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Scientific Research

Development of a magnetic ionic solvent based extraction method for determination of tetracycline, Oxytetracycline and Enrofloxacin residues in cheese sample by high performance liquid chromatography

Jalil Khandaghi[1,2], Mohammad Reza Afshar Mogaddam[3,4] And Samad Vajdi Hokmabad[5*]

1. Department of Food Science and Technology, Sarab Branch, Islamic Azad University, Sarab, Iran.
2. Department of food Biotechnology, Biotechnology Research Center, Tabriz Branch, Islamic Azad University, Tabriz, Iran.
3. Food and Drug Safety Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.
4. Pharmaceutical Analysis Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.
5. Department of Animal Science, Sarab Branch, Islamic Azad University, Sarab, Iran.

ABSTRACT

Antibiotics are used for treatment of infectious diseases in animals and also as growth promoters in animal husbandry. Therefore, especially in cases of excessive use, their residual amounts can be present in foods of animal origin such as dairy products. Since the consumption of food containing antibiotic residues has a negative effect on the human health, authorized organizations such as the European Union have set maximum residue limits (MRLs) for antibiotics in food of animal origin. This research aimed to present a deep eutectic solvent-based extraction procedure for preparation and preconcentration of tetracycline, oxytetracycline and enrofloxacin residues in cheese samples and their determination by high-performance liquid chromatography equipped with a diode array detector. For this purpose, the effect of various parameters on the extraction efficiency was investigated and optimized, and the effect of the cheese matrix on the extraction of analytes was also evaluated. The results showed that the residual amounts of oxytetracycline and tetracycline in 4 and 5 samples of the tested cheeses, respectively, were determined above the permissible limits. Enrofloxacin residue was not found in any of the samples. From the advantages of the proposed extraction method, we can point out the high separation power and the possibility of highly sensitive analyzing of mixed analytes, so that under optimized conditions, the recovery percentage ranges were 80-91 and the limits of detection and quantification were respectively less than 1.8 and 6 ng/g, which is much lower than MRLs set for target antibiotics in cheese (100 ng/g).

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*Corresponding Author E-Mail:
Samad.Vajdi@iausa.ac.ir

1. Introduction

The health and safety of milk and its products is of particular importance as one of the main food groups. Antibiotics are one of the common contaminants of milk, and different groups of them are used in the animal husbandry industry. Tetracyclines and fluoroquinolones are among the most common antibiotics [1]. Indiscriminate use of antibiotics in the livestock industry to control and treat infectious diseases or to stimulate animal growth leads to an increase in the residues of these substances in foods of animal origin, such as milk and their products, and eventually these residues find their way into the human body [2]. The adverse effects of antibiotic residues in food, such as causing allergic reactions, antibiotic resistance, toxicity, and carcinogenicity, have become an important concern today [3].

Therefore, the monitoring of antibiotic residues in milk seems necessary, and for this purpose, various methods including immunological, microbiological and analytical tests have been presented [4]. While microbiological methods remain the sameFPT [1]or immunological methods such asELISA [2] It has less sensitivity, the liquid chromatography method along with the use of different extraction techniques have the ability to identify all kinds of antibiotics with high accuracy and specificity [5].

In analytical methods, it is usually not possible to directly measure very small amounts of compounds, especially in samples with complex texture, and therefore it is necessary to use sample preparation steps and concentration of analytes in it before analyzing the sample with analytical systems [6]. Because milk has a complex matrix of fats, proteins, minerals and lactose, usually the methods used to extract and preconcentrate various compounds from milk are complex and affect all subsequent stages of analysis [7].

Liquid-liquid extraction[3] OrLLE It is one of the main methods used to prepare and extract different analytes from food [8]. Later, liquid-liquid diffusion microextraction method was introduced based on reducing the volume of the extracting solvent, automating and simplifying the extraction process, and it was widely used for many analytes in various foods [9, 10]. Nowadays, replacing chemical solvents with minimal environmental pollution such as deep tectic solvents with hazardous chemical solvents such as halogenated compounds is of great interest [11].

The aim of this study is to combine liquid-liquid extraction methods with diffusion liquid-liquid microextraction method based on magnetic ionic solvents in the extraction and preconcentration of tetracycline, oxytetracycline and enrofloxacin antibiotic residues from cheese samples and their measurement with high-performance liquid chromatography equipped with an identifier. diode array[4] Was.

2- Work method

2-1 Sampling and chemical substances

The present research tests were conducted on 30 randomly purchased cheese samples from the supply locations in Tabriz city and at the Food and Drug Safety Research Center of Tabriz University of Medical Sciences. Next, 50 grams of each The sample was crushed and homogenized, and after removing the whey, 2 grams of it was used for analysis. A cheese sample after ensuring that it is free from the studied antibiotics using an ELISA kit (BT combo test kit), was used as a blank sample in the optimization process.

[1]- Four plate test

[2]- Enzyme-linked immunosorbent assay

[3]- Liquid liquid extraction

[4]- High Performance Liquid Chromatography-diode array detector

The mother solution of tetracycline, oxytetracycline and enrofloxacin antibiotics (Sigma-Aldrich, USA) were prepared at a concentration of 50 mg/liter in methanol. Other chemicals used in this study from the companyMerck Germany and has been with analytical purity.

