IJPSR (2016), Vol. 7, Issue 1 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 23 June, 2015; received in revised form, 12 August, 2015; accepted, 19 October, 2015; published 01 January, 2016

VALIDATED RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF PARACETAMOL, PAMABROM AND DICYCLOMINE, HYDROCHLORIDE IN BULK AND PHARMACEUTICAL DOSAGE FORM

Hemraj Sharma *, Kammili Vishakha, Kondreddy Vinod Kumar and Hari Prasad Bhatta

Analytical Research Laboratory, Raghavendra Institute of Pharmaceutical Education and Research (RIPER), Anantapur (JNTUA), Andhra Pradesh – 515721, India.

Keywords:

Paracetamol, Pamabrom, Dicyclomine hydrochloride, RP-HPLC method, Standard addition.

Correspondence to Author:
Hemraj Sharma

Saigram Krishanamreddy palli cross, Chiyyedu (PO), Ananthapuramu-5155721(A.P.) India.

E-mail: sharma_raj153@yahoo.com

ABSTRACT: Simple and rapid Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) and UV-spectrophotometry method was developed for the determination of Paracetamol, Pamabrom and Dicyclomine hydrochloride, detection was carried out using photo diode array detector. Chromatographic separation of the analytes was achieved within 3.12, 4.25, 5.35 min for Paracetamol, Pamabrom and Dicyclomine hydrochloride by LC-GC Qualisil Gold-C18 (250 x 4.6 mm i.d., 5µm) column, mobile phase was methanol : water (1% TFA with pH adjusted to 3.0 with ammonia) in the ratio of (83:17), 0.4% (v/v) TEA and was filtered and degassed., flow rate was 1.0 mL/min, and the detection was carried out at 221 nm. Calibration curve was linear (r2=0.9993) in the range of 13-78 μg/mL for Paracetamol, r2=0.9991 in the range of 1-6 μg/mL for Pamabrom and r2=0.9993 in the range of 250-1500 μg/mL for Dicyclomine hydrochloride. The commonly used excipients and additives present in the pharmaceutical formulations were free from interfering components, as illustrated by Specificity of the method. Accuracy was performed as recovery study and results showed the recovery value of pure drug from the solution, between 98-102%, which indicates the method is accurate. Hence this method was better for Pharmaceutical formulations analysis.

INTRODUCTION: Paracetamol is chemically, N-(4-hydroxyphenyl) acetamide *or* N-(4-hydroxyphenyl) ethanamide **Fig.1** [1], is an Analgesic and Antipyretic agent. It is official in IP, BP & USP [2]. Pamabrom is chemically, 1:1 mixture of 2-amino-2-methylpropan-1-ol and 8-bromo-1,3-dimethyl-7H-purine-2,6-dione **Fig. 2** [3], is a potent Diuretic agent.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.7(1).316-24

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.7(1).316-24

Dicyclomine Hydrochloride is chemically 2-(diethylamino) ethyl-1-cyclohexylcyclohexane1-carboxylate **Fig.3** [4], is used as smooth muscle relaxant. Solubility profiles reveals that all the drugs are soluble in methanol, ethanol and sparingly soluble in water. Hence methanol is selected as extracting solvents for all the drugs. Literature survey reveals that spectroscopy method [5], HPLC method [6, 7, 8], for Paracetamol and Pamabrom.

Literature survey reveals that spectroscopy method, [9, 10] HPLC method [11, 12], HPTLC [13, 14] of Dicyclomine Hydrochloride in combination with other drugs. To date, there have been no published reports about the simultaneous estimation of

Paracetamol, Pamabrom and Dicyclomine Hydrochloride by HPLC in bulk drug and in pharmaceutical dosage forms. This present study, reports for the first time simultaneous estimation of Paracetamol, Pamabrom and Dicyclomine Hydrochloride by HPLC in bulk drug and in pharmaceutical dosage form.

