NRL for Cereals
DTU National Food Institute

Validation Report

Determination of isoprothiolane residues in rice by GC-MS/MS

(QuEChERS method)

Mette Erecius Poulsen &
Hanne Bjerre Christensen
16 July 2010

1. Introduction

This report describes the validation of isoprothiolane in rice by the QuEChERS method[1] with detection on GC-MS/MS. Recovery experiments were performed on spiked blank samples.

2. Principle of analysis

Sample preparation: Cold water and acetonitrile are added to the milled sample.
Extraction: The sample is shaken and a salt and buffer mixture is added and the sample is shaken again.
Clean-up: After centrifugation the supernatant is transferred to a tube with PSA and $MgSO_4$. After shaking and an additional centrifugation step the final extract is obtained.
Quantification and qualification: GC-MS/MS: The final extract was analysed GC/MS/MS (electron energy 70 eV, source temp. 180°C, transfer line GC interface 250°C). The GC was equipped with an PTV injector and the injection volume was 4 µl.
Selectivity and specificity: GC-MS/MS is a highly selective detection method, and thereby highly specific. Two MRM transitions were used, one for quantification (290>118) and another transition for qualification (290>204)

3. Validation design

The validation was performed as 5 replicates at three spiking levels; 0.01, 0.02 and 0.1 mg/kg according to Method Validation and Quality Control Procedures for Pesticide Residue Analysis in Food and Feed, Document No SANCO/10684/2010, 01/01/2010, European Commission, Brussels, 2010 [2].

4. Chromatograms and calibration curves

The calibration curve is determined by the analysis 4 calibration levels, i.e. 0.003, 0.01, 0.033 and µg/ml. The calibration curves were best fitted to a linear curve. The quantification was performed from the mean of two calibration curves surrounding the samples. The correlation coefficients (R) were 0.99. Chromatograms obtained when analysing the extracts by GC-MS/MS are presented in figure 1. Examples of calibration curves are presented in figure 2.

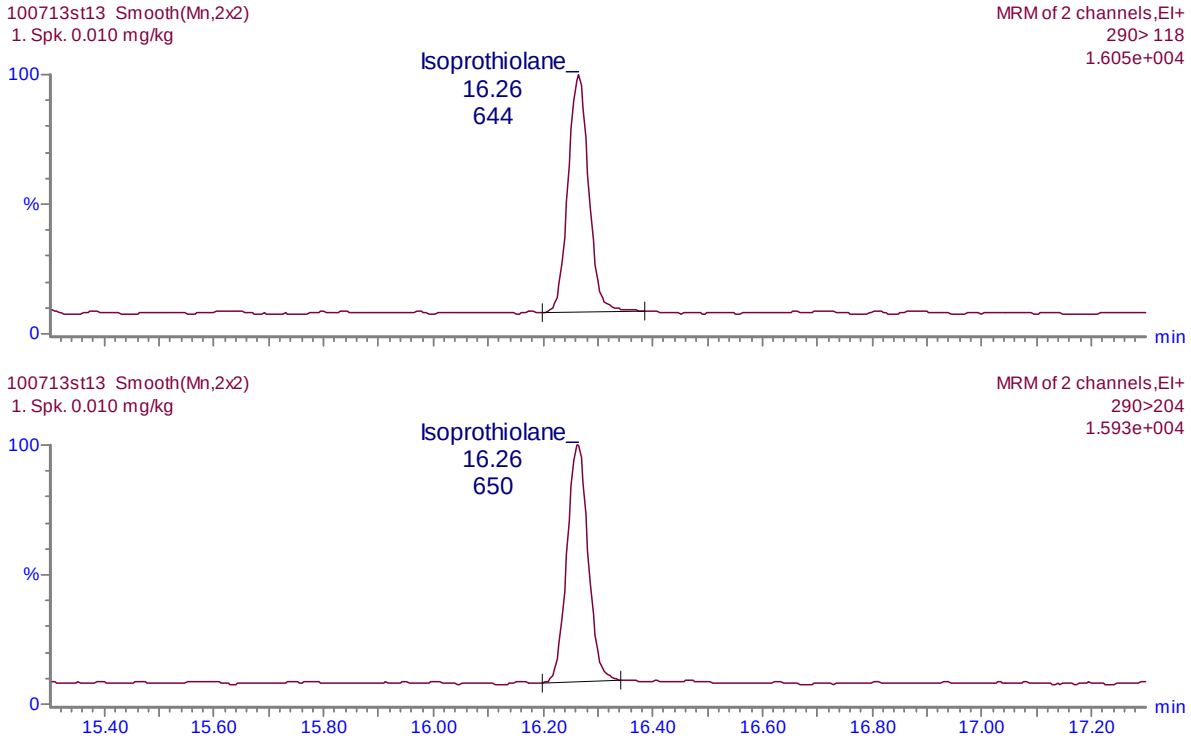

Figure 1: Chromatograms of two MRM transitions for isoprothiolane obtained when analysing extract of blank rice samples spiked with 0.01 mg/kg.

