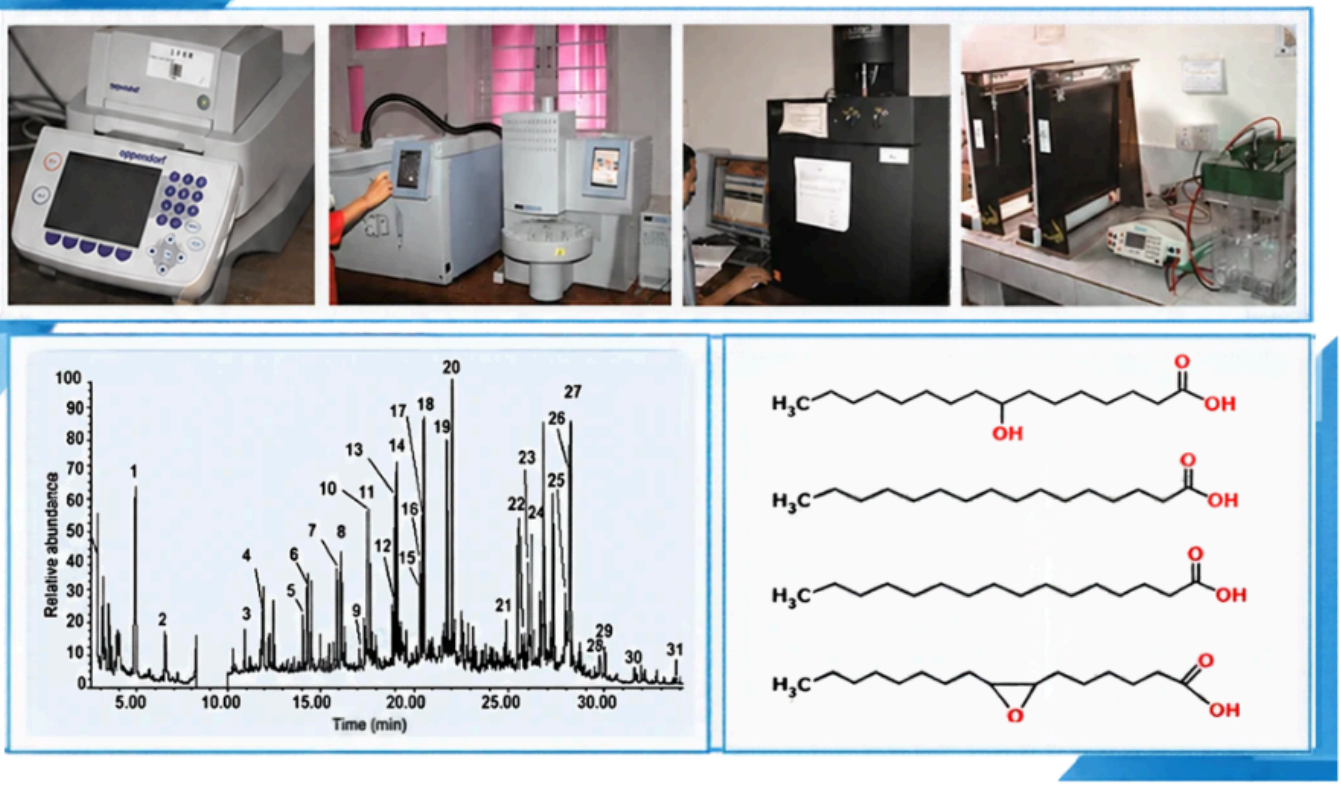
Standard Operating Procedure for

Quality Analysis of Spices



ICAR - All India Coordinated Research Project on Spices,
ICAR - Indian Institute of Spices Research, Kozhikode

2026



Standard Operating Procedure for Quality Analysis of Spices

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Foreword

India has been known to the world as the land of spices for millennia. The commodities that left our shores centuries ago still anchor a thriving export economy and sustain the livelihoods of millions of farming families. Reputation alone, however, no longer secures a place in the market. Importing countries, processing industries and discerning consumers now expect every consignment to be backed by credible numbers, and the analytical laboratory has become as important to the spice sector as the plantation or the curing yard.

The All India Coordinated Research Project on Spices (AICRPS), operating since 1971 from its headquarters at ICAR-Indian Institute of Spices Research, Kozhikode, works through a network of centres spread across the country. These centres evaluate varieties, germplasm, agro-techniques and post-harvest products of the mandated spice crops. For the results of one centre to be compared with, and combined with, those of another, the network needs a common reference for quality analysis. This publication provides that reference.

I am pleased, therefore, that this Technical Bulletin gathers the methods our scientists and technical staff use day after day and sets them down step by step, in a form that can be read, followed and audited. I particularly value its practical spirit. The procedures explain not only what is to be done but also why, and where an analyst is most likely to go wrong. This document reflects our conviction that modern instrumentation must be matched by disciplined technique if its output is to be trusted. The Standard Operating Procedure for Quality Analysis of Spices brings together the general protocols used across crops and the crop-wise protocols for black pepper, cardamom, clove, cinnamon, ginger, nutmeg and turmeric. The one-page ready reckoner at the start of each crop chapter takes the analyst from sample receipt to reporting, and the methods are aligned with national and international standards and methods of BIS, ISO, ASTA, AOAC and FSSAI.

A protocol is only as good as the people who practise it. I hope that scientists in our own institutes, state agricultural universities, commodity boards, testing laboratories and spice enterprises will adopt this bulletin, test it against their own experience and send us their observations, so that future editions may be sharper still.

I compliment the authors for the care and effort they have put into preparing this much- needed manual. I am confident that this bulletin will help the Indian spice sector and will serve the cause of quality for many years to come.

D Prasath
Project Coordinator
ICAR–All India Coordinated Research project on Spices
Kozhikode, Keralam
October 2026

Preface

Spices are among the most analysed agricultural commodities, and also among the most variable commodity with respect to its quality. The flavour profile of spices can change markedly with variety, maturity at harvest, curing, drying and storage, and depends largely on sample collection, preparation and analysis. In a multi-centre programme such as the All India Coordinated Research Project on Spices, these differences in method can easily be mistaken for differences between genotypes or locations. This manual was prepared to remove that source of doubt.

The purpose of the book is to give every AICRPS centre, and every laboratory that works on spice quality, one clear and tested set of procedures. Part A presents the general protocols that are common to all crops: estimation of essential oil by hydro-distillation, determination of moisture by the Dean and Stark method, dry recovery of fresh produce, volatile profiling by GC-MS, and sampling techniques for spice quality analysis. Part B gives the crop-wise protocols for black pepper, cardamom, clove, cinnamon, ginger, nutmeg and turmeric. Each crop chapter opens with a one-page ready reckoner that lists the tests in order, from sample receipt to reporting, and links to the detailed method, including routine and confirmatory options wherever both are available, as with spectrophotometric and HPLC estimation of its active ingredient.

We have written each protocol so that a trained technician can follow it at the bench, with the principle, apparatus and reagents, sample preparation, procedure, calculation and reporting stated plainly. Wherever possible the methods have been aligned with BIS, ISO, ASTA, AOAC and FSSAI methods, and the sources are cited so that readers can consult them. We expect the book to be useful to scientists and technical staff of the AICRPS centres, to State Agricultural Universities and research scholars, to testing and quality control laboratories, and to processors, exporters and farmer producer organisations who need dependable quality data.

This is a working document. Instruments, regulations and good practice change, and some laboratories will need to adapt a method to their own equipment. We are grateful to Dr. R. Dinesh, Director, ICAR-Indian Institute of Spices Research, for his guidance and encouragement, to the Indian Council of Agricultural Research for its support to the cause of spices, and to the scientists and staff of the AICRPS centres whose work and experience shaped this manual.

Kozhikode
October 2026

Authors

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Introduction

Spices are valued not only for their characteristic flavour, aroma, colour and pungency, but also for their diverse applications in food, nutraceutical, pharmaceutical and other value-added products. The quality of spices is determined by a combination of physical, chemical and biochemical attributes, which can vary considerably with genotype, geographical origin, maturity, harvesting practices, post-harvest handling, processing and storage. Reliable assessment of these attributes is therefore essential for research, quality assurance, product standardisation, trade, regulatory compliance and development of value-added spice products. A scientifically sound quality analysis, however, depends not only on the analytical technique employed but also on representative sampling, appropriate sample preparation, standardised operating conditions, accurate measurements, proper documentation and interpretation of results.

The present *Standard Operating Procedures for Quality Analysis of Spices* has been compiled to provide a systematic and practical reference for carrying out important quality analyses of spices in a consistent and reproducible manner. The SOPs are intended to facilitate uniformity in laboratory procedures and to minimise variations arising from differences in sampling, sample preparation, equipment, analytical conditions and reporting. The procedures are designed for use by researchers, laboratory analysts, technical personnel and students engaged in spice quality evaluation and related research activities. Wherever applicable, the methods are aligned with recognised national and international standards and analytical references.

The bulletin is broadly organised into two complementary sections. Part A: General Protocols presents analytical procedures that are applicable across more than one spice and therefore need not be repeated in individual crop chapters. These include estimation of volatile or essential oil by hydro-distillation, determination of moisture content, estimation of dry recovery, volatile profiling using gas chromatography–mass spectrometry (GC-MS), and standardised sampling procedures for spice quality analysis. The sampling protocol emphasises the importance of obtaining representative samples, while the analytical protocols provide details on principles, apparatus, reagents, sample preparation, procedures, calculations, precautions and references.

Part B: Crop-Specific Protocols brings together the quality evaluation procedures relevant to individual spices, currently covering black pepper, cardamom, clove, cinnamon, ginger, nutmeg and turmeric. Each crop chapter provides a practical workflow for sample receipt, preparation and analysis and includes crop-specific quality parameters and analytical methods. The crop chapters make use of the common procedures presented in the general protocols, thereby avoiding unnecessary duplication while maintaining a comprehensive analytical framework.

The SOP also incorporates advanced analytical approaches such as GC-MS-based volatile profiling, including sample preparation, instrumental conditions, compound identification, quantification, method validation, quality control and record-keeping. Together, these procedures provide an integrated framework for generating dependable quality data and strengthening the scientific basis for quality evaluation, standardisation and value enhancement of Indian spices.

Part A

General Protocols

These methods are not specific to a single spice. They are presented once here and referenced from every crop chapter in Part B that uses them.

General Protocol 1

Volatile oil or Essential oil (EO) estimation by hydro-distillation

Determination of EO content by modified Clevenger method is an important quality parameter for spices/herbs.

Principle

Hydro-distillation or continuous extraction process works on the principle that boiling water carries the volatile substances along with the water vapor and gets condensed by indirect cooling with water.The essential oil, most of which are lighter than water is collected over the water in the collection arm of the Clevenger trap. For EO of spices like cloves and cinnamon which is heavier than water, a modified Clevenger trap is used.

Apparatus and Reagents

1. Clevenger Hydro-distillation system – contains 1L round bottom (RB) flask, heating mantle, Clevenger trap and condenser. All glass wares should have appropriate glass joints to fit in with each other.
2. Volatile oil traps – 5 or 10 mL (Use lighter than water type except for cloves, cinnamon and allspice where heavier than water type need to be used)
3. Xylene

Sample Preparation

- Coarsely ground spice samples are recommended for EO extraction.
- Ginger and Turmeric samples are cracked before grinding.
- It is advised to use freshly ground sample as storage of ground sample may lead to loss of volatile oils.
- In case of allspice, anise and cloves, trap additive like xylene is placed to graduated portion.
- The flask with spice powder and water is heated on a heating mantle and the content is distilled for 4-5 hrs or until 2 consecutive readings taken at 1 h interval show no change of oil volume in trap.
- Once the run is over, the volume of oil collected is noted to the nearest 0.01 mL.
- The concentration of volatile oil, % (v/w) is calculated as follows:

$$\text{Volatile oil (v/w \%)} = \frac{Volume\ of\ oil\ collected\ in\ the\ trap\ (mL)}{Weight\ of\ Sample\ (g)} \times 100$$

When xylene is used,

$$\text{Volatile oil (v/w \%)} = \frac{Volume\ of\ oil\ (mL) - Volume\ of\ Xylene\ added\ in\ the\ trap\ (mL)}{Weight\ of\ Sample\ (g)} \times 100$$

Table 1.1 describes recommended solvents for volatile oil distillation of different spices

Table 1.1: Sample weight, distillation solvent and trap additives for different herbs and spices (AOAC, 2006)

Spices	Weight of sample (g)	Solvent used for distillation	Trap Additive$
Black Pepper, Turmeric, Fennel, Fenugreek, Cumin,	50	Water	-
White Pepper	40	Water	-
Ginger	50	10% NaCl solution*	-
Clove	10	10% NaCl solution*	Xylene 2.0 mL
Cinnamon	40	10% NaCl solution*	-
Nutmeg	30	Water	-
Mace	20	Water	-
Allspice	40	10% NaCl solution*	Xylene 2.0 mL
Cardamom#	20	Water	-

** - The hydro-distillation of these spices gives a whitish distillate. The salting-out process is required to separate product from the reaction mixture. The ionic compound, sodium chloride(NaCl) is taken in the distillation arm to improve the separation of essential oil and water phase by increasing the difference in polarity between two phases. If the essential oil is used for further analysis in GC-MS, a liquid – liquid extraction step is required.*

 # - Crack open the required quantity of the cardamom pod and separate the seeds from the pod. Grind the seeds and take them for essential oil estimation.

$ - Xylene is used as trap additive in volatile oil heavier than water. Two mL xylene is added above the water in the Clevenger trap and distillation is started. The volatile oil released from the material is dissolved in the xylene layer. The percentage of volatile oil is calculated using the above mentioned formula. When xylene is used as trap additive, the volatile oil cannot be used for further analysis using GC-MS.

Reference: AOAC method 962.17, ASTA method 5, FSSAI 10.013:2021

General Protocol 2

Determination of moisture content (Dean & Stark Distillation Method)

Definition: The quantity of water, expressed as a percentage by mass, distilled and collected in accordance with the method specified in this standard.

Caution [3]:

1. Toluene and hexane are highly flammable and hazardous in case of skin contact, eye contact, ingestion or inhalation.
2. Wear required personal protective equipment while working.
3. Avoid every possible source of ignition.
4. For disposal of solvent waste, follow good laboratory practices outlined by environment health and safety protocols in your institution.

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.

Apparatus

1. Analytical balance, accurate upto 0.1 mg.
2. Moisture distillation apparatus: The apparatus consists of a glass flask heated by suitable means, provided with a reflux condenser discharging into a trap and connected to the flask. The connection between the trap and the condenser and the flask should be interchangeable ground glass joints. The trap serves to collect and measure the condensed water and to return the condensed solvent to the flask.

   a) **Flask**, of capacity 500 mL, made up of heat-resistance glass, well annealed and as free as possible from striae and similar effects.
   b) **Reflux condenser**, water cooled, made of glass, having a jacket approximately 400 mm long and an inner tube of diameter 9.5 to 12.5 mm. The tip of the condenser to be inserted in the receiver may be ground off at an angle of 30° from the vertical axis of the condenser. When inserted into the receiver, the tip of the condenser is 6 to 7 mm above the surface of the liquid in the receiver after distillation conditions have been established.
   c) **Receiver**, of capacity 5 mL, made of heat-resistant glass, well annealed and as free as possible from striae and similar defects, provided with ground glass joints, with the shape, dimensions and tolerances given in the figure, and consisting essentially of the upper chamber, together with the tube and ground joint leading to the flask, and the graduated tube. The graduated portion has a capacity of 5 mL when filled to the highest graduation mark. The scale covers the range of 0 to 5 mL and is graduated at intervals of 0.1 mL. The graduation marks corresponding to each milliliter are numbered and carried completely round the tube. The graduation marks midway between the numbered marks are carried three-quarter-way, and the remaining marks are carried halfway, around the tube. The error at any indicated capacity does not exceed 0.05 mL.

d) **Heat source**, either an oil bath or an electric heater provided with a sliding rheostat or other means of heat control. The temperature of the oil in the bath should not be very much higher than the boiling point of toluene.
e) **Copper wire**, long enough to extend through the condenser and with one end twisted into a spiral. The diameter of the spiral is such that it fits snugly within the graduated portion of the receiver and yet can be moved up and down

Chemicals

1. Toluene (For most spices)
2. Hexane (For capsicum, onion, garlic, and other spices containing large amount of sugar) [1].