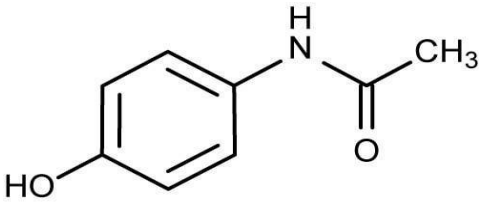

FIG. 1: PARACETAMOL

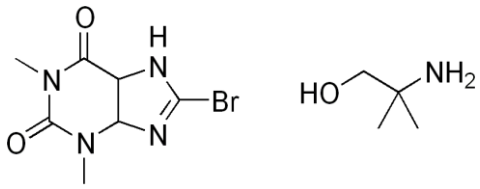

FIG. 2: PAMABROM

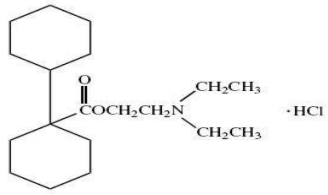

FIG.3: DICYCLOMINE HCL

MATERIALS AND METHODS:

Materials and reagents:

Pharmacopoeia grade standards of Paracetamol, Pamabrom and Dicyclomine Hydrochloride were gifted from Aurobindo Pharma Limited, Hyderabad. HPLC grade solvents Methanol, Distilled water were obtained from Merck Specialities Pvt. Ltd.

Instrumentation

A high performance liquid chromatography system consisting of Agilent LC 1200 Module with Photodiode Array detector was used with data handling system EZ chrome elite software, with 20μL loop, Rheodyne manual injector. A double beam UV/Visible spectrophotometer (Lab India 3000^{+}) using Photo diode UV enhance wide range solid state photodiode as detector with spectral width of 2 nm, Chemicals were weighed using Analytical balance Axis LC GC. All pH measurements were done on pH meter Systronics, Digital pH meter 802.

Chromatographic conditions:

Chromatographic analysis was carried out on reverse phase LC-GC Qualisil Gold-C_{18} column (250 mm ×4.6, 5 μm). The mobile phase consisted of methanol: water (1% TFA with pH adjusted to 3.0 with ammonia) in the ratio of (83:17), 0.4% (v/v). The column temperature was ambient. The content of mobile phase was filtered through 0.45µ membrane, filtered and degassed for 15 minute. The mobile phase was pumped from the solvent reservoir to the column at flow rate of 1.0 mL/ min with injection volume of 20 µl. The eluents were monitored at 221 nm. The optimized conditions were shown in table 1.

RP-HPLC method development for Paracetamol, Pamabrom and Dicyclomine Hydrochloride:

Preparation of standard drug solutions:

Accurately weighed 10 mg Paracetamol, 10 mg Pamabrom and 125 mg Dicyclomine Hydrochloride and transferred to 10 ml, 10 ml and 25 ml of volumetric flask respectively and extracted with methanol. The flasks were shaken and volume was made up to the mark with methanol to give solutions containing concentration of 1000 µg/ml Paracetamol, 1000µg/mL Pamabrom and 5000µg/mL of Dicyclomine Hydrochloride. From this stock solution, different concentrations of Paracetamol, Pamabrom and Dicyclomine Hydrochloride were prepared in the range of 13-78µg/mL, 1-6µg/mL and 250-1500 µg/mL respectively. The solutions were injected under above chromatographic conditions and peak areas were measured.

Calibration of standards:

The separate standard calibration lines were constructed for each component with Paracetamol, Pamabrom and Dicyclomine Hydrochloride, in the range of 13-78µg/mL, 1-6µg/mL and 250-1500 µg/mL respectively. Six solutions were prepared and the final volume was made up to the mark with mobile phase. The calibration curve was obtained by plotting the peak area against the concentration of drug.

Method Validation for Paracetomol, Pamabrom and Dicyclomine Hydrochloride: The developed method was validated as per ICH Guidelines

including the parameters specificity, linearity, precision, accuracy, Limit of detection, Limit of quantification and Robustness. [15]

RESULTS AND DISCUSSIONS:

Method development for the Determination of Paracetamol, Pamabrom and Dicyclomine Hydrochloride in combined dosage form:

Optimization of the chromatographic conditions: In preliminary experiments, PARA, PAMA, DH were subjected for separation by different trials by reversed phase HPLC using water as aqueous and Methanol as organic phase. Two of the drugs were separated but third drug didn't appear. Since Dicyclomine Hydrochloride is very weak chromophore in its structure, it does not show any significant peaks in the chromatogram hence 1% Trifluroacetic acid (TFA) is added, and to avoid peak broadening 0.4% Triethylamine (TEA) was added to water and the pH was maintained at 3.0 with ammonia. The various trials in optimization of chromatographic conditions were shown in the **Table 1**. The chromatogram obtained was better than all other experimental trials and shown in Figure 4. In this method development phase, phosphate buffers were avoided, to make this method as LC-MS compatible.

