⟨561⟩ ARTICLES OF BOTANICAL ORIGIN

SAMPLING

To reduce the effect of sampling bias in qualitative and quantitative results, it is necessary to ensure that the composition of the sample used be representative of the batch of drugs being examined. The following sampling procedures are the minimum considered applicable to vegetable drugs. Some articles, or some tests, may require more rigorous procedures involving more containers being sampled or more samples per container.

Gross Sample

Where external examination of containers, markings, and labels indicates that the batch can be considered to be homogeneous, take individual samples from the number of randomly selected containers indicated below. Where the batch cannot be considered to be homogeneous, divide it into sub-batches that are as homogeneous as possible, then sample each one as a homogeneous batch. It is recommended to include samples from the first, middle, and last containers where the *No. of Containers in Batch* (*N*) is 11 or more, and each container in the batch is numbered or lettered in order.

No. of Containers in Batch (*N*)	**No. of Containers to Be Sampled (*n*)**
1–10	All
11–19	11
>19	$n = 10 + (N/10)$

(Rounding was calculated as "*n*" to next highest whole number.)

Samples are taken from the upper, middle, and lower sections of each container. If the crude material consists of component parts that are 1 cm or less in any dimension, and in the case of all powdered or ground materials, withdraw the sample by means of a sampling device that removes a core from the top to the bottom of the container, NLT two cores being taken from different angles. For materials with component parts >1 cm in any dimension, withdraw samples by hand. In the case of large bales or packs, samples should be taken from a depth of 10 cm, because the moisture content of the surface layer may be different from that of the inner layers.

Prepare the gross sample by combining and mixing the individual samples taken from each opened container, taking care not to increase the degree of fragmentation or significantly affect the moisture content.

For articles in containers holding <1 kg, mix the contents, and withdraw a quantity sufficient for the tests. For articles in containers holding between 1 and 5 kg, withdraw equal portions from the upper, middle, and lower parts of the container, each of the samples being sufficient to carry out the tests. Thoroughly mix the samples, and withdraw an amount sufficient to carry out the tests. For containers holding more than 5 kg, withdraw three samples, each weighing NLT 250 g, from the upper, middle, and lower parts of the container. Thoroughly mix the samples, and withdraw a portion sufficient to carry out the tests.

Laboratory Sample

Prepare the *Laboratory Sample* by repeated quartering of the gross sample.

[NOTE—Quartering consists of placing the sample, adequately mixed, as an even and square-shaped heap and dividing it diagonally into four equal parts. The two opposite parts are then taken and carefully mixed. The process is repeated as necessary until the required quantity is obtained.]

The *Laboratory Sample* should be of a size sufficient for performing all the necessary tests.

Test Sample

Unless otherwise directed in the individual monograph or test procedure below, prepare the *Test Sample* as follows.

Decrease the size of the *Laboratory Sample* by quartering, taking care that each withdrawn portion remains representative. In the case of unground or unpowdered drugs, grind the withdrawn sample so that it will pass through a No. 20 standard-mesh sieve, and mix the resulting powder well. If the material cannot be ground, reduce it to as fine a state as possible, mix by rolling it on paper or sampling cloth, spread it out in a thin layer, and withdraw the portion for analysis.

METHODS OF ANALYSIS

Foreign Organic Matter

TEST SAMPLE

Unless otherwise specified in the individual monograph, weigh the following quantities of the *Laboratory Sample*, taking care that the withdrawn portion is representative (quartering if necessary).

Roots, rhizomes, bark, and herbs	500 g
Leaves, flowers, seeds, and fruit	250 g
Cut vegetable drugs (average weight of the pieces is <0.5 g)	50 g

Spread the sample out in a thin layer, and separate the foreign organic matter by hand as completely as possible. Weigh it, and determine the percentage of foreign organic matter in the weight of drug taken.

Total Ash

Accurately weigh a quantity of the *Test Sample*, representing 2–4 g of the air-dried material, in a tared crucible and incinerate, gently at first, and gradually increase the temperature to 675 ± 25° until free from carbon, and determine the weight of the ash. If a carbon-free ash cannot be obtained in this way, extract the charred mass with hot water, collect the insoluble residue on an ashless filter paper, incinerate the residue and filter paper until the ash is white or nearly so, then add the filtrate, evaporate it to dryness, and heat the whole to a temperature of 675 ± 25°. If a carbon-free ash cannot be obtained in this way, cool the crucible, add 15 mL of alcohol, break up the ash with a glass rod, burn off the alcohol, and again heat the whole to a temperature of 675 ± 25°. Cool in a desiccator, weigh the ash, and calculate the percentage of total ash from the weight of the drug taken.

Acid-Insoluble Ash

Boil the ash obtained as directed in *Total Ash* with 25 mL of 3 N hydrochloric acid for 5 min, collect the insoluble matter on a tared filtering crucible or ashless filter, wash with hot water, ignite, and weigh. Determine the percentage of acid-insoluble ash calculated from the weight of drug taken.

Water-Soluble Ash

Boil the ash obtained as directed in *Total Ash* with 25 mL of water for 5 min. Collect the insoluble matter in a sintered-glass crucible or on an ashless filter paper. Wash with hot water, and ignite for 15 min at a temperature not exceeding 450°. Subtract the weight of this residue, in mg, obtained in *Total Ash*, and calculate the percentage of water-soluble ash with reference to the weight of sample as determined in *Total Ash*.

Alcohol-Soluble Extractives

METHOD 1 (HOT EXTRACTION METHOD)

Transfer about 4 g of air-dried, coarsely powdered material, accurately weighed, to a glass-stoppered conical flask. Add 100 mL of alcohol, and weigh the flask. Shake, and allow to stand for 1 h. Attach a reflux condenser to the flask, boil gently for 1 h, cool, and weigh. Readjust to the original weight with alcohol. Shake, and filter rapidly through a dry filter. Transfer 25 mL of the filtrate to a tared flat-bottomed dish, and evaporate on a water bath to dryness. Dry at 105° for 6 h, cool in a desiccator for 30 min, and weigh without delay. Calculate the content, in mg/g, of alcohol-extractable matter in the test specimen.

METHOD 2 (COLD EXTRACTION METHOD)

Transfer about 4 g of air-dried, coarsely powdered material, accurately weighed, to a glass-stoppered conical flask. Add 100 mL of alcohol, insert a stopper into the flask, and macerate for 24 h, shaking frequently during the first 8 h, and then allowing to stand. Filter rapidly, taking precautions against loss of alcohol. Evaporate 25 mL of the filtrate to dryness in a tared, flat-bottomed, shallow dish, and dry at 105° to constant weight. Calculate the content, in mg/g, of alcohol-extractable matter in the test specimen.

Water-Soluble Extractives

METHOD 1 (HOT EXTRACTION METHOD)

Proceed as directed in *Method 1 (Hot Extraction Method)* in *Alcohol-Soluble Extractives*, except use water in place of alcohol.

METHOD 2 (COLD EXTRACTION METHOD)

Proceed as directed in *Method 2 (Cold Extraction Method)* in *Alcohol-Soluble Extractives*, except use water in place of alcohol.

