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A quantitative determination of pesticide residues in chili powder using GC-MS/MS

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Keywords

Quantitation, pesticides, chili powder, TraceFinder, GC-MS/MS, QuEChERS, TSQ 9000, ExtractaBrite

Goal

The objective of the experiments described here was to set up a complete analytical solution to enable commercial food testing laboratories to analyze pesticide residues in chili powder, in compliance with the requirements of the Food Safety and Standards Authority of India (FSSAI).

Introduction

Chili (*Capsicum annum* Linn.), an essential spice in Indian cuisine, is grown by farmers on a large scale, i.e., about 800,000 hectares in India. The states of Andhra Pradesh and Karnataka account for a substantial share of chili production in comparison with the other states of India. The chili crop is vulnerable to a multitude of pests such as chili thrips, scirtothriops, dorsalis hood, and yellow mite. Consequently pesticides are widely used to maintain crop quality and yield. To date, relatively few pesticides have been registered under the Central Insecticide Board and Registration Committee (CIBRC) for use on chili.[1] Current agriculture practices rely on various pesticides applications to control the insect and pest attack, but these are harmful to human health. The frequent use of these chemicals can result in the residues in the harvested crop,[2] which is a food safety concern. Therefore, monitoring of pesticide residues in chili is necessary. Effective residue monitoring requires appropriate sample preparation for optimum extraction efficiency, accuracy, and precision.

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Goon et al.[3] have reported the QuEChERS method for the extraction of pesticides residue analysis in spices including chili followed by LC-MS analysis. This technique can also be applied for GC-MS/MS amenable pesticides.

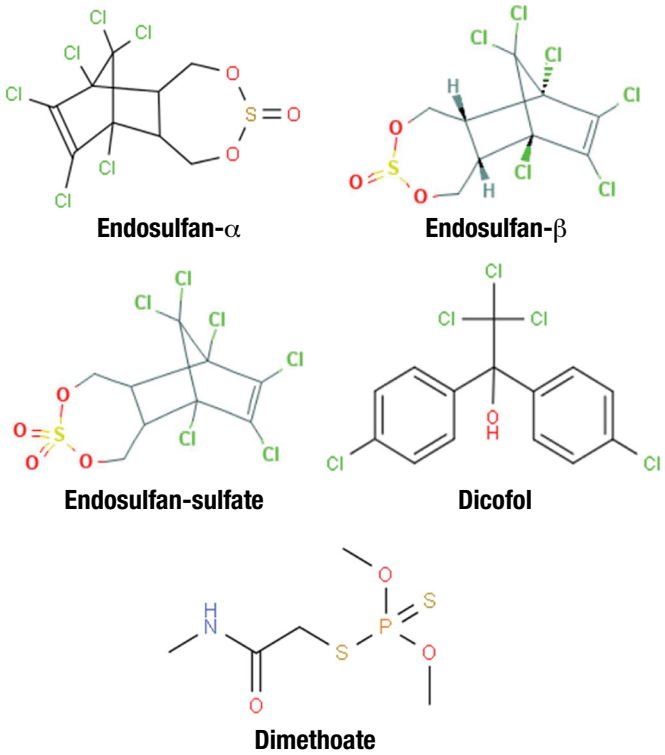

Figure 1. Structure of, endosulfan-α, endosulfan-β, endosulfan-sulfate, dicofol, and dimethoate

The aim of this work was to develop and demonstrate the sample preparation and determination of targeted pesticide residues in chili powder. Data were acquired using the Thermo Scientific™ TSQ™ 9000 GC-MS/MS (gas chromatograph-triple quadrupole mass spectrometer). The optimized method was validated at LOQ and LOQ × 2 in terms of the accuracy and precision as per the SANTE/11813/2017 guidelines and assessed for FSSAI MRL compliance. This method was also applied to the analysis of real samples for comprehensive method evaluation.

