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Development and Validation of Simultaneous Estimation of Paracetamol and Pamabrom in Bulk and Combined Pharmaceutical Dosage Form by RP-HPLC.

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Research Article

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ABSTRACT

A simple, rapid and precise Reverse Phase High Performance Liquid Chromatography (RP-HPLC) method was developed for simultaneous estimation of paracetamol and pamabrom in bulk and combined pharmaceutical dosage form by using BDS hypersil C_{18} column. The sample was analysed using phosphate buffer (pH-4): methanol (65:35v/v) as a mobile phase at a flow rate of 1.0 ml/min and detected at 270 nm. The retention time for paracetamol and pamabrom was found to be 4.04 min and 7.36 min respectively. The linearity of developed method was achieved in the range of 32.5-97.5µg/ml for paracetamol and 2.5-7.5µg/ml for pamabrom. The method was validated in terms of accuracy, precision, linearity, limit of detection, limit of quantitation and robustness as per International Conference Harmonization (ICH) guidelines.

INTRODUCTION

Paracetamol (N- 4-hydroxyphenyl acetamide) is an Analgesic-antipyretic drugs with poor Anti-inflammatory action and belongs to para-aminophenol derivative categories of NSAIDs. The main mechanism of action of paracetamol is considered to be the inhibition of cyclooxygenase (COX). Recent findings suggest that it is highly selective for COX-2 because of its selectivity for COX-2 it does not significantly inhibit the production of the pro-clotting thromboxanes [1].

Pamabrom is Diuretic drug that increases the rate of urine flow; however, clinically useful diuretics also increase the rate of excretion of Na^+ (natriuresis) and of an accompanying anion, usually Cl^- [2].

Paracetamol and pamabrom tablet is used for premenstrual dysphoric disorder, back pain, premenstrual syndrome [3].

Analytical method development is defined as development, revision and application of validated, standardized and official methods of analysis [4]. Method validation is the process of documenting or proving that selected method provides analytical data for the intended use. Method is validated by using parameters like accuracy, precision, linearity, limit of detection, limit of quantitation, system suitability, selectivity and specificity [5,6].

Paracetamol is an official in Indian Pharmacopoeia, British Pharmacopoeia and United Pharmacopoeia. Pamabrom is an official in United Pharmacopoeia. Very few analytical methods have been reported for estimation of paracetamol and pamabrom in combined pharmaceutical dosage form.

Combined formulation of paracetamol and pamabrom is approved by Central Drugs Standard Control Organization (CDSCO) dated on 07/12/2011 [7].

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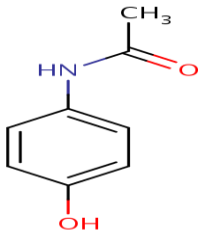
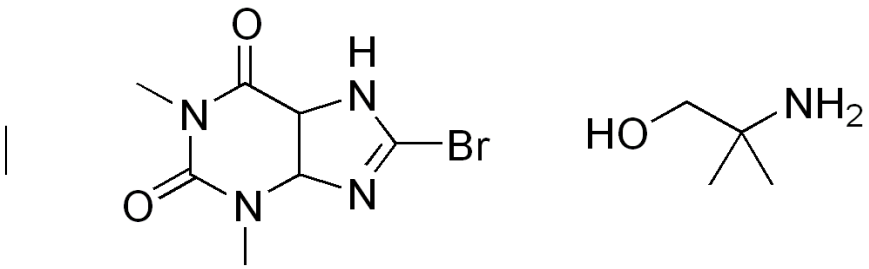
Figure 1: Chemical structure of paracetamol [6]



N-(4-hydroxyphenyl) acetamide

Figure 2: Chemical structure of pamabrom[9]



1:1 mixture of 2-amino-2-methyl-1-propanol and 8-bromotheophyllinate

MATERIALS AND METHODS [10-16]

Chemicals and solvents

HPLC grade water, methanol and acetonitrile were used for preparing mobile phase. Potassium di hydrogen phosphate (AR grade) and ortho phosphoric acid (AR grade) were used for preparing the buffer. Pure sample of paracetamol and pamabrom were gifted by Astron chemicals, Ahmadabad and Ami Life sciences Pvt Ltd, Vadodara. MENSODOL™ TAB (paracetamol-325mg, pamabrom-25mg) was gifted from Anthus Pharmaceuticals, Chennai.

