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SHORT COMMUNICATION

A HPLC-UV method for quantification of ivermectin in solution from veterinary drug products

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The HPLC conditions for analysis of ivermectin in solutions dosage forms of commercial anthelmintics are different for each product. The purpose of this study was to establish a standardized chromatographic method for the quantification of ivermectin in solution. The separation was achieved on Waters Xbridge C18 column (4.6×150 nm, 5 μm) using different kinds of mobile phase composed of water/methanol/acetonitrile (15/34/51, v/v and 19.5/27.5/53, v/v), with UV detection at wavelengths 245 nm and 254 nm. A total of five commercial ivermectin in solution samples were analyzed. In this study, the optimal chromatographic conditions for analysis of ivermectin in solution were mobile phase of water/methanol/acetonitrile (15/34/51, v/v) at a flow rate of 1.0 mL/min and a detection wavelength of 245 nm using a Waters Xbridge C18 column (4.6×250 nm, 5 μm) at a column temperature of 25℃. The linearity was observed in the concentration range of 50～150 μg/mL, with a correlation coefficient, r^2= 0.99999. The limit of detection and the limit of quantification were 0.88 and 2.68 μg/mL, respectively. The accuracy (% recovery) was found to be 98.9 to 100.3%. Intra-day and Intermediate precisions with relative standard deviations were less than 1.0%. The content of ivermectin for five market samples ranged 91.2～102.7%. The proposed method was also found to be robust, therefore, the method can be used for the routine analysis of ivermectin in solutions dosage forms.

Key Words: Ivermectin, Veterinary, HPLC, Validation

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INTRODUCTION

Ivermectin (5-O-dimethyl-22,23-dihydroaverrmectin) is an antiparasitic and anthelmintic agent that kills various parasites such as lice, mange and wireworms etc (Waldia et al, 2008; Patel et al, 2015). It has been used in domestic animals for treatment of endoparasites and ectoparasites (Bark et al, 2007; Rao et al, 2017). Many analytical methods including thin-layer chromatography (TLC), high performance liquid chromatography (HPLC), high performance liquid chromatography with mass-spectrometry (HPLC-MS) or tandem mass spectrometry (MS/MS) have been developed for the analysis of ivermectin (Waldia et al, 2008). Ivermectin can be administered orally, subcutaneously, intramusculary and applied to the skin (Bark et al, 2007). Ivermectin in solution dosage form applied to the skin is easy to use in livestock farms and its use is increasing day by day (Bark et al, 2007). Currently, there are 11 ivermectin topical solutions from 11 companies registered with the Animal Drug Associations (based on sales performance from 2010 to 2019). All 11 products use HPLC to quantify ivermectin. However, the HPLC conditions such as mobile phase, flow rate and wavelength are different for each product, quality control is not performed with the same test method.

Therefore, we aimed to establish a standardized chromatographic method for the quantification of ivermectin in solution and validate the established assay.

MATERIALS AND METHODS

HPLC apparatus

Agilent 1260 Infinity II series HPLC system equipped with a model G7111A pump, a model G7129A autosampler, a model G7117C UV-detector and Chemstation Software (Version Rev C.01.10 (236)) were used.

Reagents and chemicals

Ivermectin was purchased from the United States Pharmacopeia (USP) (Rockville, MD, USA), with 90.8% of purity. HPLC grade methanol was purchased from J. T. Baker Inc. (USA), Acetonitrile from Merck Ltd. (Germany). HPLC grade water was used from Fisher Scientific co. (Korea).

Preparation of standard solutions

Stock solution for ivermectin was prepared in mobile phase to get concentration of 1,000 μg/mL. Ivermectin standard working solution was prepared in mobile phase at a final concentration of 100 μg/mL. The stock solution was diluted with mobile phase into a series of standard solutions (50, 80, 100, 120 and 150 μg/mL).

