Research Article

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Quantification of Rafoxanide Drug Residue from Sheep Meat in Sulaymaniyah Province/Iraq Using High-Performance Liquid Chromatography Hplc-Uv

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Abstract

Contamination of edible animal products with veterinary drug residues is a potential health hazard for consumers. This study aims to apply a reliable and sensitive method for the detection of Rafoxanide anthelmintic drug residue in sheep muscle tissues in five different districts of Sulaimani city /Iraq, using HPLC - UV combined with an extraction method based on a modified QuEChERS technique. A method for extracting rafoxanide anthelmintic drug from sheep muscle was adopted based on a QuEChERS approach followed by HPLC-UV. The developed method has been fully validated according to the requirements of Eurachem-2014 by separating the standard anthelmintic drugs on a fast liquid chromatographic (FLC) reversed-phase column under the optimum separation condition. The mobile phase consists of solvents A and B, degassed with an ultrasonic bath to remove bubbles. The standard calibration curves ($R^2 \geq 0.9961$) were constructed with spiked certified reference materials (CRM) and blank tissues. The optimized approach was validated, yielding satisfactory results for all performance criteria in the tested matrix and obtaining relevant results for all performance parameters in the evaluated matrix. A multi-level calibration curve (1 to 1000 µg/kg) was used to establish instrument linearity for rafoxanide. The recoveries were 83%, the limit of quantification was 10 µg/kg, and the detection limit was 0.22 µg/kg. The results of the validation allowed for high sample throughput. The method is suitable for analyzing selected anthelmintic drug residue, and repeatability and reproducibility expressed in relative standard deviation (RSD%) were obtained at values lower than 20%. None of the testers were greater than Maximum Residual Limits (MRLs) legally established by the European Union (EU). Thirteen (26%) samples were below MRLs and 37 (74%) samples were below the LOD. Thus, we concluded that optimized approach

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was validated, yielding satisfactory results for all performance criteria in the tested matrix and obtaining relevant results for all performance parameters in the evaluated matrix. None of the testers was greater than MRLs legally established by the EU .

Keywords: Drug, HPLC-UV, rafoxanide, sheep meat.

Introduction

Globally, anthelmintic medicines are among the most widely used veterinary drugs to prevent and treat parasitic infections in livestock (1). Anthelmintic is a group of antiparasitic drugs that drive out parasitic worms, including flatworms (trematodes), tapeworms (cestodes) and roundworms (nematodes) from the body without causing damage to the host (2). Liver flukes (Fasciola spp.) are among domestic sheep/cattle's most common helminth parasites worldwide, inflicting enormous financial losses on producers (3). Fasciolosis, induced by Fasciola (F.) hepatica, has been one of the most significant helminthic infections of livestock in Iraq for a very long time (4). Fascioliasis was diagnosed in Duhok/Iraq within 2.0% (195/9757) of all slaughtered livestock, with the infection rate being highest in cattle (3.27%; 66/2021), sheep (1.76%; 122/6932), and goats (0.86%; 7/804). Local livestock had a slightly higher prevalence rate of positive cases than imported livestock (2.1% vs 1.88%) (5). In the developing countries, fasciolosis caused by F. hepatica and F. gigantica infection is mostly overlooked (6). Anthelmintic drugs are prescribed to patients with parasitic worm infections, sometimes called helminthiasis. Both humans and animals may take these medications.(7)

In a 38-year-old Kurdish woman from northern Iraq, an endoscopic retrograde cholangiopancreatography filling detected a problem in her common bile duct. Following sphincterotomy and balloon extraction, one live F. hepatica was physically removed .(8)

Fascioliasis is an unindustrialized infection in our area that should be measured in the differential diagnosis of patients with liver/biliary illness with eosinophilia and a history of watercress consumption.(9)

Salicylanilides are a class of anthelmintic drugs used frequently to treat parasitic infections in livestock. These drugs disrupt parasites' energy metabolism, resulting in death (10). Rafoxanide (Figure 1) is one of the most commonly used salicylanilides anthelmintic drugs in veterinary medicine to treat liver fluke infections in sheep and cattle, gastrointestinal nematodes and lungworms (11). Rafoxanides' IUPAC name is N-[3-chloro-4-(4-chlorophenoxy) phenyl]-2-hydroxy-3,5-diiodobenzamid with a molecular formula of $C_{19}H_{11}Cl_2I_2NO_3$ and a molecular weight of 626.0 g/mol. Rafoxanide has many synonyms, such as Disalan, Ranide, and Flukanide .

