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Novel Chromatographic Methods for Simultaneous Determination of Some Anthelmintic Drugs in Bulk Powder and Pharmaceutical Formulation

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ABSTRACT

The present study describes the development and subsequent validation of simultaneous determination of [Levamisole HCL (I) and Closantel Sodium (II) 'A'] and [Fenbendazole (III) and Rafoxanide (IV) 'B'] in two separated mixtures 'A & B', using isocratic RP-HPLC and TLC-densitometric techniques. In the HPLC technique; 'agilent eclipse XDB-C8 and thermo BDS-C18 (250 x 4.6 mm) 5 µm columns', 'with a flow rate of 1.5 ml and 2.0 $ml.min^{-1}$', 'acetonitrile: 0.02M potassium dihydrogen orthophosphate buffer (60:40, v/v) and acetonitrile: 0.02M ammonium formate (60:40, v/v) as a mobile phase' and UV measurement at '220 and 290 nm' were used for the studied mixtures 'A and B', respectively. While in TLC-densitometric technique; silica gel F_{254} aluminum sheets, 'n-butyl acetate: methanol: ammonia 13.5M (10: 1: 0.1, v/v) and ethyl acetate: toluene: methanol: ammonia (4: 2.5: 2.5: 1, v/v) as developing systems' and UV measurement at '240 and "292 and 284 nm"' were used for the studied mixtures 'A and B', respectively. The utilized chromatographic methods were validated according to the International Conference on Harmonization (ICH) guidelines and successfully applied for determination of the studied drugs in pure form, in laboratory prepared mixtures in pharmaceutical formulations with good extraction recoveries. All the results were statistically compared with the pharmacopeial and manufacturing methods for 'I, II and III' and 'IV', respectively, where there is no significant differences were found. The developed methods were satisfactorily applied to analysis of the investigated drugs and proved to be specific and accurate for quality control of them in pharmaceutical formulations.

Keywords: Levamisole HCl, Closantel Sodium, Fenbendazole, Rafoxanide, High Performance Liquid Chromatography, TLC- densitometry.

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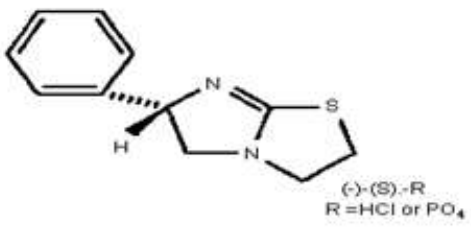
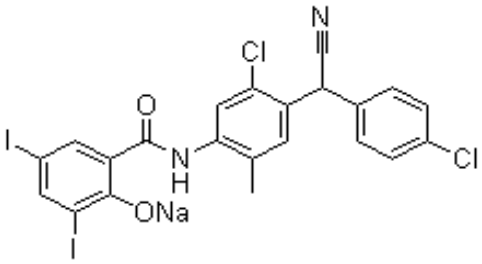
INTRODUCTION



(I)



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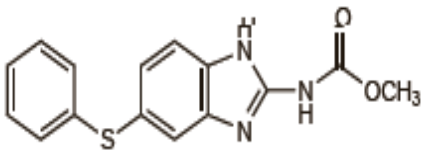
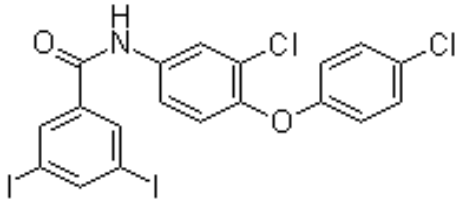


(III)



(IV)

Figure 1: Chemical structure of (I) levamisole HCl (II) closantel Sodium (III) Fenbendazole and (IV) Rafoxanide.

