Table 2.4.23.-1. – *Retention times of sterols relative to β-sitosterol for 2 different columns*

	Poly[methyl(95)-phenyl(5)]siloxane	Poly[methyl(94)-phenyl(5)vinyl(1)]siloxane
Cholesterol	0.63	0.67
Brassicasterol	0.71	0.73
24-Methylenecholesterol	0.80	0.82
Campesterol	0.81	0.83
Campestanol	0.82	0.85
Stigmasterol	0.87	0.88
Δ7-Campesterol	0.92	0.93
Δ5,23-Stigmastadienol	0.95	0.95
Clerosterol	0.96	0.96
β-Sitosterol	1	1
Sitostanol	1.02	1.02
Δ5-Avenasterol	1.03	1.03
Δ5,24-Stigmastadienol	1.08	1.08
Δ7-Stigmastenol(1)	1.12	1.12
Δ7-Avenasterol	1.16	1.16
Betulin	1.4	1.6

(1) This sterol may also be referred to as Δ7-stigmasterol in literature.

For the chromatogram obtained with the test solution, identify the peaks and calculate the percentage content of each sterol in the sterol fraction of the substance to be examined using the following expression:

$$\frac{A}{S} \times 100$$

A = area of the peak corresponding to the component to be determined,

S = sum of the areas of the peaks corresponding to the components indicated in Table 2.4.23.-1.

If required in the monograph, calculate the content of each sterol in milligrams per 100 grams of the substance to be examined using the following expression:

$$\frac{A \times m_S \times 100}{A_S \times m}$$

A = area of the peak corresponding to the component to be determined,

A_S = area of the peak corresponding to betulin,

m = mass of the sample of the substance to be examined in grams,

m_S = mass of *betulin R* added in milligrams.

01/2005:20424 corrected

2.4.24. IDENTIFICATION AND CONTROL OF RESIDUAL SOLVENTS

The test procedures described in this general method may be used:

i. for the identification of the majority of Class 1 and Class 2 residual solvents in an active substance, excipient or medicinal product when the residual solvents are unknown;

ii. as a limit test for Class 1 and Class 2 solvents when present in an active substance, excipient or medicinal product;

iii. for the quantification of Class 2 solvents when the limits are greater than 1000 ppm (0.1 per cent) or for the quantification of Class 3 solvents when required.

Class 1, Class 2 and Class 3 residual solvents are listed in general chapter *5.4. Residual solvents*.

Three diluents are described for sample preparation and the conditions to be applied for head-space injection of the gaseous sample onto the chromatographic system. Two chromatographic systems are prescribed but System A is preferred whilst System B is employed normally for confirmation of identity. The choice of sample preparation procedure depends on the solubility of the substance to be examined and in certain cases the residual solvents to be controlled.

The following residual solvents are not readily detected by the head-space injection conditions described: formamide, 2-ethoxyethanol, 2-methoxyethanol, ethylene glycol, *N*-methylpyrrolidone and sulfolane. Other appropriate procedures should be employed for the control of these residual solvents.

When the test procedure is applied quantitatively to control residual solvents in a substance, then it must be validated.

PROCEDURE

Examine by gas chromatography with static head-space injection (*2.2.28*).

Sample preparation 1. This is intended for the control of residual solvents in water-soluble substances.

Sample solution (1). Dissolve 0.200 g of the substance to be examined in *water R* and dilute to 20.0 ml with the same solvent.

Sample preparation 2. This is intended for the control of residual solvents in water-insoluble substances.

Sample solution (2). Dissolve 0.200 g of the substance to be examined in *N,N-dimethylformamide R* (DMF) and dilute to 20.0 ml with the same solvent.

Sample preparation 3. This is intended for the control of *N,N*-dimethylacetamide and/or *N,N*-dimethylformamide, when it is known or suspected that one or both of these substances are present in the substance to be examined.

Sample solution (3). Dissolve 0.200 g of the substance to be examined in *1,3-dimethyl-2-imidazolidinone R* (DMI) and dilute to 20.0 ml with the same solvent.

In some cases none of the above sample preparation procedures are appropriate, in which case the diluent to be used for the preparation of the sample solution and the static head-space conditions to be employed must be demonstrated to be suitable.

Solvent solution (a). To 1.0 ml of *Class 1 residual solvent solution CRS*, add 9 ml of *dimethyl sulphoxide R* and dilute to 100.0 ml with *water R*. Dilute 1.0 ml of this solution to 100 ml with *water R*. Dilute 1.0 ml of this solution to 10.0 ml with *water R*.

