Multiresidue determination of more than 90 pesticides by gas chromatography tandem mass spectrometry in red chili powder

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INTRODUCTION

Red chili is one of the most popular spices in the world especially in Asia. Every year, millions of tons of red chili are grown and exported from India making India as the world's largest exporter of this commodity. The extensive use of agro-chemicals has given rise to concerns over consumers' exposure to pesticides and resulting in health risks. Therefore, it is necessary to provide effective residue analysis methods.

Agro chemical residue measurement in red chili is considered difficult because of the high content of pigments such as non-volatile carotenoids[1]. These pigments usually get co-extracted with the target analytes and this necessitates frequent injection port liner replacement, column maintenance and mass spectrometer ion source cleaning.

Attempts were made to improve liner and column life by minimizing co-extracted pigments without compromising the required recoveries of target analytes. MS/MS transitions were selected from the Agilent Pesticides and Environmental Pollutants database[2] and were shown to be free from matrix interferences.

The Quick, Easy, Cheap, Effective, Rugged and safe (QuEchERS) method was used to prepare the red chili sample extracts and the effect of different dSPE sorbents on the level of co-extracted matrix components was investigated. The cleaned red chili extracts were analyzed by GC-MS/MS using the MRM mode on an Agilent 7890 GC with an Agilent 7000 Triple Quadrupole GC/MS system.

MATERIALS AND METHODS

SAMPLE PREPARATION

Modified QueEChERS sample preparation technique was followed to extract pesticides [3]

Weigh 2 g of sample in a 50 ml centrifuge tube, Add 10 ml of water, shake for 30 seconds . Then allow to stand for 30 minutes

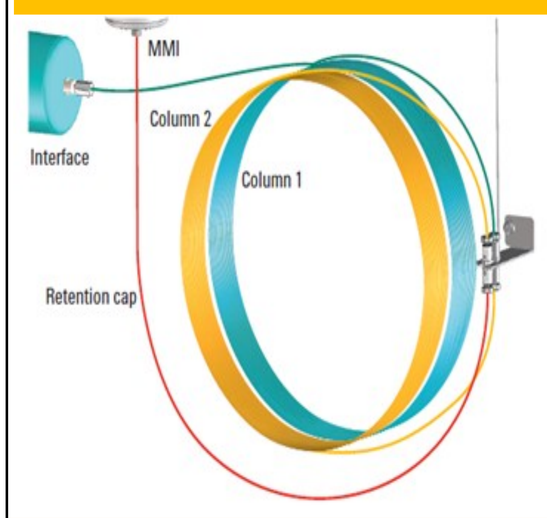
Add 10 ml of acetonitrile (containing 1% acetic acid), agitate for a minute. Add 6g Magnesium sulfate + 1.5g Sodium acetate [Agilent p/n 5982-5755]. Shake for a minute then centrifuge 6000 rpm for 5 minutes

Transfer 1 ml of acetonitrile layer to a 2 ml dispersive tube, add 50mg PSA, 50 mg C18, 7.5 mg GCB and 150 mg MgSO4 [Agilent p/n 5982-0028], shake for a minute and then centrifuge at 9000 rpm for 10 minutes

Carefully pipette out 0.5 mL of supernatant to an auto sampler vial and inject 2ul in to GC/MS/MS system

BACK FLUSH[4]

Retention gap 1m; **Column 1** : HP-5MS (15 m x 0.25 mm, 0.25 µ film thickness); **Column 2**: HP-5MS (15 m x 0.25 mm, 0.25 µ film thickness)



EQUIPMENT

Agilent 7890A GC system hyphenated to Agilent 7000B GC/MS Triple Quadrupole.

