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MP 255

Multi-Residue Pesticide Analysis in Norbixin colour additive oleoresin using GC-MS/MS

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1. Overview

Norbixin is the yellow-(red) orange carotenoid, which in combination with bixin, constitutes for 80% of red-orange annatto dye. It is extracted from the pericarp of the seeds of *Bixa Orellana* (Figure 1). The annatto pigment has global economic significance, as it is one of the most widely used natural dyes to color food, cosmetics and pharmaceutical products.

Owing to its large culinary uses and other diverse applications, use of chemical pesticides for its production in large quantities is imperative. Dye extraction process may result in concentration of pesticides and in turn contribute to adverse impact on human health when incorporated in various preparations. Hence quantitation of residual pesticides in norbixin colour additive becomes very important.

As the oleoresin is a complex matrix for extraction, it is required to develop a rugged, sensitive and efficient method for residual pesticide analysis.

Figure 1 *Bixa Orellana* seeds and it's extract

2. Introduction

This study reports a highly sensitive method for simultaneous quantification of multiple pesticides in complex matrix of norbixin using modified QuEChERS[1] with triple quadrupole gas chromatography (GC-MS/MS) system.

3. Materials and methods

For this study, customized reference standard mixtures of more than 50 most observed pesticides in colour additives were procured from Restek® Corporation.

Norbixin oleoresin sample was extracted and used to prepare matrix-matched calibration standards and spiked samples. This method was validated for criteria mentioned in SANTE Guidelines[2].

GCMS-TQ8040 NX (Figure 2), manufactured by Shimadzu Corporation Japan, was used to quantify residual pesticides in Norbixin oleoresin sample.

3-1. Method development

Instrumental method was developed based on chromatographic and mass spectrometric parameters. Smart Pesticides Database Ver.2 for GC-MS/MS enabled quick instrumental method optimization for higher throughput. For most of the pesticides, 1 target and 2 reference MRM transitions were included in the method. Shimadzu's 'LabSolutions Insight' software was used for data processing, which helped in evaluating validation parameters with ease. This greatly reduced the method development and optimization time. Pretreatment method was developed based on modified QuEChERS extraction for better and consistent recoveries.

3-2. Sample and standard preparation

Norbixin oleoresin sample was extracted using acetonitrile solvent along with sodium chloride (AR grade), anhydrous magnesium sulphate (MgSO4) (AR grade) salts in optimized proportion to maximize recoveries of pesticides. After extraction, the supernatent was subjected to clean up.

The clean up was performed using optimum combination of C18, GCB (Graphitized Carbon Back), PSA (Primary secondary amine) and anhydrous $MgSO_4$ to minimize matrix interference, reduce instrument contamination and achieve lower LOQs. The extract was filtered through 0.22 µm PTFE filter. Final reconstitution volume was adjusted such that recovery samples were diluted by 2.5 times. All samples were analyzed as per conditions shown in table 1.

• Preparation of solvent standard concentration levels

The multi-residue pesticides mixtures obtained from Restek® were diluted using ethyl acetate to prepare standard mixture stock solution of about 1000 ppb. From this, concentration levels of 10 ppb, 25 ppb, 50 ppb, 250 ppb and 500 ppb were prepared.

• Preparation of matrix matched standard linearity levels

The norbixin sample extract prepared as per section 3-2 was used as a matrix blank. It was spiked with above solvent standard levels to prepare matrix match linearity of 1, 2, 4, 10, 20 and 50 ppb.

• Preparation of spike samples (Recovery samples)

To determine the extraction efficiency of the method, recovery study was conducted. For this, 2 g sample was spiked with pesticide standard mixture to prepare recovery samples of 5, 10 and 25 ppb. The spiked pesticides were then extracted, analyzed and quantified against matrix matched linearity to study their recoveries.

Figure 2. Shimadzu GCMS-TQ8040 NX

3-3. Analytical Conditions

Table 1 Instrument configuration and Analytical Conditions: GC-MS/MS

System Configuration	
Instrument	: GCMS-TQ8040 NX
Auto-injector	: AOC™-20i + s
Column	: SH-I-5Sil MS (30 m × 0.25 mm I.D., df = 0.25 µm) (P/N:221-75954-30)
Liner	: Restek® Topaz Liner, Splitless single taper w/ wool
GC	
Injector temp.	: 250 °C
Column oven temp	: 80 °C (2 min), 20 °C/min to 180 °C (0 min), 5 °C/min to 300 °C (3.00 min)
Run time	: 34 min
Injection mode	: Splitless (High pressure at 250 kPa)
Injection volume	: 2 µL
Carrier gas	: He
Linear Velocity	: 40.4 cm/sec (Constant mode)
MS	
Interface temp.	: 280 °C
Ion source temp.	: 230 °C
Ionization mode	: EI
Solvent cut time	: 5.0 min
Loop Time	: 0.3 sec

