RJC

RASĀYAN ***J. Chem.***
Vol. 11 | No. 2 |582 - 588 | April - June | 2018
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

MODIFIED QUECHERS METHOD FOR DETERMINATION OF MULTI-PESTICIDE RESIDUES IN INDIAN TEA SAMPLES BY LC-MS/MS

C. Sivanandha Reddy[1,*], S. Kulavardhana Reddy[2] and Gopireddy Venkata Subba Reddy[3]

[1&2]Research Scholar, Faculty of Chemistry, JNTUA, Ananthapuramu- 515002 (A.P.) INDIA.
[3] Department of Chemistry, JNTUACEP, Pulivendula, Kadapa- 516390.(A.P) INDIA .
*E-mail: chanda.sivanandareddy@gmail.com.

ABSTRACT

Quantitative estimation of pesticide residues in tea samples was established by employing Liquid Chromatography with tandem mass spectrometer using electron spray ionization (LC-ESI-MS/MS) in multiple reaction modes (MRM). The modified QuEChERS (quick, easy, cheap, effective, rugged, and safe) sample extraction procedure was used in the analysis. In dispersive solid phase extraction (d-SPE), PSA+GCB (800 mg + 25mg) sorbents and 750 $MgSO_4$ were used, which enhances in clean up capabilities and obtained good recoveries. The developed method was validated by employing LC-MS/MS at the different spiking concentration levels from 5-100 $ng.mL^{-1}$. The experimental results showed and found that the matrix-matched calibration curve linearity was $r^2 > 0.99130$ for total target analytes in all samples. Better recoveries were obtained between 70% to 120% with the acceptable relative standard deviation (RSD). The limits of detection ranged from 0.03 $ng.mL^{-1}$ to 1.4 ng/mL^{-1} and limit of quantifications from 0.1 to2.6 $ng.mL^{-1}$ for all the samples under investigation. The established method was successfully applied for the estimation of pesticide residues in real tea samples. The experimental results are found to be the presence of anthraquinone pesticide residue in the concentration ranging from 7.2-8.5 $ng.mL^{-1}$ in three out of ten tea samples under investigation.

Keywords: LC-MS/MS, Multiple Reaction Modes (MRM), QuEChERS, Pesticide Residues, Tea.

INTRODUCTION

In recent years, pesticides are widely employing in Agricultural sector to raise the quality of food produced and also to increase the crop yield. Pesticides have played an important role in providing reliable supplies of agriculture products at prices inexpensive to consumers and ensuring more benefit to the agriculturalists [1]. In the process of agriculture forming practices, the general population is getting exposed to more pesticides and also the food, drinking water and air is getting contaminated with these low persistent levels of pesticides [2]. The world health organization (WHO) has stated that approximately three million pesticide poisoning cases occur yearly, consequently lead to 2220,000 deaths worldwide[3]. Tea the truly ubiquitous common man's drink in India was introduced by the British to this country from neighboring country i.e china. In India, three special varieties of tea leaves are available namely Darjeeling, Nilgris and Assam which are getting exported to worldwide. Tea crop is grown over 13 states, majorly producing from Assam, Tamil Nadu, West Bengal and Kerala. Tea crop is one of the most vulnerable crops for few diseases and weeds, and also will be affected by pests, mites, caterpillars, and leaf-eating beetles. To avoid or reduce these problems, the farmers are following a common practice of using pesticides in tea farming production. Sometimes the farmers are not maintaining a gap between the consequent sprays and dosage per hector as per agriculturalist recommendations. In such agricultural practices, the presence of pesticide residues in tea beverages could be a major path of pesticide consumption by consumers. Some international organizations like the United States Environmental Protection Agency (EPA), Food and Agricultural Organization (FAO) of the USA and European Union (EU) have existing and proposed concentration of Maximum residue limits (MRL) for some pesticides in

http://dx.doi.org/10.31788/RJC.2018.1122077

tea. Presently, the EU Pesticides database[4] based on Regulation (EC) No 396/2005[5] covers 448 pesticide residues in the list with respect to MRLs for tea. The Rapid Alert System for Food and Feed (RASFF) in Europe reported exceeding MRL s in tea leave[6].

