INTERNATIONAL STANDARD

ISO 15033

First edition
2000-04-01

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

Reference number
ISO 15033:2000(E)

ISO copyright office
Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 734 10 79
E-mail copyright@iso.ch
Web www.iso.ch

Printed in Switzerland

Contents

Page

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.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 3.

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.

Attention is drawn to the possibility that some of the elements of this International Standard may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

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

Annex A forms a normative part of this International Standard. Annex B is for information only.

Introduction

The basic method specified in this International Standard can be used for the HPLC determination of the cyclic oligomers of caprolactam up to and including the hexamer (n = 6), using UV detection. Simultaneously, 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 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).

For the determination of linear oligomers, it is assumed that the fluorescence sensitivity of the product of the reaction between the primary amino group and 1,2-phthalic dicarboxaldehyde and 3-mercaptopropionic acid is determined only by the *iso*-indole group, so that calibration with ε-aminocaproic acid (n = 1) is sufficient.

In general, the separation between ε-caprolactam and the cyclic dimer is sufficient. However, if there is a large excess of ε-caprolactam relative to the cyclic dimer, the latter cannot be determined quantitatively. For these cases, an alternative HPLC method, using the same basic equipment, is included in this International Standard.

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

WARNING — Use of this International Standard may involve hazardous chemicals, materials or operations. This standard does not purport to address all of the safety problems associated with its use. It is the responsibility of the user to establish proper safety and health practices and determine the applicability of regulatory limitations prior to use.

1 Scope

This International Standard specifies 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. However, if there is a large excess of caprolactam ($n = 1$) relative to the cyclic dimer ($n = 2$), the latter cannot be determined quantitatively. For these cases, an alternative method is specified. This method uses HPLC to determine the concentrations of caprolactam and the cyclic dimer of caprolactam at concentrations of 0,01 % by mass and higher in samples of polyamide 6, caprolactam and mixtures of rearrangement products in water.

2 Principle

2.1 Method A — Determination of the cyclic and linear oligomers of caprolactam

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. Simultaneously, the linear oligomers are 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.

2.2 Method B — Determination of caprolactam and the cyclic dimer of caprolactam

A test sample is dissolved in, or diluted with, formic acid and the two components separated by HPLC in the presence of a low-pH mobile phase using a column filled with reversed-phase packing material. Detection is by UV absorption at 200 nm. The concentrations are calculated by comparison of the measured values with those of calibration solutions.

3 Reagents

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

3.1 Distilled water, Millipore-Q or double-distilled.

3.2 Phosphoric acid, 85 % by mass.

3.3 Phosphoric acid, 1 mol/l.

Introduce 68 ml of phosphoric acid (3.2) into a 1 litre volumetric flask, make up to the mark with water (3.1) and mix well.

3.4 Acetonitrile.

3.5 Formic acid, concentrated.

3.6 Caprolactam.

3.7 Cyclic dimer of caprolactam, isolated by HPLC (see note).

3.8 Cyclic trimer of caprolactam, isolated by HPLC (see note).

3.9 Mixture of cyclic oligomers of caprolactam, isolated by HPLC (see note).

3.10 ε-Aminocaproic acid.

3.11 Helium.

3.12 Eluent for method A: add 10 ml of acetonitrile (3.4) and 10 ml of phosphoric acid (3.3) to 900 ml of water (3.1), make up to 1 litre and saturate with helium (3.11).

3.13 Eluent for method B: add 150 ml of acetonitrile (3.4) and 10 ml of phosphoric acid (3.3) to 800 ml of water (3.1), make up to 1 litre and saturate with helium (3.11).

3.14 Boric acid.

3.15 Potassium hydroxide flakes.

3.16 1,2-Phthalic dicarboxaldehyde.

3.17 Ethanol, 96 % by volume.

3.18 3-Mercaptopropionic acid.

3.18 Post-column derivatization reagent: dissolve 50 g of boric acid (3.14) in 1,5 litres of water (3.1). Raise the pH of the solution to 10 using potassium hydroxide flakes (3.15). Dissolve 1,6 g of 1,2-phthalic dicarboxaldehyde (3.16) in 20 ml of ethanol (3.17) and add this solution to the boric acid reagent. Add 2 ml of 3-mercaptopropionic acid (3.18) to the reagent, make up to 2 litres and mix well.

