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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF PARACETAMOL ANDMETOCHLOPRAMIDE HYDROCHLORIDE DRUGS IN BULK AND PHARMACEUTICAL DOSAGE FORMS USING RP-HPLC

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ABSTRACT

The present investigation describes a simple, economic, selective, accurate, precise reverse phase high performance liquid chromatographic method for the simultaneous estimation of paracetamol and Metochlopramide hydrochloride in pure and pharmaceutical dosage forms. paracetamol and Metochlopramide hydrochloride were well separated using a ZORBAX C18column of dimension 100 × 4.6, 5µm and Mobile phase consisting of Phosphatebuffer : Methanol (Adjusted with potassium dihydrogen phosphate to pH-4.5) in the ratio of 50:50v/v at the flow rate 1 ml/min and the detection was carried out at 213nm with PDA detector. The Retention time for paracetamol and Metochlopramide hydrochloride were found to be 1.9, 4.16 minutes respectively. The developed method was validated for recovery, specificity, precision, accuracy, linearity according to ICH guidelines. The method was successfully applied to paracetamol and Metochlopramide hydrochloride combination pharmaceutical dosage form.

KEY WORDS: RP-HPLC, paracetamol, Metochlopramide hydrochloride, Accuracy, Precision.

1. INTRODUCTION

Paracetamol(N-(4-Hydroxyphenyl)acetamide is a member of the drug class known as Analegesic and Antipyretic . It is used for lowering body temparature. Paracetamol (acetaminophen) is generally considered to be a weak inhibitor of the synthesis of prostaglandins (PGs). However, the in vivo effects of paracetamol are similar to those of the selective cyclooxygenase-2 (COX-2) inhibitors. Paracetamol also decreases PG concentrations in vivo, but, unlike the selective COX-2 inhibitors, paracetamol does not suppress the inflammation of rheumatoid arthritis. There is considerable evidence that the analgesic effect of paracetamol is central and is due to activation of descending serotonergic pathways, but its primary site of action may still be inhibition of PG synthesis.

Metochlopramidehydrochloride (4-amino-5-chloro-*N*-(2-(diethylamino) ethyl)-2-methoxybenzamide)[3] is a drug of the anti emetic and gastroprokinetic class. It is mainly used to reduce Nausea and Vomiting. The antiemetic action of metoclopramide is due to its antagonist activity at D_2 receptors in the chemoreceptor trigger zone (CTZ) in the central nervous system(CNS). The gastroprokinetic activity of metoclopramide is mediated by muscarinic activity, D_2 receptor antagonist activity and 5-HT_4 receptor agonist activity.

2. MATERIALS AND METHODS

Quantitative HPLC was performed on a high performance liquid chromatograph -Waters e2695Alliance HPLC system connected with PDA Detector 2998 and Empower2 Software. The drug analysis data were acquired and processed using Empower2 software running under Windows XP on a Pentium PC and ZORBAX C18column of dimension 100 × 4.6, 5µm particle size. In addition an analytical balance (DENVER 0.1mg sensitivity), digital pH meter (Eutech pH 510), a sonicator (Unichrome associates UCA 701) were used in this study.

Standards and chemicals used: Pharmaceutical grade Paracetamoland Metochlopramide hydrochloride were kindly supplied as a gift sample by Dr.Reddy's Laboratory, Hyderabad, and Andhra Pradesh, India. Methanol was of HPLC grade and Purchased from E. Merck, Darmstadt, Germany. Ortho Phosphoric Acid was analytical reagent grade supplied by Fischer Scientific Chemicals. Water HPLC grade was obtained from a Milli-QRO water purification system.Paracetamoland Metochlopramide hydrochlorideTablets available in the market as Paramet in composition of Paracetamol (325mg), Metochlopramide hydrochloride (5mg).

Preparation of mobile phase: Transfer 13.609gm of Kh_2PO_4 into 1000ml of beaker dissolve and diluted volume with water. Then adjust its pH to 4.5 with HPLC water. The Water adjusted pH to 4.5 with Water: Methanol (50:50 v/v) and filtered through 0.45µ membrane filter and degassed by sonication.