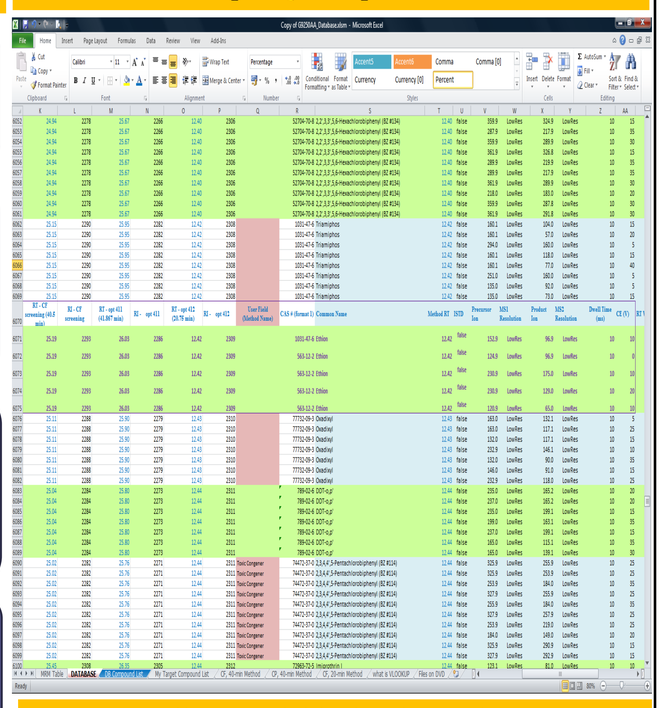


Figure 1: Agilent Pesticide data base P/N G9250AA

INSTRUMENTAL CONDITION

Oven, inlet temperature programming and Multi Reaction Monitoring (MRM) was taken from Agilent Pesticide Database above. MMI was set at cold splitless

Oven temperature

Rate (° C/ min)	Temperature (°C)	Hold (min)
Initial	70	1
25	150	0
3	200	0
8	280	8

Multi mode Inlet

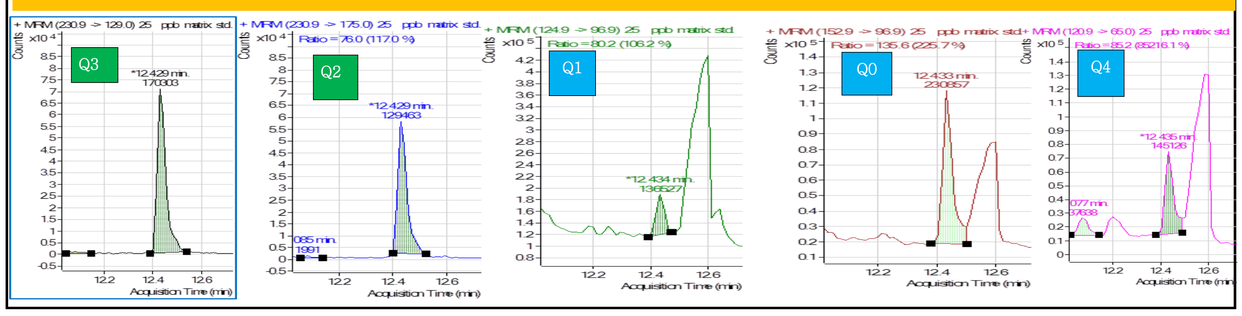
Rate (° C/ min)	Temperature (°C)	Hold (min)
	70	0.1
450	325	5
10	250	

MS/MS Conditions

Time segments were allocated by the MRM optimization tool available in Mass Hunter software.

ANALYSIS

All the transitions available for each compound in the database were analyzed and two transitions were chosen based on the ion ratio and the lack of matrix interferences. For example, the Agilent MRM data base has five transitions for Ethion and all were tested. Two transitions were chosen for use in the final method based on response and lowest matrix interference. The Q3 and Q2 transitions for Ethion were used as quantifying ion and qualifying ion, respectively.



EXPERIMENTAL DISCUSSION

Dried red chili powder contains carotenoids and the major one being beta-carotene (around 3 mg/mL) is non-volatile and will stick to a hot injection port liner if it is not removed during sample preparation. Three different sorbents (**Table 1**) were tested to eliminate beta-carotene using the modified AOAC QueEChERS extraction method.

It is evident from the **Figure 2** that the combination of PSA, C-18 and GCB (0028) is absorbing more pigments resulting in less yellow colour than 5122 and 5022. LC/MS analysis of these extracts also confirms 0028 is having the least amount of beta-carotene when compared to other cleanup strategies. The percentage reduction is given in the **Figure 3**.