Levamisole (I) is (*S*)-6-phenyl-2,3,5,6-tetrahydroimidazo[2,1-b][1,3] thiazole, refers to Levo-tetramisole, which is Levo Isomer of tetramisole. It is broad spectrum anthelmintic with high activity against roundworms, hookworms, whipworms, and strongyloises stercoralis. It is an immune modulator and effective on rheumatic disorders, also; it is a good alternative in cases of benzimidazole resistance. Closantel (II) is 5'-Chloro-alpha4-(p-chlorophenyl)-alpha4-cyano-3,5-diiodo-2',4'-salicyloxylidide; N-[5-Choloro-4- [(4-Chlorophenyl) Cyanomethyl]-2-Methylpheny]- 2-Hydroxy-3-5- Diiodobenzamide, used in veterinary medicine for treatment of fluke and nematode infections. Fenbendazole (III) is methyl [5-(phenylthio)-1H-benzimidazole-2-yl]-carbamic acid methyl ester. It is used in veterinary medicine for the treatment of nematode infections of the gastrointestinal tract and lungworm and in tapeworm infections. While Rafoxanide (IV) is 3′-Chloro-4′-(4chlorophenoxy)- 3,5-di-iodosalicylanilide, used as anthelmintics for the treatment of fascioliasis in cattle and sheep[1]. Literature review showed many methods for determination of (I); including potentiometric[2], liquid chromatographic method[3-4], ion–pair liquid chromatographic method[5], Also; literature survey reveals many chromatographic methods have been developed for determination of (II) in milk, animal tissue and multi-component anthelmintic suspensions and tablets[6-7] and a spectrophotometric method[8]. Also; literature survey reveals that (III) could be determined by liquid chromatography[9-10-11],

densitometric[12], voltametric[13] and spectrophotometric methods[14], Besides, several methods have been reported for determination of (IV) in ovine plasma by chromatographic[15] and spectrophotometric method[16]. The main goal of our work is to establish simple, accurate, rapid and precise chromatographic methods suitable for simultaneous determination of '(I) and (II)' and '(III) and (IV)' in presence of each other; to be used as a tool for routine quality control analysis of these drugs in row materials and pharmaceutical formulations.

MATERIALS AND METHOD

Chemicals and reagents

Pure sample Levamisole hydrochloride, certified to contain 99.88% was kindly supplied from Guilin pharmaceutical Co. LTD (Shanghai, China). Pure sample Closantel Sodium, certified to contain 96.90% and vermisantel complex® suspension (batch number 'C002') each 1 ml is claimed to contain 100 mg of Levamisole hydrochloride and 50 mg of Closantel Sodium were kindly supplied from pharma Divasa farmvic (Barcelona, Spain). Pure sample Fenbendazole certified to contain 99.88% was kindly supplied from Bonds chemical company (Bangkok, Thailand). Pure sample Rafoxanide certified to contain 98.00% was kindly supplied from Pharma Swede company (10th of ramadan, Egypt). Curafluke® suspension (batch number 'M095'), was kindly supplied by Univet LTD Pharmaceutical Company (Milton, Canada), each 1 ml is claimed to contain 50 mg of Fenbendazole and 50 mg of Rafoxanide. Triple distilled water, acetonitrile (POCH-Poland HPLC grade), methanol (Lab-scan analytical sciences-Poland A.R-grade), ammonia 33 % (EL- Naser Pharmaceuticals, Egypt), potassium dihydrogen orthophosphate (Adwic, EL-Naser Pharmaceuticals, Egypt), dimethyl formamide (EL-Naser Pharmaceuticals, Egypt, A.R-grade), Toluene (Adwic, EL-Naser Pharmaceuticals, Egypt), Ethyl acetate (S.d. fine-chem. Limited AR grade) and n-butyl acetate (Fisher chemical, UK).

Instruments

For HPLC

For mixture (A) Agilent RRLC SL equipped with Agilent binary pump model G1312B, connected to chem. Station software, UV detector DAD model G1315C, auto-sampler ALS model G1329B. The chromatographic separation was performed using agilent eclipse XDB- C8 (250 x 4.6 mm) 5 µm column at ambient temperature. For mixture (B) HPLC Agilent 1100 series equipped with Agilent quaternary pump G1311A, connected to chem. Station software, UV detector VWD G1314A, auto-sampler G1313A. The chromatographic separation was performed using Thermo BDS C18 (250 * 4.6 mm) 5 µm column at ambient temperature.

For TLC-densitometry

- Densitometer-Dual wave length Flying Spot CS-9301 (Shimadzu, Tokyo, Japan).
- TLC plates pre-coated with Silica gel $60F_{254}$ '10*20 cm' (Merck, Germany).
- Ultrasonic vibrator, Crest Ultrasonic-Tru/Sweep: Model 575TAE, N.Y, U.S.A.
- Disposable membrane filters, 0.45µm, (Agilent 3150-0576).

