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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS DETERMINATION OF PYRANTEL PAMOATE AND FEBANTEL IN AN ORAL DOSAGE FORM BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

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Pyrantel pamoate,
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ABSTRACT: The present manuscript describes a new simple, specific, precise and accurate RP-HPLC development and subsequent validation for simultaneous estimation of Pyrantel pamoate and Febantel in their combined dosage form. The proposed method involves the utilization of a hypersil BDS C18 (150 × 4.6 mm, 5 μ) column, mobile phase containing acetonitrile: phosphate buffer in the ratio 85:15% v/v and 55:45% v/v with apparent pH adjusted and maintained to 3.5 using 0.01M orthophosphoric acid, pumped at a flow rate of 1.0 ml/min. The injection volume was 20 μl, and detection was monitored at 286 nm. The components were eluted by gradient mode. The peaks obtained were sharp and well resolved with retention times 4.5 min (Pyrantel pamoate) and 6.5 min (Febantel). The calibration curves were linear ($r^2=0.999$) over the concentration range of 25-150 μg/mL for Pyrantel pamoate and Febantel both. Validations of the proposed method were carried out for its accuracy, precision, linearity, robustness LOD and LOQ according to ICH Q2 R1 guidelines. The statistical analysis was carried out and the results of which were found satisfactory. Based on results, the developed method could be used for routine estimation Pyrantel pamoate and Febantel in the marketed formulation.

INTRODUCTION: Helminthiasis is a parasitic disease commonly occurring in pet and domestic animals causing significant morbidity and mortality. These infections are substantial from the view of public health because the parasitic diseases are transmissible to humans affecting more than one billion population in the world¹. Owing to the narrow spectrum of anthelmintic drugs, it is needed to use combination chemotherapy to control mixed infections and to enlarge the spectrum of action².

Two active pharmaceutical ingredients, differing in mechanism of action and spectrum of activity namely Pyrantel pamoate 15 mg and Febantel 14.4 mg present in a single dosage form as a suspension in the ratio of 1:1 were used for simultaneous estimation³.

Pyrantel pamoate, a tetrahydropyrimidine that was originally developed as a veterinary anthelmintic for the treatment of hookworms, pinworm, roundworms (ascaris) in humans and domesticated animals such as horses, cattle, sheep, pigs, cats, dogs and also in *Trichostrongylus orientalis* infections^{4,5}. It acts on the cholinergic receptors of the parasite resulting in spastic paralysis, i.e. peristaltic action of the intestinal tract then the parasite is eliminated through feces³. Febantel acts as a prodrug against nematode parasites including

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whipworms. After transformation to fenbendazole, it binds to tubulin which is a structural protein of microtubules which are involved in motility, division and secretion processes of cells in all living organisms. In the worms, blocking of these microtubules perturbs the glucose uptake which eventually empties the glycogen reserves, leading to the blockage of the whole energy management mechanism of the worms. This causes paralysis and death of the worms causing them to be expelled through feces of the animal^{6, 7}. Literature survey reveals a few spectrophotometric and chromatographic methods for the estimation of Pyrantel pamoate as a single component and Pyrantel pamoate and Febantel in combination with other drugs^{1, 2, 3, 8, 9, 10}.

However, none of these techniques monitor this combination simultaneously or present adequate sensibility or selectivity so far. The objective of the present communication is to develop a simple, rapid and precise chromatographic method for the determination of Pyrantel pamoate and Febantel in an oral dosage form and to validate the method as per ICH Q2 R1/USP guidelines¹¹.

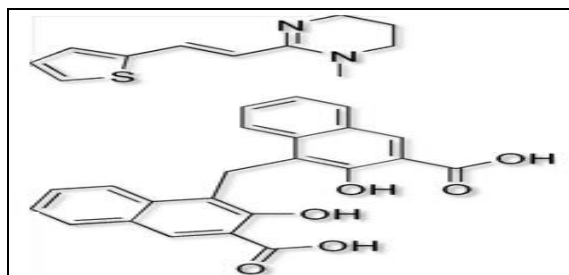


FIG. 1: STRUCTURE OF PYRANTEL PAMOATE⁴

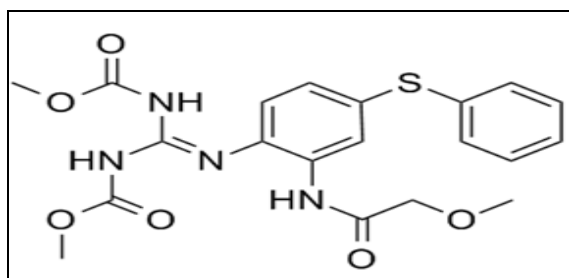


FIG. 2: STRUCTURE OF FEBANTEL⁶

MATERIALS AND METHODS:

Chemicals and Reagents: Pyrantel pamoate was obtained as a generous gift sample from IPCA Laboratories, Mumbai and Febantel were purchased from Pure Chem. Pharma Pvt. Ltd., Veterinary anthelmintic formulation worex was purchased from local pharmacy¹². All chemicals

and reagents used were of HPLC and analytical grade procured from S.D Fine Chemicals Ltd. Distilled water generated from a Millipore water purification system was used throughout the study.