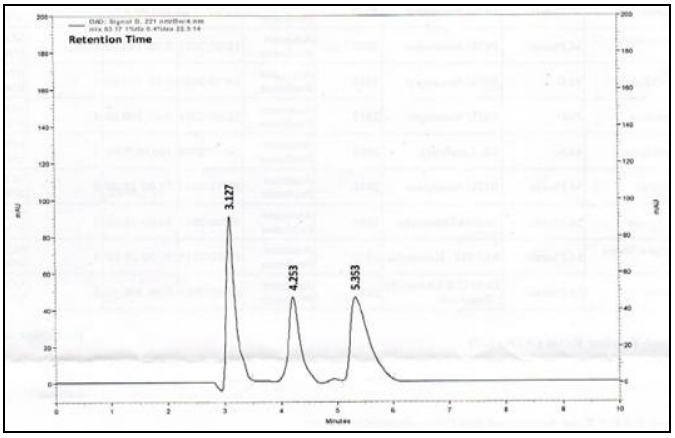

FIG. 4: OPTIMISED CHROMATOGRAM FOR PARACETAMOL, PAMABROM AND DICYCLOMINE HCL

TABLE 1: OPTIMIZED CONDITIONS FOR PARACETAMOL, PAMABROM AND DICYCLOMINE HCL

Trial No.	Mobile Phase Composition (MeOH:H_2O)	Adjusted pH to	Retention Time (Rt) (min)			No. of theoretical plates			Area			Remark
			PARA	PAMA	DH	PARA	PAMA	DH	PARA	PAMA	DH	
1	70 : 30	-	3.1	6.8	-	3320	4038	-	1240426	729005	-	DH peak didn't appear.
2	80:20 TEA- 0.2%	6.0 (OPA)	3.0	4.1	-	3319	3315	-	1205988	868391		DH peak did not appear
3	80:20 1% TFA, 0.2% TEA	6.0 (NH_3)	2.5	3.8	13.2	1368	1816	2545	639430	1082508	503065	PARA peak at void, DH peak shows fronting
4	80:20 1% TFA, 0.2% TEA	4.8 (NH_3)	3.0	4.6	7.0	1844	2827	713	1591739	1273453	613682	DH peak shows tailing
5	87:13 1% TFA, 0.2% TEA	4.8 (NH_3)	2.5	3.3	5.4	1059	1252	470	653442	1026475	320328	Broad DH peak
6	85:15 1% TFA, 0.2% TEA	4.8 (NH_3)	2.5	3.2	8.3	1692	2006	1061	727800	1003525	602716	Theoretical plates of PARA & DH are less than 2000
7	83:17 1% TFA, 0.4% TEA	3.0 (NH_3)	3.1	4.2	5.3	4306	5348	2754	5614997	257138	668094	Peaks are showing good resolution

MeOH= Methanol, PARA=Paracetamol, PAMA=Pamabrom, DH=Dicyclomine HCL, OPA=Orthophosphoric acid
TEA=Triethyl amine, TFA=Trifluroacetic acid

Validation of Analytical method for the Assay of Paracetamol, Pamabrom and Dicyclomine Hydrochloride Specificity: Specificity of the method was shown by quantifying the analyte of interest in the presence of matrix and other components. Blank injections have shown no peaks

at the retention time of 3.1 min, 4.22min and 5.31 min; hence the proposed method was specific for the detection of Paracetamol, Pamabrom and Dicyclomine Hydrochloride respectively. The represented data was shown in **Table 2**.

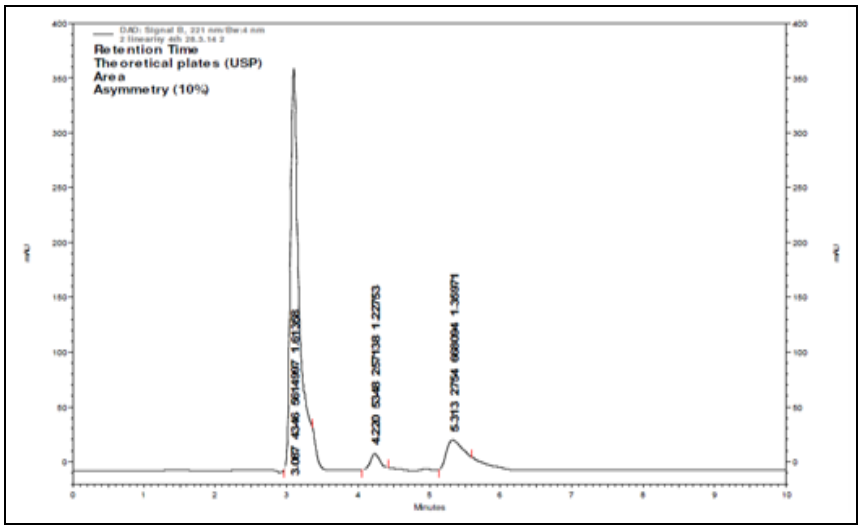

FIG.5: CHROMATOGRAM OF PARACETAMOL, PAMABROM AND DICYCLOMINEHCL (SPECIFICITY)

