

# Evaluation of fenpropathrin using quechers method and GC/MS/MS QQQ in Kenyan black tea

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## ABSTRACT

Kenyan tea has been relatively pests and diseases free apart from a few reported cases of pests. Consequently measures to control both pests and diseases have been reported by TRFK (TRFK 5<sup>th</sup> edition, 2002). Common pests and diseases in Kenya include red spider mite, Nyambene weevil, Armillaria root rot (TRFK 5<sup>th</sup> edition, 2002). Climatic changes are being experienced all over the world which means diseases and pests will also be on the rise. In order to monitor pesticide residues, European Union (EU) and Codex have regulations on food safety and have limits on residues in tea. Some of EU limits include; DDT (sum of p,p'-DDT, o,p'-DDT, p-p'-DDE and p,p'-TDE (DDD) expressed as DDT) (F) 0.2 mg/kg, deltamethrin (cis-deltamethrin) 5 mg/kg, endosulfan (sum of alpha- and beta-isomers and endosulfan-sulphate expressed as endosulfan), 30mg/kg, Fenpropathrin 2mg/kg, methidathion 0.1 mg/kg (Code of Practice – Pesticide residues in tea / Issue 16, 2/4 /12) to name but a few. Black tea samples were extracted using acetonitrile as a solvent based on the QuEChERS (Quick, Easy, Cheap, Effective Rugged and Safe) method. The extract was then analysed using a gas chromatograph fitted with an MS/MS detector (GC-MS/MS QQQ). Results of this analysis show that fenpropathrin was not detected in the samples analysed.

**Key words:** Codex, European Union, Residues, Fenpropathrin

## INTRODUCTION

Tea (*Camellia sinensis* L.) is one of the most popular non-alcoholic beverages. It is consumed by over two thirds of the world's population, mostly due to its refreshing, medicinal and stimulant effects (Tanmoy and Bhagat, 2010). However, intensive use of agrochemicals such as pesticides in some tea growing areas especially India and China have raised concerns due to the residues in the tea and thus potential health risks (Cajka *et al.*, 2012). With increasing general public awareness of adverse effects of pesticide residues in food on human health the regulators are faced with the task of ensuring that food with high levels of pesticide residues does not reach the consumer (Barooah, 1994).

Presently, it is a global concern to minimize chemical residue in tea. European union and German laws have posed stringent measures for the application of chemicals in tea and fixed maximum residue levels (MRLs) values at <0.1 mg/kg for the most commonly used pesticides (Gurusubramanian *et al.*, 2008; Belitz *et al.*, 2009).

Codex Alimentarius established by FAO and WHO in 1963 harmonised international food standards, guidelines and codes of practice to protect the health of the consumers and ensure fair practices in the food trade. Residues that have been harmonised by Codex in tea include chlorpyrifos 2 mg/kg, dicofol 50 mg/kg, methidathion 0.5 mg/kg, paraquat 0.2 mg/kg, propagite 5 mg/kg, permethrin 20 mg/kg, deltamethrin 5 mg/kg, fenpropathrin 2 mg/kg (Codex DT 1114, 2010).

Considering that tea from other parts of the world is used for blending with Kenyan tea for trade purposes, it is important to look at the global scenario of other tea growing countries. Countries

like India which have produced tea for a long time have great dependence on the use of pesticides (Gurusubramanian *et al.*, 2008). Due to this practice, the tea pests have a higher tolerance resistance status due to formation of greater amount of esterases, glutathione *s*-transferase and acetylcholinesterase (Gurusubramanian *et al.*, 2008). Overreliance on pesticides ends up with residues in made tea such as DDT, endosulfan, dicofol, ethion and cypermethrin. A report of residues from India showed levels of ethion at 8.4 mg/kg and dicofol at 6.4 mg/kg (Barooh, 1994). European Union MRLs has put in place a limit of 3.0 mg/kg ethion (European Tea Committee, 2012). Other surveys conducted in countries where India sells its tea found residues of ethion, dicofol, quinaphols and others which in some samples were above the MRLs. Residues of organochlorine pesticides were also detected in the samples. These pesticides are banned (Barooh, 1994). Central insecticide board of India has recommended ten insecticides, five acaricides, nine herbicides and ten fungicides in tea (Gurusubramanian *et al.*, 2008).

## **MATERIALS AND METHODS**

Twenty-one black tea samples were purchased from factory outlets from different parts of the country. Majority of the samples were PF1 grade as it constitutes about 60% of the total black tea production. These samples were purchased packaged ready for sale to the consumers. The samples were transferred by road to Kenya Bureau of Standards in Nairobi.

