Bull. Chem. Soc. Ethiop. **2023**, 37(4), 831-844.
DOI: https://dx.doi.org/10.4314/bcse.v37i4.3
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ISSN 1011-3924
Printed in Ethiopia
Online ISSN 1726-801X

OPTIMIZATION OF MODIFIED QuEChERS METHOD FOR EXTRACTION OF SELECTED PHARMACEUTICALS FROM VEGETABLE SAMPLES USING HPLC

Bisratewongel Tegegne[1,2,3], Bhagwan Singh Chandravanshi[2*], Feleke Zewge[2] and Luke Chimuka[1]

[1]Molecular Sciences Institute, University of Witwatersrand, Private Bag X3, Johannesburg 2050, South Africa
[2]Department of Chemistry, College of Natural and Computational Sciences, Addis Ababa University, P.O. Box 1176, Addis Ababa, Ethiopia
[3]Department of Chemistry, College of Natural Sciences, Bahir Dar University, P.O. Box 79, Bahir Dar, Ethiopia

(Received February 23, 2023; Revised April 5, 2023; Accepted April 8, 2023)

ABSTRACT. Optimized QuEChERS (quick, easy, cheap, effective, rugged and safe) method of sample preparation was employed for five selected pharmaceutical compounds on its advantage of simplicity, less extraction solvent and extraction time. Different parameters affecting the extraction efficiency of the target analyte was optimized. Since QuEChERS was developed for pesticides, uses of the method for pharmaceuticals need modification and evaluation of different parameters. Clean-up sorbents were evaluated for their matrix effect removal after extraction and a combination of d-SPE sorbent, $MgSO_4$, PSA, C_{18} and diatomaceous earth found to be selective clean-up sorbent for the targeted compounds. The addition and amount of diatomaceous earth and EDTA during the clean-up and extraction steps, respectively, were also examined and the amount was optimized. Solvent type and composition, salt type and extraction time were also optimized and methanol, $MgSO_4$ with NaCl salt and 5 min extraction time was obtained. The method was successfully applied to different vegetable samples collected from Addis Ababa, Ethiopia and Johannesburg, South Africa (carrot, cabbage and lettuce) and none of the target analytes were found in the sample investigated. The matrix effect study on vegetable samples collected was found very high that suppresses the signal during analysis.

KEY WORDS: QuEChERS, EDTA, Pharmaceuticals, Vegetable, HPLC

INTRODUCTION

Pharmaceuticals are synthetic or natural chemicals that contain active ingredients designed to have pharmacological effects and confer significant benefits. However, their possible environmental impacts are now an emerging environmental issue [1-4]. Several studies have documented that part of consumed pharmaceuticals is excreted as metabolites and unchanged parent compounds largely through the urine and feces [5]. The main sources of these compounds and their metabolites in the environments are through discharges from wastewater treatment plants, pharmaceutical companies, industrial wastes, illegal dumping of pharmaceutical waste and veterinary sources [6, 7]. Some of pharmaceutical compounds have the ability to partition to environmental solid phases including soil and sediment [8]. Many studies have recently reported that plants can take up pharmaceutical compounds from the growth media via their roots [9]. The study also reported dealing with vegetables could uptake antibiotics from contaminated water [9] and from soil [10].

As pharmaceuticals are everywhere in the environment, nowadays there is a growing demand for sample preparation methods which should be easy to perform, require a minimum volumes of solvents, provide a high selectivity without complicated clean-up solutions, rapid and of low cost,

*Corresponding author. E-mail: bscv2006@yahoo.com
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and allow that can analyze a wide range of analytes [11, 12]. Sample preparation used commonly for pharmaceutical analysis from environmental samples such as from soil, sediment, sludge and plant samples include microwave assisted extraction [13-16] and pressurized hot water extraction [17, 18]. These established methods are effective, but they are time consuming and quite expensive [19]. The quick, easy, cheap, effective, rugged and safe (QuEChERS) multi-residue procedure simplifies and reduces the time taken to complete, the extraction and clean-up processes [20].

The original QuEChERS method was successfully applied for the extraction of pesticides in cereals, fruits, vegetables, and others [21]. This method uses acetonitrile as an extraction solvent followed by a partitioning step, promoted by the addition of magnesium sulfate and sodium chloride. Due to the effectiveness of the method, it has been applied for different types of analytes and matrices through several modifications [22]. Many modifications of the QuEChERS method have been proposed in recent years for different applications. The modification of QuEChERS was done changing what is used in dispersive solid-phase extraction for clean-up [23], cartridge-based solid-phase extraction instead of dispersive solid-phase extraction for clean-up [24] with only primary secondary amine for clean-up [25] was reported. In this study, to get the best extraction result of the QuEChERS method, various parameters affecting the extraction efficiency were optimized. Several factors including clean up sorbent, the effect of diatomaceous earth, amount of diatomaceous earth, the effect of EDTA, amount of EDTA, extraction solvent, the volume of extraction solvent, salt type and extraction time were optimized which was not considered by other studies.

