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Development of a new chromatographic method for estimation of paracetamol and metoclopramide hcl in bulk and pharmaceutical dosage form by HPLC

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ABSTRACT

A new simple, accurate, economic, rapid and precise reverse phase high performance liquid chromatographic method has been developed for the validated of Paracetamol and Metoclopramide HCl, in its pure form as well as in pharmaceutical dosage form. Chromatography was carried out on X bridge C18 (4.6×150mm) 5 µ column using a mixture of Methanol: Phosphate Buffer pH-3.6 (30:70v/v) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 260nm. The retention time of the Paracetamol and Metoclopramide HCl was 2.669, 3.855±0.02min respectively. The method produce linear responses in the concentration range of 10-50µg/ml of Paracetamol and 10-50µg/ml of Metoclopramide HCl. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

Keywords: Paracetamol and Metoclopramide HCl, RP-HPLC, Validation

INTRODUCTION

Analysis may be defined as the science and art of determining the composition of materials in terms of the elements or compounds contained in them. In fact, analytical chemistry is the science of chemical identification and determination of the composition (atomic, molecular) of substances, materials and their chemical structure.

Chemical compounds and metallic ions are the basic building blocks of all biological structures and processes which are the basis of life. Some of these naturally occurring compounds and ions (endogenous species) are present only in very small amounts in specific regions of the body, while others such as peptides, proteins, carbohydrates, lipids and nucleic acids are found in all parts of the body. The main object of analytical chemistry is to develop scientifically substantiated methods that allow the qualitative and quantitative evaluation of materials with certain accuracy. Analytical chemistry derives its principles from various branches of science like chemistry, physics, microbiology, nuclear science and electronics. This method provides information about the relative amount of one or more of these components.[1]

Every country has legislation on bulk drugs and their pharmaceutical formulations that sets standards and obligatory quality indices for them. These regulations are presented in separate articles relating to individual drugs and are published in the form of book called "Pharmacopoeia" (e.g. IP, USP, and BP). Quantitative chemical analysis is an important tool to assure that the raw material used and the intermediate products meet the required specifications. Every year number of drugs is introduced into the market. Also quality is important in every product or service, but it is vital in medicines as it involves life.

There is a time lag from the date of introduction of a drug into the market to the date of its inclusion in pharmacopoeias. This happens because of the possible uncertainties in the continuous and wider usage of these drugs, report of new toxicities and development of patient resistance and introduction of better drugs by the competitors. Under these conditions standard and analytical procedures for these drugs may not be available in Pharmacopoeias. In instrumental analysis, a physical property of the substance is measured to determine its chemical composition. Pharmaceutical analysis comprises those procedures necessary to determine the

identity, strength, quality and purity of substances of therapeutic importance. [2]

Pharmaceutical analysis deals not only with medicaments (drugs and their formulations) but also with their precursors i.e. with the raw material on which degree of purity and quality of medicament depends. The quality of the drug is determined after establishing its authenticity by testing its purity and the quality of pure substance in the drug and its formulations.

Quality control is a concept which strives to produce a perfect product by series of measures designed to prevent and eliminate errors at different stages of production. The decision to release or reject a product is based on one or more type of control action. With the growth of pharmaceutical industry during last several years, there has been rapid progress in the field of pharmaceutical analysis involving complex instrumentation. Providing simple analytical procedure for complex formulation is a matter of most importance. So, it becomes necessary to develop new analytical methods for such drugs. In brief the reasons for the development of newer methods of drugs analysis are:

1. The drug or drug combination may not be official in any pharmacopoeias.
2. A proper analytical procedure for the drug may not be available in the literature due to Patent regulations.
3. Analytical methods for a drug in combination with other drugs may not be available.
4. Analytical methods for the quantitation of the drug in biological fluids may not be available.
5. The existing analytical procedures may require expensive reagents and solvents. It may also involve cumbersome extraction and separation procedures and these may not be reliable. [1, 2]

Different methods of analysis

The following techniques are available for separation and analysis of components of interest.