### **Experimental**

#### *Sample preparation*

Preparation of the black tea samples was based on QuEChERS. This method effectively extracts pesticides from tea matrix while at the same time minimizes extraction of caffeine and other co-extractives which can cause degenerative effects on chromatographic peak, shape, analyte retention, time shifts and loss of sensitivity.

Two grams of black tea samples were weighed into a 50 ml centrifuge tubes. 10 ml of distilled water was added and the sample left to hydrate for 30 minutes after agitation for 30 seconds. Acetonitrile 10 ml was added and tube agitated vigorously for one minute. Magnesium sulphate (4 g) and sodium chloride (1 g) was then added. The tubes were agitated vigorously for 1 minute then centrifuged 6000 rpm for 10 minutes at room temperature.

Upper Acetonitrile layer was transferred to a 15 ml plastic centrifuge tube. n-hexane (1 ml) was added and 20% w/w aqueous NaCl solution 5 ml was then added. The tubes were agitated vigorously for one minute then centrifuged 6000 rpm for two minutes at room temperature.

#### *Sample analysis*

Analysis was performed using Agilent GC/MS/MS 7890A combined with an Agilent 7000B quadrupole with an electron ionization (EI) source. The GC system was equipped with Electronic Pneumatics Control (EPC) and an automatic liquid sampler. Mass hunter software was used for equipment control and quantitative data analysis.

#### *GC parameters*

Column HP-5 msultra inert column (15 m × 0.25 mm ID, 0.25 μm)

Injection temperature: 230°C

Injection mode: splitless

Injection volume: 1–2 μL

*Oven temperature programme*

50°C (1 min)

50°C to 150°C 50°/min hold four min

150°C to 200°C 60°/min hold 4 min

200 °C to 280°C 16°/min hold 4 min

*MS conditions*

MS source: EI-70 eV

Transfer line: 280°C

Source temperatures: 280°C

Quadropoles temperature: 150°C

Solvent delay: 4.0 minutes

He quench gas: 2.25 ml/min

N<sub>2</sub> collision gas: 1.5 ml/min

Gain setting: 10

Acquisition mode: MRM

MS1/MS2 resolution: Wide

Dwell time: 10 ms

*Instrument calibration*

The calibration standards were prepared in acetonitrile from a standard obtained from Fluka Lot # SZBC110XV at concentrations of 5 ng/ml to 100 ng/ml.

**Method performance**

The aim of this study was to evaluate method performance using the GC/MS/MS QQQ and QuEChERS extraction protocol.

**RESULTS AND DISCUSSION**

The MS/MS conditions were optimized to obtain MRM transitions for the analyte. This optimization included a full scan and a product ion scan. The transitions were borrowed from Agilent MRM database. The transition used from precursor to production was 265.10 > 210.10 at a retention time of 24.9 minutes (Figure 1 and Figure 3).

The calibration was a 5 point with an R<sup>2</sup> of 0.9998. For the target analyte method, performance characteristics were in line with SANCO/12571/2013 which states recoveries must be within acceptable range of 70% to 120% (Figure 2). For the spiking levels 20 and 100 ng/ml, levels 20 and 100 ng/ml were at 95.85% and 97.73% respectively (Figure 3). For all the samples analysed, fenpropathrin was not detected in the black tea. This is within the EU MRLs of 2 mg/kg (Figure 4).