The objective of this study was to optimize modified QuEChERS sample preparation method for simultaneous detection of selected pharmaceuticals. The study focused on five pharmaceuticals which included trimethoprim (TRM), norfloxacin (NOR), ciprofloxacin (CIP), theophylline (THP) and caffeine (CAF). These pharmaceutical compounds were targeted and selected on the basis of commonly prescribed in the country and on the previous trend on their occurrence in environmental samples worldwide and their potential eco-toxicological impact. Vegetable samples were used to develop a modified QuEChERS extraction method as sample preparation for pharmaceutical compounds residue analysis. QuEChERS was first used for the analysis of pesticides from vegetables. However, in this study, we modified the original method in which it became suitable for the analysis of pharmaceuticals from vegetable.

EXPERIMENTAL

Chemicals and reagents

Pharmaceutical standard compounds used for QuEChERS method development and analysis CAF, THP, TRM, NOR and CIP (assigned purity > 99%) was obtained as a gift from Addis Pharmaceuticals PLC (Adigrat, Ethiopia). All chemicals and reagents used in this study were analytical grades and HPLC grade solvents. HPLC grade methanol (> 99%) and HPLC grade acetonitrile (> 99.9%) were from Fisher Scientific (UK) and formic acid (> 96%) from Sigma Aldrich (Germany) and acetic acid (99%) from Fisher Chemical (UK), ultra-pure water was prepared by using Millipore system (Direct-Q 3 UV with pump, South Africa). Extraction salts; magnesium sulfate anhydrate obtained from Sigma Aldrich (Gmbh, Japan), sodium chloride from Associated Chemical Enterprise (South Africa, Johannesburg) and ammonium acetate and sodium acetate were from Sigma Aldrich (Netherlands). Extraction clean-up, primary secondary amine (PSA) and octadecyl (C_{18}) and diatomaceous earth flux-calcinated was obtained from Sigma Aldrich (USA). Nylon syringe filters, 0.22 µm (Agela Technology, New York) was used for filtration of the sample extract prior to injection into the HPLC.

Instrumentation

Chromatographic separation was performed on an HPLC model of Agilent 1260 Series equipped with Quaternary Pump, Agilent 1260 Series, Agilent 1260 Series autosampler and Agilent 1260 Series Diode Array Detector (DAD). Data acquisition and processing were accomplished with LC Chemstation software (Agilent Technologies). Kromasil C_{18}, 5 µm (4.6 mm x 150 mm) column (Sweden) was used for separation. In the extraction of pharmaceuticals from the vegetable sample centrifugator (Hettich Zentrifugen, Rotofix 32A, Germany) and vortex mixer (Velp.Scientifica, Italy) was used.

Vegetable sample and preparation

Edible part of vegetable samples of which 500 g each (cabbage, carrot and lettuce) for this experiment were collected from Addis Ababa, vegetable market, Ethiopia and Johannesburg, vegetable market, South Africa. The samples collected from three subsampling points (small vegetable markets) were mixed together to form a bulk sample. The bulk samples were taken from both Ethiopia and South Africa vegetable sample for the study. Vegetable samples collected were washed with ultrapure water to remove any suspended wastes on the part and freeze-dried at a temperature of -50 ºC in a freeze drier (Labconco). The dried samples then ground using mortar and pestle to obtain powder and stored in a dry container for the extraction and analysis.

QuEChERS vegetable extraction procedure

A vegetable sample of each (cabbage, carrot and lettuce) mass 0.5 g was weighed and transferred to a 50 mL centrifuge tube and 25 µL of the solution of all target compounds in methanol (100 µg/mL) was spiked. Vegetable sample containing spiked mixture vortexed for 1 min and placed in a fume hood for methanol to evaporate and then extraction solvent and salt was added (5 mL methanol and 1 mL of (0.135 w/v Na_2 EDTA) (1.5 g $MgSO_4$ and 0.5 g NaCl)). Mixture solutions were vortexed for 1 min and kept for 10 min for extraction and centrifuged for 5 min at 4000 rpm. The supernatant solution was transferred to another centrifuge tube containing clean-up sorbent (20 mg C_{18} and 20 mg PSA, 250 mg $MgSO_4$, 50 mg DE) and vortexed for a minute and centrifuged for 5 min and the supernatant was filtered with a syringe filter of 0.22 µm and transferred to HPLC vial for analysis.

