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APPLICATION OF UV-SPECTROPHOTOMETRY AND RP-HPTLC METHODS FOR ESTIMATION OF PALIPERIDONE IN BULK AND TABLETS

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ABSTRACT

Two simple UV-Spectrophotometry and a Reversed-Phase High-Performance Thin-Layer Chromatography (RP- HPTLC) methods have been established for the determination of Paliperidone in bulk and in pharmaceutical formulation. Paliperidone showed absorbance maximum at 238 nm in 0.1 N HCl. In '**Method A'**, the 'Zero Order UV- spectrum' was derivatized into 'First Order Spectrum' and amplitude was recorded at 248 nm while in '**Method B**', the 'Area Under the Curve' between selected wavelengths i.e 242.0 – 253.0 nm of first order derivative were considered. In both these UV-Spectrophotometry methods, Paliperidone obeyed linearity in the concentration range of 04-28 µg/mL with coefficient correlation (r^2>0.999). In **'Method C'** (RP-HPTLC)separation of Paliperidone was achieved on 10 X10 cm RP-HPTLC plate coated with silica gel 60 F254S using *1,4 Dioxane: water: acetic acid (6:4:0.3 v/v)* as mobile phase. Paliperidone showed Rf value 0.54 ± 0.02 when detected at wavelength 283.0 nm. In Method C, Paliperidone obey linearity in the range of 100 - 600 ng/ band with r^2 > 0.99. The amounts of paliperidone estimated by all the three proposed methods were found to be in good agreement with label claimed. All these developed methods were validated as per International Conference on Harmonization (ICH) guidelines.

Key words: Paliperidone; First Order Derivative-UV –Spectrophotometry; Area Under Curve; RP-HPTLC

INTRODUCTION

Paliperidone, chemically is (*RS*)-3-[2-[4-(6-fluorobenzo[*d*]isoxazol-3-yl)-1-piperidyl] ethyl]-7-hydroxy-4-methyl-1, 5-diazabicyclo [4.4.0] deca-3, 5-dien-2-one **[Figure 1]** and used in treatment of schizophrenia[1]. The mechanism of action of paliperidone is not clear; but, it is known as antagonist of dopamine D_2, serotonin 5-HT_{2A}, and histamine, H_1, and α_1, and α_2, adrenergic receptors [2].Thorough literature survey revealed many analytical methods for estimation of paliperidone in bulk, biological fluid and pharmaceutical formulation. LC-MS/MS method for estimation of Risperidone and the enantiomers of 9 - hydroxyrisperidone in human plasma and urine[3] has been established. LC- MS [4], RP-HPLC [5,6] and RP-RRLC [7] methods for the estimation of paliperidone in bulk and in pharmaceutical formulation have been reported.

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A normal- phased HPTLC method [8] has also been established for estimation of paliperidone in tablet formulation and *in vitro* release. A rapid stability- indicating RP-UPLC method has also established for quantification of paliperidone in a depot injectable formulation [9]. A zero order UV-Spectrophotometry has also been reported for estimation of paliperidone in tablet formulation[10]. First order derivative UV-Spectroscopy methods and area under curve techniques have several advantages over that of conventional zero order spectrophotometry method such as by using increasingly higher order of data, abolition of the interference components that shows a directly proportional relationship to different orders of wavelength with the general form can be achieved. However, the resolution among the overlapping bands in spectra can be enhanced through the use of derivative spectroscopy [11]. In HPTLC method it can be simultaneously handle several samples even of divergent nature and composition supporting several analysts at a given time [12].

The aim of the aim of the present work is to establish simple rapid, economical and eco-friendly UV-Spectrophotometry using First order derivate and First Order Derivative- Area under cure technique and RP-HPTLC method and for

estimation of Paliperidone in bulk and in tablet dosage form. Further, to validate the developed analytical methods as per International conference on Harmonization (ICH) guidelines [13].

1. EXPERIMENTAL

1.1 Chemicals and Reagents

Paliperidone working standard was obtained from Dr. Reddy's Pvt. ltd. All chemicals and reagents used were of analytical grade.

1.2 Equipment and Experimental conditions

1.2.1 For UV- Spectrophotometry analysis

UV- visible double beam Spectrophotometer (UV-2450, SHIMADZU, and Japan) with 1 cm matched quartz cells and electronic balance (Model was Shimadzu AUX-120).

1.2.2 For RP- HPTLC Method

A Camag TLC system (Muttenz, Switzerland) comprising of Camag Linomat 5 automatic sample applicator, Hamilton syringe (100 µL), Camag TLC scanner 3, Camag winCATS software (version 1.3.0), Camag twin trough chamber (10 cm x 10 cm) and ultrasonicator; ENERTECH Electronics Pvt. Ltd., India was used during the study.

