Starting with solution D prepare the following sequence of dilutions:

Solution D (ml)	1.2	1.5	2.0	3.0	6.0	8.0
water R (ml)	8.8	8.5	8.0	7.0	4.0	2.0

Determine the number of millilitres of solution D which, when diluted to 10.0 ml with *water R*, still has a bitter taste (X).

Calculate the bitterness value for each panel member from the expression:

$$\left(\frac{\mathrm{Y} \times k}{\mathrm{X} \times 0.1}\right)$$

Calculate the bitterness value of the sample to be examined as the average value for all panel members.

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2.8.16. DRY RESIDUE OF EXTRACTS

In a flat-bottomed dish about 50 mm in diameter and about 30 mm in height, introduce rapidly 2.00 g or 2.0 ml of the extract to be examined. Evaporate to dryness on a water-bath and dry in an oven at 100-105 °C for 3 h. Allow to cool in a desiccator over *diphosphorus pentoxide R* or *anhydrous silica gel R* and weigh. Calculate the result as a mass percentage or in grams per litre.

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2.8.17. LOSS ON DRYING OF EXTRACTS

In a flat-bottomed dish about 50 mm in diameter and about 30 mm in height, weigh rapidly 0.50 g of the extract to be examined, finely powdered. Dry in an oven at 100-105 °C for 3 h. Allow to cool in a desiccator over *diphosphorus pentoxide R* or *anhydrous silica gel R* and weigh. Calculate the result as a mass percentage.

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2.8.18. DETERMINATION OF AFLATOXIN B_1 IN HERBAL DRUGS

CAUTION: aflatoxins are very toxic and carcinogenic. Perform manipulations under an extraction hood whenever possible. Take particular precautions, such as use of a glove box, when toxins are in dry form because of their electrostatic properties and the tendency to disperse through the working areas. Decontamination procedures for laboratory wastes of aflatoxins were developed by the International Agency for Research on Cancer (IARC).

Aflatoxins are naturally occurring mycotoxins produced mainly by *Aspergillus flavus* and *Aspergillus parasiticus*. These fungi are common and widespread in nature and are most often found when certain grains are grown under conditions of stress such as drought. The mould occurs in soil, decaying vegetation, hay, and grains undergoing microbial spoilage, and invades all types of organic substrates whenever and wherever the conditions are favourable for its growth. Favourable conditions include high moisture content and high temperature. At least 13 different types of aflatoxin are produced in nature and most of these are known to be highly toxic and carcinogenic. Aflatoxin B_1 is considered the most toxic. Herbal drugs that are subject to contamination by aflatoxins are tested by a validated method.

Unless otherwise indicated in the monograph, herbal drugs contain not more than 2 µg/kg of aflatoxin B_1. The competent authority may also require compliance with a limit of 4 µg/kg for the sum of aflatoxins B_1, B_2, G_1 and G_2.

The method described below is cited as an example of a method that has been shown to be suitable for devil's claw root, ginger and senna pods. Its suitability for other herbal drugs must be demonstrated or another validated method used.

METHOD

Liquid chromatography (*2.2.29*).

Aflatoxins are subject to light degradation. Carry out the determination protected from daylight by using UV-absorbing foil on windows in combination with subdued light, or curtains or blinds in combination with artificial light (fluorescent tubes are acceptable). Protect aflatoxin-containing solutions from daylight.

Rinse glassware before use with a 10 per cent *V/V* solution of *sulphuric acid R* and then rinse carefully with *distilled water R* until no more acid is present.

Test solution. Use an immunoaffinity column containing antibodies against aflatoxin B_1 with a capacity of not less than 100 ng of aflatoxin B_1 and which gives a recovery of not less than 80 per cent when a solution of 5 ng of aflatoxin B_1 in a mixture of 12.5 ml of *methanol R* and 87.5 ml of *water R* is passed through. Allow the immunoaffinity column to reach room temperature. To 5.00 g of the powdered drug (500) (*2.9.12*) add 100 ml of a mixture of 30 volumes of *water R* and 70 volumes of *methanol R* and extract by sonication for 30 min. Filter through folded filter paper. Pipette 10.0 ml of the clear filtrate into a 150 ml conical flask. Add 70 ml of *water R*. Pass 40 ml through the immunoaffinity column at a flow rate of 3 ml/min (not exceeding 5 ml/min). Wash the column with 2 volumes, each of 10 ml, of *water R* at a flow rate not exceeding 5 ml/min and dry by applying a slight vacuum for 5-10 s or by passing air through the immunoaffinity column by means of a syringe for 10 s. Apply 0.5 ml of *methanol R* to the column and allow to pass through by gravity. Collect the eluate in a 5 ml volumetric flask. After 1 min, apply a 2nd portion of 0.5 ml of *methanol R*. After a further 1 min, apply a 3rd portion of 0.5 ml of *methanol R*. Collect most of the applied elution solvent by pressing air through or applying vacuum to the column. Dilute to 5 ml with *water R* and shake well. If the solution is clear it can be used directly for analysis. Otherwise, pass it through a disposable filter unit prior to injection. Use a disposable filter unit (e.g. 0.45 µm pore size polytetrafluoroethylene filter) that has been shown not to cause loss of aflatoxin by retention.

Aflatoxin B_1 primary stock solution. Dissolve *aflatoxin B_1 R* in a mixture of 2 volumes of *acetonitrile R* and 98 volumes of *toluene R* to give a 10 µg/ml solution. To determine the exact concentration of aflatoxin B_1 in the stock solution, record the absorption curve (*2.2.25*) between 330 nm and 370 nm in quartz cells.