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A Green Stir Bar Sorptive Extraction of some Antiparasitic Drugs from Milk Samples and Prior to their Analysis by HPLC

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A facile and efficient stir bar sorptive extraction method was developed for the extraction of four antiparasitic drugs including eprinomectin, doramectin, ivermectin, and abamectin from cow milk samples. The extracted analytes were determined by a high-performance liquid chromatography-diode array detector. In this work, firstly, the milk sample was deproteinized and the obtained clear solution was extracted by a stir bar coated with octadecylsilane (as the sorbent). After extraction, the adsorbed analytes onto the sorbent surface were eluted by a proper volume of 1-butyl-3-methylimidazolium tetrafluoroborate as an ionic liquid. All of the effective parameters including sorbent type and amount, elution solvent type and volume, stirring time, deproteinization agent solution volume, and concentration were investigated by a design of an expert using response surface methodology. Under optimal conditions, acceptable extraction recoveries (68 to 83%) and enrichment factors (72 to 88), low limits of determination (0.05 to 0.12 ng ml^{-1}), and quantification (0.17-0.41 ng ml^{-1}) were obtained. The method precision was evaluated by analyzing the spiked samples using the introduced method on a day and different days. The obtained relative standard deviations were in the ranges of 2.3-4.1 and 2.8-4.9%, for intra- and inter-days repeatability, respectively. At last, the presence of studied drugs in milk samples was followed and ivermectin was found in some samples. The literature review verified that the method was not performed previously and can be utilized as a routine method for analyzing antiparasitic drugs.

Keywords: Extraction, Milk, High performance liquid chromatography, Antiparasitic drugs, Ionic liquid

INTRODUCTION

Different classes of drugs are broadly applied during animal husbandry to protect the animals from diverse diseases or enhance the growth of livestock [1]. The drugs are mainly administrated orally or by injection [2]. Macrocyclic drugs are used during parasitic disease treatment [3]. Avermectins, related to macrocyclic drugs, are famous compounds used in the treatment of ectoparasites, helminths, and protozoa [4,5]. These types of drugs are majorly injected into the livestock subcutaneously and proper use of the drugs like overuse leads to their residue in animal-based foods [6]. The residue of avermectin drugs in food products is the most important concern in the world because of their side effects on the health of consumers [7]. Several studies have confirmed that avermectins can play a major role in the creation of carcinogenic or teratogenic effects over a long time [8]. Due to this, a series of rules were presented by

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different commissions to control animal-based foods by avermectin drugs [9,10] and the determination of these drugs in food samples has attracted more attention.

Milk is one of the most important animal-based foods which is extensively used by humans because of its high nutritional value [11,12]. Consumption of milk and dairy products has defensive effects against stroke, diabetes, bladder cancers, and dementia due to the presence of different vitamins, proteins, ions, and fatty acids in their composition [13,14]. Due to these benefits, the safety of milk is a significant issue in the world. Thus, controlling the residue of different drugs in milk like antibiotics [11] and veterinary drugs [15] is advised by various committees. Investigation of avermectin drug residues in different samples was reported by various analytical instruments including high-performance liquid chromatography (HPLC)-fluorescence detection [16,17], enzyme-linked immunosorbent assay [16], HPLC-ultraviolet detector [18,19], HPLC-diode array detector (DAD) [20], and liquid chromatography-tandem mass spectrometry [21]. The presence of different compounds in real samples restricts the direct usage of analytical techniques in the determination of different compounds. Thus, the interfering compounds should be separated from the sample matrix by performing a sample preparation method [21].

Stir bar sorptive extraction (SBSE) is a familiar and effective sample preparation method that was introduced in 1999 by Baltussen and co-workers [22]. In SBSE, a thin layer of an adsorbent is coated on the surface of a magnetic bar and it is directly introduced into the sample solution. Then, the bar is stirred and during this step, the analytes are adsorbed onto the sorbent. After that, the analytes are desorbed thermally or eluted by a proper solvent [23,24]. Solvent elution of the analytes can be done by different solvents but the use of green solvents like ionic liquids (ILs) is preferred due to their low toxicity against human health and environment and easy handling [25,26].