2-2 Optimizing extraction conditions

In order to achieve a high-efficiency extraction method, the effect of effective factors in the first stage of extraction, including the type of extracting solvent (from acetonitrile, methanol and acetone), the amount of selected solvent (from 1 to 2.5 ml), strength Ionic (by adding 15 to 30 percent by weight/volume of saltNaCl In volumes from 0.5 to 2 ml), sonication time (within 1 to 7 minutes) as well as the effective factors in the liquid-liquid diffusion microextraction stage including the type of magnetic deep tectic solvent (among the solvents 1-hexyl 3-methylimidazolium tetrachlorofurate, 1-butyl 3-methylimidazolium tetrachlorofurate and 1-butyl 3-methylimidazolium dibromodichlorofurate) and salting effect (using zero to 10% weight/volume concentrations ofNaCl) were investigated on the extraction efficiency. "One parameter at a time" method was used to check these parameters. The effect of these factors has been evaluated by comparing the sub-peak level obtained from the analytes in different conditions[6].

2-3 extraction methods

In this research work, the combination of liquid phase extraction method and dispersion liquid-liquid microextraction method based on deep magnetic tectic solvents has been developed for the extraction and preconcentration of tetracycline, oxytetracycline and enrofloxacin antibiotics from cheese samples. For this purpose, 2 grams of the prepared cheese sample was transferred into a test tube and one milliliter of sodium chloride solution (25% by weight/volume) and one milliliter of methanol were added to it. The resulting mixture was subjected to ultrasonic waves for 4 minutes and then this mixture was centrifuged and the upper phase (methanol containing studied antibiotics) was removed and mixed with 54 microliters of magnetic ionic solvent 1-hexyl-3 methylimidazolium tetrachlorofurate. The resulting mixture was spread with a glass syringe into sodium chloride solution (2.5% weight-volume) and the spread solvent was collected with a magnet and after separating the upper phase, for analysis withHPLC-DAD (Agilent1200- DAD system, USA) Was used.

2-4 analysis conditions with chromatography device

In order to achieve high efficiency in the chromatography system, antibiotics were separated in optimal conditions. The optimal conditions of the device are listed in Table 1.

Table 1: Optimized condition of the HPLC-DAD for analysis of the selected antibiotics in cheese samples

Column	Xbridge C18 column, L=5 cm, ID= 4.6 mm, Particle size= 3.5 µm
Elution type	Gradient elution
Mobile phase	A mixture of deionized water containing 0.2% formic acid (A) and methanol: ACN (30:70, v/v) (B) was utilized as the mobile phase at a flow rate of 0.2 mL min–1 with the following gradient elution: 80% A and 20% B, a linear gradient from 20 to 80% B from 3.0 to 6.0 min, a linear gradient from 20 to 80% A from 6.0 to 8.0 min, and with 80% A and 20% B for 2 min
Injector	Temperature: 40 °C, Loop= 50 µL
Detection wavelength	Monitoring of the analytes was done at 335 nm for tetracycline and oxytetracycline, and 280 nm for enrofloxacin

5-2 Analysis of Analytical Characteristics

In order to validate the method used to extract antibiotics in this research, after drawing the benchmarking chart[5], the linear range of the method[6] (LR), thresholding[7] (LOD), measurement limit[8] (LOQ), repeatability[9] (RSD%), extraction efficiency[10] (IS) and the concentration factor[11] (IF) were calculated (12).

2-6 Investigating the effect of cheese texture in extraction

Substances such as proteins and lipids in cheese can interfere in the extraction of studied antibiotics, therefore, in order to investigate the effect of tissue[12] In cheese samples, two grams of real samples with concentrations of 10 and 25 ng/ml for each of the studied antibiotics were contaminated and extracted under the suggested conditions and withHPLC was analyzed. Then, after implementing the proposed method, the values of the analytes were calculated using the calibration curve, and the concentration values found were divided by the initial values of the analytes, and the result was reported as the percentage of relative extraction efficiency [13].

3. Results and Discussion

3-1 Results of optimization of effective factors in the first stage of extraction

3-1-1 Type and volume of extracting solvent

An ideal extracting solvent should have the characteristics of low solubility in water to form a two-phase system with water, favorable chromatographic behavior, compatibility with the environment, and even be as cheap as possible. Considering these properties, three solvents acetonitrile, methanol and acetone were studied in this work [14].

The volume of the extracting solvent plays an important role in the efficiency of the extraction methods, so that its increase will increase the volume of the collected phase after extraction, and the concentration factor and thus the analytical signals will also decrease. On the other hand, reducing the volume of the solvent reduces the extraction efficiency and reproducibility, so it is

[5]- Calibration curve
[6]- Linear range
[7]- Limit of detection
[8]- Limit of quantitation
[9]- Relative standard deviation
[10]- Extraction recovery
[11]- Enrichment factor
[12]- Matrix

necessary to optimize the volume of extracting solvents [15].