HPLC instrumentation and chromatographic conditions

The HPLC system was SPD-20AT, Shimadzu consisting of pump LC-20AT, detector SPD-20AT, column BDS hypersil C_{18}, 250mm × 4.6mm, 5µ(particle size), Thermo scientific, injector rheodyne injector (20 µl capacity) and syringe hamilton (25 µl). Data were processed using chromatographic software Spinchrom.

A freshly prepared mixture of phosphate buffer (pH-4): methanol (65:35v/v) used as the mobile phase. Mobile phase was filtered through a 0.45µm membrane filter and sonicated before use. The flow rate of the mobile phase was maintained at 1.0 ml/min.

Preparation of mobile phase

Mobile phase was prepared by mixing 350 ml of methanol and 650 ml of phosphate buffer (pH: 4.0), filtered through 0.45µm whatman filter and sonicated for 10 min.

Preparation of standard solutions

Reference standard of PCM 65 mg and PAM 50 mg were transferred to 10 ml volumetric flask separately and dissolved in methanol. Flasks were shaken for 20 min and the volume was made up to the mark with mobile phase to obtain standard stock solution of PCM (650µg/ml) and PAM (500µg/ml). Stock solutions were filtered through a 0.45µm whatman filter. 1ml PAM solution was withdrawn to 10 ml volumetric flask and diluted up to mark with mobile phase to get working standard solution of PAM (50µg/ml). The working standard solutions of PCM and PAM were prepared from suitable aliquots of stock solutions.

Preparation of sample solutions

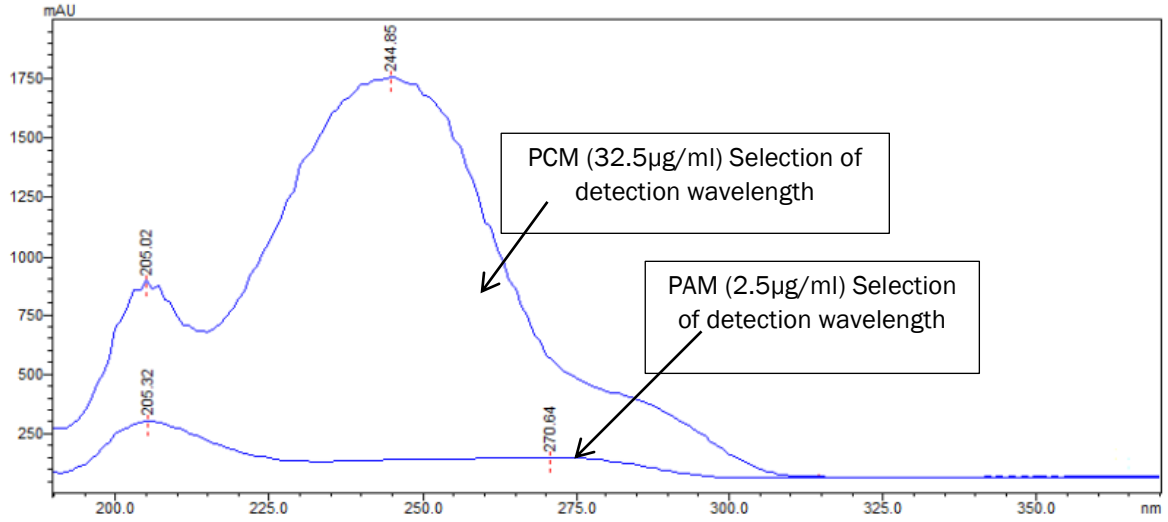
Twenty tablets were weighed, their average weight was determined and finally powdered. An accurately weighed tablet powder equivalent to 650 mg of PCM and 50 mg PAM was then transferred to 10 ml volumetric

flask containing 5 ml methanol and sonicated for 20 min. The solution was filtered through whatman filter paper and the volume was adjusted up to the mark with mobile phase. This solution is expected to contain 650µg/ml PCM and 50µg/ml PAM. This solution (0.5 ml) was transferred in to a 10 ml volumetric flask and the volume was adjusted up to mark with mobile phase to get a concentration of PCM (32.5µg/ml) and PAM (2.5µg/ml).