Spectral methods

The spectral techniques are used to measure electromagnetic radiation which is either absorbed or emitted by the sample.
E.g. UV-Visible spectroscopy, IR spectroscopy, NMR, ESR spectroscopy, Flame photometry, Fluorimetry.2

Electro analytical methods

Electro analytical methods involved in the measurement of current voltage or resistanceas a property of concentration of the component in solution mixture.
E.g. Potentiometry, Conductometry, Amperometry.[2]

Chromatographic methods

Chromatography is a technique in which chemicals in solutions travel down columns or over surface by means of liquids or gases and are separated from each other due to their molecular characteristics.
E.g. Paper chromatography, thin layer chromatography (TLC), High performance thin layer chromatography (HPTLC), High performance liquid chromatography (HPLC), Gas chromatography (GC). [2]

Miscellaneous Techniques

Mass Spectrometry, Thermal Analysis.

Hyphenated Techniques

GC-MS (Gas Chromatography – Mass Spectrometry), LC-MS (Liquid Chromatography – Mass Spectrometry), ICP-MS (Inductivity Coupled Plasma- Mass Spectrometry), GC-IR (Gas Chromatography – Infrared Spectroscopy), MS-MS (Mass Spectrometry – Mass Spectrometry).

Analytical techniques that are generally used for drug analysis also include biological and microbiological methods, radioactive methods and physical methods etc. [15]

MATERIALS AND METHODS

Paracetamol from Sura labs, Metoclopramide HCl from Sura labs, Water and Methanol for HPLC from LICHROSOLV (MERCK). Acetonitrile for HPLC from Merck, Phosphate buffer Finar chemicals**.**

HPLC METHOD DEVELOPMENT TRAILS

Mobile Phase Optimization

Initially the mobile phase tried was Water: Methanol and ACN: Methanol with varying proportions. Finally, the mobile phase was optimized to phosphate buffer (pH 3.6), Methanol in proportion 70:30 v/v respectively.

Optimization of Column

The method was performed with various columns like C18 column ODS column, Zodiac column, and Xterra C18 column. Xbridge C18 (4.6 x 150mm, 5µm) was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS

Instrument used	: Waters HPLC with auto sampler and PDA detector 996 model.
Column	: X bridge C18 (4.6×150mm) 5 µ
Buffer	: Phosphate buffer (pH-3.6)- Dissolve 1.1998g of anhydrous di hydrogen phosphate in sufficient water to produce 1000ml. Adjust the pH 3.6 by using ortho phosphoric acid.
pH	: 3.6
Mobile phase	: Methanol: Phosphate Buffer pH-3.6 (30:70v/v)
Flow rate	: 1.0 ml per min
Wavelength	: 260 nm
Injection volume	: 10 µl
Run time	: 10 min.

VALIDATION

PREPARATION OF BUFFER AND MOBILE PHASE:

Preparation of Phosphate buffer (pH-3.6):

Dissolve 1.1998g of anhydrous di hydrogen phosphate dissolved in sufficient HPLC Grade water to produce 1000mL. Adjust the pH 3.6 by using ortho phosphoric acid.

Preparation of mobile phase

Accurately measured 300 ml (30%) of Methanol and 700 ml of Phosphate buffer (70%) were mixed and degassed in digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation:

The Mobile phase was used as the diluent.