In view of this a multi-residue method for the detection and estimation of pesticides in tea is an important task for regularities as well as tea producers. Through a literature survey, it is found that the majority of research article focused on multiple pesticide residue methods subjected to less complicated matrices such as fruit and vegetables[7]. A large amount of caffeine, pigments, and polyphenols are the main cause of complexity of tea matrix. Therefore, multiple multi-residue analysis in tea is tougher owing to a complicated extraction procedure and creating tough analytical challenge[8].

In general, choice of the sample preparation method is solvent extraction method but cannot deliver an effective solution to reduce matrix effects. Solid Phase Extraction (SPE) is also one of the excellent methods for pesticide analysis[9]. Gel Permeation Chromatography (GPC) is alternative effective clean-up method which has been extensively applied[10]. Quick, easy, cheap, effective, rugged and safe (QuEChERS) is a better alternative method for multi-residue analysis which has been accepted globally, and was introduced by Anastassiades et.al[11, 12]. In recent trends, QuEChERS procedure has been followed by many governments and organization laboratories for multiresudie analysis in different samples such as, fruits, vegetables and other samples with complex matrices [11, 13, 14]. In the QuEChERS method, acetonitrile (ACN) was used in primary step followed by addition of salt mixture which leads to partitioning. An aliquot solution is added to sorbents and centrifugation is done to get the dispersive solid phase extraction (DSPE). The fine extract with acetonitrile (MeCN) is directly responsive for qualitative and quantification analysis by several analytical instruments, such as liquid chromatography (LC) or gas chromatography (GC)[15]. The gas chromatography with mass spectrometry with provided better results in different food matrices and drinking water for pesticide residue analysis[16]. Recent times, LC coupled with tandem mass spectrometry (LC-MS/MS) is a preferable method because of its more sensitivity and selectivity [17]. Wang et al., reported that 141 pesticide residues were analyzed in a tea sample by using UHPLC-TOF-MS and LC/ECI-MS/MS. The LC-ECI-MS/MS had proven superior sensitivity and good repeatability method than UHPLC-TOF-MS[18].

The present study focused on modified QuEChERS process which was developed and validated for the determination of multi-residue analysis in tea by using LC-MS/MS and applied to real samples of tea. Therefore, the developed method would be an advantage and suggested as the prescribed procedure for other multi-residue analysis in several types of tea.

EXPERIMENTAL

Reagents and chemicals

Pesticide reference standards were supplied by Sigma Aldrich (Bangalore, K.A, India). HPLC grade acetonitrile, methanol, acetic acid, and formic acid were used in sample preparation, mobile phase, and stock solution preparation. Primary secondary amine (PSA) and Graphitized carbon black (GCB) were obtained from Agilent Technologies (Hyderabad, T.S. India). Analytical reagent grade anhydrous magnesium sulfate ($MgSO_4$), Ammonium formate, and sodium chloride were used in the present work. The $MgSO_4$ was calcined at 450^0 C in a muffle furnace to remove wet content and phthalates to avoid any contaminants in materials.

Apparatus

A vortex mixture (Remi C-852) and centrifuge were used in sample extraction procedure. Liquid chromatography-mass spectrometry with ESI ionization source was used for the quantification of selected pesticides in the tea samples. Agilent1200 model HPLC was used for present analysis. The mobile phase A consisted of 90:10 water, methanol with 0.1% formic acid, B contained 80:20 methanol: water and 5 mill molar ammonium formate, 0.1% formic acid. The elution was performed in the gradient mode program analysis and parameters are listed Table-1.

