Validated liquid chromatographic method for simultaneous estimation of albendazole and ivermectin in tablet dosage form

Anil Waldia\textsuperscript{a}, Shubash Gupta\textsuperscript{b}, Roshan Issarani\textsuperscript{a} & Badri P Nagori\textsuperscript{a}*

\textsuperscript{a}Pharmacy Wing, Lachoo Memorial College of Science and Technology, Jodhpur, 342 003, India
\textsuperscript{b}Oasis Test House Ltd., 22 Godam Industrial Area, Jaipur, India

Email: bpnagori@sancharnet.in

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A reverse phased liquid chromatography (LC) method was developed and validated for simultaneous estimation of albendazole and ivermectin in tablet dosage form. The isocratic LC analysis was performed on NUCLEODUR C18 RP column (250 x 4.6 mm, 5 µ) using mobile phase composed of acetonitrile, methanol and water in ratio of 60:30:10 (v/v/v) at a flow rate of 1.8 mL/min. Quantitation was performed using UV detector at 245 nm and the run time was 20 min. The retention times were found to be 3.56 min for albendazole and 10.08 min for ivermectin. The analytical method was validated according to ICH guidelines. The linearity was observed in the range of 400–800 and 6–12 µg/mL with correlation coefficient, \( r = 0.9975 \) and 0.9969 for albendazole and ivermectin respectively. The relative standard deviation values for repeatability and intermediate precision studies were less than 2%, and the accuracy (% recovery) was greater than 98% for both the drugs. The method was successfully applied for market sample analysis and mean percentage assay values were 98.68 ± 0.50 for albendazole and 98.67 ± 0.28 and 0.9969 for ivermectin. The present work describes RP-HPLC method for the simultaneous estimation of albendazole and ivermectin in tablets.

Experimental Procedure

Apparatus

Shimadzu HPLC system equipped with LC-10 ADvp pump, with SPD-10AVvp UV detector, a NUCLEODUR C-18 RP column (250 x 4.6 mm, 5 µ), 20 µL glass syringe was used. Afcoset electronic balance HR 200 was used for weighing the materials.

Reagents and Materials

Albendazole (ALB) and ivermectin (IVM) were a gift sample from Mankind Pharma Ltd., Delhi. Acetonitrile, methanol, chloroform used were of HPLC grade. Tablets of a brand having combination of ALB and IVM were procured from the local pharmacy (Label claim: 400 mg/tablet of ALB and 6 mg/tablet of IVM, product name: Bandy-plus and manufacturer: Mankind Pharma, Ltd). The solvent system for drugs consisted of chloroform: methanol in a ratio of 70:30 (v/v).

Methods

Preparation of standard stock solutions of albendazole and ivermectin

ALB (400 mg) and IVM (6 mg) were weighed into 100 mL volumetric flasks separately and about 50 mL of solvent was added and sonicated to dissolve. The volume was made up to the mark with the solvent to achieve a concentration of 4000 µg/mL and 60 µg/mL of ALB and IVM respectively.
Standard preparation
15 mL each of ALB and IVM stock solutions were transferred to a 100 mL volumetric flask and the volume was made up with solvent and mixed well. The solution was then filtered through 0.2 µm glass nylon filter. This final solution contains 600 µg/mL and 9 µg/mL of ALB and IVM respectively.

Chromatographic conditions
Chromatographic separation was achieved at 30°C on a reversed phase column using a mobile-phase consisting of acetonitrile, methanol and water in the ratio of 60:30:10 (v/v/v). The flow rate was kept at 1.8 mL/min and detection was carried out at 245 nm.

A standard preparation containing 600 µg/mL and 9 µg/mL of ALB and IVM respectively were injected. The injection volume was 20 µL for assay level.

