A large-scale screening and quantitation of pesticide residues in cereals by using GC-(EI)-MS/MS

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ABSTRACT

A large-scale screening and quantitation solution for more than 150 pesticide residues in cereals (rice and wheat flour) by using gas chromatography tandem mass spectrometry GC-(EI)-MS/MS. A buffered QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method was used for extraction followed by GC-MS/MS analysis including electron impact (EI) ionization. The data acquisition in t-SRM and processing were performed by using Thermo Scientific TraceFinder software. Automated data processing was performed by addressing identification and quantitation in alignment with SANTE guideline. Two or more transitions per analyte were used from Thermo pesticide analyzer compound database (CDB). To harmonize the results, matrix match linearity was prepared in the range of 0.005-0.100 mg/kg for both matrices offered excellent correlation coefficient (R²=0.99). Recovery was checked at 0.010 and 0.050 mg/kg concentration level. The results obtained through this optimized protocol complies with SANTE guidelines requirements i.e. ion ratio (±30%), retention time (±0.1 min)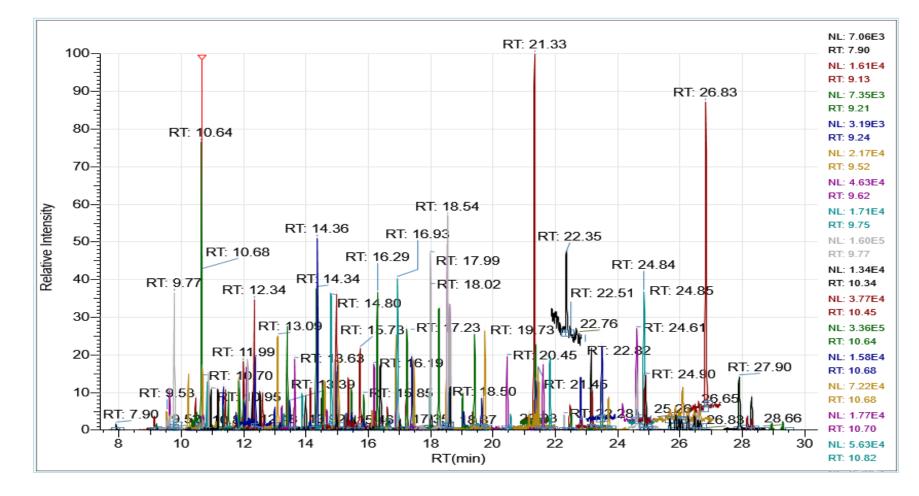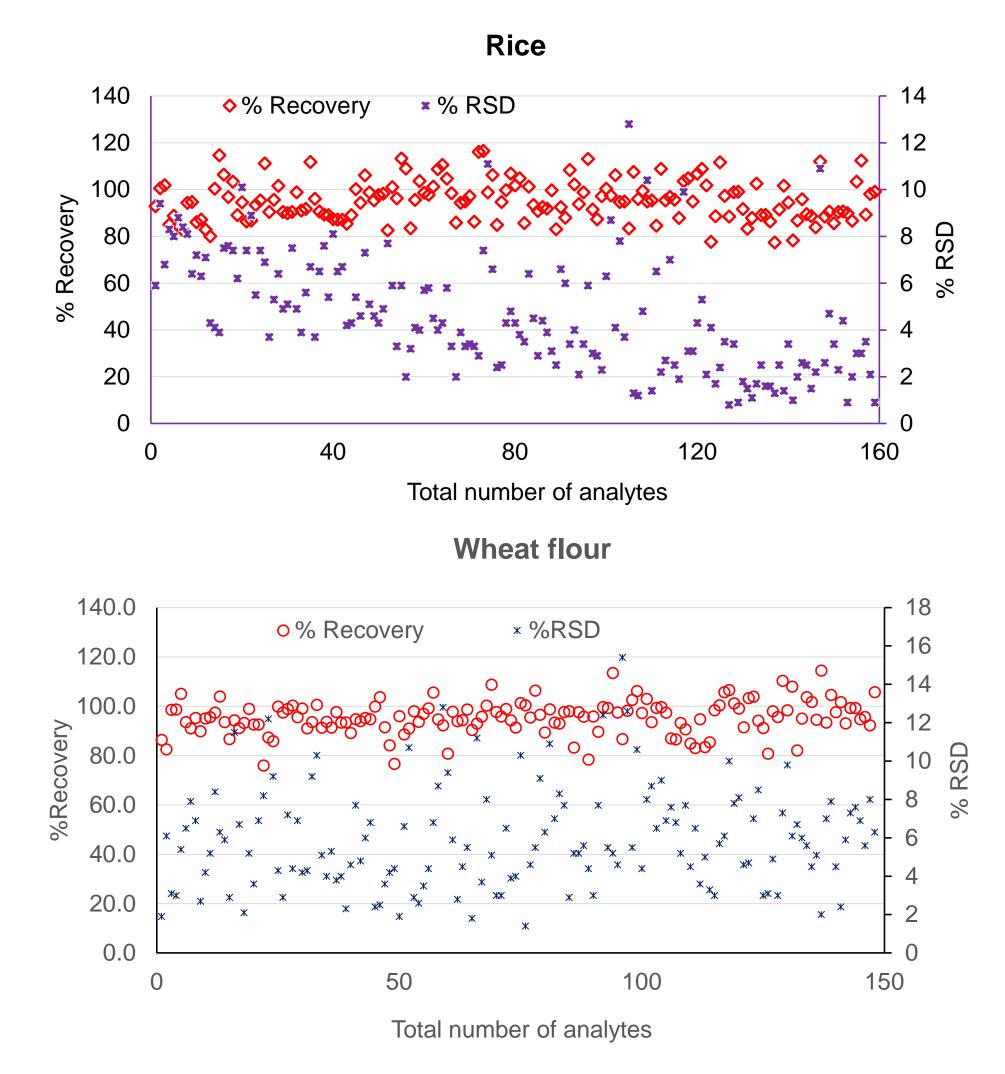, linearity (>0.99 with residuals ±20), recovery (70-120%) and precision (±20%). The optimized method fulfils the FSSAI as well as European commission (EC) MRLs requirement for pesticide residues in rice and wheat Flour.

