CHAPTER 4

RESIDUE ANALYSIS IN SPICES BY GC-MS/MS

Validation of high sensitivity, multiresidue analysis in representative matrices chosen from different categories of spices using gas chromatography and mass spectrometry is documented in this chapter. Sample homogenization, extraction, cleanup and instrumental analysis using GC-MS/MS, of residues of 25 GC-amenable pesticides that are commonly applied in spice cultivation, were optimized and validated for six spices, *viz*. cardamom, chillies, ginger, cumin, curry leaves and cinnamon.

Gas chromatographic and mass spectrometric conditions were tuned to obtain the desired high sensitivity responses for the target analytes in multiple reaction monitoring (MRM) detection. Starting from a general QuEChERS sample preparation profile as explained in Figure 1.10, specific schemes were devised to suit the different classes of spices by using various combinations of QuEChERS cleanup reagents and identifying the combination that gave best recoveries in each selected matrix. The matrix effects posed by different spices in GC-MS/MS were evaluated and addressed. An integrated methodology for high sensitivity multiresidue analysis of the GC-amenable target analytes in different spices, using an optimized sample preparation scheme, followed by GC-MS/MS analysis was developed. Validation of this analytical scheme was conducted as per SANTE guidelines[117] and measurement uncertainty was evaluated.

General analytical scheme and establishment of blanks

The analytical scheme followed in this chapter was similar to that followed in the case of LC-amenable compounds as described in Chapter 3 and followed the following sequence:

(a) The gas chromatographic and mass spectrometric parameters were optimized for 25 analytes to obtain good separation and response for all compounds.

(b) Spice samples belonging to each category were screened using a basic unoptimized QuEChERS sample preparation method and the optimized GC-MS/MS instrumentation method. Samples which were free from incidence of pesticides under consideration were selected as blanks for matrix effect and method optimization studies.

(c) The extraction and cleanup steps of the QuEChERS were then optimized for each spice matrix. For this, various combinations of extraction and cleanup reagents were studied. The combination of reagents that gave best accuracy and precision were taken as the optimized sample preparation method for each spice matrix.

(d) Using the optimized sample preparation method, extracts were prepared from blank samples of each spice matrix. These extracts were gravimetrically analysed to understand matrix load which indicated the extent of matrix interferences.

(e) Matrix effect was then assessed by comparing slopes of solvent-only and matrix-matched calibration curves. In GC-MS/MS, matrix effect observed is generally enhancement in response, and in some pesticides, solvent-based reference standards failed to give acceptable responses. Thus, in all the optimization studies, matrix matched calibration standards were used, prepared from blank extracts using the same extraction/cleanup steps used in the studies.

(f) Using the optimized sample preparation and instrumental methods, method validation was conducted for all spice matrices and fitness for intended purpose was assessed as per the criteria outlined in SANTE 12682 guidelines[112]. Measurement uncertainty at the established limit of quantification (LOQ) was

calculated using the validation data in a representative spice matrix, cumin, for all the analytes.

GC-MS/MS method optimization

After screening multiple MRM transitions for the target analytes, the transitions which showed lowest matrix interference for the spices under consideration were identified and used for analysis.

Table 1.10 Gas chromatographic and mass spectrometric conditions

Parameter	**Set Values**
Chromatographic parameters	
Injection volume	2 ml
Injector program	70°C (0.1 min), ramp at 450°C/min to 3250°C (2 min), ramp at 10°C/min to 250°C
Column	DB-5MS (15m, 250 mm, 0.25 mm) x 2, with mid-column backflush
Column flow	0.9 ml/min
Oven program	60°C (1 min), ramp at 40°C/min to 170°C (0 min), ramp at 10°C to 310°C (3 min). Total run time 20.75 min.
Mass spectrometric parameters	
Ion source	EI
Filament current	35 mA
Electron energy	70 eV
Source Temperature	300°C
Collision cell quench flow (He)	2.25 ml/min
Collision gas flow (N2)	1.5 ml/min

Once the MRMs were identified, retention time based dynamic MRM (D-MRM) was applied for each analyte, which improved response and peak shapes as shown in Figure 1.20 below. Two MRM transitions per analyte were used, with the transition giving higher response used as the quantifying transition. The second transition was used as the qualifying transition for confirming identity of the residues in samples. A mid-column backflush technique was used in which two 15 m columns were connected by a central backflush valve, which was seen to improve the method precision considerably, especially when large number of samples were analysed in a single batch run. Temperature programmes for the injector and column oven were tuned to obtain good separation and

response for the compounds under consideration. The optimized instrumental method is summarized in Table 1.10 above and the final MRMs for the 25 analytes under consideration are summarized in Table 1.11.

