

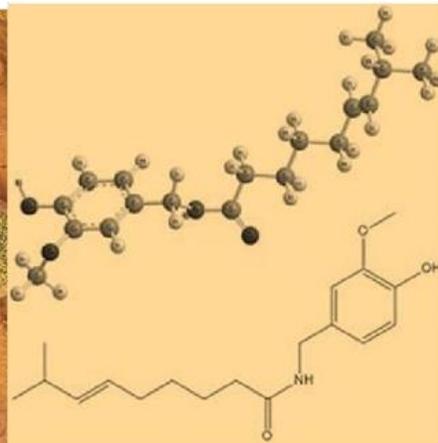
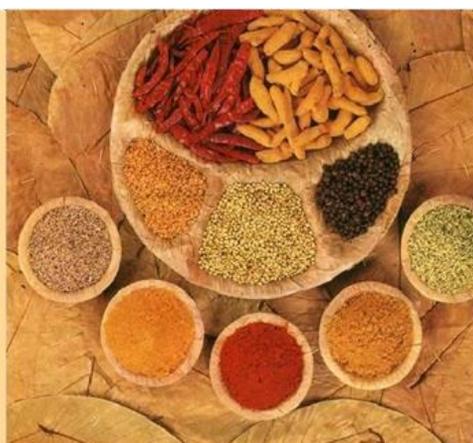
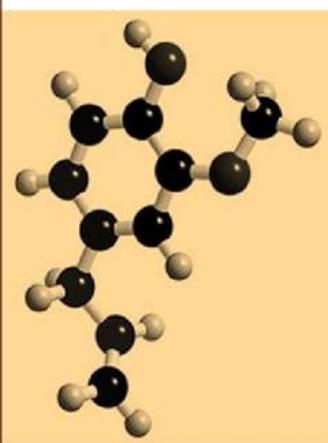


MANUAL OF METHODS

OF

ANALYSIS OF FOODS

SPICES AND CONDIMENTS



FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA
MINISTRY OF HEALTH AND FAMILY WELFARE
GOVERNMENT OF INDIA
NEW DELHI
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MANUAL FOR ANALYSIS OF SPICES AND CONDIMENTS

TABLE OF CONTENTS

S.NO.	TITLE/METHOD	PAGE NO.
1.0	Preparation of Sample	2
2.0	Determination of Extraneous Matter and other Refractions	2
3.0	Determination of Moisture	2
4.0	Determination of Total Ash	12
5.0	Determination of Ash Insoluble in dilute HCl	14
6.0	Determination of Cold Water Extract	15
7.0	Determination of Alcohol Soluble Extract	15
8.0	Determination of Calcium Oxide	17
9.0	Determination of Non Volatile Ether Extract	18
10.0	Determination of Volatile Oil	19
11.0	Determination of Crude Fibre	22
12.0	Determination of Allyl isothiocyanate in Mustard	24
13.0	Determination of p-hydroxybenzyl isothiocyanate in <i>S. alba</i> (White Mustard)	25
14.0	Black Pepper	26
15.0	Saffron	30
16.0	Turmeric Whole	34
17.0	Asafoetida	37
18.0	Microscopic Examination of Spices	39
19.0	Detection of Argemone seeds in Mustard	40
20.0	Detection of Mineral Oil in Black Pepper	40
21.0	Detection of Papaya seeds in Black Pepper	41
22.0	Detection of Turmeric in Chillies and Corriander	42
23.0	Detection of Oil Soluble Colour	42
24.0	Determination of Light and Heavy Filth in Spices and Condiments	42
25.0	Method for capsaicin content in chilli powder	42
26.0	Method for measuring colour value in chillies	48
	References	50

Note: The test methods given in the manuals are validated/ standardized test methods. However, it would be the responsibility of the respective testing laboratory to confirm that the above methods are validated in its laboratory and gives proper result in their laboratory.

MANUAL FOR ANALYSIS OF SPICES AND CONDIMENTS

Spices and condiments are added to foods in small amounts but they make important contribution to the sensory qualities due to presence of volatile and fixed oils. Standards for Spices and Condiments are laid down under Food Safety and Standards (Food Products Standards and Food Additives) Regulations, 2011.

1.0 Preparation of sample

Grind laboratory sample as quickly as possible in a grinding mill to pass sieve with 1 mm diameter aperture. Avoid undue heating of apparatus during grinding. Mix carefully to avoid stratification (layering). Store in a dry stoppered container.

(Ref: - IS Specification No IS 1797 – 1985 Methods of Test for Spices and Condiments/ AOAC 17th edn 2000, Official Method 920.164 Preparation of Test sample)

2.0 Determination of Extraneous Matter and other Refractions in Whole spices

Thoroughly mix the sample and weigh 100 – 200 gm depending on the nature of the material (10 – 20 gm in case of small sized spices), note down the exact weight and spread in an enameled tray. Separate extraneous matter and other refractions by hand. Weigh each fraction and calculate percentage.

(Ref: - IS Specification No IS 1797 – 1985 Methods of Test for Spices and Condiments)

3.0 Determination of Moisture (Dean and Stark Toluene Distillation Method)

3.1 Principle

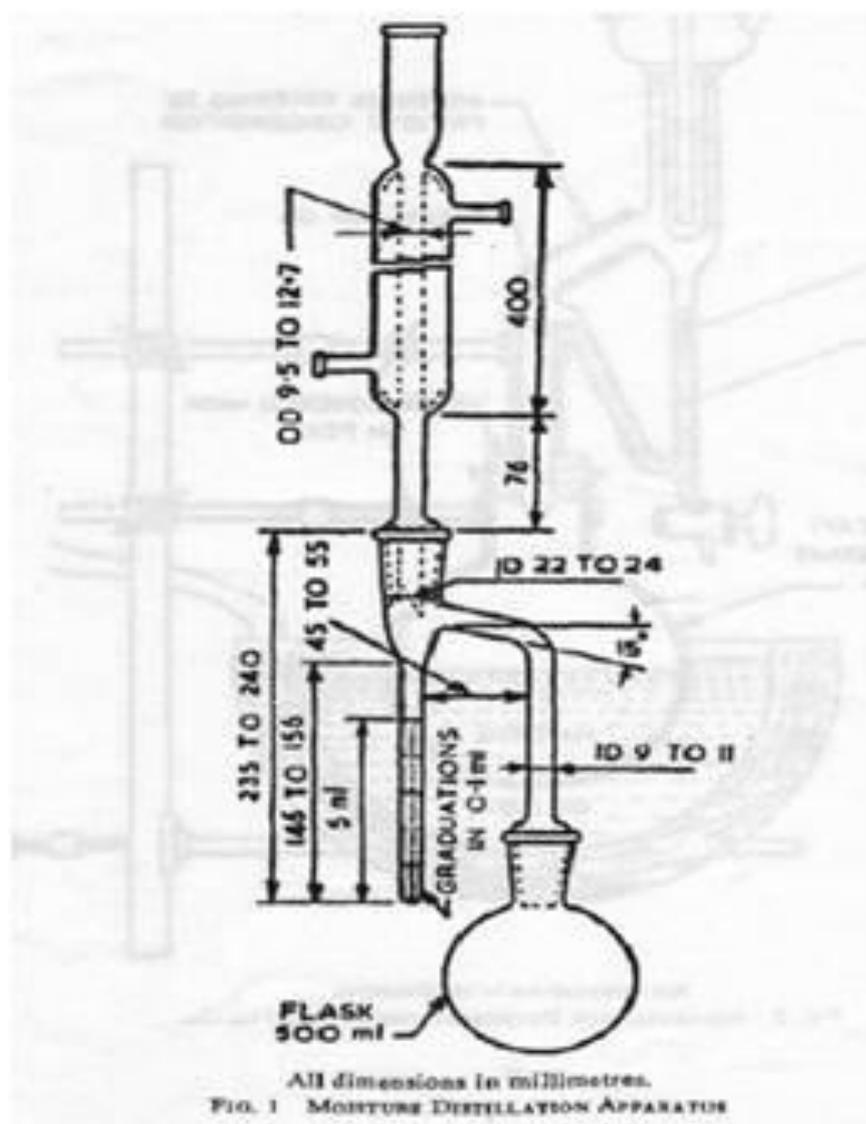
The amount of water is determined by distilling the material with an organic liquid not miscible with water and collecting the distillate in a graduated tube.

3.2 Reagents

Toluene - Saturate with small quantity of water and distill. Use the distillate for determination of moisture.

3.3 Apparatus

Moisture Distillation Apparatus - consists of a 500 mL short neck round bottomed flask heated using a heating mantle and provided with a reflux condenser discharging into a trap connected to the flask. The trap serves to collect and measure the condensed water and to return the condensed solvent to the flask (See figures below) and a copper wire long enough to extend throughout the condenser with one end twisted into a spiral. The diameter of the spiral should be such that it fits snugly within the graduated portion of the receiver and yet it can be moved up and down.



3.4 Procedure

Clean the entire apparatus with chromic acid cleaning solution to minimize adherence of water droplets to the sides of the condenser and the receiver. Rinse thoroughly with water and dry completely before use. Weigh to the nearest 0.01 gm about 20-40 gm of prepared sample (or enough to yield 2 -5 mLH₂O in the trap) and note down the exact weight. Transfer to the distilling flask with toluene. Add enough toluene to cover test portion completely (about 75 mL). Fill receiving tube with toluene pouring it through top of the condenser until it begins to overflow into the distillation flask. Insert loose cotton plug in top of the condenser to prevent condensation of atmospheric moisture in the tube. Add a few pumice stones to avoid bumping. Bring to boil and distill slowly about 2 drops

per second (about 100 drops per minute) until most of water distills over, then increase rate of distillation to 4 drops per second. Continue distilling until 2 consecutive readings 15 minutes apart show no change. Dislodge any water held up in the condensed with wire loop. Rinse condenser carefully with 5 mL toluene. Continue distillation 3-5 minutes, cool receiver to room temperature allowing it to stand in air or cooling it in water. Solvent and water layers should now be clear, if not, let stand until clearing occurs. Read volume of water estimating to nearest 0.1 mL and calculate percentage.

$$\text{Moisture Content (\% by weight)} = \frac{100 V}{M}$$

Where, V = Volume in ml of water collected

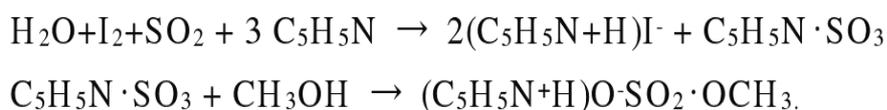
M = Weight of sample

(Ref: - AOAC 17th edn 2000 Official Method 986.21, Moisture in Spices/IS Specification No IS 1797 – 1985 Methods of Test for Spices and Condiments)

3.5 Determination of Moisture (Karl Fischer Method)

3.5.1 Principle

The Water Determination Test (Karl Fischer Method) is designed to determine water content in substances, utilizing the quantitative reaction of water with iodine and sulphur dioxide in the presence of a lower alcohol such as methanol and an organic base such as pyridine, as shown in the following formulae:



In the Volumetric Titration Method, iodine required for reaction with water is previously dissolved in water determination TS, and water content is determined by measuring the amount of iodine consumed as a result of reaction with water in a

sample.

