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DEVELOPMENT AND VALIDATION OF RP-HPLC RID METHOD FOR LACTOSE MONOHYDRATE IN SOLID DOSAGE FORMS

Narasimha S. Lakka* and Sapthagiri Y. Reddamoni

Analytical Research & Development, Dr. Reddy's Lab. Ltd, Hyderabad-500 072, India.

ABSTRACT

A RP-HPLC/RID method was developed for the quantitative determination of LM in pharmaceutical dosage forms by using RP-HPLC with RI Detector. It involved a Benson Polymeric Inc. BP-OA, Column for Organic Acid Analysis a L17 (Strong cat-ion exchange resin consisting of sulphonated cross-linked styrene-co-divinyl benzene copolymer in the calcium form, about 9 µm in diameter), 300 x 7.8 mm column. The separation was achieved in isocratic method. The mobile phase contains a mixture of water: methanol: methanol (80:20,*v/v*). The flow rate was 1.0 mL min^{-1} and the injection volume was 10 µL. The liquid chromatographic system equipped with refractive index detector. The retention time of Lactose was 5.2 min. The total run time was 10 min within which drug product and matrices were separated. The developed method was successfully applied to the determination of Lactose in pharmaceutical preparations. The developed method was validated with respect to linearity, accuracy, precision, specificity, robustness and ruggedness.

Key words: Lactose Monohydrate, RP-HPLC RID, Lactose intolerance, Development and validation.

INTRODUCTION

Lactose monohydrate is a natural disaccharide, obtained from milk, which consists of one galactose and one glucose moiety. The empirical formula and molecular weight of Lactose (S Edge *et al.,* 2005) is $C_{12}H_{22}O_{11}\cdot H_2O$ and 360.31 (Fig.1).

Lactose is composed of Beta-D-Galactose unit and alpha-D-glucose unit joined by Beta-D-glycosidic linkage between C1 of the galactose and C4 of the glucose unit (Fig.2). Lactose can exists in α and β forms. The chemical name of the "*O*-β-D-Galactopyranosyl-(1→4)-α-D-glucopyranose monohydrate".

Lactose is widely used as a filler or diluent in tablets and capsules, and to a more limited extent in lyophilized products and infant formulas. Lactose is also used as a diluent in dry-powder inhalation.

Corresponding Author

Narasimha S. Lakka
E-mail: nslakka@gmail.com

Lactose intolerance means the body cannot easily digest lactose, a type of natural sugar found in milk and dairy products. This is not the same thing as a food allergy to milk. When lactose moves through the large intestine without being properly digested (Fig.3), it can cause uncomfortable symptoms such as gas, belly pain, and bloating. Some people who have lactose intolerance cannot digest any milk products. Others can eat or drink small amounts of milk products or certain types of milk products without problems.

Digestion of lactose (catabolism), Infant mammals nurse on their mothers to drink milk, which is rich in the carbohydrate lactose. The intestinal villi secrete an enzyme called lactase (β-D-galactosidase) to digest it. This enzyme cleaves the lactose molecule into its two subunits, the simple sugars glucose and galactose, which can be absorbed. Since lactose occurs mostly in milk, in most mammals the production of lactase gradually decreases with maturity due to a lack of constant consumption. The symptoms of lactose intolerance can be mild to severe, depending on how

much lactase person body makes. The symptoms usually begin 30 minutes to 2 hours after eating or drinking milk products. The symptoms are as follows: bloating, pain or cramps, gurgling or rumbling sounds in belly, gas, loose stools or diarrhea, throwing up.

The following table contains a guide to the typical lactose levels found in various foods (Tab.1). More than 50 million Americans are lactose intolerant. Nearly two-thirds of the world's adult population has some degree of difficulty with digestion of milk sugar because of a lactase deficiency (Tab.2).

Accordingly, the scope of the present study was to determine lactose monohydrate (LM) from Bicalutamide 50 tablets under a variety of ICH recommended test conditions and to develop a stability-indicating estimation method for LM.