Crude Fiber

Exhaust a weighed quantity of the *Test Sample*, representing about 2 g of the drug, with ether. Add 200 mL of boiling dilute sulfuric acid (1 in 78) to the ether-exhausted marc, in a 500-mL flask, and connect the flask to a reflux condenser. Reflux the mixture for 30 min, accurately timed, then pass through a linen or hardened-paper filter, and wash the residue on the filter with boiling water until the effluent washing is no longer acid. Rinse the residue back into the flask with 200 mL of boiling sodium hydroxide solution, adjusted to 1.25% by titration and free from sodium carbonate. Again reflux the mixture for 30 min, accurately timed, then rapidly pass through a tared filter, wash the residue with boiling water until the last washing is neutral, and dry it at 110° to constant weight. Incinerate the dried residue, ignite to constant weight, cool in a desiccator, and weigh the ash: the difference between the weight obtained by drying at 110° and that of the ash represents the weight of the crude fiber.

[NOTE—The boiling with acid and alkali should continue for 30 min, accurately timed, from the time that the liquid (which is cooled below the boiling point by being added to the cold flask) again boils. After the solution has been brought to boiling, the heat should be turned low enough just to maintain boiling. During the boiling, the flask should be gently rotated from time to time to wash down any particles that may adhere to the walls of the flask. A slow current of air introduced into the flask during the boiling operation aids in preventing excessive frothing.]

Starch Content

METHOD 1

The following is a general procedure for all reducing sugars and may be used to determine the starch content in botanical articles.

Malt extract: Use clean new barley malt of known efficacy, and grind just before use. Prepare malt extract just before use. For every 80 mL of malt extract needed, digest 5 g of ground malt with 100 mL of water at room temperature for 2 h. [NOTE—If an electric mixer is used, stir the mixture for 20 min.] Filter to obtain a clear extract, filtering again, if necessary, and mix the infusion well.

Test solution: Extract about 5 g of the finely ground test specimen with five 10-mL portions of ether, using a filter that will completely retain the smallest starch granule. Allow the ether to evaporate from the residue, and wash with 250 mL of aqueous alcohol solution (10 in 100). Carefully wash the residue from the paper into a 500-mL beaker with about 100 mL of water. Heat to about 60° (avoiding, if possible, gelatinizing starch), and allow to stand for about 1 h, stirring frequently to effect complete solution of sugars. Transfer to a wide-mouth bottle, rinse the beaker with a little warm water, and cool. Add an equal volume of alcohol, mix, and allow to stand for NLT 1 h.

Centrifuge until the precipitate is closely packed on the bottom of the bottle, and decant the supernatant. Wash the precipitate with successive 50-mL portions of alcohol solution (50 in 100) by centrifuging and decanting through a suitable filter until the washings are sugar free. [NOTE—To test for the presence of sugar, transfer a few drops of the washings to a test tube, and add 3 or 4 drops of a 20% solution of 1-naphthol in alcohol, prepared by dissolving 200 mg of 1-naphthol in 1 mL of alcohol and 2 mL of water. Shake the test tube well to allow uniform mixing, allow 2–4 mL of sulfuric acid to flow down the sides of the test tube, and hold the test tube upright. If sugar is present, the interface of the two liquids is colored faint to deep violet, and on shaking, the whole solution becomes blue-violet.]

Transfer the residue from the bottle and hardened filter to a beaker with about 50 mL of water. Immerse the beaker in boiling water, and stir constantly for 15 min or until all of the starch is gelatinized. Cool the beaker to 55°, add 20 mL of *Malt extract*, and hold at this temperature for 1 h. Heat again to boiling for a few min, cool to 55°, add 20 mL of *Malt extract*, and hold at this temperature for 1 h or until the residue when treated with iodine TS shows no blue tinge upon microscopic examination. Cool, dilute with water to 250 mL, and filter.

General procedure: Transfer 200 mL of the *Test solution* to a flask fitted with a reflux condenser, add 20 mL of hydrochloric acid, and heat in a boiling water bath for 2.5 h. Cool, nearly neutralize with sodium hydroxide TS, complete neutralization with sodium carbonate TS, dilute with water to 500 mL, mix, and filter. The volume of aliquot taken depends on the starch content of the specimen under test (see *Table 1*). The aliquot should contain between 100 and 200 mg of dextrose. Transfer 50 mL of the filtrate to a 400-mL alkali-resistant glass beaker, add 50 mL of alkaline cupric tartrate TS, cover the beak-

er with a water glass, and heat. Adjust the flame in the burner so that the contents of the flask begin to boil in 4 min, and continue boiling for exactly 2 min. Filter the hot solution at once through a sintered-glass filter. Wash the precipitate of cuprous oxide thoroughly with water at about 60°, then with 10 mL of alcohol, and finally with 10 mL of ether.

Table 1. Determination of the Optimum Aliquot

Expected Starch Content (%)	Aliquot (mL)
60	25
50	35
40	50
30	50
20	50

For solutions of reducing sugars of comparatively high purity, proceed as directed in *Method 1A* to determine the amount of reduced copper obtained by weighing the dried cuprous oxide. For solutions of reducing sugars containing large amounts of organic impurities, including sucrose, proceed as directed in *Method 1B* to determine the amount of reduced copper obtained by titration with sodium thiosulfate.

Method 1A—Dry the precipitate obtained in *General procedure* for 30 min in an oven at 110 ± 2°, cool to room temperature in a desiccator, and weigh. Refer to *Table 2* to find the quantity of dextrose, in mg, corresponding to the weight of cuprous oxide found. Determine the percentage of dextrose and then the content of starch by the following formula:

$$\text{Percentage of dextrose} = (\text{wt. of dextrose in mg} \times 0.1 \times 500)/(\text{wt. of sample in g} \times \text{aliquot in mL})$$

$$\text{Content of starch} = \%\ \text{dextrose} \times 0.9$$

Table 2. Calculating Dextrose (mg) (applicable when Cu_2O is weighed directly)