Table 1.11 Optimized MRM transitions in GC-MS/MS

Compound	Quantifier Transition	Qualifier Transition	RT (min)	Dwell time (ms)	CE (V)
Azinphos-methyl	104.9 / 51	104.9 / 77.1	14.78	6.5	15
Bifenthrin	181.2 / 165	181.2 / 166.2	14.45	7.2	15
Chlorothalonil	263.8 / 229	265.8 / 231	8.538	5.9	20
Chlorpyrifos-methyl	285.9 / 93	287.9 / 92.9	9.525	5.2	20
Cyfluthrin isomers (sum)	162.9 / 91	162.9 / 127	16.96	6.9	15
Cyhalothrin (Gamma)	197 / 161	141 / 91.1	14.65	6.7	15
Cyhalothrin (lambda)	208 / 181	181.1 / 152	14.45	6.5	15
Cypermethrin isomers (sum)	181.1 / 152	164.9 / 91	17.06	6.3	15
Deltamethrin	181 / 152	250.7 / 172	18.82	14.5	15
Dichlorvos	109 / 79	184.9 / 93	4.92	20.79	15
Disulfoton	88 / 60	142 / 81	8.895	5.9	5
Endosulfan a	194.9 / 159	194.9 / 160	11.87	6.7	15
Endosulfan b	206.9 / 172	194.9 / 158.9	12.92	7.4	15
Esfenvalerate	167 / 125	167 / 89	18.01	11.3	15
Ethoprophos	157.9 / 97	157.9 / 114	7.5	6.3	10
Fenitrothion	277 / 260	277 / 109	10.05	4.7	5
Fenpropathrin	207.9 / 181	264.9 / 89	14.95	7.6	15
Fenvalerate	167 / 125	167 / 89	18.01	11.3	15
Fipronil	366.8 / 213	368.8 / 214.8	10.93	5.9	15
Iprodione	313.8 / 56	313.8 / 244.9	13.87	7.4	15
Parathion	290.9 / 109	138.9 / 109	10.03	4.8	10
Parathion-methyl	262.9 / 109	125 / 47	9.218	6.9	10
Phorate	121 / 65	230.9 / 128.9	7.894	7.5	10
Piperonyl butoxide	176.1 / 131	176.1 / 117.1	13.98	6.7	15
Vinclozolin	187 / 124	197.9 / 145	9.561	4.9	20

RT – retention time, CE – collision energy

Sample preparation method optimization

The spices considered under the study were representative matrices from different categories of spices, viz. cardamom (dried fruits with low pigment content), chillies (dried fruits with high pigment content), ginger (dried roots / rhizomes), cumin (dried seeds), curry leaves (dried leaves) and cinnamon (dried bark). Homogenization of the spices were performed to simulate normal culinary usage, as explained in Table 1.2.

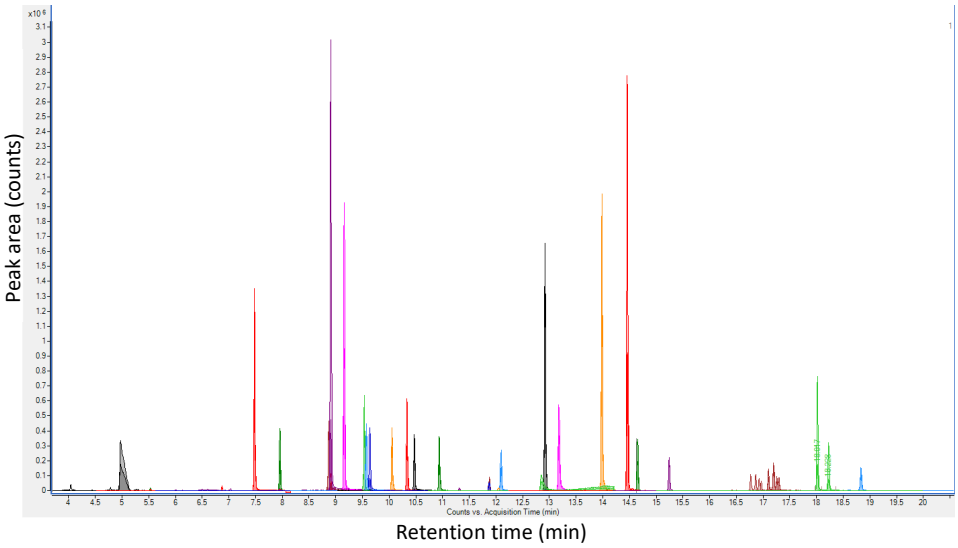

Figure 1.20 Optimized chromatogram in GC-MS/MS

The extraction steps, including sample weight, sample-water ratio, soaking time, sample-solvent ratio and use of buffering salts were adopted as optimized in Chapter 3 for UPLC-MS/MS analysis. Thus, 2 g homogenized samples were taken from each spice, soaked in 8 ml water (sample-water ratio 1:4) for 30 minutes, and extracted with 10 ml acetonitrile, with the addition of 4 g anh. $MgSO_4$, 1 g NaCl, 1g of sodium citrate tribasic dihydrate ($C_6H_5Na_3O_7$. 2 H_2O) and 0.5 g of sodium citrate dibasic sesquihydrate ($C_6H_5Na_2O_7$. 1.5 H_2O). This mixture was then vortexed thoroughly, and centrifuged at 5000 rpm for 5 minutes. From the supernatant extract, 2 ml was pipetted out and used for optimization of the cleanup steps specific for each spice.