Chromatographic condition

Chromatographic condition optimization for separations of five selected pharmaceutical compounds used for QuEChERS extraction method development was the first task that was conducted. The mobile phase composition of binary solvent (water with 1%formic acid, v/v and acetonitrile) was obtained as a good solvent for separation of the target compound. The mobile phase was prepared daily and degassed by sonication for 20 min prior to use. The analysis was carried out on an Agilent 1260 series HPLC-DAD system using Kromasil C_{18} (150 cm x 4.6 mm) 5 µm analytical columns with a detection wavelength of 270 nm. The HPLC-DAD system was allowed to warm up for nearly 30 min and the baseline was monitored until it becomes stable before the samples or standards were injected. The injection volume was 10 µL, and the flow rate was maintained at 1 mL/min with a run time of 13 min and the analysis was performed at ambient temperature.

RESULTS AND DISCUSSION

Optimization of QuEChERS method

Type of sorbent for cleanup

The effective cleanup step in the QuEChERS extraction method on the complex matrices reduces co-extracted interferences and many studies considered the use of different sorbents [26]. Particularly from plant extract, large pigment molecules may clog the chromatographic column and interfere with the separation process [27]. Dispersive solid-phase extraction (d-SPE) cleanup steps employ a different type of sorbent including, magnesium sulfate ($MgSO_4$), primary secondary amine (PSA), graphitized carbon black (GCB), and octadecyl (C_{18}). This experiment aims to see the potential of different cleanup sorbent in the QuEChERS extraction of selected pharmaceuticals from vegetable samples. Cleanup steps based on different d-SPE sorbents (PSA, PSA+C_{18} and PSA+C_{18}+GCB with $MgSO_4$) combinations and without cleanup were compared. As shown in Figure 1, the cleanup d-SPE sorbent combination of PSA+C_{18} with $MgSO_4$ gives a relatively higher peak area for all compounds except for trimethoprim. However, the addition of GCB decreases the peak area of some analyte this is most probably as GCB is planer it might bind some compounds with a similar structure.

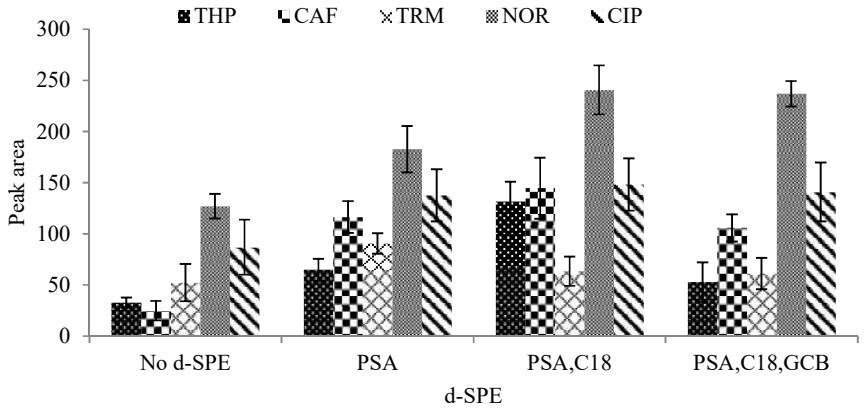

Figure 1. Effect of type of d-SPE sorbent for clean-up. Extraction condition: vegetable sample 0.5 g, extraction solvent methanol, volume of solvent 4 mL, salt $MgSO_4$:NaCl, extraction time of 8 min and spiked concentration 5 µg/g for each compound, n = 3.

Effect of diatomaceous earth (DE) as clean-up and amount in the extraction

Diatomaceous earth is used for clean-up in the QuEChERS extraction method for the vegetable matrix to avoid interferences as it is environmentally friendly natural sorbents [26]. The effect of the addition of diatomaceous earth was evaluated after the d-SPE sorbent was selected. Two sets of the experiment were conducted to compare the effect of with addition and without addition of diatomaceous earth as clean-up after extraction to reduce the interference that increases the value of the target compound in the detection (Figure 2(a)). In the same extraction condition, the clean-up of a mixture of diatomaceous earth (50 mg) together with the d-SPE sorbent selected in the section above and the other with only d-SPE sorbent was compared. The result shows the presence

of diatomaceous earth in clean up increases the peak of the target compound compared with the one without it.

As it is observed that the addition of diatomaceous earth in the clean-up step has a positive effect in extraction from the vegetable matrix, the amount of it also should be optimized to obtain a suitable amount for clean-up. Different amounts of diatomaceous earth in clean-up (5, 10, 50, 100 and 200 mg) were used to evaluate their extraction efficiency by reducing the matrix interference (Figure 2(b)). At the beginning, as the amount of diatomaceous earth increases the peak area also increases up to it reach 50 mg. However, the peak area starts to decrease after 50 mg of diatomaceous earth amount as clean-up sorbent. So that amount of 50 mg diatomaceous earth was used as an optimum amount of sorbent for clean-up also with the selected d-SPE clean-up sorbet for selected pharmaceutical compounds.