1.2.3 Selection of chromatographic conditions

Chromatographic study was carried out on aluminum-backed precoated silica gel 60-F_{254}S(10 cm × 10 cm)RP- HPTLC plates having 200 µm thickness (E.Merck, Mumbai, India). Prior to use; the HPTLC plates were pre-washed with methanol and dried in an oven at 100 ^{O}C. Densitometric detection was performed with a Camag TLC Scanner 3 (Camag, Muttenz, Switzerland) installed with winCATS software (version 1.3.0).

Drug standards and samples were applied on the HPTLC plates using Linomat 5 (Camag) applicator under nitrogen gas flow. An appropriate volume 10 µL samples were spotted 6 mm from the edge of the plates. The plates were developed in twin trough glass chamber (10 cm × 10 cm) (Camag, Muttenz, Switzerland). The volume of mobile phase was 10 mL. Linear ascending development to a migration distance 8 cm , with 1, 4, Dioxane : water : acetic acid (6:4:0.3 *v/v*)as mobile phase was performed in 10 X 10 cm twin- trough glass chamber (Camag), with tightly fitting with lid. The chamber was left for saturation for 30 min at room temperature 25 ± 2^{0}C and % Relative Humidity(%RH) 60 ± 5%. The developed plates were dried with the help of air dryer. The slit dimensions were kept at 6.00 × 0.45 mm (micro) and scanning speed employed was 20 mm s^{-1}. Densitometric scanning was done in absorbance-reflectance mode at wavelength 283 nm using a deuterium lamp emitting a continuous UV-Spectrum between 190 nm - 400 nm.

1.3 Preparation of stock standard solution

1.3.1 For UV- Spectrophotometry [Method A and B] selection of wavelengths and study of linearity curves

A stock standard solution was prepared by dissolving 10 mg of paliperidone in 100 mL of 0.1N HCl to obtain concentration 100µg/ml. For studying absorbance maximum, a fixed concentration 10µg/ ml was prepared and scanned in the UV-region i.e. 400 – 200 nm. Paliperidone showed absorbance maximum 238 nm in 0.1 N HCl.

In **Method A;** the zero order spectrum of Paliperidone was derivatized into first order using UV probe software 2.21. The amplitude was recorded at 248.0 nm while in '**Method B'**, Area under curve of first order derivative spectrum was selected in range of 242 - 253 nm. For linearity studies an appropriate volume in the range of 0.4–2.8 mL were transferred into a series of 10 mL volumetric and volume was made up to mark using 0.1N HCl to obtain concentrations in the range of 4 – 28 µg/ml. The solutions were scanned on UV-Spectrophotometer in the range of 400 – 200 nm. For **Method A and B**, the readings were ascertain by measurement at selected wavelengths **Figure 1 and 2.**

1.3.2 For RP-HPTLC [Method C]and study of linearity curve

A stock standard solution was prepared by dissolving 10 mg of Paliperidone in 10 mL methanol to obtain concentration1000 ng/µL. An appropriate volume in the range of 0.1 - 0.6 mL was transferred into series of 10 mL volumetric flask and volume was made up to the mark using methanol. A fixed volume 10 µL was applied on the RP- HPTLC plates to obtain concentration 100, 200, 300, 400, 500 and 600 ng/band of paliperidone, respectively. Each concentration was applied six times on to the RP-HPTLC plates, developed and scanned as described in earlier. Calibration curve was constructed by plotting peak areas *versus* corresponding concentrations. The detection was carried out 283nm **(Figure 3)**.

1.4 Preparation of Sample solution

Ten Paliperidone (Palido-OD) tablets were accurately weighed and ground into fine powder in mortar.

1.4.1 For UV Spectrophotometry

[Method A and B]

A quantity of powdered drug equivalent to 3 mg was transferred into 100 ml of volumetric flask containing 30 ml of 0.1 N HCl, shaken manually for 30 minutes and volume was made up to the mark using same solvent. The resulting solution was filtered through a 0.45 µm filter (Millifilter, Milford, MA, USA). An appropriate volume 3mL was further diluted to 10 ml with same solvent and volume was made up the mark. It was

subjected for analysis using **Method A and B**. The quantity of paliperidone was determined from pharmaceutical formulation was using respective linearity equations.