RESULTS AND DISCUSSION

Optimized Chromatogram (Standard)

Mobile phase : Methanol: Phosphate Buffer pH3.6 (30:70v/v)
Column : X Bridge (4.6 × 150 mm, 5 µ)
Flow rate : 1.0 ml/min
Wavelength : 260 nm
Column temp : Ambient
Injection Volume : 10 µl
Run time : 8 min

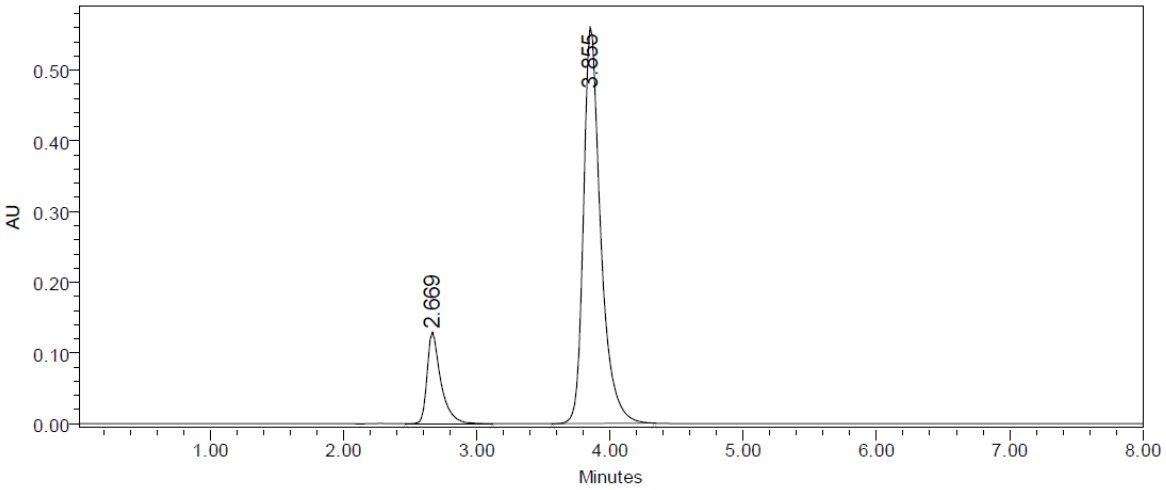

Fig 1: Optimized Chromatogram(Standard)

Table 1: Peak results for trail 7

S. No	Peak name	R_t	Area	Height	USP Resolution		USP Tailing	USP plate count
1	Paracetamol	2.669	986574	128672			1.5	3551.0
2	Metoclopramide HCl	3.855	5365216	562209	1.7		1.4	4675.7

This trial shows improper separation sample peaks, baseline and show very less plate count in the chromatogram. So it's required more trials to obtain good peaks.

From the above chromatogram it was observed that the Paracetamol and Metoclopramide HCl peaks are well separated and they shows proper retention time, resolution, peak tail and plate count. So it's optimized trial.

Optimized Chromatogram (Sample)

