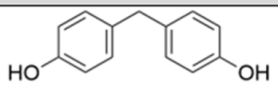
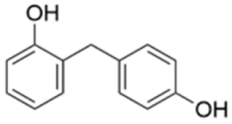
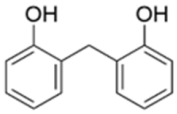
Table of contents

1 Principle and scope

This standard operating procedure describes a quantitative test method for the determination of Bisphenol-F (BPF) in epoxy resins by LC/MS.

It is based on SOP [1] that was previously developed for the determination of BPA in liquid epoxy resins. A different sample preparation to suit all types of epoxy resins was established as described in this present SOP. In addition, the analytical range was broadened to suit a wider range of epoxy resin samples types (mainly in terms molecular weight distribution of the oligomers) possessing various BPF contents. Therefore, each sample type should be spiked at a suitable level to make sure that the method works (specific SST) with a particular sample type.

The specific mass of the analytes is measured in the selected ion monitoring mode (SIM-mode).

#	IUPAC name	Molecular structure	CAS number	Abbreviation
1	4,4'-methylenediphenol		620-92-8	4,4'-BPF
2	2-(4-hydroxybenzyl)phenol		2467-03-0	2,4'-BPF
3	2,2'-methylenediphenol		2467-02-9	2,2'-BPF

All Bisphenol F (BPF), $C_{13}H_{12}O_2$, MW: 200.23 g/mol.

Parameters:

Test	Range [ppm, mg/kg] [A]	Reporting limit, LOQ [ppm, mg/kg] [A]	Limit of detection, LOD [ppm, mg/kg] [A]
Bisphenol-F (BPF)	1.0 … 300	1.0	0.5

[A] Related to 100 mg of test compound

2 Validation

The method validation is ongoing.

However, the method incorporates a number of extensive system suitability tests (SST) in order to ensure method validity and validity of each particular data set.

3 Reagents, chemicals and reference materials

The reagents, with the described quality, and reference materials used for analysis or equivalent can be seen in the below tables.

3.1 Reagents and chemicals

Reagent/Chemical	Quality	Supplier	Part. no.
Ammonium acetate	for LC-MS, ≥ 99.0%	Sigma-Aldrich	73594
Acetonitrile	LiChrosolv®, gradient grade, for HPLC, ≥ 99.9%	Merck	1.00030
Ammonium hydroxide solution	≥ 25% in H_2O, eluent additive for LC-MS	Fluka	44273
Water deionized	18.2 MΩcm	Millipore, Milli Q	n/a
Tetrahydrofuran	LiChrosolv®, for HPLC ≥ 99.9%	Merck	1.08101

3.2 Reference materials

Reagent/Chemicals	Quality	Supplier	Part. no.
Bisphenol F (BPF), 3 separate isomers	≥ 99.0%	TCI Europe	B0819 D1940 D1939

4 Equipment

The used equipment or equipment of equivalent performance can be seen in the below table.

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
Pipette	Gilson	10 µL – 100 µL
Pipette	Gilson	100 µL – 1000 µL
Eppendorf pipette	Eppendorf	500 µL – 5000 µL
pH meter	Metrohm	pH meter 780
Analytical balance	Mettler Toledo	XP205
Shaker (“Vortex”)	IKA	MS2 Minishaker
Centrifuge	Hermle	Z 300K
Standard crimp vial 2 mL, amber glass	Infochroma	8C02-CV(A)
Vial septa	Infochroma	G003-ACC-SKFK10
Filter PTFE 0.45 µm	BGB	SF1304-2
Filter syringe	Codan	1 mL, REF 62.1640

5 Solutions

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

5.1 Solvent

Mix 100 mL of water deionized with 100 mL of acetonitrile.

5.2 Preparation of reference stock solutions

Reference stock solution 1 (RSS1)

Accurately weigh 35 mg (± 5 mg) of each isomer of BPF with an accuracy of 0.01 mg in a 10 mL volumetric flask, dissolve and dilute to volume with THF.

(concentration of BPF: ≈ 3500 µg/mL)

The exact standard's purity (see certificate of analysis) as well as the actual weight has to be taken into account for the calculation of the accurate concentration.

Reference stock solution 2 (RSS2)

Pipet 1000 µL of RSS1 into a 25 mL volumetric flask. Dissolve and dilute to volume with THF.