INTRODUCTION

Rice and wheat are essential cereal based food. Every year, billions of tons of rice and wheat are grown and exported from India. Day by day it was observed an increase in demand. To meet the market need in terms of quality as well as quantity, crop protection plays an important role. In crop protection, a lot of agrochemicals were used to protect crops and to improve the efficiency of production by controlling the diseases and pest attack. Currently, there are few chemicals (insecticides, fungicides, herbicides, plant growth regulators and biocides) registered under the central insecticide board and registration committee (CIBRC) [1]. After usage of agrochemicals, the remaining quantity of these chemicals creates a residue problem and danger to human health as well as environmental risk. Therefore, it is necessary to have effective residue monitoring in rice and wheat. For that purpose, there is requirement of a method which could provide accurate and precise results. As a food safety aspect, governments, food producers and food retailers started to ensure pesticides residues in cereals (rice and wheat). The maximum residue limits (MRLs) set for pesticides in which 0.01 mg/kg observed as the minimum limit as per the European Commission (EC) and FSSAI guidelines [2, 3]. For extraction of residue, the QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method [4] has adopted in most of the food samples. On the other side, the instrument plays an important role to deliver an accurate and precise result to meet the regulatory requirement. This work aimed to optimize and method validation of a multi-residue method for pesticides in rice by using GC–MS/MS (Thermo Scientific™ TSQ 9000). The data acquisition and processing carried out by using Thermo Scientific™ TraceFinder™ software. The optimized method validated according to the SANTE/11813/ 2017 guideline [5].

MATERIALS AND METHODS

Sample Preparation

➢ Weigh 5 g homogenized sample.
➢ Add 10 mL of acidified HPLC grade water (1% acetic acid) (15 mL for wheat) and leave the sample for 10 min soaking.
➢ Add 10 mL acetonitrile to the tube (15 mL for wheat) .
➢ Shake vigorously for 1 minute on a vortex mixer at 2500 rpm.
➢ Add Buffered QuEChERS salt and again mix vigorously for 1 minute on a vortex mixer at 2500 rpm.
➢ Centrifuge at 5000 rpm for 5 min.
➢ Supernatant (1 mL) cleaned by containing 150 mg MgSO₄, 50 mg PSA.
➢ Vortex for 1 min at 2500 rpm and centrifuge samples with 10000 rpm for 5 min.
➢ Collect supernatant, transferred into a GC vial for instrumental analysis.
➢ Prepare blank (control) extract by following above protocol for matrix match calibration standards.

