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RP-HPLC Technique for the Assurance of Mebendazole In Unadulterated and In Tablet Measurements

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ABSTRACT

A new RP-HPLC method for the assay of mebendazole in in unadulterated and in tablet measurements has been developed and validated. The present chromatographic study was carried on µ Bondapak® C18 (250X4.6 mm), 5µm with a flow rate of 1.0mL/min of mobile phase and UV detection at of 218nm and ambient column temperature with mobile phase of NaH_2PO_4 buffer(pH-5.0) and acetonitrile in the ratio of 65:35%v/v as the mobile phase. The retention time for mebendazole were found to be 2.60mins respectively. The developed RP-HPLC method was validated as per ICH guidelines and was found to be suitable for pharmacokinetic studies.

Keywords: Mebendazole, tablet measurements and ICH Guidelines.

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INTRODUCTION

Mebendazole[1-3], methyl (5-benzoyl-1H-benzimidazol-2yl) carbamate[1] (Figure.1), is wide range anthelmintic operator showed for the treatment of intestinal parasites. Different brands of mebendazole are accessible in the neighborhood drug store that incorporate Eben, Exit Neomex and Mebex and so forth. It is authentic in US Pharmacopeia [4] and just a couple of techniques [5-8] were accounted for examination of mebendazole in pharmaceutical measurements shapes in dose plans. In this understanding the creator endeavored, to create and approve fewer complexes, financial, fast, exact and precise expository techniques with great affectability for quantitative examination of mebendazole in unadulterated and plans as per International Conference on Harmonization rules[9]. A writing study uncovered that lone two chromatographic strategies [7,8] were accounted for the estimation of mebendazole in tablet measurements structures. In the present area of this section, the creator endeavored and built up a straightforward, particular RP-HPLC strategy for the assurance of mebendazole in unadulterated and in plans. The present part depicts a straightforward RP-HPLC technique for the assurance of mebendazole in unadulterated and in tablet measurements structures.

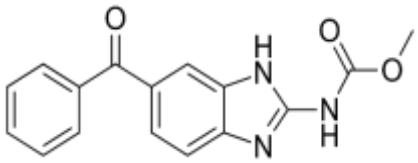

Figure 1: Chemical structure of Mebendazole

MATERIALS AND METHOD

Instrumentation:

The HPLC analysis of mebendazole was completed on a Shimadzu 2010C incorporated superior fluid chromatographic framework outfitted with a quaternary inclination siphon, 2010C UV-VIS identifier, 2010C Column Oven and 2010C programmable auto sampler constrained by CLASS-VP programming. The µ Bondapak® C18 (250X4.6 mm), 5µm was utilized as a stationary phase. The infusion volume of the sample was 10µL. The UV-locator was set to a wavelength of 255nm for the recognition and chromatographic runtime was 6.0minutes. The whole HPLC framework was equilibrated before making every infusion. Shimadzu balance (BL-220H) was utilized for all gauging.

Chemicals and solvents:

Mebendazole standard powder (99% unadulterated) was compassionately acquired as free sample from Neon Labs Ltd, India and its market plans Mebex chewable tablet (100mg mebendazole)

fabricated by Cipla Limited was secured from the neighborhood pharmacy. Dimethyl sulfoxide (DMSO), Acetonitrile (HPLC grade), Orthophosphoric acid (GR Grade), Sodium dihydrogen phosphate monohydrate (GR Grade) and Tri ethylamine (GR Grade) were obtained from Qualigens Ltd., Mumbai. The cleansed water arranged by utilizing a Milli-Q framework was utilized for the planning of buffer and different fluid arrangements.

Mobile phase preparation:

Set up a sifted and degassed blend of buffer (pH 5.0) and acetonitrile in the proportion of 65:35%v/v was utilized as mobile phase in current measure separately

Buffer preparation:

Precisely gauge and move about 2.72gms of Sodium dihydrogen phosphate (monohydrate) and 2.0mL of triethylamine in 1000mL of refined water and blend. Change pH to 5.0 (±0.05) with the weaken orthophosphoric acid arrangement. Channel the arrangement through a 0.45µm film channel.

Diluent preparation:

In the present examination DMSO and water in the proportion of 50:50%v/v is utilized as diluents.

Preparation of standard solution:

Precisely weighed about 100.0mg of mebendazole and moved into a 100mL volumetric jar at that point, include 60mL of diluent and sonicated to break up. Cool the answer for room temperature lastly weakened sufficient with a similar diluent [stock solution]. Move aliquots of the above arrangement [stock] into a progression of various 100mLvolumetric jars and weaken to the volume with the mobile phase individually to acquire working standard arrangements of focus in the scope of 10-30µg/mL separately.10µL of these arrangements were infused in triplicate in to HPLC framework and the peak zones were recorded.

