INTERNATIONAL STANDARD

**ISO 11215**

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# Modified starch — Determination of adipic acid content of acetylated di-starch adipates — Gas chromatographic method

*Amidons et fécules modifiés — Détermination de la teneur en acide adipique dans les adipates de diamidon acétylés — Méthode par chromatographie en phase gazeuse*



Reference number
ISO 11215:1998(E)

## 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.

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.

International Standard ISO 11215 was prepared by Technical Committee ISO/TC 93, *Starch (including derivatives and by-products).*

Annexes A to C of this International Standard are for information only.





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# Modified starch — Determination of adipic acid content of acetylated di-starch adipates — Gas chromatographic method



## 1 Scope

This International Standard specifies a method for the gas chromatographic determination of total adipic content and free adipic acid content of acetylated di-starch adipates.

## 2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this International Standard. At the time of the publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on the International Standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 1666:1996, *Starch — Determination of moisture content — Oven-drying method*

ISO 3696:1987, *Water for analytical laboratory use — Specification and test methods*

## 3 Principle

The test portion is dispersed in moderately concentrated sodium hydroxide solution, to hydrolyse fully the adipate from the starch. After acidification, the free adipic acid is extracted with ethyl acetate. The ethyl acetate is removed, and the dry residue is silylated. An aliquot portion of this solution is injected into a gas chromatograph equipped with a capillary column. Pimelic acid is used as internal standard.

Free adipic acid is extracted by washing out the starch with water, acidifying the extract, and extracting the free acid with ethyl acetate.

The determination is performed by silylation and gas chromatography as described above.

## 4 Reagents and materials

Use only reagents of recognized analytical grade.

**4.1** **Water**, complying with at least grade 3 in accordance with ISO 3696.

**4.2** **Waxy maize starch**, commercial grade.

NOTE Waxy maize starch is chosen as the base material, as it represents the bulk of starch adipate on the market. This may be substituted with another native starch, if appropriate.

**4.3 Adipic acid solution**, $\rho(C_6H_{10}O_4)$ = 50,0 mg/l.

**4.4 Pimelic acid solution**, $\rho(C_7H_{12}O_4)$ = 50,0 mg/l.

**4.5 Sodium hydroxide solution**, $c$(NaOH) = 4 mol/l.

**4.6 Hydrochloric acid**, concentrated, $c$(HCl) = 12 mol/l.

**4.7 Ethyl acetate** ($C_4H_8O_2$).

**4.8 Nitrogen gas**, purity 99 %.

**4.9 Acetonitrile**

**4.10 Silylation reagent**: bis(trimethylsilyl)trifluoroacetamide (BSTFA) which includes 1 % trimethylchlorosilane (TMCS).

**4.11 Helium gas**, purity 99,9999 % (e.g. grade N60).

**4.12 Hydrogen gas**, purity 99,99 % (e.g. grade N400 or better).

**4.13 Air**, purity 99,999 % (e.g. grade S).

## 5 Apparatus

Usual laboratory apparatus and, in particular, the following.

**5.1 Glass reaction tubes,** 100 mm x 16 mm, with screw caps fitted with polytetrafluoroethylene (PTFE) covered rubber seals resistant to concentrated hydrochloric acid (4.6).

**5.2 Pipettes,** adjustable, of 1,00 ml and 5,00 ml capacity, accurate to 0,01 ml.

NOTE The pipettes should be tested to see if they comply to manufacturer's tolerance. Calibration may be required.

**5.3 Rotary shaker.**

**5.4 Pasteur pipettes.**

**5.5 Heating device**, capable of being maintained at (30 ± 2) °C.

**5.6 Evaporation device**, based on solvent removal with a stream of nitrogen (e.g. Pierce Reacti-Vap III)[1)].

**5.7 Ultrasonic bath**, power 120 W.

**5.8 Gas chromatograph**, accommodating capillary columns, fitted with a flame ionization detector, on-column injector, and a computer integrator. See annex A for typical conditions for chromatography.

**5.9 Sieve,** 800 µm.

**5.10 Blade mill.**

**5.11 Laboratory centrifuge**, capable of operating at a radial acceleration of 1 100 $g_n$.

1) Pierce Reacti-Vap III is an example of suitable apparatus available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this apparatus.