3.5.2 Apparatus

- 1) An automatic burette,
- 2) A back titration flask,
- 3) A stirrer,
- 4) Equipment for amperometric titration at constant voltage or potentiometric titration at constant current.

Because water determination TS is extremely hygroscopic, the titration apparatus should be protected from atmospheric moisture. Silica gel or calcium chloride for water determination is usually used for moisture protection.

3.5.3 Direct titration method

Unless otherwise specified, proceed as directed below.

Take 25mL of methanol for water determination in a dried titration flask, and titrate with water determination TS to the end point. Unless otherwise specified, weigh accurately a quantity of the sample containing 10 to 50 mg of water, transfer it quickly into the titration flask, and dissolve by stirring. Titrate the solution with water determination TS to the end point under vigorous stirring.

When the sample is insoluble in the solvent, powder the sample quickly, weigh a suitable amount of the sample accurately, and transfer it quickly into the titration vessel, stir the mixture for 30 minutes while protecting it from moisture. Perform a titration under vigorous stirring.

When the sample interferes with the Karl Fisher reaction, water in the sample can be removed by heating and under a stream of nitrogen gas and introduced into the titration vessel by using a water-evaporation device.

$$\text{Water (H}_2\text{O)} = \frac{[\{\text{Volume (mL) of TS for water Determination consumed}\} \times f \text{ (mg/mL)}]}{\text{Weight of the sample (mg)}} \times 100(\%)$$

3.5.4 Back titration

Unless otherwise specified, proceed as directed below.

Take 20mL water determination in the dried titration vessel, and titrate with water determination TS. Weigh accurately a suitable quantity of the sample containing 10-50 mg of water, transfer the sample quickly into the titration vessel, add an excessive and definite volume of water determination TS, stir for 30 min, protecting from atmospheric moisture, and then titrate the solution with Water Methanol Standard Solution under vigorous stirring.

$$\text{Water (H}_2\text{O)} = \frac{[\text{volume of water determination TS added (mL)} \times f_1] - \frac{[\{\text{Volume of water- Methanol Standards solution consumed (mL)}\} \times f_2]}{\text{Weight of the sample (mg)}} \times 100(\%)$$

Where, f1= the number of mg of Water (H₂O) corresponding to 1mL of water determination TS,

f2= the number of mg of Water (H₂O) in 1mL of Water – Methanol standard solution.

(Ref:[http://www.ffcr.or.jp/zaidan/ffcrhome.nsf/7bd44c20b0dc562649256502001b65e/146fd852cd5e269049256f32001a133e/\\$file/b43.pdf](http://www.ffcr.or.jp/zaidan/ffcrhome.nsf/7bd44c20b0dc562649256502001b65e/146fd852cd5e269049256f32001a133e/$file/b43.pdf)).

3.6. Karl Fischer Titration Methods

Applicable to the determination of 1-15% water in dry, ground animal feed. It is not applicable for mineral mix feed. An estimated LOD is 0.25% and an estimated LOQ is 0.8% for 500 mg test portions (estimated LOD of 0.12% and estimated LOQ of 0.4% for 1g test portions).

3.6.1 Principle:

Water is extracted from the animal feed or forage material into methanol-formamide (1 + 1) directly in the Karl Fischer titration vessel by high-speed homogenization. The formamide content must not exceed 50% to maintain reaction conditions. A subsequent titration of the water is performed at 50°C with one component Karl Fischer reagent based on imidazole.

Extraction into Methanol Formamide (1 + 1) Using High-Speed homogenization.

3.6.2 Apparatus:

(a) Karl Fischer titration homogenization system—Metrohm Model 841 KFS Titrino, includes titration vessel LP with water jacket (50-150 mL), “snap-in” buret unit (10 mL), 703 titration stand with pump, cable with timer (Titrino-Polytron), or equivalent.

(b) Circulating water bath.—maintaining $50 \pm 1^\circ\text{C}$.

(c) Homogenizer—Brinkmann Polytron homogenizer with PTA 20 TSM foam-reducing generator with saw teeth and knives.

Assembled with generator extending into the titration vessel, and adjusted to be ca 1 in. (2.5 cm) from the bottom of the titration vessel. Set to provide homogenization speed of 24000 rpm.

(d) Glass weighing spoon.—With opening for dispensing test portion into the titration flask through the septum stopper (Brinkmann), or equivalent.

(e) Magnetic stirrer.

(f) Oven.— $103^\circ\text{C} \pm 2^\circ\text{C}$

3.6.3 Reagents:

(a) Karl Fischer reagent.—One component, based on imidazole, with titer ca 5 mg H₂O/mL reagent

(b) Methanol.—Anhydrous, for moisture determinations, water content not to exceed 0.05% or equivalent

- (c) Formamide
- (d) Solvent.—Methanol-formamide (1 + 1). Mix fresh each day
- (e) Sodium tartrate dehydrate —Primary standard (water content, 15.66 ± 0.05 %)
- (f) Water standard —Water standard with certificate (water content, 10 mg/g)

3.6.4 Preparation of Test sample

Grind test samples to pass a ≤ 1 mm opening sieve. Mix well and transfer to a tightly sealed container.

3.6.5 Drying or Conditioning the Cell

Dispense sufficient methanol-formamide solvent into the titration vessel to immerse the homogenizer tip about 8 mm. Close the cell to minimize the addition of atmospheric moisture. Heat to $50 \pm 1^\circ\text{C}$. Dry the cell (including solvent, cell walls, electrode walls, generator, and cell atmosphere) by performing a complete run without test portion (“blank” run), including homogenization and titration as follows:

Start the instrument and condition the solvent by titrating to remove moisture. As soon as instrument drift has stabilized, start the method. Homogenize at 24,000 rpm for 60 sec. Enter “1” for test portion weight, and titrate the solvent again to remove any traces of water remaining. The end point is reached when no change in potential is observed for 10 sec (titration system programmed for stop criterion: time; delay: 10 sec.). A dried titration cell has a maximum drift consumption of 5-10 μL Karl Fischer reagent per minute.

3.6.6 Standardization

Heat cell to $50 \pm 1^\circ\text{C}$. Dry the cell as in 3.6.5. Depending on instrument, call up calibration mode. Condition solvent by titrating back ground moisture (hit “start”). Quickly weigh 150-250 mg of sodium tartrate dihydrate standard into the glass weighing spoon and record weight of spoon and standard to the nearest 0.1 mg (S). Quickly transfer the weighed test portion into the titration flask through the septum stopper. Homogenize at 24,000 rpm

for 60 sec. Reweigh empty spoon to obtain tare weight (T) while homogenizing. Obtain the weight of standard material added by subtracting tare weight (T) from weight of spoon plus standard (S). Record weight of standard material (S - T) in mg to the nearest 0.1 mg. Stop the homogenizer after 60 sec. Enter weight into Instrument, turn on the stirrer and start the titration. Before reaching the end point of the titration, homogenize momentarily to rinse down moisture that may have been under the cap. Titrate to same end point as in E, recording volume of reagent required for the titration (mL reagent) in mL to the nearest 0.001 mL. Repeat 4 times. Calculate titer, then average the 5 values. The relative standard deviation should be <2%.

3.6.7 Calculations:

$$\text{Titer} = \frac{\text{mg H}_2\text{O}}{\text{mL reagent}} = \frac{\text{mg Na}_2\text{C}_4\text{H}_4\text{O}_6 \cdot 2 \text{ H}_2\text{O} \times 0.1566}{\text{mL reagent}}$$

where, mg Na₂C₄H₄O₆ · 2 H₂O is S-T, in mg

3.6.8 System Suitability

Heat cell to 50⁰ ± 1⁰C. Dry the cell as in 3.6.5. Check drift in the titration cell. A dried titration cell should have a maximum drift consumption of 5-10 µL Karl Fischer reagent/min.

Analyze a water standard as follows: Immediately after drying the cell, break open the standard ampoule at the white ring and take 1-2 mL of standard with syringe which has been pre-dried in an oven at 105⁰C Rinse the syringe and discard the standard solution. Draw the remaining water standard (~6 mL) into the syringe and weigh accurately by placing the syringe into a beaker on the balance pan (SS). Quickly add 2 mL water standard through the septum keeping the tip of the syringe below the surface of the solvent. Carefully withdraw the syringe tip, reweigh the syringe and record the weight (S1). Obtain the weight of standard solution (SS - S1) by subtracting the weight (S1) from the weight of the syringe plus standard (SS). Record the water standard weight to the nearest 0.1 mg. Enter the weight into the instrument, start the stirrer, and begin the titration. Record the volume of titrant (V1).