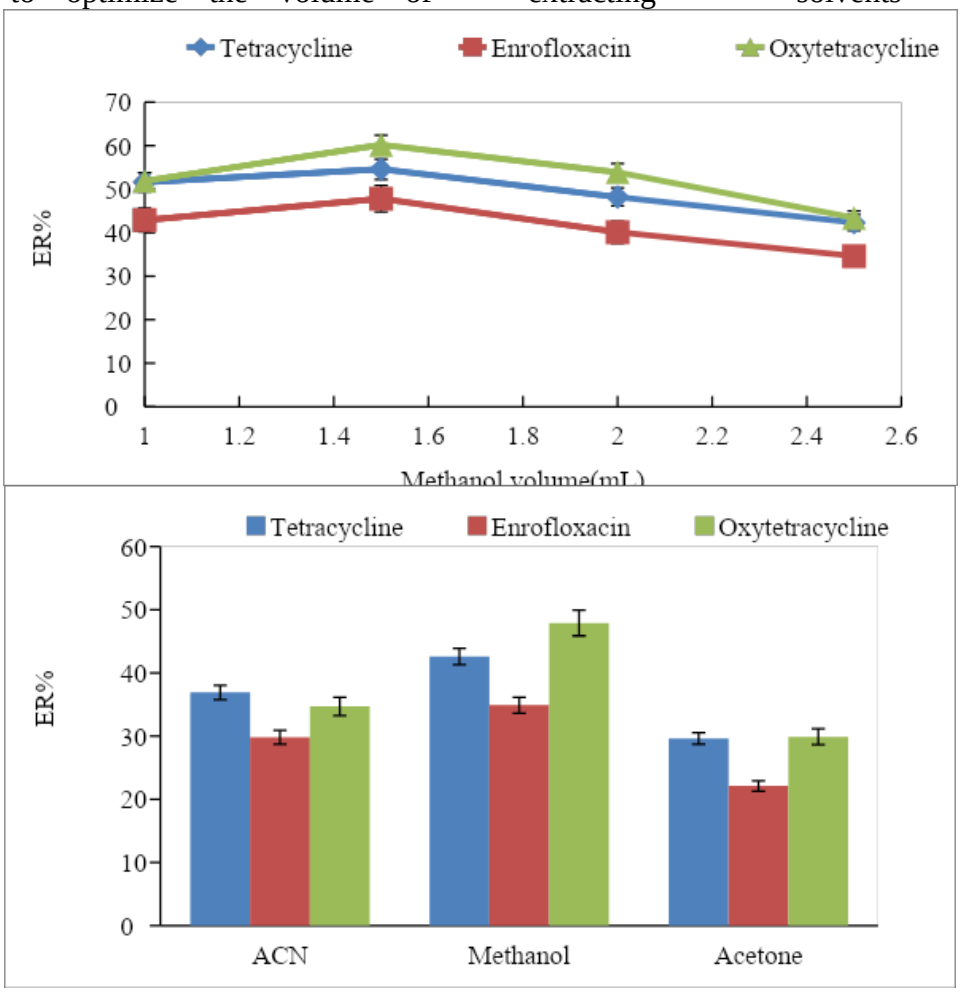

Fig1: Selection of extraction solvent type and volume in LLE step.

3-1-2 Ionic strength

Investigating the effect of ionic strength on the efficiency of the developed method was done by adding different amounts of sodium chloride salt into the aqueous phase. Adding salt to aqueous solutions in extraction methods has a double effect, that is, while the presence of salt can increase the ionic strength and decrease the solubility of analytes in the aqueous phase and thus increase the extraction efficiency, on the other hand, it can increase the viscosity of the aqueous phase and decrease the diffusion coefficient. analyte in the aqueous phase and lead to a decrease in extraction efficiency [16].

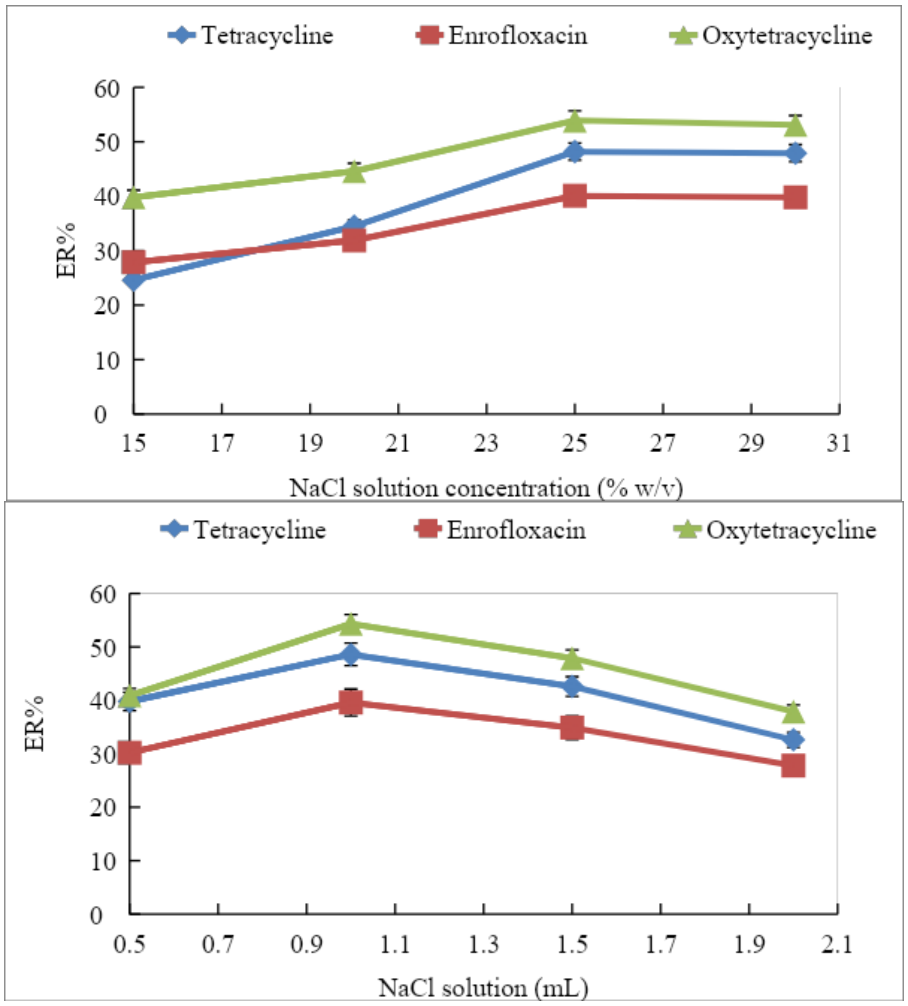

Fig2: Optimization of salt addition effect in LLE step.