Misuse of anthelmintic drugs, overdose and improper observation of withdrawal periods due to the lack of scientific knowledge may lead to their accumulation in edible tissues such as meat, either as the

parent compound or their metabolites/conjugates (12). These may transfer to the human body through the food chain and may cause a threat to public health, such as diarrhea, anemia, pulmonary edemas, allergy, teratogenic, carcinogenicity, disruption of intestinal normal microflora and the possibility of developing anthelmintic resistance ,13) .(14

To minimize the side effects of these veterinary drug residues on human health, pharmacologically active materials and their sorting regarding maximum residue limits (MRLs) in products of animal source are set and observed by many national/international institutes such as European Commission regulation (EU), the Codex Committee on Residues of Veterinary Drugs (CCRVD), and the United States Department of Agriculture. Hence it is essential to determine the concentrations of this residue in animal-derived foods, including meat .(15)

In this study, we used the QuEChERS extraction technique for the accurate determination of rafoxanide drug from meat due to its specificity/accuracy and allows validation of the analyte identity, which is essential for the resolve of trace residues of various analytes in diverse matrices, including muscle tissues (16, 17). In many studies, QuEChERS recently applied to determine anthelmintic drug residues in meat samples.(20-18 ,16)

Thus, this study aimed to apply a reliable and sensitive method for the detection, identification, and quantification of rafoxanide anthelmintic drug residue in sheep muscle tissues in five different districts of Sulaimaniyah city, Iraq, using HPLC-UV combined with an extraction method based on a modified QuEChERS technique.

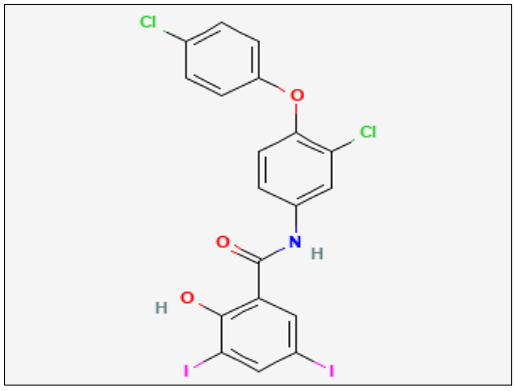

Figure 1. Rafoxanide chemical structure.

Materials and Methods

Equipment, chemicals, and reagents

This analysis was performed on the HPLC System (Shimadzu Corporation, Japan, model LC – 10AV series), which consisted of the binary pump and SPD-10A Ultraviolet (UV) detector. The chromatographic separation of anthelmintic drugs was done on HCMA-BIO 1000 C-18, 3.0 µm particle size (50x4.6 mm ID) column (Waters Corporation, Manchester, UK) under the optimum separation condition. The substances used in the QuEChERS extraction method were 1.5 g MgSO4 and 0.5 g C18. PVDF syringe filters (0.45 µm), primary, secondary amine (PSA), and octadecyl saline (C18) were obtained from MILLEX, Merck Millipore Ltd, Co. Cork, Ireland. Centrifugation was performed using a high-speed centrifuge (model LMC-56) and a Vacuum drying oven (model OD 124725, Japan). Mobile phase is composed of solvent A [0.01% acetic acid in acetonitrile: water (10:90, v/v)] and solvent B [5 mM ammonium formate in methanol: acetonitrile (75:25, v/v)]. A linear gradient was applied from 0.0% B to 100% B in 12 min. Detection UV was set at 285 nm, 30 °C, and a flow rate of 1.0 ml/min with an injection volume of 50 µl.