The main goal of the present work was the development of an SBSE approach for the extraction and preconcentration of four avermectin drugs from milk samples before their analysis by HPLC-DAD. In the suggested method, a sorbent was coated on a stir bar and it was used as the extractive phase. In the SBSE step there was no need for centrifugation and the extraction rate of the analytes was enhanced by stirring the bar. After extraction, the analytes were eluted by a water-immiscible IL to provide efficient desorption and green analysis. Response surface methodology was employed for the optimization of the effective parameters. This approach shortens the optimization time and considers the interactions between the variables. Our literature review confirmed that the method was not performed previously and can be used as a facile approach during the analysis of the studied analytes.

EXPERIMENTAL

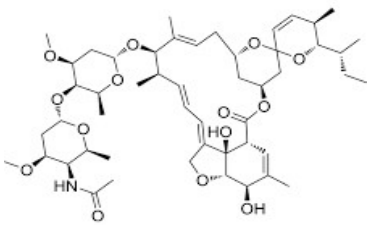
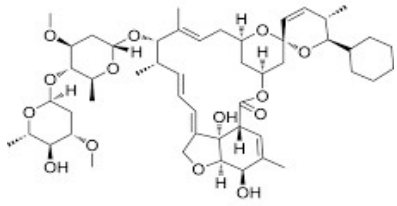
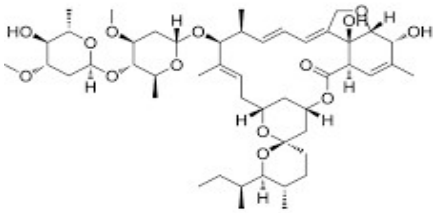
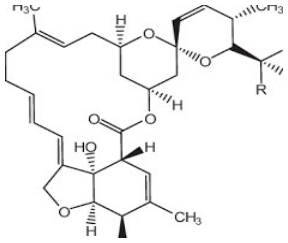
Chemical Materials

The studied drugs' standards (eprinomectin, doramectin, ivermectin, and abamectin) were bought from Sigma-Aldrich (St. Louis, MO, USA). The utilized sorbents including (primary secondary amine (PSA), octadecylsilane (ODS), and graphitized carbon black (GCB)) were bought from Merck Millipore (Darmstadt, Germany). Three ILs used for elution of the analytes from adsorbent surface consist of 1-butyl-3-methylimidazolium tetrafluoroborate ([BMIM][BF_4]), 1-hexyl-3-methyl-imidazolium-tetrafluoroborate ([HMIM][BF_4]), and 1-ethyl-3-methylimidazolium acetate ([EMIM][OAc]) were also purchased from Sigma-Aldrich. The other chemical compounds including HPLC-grade acetonitrile, methanol, water, zinc sulfate, tetrahydrofuran (THF), and sodium chloride were purchased from Merck. A stock solution (100 mg l^{-1} of each analyte) was prepared from the drugs by taking each of them at an appropriate amount and dissolving them in acetonitrile. A proper volume of stock solution was taken and added into blank milk to obtain the solutions used in optimization and validation steps. The physicochemical properties of the studied analytes are mentioned in Table 1.

Apparatus

An HPLC equipped with quaternary pumps and DAD were used for monitoring the target compounds. The HPLC system model was Agilent 1200 (Agilent Technologies, Santa Clara, CA, USA). The drugs were separated by a ZORBAX 300Å Extend-C18 column (Agilent, 150 mm × 4.6 mm). The column particle size was 3.5 µm and it was placed in an oven adjusted at 30 °C. A mixture of water: acetonitrile: and methanol (20:55:25, *v/v/v*) was used in an

Table 1. Physicochemical Properties of Studied Analytes

Analyte	Structure	Molecular weight (g mol^{-1})	LogP	pK_a	Solubility in water at 25 ºC
Eprinomectin		914.1	5.1	12.49	0.004 g l^{-1}
Doramectin		899.1	6.27	12.47	0.025 mg l^{-1}
Ivermectin		875.1	5.83	12.47	4 mg l^{-1}
Abamectin		1732.1	4.4	-	1.2

isocratic elution at a flow rate of 1.2 ml min^{-1} as the mobile phase. The DAD monitored eprinomectin and ivermectin at 245 nm, abamectin at 255 nm, and doramectin at 270 nm. All injections were done utilizing a 20-µl sample loop.

Real Samples

Thirty pasteurized and thirty raw milk samples were bought from local stores in Tabriz City (East Azerbaijan Province, Iran). The pasteurized samples were packed in tetra-pack boxes or polyethylene bottles. The raw samples were collected into glass bottles. One milk sample was obtained from a not medicated cow (Sarab, East Azarbaijan, Iran) to get drug-free milk (blank milk) that was used in optimization and validation steps.

