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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF PROMETHAZINE HYDROCHLORIDE AND PARACETAMOL IN COMBINED LIQUID FORMULATION

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ABSTRACT

A simple, efficient, and reproducible RP-HPLC method for the simultaneous determination of Promethazine hydrochloride (PMZ) and Paracetamol (PCM) in pharmaceutical dosage form has been developed and validated. The separation was carried out on Hyperchrom ODS-BP (4.6 mm X 250 mm, 5 µm) column using Methanol: Water with 1% TEA in the ratio of 30:70 v/v as eluent. The flow rate was 1 ml/min and effluent was detected at 250 nm. The retention time of Promethazine hydrochloride and Paracetamol were 3.10 and 1.72 min. respectively. The linear dynamic range was 2-10 µg/ml for Promethazine hydrochloride and 50-250 µg/ml Paracetamol, respectively. Percentage recoveries for Promethazine hydrochloride and Paracetamol were 99.00-100.33% and 99.56-101.28% respectively. All the analytical validation parameters were determined and found in the limit as per ICH guidelines, which indicates the validity of the method. The developed method is also found to be precise and robust for the simultaneous determination of Promethazine hydrochloride and Paracetamol in liquid dosage forms.

KEYWORDS

Promethazine hydrochloride, Paracetamol and High performance liquid chromatography.

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INTRODUCTION

Promethazine HCl (PMZ), is chemically10- [2-(Dimethylamino) propyl] phenothiazine monohydrochloride (Figure No.1 (a)). It is Antipruritics, Anti-Allergic Agents, Histamine H_1 Antagonists, Phenothiazine Derivatives. It acts primarily as a strong antagonist of the H_1 receptor (antihistamine) and a moderate mACh receptor antagonist (anticholinergic). Like other H_1-antagonists, promethazine competes with free histamine for binding at H1-receptor sites in the GI

tract, uterus, large blood vessels, and bronchial muscle. The relief of nausea appears to be related to central anticholinergic actions and may implicate activity on the medullary chemoreceptor trigger zone. Blocks effects but not release of histamine and exerts strong alpha-adrenergic effect. Also inhibits chemoreceptor trigger zone in medulla and alters dopamine effects by indirectly reducing reticular stimulation in CNS[1-2]. Literature survey revealed that various, UV spectroscopy[3-8], Chromatographic[9-19] methods and HPTLC method[20] have been reported for quantitative estimation of PMZ in pharmaceutical formulation and biological fluids individually or in combination with other drugs.

Paracetamol (acetaminophen, *N*-acetyl-*p*-amino-phenol) (Figure No.1 (b)) is a safe and effective analgesic and antipyretic agent although its anti-inflammatory effect is weak[21]. The antipyretic properties of paracetamol are likely due to direct effects on the heat-regulating centres of the hypothalamus resulting in peripheral vasodilation, sweating and hence heat dissipation[22]. Literature survey revealed that various; UV spectroscopy [23-30], HPLC[31-37] methods and HPTLC method[38-42] have been reported for quantitative estimation of PMZ in pharmaceutical formulation and biological fluids individually or in combination with other drugs. In combination of Promethazine HCl (PMZ) and Paracetamol (PCM) UV spectroscopy[43], HPLC[44] method has been reported.

To the best of our knowledge, there is no published HPTLC method for this combination. So, the present paper describes a simple, accurate and precise method for simultaneous estimation of PMZ and PCM in combined Pharmaceutical formulation by HPTLC method. The developed method was validated in accordance with ICH Guidelines[45] and successfully employed for the assay of PMZ and PCM in their combined pharmaceutical formulation.