The reference solutions correspond to the following limits:

- benzene: 2 ppm,
- carbon tetrachloride: 4 ppm,
- 1,2-dichloroethane: 5 ppm,
- 1,1-dichloroethene: 8 ppm,
- 1,1,1-trichloroethane: 10 ppm.

Solvent solution (b). Dissolve appropriate quantities of the Class 2 residual solvents in *dimethyl sulphoxide R* and dilute to 100.0 ml with *water R*. Dilute to give a concentration of 1/20 of the limits stated in Table 2 (see *5.4. Residual solvents*).

Solvent solution (c). Dissolve 1.00 g of the solvent or solvents present in the substance to be examined in *dimethyl sulphoxide R* or *water R*, if appropriate, and dilute to 100.0 ml with *water R*. Dilute to give a concentration of 1/20 of the limit(s) stated in Table 1 or 2 (see *5.4. Residual solvents*).

Blank solution. Prepare as described for solvent solution (c) but without the addition of solvent(s) (used to verify the absence of interfering peaks).

Test solution. Introduce 5.0 ml of the sample solution and 1.0 ml of the blank solution into an injection vial.

Reference solution (a) (Class 1). Introduce 1.0 ml of solvent solution (a) and 5.0 ml of the appropriate diluent into an injection vial.

Reference solution (a_1) (Class 1). Introduce 5.0 ml of the sample solution and 1.0 ml of solvent solution (a) into an injection vial.

Reference solution (b) (Class 2). Introduce 1.0 ml of solvent solution (b) and 5.0 ml of the appropriate diluent into an injection vial.

Reference solution (c). Introduce 5.0 ml of the sample solution and 1.0 ml of solvent solution (c) into an injection vial.

Reference solution (d). Introduce 1.0 ml of the blank solution and 5.0 ml of the appropriate diluent into an injection vial.

Close the vials with a tight rubber membrane stopper coated with polytetrafluoroethylene and secure with an aluminium crimped cap. Shake to obtain a homogeneous solution.

The following static head-space injection conditions may be used:

	Sample preparation procedure		
Operating parameters	**1**	**2**	**3**
Equilibration temperature (°C)	80	105	80
Equilibration time (min)	60	45	45
Transfer-line temperature (°C)	85	110	105
Carrier gas: *Nitrogen for chromatography R* or *Helium for chromatography R* at an appropriate pressure			
Pressurisation time (s)	30	30	30
Injection volume (ml)	1	1	1

The chromatographic procedure may be carried out using:

SYSTEM A

– a fused-silica capillary or wide-bore column 30 m long and 0.32 mm or 0.53 mm in internal diameter coated with cross-linked 6 per cent polycyanopropylphenylsiloxane and 94 per cent polydimethylsiloxane (film thickness: 1.8 µm or 3 µm),
– *nitrogen for chromatography R* or *helium for chromatography R* as the carrier gas, split ratio 1:5 with a linear velocity of about 35 cm/s,
– a flame-ionisation detector (a mass spectrometer may also be used or an electron-capture detector for the chlorinated residual solvents of Class 1),

maintaining the temperature of the column at 40 °C for 20 min, then raising the temperature at a rate of 10 °C per min to 240 °C and maintaining it at 240 °C for 20 min and maintaining the temperature of the injection port at 140 °C and that of the detector at 250 °C, or, where there is interference from the matrix, use:

SYSTEM B

– a fused-silica capillary or wide-bore column 30 m long and 0.32 mm or 0.53 mm in internal diameter coated with *macrogol 20 000 R* (film thickness: 0.25 µm),
– *nitrogen for chromatography R* or *helium for chromatography R* as the carrier gas, split ratio 1:5 with a linear velocity of about 35 cm/s.
– a flame-ionisation detector (a mass spectrophotometer may also be used or an electron-capture detector for the chlorinated residual solvents of Class 1),

maintaining the temperature of the column at 50 °C for 20 min, then raising the temperature at a rate of 6 °C per min to 165 °C and maintaining it at 165 °C for 20 min and maintaining the temperature of the injection port at 140 °C and that of the detector at 250 °C.

Inject 1 ml of the gaseous phase of reference solution (a) onto the column described in System A and record the chromatogram under such conditions that the signal-to-noise ratio for 1,1,1-trichloroethane can be measured. The signal-to-noise ratio must be at least five. A typical chromatogram is shown in Figure 2.4.24.-1.

Inject 1 ml of the gaseous phase of reference solution (a_1) onto the column described in System A. The peaks due to the Class 1 residual solvents are still detectable.