1.4.2 For RP-HPTLC [Method C]

A quantity of powered drug equivalent to 3 mg of paliperidone was transferred into 100ml of volumetric flask containing 35 ml of methanol, sonicated for 30 min and volume was adjusted to mark using same solvent. It was filtered through a 0.45 µm filter (Millifilter, Milford, MA, USA). An appropriate volume of 10 µL (containing 300 ng/band) was applied on RP-HPTLC plate, developed and scanned as described in chromatographic conditions. The experiment was repeated for five times and amount of paliperidone in tablet formulation was established by treating it with linearity equation.

2. Validation of the proposed Methods

The proposed methods are validated according to International Conference on Harmonization (ICH) guidelines.

2.1 Accuracy

To the pre analyzed sample solutions, a known amount of drug standard solution was added different levels i.e. 80 %, 100 % and 120 %. The solutions then were re-analyzed by proposed method. In UV-Spectrophotometry **(Method A and B)** methods; to the pre-analyzed sample solution (10 µg/ mL), a known amount of drug standard was added and re-analyzed by proposed respective UV-Spectrophotometry methods. In RP- HPTLC method **(Method C)**; to the pre-analyzed sample solution (200 ng/band), an amount of drug standard was over spotted and the samples were re-analyzed using proposed method.

2.2 Precision

The intra-day and inter-day precisions of the UV-Spectrophotometry and RP-HPTLC analysis were assessed using linear regression data for the calibration curves. For UV-Spectrophotometry **[Method A and B],** analysis of paliperidone was repeated at concentrations of 8, 16 and 24 µg/ml and for RP-HPTLC [**Method C],** analysis of paliperidone was performed at concentrations of 200, 400 and 600 ng/band.

2.3 Sensitivity

The sensitivity measurements of Paliperidone by the use of the proposed method were estimated in specification of the Limit of Quantification (LOQ) and Limit of Detection (LOD). The LOQ and LOD were calculated using equation LOD = 3.3 x N/B and LOQ = 10 x N/B; Where, 'N' is standard deviation of the peak areas of the drugs (n = 3), taken as a measure of noise, and 'B' is the slope of the correlative calibration curve.

2.4 Repeatability

The repeatability was determined by analyzing 16 µg/ml concentration of Paliperidone for UV-Spectrophotometry **[Method A and B]** methods while for RP-HPTLC, it was investigated by analyzing 400ng/band concentration of paliperidone solution for six times.

2.5 Ruggedness

Ruggedness of the methods was studied by two different analysts' usingsimilar experimentaland environmental conditions. UV-Spectrophotmetry **[Method A and B]** was performed by analyzing 16µg /mL of paliperione and RP-HPTLC [**Method C]** was performed using 400 ng/band of paliperidone.

2.6 Robustness

Robustness of the method was studied for RP-HPTLC method. Robustness of an analytical method is measure of its tendency to withstand purposeful variations in method parameters. The robustness was indicated by presenting test method in normal condition and each altered condition mentioned below. The composition of the mobile phase was changed slightly and the effects on the results were examined. 1, 4, Dioxane: water: acetic acid in different ratios (5.4:4.6:0.3, 6.4:3.6:0.3, *v/v*) were selected (keeping volume of acetic acid same) and chromatograms were run. The amount of mobile phase (10 ± 2 mL, i.e. 8 mL, and 12 mL), development distance (8 ± 0.5 cm, i.e. 7.5 and 8.5 cm), mobile phase composition (± 0.3 mL) and duration of saturation (20 ± 5 min, i.e. 25, 30 and 35) were varied.

3. RESULTS AND DISCUSSION

3.1 Development of optimum mobile phase

In order to obtain high resolution and reproducible peaks, different mobile phase compositions were substantiated. At the beginning, a different proportions of 1, 4, Dioxane and water were tried as mobile phase. Mobile phase consisting of 1, 4, Dioxane: water (7: 4*v/v*) gave good separation of spot but minor tailing was observed and less R_f. To succeed the problem, acetic acid was added as modifier. Thus, finally the mobile phase consisting of 1, 4, Dioxane: water: acetic acid (6:4:0.3 v/v) gave excellent resolution of spot with Rf value 0.54 ± 0.02 and chamber saturation 30 min. A typical chromatogram was shown in **Figure 3.**

3.2 Linearity studied

The linear regression data for the calibration curves showed a good linear relationship over the concentration range 4-28 µg/mL for UV-Spectrophotometry **[Method A and B]** analyses and 100 - 600 ng/band for RP-HPTLC analysis. The results are communicated in **Table 1**.

3.3 Accuracy

The accuracy of the developed methods were checked at three different levels and results are assessed as % relative standard deviation (%RSD),

Table 2. The methods evinced to be accurate as % RSD values were found to be less than 2.

3.4 Precision

The precision of the developed method was expressed in terms of % relative standard deviation (% RSD). The % RSD values found to be less than 2 indicate that the methods were precise for the determination of drugs in formulation. The data is depicted in **Table 3**.