Preparation of Reagents

Toluene: Saturate the toluene by shaking with quantity of water and distil. Use the distillate for the determination of moisture.

Procedure

Note: 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 g, about 20-40 g of prepared sample (or enough to yield 2 -5 mL H_2O 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

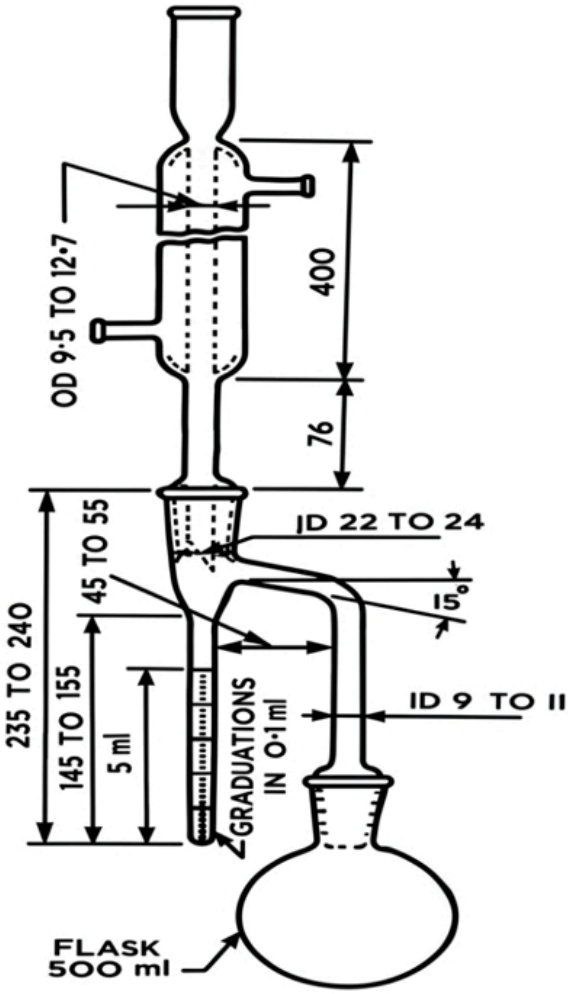


FIG:2.1: All Dimensions in millimeters
MOISTURE DISTILLATION APPARATUS

Calculation

The moisture content (percentage by mass) = $\frac{100 * V}{M}$

Where,

V = Volume of water collected in mL and

M = Mass of the test portion in g

***Note**: It is assumed that density of water is 1 g/mL exactly.*

Reference

1. A.O.A.C 17th Edn, 2000 Official Method 986.21, Moisture in spices, Distillation method.
2. I.S. Specification No I.S 1797 – Methods of Test for Spices and Condiments.
3. Nielsen, S. S. (Ed.). (2003). *Food Analysis Laboratory Manual* (p. 557). Nueva York, USA: Kluwer Academic/Plenum Publishers.
4. Alternate short-form reference — toluene distillation, ASTA 1997)

The same distillation principle, presented in the compact scope/principle/method template used elsewhere in this manual; use whichever your accreditation body specifies.

General Protocol 3

Determination of dry recovery of spices

Dry recovery of spices refers to the quantity of dried spice obtained after drying a specific quantity of fresh spice material. It is obtained as the ratio of dry weight of the sample to the specific fresh weight of the sample. The methodology holds good for all types of spices(seed, rhizomatous, tree spice etc.). Dry recovery is expressed as percentage. It is calculated using the following equation.

$$Dry\ recovery\ (\%) = \frac{Dry\ weight\ of\ sample\ (g)}{Fresh\ weight\ of\ sample\ \ (g)} \times 100$$

Principle

During drying, the weight of the fresh material which is primarily composed of water is significantly reduced as moisture is evaporated and removed, leaving behind the solid dry matter that constitutes the actual spice yield. Free and unbound moisture present in the products are evaporated during drying.

Method (Crop-Wise)

Turmeric

- Weigh 5 kg of freshly harvested spice after washing and cleaning in the ratio 60:20:20 (Primary fingers: Mother rhizomes: Secondary fingers).
- Cook the sample in boiling water for 45 min in a closed vessel.
- Allow it to cool.
- After attaining room temperature, water is drained and rhizomes are spread for sun drying on agricultural tarpaulin made from High Density Polyehylene (HDPE) sheet (150 GSM and above).
- In case, when weather conditions are unsuitable for sun drying (cloudy/rainy), mechanical drying at 55°C can be carried out.
- Mechanical drying should be carried out for 8 hours during day time and switched off and resumed on the second day. When the dryer is switched off, the samples should be stored in a desiccator or spread and kept on a neat platform until it is loaded in the dryer the next day. Likewise, the process can be repeated till constant weight of samples is attained. Constant weight of sample refers to the weight value that remains constant for two consecutive time intervals.
- Note the weight of the sample when constant weight is reached. Under sun drying, turmeric takes nearly 12 – 14 days for proper drying. (However in mechanical drying proper drying is ensured when dried sample reaches constant weight). Final moisture content of turmeric is 10-12%.
- Calculate dry recovery using the formula:

$$Dry\ recovery\ (\%) = \frac{Final\ dry\ weight\ of\ sample\ (g)}{Initial\ fresh\ weight\ of\ sample\ \ (g)} \times 100$$

Ginger

- Weigh 5 kg of freshly harvested ginger after washing and cleaning.
- Carry out partial peeling of the fresh ginger sample.
- Spread the sample for sun drying on an agricultural tarpaulin made from HDPE sheet (150 GSM and above) sheet on a neat concrete floor.
- In caseweather conditions are unsuitable for sun drying (cloudy/rainy), mechanical drying at 55°C can be carried out.
- Mechanical drying should be carried out for 8 hours during day time and switched off and resumed on the second day. When the dryer is switched off, the samples should be stored in a desiccator or spread and kept on a neat platform until it is loaded in the dryer the next day. Likewise, the process can be repeated till constant weight of samples is attained. Constant weight of sample refers to the weight value that remains constant for two consecutive time intervals.
- Note the weight of the sample when constant weight is reached. Under sun drying, turmeric takes nearly 12 – 14 days for proper drying. (However, in mechanical drying proper drying is ensured when dried sample reaches constant weight). Final moisture content of ginger is 10-12%.
- Calculate dry recovery using the formula:

$$Dry\ recovery\ (\%) = \frac{Final\ dry\ weight\ of\ sample\ (g)}{Initial\ fresh\ weight\ of\ sample\ \ (g)} \times 100$$

Black pepper

- Weigh 1 kg of freshly harvested black pepper after washing and cleaning.
- Spread the sample for sun drying agricultural tarpaulin made from HDPE sheet (150 GSM and above).on a neat concrete floor.
- In case, weather conditions are unsuitable for sun drying (cloudy/rainy), mechanical drying at 55°C can be carried out.
- Mechanical drying should be carried out for 8 hours during day time and switched off and resumed on the second day. When the dryer is switched off, the samples should be stored in a desiccator or spread and kept on a neat platform until it is loaded in the dryer the next day. Likewise, the process can be repeated till constant weight of samples is attained. Constant weight of sample refers to the weight value that remains constant for two consecutive time intervals.
- Note the weight of the sample when constant weight is reached. Under sun drying, turmeric takes nearly 12 – 14 days for proper drying. (However in mechanical drying proper drying is ensured when dried sample reaches constant weight). Final moisture content of black pepper is 10-12%.
- Calculate dry recovery using the formula:

$$Dry\ recovery\ (\%) = \frac{Final\ dry\ weight\ of\ sample\ (g)}{Initial\ fresh\ weight\ of\ sample\ \ (g)} \times 100$$

Cardamom

- Weigh 1 kg of freshly harvested cardamom after washing and cleaning.
- Direct sun drying should not be done as it causes chlorophyll degradation and affects the quality of cardamom. Sun drying is recommended under 50% shade net conditions.

- Spread the sample for sun drying on an agricultural tarpaulin made from HDPE sheet (150 GSM and above) on a neat concrete floor. Sun drying of cardamom takes nearly 5 – 6 days to attain final moisture content of 10%.
- In case, when weather conditions are unsuitable for sun drying (cloudy/rainy), mechanical or kiln drying at temperature of 45 °C can be carried out. The drying temperature can be maintained at 47°C, during the last hour of drying. Mechanical and kiln drying of cardamom takes nearly 10-12 and 18 – 22 hours, respectively.
- Calculate dry recovery using the formula:

$$Dry\ recovery\ (\%) = \frac{Final\ dry\ weight\ of\ sample\ (g)}{Initial\ fresh\ weight\ of\ sample\ \ (g)} \times 100$$

Clove

- Weigh 1 kg of freshly harvested clove after washing and cleaning.
- Direct sun drying should affects the quality of clove. Sun drying is recommended under 50% shade net conditions.
- Spread the sample for sun drying as an agricultural tarpaulin made from HDPE sheet (150 GSM and above) on a neat concrete floor. Sun drying of clove takes nearly 4 – 5 days to attain final moisture content of 8–10 %.
- In case, when weather conditions are unsuitable for sun drying (cloudy/rainy), mechanical at temperature of 55 °C can be carried out.
- Mechanical drying should be carried out for 8 hours during day time and switched off and resumed on the second day. When the dryer is switched off, the samples should be stored in a desiccator or spread and kept on a neat platform until it is loaded in the dryer the next day. Likewise, the process can be repeated till constant weight of samples is attained. Constant weight of sample refers to the weight value that remains constant for two consecutive time intervals.
- Note the weight of the sample when constant weight is reached. Final moisture content of clove is 8 – 10%.
- Calculate dry recovery using the formula:

$$Dry\ recovery\ (\%) = \frac{Final\ dry\ weight\ of\ sample\ (g)}{Initial\ fresh\ weight\ of\ sample\ \ (g)} \times 100$$

Cinnamon

- Weigh 1 kg of freshly harvested cinnamon bark.
- Cinnamon is dried under shade to prevent warping. Warping is the appearance of reddish-brown spots on cinnamon on exposure to direct sunlight. Under ambient temperature of 27 – 30 °C and relative humidity of 60 – 70%, cinnamon dries in four days to a final moisture content of 10%.
- Calculate dry recovery using the formula:

$$Dry\ recovery\ (\%) = \frac{Final\ dry\ weight\ of\ sample\ (g)}{Initial\ fresh\ weight\ of\ sample\ \ (g)} \times 100$$

Nutmeg

- Weigh 1 kg of freshly harvested nutmeg after washing and cleaning.
- Spread the sample for sun drying an agricultural tarpaulin made from HDPE sheet (150 GSM and above) on a neat concrete floor.
- In case, when weather conditions are unsuitable for sun drying (cloudy/rainy), mechanical, kiln or biomass drying at 45°C can be carried out. In biomass drying, flue gases must be circulated through pipes and ensure that smoke does not come in direct contact with the product.
- Mechanical drying should be carried out for 8 hours during day time and switched off and resumed on the second day. When the dryer is switched off, the samples should be stored in a desiccator or spread and kept on a neat platform until it is loaded in the dryer the next day. Likewise, the process can be repeated till constant weight of samples is attained. Constant weight of sample refers to the weight value that remains constant for two consecutive time intervals.
- Note the weight of the sample when constant weight is reached. Final moisture content of black pepper is 8 – 10 %.
- Calculate dry recovery using the formula:

$$Dry\ recovery\ (\%) = \frac{Final\ dry\ weight\ of\ sample\ (g)}{Initial\ fresh\ weight\ of\ sample\ \ (g)} \times 100$$

Mace

- Weigh 1 kg of freshly harvested nutmeg after washing and cleaning.
- Spread the sample for sun drying an agricultural tarpaulin made from HDPE sheet (150 GSM and above) on a neat concrete floor.
- In case, when weather conditions are unsuitable for sun drying (cloudy/rainy), mechanical, kiln or biomass drying at 55°C can be carried out. In biomass drying, flue gases must be circulated through pipes and ensure that smoke does not come in direct contact with the product.
- Mechanical drying should be carried out for 8 hours during day time and switched off and resumed on the second day. When the dryer is switched off, the samples should be stored in a desiccator or spread and kept on a neat platform until it is loaded in the dryer the next day. Likewise, the process can be repeated till constant weight of samples is attained. Constant weight of sample refers to the weight value that remains constant for two consecutive time intervals.
- Note the weight of the sample when constant weight is reached. Final moisture content of mace is 8 – 10 %.
- Calculate dry recovery using the formula:

$$Dry\ recovery\ (\%) = \frac{Final\ dry\ weight\ of\ sample\ (g)}{Initial\ fresh\ weight\ of\ sample\ \ (g)} \times 100$$

Seed spices

- Weigh 1 kg of freshly harvested spices after washing and cleaning.
- Spread the sample for sun drying an agricultural tarpaulin made from HDPE sheet (150 GSM and above) on a neat concrete floor.
- In case, when weather conditions are unsuitable for sun drying (cloudy/rainy), mechanical drying at 45 - 50°C can be carried out.

- In case, when weather conditions are unsuitable for sun drying (cloudy/rainy), mechanical drying at 45 - 50°C can be carried out.
- Mechanical drying should be carried out for 8 hours during day time and switched off and resumed on the second day. When the dryer is switched off, the samples should be stored in a desiccator or spread and kept on a neat platform until it is loaded in the dryer the next day. Likewise, the process can be repeated till constant weight of samples is attained. Constant weight of sample refers to the weight value that remains constant for two consecutive time intervals.
- Note the weight of the sample when constant weight is reached. Under sun drying, turmeric takes nearly 12 – 14 days for proper drying. (However in mechanical drying proper drying is ensured when dried sample reaches constant weight). Final moisture content of black pepper is 6 – 8%.
- Calculate dry recovery using the formula:

$$Dry\ recovery\ (\%) = \frac{Final\ dry\ weight\ of\ sample\ (g)}{Initial\ fresh\ weight\ of\ sample\ \ (g)} \times 100$$

Dry Recovery Analysis Parameters for Fresh Spices

Table 3.1 presents the standardised parameters for dry recovery analysis of freshly harvested spices. Drying temperatures and durations are based on ICAR-IISR research and applicable BIS/ISO standards.

Table 3.1 Standardised parameters for dry recovery analysis

Spice	Fresh Sample Wt. (kg)	Pre-Dry Preparation	Drying Temp. (°C)	Drying Duration	Expected Dry Recovery (%)	Reference Method
Turmeric (whole rhizome)	5	Wash, slice 3–5 mm thick	65 ± 2	8–10 h (tray dryer)	18–25	SP 18(Part 13): 1982
Ginger (fresh rhizome)	5	Wash, peel, slice 4–6 mm	60 ± 2	6–8 h	12–18	IS 3433: 2002
Clove (fresh bud)	1	Wipe with dry cloth; no washing	45–50	10–14 h (shade + forced air)	25–30	ISO 2254:2010
Cardamom (fresh capsule)	1	Remove damaged pods; intact only	50 ± 2	36–48 h	70–75 (capsule wt.)	ISO 882-1:2009
Chilli (fresh fruit)	1	Remove stem, deseed (optional)	60 ± 2	8–10 h	10–15	IS 1382: 2002
Coriander (fresh leaf)	1	Trim roots; no wash (weigh as-is)	45	4–5 h	8–12	—
Vanilla (fresh green bean)	1 (whole pods)	Wipe, weigh individually, lay flat	40–45	72–96 h (shade cure)	20–25	ISO 5565:1999
Bay leaf (fresh)	1	Lay flat; no stacking	45–50	4–6 h (shade preferred)	25–35	—
Nutmeg (fresh shelled)	1	Shell, separate kernel	55 ± 2	6–8 h	50–60	ISO 6575:2008
Mace (fresh blade)	1	Spread on tray; no overlap	50	6–8 h	20–30	ISO 6575:2008

General Protocol 4

Volatile Profiling of Spices Using Gas Chromatography–Mass Spectrometry (GC-MS)

Dry recovery of spices refers to the quantity of dried spice obtained after drying a specific quantity of fresh spice material. It is obtained as the ratio of dry weight of the sample to the specific fresh weight of the sample. The methodology holds good for all types of spices(seed, rhizomatous, tree spice etc.). Dry recovery is expressed as percentage. It is calculated using the following equation.

