



BSI Standards Publication

# Plastics — Determination of average molecular mass and mixture ratio of poly(ethylene glycol) and its derivatives by MALDI-TOF-MS

**National foreword**

This British Standard is the UK implementation of ISO 19929:2017.

The UK participation in its preparation was entrusted to Technical Committee PRI/21, Testing of plastics.

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

This publication does not purport to include all the necessary provisions of a contract. Users are responsible for its correct application.

© The British Standards Institution 2017.  
Published by BSI Standards Limited 2017

ISBN 978 0 580 89838 9

ICS 83.080.01

**Compliance with a British Standard cannot confer immunity from legal obligations.**

This British Standard was published under the authority of the Standards Policy and Strategy Committee on 31 March 2017.

**Amendments/corrigenda issued since publication**

Date	Text affected
------	---------------

---

---

---

**Plastics — Determination of average  
molecular mass and mixture ratio  
of poly(ethylene glycol) and its  
derivatives by MALDI-TOF-MS**

*Plastiques — Détermination de la masse moléculaire moyenne et  
du rapport de mélange du poly(éthylène glycol) et de ses dérivés par  
MALDI-TOF-MS*





**COPYRIGHT PROTECTED DOCUMENT**

© ISO 2017, Published in Switzerland

All rights reserved. Unless otherwise specified, no part of this publication may be reproduced or utilized otherwise in any form or by any means, electronic or mechanical, including photocopying, or posting on the internet or an intranet, without prior written permission. Permission can be requested from either ISO at the address below or ISO's member body in the country of the requester.

ISO copyright office  
Ch. de Blandonnet 8 • CP 401  
CH-1214 Vernier, Geneva, Switzerland  
Tel. +41 22 749 01 11  
Fax +41 22 749 09 47  
copyright@iso.org  
www.iso.org

# Contents

Page

<b>Foreword</b> .....	<b>iv</b>
<b>Introduction</b> .....	<b>v</b>
<b>1 Scope</b> .....	<b>1</b>
<b>2 Normative references</b> .....	<b>1</b>
<b>3 Terms and definitions</b> .....	<b>1</b>
<b>4 Principles</b> .....	<b>3</b>
<b>5 Reagents</b> .....	<b>3</b>
<b>6 Apparatus</b> .....	<b>4</b>
<b>7 Procedure</b> .....	<b>5</b>
7.1 General.....	5
7.2 Sample preparation.....	5
7.2.1 General.....	5
7.2.2 Preparation of polymer standard mixtures.....	6
7.2.3 Preparation of polymer/matrix/salt solutions.....	6
7.2.4 Deposition of the sample on the sample plate (target).....	6
7.2.5 Preparation of biopolymer/matrix solutions.....	7
7.3 Instrument settings.....	7
7.4 Spectra recording.....	8
<b>8 Data acquisition and processing</b> .....	<b>8</b>
8.1 General.....	8
8.2 Calibration of mass axis.....	8
8.2.1 General.....	8
8.2.2 Calibration of mass axis using synthetic polymer standards.....	9
8.2.3 Calibration of mass axis using biopolymer standards.....	9
8.2.4 Self-calibration method.....	9
8.3 Generation of mass calibration curve.....	9
8.4 Calibration of intensity axis.....	9
<b>9 Expression of results</b> .....	<b>10</b>
9.1 Calculation of molecular mass distribution (MMD).....	10
9.2 Calculation of the number-average molecular mass.....	10
9.3 Calculation of peak area.....	10
9.4 Constructing calibration curve for intensity.....	10
9.5 Calculation of mass ratio.....	11
<b>10 Precision</b> .....	<b>11</b>
<b>11 Test report</b> .....	<b>11</b>
11.1 General.....	11
11.2 Apparatus.....	11
11.3 Calibration.....	11
11.4 Results.....	11
<b>Annex A (normative) Calibrants</b> .....	<b>12</b>
<b>Bibliography</b> .....	<b>13</b>

## 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](http://www.iso.org/directives)).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see [www.iso.org/patents](http://www.iso.org/patents)).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation on 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 the following URL: [www.iso.org/iso/foreword.html](http://www.iso.org/iso/foreword.html).

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

## Introduction

For quality control and research of polymeric materials, it is important to know the composition of polymer mixtures with different terminal groups. In contrast to traditional methods such as liquid chromatography, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) is a rapid and effective method to characterize polymer mixtures because of its high mass resolution. It can also be applied to quantitation of mixtures of different polymers. Interlaboratory comparisons of quantitative MALDI-TOF-MS performed for mixtures of PEG and its derivatives can ensure standardized conditions of measurement. Standardization of quantitative MALDI-TOF-MS may promote increasing applications of this analytical technique.





# Plastics — Determination of average molecular mass and mixture ratio of poly(ethylene glycol) and its derivatives by MALDI-TOF-MS

## 1 Scope

This document specifies a general method for determining the average molecular mass and mixture ratio of poly(ethylene glycol) (PEG) and its derivatives with different end groups by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS). It is applicable to PEG and its derivatives with molecular masses from 500 g mol<sup>-1</sup> to 20 000 g mol<sup>-1</sup>. The composition is calculated by means of a calibration curve constructed using standard polymer mixtures, where the peak area ratio is plotted versus the mass ratio. This document can be applied to other polymers with monomeric unit similar to PEG.

