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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF PRAMIPEXOLE IN BULK AND FORMULATIONS BY USING UV SPECTROSCOPIC TECHNIQUE

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ABSTRACT

A simple, sensitive, accurate and economic UV Spectrophotometric method was developed for the estimation of Pramipexole in bulk and its tablet formulations. According to ICH guidelines this method has validated and it is proved to be economical, accurate, precise, simple, and specific in nature. Distilled water is used as solvent, it obeys beer's law in linearity range of 10 -30µg/ml and it shows maximum absorbance at 265nm The value of %RSD for intraday and interday precision was found to be less than 1.This value confirms that method is precised. This method proves that there is no interference of excipients in formulation because the value of % Recovery greater than 99 % for this method. The values of %Recovery for analysis of formulations are found within 99-101%, which shows that the method is applicable for analysis of marketed formulation.

KEYWORDS: UV spectrophotometry – Pramipexole, ICH guidelines, Recovery, Formulation.

INTRODUCTION[1-4]

Pramipexole dihydrochloride monohydrate[5-8] is a White crystalline powder which is freely soluble in water. Melting point 296.0° – 298.0°C. molecular weight 284.25 its chemical name (s)-2-amino-4, 5, 6, 7-tetrahydro-N-6-propyl-2, 6-benzothiazoledianmine dihydrochloride monohydrate. It is used as Antioxidant, Free Radical Scavenger, Antiparkinson Agent, Antidyskinetic, Dopamine Agonist.

Mechanism of action Pramipixole has ability to stimulate dopamine receptors in striatum. It is used to treat Parkinson's disease[9] but mechanism of action is unknown.

AIM AND OBJECTIVE

Aim of this work is to develop a simple accurate precise spectroscopic analytical method for the estimation of pramipixole in raw form and also tablet formulation form and to validate that method according to ICH guidelines.[10]

METHOD

Lets estimate the sample with Calibration Graph Method.[11]

- In this procedure the absorbance of number of standard solutions are noted.
  The concentration of standard solutions should encompassing the sample concentration.
- Then calibration graph is constructed.
- The concentration of analyte in the sample solution read from the graph as concentration corresponding to the absorbance of solution.

If the absorbance values & concentrations bear a linear relationship, then we obtain the regression line Y=α+βx

$$\alpha = \frac{(\sum y)(\sum x^2)-(\sum x)(\sum xy)}{N\sum x^2-(\sum x)^2}$$

$$\beta = \frac{N\sum xy-(\sum x)(\sum y)}{N\sum x^2-(\sum x)^2}$$

ANALYTICAL METHOD VALIDATION[12]

Analytical Method Validation is "the collection and evaluation of data, from the process design stage throughout production, which establishes scientific evidence that a process is capable of consistently delivering quality products.

The international Conference on Harmonization (ICH), which has been on the forefront of developing the harmonized tripartite guidelines for adoption in the US, Japan and EC, has issued two guidelines under the titles 'Text on validation of Analytical procedures(Q_2A) and validation of Analytical procedure Methodology (Q_2B)'.

Various parameters for analytical method validation according to ICH guidelines

Accuracy.[13]

The accuracy of an analytical method may be defined as the closeness of the test results obtained by the method to

the true value. The ICH documents recommend that accuracy should be assessed using a minimum of nine determinations over a minimum of three concentration levels, covering the specified range (i.e. three concentrations and three replicated of each concentration).

Closeness of aggrement between the test results with that of true value is considered as accuracy.

Precision.[13]

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility.

The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements.

- **Repeatability**

Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision.

Determination of Repeatability

Repeatability can be defined as the precision of the procedure when repeated by same analyst under the same operating conditions (same reagents, equipments, settings and laboratory) over a short interval of time. The ICH documents recommend that repeatability should be assessed using a minimum of nine determinations covering the specified range for the procedure (i.e. three concentrations and three replicates of each concentration or using a minimum of six determinations at 100% of the test concentration). (RSD below 1% for built drugs, RSD below 2% for assays in finished product).

- **Intermediate precision**

Intermediate precision expresses within-laboratories variations: different days, different analysts, different equipment, etc.

- **Reproducibility**

Reproducibility expresses the precision between laboratories (collaborative studies, usually applied to standardization of methodology).

Linearity and range.[13]

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 the analyte concentration in samples within a given range. Linearity usually expressed in terms of the variance around the slope of regression line calculated according to an established mathematical relationship from test results obtained by the analysis of samples with varying concentrations of analyte.

