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Interaction of Pesticides with Clean-up Agents during QuEChERS Dispersive-SPE Clean-up

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A method was developed using liquid chromatography tandem mass spectroscopy for the identification and quantification of multi residues of pesticides. The present study is first of this kind, destined purely to understand the interaction between the clean-up agents with 103 pesticides. The QuEChERS clean-up, employing most commonly used clean-up agents like anhyd.$MgSO_4$, PSA, C-18 and GCB in twelve combinations, was performed to assess their adsorption behavior with the pesticides. Recovery studies at $1 \mu g \cdot mL^{-1}$ showed that anhyd.$MgSO_4$ gave acceptable recovery for 100 pesticides (97.08%) in treatment T1. The PSA adsorbed some polar and acidic pesticides onto it and gave acceptable recovery for < 90% pesticides in T2 to T5 and C-18 with anhyd.$MgSO_4$ & varying amount of PSA (T6, T7) gave a lower, but acceptable recovery of 83.49% pesticides. GCB with anhyd.$MgSO_4$ & varying amount of PSA (T8, T9), adsorbed some planar pesticides like carbendazim, tricyclazole and gave a lower, but acceptable recovery for 74.75 – 77.66% pesticides. In T10 to T12, when all the adsorbents were used, adsorption of polar, acidic and planar pesticides (25.24%) was observed while rest of the pesticides (74.75%) gave acceptable recovery. The method also satisfied the single laboratory validation criteria for linearity, specificity, accuracy and precision.

Keywords: Anhydrous $MgSO_4$, C-18, GCB, Pesticides, PSA

Introduction

To ensure the safety of food and other commodities, screening methods for pesticide residues analysis are constantly a concern. But the complexity of the sample matrix is a major challenging factor since it comes in the way of extraction along with the target analytes.[1,2] Hence clean-up of the complex matrix after extraction is of utmost importance. In the development of multi-residues methods and clean-up processes, Solid Phase Extraction (SPE) is one of the most widely employed techniques. Aminopropyl, chitin, chitosan, cyanopropyl, glass beads, Graphitized Carbon Black (GCB), neutral alumina, octadecylsilane (C-18) and Primary Secondary Amine (PSA) etc. are used for a particular class of pesticides[3], however these agents are inefficient in trapping multi pesticide-residues from diverse matrices and consumes more chlorinated solvents the removal of which is a tedious process.[4] To overcome these problems, chemists across the globe were continuously challenged to develop a green, safer, simple, precise and versatile method capable of ensuring authenticity, traceability, quality and safety and of the desired analytes from the sample matrices. As a result, Anastassiades and Lehotey and co-workers introduced a fast extraction, clean-up method in 2003 called QuEChERS (Quick, easy, cheap, effective, rugged and safe) and found useful in extraction of multiclass pesticides in fruit and vegetables.[5] Later its applications in the analytical laboratories were widespread and used to extract pesticides from diverse commodities like food grains, herbs, dried commodities, fish, animal liver etc.[6–9] This method uses extraction/partitioning with acetonitrile and d-SPE (dispersive-solid phase extraction) concept which further includes clean-up step encompassing the usage of porous sorbents and or salts for the removal of complex matrix interfering substances thus guaranteeing the target analyte with greater accuracy and higher recovery.[10,11] In contrast to traditional SPE, d-SPE extracts the desired analyte in bulk solution, hence it does not require a vacuum/ column extraction, conditioning , problems with channeling, flow control and drying-out. The elution step is avoided, no dilution is required hence evaporation of solvent is not necessary.[12]

In QuChERS, depending on the specificity and property of the target analyte or pesticide and the

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matrix components, many modifications like addition of salts as buffers, and introduction of various clean-up agents were carried out in the original QuEChERS method. Later they were recognized as AOAC official method (uses acetate buffer) called AOAC 2007.01 method and the other method as a standard method of the European committee for standardization (uses citrate buffer) called CEN 15662).[13,14] In the original method, they used anhydrous magnesium sulphate (anhyd.$MgSO_4$), and/or PSA as d-SPE agents. PSA was more effective and efficient sorbent for the clean-up purpose. Apart from PSA, clean-up sorbents like GCB and C-18 were also used. Anhydrous $MgSO_4$ removes residual water from the samples after the extraction. PSA removes fatty acids, sugars, organic acids and some pigments, while C-18 effectively removes the high content of lipids, sterols and fatty matrices. Similarly, GCB is efficient in removal of co-extracted colored pigments like carotenoids, chlorophyll. But these sorbents should be used more selectively and cautiously while extracting various classes of pesticides in a mixture.[15] PSA is weak anion exchange sorbent and polar in nature, also adsorbs polar pesticides along with the complex interfering matrices[16] and GCB adsorbs planar pesticides on its surface which are present in food and other matrices.[12,16]

The above quoted studies are in relation to some matrix, but for explaining the interaction of pesticides with the cleanup agents in the absence of interfering substances are not available so far. Hence in this context, the study was initiated to understand the adsorption behavior of these clean-up agents in extracting multiclass pesticides in the absence of sample matrix. This will be the first study to be reported on direct interaction of pesticides (103 pesticides) with the d-SPE cleanup agents. For the analysis purpose, liquid chromatography- mass spectroscopy coupled with triple quadrupole was used.

Materials and Methods

Certified Reference Materials (CRM) of the 103 multiclass pesticides of 90.3 to 99.9% purity were used (Sigma-Aldrich Chemie GmbH, Germany). List of the pesticides along with their molecular weight and their use in agriculture are enlisted in Table 1. Anhydrous magnesium sulphate of > 98% purity was purchased from Thermo Fisher scientific, India. Graphitized carbon black (GCB), Octadecylsilane (C-18), Primary Secondary Amine (PSA) was employed in clean-up purpose (Agilent Technologies, Santa Clara, CA). Acetone (minimum 99.8% pure, HPLC grade, MERCK, India), acetonitrile (Hypergrade for LC-MS, Merck LiChrosolv), methanol (Gradient grade for liquid chromatography, Merck LiChrosolv) were procured. Millipore water with resistivity of 18.2 MΩ.cm was obtained from Millipore water purification system (Milli-Q, Academic, Millipore, USA) was used to obtain millipore water with resistivity of 18.2 MΩ. cm.2 mL micro-centrifuge tubes (TARSONS, India) were utilized in d-SPE clean-up.Analytical balance [0.1 g to 220 g, sensitivity 0.1 mg] was from METTLER (Switzerland TOLEDO ME-204), A-grade 10 mL volumetric flask (Borosil®, India) were used in primary stock solutions preparation. For sample preparation, a calibrated micropipette of 0.1–1 mL (Thermo Scientific, Germany), a vortex mixer (Model Spinix, Tarson, India), a sample filtration syringe (Hamilton Gastight® #1005, 5 mL capacity), a syringe filter (Qualisi/ Nylon Syringe Filter 13 mm × 0.22 m), filter (Fluro FGLP 0.22 µm × 13 mm) membrane were utilized.

Preparation of Primary Stock Solution and Working Solutions

Stock solutions of each of the 103 pesticides of concentration1000 $\mu g \cdot mL^{-1}$ were prepared by weighing precisely 10 mg of each certified reference standards in A-grade10 mL volumetric flasks and volume made with acetonitrile. Standard mixture of 103 pesticides was prepared and subsequently working solutions were made. These standards were further utilized for development of a multi residue method employing LC-MS/MS for the detection and quantification of 103 pesticides.

QuEChERS- dSPE Clean-up

Dispersive-solid phase extraction in QuEChERS, is the clean-up process involving various adsorbents after the QuEChERS extraction. For the purpose, original QuEChERS method was used with slight modifications. In 2 mL micro centrifuge tube, 1 mL of standard pesticide mixture of 1 $\mu g \cdot mL^{-1}$ concentration was taken. Different combinations of clean-up agents were added, followed by 2 min of vortex and 5 min centrifugation at 5000 rpm. Supernatant was removed with the help of syringe fitted with 0.22 µ nylon syringe filter and 0.5 mL of the filtrate is transferred to 2 mL auto-sampler vial for LC-MS/MS analysis.[5] Flow diagram for the d-SPE or clean-up procedure is

Table 1 — Molecular weight and purity of the used pesticides with their ionization mode#, LC-MS/MS parameters, regression equations, correlation coefficients and instrumental LOD and LOQ — (Contd.)

