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A Simple RP-HPLC Method for Estimation of Triclabendazole and Ivermectin in a Pharmaceutical Suspension Dosage Form.

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Abstract

A simple method has been developed for the estimation of triclabendazole and ivermectin in a pharmaceutical suspension dosage form present in 50:1 ratio respectively, by reversed-phase High-Performance Liquid Chromatography (HPLC). The isocratic LC analysis was performed on Vydac, C-18, (250 X 4.6 mm, 5 µ) using mobile phase comprising of acetonitrile, methanol and water at a flow rate of 1.5 ml/minute. Quantification was carried out by using UV detector at 254 nm and the run time was 15 minutes. Linearity was found to be 0.06-0.14 and 0.03-0.05 mg/ml with a correlation co-efficient, r= 0.998 and 0.999 for triclabendazole and ivermectin respectively. The percentage recovery values were found to be within the range of 98-102. The analytical method has been successively applied to pharmaceutical formulation and was validated according to International Conference on Harmonization (ICH) guidelines.

Key Words

Ivermectin, Triclabendazole, Pharmaceutical suspension.

Introduction

Triclabendazole (TCBZ) has been the drug of choice to treat liver fluke infections in livestock for more than 20 years, due to its high activity against both adult and juvenile flukes. TCBZ is a benzimidazole derivative and, by analogy with what is known about other benzimidazole drugs, it would be anticipated that TCBZ might bind to the β-tubulin molecule and so disrupt microtubule-based processes[1]. On the other hand ivermectin (IVR) has potent activity at GABA receptors in both invertebrates and mammals, and GABA is known to be the primary inhibitory neurotransmitter in the nematode somatic neuromuscular system. However, subsequent work by Merck scientists identified glutamate-gated Cl^- channels as the more likely physiological targets of ivermectin and related drugs[2]. Substantial *in-vivo* trials have been conducted to prove the effectiveness of this combination as flukicidal [3-5]. Three fold higher plasma availability of ivermectin was reported when co-administered with TCBZ. Similarly, Influential higher plasma concentrations of TCBZ and its metabolites were also reported on co-administration with IVR[6]. Keeping the high plasma availability of IVR in combination with TCBZ as key factor, a pharmaceutical suspension dosage form was prepared with TCBZ and IVR as actives in the ratio of 50:1 respectively as a flukicide. Thorough literature survey reveals no HPLC method is available for estimation of TCBZ and IVR in pharmaceutical suspension dosage form. However, HPLC with UV detection, liquid chromatography combined with electrospray ionization mass spectrometry, for estimation of TCBZ alone or simultaneously with its major metabolites have been reported[7-9]. Analytical methods including high-performance liquid chromatography and fluorescence detection, High-Speed Counter-Current Chromatography (HSCCC), capillary electrophoresis have been reported for the estimation of IVR alone or simultaneous with its metabolites in biological fluids/formulations[10-14]. In the current study, an analytical method by HPLC was described for estimation of TCBZ and IVR in a pharmaceutical suspension, present in the ratio of 50:1 respectively. Ivermectin contains at least 80% of 22, 23-dihydroavermectin B_{1a} and not more than 20% of 22, 23-dihydroavermectin B_{1b}[15]. The major constituent 22, 23-dihydroavermectin B_{1a} is measured and referred as ivermectin in the study.

Materials and Methods

Apparatus

Shimadzu HPLC equipped with quaternary pump, PDA detector and auto sampler, Agilent HPLC of 1100 series equipped with multi wavelength detector and manual injection port, 20 µl glass syringe,

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Vydac, C-18, (250 X 4.6 mm, 5 µ) column is used. Shimadzu Libror AEG-220 electronic balance is used for weighing the materials.

Chemicals and reagents

Triclabendazole and ivermectin were procured from Sigma Aldrich. Acetonitrile (HPLC grade), methanol (HPLC grade), propylene glycol (AR grade) and HPLC grade water prepared from Millipore water purification system were used for the study. Membrane filters (nylon 0.45 micron) and syringe filters (Hydrophilic PVDF, 0.45 micron) were purchased from Millipore (India) Pvt. Ltd., Bangalore.

Preparation of Standard solutions

Standard stock solutions of pure drugs were prepared separately in methanol containing 2 mg/ml of triclabendazole and 0.1 mg/ml of ivermectin and filtered through a 0.45 micron membrane filter.