Preparation of calibration standards:325mg Paracetamol and 5mg Metochlopramidehydrochloride was taken into a 50, 10 ml of volumetric flask and add 10ml ofDiluent and sonicated for 10 minutes and made up with Diluent. It was further diluted to get stock solution of Paracetamoland Metochlopramide hydrochloride. This is taken as a 100% concentration. Working standard solutions of Paracetamoland Metochlopramide hydrochloride was prepared with mobile phase. To a series of 10 ml volumetric flasks, standard solutions of Paracetamoland Metochlopramidehydrochloride.

System suitability: System suitabilityare an integral part of chromatographic system. To ascertain its effectiveness, certain system suitability test parameters were checked by repetitively injecting the drug solutions at 100% concentration level for Paracetamoland Metochlopramide hydrochloride to check the reproducibility of the system. At

first the HPLC system was stabilized for 40 min. One blank followed by six replicate analysis of solution containing 100% target concentration of Paracetamoland Metochlopramide hydrochloride were injected to check the system suitability. To ascertain the system suitability for the proposed method, a number of parameters such as theoretical plates, peak asymmetry, and retention time were taken and results were presented in Table 1.

Recommended procedure:

Calibration curves for ParacetamolandMetochlopramide hydrochloride: Replicate analysis of solution containing 0.325-0.975µg/mL, 0.005-0.015µg/mLof paracetamoland metochlopramide hydrochloride sample solutions respectivelywere injected into HPLC according to the procedure in a sequence and chromatograms were recorded. Calibration curves were constructed by plotting by taking concentrations on X-axis and ratio of peak areas of standards on Y-axis and regression equation were computed for both drugs and represented in Table .7

Analysis of marketed formulation: The content of ten tablets was weighed accurately. Their average weights were determined. Powder of tablets equivalent to two tabletsweight (610.5mg) were weighed and taken in a 100 ml volumetric flask, dissolved in diluents, shaken and sonicated for about 20 minutes then filtered through 0.45µ membrane filter. The filtered solution was further diluted (5 to 50ml) in the diluents to make the final concentration of working sample equivalent to 100% of target concentration. The prepared sample and standard solutions were injected into HPLC system according to the procedure. From the peak areas of paracetamol and metochlopramide hydrochloride the amount of the drugs in the sample were computed. The contents were calculated as an average of six determinations and experimental results were presented in Table 4. The representivestandard and sample chromatograms were shown in fig.2 and fig.3.

Validation study of ParacetamolandMetochlopramidehydrochloride : An integral part of analytical method development is validation. Method validation is the process to confirm that the analytical procedure employed for a specific test is suitable for its intended use. The newly developed RP-HPLC method was validated as per International Conference on Harmonization (ICH) guidelines for parameters like specificity, system suitability, accuracy, linearity, precision (repeatability), limit of detection (LOD), limit of Quantification (LOQ) and robustness.

Specificity: The effect of wide range of excipients and other additives usually present in the formulation of Metochlopramide hydrochloride and paracetamolin the determination under optimum conditions were investigated. The specificity of the RP-HPLC method was established by injecting the mobile phase and placebo solution in triplicate and recording the chromatograms. The common excipients such as lactose anhydrous, microcrystalline cellulose and magnesium state have been added to the sample solution injected and tested.

Precision: precision study of sample (Metochlopramide hydrochloride and paracetamol) was carried out by estimating corresponding responses 6 times on the same day for the 100% target concentration. The percent relative standard deviation (%RSD) is calculated which is within the acceptable criteria of not more than 2.0.

Linearity: The linearity graphs for the proposed assay methods were obtained over the concentration range of0.325-0.975µg/mL, 0.005-0.015µg/mL(50-150%) Paracetamoland Metochlopramide hydrochloriderespectively. Method of least square analysis is carried out for getting the slope, intercept and correlation coefficient, regression data values and the results were presented in Table 7. The representative chromatograms indicating the sample were shown in fig.2&3. A calibration curve was plotted between concentration and area response and statistical analysis of the calibration curves were shown in fig. 6&7.