Cuprous Oxide (Cu_2O)	Dextrose (D-Glucose)	Cuprous Oxide (Cu_2O)	Dextrose (D-Glucose)	Cuprous Oxide (Cu_2O)	Dextrose (D-Glucose)	Cuprous Oxide (Cu_2O)	Dextrose (D-Glucose)	Cuprous Oxide (Cu_2O)	Dextrose (D-Glucose)	Cuprous Oxide (Cu_2O)	Dextrose (D-Glucose)
10	4.0	90	38.9	170	75.1	250	112.8	330	152.2	410	193.7
12	4.9	92	39.8	172	76.0	252	113.7	332	153.2	412	194.7
14	5.7	94	40.6	174	76.9	254	114.7	334	154.2	414	195.8
16	6.6	96	41.5	176	77.8	256	115.7	336	155.2	416	196.8
18	7.5	98	42.4	178	78.8	258	116.6	338	156.3	418	197.9
20	8.3	100	43.3	180	79.7	260	117.6	340	157.3	420	199.0
22	9.2	102	44.2	182	80.6	262	118.6	342	158.3	422	200.1
24	10.0	104	45.1	184	81.5	264	119.5	344	159.3	424	201.1
26	10.9	106	46.0	186	82.5	266	120.5	346	160.3	426	202.2
28	11.8	108	46.9	188	83.4	268	121.5	348	161.4	428	203.3
30	12.6	110	47.8	190	84.3	270	122.5	350	162.4	430	204.4
32	13.5	112	48.7	192	85.3	272	123.4	352	163.4	432	205.5
34	14.3	114	49.6	194	86.2	274	124.4	354	164.4	434	206.5
36	15.2	116	50.5	196	87.1	276	125.4	356	165.4	436	207.6
38	16.1	118	51.4	198	88.1	278	126.4	358	166.5	438	208.7
40	16.9	120	52.3	200	89.0	280	127.3	360	167.5	440	209.8
42	17.8	122	53.2	202	89.9	282	128.3	362	168.5	442	210.9
44	18.7	124	54.1	204	90.9	284	129.3	364	169.6	444	212.0
46	19.6	126	55.0	206	91.8	286	130.3	366	170.6	446	213.1
48	20.4	128	55.9	208	92.8	288	131.3	368	171.6	448	214.1
50	21.3	130	56.8	210	93.7	290	132.3	370	172.7	450	215.2
52	22.2	132	57.7	212	94.6	292	133.2	372	173.7	452	216.3
54	23.0	134	58.6	214	95.6	294	134.2	374	174.7	454	217.4
56	23.9	136	59.5	216	96.5	296	135.2	376	175.8	456	218.5
58	24.8	138	60.4	218	97.5	298	136.2	378	176.8	458	219.6

Table 2. Calculating Dextrose (mg) (applicable when Cu_2O is weighed directly) *(Continued)*

Cuprous Oxide (Cu_2O)	Dextrose (D-Glucose)	Cuprous Oxide (Cu_2O)	Dextrose (D-Glucose)	Cuprous Oxide (Cu_2O)	Dextrose (D-Glucose)	Cuprous Oxide (Cu_2O)	Dextrose (D-Glucose)	Cuprous Oxide (Cu_2O)	Dextrose (D-Glucose)	Cuprous Oxide (Cu_2O)	Dextrose (D-Glucose)
60	25.6	140	61.3	220	98.4	300	137.2	380	177.9	460	220.7
62	26.5	142	62.2	222	99.4	302	138.2	382	178.9	462	221.8
64	27.4	144	63.1	224	100.3	304	139.2	384	180.0	464	222.9
66	28.3	146	64.0	226	101.3	306	140.2	386	181.0	466	224.0
68	29.2	148	65.0	228	102.2	308	141.2	388	182.0	468	225.1
70	30.0	150	65.9	230	103.2	310	142.2	390	183.1	470	226.2
72	30.9	152	66.8	232	104.1	312	143.2	392	184.1	472	227.4
74	31.8	154	67.7	234	105.1	314	144.2	394	185.2	474	228.3
76	32.7	156	68.6	236	106.0	316	145.2	396	186.2	476	229.6
78	33.6	158	69.5	238	107.0	318	146.2	398	187.3	478	230.7
80	34.4	160	70.4	240	108.0	320	147.2	400	188.4	480	231.8
82	35.3	162	71.4	242	108.9	322	148.2	402	189.4	482	232.9
84	36.2	164	72.3	244	109.9	324	149.2	404	190.5	484	234.1
86	37.1	166	73.2	246	110.8	326	150.2	406	191.5	486	235.2
88	38.0	168	74.1	248	111.8	328	151.2	408	192.6	488	236.3

Method 1B

SODIUM THIOSULFATE SOLUTION: Transfer 3.9 g of sodium thiosulfate, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with water to volume, and mix.

POTASSIUM IODIDE SOLUTION: Dissolve 42 g of potassium iodide in 100 mL of water.

SODIUM ACETATE SOLUTION: Dissolve 5.74 g of sodium acetate in 10 mL of water.

COPPER SOLUTION: Transfer about 0.3 g of pure electrolytic copper, accurately weighed, to a 250-mL flask, add 5 mL of nitric acid to dissolve the copper, add about 25 mL of water, and boil to expel red fumes. Add about 5 mL of bromine TS, and boil until the bromine is completely removed. Cool, add 10 mL of *Sodium acetate solution* followed by 10 mL of *Potassium iodide solution*, and titrate with *Sodium thiosulfate solution* to a light yellow color. Add enough starch TS to produce a marked blue color, and continue the titration. As the endpoint nears, add 2 g of potassium thiocyanate, and stir until completely dissolved. Continue titration until the precipitate is completely white. One mL of *Sodium thiosulfate solution* is equivalent to about 10 mg of copper. [NOTE—It is essential that the concentration of *Potassium iodide solution* be carefully regulated. If the solution contains less than 320 mg of copper at the completion of titration, add 4.2–5 g of potassium iodide to make a total solution of 100 mL. If greater amounts of copper are present, add *Potassium iodide solution* slowly, with constant agitation, from the buret in amounts proportionately greater.]

PROCEDURE: Wash the precipitated cuprous oxide obtained in *General procedure* with water, cover this filter with a watch glass, and dissolve the cuprous oxide with 5 mL of nitric acid directed under the watch glass with a pipette. Collect the filtrate in a 250-mL flask, wash the watch glass, and the filter with water. Collect all the washings in the flask. Boil the contents of the flask to expel red fumes. Add about 5 mL of bromine TS, and boil until the bromine is completely removed. Cool, and proceed as directed in *Copper solution* beginning with "add 10 mL of *Sodium acetate solution*". From the volume of *Sodium thiosulfate solution* consumed, obtain the weight of copper, in mg, and multiply the weight of copper by 1.1259 to obtain the weight, in mg, of cuprous oxide. From *Table 2*, find the quantity of dextrose, in mg, corresponding to the weight of cuprous oxide. The content of starch is equivalent to the weight, in mg, of dextrose obtained times 0.9. Conduct a blank determination, using 50 mL of alkaline cupric tartrate TS and 50 mL of *Malt extract*. If the weight of the cuprous oxide so obtained exceeds 0.5 mg, correct the result of the determination accordingly. [NOTE—The alkaline cupric tartrate TS deteriorates on standing, and the quantity of cuprous oxide obtained in the blank determination increases.]

METHOD 2

The following method is specific for dextrose (glucose), and because of its extreme sensitivity it may account for differences noted between values obtained from the same specimen. Duplicate determinations do not vary more than 2%.

Glucoamylase solution: Prepare a solution of glucoamylase in water containing 30 International Units (IU)/mL. Use glucoamylase obtained preferably from *Rhizopus delemar*. The total glucoamylase activity of the test specimen being used should be NLT 150 IU.

Acetate buffer solution: Dissolve 16.4 g of sodium acetate in 100 mL of water, add 12.0 mL of glacial acetic acid, and mix. The pH of this solution is 4.8.