Literature survey revealed that several LC methods for estimation of lactose from pharmaceutical syrups, liquids and milk methods were reported but no method has been reported for solid dosage forms (IFPMA, 2003; IFPMA, 1996; Gillianmurphy *et al.,* 1973; National institutes of health, 2006; Ian A *et al.,* 2012; Andreia AR *et al.,* 2009; Nelofar A *et al.,* 2010) and no, method was found with complete method development and validation in intravenous infusions in pharmaceutical preparations.

The present work describes the development and validation of lactose monohydrate with simple, rapid isocratic mode of a RP-HPLC method for determination of LM. Therefore aim of the present work was to develop simple, precise, accurate, linear, rugged and robust RP-HPLC method for determination of LM in tablets and application of the method for assay study. The developed method has been validated according to ICH guidelines.

EXPERIMENTAL

Materials and reagents

Standards and tablets were supplied by Dr. Reddy's laboratories limited, Hyderabad, India. The HPLC grade methanol was purchased from Merck, Darmstadt, Germany. Water was prepared by using Millipore Milli-Q Plus water purification system. Placebo mixtures were prepared in the laboratory using United States Pharmacopoeia (USP) grade excipients

Equipment

The Waters HPLC system we used consists of a quaternary solvent manager, a sample manager and a RI detector. The output signal was monitored and processed using Empower software.

Chromatographic Conditions

The chromatographic column used was a Benson Polymeric Inc. BP-OA, Column for Organic Acid Analysis a L17 (strong cat-ion exchange resin consisting of sulphonated cross-linked styrene-co-divinyl benzene copolymer in the calcium form, about 9 µm in diameter), 300 x 7.8 mm column. The separation was achieved on isocratic mode method with flow rate of mobile phase was 1.0 mL min^{-1}. The mobile phase contains a mixture of water: methanol (80:20, *v/v*). The liquid chromatographic system equipped with refractive index detector. The column and detector temperatures were 40°C and 50°C, respectively. The sensitivity and polarity of RID were 64 and "+Ve". The injection volume was 10µL. The retention time of Lactose was 5.2 min. The total run time was 10 min within which drug product and matrices were separated.

Preparation of stock solutions

A stock solution of LM standard and sample (2 mg mL^{-1}) was prepared by dissolving an appropriate amount in diluent (purified water).

Preparation of calibration standard solutions

Working solutions (0.1, 0.25, 0.50, 0.75, 1.0, 1.25 mg mL^{-1}) were prepared from above stock solution in diluent for assay determination (Fig.4).

Preparation of sample solution

Weighed and transferred one tablet equivalent amount of Bicalutamide 50mg tablets into a 100 mL standard volumetric flask. The LM was finally extracted by dissolving in 100 mL of diluent. The solution was filtered through 0.45 µm Millipore PVDF filter. Then 10 µL of these solutions were injected in the column and chromatogram was recorded and shown in Fig.5.

METHOD VALIDATION

Calibration curve

The calibration curve was plotted between concentration versus detector response. The slope and correlation coefficient values were found to be 1027 and 0.999 (Tab.3).

Precision

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 the assay method was evaluated by carrying out six independent assays of LM test samples against qualified working calibration standards of LM. Different analyst from the same laboratory evaluated the intermediate precision of the method. The percentage of RSD of six assay values was calculated (Tab.4).

Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample. Linearity solutions were prepared from stock solution at six concentration levels from 0.10, 0.25, 0.50, 0.75, 1.0 and 1.25 mg mL^{-1} to target concentrations. The slope, Y-intercept, bias at 100% level and correlation coefficient were calculated (Tab.5 and Fig.6).

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. The accuracy of the method was evaluated in triplicate at three concentration levels, i.e. 50%, 100% and 150% of target test concentration in tablets. The percentages of recoveries were calculated (Tab.6).

