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Plastics — Determination of caprolactam and its cyclic and linear oligomers by HPLC

Plastiques — Détermination du caprolactame et de ses oligomères cycliques et linéaires par CLHP

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Foreword

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The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

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ISO 15033 was prepared by Technical Committee ISO/TC 61, *Plastics*, Subcommittee SC 5, *Physical-chemical properties*.

This second edition cancels and replaces the first edition (ISO 15033:2000), which has been technically revised.

Introduction

The basic method specified in this International Standard can be used for HPLC determination of the cyclic oligomers of caprolactam up to and including the hexamer (n = 6), using UV detection. If desired, after post-column reaction of the primary amine with 1,2-phthalic dicarboxaldehyde, on-line determination of the linear oligomers up to and including the hexamer can also be carried out.

The determination is not quantitative for oligomers higher than the hexamer ($n > 6$). In the determination of cyclic oligomers the sensitivity for the tetramer and higher oligomers is constant, which means that calibration should take place up to and including the tetramer (n = 4).

The linear oligomers are determined by the fluorescence of the *iso*-indole group, which is a product of the reaction between the primary amino group, 1,2-phthalic dicarboxaldehyde and 3-mercaptopropionic acid. The calibration with the linear oligomers should be carried out up to and including the hexamer (n = 6).

Plastics — Determination of caprolactam and its cyclic and linear oligomers by HPLC

SAFETY STATEMENT — Persons using this document should be familiar with normal laboratory practice, if applicable. This document does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user to establish appropriate safety and health practices and to ensure compliance with any regulatory requirements.

1 Scope

This International Standard describes an HPLC (high-performance liquid chromatography) method for determining the concentrations of cyclic oligomers of caprolactam, from 0,01 % by mass upwards, and linear oligomers of caprolactam, from 5 mg/kg upwards, both up to and including the hexamer of caprolactam (n = 6), in samples of polyamide 6, caprolactam and mixtures of rearrangement products in water.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 472, *Plastics — Vocabulary*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 472 apply.

4 Principle

A test sample is dissolved in, or diluted with, formic acid and the oligomers separated in the presence of a low-pH mobile phase using a column filled with reversed-phase packing material. The cyclic oligomers are detected by UV absorption at 200 nm. If desired, the linear oligomers can be detected by fluorescence after post-column reaction of the primary amino group with 1,2-phthalic dicarboxaldehyde and 3-mercaptopropionic acid. The concentrations are calculated by comparison of the measured values with those of calibration solutions.

5 Reagents

During the analysis, unless otherwise stated, use only reagents of recognized analytical grade.

5.1 **Water**, ultrapure or double-distilled.

5.2 **Phosphoric acid**, 85 % by mass.

5.3 Phosphoric acid, 1 mol/l.

Introduce 68 ml of phosphoric acid (5.2) into a 1-litre volumetric flask, make up to the mark with water (5.1) and mix well.

5.4 Acetonitrile.

5.5 Formic acid, concentrated.

5.6 Caprolactam.

5.7 Cyclic dimer of caprolactam, isolated by HPLC (see Note).

5.8 Cyclic trimer of caprolactam, isolated by HPLC (see Note).

5.9 Mixture of cyclic oligomers of caprolactam, isolated by HPLC (see Note).

5.10 ε-Aminocaproic acid.

5.11 Linear dimer of ε-aminocaproic acid.

5.12 Linear trimer of ε-aminocaproic acid.

5.13 Linear tetramer of ε-aminocaproic acid.

5.14 Linear pentamer of ε-aminocaproic acid.

5.15 Linear hexamer of ε-aminocaproic acid.

5.16 Helium.

5.17 Eluent.

Add 10 ml of acetonitrile (5.4) and 10 ml of phosphoric acid (5.3) to 900 ml of water (5.1). Raise the pH of the solution to 2,6 using sodium hydroxide flakes (5.19). Make up to 1 litre and saturate with helium (5.16).

5.18 Sodium tetraborate decahydrate.

5.19 Sodium hydroxide flakes.

5.20 1,2-Phthalic dicarboxaldehyde.

5.21 Methanol, 96 % by volume.

5.22 3-Mercaptopropionic acid.