S. No.	Pesticide (ionization mode)	Mol. Wt. (amu)	Purity %	RT (min)	Quantifier (Q1)	Qualifier (Q2)	Regression Equation	Correlation coefficient (r)	LOD ($\mu g \cdot mL^{-1}$)	LOQ ($\mu g \cdot mL^{-1}$)
1	Alpha-Cypermethin (+) I	416.3	98.2	14.9	433.15 > 191.00		Y = 662301x + 816.127	1.00	0.001	0.003
2	Anilophos(+) H	367.85	99.6	17.7	368.00 > 125.00	368.00 > 199.00	Y = 9045660x 30741.4	0.99	0.001	0.003
3	Allethrin (+) I	302.41	96.5	14.92	303.00 > 135.10		Y = 2300770x−11594.5	1.00	0.005	0.015
4	Atrazine (+) H	215.68	98.1	10.97	216.00 > 174.20	216.00 > 132.00	Y = 3831650x−17872.8	1.00	0.001	0.003
5	Azimsulfuron (+) H	424.4	100	3.44	424.70 >181.95		Y = 8559190x−71205.6	0.98	0.001	0.003
6	Azoxystrobin (+) F	389.4	99.4	13.05	404.00 > 372.20	404.00 > 344.20	Y = 11163900x−32226.6	1.00	0.001	0.003
7	Bensulfuron-methyl (+) H	410.4	99	8.74	410.80 > 148.90		y=15852600x−10788.3	1.00	0.001	0.003
8	Bentazone (−) H	240.28	99.9	3.1	238.95 > 131.90	238.95 > 16.55	y=97402.8x+4329.91	0.77	0.001	0.003
9	Bifenthrin (+) I	422.87	99.87	15.22	440.00 > 181.12		Y = 1458100x−60406.5	0.95	0.005	0.015
10	Bispyribac sodium (+) H	452.3	99.4	7.58	430.65 > 274.90	430.65 > 412.95	Y = 9461080x−73707.1	1.00	0.001	0.003
11	Bitertenol (+) F	337.41	99.4	19.21	338.10 > 99.10		Y = 4264670x−17969.0	1.00	0.005	0.015
12	Bromodiolone (+) R	527.41	96.1	17.71	526.65 > 260.80		Y = 84300.5x−6689.01	0.99	0.01	0.03
13	Buprofezin (+) I	305.4	99.1	14.92	306.00 > 57.15	306.00 > 201.10	Y = 16233100x−53575.1	1.00	0.001	0.003
14	Butachlor (+) H	311.89	99	14.93	312.10 > 238.00	312.10 > 87.00	Y = 1447830x+2096.09	0.99	0.001	0.003
15	Carbaryl (+) I	201.22	97	9.2	202.10 > 145.15	202.10 > 127.10	Y = 1708100x−4484.14	0.99	0.001	0.003
16	Carbendazim (+) F	191.18	98.2	5.88	192.10 > 160.15	192.10 > 132.15	Y = 12556400x+12333	0.99	0.001	0.003
17	Carbofuran (+) I&A	221.25	99.9	8.46	222.15 > 165.20	222.15>123.15	Y = 8276220x−7437.05	0.99	0.001	0.003
18	Carboxin (+) F	235.3	99.9	9.24	236.05 > 143.05		Y = 8682090x−37544.6	1.00	0.001	0.003
19	Carfentrazone ethyl (+) H	412.19	96.7	17.22	430.60 > 413.90	430.60 > 367.85	Y = 1194220x + 35120.4	1.00	0.001	0.003
20	Carpropamid (+) F	334.7	99.9	12.9	335.80 >138.90		Y = 4185380x−28938.6	1.00	0.005	0.015
21	Chlorantraliprole (+) I	483.1	96.9	9	483.80 > 453.00	483.80 > 286.00	Y = 1614750x−6667.12	1.00	0.005	0.015
22	Chlorpyriphos (+) I	350.6	99.8	16.06	351.85 > 97.05		Y = 671515x + 15567.8	0.94	0.001	0.003
23	Chlorpyriphos-methyl (+) I	322.5	99.9	14.07	321.90 > 125.10		Y = 146981x + 4611.24	0.91	0.05	0.15
24	Chlothianidine (+) I	249.68	98.9	3.31	249.90 > 169.15	249.90 > 132.05	Y = 2408110x−2321.32	0.94	0.005	0.015
25	Clodinafop-propargyl (+) H	349.7	99.2	12.44	350.20 > 266.00		Y = 146977x + 395.537	0.99	0.001	0.003
26	Clomazone (+) H	239.7	98.3	9.42	240.00 > 125.05	240.00 > 89.10	Y = 9197700x−66492.2	0.99	0.001	0.003
27	Cyhalofop butyl (+) H	357.4	99.1	14.14	374.75 > 255.90	374.75 > 357.95	Y = 1359980x−33780.6	1.00	0.01	0.03
28	Cyhalothrin-lambda (+) I	449.8	98.4	16.79	467.10 > 18.15		Y = 446509x−3139.89	0.99	0.001	0.003
29	Cymoxanil (+) F	198.18	99.4	4.26	199.10 > 128.05		Y = 1198830x + 9972.73	0.98	0.001	0.003
30	Cyphenothrin (+) I	375.5	98.4	18.05	393.00 > 151.10		Y = 5557200x + 21850.6	0.80	0.005	0.015
31	Diafenthiuron (+) I&A	384.6	99.9	16.96	384.95 > 329.25		Y = 387529x−842.502	0.95	0.001	0.003
32	Diazinone (+)	304.35	98.5	13.19	305.00 > 169.15	305.00 > 153.20	Y = 12099300x−75894.7	0.99	0.001	0.003
33	Diclofop-methyl (+) H	341.2	99.1	15.17	358.10 > 281.00	358.10 > 120.00	Y = 490982x + 9970.85	1.00	0.01	0.03
34	Difenoconazole (+) F	406.26	95.9	13.92	405.95 > 251.00		Y = 8403410x−38345.4	1.00	0.001	0.003
35	Diflubenzuron (+) I	310.68	98.1	12	310.75 > 157.95	310.75 > 140.90	Y = 8820510x−64453.3	1.00	0.001	0.003
36	Dimethoate (+) I&A	229.26	99.5	3.79	229.85 > 199.05		Y = 5960760x−22333.6	1.00	0.001	0.003
37	Dimethomorph (+) F	387.85	99	10.52	388.10 > 301.05		Y = 7303000x−51139.8	1.00	0.001	0.003

(*Contd.*)

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S. No.	Pesticide (ionization mode)	Mol. Wt. (amu)	Purity %	RT (min)	Quantifier (Q1)	Qualifier (Q2)	Regression Equation	Correlation coefficient (r)	LOD ($\mu g \cdot mL^{-1}$)	LOQ ($\mu g \cdot mL^{-1}$)
38	Dinotefuron (+) I	202.21	99.4	2.49	202.85 > 129.05		Y = 7376020x−34128.8	1.00	0.001	0.003
39	Edifenphos (+) F	310.4	99.1	12.84	310.90 > 109.10		Y = 6643920x−35935.2	1.00	0.001	0.003
40	Ethiprole (+) I	397.2	99.5	10.02	413.90 > 397.00		Y = 2419250x + 87442.3	0.97	0.001	0.003
41	Ethoxysulfuron (+) H	398.4	97.3	6.09	399.10 > 261.10	399.10 > 218.20	Y = 2365980x−16160.9	0.99	0.001	0.003
42	Fenamidone (+) F	311.4	99.9	10.01	312.05 > 92.10		Y = 9973700x−66857.5	1.00	0.001	0.003
43	Fenarimol (+) F	331.2	99.9	11.48	331.10 > 81.10		Y = 397888x + 14388.8	0.97	0.01	0.03
44	Fenazaquin(+) I&A	306.4	99.9	18.38	307.05 > 57.15	307.05 > 161.20	Y = 26788900x−109907	1.00	0.001	0.003
45	Fenoxaprop-p-ethyl (+) H	361.76	99.1	14.8	362.20 > 288.00		Y = 129042x + 712.878	0.99	0.001	0.003
46	Fenpyroximate (+) A	421.49	98.4	17.12	421.90 > 366.13		Y = 23108600−112012	1.00	0.001	0.003
47	Fenvalerate (+) I	419.9	98	17.8	437.25 > 18.05		y= 74029.9x + 229.153	0.89	0.005	0.015
48	Fipronil (-) I	437.15	96.7	12.04	434.75 > 329.95	434.75 > 249.95	Y = 265351x + 12491.6	0.97	0.001	0.003
49	Fluazifop-p-butyl (+) H	383.36	96.1	14.95	384.10 > 282.20	384.10 > 328.00	Y = 3314260x−14620.7	1.00	0.001	0.003
50	Flubendiamide (-) I	682.4	99.9	12.32	681.05 > 254.10		Y = 25543.6x+205.171	0.98	0.1	0.3
51	Flucythrinate (+) I&A	451.5	97	16.04	468.80 > 18.20		Y = 1121180x−7921.84	0.99	0.001	0.003
52	Flufenacet (+) H	363.33	99.7	11.56	364.00 > 152.10	364.00 > 194.15	Y = 9424160x−50337.1	1.00	0.001	0.003
53	Flufenoxuron (+) I	488.8	98.4	16.19	488.90>158.05		Y = 7193540x−59843.2	1.00	0.001	0.003
54	Flusilazole (+) F	315.39	99.8	12.12	315.95 > 247.10	315.95 > 165.10	Y = 7708110x−55126.0	1.00	0.001	0.003
55	Forchlofenuron (+) PGR	247.68	99.9	8.52	248.00 > 129.05	248.00 > 93.05	Y = 12705800x−71072.1	1.00	0.001	0.003
56	Halosulfuron-methy l(+) H	434.81	99.9	4.38	435.00 > 182.10		Y = 4729960x−25849.8	1.00	0.001	0.003
57	Haloxyfop methyl (+) H	375.72	99.9	14.07	376.10 > 90.85		Y = 411301x+796.018	1.00	0.01	0.03
48	Hexaconazole (+) F	314.2	99.3	13.33	313.95 >70.15	313.95 > 159.05	Y =7172170x−42238.4	1.00	0.001	0.003
59	Hexythiazox (+) I	352.9	99.9	16.02	353.10 > 228.10		Y = 4335950x−29814.0	1.00	0.001	0.003
60	Imazamox (+) H	305.33	99.9	2.33	305.90 > 261.00		Y =1973660x−7627.33	0.97	0.001	0.003
61	Imidacloprid(+) I	255.66	99.3	3.14	256.05 > 209.10	256.05 > 175.20	Y = 2413350x−7164.85	0.99	0.001	0.003
62	Indoxacarb (+) I	527.8	93.6	13.98	527.95 > 56.05	527.95 > 150.05	Y = 1003490x−4669.42	1.00	0.001	0.003
63	Iprovalicarb (+) F	320.4	98.7	11.39	321.15 > 119.10		Y = 13508400x−62452.8	1.00	0.001	0.003
64	Isopropalin (+) H	309.36	97.2	16.9	310.20 > 226.00		Y = 240158x + 3630.66	1.00	0.001	0.003
65	Isoprothiolane (+) F	290.4	99.1	10.64	291.00 > 231.05		Y = 9527470x−33331.7	1.00	0.001	0.003
66	Kresoxim methyl (+) F	313.3	96	12.61	314.15 > 267.15	314.15 > 222.15	Y = 907479x−1445.66	0.99	0.001	0.003
67	Lactofen (+) H& F	461.77	96.2	15.04	478.75 > 343.85	478.75 > 222.75	y=11841700x−94169.2	1.00	0.001	0.003
68	Malathion (+) I	330.4	98.7	10.62	332.15 > 128.05	331.05 > 99.1	Y = 40522.0x−858.827	0.66	0.005	0.015
69	Metaflumizone (+) I	506.4	99.3	14.97	506.70 > 178.00	505.10 > 117.05	Y = 857972x−5775.14	0.99	0.005	0.015
70	Metalaxyl (+) F	279.33	99.6	8.52	280.05 > 220.15		Y = 11349200x−55289.8	1.00	0.001	0.003
71	Methabenzthiazuron (+) H	221.28	98	6.39	222.00 > 165.10	222.00 > 150.10	Y = 15540600x−26132.7	1.00	0.001	0.003
72	Methomyl (+) I	162.21	99.9	2.81	162.90 > 88.10	162.9 > 106.1	Y = 560962x+6391.55	0.91	0.001	0.003
73	Metsulfuron-methyl (+) H	381.36	98.7	2.67	382.00 > 167.10	382.00 > 141	Y = 9336590x−75760.9	0.99	0.001	0.003

(*Contd.*)