Carry out the titration procedure two additional times, recording weights of the syringe after each subsequent addition (S2, S3) and the respective volume of titrant (V2, V3). Calculate the percent recovery as follows:

$$\text{Water standard, g} = S_5 - S_1, S_1 - S_2, S_2 - S_3$$

$$\text{Rec., \%} = \frac{\frac{V_n \times \text{titer}}{\text{g standard}}}{\text{certified value, mg / g}} \times 100 = \frac{\text{mg / g H}_2\text{O found}}{\text{certified value, mg / g}} \times 100$$

Average % recovery should be $100 \pm 1\%$. If system is not within specifications, correct before continuing with determinations. If the % recovery on the water standard is within specification, it is not necessary to perform a blank run (with no material), since the water standard indicates the condition of the system and running a blank will provide no additional information.

3.6.9 Determination

Dry the cell as in 3.6.5. Depending on the instrument, call up the sample analysis mode. Quickly weigh can 0.5 gm test portion (to contain can 25 to 50 mg water) into the glass weighing spoon and record weight of the spoon plus the test portion (W). Quickly add weighed test portion into the titration flask through the septum stopper. Homogenize at 24000 rpm for 60 sec. Reweigh empty spoon and record tare weight (T) while homogenizing. Obtain the test portion weight by subtracting tare weight (T) from weight of spoon plus test portion (W). Record weight (W - T) in g to the nearest 0.1 mg. Stop homogenizer after 60 sec. Enter weight into instrument and start the titration. Before reaching the end point of the titration, homogenize momentarily to rinse down moisture that may have been under the cap. This ensures that the moisture of the particles under the cap or hanging on the glass walls above the liquid surface is titrated. The end point is reached when no change in potential is observed for 10 sec (stop criterion: time; delay: 10 sec). Record the volume of titrant (V). Repeat determination in triplicate. The relative standard deviation of replicates should be <5%. The cell need not be emptied between each titration. Usually about 3 titrations can be performed before the cell requires emptying and replenishing.

3.6.10 Calculations

$$\text{mg H}_2\text{O} = V \times \text{titer}$$

$$\% \text{ H}_2\text{O} = \frac{V \times \text{titer}}{10 \times \text{test portion wt}}$$

$$\text{Dry matter, \%} = 100 - \% \text{ H}_2\text{O}$$

where V is the volume of titrant in mL and test portion weight is W – T, in g.

[Ref. J. AOAC Int. 85, 318 (2002)]

4.0 Determination of Total Ash

4.1 Apparatus

- (1) Silica Dish – Flat bottomed having a surface area of at least 15 cm²
- (2) Muffle Furnace - regulated at 550±25°C
- (3) Filter Paper – ashless, medium fine

4.2 Procedure

Weigh to the nearest 0.001 gm, but accurately 2 gm of the prepared sample into the tared dish. Pour about 2 mL of ethanol on the material and ignite it. When the ethanol is burnt off, heat the dish carefully over a small flame to char the material. Then ignite in a muffle furnace at 550±25°C for 3-4 hours. Cool and wet the ash with a few drops of water, evaporate carefully to dryness and heat in the muffle furnace for a further 1 hour. If the wetting shows the ash to be carbon free, remove the dish to a desiccator containing an efficient desiccant, allow to cool and weigh without delay. If the wetting shows presence of carbon, repeat the wetting and heating until no specks of carbon are visible and ignite in the

muffle furnace for 1 hour after the disappearance of carbon. If carbon is still visible, leach the ash with hot water, filter through ashless filter paper, wash the filter paper thoroughly, transfer the filter paper and contents to ashing dish, dry and ignite in muffle furnace $550\pm 25^{\circ}\text{C}$ until the ash is white. Cool the dish, add the filtrate and evaporate to dryness on a water bath. Heat in muffle furnace again, cool in a desiccator and weigh as previously. Heat again in the muffle furnace for 1 hour, cool and weigh. Repeat these operations until the difference in weight between two successive weighings is less than 0.001 gm. Record the lowest weight. Reserve the total ash for determination of acid insoluble ash.

Note: - In case of Nutmeg, Mace, Ginger and Cloves the ignition should be carried out at $600\pm 25^{\circ}\text{C}$

In case of Ground Mustard proceed as above and ignite for 1 hr after disappearance of Carbon. Leach the ash with hot water, filter through ashless filter paper and wash filter paper thoroughly. Transfer the filter paper and contents to the dish, dry and ignite in muffle furnace again for 1 hour. Cool and add 5 - 10 drops of Nitric acid, evaporate to dryness on a water bath and heat in muffle furnace for 30 minutes. Repeat the addition of 5 - 10 drops of Nitric acid, evaporating to dryness and heating in muffle furnace for 30 minutes. Cool and weigh.

4.3 Calculation

$$\text{Total ash on (dry basis) \% by wt} = \frac{(W_2 - W)}{W_1 - W} \times 100 \times \frac{100}{100 - M}$$

Where, W = wt in gm of empty dish

W₁ = wt in gm of dish + sample

W₂ = wt in gm of dish + total ash

M = Percent moisture content

(Ref: - IS Specification No IS 1797 – 1985 Methods of Test for Spices and Condiments/ AOAC 17th edn , 2000 Official Method 941.12 Ash of Spices)

5.0 Determination of Ash insoluble in dilute HCl

5.1 Reagents

- (1) Dilute Hydrochloric Acid – Conc. HCl (Sp. gr. 1.19) diluted in water 2: 5 (v/v)
- (2) Silver Nitrate solution – 10 % (w/ v)

5.2 Procedure

To the dish containing total ash, add 25 mL of dilute HCl and boil covering the dish with a watch glass to prevent spattering. Allow to cool and filter the contents of the dish through an ashless filter paper (medium fine). Wash the filter paper with hot water until the washings are free from HCl as tested by silver nitrate solution, and return it to the dish. Evaporate carefully on a water bath and ignite in a muffle furnace at $550 \pm 25^\circ\text{C}$ for 1 hour. Cool the dish in a desiccator and weigh. Repeat the operation of igniting for 1 hr, cooling and weighing till the difference in weight between two successive weighings is less than 0.001 gm. Note the lowest weight.

5.3 Calculation

$$\text{Ash insol. in dil HCl (on dry basis) \% by wt} = \frac{(W_4 - W)}{W_1 - W} \times 100 \times \frac{100}{100 - M}$$

Where, W = wt of empty dish

W₁ = wt of dish + sample

W₄ = wt of dish + acid insoluble ash

M = Percent Moisture content

(Ref: - IS Specification no IS 1797 – 1985 Methods of Test for Spices and Condiments/ AOAC 17th edn 2000, Official Method 941.12 (b) Ash of Spices)

6.0 Determination of Cold Water soluble Extract

6.1 Procedure

Weigh to the nearest 0.001 gm about 2 gm sample, transfer to a 100 mL volumetric flask, add distilled water and make upto mark. Stopper the flask and shake at approximately 30 minutes interval, for 8 hours and allow to stand for 16 hours longer without shaking. Filter the extract through a dry filter paper, evaporate a 50 mL aliquot portion to dryness in the dish on the water bath and heat in an air oven at $130 \pm 2^{\circ}\text{C}$ to constant weight, that is until two consecutive weighings separated by a period of 1 hour in the oven do not differ by more than 0.001 gm. Record the lowest weight.

6.2 Calculation

$$\text{Cold Water soluble Extract (dry basis) \% by weight} = \frac{W_1}{50} \times \frac{100}{W_0} \times \frac{100}{100 - M}$$

Where, W_1 = Wt. of the dried extract (residue) obtained

W_0 = Wt. of the sample taken for test

M = Percent moisture in the sample

(Ref: - IS specification No IS 1797 – 1985 Methods of Test for Spices and Condiments)

7.0 Determination of Alcohol Soluble Extract

7.1 Reagents

(1) Ethyl Alcohol – 90 % (v/v)

7.2 Procedure

Weigh accurately about 2 gm of test sample and transfer to a 100 mL volumetric flask/250mL Conical Flask and fill to mark with ethanol. Stopper the flask and shake it approximately 30 minutes interval for 4 hours and allow to stand for 16 hours longer without shaking. Filter the extract through a dry filter paper, evaporate a 50 mL aliquot portion to dryness on a water bath and heat in an oven at $103\pm 2^{\circ}\text{C}$ to constant weight, that is until two consecutive weighings separated by a period of 1 hour in the oven do not differ by more than 0.001 gm. Record the final weight.

7.3 Calculation

$$\text{Alcohol Soluble Extract (dry basis) \% by weight} = \frac{W_1}{50} \times \frac{100}{W_0} \times \frac{100}{100 - M}$$

Where, W_1 = Wt. of the dried extract (residue) obtained

W_0 = Wt. of the sample taken for test

M = Percent moisture in the sample

(Ref: - IS specification No IS 1797 – 1985 Methods of Test for Spices and Condiments)

8.0 Determination of Calcium Oxide

8.1 Reagents

- (1) Dilute HCl – 2 volumes of conc. HCl (Sp. gr. 1.19) diluted with 5 volumes of water
- (2) Ammonium hydroxide – 1: 1
- (3) Ammonium Oxalate - Saturated solution
- (4) Standard Potassium Permanganate Solution – 0.1 N standardized against Sodium Oxalate
- (5) Dilute Sulphuric acid – 1 volume of conc. H_2SO_4 (Sp. gr. 1.84) diluted with 4 volumes of water
- (6) Bromocresol Green Indicator solution – 0.04% - Weigh accurately 0.1 gm of bromocresol green powder and grind it with 14.3 mL sodium hydroxide solution (0.01 N) in an agate mortar. Transfer the contents of the mortar quantitatively to a 250 mL graduated flask and make up the volume with water
- (7) Acetic Acid – one volume of glacial acetic acid diluted with 2 volumes of water

8.2 Procedure

Weigh accurately about 2-4 gm of test sample and ash it in a muffle furnace. Dissolve the ash with dilute HCl and evaporate to dryness. Digest the dry ash again with dilute HCl and again evaporate to dryness on a water bath. Treat the residue with 5 -10 mL

of conc. HCl, add about 50 mL water, allow to stand on water bath for few minutes and filter in a 250 mL beaker. Wash the insoluble residue with hot water and collect the washings in the same beaker. Add 0.5 mL of Bromocresol green indicator and then ammonium hydroxide till the colour of the solution is distinctly blue. Adjust the pH of the solution to 4.4 – 4.6 by adding acetic acid drop by drop until the colour changes to distinctly green. Filter and wash the filter paper with hot water. Collect the washings in the same beaker and bring the solution to boil. While still hot add saturated ammonium oxalate solution drop wise as long as any precipitate forms and then add an excess. Heat to boiling. Allow to stand for 3 hours or longer. Decant the clear solution through an ashless filter paper. Pour 15-20 mL of hot water on the precipitate and again decant the clear solution through filter paper. Dissolve any precipitate remaining on the filter paper by washing with hot dilute HCl into the original beaker. Wash the filter paper thoroughly with hot Water. Then precipitate while boiling hot by addition of ammonium hydroxide and a little of saturated ammonium oxalate solution. Allow to stand for 3 hours or longer, filter through the same filter and wash with hot water until it is chloride free. Perforate the apex of the filter cone and wash the precipitate into the beaker used for precipitation. Wash filter paper with dilute sulphuric acid and titrate at temperature not less than 70°C with standard Potassium Permanganate solution.