## 2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements 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*

ISO 10927:2011, *Plastics — Determination of the molecular mass and molecular mass distribution of polymer species by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS)*

## 3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 472, ISO 10927 and the following apply.

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

- IEC Electropedia: available at <http://www.electropedia.org/>
- ISO Online browsing platform: available at <http://www.iso.org/obp>

### 3.1

#### **matrix-assisted laser desorption/ionization time-of-flight mass spectrometry MALDI-TOF-MS**

technique in which the separation is based on different flight times in a field free flight tube depending on the mass of formed polymer ions after ionization by a laser, desorption and acceleration by high voltage

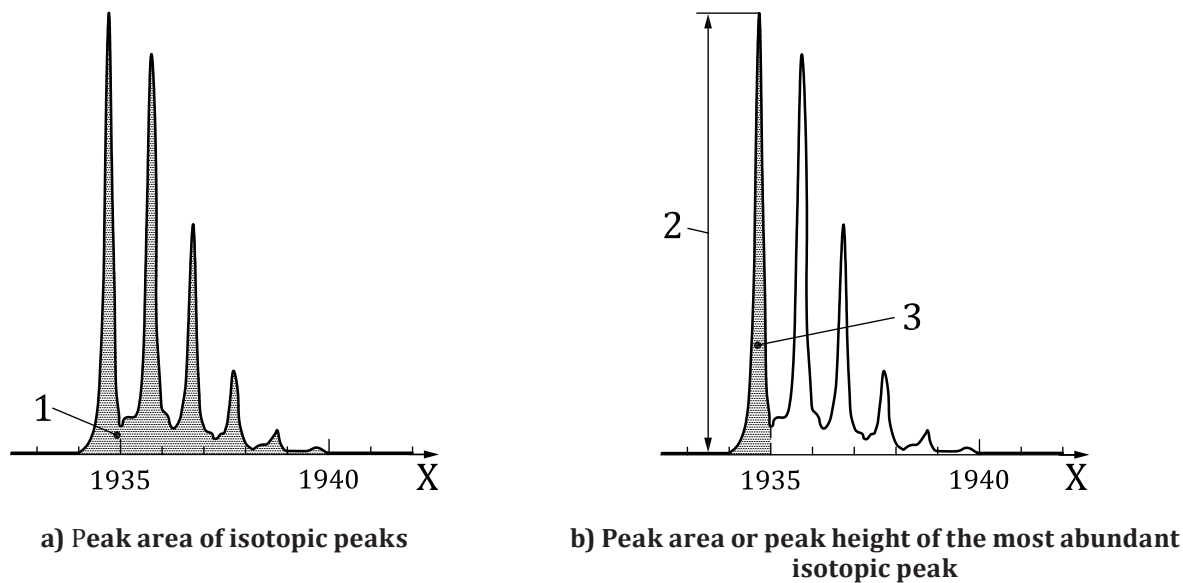
### 3.2

#### **peak area**

*A*

sum of peak areas,  $A_{ij}$ , where  $A_{ij}$  is an area under the curve of the mass,  $M_{ij}$ , associated with the  $j$ -th species of polymer  $i$

Note 1 to entry: As [Figure 1](#) a) shows, integration for peak area,  $A_{ij}$ , should be performed over all isotopes related to the  $j$ -th species. If the software is not able to integrate all isotopic peaks, the peak area of the most abundant isotopic peak can be used instead [see [Figure 1](#) b)]. For data handling, see ISO 10927:2011, 6.7.



**Key**

- 1 “peak area”,  $A_{ij}$
- 2 “peak height” of the most abundant isotopic peak of  $[\text{PEG}(n = 43)+\text{Na}]^+$
- 3 “peak area” of the most abundant isotopic peak of  $[\text{PEG}(n = 43)+\text{Na}]^+$
- X mass/charge

**Figure 1 — Definitions of peak area and peak height**

**3.3  
 molecular mass**

$M$   
 sum of the masses of atoms

Note 1 to entry: The molecular mass of the  $j$ -th species,  $M_j$ , is also calculated as the average mass of isotopes.

Note 2 to entry: The terms “molecular weight” and “molar mass” are also used instead of “molecular mass”.

**3.4  
 number-average molecular mass**

$M_n$   
 molecular mass defined as:

$$M_n \equiv \frac{\sum_j N_j M_j}{\sum_j N_j}$$

Note 1 to entry:  $N_j$  is the number of molecules of species  $j$  of molecular mass  $M_j$ .