## 6 Sampling

It is important that the laboratory receive a sample which is truly representative and has not been damaged or changed during transport or storage.

Sampling is not part of the method specified in this International Standard.

## 7 Preparation of test sample

Sieve the laboratory sample through the 800 µm sieve (5.9). If the material does not pass through the sieve, then grind the sample with a blade mill (5.10) until it passes completely through the 800 µm sieve. Homogenize the sample.

## 8 Procedure

### 8.1 Calibration for total adipic acid content

**8.1.1** Weigh, to the nearest 0,1 mg, approximately 50 mg of waxy maize starch (4.2) into each of four glass reaction tubes (5.1).

**8.1.2** Into one tube, pipette (5.2) 1,00 ml of adipic acid solution (4.3) and into the others 0,75 ml, 0,50 ml and 0,25 ml, respectively, of adipic acid solution (4.3).

**8.1.3** Adjust the volume in each tube to 1,5 ml with water (4.1) and add 1,00 ml of pimelic acid solution (4.4) to each tube. Each tube then contains 50 µg of pimelic acid, and 50,0 µg, 37,5 µg, 25,0 µg and 12,5 µg respectively of adipic acid.

NOTE It is possible that pimelic acid contains some adipic acid. If this is proven, a fifth tube should be prepared in a similar fashion but without addition of adipic acid solution (4.3).

**8.1.4** Agitate the tubes manually to disperse the starch fully. Add 2,5 ml of sodium hydroxide solution (4.5).

**8.1.5** Seal the tubes well and place on the rotary shaker (5.3) for 5 min.

**8.1.6** Remove the tubes and, with cooling, add 1,0 ml of hydrochloric acid (4.6). Mix well.

**8.1.7** Add 5 ml of ethyl acetate (4.7). Seal the tubes tightly and shake vigorously for 1 min.

**8.1.8** Let the tubes stand until good phase separation is achieved. With a Pasteur pipette (5.4), transfer the supernatant layer (ethyl acetate) to a clean screw-top tube.

Make sure that none of the aqueous layer is carried over with the organic layer.

**8.1.9** Place the tubes in the heating device (5.5) set at 30 °C, and evaporate the ethyl acetate completely under a stream of nitrogen (4.8) with the evaporation device (5.6).

**8.1.10** Repeat steps 8.1.7 to 8.1.9 three times more, accumulating the dried residue in the same tube.

**8.1.11** Dissolve the total residue in 0,6 ml of acetonitrile (4.9) and place the closed tubes in the ultrasonic bath (5.7) for 2 min.

**8.1.12** Add 0,3 ml of the silylation reagent (4.10). Close the tubes and homogenize in the ultrasonic bath for 2 min.

**8.1.13** Place the tubes in the heating device (5.5) set at 30 °C, for 30 min to complete derivatization.

**8.1.14** Inject 0,5 µl of the solution into the gas chromatograph. See annex A for typical conditions for chromatography.

## 8.2 Total adipic acid content

**8.2.1** Weigh, to the nearest 0,1 mg, approximately 50 mg of the prepared test sample into a glass reaction tube (5.1).

**8.2.2** Add 1,5 ml of water (4.1) and 1,00 ml of pimelic acid solution (4.4) and shake well to disperse fully the test portion.

**8.2.3** Proceed in accordance with 8.1.4 up to and including 8.1.14.

## 8.3 Calibration for free adipic acid content

**8.3.1** Weigh, to the nearest 0,1 mg, approximately 500 mg of waxy maize starch (4.2) into each of four glass reaction tubes (5.1).

**8.3.2** Into one tube, pipette (5.2) 1,00 ml of adipic acid solution (4.3) and into the others 0,75 ml, 0,50 ml and 0,25 ml, respectively, of adipic acid solution (4.3).

**8.3.3** Adjust the volume in each tube to 4,0 ml with water (4.1) and add 1,00 ml of pimelic acid solution (4.4) to each tube. Each tube then contains 50 µg of pimelic acid, and 50,0 µg, 37,5 µg, 25,0 µg and 12,5 µg, respectively, of adipic acid.

NOTE It is possible that pimelic acid contains some adipic acid. If this is proven, a fifth tube should be prepared in a similar fashion but without addition of adipic acid solution (4.3).

