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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<sup>[1]</sup>. 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<sup>[2]</sup> 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

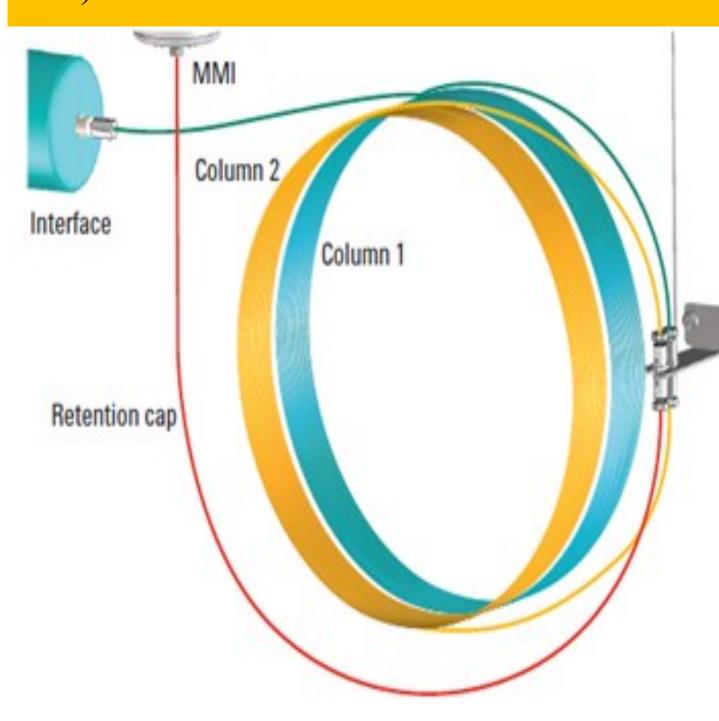
Add 10 ml of acetonitrile (containing 1% acetic acid), agitate for a minute. Add 6g Magnesium sulfate + 1.5g Sodium acetate [Agilent p/n 5982-5755]. Shake for a minute then centrifuge 6000 rpm for 5 minutes

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<sup>[4]</sup>

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  $\mu$  film thickness)



