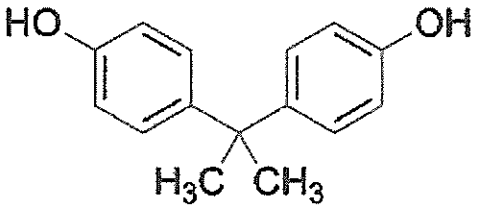
Table of Contents

1 Principle and scope

This method is used to determine Bisphenol A in different BPA-epoxy resins.

The determination of Bisphenol A is carried out by liquid chromatography-mass spectrometry (LC/MS). The specific mass of Bisphenol A is measured in the selected ion monitoring mode (SIM-mode).



$C_{15}H_{16}O_2$

MW 228,28

The method is developed as a quantitative test method:

Test	Range [ppm, mg/kg] [A]	Limit of quantification (LOQ) [ppm, mg/kg] [A]	Limit of detection (LOD) [ppm, mg/kg] [A]
Bisphenol A	0.5 - 10	0.5	0.25

[A] Related to 75 mg test sample and a final volume of 10 mL

2 Validation

This quantitative test method was validated according to the ICH Guideline Q2 (R1). It includes:

- Accuracy
- Linearity
- Specificity
- Precision (repeatability)
- Intermediate precision
- Limit of quantitation (LOQ)
- Limit of detection (LOD)
- Stability of solutions

3 Reagents, chemicals and reference materials

3.1 Reagents and Chemicals

The use of chemicals and reagents of equivalent or higher quality, also delivered by differing than mentioned suppliers, is allowed.

Reagent/Chemical	Quality	Supplier	Product Number
Ammonium acetate	for HPLC, ≥ 99.0%	Fluka	17836
Acetonitrile	CHROMASOLV®, gradient grade, for HPLC, ≥ 99.9%	Sigma Aldrich	34851
Ammonium hydroxide solution	≥ 25% in H_2O, eluent additive for LC-MS	Fluka	44273
Water deionized	18.2 MΩcm	Millipore, Milli Q	n/a

3.2 Reference Materials

Reagent/Chemical	Quality	Supplier	Part. no.
Bisphenol A	≥ 99%	Sigma-Aldrich	239658

CAS No.	Toxicity	Synonyms
80-05-7	Solvias category 2 (H317, H318, H335, H361, H411)	2,2-Bis(4-hydroxyphenyl)propane, 4,4'-Isopropylidenediphenol

4 Equipment

Use the following equipment or equipment of equivalent performance.

Instrument / Accessories	Manufacturer / Provider	Model
High performance liquid chromatograph	Agilent	1200 Series
Mass spectrometer (MS)	Agilent	MSD6140
MS Electro Spray Ion Source	Agilent	G1948A
Chromatography data system (CDS)	Agilent	LC/MSD ChemStation, Rev. B.04.03-SP2
Eppendorf pipette	Eppendorf	10 µL – 100 µL
Eppendorf pipette	Eppendorf	100 µL – 1000 µL
Eppendorf pipette	Eppendorf	500 µL – 5000 µL
pH meter	Metrohm	pH meter 780
Analytical balance	Mettler Toledo	XP205
Precision balance	Mettler Toledo	XP2002S
Standard crimp vial 2 mL, amber glass	Infochroma	8C02-CV(A)
Alu crimp cap with rubber/PTFE septa	Infochroma	G003-AC-RU/TE
UptiDisc Syringe filter	Interchim	PVDF or PTFE / 25 mm / 0.45 µm

5 Solutions

Weights and volumes may be adapted as long as the concentrations remain unchanged.

5.1 Dilution solvent

Pipette 50 mL of acetonitrile in a 100 mL volumetric flask and dilute to volume with water.

5.2 Preparation of reference stock solutions

Reference stock solution 1 (RSS1)

Weigh 48 mg to 52 mg of Bisphenol A (accuracy: 0.01 mg) in a 50 mL volumetric flask. Dissolve and dilute to volume with acetonitrile.

(Concentration of Bisphenol A: 1000 µg/mL)

Reference stock solution 2 (RSS2)

Pipette 1000 µL of reference stock solution 1 (RSS1) into a 10 mL volumetric flask and dilute to volume with acetonitrile.

(Concentration of Bisphenol A: 100 µg/mL)

Reference stock solution 3 (RSS3)

Pipette 150 µL of reference stock solution 2 (RSS2) into a 10 mL volumetric flask and dilute to volume with acetonitrile.

(Concentration of Bisphenol A: 1.5 µg/mL)

5.3 Preparation of comparison solutions and LOD solution

Pipette the amounts of reference stock solution 3 (RSS3) in a 10 ml volumetric flask according to the table below and dilute to volume with dilution solvent.

