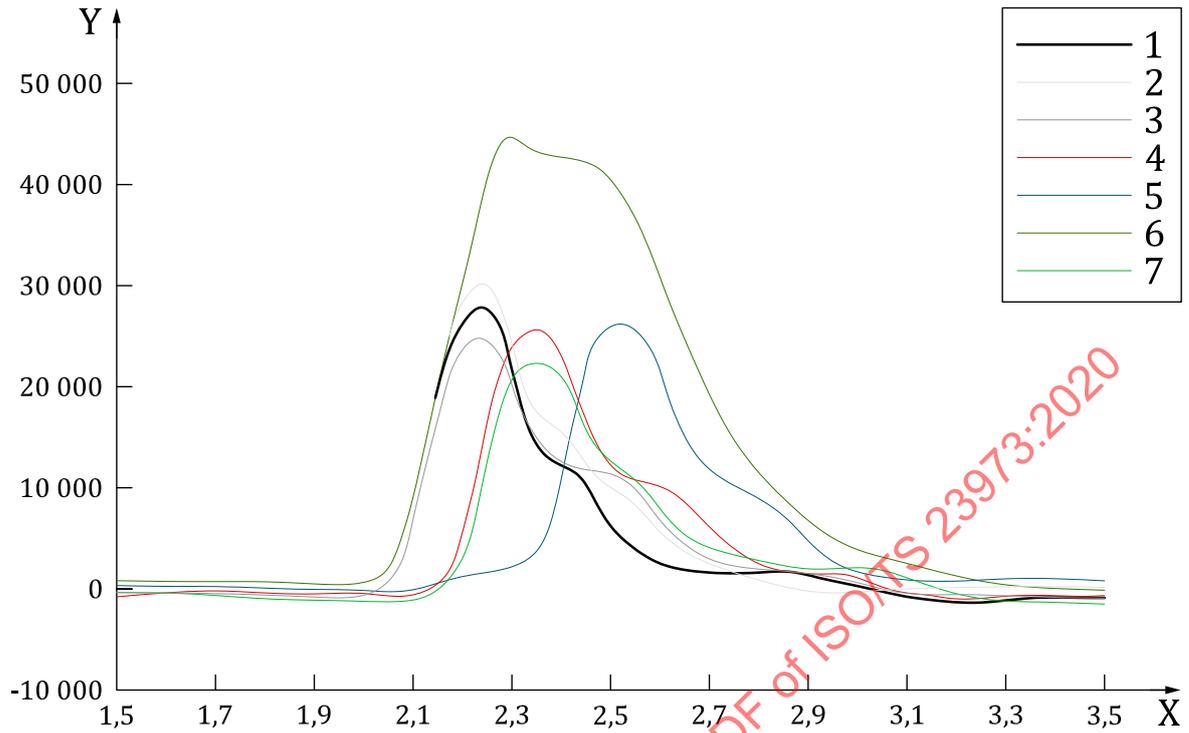
Key

X   retention volume, in millilitres

Y   intensity RI, in microvolts

1   PEG 1490

2   PEG 3060

3   PEG 6550

4   M2 PEO-M

5   A2 PEO-A

6   Mi 2 PEO-X

7   Mi 1 PEO-X

Flow rate: 1 ml/min

Concentration: 2 mg/ml

Injection volume: 20 µl

