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CHARACTERIZATION OF FORCE DEGRADATION ASSAY METHOD EVALUATION FOR SIMULTANEOUS ESTIMATION OF ALBENDAZOLE AND IVERMECTIN IN VETERINARY DOSAGE FORM USING UPLC-MS/MS^N

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ABSTRACT

In order to achieve the separation under the optimized conditions after experimental trials that can be summarized. Stationary phase like Hypersil BDS C18 (100 mm x 2.1 mm, 1.7 µm) column was most suitable one, since it produced symmetrical peaks with high resolution and a very good sensitivity and with good resolution. The flow rate was maintained 1.2 mL min-1 shows good resolution. The PDA detector response of Albendazole and Ivermectin, veterinary dosage form was studied and the best wavelength was found to be 215 nm showing highest sensitivity. The developed & validated RP-UPLC-MSMS^n methods employed here proved to be specific, fast, precise and accurate for the simultaneous estimation and stability indicating assays as well as related substance quantifications of Albendazole and Ivermectin in combine dosage form.

KEYWORDS: Veterinary dosage form, Albendazole and Ivermectin.

INTRODUCTION

Chemical stability of Albendazole and Ivermectin is a matter of great concern as it affects the safety and efficacy of the finished drug product. Forced degradation studies provide data to support identification of possible degradants; degradation pathways and intrinsic stability of the Albendazole and Ivermectin molecule and validation of stability indicating analytical procedures. (ICH Q2 (R1), 2005).

A detailed literature revealed that several analytical methods have been reported for the determination of Albendazole and Ivermectin in pharmaceutical oral dosage forms. In our present knowledge, there is no method reported for the estimation forced degradation studies of Albendazole and Ivermectin in pharmaceutical oral dosage form by UPLC-MS/MS^n.

As per the stringent regulatory requirements recommended by the ICH and regulatory agencies, it is mandatory and important to identify and structurally characterize any impurity formed during production and stability testing, exceeding the identification threshold. Various analytical instruments and advanced hyphenated techniques are routinely used to carry out the impurity profile study.

The present work aims with the development of method to separate the degradation product by preparative UPLC and subjected to ESI-MS/MS. The study describes the separation of different impurities of Albendazole and Ivermectin, as well as the development and validation of a stability-indicating RP-UPLC method for the estimation of degradation and process-related impurities of Albendazole and Ivermectin. Forced degradation studies were performed on the drug product to show the stability-indicating nature of the method. These studies were performed in accordance with established ICH guidelines.

EXPERIMENTAL METHODOLOGY

Preparation of Standard Stock Solution

Preparation of Diluent

In order to achieve the separation under the optimized conditions after experimental trials that can be summarized. Stationary phase like Hypersil BDS C18 (100 mm x 2.1 mm, 1.7 µm) column was most suitable one, since it produced symmetrical peaks with high resolution and a very good sensitivity and with good resolution. The flow rate was maintained 1.0 mL min-1 shows good resolution. The PDA detector response of ALBENDAZOLE and IVERMECTIN was studied and the best wavelength was found to be 275 nm showing highest sensitivity.

The mixture of two solutions 1 M KH_2PO_4-acetonitrile in the ratio of 80:20%v/v. Finally, the pH was adjusted to 7.65 by sodium hydroxide with gradient programming was used as mobile phase at 1.0mL/min was found to be an appropriate mobile phase for separation of ALBENDAZOLE and IVERMECTIN. The column was maintained at ambient temperature.

