

paper and wash each filter with 3 quantities, each of 15 ml, of *methylene chloride R*. Place the combined filtrate and washings from each filter separately in 3 flasks, evaporate under a stream of *nitrogen R* to 5-10 ml. Transfer to a small test tube and evaporate to dryness under a stream of *nitrogen R*.

DETERMINATION OF THE STEROLS

Gas chromatography (2.2.28). Carry out the operations protected from humidity and prepare the solutions immediately before use.

Test solution. To the sterols separated from the substance to be examined by thin-layer chromatography add a freshly prepared mixture of 0.04 ml of *chlorotrimethylsilane R*, 0.1 ml of *hexamethyldisilazane R* and 0.5 ml of *anhydrous pyridine R*. Allow to stand for at least 5 min and use the liquid phase.

Reference solution (a). To 9 parts of the sterols separated from *rapeseed oil R* by thin-layer chromatography add 1 part of *cholesterol R*. To the mixture add a freshly prepared mixture of 0.04 ml of *chlorotrimethylsilane R*, 0.1 ml of *hexamethyldisilazane R* and 0.5 ml of *anhydrous pyridine R*. Allow to stand for at least 5 min and use the liquid phase.

Reference solution (b). To the sterols separated from *sunflower oil R* by thin-layer chromatography add a freshly prepared mixture of 0.04 ml of *chlorotrimethylsilane R*, 0.1 ml of *hexamethyldisilazane R* and 0.5 ml of *anhydrous pyridine R*. Allow to stand for at least 5 min and use the liquid phase.

Column:

- *material*: fused silica,
- *size*: $l = 20\text{-}30\text{ m}$, $\varnothing = 0.25\text{-}0.32\text{ mm}$,
- *stationary phase*: *poly[methyl(95)phenyl(5)]siloxane R* or *poly(cyanopropyl)(7)(phenyl)(7)(methyl)(86)siloxane R* (film thickness 0.25 μm).

Carrier gas: *hydrogen for chromatography R* or *helium for chromatography R*.

Linear velocity: 30-50 cm/s (hydrogen) or 20-35 cm/s (helium).

Split ratio: 1:50 or 1:100.

Temperature:

- *column*: 260 °C,
- *injection port*: 280 °C,
- *detector*: 290 °C.

Detection: flame ionisation.

Injection: 1 μl .

Results: the chromatogram obtained with reference solution (a) shows 4 principal peaks corresponding to cholesterol, brassicasterol, campesterol and β -sitosterol and the chromatogram obtained with reference solution (b) shows 4 principal peaks corresponding to campesterol, stigmasterol, β -sitosterol and $\Delta 7$ -stigmastenol. The retention times of the sterols with reference to β -sitosterol are given in Table 2.4.23-1.

Table 2.4.23-1. – Retention times of sterols with reference to β -sitosterol for two different columns

	Poly(cyanopropyl)(7)-(phenyl)(7)-(methyl)(86)siloxane	Poly[methyl(95)-phenyl(5)]siloxane
Cholesterol	0.64	0.63
Brassicasterol	0.70	0.71
24-Methylenecholesterol	0.79	0.80
Campesterol	0.82	0.81
Campestanol	0.83	0.82
Stigmasterol	0.87	0.87
$\Delta 7$ -Campesterol	0.93	0.92
$\Delta 5,23$ -Stigmastadienol	0.95	0.95
Clerosterol	0.96	0.96
β -Sitosterol	1	1
Sitostanol	1.01	1.02
$\Delta 5$ -Avenasterol	1.03	1.03
$\Delta 5,24$ -Stigmastadienol	1.09	1.08
$\Delta 7$ -Stigmastenol ⁽¹⁾	1.13	1.12
$\Delta 7$ -Avenasterol	1.18	1.16
Betulin	1.4	1.4

(1) This sterol may also be referred to as $\Delta 7$ -stigmasterol in literature.