Comparison solution	Addition of RSS3 [µL]	Conc. of Bisphenol A in solution [µg/mL]	Bisphenol A [ppm, mg/kg] [A]
CS1 / LOQ	25	0.0038	0.5
CS2	50	0.0075	1.0
CS3	125	0.0188	2.5
CS4	250	0.0375	5.0
CS5	375	0.0563	7.5
CS6	500	0.0750	10.0

[A] Related to 75 mg of test sample and a final volume of 10 mL

Transfer an aliquot of the clear solution in an amber vial and analyse by LC/MS.

The comparison solutions are stable for 3 days if stored at 20 °C and daylight in the autosampler tray.

5.4 Preparation of blank solution

Transfer an aliquot of dilution solvent in an amber vial and analyse by LC/MS.

5.5 Preparation of test solutions

Accurately weigh 75 mg (± 5 mg) of test sample in a 10 mL volumetric flask, dissolve with 5 mL of acetonitrile and dilute to volume with water.

Filter the slightly clouded solution through a 0.45 µm PTFE or PVDF filter, transfer an aliquot of the clear solution in an amber vial and analyse by LC/MS.

The test solutions are stable for 3 days if stored at 20 °C and daylight in the autosampler tray.

6 Experimental

6.1 HPLC

HPLC column	Supelco Ascentis Express RP-Amide, 150 x 3.0 mm, 2.7 µm Art.-no. 53919-U or equivalent
Column thermostat	40 °C
Injection volume	50 µL
Injection mode	Injection with needle wash
Solvent needle wash	Water / acetonitrile (1:1)
Autosampler thermostat	20 °C
Eluent A	10 mM Ammonium acetate: Weigh 0.77 g ± 0.01 g ammonium acetate in a 1 L bottle, add 1 L of water deionized and agitate until the ammonium acetate is dissolved. Afterwards adjust the pH with ammonia hydroxide solution to pH 8.0 – 8.5.
Eluent B	Acetonitrile
Flow rate	0.5 mL/min

Time [Min]	A [%]	B [%]
0.0	55	45
3.0	55	45
8.0	5	95
13.0	5	95
13.1	55	45
16.0	55	45

6.2 Single quad mass spectrometer

Ionization mode	API-ES
Polarity	negative
SIM Ion	227.1 m/z Signal from 3.5 to 5.0 min From 0.0 to 3.5 min and 5.0 to 16.0 min → waste
Fragmentor	170 V
Capillary voltage	4500 V
Drying gas	12 L/min
Drying gas temperature	250 °C
Nebulizer pressure	35 psig
Gain	3.0
Dwell time	1000 msec
Expected retention time	4.0 min

6.3 Order of injections

The following order of injections is recommended:

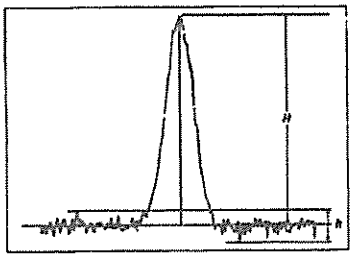
Sample description	Number of injections	Remarks
Blank solution, comparison solution respectively	n	System conditioning
Comparison solution 1 (CS1) LOQ	1	Linearity, LOQ
Comparison solution 2 (CS2)	1	Linearity
Comparison solution 3 (CS3)	1	Linearity
Comparison solution 4 (CS4)	1	Linearity
Comparison solution 5 (CS5)	1	Linearity
Comparison solution 6 (CS6)	1	Linearity
Blank solution	1	-
Test sample	n	-
Comparison solution 4 (CS4)	at least after every 10^{th} injection	SST – Performance control
Blank solution	1	-

7 System Suitability Test

7.1 System Suitability Tests

Test	Solution	Evaluation	Acceptance criteria
SST - LOQ	CS1	S/N-ratio of Bisphenol A (SIM peak)	≥ 10:1
SST - Linearity	CS1-CS6	Correlation coefficient (r^2)	≥ 0.98
SST – Performance control	CS4	Recovery of Bisphenol A	70 – 130%

7.1.1 SST - LOQ - Signal to noise ratio



$$S/N = \frac{2 \cdot H}{h}$$

7.1.2 SST - Performance control

$$R_{analyte} = \frac{M_{analyte} \times 100\%}{T_{analyte}}$$

$R_{analyte}$ Recovery analyte [%]