Preparation of samples

Among eleven ivermectin in solutions dosage forms registered with the Animal Drug Association, five commercially available anthelmintic formulations (5 solutions) were selected and purchased except for six formulations (solutions) which production was stopped. Five commercial anthelmintic formulations contain 5 g ivermectin per liter of product. 1 mL of solutions were transferred into a 50 mL volumetric flask each, and filled with mobile phase to obtain a concentration of 100 μg/mL. These samples were sonicated for 15 min and then the samples were filtered using 0.45 μm membrane.

Chromatographic conditions and HPLC method optimization

Chromatographic separation was achieved on two mobile phases - water/methanol/acetonitrile (15:34:51, v/v) and water/methanol/acetonitrile (19.5:27.5:53, v/v)

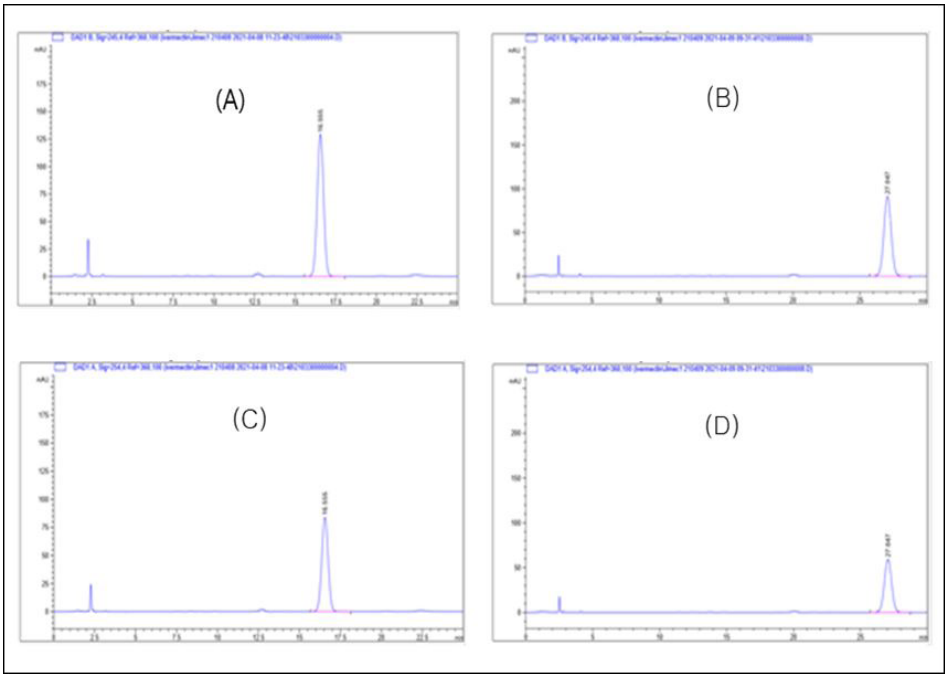

Fig. 1. Chromatograms for 100 ug/mL ivermectin standard solution: (A) Water:MEOH:ACN (15:34:51), 245 nm; (B) Water:MEOH:ACN (19.5:27.5:53), 245 nm; (C) Water:MEOH:ACN (15:34:51), 254 nm; (D) Water:MEOH:ACN (19.5:27.5:53), 254 nm.

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using Waters X Bridge C18 column (4.6×150 nm, 5 μm) at a flow rate of 1.0 mL/min, with UV detection at wavelengths 245 nm and 254 nm and a column temperature of 25℃.

System suitability parameters

System suitability test parameters such as peak area (mAU), retention time (Rt), number of theoretical plates (N) and tailing factor (T) for working solution were determined.

