RESEARCH ARTICLE

DEVELOPMENT AND VALIDATION OF A NOVEL RP-HPLC METHOD FOR RESIDUE ANALYSIS OF LUBIPROSTONE ON THE SURFACE MANUFACTURING EQUIPMENT

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Abstract

A rapid, specific, and reliable technique has been developed for the determination of lubiprostone in surface of pharmaceutical equipment's by high performance liquid chromatography. For that study, reverse phase liquid chromatography was employed, using 0.01 M Potassium dihydrogen orthophosphate buffer, pH=3.0 and Acetonitrile (50:50 v/v) and a detector wavelength of 205 nm. The instrument was set at a flow rate of 1.0 mL min^{-1} and column at ambient temperature. The method showed excellent linearity over a range of 0.5–30 μg mL^{-1}. The swabbing procedure optimized to achieve suitable accuracy in stainless still plate. The accuracy was found >90%. This method is suitable for trace level quantification of Lubiprostone in production equipment surface area and confirm the cleaning validation of the production area and equipment's for avoiding the cross contamination of Lubiprostone to the other products.

Key Wards: Lubiprostone, HPLC, Swabbing, Validation

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1. Introduction

The objective of this work was to develop a simple analytical liquid chromatography procedure, which could serve as residue method for lubiprostone. There is no analytical method for residue analysis of Lubiprostone by HPLC available. Lubiprostone does not have suitable chromophoric group, hence it is very less sensitive at ultra-violate to visible range. In the present study, an attempt was made to develop a simple, precise, and accurate method for quantification of lubiprostone in pharmaceutical cleaning validation. The manuscript describes the development and validation of an isocratic reversed-phase HPLC method for Lubiprostone residue analysis as per ICH guidelines (ICH Q2B).

Lubiprostone contents one carboxylic acid group. Its absorbance lies between 195-210 mn as in this group n electron shipment is possible to n*. It does not content any methyl substitute of double or triple bond. It has one oxo group, so pi electron can move to π*. It contents one hydroxyl group, two fluorine atom, one cyclopentyl group, bicycle group and one five alkyl chain. The cumulative wavelength would be about 300 nm, but the absorbance at the longer wavelength would be very less at low concentration.

The IUPAC name of Lubiprostone (Fig.1), 7-[(1R,3R,6R,7R)-3-(1,1-Difluoropentyl)-3-hydroxy-8-oxo-2-oxabicyclo[4.3.0]non-7-yl] heptanoic acid (Sucampo Pharmaceuticals Inc, 2008) is used for the treatment of chronic constipation of unknown cause in adults, as well as irritable bowel syndrome associated with constipation in women (Anon,2006). Lubiprostone is a bicyclic fatty acid derived from prostaglandin E1 that acts by specifically activating ClC-2 chloride channels on the apical aspect of gastrointestinal epithelial cells, producing a chloride-rich fluid secretion. These secretions soften the stool, increase motility and promote spontaneous

bowel movements (Camilleri et al, 2006; McKeage et al, 2006; Anon, 2005; Johanson at al, 2008). Cross contamination with active ingredients is a real concern. Cleaning validation is required in the pharmaceutical field to avoid potential clinically significant synergistic / antagonistic interaction between pharmacologically active chemicals. Since the issuance of the US Food and Drug Administration's "Guide to Inspection of Validation of Cleaning Process" in July 1993, cleaning validations have received increasing attention (Food and Drug Administration, 1993)

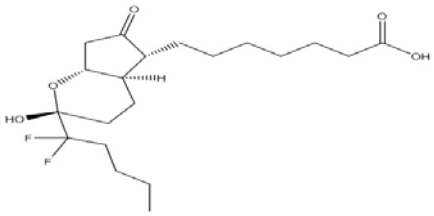

Fig.1. Structures of Lubiprostone

2. Material and Method

2.1. Chemicals and Reagents

Lubiprostone standard is obtained from Cipla (Mumbai, India). Orthophosphoric acid and acetonitrile (HPLC-grade) were procured from Rankem, RFCL Limited, New Delhi, India, di hydrogen potassium phosphate AR grade was procured from Rankem, RFCL Limited, New Delhi, India. The 0.45-mm pump nylon filter was obtained from Advanced Micro devices (Ambala Cantt, India). HPLC grade water was used throughout the study. Other chemicals used were of analytical or HPLC grade.