The peak of the internal standard (betulin) must be clearly separated from the peaks of the sterols to be determined.

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 due to the component to be determined,

S = sum of the areas of the peaks due 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:

$$\frac{A \times m' \times 100}{A' \times m}$$

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

A' = area of the peak due to betulin,

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

m' = mass of *betulin R* added in milligrams.

01/2008:20424

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 (a₁) (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:

Operating parameters	Sample preparation procedure		
	1	2	3
Equilibration temperature (°C)	80	105	80
Equilibration time (min)	60	45	45
Transfer-line temperature (°C)	85	110	105
Carrier gas: <i>Nitrogen for chromatography R</i> or <i>Helium for chromatography R</i> 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 (a₁) 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 (a₁) 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 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.

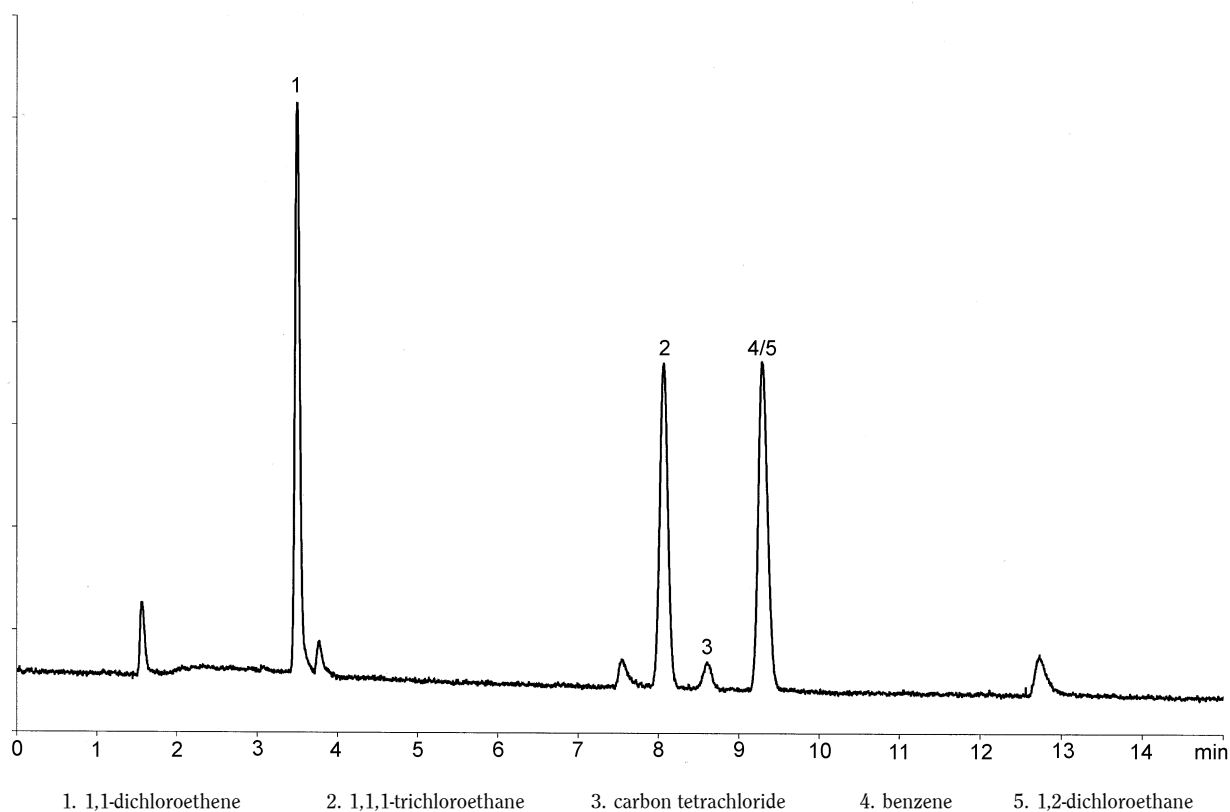


Figure 2.4.24-1. – Typical chromatogram of class 1 solvents using the conditions described for System A and Procedure 1. Flame-ionisation detector.