Inject 1 ml of the gaseous phase of reference solution (b) onto the column described in System A and record the chromatogram under such conditions that the resolution between acetonitrile and methylene chloride can be determined. The system is suitable if the chromatogram obtained resembles the chromatogram shown in Figure 2.4.24.-2 and the resolution between acetonitrile and methylene chloride is at least 1.0.

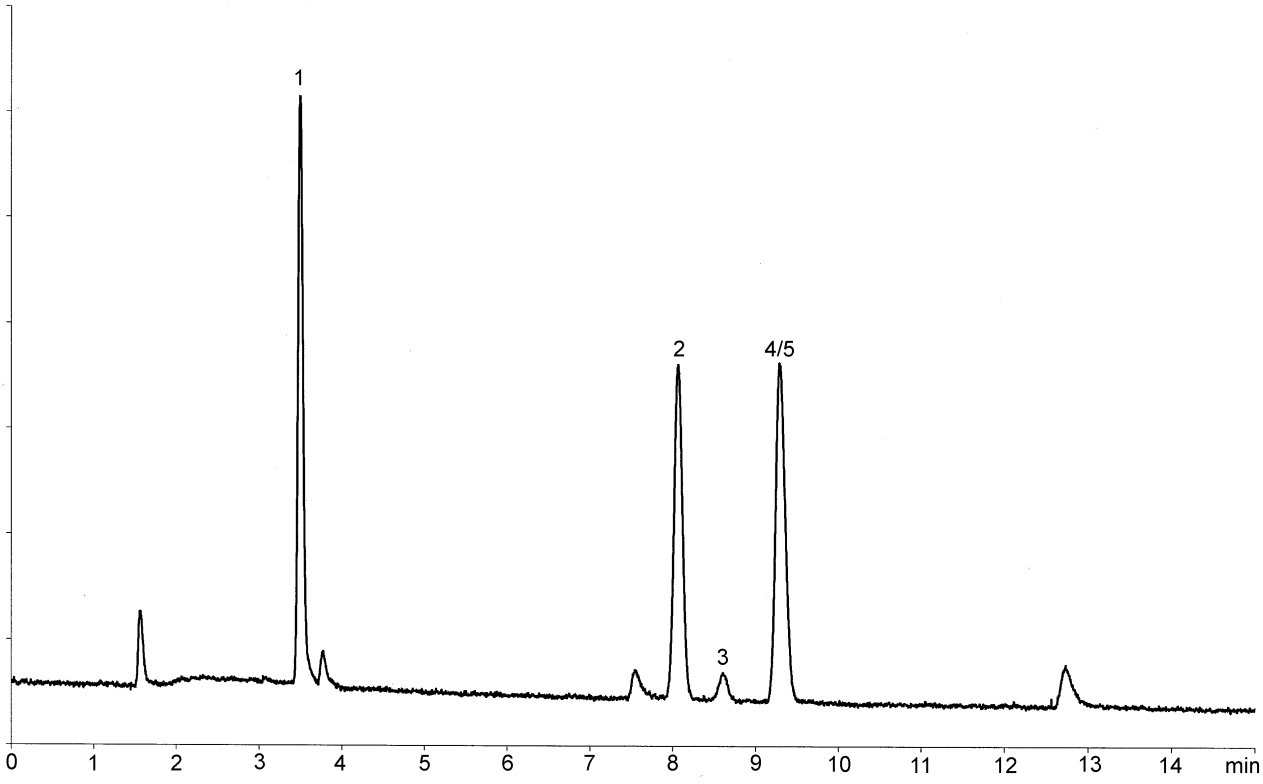
Inject 1 ml of the gaseous phase of the test solution onto the column described in System A. If in the chromatogram obtained, there is no peak which corresponds to one of the residual solvent peaks in the chromatograms obtained with reference solution (a) or (b), then the substance to be examined meets the requirements of the test. If any peak in the chromatogram obtained with the test solution corresponds to any of the residual solvent peaks obtained with reference solution (a) or (b) then System B is to be employed.

Inject 1 ml of the gaseous phase of reference solution (a) onto the column described in System B and record the chromatogram under such conditions that the signal-to-noise ratio for benzene can be measured. The signal-to-noise ratio must be at least five. A typical chromatogram is shown in Figure 2.4.24.-3.

Inject 1 ml of the gaseous phase of reference solution (a_1) onto the column described in System B. The peaks due to the Class I residual solvents are still detectable.

