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Simple Extractive Spectrophotometeric Determination of Amphetamine Sulphate by Acid Dye Complexation in Solid Dosage Form

Kalkotwar R. S[1*], Kasture A. V. [2], Pattan S. R.[1], Dengale S. S.[1] , Dighe N.S.[1]

[1]*Department of Pharmaceutical Chemistry, Pravara Rural College of Pharmacy, Pravaranagar, Loni, Ahmednagar, M.S. (India)*
[2]*Department of Pharmaceutical Sciences, RTM Nagpur University, Amravati Road, Nagpur, M.S. (India)*

Abstract

Simple, accurate, rapid and highly sensitive Extractive Spectrophotometeric determination method has been developed and validated for estimation of Amphetamine Sulphate in pharmaceutical solid dosage form. The proposed method is based on the principle that Mono and diamines in the dissociated form (Ion) combined with dissociated form of the Acid dye Solochrome black T. The formation of Acid dye complex has been evaluated by carrying TLC, Infra-red spectra. The acid dye-drug complex has λ_{max} at 520 nm. Beer's Lambert Law obeyed in the range of 20-100µg ml^{-1}. The results were validated statistically. The further scope for this method is that it can be applied to body fluid in cases of intoxication.

Keywords: Spectrophotometric method, Acid-dye complex, Amphetamine Sulphate, Solochrome black T

Introduction

Amphetamine Sulphate is symphatomimetic and central nervous system stimulant, anortic drug.[1] Chemically it is dl-α-methyl phenethylamine sulphate. Literature survey revealed the availability of the method of estimation of the drug by non-aqueous titration [2], by residual alkalimetry following solvent extraction[3] and by HPLC method [4]. Earlier work in this field have been pertaining to the like Bromothymol blue, Bromophenol blue, Bromocresol purple, Bromocresol green Methyl orange etc. Which operates in a particular range of pH. In the present study Solochrome black T a well known dye used in complexometric titration have been used, for developing Acid-dye technique to estimate Amphetamine sulphate.

The aim of this study is the direct determination of Amphetamine sulphate by extractive spectrophotometric method and the application to the solid dosage form.

Instruments:- 1) Bausch & Lamb Spectrophotometer- Spekol model.
2) Perkin Elmer 377 IR- Specord model

Chemical & Reagents:-
The Amphetamine sulphate in the pure form was made available from M/S IndyaPharma Ltd. Mumbai Central, Mumbai- 400004 India. The commercially available reputed tablets from local markets containing 5mg Amphetamine sulphate per tablet have been used for estimation. All other chemicals used are of AR grade from reputed supplier and all solvents were redistilled before use. Acid-dye Solochrome black T used was from S.D.Chemicals Mumbai.

Results and Discussion

For the present study Ion-pair Extractive Spectrophotometeric method is used because of its selectivity, sensitivity and accuracy.

The Drug dye complex shows maximum absorbance at 520 nm. The high molar absorptivity of the resulting complexes indicates the high sensitivity of this method. The Beer Lambert's law obeyed in the concentration range of 20 to100 mcg $ml^{-1}$and correlation coefficient was found to be 0.9993. Relative Standard deviation is within permissible limit. The accuracy of the method is indicated by the excellent recovery and the precision is supported by the low standard deviation <0.9.

The IR spectra of the Drug-dye complex shows important peaks at A 1040 cm-1, B 1200cm -1, C 1452 cm -1, D 1600cm-1, wave numbers, these peaks clearly show the formation of Drug-dye complex since the corresponding peaks[5] in Amphetamine sulphate A 695 cm-1,B 737 cm-1 and C1452 cm-1 has been shifted. The TLC study shows the formation of Drug-dye complex as the different Rf values are found for Pure Amphetamine sulphate, Solochrome black-T and Drug-dye complex.

Materials and Methods

Experimental: Standard solution of the drug
A stock standard solution of 100 mg ml^{-1} was prepared by dissolving Amphetamine sulphate in buffer solution pH-3. Working standard solution was then prepared by suitable dilution of the stock standard solution with buffer solution pH 3.

Selection of Wavelength
A solution of 2mg ml^{-1} concentration of Amphetamine sulphate was taken and **"**Dye solution with solvent" [i.e.5ml dye solution (1gm/100ml) and 5ml saturated solution of Sodium chloride and 10ml Chloroform containing 1% Amyl alcohol] was added and shaken well and centrifuge for 5mins. The absorbance of Chloroform layer at 520 nm was measured against reagent blank. The curve was plotted Absorbance vs Wavelength (Fig .1) The maximum Absorbance found at 520nm.