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 method A described in this International Standard.

4 Apparatus

4.1 HPLC equipment, having the following specifications:

— Eluent pump, including mixer, damper and manometric module, giving an eluent flow rate of 1,2 ml/min and a pressure drop of approximately 140 bar.

— Injector, e.g. auto-sampler for 1 µl to 50 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.

— Column:

  — stainless steel

  — inside diameter: 4 mm

  — outside diameter: 8 mm

  — length: 250 mm

  — temperature: 40 °C

  — packing: reversed-phase C18 silica or equivalent

  — particle size: 0,005 mm

— UV detector, wavelength 200 nm.

And additionally, for method A:

— Reagent pump, including manometric module and pulse damper, giving a reagent flow rate of 0,5 ml/min and a reagent pressure drop of approximately 20 bar.

— Reaction coil:

  — stainless steel or PEEK

  — length: 12 m

  — inside diameter: 0,35 mm

  — temperature: 25 °C

— Fluorescence detector:

  — excitation: 330 nm

  — emission: 420 nm

  — filter: > 390 nm

  — time constant: 0,3 s

  — detector cell: 12 µl

The resolution of the column shall be such that baseline separation of the components of interest is obtained. A schematic diagram of an HPLC apparatus is given in annex B.

4.2 Microbalance, accurate to 0,1 mg.

4.3 Ultrasonic vibration bath.

4.4 Volumetric flask, 25 ml.

5 Test sample

For polyamide 6 and caprolactam, the maximum sample size shall be 0,5 g. For mixtures of rearrangement products in water, a maximum sample size of 1,25 g shall be used.

6 Procedure

6.1 Method A — Determination of the cyclic and linear oligomers of caprolactam

6.1.1 Calibration

Prepare a series of three calibration solutions with concentrations increasing from 100 mg/l to 1 500 mg/l by dissolution in formic acid (3.5) for caprolactam, the cyclic dimer, the cyclic trimer and the oligomer mixture (3.6 to 3.9). Prepare a series of three solutions of ε-aminocaproic acid (3.10) with concentrations increasing from 2 mg/l to 15 mg/l by dissolving in formic acid (3.5). Pump eluent (3.12) through the column at a rate of 1,2 ml/min. Starting with the lowest concentration of a calibration series, inject 5 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. Immediately after UV detection, add the post-column reagent (3.19) at a rate of 0,5 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 injection of 5 µl successively for the other calibration solutions of the same component and the other calibration series.

6.1.2 Determination

Introduce a test sample (see clause 5) into a 25 ml volumetric flask. Add 20 ml of formic acid (3.5), close the flask and dissolve the sample, optionally by using ultrasonic vibration. Make up to the mark with formic acid (3.5). Pump eluent (3.12) through the column at a rate of 1,2 ml/min. Inject 5 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). Immediately after UV detection the post-column reagent (3.19) is added at a flow rate of 0,5 ml/min, mixing the eluent and the reagent in the reaction coil. Record the fluorescence chromatogram (see Figure 1). Measure the peak areas of the cyclic oligomers (UV detection) and linear oligomers (fluorescence detection) up to and including the hexamer.