### **EQUIPMENT**

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

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59	24.94	2278	25.67			2306		52704-70-8 2,2',3,3',5,6-Hexac		12.40 false	218.0	LowRes	183.0	LowRes	10	20
50 51	24.94 24.94	2278 2278	25.67 25.67			2306 2306		52704-70-8 2,2',3,3',5,6-Hexac 52704-70-8 2,2',3,3',5,6-Hexac		12.40 false 12.40 false	359.9 361.9	LowRes LowRes	287.8 291.8	LowRes LowRes	10 10	30 30
52	25.15	2270	25.95			2308		1031-47-6 Triamiphos		12.40 false	160.1	LowRes	104.0	LowRes	10	15
53	25.15	2290	25.95			2308		1031-47-6 Triamiphos		12.42 false	160.1	LowRes	57.0	LowRes	10	20
54	25.15	2290	25.95			2308		1031-47-6 Triamiphos		12.42 false	294.0	LowRes	160.0	LowRes	10	5
5	25.15	2290	25.95			2308		1031-47-6 Triamiphos		12.42 false	160.1	LowRes	118.0	LowRes	10	15
i6	25.15	2290	25.95			2308		1031-47-6 Triamiphos		12.42 false	160.1	LowRes	77.0	LowRes	10	40
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59	25.15	2290	25.95			2308		1031-47-6 Triamiphos		12.42 false	135.0	LowRes	73.0	LowRes	10	15
	RT - CF	RI - CF	RT - opt 411		RT - opt 412		User Field				Precursor M	S1	Product M	IS2	Dwell Time	
o scr	eening (40.5 min)	screening	(41.867 min)	RI - opt 411	(20.75 min)	RI - opt 412	(Method Name)	CAS # (format 1) Common Name		Method RT ISTD		esolution		esolution	(ms)	CE (V) RT
1	25.19	2293	26.03	2286	12.42	2309		1031-47-6 Ethion		12.42 false	152.9	LowRes	96.9	LowRes	10	10
2	25.19	2293	26.03	2286	12.42	2309		563-12-2 Ethion		12.42 false	124.9	LowRes	96.9	LowRes	10	0
3	25.19	2293	26.03			2309		563-12-2 Ethion		12.42 false	230.9	LowRes	175.0	LowRes	10	10
4	25.19	2293	26.03			2309		563-12-2 Ethion		12.42	230.9	LowRes	129.0	LowRes	10	20
5 6	25.19 25.11	2293 2288	26.03 25.90			2309 2310		563-12-2 Ethion 77732-09-3 Oxadixyl		12.42 loise 12.43 false	120.9 163.0	LowRes	65.0 132.1	LowRes	10 10	10 5
7	25.11	2288	25.90			2310		77732-09-3 Oxadixyl		12.43 false	163.0	LowRes	117.1	LowRes	10	25
3	25.11	2288	25.90			2310		77732-09-3 Oxadixyl		12.43 false	132.0	LowRes	117.1	LowRes	10	15
9	25.11	2288	25.90	2279	12.43	2310		77732-09-3 Oxadixyl		12.43 false	232.9	LowRes	146.1	LowRes	10	10
)	25.11	2288	25.90			2310		77732-09-3 Oxadixyl		12.43 false	132.0	LowRes	90.0	LowRes	10	35
	25.11	2288	25.90			2310		77732-09-3 Oxadixyl		12.43 false	146.0	LowRes	91.0	LowRes	10	15
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	25.04	2284	25.80			2311		789-02-6 DDT-o,p'		12.44 false	237.0	LowRes	165.2	LowRes	10	20
	25.04	2284	25.80			2311		789-02-6 DDT-o,p'		12.44 false	235.0	LowRes	199.1	LowRes	10	15
5	25.04	2284	25.80	2273	12.44	2311		789-02-6 DDT-o,p'		12.44 false	199.0	LowRes	163.1	LowRes	10	35
7	25.04	2284	25.80			2311		789-02-6 DDT-o,p'		12.44 false	237.0	LowRes	199.1	LowRes	10	15
8	25.04	2284	25.80			2311		789-02-6 DDT-o,p'		12.44 false	165.0	LowRes	115.1	LowRes	10	35
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	25.02	2282	25.76				cic Congener	74472-37-0 2,3,4,4',5-Pentachi		12.44 false	327.9	LowRes	255.9	LowRes	10	25
1	25.02	2282	25.76	2271	12.44		cic Congener	74472-37-0 2,3,4,4',5-Pentachi		12.44 false	255.9	LowRes	184.0	LowRes	10	35
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	25.02	2282	25.76				cic Congener	74472-37-0 2,3,4,4',5-Pentachi		12.44 false	253.9	LowRes	219.0	LowRes	10	25
7	25.02 25.02	2282	25.76				dc Congener	74472-37-0 2,3,4,4',5-Pentach		12.44 false	184.0	LowRes	149.0	LowRes	10	20
_	25 02	2282	25.76				cic Congener	74472-37-0 2,3,4,4',5-Pentachi	orobipnenyi (BZ #114)	12.44 false	325.9	LowRes	290.9	LowRes	10	15 15
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	25.02 25.45	2308	26.35	2305		2312		72963-72-5 Imiprothrin I	orobiphenyl (BZ #114) thod / what is VLOOKUP	12.44_ false		LowRes	292.9 81.0	LowRes LowRes	10	10

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

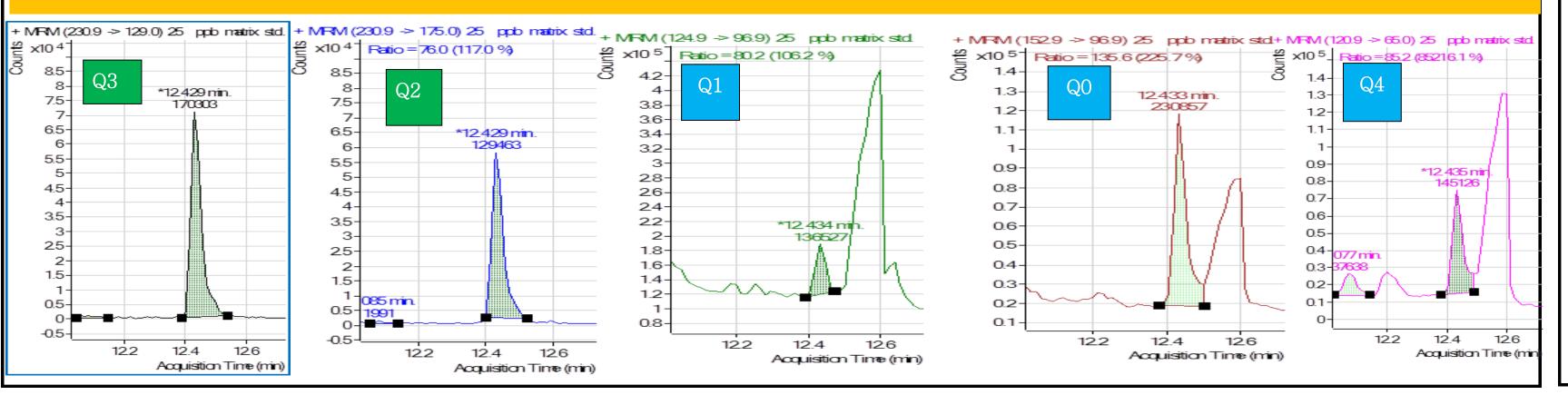
Oven	temperat	ure	Mult	i mode In	Inlet		
Rate	Tempera-	Hold	Rate	Tempera-	Hold		
(° C/	ture (°C)	(min)	(° C/	ture (°C)	(min)		
min)			min)				
Initial	<b>70</b>	1		70	0.1		
25	150	0		70	0.1		
			450	325	5		
3	200	0					
8	280	8	10	250			