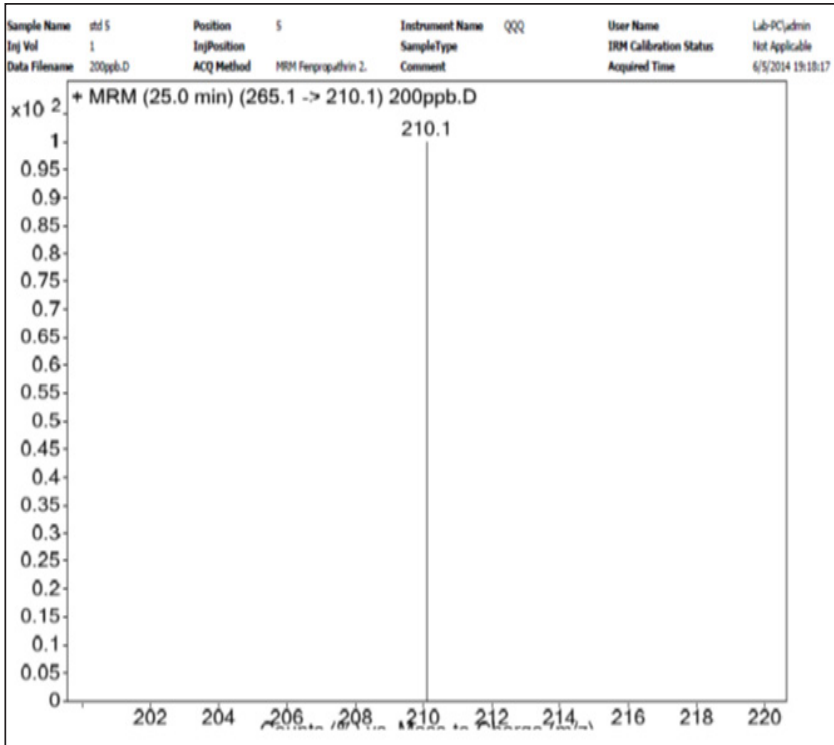


Figure 1: The transition used from precursor to production.

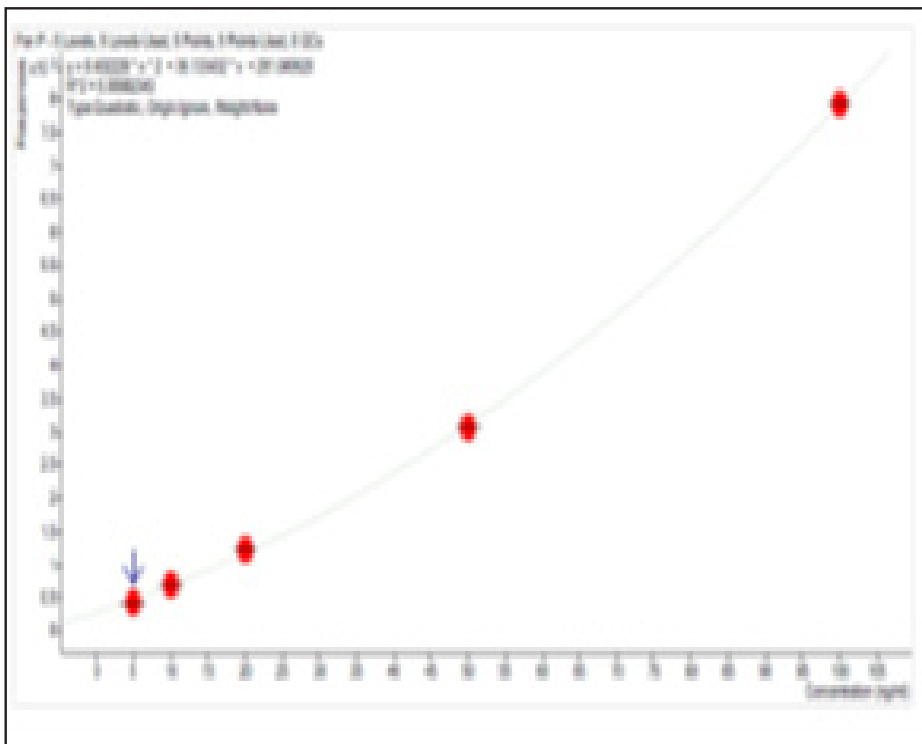


Figure 2: Performance characteristics.

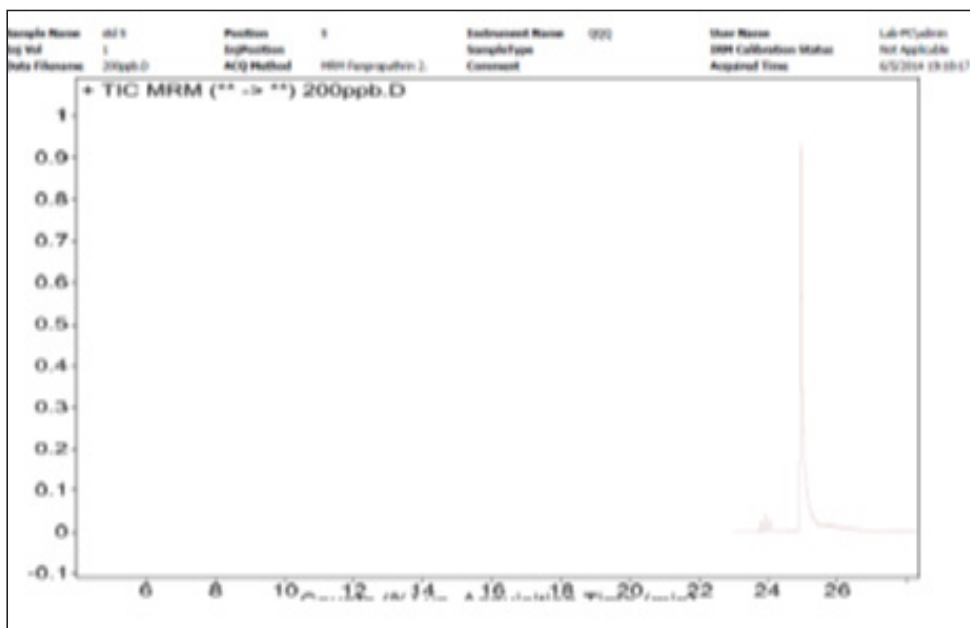


Figure 3: The transition used from precursor to production.