TABLE 2: SPECIFICITY DATA FOR PARACETAMOL, PAMABROM AND DICYCLOMINE HCL

Injection	Rt of analyte(in min)			Degradation peak Rt(in min)			Remark
	PARA	PAMA	DH	PARA	PAMA	DH	
Blank	----		----	----	----	----	No component found
Control	3.087	4.220	5.313	----	-----	----	Method is specific
Degraded sample	3.093	4.113	5.260	----	-----	----	No impurities were found

Linearity and Range:

The linearity of calibration curves (analyte peak area ratio Vs concentration) in pure solution was checked over the concentration ranges of about 13-78µg/mL for Paracetamol, 1-6µg/mL for Pamabrom and 250-1500µg/mL for Dicyclomine Hydrochloride. The total eluting time was less than 10min. The regression line relating standard concentrations of drug using regression analysis, the calibration curves were linear in the studied range and equations of the regression analysis were obtained:

Y= 44233.5451X+3434075.8038
Y=54196.8571X+42393.00
Y=678.2306X+29063.8667

The mean ± standard deviation (SD) for the slope, intercept and correlation coefficient of standard curves (N=3) were calculated. The represented data was shown in **Table 3**.

TABLE 3: LINEARITY FOR PARACETAMOL, PAMABROM AND DICYCLOMINE HYDROCHLORIDE

S.No	Con^{c}. of PARA	Peak area Mean ±SD (n=3) of PARA	Con^{c}. PAMA	Peak area Mean ±SD (n=3) of PAMA	Con^{c}. DH	Peak area Mean ±SD (n=3) of DH	% RSD		
							PARA	PMA	DH
1	13	3989842±45832	1	100256±656	250	191316±1251	1.148	0.654	0.653
2	26	4501465±5579	2	149853±1599	500	370614±718	0.123	1.067	0.193
3	39	5121779±51106	3	199946±2852	750	550125±2315	0.997	1.426	0.420
4	52	5729897±51735	4	259983±1566	1000	699051±1954	0.902	0.602	0.279
5	65	6314854±67078	5	312289±3118	1250	882963±3148	1.062	0.998	0.356
6	78	6889675±52362	6	370165±3918	1500	1041025±15654	0.760	1.058	1.532

