CHAPTER 3

RESIDUE ANALYSIS IN SPICES BY UPLC-MS/MS

Development and validation of high sensitivity, multiresidue analysis in representative matrices chosen from different categories of spices, using ultra-high performance liquid chromatography and tandem mass spectrometry (UPLC-MS/MS) is documented in this chapter. Sample homogenization, extraction, cleanup and instrumental analysis of residues of 53 LC-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.

Liquid chromatographic and mass spectrometric conditions were tuned to obtain desired high sensitivity responses for the target analytes with multiple reaction monitoring (MRM) detection. MRM transitions for each analyte which showed good response, peak shapes and low matrix interference were identified and used for quantification. 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 different combinations of QuEChERS cleanup reagents and identifying the combination that gave best recoveries in each selected matrix.

The matrix effects (MEs) posed by different classes of spices in UPLC-MS/MS were evaluated and addressed. An integrated methodology for high sensitivity multiresidue analysis of the LC-amenable pesticides for the six spices, using specifically optimized sample preparation scheme followed by UPLC-MS/MS analysis, was developed. Validation of this analytical scheme was conducted as per SANTE Guidelines[112]. Measurement uncertainty was calculated for all target analytes at the limit of quantification level (LOQ) level.

General analytical scheme and establishment of blanks

As there is considerable difference in nature and composition of spices from different groups, it is clear that the analytical methods had to be tailored and optimized to suit the different groups of spices. The general procedure followed was as follows:

(a) The liquid chromatographic and mass spectrometric parameters were optimized for the 53 analytes under consideration 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 UPLC-MS/MS method above. Samples which were free from incidence of pesticides under consideration were selected as blanks for extraction / cleanup optimization and later ME 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 recovery and precision results were taken as the optimized sample preparation method for each respective 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. ME was then assessed by comparing slopes of solvent-only and matrix-matched calibration curves.

(e) Using the optimized sample preparation and chromatographic methods, method validation was conducted for all spice matrices and fitness for intended purpose was assessed as per the acceptance criteria summarized in Table 1.3.

(f) Measurement uncertainty at the established limit of quantification (LOQ) was calculated from the validation data in a representative spice matrix, cumin, for all 53 analytes.

UPLC-MS/MS method optimization

For the UPLC mobile phase, two solvent systems were considered, viz. an acetonitrile-water system and a methanol-water system. In either case, an elution profile with gradient curve no. 6 starting with high aqueous concentration (98:2), passing through high organic concentration (1:99) and returning to the starting composition was found to give good separation of analytes on the C-18 column. This profile was then combined with a buffer system, viz. 5 mM ammonium formate / 0.1% formic acid. In all, four combinations of UPLC mobile phases were assessed: acetonitrile - water system with and without buffer, and methanol – water system with and without buffer.

Table 1.4 Optimized UPLC-MS/MS method parameters

Instrumentation	**Parameters**
UPLC	
Column	Waters XBridge® BEH C-18 2.5mm, 2.1x100mm
Mobile Phase	A: Water with 5mM ammonium formate and 0.1% formic acid B: methanol with 5mM ammonium formate and 0.1% formic acid Flow 0.5 ml/min Gradient: Initial A:B 98:2, 5 min A:B 50:50 curve 6, 7 min A:B 40:60 curve 6, 11 min A:b 25:75 curve 6, 14 min A:b 1:99 curve 6, 17 min A:B 98:2 curve 6. Total runtime 21 min.
MS/MS	
Capillary voltage	0.6 kV
Desolvation temp.	600°C
Source gas	1100 L/hr
Cone gas	50 L/hr

Of the four combinations of mobile phase studied, methanol-water composition was in general seen to be better than acetonitrile-water composition in obtaining good peak shape and resolution. It was also observed that the use of buffers improved the response

and peak shapes in general. Thus, methanol-water mobile phase containing ammonium formate / formic acid (5 mM / 0.1%) buffer was finalized as the mobile phase. The detailed mobile phase gradient profile is given in Table 1.4 above. The optimized chromatogram for 53 pesticides at 0.01 mg L^{-1} is shown in Figure 1.11.

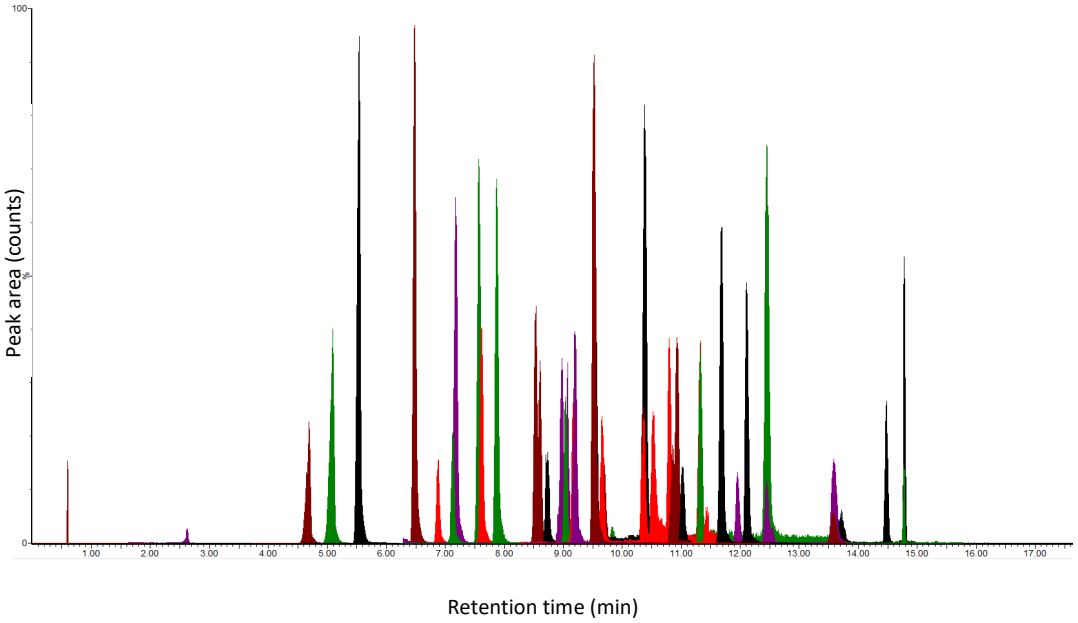

Figure 1.11 Optimized UPLC-EI-MS/MS chromatogram

As electrospray ionisation (ESI) was used for analysis, optimization of mass spectrometric conditions centred around two sets of parameters, *viz.* the compound-independent parameters which included capillary voltage, desolvation temperature, source gas flow and cone gas flow, and the compound-dependent parameters which included collision energy and cone voltage. Optimizing the compound-independent parameters was required to obtain consistent ionization of the analyte molecules and a stable spray. The optimized values of these parameters are given in Table 1.4. Two MRM transitions were used to monitor each analyte, with the transition having the higher response used for quantification, and the other transition being used as the qualifier. The compound dependant parameters were optimized individually for each MRM transition. Figure 1.12

shows the points of application of these parameters along the ion-path of the mass spectrometer.

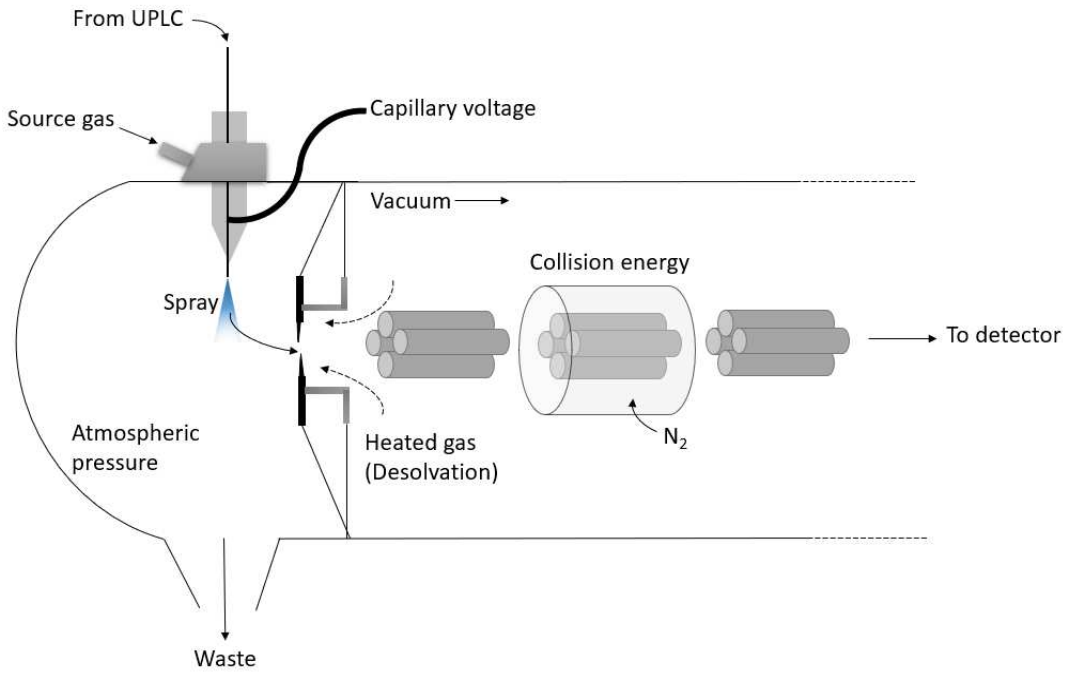

Figure 1.12 Schematic diagram showing mass spectrometric parameters: LC-MS/MS

. The retention times and the optimized compound dependant parameters for each MRM transitions of the 53 analytes are shown in Table 1.5.