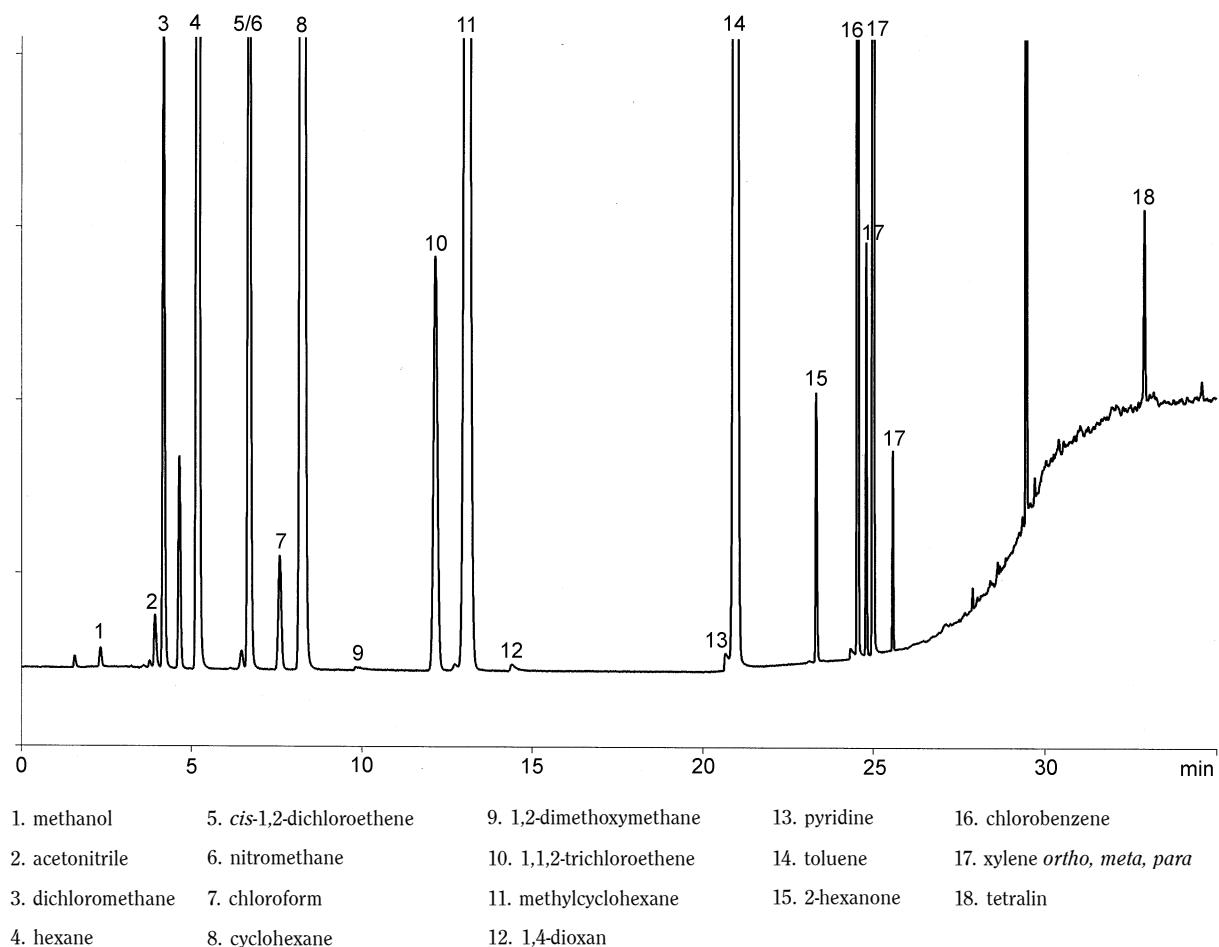


Figure 2.4.24-2. – Chromatogram of Class 2 solvents using the conditions described for System A and Procedure 1. Flame-ionisation detector.