2.2. Instrumentation

Agilent HPLC system (USA) consisting of quaternary solvent delivery pump (series 1260), a vacuum degasser, a rheodyne injector with a 500-mL loop, column, and UV-VIS detector (series 1200 UV-VIS), and Empower software was used. A YMC ODS AQ 25 cm x 4.6 mm, 5.0 µ analytical column, eluted with mobile phase at the flow rate of 1.0mL/min was used.

2.3. Standard preparation

Accurately weighed and transferred about 10 mg of Lubiprostone working standard into a 50 mL Volumetric flask, added 25 mL acetonitrile, mixed well and sonicated to dissolve then diluted to volume with water. Further 5 mL of the above solution diluted to 100 mL with diluent (water and acetonitrile mixture 50:50 v/v) and mix well.

2.4. Mobile Phase

Potassium dihydrogen phosphate of 1.36 g was taken into 1000 mL milli Q water and adjusted to pH of 3.0 with dilute orthophosphoric acid solution. A mixture of buffer and acetonitrile in the ratio of 50:50 (v/v) was used as the mobile phase. The mobile phase was degassed by sonication and filtered through 0.45µ membrane filter.

2.5. Sample preparation

The swab sticks were soaked in methanol and sonicated for 15 minutes. After decantation the ultrasonic wash, was repeated for two more times. After the last wash the swab sticks were dried under vacuum followed by storing in a screw cap bottle until usage. The swab sticks were dipped into acetonitrile before sampling. The surfaces to be sampled were swabbed from top to bottom. Then the sampled swab sticks were placed in a test tube was 5 mL of acetonitrile and sonicated for 5 min to produce complete dissolution of compound from the swab. Further added 5 ml water and mixed well and sonicated it for 10 minutes at the temperature not more than 25°C. Filter the solution through 0.45µ PVDF syringe filter.

2.6. Chromatographic condition

ODS AQ (250 mm x 4.6 mm) column, acidic 0.1mM phosphate buffer pH 3.0±0.05 with acetonitrile at the ratio of 50:50 v/v as mobile phase, at flow rate of 1 ± 0.2 ml/min, detection of elute at wavelength of 205 ± 2 nm with injection volume 300 µL, column temperature 25ºC ware considered.

2.7. Method Validation

The optimized chromatographic conditions were validated by evaluating specificity, range, linearity, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ), robustness, and system suitability parameters in accordance with the ICH guidelines Q2 (R1)

2.7.1 System Suitability Testing

For system suitability parameters, five replicate injections of standard solution were injected and parameters such as the % RSD, tailing factor,

theoretical plate, and % RSD retention time of the peaks were calculated.

2.7.2. Linearity

The range of an analytical method is the interval between the upper and lower levels of the analyte for which the method shows adequate precision, accuracy and linearity. A desired concentration range is often known before starting the validation of the method. Linearity is studied to determine the range over which analyte response is a linear function of concentration. This study was performed by preparing standard solutions at seven different drug concentrations (0.5, 5, 7.5, 10, 15, 20, and 30 mg/ml). The solutions were injected in plot was constructed. The responses were measured as peak area. The calibration curves were obtained by plotting peak area against concentration. Standard deviation (SD), slope, intercept, and coefficient of variation (R^2) of the calibration curves were calculated to assess linearity of the method. The acceptable fit to the linear regression was demonstrated by construction of residual plots and evaluation of sum of squares of residuals.

2.7.3. Specificity

Specificity is the ability of the method to accurately measure the analyte response in the presence of all potential component. The method specificity was assessed by comparing the chromatograms (HPLC) obtained from the drug, blank and excipients.

2.7.4. Accuracy

The accuracy of an analytical method is the closeness of results obtained to the true value for the sample. It is expressed as percentage of recovery, which is determined by the standard addition method. Recovery of the method was determined by spiking the API with 100% level of standard. The experiment was performed in triplicate and percentage of recovery and percentage of relative standard deviation (RSD) were calculated for each concentration.

2.7.5. Precision

The precision of an analytical method is the closeness of replicate results obtained from analysis of the same homogeneous samples. Precision was examined by the relative standard deviation (%RSD) of recovery data.

2.7.6. Limits of Detection and Limit of Quantitation

The Limits of Detection (LOD) and Limit of Quantitation (LOQ) were separately determined on the basis of a standard calibration curve. The residual standard deviation of the regression line or the standard deviation of y-intercepts of regression lines was used to calculate LOD and LOQ. Following formulae were used; LOD=(3.3xD)/S and LOQ=(10xD)/S, where, D is the standard deviation of the y-intercepts of regression line and S is the slope of the calibration curve.