3-1-3 Duration of sonication

In the current method, ultrasonic waves are used to increase the complete dissolution and increase the contact surface of the investigated analytes with the extracting solvent. Therefore, the duration of ultrasonic waves will be effective in the efficiency of the proposed method.

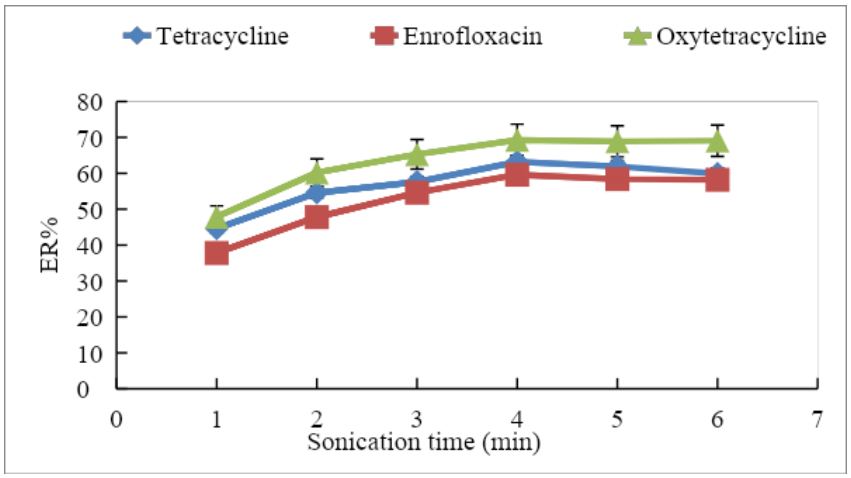

Fig3: Optimization of sonication time.

3-2 Results of optimization of effective factors in the second stage of extraction

1-3-2 The type and volume of deep tectic magnetic extracting solvent

Choosing the type of extracting solvent in the stepDLLME It is one of the important parameters. The extracting solvent must be able to disperse in the aqueous phase in order to extract and absorb the analyte well. They are also dissolved in the organic phase and can be injected into the HPLC system [17]. In addition, it should be possible to collect these solvents in some way [11]. In this research work, magnetic ionic solvents (MILs)[13] based on deep tectic solvents (DES)[14] It was chosen for this purpose because it is less toxic than other solvents and has a special application in green chemistry[15] It has [18] and it is possible to separate the solvent and the analyte dissolved in it by a magnet at the end of the extraction process easily [11].

The volume of the solvent is also an effective parameter in the proposed method, because with the increase in volume, the analytes are diluted and the concentration factor decreases [10], therefore, the volume of the solvent should be optimized. In this study, the minimum volume of tectic solvent that can be injected into the chromatographic system at the end of the DLLME process is 10 microliters (i.e. 54 microliters).

[13]- Magnetic ionic liquid solvent
[14]- Deep eutectic solvent
[15]- Green chemistry

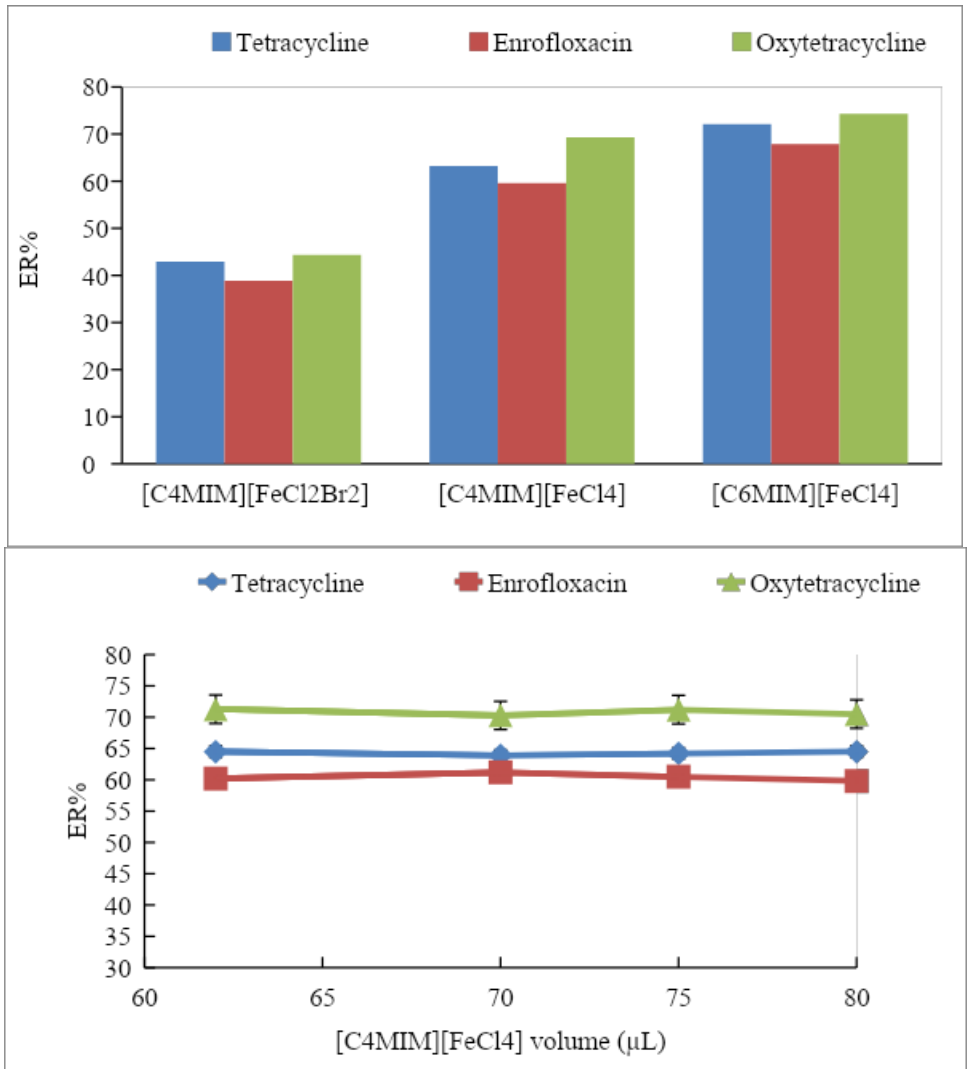

Fig4: Selection of elution solvent type and volume in DLLME step.

