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A Simple Rp-Hplc Method for Simultaneous Estimation of Paracetamol and Metoclopramide. HCl inTablet Dosage Form

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Abstract: *A simple, rapid, specific, precise and accurate reverse phase high performance liquid chromatographic (RP-HPLC) method has been developed and validated for the simultaneous estimation of Paracetamol and Metoclopramide.HCl in tablet dosage form. The chromatographic separation was achieved on AM Chemteq C18 (250mm x 4.6mm, 5µ) column at a detector wavelength of 220nm using an isocratic mobile phase comprising a mixture of Methanol: 0.5% Ammonium dihydrogen orthophosphate buffer (pH: 3) (35:65v/v) pumped at a flow rate of 1.0ml/min. Paracetamol and Metoclopramide.HCl eluted at the retention times of 4.35 and 7.5min respectively. The method was validated with respect to parameters such as specificity, linearity, precision, accuracy, robustness, limit of detection, limit of quantification, system suitability. The proposed RP-HPLC method can be used for the estimation of these drugs in tablet dosage form.*

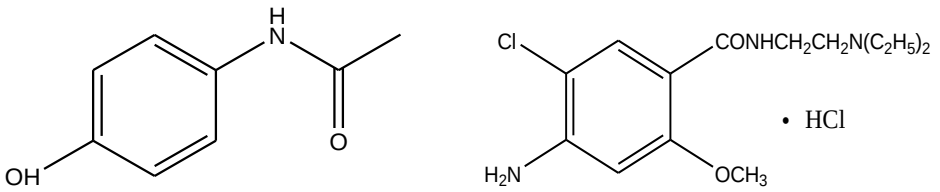
Keywords:*HPLC, Paracetamol, Metoclopramide.hcl, Validation.*

I. Introduction

Paracetamol {N-(4-hydroxyphenyl) acetamide} is analgesic and antipyretic[1]. Metoclopramide.HCl, 4-amino-5-Chloro-N-[2-(diethylamino)ethyl]-2-methoxy benzamidemono hydro-chloride[2,3].Paracetamol is thought to act primarily by increasing the pain threshold by inhibiting both isoforms of cyclooxygenase, COX-1 and COX-2, enzymes involved in prostaglandin (PG) synthesis. Unlike NSAIDs, it does not inhibit cyclooxygenase in peripheral tissues and, thus, has no peripheral anti-inflammatory effects whereas Metaclopramide. HClinhibits gastric smooth muscle relaxation produced by dopamine, therefore increasing cholinergic response of the gastrointestinal smooth muscle. It accelerates intestinal transit and gastric emptying by preventing relaxation of gastric body and increasing the phasic activity of antrum. It is used to treat nausea and vomiting. It is used for the relief of symptoms associated with acute and recurrent diabetic gastric stasis[1-3].

Literature survey revealed that analytical methods like UV, HPLC have been reported for the estimation of Paracetamol, Metoclopramide.HCl alone and in combination with other drugs. But there is only one HPLC method for the estimation of Paracetamol and Metoclopramide.HCl in combined dosage form[4-22].

The objective of the study is to develop a simple, precise, accurate and cost effective RP-HPLC method for the simultaneous estimation of Paracetamol and Metoclopramide.HCl in a tablet dosage form, with improved conditions and parameters for routine use in the laboratories. The chemical structures of the assayed compounds are given below (Fig. 1).



Paracetamol Metoclopramide.HCl

FIGURE 1: The chemical structures of paracetamol and metoclopramide.HCl

II. Experimental work

2.1Materialsand Methods

HPLC Grade Methanol and AR grade HCl were purchased from Merck. AR grade orthophosphoric acid was purchased from Merck and AR grade Ammonium dihydrogen orthophosphate

from Universal laboratories. In-house Millipore water was used throughout the study. Fixed dose combination tablets PARAMET containing 325mg PARA and 5mg of Metoclopramide.HClwere procured from local market.

2.2Instrumentation

The HPLC analysis was accomplished on WATERS high pressure liquid chromatography outfitted with 515 reciprocating dual column HPLC pump, a manually operating Rheodyne injector with 20µl sample loop, a 25cm x 4.6mm I.D. analytical column (AM Chemteq C18) containing C-18 reversed-phase material of 5µ size and a 2489 model UV-Visible detector. All the parameters of HPLC were controlled by EMPOWER – 2 software. Other instruments used were Systronics double beam UV-Vis spectrophotometer of model 2201, Shimadzu electronic balance of model AY-220, MKVI digital pHmeter and Bio-technics ultrasonic bath sonicator.