Analytical method validation

We determined parameters for method validation, including system suitability, linearity, limit of Detection (LOD), limit of quantification (LOQ), precision, accuracy and robustness according to the International Conference on Harmonization (ICH) Guidelines. System suitability was determined by six replicates of standard working solution and evaluated by calculating of the relative standard deviation (RSD) of the peak area, retention time, theoretical plates and tailing factor. Linearity was calculated by plotting peak areas versus five different concentrations (50, 80, 100, 120 and 150 ug/mL) and found out a linear equation with correlation coefficient (R^2). LOD and LOQ were calculated to determine the sensitivity of the method. Precision was evaluated for repeatability and intermediate precision. Repeatability was determined by analyzing six replicates of ivermectin standard solution in the range 50, 100 and 150 μg/mL for ivermectin. Intermediate precision was determined by analyzing six replicates of ivermectin standard solution in the range 80, 120 and 120 μg/mL for ivermectin on different days. The precision of an analytical procedure was expressed as RSD %. Accuracy was determined by calculating recovery of ivermectin and conducted by adding known amounts of ivermectin standard to the in three replicates. The percent recovery was calculated by the following formula: recovery (%)= (experimental concentration/actual concentration)× 100. The robustness was determined by assaying six replicates of standard solution (100 μg/mL of ivermectin) in minor changes of method parameters such as flow rate, column temperature, wavelength and mobile phase ratio.

Table 1. Optimized chromatographic conditions

Parameters	Chromatographic conditions
Column	Waters X bridge C_{18} 4.6×150 mm, 5 μm
Mobile phase	Water:Methanol:Acetonitrile (15:34:51)
Wavelength	245 nm
Column oven	25℃
Flow rate	1.0 mL/min
Injection volume	20 μL
apparatus	Agilent 1260 Infinity II series, USA

RESULTS AND DISCUSSION

HPLC chromatographic conditions optimization

Under the attempted chromatographic conditions, the correlation coefficients values were 0.9997 or greater.

Table 2. System suitability tests and chromatographic conditions

Parameters	Value*	RSD (%)	Acceptable limit
Retention time (min)	16.405	0.09	RSD ≤2
Peak area	3483	0.1	RSD ≤2
No. of theoretical plates	10691	0.48	>2000
Tailing factor	1.020	0.52	≤2

*Mean of six values.

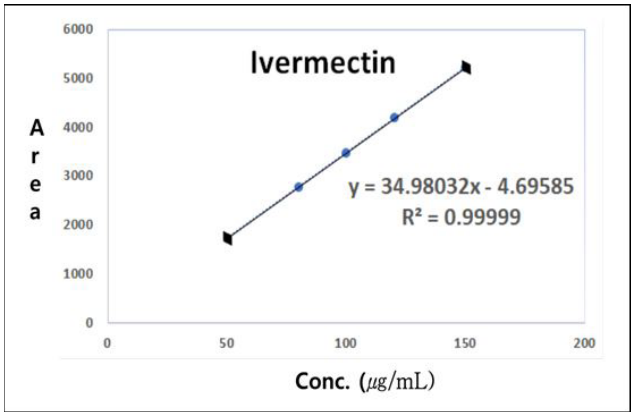

Fig. 2. Calibration graph for ivermectin standard.

The concentrations of ivermectin were close to those when tested according to the standard test methods for products, and were within the acceptable ranges (from 90 to 120%) of the labeled amount. The results show that both wavelengths (245 nm and 254 nm) can be used to measure the amount of ivermectin, but at a wavelength of 254 nm, the peak area value is smaller for the same concentration; the peak shape obtained at 245 nm was better. In the case of the mobile phase, the C18 column (4.6×150 nm, 5 μm) was used and the retention time was shorter (16.3 to 16.5 min) for the composition of water/methanol/acetonitrile (15/34/51, v/v), than that of the composition of water/methanol/acetonitrile (19.5/27.5/53, v/v) (26.9 to 27.1 min); consequently, the analysis could be conducted faster (Fig. 1). Therefore, the optimized HPLC chromatographic conditions for the analysis of ivermectin in solution were as follows: mobile phase of water/methanol/acetonitrile (15:34:51, v/v) at a flow rate of 1.0 mL/min and a detection wavelength of 245 nm using a Waters XBridge C18 column (4.6×150 nm, 5 μm) at a column temperature of 25℃ (Table 1).

