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Original Research Article

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF LANSOPRAZOLE AND PALIPERIDONE PALMITATE IN BULK DRUGS

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ABSTRACT

Objective:
An accurate and precise RP-HPLC method was developed for the simultaneous determination of Lansoprazole and Paliperidone Palmitate in bulk drugs.
Method:
Separation of the drugs was achieved on an Agilent TC 1120 RP 18 column using a mobile phase consisting of Methanol and Acetonitrile in the ratio of 60:40 v/v. the flow rate was 1.5 mL/min and detection wave length was 285 nm.
Results and conclusion:
The linearity was observed in the range of ppm for 30-70 ppm for Lansoprazole (LPZ) and 120-280 ppm for Paliperidone Palmitate (PPP) with a correlation coefficient of 0.997±0.257 and 0.998±0.359 respectively. The mean percentage recoveries for 80%, 100% and 120% accuracy were found to be 98% ±1.322, 100.86%±1.019 and 100%± 0.514 respectively for LPZ. The mean percentage recoveries for 80%, 100% and 120% accuracy were found to be 101.5%±1.473, 100.33%±1.443 and100.30%±0.128 respectively for PPP. The method showed good reproducibility and recovery with percent relative standard deviation less than 2. The proposed method was validated for its linearity, accuracy, precision and robustness. This method can be employed for routine quality control analysis of Lansoprazole and Paliperidone Palmitate bulk drugs.

Key Words: Paliperidone Palmitate, Lansoprazole, RP-HPLC Method, Simultaneous Estimation.

INTRODUCTION

Lansoprazole1 (LPZ) is a substituted benzimidazole, chemically it is 2-[[[3-methyl-4-(2, 2, 2-trifluoroethoxy) 2-pyridinyl] methyl] sulphinyl]-lH-benzimidazole. Its molecular formula and molecular weights are $C_{16}H_{14}F_3N_3O_2S$ and 369.4g/mol respectively [1]. LPZ is a proton pump inhibitor that suppresses gastric acid secretion by specific inhibition of the Enzyme system of Hydrogen/ potassium adenosine tri-phosphatase (H+/ K+ ATPase) at the secretory surface of the gastric parietal cell and is used in the treatment of various gastric disorders such as gastric and duodenal ulcers, gastro oesophageal reflux disease and pathological hyper secretory conditions. The structural formula of LPZ is in Figure 1[2]. Literature survey reveals few analytical methods for the determination of Lansoprazole alone and in combination with other drugs in Pharmaceutical preparations and biological fluids, viz. Spectrophotometry [3-7], HPLC [8-12] and HPTLC [13].

Paliperidone Palmitate (PPP) is chemically 3-{2-[4-(6-fluoro-1,2-benzoxazol-3-yl)piperidin-1-yl]ethyl}-9-hydroxy-2-methyl-4H,6H,7H,8H,9H-pyrido[1,2-a]pyrimidin-4-one. Its molecular formula and molecular weight are $C_{23}H_{27}FN_4O_3$ and 426.48g/mol, respectively. Paliperidone Palmitate is a new long-acting antipsychotic agent for the treatment of acute and maintenance therapy in schizophrenia. Paliperidone (9-hydroxyrisperidone) is the major active metabolite of risperidone and acts as an antagonist at dopamine D2 and serotonin 5HT-2A receptors. The structural formula of PPP is in Figure 1[14]. Literature survey reveals few analytical methods for the determination of Paliperidone Palmitate alone and in combination with other drugs in pharmaceutical preparations and biological fluids, viz. Spectrophotometry [15-17] and HPLC [18, 19].

The scope of developing and validating an analytical method is to ensure a suitable method for a particular analyte to be more specific, accurate and precise. The main objective for that is to improve the conditions and parameters, which should be followed in the development and validation processes. A survey of literature revealed that simultaneous analytical methods are not available for the drug combination LPZ and PPP. Hence it is proposed to develop new methods for the assay of LPZ and PPP in pharmaceutical dosage forms adapting RP-HPLC.

The objective of the study was to develop a simple and accurate method for the determination of LPZ and PPP simultaneously using RP-HPLC for bulk drug.

