New Analytical Methods and Their Validation for the Estimation of Analgin in Bulk and Marketed Formulations

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Abstract

Analgin is ampyrone sulphonate analgesic, antispasmodic and antipyretic which is most commonly given orally or parenterally to prevent and treat pain related to surgery or for the treatment of acute pain.

Purpose: There was a need for the development of newer, simple, sensitive, rapid, accurate and reproducible analytical methods for the routine estimation of analgin in bulk and pharmaceutical dosage form since the reported method make use of highly sophisticated intruments.

Methods: Two simple, sensitive, specific and validated methods (method A and method B) have been developed for the quantitative estimation of analgin in bulk and pharmaceutical dosage form. Method A is based on oxidation of analgin with ammonium molybdate to produce green colour chromogen which showed λmax at 682.8 nm. In method B, analgin oxidizes in presence of ferric chloride and subsequent complexation of resulting ferrous ion with 1,10- phenanthroline which absorbs at λmax at 511.2 nm.

Reults: The linearity was found in concentration range of 5-30µg/ml and 10-60µg/ml for method A and method B respectively. The correlation coefficient was found to be 0.9997 and 0.9995 for method A and method B respectively. The methods were validated as per ICH guidelines. The LOD and LOQ for estimation of analgin were found as 0.1390, 0.4214 for method A and 0.1596, 0.4837 for method B respectively.

Conclusions: Proposed methods were successfully applied for the quantitative estimation of analgin in marketed formulations.

Keywords: analgin, ammonium molybdate, ferric chloride hexahydrate, 1,10- phenanthroline.

Introduction:

Analgin is used for the treatment of pains of different origin and variable intensity: toothache, headache, arthralgia, neuralgia, myositis, mild to moderate pain, high fever, not responding to other drugs. The exact mechanism of analgin is not explained in detail. It is assumed that it operates in a combined central and peripheral effects[1,2]. Various methods such as spectrophotometric[3-6], HPLC[7], LC-MS/MS[8] and bioequivalence studies[9] have been reported. There was a need for development of simple, precise, accurate and which uses simple reagents for the estimation. Molecular absorption spectrophotometry is by far the instrumental technique of choice in industrial laboratories, owing mainly to its simplicity, often demanding low-cost equipment and lending itself to easy automation of trace analysis procedures.

Materials and Methods:

Analgin was obtained in highly pure form (pharmaceutical grade) from the local pharmaceutical industry. Its pharmaceutical preparations obtained from different commercial sources. All other reagents were of analytical grade. Distilled water was used for preparation of all solutions. Ultraviolet and visible spectrophotometry were carried out through Systronics UV-Visible Double beam spectrophotometer 2202.

Standard drug solution:

Accurately weighed 100mg of analgin was dissolved and volume was made up to 100ml with distilled water to give a concentration of 1mg/ml. The final concentration was brought to 50 and 100 µg/ml for Methods A and B respectively.

Reagents:

Method A:

- 3.5% ammonium molybdate
- 2.5 M HCl

Method B:

- 0.3% ferric chloride hexahydrate
- 1.5% 1,10-Phenanthroline

Assay procedure for the determination of Etodolac:

Method A: Seven 10ml volumetric flasks were taken. Then 1, 2, 3, 4, 5, 6 ml working standard solution of Analgin was added. To each flask, 2.5 ml of ammonium molybdate and 2.0 ml of 2.5M HCl were added, kept aside for 15 minutes. Made up the volume with distilled water. Absorbance was taken at 682.8 nm.

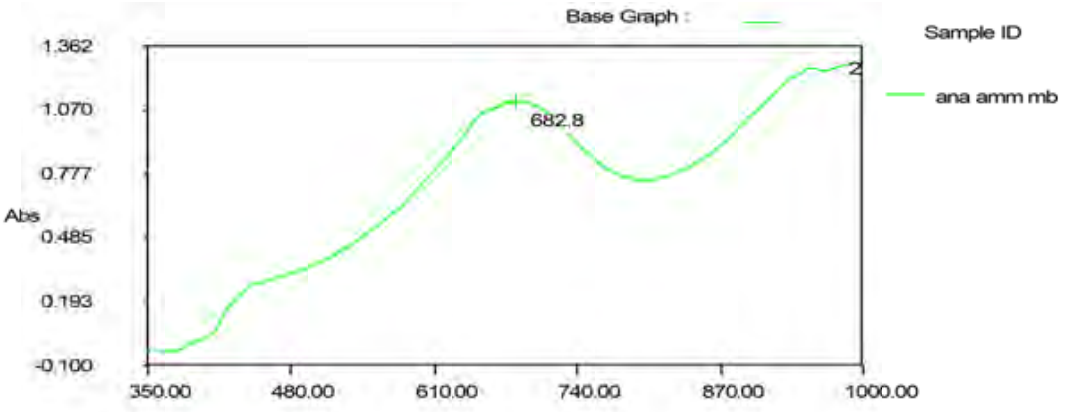

Fig: 1 Absorption spectrum of colored chromogen in method A

Method B:

Seven 10ml volumetric flasks were taken. Then 1, 2, 3, 4, 5, 6 ml working standard solution of Analgin was added. To each flask, 1.2ml of 0.3% FeCl3 and 0.9ml of 1.5% of 1,10- PTH was added and kept it for 10 minutes on boiling water bath until the completion of reaction. Then volume was made up to the mark with distilled water. Absorbance was taken at 511.2 nm.