8.3 Calculation

$$\text{Calcium (as CaO) \% by weight} = \frac{2.8 V N}{W}$$

Where, V = Volume of standard Potassium permanganate used for titration

N = Normality of standard Potassium Permanganate solution

W = Weight of the sample taken for test

(Ref: - IS specification No IS 1797 – 1985 Methods of Test for Spices and Condiments)

9.0 Determination of Non Volatile Ether Extract

9.1 Reagents

Diethyl ether – anhydrous

9.2 Procedure

Extract 2 gm of ground sample in a continuous extraction apparatus (soxhlet extractor) with diethyl ether for 18 hours. Remove the ether by distillation followed by blowing with a stream of air with the flask on a boiling water bath and dry in an oven at $110 \pm 1^\circ\text{C}$. Weigh the flask until the loss in weight between two successive weighings is less than 2 mg. Shake the residue with 2-3 mL of diethyl ether at room temperature, allow to settle and decant the ether. Repeat the extraction until no more of the residue dissolves. Dry the flask again until the loss in weight between two successive weighings is less than 2 mg. Record the lowest weight.

9.3 Calculation

$$\text{Non Volatile Ether Extract} = 100 \frac{(W_1 - W_2)}{W}$$

(% by weight)

W

Where, W_1 = Wt. of the flask with Non Volatile Extract

W_2 = Wt. of the flask with ether insoluble residue after decantation

W = Wt. of the sample taken for test

(Ref: - IS specification No IS 1797 - 1985 Methods of Test for Spices and Condiments)

10.0 Determination of Volatile Oil

10.1 Principle

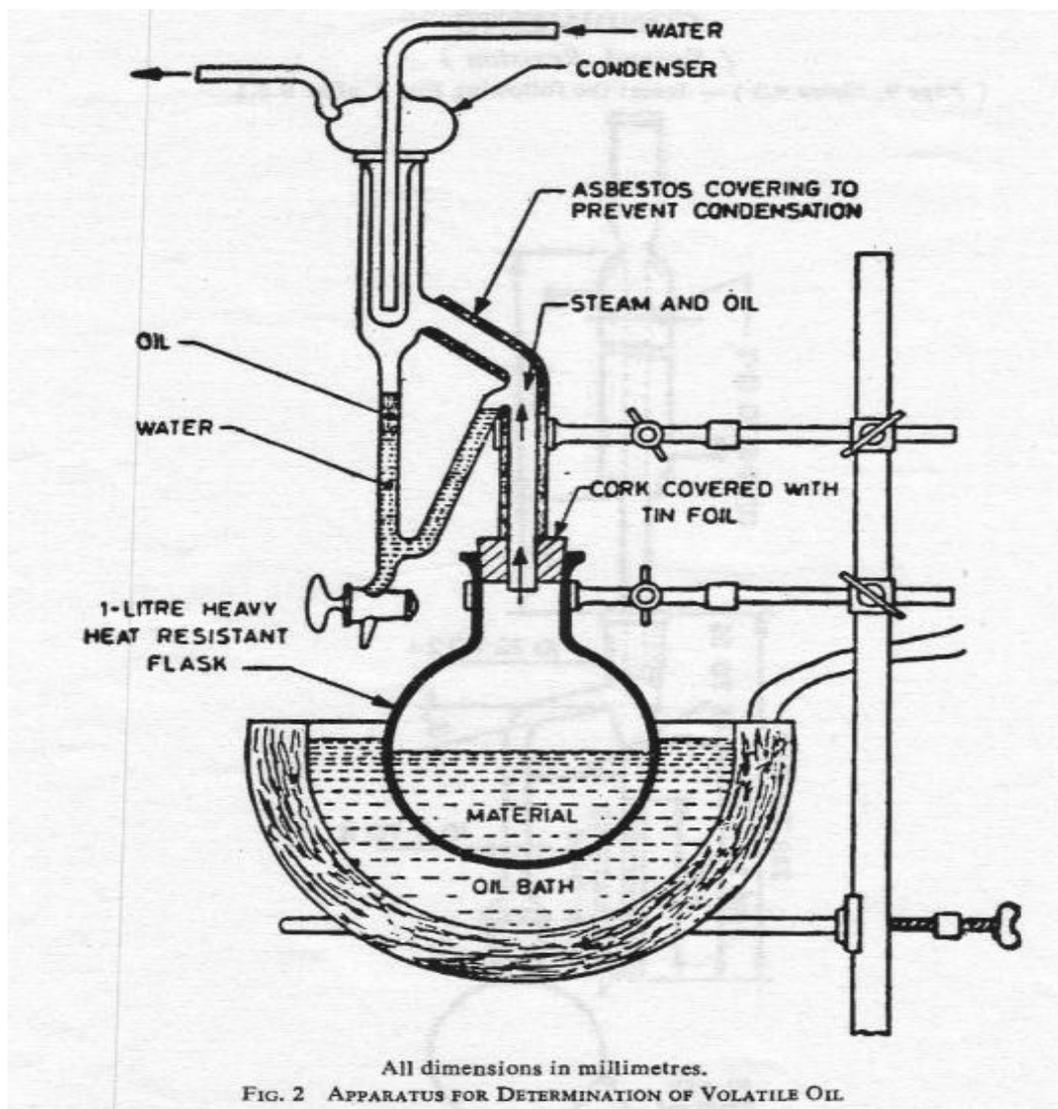
The determination of volatile oil in a spice is made by distilling the spice with water, collecting the distillate in a graduated tube in which the aqueous portion of the distillate is automatically separated and returned to the distilling flask, and measuring the volume of the oil. The content of volatile oil is expressed as a percentage v / w.

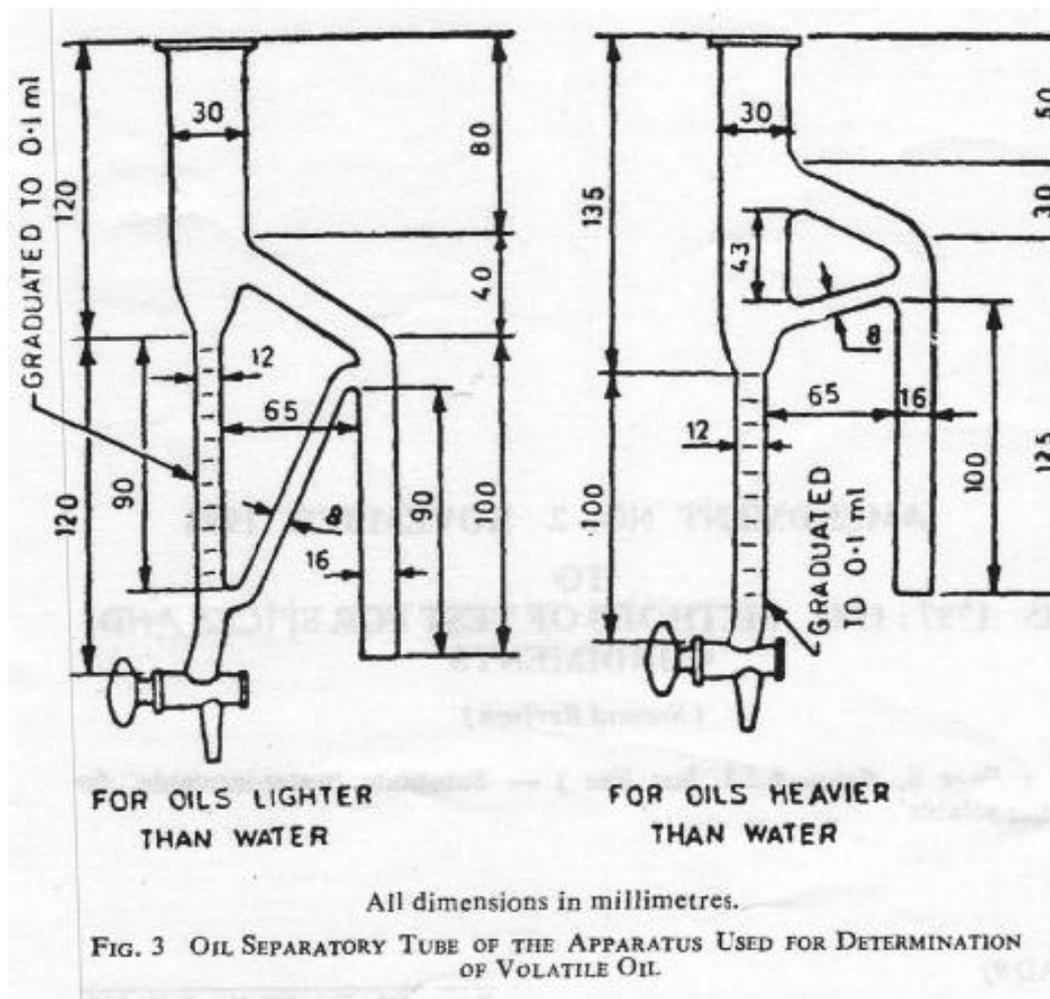
10.2 Apparatus

- (1) Flask, distilling, 1 litre capacity, preferably with magnetic stirrer.
- (2) Volatile oil traps, Clavenger type - (a) lighter than water (b) heavier than water.