Table 1.5. Optimized compound-dependent parameters in UPC-MS/MS

Pesticide	**T_R (min)**	**Quantifying transition (m/z)**	**Qualifying transition (m/z)**	**Collision Energy (V)**	**Cone Voltage (V)**
Acephate	12.62	183.9/142.95	183.9/49	20/18	10
Acetamiprid	5.09	223/126	223/56.1	15/20	30
Amectoctardin	8.53	276.16/244.07	276.16/168.06	24/14	16
Azoxystrobin	8.6	404/329	404/372	30/25	25
Bifenazate	9.55	301.1/198	301.1/170	20/10	25
Boscalid	8.92	342.9/139.9	342.9/307	20/45	25
Buprofezin	12.45	306.1/201	306.1/57.4	25/10	10

Carbaryl	6.88	202.1/145.1	202.1/127.1	25/10	25
Carbofuran	6.48	222.11/165.1	222.11/123	20/10	5
Chlorpyrifos	13.72	349.9/97	349.9/198	16/16	20
Cyantraniliprole	7.13	475.2/286	475.2/444	16/16	20
Cycloxydim	11.95	326/180	326/280	22/16	34
Cyprodinil	9.58	226/93	226/108	35/25	5
Diazinon	10.8	305.1/169	305.1/96.9	35/22	20
Dimethenamid	8.54	276/244	276/168	26/14	17
Emamectin benzoate	14.48	886.6/158	886.6/126	30/35	20
Ethion	13.59	385/199	385/142.9	25/10	30
Fenarimol	9.84	331/81	331/268	30/25	20
Fenbuconazole	10.35	337/70.1	337/125	30/20	15
Fenhexamid	9.68	301.96/55.18	301.96/97.11	35/25	35
Fenpyroximat	14.78	422.2/366.1	422.2/138.1	30/20	5
Flupicolide	8.98	383/172.999	383/109.06	66/20	40
Flutriafol	7.57	302.1/70.2	302.1/123.1	20/25	15
Fluxapyroxad	9.2	382.2/362	382.2/342	20/10	20
Hexaconazole	11.33	314/70.1	314/159	20/25	15
Imidacloprid	4.69	256.1/209.1	256.1/175.1	20/15	25
Iprobenfos	10.37	289/91	289/205	20/10	9
Malathion	9.08	331/127	331/99	20/15	10
Mandipropamid	9.04	411.8/328.1	411.8/125	35/15	35
Mehtiocarb	8.71	226/169	226/121	20/10	25
Metalaxyl	7.61	280.1/220.1	280.1/192.1	20/15	10
Methamidophos	0.6	142/93.9	142/124.9	13/13	15
Methoxyfenozide	9.2	369.2/149.1	369.2/313.23	15/10	15/5
Penthiopyrad	10.93	360.1/177.1	360.1/276	47/21	30
Phenthoate	10.52	321/79.1	321/135	40/20	9
Phosalone	11.42	367.9/181.9	367.9/110.9	42/14	12
Pirimiphos methyl	10.92	306.1/108.1	306.1/164.1	32/22	25
Procloraz	11.02	375.84/307.92	375.84/70.12	24/16	10
Profenofos	12.54	372.9/302.6	372.9/127.9	40/20	25
Pyraclostrobin	11.33	388.1/193.9	388.1/163	25/12	5
Quinalphos	10.37	299/96.9	299/162.9	30/24	15
Quinoxyfen	13.57	308/197	308/161.9	35/30	15
Spinosad A	11.68	732.6/142	732.6/98.1	35/30	35
Spinosad D	12.44	746.52/142	746.52/98.1	35/31	40
Spirodiclofen	14.76	411.14/71.16	411.14/313.1	15/10	35
Spirotetramat	9.65	374/330	374/302	30/15	20
Tebuconazole	10.85	308/70.1	308/125	20/35	10
Thiacloprid	5.54	253/126	253/90.1	35/20	40
Thiodicarb	7.17	355.08/88.1	355.08/108.1	16	17
Thiophanate	7.88	371/151	371/93.1	50/22	28
Triadimefon	9.17	294.1/69.3	294.1/197.2	20/15	25
Triazophos	9.53	314.1/161.9	314.1/118.9	35/18	22
Trifloxystrobin	12.11	409/186	409/145	40/16	10

QuEChERS sample preparation

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. Using the homogenized matrices, extraction and cleanup steps were optimized.

In the optimization experiments for general parameters like sample: water ratio and sample weight, an extraction step with sample: solvent ratio of 1:5 with 4g anh. $MgSO_4$ and 2 g NaCl, followed by vortexing for 1 minute and centrifuging at 5000 rpm for 5 minutes was followed. A basic, unoptimized cleanup profile as reported in the original QuEChERS method[35] was used, with 1 ml extract cleaned up using 150 mg anh. $MgSO_4$ and 25mg PSA, with vortexing for 30 seconds and centrifuging at 10,000 rpm for 5 minutes and injected in UPLC-MS/MS.

Five representative pesticides, viz. imidacloprid, ethion, chlorpyrifos, quinalphos and spirodeclofen were chosen to perform these initial optimizations, because of the uniformly good response obtained for these pesticides in all matrices under consideration. Subsequently the cleanup parameters were also optimized matrix-wise to obtain best recovery and precision. In all the optimization steps detailed below, matrix matched calibration (MMC) was employed in UPLC-MS/MS quantitative analysis. The calibration standards were set up using blank extracts prepared using the method steps being optimized.

Optimization of sample: water ratio

All the spices studied were low-moisture products and contained only about 8-12% average moisture content. Direct extraction of the matrices showed low accuracy and

precision, showing that rehydration of the matrices was essential to achieve efficient extractability of analytes. For this, water was added to 2 g of homogenized spice sample fortified at 50 μg kg^{-1} with the five representative pesticides, and allowed to soak in order to ensure rehydration. It was observed that the minimum soaking time required to ensure consistent results was 30 minutes. For lower soaking times, results obtained were not repeatable, and for higher soaking times, there was no significant improvement in precision. Thus 30 minutes was chosen as the optimal soaking time.

For optimizing the moisture content, the sample (g): water (ml) ratios 1:2, 1:4, 1:6 and 1:8 were used with a soaking time of 30 minutes ($n = 5$ in each case), and the accuracy and precision data for a fortification level of 50 μg kg^{-1} were compared to arrive at the optimum sample: water ratio for 5 the representative analytes chosen. It was observed when the spice samples were extracted directly without addition of moisture and using basic QuEChERS cleanup, the recovery and precision were poor, but with rehydration of the matrices, the recovery of all pesticides increased significantly.

The precision of analysis was also seen to be significantly affected by hydration. Recovery values were low when extracted without hydration for the five pesticides in all spice matrices and were in the ranges 28.2-51.8% for cardamon, 35.8-52% in cumin, 37-56% in ginger, 23.4-55.4% in chillies and 38.6-54% in curry leaves, with high standard deviations. This showed that even with proper homogenization, hydrating the matrix was important to ensure optimum extraction by the solvent. Hydration was seen to increase the recoveries by 20% or above in all cases.

These effects are shown in Figure 1.13, where the recovery values for the 5 pesticides in six spices are plotted against various sample: water ratios.