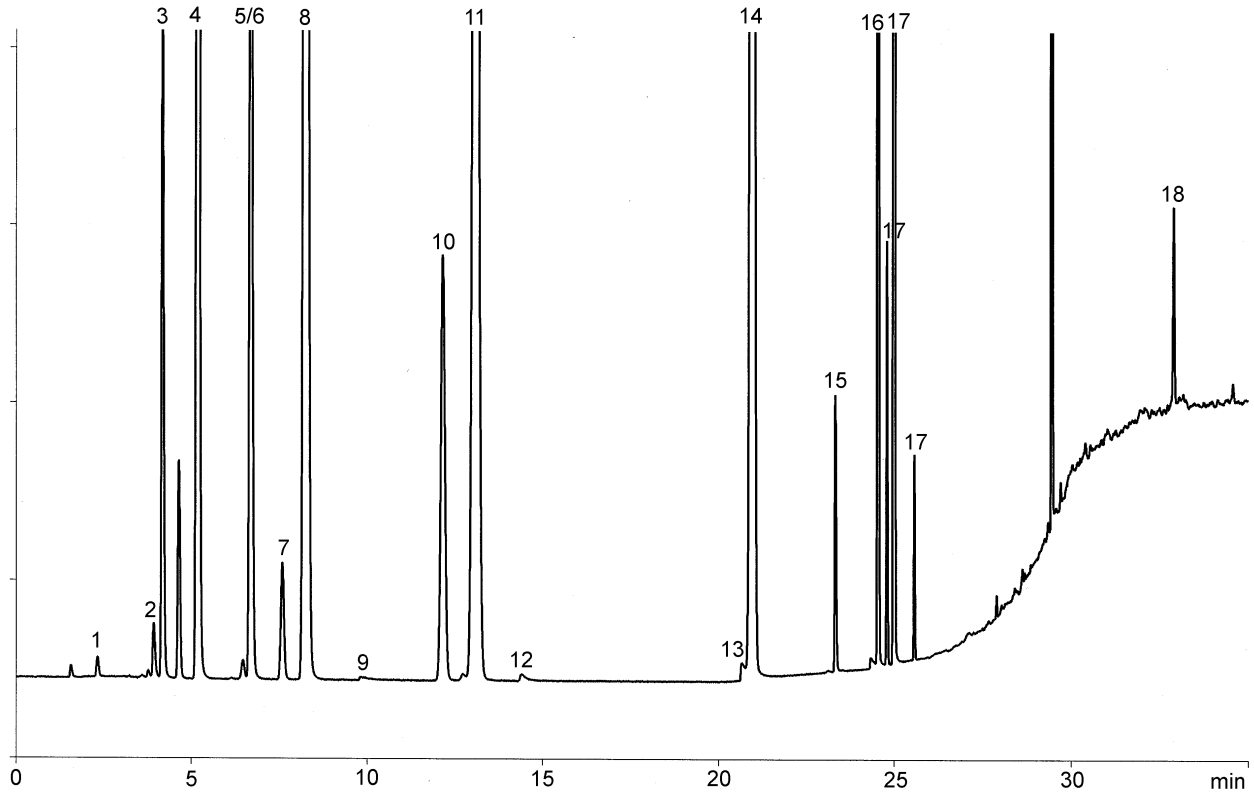
Inject 1 ml of the gaseous phase of reference solution (b) onto the column described in System B and record the chromatogram under such conditions that the resolution between acetonitrile and trichloroethene can be determined. The system is suitable if the chromatogram obtained resembles the chromatogram shown in Figure 2.4.24.-4 and the resolution between acetonitrile and trichloroethene is at least 1.0.

Inject 1 ml of the gaseous phase of the test solution onto the column described in System B. If in the chromatogram obtained, there is no peak which corresponds to any of the residual solvent peaks in the chromatogram obtained with the reference solution (a) or (b), then the substance