Analysis of Marketed sample (Dosage forms):

Ten tablets of Mebex [Label guarantee 100mg of mebendazole] oral tablets obtained from the neighborhood pharmacy were gauged and finely powdered. A precisely gauged segment of the powder, identical to about 100mg of mebendazole was moved to a 100mL volumetric flask pursued by the expansion of 70mL of diluent. The arrangement was sonicated at a controlled temperature for 30min and weakened sufficient with a similar diluent and blended completely. Channel the arrangement through a 0.45µm film channel. Get ready diverse working sample arrangements in the focus scope of 10 to 30µg/mL by weakening the above arrangement into a progression of 100mL volumetric flasks and weakened to volume with the mobile phase. 10µLof

these arrangements were infused in triplicate into HPLC framework and went before as said for the standard individually.

RESULTS AND DISCUSSION:

Method Development:

Basic parameters, for example, the wavelength of location, choice of column and creation of mobile phase and impact of stream rate on the column were considered in detail in building up this successful technique for the examine of mebendazole. To determine the absorbance maxima for the proposed technique working standard arrangement containing mebendazole of fixed focus was set up in diluent and go through a bright spectrophotometer. In the present examination, the identification wavelength of 255nm was chosen by looking over a wide scope of wavelengths 200 nm to 400nm.At first trial trails were utilized diverse stationary phases like C8 and C18, distinctive mobile phases containing buffers like phosphate buffer of pH 5.0 were made. From the above investigations, it was seen that Bondapak® C18 (250X4.6 mm), 5µm column gave the peak with better gaussian shape for mebendazole and saw as relatively superior to other C8 column and the framework reasonableness information acquired with this column.

To improve the shape and width of the peak for the above column further advancement examines were made and these outcomes affirmed that sodium dihydrogen ortho phosphate buffer (pH 5.0) and acetonitrile in the proportion of 65:35% v/v with the stream pace of 1.0mL/min and surrounding column temperature uncovered the better goals and affectability for mebendazole. The run of the mill maintenance time of the mebendazole peak was about 2.600min. individually. The approving chromatogram and the framework reasonableness consequences of the present created RP-HPLC strategy for mebendazole were introduced in Figure.2 and Table.1 separately.

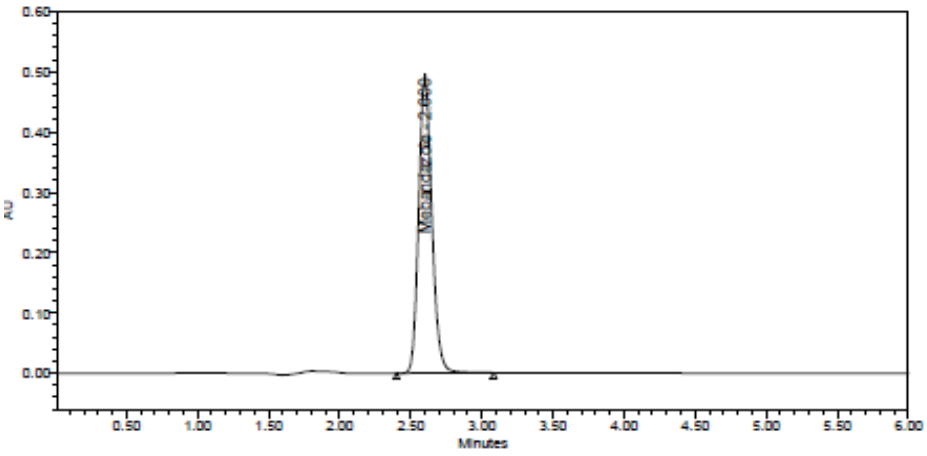

Figure 2: HPLC chromatogram of mebendazole standard solution

Chromatographic conditions:

The compound was isolated isocratically on a µBondapak® C18 (250X4.6 mm), 5µm column with a mobile phase comprising of an isocratic mobile phase containing sodium dihydrogen ortho phosphate buffer (pH 5.0) and acetonitrile in the proportion of 65:35%v/v was conveyed with the stream pace of 1.0mL/min at surrounding column temperature.

Method validation:

The created RP-HPLC strategy is broadly approved for measure of mebendazole in unadulterated and definition in understanding to ICH guidelines [9] by utilizing the accompanying parameters.

System suitability:

The framework appropriateness parameters like limit factor, asymmetry factor, following component and number of hypothetical plates were determined. It was experiential that every one of the qualities are inside the points of confinement (Table.1). These values promoted the practicality of the created strategy for routine pharmaceutical analysis for mebendazole.