2-3-2 Effect of salting

As explained in paragraph 3-1-2, adding salt to aqueous solutions has two mutual effects, and each of the effects is superior to the other, in this case, the effect of that factor will be dominant. At this stage of the experiment, the extraction efficiency increasesNaCl until the It increased by 2.5% and then remained constant, so in the continuation of the work, the addition of 2.5% sodium chloride was used for higher extraction.

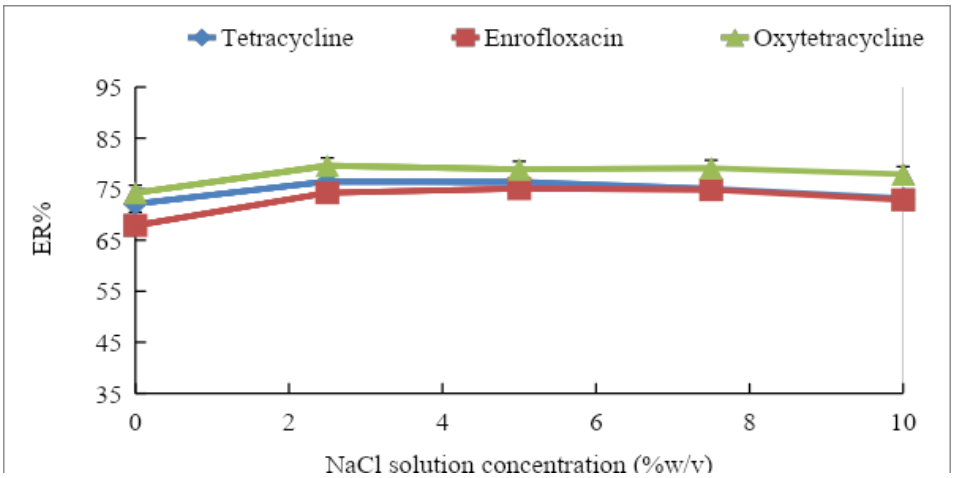

Fig5: Selection of ionic strength effect in DLLME step.

2-3 Results of the quantitative characteristics of the developed method

The analytical parameters of the present method were obtained under optimal conditions and a regression equation was drawn for the data (using standard solutions with different concentrations of the studied antibiotics). The linear range (for each analyte) was obtained from the calibration curve. The detection limit and measurement limit of the method were considered for concentrations where the signal-to-noise ratio is equal to 3 and 10, respectively. Reproducibility and accuracy of the method by calculating the relative standard deviationRSD %) was determined. The concentration factor was calculated by comparing the area under the peak of the standard solution analytes and the analytes in the milk after implementing the proposed method. The results of the merit figures of the method are presented in Table 2.

Table 2: Quantitative features of the developed method for the selected antibiotics

Analyte	LR[a]	LOD[b]	LOQ[c]	r^{2d}	RSD%[I] t is	IS ± SD[f]	EF ± SD[g]	MRLs[h]
Tetracycline	6.0-500	1.8	6.0	0.996	4.9	72 ± 5	72 ± 5	100
Enrofloxacin	4.0-500	1.2	4.0	0.995	5.9	76 ± 4	76 ± 4	100
Oxytetracycline	2.3-500	0.7	2.3	0.999	8.3	79 ± 2	79 ± 2	100

a) Linear range (of g^{-1}), b) Limit of detection (S/N = 3) (ng g^{-1}), c) Limit of quantification (S/N = 10) (ng g^{-1}), d) Coefficient of determination, e) Relative standard deviation (n = 6), f) Extraction recovery ± standard deviation (n = 3), g) Enrichment factor ± standard deviation (n = 3), h) Maximum residue limits set by the European Committee

Table 3 shows the comparison of some analytical parameters of the developed method, such as detection limit range, measurement limit, repeatability and efficiency of analyte extraction, with several methods used to extract tetracyclines from dairy samples. As can be seen, the figures of merit of the current method are comparable or even better than the mentioned methods.

Table 3: Comparison of the developed method with other methods in the extraction of drug residues in dairy products by HPLC.

Method	Sample	LOD[a]	LOQ[b]	RSD[c]	IS[d]	Reference

		(of Mr^{-1})	(of Mr^{-1})	(%)	(%)	
A	Cheese	10-200	50-600	≤7	≥69	[19]
B	Milk	1.5-8.5	5.1-28.4	≤8.4	≥70	[20]
C	Milk	4.53-9.06	-	≤10.12	≥51.3	[21]
D	Milk	17.2-21	51.5-63.7	≤6.2	≥86	[22]
AND	Cheese	07-1.8	2.3-6.0	≤8.3	≥72	This study

A) QuEChERS method coupled with HPLC-DAD, B) DLLME based on hydrophobic deep eutectic solvents coupled with HPLC-UV, C) Ionic Liquid Assisted DLLME coupled with HPLC-DAD, D) Liquid extraction method coupled with HPLC-DAD, E) Deep eutectic solvent-based DLLME method coupled with HPLC-DAD.
a) Limit of detection. b) Limit of quantification. c) Relative standard deviation. d) Extraction recovery.