Column: C18 – 120 Å, 5 µm, 250 × 4 mm

Number of plates: 1 320 per m / 330 column

Figure C.11 — Participant 11
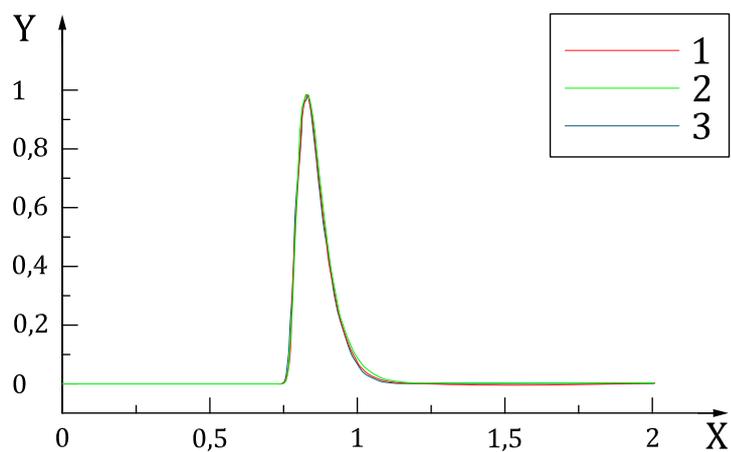
a) S 1 to S 3
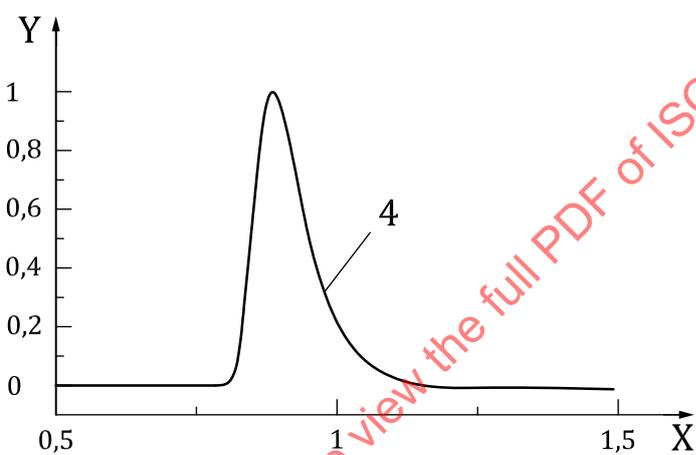
b) Mixture 1
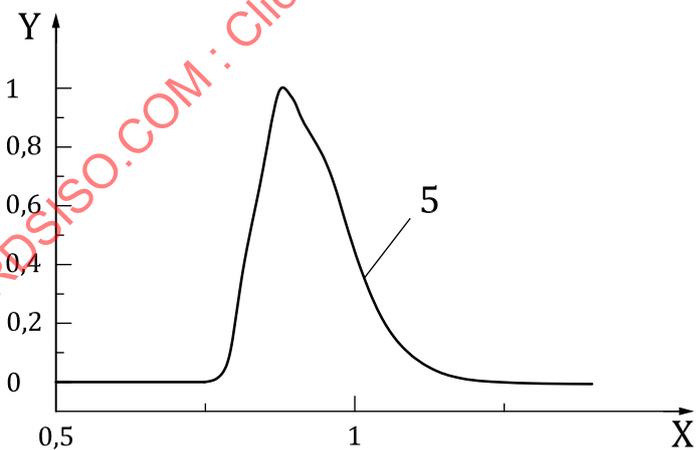
c) Mixture 2