Preparation of internal standard solution

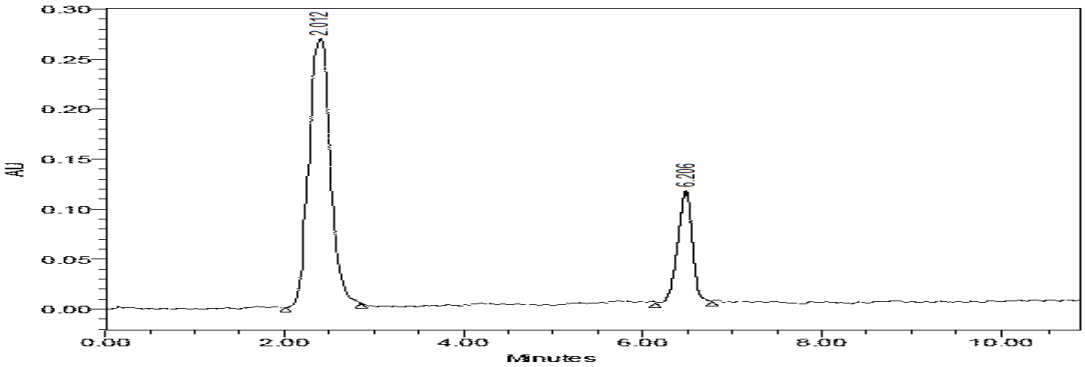
Weighed accurately about 10 mg of ALBENDAZOLE and IVERMECTIN working standard and transfer to 100 ml volumetric flask, add 50 ml of mobile phase and sonicate to dissolve it completely and then volume was made up to the mark with mobile phase to get 100 µg/ml of standard stock solution of working standard. Then it was ultrasonicated for 10 minutes and filtered through 0.20 µ membrane filter.

Preparation of ALBENDAZOLE and IVERMECTIN standard solution

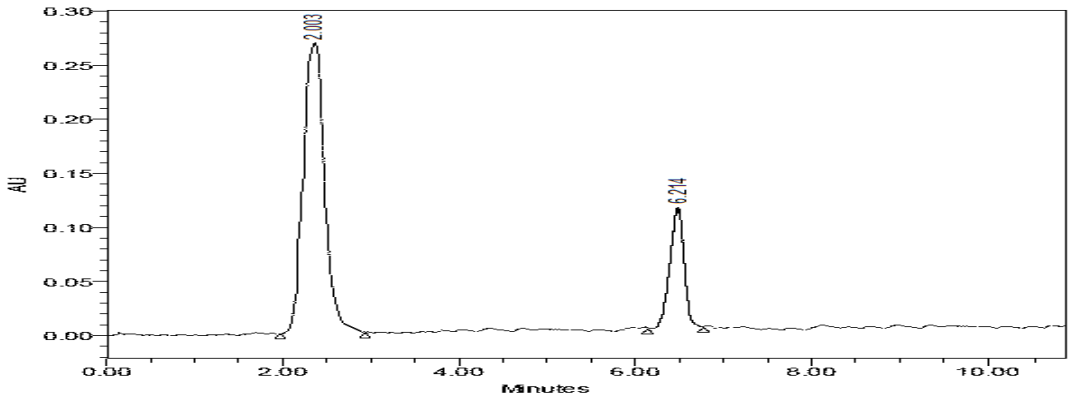
Weighed accurately about 10 mg of ALBENDAZOLE and IVERMECTIN and transfer to 100 ml volumetric flask, add 50 ml of mobile phase and sonicate to dissolve it completely and then volume was made up to the mark with mobile phase to get 100 µg/ml of standard stock solution of working standard. Then it was ultrasonicated for 10 minutes and filtered through 0.20 µ membrane filter.

ALBENDAZOLE and IVERMECTIN	
Method development by UPLC Optimised Trial	
System	UPLC
Stationary Phase	C18
"***Mobile Phase***"	"1 M KH_2PO_4-acetonitrile in the ratio of 80:20%v/v"
Injection volume	20µl
Temperature	Ambient
Flow rate	1 mL/min
UV detection	275nm
Retention Time	ALBENDAZOLE – 2.012 mins; IVERMECTIN – 6.206 mins
Inference	"Better resolution of the peaks with clear base line separation was found."