Experimental

Chemicals, reagents, and apparatus

- Acetonitrile, Optima™ LC/MS Grade, Fisher Chemical (P/N 514 L-16923 U)
- Anhydrous Magnesium Sulfate, Thermo Scientific (P/N 80020-415-500)
- Sodium Acetate, Thermo Scientific (P/N 80020-424)
- C18 Octadecyl Endcapped, Thermo Scientific (P/N 80020-413-100)
- PSA (Primary, secondary amine), Thermo Scientific (P/N 80020-416-100)
- GCB (Graphitized carbon black), Thermo Scientific (P/N 80020-417-100)
- The reference standards (dimethoate, dicofol, endosulfan-alfa, endosulfan beta, and endosulfan sulfate)
- Sample handling equipment: weighing balance (analytical and precision), benchtop centrifuge for 50 mL and 15 mL tubes, vortex mixer, micro-pipettes

Standard solutions preparations

Individual stock standard solutions were purchased from Sigma-Aldrich, Bangalore, India, and prepared by dissolving 10 mg of each in 10 mL acetonitrile corresponding to 1000 µg/mL. These were used to prepare a mixed working solution of the pesticides at 10 µg/mL in acetonitrile. The working standard solutions given in Table 1 were prepared by serial dilution of the 10 µg/mL standard.

Table 1. Matrix-matched calibration standards preparation

Working standard (µg/mL)	Volume from working standard (µL)	Acetonitrile (µL)	Final concentration (mg/kg)	Total volume (µL)
4.000	50	950	0.200	1000
3.000	50	950	0.150	1000
2.000	50	950	0.100	1000
1.500	50	950	0.075	1000
1.000	50	950	0.050	1000
0.500	50	950	0.025	1000
0.200	50	950	0.010	1000
0.100	50	950	0.005	1000

Sample preparation

Chili powder (finely homogenized powder) was purchased from a local retail outlet.

Extraction

- Weigh 2 g homogenized chili powder sample into a 50 mL extraction tube. *Note:* Spike sample at this step and wait for 10 min.
- Add 15 mL of 1% acetic acid in the water, shake, and soak for 10 min.
- Add 15 mL acetonitrile and mix vigorously for 1 min on a vortex mixer at 2500 rpm.
- Add 6 g anhydrous $MgSO_4$ and 1.5 gm of sodium acetate, again mix vigorously for 1 min on a vortex mixer.
- Centrifuge at 4000 rpm for 5 min at ambient temperature.

Dispersive solid phase extraction (dSPE) cleanup

- Decant 5 mL supernatant into 15 mL centrifuge tube and add 750 mg MgSO4, 250 mg PSA, 250 mg C18, and 50 mg of GCB.
- Vortex for 30 s and centrifuge at 10,000 rpm for 5 min.
- Collect 2.5 mL cleaned supernatant in the separate glass tube.
- Evaporate, to near dryness, under a gentle stream of nitrogen gas and then reconstitute with 1 mL acetonitrile.
- Inject 2 µL to the GC-MS/MS through the Thermo Scientific™ TriPlus™ RSH™ Autosampler.

Preparation of matrix-matched calibration standards

The blank matrix was analyzed for assuring the incurred residues of target analytes. After assuring the absence of target analytes, the same matrix was used for the further study. Following the above protocol, 2.5 mL cleaned blank matrix extract was evaporated and used to prepare matrix match calibration standards at the concentrations given in Table 1.

Recovery and precision

The mixed working standards were spiked in chili powder before extraction at 0.025 and 0.050 mg/kg (n=6 for each).

GC-MS/MS analysis

Chromatographic separation and data acquisition were carried out using the Thermo Scientific™ TRACE™ 1310 gas chromatograph coupled with a Thermo Scientific™ TSQ™ 9000 triple quadrupole mass spectrometer. The optimized instrument parameters for each compound are given in Table 2, while the compound-dependent parameters, i.e., selected reaction monitoring (SRM) parameters, are presented in Table 3.