Study area

This study was conducted in 5 slaughterhouses in Sulaimaniyah Province, Iraq, including Sulaimaniyah City (SUL), SaidSadiq District (SSQ), Chamchamal District (CHAM), Piramagrun District (PIRA), and Chwarta District (CHWA).

Stock solution

Analytical standards of rafoxanide anthelmintic drug obtained from Merck Life Science UK, and Milli-pore water from Milli-Q, Gulf scientific corporation. Methanol with purity ≥99.9%, acetonitrile with purity ≥99.9%, acetic acid with purity 99%, formic acid with purity 98%, and HPLC grade were obtained from Merck KGaA, Darmstadt, Germany.

The extraction solvent for anthelmintic drug residue was 1% acetic acid/acetonitrile and the mobile phase was component A [0.01% acetic acid in acetonitrile: water (10:90, v/v)] and component B [5.0 mM ammonium format in methanol: acetonitrile (75:25, v/v)]. The mobile phase was degassed with an ultrasonic bath to eliminate bubbles. Then, 10% Acetonitrile and 0.1% acetic acid were prepared by transferring 100 ml acetonitrile and 1.0 ml acetic acid to a 1.0 L volumetric flask; the volume was completed with water and mixed well.

Stock solution preparation

Stock solutions for rafoxanide (1000 mg/L) were made by dissolving 0.01g in a 5 ml mixture of methanol (10%) and acetic acid (0.1%), mixed, sonicated for 30 min, and stored at -20 °C. The working solution for rafoxanide (100 mg/L) was prepared by diluting 1.0 ml of rafoxanide stock solution (1000 mg/L) with 5.0 ml mixture of methanol (10%) and acetic acid (0.1%), mixed well and stored at -20°C. The working solution for rafoxanide (10 mg/L) was prepared by diluting 1.0 ml of rafoxanide working solution (100 mg/L) into a 5.0 ml mixture of methanol (10%) and acetic acid (0.1%), mixed well and

stored at -20 °C. The intermediate mixture of rafoxanide standard solution (1.0 mg/L) was prepared by diluting 1.0 ml of working solution (10 mg/L) into a 5.0 ml mixture of methanol (10%) and acetic acid (0.1%), mixed and stored at -20 °C. Multi-level calibration curves for quantitation of rafoxanide were made by serial dilutions (1,10, 50, 100, 200, 500 & 1000 µg/L).

Sample collection

About 200 g sheep (local breed, male, aged 12±3 months) neck muscle tissues with vertebrates (semispinalis capitis muscle, longissimus capitis muscle, and longissimus atlantis muscle) were collected from five slaughterhouses (10 samples from each) in Sulaimaniyah province, Iraq, including Sulaimaniyah city (SUL), SaidSadiq district (SSQ), Chamchamal district (CHAM), Piramagrun district (PIRA), and Chwarta district (CHWA). For each sample, the meat was separated from the bone. Around 90-100 g of meat left that sliced into three equivalent portions (30 g each) and tagged from 1 to 10 .

The samples were collected from December 6, 2021 to February 10 2022 in a special cool box contained ice, then transferred to Food Hygiene Laboratory, College of Veterinary Medicine, University of Sulaimani. The samples were stored in Freezer (- 23°C), till transferred to the Al-Rawabi AL-Khadhra Company for Chemical & Environmental Studies and Analysis, Baghdad, Iraq in a freeze condition .