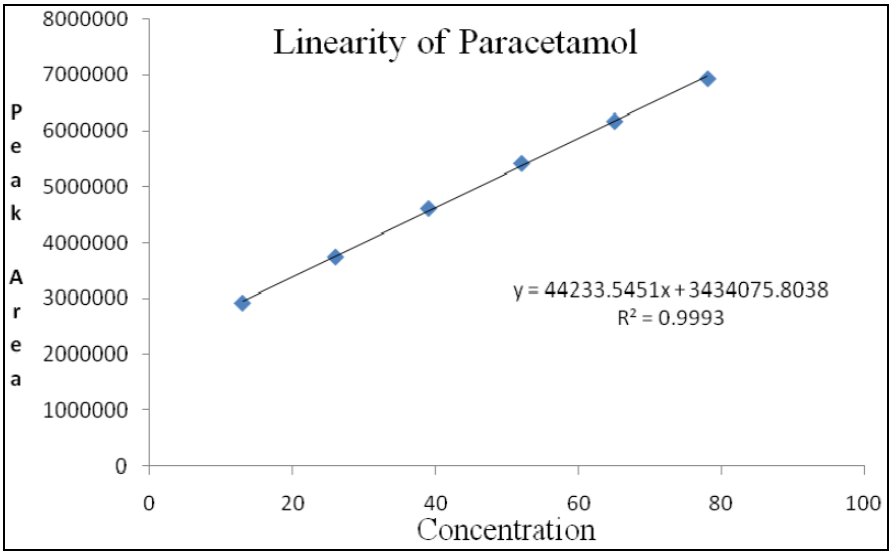

FIG.6: LINEARITY DATA FOR PARACETAMOL

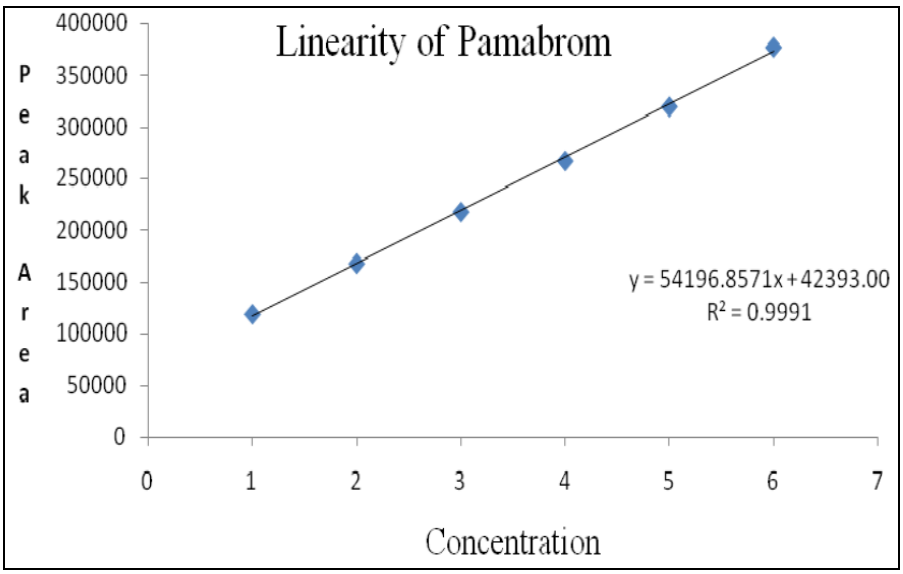

FIG. 7: LINEARITY DATA FOR PAMABROM

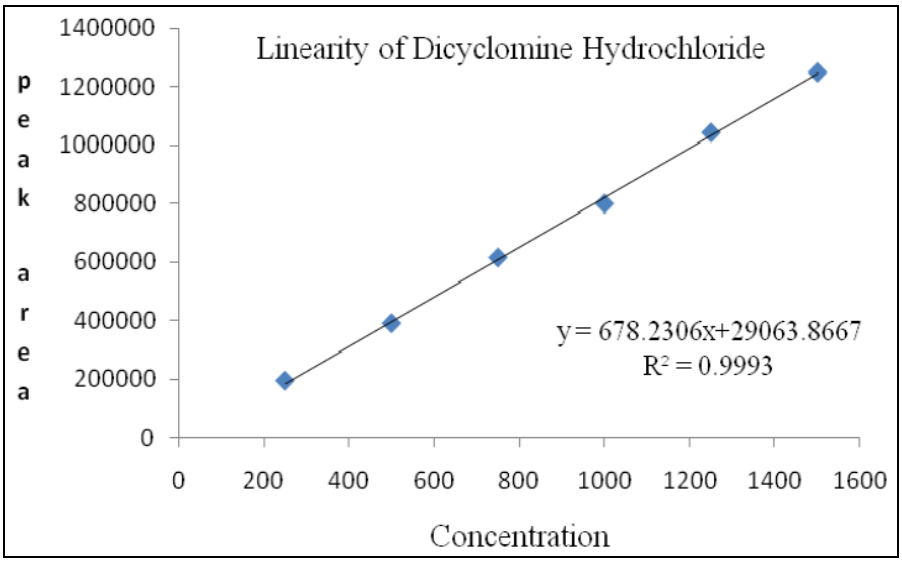

FIG.8: LINEARITY DATA FOR DICYCLOMINE HCL

Assay by standard addition method:

Twenty tablets were taken and triturated, and then a powder quantity equivalent to 32.5 mg of Paracetamol, 2.5 mg of Pamabrom and 1 mg of Dicyclomine Hydrochloride was accurately weighed and transferred to volumetric flask of 50 mL capacity. 8ml of methanol was transferred to this volumetric flask and sonicated for 15 min. The flask was shaken and volume was made up to the mark with methanol. A pale pink colour solution will appear in the volumetric flask. The above solution was filtered through Whatmann filter paper (0.45µ). From this solution 4 ml was transferred to volumetric flask of 10 ml capacity. The volume was made up to the mark with mobile phase. The solution was shaken well for 15min and then the solution was filtered using Whatmann filter paper (0.45µ). From this solution 1 ml was transferred to volumetric flask of 10 ml capacity, volume was made up to the mark with mobile phase to give a solution containing 26µg/mL of Paracetamol, 2µg/mL of Pamabrom and 0.8µg/mL of Dicyclomine Hydrochloride. Since the concentration of Dicyclomine Hydrochloride is very low a standard concentration of 500µg/mL was added to the final solution. The resulting solution was analyzed by proposed method. The quantitation was carried out by keeping these values to the straight line equation of calibration curve. The results were shown in **Table 4.**