4.1. Scope and Purpose

This section details the standard operating protocol (SOP) for the qualitative and semi-quantitative profiling of volatile organic constituents (essential-oil-type compounds: mono- and sesquiterpene hydrocarbons, oxygenated terpenoids, phenylpropanoids and related aroma compounds) of spices by gas chromatography–mass spectrometry (GC-MS). It covers sample selection and preparation, instrument set-up and operating parameters, data acquisition, compound identification and quantification, method validation, and reporting, The section also includes reference chromatograms and mass spectra for six major spices handled routinely in post-harvest and quality-evaluation work: black pepper, cardamom, clove, cinnamon, ginger and nutmeg.
The protocol is written for use by laboratory analysts, research scholars and technical staff engaged in spice quality evaluation, post-harvest processing studies, geographical-origin/adulteration screening, and nutraceutical or flavour-chemistry research. It is intended to be adapted to the make and model of GC-MS available in the laboratory, and does not replace the instrument manufacturer's own operating manual or the laboratory's document-controlled Quality Manual.

4.1.1 Applicability

- Whole and ground spices, spice oleoresins, essential oils, and spice-derived food and nutraceutical products.
- Volatile and semi-volatile analytes amenable to GC without derivatisation (typically boiling point < 300 °C, MW < 350 Da).
- Not applicable to non-volatile constituents such as piperine, curcuminoids or capsaicinoids, which require HPLC/LC-MS (referenced separately in the LC-MS SOP).

4.2. Principle

Volatile constituents are first liberated from the plant matrix (by hydro-distillation, solvent extraction or headspace sampling) and introduced into a heated GC injector, where they vaporise and are swept by an inert carrier gas (helium or hydrogen) onto a fused-silica capillary column coated with a stationary phase (commonly 5%-phenyl-95%-dimethylpolysiloxane, e.g., DB-5/HP-5/Rtx-5MS, or a polar wax phase such as DB-WAX for isomer resolution). Analytes separate along the column according to their vapour pressure and their differential affinity for the stationary phase, exiting the column at characteristic retention times.

On elution, analytes pass into the mass spectrometer's ion source, most commonly operated by electron ionisation (EI, 70 eV), where each molecule is fragmented in a reproducible manner to yield a characteristic mass spectrum (a fingerprint of ions at specific mass-to-charge, m/z, ratios and relative abundances). The quadrupole or ion-trap mass analyser records the full-scan or SIM (selected-ion-monitoring) spectrum, and the resulting total ion chromatogram (TIC) — a plot of summed ion intensity against retention time — is used together with the individual mass spectra to identify and, where a calibration or internal standard is used, to quantify each volatile constituent.
Identification rests on three converging lines of evidence: (i) comparison of the acquired mass spectrum against a reference mass-spectral library (NIST/Wiley, and the Adams essential-oil library), (ii) confirmation of the retention index (RI/Kovats index) calculated from a co-injected or bracketing homologous series of n-alkanes (typically C7–C30) against literature RI values for the same or an equivalent stationary phase, and (iii) where available, co-injection of an authentic reference standard.

4.3. Materials, Reagents and Reference Standards

4.3.1 Instrumentation

- GC-MS system: gas chromatograph coupled to a quadrupole (or ion-trap/TOF) mass-selective detector, with EI source, autosampler/autoinjector.
- Capillary column: 30 m × 0.25 mm i.d. × 0.25 µm film, 5%-phenyl-methylpolysiloxane (e.g., DB-5MS, HP-5MS, Rtx-5MS or equivalent); a polar wax-type column as a confirmatory/orthogonal column.
- Hydro-distillation apparatus: Clevenger-type apparatus (all-glass) for essential-oil isolation; round-bottom flasks, heating mantle.
- Headspace/SPME accessories (where used): SPME holder and fibres (e.g., 50/30 µm DVB/CAR/PDMS or 100 µm PDMS), headspace vials with PTFE/silicone septa, magnetic stirrer with heating block.
- Analytical balance (0.1 mg readability), oven/hot-air drier for moisture determination, mortar/mixer grinder or cyclone mill for particle-size reduction, sieve set (0.5–1.0 mm).
- Micro-syringes (10 µL, gas-tight where required), amber GC vials with micro-inserts, anhydrous sodium sulphate for drying extracts.

4.3.2 Reagents and Consumables

- Carrier gas: ultra-high-purity helium (99.9995%) or hydrogen, with inline moisture/oxygen traps.
- Solvents (GC/pesticide-residue grade): n-hexane or diethyl ether for oil dilution/extraction; dichloromethane where solvent extraction is used.
- n-Alkane homologous series standard (C7–C30 or C8–C40) for retention-index calibration.
- Authentic reference standards of key marker compounds relevant to the spice under study (e.g., limonene, β-caryophyllene, 1,8-cineole, eugenol, cinnamaldehyde, zingiberene, myristicin) for confirmatory co-injection and calibration curves.
- Internal standard for quantification (e.g., n-tetradecane, methyl nonadecanoate or another compound absent from the sample matrix and eluting in a clean region of the chromatogram).

4.3.3 Reference Libraries

- NIST/EPA/NIH Mass Spectral Library (current version) for automated spectral matching.
- Adams, R.P. — Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry — for RI cross-referencing specific to essential-oil terpenoids (see References).

- Institute/laboratory in-house RI and mass-spectral database built up from previously confirmed standards, to be preferred wherever a conflict arises with the general library.

4.4. Sample Collection and Preparation

4.4.1 Sample Selection and Pre-treatment

1. Collect representative samples following the sampling SOP (*See General Protocol 5 for sampling procedure*); record source, variety/accession, maturity stage, and post-harvest history (drying method, storage duration/conditions).
2. Determine moisture content of the raw material (hot-air oven, 105 °C, or a validated rapid moisture analyser) and report volatile yield on a dry-weight basis.
3. Reduce particle size immediately before extraction (coarse grinding for hydro-distillation; the material should not be over-comminuted, since excessive heat generation during grinding can cause loss of the most volatile fraction).

4.4.2 Isolation of Volatiles — Method Selection

Select the isolation technique according to the objective of the analysis; the technique itself materially affects the observed relative composition (see Section 11, References 6 and 9) and must therefore be reported alongside the results.

4.4.2.1 Hydro-distillation (Clevenger method) — for essential oil / bulk volatile composition

- Weigh 50–100 g of ground/crushed spice into the distillation flask with 300–500 mL distilled water.
- Reflux for 3–4 h (or until oil volume in the graduated arm stabilises); collect the oil layer, dry over anhydrous Na_2SO_4, and store at 4 °C in an amber vial under nitrogen headspace until analysis.
- Dilute an aliquot (e.g., 10–20 mg oil in 1 mL n-hexane, or 1% v/v) for GC-MS injection.

4.4.2.2 Headspace Solid-Phase Microextraction (HS-SPME) — for rapid, solvent-free, non-destructive profiling

- Place 1–2 g ground sample (or a defined volume of oleoresin/oil dilution) in a 20 mL headspace vial; equilibrate at 40–60 °C for 10–15 min with stirring.
- Expose a conditioned SPME fibre (typically DVB/CAR/PDMS for a broad polarity range) to the headspace for 20–40 min at the same temperature.
- Desorb immediately in the GC injector (splitless, 250 °C, 3–5 min) — fibre selection, extraction time and temperature should be optimised and fixed by the laboratory, since these parameters strongly influence the relative recovery of each compound class.

4.4.2.3 Direct Solvent Extraction / Oleoresin Dilution

- For oleoresins or extracted oils already available, dilute directly in GC-grade hexane or ethyl acetate (typically 1:100 to 1:500 v/v) to bring the major constituents within the linear range of the detector; filter through a 0.22 µm PTFE syringe filter into an amber GC vial.

4.5. Safety Precautions

- Handle all organic solvents (hexane, diethyl ether, dichloromethane) in a fume hood; these are flammable and, for chlorinated solvents, suspected carcinogens — use nitrile gloves and safety glasses.
- Compressed carrier-gas cylinders must be secured, leak-tested, and fitted with appropriate regulators; hydrogen carrier gas requires a gas leak sensor near the GC oven.
- Hydro-distillation involves prolonged heating of glassware under reflux — use heating mantles with thermostatic control and never leave unattended.
- Dispose of spent solvent, oil residues and SPME/GC waste as per institutional hazardous-waste protocol; label and log all reference standards (some, e.g., safrole-rich fractions, are regulated substances in some jurisdictions).
- Follow the GC-MS manufacturer's start-up/shutdown sequence to avoid source contamination, filament damage, or column bleed from oxygen ingress.

4.6. Instrumentation and Operating Conditions (Representative Parameters)

The following parameters are representative starting conditions widely used for spice essential-oil/volatile analysis and must be verified and, if necessary, re-optimised for the specific instrument and column in use. Please note that the laboratory's validated method parameters, once established per Section 9, takes precedence over these defaults.

4.6.1 Gas Chromatograph

- Column: 30 m × 0.25 mm i.d. × 0.25 µm, 5%-phenyl-methylpolysiloxane (DB-5MS/HP-5MS/Rtx-5MS or equivalent).
- Carrier gas: helium, constant flow 1.0–1.2 mL/min.
- Injector: split/splitless, 240–260 °C; split ratio 1:20–1:50 for concentrated oils, splitless for trace/HS-SPME analysis; injection volume 0.5–1.0 µL (liquid) or fibre desorption (SPME).

4.6.2 Oven Temperature Programme (representative)

- Initial: 50 °C, hold 2 min
- Ramp 1: 4 °C/min to 150 °C
- Ramp 2: 6 °C/min to 250 °C, hold 5 min
- Total run time: approx. 45 min (adjust to the resolution required and the compound range expected).

4.6.3 Mass Spectrometer

- Ionisation mode: Electron Ionisation (EI), 70 eV.
- Ion source temperature: 230 °C; quadrupole/transfer-line temperature: 250–280 °C.
- Scan range: m/z 40–400 (full scan), 2–3 scans/s, for untargeted profiling; SIM mode with 3–5 characteristic ions per analyte for targeted quantification of specific marker compounds.
- Solvent delay: 3–4 min (to protect the filament from the solvent front).

4.7. Data Acquisition, Peak Identification and Quantification

4.7.1 Retention Index Calculation

Inject the n-alkane series (C7–C30) under the identical temperature programme used for samples. Calculate the linear (Van den Dool and Kratz) retention index for every resolved peak using the retention times of the bracketing n-alkanes eluting immediately before and after the analyte of interest. Accept a tentative identification only when the experimental RI is within approximately ±5–10 RI units of the literature value for the same or an equivalent stationary phase (Section 11, Reference 10).

4.7.2 Spectral Library Matching

Search each deconvoluted mass spectrum against the NIST/Wiley library and the Adams essential-oil library. A match quality/similarity index of ≥ 85–90% is generally required, together with RI agreement, before an identification is reported with confidence; lower-confidence matches should be reported as "tentatively identified" and, where the compound is of analytical or regulatory importance, confirmed by co-injection of an authentic standard.

4.7.3 Quantification

- Area normalisation (%): the simplest approach — each peak area is expressed as a percentage of the total integrated peak area (TIC) of all identified constituents; suitable for relative composition profiling but assumes uniform detector response and is not a true concentration.
- Internal standard method: a fixed amount of internal standard is added to sample and calibration solutions; the response ratio (analyte area/IS area) versus concentration ratio is used to build a calibration curve for accurate quantification of marker compounds.
- External calibration: multi-point calibration curves (typically 5–6 levels, $R^2 \geq 0.995$) prepared from authentic standards for the key marker compounds identified for the spice of interest.

4.7.4 Data Processing Software

Use the instrument's native data system (e.g., ChemStation/MassHunter, GCMSsolution, Xcalibur or equivalent) for automated peak integration and library searching, with manual review of every automatically assigned peak — automated integration frequently mis-splits shoulders on closely eluting terpenoid pairs (e.g., α- and β-pinene; neral and geranial) and these must be checked and corrected manually.

4.8. Representative Chromatograms and Major Volatile Constituents by Spice

The chromatograms and tables below present the qualitative profile typically reported for each spice's essential oil/volatile fraction in the literature (Section 12). They are provided as a training and formatting reference for what a well-resolved TIC and constituent table should look like; they are schematic reconstructions, not raw instrument traces, and every laboratory must record and archive its own chromatograms for each batch analysed.
The reference chromatogram, constituent table and marker mass spectra for each spice covered by this protocol (black pepper, cardamom, clove, cinnamon, ginger, nutmeg) are presented in that spice's own chapter in Part B, alongside its other quality assays, rather than repeated here.

4.9. Representative Mass Spectra of Key Marker Compounds

The spectra below illustrate the principal electron-ionisation (70 eV) fragmentation pattern expected for eight marker volatiles that recur across the spices profiled in Section 8. They are schematic reconstructions of the well-documented diagnostic ions for each compound and are intended to show analysts what a correct, clean spectrum should look like when reviewing automated library-search hits. However, the laboratory's own acquired spectra (compared against NIST/Adams, Section 7.2) remain the record of reference for every reported identification.

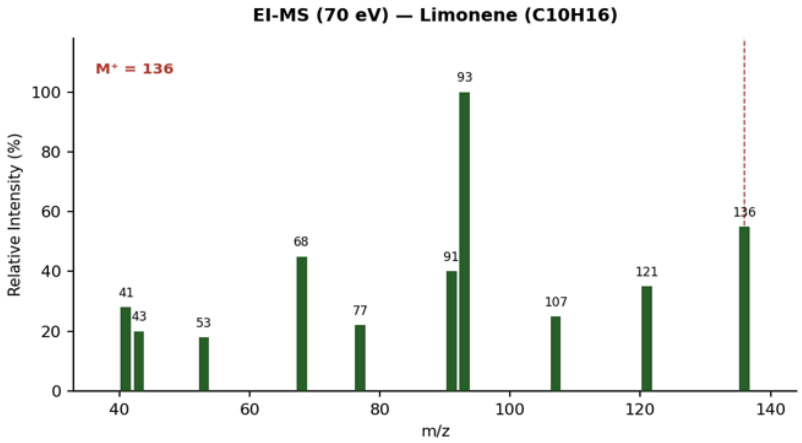


Fig. 4.1 — Limonene (C10H16, MW 136) — major marker in black pepper and nutmeg oils.

***Limonene* (*C10H16*, *MW 136*):** Molecular ion at m/z 136; retro-Diels–Alder cleavage of the cyclohexene ring gives the characteristic base peak at m/z 68 (isoprene radical cation) together with complementary fragments at m/z 93 and 67.

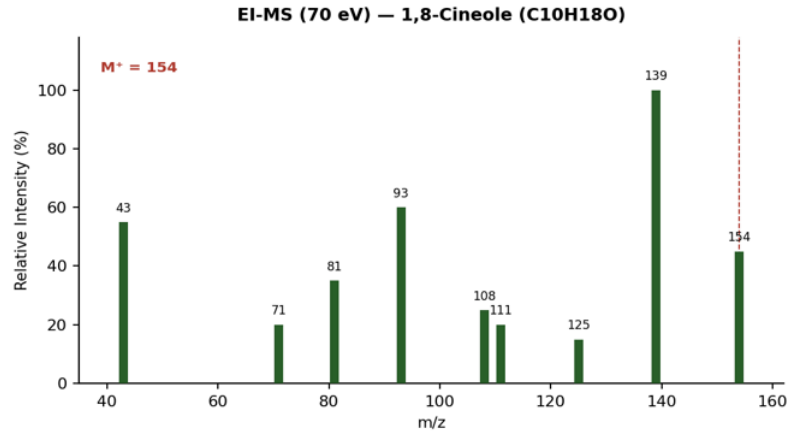


Fig. 4.2 — 1,8-Cineole (C10H18O, MW 154) — major marker in cardamom oil.