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S. No.	Pesticide (ionization mode)	Mol. Wt. (amu)	Purity %	RT (min)	Quantifier (Q1)	Qualifier (Q2)	Regression Equation	Correlation coefficient (r)	LOD ($\mu g \cdot mL^{-1}$)	LOQ ($\mu g \cdot mL^{-1}$)
74	Myclobutanil (+) F	288.77	99.4	10.85	289.05 > 70.10		Y = 5686080x−32903.6	1.00	0.001	0.003
75	Oxycarboxin (+) F	267.3	99.9	4.12	267.95 > 175.05		Y = 13474300x−48291.6	1.00	0.001	0.003
76	Penconazole (+) F	284.2	97.7	12.72	284.10 > 70.10		Y = 3303280x−18242.3	0.99	0.001	0.003
77	Pencycuron (+) F	328.8	99.7	13.76	329.05 > 125.05	329.0 > 218.15	Y = 14537200x−47534.7	1.00	0.001	0.003
78	Pendimethalin (+) H	281.31	98.8	16.21	281.90 > 211.80	281.90 > 193.85	Y = 4119120x + 20347.3	0.99	0.001	0.003
79	Permethrin (+) I	391.28	98.1	18.94	407.85 > 182.90		Y = 3016620x−16287.9	0.99	0.001	0.003
80	Phorate (+) I&N	260.36	95.8	13.65	260.85 > 75.15		Y = 482689x−1254.95	0.98	0.001	0.003
81	Phosalone (+) I&A	367.81	99.5	13.49	368.00 > 182.05	368.00 > 111.05	Y = 1896610x−12644.1	0.98	0.001	0.003
82	Phosphamidon (+) I	299.68	97.6	5.46	300.05 > 174.15		Y =1963010x−11817.3	0.99	0.001	0.003
83	Pretilachlor (+) H	311.8	98.2	14.67	311.90 > 251.95	311.90 > 175.95	Y = 23495300x−97903.1	1.00	0.001	0.003
84	Profenophos (+) I	373.63	98.6	14.91	372.80 > 302.95		Y = 1003390x−8065.71	0.99	0.001	0.003
85	Propachlor (+) H	211.7	99.8	8.47	212.00 > 169.90		Y = 4566160x−26348.6	0.99	0.001	0.003
86	Propanil (−) H	218.08	99.6	9.99	216.00 > 159.80		Y = 32607.7x + 65179.4	0.98	0.005	0.015
87	Propazine (+) H	229.71	99.3	9.893	230.00 > 146.10		Y = 6136370x−51301.7	0.99	0.001	0.003
88	Propiconazole (+) F	342.2	98.4	13.14	341.95 > 69.10	341.95 >159.05	Y = 4750940x−26129.5	1.00	0.001	0.003
89	Propoxur (+) I	209.24	99.8	6.29	209.90 > 111.05	209.90 >168.05	Y = 7863170x 1082.63	1.00	0.001	0.003
90	Pyraclostrobin (+) F	387.8	99.9	13.29	388.10 > 194.15	388.10 >163.15	Y = 4947880x−11162.2	1.00	0.001	0.003
91	Pyrazosulfuron-ethyl (+) H	414.4	99.6	4.95	415.10 > 182.10		Y = 6637720x−42284.3	1.00	0.001	0.003
92	Pyriproxyfen (+) I	321.36	99	15.77	322.00 > 91.20		Y = 423348x + 1638.04	0.97	0.005	0.015
93	Quinalphos (+) I	298.3	99.8	12.58	299.05 > 147.15	299.05 > 163.10	Y = 2937430x−3659.30	1.00	0.001	0.003
94	Quizalofop ethy l(+) H	372.8	96.3	14.94	373.10 > 299.40	373.10 > 271.10	Y = 15266.8x + 1063.42	0.95	0.01	0.03
95	Simazine (+) H	201.65	99.7	6.52	201.90 > 124.00	201.90 > 103.85	Y = 2566930x−17886.5	1.00	0.001	0.003
96	Tebuconazole(+) F	307.82	98.7	12.82	308.00 > 70.10	308.00 > 125.10	Y = 12089500x−67339.8	0.99	0.001	0.003
97	Temephos (+) I	466.5	95.6	15.2	466.80 > 124.90		Y = 1565380x−5313.37	1.00	0.001	0.003
98	Tetramethrin (+) I	331.4	90.3	15.22	333.15 > 164.10		Y = 335957x−1079.60	0.96	0.05	0.15
99	Thiacloprid (+) I	252.72	99.7	4.03	253.05 > 126.10	253.05 > 90.10	Y = 9900870x−17822.4	1.00	0.001	0.003
100	Thiamethoxam (+) I	291.71	99.1	2.77	292.00 > 211.15	253.05 > 90.10	Y = 3538720x−16060.4	0.99	0.001	0.003
101	Triadimefon (+) F	293.75	99.5	10.89	294.00 > 69.10		Y = 7140760x−48235.8	1.00	0.001	0.003
102	Triasulfuron (+) H	401.8	97.1	3.79	401.70 > 166.95		Y = 5210110x−19835.8	0.99	0.001	0.003
103	Tricyclazole (+) F	189.24	99.4	4.72	189.95 > 136.05		Y = 4173970x−31.8477	0.99	0.001	0.003

I = Insecticide, F = Fungicide, H = Herbicide, A=Acaricide, N = Nematicide, PGR = Plant growth regulator, #Ionisation mode positive (+) or negative (−) are given in parenthesis, RT = Retention time, LOD = Instrumental limit of detection, LOQ = Limit of quantification given in Fig. 1 and the treatment combinations are enlisted in Table 2.

Liquid Chromatography-Tandem Mass Spectroscopy (LC–MS/MS) and Method Development

Quantification of the target pesticides was done using Shimadzu LC-MS/MS-8030 (UPLC model-Nexera, LC-30AD Liquid Chromatography, SIL- 30AC auto-injector (Shimadzu Corporation, Kyoto, Japan) coupled with Triple Quadrupole Mass Detector. Zorbax Eclipse Plus C-18 column, 3 mm i.d., 10 cm length with 3.5 µm column particle size (Agilent Technologies, USA make) column was used. Method was operated in gradient programming (details of gradient elution are given in method development section) with the flow rate of 0.2 $mL \cdot min^{-1}$. DUIS–ESI interface was availed in Electrospray Ionization and nitrogen was utilized as nebulizing gas. Software Lab Solutions Version 5.86 was exercised. List of pesticides, ionization mode for each pesticide, LC-MS/MS conditions for method development are mentioned in Table 1.

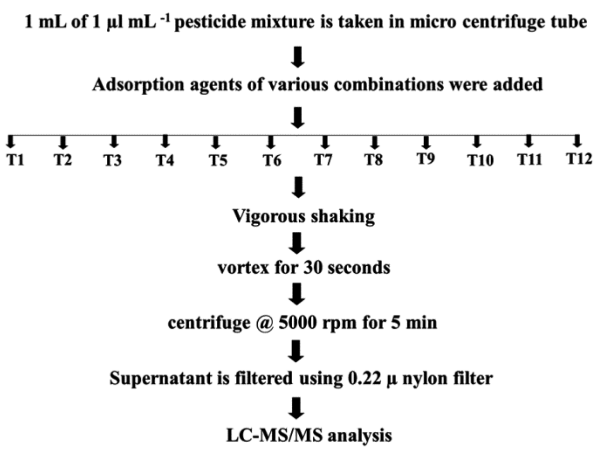

Fig. 1 — Flow diagram of d-SPE- clean-up of pesticides with various combinations of clean-up agents

Method Validation

To assess the suitability and applicability of the developed multi residues analysis method, single laboratory validation was performed as per the SANTE guidelines (SANTE/11813/2021)[17] Some of the parameters considered as per the guidelines are detailed below:

Specificity

It is the ability of a detector (supported by the selectivity of the extraction, clean-up, derivatization or separation, if necessary) to provide signals that effectively identify the analytes. In order to achieve the specificity of 103 pesticides, the detector should be able to deliver signals that detect the desired unique peak in a matrix. Detection of the target peak at a concentration of > 30 percent of the quantification/reporting limit is considered as specificity of a target analyte (SANTE/11813/2021).

Linearity

It is nothing but the ability of a detection system to produce an acceptable linear correlation between test result & concentration of analytes. To assess the linear response zone of 103 pesticides, mixed working standard solutions were prepared at 0.001, 0.005, 0.01, 0.05, 0.1, 0.5 and 1 $\mu g \cdot mL^{-1}$ concentration level and injected in LC-MS/MS using the optimized method. The concentration-response curve was generated by recoding the detector response against increasing concentration. This curve was used to determine the linear response zone, and the LC-MS/MS Lab Solution Browser software calculated the linear regression equation and coefficient of correlation.

Accuracy-Recovery

Reliability of the developed analytical method i.e., the efficiency of clean-up techniques was determined

Table 2 — List of combinations of adsorbing agents used in different treatments during d-SPE clean-up

Treatment	Adsorbent combinations
T1	150 mg anhy.$MgSO_4$
T2	40 mg PSA + 150 mg anhyd.$MgSO_4$
T3	50 mg PSA + 150 mg anhyd.$MgSO_4$
T4	75 mg PSA + 150 mg anhyd.$MgSO_4$
T5	100 mg PSA + 150 mg anhyd.$MgSO_4$
T6	25 mg C-18 + 50 mg PSA + 150 mg anhyd.$MgSO_4$
T7	25 mg C-18 + 25 mg PSA + 150 mg anhyd.$MgSO_4$
T8	10 mg GCB + 50 mg PSA + 150 mg anhyd.$MgSO_4$
T9	10 mg GCB + 25 mg PSA + 150 mg anhyd.$MgSO_4$
T10	10 mg GCB + 25 mg C-18 + 50 mg PSA + 150 mg anhyd.$MgSO_4$
T11	10 mg GCB + 25 mg C-18 + 25 mg PSA + 150 mg anhyd.$MgSO_4$
T12	7 mg GCB+20 mg C-18 + 25 mg PSA + 150 mg anhyd.$MgSO_4$

by conducting the recovery study. Recovery studies were conducted at 1 μg·mL^{-1} concentration and d-SPE clean-up was carried out, subsequently analyzed by the developed LC-MS/MS method. Samples were analyzed in triplicate and the acceptance criterion* for recovery for spike level is 70–120% according to SANTE guidelines (SANTE/11813/2021). Percentage recovery of pesticides was calculated by the following formula.

% Recovery (against solvent standard)

$$[RSS] = \left(\frac{Peak\ area\ of\ the\ spiked\ sample}{Peak\ area\ of\ the\ solvent\ standard}\right) \times 100 \quad \ldots (1)$$

*Recovery < 70% = not acceptable, 70–120% = acceptable, > 120% = not acceptable.

Precision

The precession of the method was confirmed in terms of intra-laboratory repeatability and calculated as follows

% Relative Standard deviation

$$[\%RSD] = \left(\frac{Standard\ deviation}{Mean}\right) \times 100 \quad \ldots (2)$$

Results and Discussion

Method Development

A single robust multi residue method was developed for detection and quantification of 103 target pesticides using LC-MS/MS coupled with Triple Quadrupole Mass Detector. In LC, mobile phase was mixture of A (80:20:: 5 mM ammonium formate buffer dissolved in water: methanol) and B (10: 90:: 5 mM ammonium formate buffer dissolved in water: methanol) used at a flow rate of 0.2 mL·min^{-1} under gradient programming for 22 min runtime. Mobile phase programming for liquid chromatography is depicted in Fig. 2. Each run included the injection of a 2 μL sample. In order to optimise the distinct multiple reaction monitoring (MRM) transitions for each pesticide independently, Electro Spray Ionization (ESI) operating in both positive and negative mode was used. At flow rates of 3.0 and 15 L·min^{-1}, respectively, nitrogen was employed as a nebulizing gas and a drying gas. As the Collision-Induced Dissociation (CID) gas, ultrapure argon was used. Desolvation Line (DL) temperature and heat block temperatures were maintained at 120ºC and 300ºC, respectively. Q1 pre-bias, Q3 pre-bias, collision energy, dwell time and pause times were optimized individually. Quantifier and qualifier ions, regression equation, correlation co-efficient, instrumental LOD, LOQ values of each pesticide are mentioned in Table 1. Software Lab Solutions Version 5.86, was used for data acquisition and analysis. Most of the pesticides were identified in + ESI modes while some pesticides like bentazone, fipronil, flubendiamide and propanil etc were identified in −ESI mode. Total ion chromatograms for all the 103 pesticides are given in Fig. 3.