TABLE 4: ASSAY FOR PARACETAMOL, PAMABROM AND DICYCLOMINE HYDROCHLORIDE

S. No.	Formulation (Tablet)	Labelled claim (mg)	Peak Area Mean ± S.D (n=3)	Amount found (mg)Mean± SD	Assay	%RSD
1	Paracetamol	325 mg (26 µg/mL)	4589620±5415	326.875±0.36	100.5	0.11
2	Pamabrom	25 mg (2 µg/mL)	151526±582	25.125±0.08	100.5	0.31
3	Dicyclomine Hydrochloride	10 mg (500µg/mL)	381436±1551	10.148±0.01	101.4	0.09

Accuracy:

Accuracy of the method was determined by recovery experiments. To the formulation, the reference standards of the drug were added at the level of 80%, 100%, 120%. The recovery studies were carried out three times and the percentage recovery and percentage relative standard deviation of the recovery were calculated and shown in **Table 5**.

TABLE 5: RECOVERY REPORT OF PARACETAMOL, PAMABROM AND DICYCLOMINE HYDROCHLORIDE

Drug	Pre analysed conc. taken (µg/mL)	Recovery Level	Amount of Drug Added (µg/mL)	Amount of Drug Found (µg/ml) Mean± S.D (n=6)	% Recovery	Acceptance criteria
PARA	26	80%	20.8	46.72 ± 0.309	98.91	98-102%
		100%	26.0	52.05 ± 0.203	100.12	98-102%
		120%	31.2	57.15 ± 0.08	99.87	98-102%
PAMA	2	80%	1.6	3.24±0.03	98.72	98-102%
		100%	2.0	3.98±0.04	99.01	98-102%
		120%	2.4	4.37±0.04	98.75	98-102%
		80%	0.64	1.45±0.015	101.56	98-102%
		100%	0.8	1.59±0.005	98.75	98-102%
DH	0.8	120%	0.96	1.78±0.015	101.04	98-102%

Precision:

Intermediate precision:

For Intraday studies the average RSD of Paracetamol was found to be 0.380, for Pamabrom was found to be 1.061, and for Dicyclomine Hydrochloride it was found to be 0.698. For Inter day studies the average RSD of Paracetamol was found to be 0.281, for Pamabrom was found to be 0.950, and for Dicyclomine Hydrochloride it was found to be 1.304. These results are shown Table 6.

TABLE 6: INTRA-DAY AND INTER-DAY PRECISION DATA FOR PARACETAMOL, PAMABROM AND DICYCLOMINE HCL

S. No.	Analyte	Conc. µg/mL	Intraday (n=3) Peak area mean± S.D (n=3)	Interday (n=3) Peak area mean ± S.D (n=3)	%RSD (Intraday)	% RSD (Interday)
1.	Paracetamol	13	3380742 ± 7317	3645643 ± 11163	0.216	0.306
		39	4964145 ± 10635	5105679 ± 10569	0.214	0.207
		65	6401258 ± 45512	6641256 ±22090	0.710	0.332
2.	Pamabrom	1	118452 ± 2023	116385 ± 1041	1.707	0.891
		3	162152 ± 1665	152946 ± 2090	1.026	1.366
		5	325485 ± 1492	339425 ± 2020	0.45	0.595
3.	Dicyclomine Hcl	250	193015 ± 809	263145 ±1630	0.412	1.613
		750	442494 ± 6210	562148 ± 3379	1.405	0.601
		1250	894126 ± 2488	1024527 ± 17424	0.278	1.700

Method precision:

The RSD of peak areas of three replicate injections for three different standard concentrations was calculated. The average RSD of Paracetamol was found to be 0.044, for Pamabrom was found to be 0.147, and for Dicyclomine Hydrochloride it was found to be 0.044. These results are shown **Table 7.**

Method Precision: (Reproducibility):