**8.3.4** Seal the tubes and agitate for 16 h in a shaker.

**8.3.5** Remove the tubes from the shaker and centrifuge for 5 min at a radial acceleration of 1 100 $g_n$ in the centrifuge (5.11).

**8.3.6** Transfer the clear supernatant liquid into a clean glass reaction tube (5.1). Add 50 µl of hydrochloric acid (4.6) and 5 ml of ethyl acetate (4.7).

**8.3.7** Seal the tubes tightly and shake vigorously for 1 min.

**8.3.8** Proceed in accordance with 8.1.8 up to and including 8.1.14.

## 8.4 Free adipic acid content

**8.4.1** Weigh, to the nearest 0,1 mg, approximately 500 mg of the prepared test sample into a glass reaction tube (5.1).

**8.4.2** Add 4,0 ml of water (4.1) and 1,00 ml of pimelic acid solution (4.4) and shake well to disperse fully the test portion.

**8.4.3** Proceed in accordance with 8.3.4 up to and including 8.3.8.

## 8.5 Moisture content

Determine the moisture content of the test sample in accordance with ISO 1666.

# 9 Expression of results

## 9.1 Calibration graph

Establish retention times for pimelic acid and adipic acid, and determine the peak areas for the calibrant solutions prepared (8.1.3). See annex B for a typical chromatogram.

Plot a graph with the different masses (in micrograms) of adipic acid added to the waxy maize starch on the *x*-axis, and the corresponding ratios of the area of the adipic acid peak to the pimelic acid peak on the *y*-axis. Using linear regression, derive the best fitting curve.

For each sample, calculate the ratio of the area of the adipic acid peak to the area of the pimelic acid peak, and derive the corresponding mass of adipic acid from the graph.

## 9.2 Calculation of total and free adipic acid content

Calculate the total adipic acid content and the free adipic acid content of the dry test sample by the equation:

$$w_a = \frac{m_a}{m} \times \frac{100\,\%}{(100\,\% - w_m)}$$

where

$w_a$ is the adipic acid content, in milligrams per kilogram, of the dry test sample;

$m_a$ is the mass, in micrograms, of adipic acid derived from the graph (9.1);

$m$ is the mass, in grams, of the test portion;

$w_m$ is the moisture content, in percentage by mass, of the test sample (see 8.5).

Round the result to the nearest 1 mg/kg.

## 9.3 Calculation of bound adipic acid content

Calculate the bound adipic acid content by subtracting the free adipic acid content from the total adipic acid content.

# 10 Precision

## 10.1 Interlaboratory test

Details of an interlaboratory test on the precision of the method are summarized in annex C. The values derived from this interlaboratory test may not be applicable to concentration ranges and matrices other than those given.

## 10.2 Repeatability

The absolute difference between two independent single test results, obtained using the same method on identical test material in the same laboratory by the same operator using the same equipment within a short interval of time, will in not more than 5 % of cases exceed the value of *r* mentioned in or derived from table 1.

## 10.3 Reproducibility

The absolute difference between two single test results, obtained using the same method on identical test material in different laboratories with different operators using different equipment, will in not more than 5 % of cases exceed the value of *R* mentioned in or derived from table 1.

**Table 1 — Repeatability limit (*r*) and reproducibility limit (*R*)**

| **Mean total adipic acid content** mg/kg | ***r*** mg/kg | ***R*** mg/kg |
|---|---|---|
| 90 | 5 | 33 |
| 386 | 21 | 91 |
| 405 | 7 | 114 |
| 430 | 22 | 142 |
| 659 | 36 | 175 |

## 11 Test report

The test report shall specify:

— all information necessary for the complete identification of the sample;

— the sampling method used, if known;

— the test method used;

— the test result obtained;

— if the repeatability has been checked, the final quoted result obtained;

— all operating details not specified in this International Standard, or regarded as optional, together with details of any incidents that occurred when performing the method which may have influenced the test result(s).

# Annex A
(informative)

# Typical conditions for chromatography

## A.1 Column

WCOT fused silica, 0,32 mm internal diameter, 10 m length, coating CP-SIL 5CB, thickness 0,12 µm.

## A.2 Operating conditions

| | |
|---|---|
| Helium (4.11), carrier: | 25 kPa (0,25 bar) |
| Hydrogen (4.12) : | 50 kPa (0,5 bar) |
| Air (4.13) : | 100 kPa (1,0 bar) |

## A.3 Chromatograph conditions

Detector: 300 °C.