**3.5  
 weight-average molecular mass**

$M_w$   
 molecular mass defined as:

$$M_w \equiv \frac{\sum_j (N_j \times M_j^2)}{\sum_j (N_j \times M_j)}$$

### 3.6

#### z-average molecular mass

$M_z$

molecular mass defined as:

$$M_z \equiv \frac{\sum_j (N_j \times M_j^3)}{\sum_j (N_j \times M_j^2)}$$

## 4 Principles

The MALDI process involves the ablation and the ionization of an analyte dispersed in a small organic molecule matrix. The matrix shall be able to absorb the laser energy. A metal salt may be added to cationize the analyte. A polymer is co-crystallized or co-mixed with the matrix molecule and deposited on the target. A short duration UV laser pulse is used to ablate the matrix and the analyte. The laser energy is transferred to the matrix molecules, causing them to vaporize. Analyte and matrix molecules leave the target surface in a plume. Due to the very short desorption time, polymer molecules do not degrade. The polymer in the ablation plume gains a cation and is accelerated by a high voltage, drifts down the field free flight tube and is detected at the end of the flight tube. The time-of-flight of the species depends on their molecular masses, and needs to be calibrated with standards of known molecular masses. Biopolymers such as proteins are often used to this end.

Ideally, the product of the ratio between the total sum of peak areas and that between number-average molecular mass is proportional to the mass ratio, i.e.

$$\left( \frac{A_1}{A_0} \right) \cdot \left( \frac{M_{n,1}}{M_{n,0}} \right) = k_{10} \frac{W_1}{W_0} \quad (1)$$

where  $A_i$ ,  $W_i$  and  $M_{n,i}$  are sum of peak areas in MALDI-TOF-MS spectra, mass and number-average molecular mass of polymer  $i$  ( $i = 0$  or  $1$ ), respectively. The proportionality constant,  $k_{10}$ , is experimental and generally depends on the combination of polymers with different chemical structures. Ideally, if no mass discrimination is observed,  $k_{10} = 1$ .

## 5 Reagents

### 5.1 Matrices.

$\alpha$ -Cyano-4-hydroxycinnamic acid (CHCA) and 1,8,9-trihydroxyanthracene (dithranol) are the recommended matrices for this method. Other matrices can be used after examining sufficient ionizing ability and solubility in solvents used. The procedure described in [Clause 7](#) can be applied to examination of the ability and solubility. All materials should be at least 97 % pure. They should be stored in a freezer or refrigerator. They should be warmed up to room temperature right before use. Regulated reagents shall be handled in accordance with regulations.

### 5.2 Salts.

Lithium, sodium and potassium salts, e.g. iodides or trifluoroacetates, are recommended.

### 5.3 Solvents.

Methanol and tetrahydrofuran (THF) are recommended since they are good solvents of PEG and its derivatives. They also applied to polar polymers. The solvents should be at least 97 % pure. If the solvents are regulated, they should be treated safely.

## 5.4 Molecular mass standards.

The calibration of the mass axis should be done using biopolymers and/or synthetic polymers with known repeating units and defined end groups. The molecular mass of the standards shall be within the range of the molecular mass of the investigated polymer. The software of the mass spectrometer should be used for calibration. The list of recommended biopolymers and their molecular masses in [Table A.1](#) shall be used.

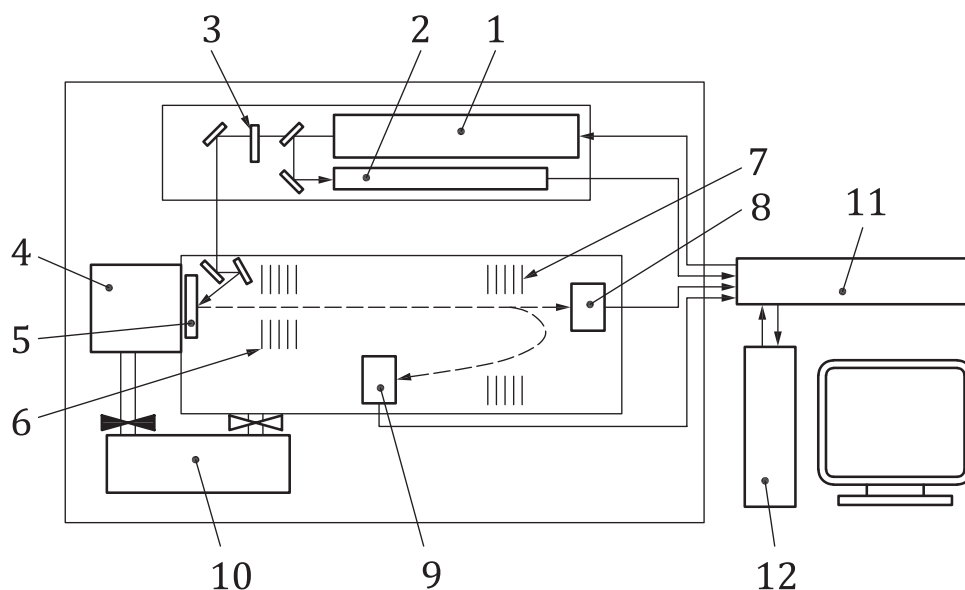
## 5.5 Mixed polymer standards.

Polymers used for the calibration of mass ratios should have identical chemical repeating unit and similar molecular mass distributions. The preparation of solutions of mixed polymer standards should be done using a balance.

# 6 Apparatus

## 6.1 General

A schematic diagram of a MALDI-TOF mass spectrometer is shown in [Figure 2](#). Essential components are sample introduction chamber, a laser source, an ion source, a flight tube with an acceleration region and an ion detector (linear detector). The instruments may have additionally an ion deflector and a reflector detector. Both commercially available TOF mass spectrometers and systems assembled in the laboratory may be used for this method, provided they meet the required levels of performance.