See figure below:

It is essential to wash the apparatus with acetone and water and leave it to stand in chromic - sulphuric acid mixture with complete rinsing prior to use.





10.3 Procedure

Grind the sample to pass No 20 (850 micron) sieve. Weigh, but accurately 20 – 50 gm of the spice enough to yield 2 - 4 mL of oil if possible and place in the flask with glass beads or porous earthenware pieces, if a magnetic stirrer is not used. Add about 300 mL water and a drop of antifoam if necessary. Fill the trap with water. Place an efficient water cooled condenser on top of the trap and heat the flask with good stirring or agitation until boiling starts and continue boiling moderately briskly, but so that the lower part of the condenser remains cold. Set the apparatus so that the condensate will not drop directly on the surface of the liquid in the trap but run down the side walls. Rotate the flask occasionally to wash down any material adhering to the upper part of the walls. Distill until two consecutive readings taken at 1 hour intervals show no change in oil content (more than 6 hours). Remove the source of heat and read the volume of oil ten minutes or so later. Calculate as v / w .

If the oil separates in the graduated portion of the trap or clings to the walls, add several drops of a saturated aqueous detergent solution through the top of the condenser. Repeat if necessary. Distill for at least 10 minutes after adding detergent in order to wash out of the trap.

Some oils (e.g Cassia) have a density close to 1 or separate into two fractions in the trap (allspice, nutmeg). For these, prior to adding sample to the flask, add 1.0 mL xylene to the trap and distill without sample for at least half an hour. Cool and read the volume of xylene after 2 minutes. Add the sample and distill for up to six hours as before. Subtract the volume of xylene from the total volume of the organic layer in the trap. Calculate as before.

The oil obtained (without the use of xylene) may be recovered, dried with a small amount of sodium sulphate and its characteristics such as density and refractive index are determined.

(Ref: - FAO Manual of Food Quality Control (1986)14 / 8 page 239) / AOAC 17th edn, 2000 Official Method 962.17 and IS 1797 - 1985)

11.0 Determination of Crude Fibre

11.1 Reagents

- (1) Dilute Sulphuric acid – 1.25 % (w / v), accurately prepared
- (2) Sodium hydroxide Solution – 1.25 % (w / v), accurately prepared
- (3) Ethanol – 95 % (v / v)

11.2 Procedure

Weigh accurately about 2 - 2.5 gm ground sample into a thimble and extract for about 1 hour with petroleum ether in a soxhlet extractor. Transfer the material in the thimble to a 1 litre flask. Take 200 mL of dilute sulphuric acid in a beaker and bring it to

boil. Transfer the whole of the boiling acid to the flask containing fat free material and immediately connect the flask to a water cooled reflux condenser and heat, so that the contents of the flask begin to boil within 1 minute. Rotate the flask frequently, taking care to keep the material from remaining on the sides of the flask and out of contact with the acid. Continue boiling for exactly 30 minutes. Remove the flask and filter through fine linen (about 18 threads to a cm) or through a coarse acid washed, hardened filter paper held in a funnel and wash with boiling water until the washings are no longer acid to litmus paper. Bring some quantity of sodium hydroxide solution to boil under a reflux condenser. Transfer the residue on the filter into the flask with 200 mL of boiling sodium hydroxide solution. Immediately connect the flask with the reflux condenser and boil for exactly 30 minutes. Remove the flask and immediately filter through the linen or filter paper. Thoroughly wash the residue with hot water and transfer to a gooch crucible prepared with a thin but compact layer of asbestos. Wash the residue thoroughly first with hot water and then with about 15 mL of ethanol and with 3 successive washings of petroleum ether. Dry the gooch crucible and contents in an air oven at $105 \pm 1^\circ\text{C}$ for 3 hours. Cool and weigh. Repeat the process of drying for 30 minutes, cooling and weighing until the difference between two consecutive weighings is less than 1mg. Incinerate the contents of the gooch in a muffle furnace at $550 \pm 25^\circ\text{C}$ until all carbonaceous matter is burnt. Cool the gooch crucible in a desiccator and weigh.

11.3 Calculation:

$$\text{Crude fibre (on dry basis)} = 100 \frac{(W1 - W2)}{W} \times \frac{100}{100 - M}$$

(% by weight)

Where, W1 = Wt. of gooch crucible + contents + asbestos before ashing

W2 = Wt. of gooch crucible + ash and asbestos after ashing

W = Wt. of sample taken for test

M = Percent moisture content

(Ref: - IS Specification No IS 1797 – 1985 Methods of Test for Spices and Condiments)

12.0 Determination of Allyl isothiocyanate in Mustard

12.1 Apparatus

(1) Gas Chromatograph – with Flame Ionisation Detector

Approx operating conditions - Temp – Column 145° C, Detector 200° C, Injector 160°C, N₂ Flow rate 100 mL/min. Optimum conditions are obtained when not less than 10 cm peak is obtained for 8 µL standard injection solution.

(2) Column and packing – 3.7 m x 4 mm i.d, Carbowax 4000 on floropak 80, 20- 40 mesh or Capillary column (30 m x 0.53 mm x 3.0 µ DB-WAX)

12.2 Reagent

Allyl Isothiocynate standard solution –30. 5 mg/100 mL.

Measure 30 µL of allyl isothiocyanate in 50 µL syringe with 0.5 % accuracy. Add to 50 mL 10 % alcohol in 100 mL volumetric flask and shake intermittently until dissolved. Dilute to volume with water.

12.3 Procedure

Grind more than 5 gm sample to pass through No. 20 sieve. Immediately weigh 6 gm into 300 mL Erlenmeyer flask, add 150 mL 5 % alcohol, stopper tightly and stir magnetically 90±5 minutes in water bath maintained at 37°C.

12.4 Gas Chromatography Method

Distill about 70 mL into 100 mL volumetric flask containing 20 mL 5% alcohol (v /v) taking care that the end of the condenser dips below surface of solution. Dilute to volume with water. Inject 4 – 10 µL into gas chromatograph. Compare peak height of sample with that from same volume of standard solution.

Distill about 60 mL into 100 mL volumetric flask containing 10 mL NH_4OH (1+2) taking care that the end of the condenser dips below surface of solution. Add 20 mL 0.1M AgNO_3 to distillate and let stand overnight, heat to boiling point on water bath (boil behind safety barrier) to agglomerate Ag_2S , cool dilute to 100 mL with water and filter. Acidify 50mL filtrate with about 5 mL HNO_3 and titrate with 0.1 M NH_4SCN using 5mL Ferrous Ammonium Sulphate as indicator.

1 mL 0.1M AgNO_3 = 0.004958 gm allyl isothiocyanate

Note: - Before discarding Ag_2S and filter paper, treat with 25 mL 0.5 M Sodium Thiosulphate in 1 M Sodium Hydroxide.

Note: - During storage mustard becomes moist – conditions which encourage production of allyl isothiocyanate, which tends to be lost by volatilization.

(Ref: - AOAC 17th edn, 2000 Official Method 970.55 Volatile oil in Mustard Seed/ Pearson's Composition and Analysis of Foods 9th edn 1991 Page417)

13.0 Determination of p-hydroxybenzyl iso- thiocyanate in *Sinapsis alba* (White Mustard)

13.1 Procedure

Weigh 5 gm of ground sample into a beaker; add 100 mL water at 70°C and 100 mL calcium carbonate. Cover, keep at 70°C for 15 minutes, add 20 mL NaOH (about 1M), mix and stand for 15 minutes. Adjust the pH to 6-6.5 with 1M HNO_3 and transfer to a 250 mL volumetric flask. Add 2mL Potassium Ferrocyanide (106gm/litre) and 2 mL Zinc acetate (219gm/litre containing 3 gm acetic acid) with shaking. Make upto mark and pipette in a further 2 mL water to take into account the insoluble matter. Mix, filter away from bright sunlight. In a 50 mL volumetric flask add 5 mL filtrate and 5 mL ferric alum (200 gm/litre in 0.5 M Sulphuric acid). Dilute to the mark, mix and measure absorbance at 450 nm. The test should be repeated with the addition of 2 drops of mercuric chloride

solution (50 gm/litre) to correct for any absorbance due to phenols present.

Dilute 5 mL of 0.1M potassium or ammonium thiocyanate to 1 litre and prepare calibration graph by taking 5- 25 mL aliquots into 50 mL volumetric flasks and develop colour as above.

13.2 Calculation

$$\text{p- hydroxylbenzyl isothiocyanate} = 2.84 \times \frac{m}{10^6} \times \frac{250}{5} \times \frac{100}{M}$$

Where, m = μg thiocyanate from calibration graph

M = Weight in gm of sample

(Ref: - Pearson's Composition and Analysis of Foods 9th edn 1991 Page418)

14.0 Black Pepper

14.1 Determination of Bulk Density (Mass/litre)

14.1.1 Procedure

Fill a cylindrical 1 litre measure with lid made of aluminum alloy, brass or stainless steel, of internal diameter 95 mm and internal height 142 mm with the test sample. Lightly shake the measure horizontally three times and fill again as much as possible to the brim. Tap the measure on a level hard surface three times by changing the position each time and fill again as much as possible to a little over the brim. By moving a thin strip of straight metal sheet of about 10 mm width and 150 mm length, in level with the top of the measure, remove the excess material. Weigh the contents in a balance to the nearest gram and record the mass.

(Ref: - IS Specification No IS 1797 – 1985 Methods of Test for Spices and Condiments)

14.2 Determination of Light Berries

14.2.1 Reagents

- (1) Alcohol –Water solution – Sp. gr. 0.80 – 0.82. The alcohol may be ethyl alcohol or denatured spirit
- (2) Prepared Sample – Remove extraneous matter and pinheads from the test sample

14.2.2 Procedure

Weigh accurately 50 gm of prepared sample and transfer to a 600 mL beaker. Add 500 mL of alcohol – water solution and stir the material with a spoon stirrer. Allow the material to settle for 2 minutes and then spoon off the berries that float on the surface. Repeat the process of stirring, settling and removal of floating berries until no more berries float on the surface in two successive stirrings.

Note: - Only berries which float on the surface should be removed and not those which may stay suspended some distance below the surface of the alcohol- water solution.