Equipment: Chromatographic separation was carried out using HPLC system (Agilent 1260 series) containing a photodiode array detector. The output signal was monitored and processed using Open Lab Control Panel software. UV spectrophotometer used was from Shimadzu, UV- 3000.

Preparation of Standard Solution: 50 mg of standard / pure drugs/ API - Pyrantel pamoate and Febantel were accurately weighed and transferred to a 100 ml volumetric flask separately and dissolved in 50 ml methanol. The flasks were shaken and sonicated for 10 min after complete dissolution of the API; volume was made up to the mark with methanol to give a solution of concentration 50 g/mL each. From each API solution, 10 ml was withdrawn and diluted up to 100 ml with the mobile phase to give a solution of 5g/mL of each Pyrantel pamoate and Febantel.

Preparation of Sample Solution: Sample quantitatively equivalent to 15 mg of Febantel and 14.4 mg of Pyrantel pamoate was weighed accurately and transferred to 100 ml amber colored volumetric flask. 70 ml of methanol was added, shaken well, sonicated for 25 min and diluted up to the mark using methanol. 1 ml of filtrate was diluted to 10 ml with the mobile phase.

HPLC Analytical Conditions: To develop the method, several trials were conducted using different diluents/solvents. Varying concentrations of the drugs were injected at their detection wavelength for simultaneous estimation using different mobile phase combinations and compositions to optimize the method.

For optimization of chromatographic conditions, the method development was initiated by selecting different columns like hypersil ODS C18 (250 × 4.6 mm, 5μ), Agilent C18 (250 × 4.6 mm, 5μ) & hypersil BDS C18 (150 × 4.6 mm, 5 μ). Both the ingredients were then well separated with hypersil BDS column (150 × 4.6 mm, 5μ). The method development and optimization were initiated with the following:

Selection of Detection Wavelength: The standard solutions of Pyrantel pamoate and Febantel were scanned individually over a range of 200-400 nm wavelength on Shimadzu's UV spectrophotometer. It was observed that the wavelength for Pyrantel pamoate was 286 nm and Febantel was 281 nm. Hence, the wavelength 286 nm was selected for the determination of Pyrantel pamoate and Febantel simultaneously.

Selection of Column: For HPLC, numerous columns are available but C18 column was preferred over other columns. Hypersil BDS C18 column 250 mm length 4.6 mm as internal diameter having a particle size of 5µ was chosen to give good peak shape and high resolution of the components.

Selection of Mobile Phase: The mobile phase was selected by best isolation of components, peak purity index, peak symmetry, and theoretical plates. Trials were carried out with solvents like water,

acetonitrile, phosphate buffer and methanol with varying composition and combination. Solvents were filtered through 0.45 membrane filter paper and sonicated for 5 min.

Selection of pH: Depending on log P and pKa value of the two drugs, pH was selected and adjusted using dilute or 1% orthophosphoric acid and triethylamine.

Buffer Preparation: Phosphate buffer of 50 mM strength was prepared by accurately weighing 6.8 g of potassium dihydrogen phosphate in 1L of volumetric flask, added 900 ml HPLC grade / Millipore grade water and degassed to sonicate and finally made up the volume with water. The pH was adjusted to the required value with dilute orthophosphoric acid using digital pH meter.

Selection of Column Temperature: The column temperature 30 °C has minimized variation of retention and made the peak sharp. It also shortened the run time.

TABLE 1: OPTIMISED CHROMATOGRAPHIC CONDITIONS

Parameters	Conditions
Instrument	Agilent 1260 series -High-Performance Liquid Chromatography
Detector	Photodiode array detector
Stationary phase/Column	Hypersil BDS column C18 (150 × 4.6 mm, 5µ)
Mobile phase	Phosphate buffer (pH 3.5): Acetonitrile
Mobile phase ratio	0-4.5 min. 85 % v/v buffer pH (3.5) & 15% v/v acetonitrile, 4.5-7 min. 55 % v/v buffer pH (3.5) and 45 % v/v acetonitrile and 7-20 min. 85 % v/v buffer pH (3.5) & 15 % v/v acetonitrile
Wavelength	286 nm
Flow rate	1 ml/min.
Mode of Elution	Gradient mode
Injection volume	20 µl
Column temperature	Ambient