2.3. Liquid Chromatographic Conditions

Chromatographic separation was obtained by isocratic elution mode which was performed using a mobile phase containing methanol and 0.5% w/v ammonium dihydrogen orthophosphate buffer (pH-3) in the ratio of 35:65 v/vat a flow rate of 1ml/min through AM Chemteq C18 (250mm x 4.6mm, 5µm) column. The selective detection of the column effluents were monitored at a wavelength of 220nm.Injection volume was 20µl. The run time was taken as 10mins.

2.4. Preparation of Stock and Working Mixed Standard Solutions

25mg of Paracetamol and 0.384mg of Metoclopramide.HCl were weighed accurately and transferred into 25ml volumetric flask and sufficient amount of 1N HCl was added to dissolve. Then the volume was made up to the mark with water. 4ml of this solution was transferred into 10ml volumetric flask and the volume was made up to the mark with water. The concentrations of Paracetamol and Metaclopramide.HCl in the final solution were 400µg/ml and 6.12µg/ml respectively. The solution was filtered through 0.45µ filter paper and then degassed by sonication.

2.5. Preparation of Test Solution

20 tablets were weighed and powdered. The tablet powder was weighed accurately which is equivalent to 25mg of Paracetamol and transferred into a 25ml volumetric flask and sufficient amount of 1N HCl was added to dissolve and sonicated for 10min. Then the volume was made up to the mark with water. 4ml of this solution was transferred into 10ml volumetric flask and the volume was made up to the mark with water. The concentrations of Paracetamol and Metoclopramide.HCl in the final solution were 400µg/ml and 6.12µg/ml respectively as per the label claim. The solution was filtered through 0.45µ filter paper and then degassed by sonication.

2.6. Assay

20µl, of blank, standard and test solutions were injected separately into HPLC system. Chromatograms were recorded and peak responses were measured and the percentage drug content was calculated by using the following formula:

$$\%\text{Drug content} = \frac{\text{test area}}{\text{std area}} \times \frac{\text{std concentration}}{\text{test concentration}} \times \frac{\text{avg wt}}{\text{label claim}} \times 100$$

III. Method Validation

The developed analytical method was validated as per ICH guidelines with respect to parameters such as, linearity, precision, accuracy, robustness, limit of detection, limit of quantification, specificity, system suitability and solution stability[23-24].

3.1. Linearity

Mixed standard stock solution (1mg/ml) was prepared by taking 25mg of each, Paracetamol and Metaclopramide. HCl into 25ml volumetric flask and required amount of 1N HCl was added to dissolve, then the volume was made up to the mark with water. Different concentrations of mixed standard solutions of Paracetamol and Metoclopramide.HCl ranging from 0.1-1000µg/ml were prepared using water as a diluent and 20µl of each of standard solution was injected into the HPLC system. Chromatograms were recorded and peak responses were measured. A calibration curve was constructed by plotting peak area against concentration and regression equation was computed(Table 1&Fig. 2-4).

Table 1:Linearity Data Of Paracetamol And Metoclopramide.HCl

S.No	Concentration (µg/ml)	Paracetamol		Metoclopramide.HCl	
		Rt(min)	Peak area	Rt(min)	Peak area
1	0.1	4.419	2358	7.66	21890
2	1	4.353	91195	7.62	178172
3	10	4.353	339143	7.621	522557
4	50	4.366	1720771	7.62	3055654
5	100	4.353	3134499	7.56	5553696
6	200	4.355	5891655	7.62	10569870
7	300	4.35	9653364	7.641	17544365
8	400	4.366	12924917	7.623	23673390
9	500	4.34	17476740	7.612	31476557
10	600	4.352	19478596	7.66	35886716
11	700	4.347	23400726	7.62	43684589
12	800	4.354	24918627	7.56	48299463
13	900	4.35	25115854	7.56	51527710
14	1000	-	-	7.562	58860655