## A.4 Temperature-time programme

| | |
|---|---|
| Initial temperature: | 100 °C |
| Hold time: | 1 min |
| Rate of increase: | 25 °C/min |
| Final temperature: | 290 °C |
| Hold time: | 5 min |
| Cool temperature: | 100 °C |

NOTE The conditions described have been successfully used for the analysis. A column of a different specification, using the same liquid phase, will also resolve the silylated acids, but probably with conditions modified from those described.

# Annex B
(informative)

## Typical chromatogram

Figure B.1 shows a typical chromatogram of an acetylated adipyl cross-linked corn starch sample. The retention time of the adipic acid derivative is 3,2 min, and of pimelic acid 3,7 min.

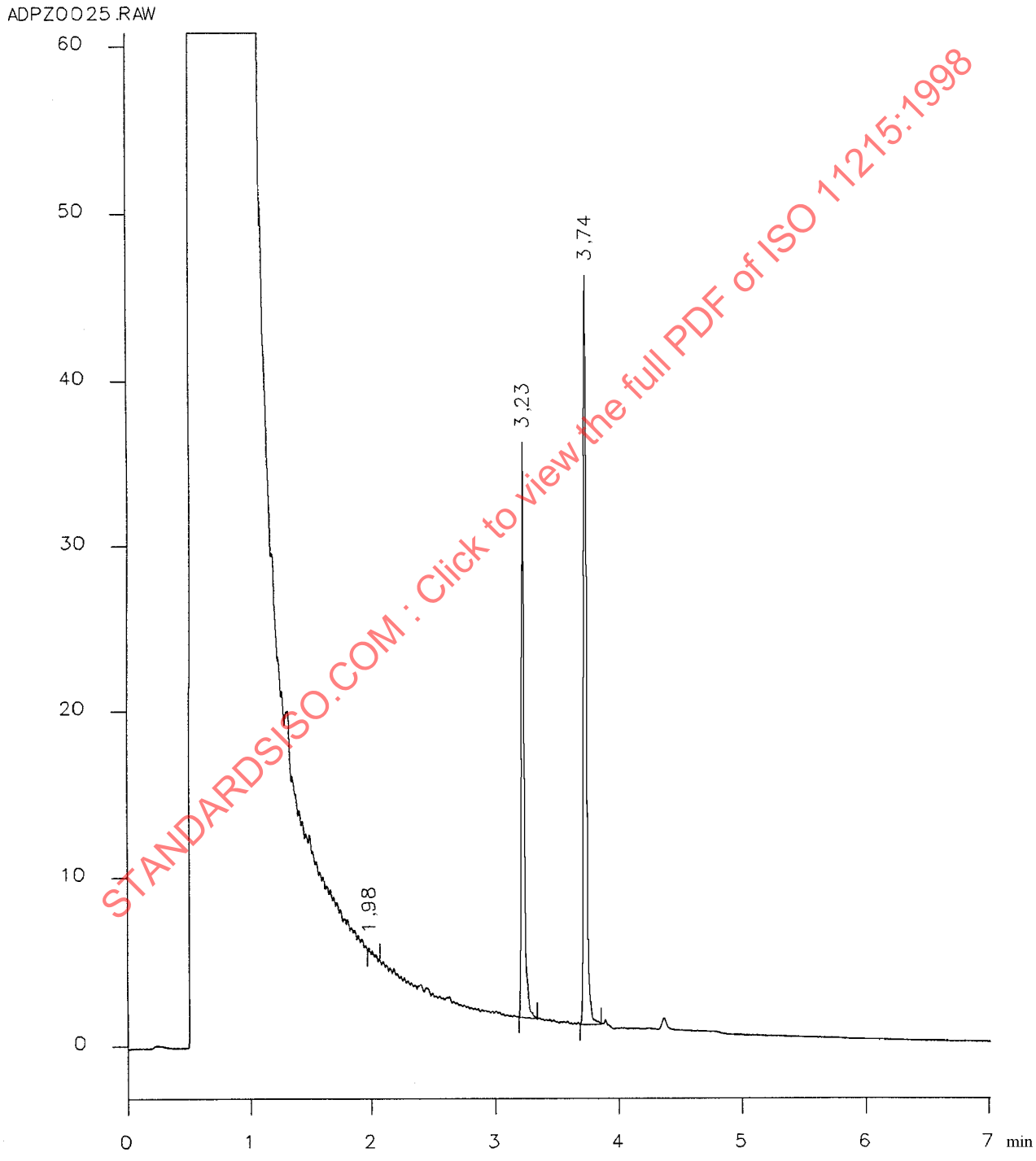




**Figure B.1 — Typical chromatogram of an acetylated adipyl cross-linked corn starch sample**

# Annex C
(informative)

# Results of an interlaboratory trial

An international collaborative test involving nine laboratories was carried out on five different samples of acetylated di-starch adipates with waxy maize starch as the base material.

The results obtained were subjected to statistical analysis in accordance with ISO 5725 [1] to give the precision data shown in tables C.1 and C.2.

**Table C.1 — Statistical results for total adipic acid content**

| Parameter | Sample[1)] | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| Number of laboratories retained after eliminating outliers | 9 | 9 | 9 | 8 | 8 |
| Number of outliers (laboratories) | 0 | 0 | 0 | 1 | 1 |
| Number of accepted results | 18 | 18 | 18 | 16 | 16 |
| Mean total adipic acid content, mg/kg | 430 | 386 | 659 | 90 | 405 |
| Repeatability standard deviation ($s_r$), mg/kg | 22 | 21 | 36 | 5 | 7 |
| Repeatability relative standard deviation, % | 5,1 | 5,3 | 5,5 | 5,4 | 1,7 |
| Repeatability limit ($r$) [$r = 2{,}8 \times s_r$], mg/kg | 61 | 58 | 102 | 14 | 19 |
| Reproducibility standard deviation ($s_R$), mg/kg | 50 | 32 | 62 | 12 | 40 |