Sample									Fen P Results		
①	▼	Name	Data File	Type	Level	Acq. Date-Time	Dil.	Amt.	RT	Final Conc.	Accuracy
		std 1	std 1.D	Cal	L1	6/6/2014 12:25	1.0		24.949	3.8693	77.4
		std 2	std 2.D	Cal	L2	6/6/2014 13:01	1.0		24.961	10.32...	103...
		std 3	std 3.D	Cal	L3	6/6/2014 13:38	1.0		24.962	21.11...	105...
		std 4	std 4.D	Cal	L4	6/6/2014 14:15	1.0		24.959	49.55...	99.1
		std 5	std 5.D	Cal	L5	6/6/2014 14:51	1.0		24.951	100.0...	100...
		C6H14	C6H14.D	Sample		6/6/2014 15:28	1.0		24.965	0.0000	
		25275/12	25275-12.D	Sample		6/6/2014 16:05	1.0		24.926	0.0000	
		10ppb spi...	10ppb spike.D	Sample		6/6/2014 16:42	1.0		24.952	0.0000	
		29042/12	29042-12.D	Sample		6/6/2014 17:19	1.0		24.936	0.0000	
		29868/12	29868-12.D	Sample		6/6/2014 17:55	1.0		24.899	0.0000	
		9873/13	9873-13.D	Sample		6/6/2014 18:32	1.0		24.909	0.0000	
		25270/12	25270-13.D	Sample		6/6/2014 19:08	1.0		24.974	0.0000	
		28985/12	28985-12.D	Sample		6/6/2014 19:45	1.0		24.928	0.0000	
		29014/12	29014-12.D	Sample		6/6/2014 20:21	1.0		24.953	0.0000	
		29003/12	29003-12.D	Sample		6/6/2014 20:58	1.0		24.853	0.0000	
		29020/12	29020-12.D	Sample		6/6/2014 21:34	1.0		24.898	0.0000	
		20ppb spi...	20ppb spike.D	Sample		6/6/2014 22:10	1.0		24.965	19.17...	
		9875/13	9875-13.D	Sample		6/6/2014 22:47	1.0		24.949	0.0000	
		9905/13	9905-13.D	Sample		6/6/2014 23:23	1.0		24.946	0.0000	
		9911/13	9911-13.D	Sample		6/6/2014 23:59	1.0		24.904	0.0000	
		29001/12	29001-12.D	Sample		6/7/2014 00:35	1.0		24.925	0.0000	
		9901/13	9901-13.D	Sample		6/7/2014 01:12	1.0		24.952	0.0000	
		9876/13	9876-13.D	Sample		6/7/2014 01:48	1.0		24.918	0.0000	
		9860/13	9860-13.D	Sample		6/7/2014 02:24	1.0		24.901	0.0000	
		25261/12	25261-12.D	Sample		6/7/2014 03:00	1.0		24.903	0.0000	
		25262/12	25262-12.D	Sample		6/7/2014 03:37	1.0		24.900	0.0000	
		28976/12	28976-12.D	Sample		6/7/2014 04:13	1.0		24.952	0.0000	
		9855/13	9855-13.D	Sample		6/7/2014 04:49	1.0		24.897	0.0000	
		28972/12	28972-12.D	Sample		6/7/2014 05:25	1.0		24.904	0.0000	
		9862/13	9862-13.D	Sample		6/7/2014 06:01	1.0		24.890	0.0000	
		100ppb	100ppb spike...	Sample		6/7/2014 06:38	1.0		24.946	97.73...	

Figure 4: The EU MRLs of 2 mg/kg.

## CONCLUSION

This method, from extraction to clean up, is not time consuming, requires less quantity of reagents as compared to other methods like soxhlet and removes most of the unwanted matrix like caffeine. This improves retention time and reduces the risk of caffeine masking analyte of interest.

The transitions employed for the MS analysis ensure that only the analyte targeted elutes at that mass. Since confirmation has already been done during method optimization, the only analyte that elutes at that mass is the target hence the name target analysis. This method has potential to do hundreds of pesticides at low levels when the lab is armed with the right mix of calibration standards after method optimization.

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