Calibration curve
The linearity of the response for ALB and IVM assay method was determined by preparing and injecting mixture of standard stock solutions suitably diluted to achieve concentrations of about 400, 500, 600, 700 and 800 µg/mL and 6.0, 7.5, 9.0, 10.5 and 12.0 µg/mL of ALB and IVM respectively. The values of coefficient of correlation, ‘r’, slope and intercept were 0.9975 and 0.9969; 243034 and 195946; and −921694 and 44317 for ALB and IVM respectively. The linear regression data for the calibration curves indicate that the response is linear over the concentration range studied for both the drugs.

Analysis of marketed formulation
Assay of tablets having combination of ALB (400 mg) and IVM (6 mg) was performed. Twenty tablets were weighed and powdered. The powder equivalent to 400 mg of ALB and 6 mg of IVM was dissolved in 100 mL of solvent and ultrasonicated for 15 min and filtered through 0.22 µm membrane filter. This solution was further diluted with the solvent to obtain concentration of 600 µg/mL of ALB and 9 µg/mL of IVM, and subjected to HPLC analysis as described. From the peak area of ALB and IVM, the amounts of drugs in tablets were determined.

Results and Discussion
Method development
Method development for the simultaneous estimation of ALB and IVM was a challenging task because of their combination ratio of approximately 67:1. Several mobile phase compositions were tried to resolve the peaks of ALB and IVM and wherein the responses could be measured simultaneously. The different mobile phase compositions tried were: methanol: acetonitrile in ratio of 50:50; acetonitrile: water in ratio of 60:40, and acetonitrile: methanol: water in ratios of 50:35:15, 60:30:10, 50:40:10 and 45:40:15. Also different flow rates for the mobile phase were tried; these were 1.4, 1.6, 1.8 and 2.0 mL/min. The optimum mobile phase containing acetonitrile: methanol: water in a ratio of 60:30:10 at a flow rate of 1.8 mL/min was selected because it was found ideal to resolve the peaks of ALB and IVM. Quantification was achieved with UV detector at 245 nm based on peak area. As per USP XXIII, system suitability tests were carried out on freshly prepared standard solution of the drugs and parameters obtained with 20 µL injection volume are summarized in Table 1. A representative chromatogram is shown in Fig. 1.

Analytical method validation

Specificity
Specificity study was performed by analyzing standard solution in the presence (C_p) and absence (C_a) of excipients/placebo. The two drug concentrations were expressed and compared as: % interference = (C_p−C_a)/ C_a x 100; acceptance criteria being % interference <0.5%. Percentage interference was found to be 0.05% and 0.02% for ALB and IVM respectively which is within the acceptance limits. Hence, the excipients do not interfere with the estimation of drugs.

Precision
Precision was measured in terms of repeatability of measurement, performed by injecting the standard solution six times and measuring the peak areas. The RSD was found to be 0.559 and 0.728 for ALB and IVM respectively. This shows that precision of the method is satisfactory as relative standard deviation is not more than 2.0%.

Intermediate precision
Intermediate precision (intermediate precision and reproducibility are the terms currently accepted/
with the expected results and expressed as percentage. The mean % recoveries of ALB and IVM were found to range between 98.73-99.71% and 97.38-98.06% respectively which are within the acceptance limit.

Robustness

Robustness of the method was determined by analyzing standard solutions at normal operating conditions and also by changing some operating analytical conditions such as flow rate, column oven temperature, detection wavelength and mobile phase methanol content.

The conditions with the variation and the results: assay, resolution factor and tailing factor are included in Table 2. Assay results were subjected to ANOVA test to see if any significant difference between the assay results obtained under varied conditions exists. No significant (p< 0.05) difference in mean assay results was found as the calculated value of $F$ is lower than the critical value of $F$. Further, the RSD values for assay were found to be <2%; the peaks of the two drugs are equally well resolved with a resolution factor of about 12; and tailing factor is around unity indicative of peak symmetry. Hence, the robustness of the method is established to the extent of variations applied to the analytical conditions. A summary of the validation parameters is presented in Table 3.

Analysis of marketed formulations

The developed method was applied for the simultaneous analysis of the drugs in tablet dosage form. The results of analysis are given in Table 4.
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References