#### **MS/MS Conditions**

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

### **ANALYSIS**

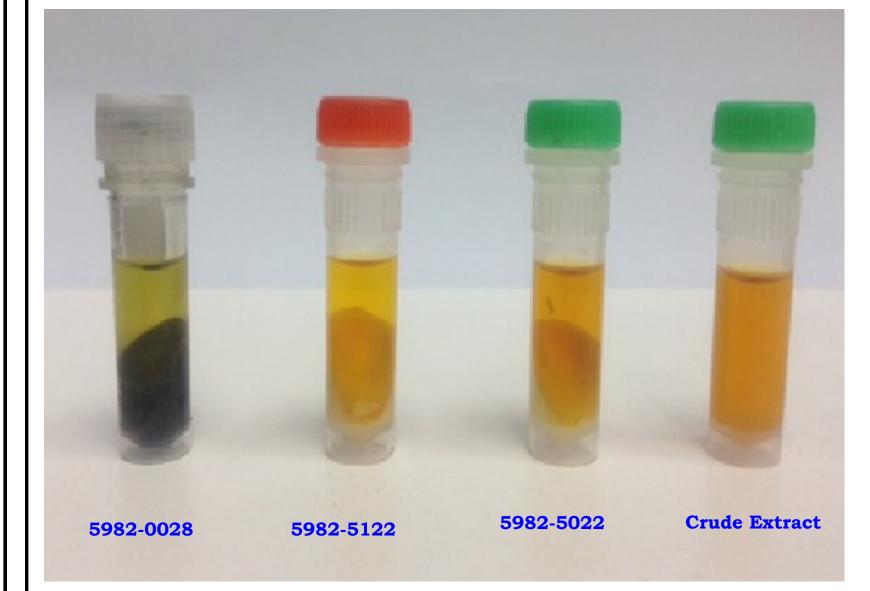
All the transitions available for each compound in the database were analyzed and two transitions were chosen based on the ion ratio and the lack of matrix interferences. For example, the Agilent MRM data base has five transitions for Ethion and all were tested. Two transitions were chosen for use in 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.

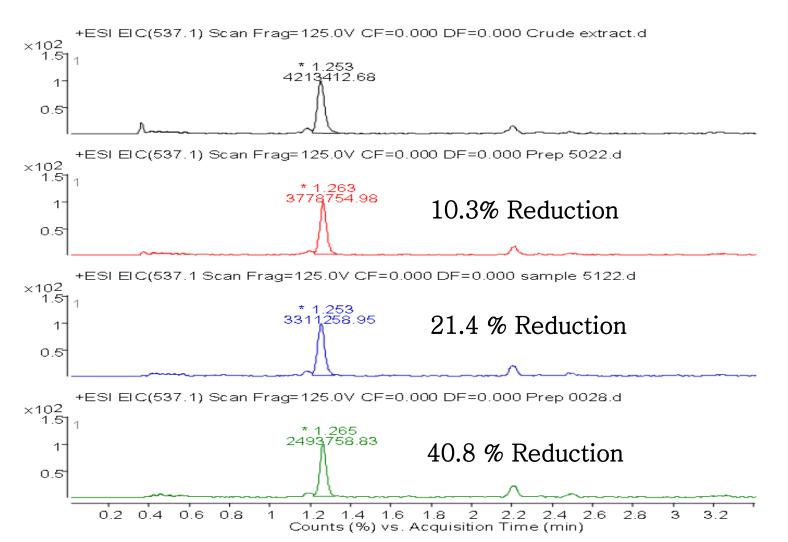
It is evident from the Figure 2 that the combination of PSA, C-18 and GCB (0028) is absorbing more pigments resulting in less yellow colour than 5122 and 5022. LC/MS analysis of these extracts also confirms 0028 is having the least amount of beta-carotene when compared to other cleanup strategies. The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