3.5 Sensitivity

In 'Method **A'**, the LOD and LOQ for Paliperidone were 0.35µg and 1.06µg, respectively while for '**Method B'**; it was found to be 0.13µg and 0.40µg, respectively. In RP-HPTLC [Method C], the LOD and LOQ were determined to be 11.44 ng and 34.68 ng, respectively.

3.6 Repeatability

For UV-Spectrophotometry **[Method A and B]** methods, repeatability was determined by analyzing 16µg/ml of concentration of Paliperidone while for RP- HPTLC method repeatability was determined by analyzing 400ng/band concentration of paliperidone solution for six times with %RSD value less than 2 for both methods. Results are depicted in **Table 4.**

3.7 Ruggedness

Ruggedness was determined for solution of paliperidone. The results are in acceptable range that is % RSD values less than 2 for all the methods as depicted in **Table 5**. The results showed no statistical variations between different operators suggesting that the developed methods are rugged.

3.8 Robustness

The % RSD values for robustness studies were found to be less than 2 denoted that the methods were robust and the results are depicted in **Table 6.**

Table 1: Optical characteristics and linearity data of Paliperidone

Parameter	Method A	Method B	Method C
Linearity Range	4 - 28 µg/ml	4 - 28 µg/ml	100 - 600 ng/band
Slope	0.0097	0.0311	8.5819
Intercept	+0.009	+0.0287	+762.17
Coefficient correlation	0.999	0.999	0.9981

Table 2: Accuracy Studies

Methods	Initial amount [µg/ml]	Amount of standard drug added	% Recovered [n=3]	% RSD
A*	10	8	99.22	0.56
		10	102.02	0.35
		12	100.17	0.39
B*	10	8	98.57	0.73
		10	100.51	0.69
		12	98.93	0.35
C#	200	160	98.56	1.69
		200	99.16	1.81
		240	98.19	1.06

n- Number of determinations at each level; * in **Method A and B** concentration is expressed in µg/ml and in# **Method C**, the concentration is expressed in ng/band

Table 3: Precision Studies

Method	Concentration	Intra-day %RSD	Inter-day %RSD
A*	8	0.15	0.55
	16	0.79	1.66
	24	0.25	0.15
B*	8	0.12	1.13
	16	0.94	0.2
	24	0.49	0.18
C#	200	1.32	1.78
	400	1.45	1.42
	500	0.9	1.17

n- Number of determinations at each level; * in **Method A and B** concentration is in µg/ml and in# **Method C**, the concentration expressed in ng/band

Table 4: Repeatability studies

Methods	Amount taken [n = 6]	Amount found	%Amount found	Mean±SD
A*	16	15.28	98.45	98.45±0.0001
B*	16	16.11	100.72	100.72±0.0005
C#	400	403.80	100.95	100.95±0.44

n- Number of determinations, * in **Method A and B** concentration is in µg/ml and in # **Method C**, the concentration is expressed in ng/band

Table 5: Ruggedness studies

Methods	Analyst-I		Analyst-II	
	%Amount found±SD[n=6]	%RSD	%Amount found ± SD [n=6]	%RSD
A	99.96±0.0002	0.14	99.72±0.0005	0.10
B	98.72±0.0001	0.06	99.91±0.0005	0.09
C	98.99±11.43	0.27	99.96±4.75	0.11

n- Number of determinations

Table 6: Robustness for HPTLC analysis of Paliperidone

Conditions	**Peak area ± SD**	**RSD [%] [n = 6]**
▪ Mobile phase composition (± 0.3 ml)	4246.13±20.34	0.47
▪ Mobile phase volume (± 2 ml)	4220.75±16.24	0.38
▪ Development distance (± 0.5 cm)	4197.41±25.50	0.60
▪ Plate saturation time (± 5 min)	4228.35±13.54	0.32

n- Number of determinations

Table 7: Summary of validation parameters

Parameter	**Method A**	**Method B**	**Method C**
λmax	248.0 nm	242.0–253.0 nm	283.0 nm
Regression equation	0.0097X+0.009	0.0311X+0.0287	8.5819X+762.17
Slope	0.0097	0.0311	8.5819
Intercept	0.009	0.0287	762.17
Correlation coefficient	0.999	0.999	0.998
Repeatability(%RSD)	0.06	0.1	0.36
Intra-day precision[%RSD]	0.402	0.524	1.23
Inter-day precision[%RSD]	0.795	0.508	1.46
LOD	0.35 µg/ml	0.13 µg/ml	11.44 ng/band
LOQ	1.06 µg/ml	0.40 µg/ml	34.68 ng/band
Accuracy [%RSD]	0.17-0.25	0.33-0.73	1.0-1.8
Ruggedness			
Analyst-I	0.14	0.06	0.27
Analyst-II	0.10	0.09	0.11
Robustness	-------	-----	Robustness