Separation was achieved using an Agilent Eclipse plus C-18 column (5µm particles, 4.6 ×250mm), maintained at 20^0C. The mass-spectrometer measurements were performed on a triple quadruple (QqQ), API 6490 MS/MS system (Agilent Technologies, Bangalore, India.) with an electrospray ionization

source (ESI) operating in positive ionization interface was used, and MRM (multi-reaction monitoring) mode was applied. The ESI source settings were gas temperature, 120^0C; gas flow, 10 L min^{-1}; nebulizer gas, 30 psi; sheath gas temperature, 375^0C; sheath gas flow, 10 L min^{-1}; capillary voltage, 4500 V and nozzle voltage, 400 V. Nitrogen was served as the nebulizer and collision gas.

Table-1: Liquid Chromatography Gradient Elution Analysis Program

Time/min	Flow rate (µL.min^{-1})	A%	B%
0.00	500	95.0	5.0
1.50	500	95.0	5.0
3.00	500	5.0	95.0
6.00	500	5.0	95.0
9.00	500	95.0	5.0
15.00	500	95.0	5.0

Extraction Procedure

Two grams of tea powder was weighed, transferred into a 50 mL PTFE centrifuge tube and 5 mL of ultra-pure water was added followed by 15 mL acetonitrile. The centrifuge was shaken vigorously by vortex shaker for 4 min and then kept settle for 45 min. Later 4 g of anhydrous $MgSO_4$, 1g NaCl were added and shaken by vortex for 2 min and cooled the tube in an ice water bath for 5 min followed by centrifugation. Then 3 mL of the upper layer was transferred to the dispersive tube which contained 800 mg PSA, 25 mg GCB, and 750 mg $MgSO_4$. The dispersive tube was conditioned with acetonitrile and centrifuged at 400 rpm about 6 minutes. The extracted eluent was concentrated to 1mL by evaporating with weak nitrogen stream at 40^0C. Finally, the residue was diluted with 1 mL MeCN and filtered with a 0.2 µm organic filter, which is ready for injection into LC-MS/MS for analysis.

Stock Solutions

Stock solutions of 1µg.mL^{-1} standard mixtures of Aldrin, Anthraquine, Atrazine, Benalaxyl, Benthiocarb, Biphenyl, Chlortenapy, Chlorpyrifos, D.D.E.o.p, Fenazaquin, Fenpropathrin, and Quinalphos were prepared by adding 1mL of each pesticide pure compound (standard) from stock solutions into 10 mL MeCN in a 10 mL volumetric flask. The standard solutions were stored at -25^0C. Thirteen (13) working standard samples were arranged using the serial dilution method (5, 10, 20, 50, and 100 ng. mL^{-1}) for above-sited reference standard pesticides. These working standard solution sets were divided into aliquots, sealed in ampoules and stored at -40 ^{0}C until required. These were used to determination of limit of quantification (LOQ), limit of detection (LOD), the linearity, and recovery experiments utilized by working standard solutions.

Preparation of Blank Samples

The tea powder commodities were used for the spiking experiments. The organic tea samples were used as blank samples for the present study. All blank Tea samples were washed with acetonitrile solvent to eliminate the contamination of pesticide residues in the sample. After that, these samples were used for spiking experiments and studied recovery values at different concentrations respectively. The fortified tea samples were used for matrix-matched calibration by LC-MS/MS.

Method Performance

Tea blank samples were used for better sensitivity and precision of the optimized method. The relative standard deviation (RSD) and recoveries were determined for four replicates at five concentration levels (5, 10, 20, 50, 100 ng.mL^{-1}) in all the 13 target analytes under investigation and obtained good recovery (≥70) and found to satisfactory.