14.2.3 Calculation

$$\text{Light Berries (\% by weight)} = \frac{w \times 100}{W}$$

Where, w = Wt. in gm of the light berries removed

W = Wt. of the sample in gm taken for the test

(Ref: - IS Specification No IS 1798 – 1982 Specification for Black Pepper, Whole and Ground)

14.3 Determination of Piperine Content

14.3.1 Principle

Piperine is extracted into Ethylene dichloride ($C_2H_4Cl_2$) and UV-Visible absorption is measured at maximum 342-345 nm. The concentration is determined from a standard curve prepared with known quantities of pure piperine. Other isomers of piperine that may be present and related compounds such as pipereddine and piperylin which also absorb at 340-345nm will also be included.

14.3.2 Apparatus and Reagents

- (a) UV spectrophotometer – any suitable model
- (b) Volumetric flasks – 100 ml, glass stoppered, amber coloured to reduce photo degradation of piperine in solution
- (c) Ethylene Dichloride – Reagent grade
- (d) Piperine - Pure

Preparation of Piperine Standard solution – Weigh 0.1000 gm piperine into 100 mL volumetric flask, add about 70 mL ethylene dichloride, shake to dissolve and make upto volume. Pipette 10 mL into 100 mL volumetric flask and dilute to volume. Pipette 1, 2, 3, 4, 5, 6 mL aliquots into six 100 mL volumetric flasks and dilute to volume with ethylene dichloride. Adjust to zero absorbance spectrophotometer with ethylene dichloride and read absorbance of each final solution at maximum 342- 345 nm with ethylene dichloride in reference cell. Plot a graph of concentration against observed absorbance.

14.3.3 Procedure

Grind sample to pass 60 mesh sieve and blend uniformly. Accurately weigh 0.5 gm test sample and transfer to 125 Erlenmeyer flask. Protect from light. Add about 70 mL

ethylene dichloride. Reflux and stir 1 hour, cool to room temperature and filter quantitatively through paper into 100 mL volumetric flask. Transfer rest of the extracted residue to filter, wash thoroughly and dilute to volume. Pipette 2 mL of this solution into 100 mL volumetric flask and dilute to volume. Record absorbance at maximum 342-345 nm. Obtain percent piperine content from the calibration curve.

(Ref: - AOAC 17th edn, 2000 Official Method Piperine in Pepper Preparations 987.07 Spectrophotometric Method)

14.3.4 An alternative method for Determination of Piperine Content by GC Method

14.3.4.1 Reagents

- 1) Dichloromethane
- 2) Piperine of purum grade (98%) from Fluka
- 3) Hexacosane (C₂₆) 99% from Fluka
- 4) GC: Dissolve 1.0 g of oleoresin in 50 mL of dichloromethane. Add 2 mL of this oleoresin solution to 1 mL of internal standard solution (hexacosane 3 gm/L in pentane).

14.3.4.2 Gas chromatography

The equipment equipped with a FID detector.

14.3.4.3 The GC analysis

Perform on a capillary polar column BP1 (50 m × 0.22 mm I.D., 0.25 µm film thickness)

14.3.4.4 The operating parameters

- 1) Carrier gas : hydrogen at 1 mL/min,
- 2) Split at 25 mL/min,

- 3) Injector temperature at 300 °C,
- 4) Detector temperature at 300 °C,

14.3.4.5 Program conditions

- 1) 250 °C to 280 °C at 0.5 °C/min.
- 2) Run time was up to 35 min,
- 3) Piperine retention time c.a. 20 min
- 4) And hexacosane retention time c.a. 13 min.

Quantify the alkaloids by internal standard method. The internal standard is hexacosane. Prepare the standard solution by adding 2 mL of pure piperine solution (4 gm/L in dichloromethane) to 1 mL of hexacosane solution (3 gm/L in pentane).

(Ref: Noyer et al. Analisis, 1999, 27, 69-74)

15.0 Saffron (Filaments and Powder)

15.1 Identification Test

Filaments - Examine visually with a magnifying glass (magnification x10 max)

Powder - Prepare a Diphenylamine solution by adding 0.1 gm diphenylamine to 20 mL sulphuric acid (Sp. gr. 1.19) and 4 mL water. Transfer to a porcelain dish with flat bottom and add 0.5 gm powder to the dish. The development of a blue colour which rapidly turns reddish brown shows presence of pure saffron.

(Ref: - IS Specification No IS 5453 (Part II) - 1966 / ISO 3632-2: 1993 Saffron – Methods of Test)

15.2 Determination of extraneous Matter

15.2.1 Apparatus

- (a) Watch glass
- (b) Small laboratory tongs
- (c) Analytical Balance

15.2.2 Procedure

Weigh to the nearest 0.01 gm, about 3 gm of homogenized sample and spread it on a sheet of neutral grey paper. With the help of a small tong separate the extraneous matter. Weigh the separated matter in a previously tared watch glass in an analytical balance and calculate the extraneous matter.

15.3 Determination of Floral waste

Reconstitute the test sample by reincorporating extraneous matter, homogenize and then weigh accurately 3 gm sample. Spread on a sheet of neutral grey paper and separate all yellow filaments attached or unattached and other floral waste that might be present. Weigh in a watch glass and calculate percentage.

(Ref: - IS Specification No IS 5453 (Part II) - 1966 / ISO 3632-2: 1993 Saffron – Methods of Test)

15.4 Determination of Moisture and Volatile Matter

15.4.1 Procedure

Weigh to the nearest 0.001 gm, accurately about 2.5 gm homogenized sample or sample reconstituted after determination and reincorporation of extraneous matter and floral waste and transfer to a moisture dish with a slip on cover. Place the dish uncovered in an oven maintained at $103 \pm 2^\circ\text{C}$ for 2 hrs and cool in a desiccator. Note down the weight of the dish. Leave for 16 hours. Cover the dish and cool it in a desiccator and

weigh to the nearest 0.001 gm. Carry out two determinations and take the arithmetic mean of both determinations.

15.4.2 Calculation

$$\text{Moisture and Volatile Matter} = \frac{(m_0 - m_1) \times 100}{m_0}$$

Where, m_0 = Wt. in gm of test portion

m_1 = Wt. in gm of the dry residue

(Ref: - IS Specification No IS 5453 (Part-II) - 1966 / ISO 3632-2: 1993 Saffron – Methods of Test)

15.5 Determination of Total Ash and Ash insoluble in dil HCl

Follow methods given in Clause 4 and 5 above. Take 2- 2.5 gm sample for test.

15.6 Determination of Picrocrocin, Safranal and Crocine

15.6.1 Apparatus

- (a) Spectrophotometer – suitable for recording optical density in the UV range between 220-480 nm.
- (b) Silica cell with an optical path length of 1 cm
- (c) Volumetric Flasks – 200 ml and 1000 ml
- (d) Pipettes – 20 ml
- (e) Filtration Membrane – made of cellulose acetate of 50 mm diameter and porosity 0.45 μm

15.6.2 Procedure

Weigh exactly 500 mg of homogenized sample crushed to pass through 500 μm

15.8 Determination of Crude Fibre

Follow method given in clause 11.0 above

16.0 Turmeric Whole and Powder

16.1 Preparation of sample – See clause 1.0 above

16.2 Determination of Moisture – See clause 3.0 above

16.3 Determination of Ash and Ash insoluble in dil HCl – See clauses 4.0 & 5.0

16.4 Determination of Curcumin Content

16.4.1 Apparatus

- (1) Extraction Flask – Flat bottom, 100 mL with TS 24/40 ground glass joint
- (2) Condenser – water cooled, drip tip 300-400 mm length TS24 /40 ground glass joint.
- (3) Volumetric Flasks – 100 and 250 mL
- (4) Spectrophotometer – any suitable type capable of measuring absorbance at 425 nm.

16.4.2 Reagents

- (1) Ethyl Alcohol – 95 %
- (2) Standard Curcumin solution – weigh 25 mg of standard curcumin into a 100 mL volumetric flask. Dissolve and dilute to mark with alcohol. Transfer 1 mL of the solution to a 100 ml volumetric flask and dilute to mark with alcohol. This standard solution contains 2.5 mg (0.0025 gm)/litre.

16.4.3 Procedure

Grind sample as quickly as possible in a grinding mill to pass sieve with 1 mm

diameter aperture. Weigh accurately about 0.1 gm, add 30 mL alcohol and reflux for two and half hours. Cool the extract and filter quantitatively into a 100 mL volumetric flask. Transfer the extracted residue to the filter. Wash thoroughly and dilute to mark with alcohol. Pipette 20 mL of the filtered extract into a 250 mL volumetric flask and dilute to volume with alcohol. Measure the absorbance of the extract and the standard solution at 425 nm in 1 cm cell against an alcohol blank.

16.4.4 Calculation

$$\text{Absorptivity of Curcumin, } A = \frac{a_1}{L \times c}$$

$$\text{Curcumin in Turmeric (\%)} = \frac{a_2 \times 125 \times 100}{L \times A \times m}$$

Where, a_1 = absorbance of standard solution at 425 nm

a_2 = absorbance of extract at 425 nm

L = cell length in cm

c = concentration in gm /litre

m = mass in gm of sample

(Ref: - IS Specification No IS 3576 – 1994 Specification for Turmeric Whole and Ground)

16.5 Determination of Starch Content

16.5.1 Reagents

(1) Ethyl ether

(2) Ethyl alcohol 10 % (v /v)

(3) Dilute Hydrochloric acid – 2.5 % prepared by mixing 20 ml of conc. HCl (sp .gr. 1.12) with 200 ml water

(4) Sodium carbonate solution – 20 % (w / v)

16.5.2 Procedure

Extract about 3 gm of the ground sample accurately weighed with five 10 mL portions of ether on a filter paper that will retain completely the smallest starch granules. Evaporate the ether from the residue and wash with 150 mL of 10 % ethyl alcohol. Carefully wash off the residue from the filter paper with 200 mL of cold water. Heat the undissolved residue with 200 mL of 2.5 % dilute HCl in a flask equipped with reflux condenser for two and half hour. Cool and neutralise with Sodium carbonate solution and transfer quantitatively to a 250 mL volumetric flask and make upto volume. Determine reducing sugars in the solution by Lane and Eynon Volumetric method using Fehling solution and methylene blue as internal indicator. Express the result as Dextrose.