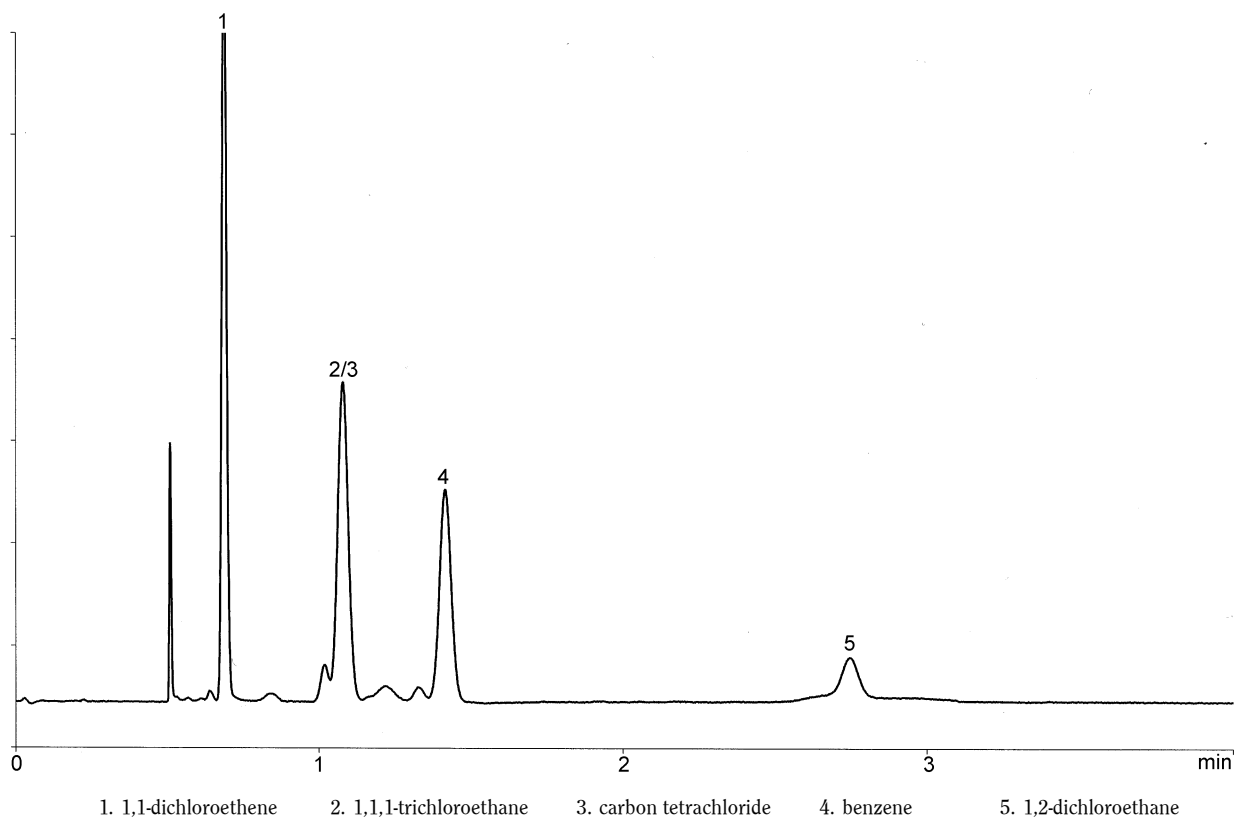


Figure 2.4.24-3. – Chromatogram of Class 1 residual solvents using the conditions described for System B and Procedure 1. Flame-ionisation detector.