### Key

1	laser source	7	reflector (ion deflector)
2	counter	8	linear detector
3	optical system with beam splitter and attenuator	9	reflector detector
4	sample introduction chamber	10	vacuum pump system
5	target (ion source)	11	data recording
6	ion acceleration optics	12	computer

**Figure 2 — Schematic diagram of a MALDI-TOF mass spectrometer**

## 6.2 Sample introduction chamber/target

A MALDI sample consists of a film of the analyte, matrix and salt mixture deposited onto a metal sample plate. The entire plate and MALDI sample are often referred to as a MALDI target. The MALDI target is introduced into the spectrometer vacuum chamber from sample introduction chamber by either a manual or an automatic operation. The target is moveable, so that all sample spots are accessible by the laser beam.

## 6.3 Laser source

The laser system comprises a pulsed laser, an attenuator which allows for the adjustment of the laser power, and a lens and mirror system to direct the laser beam onto the MALDI target. Some commercial instruments have beam splitters to direct a fraction of the laser light to a photodiode to start the timing for the TOF measurement. The wavelength of the laser should be in the absorption range of the matrices. Typically, UV-lasers are used.

## 6.4 Flight tube

The target is at a high voltage of several kV and just behind the acceleration optics. The analyte/matrix/salt mixture is deposited on this target and exposed to the pulsed laser beam. Thereby, gaseous analyte ions are formed which are accelerated by the electric field, exit the source and pass through into the flight tube. The flight tube is a field free drift region.

## 6.5 Detector

Ion detection in a TOF mass analyser is based on the fast measurement of the electrode voltage after an ion impact. This is done in a detector in which the signal is proportional to the number of ions hitting the detector.

## 6.6 Data recording

A multichannel recorder basing on the principle of “analogue-to-digital” conversion should be used.

## 6.7 Data handling

For data analysis, a computer should be used which should be able to read, store and analyse the data. Software should be able to determine a baseline, convert the data from time to mass through a calibration curve and integrate peak area of each species. It is recommended that all isotopic peaks for each species can be calculated automatically. If the software cannot integrate the peak area automatically, it is acceptable to use the peak height of the most abundant isotopic peak instead of the peak area. If some overlapping isotope patterns are observed, quit data analysis.

# 7 Procedure

## 7.1 General

The procedure includes setting up the MALDI-TOF mass spectrometer, sample preparation and calibration, data acquisition and processing. Typically, the vacuum systems and high voltage power supplies of a TOF-MS, and computers and other parts of the data collection system are left on at all times.

## 7.2 Sample preparation

### 7.2.1 General

Targets shall be prepared as described in [7.2.2](#) and [7.2.3](#). Three different sample spots should be prepared and one spectrum from each spot should be recorded. At minimum, 100 shots should be recorded for one spectrum. These spots shall be prepared using one solution of solvent, polymer, matrix

and salt (see 7.2.2). The parameters of the mass spectrometer (laser, acceleration voltage, etc.) should be kept constant during the acquisition of all three spectra. For the adjustment of optimal instrument parameters, additional spots shall be used. The adjustment of the laser attenuation is described in 7.3.

## 7.2.2 Preparation of polymer standard mixtures

It is recommended to mix two solutions of known concentrations. For their preparation, it is strongly recommended to determine the masses of each polymer using a balance.

The concentrations of polymer 0 ( $c_0'$ ) and polymer 1 ( $c_1'$ ) in a mixture are calculated using Formula (2) and Formula (3), where  $c_0$  and  $c_1$  represent the individual concentration (in mg ml<sup>-1</sup>) of polymer 0 in solution 0, and polymer 1 in solution 1, and  $V_0$  and  $V_1$  are the volumes of solution 0 and 1 (in ml).

$$c_0' = \frac{c_0 V_0}{V_0 + V_1} \quad (2)$$

$$c_1' = \frac{c_1 V_1}{V_0 + V_1} \quad (3)$$

Accordingly, the mass ratio,  $W_1/W_0$ , for polymers 0 and 1 is given by:

$$\frac{W_1}{W_0} = \frac{c_1 V_1}{c_0 V_0} \quad (4)$$

Alternatively, polymers 0 and 1 can be mixed using balance first, followed by adding the solvent. In this case, the volume of the solvent shall be measured precisely.

At least three ratios of mixtures should be prepared to perform least squares method.

To reduce the uncertainty, the concentration of the mixed polymer standard solutions should be in the same range of that of the sample solution. For example, total mass concentration may be 1 mg ml<sup>-1</sup> as shown in 7.2.3.

## 7.2.3 Preparation of polymer/matrix/salt solutions

The preparation procedure in ISO 10927:2011, 7.2.2 can be applied. Other procedures can also be applied to polymer mixtures. As an example, the following recipe has been found to work successfully for both polymer sample and mixed polymer standards through interlaboratory comparisons:

- 1 mg/ml polymer mixture dissolved in the recommended solvent;
- 10 mg/ml matrix dissolved in the same solvent;
- 1 mg/ml salt dissolved in the same solvent.