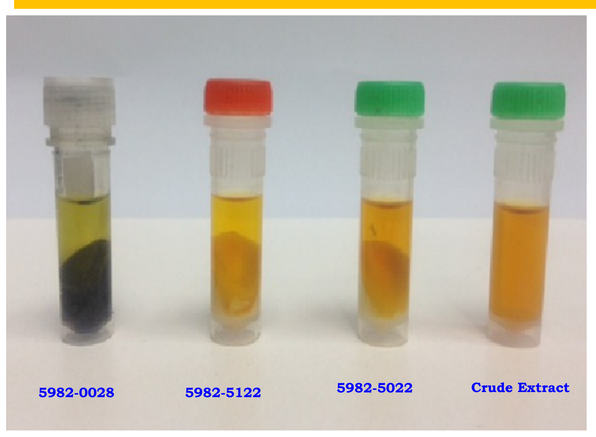


Figure 2: Effect of different cleanup strategies for the removal of pigments

Absorbent	0028	5122	5022
Primary Secondary amine	50 mg	50 mg	50 mg
Silca-C18	50 mg	50 mg	-
Graphitized Carbon Black	7.5 mg	-	-
Magnesium Sulphate	150 mg	150 mg	150 mg

Table 1: Sorbent composition of the different clean-up strategies

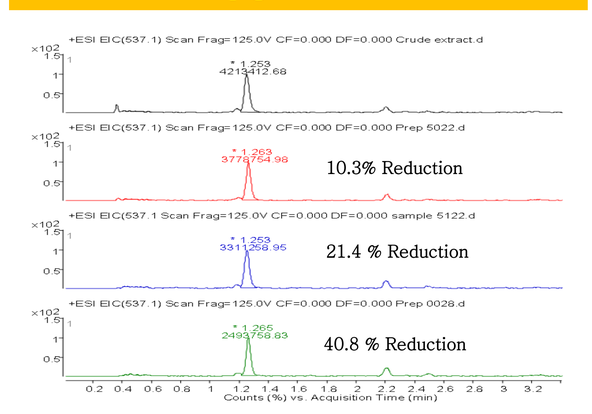


Figure 3: LC/MS chromatogram of beta-carotene showing the effectiveness of three different clean up methods

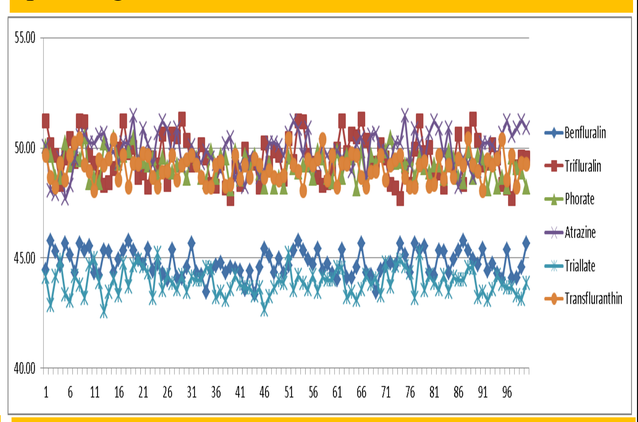


Figure 4: Stability of GC/MS/MS system responses over repeated injection of 50ng/ml spiked sample

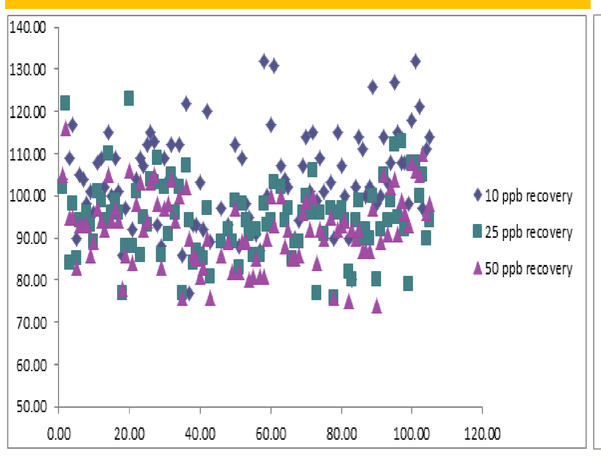


Figure 5: Percentage of recovery of pesticides at 10, 25 and 50 ng/ml concentration