Phosphate buffer: Dissolve 3.63 g of tris(hydroxymethyl) aminomethane and 5.0 g of monobasic sodium phosphate in 50.0 mL of water. At 37°, adjust with phosphoric acid to a pH of 7.0, dilute with water to 100.0 mL, and mix. [NOTE—The pH of the buffer medium is sensitive to temperature and should be adjusted to the desired pH at the temperature to be used during incubation.]

Enzyme solution: Dissolve 30 mg of glucose oxidase (Type II from *Aspergillus niger*), 3 mg of peroxidase (Type I from horseradish), and 10 mg of potassium ferrocyanide in 100 mL of *Phosphate buffer*. [NOTE—This mixture can be stored in a refrigerator for up to 10 days.]

18 N sulfuric acid: Add slowly, while stirring, 54 mL of sulfuric acid to 102 mL of water, allow to cool to 25°, and mix.

Standard solutions: Dissolve an accurately weighed quantity of USP Dextrose RS in water to obtain a solution containing 1.0 mg of USP Dextrose RS per mL. Quantitatively dilute a known volume of this solution with water to obtain *Standard solutions A*, *B*, *C*, *D*, and *E*, having known concentrations of 10, 20, 25, 40, and 50 μg/mL of USP Dextrose RS, respectively. [NOTE—Allow 4 h for complete mutarotation before use.]

Test solutions: Extract about 5 g of finely ground test specimen with five 25-mL portions of 80% alcohol, and filter. Remove all the alcohol from the residue by drying in an air oven at 105° for about 8 h. [NOTE 1—Any traces of alcohol remaining in the residue will inhibit glucoamylase.] Cool, and transfer the flask containing the dried test specimen to a desiccator. Transfer about 1 g, accurately weighed, of the test specimen to a previously tared flask, add 25 mL of water, and adjust with phosphoric acid to a pH of 5.0–7.0, if necessary. Boil the suspension for about 3 min, transfer the flask to an autoclave, and heat to 135° for 2 h. Remove the flask from the autoclave, maintain the temperature near 55°, and add 2.5 mL of *Acetate buffer solution* and sufficient water to adjust the total weight of the solution to 45 ± 1 g. Immerse the flask in a water bath maintained at 55 ± 1°, and add 5 mL of *Glucoamylase solution*. Continuously swirl the flask for 2 h to effect hydrolysis, pass through filter paper into a 250-mL volumetric flask, wash quantitatively with water, and collect all the washings in the flask. Dilute the contents of the flask with water to volume, and mix. Transfer 1 mL of an aliquot containing 20–60 μg of D-glucose to each of five test tubes. [NOTE 2—To obtain the range of concentration of glucose in the hydrolysate, quantitatively dilute, if necessary, with water to volume.] Add 2 mL of *Enzyme solution* to each of the five test tubes, and place the test tubes in the dark at 37 ± 1° for exactly 30 min to develop the color. At the end of 30 min, add 2 mL of *18 N sulfuric acid* to each of the test tubes to stop the reaction, and mix.

Control solution: Transfer an accurately weighed quantity of about 0.4 g of starch to a previously tared flask, and proceed as directed in *Test solutions* beginning with "add 25 mL of water, and adjust the pH with phosphoric acid".

Procedure: Concomitantly determine the absorbances of the *Standard solutions* and the *Test solutions* at the wavelength of maximum absorbance at about 540 nm, with a suitable spectrophotometer, using the *Control solution* as the blank to set the instrument. Plot the absorbance values of the *Standard solutions* versus concentration, in μg/mL, of dextrose, and draw the straight line best fitting the five plotted points. From the graph so obtained, determine the concentration, *C*, in μg/mL, of dextrose in each of the *Test solutions*, calculate the average concentration, in μg/mL, of the solution under test. The percentage of starch content in the weight of the test specimen taken is calculated by the formula:

$$(0.9C/10^6) \times V_1 \times (250/V_0)(100/E)(100/W) = 2.25CV_1/V_0EW$$

in which E is the weight, in g, of the test specimen taken; V_0 is the volume, in mL, of the aliquot taken from the 250-mL volumetric flask; W is the percentage of dry weight of the test specimen; and V_1 is the volume, in mL, if extra dilution is done (see *Note 2* in *Test solutions*). [NOTE—V_0 is 1.0 when no extra dilution is done.]

Volatile Oil Determination

Set up a round-bottom, shortneck, 1-L flask in a heating mantle set over a magnetic stirrer. Insert an egg-shaped stirring bar magnet in the flask, and attach a cold-finger condenser and an appropriate volatile oil trap of the type illustrated (see *Figure 1)*.

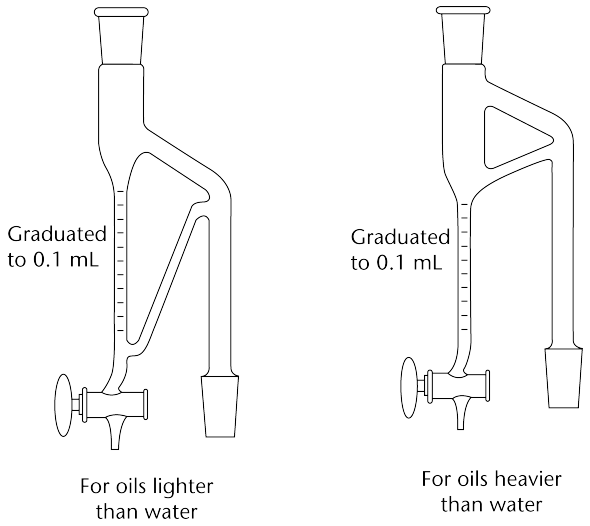

Figure 1. Traps for volatile oil apparatus.

Coarsely comminute a sufficient quantity of the drug to yield from 1 to 3 mL of volatile oil. Small seeds, fruits, or broken leaves of herbs ordinarily do not need comminution. Very fine powders are to be avoided. If this is not possible, it may be necessary to mix them with purified sawdust or purified sand. Place a suitable quantity of the drug, accurately weighed, in the flask, and fill it one-half with water. Attach the condenser and the proper separator. Boil the contents of the flask, using a suitable amount of heat to maintain gentle boiling for 2 h, or until the volatile oil has been completely separated from the drug and no longer collects in the graduated tube of the separator.

If a proper quantity of the volatile oil has been obtained in the graduated tube of the separator, it can be read to tenths of 1 mL, and the volume of volatile oil from each 100 g of drug can be calculated from the weight of the drug taken. The graduations on the separator "for oils heavier than water" are so placed that oil remains below the aqueous condensate that automatically flows back into the flask.

Water Content

For unground or unpowdered drugs, prepare about 10 g of the *Laboratory Sample* by cutting, granulating, or shredding, so that the parts are about 3 mm in thickness. Seeds or fruits smaller than 3 mm should be cracked. Avoid the use of high-speed mills in preparing the sample, and exercise care that no appreciable amount of moisture is lost during the preparation and that the portion taken is representative of the *Laboratory Sample*. Determine the water content as directed for *Procedure for Articles of Botanical Origin* in *Water Determination* ⟨921⟩*, Method III (Gravimetric)*.