5.23 Post-column derivatization reagent.

Dissolve 76 g of sodium tetraborate decahydrate (5.18) and 6 g of sodium hydroxide (5.19) in 2 litres of water (5.1). Dissolve 1,6 g of 1,2-phthalic dicarboxaldehyde (5.20) in 40 ml of methanol (5.21) and add this solution to the sodium tetraborate decahydrate reagent. Add 1,5 ml of 3-mercaptopropionic acid (5.22) and mix well.

The stability of the post-column derivatization reagent is limited. Do not keep for longer than 3 days.

NOTE The cyclic dimer, the cyclic trimer and the mixture of cyclic oligomers of caprolactam can be isolated from a methanol extract of PA6 by preparative HPLC, using the HPLC method described here. The purity of the dimer and the possible presence of other oligomers can be checked using the method described in this International Standard.

6 Apparatus

6.1 HPLC equipment, having the following specifications:

— **Eluent pump**, including mixer, damper and manometric module, giving an eluent flow rate of 0,51 ml/min and a pressure drop of approximately 100 bar.

— **Injector**, e.g. an auto-sampler capable of 1 µl to 250 µl injections, equipped to carry out a variable injector programme (see Annex A). The injector shall be capable of accommodating at least three components in the sample loop, i.e. the injector programme shall be capable of controlling the “sandwich” injection of up to three components into the sample loop plus a solvent injection in one HPLC run.

— **Column**:

  — stainless steel

  — inside diameter: 3 mm

  — length: 250 mm

  — temperature: 40 °C

  — packing: reversed-phase C18 silica or equivalent

  — particle size: 0,005 mm

  The resolution of the column shall be such that baseline separation of the components of interest is obtained.

  The lifetime of the reversed-phase C18 column is very strongly influenced by the C18-silica bonding of the packing material. Therefore, columns equipped with a packing material containing monofunctional silanes with diisobutyl side-chain groups are preferred. These side groups sterically protect the key silanes from hydrolytic attack at low pH, making the stationary phase stable at such pH (pH 1).

— **UV detector**: wavelength 200 nm and 220 nm.

And additionally for determination of the linear oligomers:

— **Reagent pump**, including manometric module and pulse damper, giving a reagent flow rate of 0,25 ml/min and a reagent pressure drop of approximately 20 bar.

— **Reaction coil**:

  — stainless steel or PEEK

  — length: 3 m

  — inside diameter: 0,25 mm

  — temperature: 25 °C

— **Fluorescence detector**:

  — excitation: 330 nm

  — emission: 420 nm

A schematic diagram of an HPLC apparatus is given in Annex B.

6.2 Microbalance, accurate to 0,1 mg.

6.3 Ultrasonic vibration bath.

6.4 Volumetric flask, capacity 25 ml.

7 Test sample

For polyamide 6 and caprolactam, the maximum test sample size shall be 0,5 g. For mixtures of rearrangement products in water, a maximum test sample size of 1,25 g shall be used. The stability of the dissolved samples is limited, since caprolactam is hydrolysed to ε-aminocapronic acid. Do not keep for longer than 6 days.

8 Procedure

8.1 Calibration

To calibrate the column for the cyclic oligomers, prepare a series of three calibration solutions with concentrations increasing from 100 mg/l to 1 500 mg/l by dissolution in formic acid (5.5) for caprolactam (5.6), the cyclic dimer (5.7), the cyclic trimer (5.8) and the oligomer mixture (5.9). To calibrate the column for the linear oligomers, prepare a series of three calibration solutions with concentrations increasing from 2 mg/l to 15 mg/l by dissolving in formic acid (5.5) for ε-aminocaproic acid (5.10) and each of the linear oligomers from the dimer (5.11) to the hexamer (5.15). Pump eluent (5.17) through the column at a rate of 0,51 ml/min. Starting with the lowest concentration of a calibration series, inject 2 µl of the calibration solution into the column in accordance with the injector programme given in Annex A. Elute in accordance with the gradient timetable in Annex A. Record the UV chromatogram. If applicable, immediately after UV detection, add the post-column reagent (5.23) at a rate of 0,25 ml/min, mixing the eluent and the reagent in the reaction coil. Record the fluorescence chromatogram. Measure the peak area of the component(s).