Method Validation

Specificity

Specificity of the pesticide for trace level identification and quantification was achieved by optimizing quantifier (Q1) and qualifier (Q2) MRM transitions for each pesticide, which unambiguously quantified target analyte pesticide in the presence of

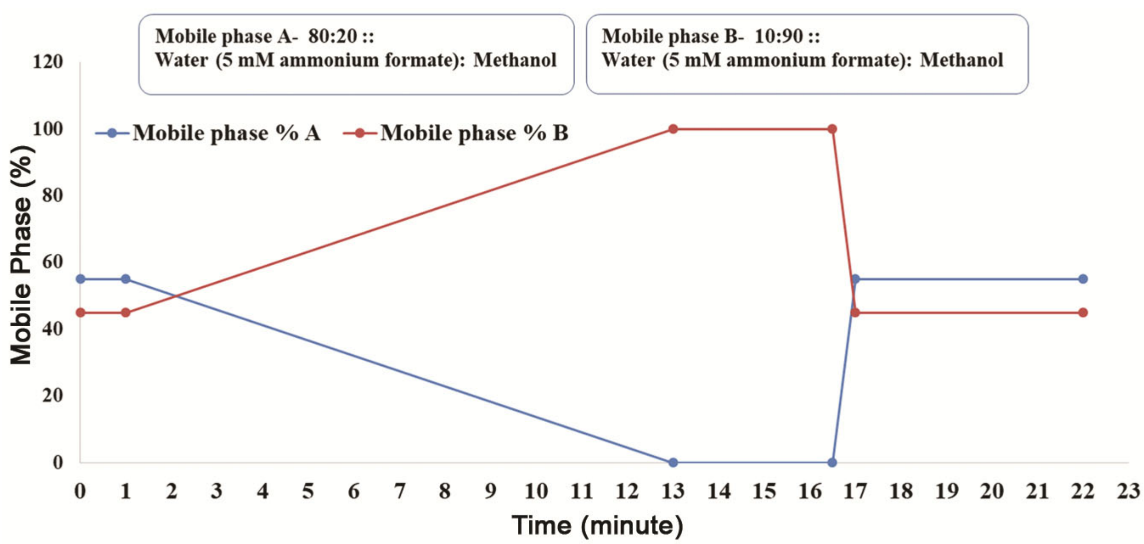

Fig. 2 — Mobile phase programming for LC-MS/MS

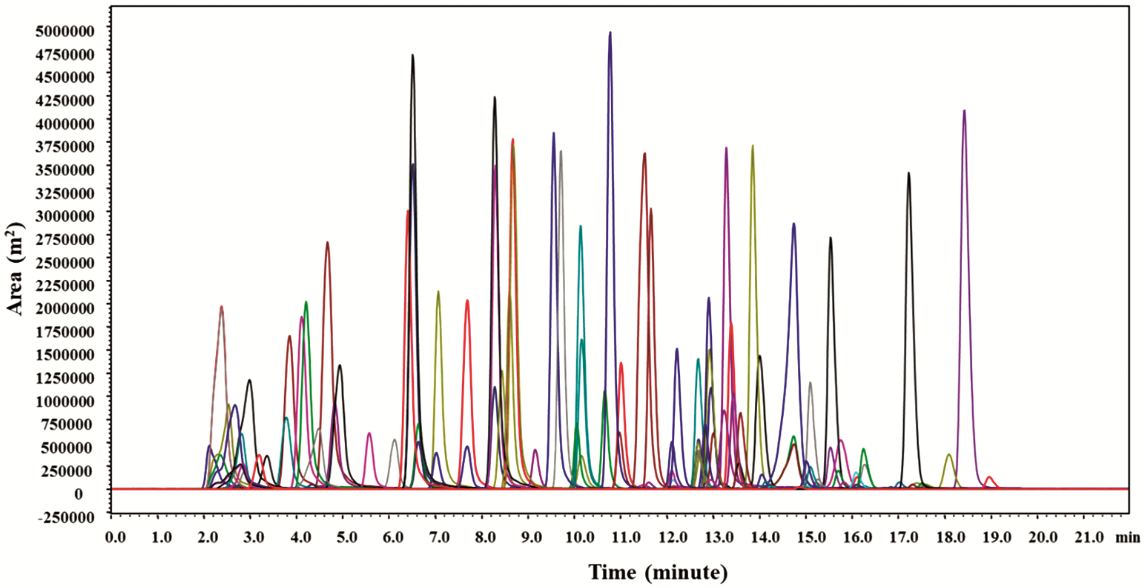

Fig. 3 — LC-MS/MS total ion chromatograms of 103 pesticides

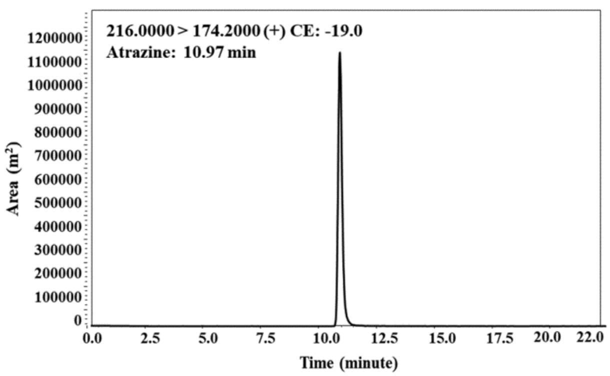

Fig. 4 — Specificity of atrazine in LC-MS/MS

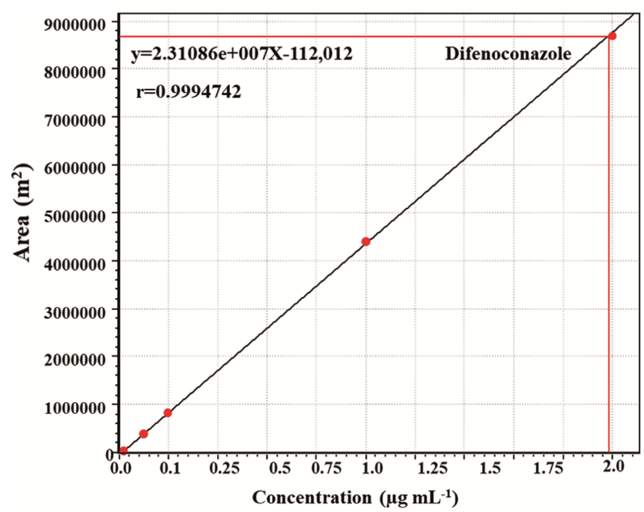

Fig. 5 — Linearity of difenoconazole obtained through LC-MS/MS operating software

other pesticides. MRM transitions for the 103 specified pesticides under the study are given in Table 2 and the specificity of a representative pesticide (atrazine) is given Fig. 4.

Linearity

In concentration range of 0.001 to 1 $\mu g{\cdot}mL^{-1}$ majority of the analyte displayed linear response with correlation coefficients with r > 0.99. Diflubenzuron (1.00), hexythiazox (1.00), propoxur (1.00), atrazine (1.00), dimethoate (1.00), pyrazosulfuron-ethyl (1.00) had showed good linear very response with r > 0.99. Correlation coefficients and regression equations are mentioned in the Table 1 and linearity curve for a representative pesticide (difenoconazole) is given in Fig. 5.

Accuracy- Recovery against Solvent Standard

The d-SPE clean-up agents mentioned in this study, are often employed even in recent studies as well in the clean-up of pesticide residues analysis from various matrices like fruits, vegetables, food and environmental samples for targeted analyte analysis.[18,19] Hence it is crucial to study the adsorption behavior of these agents onto different class pesticides. Accuracy was measured in terms of recovery at 1 $\mu g{\cdot}mL^{-1}$ concentration and the recovery results are enlisted in Table 3. Pesticides with the recovery percentage within the range of 70–120% are considered good recovery and are acceptable. In this paper, 12 combinations (T1 to T12) of most commonly used clean-up agents (C-18, GCB, PSA and anhyd.$MgSO_4$) were made and compared for the

Table 3 — Recovery of pesticides at 1 μg mL^{-1} concentration with different absorbent combinations used during QuEChERS clean-up technique — (*Cond.*)