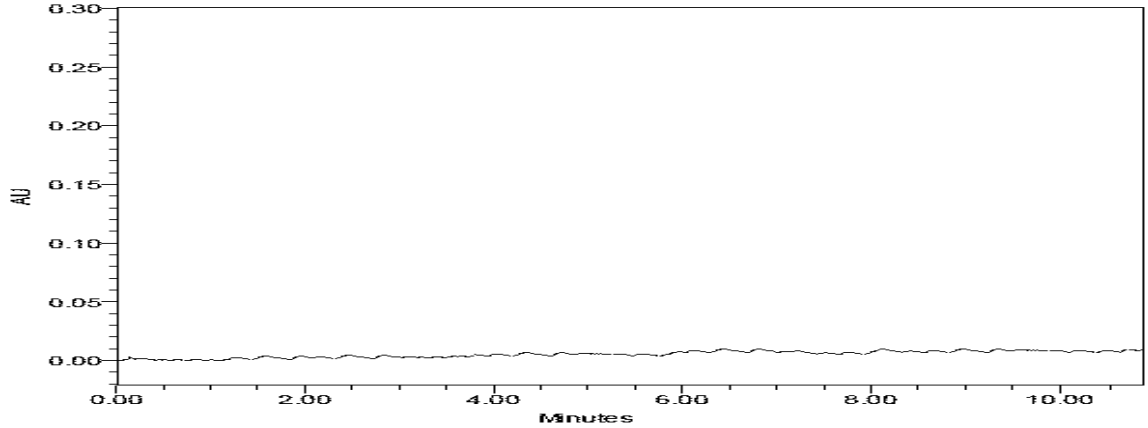
Method development of ALBENDAZOLE and IVERMECTIN in UPLC System



Chromatogram of standard preparation of ALBENDAZOLE and IVERMECTIN (1 M KH_2PO_4-acetonitrile in the ratio of 80:20%v/v)



Chromatogram of test preparation of ALBENDAZOLE and IVERMECTIN (1 M KH_2PO_4-acetonitrile in the ratio of 80:20%v/v)