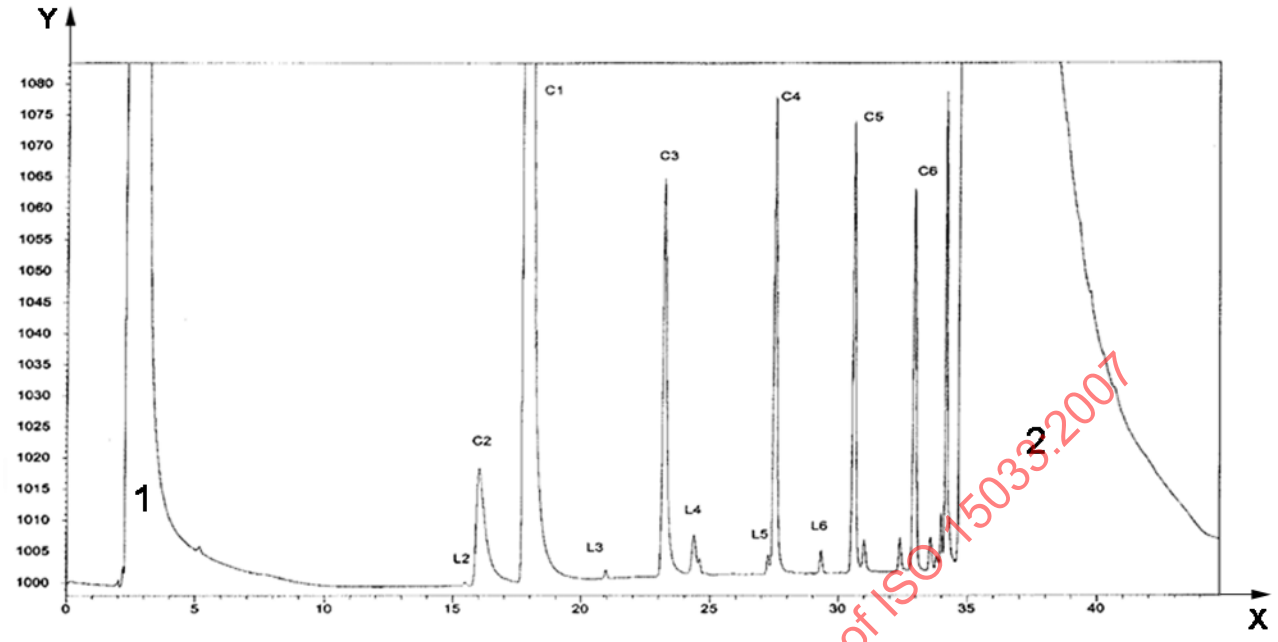
Repeat the calibration successively for the other calibration solutions of the same component and for the other calibration series.

NOTE Calibration can also be carried out using a commercially available PA6 polymer with a known concentration of cyclic and linear oligomers (see Reference [3] in the Bibliography).

8.2 Determination

Introduce a test sample (see Clause 7) into a 25 ml volumetric flask. Add 20 ml of formic acid (5.5), close the flask and dissolve the sample, optionally by using ultrasonic vibration. Make up to the mark with formic acid (5.5). Pump eluent (5.17) through the column at a flow rate of 0,51 ml/min. Inject 2 µl of the sample solution into the column in accordance with the injector programme given in Annex A. Elute in accordance with the gradient timetable in Annex A. Record the UV chromatogram (see Figure 1). If applicable, immediately after UV detection, add the post-column reagent (5.23) at a flow rate of 0,25 ml/min, mixing the eluent and the reagent in the reaction coil. Record the fluorescence chromatogram (see Figure 2). Measure the peak areas of the cyclic oligomers (UV detection) and linear oligomers (fluorescence detection) up to and including the hexamer.