RESULTS AND DISCUSSION

Extraction Procedure

In modern agriculture practices, increased availability of a number of pesticides and lack of knowledge about the proper dosage to apply for the tea plants leads to residues not only attaching the surface of tea leaves but also merged into the tea structure. Therefore, the extraction method efficiency should be high for real samples than the examining spiked sample extraction. From the literature review, it was found

that QuEChERS sample preparation method suitable for analysis of pesticide residues in different vegetable and fruits [19]. The original QuEChERS method was modified for different analytes in various food samples. QuEChERS method is required to optimize for specific sample analyte in order to reduce errors as well as to increase the extraction efficiency[20,21]. Normally, the solvent selection plays an important role in a sample preparation, based on nature of pesticides. Some solvents can be nominated as the extraction solvents namely ethyl acetate[22] acetone[23] and acetonitrile[24] for optimization. Among these solvents acetonitrile solvent has extra advantages such as less interference from proteins, lipids, and co-extracted matrix, therefore achieves high recovery efficiency. In view of the stated advantages, MeCN was selected as the extraction solvent in the QuEChERS method. In this extraction method, $MgSO_4$ and NaCl salts were used for better partitioning the layers and upper layer had given significant volume which was explored for high recoveries.

Table-2: Optimized MS/MS parameters of Pesticides

Analyte	Retention times	MRM Transitions Qualifier	Collision energy	MRM Transitions Quantifier	Collision energy	MRM Transitions Quantifier	Collision energy
Aldrin	9.789	262.9→192.9	21	262.9→190.9	16	254.9→220.0	15
Anthraquine	9.820	207.8→152.2	12	207.8→180.1	10	207.8→132.1	10
Atrazine	7.800	214.9→58.1	10	214.9→200.2	15	200.0→122.1	15
Benalaxyl	12.714	148.8→77.0	5	124.9→89.0	10	266.0→74.8.	10
Benthiocarb	9.657	100.0→72.0	20	124.9→89.0	10	256.9→100.0	10
Bifenthrin	13.916	181.9→165.2	22	181.2→166.2	10	166.2→165.2	15
Biphenyl	5.466	154.1→153.1	18	155.1→154.1	22	ND	
Chlortenapyr	11.096	137.0→102.0	20	247.1→227.1	10	328.0→247.0	10
Chlorpyrifos	9.820	196.9→169.0	10	198.9→176.2	10	313.8→257.8	15
DDE-O,P	10.933	246.0→176.2	25	248.0→176.2	10	317.8→248.0	20
Fenazaquin	14.053	145.0→117.1	15	160.0→117.1	20	160.0→145.2	15
Fenpropathrin	13.893	181.1→152.1	15	125.0→55.1	10	207.9→181.0	13
Quianalphos	10.59	146.0→118.0	16	146.0→91.0	18	157.0→129.0	15

*ND=Not detected

Several sorbents (PSA, C_{18}, GCB etc.) can be used in the dispersive method for removal of matrixes from samples in order to minimize the interference. The PSA has an ability to remove fatty acids, sugars, lipids, organic acids. C_{18} can able to eliminate the fatty compounds and other polar interferences [25]. In orderly to reduce the matrix interference further in a tea sample, GCB was added along with PSA in appropriate proportions. Few authors were investigated and reported that for good recoveries optimum quantity sorbents are essentially needed[26]. Some of the research articles reported that if > 1000 mg PSA dosages, some pesticides have not found satisfactory recoveries [27]. In tea samples caffeine also one of the co-matrix, which was removed effectively by PSA+GCB 500 mg than PSA+GCB 250 mg with NH_2-Carbon combination [28]. In present research work, the extraction efficiency, recovery percentage found to be good and matrix influence also found at less by employing of PSA+GCB (800mg, 25mg) absorbent combinations during the d-SPE step. The evaluated results for all the samples in the present study are incorporated in in table.3 and its graphical representation is shown in Fig.-1 and 2.

LC-MS/MS Conditions were optimized by using mobile phase A in the ratio of 90:10 water, methanol [29] with 0.1% formic acid, and B consist of 80:20 methanol: water and 5 mill molar ammonium formate, 0.1% formic acid used for good separation of 13 pesticides in the analysis. To improve qualifier and quantifier with respect to target analytes studies, ESI positive mode was used for ionization. The optimization parameters of the instrument are summarized in Table-1.