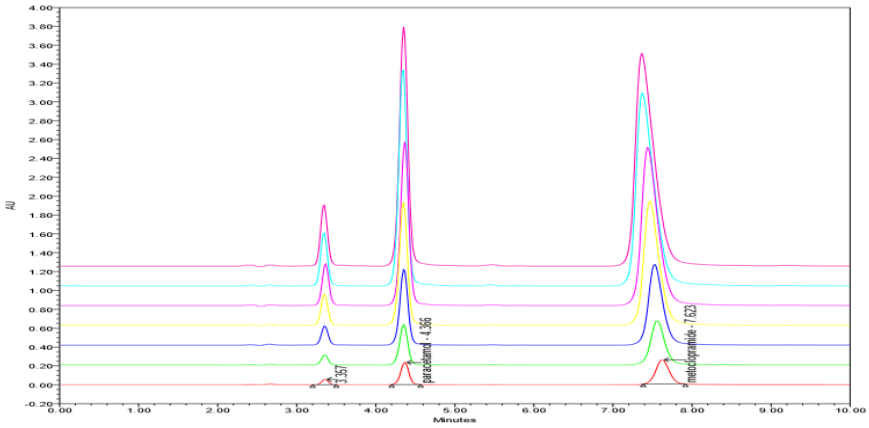


Figure 2:Linearity Overlain Chromatogram Of Paracetamol And Metoclopramide.HCl

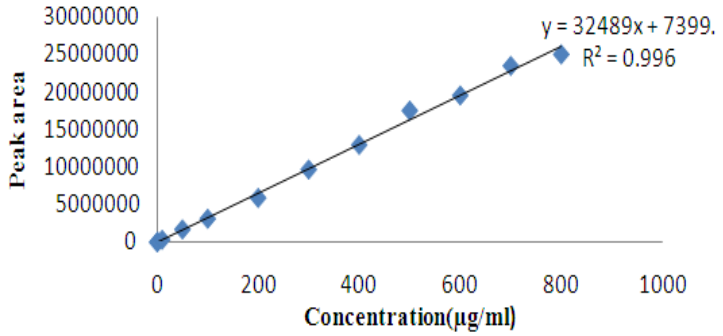


Figure 3: Calibration Curve For Paracetamol

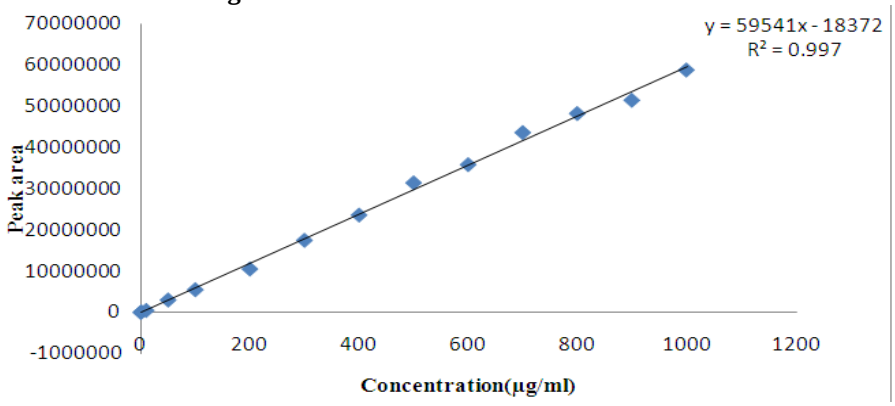


Figure 4: Calibration curve for Metoclopramide.HCl

3.2.Precision

System precision was performed by injecting six replicate injections of standard solution at (100µg/ml) concentration and the chromatograms were reviewed for the %RSD of peak areas. Method precision was demonstrated by preparing six test solutions (Paracetamol-500µg/ml and Metoclopramide.HCl -7.65 µg/ml) as per the test procedure & recording the chromatograms of six test solutions. The % RSD of %assays of six samples was calculated. Intermediate precision of the analytical method was determined by performing method precision on another day by different analysts under same experimental conditions. The results were shown in Tables2-7&Fig.5.