3-3 Analysis results of real samples

30 cheese samples were concentrated and purified according to the optimal conditions with the proposed method and then withHPLC-DAD were analyzed. Figure 3 shows the chromatograms related to the standard direct injection of the studied antibiotics (each with a concentration of 10 mg/kg), a spiked milk sample with a concentration of 50 ng/g, and an unspiked milk sample.

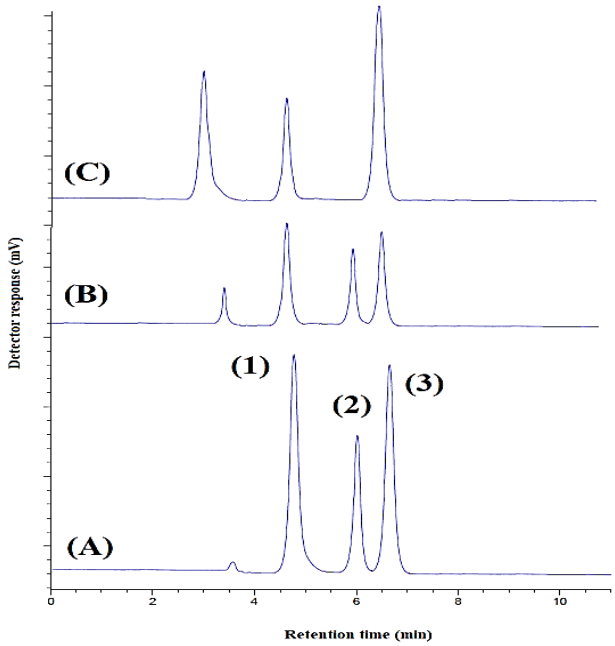

Fig 3: Typical HPLC–DAD chromatograms of direct injection of standard solution of the target analytes (A), spicked milk (B) and unspiked milk sample (C) after performing the developed method. Peak identification: (1) Tetracycline, (2) Enrofloxacin, (3) Oxytetracycline.

Comparing the chromatograms obtained from the samples with the chromatograms obtained from the standard sample showed the presence of some antibiotics, which are mentioned in Table 3. In order to measure the concentration of analytes, the standard increase method was used.

Table 3: Residual amounts of target antibiotics in cheese samples

sample	OXT	ENR	TET	sample	OXT	ENRO	TET

		O					
Cheese # 1	ND	ND	13 ± 0.5	Cheese # 16	119 ± 4	ND	15 ± 0.6
Cheese # 2	ND	ND	7 ± 0.13	Cheese # 17	ND	ND	ND
Cheese # 3	126 ± 5	ND	45 ± 2	Cheese # 18	ND	ND	ND
Cheese # 4	ND	ND	116 ± 11	Cheese # 19	ND	ND	ND
Cheese # 5	ND	ND	ND	Cheese # 20	ND	ND	17 ± 0.3
Cheese # 6	ND	ND	ND	Cheese # 21	ND	ND	55 ± 1
Cheese # 7	ND	ND	ND	Cheese # 22	116 ± 5	ND	116 ± 13
Cheese # 8	117 ± 12	ND	59 ± 3	Cheese # 23	97 ± 8	ND	52 ± 3
Cheese # 9	ND	ND	21 ± 0.5	Cheese # 24	ND	ND	31 ± 0.4
Cheese # 10	ND	ND	128 ± 2	Cheese # 25	ND	ND	118 ± 5
Cheese # 11	ND	ND	26 ± 0.1	Cheese # 26	ND	ND	26 ± 0.3
Cheese # 12	ND	ND	11 ± 0.09	Cheese # 27	ND	ND	ND
Cheese # 13	ND	ND	119 ± 4	Cheese # 28	ND	ND	99 ± 5
Cheese # 14	ND	ND	26 ± 0.3	Cheese # 29	ND	ND	46 ± 0.7
Cheese # 15	ND	ND	ND	Cheese # 30	ND	ND	ND

OXT: Oxytetracycline, ENRO: Enrofloxacin, TET: Tetracycline, ND: not detected.

4-3 Results of investigating the texture effect of cheese samples

The effect of texture in the extraction of antibiotics was evaluated by comparing the analysis signals obtained for six samples of cheese and deionized water. The results showed that the texture of the samples did not have a noticeable effect on the efficiency of the proposed method.

Table 4: Results of assays to check the cheese matrices effect for the selected antibiotics.

Analyte	Mean relative recovery ± standard deviation (n = 3)					
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
	All samples were spiked with each analyte at a concentration of 10 ng g^{-1}					
Tetracycline	94 ± 3	90 ± 5	96 ± 2	90 ± 1	98 ± 2	95 ± 4
Enrofloxacin	96 ± 5	95 ± 4	92 ± 2	91 ± 4	92 ± 5	89 ± 4
Oxytetracycline	93 ± 4	94 ± 3	92 ± 5	91 ± 2	99 ± 1	94 ± 4
	All samples were spiked with each analyte at a concentration of 25 ng g^{-1}					
Tetracycline	89 ± 4	95 ± 5	91 ± 4	90 ± 4	88 ± 2	87 ± 5
Enrofloxacin	92 ± 4	94 ± 3	95 ± 2	91 ± 2	99 ± 5	99 ± 1
Oxytetracycline	95 ± 2	94 ± 5	101 ± 3	99 ± 5	90 ± 4	97 ± 5