Three different solutions (polymer/matrix/salt) should be mixed in a ratio (v/v/v) of 1/1/1. Pre-mixed solutions shall be used within 24 h. For sample preparation, one of the methods described in 7.2.4 may be used.

## 7.2.4 Deposition of the sample on the sample plate (target)

### 7.2.4.1 General

Sample preparation is critical to the quality of the MALDI-TOF-MS data obtained. Thus, a variety of methods have been developed to deposit the sample solutions onto the sample plate surface to obtain good dispersion.

#### 7.2.4.2 Hand-spotting (air-dried droplet technique)

0,5 µl to 2 µl of the pre-mixed solution is used to deposit onto the target plate. The solvent is allowed to evaporate rapidly.

#### 7.2.4.3 Spray technique

The pre-mixed solution is pressed through a needle or capillary either manually by means of a syringe or using a syringe pump. The needle/capillary is heated. The evaporation of the solvent can be performed by spraying by means of gas (air, nitrogen, etc.) or by ultrasonic evaporation. Another method is offered by the electrospray technique. The needle/capillary of the syringe is held at a potential of between 3 kV to 7 kV against the sample target. Depending on the temperature, gas stream or potential difference, the solution is deposited at 2 µl/min up to 0,5 ml/min. Suitable conditions need be chosen, which ensure a nearly complete evaporation of the solvent and a dry deposition.

#### 7.2.5 Preparation of biopolymer/matrix solutions

1 nmol to 10 nmol of the biopolymer is dissolved in an aqueous solution of trifluoroacetic acid (TFA) (0,1 %). 10 mg of  $\alpha$ -cyano-4-hydroxycinnamic acid (CHCA) is dissolved in 1 ml of a mixture of acetonitrile/water (50/50 w/w) in 0,1 % TFA.

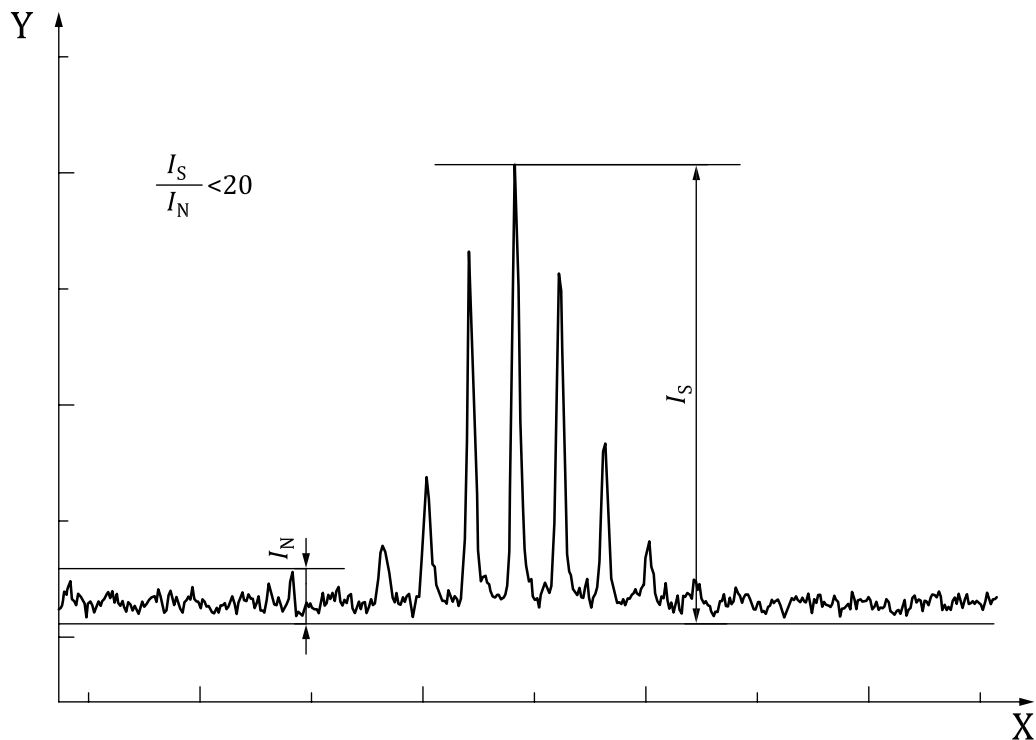
For biopolymers with masses about 5 000 g mol<sup>-1</sup>, only 0,1 nmol to 1 nmol of the biopolymer is dissolved in an aqueous solution of TFA (0,1 %). 10 mg of 3-(4-hydroxy-3,5-dimethoxy-phenyl)acrylic acid (sinapinic acid) dissolved in 1 ml acetonitrile/water (50/50 w/w) in 0,1 % TFA is used as matrix.

A 1/1 v/v mixture of both solutions is prepared and deposited on the target according to the hand-spotting method described in [7.2.4.2](#).

### 7.3 Instrument settings

Instrument parameters (except for laser energy) should be optimized for the range of expected molecular mass distribution (MMD) following instrument manufacturer's instructions.