Table 2:System Precision data of Paracetamol and Metoclopramide.HCl

S.No	Paracetamol		Metoclopramide.HCl	
	Rt	Peak area	Rt	Peak area
1	4.378	3423308	7.528	5863929
2	4.364	3489609	7.595	5993372
3	4.327	3478265	7.639	5906477
4	4.352	3494533	7.567	5954580
5	4.355	3497506	7.579	5916359
6	4.351	3427336	7.553	6032475
	Avg	3468426.167		5944532
	SD	34049.2857		56212.43
	%RSD	0.9		0.9

Table 3:Method Precision data Paracetamol and Metoclopramide.HCl

S.No	Paracetamol		Metoclopramide.HCl	
	Rt	Peak area	Rt	Peak area
1	4.524	14975494	7.570	431490
2	4.540	14993783	7.623	435743
3	4.470	15228670	7.670	438669
4	4.418	15091349	7.642	436878
5	4.378	15144959	7.570	436319
6	4.427	14993994	7.553	439740
	Avg	15113416.17		436473.16
	SD	90687.28		2865.19
	%RSD	0.6		0.66

Table 4:Interday Precision data of Paracetamol

S.No	Day 1		Day 2		Day 3	
	Rt	Peak area	Rt	Peak area	Rt	Peak area
1	4.446	3398769	4.384	3427992	4.387	3433106
2	4.418	3454702	4.402	3448818	4.346	3465123
3	4.417	3468502	4.386	3477958	4.365	3468265
4	4.435	3445888	4.330	3479636	4.382	3474522
5	4.412	3484095	4.356	3492721	4.375	3497506
6	4.436	3455244	4.380	3444706	4.374	3427336

Pooled Avg3458049
Pooled SD 25722.5
Pooled %RSD0. 74

Table 5:Interday Precision data of Metoclopramide.HCl

S.No	Day 1		Day 2		Day 3	
	Rt	Peak area	Rt	Peak area	Rt	Peak area
1	7.728	5940361	7.558	5937617	7.562	5956158
2	7.670	5964688	7.633	5886223	7.613	5892123
3	7.659	5905103	7.583	5854289	7.598	5899325
4	7.674	5916135	7.562	5876421	7.625	5946451
5	7.624	5900850	7.590	5934493	7.553	5852352
6	7.670	5950910	7.552	5987476	7.552	5925236

PooledAvg: 5916814.706

Pooled SD : 38430.3
Pooled %RSD : 0.64

Table 6:Intermediate Precision data of Paracetamol (Analyst – Analyst)

S.No	Analyst- 1		Analyst- 2	
	Rt	Peak area	Rt	Peak area
1	4.523	14975495	4.356	15173072
2	4.548	14993782	4.438	15243471
3	4.47	15228662	4.479	15058541
4	4.419	15091339	4.416	15155391
5	4.379	15144960	4.361	15148742
6	4.427	14993990	4.348	15153549
	PooledAvg :	15113416.17		
	PooledSD :	90687.28		
	Pooled %RSD :	0.6		

Table 7:Intermediate Precision data of Metoclopramide.HCl (Analyst – Analyst)

S.No	Analyst- 1		Analyst- 2	
	Rt	Peak area	Rt	Peak area
1	7.652	435956	7.573	431494
2	7.622	436361	7.620	435733
3	7.612	434652	7.669	438671
4	7.656	437516	7.624	436887
5	7.626	434940	7.569	436310
6	7.774	431601	73521	439739