Extraction and preparation of samples

The thawed samples were ground with a homogenizer, and about 5.0±0.04 g was weighed, dissolved with 10 ml de-ionized water, mixed properly, sonicated for 15 min, diluted with a 10 ml mixture of acetic acid (0.1%) in acetonitrile, vortexed for 1 min, and sonicated again for 15 min. QuEChERS kits were added, and tubes were shaken slowly and centrifuged for 10 min at 10000 rpm. Double extraction was done by transferring another 10 ml of acetic acid (0.1%) in acetonitrile, vortexed well, and centrifuged for 10 min at 10000 rpm. PSA and C18 cleaned up extracts. For the dispersive solid phase extraction, 7.0 ml from the section was removed from the SPD tube, vortexed and centrifuged at 10000 rpm for 10 min. Then, 4.0 ml extract was transferred to glass tubes for evaporation at 40 ˚C. The dry residues were dissolved in a 1.0 ml mixture of acetonitrile (10%) and formic acid (0.1%), filtered through a 0.20 µm syringe filter, then in HPLC vials, 1.0 ml samples were run with calibration standards on HPLC System (Shimadzu Corporation, Japan, LC–10AV) which consisted of the binary pump, and SPD-10A UV.

Method validation

After optimization of the procedure, a method validation was done (24). Some factors were assessed to certify the proper identification/quantification of the analytes, such as linearity, specificity, recovery, repeatability, reproducibility, and limit of quantification (LOQ).

. Linearity

Instrumental linearity (Linear range)

The linearity was evaluated using diverse concentrations to choose the calibration level for quantitation. The accurate lowest calibration level was 10 µg/L, and the

linearity ranged from 1-1000 µg/kg to cover the target analyte's LOQ, MRL and working range. The correlation coefficients (R^2) were within the acceptable limit (>0.995) (Figure 2).

Method **linearity**

method linearity was verified at four different concentrations, with LOQ and MRL, and the more frequent presence concentrations in tedious samples. Seven replicates were spiked in blank sheep meat samples for these concentrations (10, 100, 500 and 1000 µg/kg). The method was linear from LOQ 10 µg/kg to 1000 µg/kg .

Recovery study:

Table 1 showed the mean recovery of spiked sheep meat samples at concentrations of 10 to 1000 µg/kg. Method validation/quality control measures for veterinary drug residue analysis in the food of animal source specified the suitable range of recovery from 70 - 120% (21), and the other regulatory agencies reflected these values. The mean recovery of rafoxanide in sheep meat ranged from 70 - 120%. Consequently, our compound is within the acceptable range of recovery percentage.

Recovery study

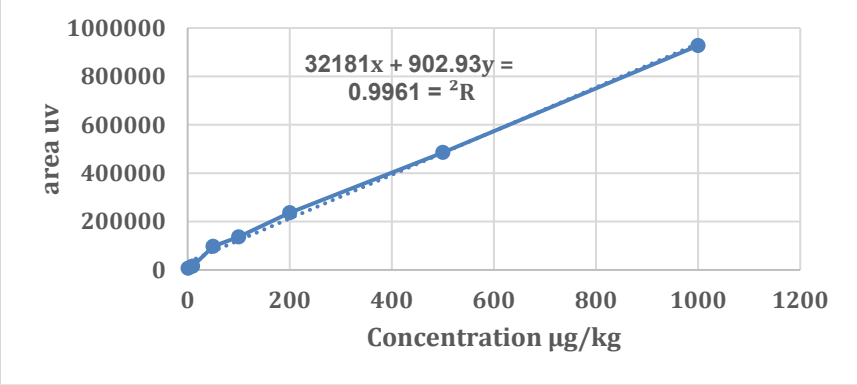

Figure 2. The linearity range and calibration curve of rafoxanide. Y-axis implies the sample area in µv, X-axis implies the sample concentration in µg/ml, and R^2 indicates the correlation coefficient.

Limit of quantification (LOQ)

LOQ is the lowest concentration of the analyte in the tester that can be found with satisfactory precision/recovery under the indicated situations of the test. The LOQ value was estimated using seven repeated spiked samples at the expected lowest quantitation level on sheep meat samples, which was ten µg/kg. The lowest quantitation levels were within accepted recoveries and precision (Table 2).

Limit of Detection (LOD)

The limit of detection is the minimum concentration of analyte in the test sample that can be measured with a stated probability when the analyte concentration is above the blank sample. The LOD is calculated as 3* Standard deviation (SD)

of 7 replicates of blank sample fortified at the lowest acceptable concentration level. The rafoxanide LOD was 0.22 (Table 2).