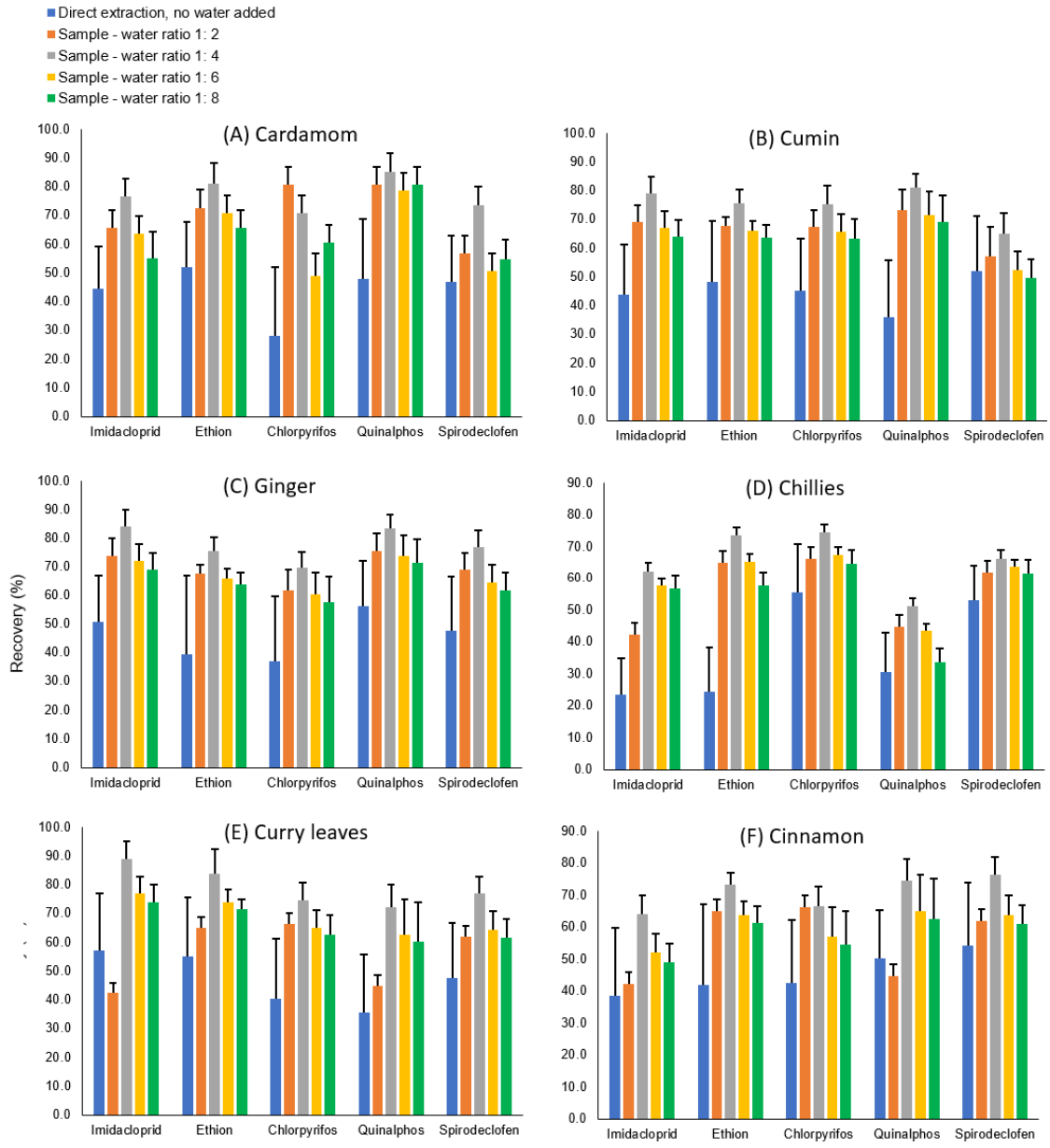

Figure 1.13 Effect of moisture content on extraction efficiency in spices

Precision (RSD_r) values were seen to be significantly improved by hydrating of the matrix, changing from 21- 84% without hydration, < 20% after hydration. It was observed that the even with unoptimized cleanup step, hydration with sample: water ratio of 1:4 could achieve recoveries in the range of 70-80%, except in the case of chillies, where recoveries of two pesticides, imidacloprid (62.2%) and spirodiclofen (66.2%) were seen

to be lower. The recoveries obtained at this sample: water ratio was consistently the highest except in one case, *viz*. chorpyrifos in cardamom, where 1:2 ratio showed a slightly higher recovery than 1:4 (+10%). However, following the major trend, the sample: water ratio of 1:4 was taken as optimal for the spices under consideration.

Optimization of sample weight

For optimizing the amount of sample taken for analysis, four sample weights were chosen, *viz.* 1 g, 2 g, 4 g and 6 g (n = 5 in each case). The homogenized samples of each of the spice matrices were first spiked with the five representative analytes at 50 μg kg^{-1}. Water was then added at the sample-water ratio of 1:4 and soaked for 30 minutes, as optimized earlier. The samples were then extracted, cleaned up and analysed in LC-MS/MS and the average recovery values were calculated.

It was observed that there were no large changes in average recovery with sample weight, but precision was seen to be significantly affected. Typical results for a representative spice, cardamom, for the five analytes are shown in Figure 1.14 where the average recoveries for the five analytes are plotted against sample weight. The same pattern was seen to recur in other matrices also.

Recovery values ranged between 69.6-88.8%, and there no significant difference in average recoveries for each compound with increase in sample weight. However, precision values showed discernible changes. The sample weight of 1 g showed high RSD_r values (14-20 %), but higher sample weights, i.e., 2 g and 4 g, showed better precision (RSD_r 3-11%). For the highest sample weight of 6g, precision was seen to decrease (RSD_r 11-17%). This is probably because spices contain significant amounts of crude fibre which makes perfect homogenization difficult, and increasing sample weight consequently would decrease the precision. As 2 g was the lowest sample weight which showed good

recovery and precision, this was chosen to be the optimal sample weight for all spices under consideration.

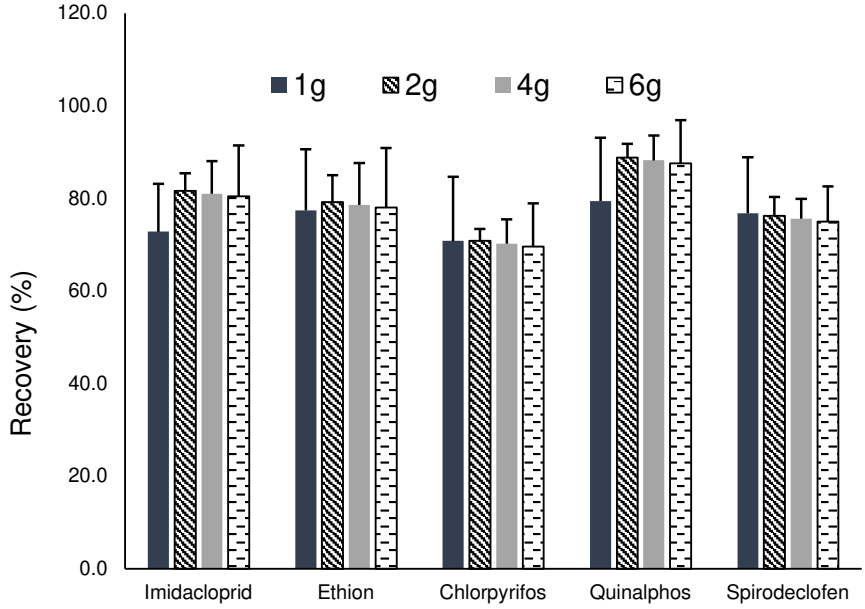

Figure 1.14 Effect of sample weight on recovery and precision in cardamom

Buffering during extraction step

It was noted during the initial optimization steps that for certain pH dependant pesticides, especially diazinon, carbaryl, chlorpyrifos, fenhexamid and malathion, there was a level of inconsistency in the repeatability of recovery values. Thus, before optimizing the cleanup step, the effect of buffer salts in the extraction efficiency in the six spices was studied. Using the optimized extraction parameters, recovery studies with and without citrate salts showed that for these pesticides, method performance improved considerably in the presence of citrate salts. For diazinon, carbaryl, chlorpyrifos and malathion, recovery values with addition of citrate salts increased by 13, 19, 17 and 24% in cardamom, 17, 18, 14 and 20% in cumin, 18, 25, 13 and 13% in ginger and 15, 12, 10 and 13% in chillies. For fenhexamid, recovery value increased by 19% in chillies. In all other cases, the variation in recovery values was minor, within ±8% for all compounds in

all spice matrices. However, it was deemed beneficial to include sodium citrate salts in the extraction step to improve overall method performance, and this was adopted to complete the optimization of the extraction step.

Optimization of cleanup step

To optimize the cleanup step, four QuEChERS reagents were considered, *viz*. anh. $MgSO_4$, PSA, C-18 endcapped sorbent and GCB. The use of $MgSO_4$ was to remove excess water from the extract and thus facilitate recovery of nonpolar residues. PSA contains primary and secondary amino groups that removed acidic interferences from the extracts. GCB acted by reducing pigments from the extracts, but it is known to affect recoveries of planar pesticides and this factor was also taken into consideration during the optimization step. The C-18 sorbent was used to remove non-polar interferences.

