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Spectrophotometric Analysis of Nicotine Bitartrate Dihydrate in Pure form and in Formulations

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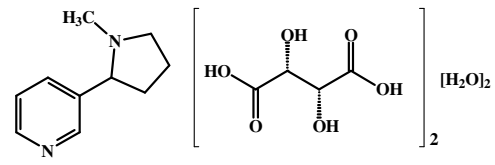
An UV spectrophometry method was developed and validated for the routine quantitative analysis of nicotine bitartrate dihydrate. The method was found to be linear (coefficient of variation *ca*. 0.9994), precise (> 1 % RSD) and accurate (average recovery *ca*.100.534 %).

Key Words: Nicotine bitartrate dihydrate, UV Spectrophotometry, Analysis, Pure, Accurate, Precision.

INTRODUCTION

Tobacco smoking related diseases constitute a major health problem all over the world[1]. Nicotine is the principle alkaloid in tobacco and is responsible for causing dependence due to its psychoactive properties and capacity to induce self administration behaviour. Various effective methods are now available which can help a person to quit nicotine addiction[2]. Nicotine replacement therapy is the use of various forms of nicotine delivery methods intended to replace nicotine obtained from smoking or other tobacco usage. These products are intended for use in smoking cessation efforts to help deal with withdrawal symptoms and cravings caused by the loss of nicotine from cigarettes[3,4]. Nicotine replacement therapy (NRT) treatments are available in many dosage forms, including chewing gum, sublingual tablets, adhesive transdermal patches, nasal sprays and oral mucosal inhalers. These NRT formulations either give a rapid, short-lived plasma level peak of nicotine (*i.e.* nasal spray), or a slow onset with prolonged, sustained plasma nicotine levels (*i.e.,* chewing gum and transdermal patches). Novel formulations of nicotine have been developed in the investigators laboratory, which are targeted at smoking cessation[5].



Structure of nicotine bitartrate dihydrate

Nicotine bitartrate dihydrate (Fig. 1) is a salt of nicotine of pharmaceutical grade and tartaric acid used for preparation of the products for smoking cessation

and as an analytical standard. It is highly soluble in solvents such as water, methanol, acetonitrile, chloroform and petroleum ether[6]. The various methods available for the analysis of nicotine bitartrate dihydrate are colorimetric[7], spectrophotometric and chromatographic methods of analysis[8-10]. Amongst these methods the chromatographic methods are widely used. The methods described above were essentially bioanalytical in nature. They have been used either to support pharmacokinetic studies on nicotine, determination of free base and its metabolites in various physiological fluids such as saliva[11], plasma/serum[12], urine[13] and nicotine determination from tobacco samples *etc.* These methods though highly specific and sensitive for nicotine, may not yield precise and accurate results during *in vitro* analysis of nicotine dosage forms.

EXPERIMENTAL

Nicotine bitartrate dihydrate was obtained from Alchem International, Pvt. Ltd., Gujarat. Concentrated HCl was obtained from S.D. Fines Chemicals, Mumbai. Distilled water was used to prepare the dilutions.

The developed method was validated for the parameters like linearity, range, limit of detection, limit of quantitation, precision and accuracy.

Linearity: Linearity is the ability of the method to test results that are directly proportional to analyte concentration within a given range. Linearity is generally reported as the variance of the slope of the regression line.

Procedure: Accurately weighed 50 mg of nicotine bitartrate dihydrate was dissolved in sufficient amount of 0.1 N HCl in a 50 mL volumetric flask and diluted to volume with the same. From the above solution, 1 mL of aliquot was withdrawn and diluted to 100 mL with the same solvent so as to obtain solution of concentration 10 µg/mL. From this solution, samples of 5, 6, 7, 8, 9 and 10 mL were withdrawn in 10 mL volumetric flasks and diluted to volume with the same solvent so as to obtain standard solutions of concentrations 5, 6, 7, 8, 9 and 10 µg/mL, respectively. The absorbance of the standard solution was determined on UV spectrophotometer Jasco V-530 at λ_{max} 259 nm. A standard plot of absorbance *versus* concentration of nicotine bitartrate dihydrate/mL was obtained.