Accuracy (Recovery studies): The accuracy of the method is determined by calculating recovery of Paracetamoland Metochlopramide hydrochlorideby the method of addition. Known amount of Paracetamoland Metochlopramide hydrochlorideat 50%, 100%, 150% is added to a pre quantified sample solution. The recovery studies were carried out in the tablet in triplicate each in the presence of placebo. The mean percentage recovery of Paracetamoland Metochlopramide hydrochlorideat each level is not less than 99% and not more than 101%.

Robustness: The robustness is evaluated by the analysis of Paracetamoland Metochlopramide hydrochlorideunder different experimental conditions such as making small changes in flow rate (±0.2 ml/min), λmax (±5), column temperature (±5), mobile phase composition (±5%), and pH of the buffer solution.

LOD and LOQ: Limit of detection is the lowest concentration in asample that can be detected but not necessarily quantified. Under the stated experimental conditions. The limit of quantification is the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy. Limit of detection and limit of quantification were calculated using following formula LOD=3.3(SD)/S and LOQ=10(SD)/S, where SD= standard deviation of response (peak area) and S= average of the slope of the calibration curve.

3. RESULTS AND DISCUSSION

Reverse phase HPLC method was preferred for the determination of Paracetamoland Metochlopramide hydrochloride. Preliminary experiments were carried out to achieve the best chromatographic conditions for the simultaneous determination of the drug substances. Several column types and lengths were tried considering other chromatographic parameters. C18 column with a 4.6 mm inner diameter and 5µm particle size was chosen. The detection wave length was selected as 213nm with PDA detector. Chromatographic conditions were optimized by changing the mobile phase composition and buffers used in mobile phase. Different experiments were performed to optimize the mobile phase but adequate separation of the drugs could not be achieved. By altering the pH of buffer results a good separation. Different proportions of solvents were tested. Eventually the best separation was obtained by the isocratic elution system using a mixture of phosphatebuffer(adjusted the pH to 4.5 with potassium dihydrogen phosphate) : Methanol (50:50, v/v) at a flow rate of 1 ml/min. a typical chromatogram for simultaneous estimation of the two drugs obtained by using a above mentioned mobile phase. Under these conditions Paracetamoland Metochlopramide hydrochloride were eluted at 1.9min and 4.16minutes respectively with a run time of 6 minutes.

The representative chromatogram of this simultaneous estimation shown in fig. 3 & 4 and results were summarized in Table.1. The Methanol and water (pH 4.5 with PotasssiumDihydrogen Phosphate) (50:50, v/v) was chosen as the mobile phase. The run time of the HPLC procedure was 6 minutes at flow rate of 1ml/min was optimized which gave sharp peak, minimum tailing factor. The system suitability parameters were shown in Table 1 were in within limit, hence it was concluded that the system was suitable to perform the assay. The method shows linearity between the concentration range of 0.325-0.975µg/mL, 0.005-0.015µg/mL. The experimental results were shown in table 6 and fig.6&7. The % recovery of Paracetamoland Metochlopramidehydrochloride wasfound to be in the range of 99.41 to 99.67 % & 99 to 100.33% respectively. As there was no interference due to excipients and mobile phase, the method was found to be specific. As both compounds pass the peak purity, the method was found to be specific. The method was robust and rugged as observed from insignificant variation in the results of analysis by changes in Flow rate, column oven temperature, mobile phase composition and wave length separately and analysis being performed by different analysts. The results were shown in Table6.The LOD and LOQ values were calculated based on the standard deviation of the response and the slope of the calibration curve at levels approximately the LOD and LOQ. The limit of detection was obtained as 0.125µg/mL for Paracetamol and 0.026µg/mL for Metochlopramide Hydrochloride. The limit of quantitation was obtained as 0.416µg/mL for Paracetamol and 0.0877µg/mL for Metochlopramide Hydrochloride which shows that the method is very sensitive. The results were shown in Table.6.