2.7.8. Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Robustness of the method was investigated under the flow and wave length variability.

2.7.9. Solution Stability and Mobile Phase Stability:

To assess the solution stability, standard, and test solutions were kept at 25°C ±2°C (laboratory temperature) for 24 hr. These solutions were compared with freshly prepared standard and test solutions. To assess the mobile phase stability a portion of mobile phase kept at bench top for different times and measured the system suitability parameters.

3. Results and Discussion

In chromatographic method development, the main focus was on the selection of mobile phase and its composition as well as other parameters such as flow rate, column, column temperature and working wave length. It was highly challenged to retain lubiprostone in reverse phase chromatography. An ODS AQ column selected for retaining the molecules and for better peak shape. A 250 mm x 4.6 mm id column selected for large column volume which is suitable for large injection volume. The acidic 0.1mM phosphate buffer pH 3.0 was selected for AQ column suitability, longer life and for better peak shape. The pKa of Lubiprostone is 4.3, hence in pH 3.0 most of the molecules remain unionized. Mobile phase of pH 3.0±0.05 obtained symmetric peak shape. Potassium di hydrogen phosphate

buffer is compatible with acetonitrile at the ratio of 50:50 v/v respectively at acidic pH. The materials and solvents are used for quantification of Lubiprostone UV cut-off <195 nm. The maximum wavelength was found in UV spectrum of drug at around 202 nm (Fig.2). At wavelength 205 nm would not have any interference for quantification of Lubiprostone.

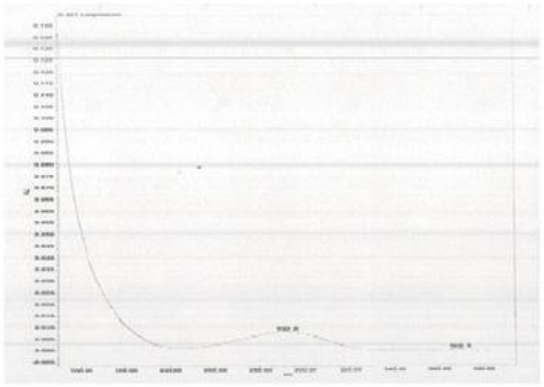

Fig.2. UV Spectrum Lubiprostone

The specificity studies proved the absence of interference, since no other peak appeared at the retention time (12.24 min) of Lubiprostone (Fig.3.). Moreover the % RSD is well within the acceptance level ±2.0%.

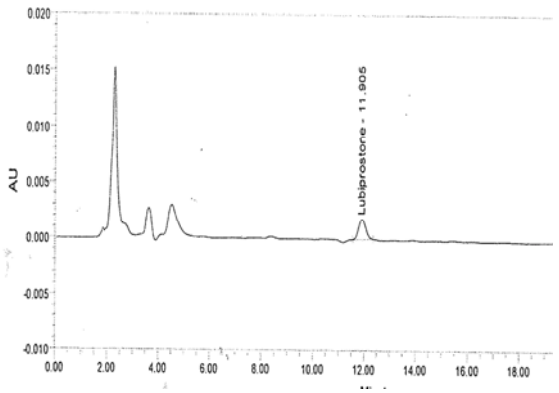

Fig.3. HPLC chromatogram of Lubiprostone.

System suitability tests are an integral part of gas and liquid chromatography methods. They are used to verify adequacy of the resolution and reproducibility of system. The system suitability results are given in Table-1. The tailing factor for each peak is not more than 2.0, the number of theoretical plates of each peak is not less than 5000 and the % RSD of peak areas generated by five injections is lower than 2.0%. The limit of Detection and Quantification value are given in Table 1.

Table.1. System Suitability Testing, limit of Detection and Quantitation of Lubiprostone

System Suitability Testing	%RSD of Response	1.7
	The USP Tailing	1.1
	The USP Plate counts	8225
	% RSD of RT	0.1
limit of Detection and Quantitation	Limit of Detection (ppm)	0.2
	Limit of Quantification (ppm)	0.6

***RSD, Relative standard deviation; ppm, parts per million; RT, room temperature; USP, united state pharmacopeia**

Seven different concentrations (0.5, 5, 7.5, 10, 15, 20, and 30 mg/ml) of Lubiprostone were prepared for linearity studies. The calibration curves obtained by plotting peak area against concentration showed linear relationship. The linear regression equation was y= 3509.4x-38.742 and The regression coefficient (R^2) values was 0.9989. Linearity curve of Lubiprostone is shown in Fig.4.