PooledAvg: 435821.7
Pooled SD : 2462.69
Pooled %RSD : 0.56

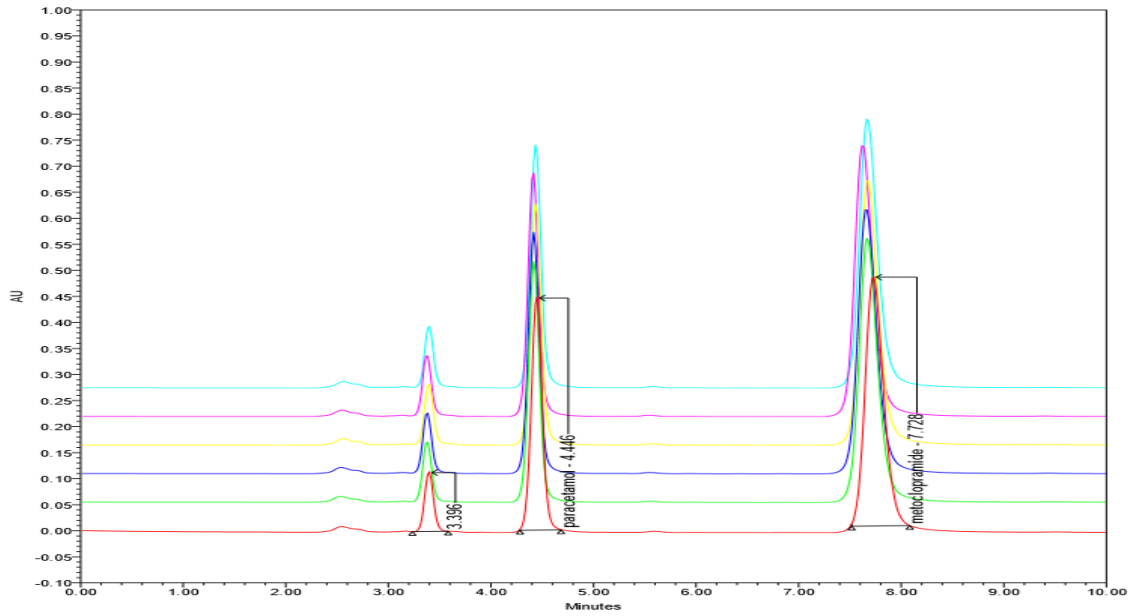


Figure 5:Overlain chromatogram for System Precision

3.3. Accuracy

The accuracy of the proposed method was evaluated by recovery studies which were carried out by standard addition method, where a known amount of test sample solution was spiked with mixed standard solution at three levels of 50%, 100% and 150%. At each level recovery studies were carried out in triplicate and expressed as percent recoveries (Tables 8-9).

Table 8:Accuracy data of Paracetamol

Spiked levels	Standard Conc (µg/ml)	Peak area	Mean peak area	Test Conc (µg/ml)	Peak area	Spiked Peak area	Mean peak area	Avg Amount recovered (µg/ml)	Avg % Recovery
50%	50	1641727 1665584 1837376	1714896	400	13146655	15183645 15192706 15168260	15181537	49.17	99.7
100%	100	3438476 3489682 3403814	3443990.6	400	13913068	17018881 16903547 17011487	16977971.7	100.53	101
150%	150	5107144 5172383 5143908	5141145	400	13324166	18565030 18593844 18618816	18592563.3	146.67	99.6

Table 9:Accuracy data of Metoclopramide.HCl

Spiked levels	Standard Conc (µg/ml)	Peak area	Mean peak area	Test Con (µg/ml)	Peak area	Spiked Peak area	Mean peak area	Avg.Amount recovered (µg/ml)	Avg % Recovery
50%	50	3158618 3137314 3466745	3254226	6.12	427599	3770048 3750323 3767303	3762558	47.76	101.8
100%	100	6600617 6679858 6699636	6660037	6.12	347813	7158966 7143707 7230513	7177728.6	96.27	101.1
150%	150	9991758 9989570 9993515	9991614.1	6.12	428141	10427704 10545289 10566688	10513227	143.12	100.36

3.3.1. Preparation Of Standard And Test Solutions

Mixed standard solutions containing 50µg/ml, 100µg/ml, 150µg/ml of each Paracetamol and Metaclopramide. HCl were prepared in triplicate from the mixed standard stock solution by appropriate dilutions. A test solution containing 400µg/ml of Paracetamol (6.12 µg/ml of Metoclopramide.HCl) was prepared by appropriate dilution of the test stock solution.

3.3.2. Procedure of Spiking

Spiking at 50% level was accomplished in triplicate, by adding 4ml of sample stock solution (1mg/ml of Paracetamol and 0.0153mg/ml of Metoclopramide.HCl) solution to the 0.5ml of mixed standard stock solution in a 10 ml volumetric flask. The contents of the volumetric flask were shaken then cautiously filtered through whatmann filter paper. The volumetric flask and filter paper were washed with small quantities of the solvent, and then the volume was made up to the mark with filtrate. The resultant solution was filtered through 0.45µ filter.

In the similar manner spiking at 100% was carried out, by adding 4ml of sample stock solution to the 1ml of mixed standard stock solution and at 150% was carried out by adding 4ml of sample stock solution to the 1.5ml of mixed standard stock solution.

3.4. Robustness

The robustness of the proposed method was determined by analysis of aliquots from homogeneous lots by differing parameters like flow rate, mobile phase composition, wavelength (Table 10).