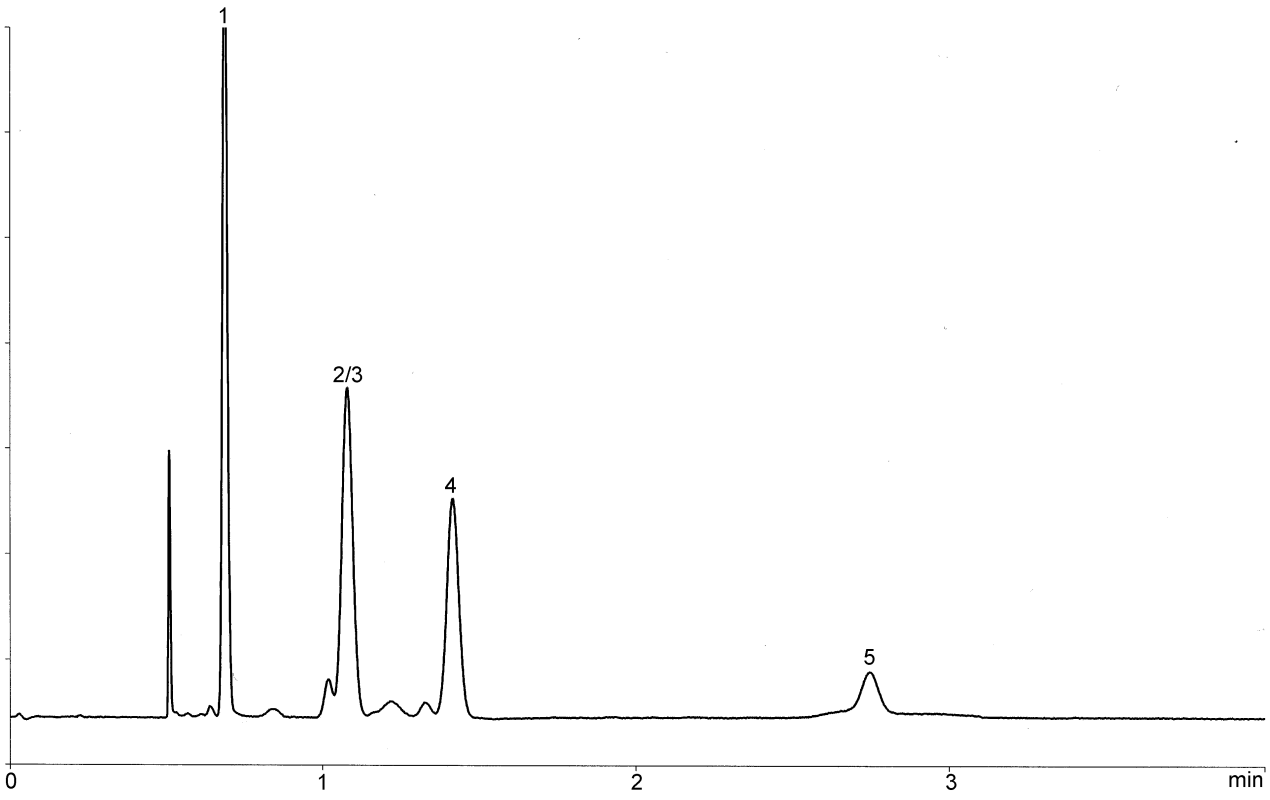
1. 1,1-dichloroethene 2. 1,1,1-trichloroethane 3. carbon tetrachloride 4. benzene 5. 1,2-dichloroethane

Figure 2.4.24.-1. – *Typical chromatogram of class 1 solvents using the conditions described for System A and Procedure 1. Flame-ionisation detector.*