Selection of analytical wavelength

The standard solution of PCM (32.5µg/ml) and PAM (2.5µg/ml) were scanned in the UV region of 200-400 nm using methanol as a blank and the overlain spectra was recorded. 270nm analytical wavelength was selected for estimation of PCM and PAM. (Figure 3).

Figure 3: Selection of detection wavelength (270 nm)



Optimization of HPLC method

The pure drug solutions of PCM (32.5µg/ml) and PAM (2.5µg/ml) were injected individually into HPLC system and allow to run in different mobile phases like methanol, water: methanol, water: acetonitrile, phosphate buffer: methanol and phosphate buffer: acetonitrile were tried in order to find the optimum conditions for the separation of PCM and PAM. It was found that mobile phase containing phosphate buffer (pH-4): methanol (65:35v/v) at a flow rate of 1.0 ml/min with detection wavelength 270 nm gave satisfactory results with sharp, well defined and resolved peaks with minimum tailing as compared to other mobile phases. Under these conditions the retention times were typically 4.04 min for PCM and 7.367 min for PAM (Figure 4) and optimized chromatographic conditions described in (Table 1).

Figure 4: Chromatogram of standard solution for PCM (tR 4.04 min) and PAM (tR 7.367 min).

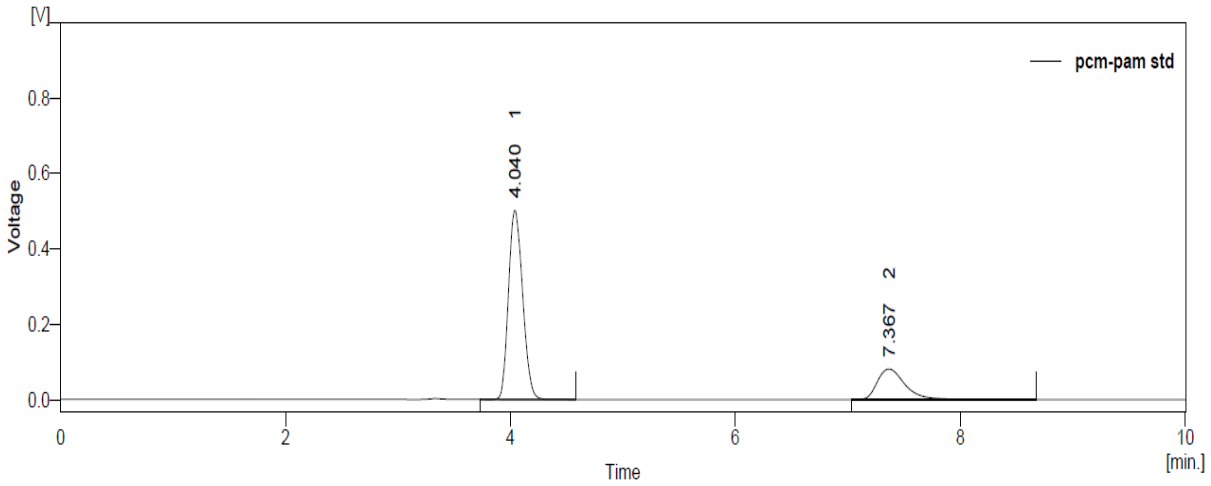


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Table 1: Optimized chromatographic conditions for simultaneous estimation of PCM and PAM

Parameters	Conditions
Mobile phase	Phosphate buffer (pH 4): Methanol (65:35 v/v)
Stationary phase	BDS hypersil C_{18}, 250mm × 4.6mm, 5µ (particle size)
Flow rate (ml/min.)	1
Run time (min.)	10
Volume of injection (µl)	20.0
Detection wavelength (nm)	270
Retention time (min.)	PCM : 4.04 PAM : 7.36

Validation of the method

Validation of the optimized RP-HPLC method was carried out with respect to the following parameters.

Linearity and range

Linearity responses for PCM and PAM were assessed in the concentration range 32.5-97.5µg/ml and 2.5-7.5µg/ml of standard solutions, respectively.