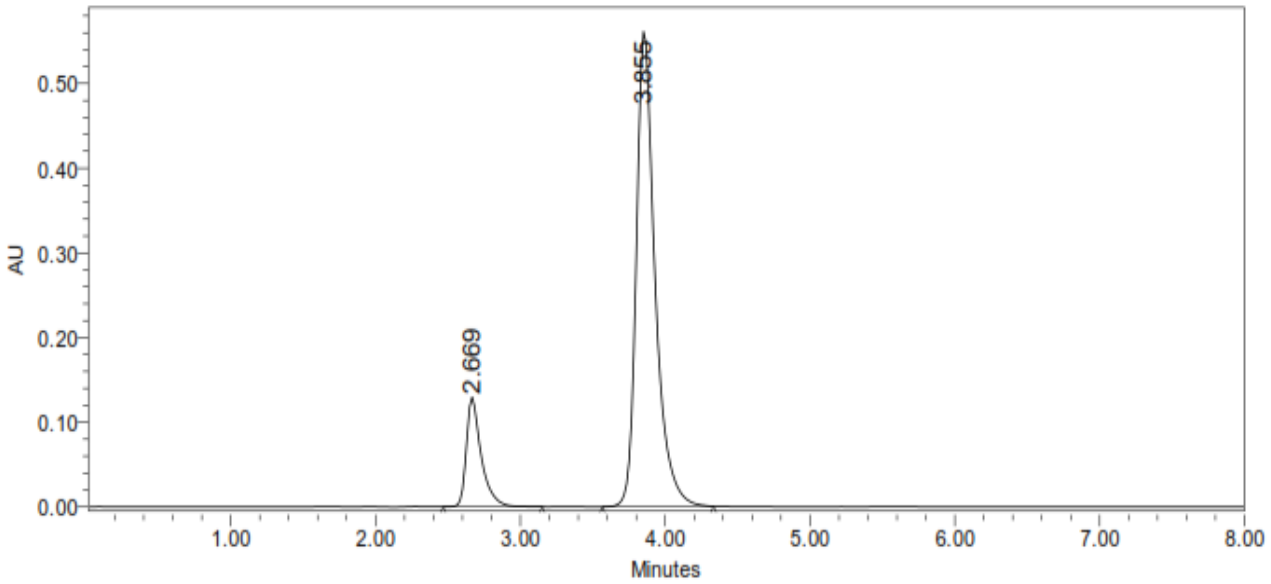

Fig 2: Chromatogram showing assay sample

Table 2: Showing assay sample results

S.No.	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Paracetamol	2.669	988626	127854		1.6	3561	1
2	Metoclopramide HCl	3.855	5387547	568541	1.7	1.4	4874	1
3	Paracetamol	2.651	989685	127841		1.5	3658	2
4	Metoclopramide HCl	3.849	5392435	563524	1.7	1.4	4641	2
5	Paracetamol	2.621	989874	127856		1.5	3854	3
6	Metoclopramide HCl	3.840	5389854	565412	1.7	1.4	4365	3

Assay (Standard)

Table 3: Results of system suitability parameters for Paracetamol and Metoclopramide HCl

S.No	Name	Retention time(min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP plate count
1	Paracetamol	2.669	979867	129658		1.6	3854
2	Metoclopramide HCl	3.855	5356471	587452	1.8	1.9	4796

- ***Resolution between two drugs must be not less than 2.,***
- ***Theoretical plates must be not less than 2000.***
- ***Tailing factor must be not less than 0.9 and not more than 2.***
- ***It was found from above data that all the system suitability parameters for developed method were within the limit.***

LINEARITY

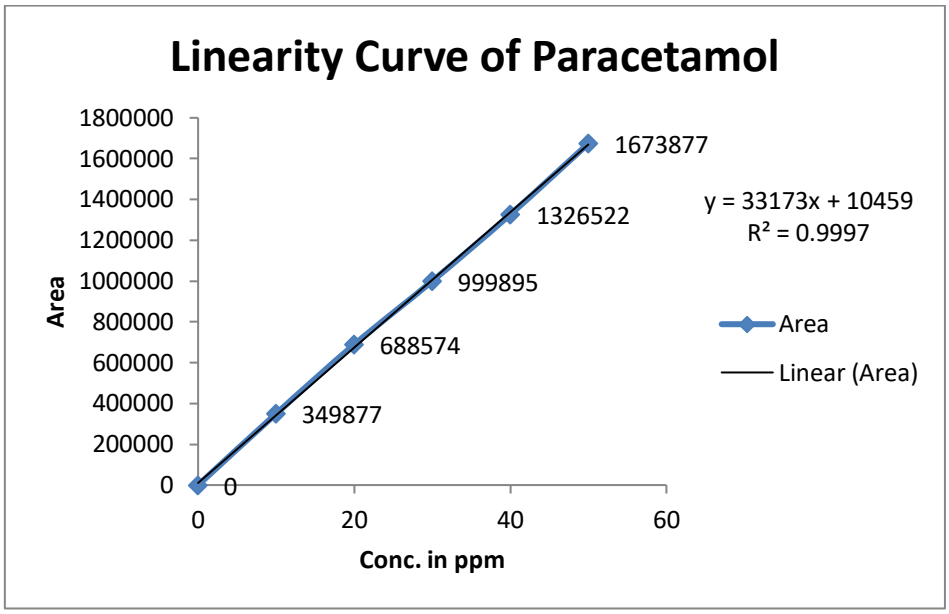

Fig 3: Calibration graph for Paracetamol

Linearity Results: (for Paracetamol)

S.No	Linearity Level	Concentration(ppm)	Area
1	I	10	349877
2	II	20	688574
3	III	30	999895
4	IV	40	1326522
5	V	50	1673877
Correlation Coefficient			0.999

Correlation coefficient should be not less than 0.999.

