Substance	Relative rentention times
β-Endosulfan	0.92
o,p'-DDT	0.95
Carbophenothion	1.00
p,p'-DDT	1.02
cis-Permethrin	1.29
trans-Permethrin	1.31
Cypermethrin*	1.40
Fenvalerate*	1.47 and 1.49
Deltamethrin	1.54

* The substance shows several peaks.

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2.8.14. DETERMINATION OF TANNINS IN HERBAL DRUGS

Carry out all the extraction and dilution operations protected from light.

In the case of a herbal drug or a dry extract, to the stated amount of the powdered drug (180) (*2.9.12*) or the extract in a 250 ml round-bottomed flask add 150 ml of *water R*. Heat on a water-bath for 30 min. Cool under running water and transfer quantitatively to a 250 ml volumetric flask. Rinse the round-bottomed flask and collect the washings in the volumetric flask, then dilute to 250.0 ml with *water R*. Allow the solids to settle and filter the liquid through a filter paper 125 mm in diameter. Discard the first 50 ml of the filtrate.

In the case of a liquid extract or a tincture, dilute the stated amount of the liquid extract or tincture to 250.0 ml with *water R*. Filter the mixture through a filter paper 125 mm in diameter. Discard the first 50 ml of the filtrate.

Total polyphenols. Dilute 5.0 ml of the filtrate to 25.0 ml with *water R*. Mix 2.0 ml of this solution with 1.0 ml of *phosphomolybdotungstic reagent R* and 10.0 ml of *water R* and dilute to 25.0 ml with a 290 g/l solution of *sodium carbonate R*. After 30 min measure the absorbance (*2.2.25*) at 760 nm (A_1), using *water R* as the compensation liquid.

Polyphenols not adsorbed by hide powder. To 10.0 ml of the filtrate, add 0.10 g of *hide powder CRS* and shake vigorously for 60 min. Filter and dilute 5.0 ml of the filtrate to 25.0 ml with *water R*. Mix 2.0 ml of this solution with 1.0 ml of *phosphomolybdotungstic reagent R* and 10.0 ml of *water R* and dilute to 25.0 ml with a 290 g/l solution of *sodium carbonate R*. After 30 min measure the absorbance (*2.2.25*) at 760 nm (A_2), using *water R* as the compensation liquid.

Standard. Dissolve immediately before use 50.0 mg of *pyrogallol R* in *water R* and dilute to 100.0 ml with the same solvent. Dilute 5.0 ml of the solution to 100.0 ml with *water R*. Mix 2.0 ml of this solution with 1.0 ml of *phosphomolybdotungstic reagent R* and 10.0 ml of *water R* and dilute to 25.0 ml with a 290 g/l solution of *sodium carbonate R*. After 30 min measure the absorbance (*2.2.25*) at 760 nm (A_3), using *water R* as the compensation liquid.

Calculate the percentage content of tannins expressed as pyrogallol from the expression:

$$\frac{62.5\,(A_1 - A_2)\,m_2}{A_3 \times m_1}$$

m_1 = mass of the sample to be examined, in grams,

m_2 = mass of pyrogallol, in grams.

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2.8.15. BITTERNESS VALUE

The bitterness value is the reciprocal of the dilution of a compound, a liquid or an extract that still has a bitter taste. It is determined by comparison with quinine hydrochloride, the bitterness value of which is set at 200 000.

Determination of the correction factor

A taste panel comprising at least 6 persons is recommended. The mouth must be rinsed with *water R* before tasting.

To correct for individual differences in tasting bitterness amongst the panel members it is necessary to determine a correction factor for each panel member.

Stock solution. Dissolve 0.100 g of *quinine hydrochloride R* in *water R* and dilute to 100.0 ml with the same solvent. Dilute 1.0 ml of this solution to 100.0 ml with *water R*.

Reference solutions. Prepare a series of dilutions by placing in a first tube 3.6 ml of the stock solution and increasing the volume by 0.2 ml in each subsequent tube to a total of 5.8 ml; dilute the contents of each tube to 10.0 ml with *water R*.

Determine as follows the dilution with the lowest concentration that still has a bitter taste. Take 10.0 ml of the weakest solution into the mouth and pass it from side to side over the back of the tongue for 30 s. If the solution is not found to be bitter, spit it out and wait for 1 min. Rinse the mouth with *water R*. After 10 min, use the next dilution in order of increasing concentration.

Calculate the correction factor *k* for each panel member from the expression:

$$k = \frac{n}{5.00}$$

n = number of millilitres of the stock solution in the dilution of lowest concentration that is judged to be bitter.

Persons who are unable to taste any bitterness when using the reference solution prepared from 5.8 ml of stock solution have to be excluded from the panel.

Sample preparation

If necessary, reduce the sample to a powder (710) (*2.9.12*). To 1.0 g of sample add 100 ml of boiling *water R*. Heat on a water-bath for 30 min, stirring continuously. Allow to cool and dilute to 100 ml with *water R*. Shake vigorously and filter, discarding the first 2 ml of the filtrate. The filtrate is labelled C-1 and has a dilution factor (DF) of 100.

If liquids have to be examined, 1 ml of the liquid is diluted with a suitable solvent to 100 ml and designated C-1.

Determination of the bitterness value

Test solutions:

10.0 ml of C-1 is diluted with *water R* to 100 ml: C-2	(DF = 1000)
10.0 ml of C-2 is diluted with *water R* to 100 ml: C-3	(DF = 10 000)
20.0 ml of C-3 is diluted with *water R* to 100 ml: C-3A	(DF = 50 000)
10.0 ml of C-3 is diluted with *water R* to 100 ml: C-4	(DF = 100 000)

Starting with dilution C-4 each panel member determines the dilution which still has a bitter taste. This solution is designated D. Note the DF of solution D is Y.