Table 2 Summary results

ID	Compound Name	Ret. Time (min)	Target MRM (m/z)	CE	Determination Coefficient (r^2)	LOQ (ppb)	Mean Recovery at LOQ (%)	Precision % RSD_R (n=6)	Precision % RSD_r (n=6)
1	Propamocarb	7.22	188.15>58.10	12	0.9988	10	117.82	2.50	0.26
2	Tetrahydrophthalimide (THPI) as Captan deg.	8.06	151.10>79.00	18	0.9940	25	77.46	24.68	17.20
3	Diazinon	10.89	304.10>179.20	19	0.9978	5	75.70	8.72	4.91
4	Pyrimethanil	11.08	198.10>118.10	30	0.9984	5	76.02	5.13	3.04
5	Metalaxyl	12.49	234.10>146.20	20	0.9976	5	86.61	8.19	5.74
6	Linuron	13.16	248.00>61.00	16	0.9994	10	76.64	7.63	11.14
7	Malathion	13.16	157.95>125.00	9	0.9986	5	82.78	4.84	5.44
8	Chlorpyrifos	13.39	313.95>257.90	17	0.9974	5	83.98	12.54	11.59
9	Cyprodinil	14.41	224.15>222.10	24	0.9969	5	75.91	9.39	8.45
10	Fipronil	14.64	367.00>213.00	29	0.9971	5	80.83	9.63	10.23
11	Triflumizole	15.06	278.05>73.10	8	0.9700	10	74.75	19.80	18.95
12	Thiabendazole	15.16	174.10>65.00	28	0.9822	5	78.00	10.67	19.61
13	Flutriafol	15.99	219.10>123.10	21	0.9945	5	87.45	12.42	4.92
14	Profenofos	16.33	339.00>268.90	15	0.9979	10	84.84	8.74	12.02
15	Fludioxonil	16.53	248.05>127.10	27	0.9959	5	75.29	4.38	5.06
16	Myclobutanil	16.78	179.05>152.00	9	0.9903	5	80.70	5.67	4.22
17	Buprofezin	16.82	172.10>57.10	21	0.9949	5	75.56	7.23	13.44
18	Chlorfenapyr	17.06	247.00>227.00	14	0.9824	10	71.85	12.95	17.79
19	Trifloxystrobin	18.81	222.05>190.10	5	0.9925	5	82.26	13.02	6.73
20	Propiconazole-1	18.85	172.95>109.00	25	0.9987	5	84.37	8.91	13.24
21	Quinoxyfen	18.90	237.00>208.10	27	0.9987	5	75.14	5.35	5.50
22	Propiconazole-2	19.07	172.95>109.00	25	0.9973	5	80.86	11.47	9.79
23	Fenhexamid	19.11	177.00>113.00	17	0.9930	5	71.02	10.07	8.15
24	Fluopicolide	19.17	209.00>182.00	19	0.9990	5	83.83	7.84	5.94
25	Tebuconazole	19.58	250.10>125.10	21	0.9956	5	78.48	10.11	12.67
26	Piperonyl-butoxide	19.84	176.05>131.10	13	0.9977	5	80.31	5.39	2.62
27	Iprodione	20.48	314.00>245.00	12	0.9990	5	69.39	17.74	13.68
28	Fluxapyroxad	20.70	381.10>159.10	16	0.9983	5	86.18	5.18	5.99
29	Bifenthrin	20.74	181.05>165.10	22	0.9963	5	84.43	5.33	4.87
30	Bifenazate	20.96	300.10>258.10	9	0.9897	5	61.30	11.86	9.26
31	Chlorantraniliprole	20.99	278.00>249.00	20	0.9927	5	63.24	13.18	12.74
32	Etoxazole	21.03	330.10>57.10	24	0.9941	10	88.50	11.16	4.68
33	Fenpropathrin	21.07	265.05>210.10	12	0.9994	5	77.59	11.35	8.58
34	Pyriproxyfen	22.24	136.10>78.00	24	0.9925	5	88.83	11.43	4.31
35	Lambda-Cyhalothrin	22.62	208.05>181.10	9	0.9983	5	88.73	5.68	4.71
36	Spirodiclofen	23.83	312.00>109.10	21	0.9863	5	74.66	15.32	6.05
37	Permethrin-1	24.10	162.95>127.00	9	0.9947	5	79.40	9.89	4.36
38	Permethrin-2	24.35	162.95>127.10	9	0.9968	5	79.42	4.44	5.73
39	Pyridaben	24.36	147.15>117.10	24	0.9961	5	85.03	8.22	9.19
40	Fenbuconazole	25.08	198.10>129.10	12	0.9986	5	83.32	4.42	3.68
41	Cyfluthrin-1	25.17	226.05>206.10	15	0.9932	10	75.65	12.42	15.97
42	Cyfluthrin-2	25.37	226.05>206.10	15	0.9798	10	85.47	13.12	10.49
43	Cyfluthrin-3	25.48	226.05>206.10	15	0.9894	10	86.09	12.40	9.76
44	Cyfluthrin-4	25.58	226.05>206.10	15	0.9975	10	88.12	9.80	6.57
45	Boscalid	25.85	140.10>76.00	24	0.9980	5	81.28	3.45	3.54
46	Cypermethrin-1	25.78	162.95>127.00	9	0.9950	5	85.96	7.36	7.41
47	Cypermethrin-2	26.00	162.95>127.00	9	0.9957	5	78.84	9.92	15.88
48	Cypermethrin-3	26.09	162.95>127.00	9	0.9986	5	72.57	7.35	10.27
49	Cypermethrin-4	26.18	162.95>127.00	9	0.9954	5	81.45	8.22	2.72
50	Pyraclostrobine	27.68	164.05>132.10	12	0.9989	5	83.86	3.69	4.20
51	Difenoconazole-1	28.35	323.05>264.90	18	0.9984	5	83.68	9.86	8.59
52	Difenoconazole-2	28.46	323.05>264.90	18	0.9977	5	76.02	10.03	5.15
53	Indoxacarb	28.79	264.05>176.00	15	0.9969	5	74.27	12.26	6.14
54	Azoxystrobin	29.26	344.10>329.00	21	0.9956	5	78.64	5.13	10.39
55	Dimethomorph-1	29.48	301.05>165.10	15	0.9950	5	84.57	6.02	4.92
56	Dimethomorph-2	30.05	301.05>165.10	15	0.9969	5	86.63	4.97	5.63