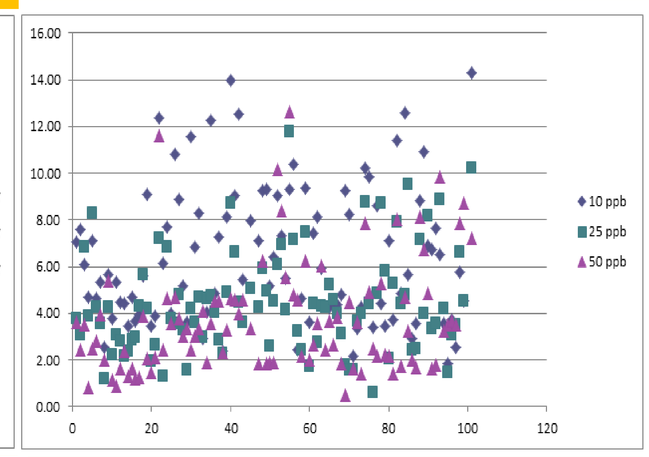


Figure 6: Relative standard deviation of the recoveries (n= 6)

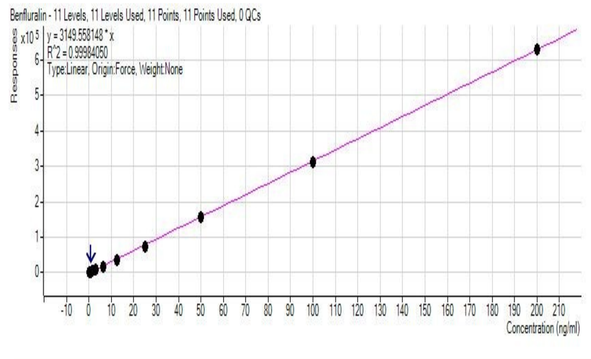


Figure 7: Calibration linearity for benfluralin [The R^2 was >0.99 for most of the compounds within 0.19–200 ng/ml (11 levels)].

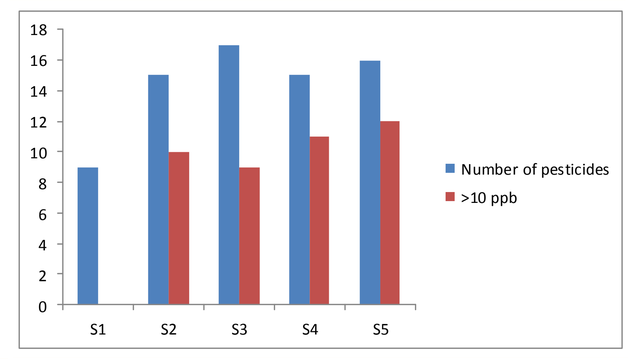


Figure 8: Analysis of red chili samples for pesticides and pesticides above 10 ng/g

CONCLUSIONS

1. The recoveries at 10, 25, 50 ng/ml were within 70– 120 % (n=6) with RSDs below 20 % indicating satisfactory intra-laboratory precision.
2. The method can be quickly setup on the instrument and the optimized clean-up employed results in reducing instrument maintenance leading to more laboratory productivity.
3. The pesticide database parameters assisted with retention time locking assures more productivity

REFERENCES

[1] Natural food flavors colorant, Mathew Attokaran Ph.D, ISBN 978-0-8138-2110-8

[2] Pesticides and Environmental pollutants MRM database. Agilent part number G9250AA

[3] Agilent's QuEChERS Sample preparation manual. Agilent publication number 5991-1057EN

[4]User Quick guide to Pressure control T (PCT) operation – post run back flush. Agilent publication number 5990-5484EN