Table.1.Optimized chromatographic conditions and system suitability parameters for proposed method

Parameter	Chromatographic conditions
Instrument	Waters e2695 Alliance HPLC with Empower2 software
Column	ZORBAX C18, (5µ, 250 x 4.6mm)
Detector	PDA Detector 2998
Diluents	Methanol
Mobile phase	Phosphatebuffer(adjusted pH 4.5 with potassium hydrogen ortho phosphate): methanolo (50:50 v/v)
Flow rate	1ml/min
Detection wavelength	213nm
Temperature	40°c
Injection volume	10µl
Retention time	
Paracetamol	1.9min
Metochlopramide hydrochloride	4.16min
Theoretical plate count	
Paracetamol	2622
Metochlopramide hydrochloride	3175
Tailing factor	
Paracetamol	1.643
Metochlopramide hydrochloride	1.631
Resolution factor	9.402

Table.2. Result of Specificity study

Name of the solution	Retention time in min
Blank	No peaks
Paracetamol	1.9min
Metochlopramide hydrochloride	4.16min

Table.3. Results of precision study

Sample	Injection number	Precission	
		RT	Peak area
Paracetamol	1	1.924	4983014
	2	1.922	4985214
	3	1.923	4989979
	4	1.918	4982656
	5	1.922	4985454
	6	1.921	4983649
	Mean		4981991
	%RSD(NMT 2.0)		0.1
Metochlopramide hydrochloride	1	4.153	2881690
	2	4.147	2880372
	3	4.169	2889639
	4	4.137	2888556
	5	4.139	2882078
	6	4.140	2888859
	Mean		2885199
	%RSD(NMT 2.0)		0.1

Table.4.Recovery data of the proposed Paracetamol and Metochlopramide hydrochloride

Sample	Spiked Amount (µg/ml)	Recovered Amount (µg/ml)	%Recovered	%Average recovery
Paracetamol	642.682	644.57	100.16	100%
	1285.363	1285.4	100	
	1927.518	1927.52	100	
Metochlopramide hydrochloride	9.917	9.917	100	100.01%
	19.835	19.835	100	
	29.744	29.73	100.04	

Table.5.Robustness results of Paracetamol and Metochlopramide hydrochloride

sample	parameters	Optimized	Used	RT	Peak area	Plate count
Atorvastatin	Flow rate (±0.2)	1ml/min	0.8	2.399	6671088	2576
			1	1.617	4016272	2543
	Temperature (±5°C)	25°C	20	1.931	4408830	2535
			30	1.924	4984239	2586
Fenofibrate	Flow rate (±0.2)	1ml/min	0.8	5.149	3896192	2808
			1	3.469	2335282	2800
	Temperature (±5°C)	25°C	20	4.158	2858998	2902
			30	4.082	2215484	3112

Table.6.Limit of Detection and Limit of Quantification

Parameter	Paracetamol	MetochlopramideHydrochloride
Limit of detection(LOD)	0.125µg/mL	0.026µg/mL
Limit of Quantification(LOQ)	0.416µg/mL	0.0877µg/mL

Table.7.Linearity results of paracetamol and metachlopromidehydrochloride

sample	Linearity level (µg/ml)	Peak area	Slope	Y-intercept	r^2
Paracetamol	0.1	2581359	192888	99780.88	0.999
	0.15	4170046			
	0.2	4989227			
	0.25	5779047			
	0.3	7896996			
Metochlopramide hydrochloride	0.01	1465678	19288	61387	0.999
	0.015	2455416			
	0.02	2921616			
	0.025	3411233			
	0.03	4689975			