TEST FOR AFLATOXINS

[CAUTION—*Aflatoxins are highly dangerous, and extreme care should be exercised in handling aflatoxin materials.*]

Where the individual monograph calls for compliance with the limits for aflatoxins, the limits are NMT 5 ppb for aflatoxin B_1 (AFB_1) and NMT 20 ppb for the sum of aflatoxins B_1 (AFB_1), B_2 (AFB_2), G_1 (AFG_1), and G_2 (AFG_2). The extent of testing may be determined using a risk-based approach that considers the likelihood of contamination. The presence of unexpected contamination with aflatoxins is to be considered in determining compliance. The following analytical procedures are provided for determining compliance. Unless otherwise specified in the individual monograph, use *Method I*. If system suitability fails, use either *Method II* or *Method III*.

Method I

This TLC test is provided to detect the possible presence of AFB_1, AFB_2, AFG_1, and AFG_2 in any material of plant origin.

ZINC ACETATE–ALUMINUM CHLORIDE REAGENT

Dissolve 20 g of zinc acetate and 5 g of aluminum chloride in sufficient water to make 100 mL.

SODIUM CHLORIDE SOLUTION

Dissolve 5 g of sodium chloride in 50 mL of water.

TEST SOLUTION 1

Grind about 200 g of plant material to a fine powder. Transfer about 50 g of the powdered material, accurately weighed, to a glass-stoppered flask. Add 200 mL of a mixture of methanol and water (17:3). Shake vigorously by mechanical means for NLT 30 min, and filter. [NOTE—If the solution has interfering plant pigments, proceed as directed for *Test Solution 2*.] Discard the first 50 mL of the filtrate, and collect the next 40-mL portion. Transfer the filtrate to a separatory funnel. Add 40 mL of *Sodium Chloride Solution* and 25 mL of solvent hexane, and shake for 1 min. Allow the layers to separate, and transfer the lower aqueous layer to a second separatory funnel. Extract the aqueous layer in the separatory funnel twice, each time with 25 mL of methylene chloride, by shaking for 1 min. Allow the layers to separate each time, separate the lower organic layer, and collect the combined organic layers in a 125-mL conical flask. Evaporate the organic solvent on a water bath. Transfer the remaining extract to an appropriate sample tube, and evaporate to dryness on a water bath. Cool the residue. If interferences exist in the residue, proceed as directed for *Cleanup procedure* in *Test Solution 2*. Otherwise, dissolve the residue obtained above in 0.2 mL of a mixture of chloroform and acetonitrile (9.8: 0.2), and shake by mechanical means if necessary.

TEST SOLUTION 2

Collect 100 mL of the filtrate from the start of the flow, and transfer to a 250-mL beaker. Add 20 mL of *Zinc Acetate–Aluminum Chloride Reagent* and 80 mL of water. Stir, and allow to stand for 5 min. Add 5 g of a suitable filtering aid, such as diatomaceous earth, mix, and filter. Discard the first 50 mL of the filtrate, and collect the next 80-mL portion. Proceed as directed for *Test Solution 1*, beginning with "Transfer the filtrate to a separatory funnel".

Cleanup procedure: Place a medium-porosity sintered-glass disk or a glass wool plug at the bottom of a 10-mm × 300-mm chromatographic tube. Prepare a slurry of 2 g of silica gel with a mixture of ethyl ether and solvent hexane (3:1), pour the slurry into the column, and wash with 5 mL of the same solvent mixture. Allow the absorbent to settle, and add to the top of the column a layer of 1.5 g of anhydrous sodium sulfate. Dissolve the residue obtained above in 3 mL of methylene chloride, and transfer it to the column. Rinse the flask twice with 1-mL portions of methylene chloride, transfer the rinses to the column, and elute at a rate NMT 1 mL/min. Add successively to the column 3 mL of solvent hexane, 3 mL of ethyl ether, and 3 mL of methylene chloride; elute at a rate NMT 3 mL/min; and discard the eluates. Add to the column 6 mL of a mixture of methylene chloride and acetone (9:1), and elute at a rate NMT 1 mL/min, preferably without the aid of vacuum. Collect this eluate in a small vial, add a boiling chip if necessary, and evaporate to dryness on a water bath. Dissolve the residue in 0.2 mL of a mixture of chloroform and acetonitrile (9.8: 0.2), and shake by mechanical means if necessary.

TEST SOLUTION 3

If interferences still exist in the residue, proceed as directed for *Cleanup procedure with IAC* in *Test Solution* in *Method II*.

AFLATOXIN SOLUTION

[CAUTION—*Aflatoxins are highly toxic. Handle with care.*]

Dilute the USP Aflatoxins RS 1:5 with acetonitrile to obtain a solution having a concentration of 0.4 μg/mL each of AFB_1 and AFG_1, and 0.1 μg/mL each of AFB_2 and AFG_2.

PROCEDURE

Separately apply 2.5, 5, 7.5, and 10 μL of the *Aflatoxin Solution* and three 10-μL applications of either *Test Solution 1*, *Test Solution 2*, or *Test Solution 3* to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Superimpose 5 μL of the *Aflatoxin Solution* on one of the three 10-μL applications of the *Test Solution*. Allow the spots to dry, and develop the chromatogram in an unsaturated chamber containing a solvent system consisting of a mixture of chloroform, acetone, and isopropyl alcohol (85:10:5) until the solvent front has moved NLT 15 cm from the origin. Remove the plate from the developing chamber, mark the solvent front, and allow the plate to air-dry. Locate the spots on the plate by examination under UV light at 365 nm.

SYSTEM SUITABILITY

The four applications of the *Aflatoxin Solution* appear as four clearly separated blue fluorescent spots. Observe any spot obtained from the *Test Solution* that coincides in hue and position with those of the *Aflatoxin Solution*. Any spot obtained from the *Test Solution* with the superimposed *Aflatoxin Solution* is not less intense than that of the corresponding *Aflatoxin Solution*.

ACCEPTANCE CRITERIA

No spot from any of the other applications of the *Test Solution* corresponds to any of the spots obtained from the applications of the *Aflatoxin Solution*. If any spot of aflatoxins is obtained in the *Test Solution*, match the position of each fluorescent spot of the *Test Solution* with those of the *Aflatoxin Solution* to identify the type of aflatoxin present. The intensity of the aflatoxin spot, if present in the *Test Solution*, when compared with that of the corresponding aflatoxin in the *Aflatoxin Solution* will give an approximate concentration of aflatoxin in the *Test Solution*. Where the individual monograph calls for compliance with the limits for aflatoxins, the limits are NMT 5 ppb for AFB_1 and NMT 20 ppb for the sum of AFB_1, AFB_2, AFG_1, and AFG_2, except when otherwise indicated.

Method II

SODIUM CHLORIDE SOLUTION

See *Method I*.

PHOSPHATE BUFFERED SALINE SOLUTION

Prepare 10 mM phosphate buffer solution containing 0.138 M sodium chloride and 0.0027 M potassium chloride in water, and adjust with 2 M sodium hydroxide to a pH of 7.4.[1]

IMMUNOAFFINITY COLUMN (IAC)

Before conditioning, adjust the IAC to room temperature. For conditioning, apply 10 mL of *Phosphate Buffered Saline Solution* onto the column and let it flow through the column by gravity force at a rate of 2–3 mL/min. Leave 0.5 mL of the *Phosphate Buffered Saline Solution* on top of the column until the *Test Solution* is applied.