Table 2A. Instrument parameters (GC)

Gas chromatography method	
Instrumentation:	TRACE 1310 GC with TSQ 9000 triple quadrupole and TriPlus RSH autosampler
Column:	Thermo Scientific™ TraceGOLD™ TG-5MS GC column (30 m × 0.25 mm i.d. × 0.25 µm) P/N 26096-1420
Liner:	Siltek™ six baffle PTV liner (P/N 453T2120)
Injector:	Programmed Temperature Vaporizing Injector (PTV)
Injector mode:	Splitless (PTV)
Splitless time:	2 min
Injection volume:	2 µL
PTV program:	90 °C for 0.1 min; 14.5 °C/min, 90–300 °C; 2.0 min (hold); 14.5 °C/min, 300–320 °C; 5 min (hold). Flow 75.0 mL/min
Cleaning phase:	On
Flow control mode:	Constant Flow
Column flow:	1.20 mL/min
Carrier gas and purity:	Helium (99.999%)
Purge flow:	5.00 mL/min
Split flow:	50.00 mL/min
Post-cycle temp.:	Cool Down
Total run time:	24.83 min
GC oven program:	70 °C, 1 min (hold); 30 °C/min, 70–150 °C; 5 °C/min, 150–200 °C; 10 °C/min, 200–280 °C; 30 °C/min, 280–300 °C, 5 min (hold)

Table 2B. Instrument parameters (MS)

Mass spectrometry method	
Instrumentation:	TSQ 9000 triple quadrupole mass spectrometer with ExtractaBrite ion source
Method type:	Acquisition-General (SRM mode)
MS transfer line temperature:	310 °C
Ion source temperature:	250 °C
Ionization:	EI (Electron Ionization)

Data acquisition and processing

Thermo Scientific™ TraceFinder™ 4.1 software was used for instrument control, data acquisition, and processing, data review, and reporting. The data was acquired in SRM mode with a minimum of two SRM transitions per analyte. The data processing included user-defined criteria as; two transitions per analytes, retention time (±0.1 min) and ion ratio (±30%) for identification, and confirmations as per the SANTE guidelines.[4]

Results and discussion

Sample preparation

Pesticide residue analysis in chili powder is challenging due to the matrix complexity. The chili powder contains red colored pigments (alkaloids) that can quickly contaminate the GC liner and column. The use of dSPE cleanup with GCB material significantly removed the amount of pigment, reducing the intensity of color more than 40% in comparison with the raw extract. After the cleanup, the red coloration of the extract became a pale yellow. This cleanup step reduced the maintenance of the liner and column therefore reducing the cost per sample. Still, matrix enhancement (>20%) was observed compared to solvent standards (Figure 2), hence the need for matrix-matched calibration standards in the range of 0.005 to 0.20 mg/kg for accurate quantitation. Also, the final acetonitrile extract carried 0.33 g/mL (3× dilution). So, the lower calibration level of 0.005 mg/kg (actual level) was used to cover the range of final diluted (3×) acetonitrile extract (0.33 g matrix/mL).