S. No	Pesticides (Clean-up combinations)	T1 % RSS	T1 % RSD	T2 % RSS	T2 % RSD	T3 % RSS	T3 % RSD	T4 % RSS	T4 % RSD	T5 % RSS	T5 % RSD	T6 % RSS	T6 % RSD	T7 % RSS	T7 % RSD	T8 % RSS	T8 % RSD	T9 % RSS	T9 % RSD	T10 % RSS	T10 % RSD	T11 % RSS	T11 % RSD	T12 % RSS	T12 % RSD
1	Alpha-Cypermethin	130.06	5.04	105.79	6.71	119.01	3.72	102.02	8.38	95.73	8.03	84.47	3.46	83.93	2.57	92.13	2.99	94.47	0.64	84.15	1.58	86.54	2.86	86.97	2.63
2	Allethrin	96.23	5.44	94.34	1.65	100.72	2.40	83.73	2.72	85.04	2.36	94.49	3.67	97.46	2.66	96.60	2.83	95.38	3.09	91.27	1.48	95.46	0.49	95.42	2.69
3	Anilophos	104.89	4.61	102.66	0.80	105.56	1.68	91.73	3.49	89.78	4.68	118.20	3.18	117.89	3.63	98.41	3.59	94.34	2.22	92.69	1.54	93.67	2.19	93.29	2.36
4	Atrazine	105.20	2.21	96.95	1.57	112.56	1.56	93.88	4.52	94.43	5.15	102.75	4.08	103.92	1.01	100.92	2.37	98.45	1.44	96.54	0.99	98.85	1.21	98.67	0.37
5	Azimsulfuron	91.55	4.75	8.38	3.10	4.80	5.63	1.92	13.68	1.13	9.30	4.40	2.78	15.26	3.53	5.77	9.18	12.38	2.98	3.17	7.82	9.05	8.17	13.83	4.38
6	Azoxystrobin	98.66	3.28	96.14	0.65	100.81	1.40	84.58	6.90	85.85	3.24	94.06	4.62	95.76	1.63	91.65	2.73	90.62	0.72	90.40	1.23	92.23	0.97	91.52	0.91
7	Bensulfuron-methyl	100.16	4.81	8.72	2.41	10.78	0.17	3.22	7.99	2.94	15.43	6.49	2.91	15.86	4.01	6.23	5.47	9.56	1.65	4.99	6.87	9.60	2.68	11.30	0.73
8	Bentazon	100.47	2.53	29.38	2.48	31.45	9.75	10.59	5.09	7.49	5.75	18.99	2.32	44.34	2.42	20.86	6.85	45.30	4.17	18.31	4.05	34.36	2.65	42.59	2.23
9	Bifenthrin	108.96	5.32	118.41	1.57	105.19	1.77	103.50	4.09	102.66	1.69	94.88	3.31	94.60	3.18	91.85	4.06	94.00	2.76	85.40	1.72	85.74	1.04	87.82	1.23
10	Bispyribac sodium	109.24	5.04	0.25	2.12	6.77	0.50	0.00	0.00	0.00	0.00	0.12	17.01	0.32	16.57	0.11	5.28	0.34	7.32	0.09	8.39	0.20	8.11	0.36	9.15
11	Bitertenol	86.09	8.72	82.33	2.41	91.79	2.34	82.77	3.55	88.73	13.29	107.68	2.20	116.45	2.64	94.97	5.00	83.98	3.78	87.19	1.69	86.23	2.84	80.62	1.86
12	Bromodiolone	128.00	8.44	5.55	8.87	8.04	15.61	0.00	0.00	0.00	0.00	9.02	16.23	8.22	4.49	1.96	19.01	1.90	14.86	0.55	0.86	1.58	13.25	2.27	6.31
13	Buprofezin	102.27	5.27	98.19	1.46	106.45	3.41	95.14	3.20	98.01	6.77	92.72	4.03	93.06	1.62	93.14	3.68	92.55	1.71	89.34	1.51	91.93	1.11	91.14	1.77
14	Butachlor	97.73	7.77	113.38	1.18	102.12	1.52	88.65	1.54	99.29	7.56	91.51	7.15	100.88	1.76	95.38	3.85	91.59	2.91	92.42	1.48	86.69	0.94	83.98	1.89
15	Carbaryl	99.54	4.70	102.90	1.12	99.02	1.46	80.56	2.40	74.70	8.76	118.20	3.97	113.72	6.35	93.41	2.53	88.14	2.61	86.75	1.25	89.77	1.20	90.36	0.85
16	Carbendazim	82.34	7.57	92.78	0.39	88.53	2.43	70.65	4.45	71.00	11.00	97.89	4.31	101.72	3.60	27.93	9.42	22.61	3.49	23.42	1.94	23.14	1.12	26.15	3.61
17	Carbofuran	92.47	6.80	105.19	0.75	94.55	1.52	88.21	7.38	86.68	4.07	116.67	4.04	115.75	4.98	97.81	3.76	92.49	3.62	91.34	0.59	93.41	0.32	91.51	0.81
18	Carboxin	95.11	6.30	103.71	1.51	94.22	0.68	86.07	8.41	82.61	5.73	117.68	5.72	117.93	5.09	95.75	5.44	89.18	1.30	87.46	0.21	86.94	1.25	86.46	0.33
19	Carfentrazone ethyl	101.44	4.50	91.52	2.06	104.72	0.92	94.71	3.06	91.96	6.64	99.85	3.70	101.20	0.70	95.83	3.27	91.35	1.11	88.55	2.21	89.39	1.27	86.01	0.62
20	Carpropamid	110.10	4.91	108.96	1.29	113.43	1.43	93.17	5.54	96.47	4.14	111.73	2.94	110.27	2.60	98.33	3.03	96.01	2.82	92.26	1.77	96.27	1.03	94.93	1.78
21	Chlorantraliprole	100.09	4.99	104.86	0.71	95.55	0.41	73.32	5.38	71.98	7.25	112.78	2.35	113.33	2.54	33.16	8.20	24.63	1.25	25.32	1.78	24.45	3.70	28.41	2.03
22	Chlorpyriphos	99.67	5.40	116.01	1.01	98.74	1.67	105.21	1.48	112.03	5.25	112.07	3.74	112.36	4.07	74.91	3.08	68.50	2.54	68.64	1.47	66.90	1.09	69.31	3.28
23	Chlorpyriphos-methyl	101.46	5.54	110.05	0.82	100.66	1.11	104.95	2.84	99.16	7.41	113.64	3.06	112.43	5.45	78.40	2.76	71.67	1.43	72.25	0.92	72.82	0.92	74.76	1.79
24	Chlothianidine	98.33	3.51	104.46	1.12	100.57	2.36	77.97	5.31	73.75	4.98	99.81	3.82	100.50	0.84	95.93	2.82	95.38	1.28	92.99	1.69	96.12	1.13	95.56	0.90
25	Clodinafop-propargyl	99.02	2.72	95.66	2.20	97.31	0.71	108.65	3.03	101.06	12.22	115.89	4.55	109.00	8.27	93.25	2.33	90.86	0.59	90.85	1.03	86.88	0.43	87.17	1.19
26	Clomazone	107.29	3.56	104.12	0.58	112.01	2.01	88.47	1.84	88.74	7.87	92.43	2.98	96.23	1.18	95.38	2.27	93.07	0.68	91.75	0.85	95.09	0.91	93.62	0.65
27	Cyhalofop butyl	112.67	1.20	124.19	1.91	126.53	3.50	128.52	5.73	145.82	16.88	85.94	5.15	90.04	4.20	91.13	4.46	89.81	5.08	88.52	3.29	91.80	1.19	91.53	2.15
28	Cyhalothrin-lambda	114.23	9.81	119.22	4.88	107.67	2.05	116.13	13.24	136.63	9.51	115.17	2.69	117.76	4.23	98.13	5.40	94.85	2.24	87.20	1.18	91.08	1.79	88.23	2.35
29	Cymoxanil	99.09	4.17	107.46	1.41	100.02	1.68	83.39	3.05	79.87	7.47	111.81	3.03	111.03	5.10	98.78	4.25	94.20	0.90	92.99	1.11	98.30	0.39	96.81	1.04
30	Cyphenothrin	90.90	12.17	88.58	5.04	80.32	3.85	103.51	3.68	106.91	5.87	100.88	1.87	104.21	1.80	97.90	3.23	98.24	2.33	87.36	0.98	92.31	2.37	89.26	3.26
31	Diafenthiuron	102.78	4.25	101.19	1.58	103.38	2.47	86.76	4.34	82.78	6.74	99.09	3.41	101.42	3.65	96.27	5.24	95.12	1.70	89.38	2.40	93.13	0.71	91.63	4.56
32	Diazinone	101.32	15.82	114.27	1.32	114.40	2.03	102.38	2.10	110.37	8.49	101.23	3.45	105.64	1.20	99.08	4.09	85.59	11.95	89.65	1.17	91.31	2.00	89.36	1.65
33	Diclofop-Methyl	101.66	3.90	97.28	2.87	105.55	3.36	101.52	2.16	91.87	12.03	92.38	6.62	92.77	4.77	88.13	4.61	90.22	3.13	86.81	1.83	89.20	1.50	85.74	2.88
34	Difenoconazole	101.56	4.78	95.63	1.61	106.57	1.47	80.85	2.51	81.83	5.22	89.06	2.76	96.30	0.82	86.44	1.98	82.32	1.35	83.93	1.55	83.50	1.04	81.41	1.73
35	Diflubenzuron	101.74	5.71	100.30	1.07	101.00	0.81	96.64	5.49	102.74	13.24	109.28	3.22	108.86	2.36	42.34	3.06	32.49	3.62	45.03	2.34	43.65	3.62	38.87	2.55
36	Dimethoate	100.58	4.02	98.93	1.06	105.90	1.69	79.57	3.99	79.46	5.89	100.19	4.74	100.02	0.31	96.32	2.34	97.98	1.27	93.34	0.88	98.78	0.94	97.99	0.90

(*Cond.*)

Table 3 — Recovery of pesticides at 1 μg mL^{-1} concentration with different absorbent combinations used during QuEChERS clean-up technique — (*Cond.*)