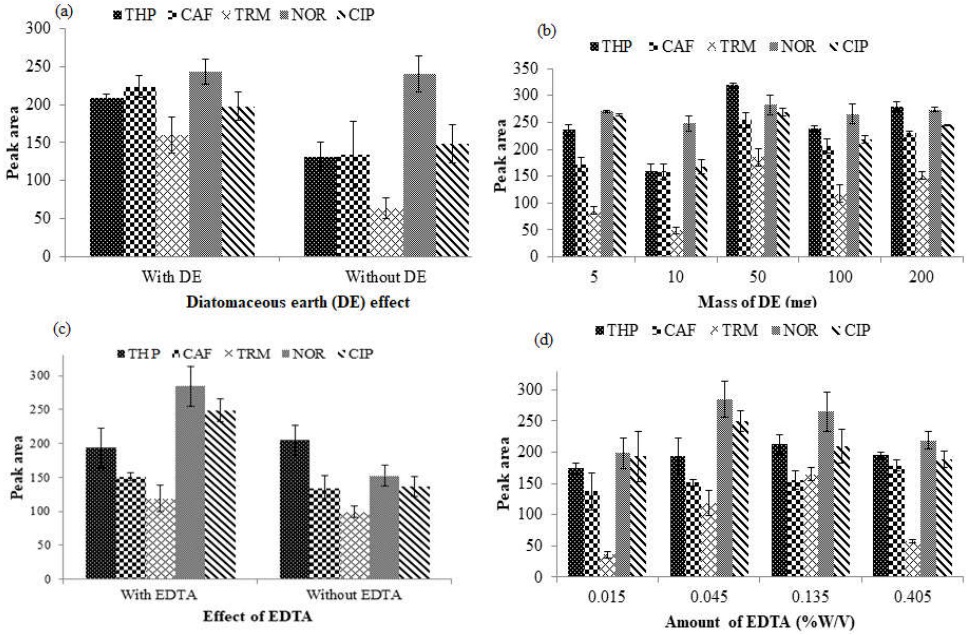

Figure 2. Effect of diatomaceous earth (DE) ((a) and (b)) and EDTA ((c) and (d)). Extraction condition: vegetable sample 0.5 g: volume of solvent 4 mL: salt $MgSO_4$: NaCl, extraction time of 8 min: clean-up $MgSO_4$ (250 mg), C_{18} (25 mg), PSA (25 mg), and spiked concentration 5 µg/g for each compound, n = 3.

Effect of EDTA on the extraction and amount in the extraction

Many studies reported the use of EDTA to increase the extraction recovery of pharmaceuticals from environmental samples to facilitate the extraction of bound compounds by avoiding the complexation of analytes with cations [28, 29]. The effect of EDTA on the extraction of pharmaceuticals from vegetables using the QuEChERS extraction technique was evaluated. The extraction with the addition and without addition of EDTA has a significant difference for extraction as the peak area improved with the presence of EDTA (Figure 2(c)). The result of extraction improvement on the addition of EDTA (highly in ciprofloxacin and norfloxacin) is in agreement with the study reported [30]. The next step could be obtaining the optimum amount of

EDTA which will be sufficient for the extraction of pharmaceuticals from the vegetable sample. Different concentration level of EDTA was evaluated (0.015, 0.045, 0.135 and 0.405, (%w/v)) for their extraction efficiency using the peak area of each compounds (Figure 2(d)). The concentration level of 0.135 %w/v EDTA was found to be sufficient for extraction in comparison with the other level. As the concentration level of EDTA increases beyond this, the peak area decrease that is because the compound might be lay on it which decreases the solubility to the extraction solvent.

Effect of type of extraction solvent and volume in the extraction

The choice of extraction solvent is one of the most important parameters in the QuEChERS sample extraction technique [19]. For extraction of selected pharmaceuticals in vegetable sample five set of different solvent was employed for the extraction efficiency. The solvents involved in this study are acetonitrile, methanol, acetonitrile with methanol (50:50, v/v), acetic acid with methanol (20:80, v/v) and formic acid with methanol (20:80, v/v). The extraction efficiency was examined with the spiked vegetable sample considering the peak area of each compound. The highest peak area was obtained when pure methanol was used as an extraction solvent for all pharmaceutical compounds as shown in Figure 3. As it is obvious pharmaceuticals are polar, so they need to have polar solvent to extract. On the other hand, acetonitrile as it is shown in the result, it cannot extract even all compounds from the vegetable sample only theophylline and caffeine appears. The addition of additives like acetic acid and formic acid in methanol and acetonitrile does not improve the extraction over the pure methanol. The volume of the selected solvent was also evaluated for the optimum amount to be sufficient for the extraction of selected pharmaceuticals from vegetable samples. The different volume of methanol 4, 5, 6 and 8 mL was employed as the extraction solvent and the extraction efficiency was evaluated by considering the peak area of the target compounds. An extracting solvent with volume of 5 mL methanol were observed to be the optimum volume for maximum extraction of pharmaceuticals.