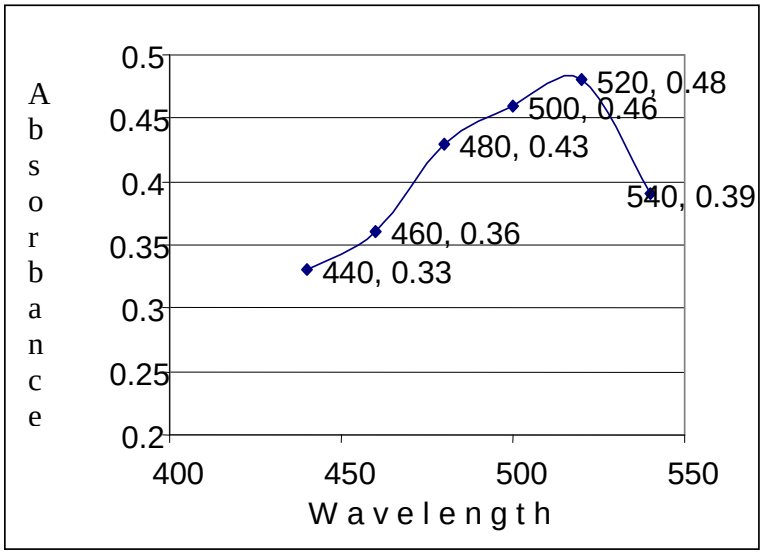

Fig. 1- Absorbance Spectra of Acid Dye-Drug complex in Amphetamine sulphate.

Procedure for the assay of bulk sample

From the 100 mg ml^{-1} solution, 0.2, 0.4, 0.6, 0.8 and 1 mg were transferred to centrifuge tubes and volume in each was made to 10 ml with pH 3 buffer. To it then added "Dye solution with solvent" and shaken well and centrifuged for 5mins. The absorbance of Chloroform layer at 520 nm was measured against reagent blank. The standard calibration plot was prepared to calculate the amount of the analyte drug in unknown samples. The results were tabulated in Tab.1 and that of official method in Tab.2

Tab. 1- Results of Estimation of Amphetamine Sulphate in powder (Sample A) by proposed method

Sr. No.	Weight of sample	Content found	%of drug	Statistical Data
1.	30.40 mg	30.59 mg	100.65	Mean 100.65 %
2.	60.80 mg	61..08 mg	100.65	S.D.* ± 0.3129
3.	40.10 mg	40.29 mg	100.49	S.E.** 0.1277
4.	80.20 mg	80. 39 mg	100.49	C.V. *** 0.3106
5.	51.70 mg	52.29 mg	101.14	
6.	103.40 mg	104.58 mg	101.14	

* Standard deviation ** Standard Error *** Coefficient of variation

Tab.2 - Results of Estimation of Amphetamine Sulphate in powder (Sample A) by Official method

Sr. No.	Weight of sample	Content found	%of drug	Mean
1.	0.2027 g.	0.2067 g.	101.87	101.82 %
2.	0.2152 g.	0.2188 g.	101.67	

Analysis of Tablets

The total content of the 20 tablets (sample AT-01 & sample AT-02 separately) was weighed and grounded to a fine powder using a glass pestle and mortar. The average weight of a tablet was calculated. An accurately weighed portion of the powder equivalent to 5mg Amphetamine sulphate was transferred into 80 ml of pH 3 buffer solution. The resulting solution was filtered; washings were given to filter and residue. Then volume was made to 100ml with pH 3 buffer in volumetric flask. Different volumes corresponding to 0.5 mg were transferred to centrifuge tube. To it then added **"**Dye solution with solvent". It was then centrifuged and Chloroform layer separated out and absorbance measured at 520 nm. The results were tabulated in Tab.3 and that of official method in Tab.4 & 5

Tab.3- Results of Estimation of Amphetamine Sulphate in tablet (Sample AT) by proposed method

Drug	Label Claim	Amount found	% Recovery* ±. % R.S.D
Sample AT-01	5mg	5.019mg*	100.38 ± 0.572
Sample AT-02	5mg	5.009mg*	100.19 ± 0.317

* Average of six determinations

Tab.4- Results of Estimation of Amphetamine Sulphate in tablet (Sample AT-01) by Official method

Sr. No.	Weight of sample	Content found	%of drug	Mean
1.	44.58 mg	44.80 mg	100.48	100.55 %
2.	50.28 mg	50.59 mg	100.62	