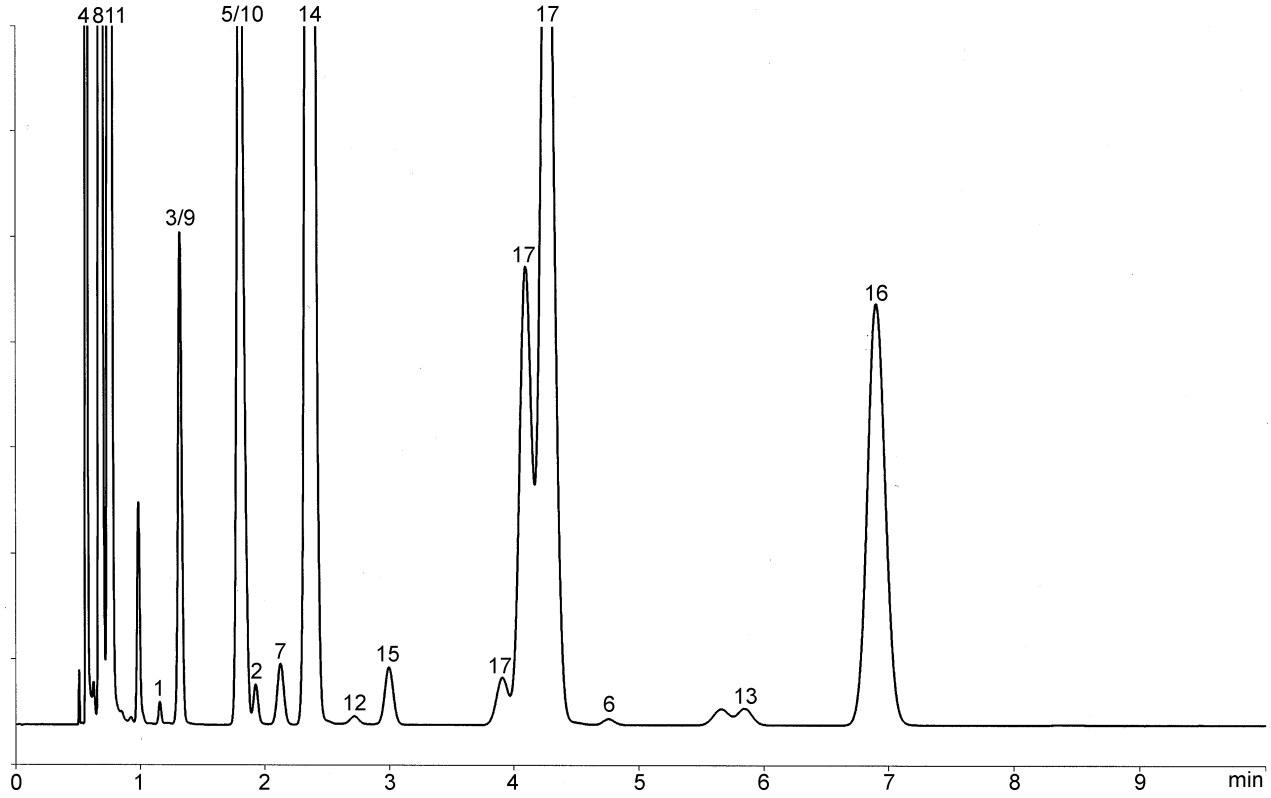
1. methanol
2. acetonitrile
3. dichloromethane
4. hexane
5. *cis*-1,2-dichloroethene
6. nitromethane
7. chloroform
8. cyclohexane
9. 1,2-dimethoxymethane
10. 1,1,2-trichloroethene
11. methylcyclohexane
12. 1,4-dioxan
13. pyridine
14. toluene
15. 2-hexanone
16. chlorobenzene
17. xylene *ortho, meta, para*
18. tetralin

Figure 2.4.24.-2. – *Chromatogram of Class 2 solvents using the conditions described for System A and Procedure 1. Flame-ionisation detector.*



1. 1,1-dichloroethene 2. 1,1,1-trichloroethane 3. carbon tetrachloride 4. benzene 5. 1,2-dichloroethane

Figure 2.4.24.-3. – *Chromatogram of Class 1 residual solvents using the conditions described for System B and Procedure 1. Flame-ionisation detector.*



1. methanol
2. acetonitrile
3. dichloromethane
4. hexane
5. *cis*-1,2-dichloroethene
6. nitromethane
7. chloroform
8. cyclohexane
9. 1,2-dimethoxyethane
10. 1,1,2-trichloroethene
11. methylcyclohexane
12. 1,4-dioxan
13. pyridine
14. toluene
15. 2-hexanone
16. chlorobenzene
17. xylene *ortho, meta, para*
18. tetralin (t_R = 28 min)

Figure 2.4.24.-4. – *Typical chromatogram of class 2 residual solvents using the conditions described for System B and Procedure 1. Flame-ionisation detector.*