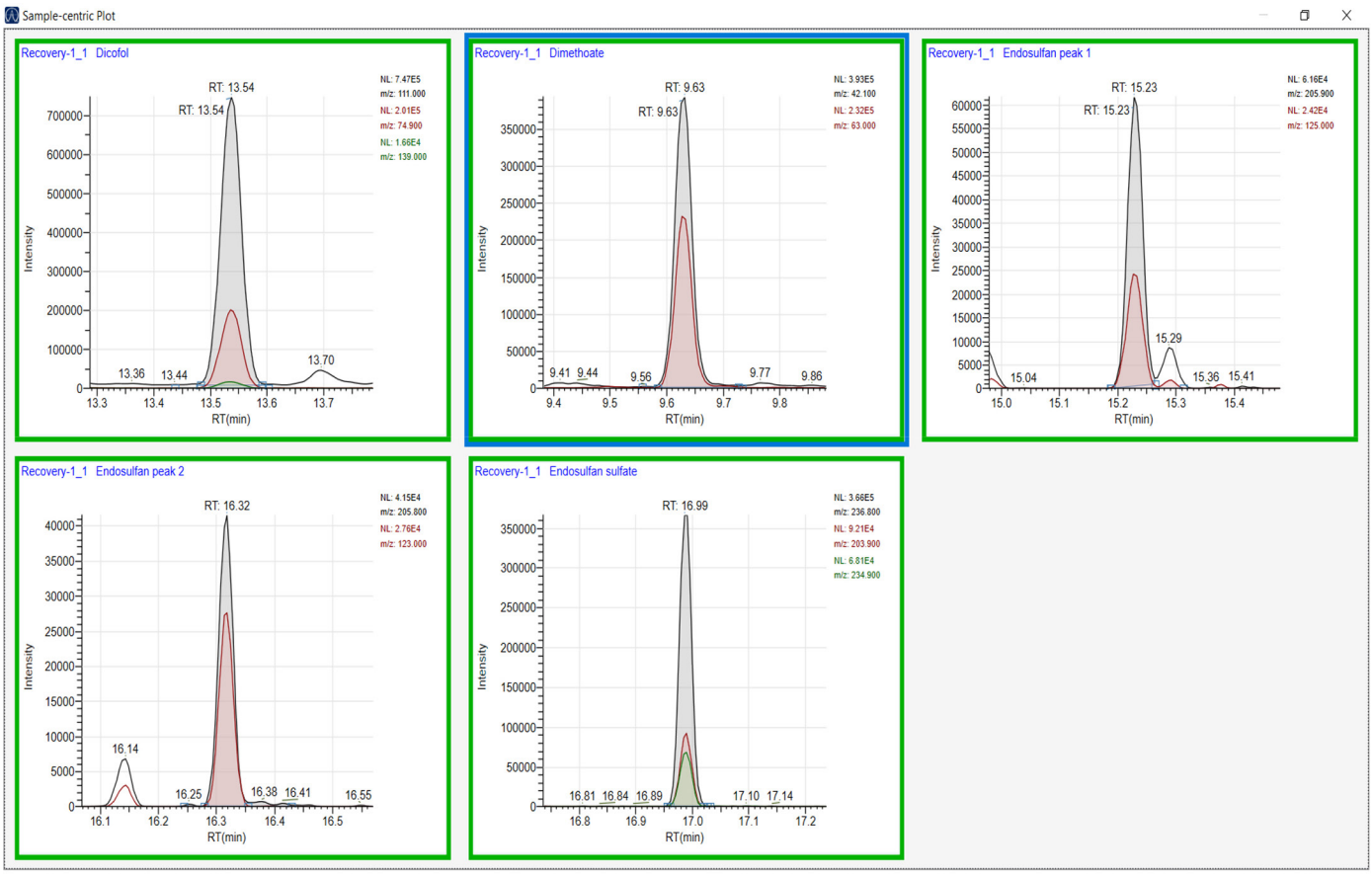

Figure 2. Extracted ion chromatogram with confirmatory ions in chili powder at 0.025 mg/kg

GC-MS/MS analysis

The optimized instrumental conditions offered Gaussian peak shapes for all target analytes for the spiked concentration, i.e., 0.025 mg/kg (Figure 2). The QuChERS method for extraction was used and the solvent exchange was avoided, i.e. from acetonitrile to any non-polar solvent, hence directly acetonitrile. Because of the high expansion volume, a 2 µL injection volume was preferred in the PTV injector, which offered symmetrical peak shapes without losing the target analyte's peak quality.

Method performance

Due to the matrix enhancement, the matrix-matched calibration curve was plotted in the range of 0.005 to 0.20 mg/kg (Figure 3). This curve offered linearity with $R^2 > 0.99$ and within 15% residuals by following the linear curve fitting and the 1/× weighting factor. Average recoveries were within 72% to 104% with <6.2% RSD at 0.025 (LOQ) and 0.05 mg/kg (n=6). For the identification, both the ions should overlap (±0.1 min retention time window) on the same retention time as per the SANTE guidelines, which has been demonstrated in Figure 2 and the retention time repeatability in Figure 4. The overall optimized method provided good ion ratios (±30%) presented in Figure 5. Recoveries and precision presented in Table 3 were within the acceptance criteria as per SANTE/11813/2017.[4] Hence, the described method can be confidently used for the routine analysis of pesticides in chili powder.