Preparation of Coated Stir Bar

The coated stir bar was prepared according to a previously published method [27]. In brief, 5 ml polyvinyl chloride solution in THF at a concentration of 0.05%, *w/v*, was transferred into a beaker, and 30 mg ODS was added. Then a cylindrical polytetrafluoroethylene stir bar (5 mm × 2 mm) was directly introduced into the mixture. The mixture

was stirred at 150 rpm and after evaporation of THF, a thin layer of sorbent was coated on the stir bar. The coated bar was washed with methanol and de-ionized water and used as a sorbent.

Optimization Steps

Selection of sorbent type and amount. In order to investigate the sorbent type, a series of experiments were done by coating the bar with three various sorbents including PSA, ODS, and GCB. In all experiments, the amount of sorbent was the same.

In the following, the amount of selected sorbent amount was changed in the range of 10-60 mg while the other experiments were the same.

Selection of elution solvent type. For the investigation of elution solvent type, various experiments were done using three ILs consisting of [BMIM][BF_4], [HMIM][BF_4], and [EMIM][OAc]. The ILs were separately used for the elution of the analytes.

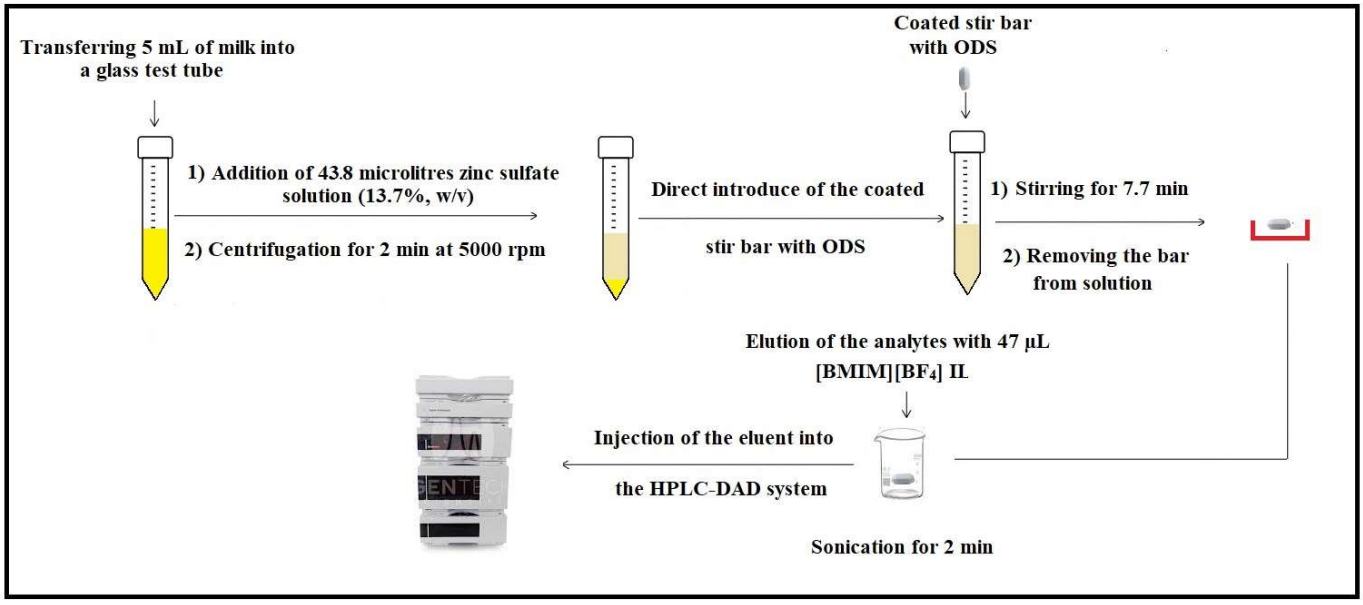
Extraction Procedure

A 5 ml blank milk spiked with the analytes (25 ng ml^{-1}, each drug) or real sample was transferred into a 10-ml glass test tube and 438 µl of zinc sulfate solution (13.7%, w/v) was added into the solution and shaken manually for 1 min. Then, added into the solution and shaken manually for 1 min. Then, the mixture was centrifuged for 2 min at 5000 rpm and the upper phase was taken and transferred into a glass beaker. After that, the coated stir bar with ODS was directly introduced into the solution and stirred for 7.7 min. By this action, the analytes were adsorbed onto the sorbent particles *via* interactions such as surface adsorption, occlusion, van der Waals, and π-π interactions. Then, the solution was placed on an external magnet, and the supernatant phase was removed. Then, the bar was placed into a microtube and adsorbed analytes were desorbed using 47 µl of [BMIM][BF_4] with the aid of sonication for 2 min. The elution solvent was removed while the bar was kept with the external magnet and 20 µl of the eluent was injected into the HPLC system. The method steps are shown in Scheme 1.