Table 1: System Suitability Parameters for Mebendazole

Parameter	Mebendazole
Retention time	2.600
Theoretical plates	3794
Tailing factor	1.16
% RSD	0.16

Particularity/selectivity:

The selectivity of the present RP-HPLC strategy was assessed by infusing the clear and unadulterated medication arrangement into the chromatographic framework under the above said advanced chromatographic conditions and their individual chromatograms were recorded. Chromatograms of clear arrangement demonstrated no peaks at the maintenance time of mebendazole peak showing that the diluent arrangement utilized in sample planning don't meddle in the examine of mebendazole. in unadulterated and plans.

Linearity:

Linearity arrangements were set up from mebendazole stock arrangements at six fixations levels from half to 150% of the focused on level of the test centralization of mebendazole. Standard arrangements containing 10-30µg/ml of mebendazole in every linearity level were arranged and were infused in triplicate. The alignment diagrams were acquired by plotting peak area stanzas the focus information were treated by least-squares linear regression analysis, the adjustment charts were seen as linear in the referenced fixations the inclines and correlation coefficients are appeared

in Table.2. These outcomes demonstrated that there was an incredible correlation between the peak area and analyte focus. The linearity bend of mebendazole was delineated in Figure.4 individually.

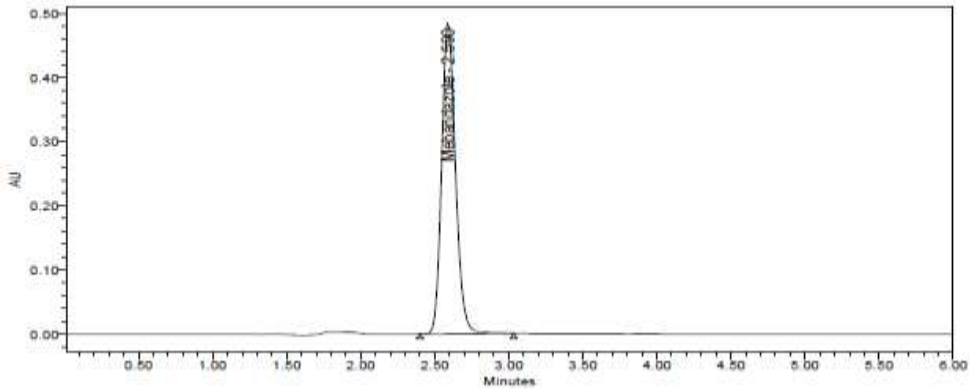

Figure.3: Chromatogram representing specificity of sample

LOD and **LOQ:** The LOD and LOQ values for mebendazole were 0.011 and 0.039µg/ml individually. The consequences of LOD and LOQ were introduced in Table. 2 separately which uncovered the affectability of the created RP-HPLC strategy.

Table 2: Linearity Studies of Mebendazole

Linearity results for mebendazole		
% Level	**Concentration (µg/mL)**	**Peak Area Ratio**
50	10	2454687
75	15	3607278
100	20	4825188
125	25	5937982
150	30	7138049
Slope, b		278722
Intercept, a		46962
r2		0.9999
LOD (µg/mL)		0.011
LOQ (µg/mL)		0.039

Precision:

The precision of the current RP-HPLC technique was evaluated by six duplicate infusions of 100% test fixation (20µg/mL) and the outcomes were communicated as far as standard deviation and %RSD. The % RSD values for the peak areas of mebendazole of accuracy consider were seen as 0.056 and 1.09% individually and these outcomes demonstrated that the current RP-HPLC technique is profoundly exact. The framework exactness results were given in Table.3.

Table 3: Results of Method Precision by The Proposed Method

S.No	Name	Rt	Area
1	Solution-1	2.590	4842481
2	Solution-2	2.589	4745303
3	Solution-3	2.587	4835711
4	Solution-4	2.588	4839851
5	Solution-5	2.587	4858648
6	Solution-6	2.586	4740994
AVG*		2.587833	4810498
STD DEV*		0.001472	52763.29
% RSD*		0.056	1.09

*Average of six determinations considered

Accuracy:

The exactness of the proposed technique was dictated by standard expansion strategy that was performed at three fixation levels of half, 100% and 150%. The standard medication arrangements were examined in triplicate at each level according to the proposed strategy and their separate chromatograms were recorded. The percent recuperation at each level was determined and their outcomes are introduced in Table. 4. These outcomes revealed the best recuperation i.e., 98.6-100.8% for mebendazole demonstrating that the created RP-HPLC strategy was exact.

