Analytical Method Development And Validation For Estimation of Withanolide A and Withanoside IV

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ABSTRACT

The RP-HPLC methods was developed and validated for estimation of Withanolide A and Withanoside IV. The RP-HPLC method has shown adequate separation of Withanolide A and Withanoside IV as Marker and its herbal formulation. The separation was achieved on a Phenomenax C18 (250mm X 4.6 mm i.d, 5 µm particle size) with an Methanol : Water in the ratio of 60:40 v/v. The mobile phase at a flow rate of 1.5 ml/min, Injection volume 20 µl and wavelength of detection used was 237 nm. The retention time for Withanoside IV and Withanolide A was obtained as 10.55±0.2min and 18.66±0.2min, respectively. The linearity of the proposed method was investigated in the range of 2-12 µg/ml and 2-12 µg/ml for Withanolide A and Withanoside IV , respectively. Correlation coefficient was 0.9952 and 0.9997 for Withanolide A and Withanoside IV , respectively. The developed method was validated as per ICH guideline, for its accuracy, precision, LOD & LOQ and the results were found to be satisfactory, thus the method is specific, rapid and simple with good for estimation of Withanolide A and Withanoside IV. These analytical methods are also applicable in ordinary laboratories. It can also be adopted for quality control tests for these markers in formulation.

Key words: Withanolide A, Withanoside IV, RP-HPLC, Validation.