TABLE 7: METHOD PRECISION DATA

Sample No.	Concentration (µg/mL)			Peak area (n=6)			Rt (min)		
	PARA	PAMA	DH	PARA	PAMA	DH	PARA	PAMA	DH
1.	26	2	500	4771101	136553	429432	3.07	4.17	5.24
2.	26	2	500	4771201	136458	429521	3.08	4.18	5.26
3.	26	2	500	4772151	136412	429754	3.04	4.12	5.22
4.	26	2	500	4776582	136124	429546	3.09	4.20	5.21
5.	26	2	500	4774517	136742	429412	3.08	4.17	5.22
6.	26	2	500	4775435	136459	429175	3.09	4.20	5.27
		Mean ± SD		4773497 ± 2133	136458 ±201	429473 ±190	3.07 ± 0.018	4.18 ± 0.029	5.23 ±0.024
		% RSD		0.044	0.147	0.044	0.586	0.693	0.458

LOD and LOQ:

LOD and LOQ were calculated from the formula 3.3 x (σ/S) and 10 x (σ/S), respectively where, σ is standard deviation of intercept and S is the mean of slope. The LOD and LOQ can also be determined by S/N. The value for LOD should be 3-5 while for LOQ 10-15. Results were shown in **Table 8.**

TABLE 8: LOD AND LOQ DATA FOR PARACETAMOL, PAMABROM AND DICYCLOMINE HCL

S. No.	Parameter	Paracetamol	Pamabrom	Dicyclomine Hydrochloride
1	LOD	0.416	0.097	3.493
2	LOQ	1.261	0.295	10.586

Robustness:

Robustness of the method was demonstrated by analyzing three different standard concentrations using the same optimized chromatographic conditions to give unaffected results for small deliberate changes in system parameters and method parameters. The changes were (a) using flow rate 0.8 and 1.2 ml/min; (b) change in volume fraction of methanol, 80% and 85% instead of 83%; (c) change in the pH of mobile phase from 3.0 to 3.5. Results were shown in **Table 9.**

TABLE 9: ROBUSTNESS DATA FOR PARACETAMOL, PAMABROM AND DICYCLOMINE HYDROCHLORIDE

Parameter	Condition	Rt (in min)			Area (n=3)			% Assay			Remark
		PARA	PAMA	DH	PARA	PAMA	DH	PARA	PAMA	DH	
Optimised	1ml/min, 83:17, pH 3.0	3.08	4.22	5.31	4709620	151526	381436	100.5	100.5	101.4	-----
Flow rate	0.8 ml/min	3.13	4.24	5.39	5553854	145995	422756	118.5	96.8	112.3	Not robust
	1.2 ml/min	3.01	4.12	5.24	4771101	136553	429432	101.8	90.5	114.5	Not robust
Mobile phase	80:20	3.09	4.11	5.26	4935612	144059	345207	105.3	95.5	91.7	Not robust
(MeOH :H_2O)	85 : 15	3.09	4.20	5.27	4964017	178152	432494	105.9	118.1	114.9	Not robust
PH	3.5	3.09	4.11	5.26	4381670	167448	241247	93.05	111.0	64.13	Not robust

TABLE 10: SYSTEM SUITABILITY PARAMETERS FOR PARACETAMOL, PAMABROM AND DICYCLOMINE HCL

Parameter	Values obtained (n = 6)			Acceptan Criteria
	Paracetamol	Pamabrom	Dicyclomine Hydrochloride	
Plate Count	4352 ± 29	5295 ± 31	2754 ± 42	> 2000
Tailing Factor	1.375±0.015	1.166±0.005	1.600±0.004	< 2.0
Capacity factor	0.232	0.688	1.124	< 2
HETP	0.05744	0.04721	0.09077	----
R_t (min)	3.08	4.22	5.31	----

SUMMARY AND CONCLUSION: Paracetamol, Pamabrom and Dicyclomine HCL were determined simultaneously by reverse phase HPLC using Methanol: Water (1%TFA) (83:17), 0.4%TEA and pH adjuseted to 3.0 with ammonia as Mobile phase and C_{18} column (250×4.6mm×5µ) as a stationary phase. Detection was carried out PDA detector at 221nm. After development of the method, it was validated as per ICH Guidelines. The specificity of method illustrates that no interference of the sample peaks with blank and degradants, all the peaks showed good resolution. This illustrates that commonly used excipients and additives present in the pharmaceutical formulations were not interfering in the proposed method. The precision was found to be within the limits.

