Development of a New Spectrophotometric Method for the Analysis of Atropine Sulphate

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A new spectrophotometric method for the assay of atropine sulphate has been developed. This utilized the well known reaction of tropic acid, Vitalimonium reaction. The sample when digested with nitric acid and treated with 3% methanolic potassium hydroxide develops a blue chromophore. The absorbance of the resulting solution was measured exactly after one minute at 570 nm. This method has a linearity range of 2-20 μg/ml and compares well with official procedure.

Atropine is an organic ester formed by the combination of tropic acid, an aromatic acid and a complex organic base, tropine[1]. A non-aqueous titrimetry method for atropine sulphate and a gas chromatographic assay method for atropine sulphate formulations were reported in Indian pharmacopoeia[2]. The other methods reported for the assay of atropine sulphate are capillary zone electrophoresis[3], derivative spectroscopy[4,5], atomic emission spectroscopy[6] and HPLC[7].

However, all these methods involve lengthy extraction procedures and expensive instruments. Atropine sulphate is known to give positive Vitali's test[8]. Hence in the present investigation we have explored this well known reaction for the quantitative estimation of atropine sulphate.

Atropine sulphate used is a reference standard supplied by Ethypharm, Cedex, France. All other chemicals like pyridine, acetone, methanol and potassium hydroxide are of AR Grade and procured from Ranbaxy Fine Chemicals S.A.S. Nagar, Punjab. A 3.0% solution of methanolic potassium hydroxide was prepared in the laboratory. Twenty milligrams of atropine sulphate was dissolved in 10 ml of concentrated nitric acid and evaporated to dryness. The residue was cooled and dissolved in 10 ml of acetone and diluted to 100 ml with pyridine.

From this, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0 ml of the solutions were pipetted in to a 10 ml volumetric flask and the volume was made up with pyridine to the mark. Then 0.2 ml of 3% of methanolic potassium hydroxide was added, shaken well and the absorbance was measured exactly after one minute in each case.

The method was optimized for solvent selection, volume of methanolic potassium hydroxide and time of absorbance measurement. The solvents selected to solubilise the residue were acetone and pyridine. The acetone alone cannot be used because when the volume is made up with acetone alone, there no color was observed. After trying various other solvents pyridine was finally selected as a solvent for the dilution.

Methanolic potassium hydroxide is must for the reaction[8]. The optimized quantity is 0.2 ml of 3% methanolic potassium hydroxide, which gave maximum intensity of the chromophore. The absorbance values for different quantity of methanolic potassium hydroxide are given in Table 1. The absorbance was measured exactly after 1 min of addition of methanolic potassium hydroxide. As this colour is known to

TABLE 1: EFFECT OF METHANOLIC POTASSIUM HYDROXIDE ON ABSORBANCE.

Methanolic potassium hydroxide(ml)	Absorbance
0.1	0.120±0.0089
0.2	0.134±0.0071
0.3	0.106±0.0115

(n=7).

*For correspondence

TABLE 2: EFFECT OF TIME ON ABSORBANCE.

Time (min)	Absorbance
1.0	0.128±0.0064
1.5	0.101±0.0062
2.0	0.099±0.0041
3.0	0.084±0.0039
5.0	0.066±0.0021

(n=7).

be unstable, time at which the maximum intensity of the colour developed is an important parameter. To different sets of solutions, methanolic potassium hydroxide was added and the absorbance was measured. Maximum absorbance was observed at 1 min as indicated in Table 2.

This method has a linearity range of 2-20 μg/ml. The straight line at this concentration range had slope of 0.131±0.00922 and r^2 value of 0.9903±0.0069 (n=5). All the absorbance were measured at 570 nm in Systronics UV/Vis spectrophotometer model 108. The method was used to analyze 5 different samples of atropine sulphate and the results obtained compare well with the pharmacopoeial method (Table 3).

This new method is simple and rapid. It explores the well known Vitali's[8] reaction for quantitative estimation of atropine sulphate. Though the method is not specific, it can be used for the routine assay of atropine sulphate successfully.

TABLE 3: COMPARISON OF THE PRESENT METHOD WITH PHARMACOPOEIAL METHOD.

I.P. method	Proposed method
99.50±0.12	99.4±0.28
99.34±0.16	99.51±0.37
99.12±0.17	99.33±0.21
99.65±0.14	99.58±0.34
99.46±0.19	99.64±0.66

(n=5).

REFERENCES

1. Brown, J.H. and Taylor, P., In; Hardman, J.G., Limbird, L.E. Molinof, P.B. and Ruddon, R.W., Eds; Goodman and Gilman's Pharmacological Basis of Therapeutics, 9th Edn., McGraw Hill Publications, New York 1996, 149.
2. *Indian Pharmacopoeia Controller of Publications, New Delhi,* 1996, 77.
3. Maleus, L., Cherkaoul, S., Christen, P. and Veethey, J.L. **J. Chromatogr. A.,** 2000, 868, 285.
4. Hassan, S.M. and Davidson, A.G., **J. Pharm. Pharmac.,** 1984, 36, 7.
5. Richard, A. and Andermann, G., **Pharmazie,** 1984, 39, 866.
6. el-Shalat, M.F., Adbul Badei, M.M. and Daifullah, A.A., **J. Chem. Technol. Biotechnol.,** 1992, 54, 175.
7. Khalil, S., **J. Pharm Biomed Anal.,** 1999, 21, 697.
8. Atherdon, L.M., Eds., In; Bentley and Driver's Text Book of Pharmaceutical Chemistry, 8th Edn., Oxford University Press, New Delhi. 1999, 816.