Spices typically have relatively high amounts of non-polar volatile oil content, of varying chemical compositions, in addition to other active chemical compounds. In cardamom the volatile oil content is around 8 - 9%, in ginger 0.7 - 4% and in cumin 2.7 - 4.3%. Chillies have capsaicinoid content, responsible for their pungency, ranging from 2000 - 5000 mg kg^{-1}. The colour in chillies, arising carotenoid content, range from 0.1 – 0.3%, or 1000 - 3000 mg kg^{-1}. All these factors contribute to matrix co-extractives which can potentially interfere with analytical performance. Also, as soaking spice samples in water was seen to be very important to obtain good recovery and precision, a natural consequence is the increased water content in the extract which has to be addressed to manage the recovery of non-polar pesticides.

In view of these factors, different combinations of cleanup chemicals were studied. After several initial trials, it was concluded that anh. $MgSO_4$ and PSA were required in the cleanup step in all spice extracts, and fine-tuning of accuracy and precision could be done based on the amounts of C18 and GCB. Thus, the following four combinations were

finalized for optimization studies: **(A)** 300 mg MgSO4 + 75 mg PSA + 50 mg C18, **(B)** 300 mg MgSO4 + 75 mg PSA + 50 mg C18 + 20 mg GCB, **(C)** 300 mg MgSO4 + 75 mg PSA + 75 mg C18 and **(D)** 300 mg MgSO4 + 75 mg PSA + 75 mg C18 + 20 mg GCB. The spice samples were first extracted with the already optimized extraction parameters like sample weight, sample-water ratio and soaking time. About 2g of the homogenized samples were extracted with 10 ml acetonitrile with 4g anh. $MgSO_4$ 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}, then average recoveries and repeatability precision (RSD_r) were assessed. Figure 1.15 shows the overall average recoveries for the five representative compounds, viz. imidacloprid, ethion, chlorpyrifos, quinalphos and spirodiclofen, obtained for the four cleanup combinations in the six spice matrices studied.

It was seen that with no cleanup, i.e., by analysis of the crude extract as such, the average recoveries ranged from ~ 20 - 65% in all the matrices studied, which is considerably below the minimum limits of acceptable method performance. It was also noted that the repeatability precision in most spices were low, with the RSD_r values clustering relatively closer to the higher limit of the acceptable criteria of 20%. This proved that cleanup was an essential step in achieving acceptable method performance in spices. In cardamom, without cleanup the average recoveries of the selected pesticides ranged from 51.4-75.0%, with RSD_r values ranging from 5-10%. Out of the four cleanup combinations studied, the best recoveries were obtained for (C), i.e., with 300 mg $MgSO_4$ + 75 mg PSA + 75 mg C18. The average recoveries (*n=5*) using this combination ranged from 83.7 - 97.8%, with RSD_r in the range 4-8%. Thus, combination **(C)** was taken as the optimized cleanup combination in cardamom. It was observed that the effect of cleanup

was in increasing the accuracy of the method, and precision values did not improve much with cleanup.

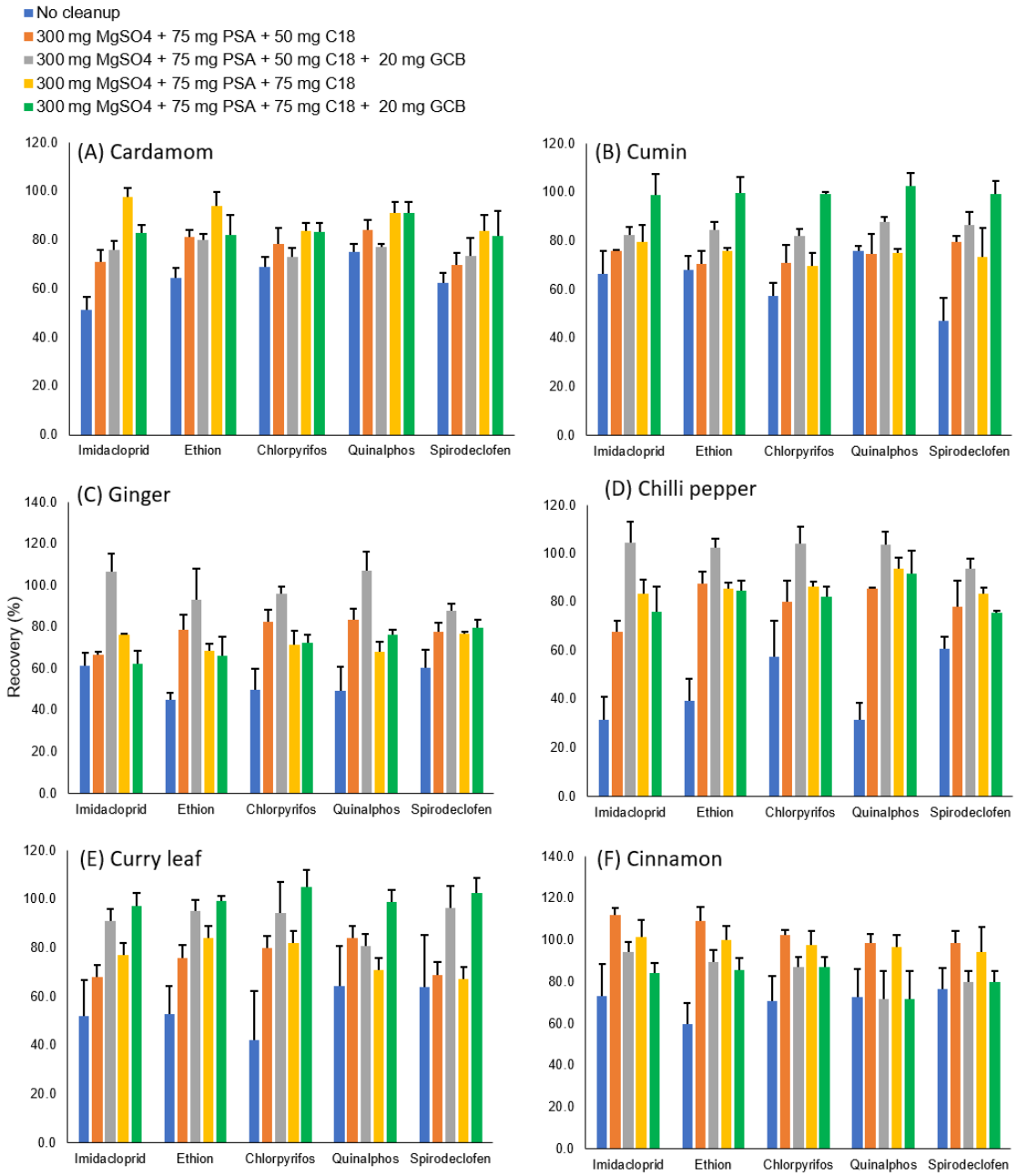

Figure 1.15 Optimization of cleanup procedures in spices: UPLC-MS/MS

In cumin, without cleanup the average recoveries of the selected pesticides ranged from 47.1-75.8%, with RSD_r values ranging from 3-20%. Out of the four cleanup

combinations studied, the best recoveries for ethion, chlorpyrifos, quinalphos and spirodiclofen were obtained for **(B),** i.e., with 300 mg $MgSO_4$ + 75 mg PSA + 50 mg C18 + 20 mg GCB, while in imidacloprid, the best recovery was obtained with combination (A), i.e. 300 mg $MgSO_4$ + 75 mg PSA + 75 mg C18 + 20 mg GCB. For the pesticides giving best performance with combination **(B)**, the recoveries ranged from 82.0-86.4% with RSD_r values from 7-11%. for imidacloprid, the average recovery with combination (B) was 82.3% with RSD_r of 1% while with combination **(D)** it was 98.7% with RSD_r of 7%. Considering that for imidacloprid the average recovery with combination (B) was within the acceptable limits of 70-120%, and had better precision than what was obtained with combination **(D)**, it was concluded that for cumin the optimal cleanup combination could be taken as combination **(B)**. It was observed that the effect of cleanup in cumin was in increasing both the accuracy and precision of the method considerably.

In ginger, without cleanup the average recoveries of the selected pesticides ranged from 45.1 - 61.4%, with RSD_r values ranging from 7-23%. Out of the four cleanup combinations studied, the best recoveries for all the five selected pesticides were obtained for **(B)**, i.e., with 300 mg $MgSO_4$ + 75 mg PSA + 50 mg C18 + 20 mg GCB. With this combination, the average recoveries obtained were in the range 87.7-107.2%, with RSD_r values ranging from 3 - 17%. It was thus concluded that for cumin the optimal cleanup could be taken as combination **(B)**. It was observed that the effect of cleanup in cumin was in increasing the accuracy, and precision was not seen to be improved significantly.