Truenes

The trueness of a method is an expression of how close the mean of a set of results is to the true value. To check the trueness of the method, seven replicates of certified reference material (CRMs) (Fera Science LTD, UK) of sheep meat samples were used (Table 3) .

. Precision

Precision measures how close results are together; the two most common precision measures are repeatability and reproducibility.

Repeatability

The closeness of agreement between successive results obtained with the same method on identical test material under the same operator, apparatus, laboratory, and short time scale conditions .

Reproducibility

Reproducibility is the precision under reproducibility conditions. This study only considered intra-laboratory reproducibility by spiking four concentrations on the long timescale and pooling the variance between all levels to calculate the pooled relative standard deviation (pooled RSD%). So, repeatability and reproducibility for the method have been studied through spiking of 4 concentration levels from 10 to 1000 µg/kg. Method validation and quality control procedures for veterinary drug residue analysis in the food of animal origin stipulated the acceptable range of RSD% (≤20%) (21), and the other regulatory agencies considered these values. RSD% and pooled RSD% were ≤ 20% for the target analytic in sheep meat (Table 4).

$$RSD_{pooled} = \sqrt{\frac{(RSD_1{}^2 (n_1 - 1) + (RSD_2{}^2 (n2 - 1) + ...}{(n_1 - 1) + (n_2 - 1) + ...}}$$

*RSD pooled: Pooled standard deviation was calculated from the following equation:

Table 1. Recovery was recorded for sheep meat fortified with different concentration levels.

Compound	Level means recovery (%)	Mean Recovery %	Qtyp recovery %
Rafoxanide	10 µg\kg low level means recovery	94.0	83.0
	100 µg\kg 2nd level mean recovery	77.0	
	500 µg\kg 3rd level mean recovery	79.0	
	10000 µg\kg 4th level mean recovery	82.0	

Qtyp: The average recovery of the four levels.

Table 2. Limit of detection (LOD) & limit of quantitation (LOQ).

	Spiked Concentration µg\kg	Recovery µg\kg
Rafoxanide	10	9.8
	10	8.4
	10	9.2
	10	8.7
	10	9.6
	10	10.6
	10	9.7
	Mean Recovery %	94
	Standard Deviation SD	0.07
	Relative Standard Deviation %	7.81
	LOD	0.22

Table 3. Trueness evaluation using different CRM for rafoxanide in sheep meat.

Matrix	Analyte	True value	Mean	RC %	SD	RSD %
Sheep	Rafoxanide	24.20	23.54	97.26	0.33	1.40

Table 4. RSD% and pooled RSD% for repeatability & reproducibility studies.

Compound	RSD % Levels	RSD%	Pooled RSD%
Rafoxanide	1st level - LOQ	7.81	7.78
	2nd level	8.47	
	3rd level	6.98	
	4th level	5.42	

Measurement of uncertainty

Relative standard uncertainty

Precision

The random error effects were estimated as the relative standard deviation of repeated spike samples for the studied analyte at different concentrations. Relative standard uncertainty due to precision experiments (Uprec), expressed as pooled RSD% (Table 5).

Bias

The bias of the analytical procedure was investigated using certified reference materials (CRM) for rafoxanide in sheep meat. The standard uncertainty (URec) was calculated as the standard deviation of the mean [URec = SD/SQRT(n)]. Relative

standard uncertainty due to bias (RSU%) is then calculated as RSU%=(URec/Mean RC%)*100 (Table 5).

Combined uncertainty (Uc)

Uc is the positive square root of the sum of the squares of different uncertainty components calculated through the following equation:

$$U_C = \sqrt{(U_p)^2 + (U_{\mathrm{Re}c})^2 + (U_{\mathrm{Re}f})^2} + (U_{Spro})^2 \ldots.$$

Table 5. Uncertainty evaluation.