RESULTS AND DISCUSSION

Optimization of Sorbent Type and Amount

Selecting a proper sorbent for coating the stir bar to adsorb the analytes is a great subject for the establishment of an effective SBSE procedure. The used sorbent should have an acceptable capability for extraction of the analytes and good stability on the stir bar surface. In this study, the extraction ability of three commercial sorbents including



Scheme 1. The developed method steps

ODS, PSA, and GCB was tested for the extraction of the analytes from the sample solution by their coating on the stir bar, independently. In all experiments, 40 mg of each sorbent was coated on the stir bar and used in the extraction procedure. The obtained results (Fig. 1a) show the better efficiency of ODS compared to other sorbents in the extraction of the analytes. It can be related to the effective interaction of the analytes with the octadecyl group of ODS *via* non-polar interactions [28]. Thus, ODS was used in all other steps for the extraction of the analytes.

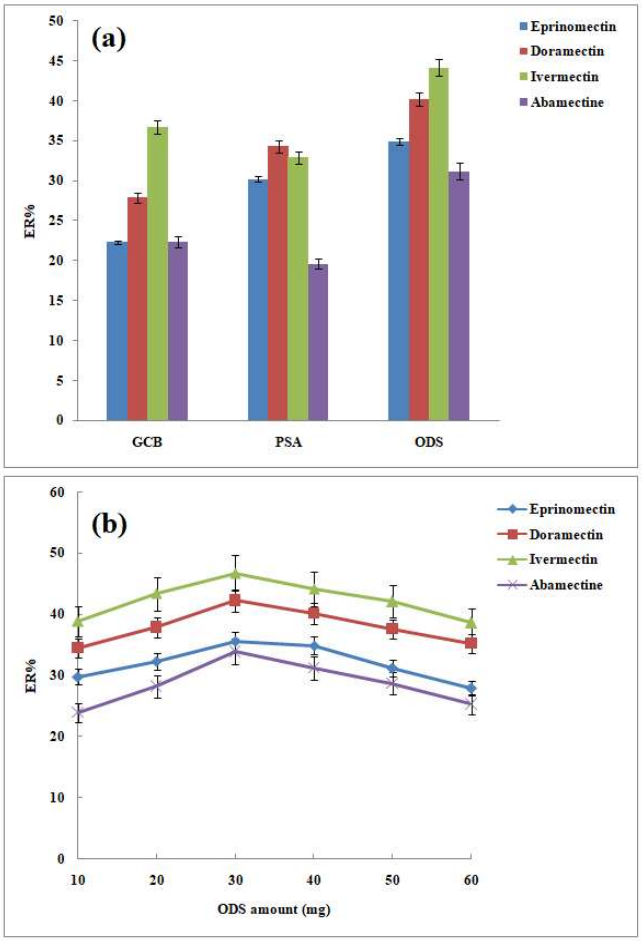


Fig. 1. Optimization of sorbent type (a) and amount (b). (a) Extraction conditions: Sample, 5 ml blank milk spiked with 25 ng ml^{-1} of each drug; zinc sulfate solution concentration (volume), 10%, w/v (1 ml); centrifugation time (speed), 2 min (5000 rpm); stirring time, 2 min; sorbent amount, 40 mg; desorption time, 2 min; and elution solvent type (volume), [HMIM][BF4] (50 µl). The error bars indicate the standard deviations of three repeated determinations. (b) Extraction conditions: are the same as those used in Fig. 1a, except ODS was chosen as the sorbent.

The amount of ODS used for the adsorption of the analytes must be optimized to obtain high extraction efficiency. For this purpose, various amounts of ODS in the range of 10-60 mg were used for coating the stir bar surface and the method efficiency was calculated. The data illustrated in Fig. 1b, depict that the method efficiency increases up to 30 mg of ODS and then decreases slowly. Increasing the sorbent amount provides more adsorption sites to the analytes and increases the method's efficacy. However, at higher ODS amounts, the sorbent adhering to the stir bar surface decreases and its release into the solution during the extraction step is occurred. This has an adverse effect on the method's efficiency. According to the results, 30 mg of ODS was used for the next tests.