Optimization of cleanup conditions

Like in the case of LC-MS/MS, cleanup of spice extracts was required in GC-MS/MS also because matrix effects are more pronounced and critical in the latter case. Because of the nature of gas chromatography, presence of large amount of pigments in the final extracts is likely to cause charring in the GC injection liner and thus result in

inconsistent responses. Also, as a result of matrix hydration in the extraction step, chances of traces of water being present in the final extract is high and this has to be removed to preserve chromatographic performance and ensure safety of the GC capillary column[118–120]. The cleanup step was designed to address these critical issues. As explained in Chapter 1, in the d-SPE step, anh. $MgSO_4$ and PSA are used for removing polar coextractives like sugars, organic acids and traces of water remaining in the extract after the extraction step. The role of C-18 is to remove nonpolar lipid interferences, while GCB is used to remove pigments. Thus, all four of the d-SPE reagents were used for optimization of the cleanup step.

Initial screening studies using various combinations of the four cleanup reagents, *viz.* PSA, GCB, C18 and $MgSO_4$, it was established that cleanup of all spice extracts needed $MgSO_4$ and C18, and fine tuning could be done based on the amounts of PSA and GCB. Accordingly, four final combinations of cleanup reagents were used for optimization, *viz*. **(A)** 100 mg $MgSO_4$ + 100 mg C18 + 25 mg PSA, **(B)** 100 mg $MgSO_4$ + 100 mg C18 + 75 mg PSA, **(C)** 100 mg MgSO4 + 100 mg C18 + 25 mg PSA + 10 mg GCB and **(D)** 100 mg MgSO4 + 100 mg C18 + 25 mg PSA + 30 mg GCB. Five representative compounds, viz. bifenthrin, disulfoton, fenitrothion, fenpropathrin and vinclozolin, with good response and peak shape in the optimized GC-MS/MS DMRM conditions, were chosen to be used for optimizing the cleanup conditions based on recovery and precision data. The spice samples were first extracted with the already optimized extraction parameters like sample weight, sample-water ratio and soaking time as detailed in Chapter 3. About 2 g of the homogenized samples were extracted with 10 ml acetonitrile with 4 g $MgSO_4$ and 2 g NaCl, followed by vortexing for 1 minute and centrifuging at 5000 rpm for 5 minutes. From the centrifugate, 2 ml extract was taken to optimize the cleanup step. Each combination from **(A)** to **(D)** were applied to 5 samples of

each of the four spices spiked at 0.01 mg kg^{-1} with the representative pesticides, then average recoveries and repeatability precision (RSD_r) were assessed. The results of optimization are summarized in Figure 1.21 below.

In all cases, extracts without cleanup showed poor recovery and precision. In cardamom, without cleanup, recoveries for the representative pesticides were in the range 48.8-71.5% with RSDr (n = 5) in the range 9-37%. In the other spices the values of recovery and precision were as follows: cumin - recoveries 51.9-69.4% (RSDr 19-25%), ginger - recoveries 50.9-65.9% (RSDr 11-28%), chillies - recoveries 37.0-54.4% (RSDr 23-44%), curry leaves - recoveries 42.0-64.0 % (RSDr 22-48%) and cinnamon - recoveries 59.8-76.6% (RSDr 13-21%).