Optimum laser energy for each polymer and matrix combination varies. Therefore, the following protocol for laser energy setting should be applied: Once all other instrument settings are made, start pulsing the laser and moving it across the surface by using the laser at the highest attenuation (lowest laser energy). Slowly decrease the attenuation (raise the laser energy) until signal from the matrix alone appears. Decrease the laser attenuation (increase the laser energy) while watching for polymer signal in the mass region where it is expected. Adjust laser attenuation so one obtains a signal-to-noise ratio of at least 20:1 for accumulations of 100 laser shots on a peak near the maximum of the distribution. This is schematically shown in [Figure 3](#). For some polymers, the use of a higher laser energy than necessary to obtain signal to noise higher than 20:1 can lead to fragmentation of the polymer and should be avoided if observed.



**Key**

X mass/charge

Y intensity

**Figure 3 — Determination of signal-to-noise ratio**

## 7.4 Spectra recording

At the attenuation obtained in 7.3, signals from a total of 100 laser shots are accumulated. This procedure should be repeated three times at three different spots or at three different locations on the same spot obtaining three spectra. Randomly chosen spots or the entire sample plate region that has been spotted with matrix and polymer and salt should be chosen. If three different sample spots were made, one spectrum from each spot should be taken. The laser or instrument settings should not be changed during the acquisition of all three spectra.

## 8 Data acquisition and processing

### 8.1 General

Due to various data systems and computer software, data acquisition can be different. The raw data file generally consists data pairs (signal intensity versus time of flight), which, through the use of a calibration curve, enables the construction of a mass spectrum (signal versus mass).

### 8.2 Calibration of mass axis

#### 8.2.1 General

Two methods for calibration of the mass axis are suggested in 8.2.2 and 8.2.3.



## 8.2.2 Calibration of mass axis using synthetic polymer standards

In this method of calibration, a synthetic polymer standard with known repeat unit and end groups is used. A previous calibration of the mass spectrometer using a biopolymer can support the correct assignment of oligomers to corresponding masses.

### 8.2.2.1 Selection of standards

A well-characterized synthetic polymer standard in the mass range of the polymer whose MMD is investigated shall be used. If a biopolymer is used in addition, its mass shall be in the mid-range of the synthetic polymer calibrant. The main peak from the biopolymer is assigned to its mass as given in [Table A.1](#).

### 8.2.2.2 Sample preparation

Solutions of polymer standards used for calibration shall be prepared according to the procedure given in [7.2](#). Synthetic polymers used for the final calibration should be run under the same conditions (matrix and laser fluence) as the test samples.

## 8.2.3 Calibration of mass axis using biopolymer standards

### 8.2.3.1 Selection of biopolymers

Biopolymers from [Table A.1](#) are used for mass axis calibration. A fresh solution of biopolymers for the mass axis calibration is prepared. For calibration, at least four masses are used. These masses should be selected to bracket the anticipated mass range of the polymer. In addition, masses of the salt and the matrix can be used for calibration.

### 8.2.3.2 Sample preparation

The preparation of the biopolymer for the calibration shall be performed following the procedure given in [7.2.4](#).

## 8.2.4 Self-calibration method

The principle of self-calibration using a polymer, which is intended to be characterized, can be applied if oligomer structure and structure of the end groups of the polymer are known. Additionally, the use of a previously done exact calibration is essential. Using this method, a single peak of the sample can be attributed to its theoretical mass.

The self-calibration method should be exceptionally used and shall be regarded as a fine-tuning of calibration methods described in [8.2.2](#) and [8.2.3](#).

## 8.3 Generation of mass calibration curve

Generally, any instrument will have a software to derive a calibration curve for the instrument. This curve shall be calculated by at least four calibration points obtained from either method described in [8.2](#).

## 8.4 Calibration of intensity axis

The calibration of the intensity axis for considered polymers with a polydispersity of less than 1,2 is not necessary.

## 9 Expression of results

### 9.1 Calculation of molecular mass distribution (MMD)

Once the MALDI spectrum of the polymer is recorded, the intensity of each species ( $i$ ) in the distribution shall be determined. The limits of calculation shall be defined by determination of those species ( $i$ ) with the lowest and highest molecular weight. A signal-to-noise ratio of at least 3:1 is used as the threshold to be used for the integration of the area.

Integration shall be performed over all the isotopes related to a peak. The mass of the peak is assigned as the apex,  $M_p$ , or the centroid,  $M_c$ , of that peak for each integral. The choice of  $M_p$  or  $M_c$  should be consistent with which choice was used in the creation of the mass calibration curve.