Specificity

The specificity of an analytical method may be defined as the ability to unequivocally determine the analyte in the presence of additional components such as impurities, degradation products and matrix. The specificity was evaluated by preparing the analytical placebo and it was confirmed that the signal measured was caused only by the analyte. A solution of analytical placebo (containing all the tablet of Bicalutamide tablets excipients (except LM) was prepared according to the sample preparation procedure and injected. To identify the interference by these excipients, a mixture of inactive ingredients (placebo), standard solutions, and the commercial pharmaceutical preparations including LM was analyzed by the developed method The representative chromatograms did not show any other peaks, which confirmed the specificity of the method (Fig. 7).

Solution stability and mobile phase stability

The solution stability of LM was carried out by leaving the test solution in tightly capped volumetric flask at room temperature for 48 h. The same sample solution was assayed for a 24 h interval up to the study period against freshly prepared standard solution of LM. The mobile phase stability was also carried out by assaying the freshly prepared standard solution for 24 h interval up to 48 h. The mobile phase preparation was kept constant during the study period. The percentage of RSD of assay of LM was calculated for the study period during mobile phase and solution stability experiments and found to be satisfactory.

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. The variations like flow rate of mobile phase, column and detector temperatures; ratio of organic content in the mobile phase etc. does not have any significant effect on the method performance (Tab.7).

Table 1. Lactose levels in foods

Sl. No.	Dairy product	Serving size	Lactose content	Percentage
1.	Milk, regular	250 ml	12 g	4.80%
2.	Milk, reduced fat	250 ml	13 g	5.20%
3.	Yogurt, plain, regular	200 g	9 g	4.50%
4.	Yogurt, plain, low-fat	200 g	12 g	6.00%
5.	Cheddar cheese	30 g	0.02 g	0.07%
6.	Cottage cheese	30 g	0.1 g	0.33%
7.	Butter	1 tsp (5.9ml)	0.03 g	0.51%
8.	Ice cream	50 g	3 g	6.00%

Table 2. The Lactose Intolerance effect people from worldwide

Sl. No.	Name of the country	% Lactose Intolerance
1	African Blacks	97-100%
2	Asians	90-100%
3	North American Blacks	70-75%
4	Mexicans	70-80%
5	Mediterraneans	60-90%
6	Jewish Descent	60-80%
7	Middle Europeans	10-12%
8	North American Caucasians	7-15%
9	Northern Europeans	1-5%

Table 3. The results of calibration curve of LM

Sl. No	Concentration in ppm	Area
1.	100.1	101984
2.	250.2	254453
3.	500.4	506711
4.	750.6	763114
5.	1000.8	1022888
6.	1251	1284851
Slope (a)		1027
Correlation coefficient (r)		0.999

Table 4. Precision results of LM

Sl. No.	Name of the test (n)	Inter day (%)	Intra-day (%)
1	Preparation-01	100.8	101.3
2	Preparation-02	101.5	101.7
3	Preparation-03	100.3	100.2
4	Preparation-04	97.8	99.7
5	Preparation-05	100.3	100.7
6	Preparation-06	100.8	100.3
Average		100.3	100.3
%RSD		1.2	0.7

(Determinants n= 6)

Table 5. The linearity of LM

Sl. No	Concentration in ppm	Area
1.	101.0	102249
2.	252.5	253094
3.	505.0	508588
4.	757.5	767170
5.	1010.0	1027444
6.	1262.5	1298739
Correlation coefficient (r)		0.9999
Slope (a)		1028
Intercept		-6876
Bias at 100% level		0.999

Table 6. The results of accuracy of LM

Sl. No	Name	Mean 'µg/mL' added	Mean 'µg/mL' found	Mean %recovery (n)
1	50%	300.2	296.7	98.8
2	100%	600.4	607.8	101.3
3	150%	900.7	914.6	101.5

Determinants 'n = 3'

