Spectrophotometric Determination of Andrographolides in *Andrographis paniculata* Nees and its Formulation

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A simple, sensitive and reproducible spectrophotometric method was developed for the estimation of andrographolides in *Andrographis paniculata* (*Kalmegh*) and its formulation. The method is based on the condensation of γ-unsaturated lactone ring of andrographolides and picric acid in alkaline medium which results in the formation of colored complex, that which could be measured at 494 nm. Linearity of the proposed method was found between 5-50 µg/ml. The powdered samples of *A. paniculata* procured from different sources were found to contain 0.9-1.5% w/w of andrographolides. Formulation containing *A. paniculata* showed 0.3% w/w of the andrographolides. Our studies clearly indicated that Baljet reagent can also be employed for estimation of andrographolides. The method was found to be reliable and precise and could be used for analyzing formulations containing andrographolides for regular quality control purposes.

Andrographis paniculata (Family: Acanthaceace) is grown widely in tropical areas of Asia and is commonly known as Kalmegh. It has been extensively used for the treatment of fever, diarrhea, inflammation, sore throat and hepatitis[1]. *A. paniculata* was reported to contain pharmacologically active diterpene lactones like andrographolide, neoandrographolide and deoxy dihydroandrographolide[2].

Many methods such as HPLC[3], HPTLC[4], gravimetric[5], spectrophotometric[6] and titrimetric[7] have been reported for quantitative estimation of andrographolides. Recently we have reported analysis of cardiac glycosides from *Nerium indicum*[8] and *Thevetia reflexa*[9] by employing Baljet reagent[10]. Since andrographolides share common structural feature with cardenolides in the form of a γ-unsaturated lactone ring, we were interested in finding out whether andrographolides can also be analyzed by employing Baljet reagent.

Andrographolide reference standard was obtained as gift from Dishman Pharmaceuticals and Chemicals Ltd., Ahmedabad. The samples of *Andrographis* powder (aerial parts) were obtained as gift from PERD Center, Ahmedabad and J and J DeChane Laboratories, Hyderabad. Herbal formulation, Amlycure, (Aimil Pharmaceutical Pvt. Ltd., Delhi) was purchased from local market. All the chemicals used in the analysis were of AR grade. Reagents include picric acid (1% in acetone free methanol, picric acid was dried at 105º for 1 h before making solution), aqueous sodium hydroxide (10% in distilled water) and Baljet reagent (95 ml picric acid reagent + 5 ml aqueous sodium hydroxide).

For the preparation of linearity curve 10 mg andrographolide were dissolved in 100 ml hot methanol (100 µg/ml). Different volumes of standard solution (0.5 to 5 ml) were taken into 10 ml volumetric flask. Five milliliter of freshly prepared Baljet reagent was added to each flask. Volume was made up to 10 ml with methanol. The flasks were

TABLE 1: METHOD VALIDATION PARAMETERS

Validation parameters	Results
λ_{max} (nm)	494
Beer's law range (µg/ml)	5 to 50
Molar absorptive (l/mol. cm.)	0.49×10^4
Regression equation	Y=0.0151 x - 0.007
Slope	0.01513
Intercept	-0.0075
Correlation coefficient (r^2)	0.999
Precision (%RSD)	6.6×10^{-3}
Sandell's sensitivity (µg/cm^2/0.001abs.unit)	0.0714

TABLE 2: ANALYSIS OF *ANDROGRAPHIS* POWDER AND FORMULATION BY PROPOSED METHOD.

Sample	% andrographolide*
Powder-1**	1.5 ± 0.19
Powder-2**	0.95 ± 0.22
Capsule-1	0.308 ± 0.03

*Each value is the mean and standard deviation of three determinations. **Powder 1 obtained from PERD, Ahmedabad, Powder 2 obtained from J&J DeChane Laboratories, Hyderabad.

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TABLE 3: PERCENT RECOVERY BY PROPOSED METHOD

Sample	Amount of Andrographolide in mg	Amount of Andrographolide added in mg	Practical value*	% Recovery*	Average % recovery
Powder-1	15.2±0.20	2.5	17.5±0.31	99.0±1.20	
Powder-2	9.5±0.23	2.5	11.8±0.25	98.9±1.09	98.9

*Each value is the mean and standard deviation of three determinations

vortexed and kept aside for 20 min. Resultant color complex was measured against blank at 494 nm using spectrophotometer (Elico, Hyderabad). The blank was prepared in a similar manner without sample. The absorbance values were plotted against their respective concentrations of andrographolides to obtain a linearity curve.

For the extraction of andrographolides from sample, dried aerial parts of *A. paniculata* (1 g) was extracted in hot methanol (4×50 ml). The filtrate was concentrated on a water bath and concentrate washed with cold toluene (3×25 ml). Excess water was added to toluene insoluble fraction and extracted with ethyl acetate (4×50 ml) in a separating funnel. Ethyl acetate was evaporated to dryness on a water bath and residue re-dissolved in 10 ml methanol. From this solution, 0.2 ml was taken in a 10 ml volumetric flask and color was developed with Baljet reagent as described above. The amount of andrographolides in the sample was calculated from the calibration curve. All the experiments were carried out in triplicate. For the estimation of andrographolides in formulation, content of two capsules (1 g) was extracted in hot methanol. The filtrate was processed and analyzed in the same manner as described above.

The proposed method is based on the condensation of butenolide ring of andrographolides with picric acid, which produces red-orange complex in alkaline solution. The intensity of the red-orange color could be measured at 494 nm. The method was validated for precision, accuracy, linearity range, slope, intercept and correlation coefficient (Table 1). The method was validated for precision by repeating the experiment five times with the same quantity of andrographolides at three different concentrations. Percent relative standard deviation was found to be 6.6×10^{-3}. Different sample of *Andrographis* have 1.5 and 0.95% w/w andrographolides. Herbal capsules(Amylcure) contained 0.308% of andrographolides (Table 2). The accuracy of the method was determined by performing the recovery study after adding known amount of andrographolides to the already analyzed powder sample. The average percentage recovery was found to be 98.95% (Table 3).

Condensation of γ–lactone ring with picric acid in alkaline medium results in the formation of red orange complex which could be measured at 494 nm, this test is widely employed for testing cardenolides. Bhavsar *et al.*[8,9] and Bell and Krantz[10] have quantified cardenolides in various herbs by employing Baljet reagent. In the present study this reagent was employed for the estimation of andrographolides in samples and formulation. It was found that like cardenolides, andrographolides give positive test with Baljet reagent and could be satisfactorily estimated by the proposed method. Statistical analyses of the results prove the efficacy of the proposed method. Recovery studies showed the reliability and suitability of the method. The andrographolides were also studied by TLC in mobile phase chloroform: methanol (7:1) and vanillin-sulphuric acid reagent was used for spot visualization. Both samples showed 3 spots with R_f 0.43 (brown), 0.70 (brown, andrographolide) and 0.88 (purple). Since *Andrographis* is brought into market in various commercial forms; this method proves to be simple and economical for routine analysis of andrographolides in *A. paniculata* and its formulations.

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