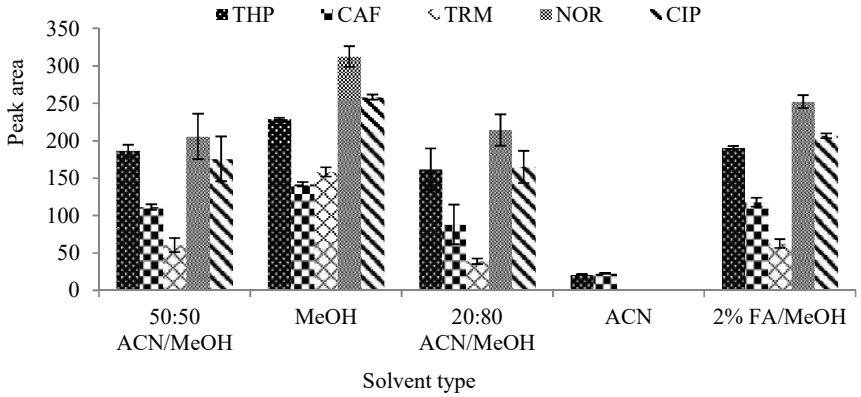

Figure 3. Effect of type of extraction solvent for extraction. Extraction condition: vegetable sample 0.5 g, volume of solvent 4 mL, salt $MgSO_4$ + NaCl, extraction time of 8 min, clean-up $MgSO_4$ (250 mg), C_{18} (25 mg), PSA (25 mg), DE (50 mg), 0.135 (% w/v) 1 mL EDTA and spiked concentration 5 µg/g for each compound, n = 3.

Effect of type of extraction salt

In QuEChERS, the extraction with solvent is followed by the addition of salts to induce phase separation [19]. Different salt was used to assess their salting-out effect for partitioning including QuEChERS common salt magnesium sulfate and sodium chloride ($MgSO_4$ + NaCl), other salts

like sodium acetate (NaOAc) alone and with sodium chloride and ammonium acetate with sodium chloride (NH_4OAc + NaCl). From the salt considered for their extraction efficiency, the QuEChERS common salt magnesium sulfate and sodium chloride was the one that gives the highest extraction efficiency in terms of peak area of the targeted compound from the vegetable sample as it is shown in Figure 4.

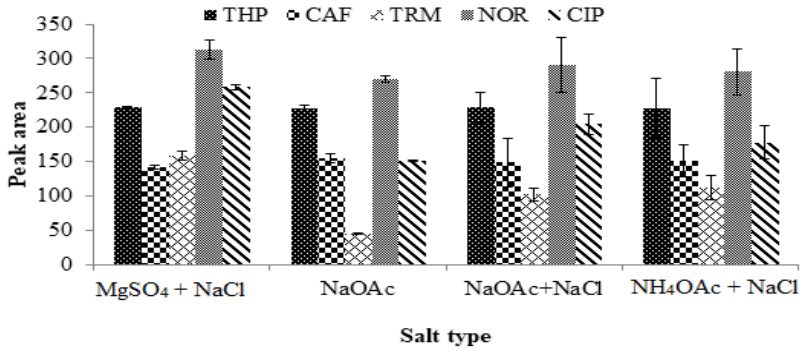

Figure 4. Type of salt for extraction: Extraction condition: vegetable sample 0.5 g, extraction solvent methanol 5 mL, extraction time of 8 min, clean-up $MgSO_4$ (250 mg), C_{18} (25 mg), PSA (25 mg), DE (50 mg), 0.135 (%w/v) 1 mL EDTA and spiked concentration 5 µg/g for each compound, n = 3.

Effect of extraction time

Extraction time in the QuEChERS extraction method plays a role as the mass transfer takes time to transfer from one phase to the other phase. This parameter is reported by [19] found to be the basic to be optimized in the extraction method optimization. The extraction time was the time after mixing the extraction solvent with the extraction salt together with the spiked vegetable sample. Extraction time from 5, 10, 15 and 20 min was employed for the extraction time to extract the analyte from the vegetable sample. As it is presented in (Figure 5) 10 min extraction time was the maximum time that gives the highest peak area for all compounds.

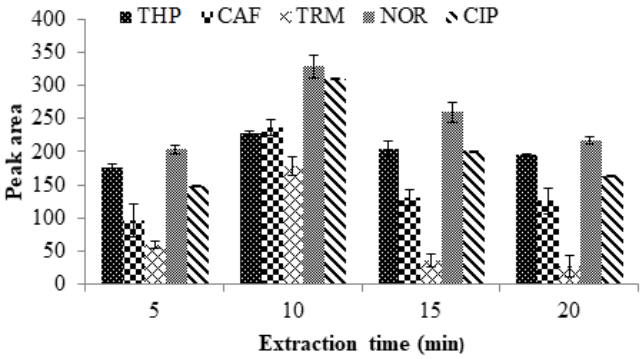

Figure 5. Effect of extraction time. Extraction condition: vegetable sample 0.5 g, extraction solvent methanol, volume of solvent 5 mL, extraction time of 8 min, clean-up $MgSO_4$ (250 mg), C_{18} (25 mg), PSA (25 mg), DE (50 mg), 0.135 (%w/v) 1 mL EDTA and spiked concentration 5 µg/g for each compound, n = 3.

