

Foodstuffs — Determination of acesulfame-K, aspartame and saccharin — High performance liquid chromatographic method

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ICS 67.040

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Summary of pages

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**Foodstuffs — Determination of acesulfame-K, aspartame and saccharin —
High performance liquid chromatographic method**

Produits alimentaires — Dosage d'acésulfame-K,
d'aspartame et du saccharine — Méthode par
chromatographie liquide haute performance

Lebensmittel — Bestimmung von Acesulfam-K,
Aspartam und Saccharin — Hochleistungs-
flüssigchromatographisches Verfahren

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Comité Européen de Normalisation
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Foreword

This European Standard has been prepared by Technical Committee CEN/TC 275, Food analysis — Horizontal methods, the Secretariat of which is held by DIN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by October 1999, and conflicting national standards shall be withdrawn at the latest by October 1999.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Luxembourg, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and the United Kingdom.

1 Scope

This European Standard specifies a high performance liquid chromatographic (HPLC) method for the determination of acesulfame-K, aspartame and saccharin, see [1], [2] and [3]. It also allows the determination of caffeine, sorbic acid and benzoic acid in foodstuffs. Interlaboratory tests have been carried out with acesulfame-K in marzipan, yogurt, fruit yogurt, orange juice beverage, cola, cream and jam, with aspartame in marzipan, fruit yogurt, orange juice beverage, orange flavoured beverage, cola, jam, and preparation for flan, and with sodium saccharin in marzipan, yogurt, fruit yogurt, orange juice, orange juice beverage, cola, cream and jam.

2 Normative references

This European Standard incorporates by dated or undated reference, provisions from other publications. These normative references are cited at the appropriate places in the text and the publications are listed hereafter. For dated references, subsequent amendments to or revisions of any of these publications apply to this European Standard only when incorporated in it by amendment or revision. For undated references the latest edition of the publication referred to applies.

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

3 Principle

The sample is extracted or diluted with water. If necessary, the sample solution with the intense sweeteners is purified on a solid phase extraction column or with Carrez reagents. The intense sweeteners in the sample test solution are separated on an HPLC-reversed phase chromatography column and determined spectrometrically at a wavelength of 220 nm.

4 Reagents

During the analysis, unless otherwise stated, use only reagents of recognized analytical grade for HPLC analysis and water of at least grade 1 as defined in EN ISO 3696. When preparing solutions, the purity of the substances shall be taken into account.

4.1 Acetonitrile, for HPLC.

4.2 Methanol, for HPLC.

4.3 Potassium dihydrogen orthophosphate (KH_2PO_4).

4.4 Dipotassium hydrogen orthophosphate (K_2HPO_4).

4.5 Phosphoric acid, $r_{20}(\text{H}_3\text{PO}_4) = 1,71 \text{ g/ml}$, $w(\text{H}_3\text{PO}_4) = 85 \text{ \%}^{1)}$.

4.6 Phosphoric acid, $w(\text{H}_3\text{PO}_4) = 5 \text{ \%}$.

Carefully pipette 6 ml of phosphoric acid (4.5) into a 100 ml volumetric flask, which already contains 80 ml of water. Dilute to the mark with water.

4.7 Carrez solution No. 1

Dissolve 15 g potassium hexacyanoferrate (II) ($\text{K}_4[\text{Fe}(\text{CN})_6] \cdot 3\text{H}_2\text{O}$) in water and dilute to 100 ml.

4.8 Carrez solution No. 2

Dissolve 30 g zinc sulfate ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$) in water and dilute to 100 ml.

¹⁾ w is the mass fraction.

4.9 Phosphate buffer solution I, $c(\text{KH}_2\text{PO}_4) = 0,02 \text{ mol/l}$, $\text{pH} \approx 4,3^{2)}$

Dissolve 2,72 g of potassium dihydrogen orthophosphate (4.3) and 800 ml of water in a 1 000 ml beaker. Adjust to pH 4,3 with phosphoric acid (4.6). Transfer the solution to a 1 000 ml volumetric flask and dilute to the mark with water.

4.10 Phosphate buffer solution II, $c(\text{KH}_2\text{PO}_4) = 0,0125 \text{ mol/l}$, $\text{pH} \approx 3,5$

Dissolve 1,70 g of potassium dihydrogen orthophosphate (4.3) and 800 ml of water in a 1 000 ml beaker. Adjust to pH 3,5 with phosphoric acid (4.6). Transfer the solutions to a 1 000 ml volumetric flask and dilute to the mark with water.

4.11 Mobile phase, phosphate buffer and acetonitrile

Add, carefully measured, the required amounts of the selected phosphate buffer to acetonitrile as given in A.5 and mix. Filter through suitable membrane filters, e.g. of pore size 0,45 μm , and degas, e.g. for 5 min in an ultrasonic bath. Prepare the mobile phase on the day of use.

4.12 Control solution (optional)

The control solution contains acesulfame-K, sodium saccharin, aspartame, 5-benzyl-3,6-dioxo-2-piperazine acetic acid (diketopiperazine), aspartylphenylalanine, phenylalanine, caffeine, sorbic acid, benzoic acid, theobromine, hydroxymethylfurfural and vanillin.

In a 100 ml volumetric flask, weigh to the nearest 0,1 mg, 30 mg of acesulfame-K, 20 mg of sodium saccharin, 220 mg of aspartame, 60 mg of caffeine, 100 mg of sorbic acid, 100 mg of benzoic acid, 100 mg of vanillin, 10 mg of diketopiperazine, 10 mg of phenylalanine, 10 mg of aspartylphenylalanine, 20 mg of hydroxymethylfurfural and 70 mg of theobromine. Dissolve and dilute to the mark with water.

Pipette 20 ml of this solution into a 100 ml volumetric flask and dilute to the mark with water.

4.13 Stock solution

Weigh, to the nearest 0,1 mg, 100 mg of acesulfame-K, 100 mg of sodium saccharin and 100 mg of aspartame, in the same 100 ml volumetric flask. Dissolve and dilute to the mark with water. This solution contains 1g/l of each sweetener.

4.14 Standard solutions (optional)

4.14.1 Standard solution I

Pipette 10 ml of the stock solution (4.13) into a 100 ml volumetric flask and dilute to the mark with water. This solution contains 100 mg/l of each sweetener.

4.14.2 Standard solution II

Pipette 5 ml of the stock solution (4.13) into a 100 ml volumetric flask and dilute to the mark with water. This solution contains 50 mg/l of each sweetener.

4.14.3 Standard solution III

Pipette 1 ml of the stock solution (4.13) into a 100 ml volumetric flask and dilute to the mark with water. This solution contains 10 mg/l of each sweetener.

²⁾ c is the substance concentration.