According to Beer's and Lambert's law concentration of analyte is directly proportional to absorbance of analyte. When we construct a graph between concentration and absorbance we get a straight line passing mostely through the origin and it is expressed in terms of slope and coefficient of regression. The concentration range of analytical samples which obeys beer's lambert's law is considered as linearity range of that particular method. Range is defined as difference between upper and lower concentrations of analyte in linearity

Limit of detection.[13]

The limit of detection is the parameter of limit tests. It is the lowest level of analyte that can be detected, but not necessarily determined in a quantitative fashion, The determination of the limit of detection of instrumental procedures is carried out by determining the signal-to-noise ratio by comparing test results from the samples with known concentration of analyte with those of blank samples and establishing the minimum level at which the analyte can be reliably detected. A signal-to-noise ratio of 2:1 or 3:1 is generally accepted. For spectroscopic techniques or other methods that rely upon a calibration curve for quantitative measurements, the IUPAC approach employs the standard deviation of the intercept (S_a) which may be related to LOD and the slope of the calibration curve, b, by LOD = 3 S_a / b.

The lowest amount of analyte which we can detect but not quantify through our technique is considered as limit of detection. Limit of detection of any analytical technique is determined by fallowing ways 1.By using signal to noise ratio value (2:1 or 3:1 acceptable).

2. By using formula in case of spectroscopic technique by the usage of slope and intercept (LOD=3S_a/b).

Limit of quantification.[13]

The limit of quantization is the lowest concentration of analyte in a sample that may be determined with acceptable accuracy and precision when the required procedure is applied.

The lowest concentration of analyte which we can determine accurately and precisely is considered as limit of quantification.

Selectivity and Specificity.[13]

The selectivity of an analytical method is its ability to measure accurately and specifically the analyte of interest in the presence of components that may be expected to be present in the sample matrix.

We can say any method as selective and specific when we are able to measure our analyte of interest even in the presence of some other substance

Robustness.[13]

"The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate

variation in method parameters and provides an indication of its reliability during normal usage. The determination of robustness requires that methods characteristic are assessed when one or more operating parameter varied".

The developed method should not be effected by small but deliberate changes that happens in method parameters so the method is considered as robust

Stability and System suitability tests.[13]
Stability of the sample, standard and reagents is required for a reasonable time to get reproducible and reliable results. For example, 24 hour stability is desired for solutions and reagents that need to be prepared for each analysis.

Experimental
Apparatus and Software
Double beam spectrophotometer was used which is having UV spectra software. And quartz cells(1cm) 10ml capacity over the range of 400-800 nm are used.

Reagents and Pharmaceutical Preparations
Analytical grade chemicals are used.

"Zydus cadila healthcare limited (Ahmedabad, India)" has provided required Pramipexole sample.

Solvent: "Distilled water"
Formulation: "Pramipex 0.5mg"
"Pramirol SR 0.26"

Preparation of Standard solutions
Pramipexole stock solution: 1mg ml^{-1}, prepared by dissolving 50 mg Pramipexole in 50 ml distilled water.

Preparation of calibration curve
"For preparation of calibration curve of Pramipexole, different dilutions of Pramipexole stock solution taken into 10 ml volumetric flask and added distilled water" as given in table 6.1.

For Pramipex 0.5
A total of tablets were accurately weighed and transferred in to a mortor and triturated well to fine powder. An amount equivalent to one tablet content (Containing 500mg of Pramipexole for Pramipex 0.5) was taken to a calibrated volumetric flask containing 50ml of distilled water, sonicate it for 15 mins & finally dilute upto 100ml with distilled water. A solution was filtered through Whatman filter paper number 41 and make further dilution with distilled water. The above solutions were analyzed for the content of Pramipexole.

For Pramirol SR 0.26
A total of tablets were accurately weighed and transferred in to a mortor and triturated well to fine powder. An amount equivalent to one tablet content (Containing 250mg of Pramipexole for Pramipex 0.5) was taken to a calibrated volumetric flask containing 50ml of distilled water, sonicate it for 15 mins & finally dilute upto 100ml with distilled water. A solution was filtered through Whatman filter paper number 41 and make further dilution with distilled water. The above solutions were analyzed for the content of Pramipexole.

RESULTS AND DISCUSSION

Optimization and selection of method parameters
All the optimized method parameters are summarized in table 2. Distilled water was selected as solvent because Pramipexole was freely soluble in distilled water. 260 nm were selected as maximum wavelength of absorptionfor determination of Pramipexole.

Method validation
1. Linearity and range
The proposed spectroscopic method shows good linearity in the concentration range of 10 to 30 µg ml^{-1} with correlation co-efficient, slope and intercepts 0.9997, 0.022 and 0.004 respectively for Pramipexole (Figure 3).

2. Precision
"Inter day and intraday precision for spectroscopic method were measured in terms of % RSD". Repeat the experiment for five times in a day and also in five different days to get intraday and inter day precisions. The average % RSD of intraday and inter day measurements for determination of Pramipexole was found to be 0.0012793 and 0.001528 respectively. The values confirm the precision of the method.

3. Accuracy
Through recovery studies we can conform accuracy of the method in which marketed formulation solution have to be spiked with 80 %, 90% and 120 % standard drug as label claim. If recovery greater than 100 % that justifies the accuracy of the method.