Analytical performance characteristics

The optimized QuEChERS method for analysis of selected pharmaceutical compounds was examined using analytical parameters. The linearity (using the standard solution and matrix-matched calibration curve), the limit of detection (LOD), limit quantification (LOQ), accuracy and precision were parameters considered in this study.

Linearity, the limit of detection and quantification

Linearity was evaluated using matrices matched method using spiked cabbage at a different level in the range 0.5-10 µg/g. The peak areas of each analyte were plotted against the concentrations and linear regression analysis was performed to determine the linearity equation and correlation coefficient (R^2). The result obtained (Table 1) confirmed relatively good linearity between analytical signal and analyte concentration for all selected pharmaceutical compounds considered in this study. The sensitivity of the optimized QuEChERS method for the determination of selected pharmaceutical compounds from the vegetable sample was evaluated using the limit of detection (LOD) and limit of quantification (LOQ) of each analyte. The LOD was calculated as LOD = 3.3σ/S, where σ is the standard deviation of the response and S is the slop of the calibration curve for each analyte. LOQ on the other hand was calculated as LOQ = 10σ/S, where σ is the standard deviation of the response and S is the slope of the calibration curve for each analyte [31].

Table 1. Analytical performance of QuEChERS for the determination of selected pharmaceuticals in vegetables using matrices matched analysis.

Compound	Range (µg/g)	R^2	Equation	LOD (µg/g)	LOQ (µg/g)
Caffeine	0.5-10	0.9401	y = 14.741x+26.453	0.010	0.030
Ciprofloxacin	0.5-10	0.9558	y = 24.94x+41.693	0.005	0.014
Norfloxacin	0.5-10	0.9973	y = 33.78x+30.535	0.001	0.003
Theophylline	0.5-10	0.9342	y = 16.348x+28.51	0.006	0.018
Trimethoprim	1-10	0.9434	y = 8.286x+6.153	0.003	0.009

Precision

The precision of the optimized method was investigated by calculating the relative standard deviation (% RSD) in terms of reproducibility (between-day) and repeatability (within-day) using cabbage sample spiked with two different concentration levels. In each case, three replicate spiked cabbage samples were analyzed in the same optimized parameters. The precision result in repeatability and reproducibility was obtained which was satisfactory for all selected pharmaceutical compounds which is < 15% (Table 2).

Selectivity

The selectivity of the method was evaluated by analyzing extracted cabbage, which was spiked previously by a mixture of the standard solution. The selectivity study was used to observe the interference compound in the vegetable matrix. The chromatogram in Figure 6 (a), (b) and (c) shows about high selectivity of the optimized method towards the selected compound which was promising for further analysis of the vegetable sample.

Table 2. Analytical performance precision of the optimized QuEChERS for the determination of pharmaceuticals from vegetables.

Pharmaceuticals	Intra-day, %RSD (n = 3)		Inter-day, %RSD (n = 6)	
	Level 1	Level 2	Level 1	Level 2
Theophylline	2.83	4.56	5.41	8.45
Caffeine	1.63	5.66	4.86	4.39
Trimethoprim	0.64	6.44	4.43	6.31
Norfloxacin	4.60	6.56	5.85	11.33
Ciprofloxacin	0.78	11.81	6.85	8.73

(Level 1 = 1 µg/g, Level 2 = 5 µg/g).