Selection of Elution Solvent Type

The success of the introduced method is completely related to the complete desorption of the analytes from the employed sorbent surface. It is clear that this step can be affected by the type of elution solvent due to the different solubility of the analytes in diverse solvents. In the present study, different ILs which are known as green solvents were employed. To find the best solvent, three ILs including [BMIM][BF_4], [HMIM][BF_4], and [EMIM][OAc] were investigated as the possible eluent. The results in Fig. 2, show the priority of [BMIM][BF_4] compared to other solvents in elution of the analytes. It can be related to the higher solubility of the analytes in [BMIM][BF_4] or its lower viscosity compared to the other tested elution solvents. Therefore, [BMIM][BF_4] was used in the following experiments.

Desing of Expert

Fractional factorial design. The effectiveness of the parameters in the efficacy of the developed method was screened by fractional factorial design (FFD). In FFD, l^{k-p}, where: l is the number of levels in each treatment factor. k is the number of treatment factors. p is the number of interactions that are confounded. In this work, the experiments were done considering [BMIM][BF_4] volume,

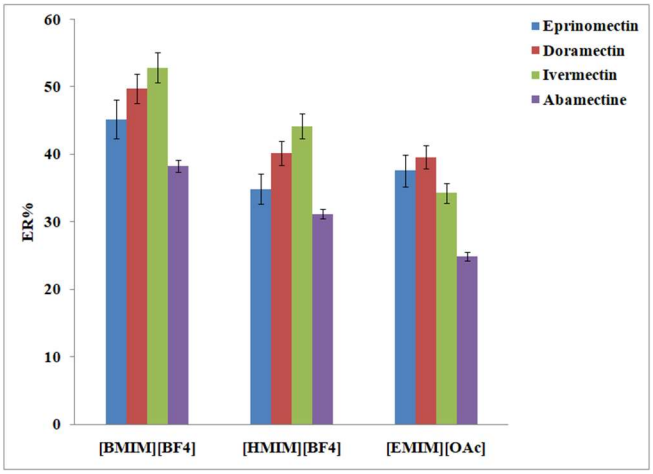


Fig. 2. Selection of elution solvent. Extraction conditions: are the same as those used in Fig. 1b, except 30 mg ODS was used as the sorbent.

zinc sulfate solution volume and concentration, sodium chloride concentration, and stirring time as the factors that can be optimized. The obtained Pareto chart (Fig. S1) depicts that all factors are statistically significant, except sodium chloride addition. Therefore, a central composite design (CCD) was established by [BMIM][BF_4] volume, zinc sulfate solution volume and concentration, and stirring time.

Central composite design. According to FFD results, optimization of the developed method was done. The volume and concentration of zinc sulfate alter the precipitated proteins amounts and may change the adsorption of the analyte onto the produced precipitate. The volume of the elution solvent affects the analytes' desorption from the sorbent surface and the method efficiency due to altering the ratio of sorbent to the eluent. Stirring time is an essential parameter for the adsorption of analytes onto the SBSE coating to reach the equilibrium contact time. At high stirring times, the adsorption of the analytes can be improved by contacting the sorbent with the whole solution. However, at a high stirring time, the sorbent may be plucked from the sorbent surface and the method failed to work. In this study, the above-mentioned factors at five levels were designed in CCD using Design Expert Software 11 with a total of 30 experimental runs. The designed CCD and the obtained results for the analytes are summarized in Table 2. For evaluation of these data, the ANOVA results obtained for each analyte are given in Tables S1-S4. The results illustrate that all of the studied factors are significant. The obtained results confirm that there is a quadratic relationship between the extraction recovery (ER) and four factors through a second-order polynomial equation (the equations are presented in Table S5). Comparison of coefficients of determination (R^2 = 0.9808, R^2-predicted = 0.9628, and R^2-adjusted = 0.9303) show that the results are in good agreement with the equations and that the model has acceptable reliability and accuracy. Figures S2-S5 depict the effect of diverse factors on the method efficacy as 3D-response surface and contour plots. According to the obtained data, 43.8 µl, 13.7% (w/v), 7.7 min, and 47.5 µl are the optimum amounts of zinc sulfate solution volume, zinc sulfate concentration, stirring time, and [BMIM][BF_4] volume, respectively. At these values, the highest desirability factors were obtained.