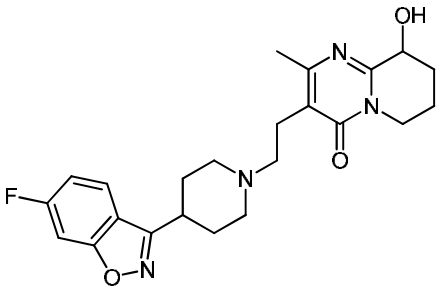

Figure 1: Chemical Structure of Paliperidone

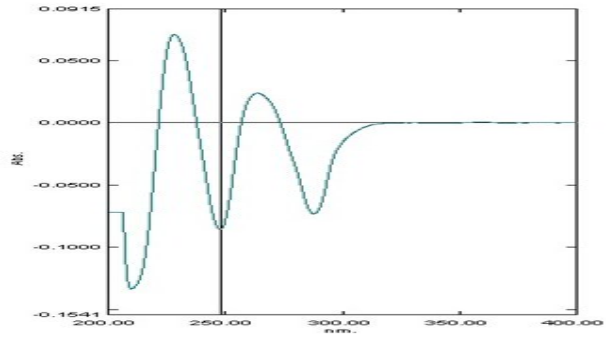

Figure 2: First Order Derivative UV- Spectrum of Paliperidone

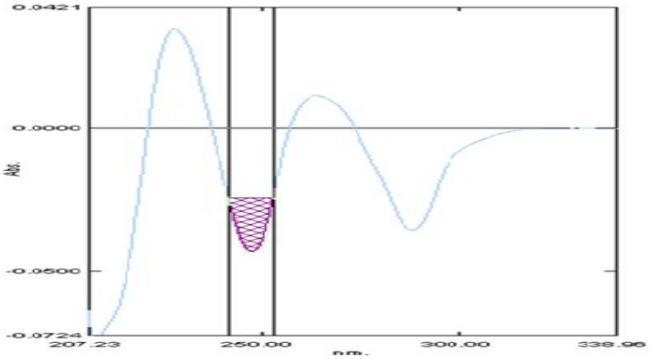

Figure 3: First order derivative UV-Spectrum of Paliperidone showing AUC between Selected wavelengths

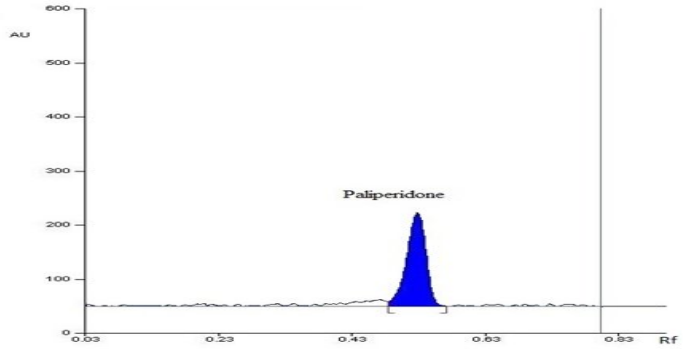

Figure 4: RP-HPTLC chromatogram for Paliperidone standard 400ng/band) in 1, 4, dioxane-water-acetic acid (6:4:0.3*v/v*) as mobile phase with R_f Value 0.54 ±0.02

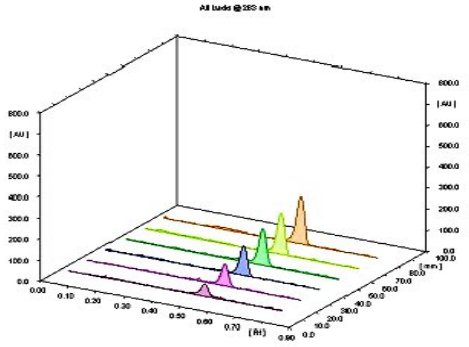

Figure 5: Linearity studies for Paliperidone in *1, 4, Dioxane-water-acetic acid (*6:4:0.3*v/v*) as mobile phase with $R_{f=}$0.54 ± 0.02

4. CONCLUSION

All the proposed developed methods are simple, economical, accurate, precise and rugged. These methods can be used for the regular analytical studies of Paliperidone from its pharmaceutical formulations. The methods are developed for quantification of Paliperidone in tablets. It is also used in normal quality control of the formulations containing Paliperidone.

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