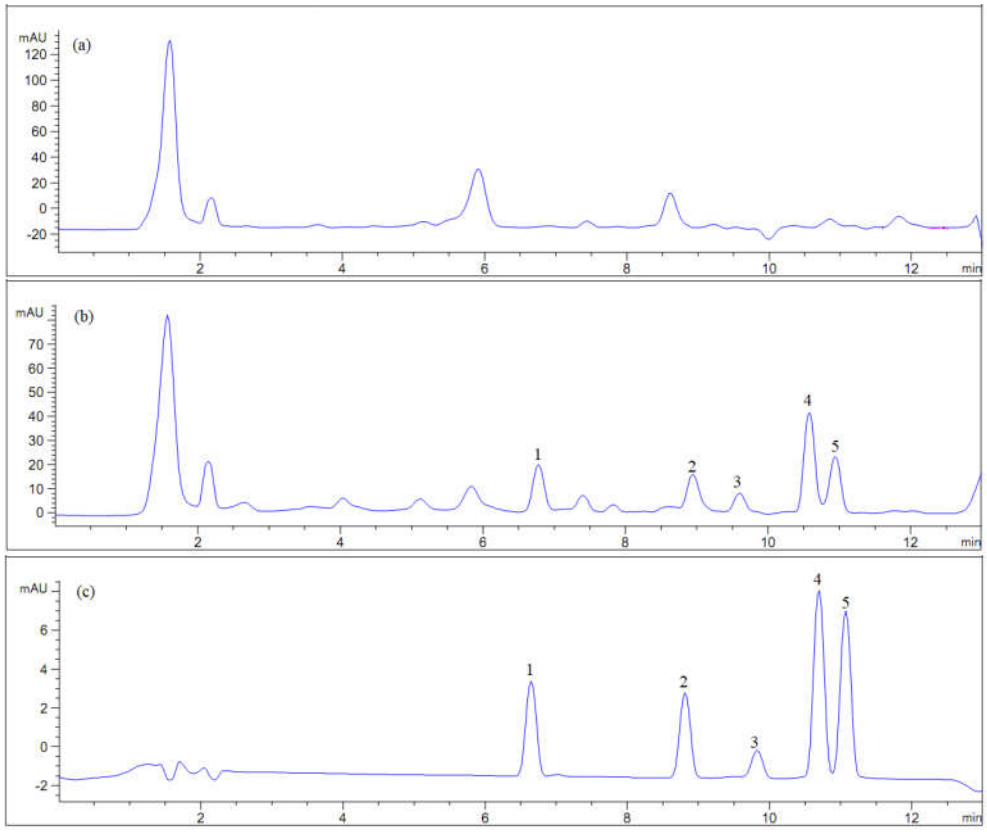

Figure 6. HPLC chromatogram of the selected compound (1: theophylline, 2: caffeine, 3: trimethoprim, 4: norfloxacin and 5: ciprofloxacin), and vegetable (a) cabbage extract, (b) spiked cabbage and extract using optimized QuEChERS and (c) standard mixture solution.

Matrix effects evaluation

Matrix effects that can cause suppression or enhancement of analytical signals are frequently observed in the chemical analysis field. This phenomenon is due to matrix compounds that are

eluted with the same retention time as the target compounds. Matrix effects depend on the nature of the matrix and the efficiency of the sample preparation step. Therefore, the sample preparation step should eliminate interfering compounds while retaining the target analytes [32-34]. To study this phenomenon, solvent without matrix and with matrix were spiked by adding 1 µg/g of the target compounds and the blank without spiking were analyzed using the optimized QuEChERS procedure and the result are presented in Figure 7(a) and (b).

$$\text{Matrix effect (\%)} \quad \left(\frac{\text{A(Spiked)}-\text{A(Blank)}}{\text{A(Solvent)}} - 1\right) \text{x } 100 \qquad (1)$$

where, A (matrix) is the area in the spiked matrix, A (blank) is the area in the non-spiked matrix and A (solvent) is the area in the spiked solvent without sample matrix.

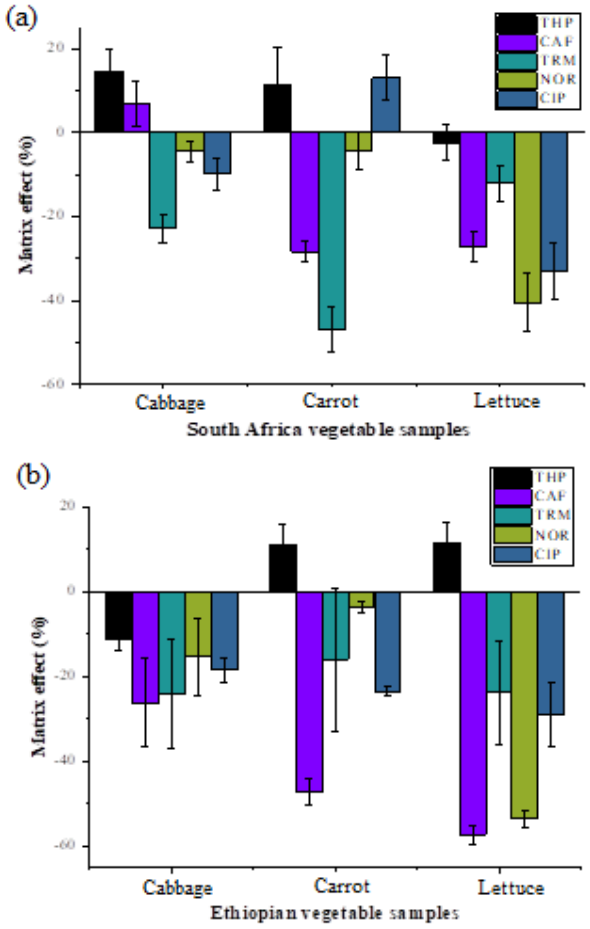

Figure 7. Matrix effect (%) calculated for all target analytes in vegetable samples from South Africa (a) and Ethiopia (b).