In chilli pepper also, the best recoveries were obtained with combination **(B)**, which was taken as the optimal cleanup combination for this spice. Here, the recoveries improved from 31.6-60% (RSDr 8-30%) without cleanup, to 93.8-104.6% (RSD_r 5-8%) with cleanup combination **(B)**. In curry leaves, the optimal cleanup combination turned out to be combination **(D)**, i.e., 300 mg $MgSO_4$ + 75 mg PSA + 75 mg C18 + 20 mg GCB.

Here, the recoveries improved from 42-64.5 (RSD_r 25 - 48%) without cleanup to 97.3-104.9% (RSDr 2-7%) with cleanup combination **(D).** Finally, for cinnamon, the optimal cleanup combination was identified as combination **(A)**, i.e., 300 mg $MgSO_4$ + 75 mg PSA + 50 mg C18. Here, recovery improved from 59.8-76.6% (RSD_r 13-21%) without cleanup to 98.6-112% (RSD_r 2-7%) with cleanup combination **(A)**. 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.

Table 1.6 Optimized extraction and QuEChERS cleanup scheme for LC-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)	2	2	2	2	2	2
Add PSA (mg)	75	75	75	75	75	75
Add C18 sorbent (mg)	75	50	50	50	75	50
Add GCB (mg)	0	20	20	20	20	0
Add $MgSO_4$ anh (mg)	300	300	300	300	300	300
	Vortexed 30 sec, centrifuged 10000 rpm 5 min.					
Concentration and reconstitution						
Cleaned extract evaporated to dryness (ml)	2	2	2	2	2	2
Reconstituted in 1:1 MeOH:H_2O (ml)	1	2	2	2	2	2

Concentration and reconstitution

The solution obtained after extraction and cleanup is in acetonitrile, whereas the mobile phase used in LC-MS/MS analysis is methanol-water. It was observed that changing the final extract from acetonitrile to methanol enhanced method performance and

also improved peak shapes. Thus, at the end of the optimized cleanup step, 2 ml of the extract was evaporated under nitrogen to near dryness and reconstituted with 1 ml, 1:1 methanol water solution. This introduced a concentration of the residues thus considerably enhancing the sensitivity of the method. The presence of water in the final injection solution was also seen to improve the peak shapes in some of the pesticides like acetamiprid. Table 1.7 above summarizes the optimized extraction, cleanup and concentration methodologies for all the spices studied, for analysis of the 53 pesticides using LC-M/MS.

Matrix load with optimized cleanup

The effect of the optimized cleanup step on the matrix load in the final solution is evident from the results of the gravimetric studies shown in Figure 1.16. The load of potentially interfering matrix co-extractives (mg ml^{-1}) in the extract was reduced after cleanup by 53% in cardamom, 51% in cumin, 50% in ginger, 57% in chillies, 39% in curry leaves and 57% in cinnamon.

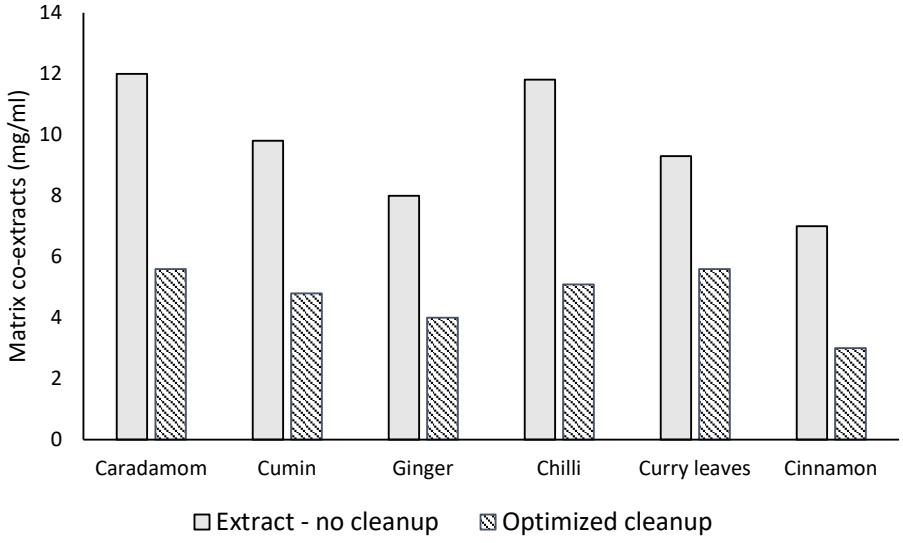

Figure 1.16 Matrix load in cleaned extracts: UPLC-MS/MS

Evaluation of matrix effects

In spite of the efficient cleanup steps which were optimized of all spices, it is evident from Figure 1.16 above that there is still considerable amount of matrix components remaining in the extract to cause interference to quantification. The assessment of matrix effects (MEs) was thus considered to be of importance in optimizing overall method performance.

The MEs were calculated using the following equation:

$$ME(\%) = \frac{Slope_{matrix-matched}}{Slope_{Solvent}} \times 100$$

ME between 80-120% are considered negligible, or soft ME, and does not require matrix matched calibration for reliable quantitative results. ME between 50-80% (suppression) and 120-150% (enhancement) are considered medium. ME lower than 50% (suppression) and higher than 150% (enhancement) are considered strong[52,115].

The ME posed by the spice matrices were uniformly suppressive and ranged from medium to strong. In cardamom, the ME ranged from 25-80%, in cumin between 10-46%, in ginger between 35-89% in chillies between 11-67%, in curry leaves from 40-83% and in cinnamon 45-79%. Thus, the highest suppression was observed in cumin and chillies.

Only 4 pesticides showed matrix suppression in the low ranges (ME > 80%), viz. fenhexamid (88%), fenpyroximat (89%) ad flutriafol (87%) in ginger matrix and pyroaclostrobin (80%) in cardamom matrix. When matrix suppression is low, i.e., ME is between 80 - 100%, results estimated using solvent-only calibration curves will not have large errors. However, with ME < 80%, using solvent-only calibration curves will lead to considerable underestimation of results.

In spices, the ME values were > 80% only in 1.8% cases in all the spice - pesticide combinations studied. This meant that for 98.2% of the analytes studied, ME manifested as response suppression in the medium and high ranges. Thus, it was concluded that matrix

matched calibration could not be avoided in all four spices so as to obtain reliable results. Table 1.7 shows the comparison of calibration equations ($y = mx + c$, where y represents the response, x the concentration of analyte, m the slope and c the y-intercept) and regression coefficients (R^2) for the analytes studied, in solvent and spice matrices. The matrix effects observed in the analytes in four representative spices are shown in Figure 1.17.

From the above data, it is evident that matrix effect is a significant aspect of pesticide residue analysis in spices using LC-MS/MS, and without addressing this issue, reliable method performance is not possible. Thus, matrix-matched calibration was fixed as a necessary requirement in the optimized methods. This posed the additional difficulty of ensuring the availability of blank matrices for the preparation of matrix matched calibration solutions. An attempt to address this issue to some extent is made in the studies outlined in Chapter 5.

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 between 0.98-0.99, as shown in Table 1.7. 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, $n \geq 5$), 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 1.7 Linearity equations and correlation coefficient values for pesticides analyzed by LC-MS/MS