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

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

Table 1: Sorbent composition of the different cleanup strategies



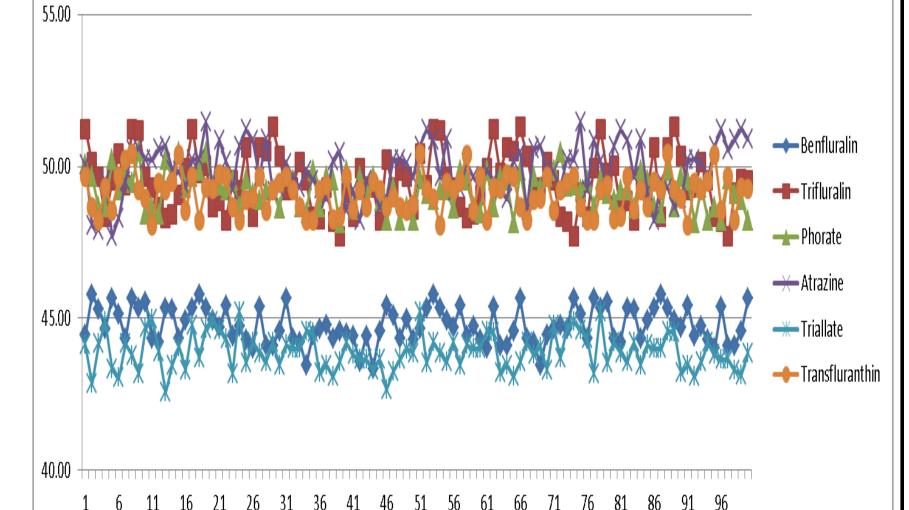
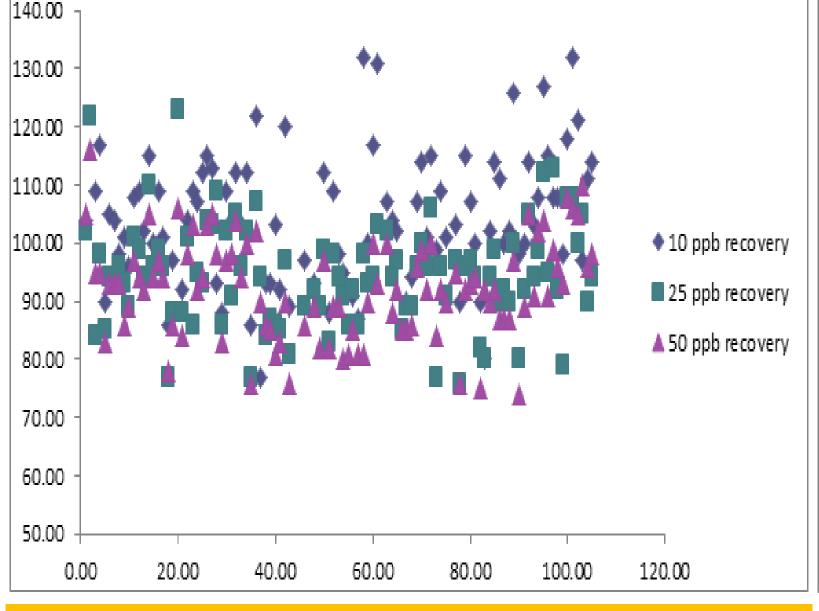
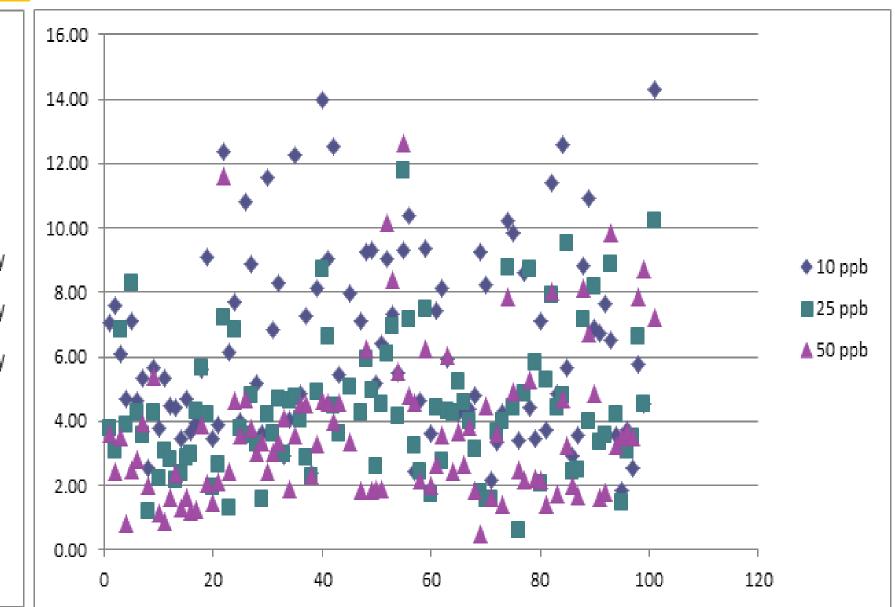