3-5 general conclusions

In the implementation of the proposed method in the present study, the combination of liquid phase extraction and liquid-liquid diffusion microextraction based on deep magnetic tectic solvents was used to extract the remaining antibiotics of tetracycline, oxytetracycline and enrofloxacin from cheese samples. Among the advantages of the developed method, we can mention the simplicity and short analysis time and the greenness of the method. Desirable quantitative features of the presented method Such as extraction efficiencies of more than 72% and detection limits in the range of 0.7-1.8 ng/ml and ideal reproducibility, it emphasizes the appropriate efficiency of the present method in the extraction and analysis of target antibiotics in cheese samples. In addition, the mentioned method was successfully performed on the studied antibiotics in cheese samples, and the analysis of real

samples showed that the residual amounts of oxytetracycline and tetracycline were determined above the permissible limits in 4 and 5 samples of the tested cheeses, respectively. Enrofloxacin residue was not found in any of the samples.

4- Conflict of benefits

The authors have no conflict of interest.

5- References

[1] Landers, T.F., Cohen, B., Wittum, T.E. and Larson, E.L. 2012. A review of antibiotic use in food animals: perspective, policy, and potential. Public health reports,127(1), 4-22.

[2] Rasi, H., AfsharMogaddam, M. and Khandaghi, J. 2021. Application of a new extraction method coupled to high performance liquid chromatography for tetracyclines monitoring in cow milk. Journal of food science and technology (Iran), 8(113), 339-349. [In Persian]

[3] Bacanlı, M. and Başaran, N. 2019. Importance of antibiotic residues in animal food. Food and Chemical Toxicology, 125:462-466.

[4] Zarean Baniasadi, F., Ahmadi, M., Rokni, N., Golestan, L. and Shahidi, S.A. 2019. Evaluation of four common antibiotic classes in the muscle and liver of chickens slaughtered tehran by LC-MS/MS. Veterinary Researches & Biological Products, 32(3):55-63.

[5] Ghasemi, R., Mirzaei, H., Afshar Mogaddam, M.R., Khandaghi, J. and Javadi, A. 2022. Application of magnetic ionic liquid-based air–assisted liquid–liquid microextraction followed by back-extraction optimized with centroid composite design for the extraction of antibiotics from milk samples prior to their determination by HPLC–DAD. Microchemical Journal, 181:107764.

[6] Mitra, S. and Brukh, R. 2003. Sample Preparation: An Analytical Perspective. In: Winefordner, J.D. Sample Preparation Techniques in Analytical Chemistry. Volume 162, 1st edition, John Wiley & Sons, Inc., Hoboken. pp. 100-1 12-3

[7] Mohebi, A., Samadi, M., Tavakoli, H.R. and Parastouei, K. 2020. Homogenous liquid–liquid extraction followed by dispersive liquid–liquid microextraction for the extraction of some antibiotics from milk samples before their determination by HPLC. Microchemical Journal, 157:104988.

[8] Amini, R., Khandaghi, J. and Afshar Mogaddam, M.R. 2018. Combination of vortex-assisted liquid–liquid extraction and air-assisted liquid–liquid microextraction for the extraction of bisphenol A and bisphenol B in canned doogh samples. Food Analytical Methods, 11(11):3267-3275.

[9] Meshkini, K., AfsharMogaddam, M. and Khandaghi, J. 2021. Development of Homogeneous Liquid-Liquid Extraction in Combination with Dispersive Liquid-Liquid Microextraction Based on Deep Eutectic Solvents for the Extraction and Assessment of Phytosterols in Animal Cream Samples using Gas Chromatography Equipped with Flame Ionization Detector. Iranian Journal of Nutrition Sciences & Food Technology, 16(2):57-67. [In Persian]

[10] Rastpour, N., Khandaghi, J., Farajzadeh, M.A. and Afshar Moghaddam, M.A. 2022.Deep eutectic solvent-based QuEChERS method combined with dispersive liquid–liquid microextraction for extraction of benzoylurea insecticides in cabbage leaves samples. International Journal of Environmental Analytical Chemistry,102(12):2778-2791.

[11] Gholizadeh, S., Mirzaei, H., Khandaghi, J., Afshar Mogaddam, M.R. and Javadi, A. 2022. Ultrasound–assisted solvent extraction combined with magnetic ionic liquid based-dispersive liquid–liquid microextraction for the extraction of mycotoxins from tea samples. Journal of Food Composition and Analysis, 114:104831.

[12] Chandran, S. and Singh, R. 2007. Comparison of various international guidelines for analytical method validation. International Journal of Pharmaceutical Sciences, 62, 4-14.

[13] https://www.idbs.com/blog/2019/07/ich-m10-bioanalytical-method-validation-guideline-

what-the-new-draft-means-for-bioanalysis. Access date: 11/24/2022.

[14] Jalili, V., Barkhordari, A. and Ghiasvand, A. 2020. New extraction media in microextraction techniques. A review of reviews. Microchemical Journal, 153:104386.

[15] Jeddy, M. and Khandaghi, J. 2019. Detection and quantification of phytosterols in yogurt using gas chromatography. Journal of food hygiene, 9, 59-71. [In Persian]

[16] Farajzadeh, M.A., Afshar Mogaddam, M.R. and Alizadeh Nabil A.A. 2015. Polyol-enhanced dispersive liquid–liquid microextraction coupled with gas chromatography and nitrogen phosphorous detection for the determination of organophosphorus pesticides from aqueous samples, fruit juices, and vegetables. Journal of separation science, 38(23):4086-4094.