1,8-Cineole (C10H18O, MW 154):The cyclic ether ring opens readily under EI, giving the base peak at m/z 139 (loss of ·CH3) and a further diagnostic ion at m/z 43 (acylium-type fragment); the molecular ion at m/z 154 is usually of low intensity.

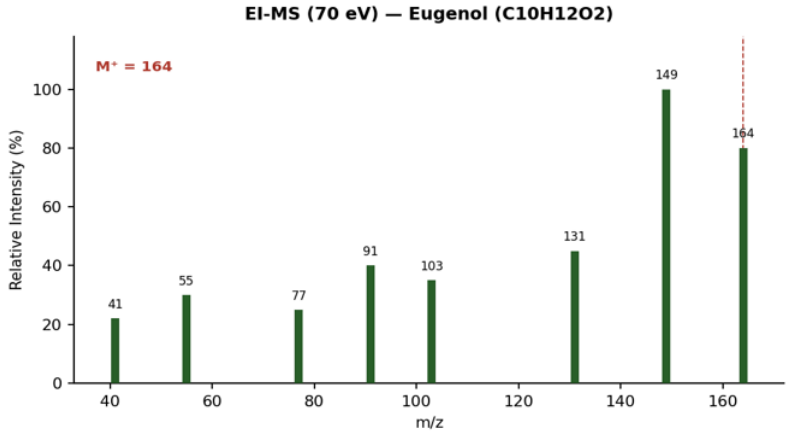


Fig. 4.3 — Eugenol (C10H12O2, MW 164) — major marker in clove and a co-constituent in cinnamon.

Eugenol (C10H12O2, MW 164): The molecular ion at m/z 164 is prominent (phenolic allyl ethers are comparatively stable under EI); loss of ·CH3 gives m/z 149, and further loss of CO/CH2O gives the m/z 131 and 103 series.

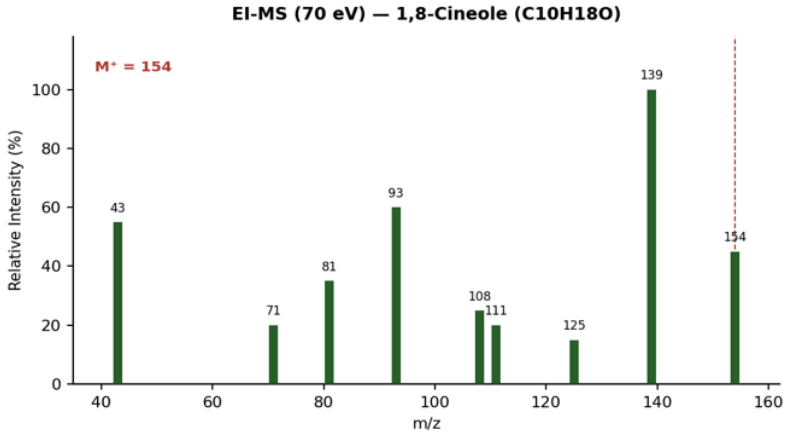


Fig. 4.4 — trans-Cinnamaldehyde (C9H8O, MW 132) — major marker in cinnamon bark oil.

trans-Cinnamaldehyde (C9H8O, MW 132): Molecular ion at m/z 132 with loss of ·CHO giving the base-peak-adjacent ion at m/z 131 (cinnamyl/styryl cation), and further fragmentation to the phenyl-vinyl cation at m/z 103 and phenyl cation at m/z 77.

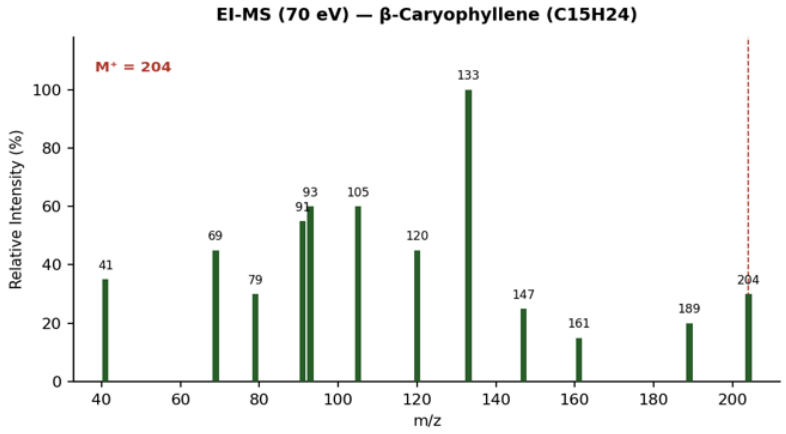


Fig. 4.5 — β-Caryophyllene (C15H24, MW 204) — the dominant sesquiterpene of black pepper and clove oils.

β-Caryophyllene (C15H24, MW 204): A weak molecular ion at m/z 204 is typical of bicyclic sesquiterpene hydrocarbons; the characteristic base peak at m/z 133 arises from cleavage of the cyclobutane ring, with supporting ions at m/z 91, 105 and 120.

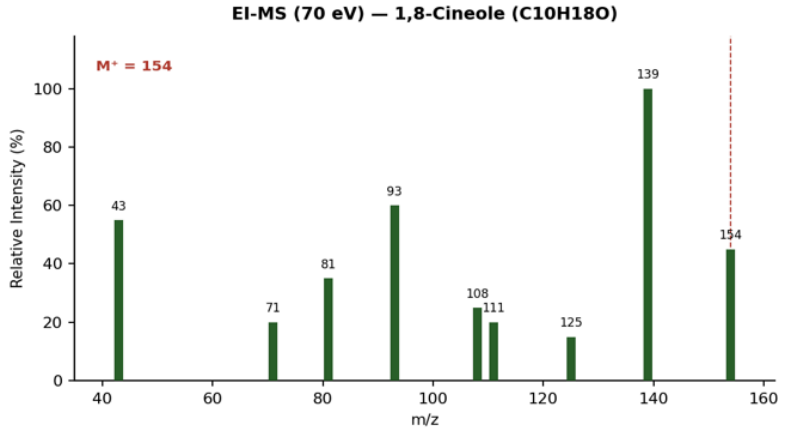


Fig. 4.4 — trans-Cinnamaldehyde (C9H8O, MW 132) — major marker in cinnamon bark oil.

trans-Cinnamaldehyde (C9H8O, MW 132): Molecular ion at m/z 132 with loss of ·CHO giving the base-peak-adjacent ion at m/z 131 (cinnamyl/styryl cation), and further fragmentation to the phenyl-vinyl cation at m/z 103 and phenyl cation at m/z 77.

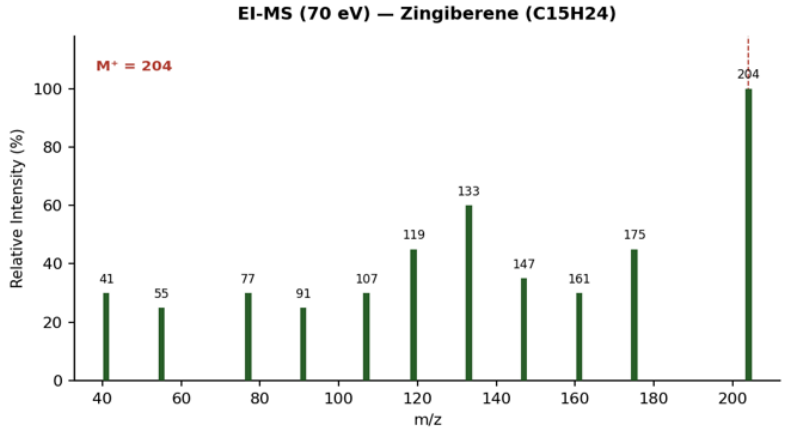


Fig. 4.6 — Zingiberene (C15H24, MW 204) — the principal sesquiterpene marker of ginger oil.

Zingiberene (C15H24, MW 204):Zingiberene shows a comparatively more intense molecular ion at m/z 204 than the caryophyllene-type skeleton, with a characteristic descending ion series at m/z 175, 161, 133 and 119 reflecting sequential methyl and side-chain losses.

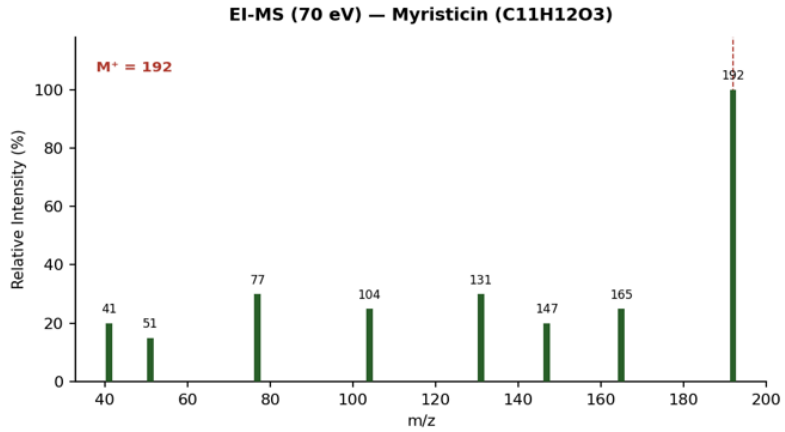


Fig. 4.7 — Myristicin (C11H12O3, MW 192) — the principal phenylpropanoid marker of nutmeg oil.

Myristicin (C11H12O3, MW 192):The methylenedioxy-substituted allylbenzene gives a prominent molecular ion at m/z 192; characteristic losses include the methylenedioxy-related ion at m/z 131 and lower-mass aromatic fragments at m/z 77 and 51.

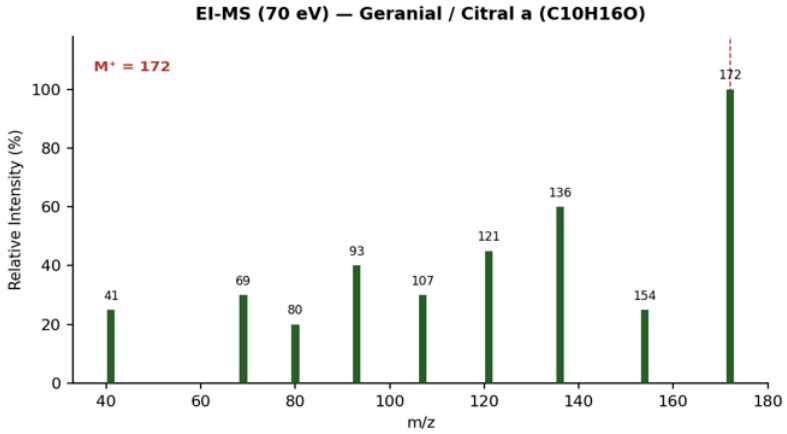


Fig. 4.8 — Geranial / Citral a (C10H16O, MW 152) — a marker acyclic monoterpenal in ginger oil.

Geranial / Citral a (C10H16O, MW 152):The molecular ion at m/z 152 is weak; the base peak at m/z 69 corresponds to the resonance-stabilised C5H9+ fragment common to acyclic monoterpenes, with supporting ions at m/z 41, 84 and 109.

4.10. Method Validation and Quality Control

4.10.1 Parameters to Establish

- ***Linearity***: calibration curve for each targeted marker compound over a concentration range bracketing the expected sample content; $R^2 \geq 0.995$ acceptance criterion.
- ***Limit of Detection (LOD) and Limit of Quantification (LOQ)***: typically estimated from the calibration curve's residual standard deviation and slope (LOD ≈ 3.3σ/S; LOQ ≈ 10σ/S), or from signal-to-noise ratio (S/N ≥ 3 for LOD, S/N ≥ 10 for LOQ).
- ***Precision***: repeatability (intra-day, n ≥ 6 replicate injections) and intermediate precision (inter-day, ≥ 2 days) expressed as % RSD of retention time and peak area/quantified concentration; RSD ≤ 5% for RT and ≤ 10–15% for peak area is a common acceptance target for essential-oil GC-MS work.
- ***Accuracy/recovery***: spike-and-recovery experiments on a representative matrix, targeting 80–120% recovery for marker compounds.

4.10.2 Routine Quality-Control Checks

- Inject a mid-level calibration standard or a well-characterised reference oil at the start and end of each analytical batch to verify instrument response has not drifted.
- Run a solvent blank between samples of widely differing concentration to check for carry-over.
- Monitor column performance (tailing factor, resolution between critical pairs such as α-/β-pinene or neral/geranial) and trim/replace the column when resolution degrades.
- Re-run the n-alkane RI ladder periodically (e.g., at the start of each week of analysis, or after any column/method change) since RI drifts with column ageing.

4.10.3 Record-Keeping

For every batch, archive: raw data files, the integrated TIC with peak table, the library-search report for each identified peak, the RI calculation worksheet, instrument tune/performance check results, and the analyst's log of sample preparation (weights, dilutions, extraction conditions).

This documentation is what converts the illustrative material in Sections 8–9 of this manual into a laboratory's own validated, audit-ready reference dataset.

4.11. Common Problems and Troubleshooting

- ***Poor peak shape/tailing***: check injector liner for contamination, inlet septum for leaks, and column for activity/degradation near the inlet end; consider trimming the column.
- ***Retention-time drift between runs***: verify carrier-gas flow/pressure stability, oven temperature calibration, and that the n-alkane ladder was run under identical conditions.
- ***Ghost peaks / column bleed***: check for oxygen ingress (septum, ferrules), condition the column, verify carrier-gas purity and trap integrity.
- ***Co-elution of closely related terpenoids*** (e.g., limonene/1,8-cineole, neral/geranial): consider an orthogonal polar column (DB-WAX) or adjust the temperature programme's ramp rate in the affected region.
- ***Low/inconsistent recovery by HS-SPME***: re-optimise fibre type, equilibration time, extraction temperature and time, and salt addition (e.g., NaCl) to the sample matrix.
- ***Ambiguous library match***: cross-check RI against at least two independent literature sources (Section 12) and, wherever the compound is analytically significant, confirm with an authentic standard.

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General Protocol 5

Sampling Techniques and Protocols for Spice Quality Analysis

5.1. Introduction

Sampling is the most critical — and often most underestimated — step in the quality analysis of spices. A correctly designed and executed sampling protocol determines whether the test result from a laboratory truly represents the lot under inspection. Errors introduced at the sampling stage cannot be corrected by even the most precise analytical methods downstream. Spices present unique sampling challenges owing to the heterogeneity of raw material lots, the highly volatile nature of key quality constituents (essential oils, oleoresins), and the susceptibility of fresh material to enzymatic and microbial degradation between collection and analysis. This chapter provides standardised, spice-specific sampling procedures that are consistent with national and international regulatory requirements, covering fresh material received at a processing facility, material at the farm gate, and processed/ground product sampled at the factory level.

5.2. Objectives of Sampling

The primary objectives of a scientifically sound sampling programme are:
·To obtain a representative sample of the lot for physical, chemical, and microbiological quality characterisation.

- To determine dry recovery (outturn) of fresh spice raw material.
- To ensure compliance with statutory standards prescribed by regulatory bodies.
- To detect adulteration, contamination, and off-quality material before processing.
- To generate authentic data for traceability documentation and supply chain certification.

5.3. Key Terminology

Lot: A defined quantity of spice from a single origin, handled as a single consignment, from which the sample is drawn.
Primary (Increment) Sample: A small portion taken from a single sampling point in the lot. Also called a 'grab sample' or 'increment'.
Composite Sample: The aggregate of all increment/primary samples from a single lot, mixed thoroughly before sub-division.
Laboratory Sample: The final reduced quantity of composite sample submitted for analysis, obtained by quartering or riffle dividing.
Dry Recovery (Outturn): The percentage by weight of dried product obtained from a known weight of fresh spice material after standardised drying. Expressed as: Dry Recovery (%) = (Weight after drying / Weight before drying) × 100.
Quartering: A sample reduction technique in which the composite sample is spread into a flat circle, divided into four equal quarters, and two opposite quarters are discarded. The remaining two quarters are combined and the process repeated until the required quantity is achieved.
Riffle Divider: A mechanical device used to divide a sample into equal halves by passing material alternately into two receivers through a series of chutes.
Mesh Size / Sieve Aperture: The size of openings in a wire mesh sieve, expressed in micrometres (µm) for fine ground spices or millimetres (mm) for whole/coarse spices. The mesh size determines powder fineness and extraneous matter separation.

