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,

M_R = mass of the substance to be examined in the reference solution, in grams,

C = the amount of ethylene oxide added to reference solution (a), in micrograms.

$$\frac{D_T \times C}{(D_R \times M_T) - (D_T \times M_R)}$$

D_T = area of the peak corresponding to dioxan in the chromatogram obtained with the test solution,

D_R = area of the peak corresponding to dioxan in the chromatogram obtained with reference solution (a),

C = the amount of dioxan added to reference solution (a) in micrograms.

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2.4.26. *N,N*-DIMETHYLANILINE

METHOD A

Examine by gas chromatography (*2.2.28*), using *N,N-diethylaniline R* as the internal standard.

Internal standard solution. Dissolve 50 mg of *N,N-diethylaniline R* in 4 ml of *0.1 M hydrochloric acid* and dilute to 50 ml with *water R*. Dilute 1 ml of this solution to 100 ml with *water R*.

Test solution. Dissolve in a ground-glass-stoppered tube 0.50 g of the substance to be examined in 30.0 ml of *water R*. Add 1.0 ml of the internal standard solution. Adjust the solution to a temperature of 26 °C to 28 °C. Add 1.0 ml of *strong sodium hydroxide solution R* and mix until

completely dissolved. Add 2.0 ml of *trimethylpentane R*. Shake for 2 min and allow the phases to separate. Use the upper layer.

Reference solution. Dissolve 50.0 mg of *N,N-dimethylaniline R* in 4.0 ml of *0.1 M hydrochloric acid* and dilute to 50.0 ml with *water R*. Dilute 1.0 ml of this solution to 100.0 ml with *water R*. Dilute 1.0 ml of this solution to 30.0 ml with *water R*. Add 1.0 ml of the internal standard solution and 1.0 ml of *strong sodium hydroxide solution R*. Add 2.0 ml of *trimethylpentane R*. Shake for 2 min and allow the phases to separate. Use the upper layer.

The chromatographic procedure may be carried out using:

– a fused-silica capillary column 25 m long and 0.32 mm in internal diameter coated with cross-linked *polymethylphenylsiloxane R* (film thickness 0.52 μm),
– *helium for chromatography R* as the carrier gas with a split ratio 1:20, a column head pressure of 50 kPa and a split vent of 20 ml/min,
– a flame-ionisation detector,
– a split-liner consisting of a column about 1 cm long packed with *diatomaceous earth for gas chromatography R* impregnated with 10 per cent *m/m* of *poly(dimethyl)siloxane R*,

maintaining the temperature of the column at 150 °C for 5 min, then raising the temperature at a rate of 20 °C per min to 275 °C and maintaining it at 275 °C for 3 min and maintaining the temperature of the detector at 300 °C and that of the injection port at 220 °C.

The retention times are: *N,N*-dimethylaniline about 3.6 min, *N,N*-diethylaniline about 5.0 min.

Inject 1 μl of the test solution and 1 μl of the reference solution.

METHOD B

Examined by gas chromatography (*2.2.28*), using *naphthalene R* as the internal standard.

Internal standard solution. Dissolve 50 mg of *naphthalene R* in *cyclohexane R* and dilute to 50 ml with the same solvent. Dilute 5 ml of this solution to 100 ml with *cyclohexane R*.

Test solution. To 1.00 g of the substance to be examined in a ground-glass-stoppered tube add 5 ml of *1 M sodium hydroxide* and 1.0 ml of the internal standard solution. Stopper the tube and shake vigorously for 1 min. Centrifuge if necessary and use the upper layer.

Reference solution. To 50.0 mg of *N,N-dimethylaniline R* add 2 ml of *hydrochloric acid R* and 20 ml of *water R*, shake to dissolve and dilute to 50.0 ml with *water R*. Dilute 5.0 ml of this solution to 250.0 ml with *water R*. To 1.0 ml of the latter solution in a ground-glass-stoppered tube add 5 ml of *1 M sodium hydroxide* and 1.0 ml of the internal standard solution. Stopper the tube and shake vigorously for 1 min. Centrifuge if necessary and use the upper layer.

The chromatographic procedure may be carried out using:

– a glass column 2 m long and 2 mm in internal diameter packed with *silanised diatomaceous earth for gas chromatography R* impregnated with 3 per cent *m/m* of *polymethylphenylsiloxane R*,
– *nitrogen for chromatography R* as the carrier gas at a flow rate of 30 ml/min,
– a flame-ionisation detector,

maintaining the temperature of the column at 120 °C and that of the injection port and of the detector at 150 °C.

Inject 1 μl of the test solution and 1 μl of the reference solution.

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2.4.27. HEAVY METALS IN HERBAL DRUGS AND FATTY OILS

Examine by atomic absorption spectrometry (*2.2.23*).

CAUTION: when using closed high-pressure digestion vessels and microwave laboratory equipment, be familiar with the safety and operating instructions given by the manufacturer.

APPARATUS

The apparatus typically consists of the following:

– as digestion flasks, polytetrafluoroethylene flasks with a volume of about 120 ml, fitted with an airtight closure, a valve to adjust the pressure inside the container and a polytetrafluoroethylene tube to allow release of gas,
– a system to make flasks airtight, using the same torsional force for each of them,
– a microwave oven, with a magnetron frequency of 2450 MHz, with a selectable output from 0 to 630 ± 70 W in 1 per cent increments, a programmable digital computer, a polytetrafluoroethylene-coated microwave cavity with a variable speed exhaust fan, a rotating turntable drive system and exhaust tubing to vent fumes,
– an atomic absorption spectrometer, equipped with hollow-cathode lamps as source of radiation and a deuterium lamp as background corrector; the system is fitted with:

(a) a graphite furnace as atomisation device for cadmium, copper, iron, lead, nickel and zinc.

(b) an automated continuous-flow hydride vapour generation system for arsenic and mercury.

METHOD

In case alternative apparatus is used, an adjustment of the instrument parameters may be necessary.

Clean all the glassware and laboratory equipment with a 10 g/l solution of *nitric acid R* before use.

Test solution. In a digestion flask place the prescribed quantity of the substance to be examined (about 0.50 g of powdered drug (1400) (*2.9.12*) or 0.50 g of fatty oil). Add 6 ml of *heavy metal-free nitric acid R* and 4 ml of *heavy metal-free hydrochloric acid R*. Make the flask airtight.

Place the digestion flasks in the microwave oven. Carry out the digestion in 3 steps according to the following programme, used for 7 flasks each containing the test solution: 80 per cent power for 15 min, 100 per cent power for 5 min, 80 per cent power for 20 min.

At the end of the cycle allow the flasks to cool in air and to each add 4 ml of *heavy metal-free sulphuric acid R*. Repeat the digestion programme. After cooling in air, open each digestion flask and introduce the clear, colourless solution obtained into a 50 ml volumetric flask. Rinse each digestion flask with 2 quantities, each of 15 ml, of *water R* and collect the rinsings in the volumetric flask. Add 1.0 ml of a 10 g/l solution of *magnesium nitrate R* and 1.0 ml of a 100 g/l solution of *ammonium dihydrogen phosphate R* and dilute to 50.0 ml with *water R*.

Blank solution. Mix 6 ml of *heavy metal-free nitric acid R* and 4 ml of *heavy metal-free hydrochloric acid R* in a digestion flask. Carry out the digestion in the same manner as for the test solution.