GC-MS/MS conditions

Instrumentation	TRACE 1310 GC with TriPlus RSH autosampler (Thermo Scientific™)
Column	TG-5SIL MS (30 m x 0.25 mm ID x 0.25µm)
Injector	Spit/Spitsless (SSL)
Liner	SSL splitless liner, single taper, deactivated
Injector Mode	Splitless
Splitless Time	2 min
Injection Volume	1.5 µL
Injector Temp	280° C
Column Flow	1.20 mL/min
Carrier gas	Helium (99.999%)
Purge Flow	5.00 mL/min
Split Flow	50.00 mL/min
Total run time	32.0 min
Oven Program	90° C, 5 min, 25° C/min to 180° C, 5° C/min to 280° C, 10° C/min to 300° C, 1.4 min
Instrumentation	TSQ 9000 Triple Quadrupole Mass spectrometer (Thermo Scientific™)
Method type	Acquisition-Timed (SRM mode)
MS transfer line temp	310° C
Ion source temp	280° C
Ionization	Electron Impact (EI) ionization

RESULTS

Figure 1: TIC for 159 pesticides at 0.05 mg/kg with overlay extraction ions.
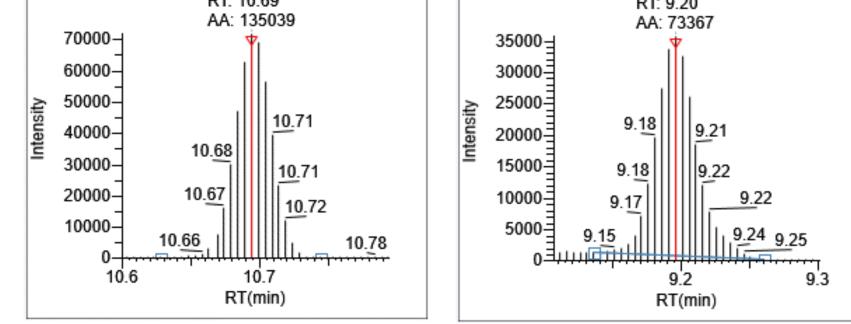

Figure 2. Impact of optimized dwell time on the data points per peak.

Identification and quantitation

User-Defined parameters for data processing includes two transitions per analytes, retention time (±0.1 min), correlation coefficient (>0.99), residuals (±20%) set in the data processing method of TraceFinder (Master method). Based on these parameters, the data was processed automatically with flagging. These automated flags indicated the through color codes whether results pass or fail as per acceptance/ user-defined criteria (SANTE guideline) are given in the processing method (figure3).

Figure 3. Trace finder processed data in the form of results showing the flagging for identification and quantitation.

Figure 4. (A) Extracted ion chromatogram for quantifier ion of parathion-methyl (B) Identification based on the selectivity of confirmatory ions separated from isobaric interference caused by chlorpyrifos (CPP) methyl and fenchlorphos peaks and confirmed with ion ratio, and (C) calibration curve in rice.

Figure 5: Area repeatability at 0.01 mg/kg for 2,3,5,6 tetrachloroaniline, cycloate, oxadiazon and vinclozolin in rice (n=50)

Figure 5: Area repeatability at 0.01 mg/kg for fluazifop p-butyl, fluquinconazole and myclobutanil in wheat (n=38).

Method performance

Figure 7. Percentage recoveries (% RSD) obtained at 0.01 mg/kg in rice and wheat flour