6.2 Method B — Determination of caprolactam and the cyclic dimer of caprolactam

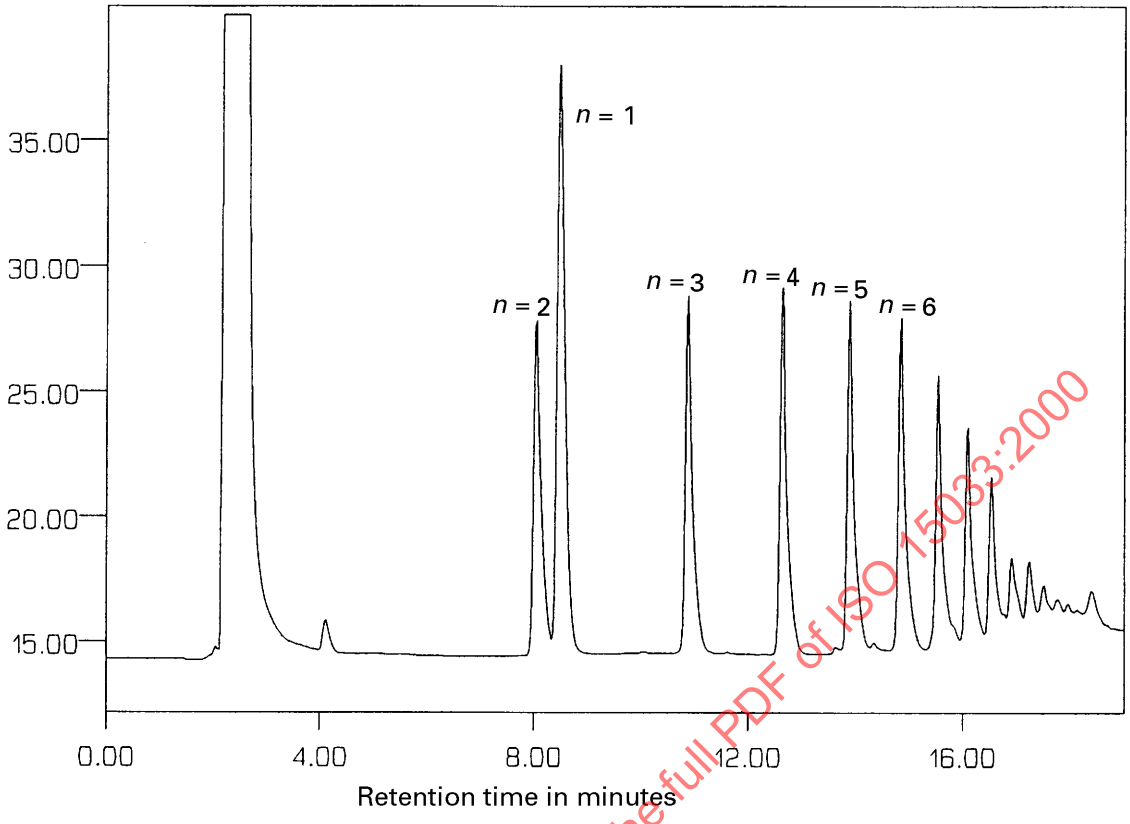
6.2.1 Calibration

Prepare a series of three calibration solutions of caprolactam (3.6) with concentrations increasing from 100 mg/l to 1 500 mg/l by dissolving in formic acid (3.5). Prepare a series of three solutions of the cyclic dimer of caprolactam (3.7) with concentrations increasing from 100 mg/l to 1 500 mg/l by dissolving in formic acid (3.5). Pump eluent (3.13) through the column at a flow rate of 1,2 ml/min. Starting with the lowest concentration of a calibration series, inject 5 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. Measure the peak area of the component.