Reusability of sorbent. The capability of ODS in the extraction of target compounds from milk samples in the repeated applications was evaluated based on section 2.6 and the obtained results verified that there was no memory effect, therefore, the adsorbed compounds were desorbed totally from the sorbent surface in the first extraction. The data illustrate that the method efficiency is the same after using the same sorbent 7 times with relative standard deviations (RSDs) ≤ 8.6%.

Validation of the Offered Method

Several quantitative criteria of the developed method such as limit of detection (LOD), limit of quantification (LOQ), linear range (LR), coefficient of determination (r^2), ER, enrichment factor (EF), and relative standard deviation (RSD) were explored to assess the method validity. Based on the data, presented in Table 3, LODs (calculated considering signal (S)/noise (N) ratio of 3) and LOQs (S/N = 10) were in the ranges of 0.05-0.12 and 0.17-0.41 ng ml^{-1}, respectively. The calibration curves were linear in the ranges of 0.41-500, 0.26-500, 0.17-500, and 0.29-500 ng ml^{-1} for eprinomectin, doramectin, ivermectin, and abamectin, respectively. The r^2 for the calibration curves was ≥ 0.994. For assessing the approach precision, blank milk samples were spiked with the analytes at two concentrations (0.5 and 5 ng ml^{-1}) and they were analyzed repeatedly on one day and different days.

Table 2. Experimental Factors, Levels, and Results for CCD Design

Variables						Levels				
						-α	-1	0	+1	+α
(A) [BMIM][BF_4] volume (µl)						30	47.5	65	82.5	100
(B) Zinc sulfate solution volume (ml)						0.25	0.43	0.62	0.81	1.0
(C) Zinc sulfate concentration (%w/v)						10	13.75	17.5	21.25	25
(D) Stirring time (min)						1.0	3.25	5.5	7.75	10
Std	Run	A	B	C	D	Average ER (%) obtained from three repeated determinations of				
						Eprinomectin		Doramectin	Ivermectin	Abamectin
26	1	65	0.625	17.5	5.5	45.5		49.6	55.0	51.2
7	2	47.5	0.8125	21.25	3.25	41.6		45.3	50.3	46.8
27	3	65	0.625	17.5	5.5	46.7		50.9	56.5	52.5
30	4	65	0.625	17.5	5.5	45.6		49.7	55.2	51.3
18	5	100	0.625	17.5	5.5	55.1		60.1	66.7	62.0
28	6	65	0.625	17.5	5.5	43.6		47.5	52.7	49.0
15	7	47.5	0.8125	21.25	7.75	35.1		38.2	42.4	39.5
6	8	82.5	0.4375	21.25	3.25	52.2		56.9	63.2	58.8
1	9	47.5	0.4375	13.75	3.25	36.7		40.0	44.4	41.3
3	10	47.5	0.8125	13.75	3.25	36.4		39.7	44.1	41.0
21	11	65	0.625	10	5.5	50.1		54.6	60.6	56.3
11	12	47.5	0.8125	13.75	7.75	58.5		63.8	70.8	65.8
14	13	82.5	0.4375	21.25	7.75	43.6		47.5	52.8	49.1
13	14	47.5	0.4375	21.25	7.75	48.5		52.9	58.7	54.6
29	15	65	0.625	17.5	5.5	41.6		45.3	50.3	46.8
9	16	47.5	0.4375	13.75	7.75	69.1		75.3	83.6	77.8
23	17	65	0.625	17.5	1	42.6		46.5	51.6	48.0
16	18	82.5	0.8125	21.25	7.75	38.2		41.6	46.2	43.0
22	19	65	0.625	25	5.5	37.9		41.3	45.9	42.7
2	20	82.5	0.4375	13.75	3.25	39.9		43.5	48.3	44.9
8	21	82.5	0.8125	21.25	3.25	51.3		56.0	62.1	57.8
17	22	30	0.625	17.5	5.5	44.8		48.8	54.2	50.4
20	23	65	1	17.5	5.5	44.5		48.5	53.8	50.1
12	24	82.5	0.8125	13.75	7.75	61.3		66.8	74.2	69.0
10	25	82.5	0.4375	13.75	7.75	68.3		74.4	82.6	76.8
5	26	47.5	0.4375	21.25	3.25	47.5		51.8	57.5	53.5
4	27	82.5	0.8125	13.75	3.25	43.1		47.0	52.2	48.5
24	28	65	0.625	17.5	10	61.0		66.5	73.8	68.7
25	29	65	0.625	17.5	5.5	41.9		45.7	50.7	47.2
19	30	65	0.25	17.5	5.5	51.2		55.8	62.0	57.6