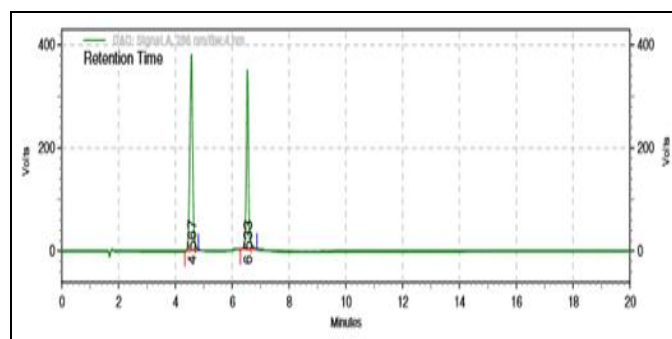


FIG. 3: CHROMATOGRAM OF STANDARD PYRANTEL PAMOATE AND FEBANTEL

TABLE 2: RESULTS OF SYSTEM SUITABILITY

Parameters	Proposed method		Standard value
	Pyrantel pamoate	Febantel	
Retention time (Rt), min	4.5	6.5	--
Resolution (Rs)	--	12.06	Rs >2
Theoretical plates (N)	9256	35629	N > 2000
Tailing factor (Tf)	0.9%	1%	T <= 2

RESULTS AND DISCUSSION:

Validation of RP-HPLC Method: As per ICH Q2 R1 guidelines, the method validation parameters checked were linearity and range, LOD, LOQ, accuracy, precision, robustness and specificity¹¹.

Linearity and Range: Appropriate aliquots of Pyrantel pamoate and Febantel working standard solutions were taken in different 10 ml volumetric flasks and diluted up to the mark with mobile phase to obtain final concentrations of 25, 50, 75, 100, 125 and 150 µg/mL of Pyrantel pamoate and Febantel each. Chromatograms were recorded. Calibration curves were constructed by plotting peak area versus concentration, and regression equations were computed for both the drugs. The correlation coefficient value of Pyrantel pamoate was found to be 0.999 and Febantel was 0.995, and

the equation for regression line was $y=53734x+12399$ for Pyrantel pamoate and $y=36047x+23942$ for Febantel. The results show that an excellent

correlation exists between the response factor and the concentration of drugs within the concentration range stated above.

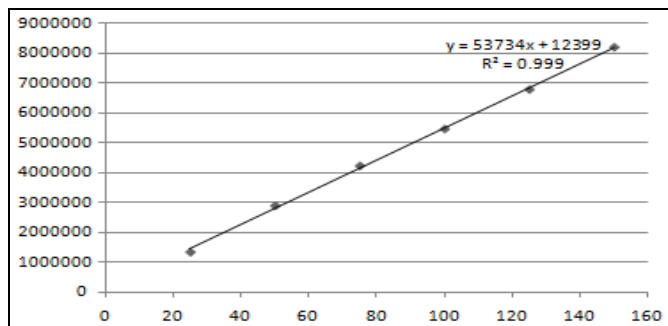


FIG. 4: STANDARD CALIBRATION CURVE FOR PYRANTEL PAMOATE

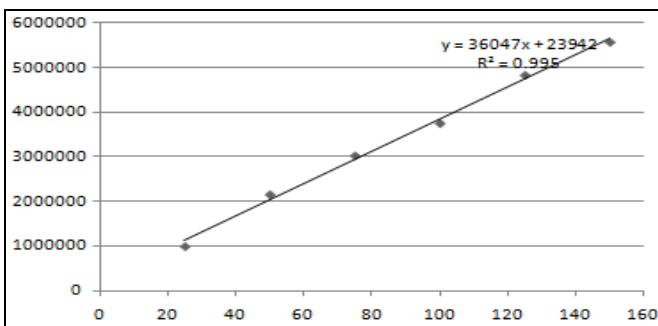


FIG. 5: STANDARD CALIBRATION CURVE FOR FEBANTEL

LOD and LOQ: The limit of detection (LOD) and limit of quantification (LOQ) were calculated using signal-to-noise (*i.e.*, 3.3 for LOD and 10 for LOQ) ratio using following equations designated:

$$DL = 3.3 \sigma / S \text{ and } QL = 10 \sigma / S$$

Where, σ = the standard deviation of the response S = the slope of the Calibration curve.

The results are summarized below:

TABLE 3: RESULT FOR LOD AND LOQ

Parameters	Pyrantel pamoate	Febantel
LOD ($\mu\text{g/mL}$)	0.797	5.95
LOQ ($\mu\text{g/mL}$)	2.41	18.032

Accuracy: The accuracy of an analytical method expresses the nearness between the reference value and experimental value. The accuracy of the method was evaluated in triplicate at three

concentration levels, *i.e.*, 80%, 100%, and 120% and the percentage recovery was calculated and presented in **Table 4**. The results obtained accurately fall within the limit of acceptance criteria.