A (Jenway 3510, UK) pH-meter, equipped with combined glass electrode for pH adjustment.

Chromatographic conditions

HPLC

Isocratic RP-chromatographic elution was achieved at ambient temperature on a 'agilent eclipse XDB-C8 and thermo BDS-C18 (250 x 4.6 mm) 5 µm columns', using 'acetonitrile: 0.02M potassium dihydrogen orthophosphate buffer (60:40, v/v) and acetonitrile: 0.02M ammonium formate (60:40, v/v) as mobile phases' with UV detection at '220 and 290 nm' for mixtures 'A & B', respectively. The mobile phases were filtered through a 0.45-mm membrane filter and degassed for 30 min in an ultrasonic bath prior to use. The mobile phases were pumped through the columns at a flow rate of 1.5 ml and 2.0 $ml.min^{-1}$', with the injection volume of 20 µL.

TLC-densitometric

Silica gel F_{254} aluminum sheets, 'n-butyl acetate: methanol: ammonia 13.5M (10:1:0.1, v/v) and ethyl acetate: toluene: methanol: ammonia (4:2.5:2.5:1, v/v) as developing systems' and UV measurement at '240 and "292 and 284 nm"' were used for the studied mixtures 'A & B', respectively.

Standard Solutions

For mixture (A)

Stock standard solution of (I) and (II), each having concentration of 1.0 $mg.ml^{-1}$ was prepared in 50% acetonitrile in water, which further diluted to obtain a concentration of 0.1 $mg.ml^{-1}$ was with the same solvent to be used as working standard solution for HPLC technique.

For mixture (B)

Stock standard solution of (III) and (IV), each having concentration of 1.0 $mg.ml^{-1}$ was prepared in dimethyl formamide, which further diluted to obtain a concentration of 0.1 $mg.ml^{-1}$ was with methanol to be used as working standard solution for both HPLC and TLC-densitometric technique.

Procedures

For HPLC technique

For mixture (A) an accurately measured aliquots of each working standard solutions equivalent to (100–900 µg) and (50-700 µg) for (I) and (II) were separately transferred into a series of 10-mL volumetric flasks and then completed to volume with the mobile phase. While; for mixture (B) aliquots of each working standard solution of (III) and (IV) equivalent to (100 –900 µg) were separately transferred into a series of 10-mL volumetric flasks and then completed to volume with the mobile phase. A volume of 20 µl of each solution was injected and the mentioned chromatographic conditions (2-3.1.) were adapted. The calibration curve for each drug was obtained by plotting area under the peaks (AUP) against concentrations (C).

For TLC-densitometric

Calibration curve was performed by applying separately ten µl of different aliquots equivalent to (0.1-0.6 $mg.ml^{-1}$), (0.1-0.5 $mg.ml^{-1}$) and (40-180$µg.ml^{-1}$) of (I), (II) and (III and IV) of each working standard solution as separate compact spots 1.0 - cm apart from each other and 1.0 - mm from the bottom of the plate, using 25 µl micro syringe, and the mentioned chromatographic conditions (2-3.2.) were adapted. The plate was developed, by ascending chromatography to a distance of about 8.0 - cm in a chromatographic glass chamber pre-saturated for 30 - minutes, then removed, dried in air, scanned at ‘240 and "292 and 284 nm"’. Then the peak area corresponding to each concentration was measured. The average peak area obtained for each concentration was plotted versus concentration and the regression equation was then computed.

Assay of the pharmaceutical formulations

For mixture (A)

Into 50-ml volumetric flask, 1.0 ml of suspension containing (I and II) was quantitavely transferred, 30 ml methanol was added, sonicated for 15 min. and the volume was completed with methanol to obtain a concentration of ‘2 and 1 $mg.ml^{-1}$’ for the 2-investigated drugs, respectively, which further diluted to obtain a concentration of 0.1 $mg.ml^{-1}$ with 50% acetonitrile in water to be used as working standard solution for HPLC technique. Also, the prepared stock standard solution was further diluted to obtain a concentration of 0.1–0.6 mg.ml-1 and 0.1-0.5 mg.ml-1 was with methanol to be used as working standard solution for TLC-densitometric technique, to be used for determinations (I) and (II), respectively.as mentioned under (2-5.1) and (2-5.2).