Table 10:Robustness Study Data of Paracetamol And Metoclopramide.HCl

Parameter		Paracetamol			Metoclopramide.HCl		
		Rt	Mean Area	%RSD of Peak Areas	Rt	Mean Area	%RSD of Peak Areas
Flow rate (ml/min)	0.9	4.8	11511776	1.1	8.51	21328415	0.72
	1.0	4.46	10451114	0.65	7.76	19096721	0.92
	1.1	3.93	9531102	0.3	6.55	17362569.8	0.41
Wavelength (nm)	217	4.39	6149906.1	0.167	7.67	14361337.5	0.90
	220	4.39	6735644.3	0.030	7.68	12167739.8	0.84
	223	4.38	7908634.3	0.219	7.66	10077804	1.10
Mobile Phase	33:67	4.49	13737260	0.47	8.05	24692164	0.74
	35:65	4.48	13589198	0.53	7.64	24745677	0.73
	37:63	4.19	13608012	1.2	6.7	24438978	0.77

3.4.1 Effect of Variation In Flow Rate

A study was conducted to determine the effect of variation in flow rate. Mixed standard solution (300 µg/ml of each Paracetamol and Metoclopramide.HCl) was prepared according to the test procedure and injected six times into the HPLC system by changing the flow rate from 1ml/min to 0.9ml/min and 1.1ml/min.

3.4.1 Effect of Variation In Mobile Phase Composition

A study was conducted to determine the effect of variation in mobile phase composition. Mixed standard solution (400 µg/ml of each Paracetamol and Metoclopramide.HCl) was prepared according to the test procedure and injected six times into the HPLC system by changing the ratio of mobile phase i.e. methanol: buffer from 35:65 to 37:63 and 67:33.

3.4.2 Effect Of Variation In Wavelength

A study was conducted to determine the effect of variation in wavelength. Mixed standard solution (200 µg/ml of each Paracetamol and Metoclopramide.HCl) was prepared according to the test procedure and injected six times into the HPLC system by changing the wavelength from 220nm to 223nm and 217nm.

3.5.Limit of Detection And Limit of Quantitation

Limit of Detection (LOD) is calculated based on the standard deviation of the y-intercept (σ) and the slope of the calibration curve (S) at levels approximating the LOD according to the formula: LOD = 3.3(σ/S). LOQ is calculated based on the standard deviation of the intercept (σ) and the slope of the calibration curve (S) at levels approximating the LOQ according to the formula: LOQ = 10(σ/S). The standard deviation of y-intercept obtained from the LINEST function (Table 11).

Table 11: LOD and LOQ Data Of Paracetamol And Metoclopramide.HCl

Paracetamol			**Metoclopramide.HCl**		
Conc.(x) (µg/ml)	**Peak Area (Y)**	**Statistical Analysis**	**Conc.(x) (µg/ml)**	**Peak Area (Y)**	**Statistical Analysis**
0.1	2358	S = 34347.8	0.1	30859	S = 47225.7
0.5	18126	c = -470.14	0.5	50569	c = 27188.5
1	34195	σ = 945.3	1	751172	σ = 727.3
2.5	83521	LOD = 0.09µg/ml	2.5	146521	LOD = 0.05µg/ml
5	172015	LOQ = 0.27µg/ml	5	262576	LOQ = 0.15µg/ml

3.6. Specificity

The blank, standard and test solutions were injected into HPLC system and the chromatograms were recorded. As there were no peaks were found at the retention time of Paracetamol and Metoclopramide.HCl the proposed method was specific for the detection of the same (Fig. 6-8).