Sensitivity

The sensitivity measurement of PAM and PCM by the use of proposed method was estimated in terms of Limit of Detection (LOD) and Limit of Quantitation (LOQ). The LOD and LOQ were calculated using following equations.

$$LOD = 3.3 \times \sigma/S$$
$$LOQ = 10 \times \sigma/S$$

Where,
σ = the standard deviation of the response
S = slope of the calibration curve.

Precision

The precision of the method was verified by repeatability, interday and intraday precision. Repeatability study was performed by analysis of three different concentrations of the drug in six replicates on the same day. Intraday precision was determined by analysing sample solutions at different time intervals on the same day and on different day for interday precision.

Accuracy

To the pre-analysed sample, a known amount of standard solution of pure drug (PCM and PAM) were spiked at three different levels. These study was carried out at 80%, 100% and 120% level.

Robustness

Robustness was performed by deliberately changing the chromatographic conditions. The important parameter to be studied was the resolution factor between two peaks. The robustness was checked by changing following parameters one by one:

Change in the ratio of mobile phase by ± 0.2 ml [phosphate buffer (pH 4): methanol (67:33v/v) and phosphate buffer (pH 4): methanol (63:37v/v)]

Change in flow rate by ± 0.2 ml/minute (0.8 ml/min. and 1.2 ml/min.) After each change, sample solution was injected and % assay with system suitability parameters were checked.

Change in pH of mobile phase by ± 0.2 pH [phosphate buffer (pH 4.2): methanol (65:35v/v) and phosphate buffer (pH 3.8): methanol (65:35v/v)].

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System suitability parameters

To check System suitability, number of theoretical plates, resolution, retention time and tailing factor were determined [17].

Quantitative determination in pharmaceutical formulation

Twenty tablets were weighed, their average weight was determined and finally powdered. An accurately weighed tablet powder equivalent to 650 mg of PCM and 50 mg PAM was then transferred to 10 ml volumetric flask containing 5 ml methanol and sonicated for 20 min. The solution was filtered through 0.45µm filter and the volume was adjusted up to mark with methanol. From the above solution 1 ml was taken into a 10 ml volumetric flask and the volume was adjusted up to mark with methanol to get a final concentration of PCM (65µg/ml) and PAM (5µg/ml). 20 µl of the test solution was injected and chromatogram was recorded for the same and the amount of the drug was calculated [18].

RESULTS AND DISCUSSION

The results of method development and validation studies on simultaneous estimation of PCM and PAM in the current study involving phosphate buffer (pH-4) and methanol (65:35v/v) as the mobile phase for RP-HPLC are given below.

Method development

PCM and PAM were completely separated on C_{18} column by RP-HPLC using the iso-cratic elution of phosphate buffer and methanol as mobile phase. When the methanol percentage was reduced starting from 80% by a decrement of every 5%, broadening, fronting and tailing of peaks were observed. As a result of decrease in the percentage of methanol and using phosphate buffer (pH-4) a sharp pointed and well separated peak was observed. As methanol concentration gradually decreases the peak broadening, fronting and tailing were remarkably reduced. Eventually proper resolution was achieved at flow rate of 1ml/min and using phosphate buffer (pH-4): methanol (65:35 v/v) as the mobile phase for RP-HPLC. (Figure 4) (Table 1)

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.[6] The drug response was linear (R^2 = 0.9995 for PCM and 0.9995 for PAM) over the concentration range between 32.5-97.5µg/ml for PCM and 2.5-7.5µg/ml for PAM. The result is shown in (Table 2).

Table 2: Linearity data of PCM and PAM by proposed method

PCM		PAM	
Concentration (µg/ml)	Mean Peak Area (n=3)	Concentration (µg/ml)	Mean Peak Area (n=3)
32.5	2171.289	2.5	682.424
48.75	3205.757	3.75	1015.305
65	4383.295	5	1388.885
81.25	5397.235	6.25	1710.713
97.5	6567.476	7.5	2081.916
Correlation coefficient : 0.9995 Intercept : 48.53 Slope : 67.59 Regression Equation : y=67.593x-48.53 LOD (µg/ml) : 2.26 LOQ (µg/ml) : 6.86		Correlation coefficient : 0.9995 Intercept : 21.90 Slope : 279.55 Regression Equation : y=279.55x-21.908 LOD (µg/ml) : 0.16 LOQ (µg/ml) : 0.55	

Sensitivity

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy [6]. The LOD and LOQ were calculated by respective equations. The LOD values were found to be 2.26 and 6.86µg/ml for PCM and PAM respectively. The LOQ values were found to be and 0.16 and 0.55µg/ml for PCM and PAM respectively. (Table 2)

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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 [6]. The results of the repeatability, intra-day and inter-day precision experiments are shown respectively as given in (Table 3) and (Table 4). The developed method was found to be precise as the RSD values for repeatability of intra-day and interday precision studies were < 2 %.