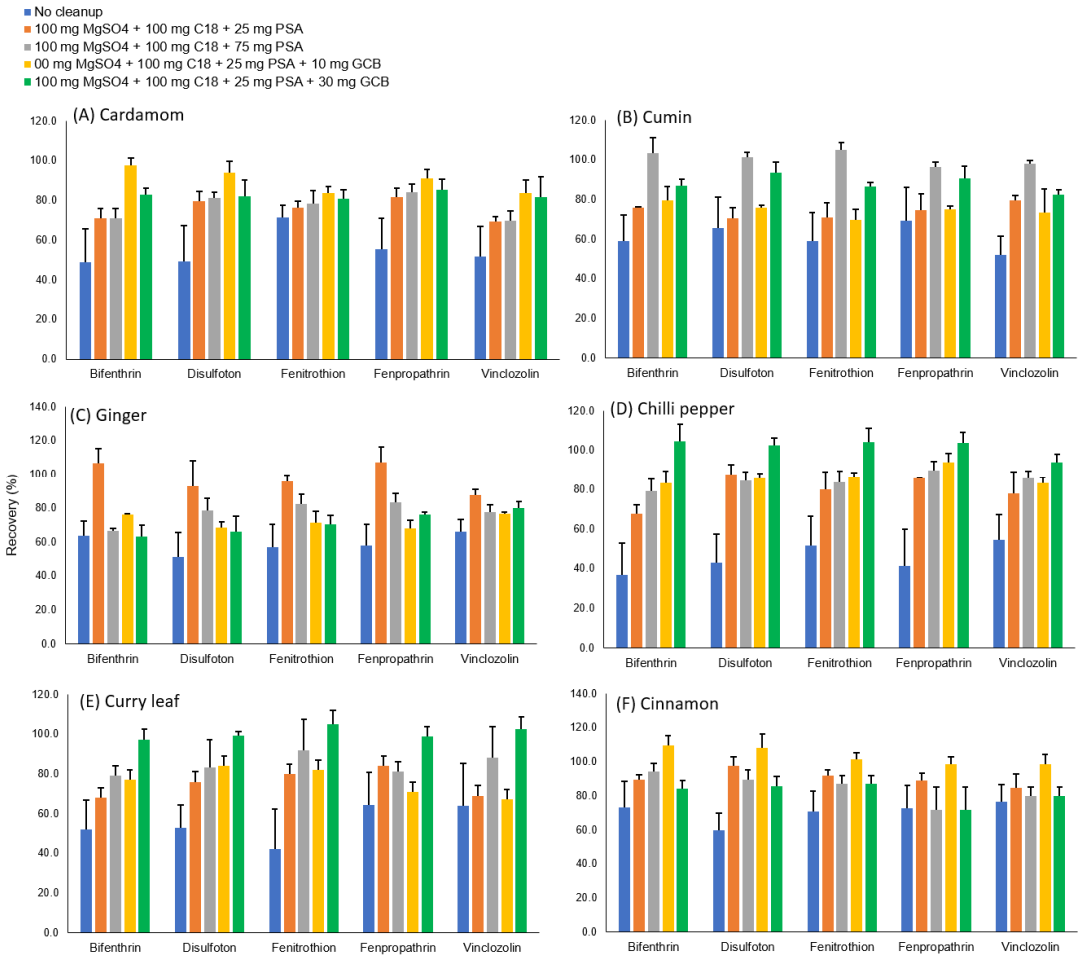

Figure 1.21 Optimization of cleanup procedure in spices: GC-MS/MS

The uniformly low recovery results in all cases without cleanup indicates that cleanup was an essential step for good method performance in analysing spices with GC-MS/MS. The precision values without cleanup were especially poor in the case of the two spices with most pigments, *viz.* chillies and curry leaves. This is possibly due to the deposition of pigments in the GC injector liner which gets charred on heating and cause response variations. Both the accuracy (recovery %) and the precision values were seen to considerably improve with the introduction of the cleanup step.

In cardamom and cinnamon, out of the four cleanup combinations studied, the best recoveries were obtained for **(C),** i.e., with 100 mg $MgSO_4$ + 100 mg C18 + 25 mg PSA + 10 mg GCB. In cardamom, the average recoveries for the five representative pesticides, *viz.* bifenthrin, disulfoton, fenitrothion, fenpropathrin and vinclozolin, using this combination ranged from 86.2-99.7%, with RSD_r in the range 4-11%. In cinnamon, the average recoveries were in the range 98.5-108.1% with RSDr in the range 4-7%. Thus, combination **(C)** was taken as the optimized cleanup combination in cardamom and cinnamon.

In cumin, out of the four cleanup combinations studied, the best recoveries for the five representative pesticides were obtained for **(B)**, i.e., with 100 mg $MgSO_4$ + 100 mg C18 + 75 mg PSA. The recovery values for this combination ranged from 96.2-104.8% with RSD_r values in the range 2-8%, so this combination was considered as the optimum cleanup step for cumin. In ginger, the optimized cleanup combination was **(A)**, i.e., 100 mg $MgSO_4$ + 100 mg C18 + 25 mg PSA, with average recoveries in the range 87.7-107.2% and RSD_r in the range 3-17%. In chillies and curry leaves, the optimized cleanup combination turned out to be **(D)**, i.e., 100 mg MgSO4 + 100 mg C18 + 25 mg PSA + 30 mg GCB. The average recoveries for chillies were in the range 93.8-104.6% with RSDr in

the range 5-8%, while in curry leaves the average recoveries in the range 99.2-104.9% with RSDr in the range 2-7%.

In all the spice matrices, accuracy (recovery %) and precision (RSD_r)values obtained using the optimized cleanup combination were well within the acceptable criteria of 70-120% and ≤ 20% respectively. In cumin and ginger, use of GCB, which reduces pigmentation in the extract, was not seen to be required. The requirement for PSA, which limits acidic cooextractives in the extract, turned out to be higher in cumin (75 mg), owing to the nature of this matrix. Cardamom and cinnamon required 10mg of GCB in the cleanup step. As expected, the requirement of GCB was seen to be highest in chillies and curry leaf (30 mg). Using higher amounts GCB for removing pigmentation is not generally advisable as it can adsorb planar pesticides and reduce recovery of such compounds, but this effect was not observed in the case of the target analytes used in the present study.

The effect of the optimized cleanup procedure in each of the spices, in terms of the matrix load (mg/ml) measured gravimetrically in the spice extracts is shown in Figure 1.22.