Table 4: Accuracy Results of Mebendazole

S.No	Accuracy Level	Injection	*%Recovery
1	50%	1	99.0
		2	99.6
		3	98.6
2	100%	1	99.8
		2	99.5
		3	99.4
3	150%	1	100.8
		2	100.0
		3	100.7

*Average of three determinations considered

Robustness:

The power of the present proposed strategy was finished by adjusting the exploratory conditions that incorporate (i) the impact of progress in stream rate (±0.2 mL/min) and (ii) the impact of location wavelength (±5.0nm). The stream pace of the mobile phase and the recognition wavelength in the current measure was 1.0mL/min and 255nm individually. In the above said fluctuated conditions, the segments of the mobile phase stayed steady. The aftereffects of the power investigation of the proposed technique are exhibited in Table.5 and these outcomes indicated that the measure estimation of the test readiness arrangement was marginally influenced

and was as per that of genuine during the above-said change conditions. Furthermore, increasingly over the framework appropriateness parameters were additionally discovered agreeable end that the created strategy as powerful.

Table 5: Results of Robustness Study

Robust conditions		Mebendazole		
		Rt	Theoretical plates	peak area
Flow Rate	0.8 mL/min	2.853	3968	1.17
	1.2 mL/min	2.352	3533	1.15
Wavelength in nm	250nm	2.593	3683	1.16
	260nm	2.600	3932	1.15

Ruggedness:

The toughness of the proposed RP-HPLC strategy was assessed by two distinct examiners with various instruments in a similar research centre. The % RSD of peak areas of mebendazole were determined and the trial results are appeared in Table. 6. These outcomes uncovered that the %RSD was inside the points of confinement showing that the created RP-HPLC technique was seen as rough.

Table 6: Results of Ruggedness Studies of Mebendazole

S No	Name	Analyst-1 Area	Analyst -2 Area
1	Injection-1	4842481	4841565
2	Injection-2	4745303	4839924
3	Injection-3	4835711	4855439
4	Injection-4	4839851	4851322
5	Injection-5	4858648	4860188
6	Injection-6	4740994	4853432
AVG*		5846001	4850312
STD DEV*		27017.8	7987.447
% RSD*		0.46	0.164

*Average of six determinations considered

Measure of Mebendazole in Pharmaceutical formulations:

Analysis of promoted tablets of mebendazole (Mebex; Label guarantee 100mg) was done utilizing the above said advanced mobile phase and HPLC conditions. The % tranquilize substance of tablets got by the proposed strategy for mebendazole (Figure.3) was seen as 99.99% separately. This indicated the estimation of measurements structures was precise inside the acknowledgment level. The outcomes are given in Table.7.

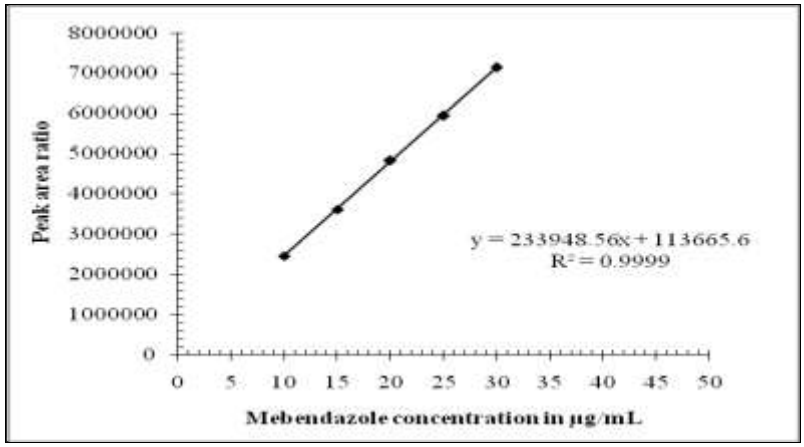

Figure.4: Linearity plot of Mebendazole by the proposed method

Table 7: Analysis of Marketed Tablets [MEBEX]

Drug	Label claim	Quantity found	*%Assay
Mebendazole (MEBEX)	100mg	99.99	99.99

*Average of three determinations

CONCLUSION

Exceptionally dependable and cost proficient HPLC strategy was created and approved for the quantitative estimation of mebendazole in tablet measurements structure. The chromatographic partition of mebendazole was completed on a Bondapak® C18 (250X4.6 mm), 5µm scientific column. The mobile phase included sodium dihydrogen ortho phosphate buffer (pH 5.0) and acetonitrile in the proportion of 65:35% v/v. The stream rate was 1.0mL/min with surrounding column temperature. Results were resolved at 255nm with a fixed wavelength PDA identifier with a run time was 6.0minutes separately. The legitimacy and accuracy of the created RP-HPLC technique were clear from the factual and scientific parameters got. It is finished up, that the RP-HPLC technique created is exact, straightforward, quick, explicit and exact consequently appropriate for application in routine analysis of mebendazole in pharmaceutical arrangements.

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