Tab.5- Results of Estimation of Amphetamine Sulphate in tablet (Sample AT-02) by Official method

Sr. No.	Weight of sample	Content found	% of drug	Mean
1.	44.68 mg	44.75 mg	100.16	100.24 %
2.	50.30 mg	50.46 mg	100.32	

Validation data of proposed method

Optical characteristics, Precision and Accuracy

The data regarding Optical characteristics, Precision and Accuracy has been tabulated in Tab.6

Tab.6- Optical characteristics, precision and accuracy of the proposed method

Parameters	**Values**
λmax	nm 520
Beer's law limit	mcg ml^{-1} 20-100
Molar absorptivity	1 mol^{-1} cm^{-1} 5.3388×10^3

Linear regression equation	
Y = mX + C	*Slope m 2.7×10-3
Standard Deviation of Slope	0.12×10-2
Intercept (C)	1.81×10-2
Standard Deviation of Intercept	0.012×10-3
Correlation coefficient (R^2)	0.9993
% Range of error (Confidence limits) [a] 0.05 level 0.01 level	 0.131 0.060

Where Y is absorbance and X is the concentration (mcg ml^{-1}); [a] Average of five determination

Linearity and range

Beer's law range, molar absorptivity, regression equation, and correlation coefficient determined for this method are given in tab.6 A linear relationship was obtained in the concentration range of 20 to100 mcg ml^{-1}. Regression analysis of the Beer's law plots reveals a good correlation. The graphs show negligible intercept and are described by the regression equation, Y = mX + C (where Y is the absorbance of 1 cm layer, m is the slope, C is the intercept and X is the concentration of the measured solution in mcg ml^{-1}. The high molar absorptivity of the resulting complexes indicates the high sensitivity of this method.

RSD and % Recovery

The standard deviation, relative standard deviation, recovery and were determined from the calibration curve, as recorded in Tab.7. The accuracy of the method is indicated by the excellent recovery and the precision is supported by the low standard deviation <0.9.

Tab.7- Determination of Amphetamine Sulphate in Tablet by Proposed method

Drug	Label Claim	Amount found	% Recovery* ±. % R.S.D
Sample AT-01	5mg	5.019mg*	100.38 ± 0.572
Sample AT-02	5mg	5.009mg*	100.19 ± 0.317

* Average of six determinations.

Isolation and characterization of acid dye complex

i) Isolation of Drug-dye complex:-

About 0.2g Amphetamine sulphate was dissolved in buffer pH 3 and volume made to 100ml and it was mixed with 100ml dye solution (1g/100ml) in separator and shaken and allowed to stand for 15 minutes. It was then extracted with several portions of Chloroform (~500ml) The Chloroform extract was combined and the Chloroform distilled off. The residue was taken in suitable container and dried in an oven at 85^0C . The brownish colored powder was obtained.

ii) TLC of Drug-dye complex:-

The Amphetamine sulphate-dye complex solution in Chloroform was spotted on Silicagel G TLC plates along with the spots of Amphetamine sulphate and Dye solution. The plates were developed in Chloroform- methanol (85+15) solvents mixture, solvent front was run to 14cm and spots visualized in Iodine vapor. The Drug-dye complex has different Rf value than Amphetamine sulphate and Solochrome black-T. The results are tabulated in Tab.8

Tab.8- Results of TLC

Sr. No.	Sample	Rf.
1	Standard Amphetamine sulphate	0.20
2	Solochrome black T dye	0.014
3	Amphetamine-dye complex	0.03

iii) UV and Infra-red spectra of Drug-dye complex :-

UV Spectra for Amphetamine sulphate and its dye complex were recorded on Perkin Elmer Spekol spectrophotometer and λmax found were 259nm and 520nm respectively. IR spectra for

Amphetamine sulphate and its drug-dye complex were recorded on Perkin Elmer Specord using Nujol-mull method.

The IR spectra of the above Drug-dye complex shows important peaks at A 1040 cm-1, B 1200cm -1, C 1452 cm -1, D 1600cm-1, wave numbers, these peaks clearly show the formation of Drug-dye complex since the corresponding peaks[5] in Amphetamine sulphate A 695 cm-1,B 737 cm-1 and C1452 cm-1 has been shifted. Furthermore, had there been no drug-dye complex formation, the peaks in the region 1600-900 cm-1 wavelength for drug-dye complex would have shown superimposition on the peaks of Amphetamine sulphate.

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