Matrix Effect

The matrix effect was calculated by comparing the slopes of calibration curves in respect of matrix and solvent. In this study 5,10,20,50,100 ng.mL^{-1} concentrations were used for calibration curves for matrix and solvent. The ME evaluated by the following equation:

$$\mathrm{ME}(\%) = \frac{Slope\ of\ matrix\ matched\ curve}{slope\ of\ solvent\ curve} \times 100$$

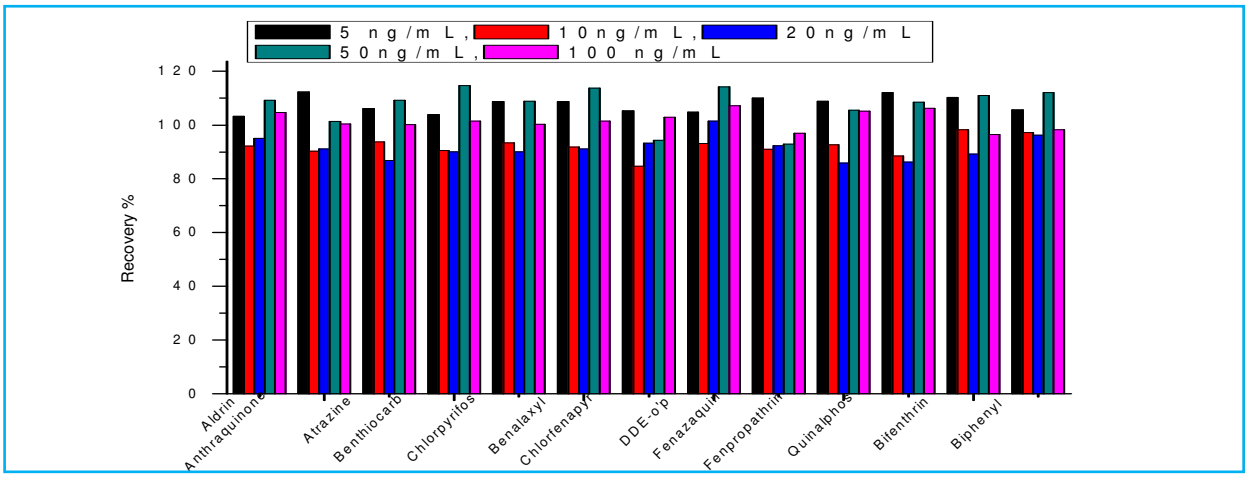

Fig.-1: Recovery percentages of pesticides from 5 to 100 ng.mL^{-1} concentrations.

Ferrer et.al reported that, the percentage of ME might be positive or negative and classified three categories of Matrix effects on the basis of ME percentage ranges. When the ME% values found to be between -20 and +20 it is considered as low matrix effect, if these values found in the ranges between –50 and -20; +50 and +20 it is considered as medium matrix effect. If ME% are above -50 and +50 then it is considered as strong matrix effect. The ME values were calculated using a calibration curve slope of solvent and calibration curve of matrix depicted in Table-5 and Fig.-2. A signal enhancement would occur if the percentage of the difference between these slopes were positive. If it was negative, it would be indicative of signal suppression[30]. In this study out of 13 pesticides,12 pesticides had low matrix effect ranging from -5.4 to 4.9 except bifenthrin showed medium matrix effect and hence the optimized extraction procedure as well as established analytical conditions were found to in good agreement and satisfactory.