For mixture (B)

Into 50-ml volumetric flask, 1.0 ml of suspension was taken is transferred, followed by 30 ml dimethyl formamide, then sonicated for 15 min. and the volume was completed with methanol to

obtain a concentration of 1.0 mg.ml^{-1} for the 2-investigated drugs which further diluted to obtain a concentration of 0.1 mg.ml^{-1} with methanol to be used as working standard solution for both HPLC and TLC-densitometric techniques, for determination of (III and IV) by HPLC and TLC techniques, as mentioned under (2-5.1) and (2-5.2.)

RESULTS AND DISCUSSION

Method development

For HPLC technique

Simultaneous determination of (I) and (II) in presence of each other either in bulk powder or in pharmaceutical formulation has been performed on agilent eclipse XDB- C8 (250 x 4.6 mm) 5 µm column, while for (III) and (IV) Thermo BDS C18 column (5 µm, 250 x 4.6 mm) was used. The proportion of the mobile phase components was optimized to reduce each of 'retention time and tailing' and to enable obtain a good resolution of both investigated drugs. At high acetonitril ratio, retention time of different components decrease, but with excessive tailing of its peak. High resolution was obtained by using ‘acetonitrile: 0.02M potassium dihydrogen orthophosphate buffer (60:40, v/v) and acetonitrile: 0.02M ammonium formate (60:40, v/v) as a mobile phase’ and UV measurement at ‘220 and 290 nm’ with a flow rate of 1.5 ml and 2.0 ml.min^{-1}, where the maximum sensitivity was observed as shown in (Figure 2) and (Figure 3) for the studied mixtures ‘A and B’, respectively.

For TLC-densitometric technique

Experimental conditions, such as developing system, scan mode and wavelength of detection were optimized to provide accurate, precise and reproducible results. A variety of developing systems were evaluated for mixture (A and B), where for mixture (A) ‘tolune: acetone: ammonia (6:4:1, v/v/v), chloroform: methanol: ammonia (4:2:2 v/v/v), butylacetate: methanol: ammonia (10:1:0.1, v/v/v), butyl acetate: methanol: ammonia (8:2:0.2, v/v/v) ,butyl acetate: methanol: ammonia (8:2:0.3, v/v/v) ’ were tested as development systems, also; for mixture (B) ‘ethylacetate: tolune: ammonia (5.5: 3.5: 1, v/v/v), ethylacetate: tolune: ammonia (6.5: 3: 0.5, v/v/v) ethylacetate: cyclohexane: ammonia (5.5: 3.5: 1, v/v/v)’. But, good resolution with minimum tailing of the studied drugs was achieved; by using butyel acetate: methanol: ammonia 13.5M (10: 1: 0.1, v/v/v) and ethylacetate: tolune: methanol: ammonia (33%) (4: 2.5: 2.5:1, v/v/v) for the investigated mixtures, where the R_f-values were found to be '0.670 and 0.250' and '0.445 and 0.789' for the components of mixture (A and B), respectively, at ‘240 and "292 and 284 nm"’, as shown in (Figure 4-5).

Methods validation

ICH-guidelines for method validation were followed as shown in (Table 1).

Linearity

For HPLC method, a linear correlation was obtained in the range of '(10–90 μg.ml^{-1}) and (5-70 μg.ml^{-1})' and (10–90 μg.ml^{-1}) for (I), (II) and (III and IV), respectively, with correlation coefficient [r] = 0.9990. While, for TLC-densitometric method, linear correlation was obtained in the range of (2–6 μg.spot^{-1}),(1–5 μg.spot^{-1}) and (0.4–1.8 μg.spot^{-1}) for (I), (II) and (III and IV)), respectively, with correlation coefficient [r] = 0.9990.

Accuracy

Accuracy of the proposed methods was tested by analyzing freshly prepared solutions of the studied drugs in triplicate. The recovery percent and standard deviation revealed excellent accuracy. The results obtained by applying the proposed chromatographic methods were statistically compared with those results obtained by the pharmacopeial[17] and manufacturing method[18]. It was concluded that with 95% confidence, there is no significant difference between them, since the calculated *t* and F values are less than the theoretical values (Table 2).