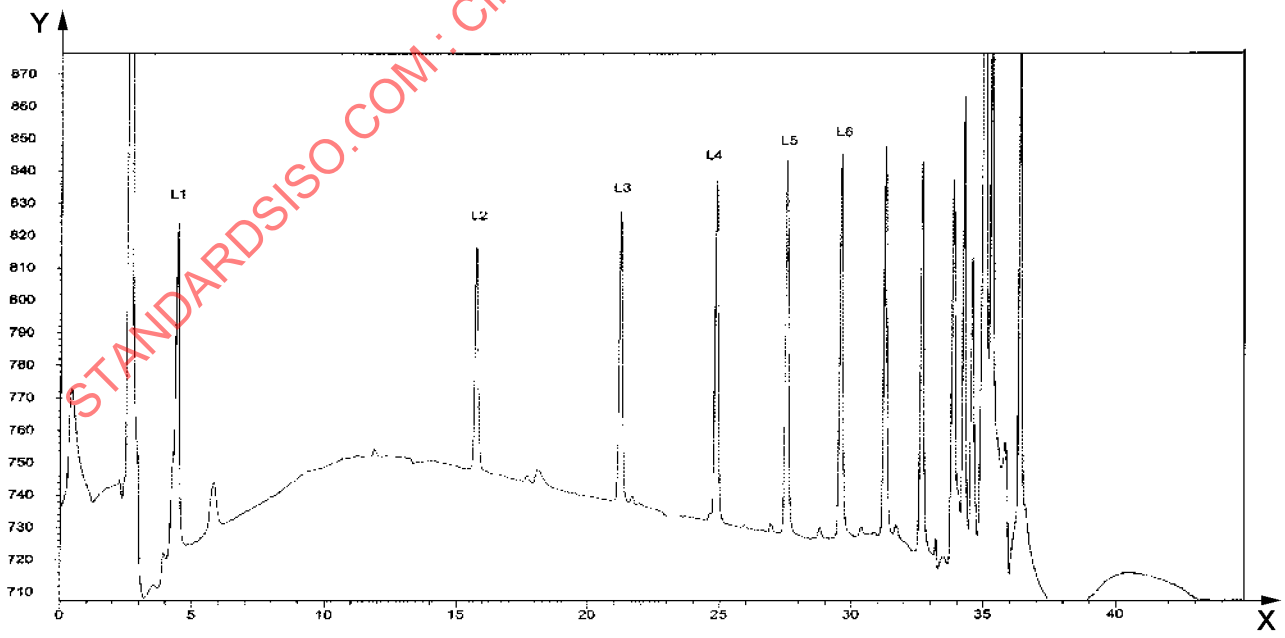
NOTE If necessary, e.g. if lower sensitivity is necessary due to high(er) concentrations, caprolactam can be detected by UV absorption at 220 nm instead of 200 nm.



Key

X	retention time (min)
Y	response (mV)
1	formic acid sandwich injection
2	250 µl formic acid injection
C1 to C6	cyclic oligomers
L2 to L6	linear oligomers

Figure 1 — Determination of the cyclic oligomers of caprolactam



Key

X	retention time (min)
Y	response (mV)
L1 to L6	linear oligomers

Figure 2 — Determination of the linear oligomers of caprolactam

9 Calculations

9.1 Calculation of calibration factors

9.1.1 Calibration factors for cyclic oligomers

Calculate the calibration factor for the relevant component for each solution using the following equation (see Note 1):

$$\frac{A_c}{C_c} = f_c \quad (1)$$

where

A_c is the peak area of the relevant component;

C_c is the concentration of the relevant component in the calibration solution, in mg/l;

f_c is the calibration factor for the relevant component.

Calculate the calibration factors for n = 1 up to and including n = 3 by taking the mean value of the three calculated calibration factors in the series of the relevant component.

The calibration factors for the tetramer (n = 4) up to and including the hexamer (n = 6) are assumed to be constant. The calibration factor for the oligomers from n = 4 up to and including n = 6 is calculated from the chromatogram of the oligomer mixture, the calibration factors for n = 1 up to and including n = 3 being known. Calculate the calibration factor for n = 4 to n = 6 by taking the mean value of the three calculated calibration factors in the series.

The calibration shall be repeated if the relative difference between the calibration factors for two solutions is more than 5 %.

NOTE 1 The calibration factor for e.g. n = 3 can also be calculated by using for instance a calibration solution also containing caprolactam and/or the cyclic dimer if the latter are known.

NOTE 2 Usually, the linear oligomers are present in polyamide 6 at a concentration lower than that of the cyclic oligomers by approximately two orders of magnitude, and do not therefore interfere with the determination.