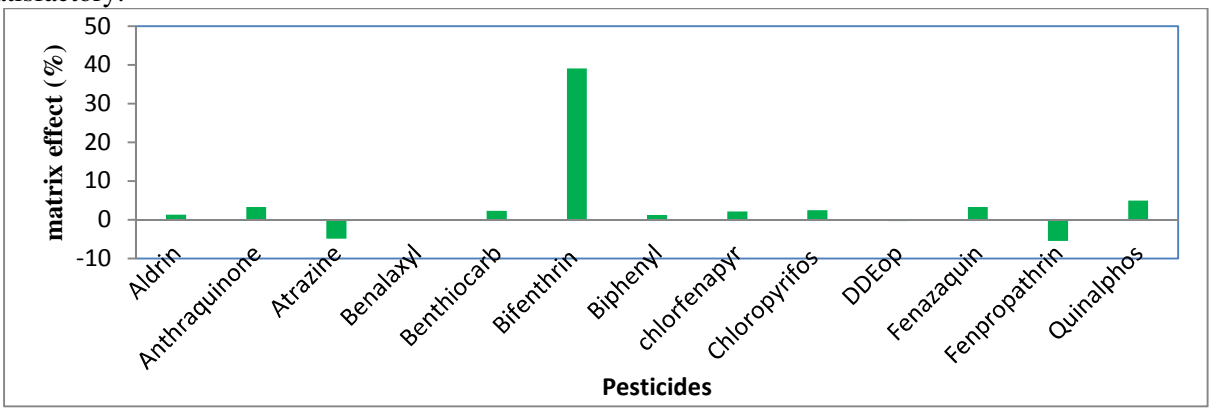

Fig.-2: The ME on pesticides analyzed by LC-MS/MS in tea matrixes: positive ME (suppression); negative ME (enhancement).

Method Validation

Good linearity results with the regression coefficient more than0.99130 for all pesticides were obtained. The limit of detection (LOD) and limit of quantification measured by signal to noise ratio (S/N) are 3×S/N and 10×S/N, respectively[31]. The evaluated LOD and LOQ values were shown in table.3. The chromatograms of qualifier and quantifier of all title compounds at different concentrations were represented in supplementary data.

The recovery and precision of the method for the 13 pesticides were evaluated by using five replicates (n=5) of spiked tea samples at five concentration levels (5,10,20,50,100 ng.mL^{-1}). The external calibration method was used for calculation of recovery values obtained, which were between 84.67 to 114.31 % are shown in Table-3. The relative standard deviations (RSDs) for the all spiked levels were lower than 20% as shown in Table-4.

Real samples

Ten tea samples (not a branded) were obtained from a local market in Pulivendula, Y.S.R Dist Andhra Pradesh (A.P), India. The validated method was applied to all these real samples. Anthraquinone pesticide residue was found in three out of 10 tea samples with the concentrations of 7.6, 7.9, 8.5 ng.mL^{-1} respectively which is found to be below MRL value as per EU legislation.

Table-3: Linearity and limit of detection (LOD) and limit of quantification (LOQ)

Analyte	Retention times	Linearity	r^2	LOD(ng.mL^{-1})	LOQ(ng.mL^{-1})
Aldrin	9.789	y = 55537.60 x-62511.76	0.99909	0.6	2.2
Anthraquine	9.820	y =23078.99 x-93315.31	0.99420	0.5	1.8
Atrazine	7.800	y =54624.12 x-18273.63	0.99255	0.5	1.8
Benalaxyl	12.714	y =47637.30 x-20434.31	0.99130	0.1	0.3
Benthiocarb	9.657	y =36892.55 x-10587.12	0.99672	1.4	4.7
Bifenthrin	13.916	y =20773.25 x- 65959.83	0.9948	0.2	0.6
Biphenyl	5.466	y = 13081.34 x 10705.66	0.9993	0.4	1.3
Chlortenapyr	11.096	y =29047.02 x-10751.56	0.9954	0.2	0.6
Chlorpyrifos	9.820	y = 11122.54 x-31622.35	0.99660	0.5	1.7
DDE-O,P	10.933	y =39044.89 x-24930.94	0.99915	0.1	0.4
Fenazaquin	14.053	y =40721.57 x-19492.85	0.99370	0.03	0.1
Fenpropathrin	13.893	y =75725.96 x-34861.28	0.99485	0.8	2.6

Table-4: Average recoveries and Relative standard deviation (RSD) of analytes at 5,10,20,50,100 ngmL^{-1}in tea samples.