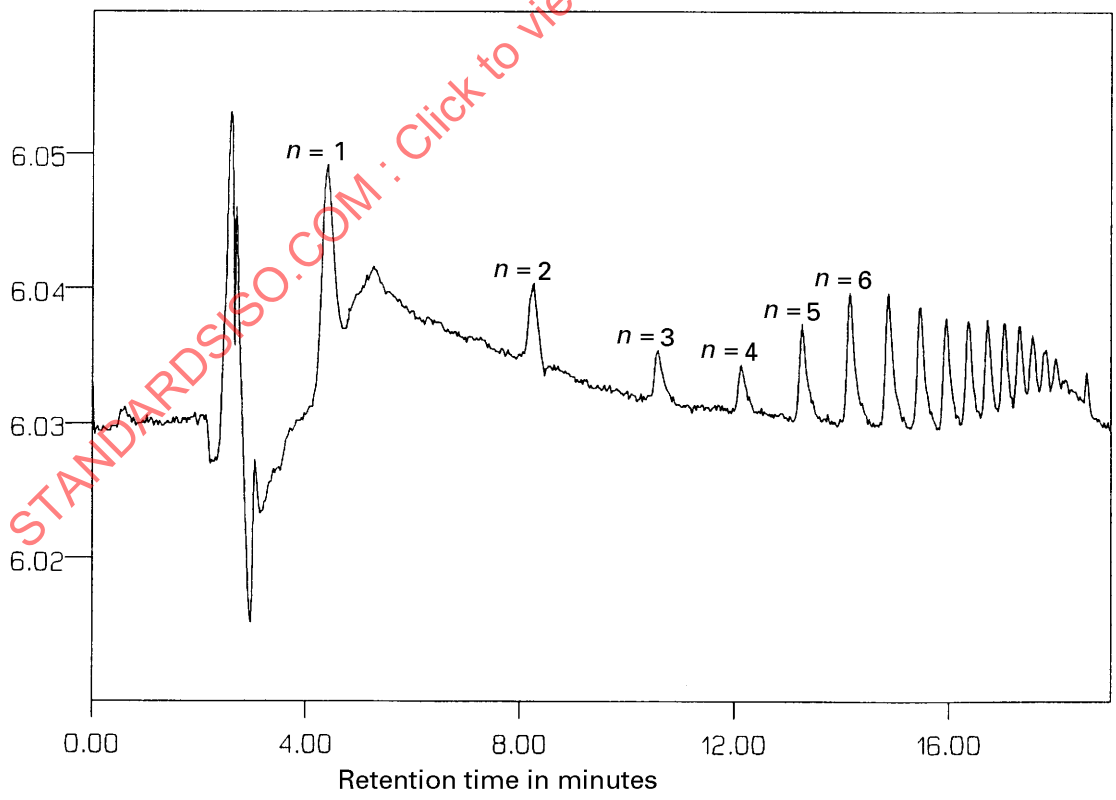
Repeat the injection of 5 µl successively for the other two calibration solutions of the same component and the second calibration series.

6.2.2 Determination

Introduce a test sample (see clause 5) into a 25 ml volumetric flask. Add 20 ml of formic acid (3.5), close the flask and dissolve the sample, optionally by using ultrasonic vibration. Make up to the mark with formic acid (3.5). Pump eluent (3.13) through the column at a rate of 1,2 ml/min. Inject 5 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 2). Measure the peak areas of caprolactam and the dimer of caprolactam.