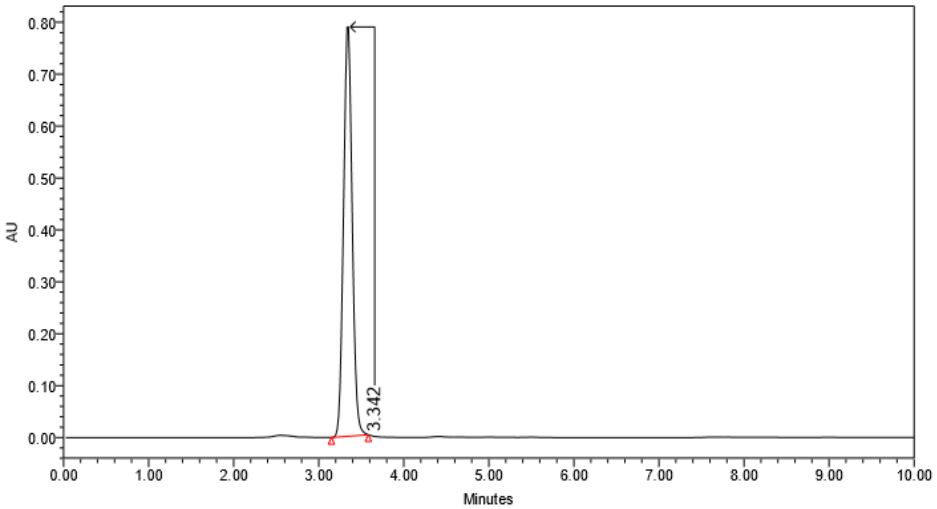


Figure 6:Chromatogram Of Blank

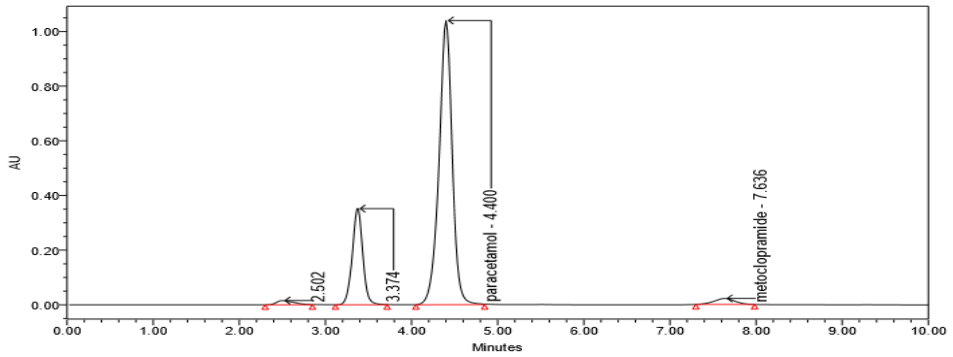


Figure 7:Chromatogram Of Test Solution

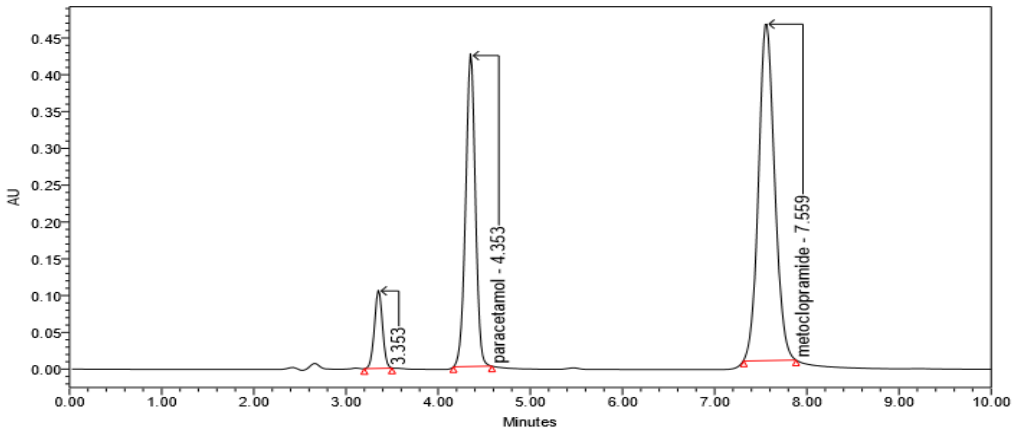


Figure 8: Standard Chromatogramof Paracetamol And Metoclopramide.HCl

3.7. System Suitability

Mixed standard solution (200µg/ml) of Paracetamol and Metoclopramide.HCl was prepared from the mixed standard stock solution (1mg/ml) and injected six times into the HPLC system. The system suitability parameters were evaluated from standard chromatograms obtained by calculating the % RSD of retention times and peak areas, average of tailing factor and theoretical plates from six replicate injections. The results were given in the Tables 12-13.

Table 12: System Suitability Data of Paracetamol

S.No	Retention time	Peak area	Theoretical plate number	Tailing factor
1	4.372	6527850	3659.54	0.94
2	4.386	6684616	3382.45	0.92
3	4.379	6750163	3313.91	1.0
4	4.372	6707196	3145.52	0.98
5	4.384	6788723	3238.400	0.9
6	4.356	6527820	3340.13	0.91
Mean	4.374	6664395	3346.658	0.94
SD	0.0109	111670.7	-	-
%RSD	0.2	1.6	-	-