Pesticide	Regression equation, R^2 value						
	Solvent	**Cardamom**	**Cumin**	**Ginger**	**Chillies**	**Curry leaves**	**Cinnamon**
Acephate	874x - 233, 0.9952	454x - 205, 0.9932	192x - 184, 0.9922	507x - 182, 0.9902	297x - 238, 0.9862	103x - 529, 0.9864	166x + 1803, 0.9871
Acetamiprid	19728x + 24531, 0.9952	13218x + 21588, 0.9912	1973x + 19380, 0.9872	13218x + 19134, 0.9862	6116x + 25022, 0.9912	12733x - 285, 0.9939	1358x + 7249, 0.9868
Amectoctardin	22375x - 353, 0.9981	9845x - 311, 0.9921	5146x - 279, 0.9931	14320x - 275, 0.9911	9397x - 360, 0.9891	8388x - 601, 0.9873	1280x + 29450, 0.9913
Azoxystroin	12353x + 1181, 0.9941	7165x + 1040, 0.9881	4200x + 933, 0.9881	7659x + 922, 0.9921	3459x + 1205, 0.9871	5561x + 14936, 0.9882	3229x + 5198, 0.9903
Bifenazate	23099x - 593, 0.9896	15476x - 522, 0.9866	7392x - 468, 0.9806	12704x - 463, 0.9876	15476x - 605, 0.9826	531x + 4225, 0.9815	3803x - 137, 0.9882
Boscalid	3380x - 35, 0.9933	2602x - 31, 0.9843	1048x - 28, 0.9923	1521x - 27, 0.9873	777x - 36, 0.9893	11534x + 10545, 0.9831	13868x - 524, 0.9901
Buprofezin	49527x - 663, 0.9951	33183x - 583, 0.9901	13868x - 524, 0.9901	17335x - 517, 0.9931	17830x - 676, 0.9881	7471x + 5544, 0.9878	432x + 27305, 0.9924
Carbaryl	1728x + 34564, 0.9914	933x + 30416, 0.9924	432x + 27305, 0.9924	1158x + 26960, 0.9924	639x + 35255, 0.9894	9496x + 22521, 0.9963	13009x + 1396, 0.9941
Carbofuran	37168x + 1767, 0.9951	21558x + 1555, 0.9891	13009x + 1396, 0.9941	27876x + 1378, 0.9941	12266x + 1803, 0.9871	10530x + 3975, 0.9913	787x + 8594, 0.9829
Chlorpyrifos	1789x + 10878, 0.9819	876x + 9573, 0.9669	787x + 8594, 0.9629	1180x + 8485, 0.9699	751x + 11096, 0.9659	4839x - 692, 0.9924	913x + 19532, 0.9882
Cyantraniliprole	9938x - 569, 0.9988	3677x - 501, 0.9918	2783x - 450, 0.9908	6361x - 444, 0.9898	1590x - 580, 0.9958	3844x - 372, 0.9939	385x + 5525, 0.9835
Cycloxydim	8267x - 156, 0.9952	3803x - 137, 0.9882	1653x - 123, 0.9872	4960x - 122, 0.9872	1819x - 159, 0.9912	1980x - 785, 0.9923	5592x + 13790, 0.9841
Cyprodinil	236x + 11621, 0.9877	130x + 10226, 0.9847	52x + 9181, 0.9847	139x + 9064, 0.9887	57x + 11853, 0.9997	913x + 19532, 0.9882	1358x + 7249, 0.9868
Diazinon	21039x - 678, 0.9954	11151x - 597, 0.9884	5049x - 536, 0.9874	10309x - 529, 0.9864	4839x - 692, 0.9924	385x + 5525, 0.9835	1280x + 29450, 0.9913
Dimethenamid	24025x - 365, 0.9979	14895x - 321, 0.9909	6006x - 288, 0.9909	12733x - 285, 0.9939	3844x - 372, 0.9939	5592x + 13790, 0.9841	5146x - 279, 0.9931
Emamectin benzoate	11650x + 770, 0.9953	6291x - 678, 0.9873	3961x - 608, 0.9893	8388x - 601, 0.9873	1980x - 785, 0.9923	1358x + 7249, 0.9868	4200x + 933, 0.9881
Ethion	8300x + 19149, 0.9962	5312x + 16851, 0.9932	3652x + 15127, 0.9912	5561x + 14936, 0.9882	913x + 19532, 0.9882	5592x + 10680, 0.9831	7392x - 468, 0.9806
Fenarimol	856x + 5417, 0.9905	368x + 4767, 0.9865	300x + 4279, 0.9875	531x + 4225, 0.9815	385x + 5525, 0.9835	2207x + 5615, 0.9858	1048x - 28, 0.9923
Fenbuconazole	17476x + 13519, 0.9911	7864x + 11897, 0.9821	5592x + 10680, 0.9831	11534x + 10545, 0.9831	5592x + 13790, 0.9841	2667x + 22809, 0.9923	13868x - 524, 0.9901
Fenhexamid	8489x + 7107, 0.9918	4584x + 6254, 0.9848	2207x + 5615, 0.9858	7471x + 5544, 0.9878	1358x + 7249, 0.9868	3370x + 4026, 0.9923	432x + 27305, 0.9924
Fenpyroximat	10669x + 28873, 0.9993	6722x + 25408, 0.9953	2667x + 22809, 0.9923	9496x + 22521, 0.9963	1280x + 29450, 0.9913	8040x + 18755, 0.9904	1358x + 7249, 0.9868
Flupicolide	14041x + 5096, 0.9953	10952x + 4485, 0.9903	3370x + 4026, 0.9923	10530x + 3975, 0.9913	3229x + 5198, 0.9903	4153x + 5977, 0.9919	1280x + 29450, 0.9913
Flutriafol	30923x + 23741, 0.9974	19791x + 20892, 0.9904	8040x + 18755, 0.9904	26903x + 18518, 0.9934	7422x + 24215, 0.9944	8300x + 19149, 0.9962	3229x + 5198, 0.9903
Fluxapyroxad	18056x + 7566, 0.9939	10111x + 6658, 0.9919	4153x + 5977, 0.9919	13361x + 5901, 0.9919	3070x + 7717, 0.9919	856x + 5417, 0.9905	1848x + 5374, 0.9828
Hexaconazole	23678x - 789, 0.9934	13023x - 694, 0.9924	4262x - 623, 0.9884	17048x - 615, 0.9904	8051x - 805, 0.9884	17476x + 13519, 0.9911	282x + 970, 0.9947
Imidacloprid	15187x - 266, 0.9964	10175x - 234, 0.9944	3341x - 210, 0.9884	9416x - 207, 0.9884	5467x - 271, 0.9874	8489x + 7107, 0.9918	5684x + 36169, 0.9968

Iprobenfos	44698x + 194, 0.9966	24584x + 171, 0.9896	14303x + 153, 0.9896	28607x + 151, 0.9906	13856x + 198, 0.9896	10669x + 28873, 0.9993	6369x - 572, 0.9961
Malathion	14856x + 1308, 0.9839	10102x + 1151, 0.9869	6091x + 1033, 0.9819	9656x + 1020, 0.9879	5348x + 1334, 0.9889	14041x + 5096, 0.9953	1592x - 515, 0.9924
Mandipropamid	9253x + 11353, 0.9902	5644x + 9990, 0.9862	3516x + 8969, 0.9852	5089x + 8855, 0.9842	1481x + 11580, 0.9872	1748x + 3310, 0.9872	7981x - 370, 0.9935
Mehtiocarb	4483x + 33510, 0.9867	2331x + 29489, 0.9887	1435x + 26473, 0.9147	2869x + 26138, 0.9887	1524x + 34180, 0.9847	10827x + 40289, 0.9938	1593x - 5298, 0.9899
Metalaxyl	18905x - 810, 0.9960	8318x - 713, 0.9930	8318x - 640, 0.991	10209x - 632, 0.9870	6239x - 826, 0.9870	8861x - 637, 0.9951	1356x + 5668, 0.9885
Methamidophos	198x + 14601, 0.9639	133x + 12849, 0.9829	46x + 11535, 0.9869	109x + 11389, 0.9809	73x + 14893, 0.9869	1137x - 574, 0.9954	4928x + 5976, 0.9963
Methoxyfenozide	2731x + 4244, 0.9882	1803x + 3734, 0.9832	546x + 3353, 0.9802	1748x + 3310, 0.9872	1038x + 4329, 0.9822	12144x - 412, 0.9915	1632x + 24504, 0.9962
Penthiopyrad	8586x + 3839, 0.9966	5409x + 3379, 0.9936	1631x + 3033, 0.9886	5238x + 2995, 0.9956	2662x + 3916, 0.9926	4249x - 5901, 0.9929	2003x + 128, 0.9893
Phenthoate	9306x + 76635, 0.9886	5956x + 67439, 0.9876	2419x + 60542, 0.9956	4839x + 59776, 0.9896	2140x + 78168, 0.9866	2035x + 6314, 0.9905	13883x - 170, 0.9925
Phosalone	3230x + 146, 0.9913	2003x + 128, 0.9893	1421x + 115, 0.9833	2519x + 114, 0.9853	807x + 149, 0.9843	7008x + 6657, 0.9943	4467x + 5987, 0.9878
Pirimiphos methyl	23530x - 193, 0.9955	13883x - 170, 0.9925	2588x - 152, 0.9905	17412x - 150, 0.9875	6118x - 197, 0.9865	5238x + 2995, 0.9956	883x + 1081, 0.9907
Procloraz	7702x + 6803, 0.9918	4467x + 5987, 0.9878	1848x + 5374, 0.9828	5314x + 5306, 0.9898	1617x + 6939, 0.9888	4839x + 59776, 0.9896	10827x + 40289, 0.9938
Profenofos	1226x + 1228, 0.9977	883x + 1081, 0.9907	282x + 970, 0.9947	748x + 958, 0.9887	417x + 1253, 0.9917	2519x + 114, 0.9853	8861x - 637, 0.9951
Pyraclostrobin	13534x + 45783, 0.9978	10827x + 40289, 0.9938	5684x + 36169, 0.9968	8662x + 35711, 0.9938	2707x + 46699, 0.9908	2707x + 46699, 0.9908	1137x - 574, 0.9954
Quinalphos	13845x - 724, 0.9981	8861x - 637, 0.9951	6369x - 572, 0.9961	9138x - 565, 0.9971	2354x - 738, 0.9921	2354x - 738, 0.9921	12144x - 412, 0.9915
Quinoxyfen	4550x - 652, 0.9964	1137x - 574, 0.9954	1592x - 515, 0.9924	3367x - 509, 0.9934	2002x - 665, 0.9884	1356x + 5668, 0.9885	4249x - 5901, 0.9929
Spinosad A	34698x - 469, 0.9985	12144x - 412, 0.9915	7981x - 370, 0.9935	22207x - 366, 0.9905	14920x - 478, 0.9935	4928x + 5976, 0.9963	2035x + 6314, 0.9905
Spinosad D	6640x - 6706, 0.9979	4249x - 5901, 0.9929	1593x - 5298, 0.9899	4382x - 5231, 0.9959	2722x - 6840, 0.9949	1632x + 24504, 0.9962	7008x + 6657, 0.9943
Spirodiclofen	3083x + 7175, 0.9915	2035x + 6314, 0.9905	1356x + 5668, 0.9885	2065x + 5597, 0.9855	1079x + 7319, 0.9835	12393x - 126, 0.9962	2888x + 27296, 0.9902
Spirotetramat	10950x + 7564, 0.9973	7008x + 6657, 0.9943	4928x + 5976, 0.9963	7008x + 5900, 0.9953	3723x + 7716, 0.9893	9538x - 3155, 0.9898	29836x - 141, 0.9942
Tebuconazole	6278x + 31018, 0.9982	2888x + 27296, 0.9902	1632x + 24504, 0.9962	4144x + 24194, 0.9932	3390x + 31638, 0.9902	10897x + 12613, 0.988	14471x - 3514, 0.9918
Thiacloprid	45901x - 160, 0.9972	29836x - 141, 0.9942	12393x - 126, 0.9962	36262x - 125, 0.9892	18820x - 163, 0.9952	4138x + 5065, 0.9873	11822x + 6412, 0.9933
Thiodicarb	32888x - 3993, 0.9988	14471x - 3514, 0.9918	9538x - 3155, 0.9898	23351x - 3115, 0.9978	7564x - 4073, 0.9928	12366x - 299, 0.9951	51525x - 378, 0.9961
Thiophanate	26577x + 15966, 0.997	17807x + 14050, 0.993	10897x + 12613, 0.988	11960x + 12454, 0.991	5050x + 16286, 0.992	18905x - 810, 0.9960	17890x + 36481, 0.9988
Triadimefon	11822x + 6412, 0.9933	6502x + 5642, 0.9913	4138x + 5065, 0.9873	7566x + 5001, 0.9873	3783x + 6540, 0.9843	198x + 14601, 0.9639	23530x - 193, 0.9955
Triazophos	51525x - 378, 0.9961	22671x - 333, 0.9891	12366x - 299, 0.9951	32461x - 295, 0.9981	13396x - 386, 0.9931	2731x + 4244, 0.9282	7702x + 6803, 0.9918
Trifloxystrobin	17890x + 36481, 0.9988	13239x + 32103, 0.9918	4830x + 28820, 0.9908	12702x + 28455, 0.9938	5367x + 37211, 0.9978	8586x + 3839, 0.9966	1226x + 1228, 0.9977