	5ng/mL	RSD%	10ng/mL	RSD%	20ng/mL	RSD%	50ng/mL	RSD%	100ng/mL	RSD%
Aldrin	103.33	4.33	92.23	3.26	95.07	1.68	109.19	8.18	104.70	4.25
Anthraquinone	112.33	7.70	90.30	4.61	91.13	5.71	101.34	12.95	100.43	2.90
Atrazine	106.07	10.97	93.77	5.01	86.77	5.53	109.26	8.18	100.09	13.12
Benthiocarb	103.80	8.86	90.57	7.78	90.02	6.59	114.61	4.89	101.49	4.91
Chlorpyrifos	108.73	12.64	93.40	7.23	90.05	1.17	108.83	9.14	100.24	6.55
Benalaxyl	108.67	9.92	91.77	3.64	91.10	5.82	113.69	8.97	101.41	7.45
Chlorfenapyr	105.27	13.62	84.67	13.25	93.22	2.89	94.35	4.92	102.95	10.29
DDE-o'p	104.80	11.87	93.10	10.63	101.43	4.35	114.31	5.39	107.23	8.32
Fenazaquin	110.07	9.59	91.00	8.21	92.27	13.02	92.90	11.98	96.93	8.47
Fenpropathrin	108.93	8.50	92.67	3.41	85.82	3.99	105.52	14.02	105.21	2.48
Quinalphos	112.00	10.86	88.43	5.92	86.25	6.04	108.51	12.09	106.25	3.19
Bifenthrin	110.27	9.54	98.22	4.28	89.25	7.02	111	6.25	96.52	4.21
Biphenyl	105.64	10.62	97.22	7.21	96.25	1.89	112.1	7.25	98.25	3.91

CONCLUSION

A simple and fast analytical procedure for the determination of 13 pesticide residues in tea samples has been developed and validated. The use of dosage sorbents combinations (PSA800mg, GCB25mg) used in cleanup for the d-SPE step of QuEChERS methodology provided a significant removal of co-extractives interferences and satisfactory recoveries were obtained with acceptable RSD% for 13 pesticide residues in even under complex matrix. The applicability of the method was checked by analysis of 10 real tea samples and found positive results for three tea samples. Finally, the results presented in this study are reliable and established method is optimal which can be applied for real tea sample analysis for quantification of pesticide residues.

REFERENCES

1. E. C. Oreke, H. W. Dehne, *Crop Protection.*, **23**, 275(2004).
2. C. A. Damalas, I.G.Eleftherohorinos, *International Journal of Environment Research and Public Health.*, **8 (5),** 1402(2011), **DOI:** 10.3390/ijerph8051402.