Figure 3: LC/MS chromatogram of beta-carotene Figure 4: Stability of GC/MS/MS system responses showing the effectiveness of three different clean up methods

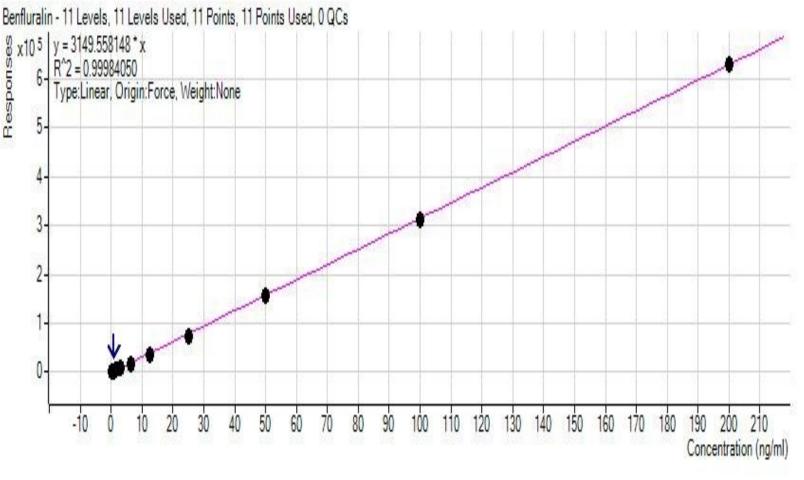
over repeated injection of 50ng/ml spiked sample

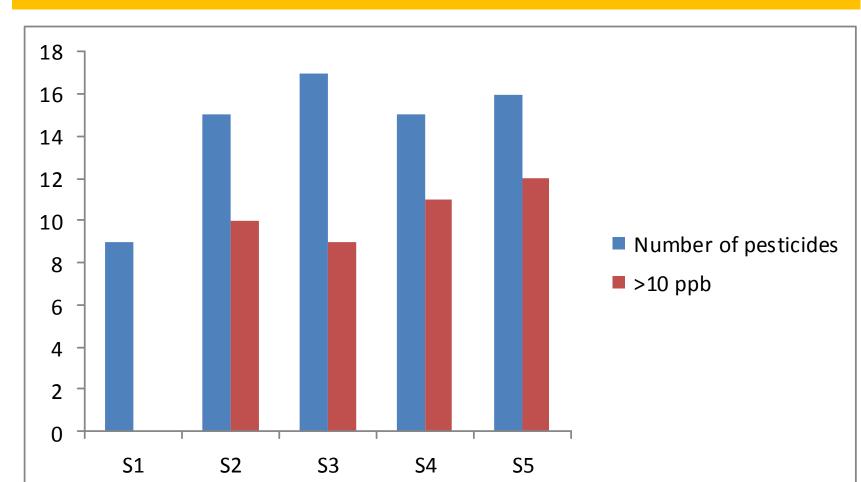




25 and 50 ng/ml concentration

Figure 5: Percentage of recovery of pesticides at 10, Figure 6: Relative standard deviation of the recoveries (n=6)





[The R<sup>2</sup> was >0.99 for most of the compounds pesticides and pesticides above 10 ng/g within 0.19–200 ng/ml (11 levels)].

Figure 7: Calibration linearity for benfluralin Figure 8: Analysis of red chili samples for

## CONCLUSIONS

- . 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