4. LOD and LOQ
To determine LOD/LOQ estimate standard deviation(SD) of intercepts which are obtained by repeating Calibration curve for 5 times and Then LOD and LOQ were measured as follows
LOD=3 * SD/slope
LOQ=10 * SD/slope
SD = Standard deviation of intercepts
The values of LOD and LOQ are given in table 7.

Applicability of the method
1. Analysis of market formulation[14]
The proposed method was tested by analyzing the commercially available two formulations i.e. Pramipex 0.5, Pramirol SR 0.26. The results are shown in Table 8.

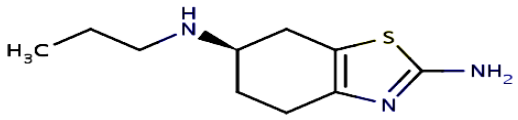

Fig. 1: structure of pramipixole

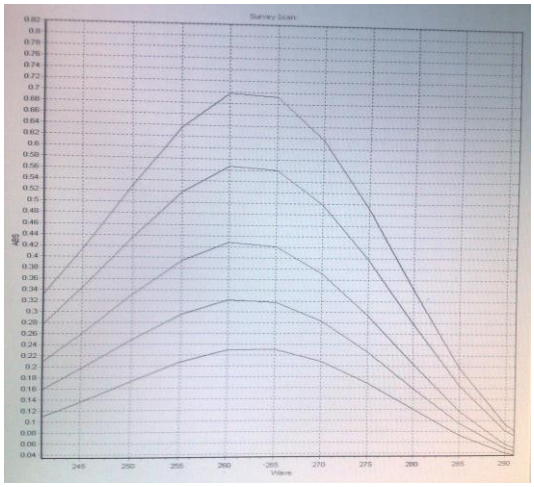

Fig.2: Calibration spectrum for Pramipexole

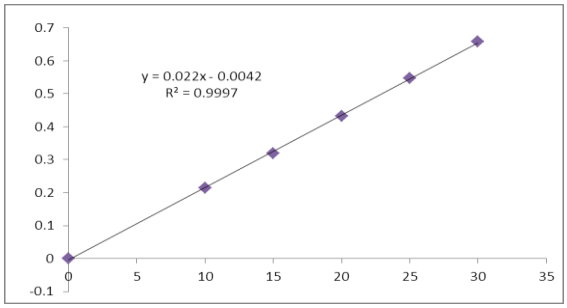

Fig.3: Calibration curve for Pramipexole

TABLE 1: FOR CALIBRATION CURVE OF PRAMIPEXOLE

Concentration (µg ml^{-1})	Stock solution (ml)	Distilled water make up to
10	1.0	10.0
15	1.5	10.0
20	2.0	10.0
25	2.5	10.0
30	3.0	10.0
Blank	0.0	0.0

TABLE 2: OPTIMIZED METHOD PARAMETERS

Method parameters	Optimized parameters
Solvent	Distilled water
Scanning range	200 nm to 300 nm
Scan speed	fast
Analytical wavelength for determination of Pramipexole	260 nm

TABLE 3: INTRADAY PRECISION FOR DETERMINATION OF PRAMIPEXOLE

Pramipexole (µg ml^{-1})	Absorbance At 260 nm				
	Trail 1	Trail 2	Trail 3	Mean	%RSD
Day 1	0.432	0.435	0.433	0.4333	0.001528
Day 2	0.430	0.432	0.430	0.4306	0.001155
Day 3	0.431	0.433	0.431	0.4316	0.001155
Average %RSD					0.0012793

TABLE 4: INTER DAY PRECISION FOR DETERMINATION OF PRAMIPEXOLE

Pramipexole (µg ml^{-1})	Absorbance at 260 nm				
	Day 1	Day 2	Day 3	Mean	% RSD
20	0.435	0.432	0.433	0.4333	0.001528

TABLE 5: RECOVERY FROM FORMULATION (PRAMIPEX 0.5)

Pramipexole In Dosage Form (µg ml^{-1})	% Pure Pramipexole added	Pure Pramipexole Added (µg ml^{-1})	*Pure Pramipexole Recovered %±RSD
5	80	4	99.88±0.69
5	100	5	100.18±0.94
5	120	6	100.10±1.18

TABLE 6: RECOVERY FROM FORMULATION (PRAMIROL SR 0.26)

Pramipexole In Dosage Form (µg ml^{-1})	% Pure Pramipexole added	Pure Pramipexole Added (µg ml^{-1})	*Pure Pramipexole Recovered %±RSD

5	80	4	99.67±0.93
5	100	5	100.17±1.12
5	120	6	99.73±0.72

TABLE 7: LOD AND LOQ OF PRAMIPEXOLE

Parameter	Pramipexole (µg ml^{-1})
SD	0.001703
LOD (µg ml^{-1})	1.5965
LOQ (µg ml^{-1})	5.3216

TABLE 8: ANALYSIS OF MARKET FORMULATION

Formulation	Label claim	Concentration found	Assay
PRAMIPEX 0.5	0.5 mg	0.497	99.40±0.89
PRAMIROL SR 0.26	0.26 mg	0.261	100.38±1.27

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