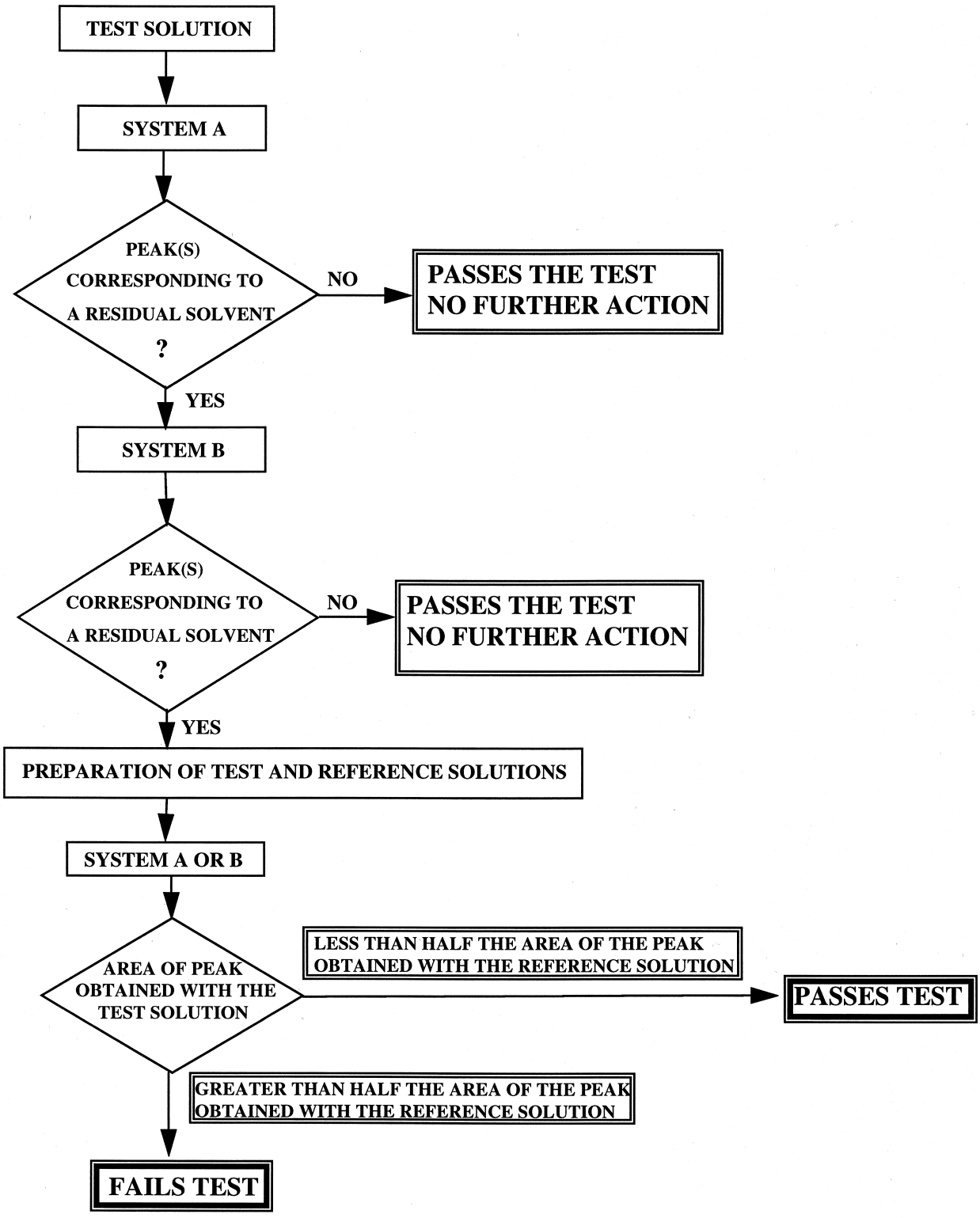


Figure 2.4.24.-5. – *Diagram relating to the identification of residual solvents and the application of limit tests*

to be examined meets the requirements of the test. If any peak in the chromatogram obtained with the test solution corresponds to any of the residual solvent peaks obtained with reference solution (a) or (b) and confirms the correspondence obtained when using System A, then proceed as follows.

Inject 1 ml of the gaseous phase of reference solution (c) onto the column described for System A or System B. If necessary, adjust the sensitivity of the system so that the height of the peak corresponding to the identified residual solvent(s) is at least 50 per cent of the full scale of the recorder.

Inject 1 ml of the gaseous phase of reference solution (d) onto the column. No interfering peaks should be observed.

Inject 1 ml of the gaseous phase of the test solution and 1 ml of the gaseous phase of reference solution (c) on to the column. Repeat these injections twice more.

The mean area of the peak of the residual solvent(s) in the chromatograms obtained with the test solution is not greater than half the mean area of the peak of the corresponding residual solvent(s) in the chromatograms obtained with reference solution (c). The test is not valid unless the relative standard deviation of the differences in areas between the analyte peaks obtained from three replicate paired injections of reference solution (c) and the test solution, is at most 15 per cent.

A flow diagram of the procedure is shown in Figure 2.4.24.-5.

When a residual solvent (Class 2 or Class 3) is present at a level of 0.1 per cent or greater then the content may be quantitatively determined by the method of standard additions.

01/2005:20425

2.4.25. ETHYLENE OXIDE AND DIOXAN

The test is intended for the determination of residual ethylene oxide and dioxan in samples soluble in water or dimethylacetamide. For substances that are insoluble or insufficiently soluble in these solvents, the preparation of the sample solution and the head-space conditions to be employed are given in the individual monograph.

Examine by head-space gas chromatography (*2.2.28*).