The optimization of chromatographic conditions led to more efficient and faster separation of ivermectin from commercial ivermectin topical solutions with different HPLC conditions.

System suitability

RSD % of peak areas, retention times and tailing factors were less than 1.0. The theoretical plates were >10,000 (Table 2). The values of system suitability parameters were all within the acceptable limits and indicated that the system was suitable for the quantification of ivermectin.

Linearity

The calibration curve was obtained in the concen-

Table 3. Summary of validation parameters

Parameters	Ivermectin
Linear range (μg/mL)	50～150
Correlation coefficient (r^2)	0.99999
LOD (μg/mL)	0.88
LOQ (μg/mL)	2.68
Intra-day precision (repeatability, RSD %)	0.09～0.17
Intermediate precision (inter-day precision, RSD %)	0.07～0.20
Accuracy (% Recovery)	98.9～100.3

Table 5. Concentration of ivermectin in marketed solutions

Sample No.	Label concentration (g/L)	The standard test method (μg/mL)	The proposed method	
			Quantity in sample (μg/mL)	Concentration (%)
1	5	4.99	4.96	99.5
2	5	5.11	5.13	102.7
3	5	4.75	4.78	95.6
4	5	4.70	4.56	91.2
5	5	4.80	5.08	101.5

Table 4. Robustness tests for Ivermectin

Parameters	Variation	Tailing factor		Theoretical plates	
		Value*	RSD (%)	Value*	RSD (%)
Flow rate (mL/min)	0.9	1.13	0.45	7225	0.49
	1.1	1.13	0.59	6577	0.55
Column temperature (℃)	23	1.13	0.47	7355	0.35
	27	1.14	0.63	6728	0.22
Detection wavelength (nm)	243	1.14	0.47	6761	0.99
	247	1.14	0.46	6757	1.00
Mobile phase composition-Methanol content (% v)	32	1.14	0.34	7254	0.89
	36	1.13	0.42	7254	0.29

*Mean of three values.

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tration range of 50~150 μg/mL. The linear regression equation for ivermectin was Y=34.98032x-4.69585 and the correlation coefficient (R^2) was 0.99999 (Fig. 2). The correlation coefficient was satisfactory (R^2 >0.999) and indicated that this method was reliable for quantitative detection.

LOD and LOQ

Limit of detection (LOD) is the lowest concentration of analyte that can be detected, and limit of quantification (LOQ) is the lowest concentration of analyte that can be quantified with acceptable accuracy and precision. The LOD and LOQ for ivermectin were 0.88 μg/mL and 2.68 μg/mL, respectively (Table 3). The results showed the good sensitivity of the proposed method.

Precision and accuracy

For the accuracy of the method, a recovery test was conducted by adding known amounts of ivermectin standard (80, 100 and 120 μg/mL) to the sample (19.66 μg/mL of ivermectin) and analyzing three replicates. To determine the precision of the method, repeatability (intra-day precision) and intermediate precision (inter-day precision) were evaluated. In this study, the mean recovery rate of ivermectin was 98.9% to 100.3% and the % RSD was not >2% (Table 3), indicating that the method was capable of showing good accuracy and precision.

Robustness

The values of tailing factor, theoretical plates and % RSD were within the acceptance ranges (Table 4). So, the method was shown to be robust for minor changes in method parameters such as flow rate (±0.1 mL/min), column temperature (±2℃), wavelength (±2 nm) and mobile phase composition (MEOH content ±2% v).

Analysis of Sample

The optimized HPLC procedure was applied for the analysis of 5 commercially available products. The contents of ivermectin were found to be in the range from 91.2% to 102.7% and were within the acceptable ranges (90~120%) for the percent of the labeled amount (Table 5).

CONCLUSIONS

The optimized HPLC chromatographic method was established and validated for the assay of ivermectin in solutions. The results of this study showed that this HPLC method is simple, rapid, accurate, precise, sensitive and robust. Therefore, this method can be performed for routine quality control analysis of ivermectin from commercial ivermectin topical solutions with different HPLC conditions.

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CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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