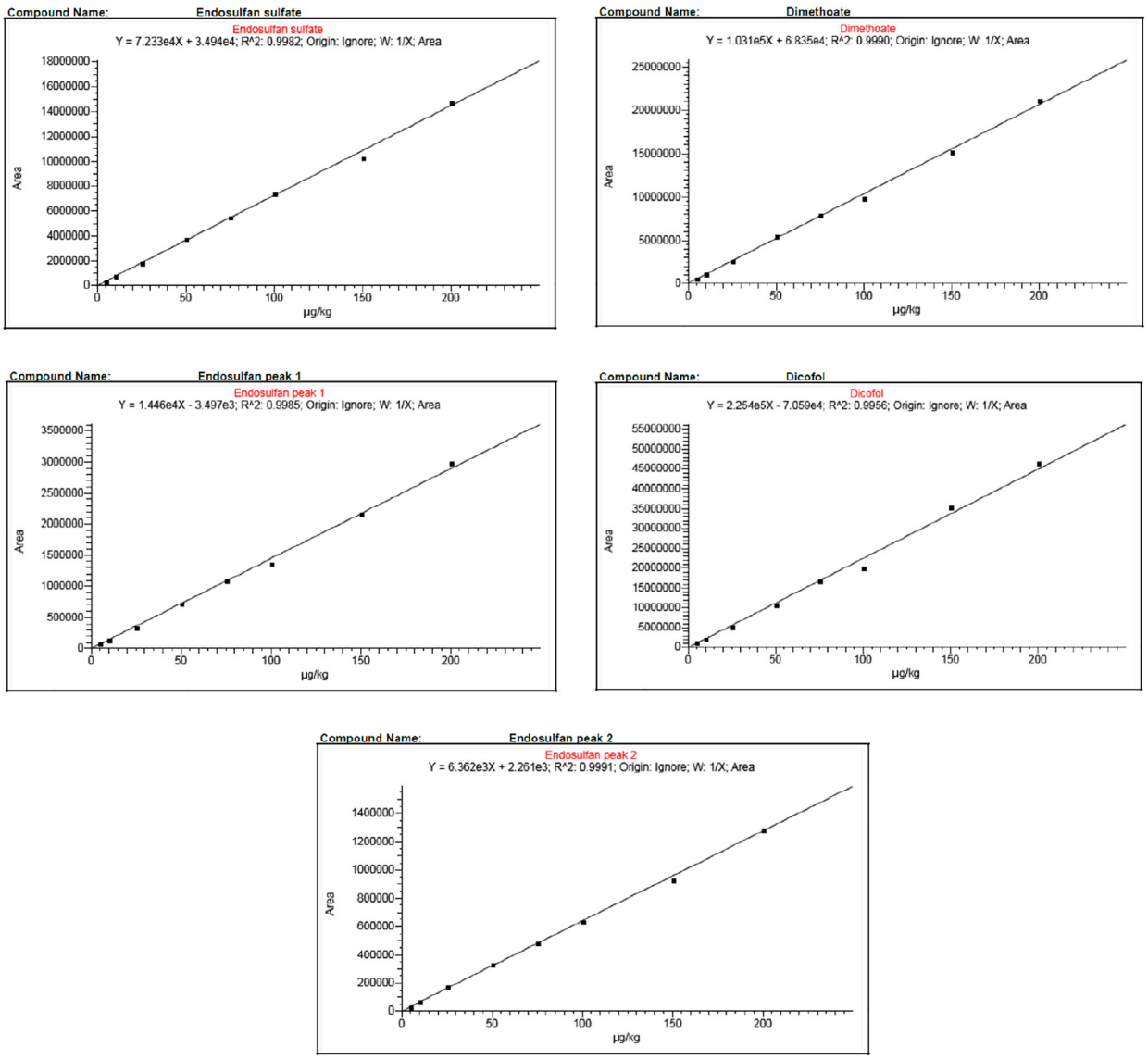

Figure 3. The matrix-matched calibration standards linearity for target analytes

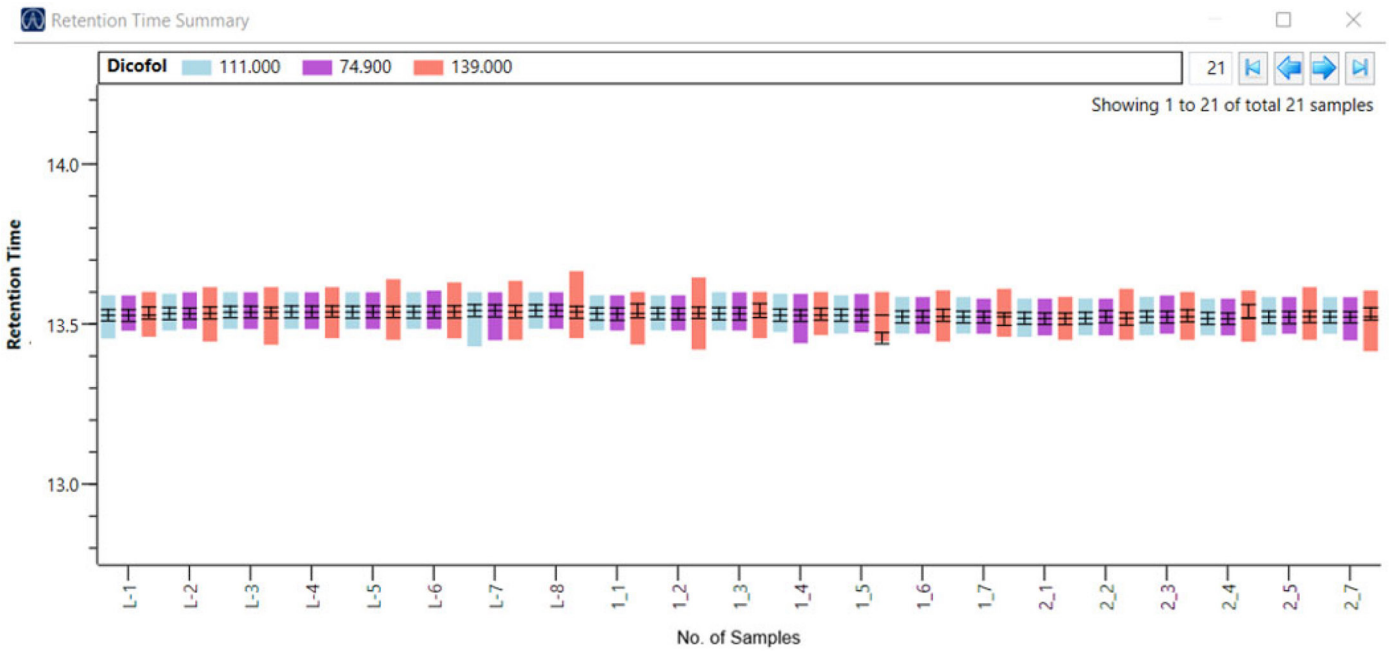

Figure 4. Retention time stability of dicofol in chili powder as assessed across n=25 consecutive matrix injections in a batch