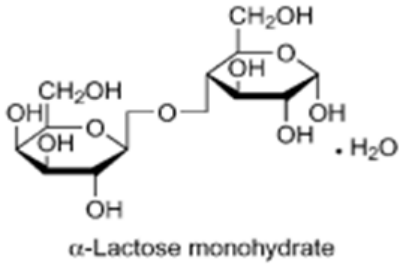
Table 7. The results of robustness of LM

Robustness of LM		
Flow rate	1.0 mL/min	± 0.2 mL/min
Column temperature	40 °C	± 0.5 °C
Detector Temperature	50 °C	± 0.5 °C
Organic phase composition in mobile phase	100 %	± 10 %

Table 8. The solubility profile of Lactose monohydrate

Sl. No.	Solvent	Solubility at 20°C unless otherwise stated
1.	Chloroform	Practically insoluble
2.	Ethanol	Practically insoluble
3.	Ether	Practically insoluble
4.	Water	1 in 5.24 at 25°C
		1 in 3.05 at 40°C
		1 in 2.30 at 50°C
		1 in 1.71 at 60°C
		1 in 0.96 at 80°C

Fig 1. Chemical structure of Lactose monohydrate



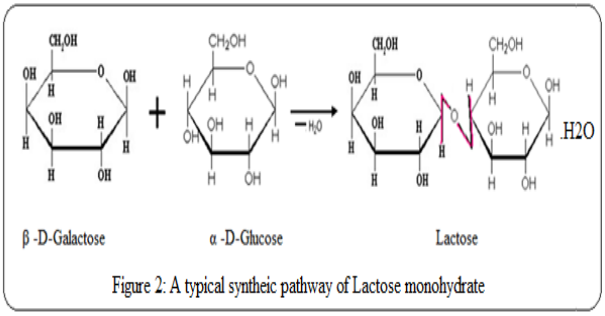


Figure 2: A typical syntheic pathway of Lactose monohydrate

Fig 3. Lactose Intolerance in Human Intestine

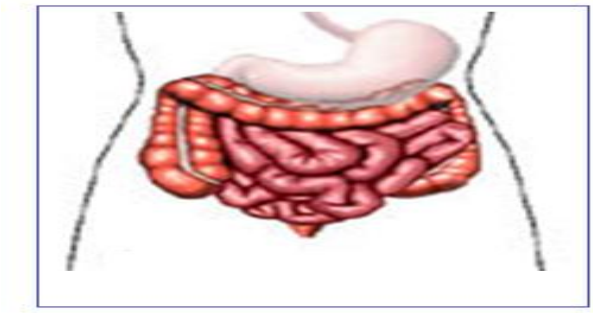


Fig 4. A typical chromatogram of LM from standard preparation

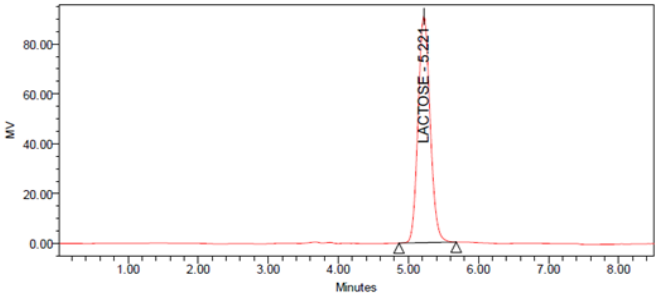


Fig 5. A typical chromatogram of the LM from test preparation

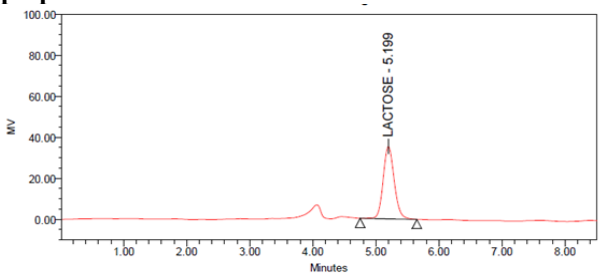


Fig 6. Linearity graph of LM

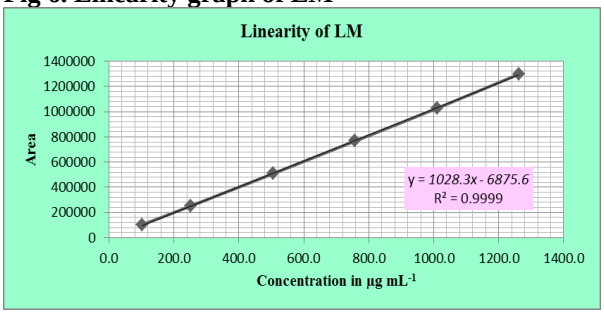
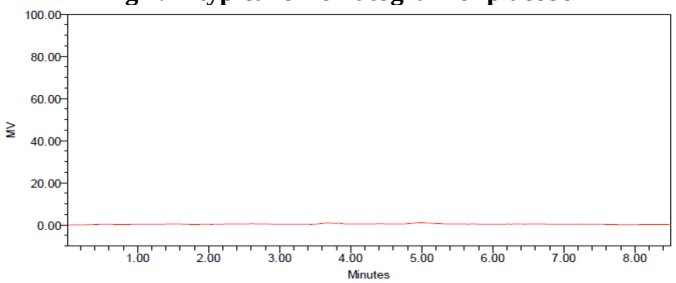


Fig 7. A typical chromatogram of placebo



RESULTS AND DISCUSSION

Method development and optimization

For HPLC analysis, initially various mobile phases and stationary phases were tried in attempts to obtain the best separation from other peaks. The different ratios of water and methanol and water acetonitrile were used for method development. The chromatographic method was optimized with a mixture of water and methanol (80:20,*v/v* respectively). The method was found to be an appropriate mobile phase allowing adequate separation for LM using a L17 column using refractive index detection.

The sensitivity and temperature of RID were optimized for LM. From this study, the sensitivity of LM was selected 64. The sensitivity 64 was used for getting good peak response, peak intensity, peak shape and height of LM.

Interference of excipients was also checked by injecting sample solutions of these excipients. There was no interference of excipients with LM peak.