Figure 8. Retention time repeatability for vinclozolin in rice (n=50)
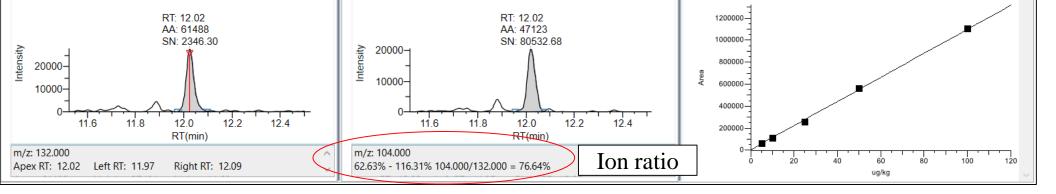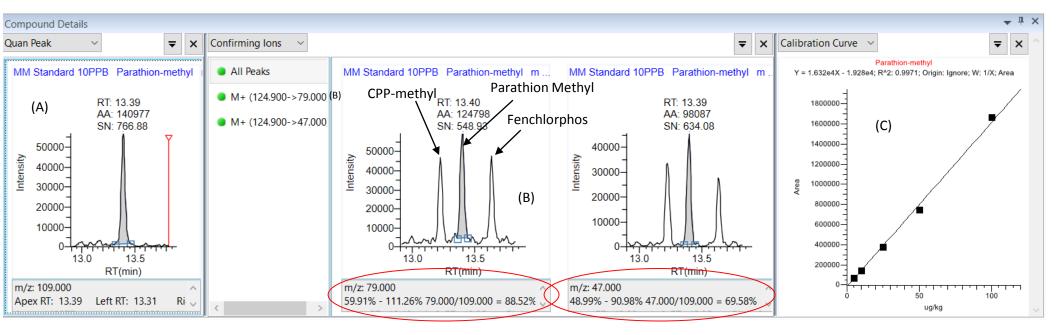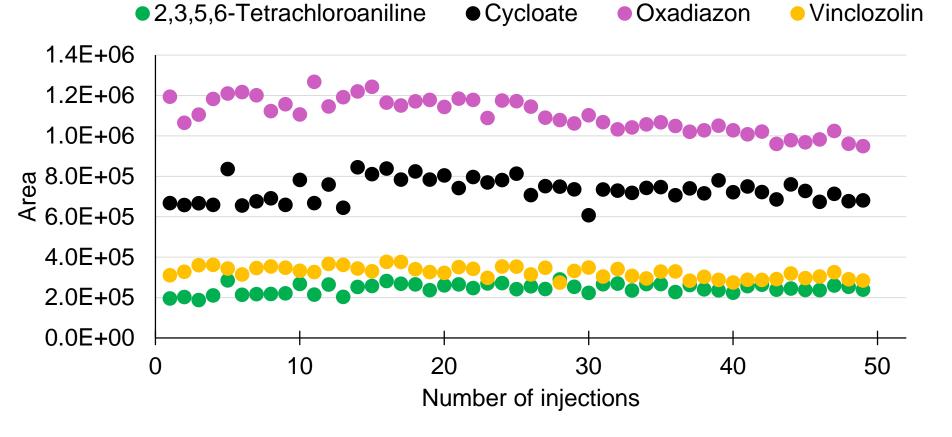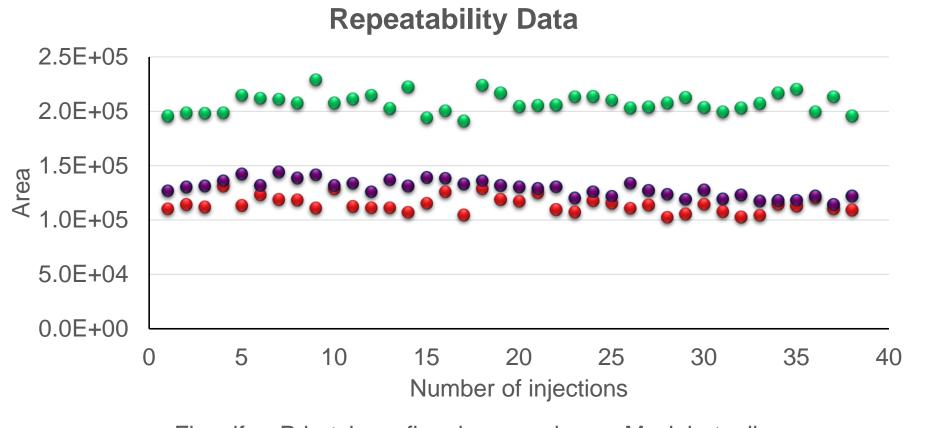
CONCLUSION

➢ Two transitions were observed per analyte with same retention (±0.1 min) in standard as well as in matrix.

➢ The ion ratio found in both matrices in comparison with solvent standard were within 30% acceptance criteria.

➢ The matrix effect was >20% both matrices hence matrix matched calibration standards were used.

➢ Demonstrated good calibration coefficients of determination (R²) >0.99 and residuals within <15% for matrix match calibration.

➢ The limit of quantification (LOQ) were in the range of 0.005-0.01 mg/kg.

➢ The method performance was verified at defaults reporting limit (RL) 0.01 mg/kg with recoveries within 70-120% with <20% RSD for all target analytes.

➢ These results indicates the fulfillment of the SANTE guideline requirements.

➢ This method complies the EU as well as FSSAI MRLs requirement..

➢ The optimized protocol improved the throughput of commercial food testing laboratories.

REFERENCES

1) Insecticides / Pesticides Registered under section 9(3) of the Insecticides Act, 1968 for use in the Country:(As on 31/12/2018) http://ppqs.gov.in/insecticides-pesticides-registered-under-section-93-insecticides-act-1968-use-country-31122018.

2) FSSAI Manual for food safety, 17thEdition-2017 (THE FOOD SAFETY AND STANDARDS ACT, 2006)

3) http://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=product.resultat&language=EN&selectedID=237

4) S.J Lehotay, K. Maštovská, & A. R. Lightfield. Use of buffering and other means to improve the results of problematic pesticides in a fast and easy method for residue analysis of fruits and vegetables. J. AOAC Int. 2005, 88, 615–630; (Note: Basis of the AOAC Method 2007.01).

5) SANTE guideline https://ec.europa.eu/food/sites/food/files/plant/ docs /pesticides_mrl_guidelines_wrkdoc_2017-11813.pdf.

TRADEMARKS/LICENSING

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