Repeatability and reproducibility

The intra- and inter-day precision was evaluated by assaying freshly prepared samples in triplicate, as shown in (Table 1).

Specificity

The specificity of the adopted methods was illustrated by complete separation of the studied drugs from each other, as shown in (Figure 1-4). In the HPLC method the Rs-value between (I), (Π) and (III), (IV) was above 2.0, while in the TLC-densitometric method the R_f-values were found to be '0.67 , 0.25' and '0.445 , 0.789', respectively, which ensure complete separation as shown in (Table 3).

Robustness and system suitability of the proposed methods

Robustness of the proposed method was assessed by evaluating the influence of small but deliberate variations of experimental variables as developing system composition, saturation time, and wavelength on the reliability of the method. For the HPLC methods small variations of in the organic strength (65&55%) and flow rate (1.5ml/ min) for mixture A or (2 ml/min) for mixture B had no significant effect on the results. For the TLC-densitometric methods small changes in saturation time (35 & 25 min), and wavelength (238 & 242 nm.) for mixture A and (290 & 295 nm.) for mixture B didn't significantly affect results.

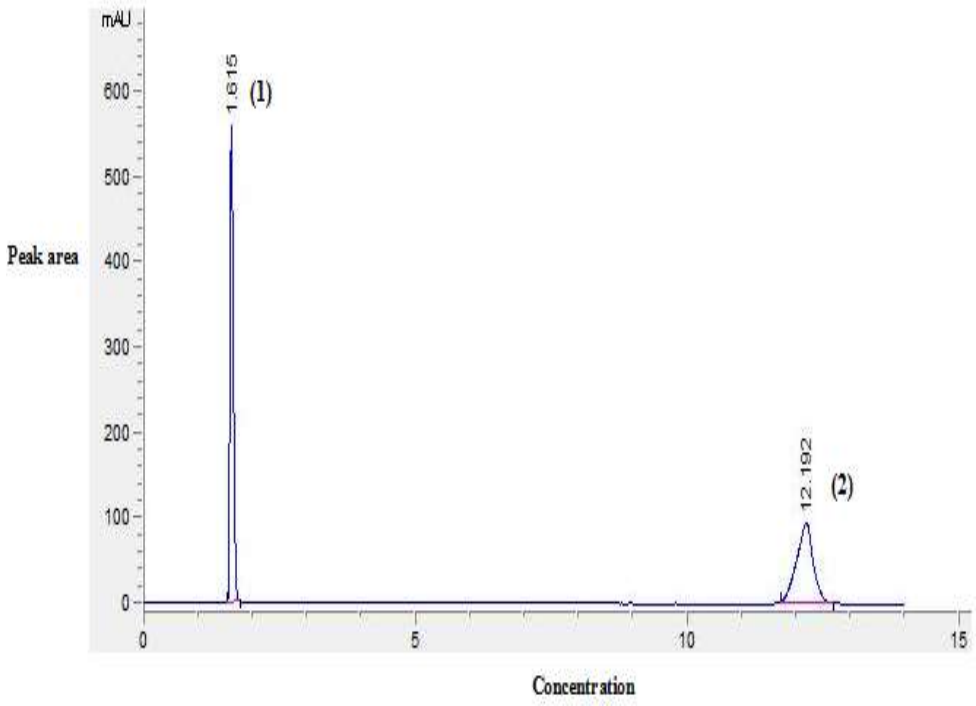


Figure 2: HPLC chromatogram of mixture solution of containing Levamisole HCl (1) and Closantel Sodium (2), 40.0 µg.ml^{-1}.