Table 3: Repeatability study of PCM and PAM

Concentration	PCM (65µg/ml)	PAM (5µg/ml)
Area	4365.93	1383.33
	4374.54	1386.11
	4310.80	1388.88
	4392.08	1333.75
	4370.31	1384.71
	4378.92	1387.51
Mean	4365.43	1377.38
± SD	28.22	21.46
%RSD	0.64	1.55

Table 4: Intra-day and inter-day study of PCM & PAM

Drug	Concentration (µg/ml)	Intra-day area mean (n=3) ± SD	%RSD	Inter-day area mean (n=3) ± SD	%RSD
PCM	32.5	2154.009± 17.03	0.79	2152.55±13.78	0.64
	65	4353.897± 25.29	0.58	4349.53± 21.07	0.48
	97.5	6517.281± 20.10	0.30	6514.32± 23.33	0.35
PAM	2.5	673.9233± 9.623	1.42	674.284± 7.07	1.04
	5	1373.856± 15.63	1.13	1371.217± 16.21	1.18
	7.5	2057.512± 29.88	1.45	2050.54± 32.81	1.60

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.[6] As shown in (Table 5) and (Table 6), good recoveries of the PCM and PAM in the range from 99 to 101 % were obtained at various added concentrations.

Table 5: Determination of accuracy for PCM

% Level of Recovery	Conc. of sample solution (µg/ml)	Conc. of standard Solution (µg/ml)	Total conc. (µg/ml)	Mean peak area (n=3)	Conc. found (µg/ml) (n=3)	% Recovery mean(n=3)
80	32.5	26	58.5	3912.93	58.6	100.17
100	32.5	32.5	65	4346.82	65.02	100.04
120	32.5	39	71.5	4786.03	71.08	99.41

Table 6: Determination of accuracy for PAM

% Level of Recovery	Conc. of sample solution (µg/ml)	Conc. of standard Solution (µg/ml)	Total conc. (µg/ml)	Mean peak area (n=3)	Conc. found (µg/ml) (n=3)	% Recovery mean (n=3)
80	2.5	2	4.5	1255.55	4.56	101.54
100	2.5	2.5	5	1365.23	4.96	99.24
120	2.5	3	5.5	1508.11	5.47	99.51

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Robustness

The standard deviation of the peak areas was calculated for each parameter and the % RSD was found to be less than 2 %. Result shows low values of % RSD as shown in (Table 7) and signify the robustness of the method.

Table 7: Robustness study for PCM and PAM

Parameters	Normal Condition	Change in Condition	Drug	Conc. (µg/ml)	Mean area (n=3) ± SD	% RSD	Retention time (min)	Theoretical plates
Mobile phase ratio (phosphate buffer:methanol 65:35 v/v)	65:35	63:37	PCM	65	4471.36±22.426	0.50	4.14	4490
			PAM	5	1346.854±16.639	1.234	7.55	4482
		67:33	PCM	65	4265.50±43.522	1.02	3.94	4388
			PAM	5	1406.603±26.65	1.894	7.18	4454
Change in flow rate	1.0 ml/min	0.8 ml/min	PCM	65	4536.65±31.975	0.70	4.18	4507
			PAM	5	1425.411±26.147	1.834	7.62	4458
		1.2 ml/min	PCM	65	4273.961±39.53	0.924	3.94	4387
			PAM	5	1356.969±8.286	0.61	7.18	4454
Change in pH	4	4.2	PCM	65	4186.131±31.242	0.746	3.86	4643
			PAM	5	1317.18±13.663	1.037	7.02	4468
		3.8	PCM	65	4486.737±42.149	0.939	4.14	4490
			PAM	5	1416.934±15.44	1.09	7.55	4482

System suitability studies

System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such.[6] The column efficiency, resolution and peak asymmetry were calculated for the standard solutions and the results are mention in (Table 8) [17].