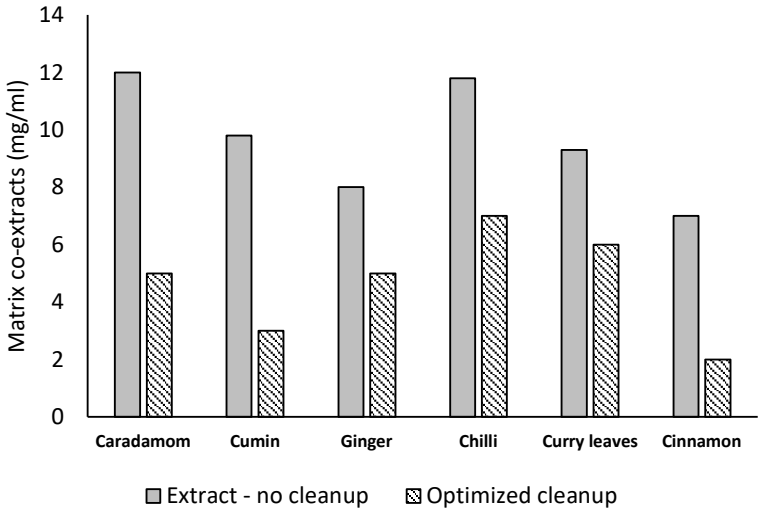

Figure 1.22 Matrix load in cleaned extracts: GC-MS/MS

The highest matrix load was in the extract was observed in the case of cardamom (12 mg/ml) and the least was for cinnamon (7 mg/ml). After cleanup, the highest reduction in matrix load was observed in cinnamon (71.4%), followed by cumin (69.3%), cardamom (58.3%), chillies (40.7%), ginger (37.5%) and curry leaves (35.4%). The reduction in matrix load is seen to translate directly into the considerable increase in accuracy and precision in the results in the cleaned-up extracts. The summary of the optimized sample preparation method for the 25 target analytes in six spices for analysis by GC-MS/MS is given in Table 1.12 below.

Table 1.12 Optimized extraction and QuEChERS cleanup scheme for GC-MS/MS

Process	**Cardamom**	**Cumin**	**Ginger**	**Chillies**	**Curry leaves**	**Cinnamon**
Extraction						
Sample weight (g)	2	2	2	2	2	2
Add water (ml) / soak time (min)	8/30	8/30	8/30	8/30	8/30	8/30
Add acetonitrile (ml)	10	10	10	10	10	10
Add $MgSO_4$ anh. (g)	4	4	4	4	4	4
Add NaCl (g)	1	1	1	1	1	1
Add Sodium citrate tribasic dihydrate (g)	1	1	1	1	1	1
Add sodium citrate dibasic sesquihydrate (g)	1	1	1	1	1	1
	Vortexed 30 sec, centrifuged 5000 rpm 5 min.					
Cleanup						
Volume taken for cleanup (ml)	1	1	1	1	1	1
Add PSA (mg)	25	75	25	25	25	25
Add C18 sorbent (mg)	100	100	100	100	100	100
Add GCB (mg)	10	0	0	30	30	10
Add $MgSO_4$ anh. (mg)	100	100	100	100	100	100
	Vortexed 30 sec, centrifuged 10000 rpm 5 min.					

Matrix effects in GC-MS/MS

In GC-MS/MS, the matrix effect manifests as response enhancement of analytes in the matrix extract as compared to pure solvent, and is considered to originate because of competition for active sites in the injection system of the GC between analytes and the compounds coextracted from the matrix[83]. Although modern developments in GC

technology have enhanced the inertness of the various components of the injection system, some active sites remain in these components. When an analyte is injected in solvent, the analytes get adsorbed on these active sites, and as a consequence the amount of analyte molecules that goes into the column gets reduced, resulting in diminished response. However, in matrix, the coextractives from the matrix will compete with the analyte for these active sites. Being in much higher concentration than the trace level analytes, the matrix compounds will saturate the active sites, thereby resulting in a much higher fraction of the analyte molecules entering the column culminating in enhanced response. On the whole, this means that matrix matched calibration is mostly unavoidable in GC-MS/MS, as solvent based calibration standards might offer poor response below acceptability criteria. However, the downside of this approach is that injecting large number of matrix matched calibration standards in the GC will result in deposition of the matrix in the injection liner and cause charring, and this will result in unexpected drop or inconsistency in response. Thus, optimized cleanup is a critical step in GC-MS/MS analysis of pesticide residues in spices, as it removes the matrix coextractives to a substantive extent, thereby extending life of the injection liner and still provides considerable extent of response enhancement due to the remaining matrix coextractives.

Figure 1.23 shows the effect of cleanup in removing matrix components in two representative spices, cardamom and chillies. From the MS full-scan total ions chromatogram (TIC) of spice extracts with and without cleanup, it is seen that the high boiling, early eluting compounds are not much affected by cleanup, but there is reduction in amount of matrix coextractives at later retention times. From the recovery and precision studies using the optimized cleanup methods specific to each spice, it is evident that the reduction in matrix coextractives thus achieved by cleanup is enough to bring the method

performance within acceptable criteria. Further studies on ME in GC-MS/MS and alternate methods for mitigating these effects are further addressed in detail in Chapter 5.

