# CHAPTER 2

# MATERIALS AND METHODS

This chapter describes the chemicals, reagents, certified reference materials, sample preparation techniques and instrumentation methods employed for multiresidue analysis in real samples of six spices, *viz.* chillies, cardamom, cumin, ginger, cinnamon and curry leaves. Performing compliance evaluation against FSSAI MRLs for these spices using the results obtained, and characterization of food safety hazards in the tested samples based on these results, are also described.

## Materials

The mass spectrometry grade solvents used for mobile phase preparation in UPLC, *viz.* methanol and acetonitrile, were obtained from Biosolv, USA. The QuEChERS chemicals, principally primary secondary amine (PSA), graphatized carbon black (GCB), and C-18 bulk sorbent were procured from Agilent, India. All other analytical grade chemicals like isooctane, acetic acid, formic acid, sodium chloride, anhydrous magnesium sulphate, ammonium formate, formic acid, sodium citrate dibasic trihydrate, sodium citrate dibasic sesquihydrate etc. were procured from Merck, India. All pesticide residue certified reference materials (CRMs) were procured from Dr. Erhenstorfer, Germany. Carrier gas for GC was 99.9995% pure helium obtained from Bhuruka gases, India.

## Instrumentation

A 3-digit precision balance (Sartorius BSA223S) was used for weighing all samples for analysis. For reference standard preparations a 5-digit precision balance (Shimadzu AUW220D) was used. Homogenization was carried out in all spices using a kitchen blender. Certified reference material and stock standards were stored at -20°C in a freezer (Remi RQV-300 plus), and intermediate standards were stored at 4°C in a low temperature cabinet (Remi CC-19 plus). Centrifuges for sample preparation with two

speeds were used, *viz.* 5000 rpm (Remi CM-8 plus) and 10,000 rpm (Remi C-24 plus). Vortex shaker used was Remi CM-101. For concentration of extracts, a nitrogen-based evaporator from PCI Analytics ($N_2$ Fastvap) with a Peak nitrogen generator was used. For detection and quantification of analytes, Agilent GC-MS/MS (7890 GC / 7000 C MS) and Waters UPLC-MS/MS (Xevo TQS Micro) were used.

**Sample collection**

Samples were collected from local markets in Kochi, Kerala in whole, dried forms. Branded spices in retail packs were not considered in this study. Instead, samples were procured in loose from as detailed in Table 2.2. Ten samples of each spice were collected. The curry leaf samples were obtained in fresh and then sun-dried to constant weight before commencing analysis, as the method development had been performed in the dried form of this spice.

**Table 2.2** Details of samples collected for survey

| Spice samples | Number of samples | Weight collected (kg) |
|---|---|---|
| Cardamom (whole) | 10 | 1 |
| Cumin (whole) | 10 | 1 |
| Ginger dried (whole) | 10 | 0.5 |
| Cinnamon (whole) | 10 | 0.25 |
| Curry leaves (fresh) | 10 | 0.5 |

**Sample preparation and extraction**

The samples of the different spices were homogenized as described in Part 1 (Table 1.2) before analysis. Each sample was analysed in duplicate. QuEChERS based sample preparation, as optimized for each of the six spices in Part 1, Chapters 3 (LC-MS/MS) and 4 (GC-MS/MS), were used for analyses.

**Instrumental analysis**

For all analyses, reference standards were prepared as described in Part I, Chapter 2. A total of 78 residues were analysed in the six spices considered for the study, with GC-MS/MS being used for analysis of 25 residues and LC-MS/MS being used for the analysis of 53 residues. Instrument conditions used were as described in Part I, Chapters 3 and 4. Matrix matched calibrations were used in all analyses. Average of the results from duplicate analysis was used to perform compliance evaluations and hazard characterization calculations.

**Assessing compliance with Indian MRLs**

In India, the national MRLs are issued by the FSSAI, in the Food safety and standards (contaminants, toxins and residues) regulation[6]. This regulation is frequently updated, and in the latest version available online, there are MRLs issued for 213 pesticides. However, the number of MRLs issued for spices is only 23. Out of the six spices considered in the present study, specific MRLs are available only for 3 spices, *viz.* chillies, cumin and cardamom. MRLs are available for a class named leafy vegetables, in which curry leaves could be included. However, in this study, only dried curry leaf was considered. Thus, dehydration factor would need to be applied in this case while considering compliance with MRLs for leafy vegetables. Out of the 78 pesticides commonly used in spices covered in this study, MRLs are available only for 23, and these are summarized in Table 2.3.

For compliance evaluation, the concentrations of the pesticides observed in the study were compared against the MRL values as described above, considering decision rule C (see Figure 2.1). In cases were FSSAI has not fixed MRLs for a spice-pesticide combination, the default MRL of 0.01 mg $kg^{-1}$ was used for evaluating compliance.

**Table 2.3** Available national MRLs for pesticides for various spices

| No. | Pesticide | FSSAI MRL (mg $kg^{-1}$) |
|---|---|---|
| 1 | Fipronil | Chillies: 0.1 |
| 2 | Spinosad A | Chillies: 0.1 |
| 3 | Spinosad D | Chillies: 0.1 |
| 4 | Thiodicarb | Chillies: 0.1 |
| 5 | Thiacloprid | Chillies: 0.2 |
| 6 | λ Cyhalothrin | Chillies: 0.5 |
| 7 | Deltamethrin | Chillies: 0.5 |
| 8 | Pyraclostrobin | Cumin: 0.02, Chillies: 0.5 |
| 9 | Fenpropathrin | Chillies: 2 |
| 10 | Quinalphos | Cardamom: 0.01, Chillies: 2 |
| 11 | Triazophos | Chillies: 2 |
| 12 | Imidacloprid | Chillies: 3 |
| 13 | Tebuconazole | Chillies: 4 |
| 14 | Triadimefon | Chillies: 4 |
| 15 | Trifloxystrobin | Chillies: 4 |
| 16 | Cyantraniliprole | Chillies: 5 |
| 17 | Hexaconazole | Chillies: 5 |
| 18 | Azoxystrobin | Cumin: 0.03, Chillies: 10 |
| 19 | Fenpyroximate | Chillies: 10 |
| 20 | Acetamiprid | Chillies: 20 |
| 21 | Buprofezin | Chillies: 20 |
| 22 | Spirotetramat | Chillies: 20 |
| 23 | Carbaryl | Chillies: 50, Leafy vegetables: 10 |

**Characterization of food safety hazards**

For each spice, the pesticide with the highest incurred residue concentration was used to perform the hazard assessment study. The theoretical maximum daily intake (TMDI) was calculated as the average incidence level of the residue (mg $kg^{-1}$) multiplied by the average consumption of the respective spice (g $person^{-1}$ $day^{-1}$) taken from FSSAI guidelines for calculating MRLs[2,7], as summarized in Table 2.1. The acceptable daily intake (ADI) values assigned for these pesticides by the Codex Joint Meeting of Pesticide Residues (JMPR) was used as the threshold criteria. The ADI multiplied by the average body weight of a child, taken as 16 kg, gave the maximum permissible intake (MPI, mg $person^{-1}$ $day^{-1}$)[7]. The TMDI values were then compared with the MPI values to arrive at a

characterization of hazards associated with the incidence of the residues in the tested samples of spices. When the TMDI value calculated for a pesticide based on the average incurred residues in a sample was found to be less than the MPI value for that pesticide, the incurred residue was considered as not posing significant food safety hazard to humans. If the TMDI value exceeded the MPI value, the food safety hazard posed by the incurred residue was considered as significant.