Table 8: System suitability parameters

Parameters	PCM	PAM
Theoretical plates	4401	4447
Retention time (min)	4.04 ± 0.008	7.36 ± 0.012
Tailing factor	1.273	1.632
Resolution	9.707	

Quantitative determination in pharmaceutical formulation

When dosage form was analysed, PCM and PAM gave sharp and well defined peaks at retention time 4.04 min. and 7.36 min. respectively, when scanned at 270 nm. Assay result of marketed formulations is shown in (Table 9) [18].

Table 9: Quantitative determination in pharmaceutical formulation

Parameters	MENSODOL™ TAB	
	PCM	PAM
Actual Concentration (µg/ml)	65	5
Concentration Obtained (µg/ml)	62.89	4.97
% Assay	96.75	99.43
%RSD	0.57	1.81
Limit	90-110 %	90-110 %

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CONCLUSION

Development and validation of RP-HPLC method was found to be simple, accurate, precise and economical. These methods can be applied for routine quantitative analysis of paracetamol and pamabraom in bulk and pharmaceutical dosage forms.

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REFERENCES

1. Tripathi KD. Nonsteroid Antiinflammtory Drugs and Antipyretic Analgesics. In: Tripathi M, Editor. Essentials of medical pharmacology. New Delhi, p. 184-201; 2008.
2. Tripathi KD. Diuretics. In: Tripathi M, Editor. Essentials of medical pharmacology. New Delhi, p. 561-573; 2008.
3. http://www.drugs.com/search.php?searchterm=paracetamol+and+pamabrom+adavntages
4. http://www.pharmainfo.net/reviews/introduction-analytical-method-developmentpharmaceutical-formulations
5. ICH Q2B (2005) Validation of Analytical Procedure: Methodology, International Conference on Harmonization, IFPMA, Geneva, Switzerland.
6. ICH Q2 (R1) (2005) Validation of Analytical Procedure: Text & Methodology, International Conference on Harmonization, IFPMA, Geneva, Switzerland.
7. http://www.cdsco.nic.in/writereaddata/FDC_approved_dcgi%281%29_2013.pdf
8. http://www.drugbank.ca/drugs/DB00316#properties
9. http://www.chemnet.com/India/Products/pamabrom/Suppliers-0-0.html
10. Beckett AH, Stenlake, JB. Practical Pharmaceutical Chemistry Part-II. In:CBS Publishers and distributors: India, p. 275-337; 1997.
11. Lough WJ. and Wainer IW. HPLC fundamental principles and practices, Blackie Academic and professional, p. 52-67; 1991.
12. Phazna TA, Aravind S, Srikanth S, Sivaramaiah N, Smita CP, Venkateshwara R. Method Development and Validation of Paracetamol drug by RP-HPLC. J Med Allied Sci. 2013;3(1):08-14.
13. Roshni JH, Vibhuti C, Ashwin A. A validated rp- hplc method for the simultaneous estimation of paracetamol and dicyclomine hydrochloride in pharmaceutical formulations. Int J Pharm Sci Res. 2013; 4(6):2400-2405.
14. Deepak K, Pratibha P, Chandel HS, Abhay K, Nilesh J. Development and validation of reversed phase–high-performance liquid chromatography method for determination of paracetamol and lornoxicam in tablet dosage form. Pharma methods. 2011 Jan [cited 2011 March];2(1): [about 1 p.]. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3658034/
15. Karunakaran K, Navaneethan G, Elango KP. Development and validation of a stability indicating RP-HPLC method for simultaneous determination of paracetamol, tramodol HCL and domperidone in a combined dosage form. TJPR. 2012;11(1): 99-106.
16. Samson IS, Krishna CK, Gowri SD, Vijayalakshmi A. Method development and validation for simultaneous determination a multiple drug dosage form of paracetamol, orphenedrine, ibuprofen by RP-HPLC. JGTPS. 2013;4(3):1153-1162.
17. http://www.sge.com/uploads/db/Rl/dbr/y8NOG4YTZB2MDHivmfg/Ta-0010-h.pdf
18. Indian Pharmacopoeia, government of India, ministry of health and welfare,volume-III,Gaziabad,2007,902.