5.4. Equipment and Materials Required

5.4.1 General Sampling Equipment

- Grain probe / spear sampler (stainless steel, 80–120 cm length): for bagged lots
- Riffle divider (stainless steel, slot width 12–19 mm)
- Sample splitter / quartering board (stainless steel, 50 × 50 cm)
- Tared sampling containers: sealed polyethylene (PE) bags, paper bags, glass jars with lids
- Top-pan analytical balance (± 0.1 g) and platform balance (± 5 g for bulk)
- Insulated cold box or portable refrigerator (4–10 °C) for fresh material transport
- Sampling labels: tamper-evident, moisture-proof
- Personal protective equipment (PPE): gloves (nitrile), face mask, apron

5.4.2 Equipment for Dry Recovery Analysis

- Hot air oven (tray dryer) with forced air circulation (temperature range: 40–80 °C)
- Silica gel / aluminium alloy drying trays (stainless steel preferred)
- Desiccator with silica gel
- Analytical balance (± 0.01 g) for final weight
- Slicing blade / mandoline slicer (for turmeric, ginger): thickness 3–6 mm
- Vegetable peeler (for ginger)

5.4.3 Sieve Equipment

- IS test sieves (woven wire, stainless steel): 40, 50, 60, 80 mesh (see Table 3 for specifications)
- Rotary / vibratory sieve shaker with timer
- Collection receivers and brush for cleaning

5.5. General Sampling Principles

5.5.1 Random Sampling

All sampling points within a lot must be selected randomly to ensure that every unit in the lot has an equal probability of inclusion. For large lots (>1000 kg), structured random sampling using a systematic grid of sampling points is preferred over purely ad hoc grab sampling.

5.5.2 Minimum Number of Sampling Points

The minimum number of sampling points (n) as a function of lot size is given in Table 5.1 (based on IS 2649 and ISO 948 guidelines):

Table 5.1: Minimum number of sampling points

Lot Size (Packages)	Minimum Sampling Points	Reference Standard
1 – 5	All packages	IS 2649
6 – 50	5	IS 2649
51 – 100	7	IS 2649
101 – 500	10	ISO 948
> 500	15 or √n (whichever is greater)	ISO 948

5.5.3 Composite Sample Formation and Reduction

- Collect all primary (increment) samples in a clean stainless-steel tray or container.
- Mix thoroughly to form the composite sample.
- Use a riffle divider or quartering method to reduce the composite sample to the required aboratory sample size (see Table 1).
- Retain the remaining portion as the reserve/referee sample, sealed and labelled.

5.5.4 Labelling

- Each sample container must be labelled immediately after filling. The label must include:
- Spice name and variety (if known)
- Lot identification number / consignment number
- Date, time, and place of sampling
- Name and designation of sampler
- Sampling stage (farm gate / reception / processing / finished product)
- Intended analysis
- Storage condition requirement

5.6. Spice-Specific Sampling Procedures for Fresh Material

The following sub-sections describe the sampling procedure individually for each major spice, with particular emphasis on parameters relevant to dry recovery analysis. Refer to Table 5.4 for a consolidated summary.

5.6.1 Turmeric (*Curcuma longa* L.)

6.1.1 Fresh Rhizome (Mother and Finger Rhizomes)

Turmeric arrives at processing units as freshly harvested rhizomes, often in gunny bags or loose heap loads. The lot is typically heterogeneous in terms of size, maturity, and surface moisture.
Sampling Procedure:

- Visually inspect the consignment and classify into lots by variety and harvest date.
- For bag lots: insert a grain probe diagonally from the top corner to the bottom of each sampled bag and collect the core as the primary sample.
- For heap / open lots: select a minimum of 10 sampling points in a grid pattern covering top, middle, and bottom layers.
- Collect ≥500 g per sampling point. Combine all primary samples to form the composite.
- Reduce to 500 g laboratory sample using the quartering method.
- For dry recovery analysis: wash rhizomes with clean water, pat dry with a clean cloth, slice transversely to 3–5 mm thickness (use a mandoline slicer for uniformity), and weigh the fresh slices accurately to the nearest 0.1 g before drying.

NOTE: *Separate mother rhizomes from finger rhizomes before weighing if the dry recovery is to be reported for each fraction separately, as mother rhizomes have a lower dry recovery (~18%) compared to fingers (~22–25%).*

5.6.2 Ginger (*Zingiber officinale* Roscoe)

5.6.2.1 Fresh Rhizome

- Fresh ginger is received at processing units with high moisture content (80–82%), requiring prompt handling to prevent enzymatic browning and fibre development.
- Inspect and separate fibrous / old material from fresh market-grade rhizomes.
- Sample from ≥8 points from crates, jute bags, or open heaps.
- Collect ≥500 g composite sample; reduce to 500 g laboratory sample.
- For dry recovery analysis: wash gently under running water, peel using a vegetable peeler or blunt edge (the skin constitutes ~10% of the weight and is normally included in commercial dry recovery), slice to 4–6 mm thickness. Weigh accurately before loading onto drying trays.

NOTE: *In commercial dry recovery, peeled and unpeeled ginger give significantly different results (typically 12–15% peeled vs. 15–18% unpeeled on a fresh weight basis). The procedure must be specified in the report.*

5.6.2.2 Dried Ginger (Sonth)

Dried ginger is sampled using a grain probe from bags, selecting 5–8 points per lot. A 200 g laboratory sample is used for moisture, volatile oil, and oleoresin determination. Mesh: 30–40 for coarse ground; 60 for fine/pharmaceutical grade.

5.6.3 Black Pepper (*Piper nigrum* L.)

5.6.3.1 Fresh Berry (Spike-harvested)

- Fresh black pepper berries are harvested as spikes (drupes attached to the spike). The spikes must be separated before sampling for dry recovery.
- Separate berries from spikes by hand-threshing or rubbing gently. Do not use metal implements (may cause bruising and essential oil loss).
- Sample berries from ≥10 points in the harvested heap, selecting from three vertical levels.
- Form a 300 g composite laboratory sample. Do not wash.
- For dry recovery: fresh berries are placed on stainless-steel trays and shade-dried or mechanically dried at 55 °C until constant weight. Expected dry recovery: 30–35%.

NOTE: *Never use polythene bags for fresh pepper. Jute or cloth bags allow aeration and prevent anaerobic fermentation, which would otherwise elevate apparent moisture loss and distort dry recovery values.*

5.6.3.2 Dried / Cured Black Pepper

Use a grain probe to collect samples from 10 positions in the lot (top, middle, and bottom of each sampled bag). Obtain a 200 g laboratory sample by quartering. Record 1000-berry weight as a quality index alongside moisture and piperine content. Sieve through 40 mesh for ground pepper QC.

5.6.4 Clove (*Syzygium aromaticum* (L.) Merrill & L.M.Perry)

5.6.4.1 Fresh Bud

Clove buds are harvested at the pink bud stage (before anthesis). At this stage, the eugenol content is at its peak. Any delay in handling leads to rapid moisture loss and oil volatilisation.

- Collect samples within 6–8 hours of harvest. Use gloves throughout to minimise handling.
- Wipe each bud cluster gently with a dry cloth. Do NOT wash fresh buds.
- Sample from ≥5 designated points (corners and centre) of the harvested pile or collection trays.
- Form a 200 g composite laboratory sample.
- Immediately record: (a) stem-to-bud ratio by weight, (b) percentage of open (post-anthesis) buds, (c) presence of insect damage.
- For dry recovery: spread buds on a single layer on stainless-steel trays. Dry at 45–50 °C with forced air for 10–14 h, or in shade with good ventilation for 2–3 days. Weigh after cooling in a desiccator. Typical dry recovery: 25–30%.

NOTE: *The calyx (clove head) and stem (clove nail) must both be included in the dry recovery analysis. A high stem-to-bud ratio in the lot is a quality defect and must be reported separately.*

5.6.5 Cardamom (*Elettaria cardamomum* (L.) Maton)

5.6.5.1 Fresh Capsule

Cardamom capsules are harvested individually at the three-quarter ripening stage (pale green colour) to maximise seed content and essential oil.

- At the collection centre / curing unit, inspect the lot for maturity uniformity. Segregate ‘bold’ capsules (>8 mm) from ‘pinhead’ (<5 mm) and ‘medium’ (5–8 mm). Record the proportion of each grade.
- From each grade class, sample randomly from all layers of the collection bag or pile. Minimum 5 sampling points.
- Combine into a 300 g composite sample. Reduce to 300 g laboratory sample (no reduction needed if lot is small).
- Do not wash. For dry recovery: record fresh weight of intact capsules. Arrange on curing trays in a single layer. Cure at 50 ± 2 °C in a mechanical curer for 36–48 h. Reweigh after cooling. Expected dry recovery on capsule weight basis: 70–75%. Seed content within dry capsule: 65–70% of capsule weight.

NOTE: *Dry recovery in cardamom is typically expressed as: (i) capsule dry recovery (fresh capsule to dry capsule), and (ii) seed ratio (dry seed to dry capsule). Both must be reported for commercial purposes.*

5.6.6 Chilli / Red Pepper (*Capsicum annuum* L. and related spp.)

5.6.6.1 Fresh Fruit

- Inspect consignment for uniformity of colour (deep red = mature, optimum for processing).
- Sample from ≥10 points in the bulk heap, using a diagonal grid pattern.
- Form a 500 g laboratory sample by quartering.

- Record colour at this stage using a Minolta chromameter or Lovibond comparator (L*, a*, b* values).
- For dry recovery: remove stems. Optionally remove seeds if seed-free dry recovery is required. Record weight. Dry at 60 ± 2 °C for 8–10 h. Expected dry recovery: 10–15% (whole fruit with seeds) or 8–12% (deseeded flesh only).

NOTE: *Aflatoxin risk is high in chilli. Fresh samples must be analysed within 24 h. Bulk dried chilli for analysis should be kept in cold storage (<8 °C) if the analysis is to be delayed beyond 48 h.*

5.6.7 Coriander (*Coriandrum sativum* L.)

5.6.7.1 Fresh Green Leaf

- Trim roots to 1 cm above the base. Do not wash before weighing (washing introduces surface water and inflates fresh weight).
- Sample 5 bunches randomly from each designated crate or pile. Weigh all sampled material together.
- For dry recovery: spread leaves on trays in a thin layer. Dry at 45 °C for 4–5 h. Expected dry recovery: 8–12%.

5.6.7.2 Dried Seed

Use a grain probe to sample from ≥5 bag positions per lot. Obtain a 300 g laboratory sample by quartering. Pass through a 3 mm round-hole sieve to separate stem/chaff before analysis. Moisture: dried coriander seed should not exceed 9% (FSSAI).

5.6.8 Vanilla (*Vanilla planifolia* Jacks.)

Fresh vanilla beans are rare in commerce as they require immediate curing post-harvest. However, dry recovery determination is critical at the primary curing stage to estimate vanillin yield.
·Collect all pods from a single harvest. Weigh each pod individually. Record pod length (minimum commercial: 10 cm for export, 6 cm for domestic). This constitutes 100% sampling due to the high unit value.

- For the laboratory sample: select 20 pods representing the full length distribution of the lot.
- For dry recovery analysis: wipe pods with a dry cloth. Lay individually on wax-paper-lined trays without overlapping. Dry at 40–45 °C for 72–96 h (or follow traditional Bourbon curing cycle). Expected dry recovery: 20–25% by weight.

5.6.9 Nutmeg (*Myristica fragrans* Houtt.) and Mace

5.6.9.1 Fresh Shelled Nutmeg Kernel

- After dehusking and removal of mace, collect shelled kernels from ≥5 sampling points in the drying yard or fresh collection pile.
- Form a 300 g composite sample.
- For dry recovery: record fresh kernel weight. Dry at 55 ± 2 °C for 6–8 h. Expected dry recovery: 50–60%.

5.6.9.2 Mace (Fresh Aril)

- Collect fresh mace blades immediately after dehusking. Sample across the entire freshly harvested lot.
- Form a 150 g laboratory sample. Spread on a single layer on stainless-steel trays.
- Dry in shade with good ventilation (preferred) or at 50 °C for 6–8 h. Expected dry recovery: 20–30%.

NOTE: *Mace and nutmeg from the same fruit must be processed and recorded as separate samples. They have distinct economic value and distinct dry recovery rates.*

5.6.10 Bay Leaf (Laurus nobilis L.)

- Sample 5–10 leaves from each sampled bundle or tray. Lay flat; do not stack or fold.
- Discard damaged or insect-eaten leaves from the laboratory sample.
- For dry recovery: weigh fresh leaves. Dry at 45–50 °C for 4–6 h. Shade drying preferred for colour retention. Expected dry recovery: 25–35%.

5.7. Master Sampling Requirements for Major Spices

The following table consolidates sampling quantities, container types, storage conditions, and other key parameters for major spices in their common commercial forms.

Table 5.2: Master sampling requirements for major spices

Spice	Market Form	Primary Sample (lot unit)	Lab Sample Qty	Sample Container	Storage Condition	Holding Period	Remarks
Turmeric	Whole rhizome (fresh)	5 kg from each lot	500 g	Polyethylene bag	4 +/- 1 oC	24 h max	Avoid bruising
Turmeric	Cured fingers	3 kg	300 g	Sealed PE bag	Ambient dry	7 days	Wipe surface moisture before weighing
Turmeric	Dried or ground	500 g	100 g	Airtight glass jar	Ambient, dark	30 days	Mesh: 40-60 mesh for powder QC
Ginger	Fresh rhizome	5 kg	500 g	Perforated PE bag	10 +/- 2 oC	24 h max	Peel before dry recovery analysis
Ginger	Dried slices	500 g	200 g	Airtight PE bag	Ambient, dry	30 days	Mesh: 30-40 mesh
Ginger	Ground powder	300 g	100 g	Airtight glass jar	Cool, dark	30 days	Mesh: 60-80 mesh
Black Pepper	Whole berry (fresh)	3 kg from 10 pts	300 g	Jute or cloth bag	Ambient	48 h max	Avoid polythene; aerobic storage preferred
Black Pepper	Dried whole berry	1 kg	200 g	Cloth bag	Cool, dry, dark	60 days	1000-berry weight to be recorded
Black Pepper	Ground pepper	500 g	100 g	Airtight container	Cool, dark	30 days	Mesh: 40 mesh (IS 2645)

Clove	Fresh bud	2 kg from 5 pts	200 g	Perforated paper bag	Ambient, shade	12 h max	Handle with gloves; avoid essential oil loss
Clove	Dried bud	500 g	150 g	Sealed PE bag	Cool, dry	60 days	Stem to bud ratio to be noted
Clove	Ground	300 g	100 g	Airtight dark glass jar	Cool, dark	30 days	Mesh: 50-60 mesh
Cardamom	Fresh capsule	3 kg	300 g	Perforated PE bag	10 +/- 2 oC	24 h max	Record % bold vs. pinhead ratio
Cardamom	Dried capsule	1 kg	200 g	Paper bag	15 +/- 2 oC, RH<65%	60 days	AGMARK grade designation required
Cardamom	Decorticated seeds	300 g	100 g	Airtight dark vial	Cool, dark	30 days	Mesh: 40 mesh
Chilli	Fresh fruit	5 kg	500 g	Vented PE bag	8 +/- 2 oC	24 h max	Record colour (ASTA units)
Chilli	Dried whole	1 kg	300 g	Polythene-lined bag	Ambient, dark	30 days	Aflatoxin risk; cold chain preferred
Chilli	Ground powder	500 g	100 g	Airtight container	Cool, dark	30 days	Mesh: 40-60 mesh
Coriander	Fresh green leaf	1 kg	200 g	Vented PE bag	4 +/- 1 oC	12 h max	Trim roots before weighing
Coriander	Dried seed	1 kg	300 g	Cloth bag	Cool, dry	60 days	Sieve: 3 mm (IS sieve)
Coriander	Ground	500 g	100 g	Airtight container	Cool, dark	30 days	Mesh: 40-60 mesh
Nutmeg	Whole nut (shelled)	3 kg	300 g	Cloth bag	Ambient, dark	7 days	Separate mace before sampling
Mace	Fresh or dried blade	500 g	150 g	Sealed PE bag	Ambient, dark	7 days	Dry in shade before dry recovery
Vanilla	Fresh bean	All pods from lot	20 pods (200 g)	Wax paper + cloth	15-18 oC, RH 60-70%	24 h max	Avoid direct sunlight; measure pod length
Vanilla	Cured bean	20 pods	10 pods (100 g)	Sealed, dark	15 oC, dark	30 days	Vanillin content assay specimen
Fenugreek	Dried seed	1 kg	300 g	Cloth bag	Ambient, dry	60 days	Sieve: 2 mm; record seed weight uniformity
Cumin	Dried seed	1 kg	200 g	Cloth bag	Cool, dark	30 days	Sieve: 2.5 mm (IS standard)
Fennel	Dried seed	1 kg	200 g	Paper bag	Cool, dark	30 days	Mesh: 2 mm; check % stem admixture
Mustard	Fresh seed	2 kg	500 g	Cloth bag	Cool, dry	48 h max	Grade by bold/thin seed ratio
Allspice	Dried berry	1 kg	200 g	Sealed PE bag	Cool, dry	60 days	Mesh: 40 mesh
Bay Leaf	Fresh leaf	500 g	200 g	Perforated bag	4 +/- 1 oC, shade	24 h max	Lay flat; avoid crushing
Bay Leaf	Dried leaf	500 g	150 g	Paper or cloth bag	Ambient, dark	60 days	Whole leaves; mesh not required

5.8. Mesh / Sieve Size Reference for Ground and Whole Spices

The following table (Table 5.3) provides recommended IS sieve numbers, mesh apertures, Tyler equivalents, and intended analytical purpose for ground and whole spice quality analysis. Sieve numbers and apertures conform to IS 460 (Part 1): 1985.