Table 3. Figures of Merit of the Proposed Method for the Studied Drugs

Analyte	LOD	LOQ	LR	r^{2d}	RSD (%)[e]				EF ± SD[f]	ER ± SD[g]
					0.5 ng ml^{-1}		5 ng ml^{-1}			
	(ng ml^{-1})[a]	(ng ml^{-1})[b]	(ng ml^{-1})[c]		Intra-day (n = 6)	Inter-day (n = 4)	Intra-day (n = 6)	Inter-day (n = 4)		
Eprinomectin	0.12	0.41	0.41-500	0.994	4.1	4.9	3.1	3.9	72 ± 6	68 ± 5
Doramectin	0.07	0.26	0.26-500	0.996	3.9	4.6	2.3	3.1	80 ± 4	75 ± 4
Ivermectin	0.05	0.17	0.17-500	0.995	3.4	3.6	2.0	2.8	88 ± 3	83 ± 3
Abamectin	0.08	0.29	0.29-500	0.999	3.2	4.9	2.2	2.9	82 ± 2	77 ± 2

[a]Limit of detection (S/N = 3). [b]Limit of quantification (S/N = 10). [c]Linear range. [d]Coefficient of determination. [e]Relative standard deviation for intra- and inter-day precisions. [f]Enrichment factor ± standard deviation (n = 3). [g]Extraction recovery ± standard deviation (n = 3).

The calculated RSDs obtained for repeated analysis were less than 4.9%. The selectivity of the method was evaluated by analyzing three blank milk samples and the apparent response at the retention time of the analytes was compared with the response of the analyte at LOQ. The results confirmed that there was no significant interference. Also, the ERs ($\frac{Migrated\ amount\ of\ analyte\ to\ elution\ solvent\ (n_{fin})}{total\ amount\ of\ analyte(n_0)} \times 100$) and EFs ($\frac{ER}{100} \times \frac{Initial\ volume\ of\ solution\ (V_{aq})}{Volume\ of\ elution\ solvent\ (V_{fin})}$) and were in the ranges of 68-83% and 72-88, respectively.

Analysis of Real Samples

The successfulness of the method in the analysis of the selected drugs was studied by applying it to different milk samples. For this purpose, thirty pasteurized and thirty raw milk samples were randomly bought from local stores in Tabriz City and analyzed by the introduced method. The obtained chromatograms showed that ivermectin was found in four pasteurized and eight raw milk samples at the concentration range of 39-83 and 64-96 ng ml^{-1}, respectively. In the following, to acquire data about the matrix effect of the investigated samples, added-found method was utilized. For this purpose, two pasteurized milk samples, two raw milk samples, and blank milk were spiked with the analytes at two concentrations (0.5 and 5.0 ng ml^{-1}, each drug). After performing the method on them, the concentrations of the analytes were found and they were divided into the initial concentration in the blank sample. The obtained ratio for each analyte was multiplied by 100 and reported as mean relative recoveries ($RR = \frac{Cf}{C_0} \times 100;$) ($C_f$ = the analyte concentration after performing the method and C_0 = the initial concentration of the analyte in the sample) [11]. The data (Table 4) verified the negligible effect of the samples matrix on the developed approach. Figure S6 shows typical HPLC-DAD chromatograms of standard solution, and spiked and unspiked milk samples after performing the developed method.

Comparison of the Method with other Approaches

The established method analytical figures of merit (RSD, LOD, LOQ, LR, and ER), sample type and amount, and extraction time were compared with other previously published methods, and the results were summarized in Table 5. Wider LRs and lower RSDs are obtained by this method. The LODs and LOQs obtained by the present work for the target drugs are less than those of the other approaches. Comparable ERs were obtained by this method

Table 4. Results of Assays to Check the Sample Matrices Effect for the Selected Analytes. Data are Mean Relative Recovery ± Standard Deviation Obtained from Three Repeated Determinations