Key

X   elution time, in minutes

Y   ELSD response

1   PEG 1500-56/44-AC/H20

2   PEG 3000-56/44-AC/H20

3   PEG 6000-56/44-AC/H20

4   Mi1-PEO-X-A

5   Mi2-PEO-X

Column: C18 – 100 Å, 5 µm, 125 × 4 mm

Number of plates: 8 640 per m / 1 080 column

Figure C.12 — Participant 12
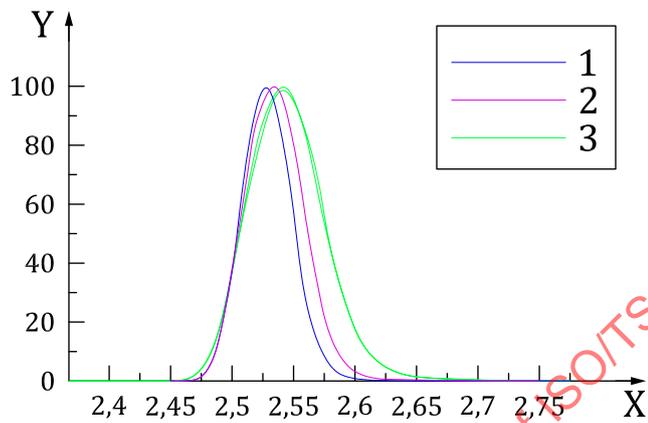
a) S 1 to S 3
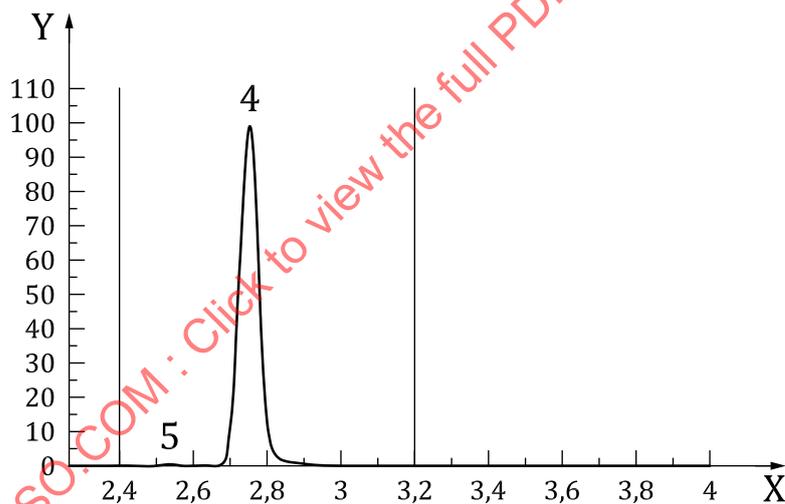
b) Mixture 1
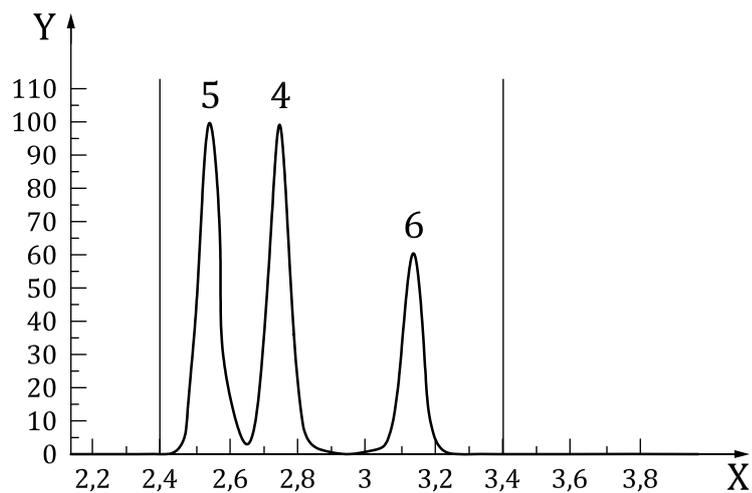
c) Mixture 2