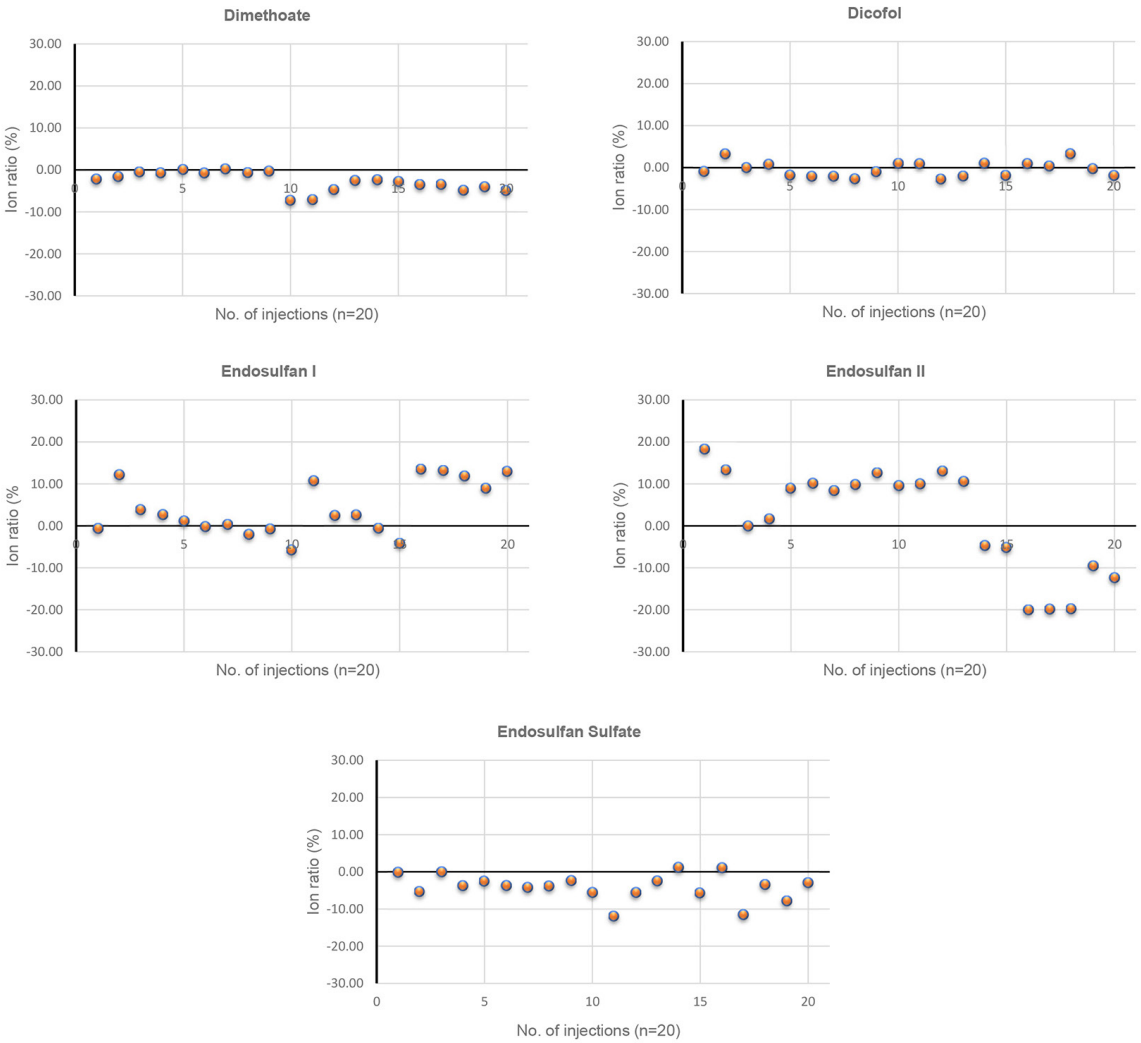

Figure 5. Ion ratio % difference observed range (±30%) for the above target analytes in chili powder spiked at 0.025 mg/kg in replicate injections (n=20)

Table 3. SRM transitions, retention time, R^2, LOQ, recovery and precision, and FSSAI MRLs for target analytes

Name of compound	RT (min)	Q1 (*m/z*)	Q3 (*m/z*)	CE (V)	R^2	LOQ (mg/kg)	% Recovery (%RSD) 0.025 (mg/kg)	% Recovery (%RSD) 0.05 (mg/kg)	FSSAI MRLs (mg/kg)
Dimethoate	9.63	87 93 125	42 63 79	10 8 8	0.999	0.025	90 (2.3)	96 (6.2)	5.00
Dicofol	13.54	139 111 251	111 75 139	12 12 12	0.995	0.025	104 (2.4)	97 (3.8)	10.00
Endosulfan-alpha	15.24	241 195 195	206 125 159	14 22 8	0.998	0.025	90 (5.5)	72 (2.7)	0.025*
Endosulfan-beta	16.31	159 195 241	123 159 206	12 8 12	0.999	0.025	85 (5.3)	86 (6.0)	
Endosulfan-sulfate	16.99	272 239 272	237 204 235	12 12 12	0.998	0.025	85 (3.5)	75 (3.5)	

Q1=precursor ion, Q3=product ion, RT=retention time, CE=collision energy
* MRL set at LOQ

Conclusion

The developed method successful complies with the MRL requirements of the FSSAI and the method performance criteria of the EU SANTE guidelines for the determination of pesticides in chili powder by GC-MS/MS. The optimized method demonstrated that the LOQ (0.025 mg/kg) is much lower than the MRLs, except for endosulfan, without compromising data quality. Even at the LOQ level, identification and confirmation with retention time, ion ratio, recoveries (70–120%) and precision (<20%) offered by the method are within acceptance criteria of SANTE. This method was fast, allowing a batch of 10 samples to be analyzed within eight hours. This method can be implemented for routine pesticide residue analysis and target analytes sensitivity meeting the FSSAI MRLs requirement (The Food Safety Standard Act, 2006), in the chili powder.

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