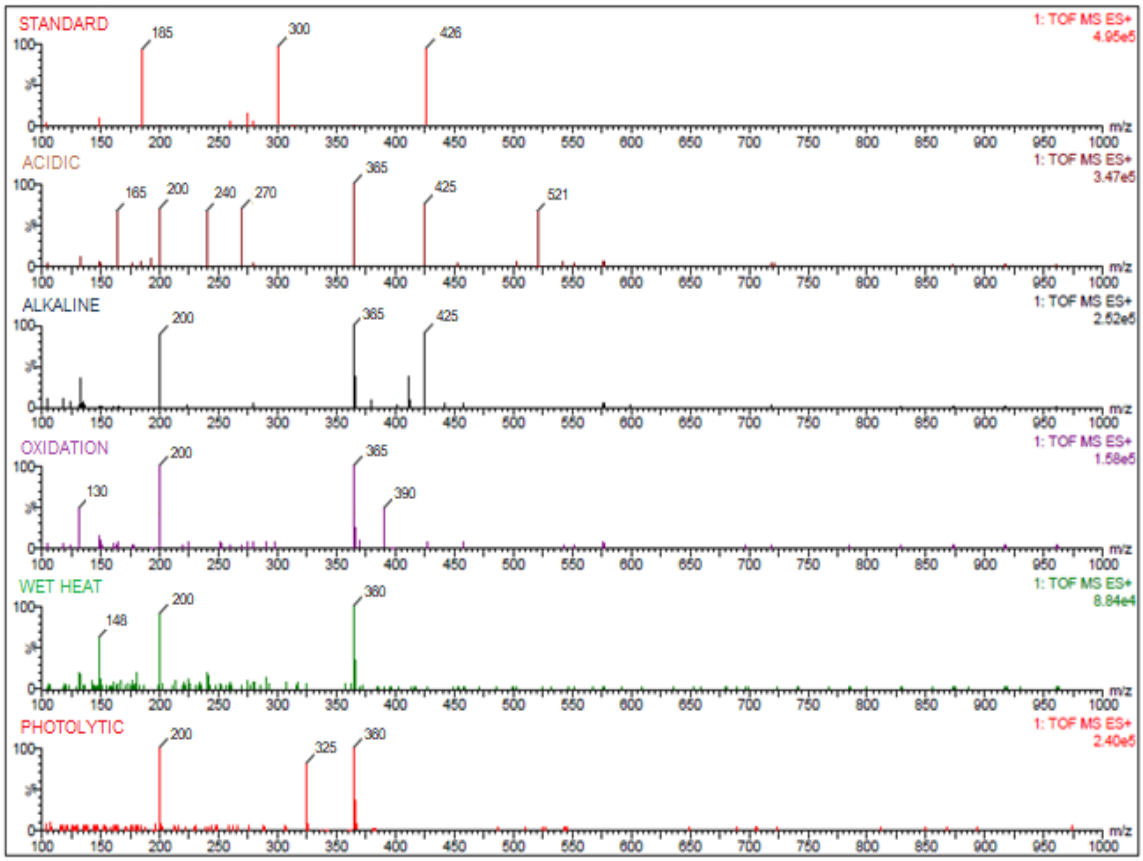


Blank Chromatogram of ALBENDAZOLE and IVERMECTIN ("1 M KH_2PO_4-acetonitrile in the ratio of 80:20%v/v")

Gradient Composition of ALBENDAZOLE and IVERMECTIN

Time –Interval (Mins.)	Solvent – A% (Potassium Phosphate buffer)	Solvent – B% (Mobile Phase)
0-2	0	100
2-4	25	75
4-6	15	85
6-8	10	90
8-10	0	100

Gradient Composition of ALBENDAZOLE and IVERMECTIN in UPLC System



LCMS

Analyte	Observedion mass (Da)	Proposed formula	Calculated mass (Da)
Unknown	148.38	C8H19NO	145.24
Unknown	185.54	C9H18N2O2	186.25
Unknown	200.02	C10H20N2O2	200.28
Unknown	300.42	C13H23N3O3S	301.40
Unknown	325.46	C14H19N3O4S	325.38
Unknown	365.36	C17H23N3O4S	365.45
Unknown	425.45	C17H23N5O4S2	425.52
Unknown	426.87	C17H24N5O4S2	426.53
Unknown	521.72	C17H27N7O6S3	521.63

LCMS INTERPRETATION
SUMMARY OF RESULTS

Parameters	Acceptance Criteria	Value Found	Inference
Linearity Range (µg/ml)	Linear Regression	*ALB* - 250-750 (µg/ml) *IVE* - 62.5-187.5 (µg/ml)	The concentration of linearity range was found to be Linear.
Correlation Coefficient	Correlation Coefficient of $r^2 \geq 0.995$	*ALB* - 0.999 *IVE* - 0.999	It is found to be under the acceptance criteria.
Method Precision (%RSD)	%RSD nmt 2%	*ALB* - 0.01%, *IVE* - 0.02%	The Method Precision is found to be precised.
Accuracy (%Recovery)	The % recoveries should be in between 98-102% w/w	*ALB* - 98.69% *IVE* - 98.98%	The good recoveries shows the method is Accurate.
LOD (µg/ml)	Signal to noise ratio (S/N) ≥ 3.3	*ALB* - 0.0029 µg/mL *IVE* - 0.0052 µg/mL	It is found to be under the acceptance criteria.
LOQ (µg/ml)	Signal to noise ratio (S/N) ≥ 10.1	*ALB* - 0.0091 µg/mL *IVE* - 0.0160 µg/mL	It is found to be under the acceptance criteria.
Robustness (Low, Actual, High) (%RSD)	%RSD nmt 2%	*ALB – (0.03%,* 0.02%, 0.02%) *IVE – (0.04%,* 0.02%, 0.03%)	With small deliberate variations in the method parameters has proven that the method is robust.
Intra Day (%RSD)	%RSD nmt 2%	*ALB* - 0.02% *IVE* - 0.04%	The repeatability is excellent, indicating the ruggedness of the method.
Inter Day (%RSD)	%RSD nmt 2%	*ALB* - 0.01% *IVE* - 0.03%	The reproducibility is excellent, indicating the ruggedness of the method.
Analysis of Formulation (% Assay)	The % Assay should be in between 98-102% w/w	*ALB -99.28* % *IVE -99.41* %	Satisfactory quantitative detection of the analytes

CONCLUSION

The RPUPLC techniques used in this study satisfy all of the needed criteria. With future refinement, the approaches proposed will boost excitement for evaluating inadequate medical goods and job scheduling. Unexpected recoveries were detected on all instances, and the Convention suggested that tactics for examinations employing vet dosage approaches may be applied.

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