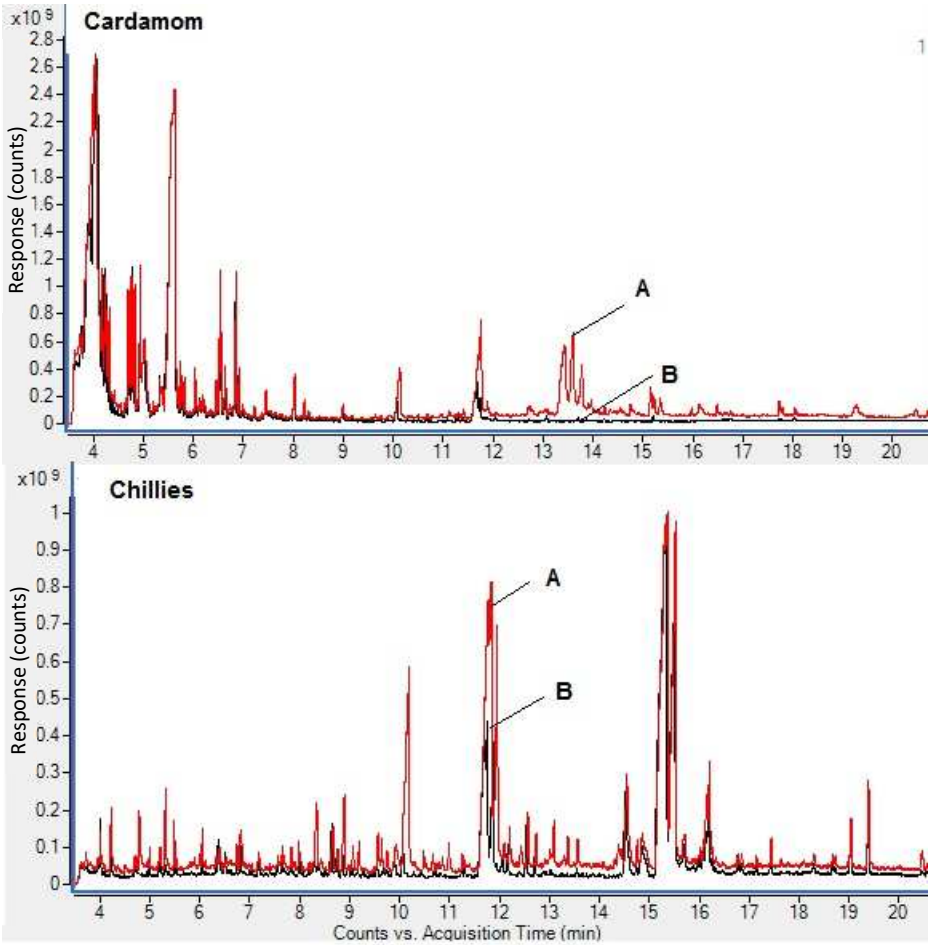

Figure 1.23 Full-scan TIC for extracts of chillies and cardamom, without cleanup (A) and with cleanup (B)

Method performance

The method performance evaluation was performed based on the criteria given in Table 1.3. For all pesticides and spice matrices, good linearity could be established with R^2 values 0.98 or better. All the optimized methods achieved the criteria of ≤ 20 % deviation in back-calculated concentrations from the true concentrations using five-point

calibration curves. Average recoveries obtained were well within the acceptability criteria of 70-120%. Repeatability Precision (RSD_r, same analyst, same day, n = 5), and within-laboratory reproducibility precision (RSD_R, of 3 replicates of each spike level performed on 3 non-consecutive days, different analysts, n = 9) met the acceptability criteria of ≤ 20 % in all spike levels for all pesticides and spice matrices. Table 1.13 summarizes the key validation parameters using the optimized sample preparation and instrumentation methods in a representative spice matrix, cumin.

Table 1.13 Validation parameters for target analytes in cumin as a representative matrix