TEST SOLUTION

Sample extraction: Transfer about 5 g of a representative powdered sample, accurately weighed, to a glass-stoppered flask. Add 20 mL of a mixture of methanol and water (17:3). Shake vigorously by mechanical means for NLT 30 min, and filter. Discard the first 5 mL of the filtrate, and collect the next 4-mL portion. Transfer the filtrate to a separatory funnel. Add 4 mL of *Sodium Chloride Solution* and 2.5 mL of hexane, and shake for 1 min. Allow the layers to separate, and transfer the lower aqueous layer to a second separatory funnel. Extract the aqueous layer in the separatory funnel twice, each time with 2.5 mL of methylene chloride, by shaking for 1 min. Allow the layers to separate each time, separate the lower organic layer, and collect the combined organic layers in a 50-mL conical flask. Evaporate the organic solvent on a water bath. Transfer the remaining extract to an appropriate sample tube, and evaporate to dryness on a water bath. Cool the residue. If interferences exist in the residue, proceed as directed for *Cleanup procedure with IAC*. Otherwise, dissolve the residue obtained above in 200 µL of acetonitrile, and shake by mechanical means if necessary.

Cleanup procedure with IAC: The residue is dissolved in 5 mL of a mixture of methanol and water (60:40) and then diluted with 5 mL of water. This extract is applied onto a conditioned IAC. The IAC is rinsed twice with 10 mL of *Phosphate Buffered Saline Solution*, and the elution is performed slowly with 2 mL of methanol. Evaporate the eluate with nitrogen, and dissolve the residue in 200 µL of acetonitrile.

AFLATOXIN SOLUTION

[CAUTION—*Aflatoxins are highly toxic. Handle with care.*]

Dilute quantitatively the USP Aflatoxins RS 1:50 with acetonitrile to obtain a solution containing 0.04 µg/mL each of AFB_1 and AFG_1, and 0.01 µg/mL each of AFB_2 and AFG_2.

ANALYSIS

Separately apply 5, 7.5, and 10 µL of *Aflatoxin Solution* and three 10-µL applications of the *Test Solution* to a suitable HPTLC plate (see *Chromatography* ⟨621⟩) coated with a 200-µm layer of chromatographic silica gel mixture. Superimpose 5 µL of *Aflatoxin Solution* on one of the three 10-µL applications of the *Test Solution.* Allow the spots to dry, and develop the chromatogram in a saturated chamber containing a solvent system consisting of a mixture of chloroform, acetone, and water (140: 20: 0.3) until the solvent front has moved NLT 72 mm from the origin (80 mm from the lower edge of the plate). Remove the plate from the developing chamber, mark the solvent front, and allow the plate to air-dry for 5 min. Locate the spots

[1] A suitable powder mixture is available from Sigma as PBS P-3813.

on the plate by scanning fluorescence density (>400 nm) under UV light at 366 nm. Match the position of each fluorescent spot of the *Test Solution* with those of *Aflatoxin Solution* to identify the type of aflatoxin present. The concentration of aflatoxins in the *Test Solution* can be calculated from the calibration curve obtained from the scan data with *Aflatoxin Solution*.

SYSTEM SUITABILITY

The four applications of *Aflatoxin Solution* appear as four clearly separated blue fluorescent spots. Observe any spot obtained from the *Test Solution* that coincides in hue and position with those of *Aflatoxin Solution*. Any spot obtained from the *Test Solution* with the superimposed *Aflatoxin Solution* is not less intense than that of the corresponding *Aflatoxin Solution*. The mean recovery of spiked AFB_1 and AFG_1 is NLT 70%.

ACCEPTANCE CRITERIA

Where the individual monograph calls for compliance with the limits for aflatoxins, the limits are NMT 5 ppb for AFB_1 and NMT 20 ppb for the sum of AFB_1, AFB_2, AFG_1, and AFG_2, except when otherwise indicated.

Method III

This test method is provided as an example for the detection of the possible presence of AFB_1 and total aflatoxins (AF: sum of AFB_1, AFB_2, AFG_1, and AFG_2). It has been shown to be suitable for powdered ginseng and ginger. Its suitability to other articles of botanical origin must be demonstrated.

0.1 M PHOSPHATE BUFFER SOLUTION

Dissolve 8.69 g of anhydrous disodium phosphate and 4.66 g of anhydrous monosodium phosphate or 5.36 g of monosodium phosphate monohydrate in 800 mL water, adjust with 2 M sodium hydroxide to a pH of 7.4, add 10 mL of polysorbate 20, and dilute to 1 L.

PHOSPHATE BUFFERED SALINE SOLUTION

Prepare as directed in *Method II*.

WORKING AFLATOXIN STANDARD SOLUTIONS

Prepare six solutions in separate 10-mL volumetric flasks according to *Table 3*. Dilute with methanol and water (1:1, v/v) to volume. Store in a refrigerator, and equilibrate to room temperature before use. Prepare the solutions daily.

Table 3. Preparation of Working Aflatoxin Standard Solutions

Working Aflatoxin Standard Solutions	USP Aflatoxins RS (μL)	Final Aflatoxin Concentration of Working Aflatoxin Standard Solution (ng/mL)				
		AFB_1	AFB_2	AFG_1	AFG_2	ΣAF
1	0	0	0	0	0	0
2	12.5	0.25	0.0625	0.25	0.0625	0.625
3	25	0.5	0.125	0.5	0.125	1.25
4	50	1	0.25	1	0.25	2.5
5	100	2	0.5	2	0.5	5
6	200	4	1	4	1	10

IMMUNOAFFINITY COLUMN (IAC)[2]

Use an immunoaffinity column that contains monoclonal antibodies cross reactive toward AFB_1, AFB_2, AFG_1, and AFG_2. The immunoaffinity columns have a minimum capacity of NLT 100 ng of total aflatoxin and give a recovery of NLT 80% for AFB_1, AFB_2, AFG_1, and AFG_2 when 5 ng of each AFB_1, AFB_2, AFG_1, and AFG_2 is applied in 10 mL of 10% methanol in *Phosphate Buffered Saline Solution* (v/v).

[2] AflaOchraTest column (G1017; Vicam, Watertown, MA, USA) or equivalent. Aflatoxin/OTA immunoaffinity columns are suitable.

TEST SOLUTION

Extraction: Weigh 5 g of a representative test sample in a 50-mL centrifuge tube. Add 1 g of sodium chloride and 25 mL of a mixture of methanol and 0.5% sodium bicarbonate (700:300). Mix on a vortex mixer until sample particles and extract solvent are well mixed. Shake at 400 rpm for 10 min. Centrifuge for 10 min at 7000 rpm (*g* value = 5323 mm/s^2) or at a speed that can result in a firm pellet of residues. Immediately pipette 7 mL into a 50-mL centrifuge tube, add 28 mL of *0.1 M Phosphate Buffer Solution*, mix, and filter through glass microfiber paper. Collect 25 mL of filtrate (equivalent to 1 g of test sample) into a 25-mL graduated cylinder, and proceed immediately with IAC chromatography.