Range is the interval between the upper and lower levels of analyte (inclusive) that have been demonstrated to be determined with precision, accuracy and linearity. The ICH guidelines specify a minimum of five concentration levels for the purpose of linearity studies.

Standards: The co efficient of correlation should be more than 0.995.

Range of the analytical method was determined as the upper and the lower limit of the linearity curve. The results for linearity range are given in the Table-1.

Limit of detection (LOD): The limit of detection is the lowest concentration of analyte in a sample that can be detected, but necessarily quantitated, under the stated experimental conditions. The lowest limit of detection measured for stock solution.

TABLE-1
LINEARITY STUDIES ON NICOTINE BITARTRATE DIHYDRATE
TO BE ASSAYED SPECTROPHOTOMETRICALLY

Concentration (μg/mL)	Absorbance at 290 nm
0	0.0000
5	0.1750
6	0.2040
7	0.2452
8	0.2805
9	0.3193
10	0.3503

Limit of quantitation (LOQ): Limit of quantitation is a parameter of quantitative assay for levels of compounds in sample matrices, such as impurities in bulk drug substances and degradation products in finished pharmaceuticals. It is the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions. The limit of quantitation is the minimum amount that gives precise measurements times the detection limit. The lowest limit of quantitation was measured for stock solution.

Precision: Precision is the measure of the degree of repeatability of an analytical method under normal operation and is normally expressed as the per cent relative standard deviation for a statistically significant number of samples.

Repeatability: Repeatability of the method was checked by analyzing 6 replicate samples of nicotine (at the 100 % of the test solution *i.e.*, 7 μg/mL) and calculating per cent relative standard deviation (% RSD).

Intra-day-precision: Inter-day precision of the method was checked by repeating the entire procedure at 3 various intervals of the same day and calculating the RSD between 3 intervals for measuring three different concentrations (2, 5 and 10 μg/mL) and three replicates of each concentration of the solution from the above prepared stock solution.

Inter-day precision: Inter-day precision of the method was checked by repeating the entire procedure for 3 consecutive days and calculating the % RSD between 3 days for measuring three different concentrations (2, 5 and 10 μg/mL) and three replicates of each concentration of the solution from the above prepared stock solution. Precision evaluated by measuring the % RSD.

Accuracy: Accuracy may often be expressed as per cent recovery by the assay of known, added amount of analyte. The accuracy of an analytical method may be determined by applying that method to samples or mixtures of excipients to which known amounts of analyte recovered by the assay.

Procedure

Standard stock solution: Accurately weighed 50 mg of nicotine bitartrate dihydrate was dissolved in sufficient amount of 0.1 N HCl in a 50 mL volumetric flask and diluted to 50 mL with the same solvent. From this solution 1 mL of aliquot was

withdrawn in 100 mL volumetric flask and diluted to 100 mL again with the same so as to yield the solution of concentration 10 µg/mL. (solution A).

Sample stock solution: Nicotine bitartrate dihydrate equivalent to 10 mg was weighed and transferred to a 100 mL volumetric flask. The weighed sample was shaken with 50 mL of 0.1 N HCl and diluted to volume with the same. This solution was shaken with 50 mL of 0.1 N HCl and diluted to volume with the same. This solution was filtered through Whatmann filter paper to yield a solution of concentration 100 µg/mL (solution B).

Preparation of sample solutions: Set I: Solution B (5 mL) diluted to 50 mL with 0.1 N HCl, concentration = 10 µg/mL. **Set II:** Solution A (5 mL) + solution B (5 mL), diluted to 50 mL to yield a solution of concentration = 11 µg/mL. **Set III:** Solution A (10 mL) + solution B (5 mL) diluted to 50 mL to yield a solution of concentration = 12 µg/mL. **Set IV:** Solution A (15 mL) + solution B (5 mL) diluted to 50 mL to yield a solution of concentration =13 µg/mL.