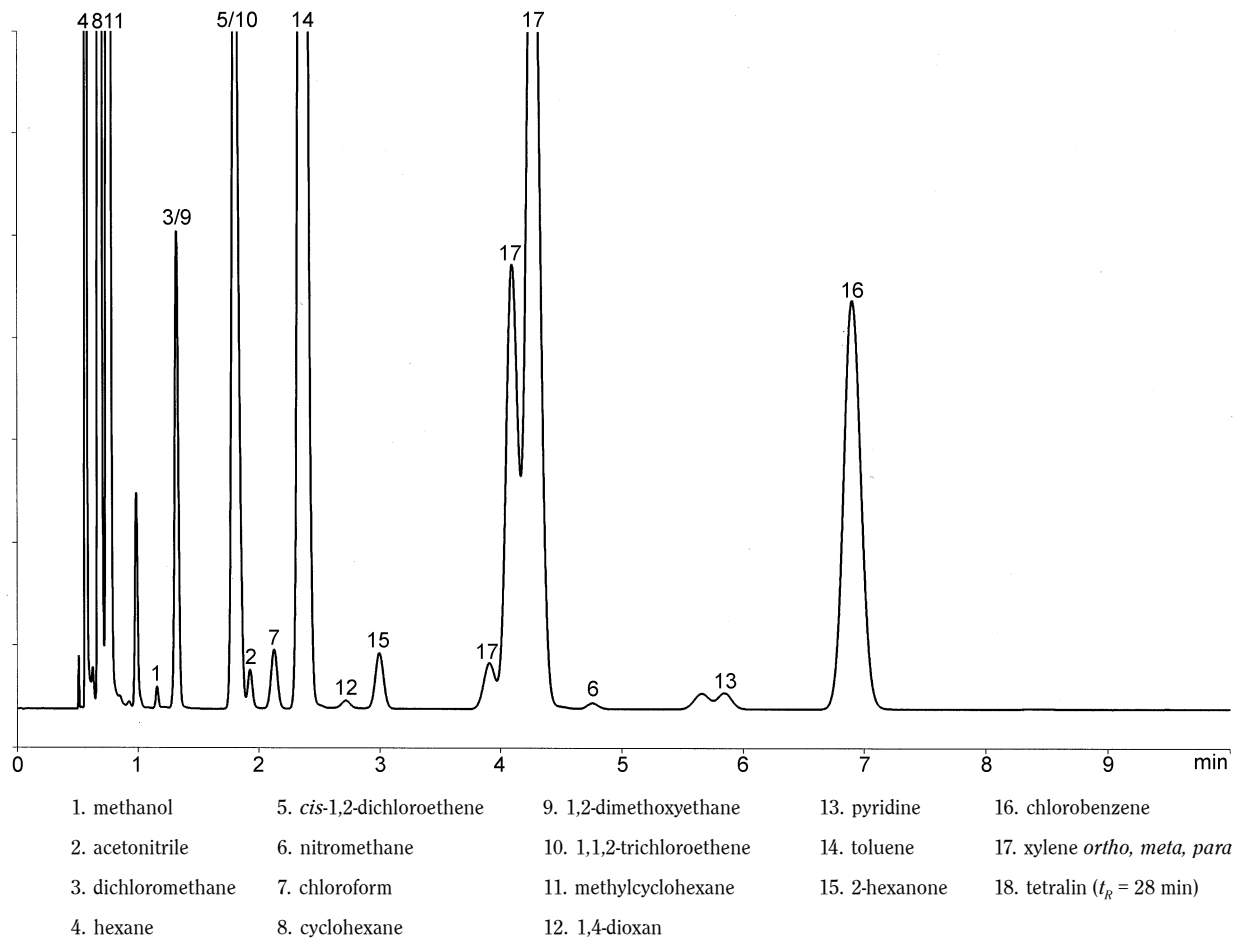


Figure 2.4.24-4. – Typical chromatogram of class 2 residual solvents using the conditions described for System B and Procedure 1. Flame-ionisation detector.