IAC cleanup: [NOTE—For IAC cleanup, columns must be kept at room temperature for at least 15 min before use.] Remove the top cap from the column, and connect it with the reservoir. Remove the end cap from the column, and attach it to the column manifold (the fit must be tight). Let the liquid in the column pass through until the liquid is about 2–3 mm above the column bed. Pass 25 mL of filtrate into the reservoir. Let the filtrate flow through the column by gravity force. Let the column run dry. To start the flow again easily, remove the column from the manifold, add about 2 mL of *Phosphate Buffered Saline Solution* into the column, reattach the column to the reservoir, and wash the column with an additional 3 mL of *Phosphate Buffered Saline Solution* and then with 5 mL of water (the 5 mL of *Phosphate Buffered Saline Solution* can be added directly to the column reservoir if other techniques are used to dislodge the air bubble at the end of the column and to start flow again easily). Let the column run dry, then force 3 mL of air through the column with a syringe. Elute with 1 mL of methanol, and collect the analytes in a 3-mL volumetric flask, letting the eluate drip freely. Let the column run dry. Let stand for 1 min, then elute with an additional 1 mL of methanol, and collect in the same volumetric flask. Let the column run dry, and force 10 mL of air through the column. Dilute the eluate with water to volume. Use this as the *Test Solution*, and perform the analysis of aflatoxins immediately.

SYSTEM SUITABILITY SOLUTION

Prepare a spiked sample by adding 5 mL of *Working Aflatoxin Standard Solution 5* to a 5-g sample and repeating the procedure for the *Test Solution*, using 20 mL instead of 25 mL of the mixture of methanol and 0.5% sodium bicarbonate (700:300).

CHROMATOGRAPHIC SYSTEM

Flow rate: 0.8 mL/min

Detection: Fluorescence detector set at excitation wavelength (Ex) 362 nm and emission wavelength (Em) 440 nm

Column: 4.6-mm × 15-cm containing 3-μm packing L1

Mobile phase: Isocratic

For post-column derivatization with PHRED cell[3]—Water, methanol, and acetonitrile (600:250:150)

For post-column derivatization with Kobra cell[4]—A solution prepared by mixing 1 L of a mixture of water, methanol, and acetonitrile (600:250:150); 350 μL of 4 M nitric acid; and 120 mg of potassium bromide

Post-column derivatization (PCD) systems

PHRED cell—Post-column photochemical derivatization cell

Kobra cell—Electrochemical cell, post-column bromination derivatization cell

ANALYSIS

Post-column derivatization for aflatoxins: Use a UV or Kobra cell. Inject 50 μL of reagent blank *(Working Aflatoxin Standard Solution 1)*, *Working Aflatoxin Standard Solutions 2–6*, or the *Test Solution* into the LC column. Identify the aflatoxin peaks in the *Test Solution* by comparing the retention times with those of the working standards. The aflatoxins elute in the order AFG_2, AFG_1, AFB_2, and AFB_1. After passing through the PHRED or Kobra cell, the AFG_1 and AFB_1 have been derivatized to form AFG_{2a} (derivative of AFG_1) and AFB_{2a} (derivative of AFB_1). [NOTE—The chemical structures of the derivatives resulting from electrochemical bromination and photolysis are not the same. The structures of AFB_1 and AFG_1 photolysis products have not been established.] The retention times of AFG_2, AFG_{2a}, AFB_2, and AFB_{2a} are between about 14 and 27 min using the PHRED cell; retention times are shorter using the Kobra cell. The peaks should be baseline resolved. Construct standard curves for each aflatoxin. Determine the concentration of each aflatoxin in the *Test Solution* from the calibration curve.

Aflatoxins calibration curves: Calibration curves are prepared for each of the aflatoxins using the *Working Aflatoxin Standard Solutions* containing the four aflatoxins described. These solutions cover the range of 0.25–4 ng/mL for AFB_1 and AFG_1, and the range of 0.0625–1 ng/mL for AFB_2 and AFG_2. Make the calibration curves before analysis according to *Table 3*, and check the plot for linearity. If the test portion area response is outside (higher) the calibration range, then the *Test Solution* should be diluted with a mixture of methanol and water (1:1, v/v) and reinjected into the LC column.

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[3] PHRED™ Photochemical Reactor (AURA Industries, New York, NY, USA) or equivalent. Avoid looking at the UV lamp.

[4] Kobra Cell™ (R-Biopharm Inc., Marshall, MI, USA) or equivalent. Set at 100 mA. Do not turn on the current until the LC pump is operating to avoid overheating the cell membrane.

Quantitation of aflatoxins: Quantitation of aflatoxins is performed by measuring peak areas at each aflatoxin retention time and comparing them with the corresponding calibration curve.

SYSTEM SUITABILITY

The mean recovery of spiked AFB_1 (2 μg/kg) and the total of aflatoxins (5 μg/kg) is NLT 68% and 70%, respectively. The relative standard deviation (RSD) is NMT 10% for AFB_1 and for the total of aflatoxins.

CALCULATIONS

Plot the peak area (response, *y*-axis) of each of the toxin standards against the concentration (ng/mL, *x*-axis) and determine the slope (*S*) and *y*-intercept (*a*). Calculate the level of toxin in the sample by the following formula:

$$\text{Toxin } (\mu g/kg) = \{[(R - a)/S] \times V/W\} \times F$$

where *R* is the *Test Solution* peak area; *V* is the final volume of the injected *Test Solution* (mL); and *F* is the dilution factor. $F = 1$ when $V = 3$ mL. *W* is 1 g of test sample passed through the immunoaffinity column. The total of aflatoxins is the sum of AFG_2, AFG_1, AFB_2, and AFB_1.

ACCEPTANCE CRITERIA

Where the individual monograph calls for compliance with the limits for aflatoxins, the limits are NMT 5 ppb for AFB_1 and NMT 20 ppb for the sum of AFB_1, AFB_2, AFG_1, and AFG_2, except when otherwise indicated.

PESTICIDE RESIDUE ANALYSIS

Definition

Where used in this Pharmacopeia, the designation "pesticide" applies to any substance or mixture of substances intended to prevent, destroy, or control any pest, unwanted species of plants, fungus, or animals causing harm during or otherwise interfering with the production, processing, storage, transport, or marketing of pure articles. The designation includes substances intended for use as growth regulators, defoliants, or desiccants, and any substance applied to crops before or after harvest to protect the product from deterioration during storage and transport.