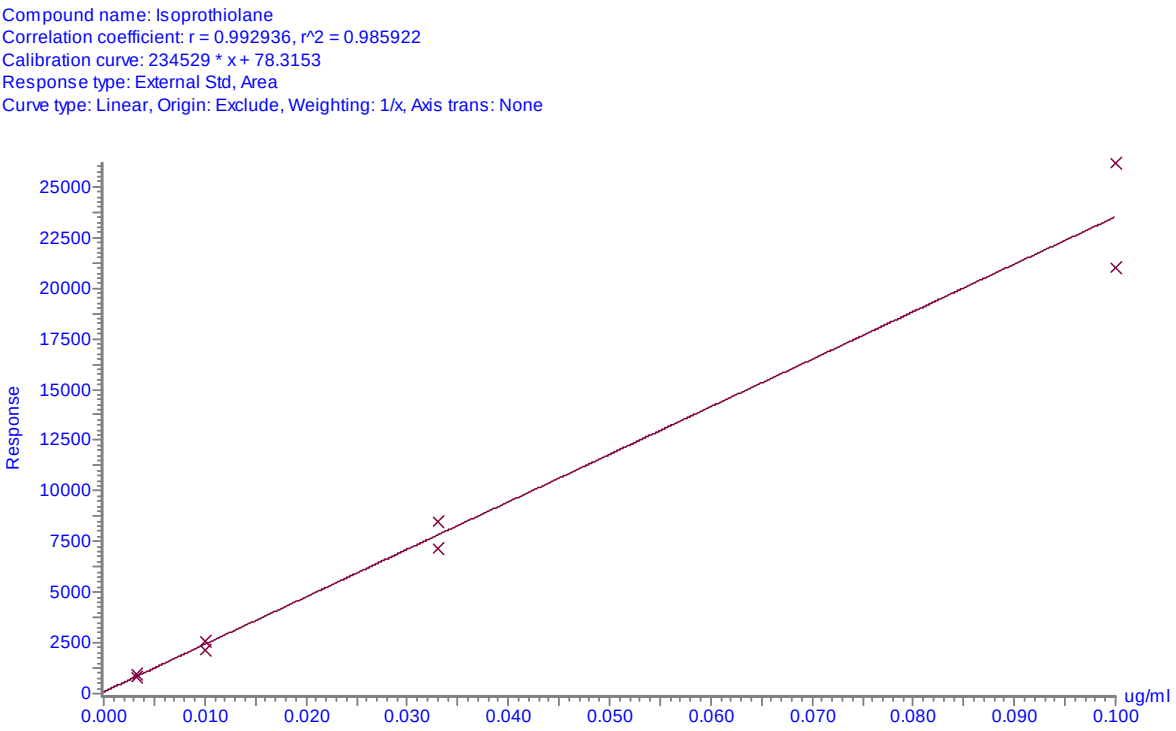

Figure 2. Calibration curves for isoprothiolane (concentrations from 0.003-0.1µg/ml)

5. Validation parameters

Precision – Repeatability
Repeatability (Table 1) was calculated for all three spiking levels. Repeatability in this validation was calculated from the 5 replicate determinations. Repeatability were calculated as given in ISO 5725-2[3].

Accuracy – Recovery
The accuracy was determined by recovery samples spiked at three concentration levels (Table 1)

Limit of quantification, LOQ
Quantification limits (LOQ) are calculated from the results at the lowest accepted spike level, as 6 times the standard deviation (absolute recovery) (Table 1).

Robustness
The QuEChERS method has earlier by Anastassiades et al. 2003[1] in connection with the development of the method been shown to be robust.

6. Criteria for the acceptance of validation results

For the pesticides to be accepted as validated the following criteria for precision and trueness must to be fulfilled:
1. The relative standard deviation of the repeatability must be less than or equal to the standard deviation proposed by Horwitz[4].
2. The average relative recovery must be between 70 and 120%[2].
If the above mentioned criteria have been meet, the detection limits have been calculated.

7. Results

The results from the validation is shown in Table 1

Table 1. Repeatability, recovery for spike levels 0.01, 0.02 and 0.1 mg/kg

Spike level, mg/kg	**0.01**	**0.02**	**0.1**
Repeatability, RSD_r, %	7.8	8.8	6.7
Recovery, %	105	88	88
LOQ, mg/kg	0.005		

Isoprothiolane is easy to detect with good sensitivity at low levels (Figure 1). No problems with interfering matrix compounds were seen. Repeatability was between 6.7-8.8 and recoveries between 88-105%. The LOQ was calculated as 6 times the standard deviation on the results at the lowest spike level. The blank samples used for the recovery experiments, contained probably

low levels of isoprotiolane (10% response of the response at the lowest calibration level). However, the residue level has not affected the result of the validation. An organically produced rice samples was analysed in parallel with the validation and contained not residues of isoprotiolane.

8. Conclusions

Isoprotiolane was validated in rice matrix at three spike levels, 0.01, 0.02, 0.1 mg/kg with relative repeatability of 7-8 %. Recoveries obtained were 88-105 %. LOQ was calculated to be 0.005 mg/kg.

9. References

1 http://www.quechers.com/ or Anastassiades et al., J. AOAC Int., vol. 86, no. 2, p. 412, 2003

2 Method Validation and Quality Control Procedures for Pesticide Residue Analysis in Food and Feed, Document No SANCO/10684/2010, 01/01/2010, European Commission, Brussels, 2010.

3 ISO 5725-2:1994. Accuracy (trueness and precision) of measurement methods and results – Part 2. Basic method for the determination of repeatability and reproducibility of standard measurement method. First edition. December 1994.

4 W. Horwitz, Anal. Chem., 1982; 54, 67A.