Compound	(U) due to precision	Pooled RSD%	7.78 %
Rafoxanide	(U) due to Bias	RSU %	0.13 %
		Others (Sample processing)	10.00 %
		Combined Uncertainty (Ucom)	12.67 %
		Expanded uncertainty (Uexp)	25.34 %

Expanded uncertainty (Uexp)

Uexp is obtained by multiplying the Uc by a coverage factor k at a 95% confidence level, k is two.

Ethical approval

Appropriate guidelines and regulations belonging to the Declaration of Helsinki were followed to conduct this research study on animal tissue samples. The Scientific and Ethics Committee of the College of Veterinary Medicine, University of Sulaimani, Sulaimaniyah, Iraq, revised and approved the study protocol (No. 78/18/03/2020/UoS).

Statistical analysis

The results of this project were statistically evaluated using IBM SPSS, version 28. Then, One-way ANOVA, Student t-test, Excel (XLSTAT), and Graph Pad Prism 8 were used. Anthelmintic drug residue concentrations, HPLC-UV data, pressure cooking/barbeque data, and slaughterhouses (district)/anthelmintic drug types were substantially compared using multiple ranges.

Results

Figure 3A appeared the peak area (927388 µv) and retention time (3.58 min) of the rafoxanide standard. However, Figure 3B demonstrated the blank sample from FAPAS that has been used with no response at the retention time. Figures 3C & 3D showed SSQ1 and CHWA7 sheep muscles, respectively. Rafoxanide drug residue was found in 13 samples (26%) (in a total of 50 samples); 4 samples of SUL, 3 samples of PIRA, 1 sample of CHAM, 1 sample SSQ, and 4 samples of CHWA, all of them below the MRLs set by EU (No. 37/2010), with no significant difference between locations (Table 6) .

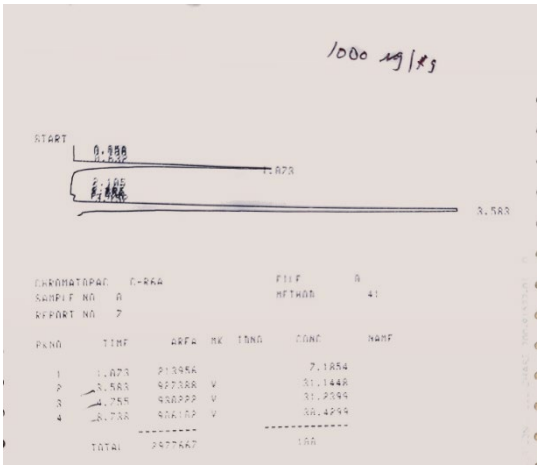

Figure 3A. HPLC peak area result and retention time of the rafoxanide standard.

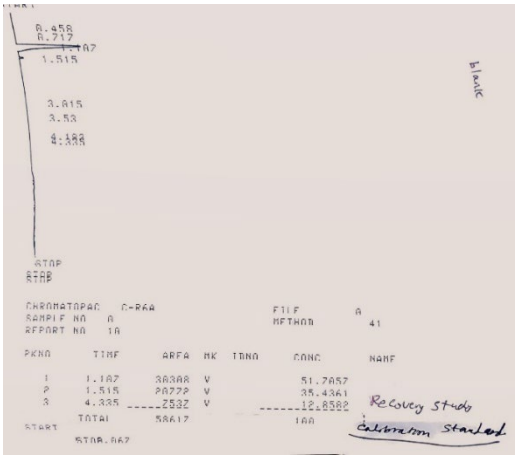

Figure 1B. HPLC peak area result and retention time of the blank sample.

Figure 3C. HPLC peak area result and retention time of Said Sadiq sample.

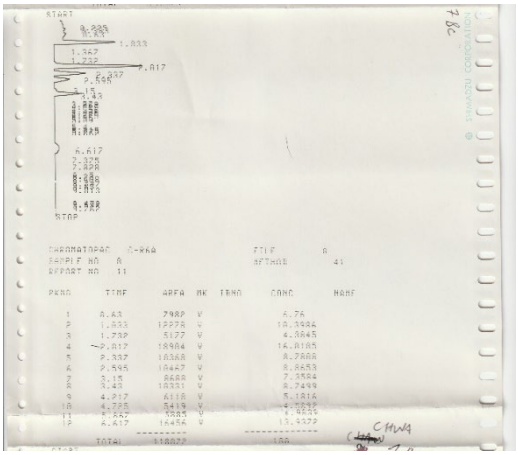

Figure 3D. HPLC peak area result and retention time of Chwarta samples.