MATERIALS AND METHODS

Materials

Reference Standard drugs and sample bulk drugs of LPZ and PPP obtained from pharmaceutical market were of analytical grade. Methanol (Specialties Private Ltd, Mumbai), and Acetonitrile (Thermo Electron LLS India Private Ltd, Mumbai) were of HPLC grade.

Instrumentation and chromatographic conditions

Quantitative HPLC was performed on Agilent TC (model no: 1120), with UV-Visible detector. Symmetry Shield RP 18(250x 4.6 mm, packedwith5microns) column used for the chromatographic separation. Manual injections (20µl) were used. The column was maintained at ambient temperature. To develop a suitable and robust HPLC method for the determination of LPZ and PPP, different mobile phases Methanol: Acetonitrile, were used in different compositions of mobile phases at different flow rates. Finally, the mobile phase Methanol: Acetonitrile at the ratio of 60:40v/v at a flow rate of 1.5 ml/min gave peaks good resolution for LPZ and PPP. LPZ and PPP were eluted at retention times around 2.2 min and 5.76 min, respectively with symmetric peak shape.

The mobile phase was degassed and filtered through 0.25µm membrane filter before pumping into the HPLC system. The column was equilibrated by pumping the mobile phase through the column for at least 30 min prior to the injection of the drug solution. The run time was set at 10 minutes. The data were collected and analyzed with Ezchrome software in a computer system.

A typical chromatogram showing these parathion of the drug is given in Figure 2. Detection wavelength was set at 285 nm since both the components showed reasonable absorbance at this wavelength. The optimum wavelength of detection was determined by scanning standard solutions of the analyte and internal standard from 200 to 400 nm using Labindia UV 3000+ double beam UV visible spectrophotometer (Maharashtra, India) with matched 1 cm path-length quartz cells and Labindia UV-Win software.

Trial and error method:

To develop a suitable and robust High performance liquid chromatographic method for the determination of LPZ and PPP, different mobile phase ratios of Methanol and Acetonitrile, were tried based on the solubility and functional group present in the compound. Finally Methanol: Acetonitrile in the ratio of 60:40v/v were selected as the mobile phase.

Preparation of mobile phase

Mobile phase was prepared by mixing Methanol (HPLC grade) and acetonitrile (HPLC grade) in 60:40 (v/v) proportions. Mixture was shaken vigorously and sonicated for 30 min prior to use.

Preparation of Standard stock solutions:

The standard stock solutions of 1000µg/ml of LPZ and PPP were prepared by dissolving 100 mg drugs separately in 100ml volumetric flask containing 50ml of methanol, Sonicated for 20 min. Make up to the mark with diluents to get 1000µg/ml concentration.

The working dilutions of binary mixture were in the range of 30-70 µg/ml for LPZ and 120-280 µg/ml for PPP respectively were prepared by further dilutions for calibration curves.

Preparation of Sample stock solutions:

The sample stock solutions of 1000µg/ml of LPZ and PPP were prepared by dissolving 100 mg drugs separately in 100ml volumetric flask containing 50ml of methanol, Sonicated for 20 min. Make up to the mark with diluents to get 1000µg/ml concentration. The working dilutions of binary mixture were in the range of 30-70 µg/ml for LPZ and 120-280 µg/ml for PPP respectively were prepared by further dilutions.

METHOD VALIDATION

The described method has been validated for the assay of PPP and LPZ using following parameters. System-suitability tests are an integral part of method development and are used to ensure adequate performance of the chromatography system. Peak area (A), Number of theoretical plates (N), Retention time (Rt) and resolution were evaluated for five replicate injections of the drugs at a concentration of 50µg/ml for LPZ and 200µg/ml for PPP respectively. The linearity range was found to be 60- 140% and 120-280% concentrations of LPZ and PPP respectively.

To obtain proportionality, the slope of the regression line was calculated statistically by the method of average peak area Vs concentrations for LPZ and PPP and Correlation Coefficient was calculated. Precision was studied to find out variations in the test methods of 50µg/ml for LPZ and 200µg/ml for PPP concentration when analysis carried out on the same day and on different day (Ruggedness) by using different analyst. The standard solution was injected for six times and measured the area for all five injections in HPLC.