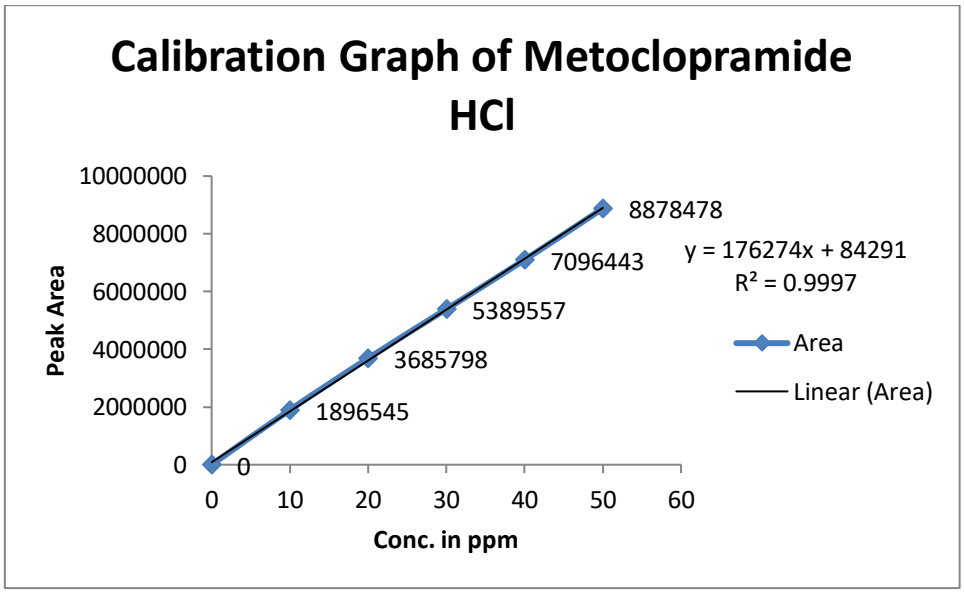

Fig 4: Calibration graph for Metoclopramide HCl

S.No.	Linearity Level	Concentration (ppm)	Area
1	I	10	1896545
2	II	20	3685798
3	III	30	5389557
4	IV	40	7096443

5	V	50	8878478
Correlation Coefficient			0.999

Correlation coefficient should be not less than 0.99.

REPEATABILITY

Table 4: Results of repeatability for Paracetamol and Metoclopramide HCl

Sno	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Paracetamol	2.669	986587	127854		1.5	3552	1
2	Metoclopramide HCl	3.855	5387451	561414	1.7	1.4	4654	1
3	Paracetamol	2.669	987824	126985		1.5	3571	2
4	Metoclopramide HCl	3.855	5378475	568951	1.7	1.4	4635	2
5	Paracetamol	2.654	986541	127894		1.5	3841	3
6	Metoclopramide HCl	3.849	5369875	568475	1.7	1.4	4684	3

Table 5: Results of method precession for Metformin

S.No	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Metformin	2.486	3233700	59095	6654	1.2
2	Metformin	2.484	3241323	57552	6524	1.3
3	Metformin	2.482	3245927	57213	6440	1.3
4	Metformin	2.483	3245927	57096	6411	1.4
5	Metformin	2.483	3222194	54363	6260	1.4
Mean			3237814			
Std. Dev			10060.62			
% RSD			0.310722			