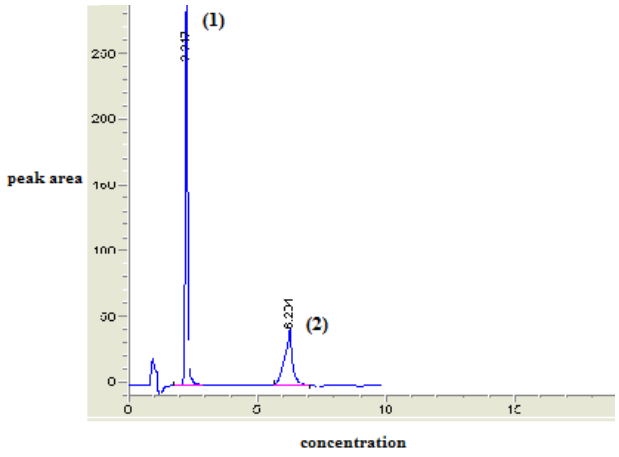


Figure 3: HPLC chromatogram of mixture solution of containing Fenbendazole (1) and Rafoxanide (2), (each, 20.0 µg.ml^{-1}).

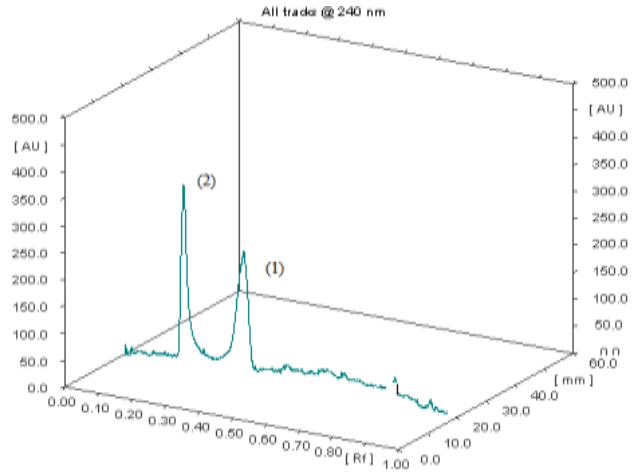


Figure 4: TLC chromatogram of mixture solutions of Levamisole HCl (1) and Closantel Sodium (2) (each, 300 µg.ml^{-1}), at 240 nm.

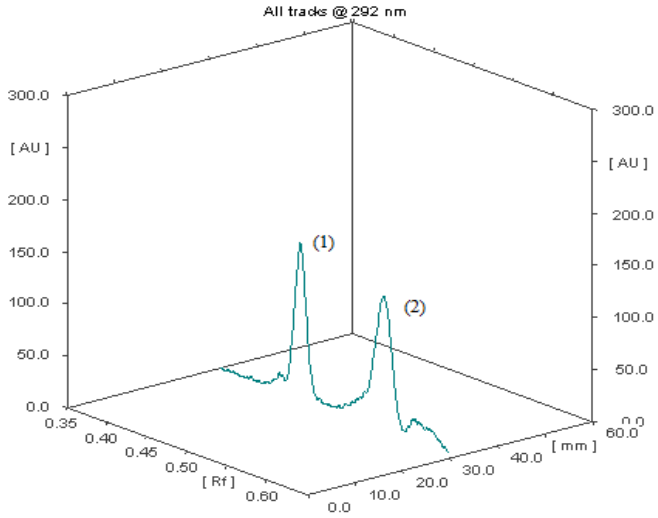


Figure 5: TLC chromatogram of mixture solutions of Fenbendazole (1) and Rafoxanide (2), (each, 100 µg.ml^{-1}), at 292 nm.

Table 1: Validation report of proposed HPLC and TLC-densitometric methods for determination of Levamisole HCl (I), Closantel sodium (II), Fenbendazole (III) and Rafoxanide (IV)

Parameters	HPLC method				TLC-densitometric method			
	(I)	(II)	(III)	(IV)	(I)	(II)	(III)	(IV)
	Concentration in µg.ml^{-1}				Concentration in µg.spot^{-1}			
Linearity	10-90	5-70	10-90	10-90	2-6	1-5	0.4 -1.8	0.4 -1.8
Slope[(b)]	1.27	107.2	31.773	15.229	6.302	8.513	48.648	15.67
Intercept[(a)]	-0.058	-85.95	30.62	1.454	447.5	16	66.433	564.8
Correlation coefficient(r)	*0.9995*	*0.9999*	*0.9997*	*0.9999*	*0.9990*	*0.9990*	*0.9994*	*0.9991*
Accuracy [(b)]	99.75 ±1.14	101.1 ±0.95	99.89 ±0.69	99.87 ±0.96	100.72 ±0.53	99.1 ±0.35	100.76 ±1.39	98.2 ±0.52
Precision:								
Repeatability[b]	99.4 ±1.6	100.0 ±1.33	99.5 ±1.52	99.65 ±0.19	99.2 ±0.79	99.03 ±1.5	98.96 ±1.3	97.4 ±0.38
Intermediate precision[b]	99.98 ±1.2	99.6 ±1.3	100.3 ±1.87	99.51 ±0.52	99.96 ±1.8	100.7 ±0.87	100.83 ±1.61	98.7 ±1.5