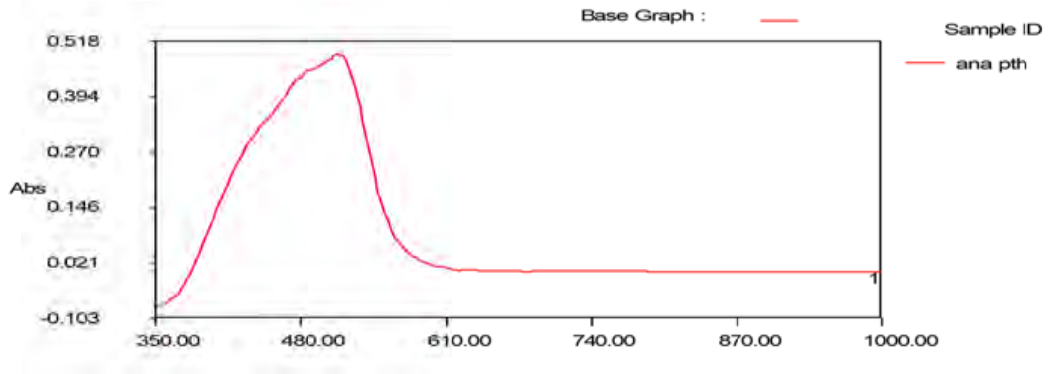

Fig: 2 Absorption spectrum of colored chromogen in method B

Assay of pharmaceutical formulations

Weighed accurately tablet powder equivalent to 100mg of analgin and transferred into 100ml volumetric flask and made up the volume with distilled water to get 1000µg/ml concentration. This solution was further diluted to get concentration range required. To keep an additional check on accuracy of developed assay method, analytical recovery experiments were performed. The different solutions of different concentration were prepared in case of both pure drug solution and formulation extract solution and these solutions were subjected to analysis by above developed methods. Results of recovery studies are reported in table 2.

TABLE - 2.Assay and Recovery of Analgin in Pharmaceutical Formulations

Method	Sample	Labeled amount (mg)	Amount found (mg)	% Recovery
Method A	Analgin I.P.	500	492.34	98.46
Method B			489.32	97.86

Results and Discussion:

In method A, Analgin was estimated based on the reaction of ammonium molybdate in the presence of acidic medium. Ammonium molybdate behaves as an oxidizing agent in acidic medium and it oxidizes analgin. Reduced molybdate moiety forms complex with oxidized form of analgin. Ammonium molybdate has been used as oxidizing agent for spectrometric determination of pharmaceutical substances. The colored solution is obtained as a result of reduction of acidified molybdenum [Mo(VI)]. It has several oxidation states, the most stable being +4 and +6. The proposed method B involves the oxidation of analgin with ferric chloride and subsequent complexation of resulting ferrous ion (Fe^{2+}) with 1, 10- phenanthroline to form orange red coloured chromogen. Fe^{3+} oxidizes analgin and the produced Fe^{2+} forms orange red coloured complex by reacting with 1, 10- phenanthroline. The absorbance of the coloured solution increases linearly with an increasing concentration of the analgin.

TABLE – 1 Optical Characteristics, Precision and Accuracy of Proposed Methods

Parameters	***Method A***	***Method B***
λmax (nm)	682.8	511.2
Beer's law limit (µg/ml)	5-30	10-60
Regression Equation* (y)	0.0163x+0.0023	0.0154x+0.0059
Slope(b)	0.0163	0.0154
Intercept(a)	0.0023	0.0059
Correlation coefficient (R2)	0.9997	0.9995
Accuracy (% Recovery)	97.67	97.92
Precision (% RSD)		
I. Method Precision	0.70	0.47
II. Intermediate Precision		
a. Intra-day	0.73	0.35
b. Inter-day	1.3	0.70
Limit of Detection (µg/ml)	0.139	0.1596
Limit of Quantitation (µg/ml)	0.4212	0.4837

y = bx + a, where y is the absorbance and x is the concentration of analgin in µg/ ml.

Conclusion

The proposed validated spectrophotometric methods are simple, rapid, accurate, precise and inexpensive and hence can be used for the routine analysis of analgin in tablet. The sample recovery for both the methods were in good agreement with their respective label claims, which suggested non- interference of formulation additives in estimation.

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