The calculated matrix effect results showed variability among the targeted compounds and also among the different types of vegetable samples and between the sampling areas. Matrix effect, signal suppression has been observed to be higher in the vegetable lettuce followed by carrot and cabbage in both sampling countries. Matrix effects values ranged from −46.7% to +14% in the South Africa sample, while values ranged from −57.4% to +16.9% in the Ethiopian vegetable sample. The addition of deuterated internal standards is therefore recommended to correct matrix effects.

Application and recovery studies

Vegetables commercially available (cabbage, carrot and lettuce) were considered for application to the proposed method. The selected analytes were not detected in any of the studied vegetable samples. To evaluate the accuracy of the proposed method, recovery studies were carried out with spiked in two different concentration level using vegetable samples (cabbage, carrot and lettuce) collected from two countries (Ethiopia and South Africa) from the main vegetable site and the result obtained is presented in Table 3. In both concentration levels, the recoveries obtained with the current method were in the range of 33.5 to 115%. The lower and the higher recovery value obtained from South Africa, Johannesburg vegetable samples. The reason for having such low %recovery result is because of the vegetable matrix suppression effect. As it is known vegetables contain different components and colorants with affects the extraction and the detection of a real sample. This one can be understood by observing the result of the chromatogram (Figure 5) intensity both for standard and sample.

Table 3. Analytical performance recovery (%R) and relative standard deviation (%RSD) for each selected pharmaceutical compounds in vegetable (n = 3) (Level 1 = 1 µg/g, Level 2 = 5 µg/g).

			Theophylline		Caffeine		Trimethoprim		Norfloxacin		Ciprofloxacin	
Vegetable sample		Spiked (µg/g)	%R	%SD	%R	%RSD	%R	%RSD	%R	%RSD	%R	%RSD
South Africa	Cabbage	0	-	-	-	-	-	-	-	-	-	-
		1	115	5.1	106	5.5	77.2	3.4	95.6	2.4	90.2	3.90
		5	93.8	4.1	33.5	6.8	88.8	11.9	97.7	12.0	81.5	9.8
	Carrot	0	-	-	-	-	-	-	-	-	-	-
		1	111	9.1	71.6	2.6	53.3	5.4	95.8	4.4	113	5.3
		5	113	11.8	69.9	7.1	66.1	11.1	79.5	12.6	77.9	0.8
	Lettuce	0	-	-	-	-	-	-	-	-	-	-
		1	97.7	4.4	72.8	3.8	85.9	4.2	59.4	6.9	66.9	6.8
		5	90.9	10.9	87.9	14.7	94.2	12.1	52.3	2.2	63.1	2.5
Ethiopia	Cabbage	0	-	-	-	-	-	-	-	-	-	-
		1	88.9	2.9	73.8	10.5	75.7	12.9	84.7	9.1	81.5	3.1
		5	75.2	13.5	65.0	10.8	58.5	8.5	75.5	7.5	84.5	5.0
	Carrot	0	-	-	-	-	-	-	-	-	-	-
		1	111	3.4	52.8	2.1	84.0	11.9	96.3	0.9	76.6	0.8
		5	94.6	3.2	40.4	2.9	57.5	1.2	65.5	3.4	47.2	1.7
	Lettuce	0	-	-	-	-	-	-	-	-	-	-
		1	107	5.1	40.9	2.3	67.6	12.1	45.1	2.0	65.8	7.6
		5	113	3.9	51.9	8.0	42.9	4.6	72.4	1.1	66.4	5.3

CONCLUSION

In this work, QuEChERS method of sample preparation was optimized using selected pharmaceutical compounds. Different clean-up sorbents were evaluated for their matrix effect

removal after extraction and a combination of d-SPE sorbent $MgSO_4$, PSA, C_{18} and DE were found to be selective clean-up sorbent for the targeted compounds. The addition and amount of diatomaceous earth and EDTA during the clean-up and extraction steps were also examined and the amount was optimized. Solvent type and composition, salt type and extraction time were also optimized and the optimum condition was obtained. The method was successfully applied to different vegetable samples collected from Addis Ababa, Ethiopia and Johannesburg, South Africa (carrot, cabbage and lettuce) and none of the target analytes were found in the sample investigated. The matrix effect study on vegetable samples collected was found very high that suppresses the signal during analysis.

ACKNOWLEDGEMENTS

The authors acknowledge the Organization for Women Scientists for Developing World (OWSD Postgraduate Fellowship) and SIDA (Swedish International Development Cooperation Agency) for funding this research and the University of Witwatersrand, Johannesburg, South Africa, for access to their laboratory.

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