Preparation of Calibration Curve

Triclabendazole For preparation of the calibration curve of TCBZ, aliquots 0.3, 0.4, 0.5, 0.6 and 0.7 ml of the standard stock solution of TCBZ (2 mg/ml) were transferred to a series of 10 ml volumetric flasks and the volume was made up to the mark with methanol.

Ivermectin

For preparation of the calibration curve of IVR, aliquots 1.5,1.75, 2.0, 2.25 and 2.5 ml of the standard stock solution of IVR (0.1 mg/ml) were transferred to a series of 5 ml volumetric flasks and the volume was made up to the mark with methanol.

Analysis of suspension dosage form

Preparation of sample solutions (Triclabendazole)

An accurately weighed quantity of suspension equivalent to 50 mg of TCBZ was dissolved in 10 ml of methanol and sonicated for 5 minutes. The solution was filtered through Whatmann filter paper no.41.The residue was washed with 5 ml portions of methanol twice and the combined filtrate was made up to 25 ml with methanol (2mg/ml). Further, 0.5 ml of the above solution was made up to 10 ml with methanol (0.1mg/ml). The solution was then filtered through 0.45 µ syringe filter and analyzed by RP-HPLC.

Preparation of sample solutions (Ivermectin)

An accurately weighed quantity of suspension equivalent to 2.5 mg of IVR was transferred into 25 ml volumetric flask and dissolved in 2.5 ml of propylene glycol and sonicated for 5 minutes. The solution was made up to the mark with water and sonicated for 10 minutes. The above solution was centrifuged for 5 minutes at around 5000 RPM. 2 ml of the supernatant was taken into a 5ml volumetric flask and the final concentration was brought to 0.04 mg/ml with methanol. The solution was then filtered through 0.45 µ syringe filter and analyzed by RP-HPLC.

Results and Discussion

Method development

It was challenging to develop a method for simultaneous estimation of TCBZ and IVR as they are present in the ratio of 50:1 in the formulation. In order to overcome the huge differences occurred in the peak heights due to the concentration of TCBZ and IVR in the formulation, a selective sample preparation method is developed for IVR. Mobile phase containing Acetonitrile: Methanol: Water in the ratio of 60:30:10 at a flow rate of 1.5 ml/minute were selected as it was found ideal to resolve TCBZ and IVR. HPLC conditions were mentioned in Table No. 1.

System suitability

System suitability studies were carried out on a freshly prepared solution of standards and the results are tabulated in Table No. 2.

Analytical method validation

Specificity

Spiked a known quantity of working standard into placebo and analyzed using PDA detector. No interfering peaks observed at the retention time of TCBZ and IVR. Percentage recovery of TCBZ and IVR spiked quantity was found to be in the range of 98-102%.

Precision

Precision of the method was tested by six repeated injections of standard and sample solutions and measuring the peak areas and calculated the percentage assay. Intermediate precision was established by performing the analysis of the formulation in replicates by different analyst and reproducibility, using two different instruments in different labs. The results were found to be satisfactory as the RSD values were found to be less than 2%. The results of repeatability, intermediate precision and reproducibility were tabulated in Table No. 3.

Accuracy

To check the accuracy of the developed methods and to study the interference of formulation additives,

analytical recovery experiments were carried out by standard addition method at three different levels. From the total amount of drug found, the percentage recovery was calculated and the results were found to be within the acceptable limit of 98-102%. The results were reported in Table No. 4.

Robustness

The robustness of a method is its ability to remain unaffected by small deliberate variations in the method parameters. Robustness of the method was checked by making slight changes in the composition of mobile phase and by changing the column temperature. The results were found to be within the specified limits for triclabendazole (4.62 – 5.37 %W/V) and ivermectin (0.092 - 0.107 %W/V). The results were reported in Table No. 5.

Conclusion

The validated RP-HPLC method employed here proved to be simple, fast, accurate, precise and sensitive enough. Selectivity experiment showed that there is no interference or overlapping of the peaks either due to diluents with the main peak of TCBZ and IVR. The percentage RSD for precision is <2 which confirms that method is sufficiently precise. The proposed method is simple, fast, accurate, and precise and can be used for routine analysis in quality control of TCBZ and IVR in pharmaceutical suspension dosage form.