Precision: The precision of an analytical method gives information on random error. It agrees to a series of measurements obtained from multiple sampling of the same homogeneous sample under prescribed conditions.

The percentage RSD values for the precision study was 0.237%, 1.69% (intra-day precision) and 0.437%, 1.491% (inter-day precision) for Pyrantel pamoate and Febantel, respectively. This is confirming good precision of the method. The results are summarized in **Table 5**.

TABLE 4: RESULTS FOR ACCURACY

Analyte	Concentration levels	Peak area	Amount added (mg)	Amount found (mg)	% recovery
Pyrantel pamoate	80%	880021	11.6	11.58	99.82
	100%	905637	14.5	14.21	98
	120%	926654	17.4	17.39	99.94
Febantel	80%	892348	12	12.11	100.91
	100%	910233	15	15.05	100.33
	120%	944622	18	17.92	99.55

TABLE 5: INTRADAY PRECISION / REPEATABILITY STUDY DATA

S. no. (100 $\mu\text{g/mL}$)	Peak area	
	Pyrantel pamoate	Febantel
1	5479373	3788416
2	5462076	3861409
3	5454586	3884205
4	5482013	3908150
5	5482871	3755243
Mean	5472184	3839485
Standard deviation	12984.26	65000.35
% RSD	0.237	1.69

TABLE 6: INTERDAY PRECISION DATA FOR ANALYSIS

Sr. no. (100 µg/mL)	Peak area	
	Pyrantel pamoate	Febantel
1	5467321	3722345
2	5468762	3844563
3	5523345	3725443
4	5467889	3705311
5	5483574	3734352
Mean	5482178	3746403
Standard deviation	23987.32	55873.24
% RSD	0.437	1.491

Robustness: The robustness of a method is its capacity to remain unaffected by small changes in chromatographic condition. To determine the robustness of the method, the experimental condition, *i.e.* pH of mobile phase was deliberately altered and system suitability parameter like relative standard deviation for replicate injections

of Pyrantel pamoate and Febantel peaks were evaluated.

Specificity: No peaks were observed at the retention time of both the drugs; hence, it complies with acceptance criteria.

TABLE 7: RESULT OF ROBUSTNESS FOR VARIATION IN pH OF MOBILE PHASE (PYRANTEL PAMOATE)

Variation in pH	Retention time 1 (min)	Retention time 2 (min)	Retention time 3 (min)	Mean retention time (min)	Standard deviation	% Relative standard deviation	Mean % relative standard deviation
3.3	4.56	4.51	4.55	4.54	0.0264	0.572	
3.5	4.52	4.55	4.58	4.55	0.031	0.681	0.881
3.7	4.61	4.63	4.51	4.58	0.064	1.39	

TABLE 8: RESULT OF ROBUSTNESS FOR VARIATION IN pH OF MOBILE PHASE (FEBANTEL)

Variation in pH	Retention time 1 (min)	Retention time 2 (min)	Retention time 3 (min)	Mean retention time (min)	Standard deviation	% Relative standard deviation	Mean % relative standard deviation
3.3	6.58	6.54	6.54	6.55	0.023	0.351	
3.5	6.52	6.55	6.51	6.52	0.020	0.306	0.382
3.7	6.49	6.50	6.55	6.51	0.032	0.491	

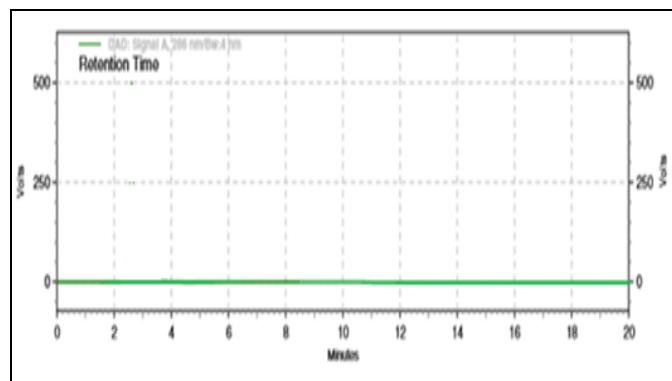


FIG. 6: CHROMATOGRAM FOR MOBILE PHASE

CONCLUSION: From the results obtained, it is obvious that the proposed method is applicable for the determination of Pyrantel pamoate and Febantel without interference and with good sensitivity. The method was validated entirely showing adequate data for all the method validation parameters tested proving it to be specific, rapid, linear, accurate, precise, robust and suitable for intended use.

These merits suggest the use of the proposed method in routine and quality control analysis of Pyrantel pamoate and Febantel without interference from commonly encountered excipients and additives.

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CONFLICT OF INTEREST: Nil

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