Samples of each set were prepared in triplicate and an average of three absorbance readings was taken for each set and percentage recovery was calculated the using the formula:

$$\text{Recovery}\,(\%) = \frac{n(\Sigma XY - \Sigma X\Sigma Y)}{n(\Sigma X^2 - (X)^2)} \times 100 \quad (1)$$

RESULTS AND DISCUSSION

Table-1 represents the absorbance obtained for the respective concentrations in the linearity curve plotted for nicotine bitartrate dihydrate at λ_{max} 259 nm.

Linearity: Table-2 represents the regression statistics obtained from the linearity test. The linearity of an analytical method is its ability to elicit test results that are directly or by a well-defined mathematical transformation, proportional to concentration of analyte within a given range. The linearity of the method was observed in the expected concentration range, demonstrating its suitability for analysis. The goodness of fit (R^2) was found to be 0.9994. Table-1 reveals that the lowest limit of quantification was found to be 5 µg/mL whereas the lowest limit of detection was found to be 0.1 µg/mL.

TABLE-2
REGRESSION STATISTICS FOR NICOTINE BITARTRATE DIHYDRATE

Parameter	Range	Goodness of fit (R^2)	Slope	Intercept
Linearity	0- 10 µg/mL	0.9994	0.0352	-0.0016

Precision: The precision of an analytical method is the degree of agreement among the individual test results when the method is applied repeatedly to multiple sampling of homologous sample.

Repeatability: It refers to the use of analytical procedure within a laboratory over a short period of time using the same analyte with the same equipment and is expressed as the per cent RSD. The method passed the test for repeatability as determined by % RSD (Table-3).

TABLE-3
REPEATABILITY STUDIES

Concentration (µg/mL)	Absorbance	RSD (%)
7	0.2446	0.649
	0.2502	
	0.2415	
	0.2519	
	0.2476	

Intermediate precision: Intermediate precision involves estimation of variations in analysis when a method is used within laboratories, on different days, by different analysts and on different equipments. The intermediate precision was studied by preparing the standard curve for 3 different days and the results of interday variation are given in Table-4. The method passed the test for intermediate precision as per cent RSD of the absorbance obtained with 3 different days were within the limits of 2 %.

Accuracy: The accuracy of an analytical method is the closeness of test results obtained by the method to the true value. The results of per cent recovery of the drug from solution is given in the Table-5. The per cent drug recovered from the solution was calculated using the formula 1 was found to be 100.53 % reveals that the method is evident within the desired range.

TABLE-4
INTERMEDIATE PRECISION

Concentration (µg/mL)	RSD Intraday precision (%)	RSD Interday precision (%)
2	0.525	0.165
5	0.703	0.306
10	0.217	0.157

TABLE-5
ACCURACY/RECOVERY DATA FOR NICOTINE BITARTRATE DIHYDRATE

Amt. of Std added (µg/mL)	X2	Absorbance (Avg. of 3 readings for each set)	Concentration (µg/mL) Y = abs/K*	XY
0	0	0.3489	9.9500	0
1	1	0.3850	10.9800	10.9800
2	4	0.4209	12.0028	24.0056
3	9	0.4546	12.9602	24.0056

Conclusion

A simple method using UV spectrophotometer was developed successfully for the quantitative determinations of nicotine bitartrate dihydrate. The method was validated with respect to various analytical parameters. The method was found to be linear, accurate and precise for the desired range of concentrations.

However, the proposed method should be evaluated for its ability to separate various degradation products of nicotine bitartrate dihydrate that may arise during stability studies/stressed conditions on the formulations. Experiments should also be carried out to evaluate stability-indicating potential of this method.

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