A. For samples soluble in or miscible with water, the following procedure may be used.

Test solution. Weigh 1.00 g (M_T) of the substance to be examined in a 10 ml vial (other sizes may be used depending on the operating conditions) and add 1.0 ml of *water R*. Close and mix to obtain a homogeneous solution. Allow to stand at 70 °C for 45 min.

Reference solution (a). Weigh 1.00 g (M_R) of the substance to be examined into an identical 10 ml vial, add 0.50 ml of *ethylene oxide solution R3* and 0.50 ml of *dioxan solution R1*. Close and mix to obtain a homogeneous solution. Allow to stand at 70 °C for 45 min.

Reference solution (b). To 0.50 ml of *ethylene oxide solution R3* in a 10 ml vial add 0.1 ml of a freshly prepared 10 mg/l solution of *acetaldehyde R* and 0.1 ml of *dioxan solution R1*. Close and mix to obtain a homogeneous solution. Allow to stand at 70 °C for 45 min.

B. For samples soluble in or miscible with dimethylacetamide, the following procedure may be used.

Test solution. Weigh 1.00 g (M_T) of the substance to be examined in a 10 ml vial (other sizes may be used depending on the operating conditions) and add 1.0 ml of *dimethylacetamide R* and 0.20 ml of *water R*. Close and mix to obtain a homogeneous solution. Allow to stand at 90 °C for 45 min.

Reference solution (a). Weigh 1.00 g (M_R) of the substance to be examined into a 10 ml vial, add 1.0 ml of *dimethylacetamide R*, 0.10 ml of *dioxan solution R* and 0.10 ml of *ethylene oxide solution R2*. Close and mix to obtain a homogeneous solution. Allow to stand at 90 °C for 45 min.

Reference solution (b). To 0.10 ml of *ethylene oxide solution R2* in a 10 ml vial, add 0.1 ml of a freshly prepared 10 mg/l solution of *acetaldehyde R* and 0.10 ml of *dioxan solution R*. Close and mix to obtain a homogeneous solution. Allow to stand at 70 °C for 45 min.

The following static head-space injection conditions may be used:

- equilibration temperature: 70 °C (90 °C for solutions in dimethylacetamide),
- equilibration time: 45 min,
- transfer-line temperature: 75 °C (150 °C for solutions in dimethylacetamide),
- carrier gas: *helium for chromatography R*,
- pressurisation time: 1 min,
- injection time: 12 s.

The chromatographic procedure may be carried out using:

- a capillary glass or quartz column 30 m long and 0.32 mm in internal diameter the inner surface of which is coated with a 1.0 µm thick layer of *poly(dimethyl)siloxane R*,
- *helium for chromatography R* or *nitrogen for chromatography R* as the carrier gas with a linear velocity of about 20 cm/s and a split ratio of 1:20,
- a flame-ionisation detector,

maintaining the temperature of the column at 50 °C for 5 min, then raising the temperature at a rate of 5 °C per minute to 180 °C and then raising the temperature at a rate of 30 °C per minute to 230 °C and maintaining at 230 °C for 5 min; maintaining the temperature of the injection port at 150 °C and that of the detector at 250 °C.

Inject a suitable volume, for example 1.0 ml, of the gaseous phase of reference solution (b). Adjust the sensitivity of the system so that the heights of the peaks due to ethylene oxide and acetaldehyde in the chromatogram obtained are at least 15 per cent of the full scale of the recorder. The test is not valid unless the resolution between the peaks corresponding to acetaldehyde and ethylene oxide is at least 2.0 and the peak of dioxan is detected with a signal-to-noise ratio of at least 5.

Inject separately suitable volumes, for example 1.0 ml (or the same volume used for reference solution (b)), of the gaseous phases of the test solution and reference solution (a). Repeat the procedure twice more.

Verification of precision

For each pair of injections, calculate for ethylene oxide and for dioxan the difference in area between the peaks obtained with the test solution and reference solution (a). The test is not valid unless the relative standard deviation of the 3 values obtained for ethylene oxide is not greater than 15 per cent and the relative standard deviation of the 3 values obtained for dioxan is not greater than 10 per cent. If the weighings used for the test solution and reference solution differ from 1.00 g by more than 0.5 per cent, the appropriate corrections must be made.

The content of ethylene oxide or dioxan in parts per million is calculated from the expressions:

$$\frac{A_T \times C}{(A_R \times M_T) - (A_T \times M_R)}$$

A_T = area of the peak corresponding to ethylene oxide in the chromatogram obtained with the test solution,

A_R = area of the peak corresponding to ethylene oxide in the chromatogram obtained with reference solution (a),

M_T = mass of the substance to be examined in the test solution, in grams,