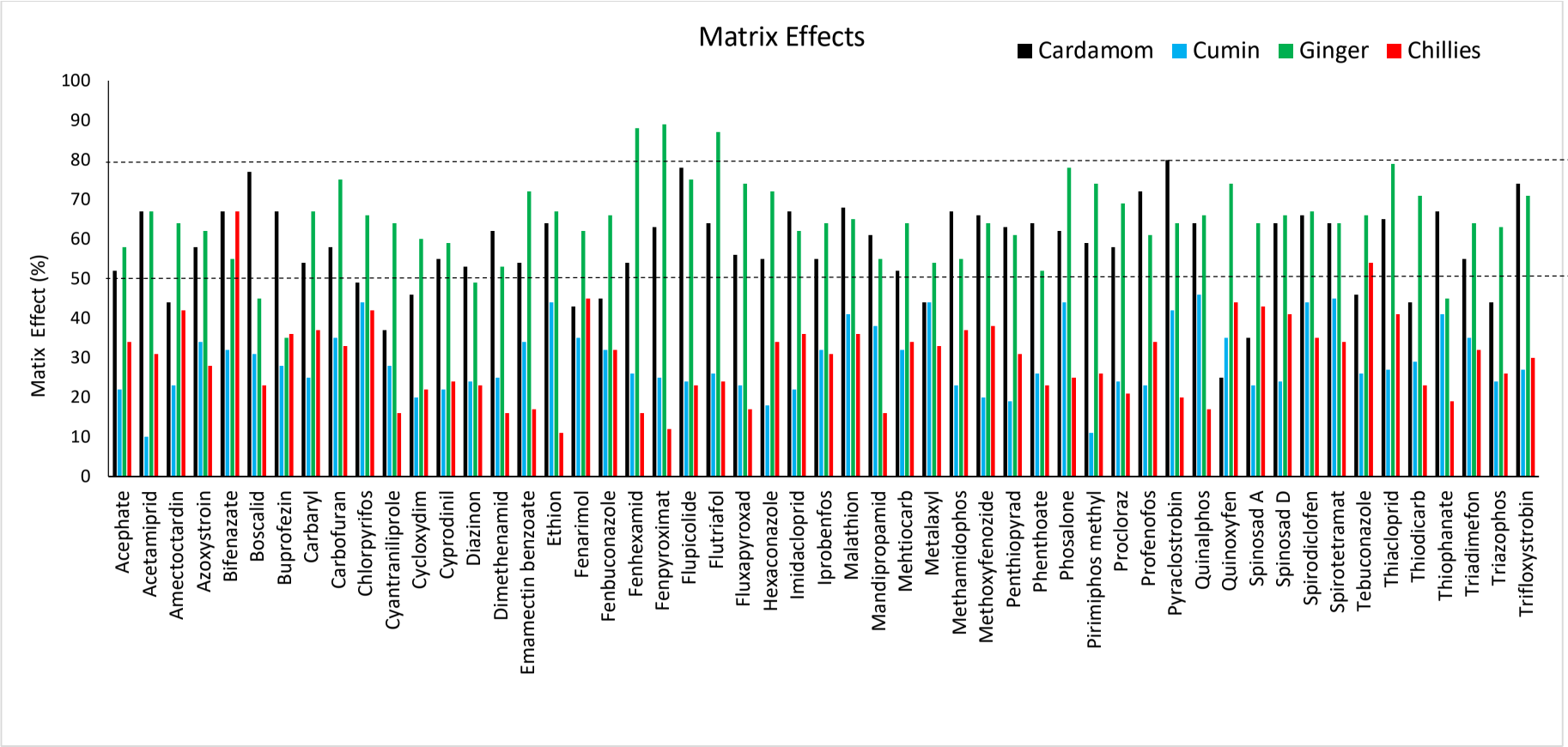

Figure 1.17 Matrix effects (%) of pesticides in four representative spices

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}, although in some cases limits of 0.005 mg/kg could be demonstrated. 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 in all the optimized methods. For the study of method ruggedness, three variables in the sample preparation method, *viz*. sample weight, sample: water ratio and extraction solvent volume were chosen. By varying these three variables by 20%, five different combinations were created, and each combination was applied in duplicate to a blank cardamom sample spiked with all analytes at 0.03 mg kg^{-1} (n = 10). The RSD value obtained was 14.36%, which was within the acceptability criteria, indicating that the method was sufficiently rugged to withstand small changes in the optimized method conditions.

Measurement uncertainty calculation

Uncertainty of measurement is defined as a value associated with a result that characterises the dispersion of the values that can be reasonably attributed to the measurand[116]. It is typically measured by first identifying the various components that can contribute to the uncertainty of the method using a cause-and-effect diagram, and then quantifying the uncertainties associated with each step.

Type A uncertainties are those arising from repeated measurements and Type B comprise of all other measurements. For the study, cumin was taken as a reference matrix for spices. Figure 1.18 shows the factors considered in this study for assessing method uncertainty.