	Mean relative recoveries ± SD (Matrix effect)[a]			
Analyte	Pasteurized milk #1	Pasteurized milk #2	Raw milk #1	Raw milk #2
All samples were spiked at a concentration of 0.5 ng ml^{-1} of each analyte				
Eprinomectin	82 ± 4 (18)	92 ± 2 (8)	92 ± 4 (8)	92 ± 4 (8)
Doramectin	93 ± 2 (7)	94 ± 4 (6)	91 ± 3 (9)	94 ± 3 (6)
Ivermectin	92 ± 3 (8)	92 ± 3 (8)	99 ± 4 (1)	93 ± 2 (7)
Abamectin	97 ± 4 (3)	99 ± 4 (1)	89 ± 2 (11)	102 ± 1 (2)
All samples were spiked at a concentration of 5 ng ml^{-1} of each analyte				
Eprinomectin	93 ± 3 (7)	106 ± 2 (6)	89 ± 4 (11)	95 ± 4 (5)
Doramectin	91 ± 4 (9)	101 ± 3 (1)	97 ± 3 (3)	92 ± 3 (8)
Ivermectin	92 ± 4 (8)	92 ± 4 (8)	99 ± 4 (1)	94 ± 2 (94)
Abamectin	96 ± 3 (4)	94 ± 5 (6)	91 ± 2 (9)	95 ± 2 (95)

[a]%Matrix effect = [1 - (Peak area of post-spike)/(average peak area of neat blanks)] × 100.

compared to other methods. The method has a short extraction time compared to the other methods. The needed sample amount for performing the method was lower or comparable with other methods.

CONCLUSIONS

Herein, an SBSE method was utilized for the simultaneous extraction of four avermectin drugs from milk samples before their quantification by HPLC-DAD. In this study, firstly, an ODS-coated stir bar was prepared and directly introduced into the solution after the precipitation of proteins. After adsorption of the analytes onto the sorbent surface, they were eluted by an IL to analyze with an HPLC-DAD system. The suggested method provided satisfactory figures of merit including low LODs and LOQs, acceptable ERs and EFs, wide LRs, and good precision. Also, having no serious matrix effect assisted the method to be applied on milk samples. These points show the high potential as a reliable analytical method for the determination of the target compounds in milk samples.

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Table 5. Comparison of the Offered Approach with the Previously Reported Methods Used in Preconcentration and Determination of the Studied Drugs

Method	Sample	Analyte	RSD[a]	LOD[b]	LOQ[c]	ER[d]	LR[e]	Extraction time (min)	Sample amount	Ref.
SPE-HPLC-MS/MS[f])	Cow milk	Eprinomectin	≤10	0.21	0.85		1-60	~50	5 ml	[21]
		Doramectin		0.21	0.57	-				
		Ivermectin		0.26	0.72					
		Abamectin		0.21	0.63					
RAMIP-BSA-HPLC-UV[g]	Meat	Ivermectin	≤ 10.6	30	50	-	50-500	~60	2 g	[22]
LLE-SPE-HPLC-FLD[h]	Bovine milk	Abamectin	≤14.9	0.5	1.0	75	1-100	~20	5 ml	[23]
		Doramectin		0.5	1.0	74	1-100			
		Eprinomectin		0.5	1.0	76	1-100			
		Ivermectin		0.3	1.0	73	1-100			
MAE-DμSPE-HPLC-DAD[i]	Cow tissues	Eprinomectin	≤6.6	0.07	0.24	72	0.24-500	~15	15 g	[13]
		Doramectin		0.06	0.19	86	0.19-500			
		Ivermectin		0.10	0.32	77	0.32-500			
		Abamectin		0.08	0.27	82	0.27-500			
SBSE-HPLC-DAD[j]	Cow milk	Eprinomectin	≤4.9	0.12	0.41	68	0.41-500	~12	5 ml	This method
		Doramectin		0.07	0.26	75	0.26-500			
		Ivermectin		0.05	0.17	83	0.17-500			
		Abamectin		0.08	0.29	77	0.29-500			

[a]Relative standard deviation (%). [b]Limit of detection (ng ml^{-1}). [c]Limit of quantification (ng ml^{-1}). [d]Extraction recovery (%). [e] Linear range (ng ml^{-1}). [f]Solid phase extraction-high performance liquid chromatography-tandem mass spectrometry. [g]Restricted access molecularly imprinted polymer-coated with bovine serum albumin-high performance liquid chromatography-ultraviolet detector. [h]Liquid-liquid extraction-solid phase extraction-high performance liquid chromatography-fluorescence detector. [i]Microwave-assisted extraction-dispersive micro solid phase extraction-high performance liquid chromatography-diode array detection. [j]Stir bar sorptive extraction-high performance liquid chromatography-diode array detection.

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