From the linearity table, it was found that, the drug obeys beer's law. The calibration plot for Paracetamol was observed as linear in the range of 6.5-39µg/mL with R^2 value of 0.999. The calibration plot for Pamabrom was linear in the range of 0.5-3µg/mL with R^2 value of 0.999. The calibration plot for Dicyclomine HCL was linear in the range of 100-600µg/mL with R^2 value of 0.998. Accuracy was performed as recovery study and results showed the recovery value of pure drug from the solution, between 98-102%, which indicates the method, is accurate. Hence this method was better for Pharmaceutical formulations analysis. Finally the data from solution stability, both standard and sample solution was stable up to 24 hours, with %RSD less than 2.

ACKNOWLEDGMENT: The authors wish to thank the Department of Pharmaceutical analysis and quality assurance, Raghavendra Institute of Pharmaceutical Education and Research, Anantapur, India for providing research facility to carry out the work.

REFERENCES:

1. Indian pharmacopoeia: Govt. Of India, Ministry of Health and Family welfare, controller and publication: Delhi, 2010; Volume 3, page no:1859-1861
2. http://www.indianpharmacopoeia.in
3. The United States Pharmacopoeia: Monograph development -cough, cold and analgesic,USP29-NF24 page 18,
4. http://pubchem.ncbi.nlm.nih.gov/compound/pamabrom
5. http://pubchem.ncbi.nlm.nih.gov/compound/dicyclomine
6. Bambhrolia, Satish, and S. J. Rajput. Simultaneous estimation of Paracetamol and Pamabrom in bulk drugs and in pharmaceutical formulation by spectrophotometry. International Journal of Chemtech Research, 2013; 5: 1802-1807
7. Shah, Dhruvi, Bhagirath Patel, and Ankita Bhavsar. Stability Indicating Rp-Hplc Method for Simultaneous Estimation of Paracetamol and Pamabrom in Bulk and Combined Pharmaceutical Dosage Form. World journal of pharmacy and pharmaceutical science, 2014; 3: 1009-1020
8. Abdelaleem, Eglal A., et al. HPTLC and RP-HPLC methods for simultaneous determination of Paracetamol and Pamabrom in presence of their potential

impurities. Journal of pharmaceutical and biomedical analysis, 2015; 114: 22-27.
9. http://www.ncbi.nlm.nih.gov/pubmed/26001162
10. Li-hua, Zhou Ling-jie GU, and Wang Yi-jian Liang Jian-ying. Determination of two constituents in compound acetaminophen and pamabrom tablets in human plasma by high performance liquid chromatography. Chinese Journal of New Drugs and Clinical Remedies, 2007; 3: 008.
11. Raghunath, Malathi, et al. Validated UV Spectrophotometric Method for Estimation of Fixed Dose Combination of Ciprofloxacin, Tinidazole and Dicyclomine in Bulk and Tablet Formulation. World journal of pharmacy and pharmaceutical science, 2014; 4: 1117-1127.
12. Prajapati, Dipal, and Hasumati Raj. Simultaneous estimation of mefenamic acid and dicyclomine hydrochloride by spectroscopic methods. International Journal of Pharmaceutical Science and Research, 2012; 3:3766-76.
13. Prajapati, D., & Raj, H. Simultaneous estimation of mefenamic acid and dicyclomine hydrochloride by RP-HPLC method. International Journal of Pharma and Biosciences, 2012; 3: 611-25.
14. Neelima, K., and Y. Rajendra Prasad. Analytical Method Development and Validation for Simultaneous Estimation of Dextropropoxyphene Hcl, Dicyclomine and Paracetamol in Bulk and Capsule Formulation by RP-HPLC. Indo American Journal of Pharmaceutical Research, 2013; 3:1225-1232.
15. Keer, Amey R., et al. Application of High performance thin layer chromatography-densitometry for the determination of Dicyclomine HCL in bulk drug and injection formulation. Scholars Research Library 2011; 3: 549-556.
16. Shah, Dimal A., et al. High-performance thin-layer chromatographic method for the estimation of Paracetamol, Dicyclomine Hydrochloride, and Mefenamic acid in combined tablet dosage form. JPC-Journal of Planar Chromatography-Modern TLC, 2014; 27: 52-57.
17. ICH (International Conference on Harmonization) – Guidelines Q2A, Validation of Analytical Procedures: Definition, terminology and methodology; Geneva, Switzerland. Journal of AOAC International 1995,1996

How to cite this article:
Sharma H, Vishakha K, Kumar KV and Bhatta HP: Validated RP-HPLC method for simultaneous estimation of paracetamol, pamabrom and dicyclomine, hydrochloride in bulk and pharmaceutical dosage form. Int J Pharm Sci Res 2016; 7(1): 316-24.doi: 10.13040/IJPSR.0975-8232.7 (1).316-24.