	Clean-up combinations	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12												
S. No	Pesticides	% RSS	% RSD	% RSS	% RSD	% RSS	% RSD	% RSS	% RSD	% RSS	% RSD	% RSS	% RSD	% RSS	% RSD	% RSS	% RSD	% RSS	% RSD	% RSS	% RSD	% RSS	% RSD	% RSS	% RSD
37	Dimethomorph	94.39	6.73	101.76	2.50	92.83	0.93	84.89	4.00	85.06	5.08	129.20	3.66	124.67	4.46	95.79	6.38	90.45	2.79	88.02	1.74	87.02	1.96	86.53	1.07
38	Dinotefuron	100.29	4.56	108.97	0.31	103.93	0.81	84.68	2.94	82.99	3.70	117.20	2.72	118.77	2.39	100.02	3.85	95.46	0.98	92.27	0.52	97.00	1.05	93.24	1.76
39	Edifenphos	112.08	2.93	104.66	1.09	116.72	1.29	87.72	2.61	92.55	5.08	93.06	1.80	96.63	0.59	91.98	3.35	87.76	2.67	86.81	1.11	91.59	1.35	91.64	1.34
40	Ethiprole	103.38	4.76	101.44	0.41	105.51	1.05	87.21	4.27	88.20	5.42	97.10	3.90	99.52	1.13	95.98	3.95	90.88	3.02	90.30	0.89	92.56	0.88	90.19	1.01
41	Ethoxysulfuron	86.29	8.62	6.06	5.43	4.00	3.73	1.83	15.18	1.22	8.24	4.04	5.32	13.50	7.36	4.26	4.64	8.78	5.39	2.51	14.48	5.42	4.55	8.67	1.05
42	Fenamidone	100.05	7.97	98.09	1.15	101.99	1.33	87.34	3.91	91.15	6.06	119.04	4.21	118.08	2.74	99.87	4.89	88.27	4.07	90.47	2.01	89.37	1.46	86.13	1.56
43	Fenarimol	98.97	5.33	98.59	3.75	94.70	2.34	81.02	4.30	89.41	7.29	134.07	5.34	127.79	4.13	100.03	5.85	90.11	3.53	83.26	3.49	84.99	2.34	79.26	1.73
44	Fenazaquin	115.26	6.00	108.55	0.31	119.26	0.80	91.66	2.33	94.57	3.86	87.35	3.94	94.78	0.48	45.34	4.49	38.98	2.10	40.19	1.19	37.07	0.85	42.32	4.23
45	Fenoxaprop-p-ethyl	111.90	4.43	105.03	1.27	110.62	1.11	96.75	3.62	100.56	8.00	118.98	8.46	117.38	3.99	45.39	1.98	36.35	1.65	37.18	1.23	33.59	0.30	37.79	1.43
46	Fenpyroximate	118.69	6.30	101.85	1.44	119.51	1.89	85.16	3.02	89.79	18.47	95.76	7.00	106.29	1.50	68.25	1.19	67.95	1.49	60.83	1.64	64.37	0.78	60.69	5.78
47	Fenvalerate	127.96	12.31	117.15	7.29	116.56	3.11	103.35	5.03	117.44	8.10	108.35	2.74	107.65	1.36	96.73	4.84	90.07	1.84	81.46	2.79	83.18	2.91	81.19	1.75
48	Fipronil	76.31	5.93	96.46	1.29	73.97	3.66	70.80	13.66	79.25	11.49	111.59	3.81	111.05	4.10	93.51	4.62	91.20	1.56	92.23	4.72	92.18	3.46	87.34	8.23
49	Fluazifop-p-butyl	98.17	4.74	101.39	0.66	99.50	2.09	94.75	3.52	101.55	6.18	118.98	3.38	116.05	9.34	98.83	5.47	92.41	0.98	89.41	1.11	90.30	1.54	86.61	2.33
50	Flubendiamide	81.37	4.00	105.79	0.75	78.29	1.28	71.34	2.52	71.29	6.14	122.65	4.60	129.33	2.70	93.82	6.47	89.53	2.74	87.22	5.01	91.17	1.71	86.05	1.32
51	Flucythrinate	102.80	4.53	112.01	1.50	108.34	2.48	89.40	4.61	79.39	6.58	83.25	2.50	84.62	2.60	92.77	3.93	96.43	1.39	86.99	1.88	94.29	2.54	93.68	2.78
52	Flufenacet	105.60	4.60	103.58	0.73	108.89	1.76	89.01	5.33	93.28	11.59	106.97	2.99	106.56	1.17	96.96	3.36	94.81	0.23	92.40	1.87	92.02	0.74	88.93	0.98
53	Flufenoxuron	86.27	5.68	110.57	5.07	81.44	2.88	118.06	1.74	117.24	4.15	89.04	2.94	89.93	1.24	57.40	4.48	55.31	3.23	51.96	2.32	51.70	3.25	57.50	3.30
54	Flusilazole	109.37	5.03	108.67	0.47	116.51	1.28	93.42	3.33	98.79	3.25	94.26	2.59	97.53	1.50	95.16	2.60	92.15	2.34	89.40	0.65	96.26	1.14	95.30	1.41
55	Forchlofenuron	102.10	4.98	82.15	1.33	70.92	2.46	71.70	3.40	76.98	1.49	90.40	2.65	103.69	4.25	29.90	9.33	18.65	1.77	19.86	1.56	17.29	2.30	20.48	2.22
56	Halosulfuron-methyl	89.72	4.38	18.25	2.58	10.70	17.20	5.01	4.46	3.38	7.88	14.22	2.29	38.32	7.51	13.16	11.39	25.96	3.44	7.87	10.27	18.64	2.21	24.08	4.21
57	Haloxyfop methyl	113.95	5.10	108.22	1.61	115.91	1.04	100.84	4.36	100.04	6.08	84.50	3.67	87.67	0.33	91.60	2.24	92.15	1.32	90.50	1.41	93.21	0.80	91.91	1.79
58	Hexaconazole	86.76	11.46	97.80	1.23	102.14	0.68	85.79	3.18	86.04	3.68	96.89	3.59	97.49	2.26	97.74	1.92	89.95	7.24	91.44	1.05	96.70	0.64	94.78	1.07
59	Hexythiazox	89.15	6.61	115.51	1.82	87.88	2.06	89.87	3.79	86.11	8.51	113.75	2.88	112.35	5.80	93.54	4.18	95.02	1.83	89.05	1.82	88.98	0.70	88.06	3.84
60	Imazamox	80.27	12.71	0.58	8.88	3.07	14.73	0.20	18.47	0.00	0.00	0.40	9.54	0.80	12.93	0.33	16.28	0.60	17.73	0.20	14.03	0.59	17.94	0.72	10.15
61	Imidacloprid	92.30	5.45	113.81	1.55	92.93	1.28	83.21	5.74	79.26	4.89	137.58	3.36	131.21	4.83	102.19	5.29	94.42	2.51	92.53	1.26	96.27	0.26	91.97	2.37
62	Indoxacarb	89.98	4.58	86.23	2.31	92.58	4.19	93.05	2.03	85.83	3.99	90.97	3.69	98.33	0.97	90.19	2.25	88.63	1.73	90.37	1.55	87.89	1.23	85.19	2.60
63	Iprovalicarb	97.11	5.13	98.81	1.75	99.33	1.21	88.70	4.26	92.30	8.05	118.04	4.29	118.28	2.44	97.63	5.78	93.69	2.06	92.59	1.02	93.43	1.50	87.32	1.27
64	Isopropalin	114.48	4.33	102.52	1.82	118.98	1.58	91.63	2.37	82.00	12.33	89.15	3.22	86.11	1.76	94.59	2.47	99.96	4.29	91.63	2.17	96.18	2.50	98.87	3.71
65	Isoprothiolane	102.51	5.59	97.16	0.46	105.46	0.79	95.02	2.29	91.03	8.76	106.33	4.31	108.83	1.06	97.04	4.08	91.03	1.53	89.95	2.23	91.04	1.78	88.19	0.75
66	Kresoxim methyl	99.81	3.87	106.02	1.15	98.65	0.47	97.91	2.80	93.09	9.21	111.94	4.06	109.25	4.13	96.07	3.47	92.36	0.97	91.37	1.63	96.21	0.93	94.56	1.72
67	Lactofen	105.47	3.76	106.15	0.69	109.24	3.33	93.11	2.89	151.90	5.67	96.94	5.69	101.58	0.43	101.00	2.43	96.38	3.43	90.71	1.54	94.44	2.07	93.33	3.38
68	Malathion	102.27	1.23	102.26	2.23	101.27	2.24	91.49	2.88	95.20	6.65	129.71	3.80	127.28	3.23	97.93	7.36	92.47	0.89	90.84	3.74	90.49	2.07	86.53	2.03
69	Metaflumizone	108.02	2.52	100.03	2.01	111.52	4.27	91.88	4.86	142.87	0.61	86.09	3.32	89.23	2.19	15.89	20.89	10.74	1.20	9.99	4.83	9.76	7.02	12.49	2.84
70	Metalaxyl	101.40	4.20	98.74	1.33	107.55	1.24	85.76	7.91	90.59	8.99	95.84	3.95	99.12	1.04	97.00	3.67	93.75	2.46	93.05	1.63	92.76	2.56	92.94	2.26
71	Methabenzthiazuron	106.45	2.91	104.33	0.67	117.27	0.61	89.04	3.87	84.53	5.96	89.62	1.96	94.80	2.15	95.86	2.04	36.08	4.64	38.32	3.14	34.71	2.58	40.96	1.82
72	Methomyl	98.49	3.83	100.54	1.44	106.98	3.36	76.58	7.46	73.96	2.52	109.81	4.26	108.03	3.41	96.90	3.20	95.62	0.71	94.53	0.32	98.36	1.12	96.22	1.28

(*Cond.*)

Table 3 — Recovery of pesticides at 1 μg mL^{-1} concentration with different absorbent combinations used during QuEChERS clean-up technique — (*Cond.*)

	Clean-up combinations	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12												
S. No	Pesticides	% RSS	% RSD	% RSS	% RSD	% RSS	% RSD	% RSS	% RSD	% RSS	% RSD	% RSS	% RSD	% RSS	% RSD	% RSS	% RSD	% RSS	% RSD	% RSS	% RSD	% RSS	% RSD	% RSS	% RSD
73	Metsulfuron-methyl	98.06	3.95	4.52	5.73	3.59	1.73	1.12	15.29	0.77	15.66	2.45	10.03	8.38	5.09	2.91	13.23	6.39	4.01	1.65	11.19	4.65	1.77	7.52	3.61
74	Myclobutanil	97.11	7.04	104.34	1.24	97.02	1.53	92.69	2.44	96.89	3.26	112.61	12.17	119.51	10.57	98.75	4.99	91.96	1.63	89.89	1.18	90.11	0.78	85.88	1.42
75	Oxycarboxin	100.80	3.07	91.63	0.81	91.18	1.91	77.66	3.67	77.13	6.09	89.99	4.09	93.51	0.62	87.13	2.63	88.35	0.48	75.55	1.13	85.65	0.53	84.93	1.64
76	Penconazole	101.78	2.93	98.24	1.47	106.67	2.25	92.72	1.94	85.97	9.19	98.58	4.30	97.92	1.30	95.34	2.69	94.23	1.46	92.23	0.47	95.28	0.54	95.77	0.11
77	Pencycuron	106.26	3.91	107.56	1.04	108.56	2.74	95.65	2.22	102.91	6.58	108.93	2.79	112.89	2.24	95.52	2.95	86.82	1.42	85.99	1.00	83.82	1.49	84.06	1.94
78	Pendimethalin	117.78	5.82	108.07	4.47	118.25	1.52	112.77	5.24	112.98	6.67	99.89	3.48	99.14	5.34	80.62	3.70	79.24	2.79	74.63	2.33	74.87	1.94	77.20	1.42
79	Permethrin	108.53	6.31	103.43	4.72	123.87	2.94	128.12	3.84	130.96	9.77	99.01	4.57	101.21	0.64	94.94	3.59	92.34	2.05	81.57	1.12	86.82	3.03	87.29	4.17
80	Phorate	112.25	3.64	114.66	0.39	117.26	2.11	100.99	3.59	103.42	3.80	96.22	3.85	98.76	1.57	97.15	2.71	94.22	1.59	91.81	1.22	98.18	0.95	96.97	0.75
81	Phosalone	101.69	5.73	104.51	0.94	101.66	1.89	101.21	3.98	92.55	9.35	102.35	3.28	100.45	3.68	71.32	1.51	45.67	2.82	48.02	0.88	47.92	1.89	48.92	1.65
82	Phosphamidon	90.99	7.05	106.70	2.25	88.40	2.15	77.97	4.12	77.46	5.92	118.89	8.47	117.19	4.49	97.90	6.01	91.90	2.51	90.37	2.19	92.82	1.47	89.45	1.82
83	Pretilachlor	115.60	4.48	112.92	1.28	118.14	1.08	93.04	0.94	96.02	3.73	105.78	2.94	111.52	2.10	97.86	4.57	93.60	2.11	88.89	0.79	90.32	0.71	90.62	1.71
84	Profenophos	115.30	3.16	101.73	0.55	116.47	1.66	91.22	2.58	93.16	6.38	89.11	4.23	89.10	1.57	85.41	2.23	87.88	1.13	82.74	2.31	86.57	1.86	90.31	2.30
85	Propachlor	103.64	3.20	93.28	0.45	108.54	2.80	84.79	7.03	85.70	8.29	92.81	5.37	95.00	0.78	95.05	2.08	96.73	0.74	93.87	1.72	97.80	0.48	97.56	1.04
86	Propanil	86.46	8.64	103.35	2.60	84.10	3.95	83.44	3.10	78.36	7.62	114.62	4.24	113.41	5.23	78.62	5.33	70.50	4.91	72.77	3.40	75.57	4.92	74.76	1.84
87	Propazine	104.62	6.73	98.69	1.18	109.80	2.00	86.05	1.71	91.63	8.29	93.41	4.10	94.44	1.47	94.73	2.42	94.58	2.96	93.29	1.41	97.33	1.73	95.68	1.64
88	Propiconazole	85.30	16.23	98.88	0.93	97.59	0.96	91.27	2.23	90.80	4.07	95.45	3.03	96.84	0.25	94.80	3.44	84.03	10.87	90.99	2.26	94.49	0.62	92.88	1.96
89	Propoxur	96.26	7.00	107.09	0.85	98.96	1.10	83.04	3.38	79.49	4.39	119.53	3.55	119.88	4.46	101.02	4.53	90.22	2.69	89.74	2.31	95.33	0.91	91.92	1.97
90	Pyraclostrobin	91.84	13.70	103.19	1.14	95.21	1.46	100.42	6.39	96.47	10.00	110.59	4.63	107.33	3.60	33.50	6.71	25.17	9.04	27.95	1.41	26.11	1.51	32.31	2.85
91	Pyrazosulfuron-ethyl	90.12	5.88	10.99	2.56	5.90	0.72	2.98	11.72	1.90	6.72	7.41	2.46	24.87	7.07	8.16	8.88	18.65	1.67	4.81	9.35	13.85	3.23	20.06	1.34
92	Pyriproxyfen	115.93	4.91	117.79	0.87	119.09	2.42	105.26	5.69	99.46	7.27	99.16	3.10	100.92	3.65	84.27	2.66	80.41	3.42	80.38	1.59	78.01	3.77	78.09	1.44
93	Quinalphos	96.75	4.06	110.29	2.42	97.98	2.18	94.00	5.86	86.96	12.24	118.11	3.20	116.46	8.05	76.36	4.47	71.87	1.54	72.75	0.93	70.29	2.27	70.39	1.68
94	Quizalofop ethyl	110.59	6.16	107.43	2.96	111.15	1.92	90.71	5.17	86.59	4.90	117.48	18.35	108.46	13.01	43.54	3.82	32.26	4.31	32.25	4.67	34.93	2.52	40.80	4.36
95	Simazine	106.82	5.65	105.78	0.79	112.75	1.61	81.67	7.32	77.26	4.83	112.89	4.08	115.00	4.13	93.26	4.69	83.94	3.13	84.76	2.01	89.37	2.64	88.26	1.51
96	Tebuconazole	96.84	3.51	105.08	0.25	108.68	2.17	89.78	3.90	91.02	3.37	97.27	2.37	101.06	1.70	88.61	2.23	84.03	1.81	84.32	1.00	85.92	0.85	88.16	0.85
97	Temephos	105.07	4.09	99.62	2.05	104.99	3.31	104.72	3.57	112.24	4.96	103.94	3.07	109.55	5.46	75.86	4.04	70.66	2.32	73.35	1.62	70.95	0.91	74.67	3.02
98	Tetramethrin	95.46	4.19	100.30	2.47	93.37	3.86	92.76	1.36	93.53	2.13	112.89	5.12	119.87	5.09	99.48	6.25	94.15	2.13	89.76	4.31	93.28	1.15	88.49	3.36
99	Thiacloprid	92.09	5.34	109.66	0.21	91.66	0.91	82.80	3.70	79.65	7.24	124.72	3.12	122.72	5.03	97.47	4.67	92.76	0.86	91.75	0.72	90.93	0.70	88.20	1.64
100	Thiamethoxam	92.00	4.97	117.13	0.69	98.34	1.87	93.97	4.48	89.16	3.79	142.23	3.58	137.13	5.26	106.39	4.87	98.41	1.17	96.99	1.50	99.62	1.77	96.00	1.69
101	Triasulfuron	100.50	3.98	4.12	2.93	4.07	0.24	1.30	17.82	0.28	18.25	2.36	5.65	6.79	4.77	2.93	11.12	5.75	3.35	2.27	7.82	5.95	5.18	7.32	8.31
102	Tricyclazole	99.76	4.21	91.78	2.43	111.90	2.98	77.63	2.86	81.24	7.50	92.88	4.96	94.56	0.82	64.62	1.57	61.05	1.93	61.81	1.65	60.93	2.28	66.51	1.69
103	Triadimefon	103.84	4.39	98.29	1.47	108.94	1.70	87.68	1.35	98.15	7.17	97.19	3.49	98.25	0.76	96.30	2.18	92.18	1.24	90.99	2.21	93.53	0.96	90.63	1.22