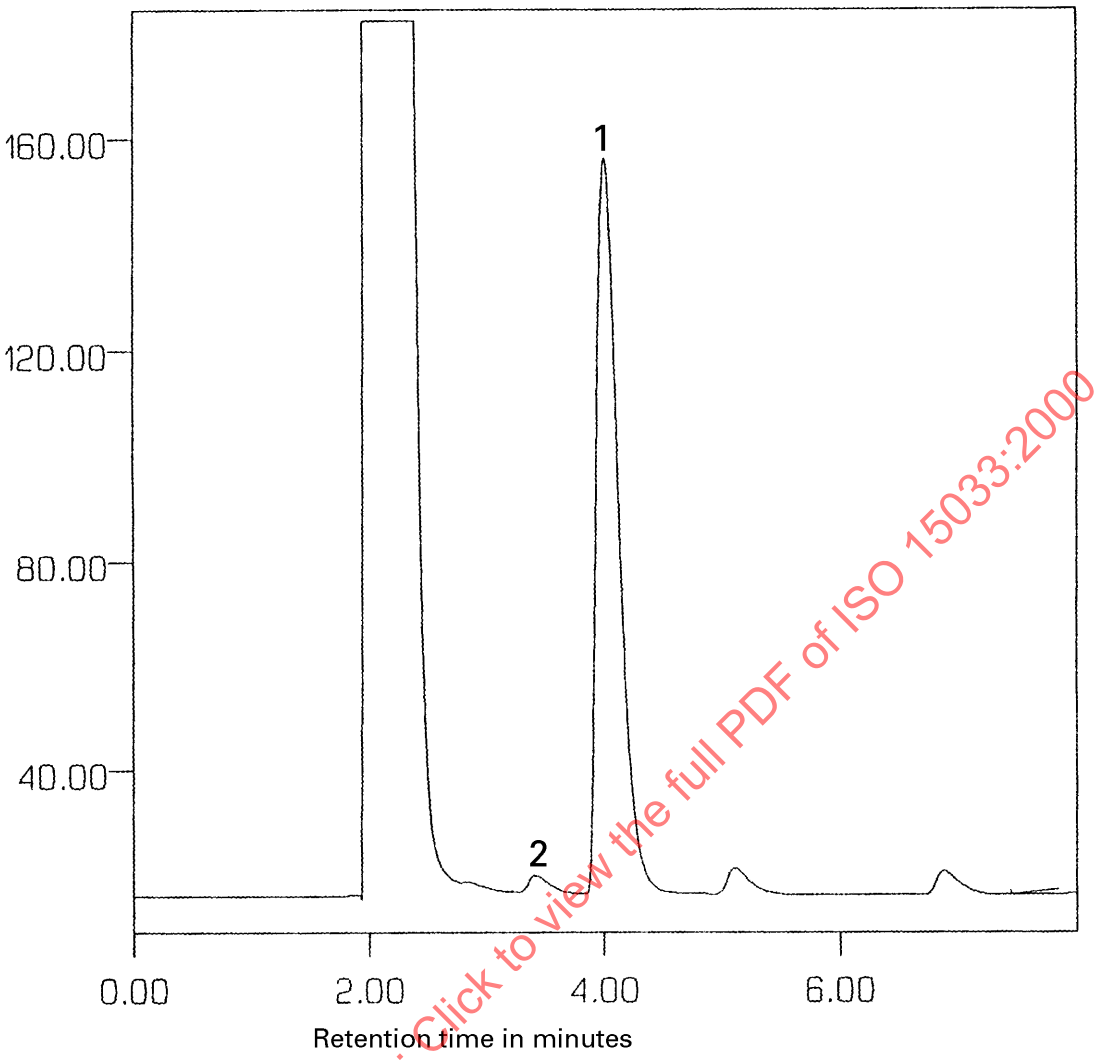


a) Cyclic oligomers



b) Linear oligomers

Figure 1 — Chromatograms obtained with method A
(determination of the cyclic and linear oligomers of caprolactam)



Key

1 Caprolactam
2 Cyclic dimer

Figure 2 — Chromatogram obtained with method B
(determination of caprolactam and the cyclic dimer of caprolactam)

7 Calculations

7.1 Method A — Determination of the cyclic and linear oligomers of caprolactam

7.1.1 Calculation of calibration factors

7.1.1.1 Calibration factors for cyclic oligomers

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

$$\frac{c_c - \sum(f \cdot A)}{A_c} = f_c \qquad (1)$$

where

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

A_c is the peak area of the relevant component;

$\sum(f \cdot A)$ is the sum of the concentrations of any other cyclic oligomers 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 for 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 three orders of magnitude, and do not interfere with the determination.

7.1.1.2 Calibration factors for linear oligomers

Calculate the calibration factor for ε-aminocaproic acid for each solution using the following equation:

$$\frac{c_{c1}}{A_{c1}} = f_{c1} \qquad (2)$$

where

c_{c1} is the concentration of ε-aminocaproic acid in the calibration solution, in mg/l;

A_{c1} is the peak area of ε-aminocaproic acid;

f_{c1} is the calibration factor for ε-aminocaproic acid.

Calculate the calibration factor for ε-aminocaproic acid 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 %.

Calculate the calibration factors for the linear oligomers for $n = 1$ up to and including $n = 6$ from the calibration factor for ε-aminocaproic acid on the basis of the ratio of their molecular mass to that of caprolactam (see Table 1), using the following equation:

$$f_{c1} \times M_r = f_c \qquad (3)$$

where

M_r is the molecular mass ratio for the relevant component in Table 1;

f_{c1} is the calculated calibration factor for ε-aminocaproic acid;

f_c is the calibration factor for the relevant component.

Table 1 — Molecular mass of linear oligomers relative to that of ε-aminocaproic acid

Component	Molecular mass, M	Molecular mass ratio, M_r
ε-Aminocaproic acid	131	1
Dimer	244	1,86
Trimer	357	2,73
Linear tetramer	470	3,59
Linear pentamer	583	4,45
Linear hexamer	696	5,31

7.1.2 Test sample

7.1.2.1 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, rounding the result to 0,1 % for concentrations greater than or equal to 10 % and to 0,01 % for concentrations below 10 %:

$$\frac{f_c \times A_{cs}}{400 \times m_g} = c_{cs} \quad (4)$$

where

f_c is the calibration factor for the relevant component, calculated in accordance with 7.1.1.1;