[17] Khani, F., Khandaghi, J., Farajzadeh, M.A. and Afshar Mogaddam, M.R. 2021. Cold-induced Homogenous Liquid–Liquid Extraction Performed in a Refrigerated Centrifuge Combined with Deep Eutectic Solvent-based Dispersive Liquid–Liquid Microextraction for the Extraction of Some Endocrine Disrupting Compounds and Hydroxymethylfurfural from Honey Samples. Food Analytical Methods, 14(10):2063-2075.

[18] Santos, L.B., Assis, R.S., Barreto, J.A., Bezerra, M.A., Novaes, C.G. and Lemos, V.A. 2022. Deep eutectic solvents in liquid-phase microextraction: Contribution to green chemistry. TrAC Trends in Analytical Chemistry, 146:116478.

[19] Amelin, V., Volkova, N., Timofeev, A. and Tret'yakov, A. 2015. QuEChERS sample preparation in the simultaneous determination of residual amounts of quinolones, sulfanilamides, and amphenicols in food using HPLC with a diode-array detector. Journal of analytical chemistry, 70(9):1076-84.

[20] Sereshti, H., Semnani Jazani, S., Nouri, N. and Shams, G. 2020. Dispersive liquid–liquid microextraction based on hydrophobic deep eutectic solvents: Application for tetracyclines monitoring in milk. Microchemical Journal, 158, 105269.

[21] Antep, M., Kaynaker, M. and Merdivan, M. 2018. Determination of Tetracycline Residues After Ionic Liquid Assisted Dispersive Liquid Liquid Microextraction in Dairy Foods. Journal of Science and Engineering, 20(59):645-662.

[22] Moudgil, P., Bedi, J.S., Aulakh, R.S., Gill, J.P.S. and Kumar, A. 2019. Validation of HPLC multi-residue method for determination of fluoroquinolones, tetracycline, sulphonamides and chloramphenicol residues in bovine milk. Food Analytical Methods,12(2):338-346.

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توسعه یک روش استخراج مبتنی بر حلال‌های یونی مغناطیسی برای اندازه‌گیری باقیمانده آنتی‌بیوتیک‌های تتراسایکلین، اکسی تتراسایکلین و انروفلوکساسین در نمونه پنیر به‌وسیله کروماتوگرافی مایع با کارایی بالا

جلیل خندقی[۱و۲]، محمدرضا افشار مقدم[۳و۴] ، صمد وجدی حکم‌آباد [۵*]

۱.گروه علوم و صنایع غذایی، واحد سراب، دانشگاه آزاد اسلامی، سراب، ایران.

۲.گروه بیوتکنولوژی مواد غذایی، مرکز تحقیقات بیوتکنولوژی، واحد تبریز، دانشگاه آزاد اسلامی، تبریز، ایران.

۳.مرکز ایمنی غذا و دارو، دانشگاه علوم پزشکی تبریز، تبریز، ایران.

۴.مرکز آنالیز دارویی، دانشگاه علوم پزشکی تبریز، تبریز، ایران.

۵. گروه علوم دامی، واحد سراب، دانشگاه آزاد اسلامی، سراب، ایران.

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* مسئول مکاتبات:

Samad.Vajdi@iausa.ac.ir

چکیده

آنتی بیوتیک ها برای درمان بیماری‌های عفونی در حیوانات و همچنین به عنوان محرک‌های رشد در پرورش دام استفاده می‌شوند. بنابراین بخصوص در موارد استفاده بیش از حد از آنها، مقادیر باقیمانده آنها می‌تواند در مواد غذایی با منشاء حیوانی مانند محصولات لبنی وجود داشته باشد. از آنجائیکه مصرف مواد غذایی حاوی باقیمانده آنتی بیوتیک بر سلامتی انسان تأثیر منفی می‌گذارد لذا سازمان‌های نظارتی مانند اتحادیه اروپا حداکثر مجاز باقیمانده (MRLs) برای آنتی‌بیوتیک‌ها در مواد غذایی با منشاء دامی تعیین کرده‌اند. هدف این تحقیق ارائه یک روش میکرواستخراج مبتنی بر حلال‌های یونی مغناطیسی برای پیش تغلیظ و آماده‌سازی باقیمانده آنتی‌بیوتیک‌های تتراسایکلین، اکسی تتراسایکلین و انروفلوکساسین در پنیر و اندازه‌گیری آن‌ها به‌وسیله کروماتوگرافی مایع با کارایی بالا مجهز به شناساگر آرایه دیودی بوده است. برای این منظور اثر پارامترهای مختلف موثر در کارایی استخراج بررسی و بهینه‌سازی شده و همچنین اثر ماتریکس پنیر در استخراج آنالیت‌ها مورد ارزیابی قرار گرفت. نتایج تحقیق نشان داد که مقادیر باقیمانده اکسی‌تتراسایکلین و تتراسایکلین به ترتیب در ۴ و ۵ نمونه از پنیرهای آزمایش شده بیش از حدمجاز تعیین شده بود. باقیمانده انروفلوکساسین نیز در هیچ‌یک از نمونه‌ها یافت نشد. از مزایای روش استخراج پیشنهادی می‌توان به قدرت جداسازی بالا و امکان آنالیز مخلوط آنالیت ها با حساسیت بالا اشاره کرد به‌طوریکه تحت شرایط بهینه، درصد بازیافت آنالیت‌ها ۹۱-۸۰ و حدود تشخیص و اندازه‌گیری به ترتیب کمتر از ۱/۸ و ۶ نانوگرم در گرم و بسیار کمتر از حد مجاز تعیین شده برای آنتی‌بیوتیک‌های هدف در پنیر (۱۰۰ نانوگرم در گرم) بود.