Table 13: System Suitability Data of Metoclopramide.HCl

S.No	Retention time	Peak area	Theoretical plate number	Tailing factor	Resolution
1	7.509	12345419	3206.73	1.16	7.8
2	7.583	12280338	2672.73	1.15	7.9
3	7.567	12499392	2817.13	1.08	8.0
4	7.539	12333966	2613.24	1.05	7.9
5	7.558	12357710	2585.34	1.15	8.0
6	7.50	12451987	2674.21	1.0	8.1
Mean	7.54	12378135	2771.563	1.09	7.95
SD	0.0334	81437	-	-	-
%RSD	0.4	0.65	-	-	-

3.8. Solution Stability

Stability was estimated with standard and sample solutions. The standard solution (100µg/ml) and the test sample solution (300µg/ml) were prepared as per the test procedure and then injected into the HPLC. Chromatograms were recorded and peak responses were measured. The same procedure was repeated at an interval of 24 hours until there was a significant change in the peak area values(Tables 14-15).

Table 14: Stability Study Data of Paracetamol

Standard solution			**Test solution**		
Time (hours)	**Peak area**	**% Assay**	**Time (hours)**	**Peak area**	**% Assay**
Initial	3588404	100	Initial	8104703	100
24	3566988	99.4	24	8026004	99.02
48	3499191	97.5	48	7875735	97.1

Table 15: Stability Study Data of Metoclopramide.HCl

Standard solution			**Test solution**		
Time (hours)	**Peak area**	**% Assay**	**Time (hours)**	**Peak area**	**% Assay**
Initial	6648675	100	Initial	251887	100
24	6638559	99.8	24	250134	99.3
48	6592911	99.1	48	249346	98.99
72	6456731	97.1	72	242180	96.1

IV. Results And Discussion

Paracetamol is an anti-pyretic and analgesic drug and Metoclopramide.HCl is an anti-emetic drug. The combination of Paracetamol and Metoclopramide.HCl is used to relieve the pain and nausea which may occur during migraine attacks. In the present work, an attempt was made to provide a simple, precise, accurate RP- HPLC method which is successfully applied for the determination of Paracetamol and Metoclopramide.HCl in tablet dosage form.HPLC conditions were optimized to obtain an adequate separation of eluted compounds. Initially, various mobile phase compositions were tried to get good optimum results. Mobile phase and flow rate selection was based on peak parameters (tailing factor, theoretical plates, and run time.). The mobile phase of a mixture of methanol and 0.5% ammonium dihydrogen orthophosphate (adjusted to pH-3 using orthophosphoric acid) in the ratio of 35:65v/v was delivered at a flow rate of 1.0 ml/min. The optimum wavelength for detection was 220nm and a run time of 10min. The drugs were first dissolved in (solvent) sufficient quantity of 1N HCl and water was used as diluent for further dilutions. The retention time for Paracetamol and Metoclopramide.HCl were found to be 4.4 and 7.6min respectively. The calibration curve was linear in the concentration range of 0.1-800µg/ml and 0.1- 1000µg/ml with correlation coefficient values of 0.996 and 0.997 for Paracetamol and Metoclopramide.HCl respectively.System suitability tests are an integral part of chromatographic method. They are used to verify the reproducibility of the chromatographic system and to ascertain its effectiveness. From the system suitability studies it was observed that all the parameters within the limits.The %recoveries were found in the range of 98- 102% indicates that the method was accurate.Precision of the proposed method was done at the levels of repeatability (intraday) and intermediate precision. The values of %RSD were within the limits indicates that the method was precise.The chromatogram of blank was recorded and no peak was observed at the retention time of drug peaks. So the developed method was specific for assay of titled drugs.Robustness of the method was done by differing parameters like flow rate, mobile phase composition and wavelength. From the results it was concluded that in spite deliberate changes made, responses were within the limits therefore the developed method was robust.By performing solution stability studies it was observed that standard and test solutions were found to be stable for 48 hours.The lowest possible concentrations of Paracetamol and Metoclopramide.HCl that can be detected were found to be 0.06µg/ml and 0.04µg/ml respectively and that can be quantities were found to be 0.12µg/ml and 0.13µg/ml respectively.

V. Conclusion

The RP- HPLC method was developed and validated for simultaneous estimation of Paracetamol and Metoclopramide.HCl in combined dosage form. The method was found to be simple, sensitive, accurate, precise, robust, and economical. It can be used in laboratories for routine analysis of titled drugs individually and in combined tablet dosage forms.

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