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

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

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

7.1.2.2 Calculation of linear oligomer concentrations

Calculate the concentration, in mg/kg, of each 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:

$$\frac{f_c \times A_{cs} \times 25}{m_g} = c_{cs} \quad (5)$$

where

f_c is the calibration factor for the relevant component, calculated in accordance with to 7.1.1.2;

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

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

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

7.2 Method B — Determination of caprolactam and the cyclic dimer of caprolactam

7.2.1 Calculation of calibration factors

Calculate the calibration factor for the relevant component for each solution using the following equation:

$$\frac{c_c}{A_c} = f_c \qquad (6)$$

where

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

A_c is the peak area of the relevant component;

f_c is the calibration factor for the relevant component.

Calculate the calibration factors for caprolactam and the dimer of caprolactam by taking the mean value of the three calculated calibration factors in the series for the relevant component.

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

7.2.2 Test sample

Calculate the concentration, in % by mass, of caprolactam and the cyclic dimer of caprolactam using the following equation, rounding the result to 0,1 % for concentrations greater than or equal to 10 % and to 0,01 % for concentrations below 10 %:

$$\frac{f_c \times A_{cs}}{400 \times m_g} = c_{cs} \qquad (7)$$

where

f_c is the calibration factor for the relevant component, calculated in accordance with 7.2.1;

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

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

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

8 Precision

The precision of this method is not known because inter-laboratory data are not available. However, the precision of the method is expected not to deviate significantly from the precision already determined in one laboratory and given below. Inter-laboratory data are being obtained and will be added at the next revision.

Precision data within one laboratory were determined by analysis of a reference polyamide 6. The reproducibility of the determination of the cyclic and linear oligomers of caprolactam using method A was determined by analysis of 68 samples and carried out by six laboratory employees within the laboratory over an extended period of time. In Tables 2 to 4, X is the mean, s the standard deviation and CV the coefficient of variation.

Table 2 — Cyclic oligomers — Reproducibility (% by mass)

	$n = 1$	$n = 2$	$n = 3$	$n = 4$	$n = 5$	$n = 6$
X	0,13	0,29	0,27	0,30	0,32	0,33
s	0,007 7	0,013 4	0,009 3	0,008 9	0,013 6	0,014 5
CV (%)	5,99	4,61	3,49	2,97	4,29	4,33

In general, the reproducibility of the determination of cyclic oligomers at a concentration of < 0,4 % by mass, expressed as a coefficient of variation, would be expected to be between 3 % and 6 %.

Table 3 — Linear oligomers — Reproducibility (mg/kg)

	$n = 1$	$n = 2$	$n = 3$	$n = 4$	$n = 5$	$n = 6$
X	< 4	21	34	68	133	202
s	—	2	3	7	7	11
CV (%)	—	9,4	9,2	10,6	5,6	5,4

In general, the reproducibility of the determination of linear oligomers at a concentration of < 100 mg/kg (ppm), expressed as a coefficient of variation, would be expected to be between 9 % and 10 %, and at concentrations higher than 100 mg/kg (ppm) lower than 6 %.

The repeatability was determined by analysis of nine samples in triplicate during a single analysis run.

Table 4 — Cyclic oligomers — Repeatability (% by mass)

	$n = 1$	$n = 2$
X	8,88	0,54
s	0,075	0,015 8
CV (%)	0,85	2,90

In general, the repeatability, expressed as a coefficient of variation, would be expected to be

— approximately 0,9 % at a concentration of 8 % to 9 % by mass for caprolactam;

— approximately 3 % at a concentration of 0,5 % to 0,6 % by mass for the cyclic dimer of caprolactam.

9 Test report

The test report shall include the following particulars:

a) a reference to this International Standard;

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

c) any deviation from the specifications for the HPLC equipment or from the procedure specified in this International Standard;

d) any treatment of the sample prior to the analysis;

e) the mass of the test sample used;

f) the content of the cyclic and linear oligomers of caprolactam in, respectively, % by mass and mg/kg (method A) or the content of caprolactam and the cyclic dimer of caprolactam in % by mass (method B);

g) a representative chromatogram;

h) the date of the analysis.