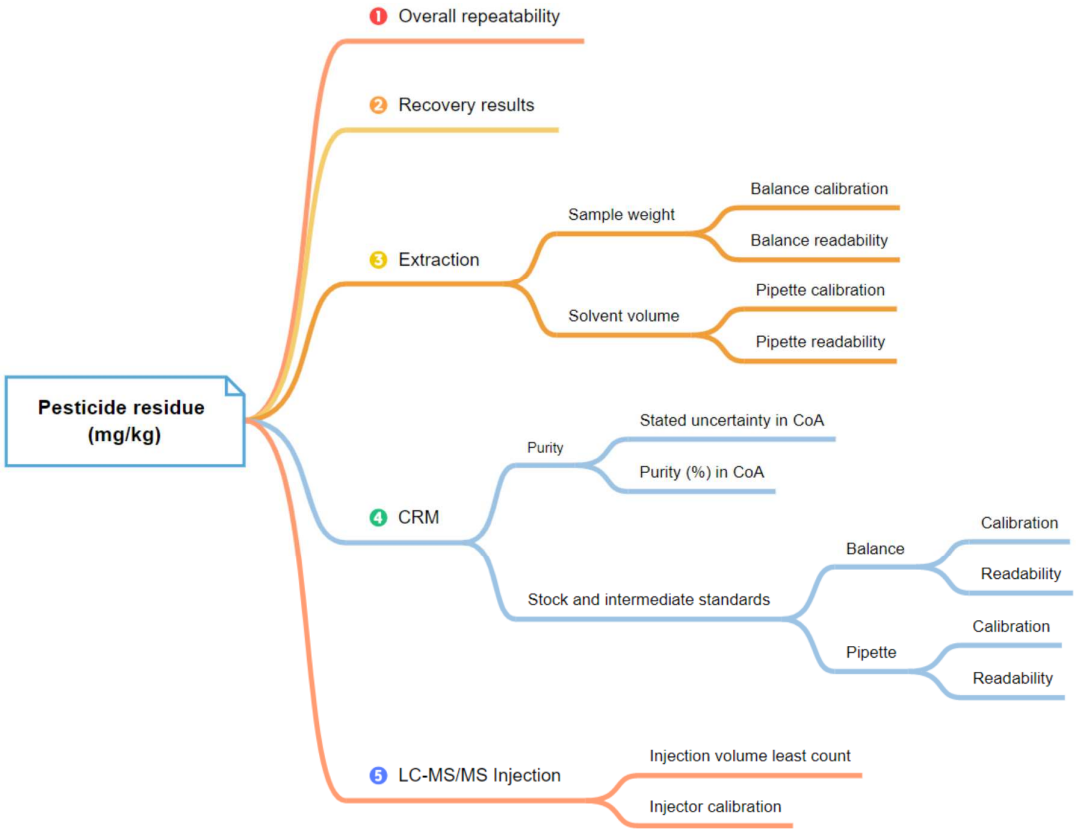

Figure 1.18 Uncertainty components for residue analysis

Uncertainty was evaluated at the LOQ level of 10 µg kg^{-1} (0.01 mg kg^{-1}) which was achieved using the optimized methods developed. Table 1.9 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^{-1}, 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 $\frac{U_{CRM}}{P \times \sqrt{3}}$.

Tale 1.8 Relative standard uncertainty components at reference value 10 µg kg^{-1}

Compound	U (precision)	U (trueness)	U (CRM purity)
Acephate	0.1538	0.1910	0.0029
Acetamiprid	0.0806	0.3321	0.0029
Amectoctardin	0.1397	0.0907	0.0029
Azoxystrobin	0.1669	0.4393	0.0029
Bifenazate	0.1397	0.0907	0.0029
Boscalid	0.1704	0.2629	0.0029
Buprofezin	0.1426	0.1608	0.0029
Carbaryl	0.1704	0.2629	0.0029
Carbofuran	0.0700	0.3335	0.0029
Chlorpyrifos	0.0597	0.2436	0.0010
Cyantraniliprole	0.0769	0.0593	0.0029
Cycloxydim	0.1669	0.4393	0.0029
Cyprodinil	0.1397	0.0907	0.0029
Diazinon	0.0841	0.2216	0.0029
Dimethenamid	0.1048	0.0371	0.0029
Emamectin benzoate	0.1114	0.1824	0.0030
Ethion	0.0455	0.4223	0.0030
Fenarimol	0.1704	0.2629	0.0030
Fenbuconazole	0.0894	0.2084	0.0029
Fenhexamid	0.1274	0.1495	0.0030
Fenpyroximate	0.0769	0.0593	0.0029
Flupicolide	0.1877	0.1202	0.0029
Flutirafol	0.0675	0.2443	0.0030
Fluxapyroxad	0.1361	0.0018	0.0014
Hexaconazole	0.1114	0.1824	0.0029
Imidacloprid	0.0860	0.1998	0.0029
Iprobenfos	0.1274	0.1495	0.0029
Malathion	0.1717	0.3345	0.0030
Mandipropamid	0.0561	0.2230	0.0029
Mehtiocarb	0.0860	0.1998	0.0030
Metalaxyl	0.1704	0.2629	0.0029
Methamidophos	0.1336	0.2302	0.0030
Methoxyfenozide	0.1710	0.3029	0.0030
Penthiopyrad	0.0660	0.3966	0.0029
Phenthoate	0.1336	0.2302	0.0011
Phosalone	0.2239	0.1119	0.0011
Pirimiphos methyl	0.1710	0.3029	0.0030
Procloraz	0.0845	0.0906	0.0029
Profenofos	0.4064	0.0389	0.0010
Pyraclostrobin	0.1107	0.1462	0.0029
Quinalphos	0.1336	0.2302	0.0029
Quinoxyfen	0.1361	0.0018	0.0030
Spinosad A	0.0661	0.0149	0.0030

Spinosad D	0.2239	0.1119	0.0030
Spirodiclofen	0.1286	0.3805	0.0011
Spirotetramat	0.0860	0.1998	0.0030
Tebuconazole	0.1313	0.1665	0.0059
Thiacloprid	0.0740	0.0347	0.0029
Thiodicarb	0.0612	0.0801	0.0029
Thiophanate	0.1710	0.3029	0.0030
Triadimefon	0.2370	0.0369	0.0029
Triazophos	0.1278	0.1410	0.0029
Trifloxystrobin	0.1806	0.1838	0.0010

For the standard preparation and extraction steps, the uncertainty components (U_x) 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 $U_s = U_x / \sqrt{3}$, and relative uncertainty was then calculated as $RU_s = U_s/R$ where R is the reference value.

Tale 1.9 Common relative standard uncertainty components – UPLC-MS/MS analysis

Activity	**Step**	**Ref. value**	**Parameter**	**U_X**	**Type**	**U_S**	**RU_S**
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

Table 1.9 shows above 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. In reporting results, the format used was $X \pm U_E$ *@ 95% CL.*

Figure 1.19 below shows the expanded uncertainty values in percentage for the reference value of 10 mg kg^{-1}, for various pesticides studied. The calculated expanded uncertainty values ranged from 3.40 - 9.90%. For the purpose of reporting results, a uniform expanded uncertainty of ± 10% at the reference value of 10 mg kg^{-1} was adopted.

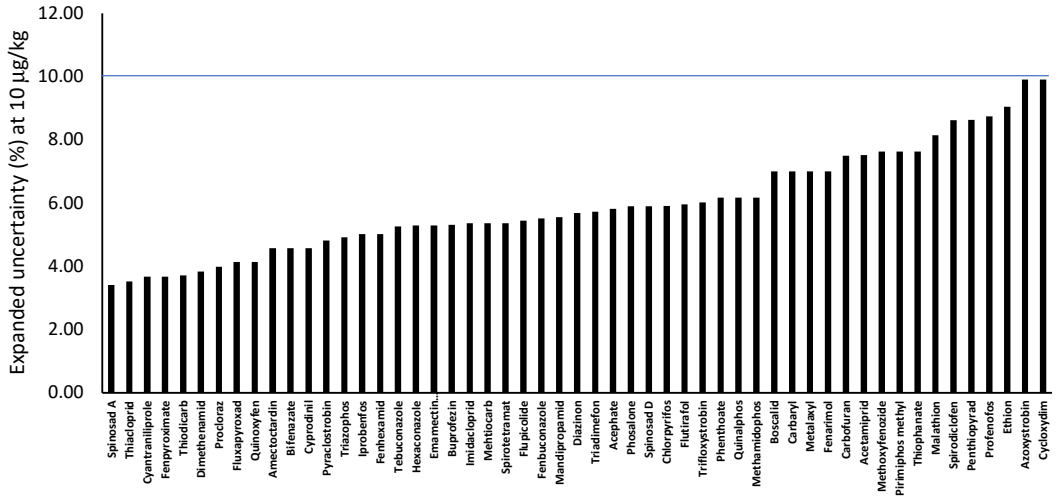

Figure 1.19 Expanded uncertainty for 95% confidence limit for UPLC-MS/MS analysis

Conclusions

A versatile, efficient and sensitive analytical method for pesticide residues using UPLC-MS/MS in six selected spices was developed, optimized specifically for different 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 concluded that with medium to high matrix suppression noted in all spices, matrix-matched calibration was an essential requirement to obtain trouble-free quantitation at low concentration levels. Limit of quantification of 10 mg kg^{-1} or better were obtained in all analytes and matrices. Measurement uncertainty at limit of quantification was calculated as ±10% with 95% confidence limit for all analytes. The developed method can be used for regulatory compliance evaluation of spices as per national and international maximum residue limit requirements.