### 9.2 Calculation of the number-average molecular mass

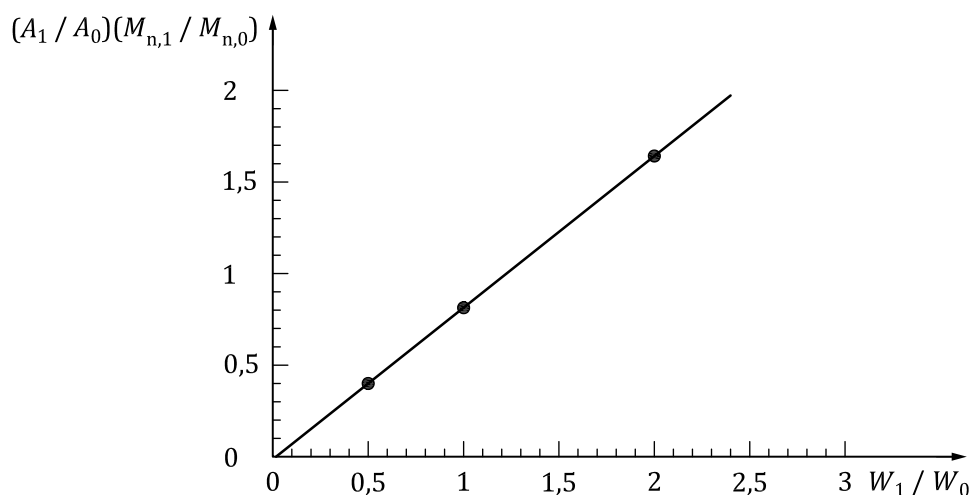
The number-average,  $M_{n,i}$ , of each polymer  $i$  should be computed using the formula shown in [3.4](#).

### 9.3 Calculation of peak area

The peak area,  $A_{ij}$ , of polymer  $i$  is calculated as a sum of peak areas of  $j$ -species of polymer  $i$ .

### 9.4 Constructing calibration curve for intensity

Construct a calibration curve (see [Figure 4](#)) by plotting the ratio of  $(A_1/A_0)(M_{n,1}/M_{n,0})$  versus  $W_1/W_0$  for different concentrations of the mixed polymer standard solutions. To obtain the calibration curve, first-order least squares fit is recommended.



#### Key

$(A_1/A_0)(M_{n,1}/M_{n,0})$  ratio shown in [Formula \(1\)](#)  
 $W_1/W_0$  mass ratio shown in [Formula \(4\)](#)

**Figure 4 — Calibration curve for peak area ratio as a function of mass ratio**

## 9.5 Calculation of mass ratio

Calculate the mass ratio of polymers 0 and 1 using the calibration curve constructed in 9.4 using [Formula \(5\)](#):

$$\frac{W_1}{W_0} = \frac{1}{k_{10}} \cdot \frac{A_1}{A_0} \cdot \frac{M_{n,1}}{M_{n,0}} \quad (5)$$

where  $k_{10}$  is the slope of the calibration curve.

## 10 Precision

At the time of publication, there are no precision data available for this method.

## 11 Test report

### 11.1 General

The test report shall contain all details necessary for complete identification of the polymer analysed and the following information, as applicable.

### 11.2 Apparatus

- a) the sample preparation method (hand spotting, spray or grinding) and the exact recipe used for sample preparation;
- b) the date of the analysis and that of the calibration;
- c) the type of MALDI-TOF mass spectrometer, the model and the manufacturer (if instrument is home built, a description of the apparatus);
- d) instrument settings;
- e) the data-processing system, software version, the model and the manufacturer.

### 11.3 Calibration

- a) the compounds used for mass axis calibration;
- b) the method used for mass axis calibration;
- c) the calibration data for mixture ratio versus peak area ratio;
- d) the calibration curve.

### 11.4 Results

- a) the mass spectrum;
- b) the table of number-average MMD;
- c) the number-average molecular mass,  $M_n$ ;
- d) the ratio of mass of the polymer mixture.

## Annex A (normative)

### Calibrants

**Table A.1 — Calibrants**

Calibrant [CAS No.]	Average molecular mass of M <sup>+</sup> ion <sup>a</sup> u	Monoisotopic (exact) molecular mass of M <sup>+</sup> ion <sup>b</sup> u	Average molecular mass of MH <sup>+</sup> ion <sup>a</sup> u	Monoisotopic (exact) molecular mass of MH <sup>+</sup> ion <sup>b</sup> u
Dithranol [1143-38-0]	226,227 400	226,062 994	227,234 791 4	227,070 271
2,5-Dihydroxybenzoic acid [490-79-9]	154,120 140	154,026 609	155,127 531 4	155,033 885
Sinapinic acid [530-59-6]	224,209 98	224,068 473	225,217 371 4	225,075 75
Angiotensin II, human [68521-88-0]	1 046,197 2	1 045,534 5	1 047,205 2	1 046,542 3
ACTH(18-39) (CLIP), human [53917-42-3]	2 465,708 7	2 464,191 1	2 466,716 6	2 465,198 9
Insulin, bovine [11070-73-8]	5 733,581 5	5 729,600 9	5 734,589 5	5 730,608 7
Ubiquitin [79586-22-4]	8 564,877 1	8 559,616 7	8 565,885 1	8 560,624 5
C8857 cytochrome c, equine [9007-43-6]	12 360,142 6		12 361,150 6	
Myoglobin, equine [9047-17-0]	17 568,0			
Myoglobin, apomyoglobin [9008-17-0]	16 951,543 3		16 952,551 3	
Trypsin, bovine [9002-07-7]	23 311,5		23 312,5	
Bovine serum albumin [9048-46-8]	66 430,069 4		66 431,077 4	
The use of silver salts can result in the formation of clusters, and silver salts shall not therefore be used for calibration.				
<sup>a</sup> The mass of the ion is calculated by summing the atomic masses of the constituent elements (weighted average of all isotopes of an element), i.e. C = 12,011 15, H = 1,007 97, O = 15,999 4.				
<sup>b</sup> The mass of the ion is calculated by summing the exact atomic masses of the main isotopes of the constituent elements, i.e. C = 12,000 0, H = 1,007 825, O = 15,994 915 (taking the missing electron into consideration).				