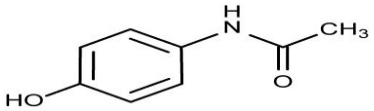

Figure.1.Structure of Paracetamol

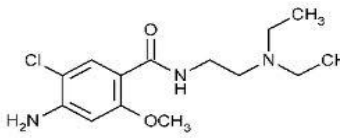

Figure.2.Structure of Metochlopramide Hydrochloride

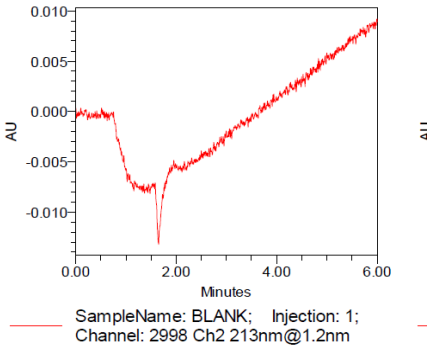

Figure.3.Chromatogram of Blank solution

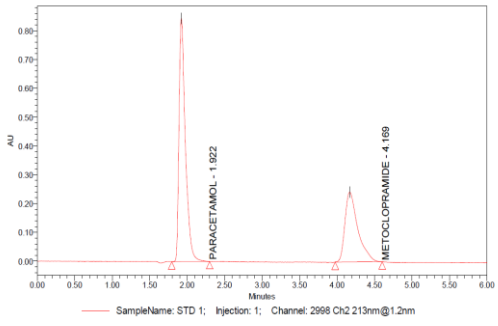

Figure.4.Typical Chromatogram of standard

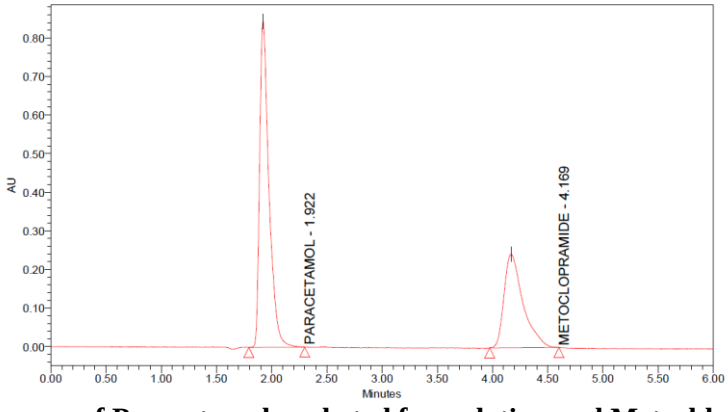

Figure.5.Typical chromatogram of Paracetamolmarketed formulation and Metochlopramide Hydrochloride in marketed formulation

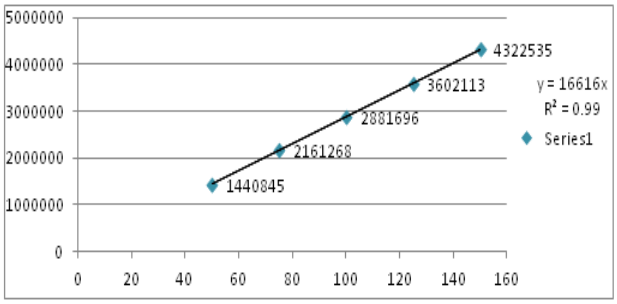

Figure.6.Linearity of Paracetamol

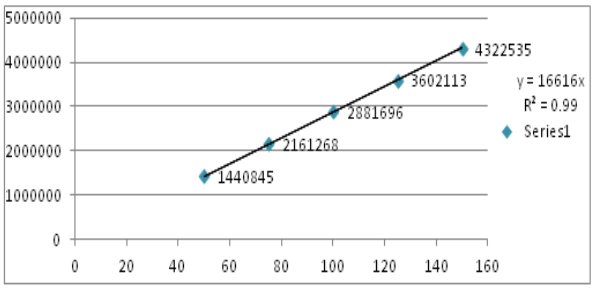

Figure.7.Linearity of Metochlopramide Hydrochloride

4. CONCLUSION

A new validated RP-HPLC method has been developed for the quantitative and Qualitative determination of paracetamoland Metochlopramide hydrochloride in tablet dosage forms in bulk and pharmaceutical dosage forms was established. The method was completely validated shows satisfactory results for all the method validation parameters tested and method was free from interferences of the other active ingredients and additives used in the formulation. Infact results of the study indicate that the developed method was found to be simple, reliable, accurate, linear, sensitive, economical and reproducible and have short run time which makes the method rapid. Hence it can be concluded that the proposed method was a good approach for obtaining reliable results and found to be suitable for the routine analysis of paracetamol and Metochlopramide hydrochloride in Bulk drug and Pharmaceutical formulations.

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