Compound	R^2 (matrix matched)	Repeatability[a]		Reproducibility[b]	
		Av. Rec (%)	RSD_r	Av. Rec (%)	RSD_R
Azinphos methyl	0.9904	92.3	12	86.3	16
Bifenthrin	0.9921	88.9	4	83.4	7
Chlorothalonil	0.9836	92.3	3	90.6	14
Chlorpyrifos-methyl	0.9811	93.6	6	93.2	12
Cyfluthrin isomers (sum)	0.9901	104.1	7	96.0	12
Cyhalothrin (Gamma)	0.9812	103.1	4	92.9	8
Cyhalothrin (lambda)	0.9813	102.9	3	92.5	15
Cypermethrin isomers (sum)	0.9932	102.1	8	97.5	16
Deltamethrin	0.9866	89.6	7	77.9	13
Dichlorvos	0.9803	92.1	3	110.1	7
Disulfoton	0.9932	96.1	4	94.5	6
Endosulfan a	0.9904	100.8	5	96.3	5
Endosulfan b	0.9812	110.3	7	98.3	8
Esfenvalerate	0.9865	113.3	2	114.0	5
Ethoprophos	0.9932	93.8	4	94.9	4
Fenitrothion	0.9963	90.0	9	85.6	12
Fenpropathrin	0.9839	98.1	5	79.0	6
Fenvalerate	0.9932	115.2	2	114.3	6
Fipronil	0.9869	113.0	6	106.0	10
Iprodione	0.9811	112.3	4	98.3	6
Parathion	0.9899	86.6	6	75.6	7
Parathion-methyl	0.9951	91.7	6	99.2	3
Phorate	0.9887	107.6	3	94.1	5
Piperonyl butoxide	0.9937	97.6	5	85.3	7
Vinclozolin	0.9961	108.8	8	102.2	9

[a]Spike level 10 μg kg^{-1}, same analyst, same day, n = 5.

[b]Spike level 10 μg kg^{-1}, 3 replicates performed on 3 non-consecutive days, different analysts, n = 9.

The stated limit of quantification (LOQ) of the method, taken as the lowest spike level which could achieve the performance criteria for accuracy and precision, was fixed uniformly at 0.01 mg kg^{-1}. Specificity, assessed as the response in reagent blank and blank control samples in the same MRM and at the same retention time as the analyte, could meet the requirement of ≤ 30 % of LOQ.

Assessment of Measurement Uncertainty

For measurement uncertainty calculations in GC-MS/MS analysis, the same sequence of steps outlined in Chapter 3 was followed.

Table 1.14 Relative standard uncertainties specific to each analyte compound, at a reference value of 10 μg kg^{-1}

Compound	U (precision)	U (trueness)	U (CRM purity)
Azinphos methyl	0.1607	0.3707	0.0029
Bifenthrin	0.1063	0.3770	0.0010
Chlorothalonil	0.4487	0.0890	0.0029
Chlorpyrifos-methyl	0.2689	0.2491	0.0029
Cyfluthrin isomers (sum)	0.1308	0.1597	0.0002
Cyhalothrin (Gamma)	0.0878	0.1686	0.0002
Cyhalothrin (lambda)	0.4582	0.0555	0.0030
Cypermethrin isomers (sum)	0.1628	0.4942	0.0029
Deltamethrin	0.0928	0.1230	0.0030
Dichlorvos	0.0952	0.2129	0.0030
Disulfoton	0.1041	0.1149	0.0031
Endosulfan a	0.1882	0.3211	0.0013
Endosulfan b	0.0892	0.4687	0.0029
Esfenvalerate	0.0492	0.3207	0.0029
Ethoprophos	0.0626	0.3130	0.0029
Fenitrothion	0.2314	0.1333	0.0030
Fenpropathrin	0.1276	0.0783	0.0029
Fenvalerate	0.0898	0.5465	0.0029
Fipronil	0.1171	0.0174	0.0029
Iprodione	0.0494	0.1324	0.0015
Parathion	0.0876	0.3296	0.0031
Parathion-methyl	0.0779	0.2138	0.0029
Phorate	0.1645	0.0501	0.0029
Piperonyl butoxide	0.2314	0.3207	0.0029
Vinclozolin	0.1276	0.3130	0.0029

Cumin was chosen as the representative matrix for the study. The uncertainty components detailed in Figure 1.18 in Chapter 3 holds good in the present case also. Uncertainty was evaluated at the limit of quantification level of 10 µg kg^{-1} (0.01 mg/kg) which was achieved using the optimized methods developed. Table 1.14 shows the relative standard uncertainties specific to each analyte. Uncertainty component related to precision was assessed from the repeatability results of spike level 10 µg/kg, n = 5 as (standard deviation of measurements)/$\sqrt{n}$.

The uncertainty component related to accuracy was calculated from the average recovery value R as (100-R)/ $\sqrt{3}$, considering recovery error as Type B uncertainty with rectangular distribution. The uncertainty component with respect to standard purity is calculated from the percentage of purity P and uncertainty value U_{CRM} stated on the certificate, as $\frac{U_{CRM}}{P \times \sqrt{3}}$. For the standard preparation and extraction steps, the uncertainty components were taken as common for all analytes. These were all Type B components, so rectangular distribution was assumed and the standard uncertainty was calculated as U_s = U / $\sqrt{3}$, and relative uncertainty was then calculated as U_s/R where R is the reference value. Table 1.15 below shows these relative standard uncertainty components.