4. Results

Validation parameters like linearity, recovery and precision were studied to establish LOQs. The summary results are shown in Table 2.

4-1. Linearity

For linearity study and quantifying spiked samples, matrix matched calibration standards were used. Multilevel calibration curve included 1, 2, 4, 10, 20, and 50 ppb concentration levels. All calibration standards were found within 80 to 120% accuracy range which is well within the criteria mentioned in SANTE guidelines.[2]

4-2. Recovery

Recovery was evaluated by analyzing spiked samples at 5, 10 and 25 ppb (six spiked samples at each level) against matrix matched linearity plotted between 1 to 50 ppb. Mean recoveries were found to be within 60-120% at LOQ level (Refer Table 2). As mentioned previously, spiked samples were diluted 2.5 times, so that final concentrations analyzed against linearity were 2, 4 and 10 ppb.

4-3. Precision (% RSD)

For precision, repeatability and within-laboratory reproducibility studies were carried out.

RSD_r : Repeatability experiment was performed by injecting 6 replicates of spiked samples at 5, 10 and 25 ppb concentration levels. The % RSD for 6 injections at their respective LOQ levels was found to be less than 20 % (Refer Table 2).

RSD_R : Reproducibility experiment for recoveries was performed on 6 different spiked samples at 5, 10 and 25 ppb concentration levels. The % RSD of 6 spiked samples at their respective LOQ level was found to be less than 30 % (Refer Table 2).

4-4. LOQ

Out of 56 pesticides analyzed, this method successfully achieved LOQs of 5, 10 and 25 ppb for 45, 10 and 1 pesticides, respectively. Captan was detected in the form of it's degradant i.e. Tetrahydrophthalamide (THPI) at 25 ppb. List of LOQs of individual pesticides is shown in Table 2.

5. Conclusion

- This study shows that the modified QuEChERS method combined with GC-MS/MS system is a reliable and efficient tool to quantify residual pesticides in norbixin sample. Although oleoresin is a complex matrix, the modified QuEChERS method significantly reduces interference.
- Also, highly sensitive Shimadzu GC-MS/MS allows trace level detection even after multifold dilution of sample. This helps in reducing contamination and enhancing ruggedness resulting in reproducible detection of analytes.
- The combination of sensitive instrument and reliable method enables its use in testing laboratories for multi-residue analysis of Norbixin oleoresin.

6. References

1. M. Anastassiades, S. J. Lehotay, D. Štajnbaher, F. J. Schenck, Fast and Easy Multiresidue Method Employing Acetonitrile Extraction/Partitioning and "Dispersive Solid-Phase Extraction" for the Determination of Pesticide Residues in Produce, J. AOAC Int., 86: 412–431, 2003
2. Guidance document on analytical quality control and method validation procedures for pesticide residues and analysis in food and feed. SANTE/11312/2021

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