Key

X   elution volume, in millilitres

Y   detection signal, in volts

1   S1 PEG 1500

2   S2 PEG 3000

3   S3 PEG 6000
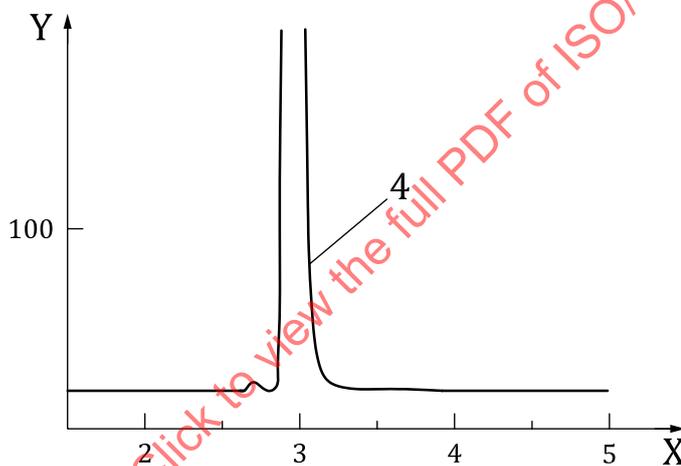
4 to 6   peaks

Column: C18 – 120 Å, 5 μm, 150 × 4 mm 2×

Number of plates: 47 000 per m / 14 100 column
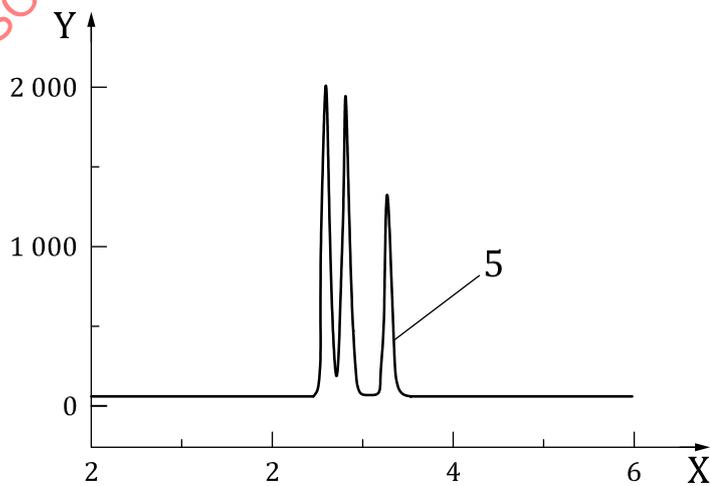
Figure C.13 — Participant 13
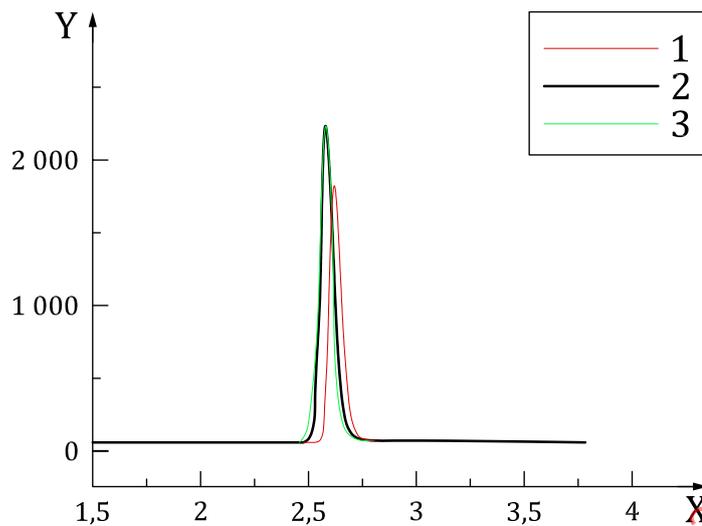
a) S 1 to S 3

b) Mixture 1

c) Mixture 2

Key

X   retention time, in minutes

Y   detector signal

1   PEG1500
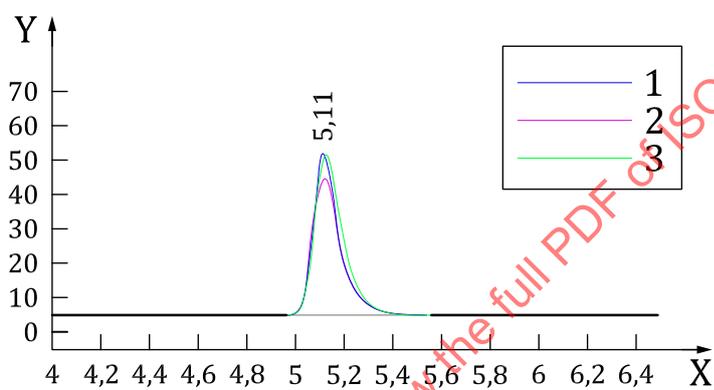
2   PEG3000

3   PEG6000

4   mixture 1

5   mixture 2
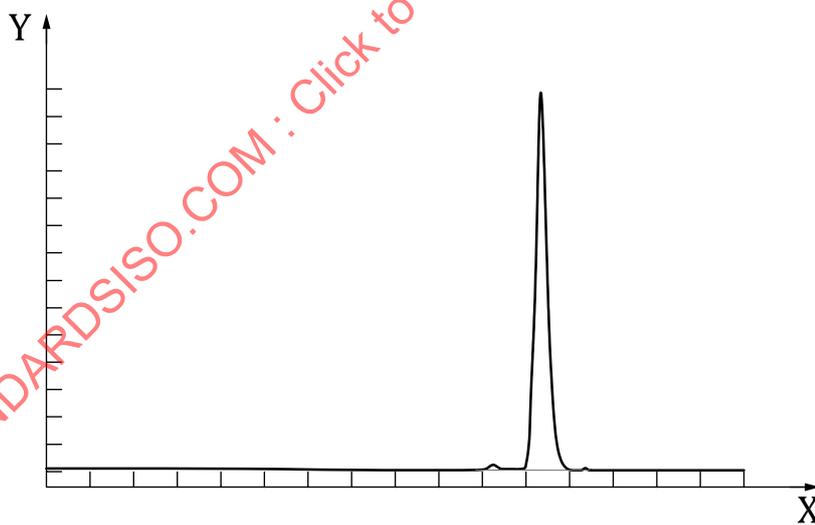
Column: C18 – 120 Å, 5 µm, 250 × 4,6 mm

Number of plates: 39 000 per m / 9 750 column

Figure C.14 — Participant 14

a)   1 S to 3 S

b)   Mixture 2