[a] Regression equation : A= a + bc, where A = peak area and c = the concentration (µg.ml^{-1})

[b] Mean ± S.D.

Table 2: statistical comparison between the proposed methods and the comparative methods[(17,18)]

parameters	HPLC method				TLC-densitometric method				comparative methods			
	(I)	(II)	(III)	(IV)	(I)	(II)	(III)	(IV)	(I)[a]	(II)[a]	(III)[a]	(IV)[c]
Mean%	99.94	100.4	99.89	99.78	100.9	100.48	100.76	99.06	100.25	99.12	100.04	100.73
S.D.	0.83	1.6	0.69	0.96	1.38	1.33	1.39	1.25	0.39	0.72	0.46	1.5
n	**5**											
Variance	0.68	2.6	0.48	0.92	1.93	1.78	1.95	1.58	0.15	0.52	0.213	2.4
Student's *t*-test (1.86)	0.599	0.213	0.772	0.47	0.509	0.136	0.47	0.23	-	-	-	-
F-test (6.39)	0.036	0.215	0.615	0.55	0.144	0.336	0.196	0.79	-	-	-	-

Values in parenthesis are the theoretical values of *t* and *F* at P=0.05.

Table 3: Determination of Levamisole (I), Closantel (II), Fenbendazole (III) and Rafoxanide (IV) in laboratory prepared mixtures by proposed methods.

HPLC method								TLC-densitometric method							
Conc. in µg.ml^{-1}		% Recovery*		Conc. in µg.ml^{-1}		% Recovery*		Conc. in µg.ml^{-1}		% Recovery*		Conc. in µg.ml^{-1}		% Recovery*	
I	**II**	**I**	**II**	**III**	**IV**	**III**	**IV**	**I**	**II**	**I**	**II**	**III**	**IV**	**III**	**IV**
40.0	40.0	98.5	98.4	50.0	50.0	97.80	98.28	600	100	99.9	101	1.2	1.2	100.36	98.16
20	50	101.4	98.6	20	60	101.7	98.47	300	300	101.4	98.9	0.4	1.8	100.52	99.01
80	20	98.0	98.0	60	20	99.72	99.16	200	500	99.8	98.5	1.8	0.4	99.13	99.25
Mean		99.3	98.33			99.51	98.63			100.4	99.5			100.0	98.8
±SD		1.8	0.3			1.62	0.46			0.89	1.4			0.76	0.57

*Mean of three determinations.

Table 4a: Determination of Levamisole HCl(I) , Closantel Sodium (II) in pharmaceutical formulation, using the proposed HPLC method and application of standard addition technique

Pharmaceutical formulation	%Found* ±S.D.		Standard addition technique							
			Taken µg.ml^{-1}		Added µg.ml^{-1}		Found µg.ml^{-1}		%Recovery	
	I	**II**	**I**	**II**	**I**	**II**	**I**	**II**	**I**	**II**
Vermisantel complex® suspension batch number: 'C002'	99.26 ±0.55	99.13 ±0.58	40	20	10	10	9.89	10.2	98.9	102
					20	20	19.72	20.28	98.6	101.4
					30	30	29.67	30.57	98.9	101.9
Mean ± S.D									98.8± 0.17	101.7± 0.32

*The mean percentage of three separate determinations of pharmaceutical the preparation.