For ruggedness and precision, the %RSD and %content results were calculated. The accuracy of the method was shown by analyzing model mixtures which were obtained by spiking known amounts of LPZ and PPP to the placebo. The model mixtures contained 80%, 100%, and 120% of LPZ and PPP compared to the drug amount. After injection, individual recovery values were calculated. Specificity is the ability of a method to discriminate between the analyte(s) of interest and other components that are present in the sample. A study of placebo interference from excipients was conducted. The equivalent weight of placebo taken as per the test method and placebo interference was conducted in duplicate. Robustness of the method were determined by varying the chromatographic conditions such as the changing flow rate (± 0.1ml/min), mobile phase ratio (± 5 ratio) and the wavelength of detection (±5nm). Refrigerator solution stability was performed on 1st day.

RESULTS

Table 1. Precision data of Lansoprazole and Paliperidone Palmitate.

	Lansoprazole (Conc. 50µg/ml)		Paliperidone Palmitate (Conc. 200µg/ml)	
	peak area	%content	peak area	%content
	21082527	101.73777	26928032	97.26953351
	21072516	101.7861	27844917	100.5815088
	21815921	98.317609	27615418	99.75251168
	21680498	98.931731	28526634	103.0440094
	21678377	98.941411	27544268	99.49550339
	21363513	100.39965	27644326	99.85693326
MEAN	21448892	100.01904	27683932.5	100
SDEV	323785.96	1.5139202	516375.149	1.865252164
%RSD	1.5095696	1.5136319	1.86525216	1.865252164

Table 2.Accuracy data of Lansoprazole and Paliperidone Palmitate.

N=3	Peak Area		Percentage Content		Percentage Recovery	
	LPZ	PPP	LPZ	PPP	LPZ	PPP
	Accuracy 80%					
MEAN	16269313.6	23577750.3	76.666	84.029	98	101.5
SD	204351.206	322615.144	0.962	1.149	1.322	1.473
%RSD	1.2560530	1.36830333	1.256	1.368	1.349	1.451
	Accuracy 100%					
MEAN	21220830	28059636	100	100	100.86	100.33
SD	131193.132	373853.407	0.618	1.332	1.019	1.44
%RSD	0.6182280	1.33235301	0.618	1.332	1.010	1.438
	Accuracy 120%					
MEAN	25253338.	33033424.3	119.00	117.72	100	100.30
SD	129874.72	568695.261	0.612	2.026	0.514	0.128
%RSD	0.5142873	1.72157526	0.514	1.721	0.514	0.127
SD- Standard deviation; %RSD- percentage relative standard deviation PPP-Paliperidone Palmitate, LPZ-Lansoprazole						

Table 3.Linearity data of Lansoprazole and Paliperidone Palmitate.

Lansoprazole			Paliperidone Palmitate		
Concentration (µg/ml)	**Mean Peak Area**	**Standard deviation**	**Concentration (µg/ml)**	**Mean peak area**	**Standard deviation**
30	12458656	142092	120	18418593	340811
40	16569018	54523.5	160	23140684	87416
50	20551252	529246	200	28306057	220577
60	23403142	159908.5	240	31800916	437764
70	26698405	237593.5	280	36655169	316489
Slope	353136.2	4054.35	**Slope**	112833.5	754.26
Intercept	2279284	211552.6	**Intercept**	5097592	129759.4
Correlation Coefficient	0.9973	0.2571	**Correlation Coefficient**	0.9985	0.3592

Table 4.Summary of validation parameters of Lansoprazole and Paliperidone Palmitate by RP HPLC method.