9.1.2 Calibration factors for linear oligomers

Calculate the calibration factor for the linear oligomers for each solution using the following equation:

$$\frac{A_{c1}}{C_{c1}} = f_{c1} \quad (2)$$

where

A_{c1} is the peak area of the relevant component;

C_{c1} is the concentration of the relevant component in the calibration solution, in mg/l;

f_{c1} is the calibration factor for the relevant component.

Calculate the calibration factor for the linear components by taking the mean value of the three calculated calibration factors in the series. The calibration shall be repeated if the relative difference between the calibration factors for two solutions is more than 5 %.

9.2 Test sample

9.2.1 Calculation of cyclic oligomer concentrations

Calculate the concentration, in % by mass, of each of the cyclic oligomers from n = 1 up to and including n = 6 using the following equation and rounding the result to 0,1 % for concentrations greater than or equal to 10 % and to 0,01 % for concentrations below 10 %:

$$\frac{A_{cs} \times 2{,}5}{f_c \times m_g} = C_{cs} \tag{3}$$

where

A_{cs} is the peak area of the relevant component;

f_c is the calibration factor for the relevant component, calculated in accordance with 9.1.1;

m_g is the sample size, in mg/25 ml;

C_{cs} is the concentration of the relevant component in the sample, in % by mass.

9.2.2 Calculation of linear oligomer concentrations

Calculate the concentration, in mg/kg, of the linear oligomers from n = 1 up to and including n = 6 using the following equation, rounding the result to 1 mg/kg in each case:

$$\frac{A_{cs} \times 25\,000}{f_c \times m_g} = C_{cs} \tag{4}$$

where

A_{cs} is the peak area of the relevant component;

f_c is the calibration factor for the relevant component, calculated in accordance with 9.1.2;

m_g is the sample size, in mg/25 ml;

C_{cs} is the concentration of the relevant component in the sample, in mg/kg.

10 Precision

The reproducibility of this analytical method is not known because interlaboratory data are not available.

However, precision data within one laboratory have been determined by analysis of two reference polyamide 6 samples over an extended period of time. The precision of this method is not expected to deviate significantly from the repeatability thus determined, the data for which is summarized in Tables 1 to 4.

Table 1 — Repeatability data for determination of cyclic oligomers in a PA6 sample with a low cyclic oligomer content

	n = 1	n = 2	n = 3	n = 4	n = 5	n = 6
Mean result, x (% by mass)	0,11	0,05	0,18	0,25	0,30	0,34
Standard deviation, s	0,007	0,008	0,008	0,01	0,01	0,02
Coefficient of variation (%)	6,9	15,6	4,5	3,9	4,4	6,9

Table 2 — Repeatability data for determination of cyclic oligomers in a PA6 sample with a high cyclic oligomer content

	n = 1	n = 2	n = 3	n = 4	n = 5	n = 6
Mean result, x (% by mass)	8,37	0,33	0,50	0,47	0,40	0,34
Standard deviation, s	0,28	0,03	0,02	0,03	0,03	0,03
Coefficient of variation (%)	3,4	8,4	4,8	6,3	5,3	5,3

Table 3 — Repeatability data for determination of linear oligomers in a PA6 sample with a low linear oligomer content

	n = 1	n = 2	n = 3	n = 4	n = 5	n = 6
Mean result, x (mg/kg)	< 4	23	50	116	206	292
Standard deviation, s	—	5	9	19	25	42
Coefficient of variation (%)	—	23	18	17	12	14

Table 4 — Repeatability data for determination of linear oligomers in a PA6 sample with a high linear oligomer content

	n = 1	n = 2	n = 3	n = 4	n = 5	n = 6
Mean result, x (mg/kg)	141	59	82	148	203	252
Standard deviation, s	—	11	13	24	33	37
Coefficient of variation (%)	—	18	15	16	16	15

11 Test report

The test report shall include the following particulars:

— a reference to this International Standard;

— all details necessary for complete identification of the sample, including type, manufacturer's code number, source, trade name, etc.;

— any deviation from the specifications for the HPLC equipment or from the procedure given in this International Standard;

— any treatment of the sample prior to the analysis;

— the mass of test sample used;

— the contents of the cyclic and linear oligomers of caprolactam, in % by mass and mg/kg, respectively;

— the relevant chromatograms.