T1 to T12 – various combinations of clean-up agents as given in Table 2, RSS – Recovery using solvent standard (mean of 3 replicates), RSD - Relative standard deviation (n = 3) recovery (Fig. 6 & Table 3). Many pesticides in various treatment combinations in the study had the acceptable recovery. Treatment, T1 (150 mg anhyd.$MgSO_4$) gave the acceptable recovery in the range of 70–120% for 100 pesticides (97.08% pesticides) out of 103 pesticides and 3 pesticides (alpha cypermethrin, bromodiolane and fenvelerate) showed recoveries greater than 120%. Since anhyd.$MgSO_4$ was used alone in this treatment, it gave good amount of acceptable recovery for the highest number of pesticides. Anhyd.$MgSO_4$, did not adsorbed any pesticides onto it thus ensuring good clean-up and recovered maximum number of pesticides. RSD of the most of the pesticides were less than 10% and a few had up to 20%, which shows the good precession of the method (Table 3).

In case of T2 where 40 mg PSA is used along with 150 mg anhyd.$MgSO_4$ gave acceptable recovery for 88.34% pesticides (91 pesticides) and > 120% of recovery for cyhalofop butyl. Here, 10.67% herbicides (11 herbicides) gave less recovery i.e., < 70%. Less recovery of pesticides can be attributed to the fact that, PSA being weak anion exchange sorbent, adsorbed these herbicides which are acidic and polar in nature belonging to the class of sulfonylureas (azimsulfuron, bensulfuron-methyl, ethoxysulfuron, halosulfuron-methyl, metsulfuron-methyl, pyrazosulfuron-ethyl, triasulfuron) and other class of herbicides like bentazone, bispyribac sodium, bromodiolane, imazamox.

Varela-Martinez *et al*. (2020)[20] also quoted that PSA caused adsorption of polar pesticides, hence our results were best explained since, in our study also polar pesticides were adsorbed by PSA giving lesser recovery of < 70%. Similar observations were noticed by Lehotay *et al*. (2005)[21], where when PSA was used as an adsorbent, acidic pesticides were not recovered. In other study made by He and Liu (2007)[22], PSA adsorbed acidic and planar pesticide like chlorpyriphos in apple and cucumber resulting in poor recovery and false negative results. Koesukwiwat *et al*. (2010)[23], also found retaining of acidic pesticides by anion exchange sorbent PSA.

In the treatment T3, consisted the combination of 50 mg PSA is used along with 150 mg anhyd.$MgSO_4$ recovered 87.37% pesticides (90 pesticides) in acceptable range, 11 pesticides with acidic and polar nature were recovered with < 70% like in T2 (azimsulfuron, bensulfuron-methyl, bentazone, bispyribac sodium, bromodiolone, ethoxysulfuron, halosulfuron-methyl, imazamox, metsulfuron-methyl, pyrazosulfuron-ethyl, triasulfuron), 2 pesticides (cyhalofop butyl and permethrin) recovery was > 120%.

Treatment T4, where 75 mg PSA was used, recovered 85.43% pesticides (88 pesticides) in acceptable range, and as like in T2 & T3, T4 also gave < 70% recovery for the 11 polar and acidic pesticides. Highly water soluble pesticides like bispyribac sodium and bromodiolane were not recovered hence not detected in LC-MS/MS and pesticides like cyhalofop butyl and permethrin had shown > 120% recovery.

By increasing the amount of PSA to 100 mg and anhyd.$MgSO_4$ kept constant at 150 mg in treatment 5, acceptable recovery of the pesticides further fell to 81.55 % (84 pesticides) and 10.67% of (11 pesticides) pesticides of polar nature were recovered in < 70%, 3 highly water soluble pesticides like bispyribac sodium, bromodiolone and andimazamox were not recovered. PSA presence had caused the adsorption of

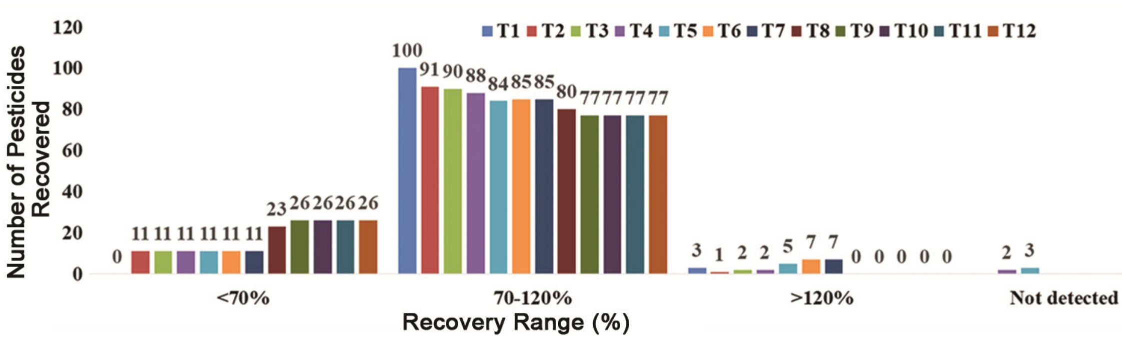

Fig. 6 — Recovery percentage of pesticides by different QuEChERS clean-up combinations at 1 µg·mL^{-1} concentration [**T1**: 150 mg anhyd.$MgSO_4$, **T2**: 40 mg PSA + 150 mg anhyd.$MgSO_4$, **T3**: 50 mg PSA+150 mg anhyd.$MgSO_4$, **T4**: 75 mg PSA + 150 mg anhyd.$MgSO_4$, **T5**: 100 mg PSA + 150 mg anhyd.$MgSO_4$, **T6**: 25 mg C-18 + 50 mg PSA+150 mg anhyd.$MgSO_4$, **T7**: 25 mg C-18 + 25 mg PSA+150 mg anhyd.$MgSO_4$, **T8**: 10 mg GCB + 50 mg PSA + 150 mg anhyd.$MgSO_4$, **T9**: 10 mg GCB + 25 mg PSA + 150 mg anhyd.$MgSO_4$, **T10**: 10 mg GCB + 25 mg C-18 + 50 mg PSA + 150 mg anhyd.$MgSO_4$, **T11**: 10 mg GCB + 25 mg C-18 + 25 mg PSA + 150 mg anhyd.$MgSO_4$, **T12**: 7.5 mg GCB + 20 mg C-18 + 25 mg PSA + 150 mg anhyd.$MgSO_4$]

polar pesticides thus giving lesser recovery. Pesticides like cyhalofop butyl, cyhalothrin-lamda, lactofen, metaflumizone and permethrin gave more than 120% recovery. We could see the trend that, by increasing the amount of PSA, recovery % of number of pesticides was reduced (Table 3).

When 25 mg C-18 combined with PSA and anhyd.$MgSO_4$ in treatment T6 & T7, 82.52% pesticides (85 pesticides) were recovered in acceptable range and 11 pesticides were recovered < 70%. Non-polar sparingly water soluble pesticides namely dimethomorph, fenarimol, flubendiamide and medium to high water soluble pesticides like imidacloprid, malathion, thiacloprid, thiamethoxam gave higher recoveries of > 120%. Here more recovery can be explained with the matrix enhancement caused due to the use of C-18.