Table. 5.3: Mesh / Sieve size reference for ground and whole spices

Spice / Product	IS Sieve No.	Mesh Size (µm)	Aperture (mm)	Tyler Equiv.	Purpose / Quality Parameter	Standard	Pass Min. (%)
Turmeric powder	60	250	0.25	60	Fineness; curcumin extraction efficiency	IS 1423: 2018	95
Ginger powder	40	425	0.425	35	General quality; oleoresin yield	IS 3433: 2002	90
Ginger powder (special)	60	250	0.25	60	Pharmaceutical / food grade	IS 3433: 2002	85
Black pepper (ground)	40	425	0.425	35	IS quality; piperine content	IS 2645: 2020	90
Cardamom powder	40	425	0.425	35	Essential oil retention	IS 1805: 2011	90
Clove powder	50	300	0.3	48	Eugenol content; surface area	IS 1387: 2015	90
Chilli powder	40	425	0.425	35	ASTA colour units; capsaicin	IS 1382: 2002	95
Chilli powder (extra fine)	60	250	0.25	60	Colour extraction; pharma use	—	90
Coriander powder	40	425	0.425	35	Volatile oil; adulteration check	IS 1575: 2003	90
Coriander seed (whole)	3 mm round hole sieve	3000	3	—	Admixture / extraneous matter	IS 1575: 2003	—
Cumin seed (whole)	2.5 mm round hole	2500	2.5	—	Size grading; impurity	IS 1983: 2009	—
Fenugreek seed	2 mm round hole	2000	2	—	Grading; admixture	IS 5100: 2002	—
Nutmeg (powdered)	40	425	0.425	35	Fixed oil (chloroform extract)	IS 1804: 2015	90
Fennel seed (whole)	2 mm round hole	2000	2	—	Impurity; stem removal	—	—
Allspice (ground)	40	425	0.425	35	Essential oil	ISO 973:1999	90
Vanilla (ground/paste)	60	250	0.25	60	Vanillin; adulteration check	ISO 5565:1999	85

5.9. Spice-Specific Fresh Material Sampling Protocol

A consolidated quick reference for sampling stage, sampling unit, number of sampling points, sub-sampling method, composite sample weight, and critical quality parameters for fresh spice material.

Table 5.4: Quick reference for sampling fresh spices

Spice	Sampling Stage	Sampling Unit	No. of Sampling Points	Sub-sample Method	Composite Sample Wt.	Critical Parameters
Turmeric	At harvest / reception	Gunny bag / heap	≥ 10 points (top, middle, bottom)	Riffle divider or quartering	500 g	Moisture, curcumin, dry recovery
Ginger	Post-harvest / market	Crate / heap / gunny	≥ 8 points	Random grab + quartering	500 g	Moisture, oleoresin, fibre content
Black Pepper	Bunch / spread heap	Cluster of berries	10 sampling points from 3 levels	Grab + riffle divider	300 g	Moisture, bulk density, piperine
Clove	Post-harvested cluster	Polybag / tray	5 sampling points	Random grab (gloved)	200 g	Moisture, eugenol, stem:bud ratio
Cardamom	Freshly harvested lot	Sack / pile	All-layer random grab	Manual selection (20% undamaged)	300 g	Moisture, bold:pinhead ratio, essential oil
Chilli	Bulk heap / lorry	Heap	≥ 10 diagonal points	Grab + quartering	500 g	Moisture, ASTA colour, capsaicin
Coriander (green)	Bundle / tray	Loose bunch	Entire lot (small volume)	Weigh intact; discard roots only	200 g	Dry recovery, essential oil
Vanilla	Harvesting basket	Individual pod	100% inspection (each pod)	Individual pod weigh-in	All pods / 20 for lab	Pod length, moisture, vanillin
Nutmeg	Drying yard / sack	Open bag	5 sampling points	Random grab, separate mace	300 g	Moisture, fixed oil, volatile oil
Mace	Drying tray / heap	Loose blade	Entire thin spread	Random grab across tray	150 g	Moisture, colour, essential oil
Bay leaf	Bundle / tray	Individual leaf / bundle	5 points from bundle	Random leaf selection	200 g	Dry recovery, moisture, volatile oil
Fenugreek	Drying heap / sack	Sack	≥ 5 sampling points	Grain probe / spear sampler	500 g	Moisture, seed uniformity, diosgenin

5.10. Sample Preservation and Chain of Custody

5.10.1 Fresh Material

- Place in food-grade polyethylene bags (perforated for fresh material; sealed for dried material).
- Transport in cold box at 4–10 °C. Analysis must begin within 2–24 h (spice-specific; see Table 5.1).
- Do NOT use chemical preservatives for physical quality parameters (dry recovery, moisture).
- 5.10.2 Dried and Ground Spices
- Store in airtight glass or food-grade PE containers away from light, heat, and moisture.
- Maintain temperature < 25 °C; RH < 60%.
- Label with sample ID, date, and required analyses.

5.10.3 Referee / Reserve Sample

A duplicate of the laboratory sample (equal quantity) must be retained as a referee sample in case of dispute. The referee sample must be:

- Sealed with tamper-evident tape and signed by both the sampler and a witness.
- Stored under the same conditions as the original analytical sample.
- Retained for a minimum of 90 days (fresh) or 6 months (dried / processed) after the analysis report is issued.

5.10.4 Documentation

All samples must be logged in the Laboratory Sample Register (LSR) immediately upon receipt. The LSR entry must capture: sample ID, source/lot number, date received, condition of sample on receipt, assigned analyst, and date of analysis commencement. A chain-of-custody form must accompany each sample from point of collection to final report.

5.11. Applicable Quality Standards and Regulatory References

Spice	BIS Standard	ISO Standard	FSSAI / Regulatory Reference
Turmeric	IS 1423: 2018	ISO 5562: 1984	FSSAI Food Safety Standards, 2011
Ginger	IS 3433: 2002	ISO 1003: 1980	FSSAI 2.10.19 (dried ginger)
Black Pepper	IS 2645: 2020	ISO 959-1: 1998	FSSAI 2.10.4
Cardamom	IS 1805: 2011	ISO 882-1: 1993	Spices Board Cardamom Grade Rules
Clove	IS 1387: 2015	ISO 2254: 2010	FSSAI 2.10.7
Chilli	IS 1382: 2002	ISO 7540: 1984	FSSAI 2.10.6
Coriander	IS 1575: 2003	ISO 2255: 1987	FSSAI 2.10.8
Nutmeg / Mace	IS 1804: 2015	ISO 6575: 2008	FSSAI 2.10.13
Vanilla	—	ISO 5565-1: 1999	FSSAI 2.10.23
Cumin	IS 1983: 2009	ISO 6465: 1985	FSSAI 2.10.9
Fennel	IS 6263: 2002	ISO 5567: 1982	FSSAI 2.10.11
Fenugreek	IS 5100: 2002	—	FSSAI 2.10.12

5.12. Safety and Good Laboratory Practice

- Always wear nitrile gloves and a face mask when handling chilli, clove, and black pepper samples (mucous membrane and skin irritants).
- Use a fume hood when working with volatile oil-rich spices (clove, cardamom, nutmeg) at elevated temperatures.
- Ground spice powders (especially chilli and black pepper) can cause severe respiratory irritation; handle in a laminar flow hood or well-ventilated area.
- Hot-air ovens must be calibrated annually, and temperature uniformity across the tray area must be verified at the beginning of each season.
- Sieves must be cleaned, dried, and inspected for damage before each use. Damaged sieve cloth invalidates results.
- Do not use the same balance for fresh and dried material without zeroing and checking calibration between weighings.

5.13. Common Errors in Spice Sampling and How to Avoid Them

Common Error	Effect on Result	Corrective Action
Sampling only from the top layer of heap or bag	Biased towards surface material; misses bulk quality	Use a grain probe to sample from all depth levels
Washing fresh spice before weighing for dry recovery	Inflates fresh weight; underestimates dry recovery	Wipe only; wash is permissible for turmeric and ginger but pat-dry thoroughly before weighing
Insufficient cooling time before final dry weight reading	Residual heat causes falsely low dry weight	Always cool in a desiccator for a minimum of 30 minutes before weighing
Overlapping material on drying tray	Uneven drying; surface moisture trapped; underestimates dry recovery	Spread material in a single, thin layer; use additional trays if volume is large
Using polythene bags for fresh pepper storage	Anaerobic conditions cause fermentation and off-odour	Use cloth or jute bags; maintain aeration
Failure to separate mace from nutmeg before dry recovery	Mixed recovery figure is commercially meaningless	Always separate aril (mace) from kernel (nutmeg) before analysis
Ignoring stem-to-bud ratio in clove	Dry recovery figure misleadingly high due to heavy stems	Always record stem and bud weights separately
Not recording grade (bold vs. pinhead) in cardamom	Recovery figures not comparable across lots or buyers	Grade before sampling; report dry recovery per grade class
Inconsistent sieve cleaning between samples	Cross-contamination; incorrect particle size distribution	Clean sieve with brush and compressed air between each sample
Using the wrong mesh size for a given spice	Incorrect fineness; non-compliance with IS specification	Refer to Table 3 before setting up sieve analysis

5.14. References

1. Bureau of Indian Standards (BIS). IS 2649: Methods of Test for Spices and Condiments. BIS, New Delhi.
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Part B

Crop-wise Quality Evaluation Protocols

Every assay specific to a spice is collected under this section. Common methods are given in Part A as General Protocol.

1. Black Pepper (*Piper nigrum*)

Ready Reckoner — Black Pepper

One-page workflow from sample receipt to reporting. Test names are hyperlinked to the full, detailed protocol.

Tests/Activity	What to do / Test
Sample receipt	Log sample; record source, variety, moisture history.
Sample prep	Grind coarsely for EO/moisture; grind to pass 60-mesh sieve separately for piperine estimation.
Moisture	Moisture Content (General protocol 2)
Dry recovery	Dry Recovery (General protocol 3)
Essential oil	EO Estimation (General protocol 1)
Bulk density	Bulk Density of Black Pepper
Piperine (routine)	Piperine — Spectrophotometric Method
Piperine (confirmatory)	Piperine — HPLC Method
Volatile profile	GC-MS Reference Chromatogram (full method: General protocol 4)

A.Determination of Bulk Density of Black Pepper

This method is applicable for determining the bulk density of black pepper. The method is based on the basic principle of density which is defined as mass per unit volume.

Apparatus

- One litre measuring cylinder
- A thin strip of straight metal sheet of about 10 mm width and 150 mm length.

Method of analysis

- Fill the 1-liter cylindrical measure with the test sample.
- Tap the measure on a level hard surface three times and fill again as much as possible till the marking.
- Remove the excess material using forceps or spatula and tap again to make it even on the litre marking.
- Weigh the contents in a balance to the nearest g and record themass.
- The mass of one litre of sample provides the Bulk density.

Calculation

Bulk density (g/L) = Mass of sample in gram
Reference: FSSAI 10.018:2021

B.Piperine content in black pepper

The test is specifically done for ground peppers (Black or white) and pepper oleoresin.

Principle

Piperine and its associated piperamides (isochavicine, piperettine and piperyline) are responsible for pungency and tingling sensation in tongue when we consume it. Piperine content of black pepper, white pepper and pepper oleoresin is estimated using spectrophotometric method. These alkaloids are extracted in organic solvents like methanol and ethanol and their absorbance is measured at 343 nm and quantified as compared with standard piperine.

Apparatus and Reagents

- UV- Vis Spectrophotometer
- Volumetric flasks – 100 mL (Amber colour flask is required to reduce photo degradation of piperine in solution)
- Standard denatured alcohol (SDA) – prepared by mixing one litre of 95% ethyl alcohol with 50 mL of methyl alcohol. All dilutions are made with this solvent.
- Standard piperine

Standard preparation and determination of Absorbance Factor

Stock solution of standard piperine is prepared in SDA at 1 mg/mL concentration. First working standard is prepared by diluting stock with SDA at 1:10 ratio. A range of working standards is prepared by further diluting 1, 2, 3, 4, 5, 6 mL of aliquots into 100 mL respectively and made up to volume with SDA. The absorbance (A) of the solution is measured in spectrophotometer at 343 nm using denatured alcohol as blank. Each absorbance value is divided by quantity of mL used in final dilution to convert absorbance to concentration ($1/10^6$ µg/mL). The six values obtained are averaged to obtain average absorbance of standards (A_{avg}) normalized to 1µg/mL for each standard.

$$A_{avg} = \left(\frac{A1}{1} + \frac{A2}{2} + \frac{A3}{3} + \frac{A4}{4} + \frac{A5}{5} + \frac{A6}{6}\right) \div 6$$

Sample measurement

a)Black and white pepper

Grind sample to pass 60-mesh sieve and blend uniformly. Sample test portion (0.5 g) is transferred to round bottom flask or Erlenmeyer flask and 70 mL of denatured alcohol is added and is refluxed for 1h.The content is cooled and brought to room temperature, and filtered into 100mL volumetric flask. The extract is washed thoroughly and made up to volume with SDA. From this 2mL is diluted to 100 mL and theabsorbance is measured at 343 nm.

b)Pepper Oleoresin

The oleoresin samples are analysed as the same way as before by dissolving the oleoresin directly in SDA and made dilution further before taking spectrophotometric reading at 343 nm.

Calculation

$$\text{Piperine \%} = \frac{As}{Aavg} \times \frac{V}{Ws\ x10^6} \times 100$$

Where As = absorbance of test solution; Aavg = Average of standard absorbances, each normalized to 1µg/mL; V = dilution volume, mL; Ws= test portion weight, g.

Reference: *ASTA method 12.1; FSSAI 10.020:2021*

C.HPLC quantification of Piperine

As spectrophotometric method estimates piperine and its analogues as well, advanced instruments like High Performance Liquid Chromatography (HPLC) which could separately detect piperine is adopted for selected analysis. UV spectrophotometric method is quite fast and hence adopted for routine estimation of large number of samples.

Principle

Piperine in the sample is separated using reverse phase analysis system and the concentration is calculated in comparison with peak area of piperine standard

Reagents

- HPLC grade acetonitrile
- HPLC grade water
- HPLC grade acetic acid
- The Mobile phase used in this method are A: 1% aqeous acetic acid; B: Acetonitrile. Solvents should be degassed before use.
- Standard Piperine of at least 98% purity

HPLC run condition

The piperine extracted in ethanol is separated in a RP-C18 column in an isocratic elution of 50:50 of mobile phase A and B with injection volume of 20 µL; flow rate – 1.5 mL/minute; Run time varies from 15-20 min depending on make of HPLC. Conditions have to suitably modified for UHPLC and other liquid chromatographic gadgets.