References

1. Brennan G P, Fairweather I, Trudgett A, Hoey E, McCoy, McConville M, Meaney M, Robinson M, McFerran N, Ryan L, Lanusse C, Mottier L, Alvarez L, Solana H, Virkel G, Brophy P M, Understanding triclabendazole resistance, Experimental and Molecular Pathology, 2007, 82,104–109.
2. Timothy G. Geary, ivermectin 20 years on: maturation of a wonder drug, Trends in Parasitology, 2005, 21, 11.
3. Lifschitz A, Virkel G, Ballent M, Sallovitz J, Lanusse C, Combined use of ivermectin and triclabendazole in sheep: in vitro and in vivo characterisation of their pharmacological interaction, Vet J. 2009, 182,2, 261-8.
4. Nasreen S, Khan M. R, Peerzada S, Andrabi S A, Efficacy of different anthelmintic formulations against helminth Infestation in Sheep, vet scan, 2008,3, 2, 29.
5. Stevenson C R, Mahoney R H, Fisara P, Strehlau G, Reichel M P, The efficacy of formulations of triclabendazole and ivermectin in combination against liver fluke (Fasciola hepatica) and gastro-intestinal nematodes in cattle and sheep and sucking lice species in cattle., Aust Vet J. 2002, 80,11, 698-701.
6. Adrian Lifschitz, Guillermo Virkel, Mariana Ballent, Juan Sallovitz , Carlos Lanusse, Combined use of ivermectin and triclabendazole in sheep: In vitro and in vivo characterisation of their pharmacological interaction, The Veterinary Journal, Volume, 2009, 182,2, 261-268.
7. Kazue Takeba, Kenji Fujinuma, Miho Sakamoto, Tomoyuki Miyazaki, Hisao Oka, Yuko Itoh and Hiroyuki Nakazawa., Simultaneous determination of triclabendazole and its sulphoxide and sulphone metabolites in bovine milk by high-performance liquid chromatography, Journal of Chromatography A, 2000,882,1-2,99-107.
8. Mestorino N, Formentini E A, Lucas M F, Fernandez C, Modamio P, Marino Hernández E, Errecalde J O, Pharmacokinetic disposition of triclabendazole in cattle and sheep; discrimination of the order and the rate of the absorption process of its active metabolite triclabendazole sulfoxide, Vet Res Commun, 2008, 32, 21–33.
9. http://www.ema.europa.eu/docs/en_GB/document_library/Maximum_Residue_Limits_-_Report/2009/11/WC500015673.pdf
10. Kjetil Asbakk, Heidi R. Bendiksen, Antti Oksanen, ivermectin in Reindeer Feces: Determination by HPLC, J. Agric. Food Chem., 1999, 47, 3, 999–1003.
11. Dennis Kitzman, She-Yi Wei and Lawrence Fleckenstein, Liquid chromatographic assay of ivermectin in human plasma for application to clinical pharmacokinetic studies, Journal of Pharmaceutical and Biomedical Analysis, 2006, 40, 4,1013-1020.
12. Hisao Oka, Yoshimoto Ikai, Junko Hayakawa, Ken-ichi Harada, Mokoto Suzuki, Atsuko Shimizu, Tomoko Hayashi, Kazue Takeba, Hiroyuki Nakazawa and Yoichiro Ito, Separation of ivermectin components by high-speed counter-current chromatography,

Journal of Chromatography A, 1996, 723, 1,61-68.

13. Degroodt J M, Wyhowski De Bukanski B, Srebrnik S, Determination of ivermectin Residues in Meat and Liver by HPLC and Fluorometric Detection, Journal of Liquid Chromatography & Related Technologies, 1994,17, 6, 1419 – 1426.
14. Kowalski P, Bieniecki M, Oledzka I, Lamparczyk H, Validated capillary electrophoretic method for the analysis of ivermectin in plasma after intragastric administration in pigs and horses, Biomed Chromatogr, 2004, 18, 5, 302-10.
15. The Merck Index, An encyclopedia of chemicals, drugs and biologicals; Thirteenth edition, Merck research laboratories, NJ, USA,2001,939
16. ICH-Q2B Validation of Analytical procedure: Geneva, Switzerland, 1996BS 7501 EN 45001. General Criteria for the operation of Testing Laboratories 1989.
17. ICH, Q2A validation of analytical procedure, Methodology International Conference on Harmonization, Geneva, October 1994.
18. Validation of Analytical procedure Methodology, ICH Harmonized tripartite guidelines, 1996, 1-8.