In T8 where GCB was used along with PSA & anhyd.$MgSO_4$, 75.72% pesticides (80), were recovered in acceptable range, while 23 pesticides were recovered below 70%. In T9 GCB was used along with increased amount of PSA & anhyd.$MgSO_4$ and number of recovered pesticides below 70% further fell to 26. In T10, T11, T12, which are the combinations of all the clean-up agents namely C-18, GCB, PSA & anhyd.$MgSO_4$, 77.66% (77 pesticides) were recovered in 70–120% range, while 25.24% pesticides (26 pesticides) had recoveries of less than 70%.

Planar pesticides like carbendazim, chlorantraniliprole, diflubenzuron, fenazaquin, fenaxaprop-p-ethyl, phosalone were strongly adsorbed by GCB indicated by lesser recovery ranging from 0 to 50% as shown in Table 3. Whereas, chlorpyriphos, fenpyroximate, flufenoxuron, phorate, tricyclazole were moderately adsorbed by GCB indicating the recovery ranging from 50 to 70%. Other pesticides like flufenoxuron, forchlofenuron, metaflumizone, methabenzthiazuron, pyraclostrobin, quizalofop-ethyl were also adsorbed by GCB.

Lehotay *et al*. (2011)[24] observed that GCB strongly retains some pesticides, like cyprodinil, hexachloro-benzene, quintozene, thiabendazole, and some other structurally planar pesticides. Similar results were observed by Mol *et al*. (2007)[25], where GCB adsorbed planar pesticides like carbendazim, clofentazine, difluben-zuron, thiobendazole, tricyclazole, fenpyroximate, flufenoxuron, pymetrozine, triflumuron, thiophenate-methyl when analyzed in fruits and vegetables using liquid chromatography-mass spectroscopy.

Pesticides like azimsulfuron, bensulfuron-methyl, bentazone, bispyribac sodium, bromodiolane, ethoxysulfuron, halosulfuron-methyl, imazamox, metsulfuron-methyl, pyrazosulfuron-ethyl, triasulfuron were also given recovery of < 70%. This is due to adsorption of polar pesticides by PSA as seen in the treatments T2 to T6.

Our results can be comparable with the work of Varela-Martinez *et al*. (2020)[20], Lehotay *et al*. (2005)[21], where polar pesticides were adsorbed by PSA hence gave lesser recovery of < 70%.

Precision

Precision as relative standard deviation (%RSD), the intra-laboratory repeatability for each pesticide at three replicates was calculated and enlisted in Table 3. With some exceptions, the majority of the pesticides had percent RSD of < 20% indicating the method's acceptable repeatability and robustness.

Conclusions

Identification and quantification of multi-residues was achieved using LC-MS/MS method. The d-SPE employing various combinations of clean-up agents were studied and compared for the recovery. Anhyd.$MgSO_4$ used alone gave good recovery for 100 pesticides at 1 $\mu g \cdot mL^{-1}$ fortification, while rest of the combinations retained some pesticides onto them. Method was single laboratory validated with SANTE 2021 guidelines for specificity, linearity, accuracy, precession and fulfilled the same. Despite the fact that anhyd.$MgSO_4$ alone as an adsorbent gave negligible interactions with the pesticides yielding acceptable recovery for more pesticides, it will be erroneous to conclude that the presence of clean-up agents adversely affected the accuracy in pesticide residue study. On the contrary, in presence of food or environmental matrix, these agents contribute significantly to obtain accurate results. The study will help in judicious selection and optimization of absorbents in multi residue estimation depending on nature of matrix and analyte concerned.

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References

1 Wilkowska A & Biziuk M, Determination of pesticide residues in food matrices using the QuEChERS methodology*,* *Food Chem*, **125(3)** **(**2011) 803–812, https://doi.org/10.1016/j.foodchem.2010.09.094.

2 Souza-Silva E A, Jiang R, Rodríguez-Lafuente A, Gionfriddo E & Pawliszyn J, A critical review of the state of the art of solid-phase microextraction of complex matrices I. environmental analysis, *Trends Analyt Chem*, **71** **(**2015) 224–235, https://doi.org/10.1016/j.trac.2015.04.016.

3 Akiyama Y, Yoshioka N, Mitsuhashi T, Takeda N & Tsuji M, Multi-pesticide screening method for agricultural products with an automated sample preparation instrument, *J Food Hyg Saf*, **38(5)** **(**1997) 363–371, https://doi.org/10.3358/shokueishi.38.5_363.

4 Lehotay S J & Eller K I, Development of a method of analysis for 46 pesticides in fruits and vegetables by supercritical fluid extraction and gas chromatography/ion TVap mass spectrometry, *J AOAC Int*, **78(3)** (1995) 821–830, https://doi.org/10.1093/jaoac/78.3.821.

5 Anastassiades M, Lehotay S J, Štajnbaher D & Schenck F J, Fast and easy multi residue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce, *J AOAC Int*, **86(2)** **(**2003) 412–431, https://doi.org/10.1093/jaoac/86.2.412.

6 Perestrelo R, Silva P, Porto-Figueira P, Pereira J A, Silva C, Medina S & Câmara J S, QuEChERS-Fundamentals, relevant improvements, applications and future trends, *Anal Chim Acta*, **1070** (2019) 1–28, https://doi.org/10.1016/j.aca.2019.02.036.

7 Wang Y, Nie J, Yan Z, Li Z, Cheng Y & Chang W, Occurrence and co-occurrence of mycotoxins in nuts and dried fruits from China, *Food Control*, **88** (2018) 181–189, https://doi.org/10.1016/j.aca.2019.02.036.

8 Rutkowska E, Łozowicka B & Kaczyński P, Modification of multi residue QuEChERS protocol to minimize matrix effect and improve recoveries for determination of pesticide residues in dried herbs followed by GC-MS/MS, *Food Anal Methods*, **11(3)** (2018) 709–724, https://doi.org/10.1007/s12161-017-1047-3.

9 Sell B, Sniegocki T, Zmudzki J & Posyniak A, Development of an analytical procedure for the determination of multiclass compounds for forensic veterinary toxicology, *J Anal Toxicol*, **42(3)** (2018) 183–191, https://doi.org/10.1093/jat/bkx093.

10 Luke M A, Froberg J E & Masumoto H T, Extraction and cleanup of organochlorine, organophosphate, organonitrogen, and hydrocarbon pesticides in produce for determination by gas-liquid chromatography, *J AOAC Int*, **58(5)** (1975) 1020–1026, https://doi.org/10.1093/jaoac/58.5.1020.

11 Cook J, Beckett M P, Reliford B, Hammock W & Engel M, Multi residue analysis of pesticides in fresh fruits and vegetables using procedures developed by the florida department of agriculture and consumer Services, *J AOAC Int*, **82(6)** (1995) 1419–1435, https://doi.org/10.1093/jaoac/82.6.1419.

12 Lehotay S J, Quick, easy, cheap, effective, rugged, and safe approach for determining pesticide residues. in *Methods in Biotechnology-Pesticide Protocols*, edited by J L M Vidal & A G Frenich (Humana Press, Totowa, New Jersey, USA) **19** (2006) 239–261, https://doi.org/10.1385/1-59259-929-X:239-261.

13 Rejczak T & Tuzimski T, A review of recent developments and trends in the QuEChERS sample preparation approach, *Open Chem*, **13(1)** (2015) 980–1010, dx.doi.org/10.1021/jf304648h.

14 Lehotay S J, Anastassiades M & Majors R E, The QuEChERS revolution, *LcGc Eur*, **23(9)** (2010) 418–428.

15 Majors R E, Modern techniques for the extraction of solid materials-an update, *LC GC North America*, **20(2)** **(**2007), 71–81.

16 Zhao L & Stevens J, Optimizing recoveries of planar pesticides in spinach using toluene and the AOAC QuEChERS kits with graphitized carbon, In *123rd AOAC Int Ann Meet*, (2008) 141.

17 SANTE guidelines, Analytical quality control and method validation procedures for pesticide residues analysis in food and feed, SANTE 11312/2021 (European Commission) 2021, 155, [https://www.accredia.it/app/uploads/2021/02/SANTE_11312_2021.pdf, Accessed on 30.08.2023]

18 Damale R D, Dutta A, Shaikh N, Pardeshi A, Shinde R, Babu K D & Banerjee K, Multi residue analysis of pesticides in four different pomegranate cultivars: Investigating matrix effect variability by GC-MS/MS and LC-MS/MS, *Food Chem*, **407** (2023) 135179, https://doi.org/10.1016/j.foodchem.2022.135179.

19 Jankar J, Taynath B, Hingmire S, Patil R & Banerjee K, Method development and validation in the curry leaf matrix employing advanced mass spectrometry: Quantitative screening of 490 multiclass pesticides by buffered ethyl acetate technique, *J Env Sci Health, Part B*, **58(5)** (2023) 1–17, https://doi.org/10.1080/03601234.2023.2221994.

20 Varela-Martinez D A, Gonzalez-Curbelo M A, Gonzalez-Salamo J & Hernandez-Borges J, Analysis of multiclass pesticides in dried fruits using QuEChERS-gas chromatography tandem mass spectrometry, *Food Chem*, **297** (2019) 124961, https://doi.org/10.1016/j.foodchem.2019.124961.

21 Lehotay S J, Kok A D, Hiemstra M & Bodegraven P V, Validation of a fast and easy method for the determination of residues from 229 pesticides in fruits and vegetables using gas and liquid chromatography and mass spectrometric detection, *J AOAC Int*, **88(2)** (2005) 595–614, https://doi.org/10.1093/jaoac/88.2.595.

22 He Y & Liu Y H, Assessment of primary and secondary amine adsorbents and elution solvents with or without graphitized carbon for the SPE clean-up of food extracts in pesticide residue analysis, *Chromatographia*, **65** (2007) 581–590, https://doi.org/10.1365/s10337-007-0198-6.

23 Koesukwiwat U, Lehotay S J, Mastovska K, Dorweiler K J & Leepipatpiboon N, Extension of the QuEChERS method for pesticide residues in cereals to flaxseeds, peanuts, and doughs, *J Agric Food Chem*, **58(10)** (2010) 5950–5958, https://doi.org/10.1021/jf902988b.

24 Lehotay S J, 2011). QuEChERS Sample Preparation Approach for Mass Spectrometric Analysis of Pesticide Residues in Foods, In *Mass Spectrometry in Food Safety-Methods in Molecular Biology* edited by J Zweigenbaum (Humana Press) 2011, 747, https://doi.org/10.1007/978-1-61779-136-9_4

25 Mol H G, Rooseboom A, Van D R, Roding M, Arondeus K & Sunarto S, Modification and re-validation of the ethyl acetate-based multi-residue method for pesticides in produce, *Anal Bioanal Chem*, **389** (2007) 1715–1754, https://doi.org/10.1007/s00216-007-1357-1.