Preparation of Calibration curve

Standard piperine is prepared by dissolving 100 mg of piperine in 100 mL of ethanol and used immediately. A standard curve is prepared using piperine dilution from 0.05 mg/mL to 0.5mg/mL concentrations. The calibration curve is plotted using peak area versus piperine concentration and and linear regression equation, y = mx + c is obtained. The constant m is the slope of the curve, c is the y intercept and can be determined by extrapolating the straight line to the y axis.
From this piperine content of the sample is calculated and expressed as % piperine.
Reference: Wood *et al*, 1988

GC-MS Reference Chromatogram and Major Volatile Constituents

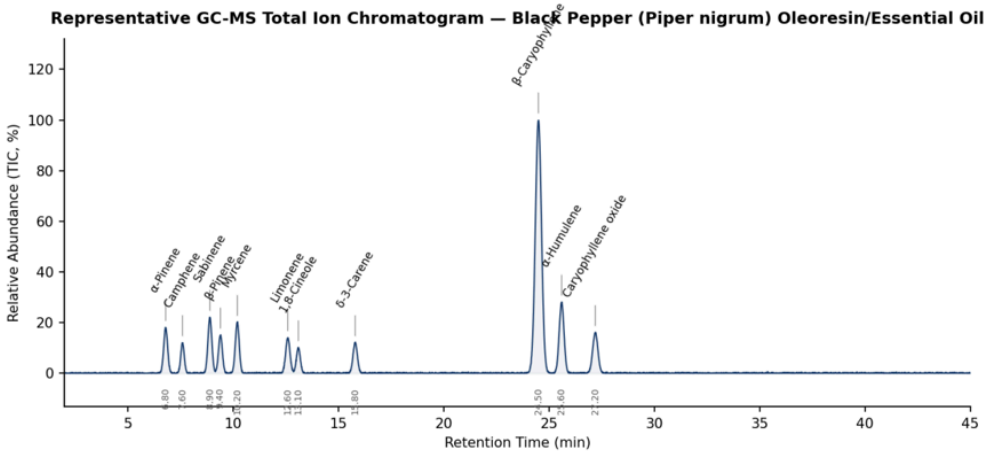


Fig. 1. Representative TIC of black pepper essential oil, dominated by the sesquiterpene β-caryophyllene.

Black pepper (Piper nigrum) essential oil composition is strongly influenced by geographical origin and post-harvest processing: β-caryophyllene is consistently reported as the principal component (approx. 20–42%), with limonene, sabinene, β-pinene and δ-3-carene as major co-constituents; piperine, the pungency principle, is non-volatile and is not seen in the GC-MS volatile-oil profile — it is analysed separately by HPLC/LC-MS (Section 1.1).

Compound	RT (min)*	RI (DB-5, lit.)	Class	Typical % (range)	Key diagnostic ions (m/z)
α-Pinene	6.8	932–939	Monoterpene HC	1–5	93, 91, 77, 41, 136
Sabinene	8.9	969–975	Monoterpene HC	3–9	93, 91, 77, 41, 136
β-Pinene	9.4	974–979	Monoterpene HC	5–8	93, 69, 41, 136
Myrcene	10.2	988–991	Monoterpene HC	2–7	93, 69, 41, 136
Limonene	12.6	1024–1029	Monoterpene HC	10–15	68, 67, 93, 136
δ-3-Carene	15.8	1008–1011	Monoterpene HC	up to 23 (origin-dependent)	93, 91, 136, 121
β-Caryophyllene	24.5	1417–1420	Sesquiterpene HC (major)	20–41	133, 91, 105, 120, 204
α-Humulene	25.6	1452–1455	Sesquiterpene HC	3–9	93, 80, 121, 204
Caryophyllene oxide	27.2	1580–1583	Oxygenated sesquiterpene	1–5	79, 91, 109, 220

**Retention times are illustrative, generated for a 30 m × 0.25 mm × 0.25 µm 5%-phenyl-methylpolysiloxane (DB-5/HP-5 equivalent) column under the temperature program in Section 6.2; actual RT will shift with column, flow and oven program and must always be confirmed against the laboratory's own n-alkane RI ladder and NIST/Adams library match.*

2. Cardamom (*Elettaria cardamomum*)

Ready Reckoner — Cardamom

One-page workflow from sample receipt to reporting. Test names are hyperlinked to the full, detailed protocol.

Tests/Activity	What to do / Test
Sample receipt	Log sample; note capsule maturity/curing method.
Sample prep	Crack pods, separate seeds and grind (see Notes, General protocol 1)
Moisture	Moisture Content (General protocol 2)
Dry recovery	Dry Recovery (General protocol 3)
Essential oil	EO Estimation (General protocol 1) — 20 g seed sample, water distillation.
Bulk density	Bulk Density measurement of Cardamom
Volatile profile	GC-MS Reference Chromatogram (full method: General protocol 4)

GC-MS Reference Chromatogram and Major Volatile Constituents

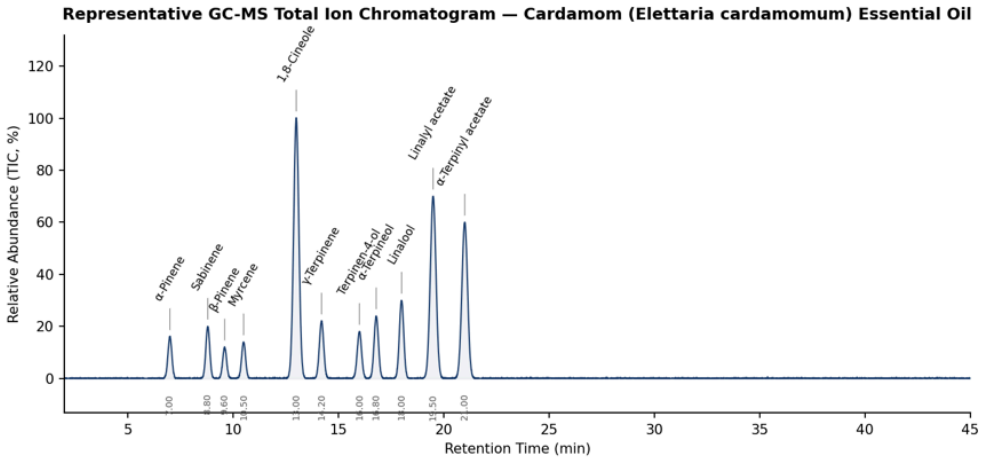


Fig.2. Representative TIC of cardamom essential oil, dominated by 1,8-cineole with linalyl/α-terpinyl acetate

Cardamom oil is characterised by two dominant classes — the ether 1,8-cineole and the ester α-terpinyl acetate — whose relative proportion varies with capsule maturity at harvest and curing method, making this ratio a useful post-harvest quality/maturity marker.

Compound	RT (min)*	RI (DB-5, lit.)	Class	Typical % (range)	Key diagnostic ions (m/z)
α-Pinene	7	932–936	Monoterpene HC	1–3	93, 91, 136
Sabinene	8.8	969–973	Monoterpene HC	2–5	93, 91, 136
1,8-Cineole (Eucalyptol)	13	1026–1031	Oxygenated monoterpene (major)	20–47	43, 81, 93, 108, 154
Linalool	18	1095–1099	Oxygenated monoterpene	2–8	71, 93, 121, 136
Linalyl acetate	19.5	1253–1257	Ester	5–20	80, 93, 121, 136
Terpinen-4-ol	16	1174–1178	Oxygenated monoterpene	1–4	71, 93, 111
α-Terpineol	16.8	1186–1190	Oxygenated monoterpene	1–5	59, 93, 121, 136
α-Terpinyl acetate	21	1346–1350	Ester (major)	15–47	80, 93, 121, 136

**Retention times are illustrative, generated for a 30 m × 0.25 mm × 0.25 µm 5%-phenyl-methylpolysiloxane (DB-5/HP-5 equivalent) column under the temperature program in Section 6.2; actual RT will shift with column, flow and oven program and must always be confirmed against the laboratory's own n-alkane RI ladder and NIST/Adams library match.*

3. Clove (*Syzygium aromaticum*)

Ready Reckoner — Clove

Tests/Activity	What to do / Test
Sample receipt	Log sample; record bud vs stem fraction.
Sample prep	Coarsely grind fresh sample.
Moisture	Moisture Content (General protocol 2)
Dry recovery	Dry Recovery (General protocol 3)
Essential oil	EO Estimation (General protocol 1) — 10 g sample, 10% NaCl solution, xylene trap additive 2.0 mL.
Bulk density	Bulk Density of Clove
Volatile profile	GC-MS Reference Chromatogram (full method: General protocol 4)

GC-MS Reference Chromatogram and Major Volatile Constituents

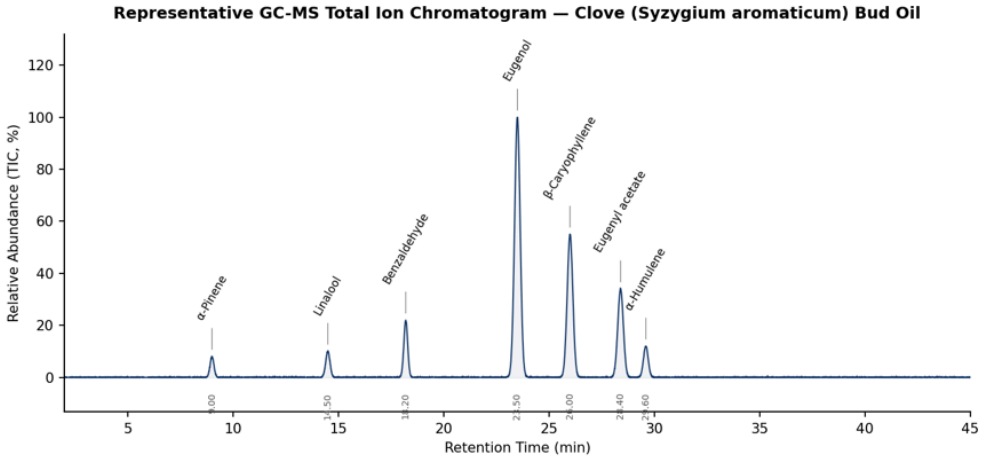


Fig. 3. Representative TIC of clove bud oil, showing the characteristic eugenol-dominated profile.

Clove bud oil is one of the simplest and most reproducible spice-oil profiles for method verification: eugenol alone typically contributes 70–90% of the total peak area, making it an ideal marker compound for injector/column performance checks and for HS-SPME fibre comparison studies.

Compound	RT (min)*	RI (DB-5, lit.)	Class	Typical % (range)	Key diagnostic ions (m/z)
Benzaldehyde	18.2	960–963	Aromatic aldehyde	0.2–1	77, 105, 106
Eugenol	23.5	1356–1360	Phenylpropanoid (major)	70–90	164, 149, 103, 77
β-Caryophyllene	26	1417–1420	Sesquiterpene HC	5–15	133, 91, 105, 204
Eugenyl acetate	28.4	1523–1527	Ester	2–15	163, 164, 121
α-Humulene	29.6	1452–1456	Sesquiterpene HC	0.5–2	93, 80, 121, 204

**Retention times are illustrative, generated for a 30 m × 0.25 mm × 0.25 µm 5%-phenyl-methylpolysiloxane (DB-5/HP-5 equivalent) column under the temperature program in Section 6.2; actual RT will shift with column, flow and oven program and must always be confirmed against the laboratory's own n-alkane RI ladder and NIST/Adams library match.*

4. Cinnamon (*Cinnamomum verum*)

Ready Reckoner — Cinnamon

Stage	What to do / Test
Sample prep	Coarsely grind fresh sample (General protocol 5)
Moisture	Moisture Content (General protocol 2)
Dry recovery	Dry Recovery (General protocol 3)
Essential oil	EO Estimation (General protocol 1) — 40 g sample, 10% NaCl solution.
Volatile profile	GC-MS Reference Chromatogram (full method: General protocol 4)

GC-MS Reference Chromatogram and Major Volatile Constituents

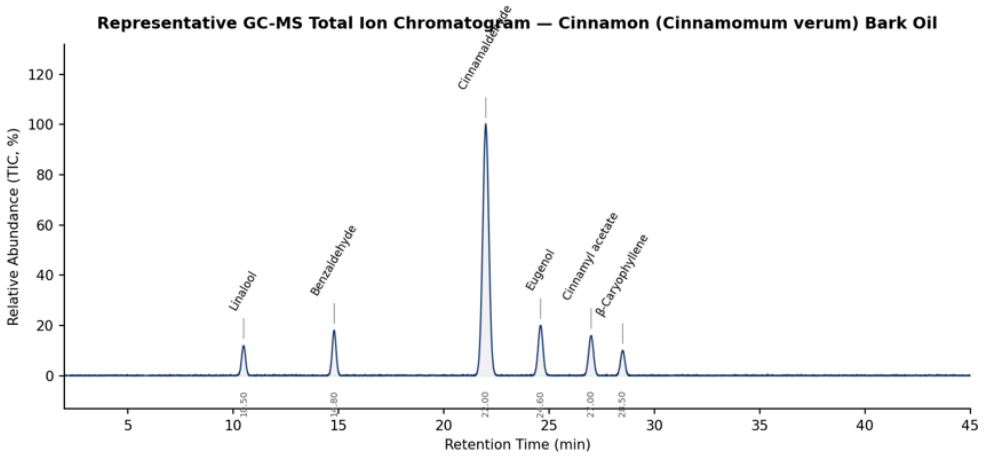


Fig.4. Representative TIC of true cinnamon bark oil, dominated by trans-cinnamaldehyde.

***Note**: Bark oil (true cinnamon, C. verum) is cinnamaldehyde-dominant, whereas cassia (C. cassia/aromaticum, often traded as "cinnamon") shows an even higher relative cinnamaldehyde content (commonly >80%) — a distinction routinely used for species/authenticity screening of commercial cinnamon.*

Compound	RT (min)*	RI (DB-5, lit.)	Class	Typical % (range)	Key diagnostic ions (m/z)
Linalool	10.5	1095–1099	Oxygenated monoterpene	1–5	71, 93, 121, 136
Benzaldehyde	14.8	960–963	Aromatic aldehyde	0.5–2	77, 105, 106
trans-Cinnamaldehyde	22	1267–1271	Phenylpropanoid (major)	60–85	131, 103, 77, 132
Eugenol	24.6	1356–1360	Phenylpropanoid	2–10	164, 149, 103
Cinnamyl acetate	27	1445–1449	Ester	1–8	131, 91, 43

**Retention times are illustrative, generated for a 30 m × 0.25 mm × 0.25 µm 5%-phenyl-methylpolysiloxane (DB-5/HP-5 equivalent) column under the temperature program in Section 6.2; actual RT will shift with column, flow and oven program and must always be confirmed against the laboratory's own n-alkane RI ladder and NIST/Adams library match.*

5. Ginger (*Zingiber officinale*)

Ready Reckoner — Ginger

Stage	What to do
Sample receipt	Log sample; record fresh vs dried/split.
Sample prep	Crack before grinding (see General protocol 1).
Moisture	Moisture Content (General protocol 2)
Dry recovery	Dry Recovery (General protocol 3)
Essential oil	EO Estimation (General protocol 1) — 50 g sample, 10% NaCl solution.
Fibre content	Crude Fibre Content of Ginger — maturity/quality indicator.
Volatile profile	GC-MS Reference Chromatogram (full method: General protocol 4)

Crude Fibre Content of Ginger

Crude fibre content is a key maturity and quality indicator for dried ginger: rhizomes harvested before or after the optimum 8–9-month window, or ginger held in storage too long, develop higher lignified fibre, which lowers culinary/processing value and is penalised in trade grading.

Principle

Crude fibre is the organic residue remaining after sequential digestion of a defatted, dried sample with dilute sulphuric acid and dilute sodium hydroxide under reflux, filtered, dried and weighed; the loss in weight on subsequent ashing of this residue gives the crude fibre content (AOAC 962.09; ISO 5498).