Validation	Parameters	Lansoprazole	Paliperidone Palmitate
System suitability	%RSD	1.408408	1.44549
	Theoretical plates	9667	12011
	Resolution	8.91	
Linearity	Slope	353136.2	112833.5
	Intercept	2279284	5097592
	Correlation coefficient	0.997317	0.998523
Precision	%RSD	1.5136319	1.865252164
Ruggedness	%RSD for Analyst 1 variation	1.570178	1.205959
	%RSD for Analyst 2 variation	1.430015	1.391591
Accuracy	Mean % recovery for 80%, 100, 120% respectively	98%, 100.86% & 100%	101.5%, 100.33% & 100.30
LOD	3.3 SD/S	0.0521	0.2088
LOQ	10 SD/S	0.1581	0.6328
Assay	Mean % content	100.12	100.43
Robustness	%RSD for Flow rate 1.6ml/min	0.271601	0.111287
	%RSD for Flow rate 1.4ml/min	0.146431	0.112992
	%RSD for Mobile phase 65:35	1.493556	0.746618
	%RSD for Mobile phase 55:45	0.980128	0.980557
	%RSD for wavelength 280nm	1.119994	1.219841
	%RSD for wavelength290nm	1.805232	1.874191

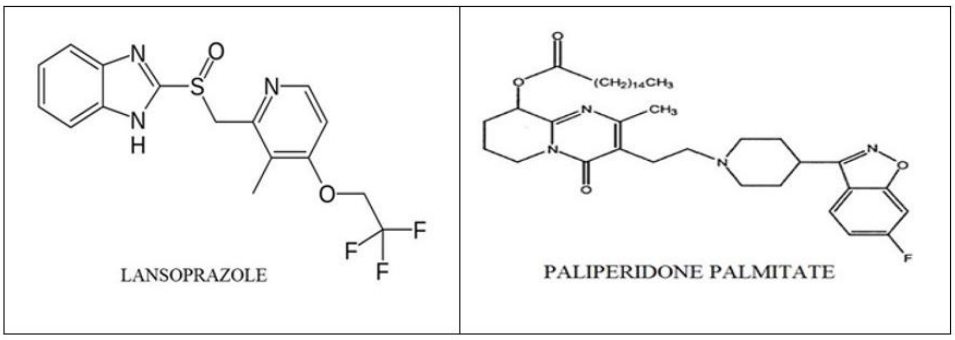

Figure 1.Structures of Lansoprazole and Paliperidone Palmitate.

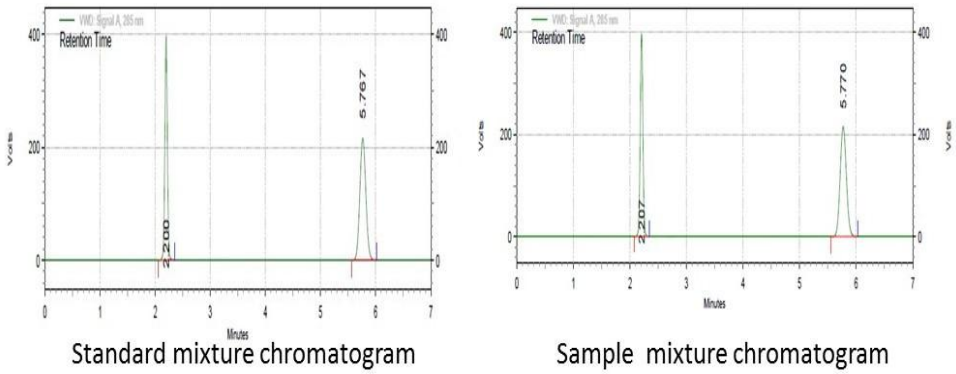

Figure 2. Chromatograms of Standard and Sample mixture of Lansoprazole and Paliperidone Palmitate.

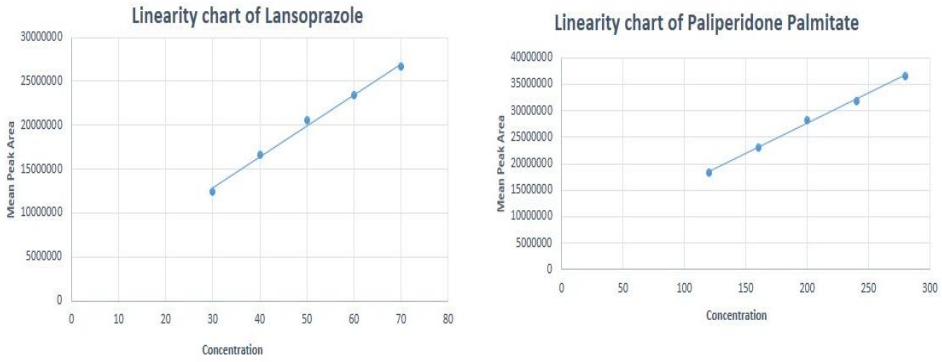

Figure 3.Linearity Charts of Lansoprazole and Paliperidone Palmitate.