(concentration of BPF: ≈ 140 µg/mL)

Reference stock solution 3 (RSS3)

Pipet 900 µL of RSS2 into a 25 mL volumetric flask. Dissolve and dilute to volume with THF.

(concentration of BPF: ≈ 5 µg/mL)

5.3 Preparation of comparison solutions

Prepare the solutions in 10 mL roundbottom tubes according to the pattern below:

Solution	Addition of RSS2 [µL]	Addition of RSS3 [µL]	Addition of THF [µL]	Conc. of analyte [A] [µg/mL]	Conc. of analyte [A] [ppm]
CS1 '	-	20	980	≈ 0.020	≈ 1.0
CS2 '	-	60	940	≈ 0.060	≈ 3.0
CS3 '	-	200	800	≈ 0.20	≈ 10
CS4 '	-	600	400	≈ 0.60	≈ 30
CS5 '	80	-	920	≈ 2.2	≈ 110
CS6 '	220	-	780	≈ 6.2	≈ 310

[A] Related to 100 mg of test compound and 5.0 mL of final volume

Each solution (CS1' through CS6'): pipet 4.0 mL of solvent into roundbottom tube. Shake thoroughly.
→ CS1 through CS6

5.4 Preparation of blank solution

Pipet 1.0 mL of THF into a 10 mL roundbottom tube. Add 4.0 mL of solvent and shake thoroughly. Filter an aliquot of the solution over a 0.2 µm PTFE syringe filter into an autosampler vial.

5.5 Preparation of test solutions (n=2)

Prepare two test solutions per sample (double determinations).

Accurately weigh 100 mg (± 5 mg) of test sample with an accuracy of 0.01 mg into a 10 mL roundbottom tube. Add 1.0 mL THF and thoroughly shake tube for app. 2min (e.g. using a Vortex shaker at about 1800 rpm) until a solution is obtained.

Add 4.0 mL of solvent to the roundbottom tube; solution turns cloudy. Shake thoroughly for app. 1min (e.g. using a Vortex shaker at about 1800 rpm). Centrifuge at 2500 rcf for 2min; matrix separates at the tube's bottom. Filter an aliquot of the top solution over a 0.45 µm PTFE syringe filter into an autosampler vial.

5.6 Preparation of spiked test solutions

Prepare at least one spiked test solution per sample/matrix type. Choose a suitable spike level; this should be of a similar magnitude as the sample's unspiked content. E.g. if you had a sample containing about 30 ppm, ideally you would spike 30 ppm. The following shows a 30 ppm spike:

Accurately weigh 100 mg (± 5 mg) of test sample with an accuracy of 0.01 mg into a 10 mL roundbottom tube. Add 400 µL THF as well as 600 µL RSS3 (see table comparison solutions) and thoroughly shake tube for app. 2min (e.g. using a Vortex shaker at about 1800 rpm) until a solution is obtained.

Add 4.0 mL of solvent to the roundbottom tube; solution turns cloudy. Shake thoroughly for app. 1min (e.g. using a Vortex shaker at about 1800 rpm). Centrifuge at 2500 rcf for 2min; matrix separates at the tube's bottom. Filter an aliquot of the top solution over a 0.45 µm PTFE syringe filter into an autosampler vial.

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	5 µL (or more, e.g. 10 µL, to obtain more sensitivity if necessary)
Injection mode	Injection with needle wash
Solvent needle wash	THF / Water / acetonitrile (1:2:2)
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
16.5	55	45

6.2 Quadrupole mass spectrometer

Ionization mode	API-ES
Polarity	Negative
Mode	SIM (Selected ion monitoring)
Ion	199.2 m/z Collect signal from 1.5 to 5.5 min From 0.0 to 1.5 min and 5.5 to 16.5 min → direct eluent into 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 ms
Expected retention time	2.5 – 3.5 min
UV detector (DAD)	Used for information only (matrix visible, possibly confirmation of analyte)
UV wavelength	280 nm

6.3 Order of injections

The following order of injections is recommended:

Sample description	Number of injections	Remarks
Blank solution, comparison solution, test solution respectively	n	System conditioning
Comparison solution 1 (CS1)	1	SST regression, SST LOQ, SST CS1 bias
Comparison solution 2 (CS2)	1	SST regression
Comparison solution 3 (CS3)	1	SST regression
Comparison solution 4 (CS4)	1	SST regression
Comparison solution 5 (CS5)	1	SST regression
Comparison solution 6 (CS6)	1	SST regression
Blank solution	1	SST blank
Test solution (first determination)	1	-
Test solution (second determination)	1	-
Spiked test solution	1	SST recovery spiked sample
Comparison solution of own choice	at least after every 10^{th} injection	SST performance control
Blank solution	1	-

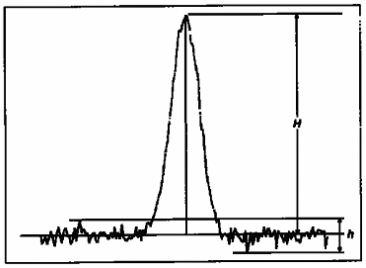
7 System Suitability Test

7.1 System Suitability Tests

Test	Solution	Evaluation	Acceptance criteria
SST - LOQ	CS1	S/N-ratio of Bisphenol F (SIM peak)	≥ 10:1
SST - Regression	CS1-CS6	Correlation coefficient (r^2)	≥ 0.98
SST - Blank	Blank solution	Area compared to CS1	NMT 30%
SST - CS1 bias	CS1	Recovery of Bisphenol F	70 – 130%
SST - Performance control	CS of choice	Recovery of Bisphenol F	70 – 130%
SST - Recovery spiked sample	Spiked test sol.	Recovery of Bisphenol F	70 – 130%

7.1.1 SST - LOQ - Signal to noise ratio

The signal to noise ratio of the analyte in the LOQ solution (CS1, n=1) must be S/N ≥ 10:1. The calculation is performed according to USP/Pharm. Eur., e.g. by the use of the chromatography data system or by graphically evaluation in conjunction with following formula.



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

7.1.2 SST – CS1 bias

The recovery of the analyte in the CS1 solution (calibration point) must be between 70% and 130% related to theoretical content. This value can be called bias and describes the regression-influenced difference between theoretical and analyzed contents. The lowest calibration point is chosen because it is most prone to regression-influenced error and also since the LOQ is important in general. The measured analyte content must be calculated by reprocessing using the complete regression with all calibration points considered (just like all unknowns in the sequence).

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

$R_{analyte}$ Recovery analyte [%]

$M_{analyte}$ Measured analyte content in comparison solution CS1 (calibration point)

$T_{analyte}$ Theoretical analyte content in comparison solution CS1

7.1.3 SST – Performance control

The recovery of the analyte in a particular comparison solution of the performance control must be between 70% and 130% related to theoretical content.

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

$R_{analyte}$ Recovery analyte [%]

$M_{analyte}$ Measured analyte content in comparison solution of choice

$T_{analyte}$ Theoretical analyte content in comparison solution of choice

7.1.4 SST – Recovery spiked sample

The recovery of the analyte in a particular spike solution must be between 70% and 130% related to theoretical content. The spike levels are individually different.

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

$R_{analyte}$ Recovery analyte [%]

$M_{analyte}$ Measured analyte content in a particular spike solution

$T_{analyte}$ Theoretical analyte content in a particular spike solution

7.1.5 SST – Blank

No evidence of interfering and overlapping peaks ≥ LOQ at the retention time window of the analyte peak in the blank solution. The analyte peak area should be no more than 30% of the CS1.

8 Evaluation and reporting

The concentration of Bisphenol F is calculated with the external standard method (calibration function: linear or quadratic, weight: 1/x or equal).
This calculation is usually performed by the CDS.

The results will be reported in ppm (mg/kg). Each sample's result (double determinations) can be reported individually and/or its corresponding mean value.

The spike recoveries can be reported optionally.

9 Abbreviations

CDS	Chromatography data system
CS	Comparison solution
HPLC	High pressure liquid chromatography
DAD	Diode array detector
MS	Mass spectrometer
SIM	Selected ion monitoring
LOD	Limit of detection
ppm	Parts per million (here mass fraction, e.g. µg/g or mg/kg)
RSD	Relative standard deviation
S/N	Signal to noise
SOP	Standard operation procedure
SST	System suitability test
THF	Tetrahydrofuran
BPF	Bisphenol-A

10 References

[1] C.52.S1593_01: BPA-epoxy resins: Determination of Bisphenol A by LC-MS