Starch content = Dextrose x 0.9

(Ref: - IS Specification No IS 3576 – 1994 Specification for Turmeric Whole and Ground)

16.6 Test for presence of Chromate

16.6.1 Reagents

(1) Dilute Sulphuric acid – 1: 7 (v / v)

(2) Diphenyl Carbazide solution – 0.2 % (w / v) in 95 % ethyl alcohol

16.6.2 Procedure

Ash about 2 gm of the ground sample. Dissolve the ash in 4-5 mL of dilute sulphuric acid in a test tube and add 1 mL of diphenyl carbazide solution. The development of a violet colour indicates the presence of Chromate.

(Ref: - IS Specification No IS 3576 – 1994 Specification for Turmeric Whole and Ground)

17.0 Asafoetida

17.1 Determination of Moisture – Follow Toluene Distillation Method – Refer clause 3.0 above

17.2 Determination of Total Ash and Ash insoluble in dil HCl – Refer clause 4.0 and 5.0 above

17.3 Determination of Alcohol Extract

Follow the procedure as given under clause 7.0

17.4 Detection of Galbnum, Ammoniacum and other Foreign Resins

17.4.1 Galbnum

Add dilute HCl drop wise to 10 mL of alcoholic extract of the sample until a faint turbidity appears. Appearance of a bluish green colour in the mixture which fades on standing indicates absence of galbanum.

17.4.2 Ammoniacum

17.4.2.1 Reagent

Sodium Hypobromite solution – Prepare fresh by dissolving 20 gm Sodium Hydroxide in 75 mL water, adding 5 mL Bromine and making upto 100 mL with water.

17.4.2.2 Procedure

Mix well about 4 gm asafoetida (20 gm of compounded asafoetida) with 90 mL of distilled water in a mortar. Filter and make up the filtrate to 100 mL. Mix 2 mL of the extract with 5 mL water in a test tube and add 5 mL of hypobromite reagent cautiously down the side of the test tube so as to form a separate layer. Non appearance of red colour in the mixture shows absence of ammoniacum.

17.4.3 Foreign Resins

Add a few drops of 9% aqueous ferric chloride solution to 5 mL of alcoholic extract. Appearance of olive green colour in the mixture shows absence of foreign resins. Appearance of blackish precipitate or coloration in the mixture shows absence of foreign resins in compounded asafoetida.

(Ref: - IS Specification No IS 7807 – 1975 Methods of Test for Asafoetida)

17.5 Test for Presence of Colophony Resin

17.5.1 Thin Layer Chromatography Method

17.5.1.1 Principle

The test is based on isolation and identification of abietic acid which is a major constituent of colophony resin. Abietic acid spots are visualised by spraying with Halphen - Hicks reagent.

17.5.1.2 Reagents

- (1) Petroleum ether - B.P. 40 - 60 °C
- (2) Solvent – benzene – methanol (9:1)
- (3) Spray reagent (Halphen – Hicks Reagent) Carbon Tetrachloride and Phenol (2:1)
- (4) Standard Abietic acid solution – Dissolve good quality colophony resin in 98 % acetic acid and reflux for two hours. Filter, cool and allow the crystals to separate out. Recrystallise in 95% alcohol Dissolve 0.1 gm of recrystallised abietic acid in 100 mL of petroleum ether. 1 µl of solution is equivalent to 1 µg of abietic acid.
- (5) Silica gel
- (6) Bromine

17.5.1.3 Apparatus

- (1) Glass Plates 20 x 10 cm
- (2) TLC Chamber

17.5.1.4 Procedure

Take about 0.1 gm asafoetida or 0.5 gm compounded asafetida and mix with 10 mL of petroleum ether for 5 minutes. Filter and keep filtrate in a stoppered test tube. Coat glass plates with a slurry of silica gel in water (1:2) to thickness of 250 μm . Allow to set and activate in an air oven at 100°C for 1 hr. Store the plates in a dessicator.

Spot the plate with 10 μL of sample extract, 10 μL of standard abietic acid and 10 μL of sample + abietic acid (co - spotting). Develop the plate in an ascending manner in a TLC chamber. When the solvent front reaches 10 cm remove the plate, dry in air and spray with halphens- hicks reagent. Expose the plates to bromine vapour in a saturated bromine chamber. The presence of blue - purple spots at an approximate Rf of 0.75 shows presence of abietic acid/colophony. 5 μg of abietic acid can be detected by TLC.

(Ref: - IS Specification No IS 7807 - 1975 Methods of Test for Asafoetida)

18.0 Microscopic Examination of Spices

18.1 Procedure

A water slide should be first prepared by dissolving finely powdered sample with a drop of alcohol and then adding one or two drops of glycerol solution (30% in water) before sliding on the cover slip. The water slide is particularly suitable for detecting starch. The presence of starch can be confirmed by adding a drop of very dilute solution of iodine which produces the usual dark blue colour. Some spices namely cumin, coriander, chillies and cloves do not contain true starch and the presence of extraneous starch can be easily detected in these powdered spices.

A cleared slide is prepared by gently boiling the material with chloral hydrate solution (prepared by warming 80 gm of crystals in 50 mL water) in a tube until the particles look fairly transparent. Chloral hydrate has two fold action (1) it removes starch thereby concentrating other tissues and (2) it removes colouring matter from the tissues so that the outlines can be seen much more clearly. Sclerenchymatous matter can be stained red by warming the cleared material with excess of phloroglucinol solution (1% in 90 percent alcohol) followed by a drop of conc. Hydrochloric acid.

(Ref: - Pearson's Composition and Analysis of Foods 9th edn 1991 page 394)

19.0 Detection of Argemone seeds in Mustard

19.1 Procedure

Weigh 50 gm of powdered sample sufficient to yield 5- 10 gm non volatile ether extract and extract with 125 mL ethyl ether in a closed Erlenmeyer flask for 24 hrs with occasional shaking. Filter the contents through Whatman filter paper No. 1 and wash the residue with two 50 mL portions of ethyl ether. Evaporate the combined ether extract and dry the residual oil at 100°C. Test the extracted oil for argemone oil as per the procedure in the manual on oils and fats.

(Ref: - Manual Methods of Analysis for Adulterants and Contaminants in Foods I.C.M.R. 1990, page24)

20.0 Detection of Mineral oil in Black Pepper

20.1 Procedure

Take about 10 gm sample in a 100 mL glass stoppered conical flask and add 25 mL petroleum ether. Shake well for 30 seconds and immediately filter in a 50 mL beaker. Evaporate the solvent in the beaker on a water bath. Dissolve the residue in 1 mL chloroform and spot about 10 µL on activated silica gel G plate using capillary tube. Place

the plate in a chromatographic chamber containing Petroleum ether (40-60°C) as developing solvent. Develop the plate to 10 cm from the point of application. Take out the plate and air dry. Spray the plate with 0.1 % 2, 6 dichlorofluorescein in ethyl alcohol and view the plate under UV light. Bright yellow fluorescent spot near solvent front indicates mineral oil in the sample. It is advisable to run a standard of mineral oil alongside the sample.

(Ref: - Manual Methods of Analysis for Adulterants and Contaminants in Foods I.C.M.R. 1990, page23)

21.0 Detection of Papaya seeds in Black Pepper

21.1 Principle

Papaya seeds float in ethyl alcohol of 0.8 Sp. gr. along with immature seeds and light berries whereas mature seeds of black pepper sink.

21.2 Procedure

Float the sample in ethyl alcohol of Sp. gr. 0.8, separate all the floaters and examine them as under:

The morphological characteristics of papaya seeds are quite different from black pepper. The papaya seed is a dicotyledon and pepper is a monocotyledon. Cut the seed into two halves and put a drop of iodine solution. The pepper seed gives blue colour due to presence of starch while papaya seed gives pale colour due to presence of dextrins.

(Ref: - Manual Methods of Analysis for Adulterants and Contaminants in Foods I.C.M.R. 1990, page23)

22.0 Detection of Turmeric in Chillies and Coriander

22.1 Principle

Boric acid gives characteristic colour with turmeric.

22.2 Reagents

- (1) Hydrochloric acid
- (2) Ethyl alcohol
- (3) Boric acid
- (4) Ammonium Hydroxide

22.3 Procedure

Slightly acidify the aqueous or dilute ethanolic extract of the sample with hydrochloric acid and add a few boric acid crystals. A brown red coloration is formed in the presence of turmeric.

(Ref: - Manual Methods of Analysis for Adulterants and Contaminants in Foods I.C.M.R 1990 page 24)

23.0 Detection of oil soluble colour

Refer to manual on Food additives.

24.0 Determination of Light and Heavy Filth in Spices and Condiments

Refer to manual on Cereal and Cereal Products.

25.0 Method for capsaicin content in chilli powder

Capsaicinoids in Capsicums and Their Extractives by Liquid Chromatographic Method (AOAC 995.03)

[Applicable for determination of 750–650000 Scoville Heat Units (SHU) of capsaicinoids in ground and crushed red pepper, chili pepper, ground cayenne pepper, ground jalapeno pepper, and red pepper oleoresins. Not applicable to chili powders or products containing oregano or thyme.]

(*Caution: See Appendix B, safety notes for the safe handling of organic solvents and special chemical hazards—acetone, acetonitrile, and ethanol. See Material Safety Data Sheets, or equivalent, for each reagent. N-Vanillyl-n-nonanamide is an extreme irritant, do not inhale. Dispose of waste solvents in an appropriate manner compatible with applicable environmental rules and regulations.*)

Method Performance: See Table 995.03 for method performance data.

25.1 Principle

Test sample is extracted in warm ethanol using reflux condenser. Extract is filtered and injected into liquid chromatography equipped with UV or fluorescence detector.

