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Certificate

This is to certify that this thesis entitled "**Multicomponent Pesticide Residue Analysis on Selected Spices**" is an authentic record of research work carried out by **Mr RAMESH BABU N** under my supervision in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Chemistry of University of Calicut and further that no part thereof has been presented before for any other degree.

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DECLARATION

I hereby declare that the thesis entitled "**Multicomponent Pesticide Residue Analysis on Selected Spices**", submitted to the University of Calicut in partial fulfilment of the requirements for the award of the Degree of Doctor of Philosophy in Chemistry is a bonafide research work done by me under the supervision and guidance of Dr. Joby Thomas K., Associate Professor (Retd.), Department of Chemistry, St. Thomas' College, Thrissur.

I further declare that this thesis has not previously formed the basis of any degree, diploma or any other similar title.

27-07-2022 **RAMESH BABU N**

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Ramesh Babu N

To

My Family

PREFACE

From historical times when human beings started relying on agriculture for food, protection of crops against pests have been a persistent concern. Controlling pest population and mitigating their adverse effects on crops have been a constant challenge. The use of chemical pesticides as crop protection agents have evolved over time following a path of increasing sophistication, culminating in modern synthetic pesticides which are highly effective against pests and less persistent in nature. These pesticides have played a significant role in ensuring global food security in the modern era.

Since synthetic pesticides function by inhibiting or interfering with biochemical processes in the body of the pests, these are potentially harmful to other living beings as well, including humans. Extended and indiscriminate use of these pesticides results in the accumulation of traces of these chemicals in the agricultural produce, termed as pesticide residues, which in turn cause harmful effects upon consumption of such produce. Health issues like cancer and disorders of the immune, reproductive and nervous systems have been attributed to the presence of pesticide residues in food. This makes pesticide residues a major food safety concern. Many countries across the world have issued increasingly stringent regulations of maximum residue limits (MRLs) for pesticide residues in various food commodities to ensure consumer protection. In this context, testing of pesticide residues in food is important to ensure compliance of food commodities with such regulations.

Analysis of pesticide residues have also evolved over time. For many years, chromatographical techniques with conventional detectors have been the preferred method for trace analysis. With the advent of highly sensitive and selective mass spectrometric techniques, hyphenated instrumentation where gas and liquid chromatography were

coupled with tandem mass spectrometry became the tool of choice for the analytical chemist in testing pesticide residues in food. The sample preparation techniques for pesticide residue analysis have also undergone considerable changes. The classical techniques which relied on solvent extraction and partitioning were time intensive and tedious, and have given way to the modern 'quick, easy, cheap, effective, rugged and safe' (QuEChERS) sample preparation technique which offers simplicity without sacrificing analytical performance.

Spices are considered difficult matrices to analyse because of their complex chemical composition. All spices have some active chemical compounds present in significant concentrations which contribute to their special properties like colour, flavour and aroma. These compounds can potentially interfere with analysis of pesticide residues. Spices are also very diverse, and belong to different classes like dried fruits (e.g., chillies, black pepper), dried seeds (e.g., cumin, fennel), dried floral parts (e.g., saffron), dried roots (e.g., ginger, turmeric) etc. Each class of spices have distinct properties and it is practically difficult to use a single analytical method to cover all major classes of spices. Thus, modern analytical methods for spices need to be sufficiently general to aid easy implementation but also have to be fine-tuned with respect to different classes of spices to ensure analytical performance. This is a gap area which is addressed in this thesis. For convenience, the work presented in this thesis is divided into two parts.

Part I of the thesis deals with developing, optimizing and validating pesticide residue analysis for different classes of spices. The pesticides most commonly used for cultivation of spices in India are covered. Two main instrumentation techniques are used, viz. ultra-high performance liquid chromatography coupled to tandem mass spectrometry (UPLC-MS/MS) and gas chromatography coupled to tandem mass spectrometry (GC-MS/MS). One of the most important problems faced in using mass spectrometric

techniques for quantitative analysis is the matrix effect (ME), which makes response of a target analyte different in solvent and matrix extracts. This issue poses significant challenges in high sensitivity trace analysis for pesticide residues, especially in complex matrices like spices. The causes of ME are different in UPLC-MS/MS and GC-MS/MS and have to be addressed differently in developing analytical methods. This is also addressed in Part I of the thesis.

In Part I, the first chapter presents an overview of classical and modern pesticide residue analysis methodology and instrumentation, the origins of ME in LC-MS/MS and GC-MS/MS with different approaches to mitigating these effects, and the processes used for method validation. The analytical protocols and instrumentation methods used for pesticide residue analysis in spices is described in Chapter 2. In Chapter 3, the development, optimization and validation of a multiresidue method for 53 pesticides in six representative spices using UPLC-MS/MS is documented, along with studies on matrix effect and measurement uncertainty calculations. In Chapter 4, the development, optimization and validation of a multiresidue method for 25 pesticides in six representative spices using GC-MS/MS is covered, along with evaluation of matrix effect measurement uncertainty calculations. In Chapter 5, two novel methods for mitigating ME in pesticide residue analysis in spices is explored, viz. use of analyte protectants in GC-MS/MS, and use of surrogate matrix compounds in solvent-based reference standards in LC-MS/MS. In Chapter 6, analysis of a class of broad-spectrum fungicides called dithiocarbamates, which are extensively used in cultivation of spices, using GC-MS is documented. This is followed by select bibliography.

Part II of the thesis deals with application of the methods developed in Part I to real samples for the purpose of evaluation of compliance with national MRLs as well as characterization of food safety hazards due to presence of pesticide residues in commonly

consumed spices. Chapter 1 presents a review of the regulations in India with respect to pesticide residues, the extant MRL regulations, evaluating compliance with MRLs and performing food safety hazard characterizations based on results of analysis. The methodology and instrumentation used in the study is depicted in Chapter 2. In Chapter 3, the results of application of the methods developed in Part I to real samples of six representative spices collected from local markets is covered. A total of 60 samples were analysed for 78 pesticides using UPLC-MS/MS and GC-MS/MS. Based on the results obtained, compliance with the national MRLs and food safety hazard characterization calculations were performed. This is followed by select bibliography.

ABBREVIATIONS

ADI	Acceptable daily intake
AP	Analyte protectants
AQC	Analytical quality control
ASTA	American Spice Trade Association
CRM	Certified reference material
d-SPE	Dispersive solid phase extraction
EI	Electronic ionization
ESI	Elecrospray ionization
FSSAI	Food Safety and Standards Authority of India
GAP	Good agricultural practices
GC	Gas chromatography
GCB	Graphitized carbon black
LOD	Limit of Detection
LOQ	Limit of Quantification
MMC	Matrix matched calibration
MPI	Maximum permissible intake
MrM	Multiresidue method
MRM	Multiple reaction monitoring
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
NEDI	National estimated dietary intake
NVNA	N-vanillyl nonanamide
PSA	Primary secondary amine
QuEChERS	Quick, easy, cheap, effective, rugged, safe sample preparation method
RSD_r	Relative standard deviation - repeatability precision
RSD_R	Relative standard deviation - reproducibility precision
TMDA	Theoretical maximum daily intake
UPLC	Ultra-high performance liquid chromatograph