From the uncertainty components, the combined uncertainty was then calculated as

$$U_C = \sqrt{U_1^2 + U_2^2 + \cdots + U_n^2}$$

The expanded uncertainty was then calculated as $U_E = k \times U_C$. For 95% confidence limit (CL), the value of k was taken as 2. Figure 1.24 below shows the expanded uncertainty values in percentage for the reference value of 10 mg kg^{-1}, for various pesticides studied. In reporting results, the format used was $X \pm U_E$ *@ 95% CL.*

Tale 1.15 Common relative standard uncertainty components: GC-MS/MS analysis

Activity	Step	Ref. value	Parameter	Ux	Type	SUx	RSUx
Stock standard Preparation	Weighing	0.01 g	Balance readability	0.0001 g	B	0.00006	0.00577
Stock standard Preparation	Weighing	0.01 g	Balance calibration	0.0002 g	B	0.00009	0.00866
Stock standard Preparation	Measuring volume	10 ml	Pipette readability	0.1 ml	B	0.05774	0.00577
Stock standard preparation	Measuring volume	10 ml	Pipette calibration	0.013 ml	B	0.00751	0.00075
Intermediate standard Preparation	Measuring volume	1 ml	Pipette readability	0.1 ml	B	0.05774	0.05774
Intermediate Std Prep	Measuring volume	10 ml	Pipette calibration	0.013 ml	B	0.00751	0.00075
Sample Weight	Weighing	2 g	Balance readability	0.001 g	B	0.00058	0.00029
Extraction volume	Measuring volume	10 ml	Balance calibration	0.013 ml	B	0.00751	0.00075
Sample injection	Measuring volume	2 ml	Injector readability	0.5 ml	B	0.28868	0.14434

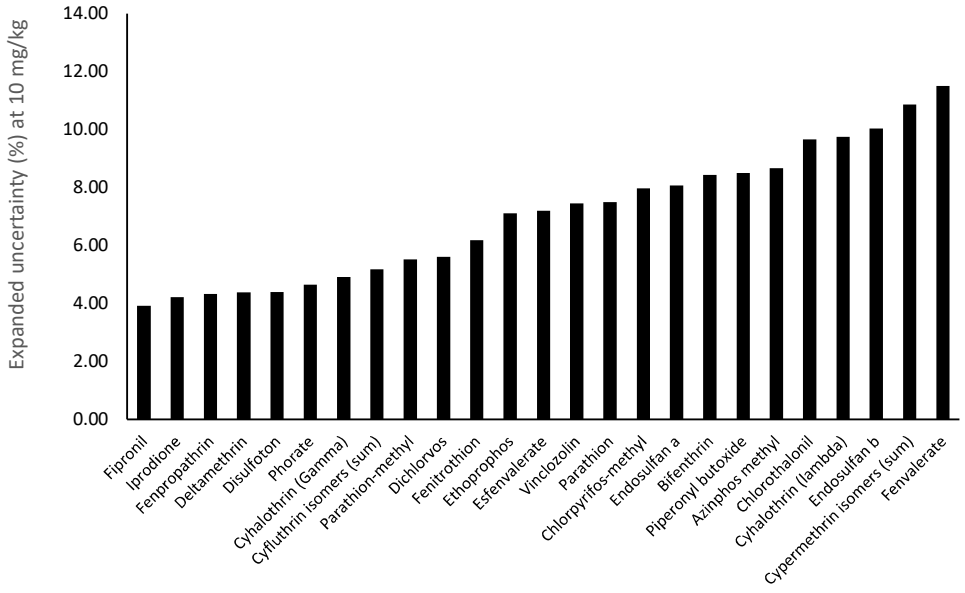

Figure 1.24 Expanded uncertainty at 95% confidence limit for GC-MS/MS analysis

For all the analytes studied with GC-MS/MS, the expanded measurement uncertainty values at the LOQ of 10 μg kg^{-1} was below 10%, except in the cases of cypermethrin isomers (10.9%) and fenvalerate (11.5%). The lowest measurement uncertainty obtained was for Fipronil (4.9%).

Conclusions

An efficient and sensitive analytical method for analysis of residues of 25 pesticides using GC-MS/MS in six selected spices was developed, optimized for different spice matrices, and validated. The matrices selected were representatives from different categories of spices, viz. cardamom (dried fruits with low pigment content), chillies (dried fruits with high pigment content), ginger (dried roots / rhizomes), cumin (dried seeds), curry leaves (dried leaves) and cinnamon (dried bark). Extraction parameters were optimized to obtain efficient transfer of analytes from the spice matrices to solvent, and spice-specific cleanup steps were optimized to obtain accuracy and precision levels meeting internationally accepted method performance requirements. Matrix effects were assessed in various spices, and it was noted that high matrix effects, mostly manifesting as response enhancement, was present in all cases. Thus matrix-matched calibration was an essential requirement to obtain trouble-free quantitation at low concentration levels. Limit of quantification of 10 μg kg^{-1} was obtained in all analytes and matrices. Expanded measurement uncertainty at limit of quantification was calculated in the range of 4.9-11.5% with 95% confidence limit for all analytes at LOQ. A common measurement uncertainty value of 12% at LOQ was adopted, covering all the compounds studied. The developed method can be used for regulatory compliance evaluation of spices as per international maximum residue limit requirements.