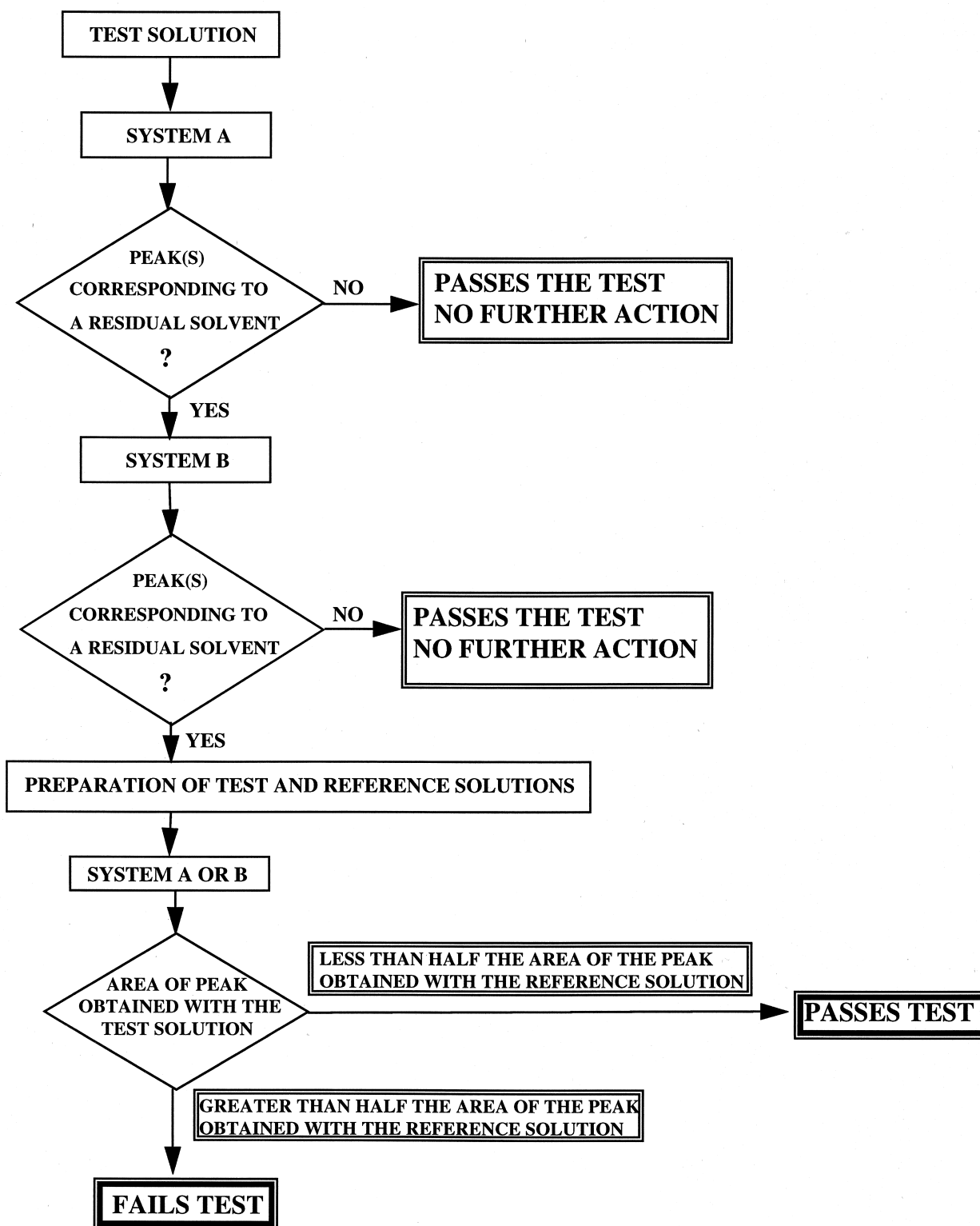


Figure 2.4.24-5. – Diagram relating to the identification of residual solvents and the application of limit tests

01/2008:20425 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.

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