Apparatus

- Fibre digestion (reflux) apparatus with 600 mL tall-form beakers/flasks and water-cooled condensers (or an equivalent Soxhlet-type crude-fibre extraction unit)
- Buchner funnel or sintered-glass crucible (porosity 1) with vacuum filtration assembly, or muslin cloth for filtration
- Muffle furnace capable of 550 ± 25 °C
- Hot-air oven (130 °C), desiccator, analytical balance (0.1 mg)
- Soxhlet apparatus for pre-defatting (recommended for ginger, given its oleoresin content)

Reagents

- Sulphuric acid, 1.25% w/v (0.255 N)
- Sodium hydroxide, 1.25% w/v (0.313 N)
- Petroleum ether (40–60 °C), for defatting
- Acetone, for the final wash

Sample Preparation

- Grind dry ginger to pass an 18-mesh (1 mm) sieve; use a freshly ground sample, as for EO estimation (General protocol 1).
- Where the oleoresin/oil content is high, defat approximately 2–5 g of the ground sample by Soxhlet extraction with petroleum ether for 1 hour before digestion, and air-dry to remove residual solvent.

Method of Analysis

- Accurately weigh 2 g of the defatted, dried sample (W_0) into the digestion flask.
- Add 200 mL of pre-boiled 1.25% H_2SO_4; reflux for exactly 30 minutes from the onset of boiling, swirling periodically.
- Filter hot; wash the residue with boiling water until the washings are acid-free (check with pH paper).
- Return the washed residue to the flask; add 200 mL of pre-boiled 1.25% NaOH and reflux for a further 30 minutes.
- Filter hot as before; wash successively with boiling water, then a small volume of acetone.
- Transfer the residue to a pre-weighed crucible and dry to constant weight in a hot-air oven at 130 °C (~2 h); cool in a desiccator and weigh (W_1).
- Ash the dried residue in a muffle furnace at 550 ± 25 °C for 30 minutes to constant weight; cool in a desiccator and weigh (W_2).

Calculation

$$\textit{Crude fibre (\%, on the sample basis)} = [(W_1 - W_2) / W_0] \times 100$$

Where
W_0 = initial sample weight (g);
W_1 = weight of the oven-dried residue (g); and
W_2 = weight of the residue after ashing (g).
Report on a dry-weight basis using the moisture content obtained in parallel by General protocol 2.
Typical crude fibre content of well-matured dry ginger (harvested at 8–9 months) falls in the range of about 2.5–6% on a dry-weight basis; material well above this range should be graded accordingly.

Reference: AOAC 962.09; ISO 5498:2020; Jayashree, E., Visvanathan, R. (2011). Physical and biochemical parameters of fresh and dry ginger (*Zingiber officinale* Roscoe). Journal of Spices and Aromatic Crops, 20(1), 14–21.

GC-MS Reference Chromatogram and Major Volatile Constituents

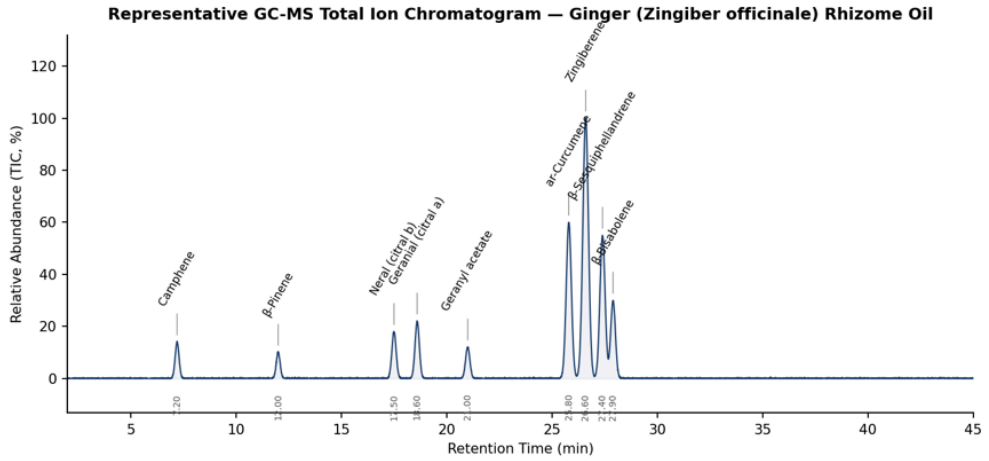


Fig.5. Representative TIC of ginger rhizome oil, dominated by the sesquiterpenes zingiberene and ar-curcumene.

Zingiberene content is markedly method-dependent: HS-SPME with a PDMS fibre has been reported to recover a substantially higher relative proportion of zingiberene than petroleum-ether extraction or steam distillation on the same rhizome material (Section 12, Reference 8), reinforcing the requirement to fix and report the isolation method used.

Compound	RT (min)*	RI (DB-5, lit.)	Class	Typical % (range)	Key diagnostic ions (m/z)
Camphene	7.2	946–950	Monoterpene HC	5–10	93, 121, 136
Neral (citral b)	17.5	1225–1229	Monoterpene aldehyde	5–15	69, 94, 109, 152
Geranial (citral a)	18.6	1255–1259	Monoterpene aldehyde	8–20	69, 94, 109, 152
ar-Curcumene	25.8	1479–1483	Sesquiterpene HC	10–18	119, 132, 157, 202
Zingiberene	26.6	1493–1497	Sesquiterpene HC (major)	20–53	93, 119, 161, 204
β-Sesquiphellandrene	27.4	1518–1522	Sesquiterpene HC	8–20	93, 119, 161, 204
β-Bisabolene	27.9	1508–1512	Sesquiterpene HC	3–10	69, 93, 204

**Retention times are illustrative, generated for a 30 m × 0.25 mm × 0.25 µm 5%-phenyl-methylpolysiloxane (DB-5/HP-5 equivalent) column under the temperature program in Section 6.2; actual RT will shift with column, flow and oven program and must always be confirmed against the laboratory's own n-alkane RI ladder and NIST/Adams library match.*

6. Nutmeg (*Myristica fragrans*)

Ready Reckoner — Nutmeg

Stage	What to do / Test
Sample receipt	Log sample; record whole/broken kernel or mace.
Sample prep	Coarsely grind fresh sample.
Moisture	Moisture Content (General protocol 2)
Dry recovery	Dry Recovery (General protocol 3)
Essential oil	EO Estimation (General protocol 1) — 30 g kernel / 20 g mace, water distillation.
Volatile profile	GC-MS Reference Chromatogram (full method: General protocol 4)
Report	Compile results and issue Certificate of Analysis.

GC-MS Reference Chromatogram and Major Volatile Constituents

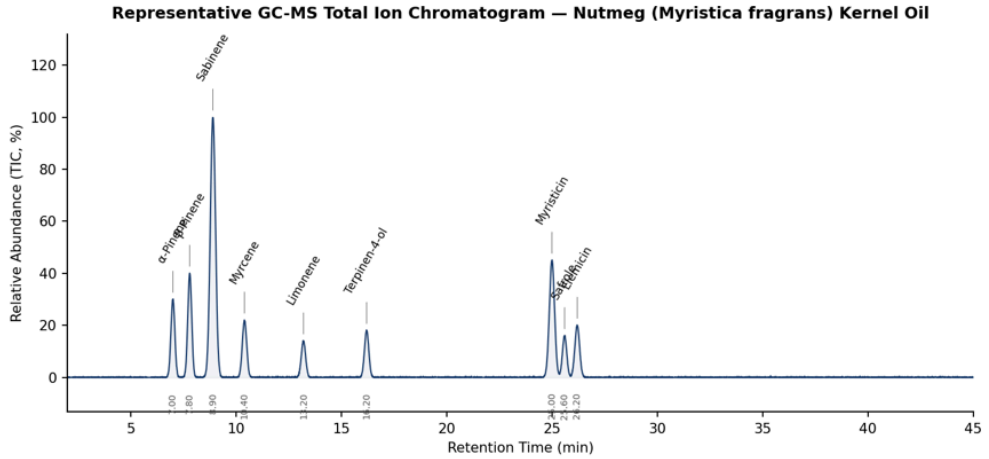


Fig.6. Representative TIC of nutmeg kernel oil, showing the characteristic monoterpene-hydrocarbon-rich profile with the phenylpropanoid myristicin.

Myristicin is the principal aromatic/psychoactive marker in nutmeg oil and, together with the sabinene/pinene monoterpene fraction, is routinely monitored for quality and safety screening given myristicin's reported physiological activity at high doses.

Compound	RT (min)*	RI (DB-5, lit.)	Class	Typical % (range)	Key diagnostic ions (m/z)
α-Pinene	7	932–936	Monoterpene HC	8–15	93, 91, 136
β-Pinene	7.8	974–978	Monoterpene HC	8–15	93, 69, 136
Sabinene	8.9	969–973	Monoterpene HC (major)	15–30	93, 91, 136
Myrcene	10.4	988–992	Monoterpene HC	1–3	93, 69, 136
Limonene	13.2	1024–1029	Monoterpene HC	2–5	68, 93, 136
Terpinen-4-ol	16.2	1174–1178	Oxygenated monoterpene	2–8	71, 93, 111
Safrole	25.6	1284–1288	Phenylpropanoid	0.5–3	104, 135, 162
Myristicin	25	1517–1521	Phenylpropanoid (major aromatic)	5–20	192, 91, 77, 131
Elemicin	26.2	1553–1557	Phenylpropanoid	1–6	208, 193, 165

**Retention times are illustrative, generated for a 30 m × 0.25 mm × 0.25 µm 5%-phenyl-methylpolysiloxane (DB-5/HP-5 equivalent) column under the temperature program in Section 6.2; actual RT will shift with column, flow and oven program and must always be confirmed against the laboratory's own n-alkane RI ladder and NIST/Adams library match.*

7. Turmeric (*Curcuma longa*)

Ready Reckoner — Turmeric

Stage	What to do / Test
Sample receipt	Log sample; record cured/polished status.
Sample prep	Crack before grinding (see General protocol 1).
Moisture	Moisture Content (General protocol 2)
Dry recovery	Dry Recovery (General protocol 3)
Essential oil	EO Estimation (General protocol 1)
Curcuminoids (routine)	Curcuminoid Content — Spectrophotometric Method
Curcuminoids (confirmatory)	Curcuminoid Content — HPLC Method

Curcuminoid Content — Spectrophotometric Method

Curcuminoids (curcumin, demethoxycurcumin and bisdemethoxycurcumin) are the principal colouring and bioactive constituents of turmeric and the primary chemical basis for its trade grading (Chapter 9). This spectrophotometric method is a fast, routine screening method; it does not resolve the individual curcuminoids and tends to read marginally higher than HPLC because other ethanol-soluble pigments absorb at the same wavelength (Taylor & McDowell, 1992) — confirm borderline or disputed results by the HPLC method that follows.

Principle

Curcuminoids are extracted from turmeric powder with ethanol and the absorbance of the extract is measured at 425 nm against an ethanol blank. Content is calculated either from a curcumin reference calibration curve or from the internationally recognised specific absorbance of curcumin in ethanol at 425 nm ($E^{1\%}_{1cm}$ = 1607), applied as the reference value for total curcuminoids (JECFA).

Apparatus

- UV-Vis spectrophotometer with matched 1 cm quartz or glass cuvettes
- Analytical balance (0.1 mg); volumetric flasks (50/100 mL), amber or foil-wrapped
- Magnetic stirrer/orbital shaker or ultrasonic bath
- Whatman No. 1 filter paper / 0.45 µm membrane filter

Reagents

1. Absolute (or 95%) ethanol, spectroscopic grade
2. Curcumin reference standard (≥95% purity), for the optional calibration-curve approach

Sample Preparation

Work in subdued light throughout, since curcuminoids are photolabile; use amber glassware or foil-wrapped flasks.

- Grind the turmeric sample to pass a 60-mesh sieve.
- Weigh 0.1 g (± 0.001 g) of the powder into a 100 mL amber volumetric flask.

Method of Analysis

- Add approximately 70 mL of ethanol and extract by stirring/sonicating in the dark for 1–2 hours (or by reflux for 2.5 hours at moderate heat), until the residue is exhausted of colour.
- Make up to volume (100 mL) with ethanol; mix and filter through Whatman No. 1 paper, discarding the first few mL of filtrate.
- Dilute an aliquot of the filtrate with ethanol as needed to bring the absorbance within the 0.2–0.8 range.
- Measure the absorbance (A) at 425 nm against an ethanol blank.
- If using the calibration-curve approach, prepare a curcumin standard curve over 1–7 µg/mL in ethanol at 425 nm and read the sample concentration from it.

Calculation

$$\textit{Total curcuminoid content (\%, w/w, on the basis analysed)} = (A \times V \times DF \times 100) / (1607 \times W \times 100)$$

Where,
A = absorbance at 425 nm;
V = total volume of extract (mL);
DF = additional dilution factor, if any;
W = sample weight (g);
1607 = specific absorbance (E^{1}%,1cm) of curcumin in ethanol at 425 nm.
Report on a dry-weight basis using the moisture content obtained by General protocol 2.

Reference: *JECFA specification for curcumin (INS 100(i)); Rohman, A. (2012); Taylor, S.J., McDowell, I.J. (1992). Comparison of HPLC and spectrophotometric methods for curcuminoid analysis. Chromatographia.*

Curcuminoid Content — HPLC Method (Confirmatory / Individual Curcuminoids)

For accredited reporting, export certification, or wherever the individual proportions of curcumin, demethoxycurcumin and bisdemethoxycurcumin are required, quantify by reversed-phase HPLC with UV/DAD detection at 425 nm.

Principle

The three curcuminoids are separated on a C18 reversed-phase column by differing polarity and quantified against external calibration curves of each reference standard, monitored by UV/DAD absorbance at 425 nm, their common absorbance maximum.

Apparatus

- HPLC system with quaternary/binary pump, autosampler, column oven and UV or photodiode-array (DAD) detector
- C18 reversed-phase column (e.g., 250 × 4.6 mm, 5 µm, or an equivalent validated column)
- 0.45 µm PTFE/nylon syringe filters; ultrasonic bath; analytical balance (0.1 mg)

Sample Preparation

- Weigh 0.1 g of 60-mesh turmeric powder into an amber volumetric flask; extract with ethanol/methanol by sonication for 20–30 minutes in the dark; make up to volume, filter through Whatman No. 1, then through a 0.45 µm membrane filter directly into an amber HPLC vial.

Chromatographic Conditions (Representative-optimise & validate for the instrument in use)

- Mobile phase: acetonitrile : water (with 1–2% acetic acid), isocratic 55:45 v/v, or an equivalent validated gradient
- Flow rate: 1.0 mL/min; column temperature: 40–45 °C; injection volume: 10–20 µL
- Detection: UV/DAD at 425 nm; typical run time 15–20 minutes — curcumin, demethoxycurcumin and bis-demethoxycurcumin elute within this window, well resolved from each other

Method of Analysis

- Construct individual external calibration curves (minimum 5 points, $R^2 \geq 0.999$) for curcumin, demethoxycurcumin and bisdemethoxycurcumin using the certified reference standards.
- Inject the sample extract under the validated conditions; identify each curcuminoid peak by retention-time match against the standards and, where available, by co-injection/spiking.
- Quantify each curcuminoid against its own calibration curve; sum the three to obtain total curcuminoid content.

Calculation

Individual curcuminoid content (%, w/w) = $(C \times V \times 100) / (W \times 10^6)$

Where,
C = concentration read from the calibration curve (µg/mL);
V = total extract volume (mL);
W = sample weight (g).
Total curcuminoid content = curcumin + demethoxycurcumin + bisdemethoxycurcumin.
Report on a dry-weight basis using the moisture content obtained by General protocol 2.

Reference: *Poudel, A., Pandey, J., Lee, H.-K. (2019). Geographical discrimination in curcuminoids content of turmeric assessed by rapid UPLC-DAD validated analytical method. Molecules, 24(9), 1805; Taylor, S.J., McDowell, I.J. (1992). Chromatographia.*

For Further Information

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