Table 4b: Determination of Levamisole HCl(I) , Closantel Sodium (II) in pharmaceutical formulation, using the proposed TLC-densitometric method and application of standard addition technique:

Pharmaceutical formulation	%Found* ±S.D.		Standard addition technique							
			Taken µg.ml^{-1}		Added µg.ml^{-1}		Found µg.ml^{-1}		%Recovery	
	I	II	I	II	I	II	I	II	I	II
Vermisantel complex® suspension	100.26	98.8	200	100	200	100	200.24	101	100.12	101
batch number: 'C002'	±1.3	±1.2			300	200	299.7	199.2	99.9	99.6
					400	300	39.2	301.5	98	100.5
Mean ± S.D									99.34 ± 1.1	100.4 ± 0.71

*The mean percentage of three separate determinations of pharmaceutical the preparation.

Table 4c: Determination of Fenbendazole (III) and Rafoxanide (IV) in pharmaceutical formulation, using the proposed HPLC method and application of standard addition technique

Pharmaceutical formulation	%Found* ±S.D.		Standard addition technique							
			Taken µg.ml^{-1}		Added µg.ml^{-1}		Found µg.ml^{-1}		%Recovery	
	III	IV	III	IV	III	IV	III	IV	III	IV
Curafluke®	99.755 ±2.18	98.63 ±0.339	20	20	20	20	20.39	19.72	101.97	98.60
suspension 5%					50	50	50.89	50.68	101.79	101.36
B.N.(MO95)					70	70	69.29	71.26	98.98	101.8
Mean ± S.D									100.91 ±1.67	100.58 ± 1.73

*The mean percentage of three separate determinations of pharmaceutical the preparation.

Table 4d: Determination of Fenbendazole (III) and Rafoxanide (II) in pharmaceutical formulation, using the proposed TLC-densitometric method and application of standard addition technique.

Pharmaceutical formulation	%Found* ±S.D.		Standard addition technique							
			Taken µg.ml^{-1}		Added µg.ml^{-1}		Found µg.ml^{-1}		%Recovery	
	III	IV	III	IV	III	IV	III	IV	III	IV
Curafluke®	99.09 ±0.72	99.27 ±0.43	50	50	20	20	19.53	19.59	97.63	97.99
suspension 5%					50	50	51	50.23	102%	100.46%
B.N.(MO95)					70	70	68.39	69.23	97.7%	98.9%
Mean ± S.D									98.97 ± 2.72	99.08 ± 1.29

[a] British pharmacopoeia(vet)2014

(I) dissolve 0.2 gm in 30-ml of anhydrous acetic acid R, titrate with 0.1 M perchloric acid, determine the End point potentiometrically (1-ml of 0.1 M perchloric acid is equivalent to 29.94 mg of $C_{15}H_{13}N_3O_2S$).

(II) dissolve 0.500 gm in 50-ml of a mixture of 1 volume of anhydrous acetic acid R and 7 – volumes methyl ethyl ketone R, titrate with 0.1 M perchloric acid, determine the end point potentiometrically (1-ml of 0.1 M perchloric acid is equivalent to 68.5 mg of $C_{22}H_{13}Cl_2I_2N_2NaO_2$).

(III) dissolve 0.15gm in 50-ml of a mixture of 1-volume of anhydrous acetic acid R and 7-volumes methyl ethyl ketone R, titrate with 0.1 M perchloric acid , using 0.2 ml of naphtholbenzein solution R as indicator (1-ml of 0.1 Mperchloric acid is equivalent to 20.43 mg of $C_{11}H_{12}N_2S$).

[b] manufacturing method

(IV) dissolve 0.01 gm of Rafoxanide in [50% dimethyl formamide and 50% ethanol]as a solvent then take 5-ml in 50-ml volumetric flask and measure at 282 nm.

Standard addition technique

The proposed methods were applied for determination of the studied drugs in their commercial products, where satisfactory results with good agreement with the labeled amounts were obtained as shown in (Table 4a-4d). Moreover; to check the validity of the adopted methods, the standard addition technique was applied by adding known amounts of the studied drugs to a fixed concentrations of analyzed suspension, as shown also in (Table 5a-5d), suggesting that; there is no interference from any excipients, which are normally present in the commercial ones.

CONCLUSION

The proposed methods are precise, specific, accurate and reproducible, where the studied drugs can be determined in bulk powder and in pharmaceutical formulation in presence of each other by the proposed HPLC and TLC-densitometric methods. ICH-guidelines were followed throughout the study for method validation and the suggested methods can be applied for routine quality and control analysis.

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