3. World Health Organization (WHO) our planet, our health .Report of the WHO Commission on Health and Environment .Geneva, Switzerland. WHO. (1992).
4. EU pesticides database, http://ec.europa.eu/sancopesticides/public/index (accessed 20.03.2012).
5. Regulation (EC) No 396/2005 of The European Parliament and of The Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC,Off. J. Eur. Union L 70, 1–16, (2005).
6. Rapid Alert System for Food and Feed, http://ec.europa.eu/food/food/rapidalert/index en.htm (accessed 20.03.2012).
7. C. Tomas, B. Chris Sandy, B. Veronika, D. Lucie, K. Kamila, P. Jana, H. Jana, *Analytica Chimica Acta.,* **743**, 51(2012), **DOI:**10.1016/j.aca.2012.06.051.
8. D. Steiniger, G. Lu, J. Butler, E. Phillips, T. Fintschenko, *J. AOAC Int*., **93**,1169(2010).
9. Z. Huang, Y. Li, B. Chen, S. Yao, *Journal of Chromatography.*, **853**, 154(2007), **DOI:**10.1016/j.jchromb.2007.03.013.
10. H. Kerkdijk, H. G. J. Mol, B. V. D. Nagel, *Analytical Chemistry.*, **79**, 7975(2007).
11. M. Anastassiades, S. J. Lehotay, D. Stajnbaher, F. J. Schenck, *J. AOAC Int.,* **86**, 412(2003).
12. P.Y. Zhao, L. Wang, L. Zhou, F. Z. Zhang, S. Kang, C.P. Pan, *J. Chromatography. A.,* **1225**, 17(2012).
13. P. Paya, M. Anastassiades, D. Mack, I. Sigalova, B. Tasdelen, J. Oliva, A. Barba, *Anal. Bioanal. Chem.,* **389 (6)**, 1697(2007), **DOI:**10.1007/s00216-007-1610-7.
14. S. J. Lehotay, K. A. Son, H. Kwon, U. Koesukwiwat, W.S. Fu, K. Mastovska, E. Hoh, N. Leepipatpiboon, *Chromatography. A.,* **1217**, 2548(2010), **DOI:** 10.1016/j.chroma.2010.01.044.
15. S.N. Sinha, K. Vasudev, M.V.V. Rao, *Food Chem.,***132,** 1574(2012), **DOI:**10.1016/ j.foodchem. 2011.11.102.
16. A. Vijaya Bhaskar Reddy, Z. Yusop,J.Jaafar,A. Bin Aris, Z.Abdul Majid, K. Umar,J. Talib. *Journal of Separation Science*., **39**,2276(2016), **DOI:**10.1002/jssc.201600155.
17. C. Hao, X. Zhao, D. Morse, P. Yang, V. Taguchi, F. Morra, *J.Chromatography. A.,* **1304**, 169(2013), **DOI:**10.1016/**j**.chroma.2013.07.033.
18. J. Wang, W. Chow, D. Leung, *J. AOAC Int.,* **94**, 1685(2011).
19. C. Guoqiang, C. Pengying, L. Renjiang, *Food Chemistry.,***125**, 1406(2011).
20. R.P.Carneiro, F. A. S. Oliveira, F. D. Madureira, G. Sliva, W. R. Desouza, R. P. Lopes, *Food Control.,***33**,413(2013).
21. A. R. Restrepoa, A. F. G. Ortiza, D. E. H. Ossaa, G. A. P. Mesaa, *Food Chemistry.,* **158**, 153(2014).
22. A. G. Frenich, M. J. Gonzaleguez-Rodriguez, F. J. Arrebola, J. M. Vidal, *Analytical Chemistry.,* **77**, 4640(2005).
23. Germany DFG Methods19, Modular Multiple Analytical Method for the Determination of Pesticides, L-00-34. (2001).
24. R.M. K. Hajou, F. U. Afifi, A. H. Battah, *Food Chemistry,* **88**,469(2004).
25. R. Tomasz and T. Tomasz, *Journal of AOAC Int.*, **98**, 5, (2015).
26. A. N. Edouard, M. G. David, P. T. Faustin, G. G. Laura, M. G. Ana, *Food Chemistry*, **216**, 334(2017).
27. G. Yaqian, T. Hua, C. Dazhou, X. Ting, L. Lei, *Anal. Methods.*, **5**, 30(2013).
28. H. Xue, R. L. Shao, G. LingAn, Q. ShiTing, *Rev. Bras. Farmacogn*., **26**, (2016).
29. V. Camel, TrAc, *Trends*. *Anal. Chem.*, **19**, 229(2000).
30. C. Ferrer, A. Lozano, A. Aguera, A. Jimenez Giron, A. R. Fernandez-Alba, *Journal of Chromatography. A.*, **1218**, 7634-7639, (2011), **DOI:**10.1016/j.chroma.2011.07.033.
31. Method Validation and Quality Control Procedures for Pesticide Residues Analysis in Food and Feed, European Commission, 2011, SANCO /12495/2011.

[RJC-2077/2018]