| Reproducibility relative standard deviation, % | 11,7 | 8,4 | 9,4 | 13,0 | 10,0 |
| Reproducibility limit ($R$) [$R = 2{,}8 \times s_R$], mg/kg | 142 | 91 | 175 | 33 | 114 |
| 1) All samples were acetylated di-starch adipates with waxy maize starch as the base material. | | | | | |
| NOTE Reference [2] shows that better precision can be obtained with the method in certain cases. | | | | | |

**Table C.2 — Statistical results for free adipic acid content**

| Parameter | Sample[1)] | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| Number of laboratories retained after eliminating outliers | 9 | 9 | 9 | 9 | 8 |
| Number of outliers (laboratories) | 0 | 0 | 0 | 0 | 1 |
| Number of accepted results | 18 | 18 | 18 | 18 | 16 |
| Mean free adipic acid content, mg/kg | 33 | 110 | 17,8 | 14,6 | 100 |
| Repeatability standard deviation ($s_r$), mg/kg | 3,4 | 6,1 | 2,0 | 2,3 | 2,5 |
| Repeatability relative standard deviation, % | 10,3 | 5,5 | 11,3 | 16,1 | 2,5 |
| Repeatability limit ($r$) [$r = 2{,}8 \times s_r$], mg/kg | 9,6 | 17,1 | 5,7 | 6,6 | 7,0 |
| Reproducibility standard deviation ($s_R$), mg/kg | 11,4 | 29,3 | 6,0 | 6,0 | 31,2 |
| Reproducibility relative standard deviation, % | 35,0 | 26,7 | 33,7 | 41,0 | 31,2 |
| Reproducibility limit ($R$) [$R = 2{,}8 \times s_R$], mg/kg | 32,4 | 82,8 | 17,0 | 17,0 | 88,3 |
| 1) All samples were acetylated di-starch adipates with waxy maize starch as the base material. | | | | | |

# Annex D
(informative)

# Bibliography

[1] ISO 5725:1986, *Precision of test methods — Determination of repeatability and reproducibility for a standard test method by inter-laboratory tests* (now withdrawn) was used to obtain the precision data.

[2] Sanders, P. and Brunt, K., Improved method for the determination of the total adipyl content in acetylated adipyl cross-linked starches, *Starch/Stärke*, **46** (1994) No. 7, pp. 255-259.

[3] Sanders, P. and Brunt, K., Gas chromatographic determination of free adipic acid and adipyl cross-linked starches, *Starch/Stärke*, **48** (1996) No. 11/12, pp. 448-452.