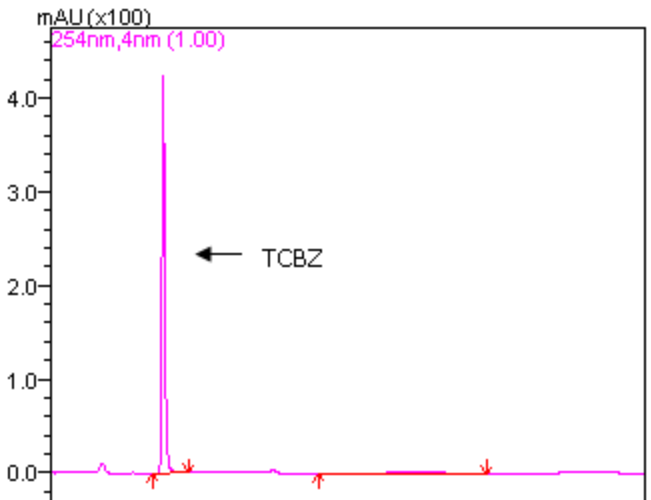

Fig -1(a): Representative chromatogram of TCBZ in the formulation.

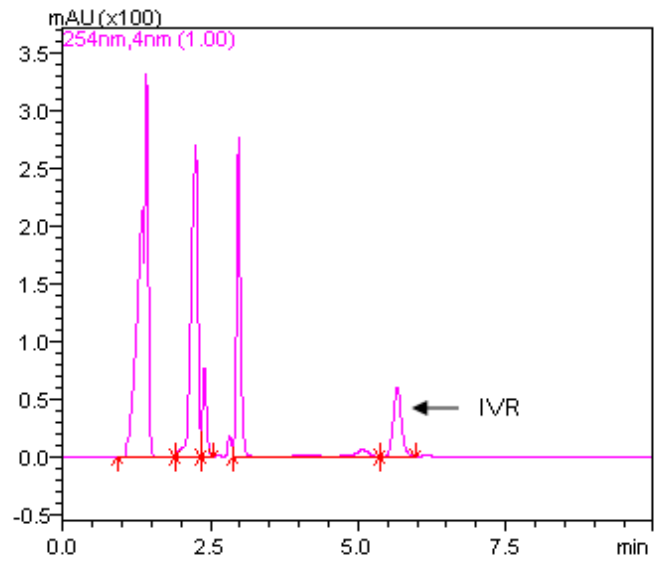

Fig -1(b): Representative chromatogram of IVR in the formulation.

Table No. 1: Chromatographic condition.

Column	Vydac™, RP- C18 column (250 mm x 4.6 mm, 5 micron)
Mobile phase	Acetonitrile:Methanol:Water (60:30:10)
Column Temperature	30 °C
Detector	254 nm
Flow rate	1.5 mL.min^{-1}
Injection volume	20 µL

Table No. 2: System suitability.

Parameters	**TCBZ**	**IVR**
Retention time	2.80	5.66
Theoretical plates	5549.06	7233.3
Tailing factor	1.48	1.14

Table No. 3: Precision.

Parameters		**TCBZ**	**IVR**
Repeatability RSD (n=6)		0.34	0.33
Intermediate precision RSD (n=6)	Analyst-1	0.21	0.33
	Analyst-2	0.19	0.35
Reproducibility RSD (n=6)	HPLC System-1	0.04	0.33
	HPLC System-2	0.74	1.77

n-Number of replicates.

Table No. 4: Accuracy.

Drug	TEST	% Recovery (n=3)	% RSD
TCBZ	Spiked 80 %	99.57	0.17
	Spiked 100%	98.82	0.29
	Spiked 120 %	99.51	0.20
IVR	Spiked 75 %	98.31	0.96
	Spiked 100%	100.27	0.03
	Spiked 125 %	99.20	0.34

n-Number of replicates.

Table No. 5: Robustness.

Parameters	Composition	Assay % W/W	
		TCBZ	**IVR**
Mobile phase-1	Acetonitrile: Methanol: Water (58:32:10)	5.26	0.096
		5.25	0.097
		5.18	0.096
Mobile phase-2	Acetonitrile: Methanol: Water (64:26:10)	5.16	0.094
		5.17	0.095
		5.18	0.095
Column Temperature -1	32 °C	5.27	0.096
		5.22	0.095
		5.23	0.096
Column Temperature -2	28 °C	5.16	0.096
		5.17	0.096
		5.18	0.096

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