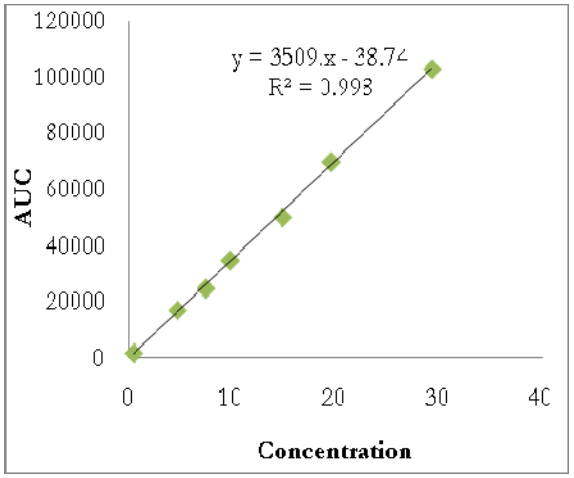

Fig.4. Linearity of Lubiprostone

A study of Accuracy was conducted. The recovery was performed on a 4"x4" Stainless steel plate, by analyzing three samples at 100% level of target concentration. Accuracy was performed by spiking Lubiprostone API on the plate and swab separately for each spike level to get the concentration of Lubiprostone equivalent to 100% of the target concentration of Lubiprostone. The mean %

recovery of Lubiprostone at 100% level was found to be within the acceptance criteria (Table 2).

Table.2. The 100% accuracy level on Stainless steel plate; Bench top stability of Standard and Test preparation and Bench top stability of Mobile Phase

100% accuracy level on Stainless steel plate				
S.No.	Spike level	'mg' added	'mg' found	Individual recovery
1	100%	1.01	1.056	104.6
2	100%	1.01	1.018	100.8
3	100%	1.01	1.015	100.5
Bench top stability of Standard and Test preparation				
Time	% Assay of drug	% Difference	Time	Standard Similarity factor
Initial	104.5	-	Initial	-
3rd hour	106.1	1.6	6th hour	0.99
9th hour	102.1	2.4	12th hour	0.95
15th hour	103	1.5	18th hour	0.97
24th hour	106.7	2.2	24th hour	0.96
Bench top stability of Mobile Phase				
System suitability	Observed value			
	Initial	Day-1	Day-4	
%RSD of Lubiprostone from five replicate standard injections	0.1	0.1	0.1	

The mean accuracy (recovery) are acceptable for this type of analysis (recovery >80%), a recovery factor is applying during the routine analysis calculation.

The Intermediate precision was examined by repeated recovery test by another analyst. The % RSD of recovery results were < 5% which is within the acceptance value (< 10%)

The robustness was investigated by varying the chromatographic conditions with respect to flow rate, organic modifier and wavelength. The study was conducted at different flow rates of 0.9 ml/min, and 1.1 ml/min (i.e. ± 10% of actual flow of 1.0 ml/min and the wavelength was altered to 203 nm and 207 nm (i.e. ± 2 nm of actual wavelength of 205 nm) for each of the analyte. Standard solutions were injected and the system suitability parameters were evaluated for these modified conditions. The method was found to be robust with respect to small changes in flow rate and small changes in wavelength. The system suitability parameters such as tailing, resolution and number of theoretical plates were within the specified limits.

The results of both bench top stability studies of standard and test preparation and mobile phase (table 2) signifies, analytical solutions and mobile phase were found to be stable for a reasonable period of time.

3. Conclusion

The purpose of this study was to develop a simple and sensitive method for the cleaning validation process in the pharmaceutical manufacturing equipment. A fast, isocratic HPLC method has been developed can be applied for the cleaning control analysis of the Lubiprostone in the manufacturing equipment. The HPLC method was validated. For system precision and suitability, five repetitions of injection from standard solution was used. The tailing factor for each peak is not more than 2.0, the number of theoretical plates of the each of peak is not less than 5000 and the % RSD of peak areas generated by five injections is lower than 2.0% for specificity, linearity, accuracy, precision, limit of quantitation and detection were determined. The mean accuracy (recovery) are acceptable for this type of analysis (recovery >80%), a recovery factor is applying during the routine analysis calculation. The results of robustness show that the small changes in the method does not have major impact on the chromatographic parameters such as tailing, % RSD and number of theoretical plates. The analytical solutions and mobile phase were found to be stable for a reasonable period of time.

4. Acknowledgement

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