Table 6. Residue levels of the 50 random samples; 10 from each location with MRL of 100 µg/kg.

No.	Slaughter Location	Detected Less than MRLS	Non detected
1	Sulaimaniyah	4.0 (40%)	6.0 (60%)
2	Piramagrun	3.0 (30%)	7.0 (70%)
3	Chamchamal	1.0 (10%)	9.0 (90%)
4	SaidSadiq	1.0 (10%)	9.0 (90%)
5	Chwarta	4.0 (40%)	6.0 (60%)
	Total	13.0 (26%)	37.0 (74%)

Discussion

In Sulaimaniyah province, the farmer administers anthelmintic drugs to sheep through oral drenching, subcutaneous injection, tablet, feed additive, or topical pour on, and at least four times per year or randomly during appearing of some clinical signs .

Neima (22) highlighted the significant development and changes in the red meat production sector during nine years, from 2009 to 2018, in Sulaimaniyah governorate, aiming to determine the trends in production and consumption patterns of red meat, especially the number of slaughtered sheep from 2009 equal to 746,515 heads increased to 1,457,645 heads in 2018.

In addition, rafoxanide is extensively used against fasciolosis and haemonchosis, bunostomosis, and nasal bot in sheep (23). Therefore, rafoxanide administer to sheep mainly by an oral drenching from the last of spring to the final of autumn, especially when the sign of liver fluke appears that including emaciation, easily remove wool, and bottle jaw appears in sheep or herd. The animal's physiological status also affects the amount of residue in tissues. For instance, when an animal loses weight, the drug residues that have built up and been stored in fat are progressively released into the bloodstream and may appear as more residues and metabolites in the meat and blood tissues.(24)

A study conducted in South Korea proved that rafoxanide was one of the most commonly detected residues in beef and chicken meat (20). A study from seven European countries also reported that 2.45% of detectable residues contained rafoxanid.(25).

Anthelmintic drug residues accumulating in the human body may result in several adverse effects, including abdominal discomfort, diarrhea, pain, headache, dizziness, fever, shaking, chills, rash, pruritus, and hair (23).

Anthelmintic resistance is a ‘heritable change’ in the ability of individual parasites to survive the recommended therapeutic dose of anthelmintic for decades of indiscriminate use. The incorrect dosing, widespread use, and increased frequency of treatment led to the development of anthelmintic resistance, including multi-drug resistance (26). Anthelmintic resistance is evident in different helminths of almost every animal species and other anthelmintic groups on several continents. Frequent treatment, under-dosing, genetics of the parasite, and targeting and timing of mass treatment are predisposing factors for anthelmintic resistance.(27)

Since this study is the first research on quantifying rafoxanide drug residue in sheep meat using HPLC in Iraq, the Central Organization of Standardization and Quality Control (COSQC), housed within the Ministry of Planning, is responsible for monitoring product standards in Iraq does not keep track accurately and has not officially set a maximum dose for this drug residues. Thus, the results depend on international standards such as EU, USAID, and Codex Alimentarius. The HPLC testing system was selected for this research due to its

high sensitivity, accuracy, and specificity level (16, 28). In this research, only 26% contained residues. Out of the 13 samples containing residues, the residues didn't exceed the MRLs, but some were near to MRLs .