25.2 Apparatus

(a) Liquid Chromatography (LC) - Equipped with 1 V integrator, 20 μ L sample injector, and with UV detector set at 280 nm Wave-length or fluorometer with excitation 280 nm and emission 325 nm. Operating conditions: temperature, ambient (20–25°C); flow rate, 1.5 mL/min., isocratic; relative retention times: N-vanillyl-n-nonanamide, 1.00; nordihydrocapsaicin, 0.90; capsaicin, 1.00; dihydrocapsaicin, 1.58. See Figure 995.03 for baseline separation of major Capsaicinoids.

(b) LC column- Stainless, C₁₈, 150 x 4.6 mm id, packed with 5 μ m particle size. Use guard column, if desired.

(c) Reflux condenser.

(d) Syringe filter- 0.45 μ m.

(e) C₁₈ solid-phase extraction cartridge.

25.3 Reagents

(a) Ethanol- 95% or denatured, suitable for chromatography.

(b) Acetone- ACS grade.

(c) LC mobile phase- Acetonitrile-water, Use LC grade solvents, or equivalent. Mix 400 mL acetonitrile with 600 mL H₂O containing 1% acetic acid (v/v). De-gas with helium or by other suitable technique.

(d) *N*-Vanillyl-*n*-nonanamide standard solutions—*N*-Vanillyl-*n*-nonanamide standard, 99%, is available as synthetic capsaicin from Penta International Corp., 50 Okner Pkwy, Livingston, NJ 07039. Keep solutions out of direct sunlight.

(1) Standard solution A.—0.15 mg/mL. Accurately weigh 75 mg *N*-vanillyl-*n*-nonanamide and transfer it into 500 mL volumetric flask. Dilute to volume with ethanol, and mix. Use standard solution A for analyzing all peppers except chili pepper.

(2) Standard solution B.—0.015 mg/mL. Transfer 10 mL standard solution A into 100 mL volumetric flask, dilute to volume with ethanol, and mix. Use standard solution B when analyzing chili peppers.

25.4 Extraction

(a) Ground or crushed peppers— Accurately weigh ca 25 gm pepper into 500 mL boiling flask. Place 200 mL ethanol into same flask, add several glass beads, and attach flask to reflux condenser. Gently reflux test sample 5 h and then allow to cool. Filter 1–4 mL sample through 0.45 μ m syringe filter into small glass vial. Use for LC analysis.

(b) Red pepper oleoresin — Accurately weigh 1–2 gm oleoresin into 50 mL volumetric flask. Increase weight of sample, if total capsaicinoid concentration is <1%.

Note: Do not allow any oleoresin to coat sides of flask.

Add 5 mL acetone, C(b), to flask and swirl contents of flask until test sample is completely dispersed (no oleoresin can coat bottom of flask when turning flask neck at 45° angle). Add five 3–5 mL portions ethanol, swirling flask during each addition. Dilute contents of flask to volume with ethanol and mix well.

Hold C₁₈ solid-phase extraction cartridge over 25 mL volumetric flask or place cartridge on 10 mL glass syringe and hold over 25 mL volumetric flask. Transfer 5 mL solution from flask to cartridge or syringe. (Note: When using syringe, deliver solution to bottom of syringe so that sides of syringe are not coated with sample.) Pass aliquot through cartridge and collect in 25 mL flask. Wash cartridge 3 times with 5 mL ethanol, collecting washings in same flask. Dilute contents of flask to volume with ethanol and mix. Filter 1–4 mL solution through 0.45µm syringe filter into small glass vial. Use for LC analysis.

Table 995.03 Method Performance for Determination of Capsaicinoids in Capsicums and Their Extractives by Liquid Chromatography (SHU)

Sample	Mean	N ^a	s _r	s _R	RSD _r , %	RSD _R , %	r ^b	R ^c
Chili peper	900-2750	9	40-80	100-220	3.5-4.5	9.6-11.6	110-220	280-620
Red pepper	30140-41600	11	370-840	1600-1860	1.0-2.3	4.5-5.2	1040-2350	4480-5210
Red pepper oleoresins	305690-644060	13	6770-86870	23100-86870	2.2-14.8	7.6-14.8	18960-243200	64680-243240

^a N = No. of laboratories.

^b r = 2.8 × s_r.

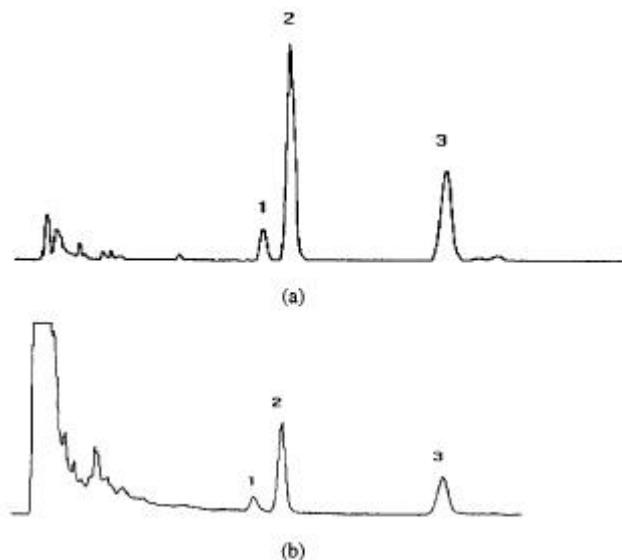


Figure 995.03—Red pepper extract analyzed by (a) fluorescence detection, and (b) UV detection. Peak 1 = nordihydrocapsaicin; peak 2 = capsaicin; Peak 3 = dihydrocapsaicin

25.5 LC Determination

Inject 20 μ L standard solution B, C(d)(2), onto LC column, when analyzing chili peppers. When analyzing other matrices inject 20 μ L standard solution A, C (d)(1). Re-inject standard solution at intervals of 6 sample injections, or less.

Inject in duplicate 20 μ L test sample from D onto LC column.

After \leq 30 sample injections, purge LC column 30 min with 100% acetonitrile at 1.5 mL/min flow rate. Use LC mobile phase, C(c), for further analysis.

25.6 Calculation

Capsaicinoids contain 3 major compounds: nordihydrocapsaicin (N), capsaicin (C), and dihydrocapsaicin (D). Calculate capsaicinoids as sum of these compounds [N + C + D; in Scoville Heat Units (SHU); 1 μ g total capsaicinoids/gm = ca 15 SHU], as follows:

(a) UV detection:

(1) *Ground peppers and chili pepper:*

$$N = (P_N/P_S) \times (C_S/W_T) \times (200/0.98) \times 9300$$

$$C = (P_C/P_S) \times (C_S/W_T) \times (200/0.89) \times 16100$$

$$D = (P_D/P_S) \times (C_S/W_T) \times (200/0.93) \times 16100$$

Where P_N , P_C , and P_D = average peak areas for nordihydrocapsaicin, capsaicin, and dihydrocapsaicin, respectively, from duplicate injections; P_S = average peak area of appropriate standard solution; C_S = Concentration of standard solution, mg/mL; W_T = weight of test sample, gm.

(2) Red pepper oleoresins:

$$N = (P_N/P_S) \times (C_S/W_T) \times (250/0.98) \times 9300$$

$$C = (P_C/P_S) \times (C_S/W_T) \times (250/0.89) \times 16100$$

$$D = (P_D/P_S) \times (C_S/W_T) \times (250/0.93) \times 16100$$

(b) Fluorescence detection:

(1) Ground peppers and chili pepper:

$$N = (P_N/P_S) \times (C_S/W_T) \times (200/0.92) \times 9300$$

$$C = (P_C/P_S) \times (C_S/W_T) \times (200/0.88) \times 16100$$

$$D = (P_D/P_S) \times (C_S/W_T) \times (200/0.93) \times 16100$$

(2) Red pepper oleoresins:

$$N = (P_N/P_S) \times (C_S/W_T) \times (250/0.92) \times 9300$$

$$C = (P_C/P_S) \times (C_S/W_T) \times (250/0.88) \times 16100$$

$$D = (P_D/P_S) \times (C_S/W_T) \times (250/0.93) \times 16100$$

[Reference: J. AOAC Int. 79, 738(1996)]

26.0 Method for measuring color value in chillies (AOAC 971.26)

26.1 Apparatus and Reagents

- a) Spectrophotometer- Capable of accurately measuring A at 460nm; with 1cm stoppered cells.
- b) Glass reference standard-NIST SRM 2030 or 930, glass filter with A specified by NIST in range 0.4-0.6 at 465 nm.

26.2 Determination

a) Capsicums- Grind capsicums to pass No. 18 sieve. Place accurately weighted sample containing 70-100mg ground capsicums in 100mL, volumetric flask, dilute to volume with acetone, and stopper tightly. Shake flask and let stand 16 h at room temperature in dark. Shake flask again and let particles settle 2 min. Transfer portion of extract to spectrophotometer cell with 10mL piper.

Determine A of sample at 460nm, using acetone as blank. Determine A of NIST standard at 465nm.

B) Oleoresin- Weigh, to nearest: 0.1mg, 70-100mg sample and transfer to 100mL volumetric flask, dilute to volume with acetone, shake and let stand 2 min. Piper 10mL extract into another 100mL, volumetric flask, dilute to volume with acetone and shake. Transfer portion to cell and measure A at 460nm against acetone.

26.3 Calculations

To correct for instrument and cell variations calculate correction factor, $I_f = \text{declared A of NIST standard at 465 nm} / \text{actual A of NIST standard at 465nm}$. Re-determine I_f each time spectrophotometer is turned on.

Range of A should be 0.30 to 0.70. Dilute extracts with $A > 0.70$ with acetone to $\frac{1}{2}$ original concentration. Discard extracts with $A < 0.30$ and extract larger sample.

ASTA color value for capsicum = $\{(A_{\text{extract at 460nm}}) \times (16.4 I_f)\} / \text{gm sample}$

ASTA color value for oleoresin= $\{A_{\text{extract at 460nm}} \times (164 I_f)\} / \text{gm sample}$

Where 16.4 and 164 are conversion factors to American spice Trade Association (ASTA) color values.

Reference:

- J. AOAC 54, 37(1971):55,428(1972):60, 1(1977):67,414(1984)
- AOAC official method 971.26 color (extractable) in Spices

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