DISCUSSION

A reverse phase isocratic procedure was proposed as a suitable method for the analysis of LPZ and PPP in bulk drugs. Methanol: Acetonitrile at the ratio of 60:40v/v at a flow rate of 1.5ml/min was found to be an appropriate mobile phase allowing adequate and rapid separation of analyte. The retention time was found to be 2.2 min and 5.76 min for LPZ and PPP respectively. System suitability for the LPZ and PPP reported that theoretical Plates obtained from the standard injections was not less than 2500. System suitability studies for the LPZ and PPP reported that the %Relative standard deviation (%RSD) values of five replicate injections for LPZ and PPP were found to be 1.408 and 1.445 respectively. The theoretical plates for LPZ and PPP were found to be 9667 and 12011 respectively. The resolution was found to be 8.91. As shown in the Figure 2, the substances were eluted forming well shaped, symmetrical single peaks, well removed from the solvent front. The precision of the HPLC system was determined using the %RSD of the peak areas for six injections of the standard solution of LPZ and PPP. %RSD for LPZ and PPP were found to be 1.51 and 1.86 respectively. The %RSD of both the drug under this method was less than 2. Precision data were presented in table 1. In order to verify the accuracy of the described method, recovery studies were carried out by analyzing model mixtures of PPP and LPZ. The recovery of LPZ and PPP was evaluated from 80%, 100% and 120%. The mean percentage recoveries for 80% and 120% accuracy were found to be 98% and 100% for LPZ. The mean percentage recoveries for 80% and 120% accuracy were found to be101.5% and 100.30% for PPP. The results of percentage recovery data were within the limit. Accuracy data were presented in table 2. For quantitative application a linear calibration curve was obtained over the concentration range from 60 to 140 % and 120 to 280 % for LPZ and PPP respectively. The correlation coefficient for LPZ and PPP were found to being 0.997 and 0.998 respectively.

The regression equation of LPZ was found to be y= 353136.2017x+ 2279284.383 with coefficient of correlation 0.9973 where x is concentration, y is absorbance, 353136.2017 is slope and 2279284.383 is intercept. The regression equation of PPP was found to be y= 353136.20x +2279284.38 with coefficient of correlation 0.998 where x is concentration, y is absorbance, 353136.20 is slope and 2279284.38 is intercept. Percentage curve fitting of LPZ and PPP was found to be 99.73% and 99.85 % respectively. The calibration curve of both drugs was present in figure 3 and related linearity data in table 3. Ruggedness for LPZ and PPP were determined by varying analysts carrying out the procedure. Totally 2 analysts carried out the procedure and the results were within the limits. Limit of detection of LPZ and PPP were found to be 0.0521 and 0.2088 mcg/mL respectively. Limit of quantitation of LPZ and PPP were found to be 0.1581 and 0.6328 mcg/mL respectively. The results of robustness indicate that the variation in chromatographic conditions the method was not affected. Variation in parameters for robustness studies was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed was robust. Stability studies (Refrigerator stability and Bench top stability) have expressed the percentage deviation from the true value within the limit for the both drugs. The results of validation parameters of proposed method were presented in table 4.

CONCLUSION

The presented method is precise, sensitive and accurate. The advantages of the proposed method are its short analysis time and a simple procedure for sample preparation. The satisfying recoveries and low coefficient of variation confirmed the suitability of the proposed method for the routine analysis of mixtures of Paliperidone Palmitate and Lansoprazole and in pharmaceuticals.

CONFLICT OF INTEREST

Authors declare no Conflict of Interest.

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