Conclusion

The proposed chromatographic method was accurate, quick, and reproducible, and it can be used efficiently for routine quality inspection of rafoxanide in bulk, single, or combined dosage forms with no interference with an HPLC instrument equipped with a UV detector. Rafoxanide was found in samples as residue, and their contamination levels were mostly near the MRLs. This study's designed and confirmed analytical approach is sufficient for quantifying anthelmintic drug residues in sheep muscle tissues. The adaptability of the technique is demonstrated by the fact that the QuEChERS extraction (Double extraction) method and dispersive solid phase extraction (d-SPE) for extract cleanup were applied to the diverse matrices investigated. Therefore, it is appropriate for quantifying anthelmintic drug residues following the MRL values defined by the EU for sheep muscle tissues. Data from actual samples revealed the precision of the approach in really contaminated samples, assuring that it is suitable for identifying anthelmintic drug residues in sheep muscle tissues in slaughterhouses and sold at retail.

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Conflict of Interest

There is no conflict of interest.

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القياس الكمي لبقايا رافوكسنايد في لحوم الضأن (الخروف) في محافظة السليمانية/ العراق

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الخلاصة

يعد تلوث المنتجات الحيوانية الصالحة للأكل بمخلفات الأدوية البيطرية خطراً محتملاً على صحة المستهلكين. تهدف هذه الدراسة إلى تطبيق طريقة موثوقة وحساسة للكشف عن بقايا عقار رافوكسانيد الطارد للديدان في أنسجة عضلات الأغنام في خمس مناطق مختلفة من مدينة السليمانية / العراق، وذلك باستخدام HPLC-UV مع طريقة استخلاص تعتمد على تقنية QuEChERS المعدلة. تم اعتماد طريقة لاستخلاص دواء رافوكسانيد الطارد للديدان من عضلات الأغنام بناءً على طريقة QuEChERS متبوعة باستخدام HPLC-UV. تم التحقق من صحة الطريقة المطورة بالكامل وفقًا لمتطلبات Eurachem-2014 من خلال فصل الأدوية الطاردة للديدان القياسية على عمود الطور المعكوس الكروماتوجرافي السائل السريع (FLC) في ظل ظروف الفصل المثلى. يتكون الطور المتحرك من المذيبات A وB، ويتم تفريغهما من الغاز باستخدام حمام بالموجات فوق الصوتية لإزالة الفقاعات. تم إنشاء منحنيات المعايرة القياسية ($R^2 \geq 0.9961$) باستخدام مواد مرجعية معتمدة مسننة (CRM) وأنسجة فارغة. تم التحقق من صحة النهج الأمثل، مما أدى إلى نتائج مرضية لجميع معايير الأداء في المصفوفة التي تم اختبارها والحصول على النتائج ذات الصلة لجميع معلمات الأداء في المصفوفة التي تم تقييمها. تم استخدام منحنى معايرة متعدد المستويات (من 1 إلى 1000 ميكروجرام/كجم) لتحديد الخطية للأداة الخاصة بالرافوكسانيد. وكانت نسبة الاسترداد 83%، وكان حد القياس الكمي 10 ميكروجرام/كجم، وكان حد الكشف 0.22 ميكروجرام/كجم. سمحت نتائج التحقق بإنتاجية عالية للعينة. هذه الطريقة مناسبة لتحليل بقايا الأدوية الطاردة للديدان المختارة، وتم الحصول على التكرار والتكاثر المعبر عنه بالانحراف المعياري النسبي (RSD٪) بقيم أقل من 20٪. لم يكن أي من المختبرين أكبر من الحدود القصوى المتبقية (MRLs) التي وضعها قانونًا الاتحاد الأوروبي. (EU)وكانت ثلاثة عشر (26%) عينة أقل من الحدود القصوى للحدود و37 (74%) عينة كانت أقل من الحدود القصوى للحدود. وهكذا، خلصنا إلى أنه تم التحقق من صحة النهج الأمثل، مما أدى إلى نتائج مرضية لجميع معايير الأداء في المصفوفة التي تم اختبارها والحصول على النتائج ذات الصلة لجميع معلمات الأداء في المصفوفة التي تم تقييمها. لم يكن أي من الاختبارات أكبر من الحدود القصوى للمخلفات التي حددها الاتحاد الأوروبي بشكل قانوني.

الكلمات المفتاحية : دواء ، HPLC-UV ، رافوكسانيد ، لحم غنم.