Limits

Within the United States, many botanicals are treated as dietary supplements and are subject to the statutory provisions that govern foods but not drugs in the Federal Food, Drug, and Cosmetic Act. Limits for pesticides in foods are determined by the Environmental Protection Agency (EPA) as indicated in the Code of Federal Regulations (40 CFR Part 180) or the Federal Register (FR). In addition, the Food and Drug Administration (FDA) establishes action levels for unavoidable pesticide residues (21 CFR Part 109 and 21 CFR Part 509). For pesticide chemicals without EPA-established tolerance levels or FDA action levels, the residues should be below the detection limit of the specified method. Results less than the EPA detection limits are considered zero values. The limits contained herein, therefore, are not applicable in the United States when articles of botanical origin are labeled for food purposes. The limits, however, may be applicable in other countries. Unless otherwise indicated in the monograph, the article to be examined complies with the limits given in *Table 4*. The limits for suspected pesticides that are not listed in *Table 4* must comply with the regulations of the EPA. For instances in which a pesticide is not listed in *Table 4* or in EPA regulations, calculate the limit by the formula:

$$\text{Limit (mg/kg)} = AM/100B$$

where *A* is the acceptable daily intake (ADI), as published by FAO-WHO, in mg/kg of body weight; *M* is body weight, in kg (60 kg); and *B* is the daily dose of the article, in kg.

If the article is intended for the preparation of extracts, tinctures, or other pharmaceutical forms of which the preparation method modifies the content of pesticides in the finished product, calculate the limits by the formula:

$$\text{Limit (mg/kg)} = AME/100B$$

where *E* is the extraction factor of pesticide in the preparation method, determined experimentally as the ratio between the original pesticide content in the plant material and the final pesticide content in the preparation; *B* is the daily dose of the preparation in kg; and *A*and *M* are as defined above.

A total or partial exemption from the test may be granted when the complete history (nature and quantity of the pesticides used, date of each treatment during cultivation and after harvest) of the treatment of the batch is known and can be checked precisely according to good agricultural and collection practice (GACP).

Table 4

Substance	Limit (mg/kg)
Acephate	0.1
Alachlor	0.05
Aldrin and dieldrin (sum of)	0.05
Azinphos-ethyl	0.1
Azinphos-methyl	1
Bromide, inorganic (calculated as bromide ion)	125
Bromophos-ethyl	0.05
Bromophos-methyl	0.05
Bromopropylate	3
Chlordane (sum of *cis*-, *trans*-, and oxychlordane)	0.05
Chlorfenvinphos	0.5
Chlorpyriphos-ethyl	0.2
Chlorpyriphos-methyl	0.1
Chlorthal-dimethyl	0.01
Cyfluthrin (sum of)	0.1
λ-Cyhalothrin	1
Cypermethrin and isomers (sum of)	1
DDT (sum of *o,p*′-DDE, *p,p*′-DDE, *o,p*′-DDT, *p,p*′-DDT, *o,p*′-TDE, and *p,p*′-TDE)	1
Deltamethrin	0.5
Diazinon	0.5
Dichlofluanid	0.1
Dichlorvos	1
Dicofol	0.5
Dimethoate and omethoate (sum of)	0.1
Dithiocarbamates (expressed as CS_2)	2
Endosulfan (sum of isomers and endosulfan sulphate)	3
Endrin	0.05
Ethion	2
Etrimphos	0.05
Fenchlorophos (sum of fenchlorophos and fenchlorophos-oxon)	0.1
Fenitrothion	0.5
Fenpropathrin	0.03
Fensulfothion (sum of fensulfothion, fensulfothion-oxon, fensulfothion-oxon sulfone, and fensulfothion sulfone)	0.05
Fenthion (sum of fenthion, fenthion-oxon, fenthion-oxon sulfone, fenthion-oxon sulfoxide, fenthion sulfone, and fenthion-sulfoxide)	0.05
Fenvalerate	1.5
Flucythrinate	0.05
τ-Fluvalinate	0.05
Fonophos	0.05
Heptachlor (sum of heptachlor, *cis*-heptachlorepoxide, and *trans*-heptachlorepoxide)	0.05
Hexachlorbenzene	0.1
Hexachlorocyclohexane (sum of isomers α-, β -, δ-, and ε-)	0.3
Lindan (γ-hexachlorocyclohexane)	0.6
Malathion and malaoxon (sum of)	1
Mecarbam	0.05
Methacriphos	0.05
Methamidophos	0.05
Methidathion	0.2
Methoxychlor	0.05
Mirex	0.01
Monocrotophos	0.1

Table 4 *(Continued)*

Substance	Limit (mg/kg)
Parathion-ethyl and paraoxon-ethyl (sum of)	0.5
Parathion-methyl and paraoxon-methyl (sum of)	0.2
Pendimethalin	0.1
Pentachloranisole	0.01
Permethrin and isomers (sum of)	1
Phosalone	0.1
Phosmet	0.05
Piperonyl butoxide	3
Pirimiphos-ethyl	0.05
Pirimiphos-methyl (sum of pirimiphos-methyl and *N*-desethyl-pirimiphos-methyl)	4
Procymidone	0.1
Profenophos	0.1
Prothiophos	0.05
Pyrethrum (sum of cinerin I, cinerin II, jasmolin I, jasmolin II, pyrethrin I, and pyrethrin II)	3
Quinalphos	0.05
Quintozene (sum of quintozene, pentachloraniline, and methyl pentachlorphenyl sulfide)	1
S-421	0.02
Tecnazene	0.05
Tetradifon	0.3
Vinclozolin	0.4

Qualitative and quantitative analysis of pesticide residues: Use analytical procedures validated e.g. in accordance with the latest version of the EU guideline on analytical quality control and validation procedures for pesticide residue analysis [NOTE—Current version Document No. SANCO/12571/2013, http://ec.europa.eu/food/plant/resources/qualcontrol_en.pdf] or the EPA method validation principles (OPPTS 860.1340) that satisfy the following criteria. The method, especially with respect to its purification steps, is suitable for the combination of pesticide residue and substance under test, and is not susceptible to interference from co-extractives; the limit of quantification for each pesticide matrix combination to be analyzed is NMT the corresponding tolerance limit: the method is shown to recover between 70% and 120% of each pesticide with a repeatability NLT 20% RSD [NOTE—lower recoveries may be acceptable in certain cases as discussed in SANCO/12571/2013]; and the concentrations of test and reference solutions and the setting of the apparatus are such that a linear response is obtained from the analytical detector.

LIMITS OF ELEMENTAL IMPURITIES

The levels of elemental impurities should be restricted as shown in *Table 5* unless otherwise stated in the individual monograph. Specific monographs may provide different limits for articles that are typically used in large quantities.

Table 5. Limits of Elemental Impurities

Element	Limits (μg/g)
Arsenic (inorganic)[a]	2
Cadmium	0.5
Lead	5
Mercury (total)	1
Methylmercury (as Hg)[b]	0.2

[a] Arsenic may be measured using a nonspeciation procedure under the assumption that all arsenic contained in the supplement is in the inorganic form. Where the limit is exceeded using a nonspeciation procedure, compliance with the limit for inorganic arsenic shall be demonstrated on the basis of a speciation procedure.

[b] Methylmercury determination is not necessary when the content for total mercury is less than the limit for methylmercury.

Articles are tested according to the procedures set forth in *Elemental Impurities—Procedures* ⟨233⟩. Where speciation is required, the procedures given in *Elemental Contaminants in Dietary Supplements* ⟨2232⟩ are used for testing.