The solubility profile of the LM data has been is helped a lot in the section of diluent. Lactose monohydrate and its solubility profile in different solvents was mentioned in the below table (Tab.8).

Validation

The percentage RSD value for the precision study was 1.2% (inter-day precision) and 0.7% (intra-day precision). This is confirming good precision of the method.

Linearity calibration plots for this method was obtained over the calibration ranges tested; i.e. 101 to 1262.5 µg mL^{-1} LM and the correlation coefficient obtained was greater than 0.999. The results show that an excellent correlation existed between the peak area and concentration of the analyte. The slope and Y-intercept of the calibration curve were calculated. The mean regressions equation was found as "y = *1028.3x - 6875.6"* (R^2 = 0.9999, *n* = 6). "y = *aC + b"*, where 'y' is the peak area ratio of the drugs, '*a'* is the slope, '*b'* is the intercept and '*C'* is concentration of the measured solution in µg mL^{-1}. The results show that an excellent correlation existed between the peak area and concentration of the analyte.

The percentage recovery of LM in pharmaceutical dosage forms ranged from 98.8 to 101.5. Excellent recoveries were made at each added concentration. The solution stability and mobile phase stability experiment data confirms that sample solutions and mobile phase used during the assay were stable up to 48 hours.

The variations like flow rate of mobile phase, temperatures of column and detector, ratio of organic content in the mobile phase does not have any significant effect on the method performance.

CONCLUSION

A simple, rapid, cost effective and accurate RP-HPLC method was developed for the determination of LM in pharmaceutical formulations by isocratic mode elution. The analytical conditions and the solvent system developed provided good separation for LM within a short run time. The HPLC method was validated and demonstrated good linearity, precision, accuracy, ruggedness, robustness and specificity. Thus, the developed HPLC method can be utilized for routine analysis during the analysis of LM.

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