$M_{analyte}$ Measured analyte content in comparison solution 1

$T_{analyte}$ Theoretical analyte content in comparison solution 1

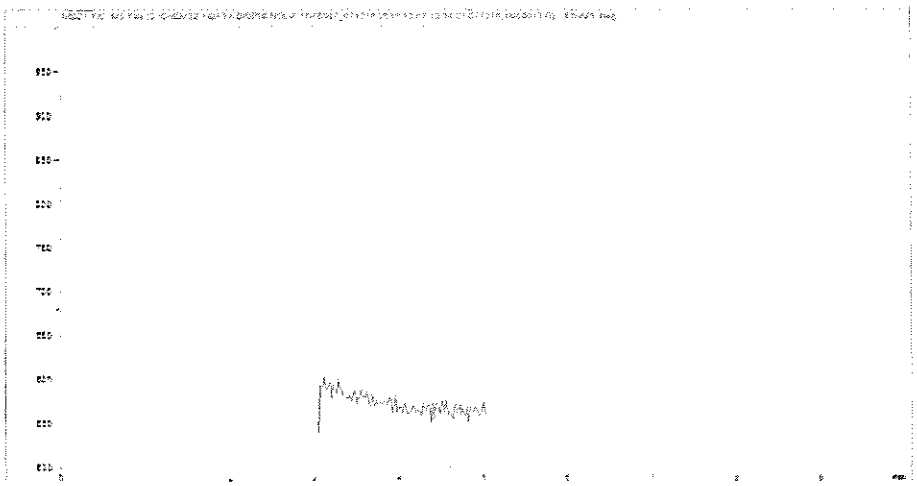
8 Evaluation and reporting

The concentration of Bisphenol A in the test sample is calculated with the external standard method (calibration function: linear).
This calculation is usually performed by the CDS.

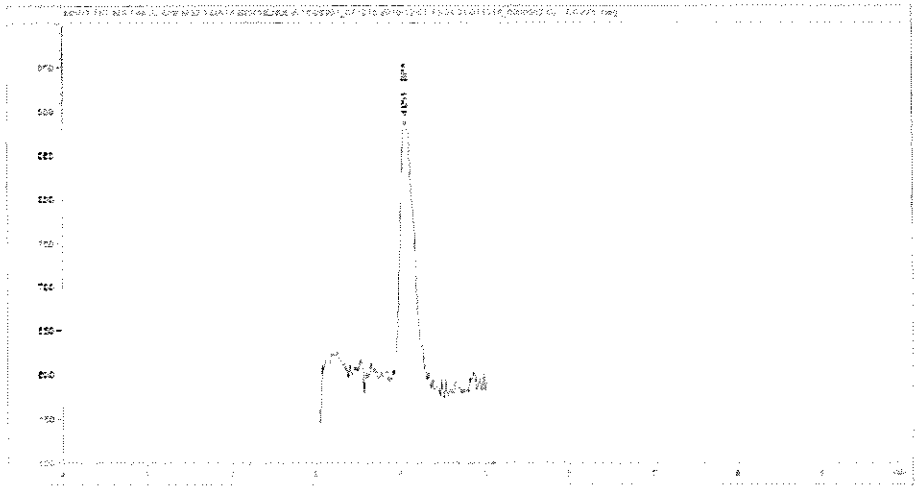
The results will be reported in ppm (mg/kg).

9 Chromatograms

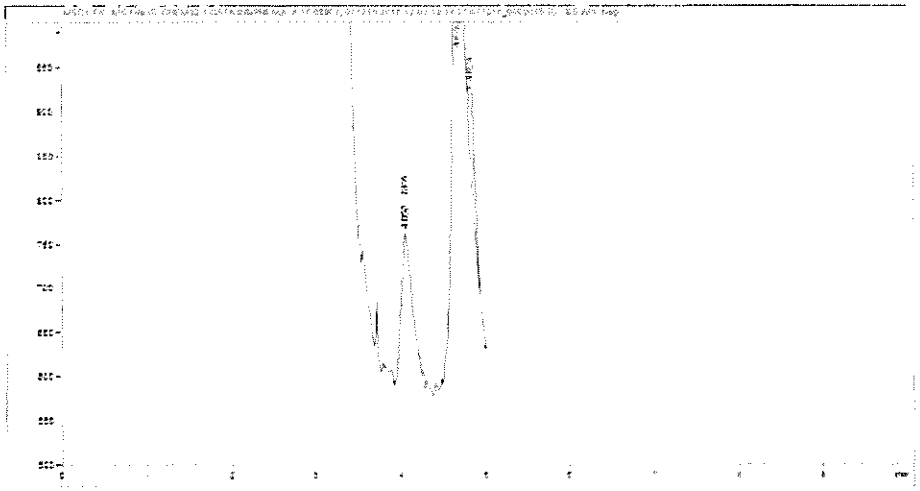
9.1 Blank solution



9.2 Comparison solution 1 (CS1) / LOQ / 0.5 ppm



9.3 Test solution (epoxy resin)



10 Abbreviations

API-ES	Atmospheric pressure ionization – Electrospray
HPLC	High performance liquid chromatography
MS	Mass spectrometer
m/z	Mass to charge ratio
CDS	Chromatography data system
CS	Comparison solution
RSD	Relative standard deviation
LOD	Limit of detection
LOQ	Limit of quantification
ppm	Parts per million (also mg/kg)
SIM	Selected (single) ion monitoring mode
S/N	Signal to noise
SOP	Standard operation procedure
SST	System suitability test

11 Changes to previous edition

Changes

Validation completed and results considered
Chapter 5.3 and 5.5: Stability of comparison solutions and test solutions considered

Justification of changes

New edition after successful limit test validation

Impact on validation status

Limit test method is fully validated according to ICH Guideline Q2 (R1)