%RSD for sample should be NMT 2

Intermediate precision

Table 6: Results of method precision Day 1 for Paracetamol

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Paracetamol	2.669	986857	128231	3653	1.5
2	Paracetamol	2.659	987854	129852	3541	1.5
3	Paracetamol	2.671	985474	128145	3635	1.5
4	Paracetamol	2.669	986589	129611	3595	1.5
5	Paracetamol	2.669	985213	128321	3698	1.5
Mean			986397.4			
Std. Dev			1075.302			
% RSD			0.109013			

Table 7: Results of method precession Day 1 for Metoclopramide HCl

Sno	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Metoclopramide HCl	3.855	5378559	565621	4675	1.4	1.7
2	Metoclopramide HCl	3.842	5386231	564587	4696	1.4	1.7
3	Metoclopramide HCl	3.850	5385411	563651	4684	1.4	1.7
4	Metoclopramide HCl	3.845	5369874	563544	4763	1.4	1.7
5	Metoclopramide HCl	3.855	5389745	578547	4954	1.4	1.7
Mean			5381964				
Std. Dev			7880.279				
% RSD			0.14642				

- *%RSD for sample should be NMT 2.*
- *The %RSD for the standard solution is below 1, which is within the limits hence method is precise.*

Table 8: Results of Intermediate precision Day 2 for Paracetamol

Sno	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Paracetamol	2.669	978985	128874	3686	1.5
2	Paracetamol	2.529	975686	128365	3654	1.5
3	Paracetamol	2.669	969876	128471	3536	1.5
4	Paracetamol	2.569	975487	128698	3682	1.5
5	Paracetamol	2.569	978546	128365	3598	1.5
6	Paracetamol	2.669	976898	128241	3536	1.5
Mean			975913			
Std. Dev			3286.897			
% RSD			0.336802			

Table 9: Results of Intermediate precision Day 2 for Metoclopramide HCl

S.No	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Metoclopramide HCl	3.845	5352141	563658	4685	1.4	1.7
2	Metoclopramide HCl	3.795	5365847	564587	4665	1.4	1.7
3	Metoclopramide HCl	3.855	5378412	563652	4654	1.4	1.7
4	Metoclopramide HCl	3.840	5378543	563547	4641	1.4	1.7
5	Metoclopramide HCl	3.855	5363598	565811	4669	1.4	1.7
6	Metoclopramide HCl	3.855	5386879	562541	4658	1.4	1.7
Mean			5370903				
Std. Dev			12656.43				
% RSD			0.235648				

- *%RSD of five different sample solutions should not more than 2*
- *The %RSD obtained is within the limit, hence the method is rugged.*

SUITABILITY

System suitability results for Paracetamol

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	4789.4	1.5
2	*Actual	3551.0	1.5
3	10% more	4635.6	1.5

System suitability results for Metoclopramide HCl

S.No.	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	5865.8	1.4
2	*Actual	4675.7	1.4
3	10% more	5342.4	1.4

***** Results for actual mobile phase have been considered from Assay standard.

CONCLUSION

High performance liquid chromatography is at present one of the most sophisticated tool of the analysis. The estimation of Paracetamol and Metoclopramide HCl was done by RP-HPLC. The Phosphate buffer was p^H 3.6 and the mobile phase was optimized with consists of Methanol: Phosphate buffer (pH-3) mixed in the ratio of 30:70 % v/ v. An Xbridge column C18 (4.6 x 150mm, 5µm) or equivalent chemically bonded to porous silica particles was used as stationary phase. The solutions were chromatographed at a constant flow rate of 1.0 ml/min. The linearity range of Paracetamol and Metoclopramide HClwere found to be from 10-50µg/ml, 10-50µg/ml respectively. Linear regression coefficient was not more than 0.999, 0.999.

The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 98-102% of Paracetamol and Metoclopramide HCl. LOD and LOQ were found to be within limit.

The results obtained on the validation parameters met ICH and USP requirements. It inferred the method found to be simple, accurate, precise and linear. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

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