## Bibliography

- [1] ASTM D7134-05, *Standard Test Method for Molecular Mass Averages and Molecular Mass Distribution of Atactic Polystyrene by Matrix Assisted Laser Desorption/Ionization (MALDI)-Time of Flight (TOF) Mass Spectrometry (MS)*





# British Standards Institution (BSI)

BSI is the national body responsible for preparing British Standards and other standards-related publications, information and services.

BSI is incorporated by Royal Charter. British Standards and other standardization products are published by BSI Standards Limited.

## About us

We bring together business, industry, government, consumers, innovators and others to shape their combined experience and expertise into standards-based solutions.

The knowledge embodied in our standards has been carefully assembled in a dependable format and refined through our open consultation process. Organizations of all sizes and across all sectors choose standards to help them achieve their goals.

## Information on standards

We can provide you with the knowledge that your organization needs to succeed. Find out more about British Standards by visiting our website at [bsigroup.com/standards](http://bsigroup.com/standards) or contacting our Customer Services team or Knowledge Centre.

## Buying standards

You can buy and download PDF versions of BSI publications, including British and adopted European and international standards, through our website at [bsigroup.com/shop](http://bsigroup.com/shop), where hard copies can also be purchased.

If you need international and foreign standards from other Standards Development Organizations, hard copies can be ordered from our Customer Services team.

## Copyright in BSI publications

All the content in BSI publications, including British Standards, is the property of and copyrighted by BSI or some person or entity that owns copyright in the information used (such as the international standardization bodies) and has formally licensed such information to BSI for commercial publication and use.

Save for the provisions below, you may not transfer, share or disseminate any portion of the standard to any other person. You may not adapt, distribute, commercially exploit, or publicly display the standard or any portion thereof in any manner whatsoever without BSI's prior written consent.

## Storing and using standards

Standards purchased in soft copy format:

- A British Standard purchased in soft copy format is licensed to a sole named user for personal or internal company use only.
- The standard may be stored on more than 1 device provided that it is accessible by the sole named user only and that only 1 copy is accessed at any one time.
- A single paper copy may be printed for personal or internal company use only.

Standards purchased in hard copy format:

- A British Standard purchased in hard copy format is for personal or internal company use only.
- It may not be further reproduced – in any format – to create an additional copy. This includes scanning of the document.

If you need more than 1 copy of the document, or if you wish to share the document on an internal network, you can save money by choosing a subscription product (see 'Subscriptions').

## Reproducing extracts

For permission to reproduce content from BSI publications contact the BSI Copyright & Licensing team.

## Subscriptions

Our range of subscription services are designed to make using standards easier for you. For further information on our subscription products go to [bsigroup.com/subscriptions](http://bsigroup.com/subscriptions).

With **British Standards Online (BSOL)** you'll have instant access to over 55,000 British and adopted European and international standards from your desktop. It's available 24/7 and is refreshed daily so you'll always be up to date.

You can keep in touch with standards developments and receive substantial discounts on the purchase price of standards, both in single copy and subscription format, by becoming a **BSI Subscribing Member**.

**PLUS** is an updating service exclusive to BSI Subscribing Members. You will automatically receive the latest hard copy of your standards when they're revised or replaced.

To find out more about becoming a BSI Subscribing Member and the benefits of membership, please visit [bsigroup.com/shop](http://bsigroup.com/shop).

With a **Multi-User Network Licence (MUNL)** you are able to host standards publications on your intranet. Licences can cover as few or as many users as you wish. With updates supplied as soon as they're available, you can be sure your documentation is current. For further information, email [subscriptions@bsigroup.com](mailto:subscriptions@bsigroup.com).

## Revisions

Our British Standards and other publications are updated by amendment or revision.

We continually improve the quality of our products and services to benefit your business. If you find an inaccuracy or ambiguity within a British Standard or other BSI publication please inform the Knowledge Centre.

## Useful Contacts

### Customer Services

**Tel:** +44 345 086 9001

**Email (orders):** [orders@bsigroup.com](mailto:orders@bsigroup.com)

**Email (enquiries):** [cservices@bsigroup.com](mailto:cservices@bsigroup.com)

### Subscriptions

**Tel:** +44 345 086 9001

**Email:** [subscriptions@bsigroup.com](mailto:subscriptions@bsigroup.com)

### Knowledge Centre

**Tel:** +44 20 8996 7004

**Email:** [knowledgecentre@bsigroup.com](mailto:knowledgecentre@bsigroup.com)

### Copyright & Licensing

**Tel:** +44 20 8996 7070

**Email:** [copyright@bsigroup.com](mailto:copyright@bsigroup.com)

### BSI Group Headquarters

389 Chiswick High Road London W4 4AL UK