MATERIALS AND METHOD

Materials

PMZ reference standard was procured from Cliantha Research Ltd. Ahmedabad, Gujarat, India, as gift sample for research purpose and PCM reference standard was procured from Vital Pharmaceuticals Pvt. Ltd., V.V Nagar G.I.D.C., Gujarat, India. PHENA-P Syrup containing PMZ 5 mg and PCM 125 mg per 5 ml was procured from local market. The HPLC System in Analytical technologies limited, Pump: P2230 plus HPLC pump, Injector: Rhenodyne valve with 20µl fixed loop Detector: UV 2230 plus detector Software: Analchrom 2006. Column: Hyperchrom ODS-BP (4.6 mm X 250mm) Mumbai. HPLC grade water, methanol, Triethyl amine was purchased from Rankem, Ahmedabad, Gujarat.

Instrument and Experimental Conditions

Column Hyperchrom ODS-BP (4.6 mm X 250mm), 5 µm) was used for separation. The mobile containing Methanol: Water with 1% TEA in the ratio of 30:70 v/v was delivered at a flow rate of 1.0 ml/min with detection at wavelength 250 nm. The Injection volume was 20 µl and the analysis was performed at ambient temperature.

Standard stock solution

Based upon trial and error at laboratory scale finally it was decided to prepare stock solution of 100 µg/ml and 1000 µg/ml of PMZ and PCM respectively.

Analysis of Promethazine hydrochloride and Paracetamol in combined liquid dosage form

Accurately measured 5 ml of syrup sample solution taken and transferred in to a 25 ml volumetric flask. After addition of 10 ml of methanol, flask was sonicated for 15 min. volume was made up to the mark with diluent to give a solution containing 200 µg/ml of PMZ and 5000 µg/ml of PCM. Solution thereafter was filtered through 0.45 µm filter. First few ml of filtrate was discarded to saturate the filter. This filtrate was used for the estimation of PMZ and PCM.

Analytical Method validation

Preparation of calibration curves/ Linearity and range

5 mg of standard PMZ and 125 mg of standard PCM were accurately weighed and transferred to 25 ml volumetric flask and dissolved in 10 ml methanol. The flask was sonicated for 10 min. The flask was shaken and volume was made up to the mark with methanol. From this standard stock the appropriate

aliquots were transferred to volumetric flask of 10 ml capacity. The volume was made up to the mark with mobile phase to give solution containing 2, 4, 6, 8 and 10 µg/ml of PMZ; 50, 100, 150, 200 and 250 µg/ml of PCM. The mixed standard solution was chromatographed for 7 minutes using mobile phase at a flow rate of 1.0 ml/min

Procedure was repeated for further 5 times (Total n=6) (Figure No.2). Finally mean area was plotted against concentration (µg/ml) (Table No.1 and Table No.2).

Accuracy studies (Recovery)

Accuracy studies were performed by spiking test solution with standard solution. Accuracy studies were performed at spiking level of 50, 100 and 150% of target concentration. Here sample solution containing 200 µg/ml of PMZ and 5000 µg/ml of PCM was prepared from syrup formulation. Resulting solution was filtered and 0.2 ml of solution was transferred to each four 10 ml volumetric flask. Now from standard stock solution of 100 µg/ml of PMZ and 1000 µg/ml of PCM various aliquots were transferred to each 10 ml volumetric flask. Volume was made upto mark with methanol. Procedure was repeated for further 2 times and mean recovery for each level was calculated (n=3) (Table No.3).

Precision

Intraday precision

The result of intraday precision for PMZ and PCM was found to be C.V. 1.58-1.71 for intraday (n=3) for PMZ and C.V. 1.18-1.61 for intraday (n=3) for PCM (Table No.4).

Interday precision

The result of interday precision for PMZ and PCM was found to C.V. 1.55 - 1.71 for PMZ and C.V. 1.01-1.58 for interday (n=3) for PCM respectively (Table No.4 and Table No.5).

Repeatability

Standard solution mixture containing PMZ (2-10 µg/ml) and PCM (50-250 µg/ml) were prepared and chromatograms were recorded and area was measured and C.V. was calculated. Sample solution of 4 µg/ml PMZ 100 µg/ml PCM was prepared and chromatograms were recorded. Area was measured of the same concentration solution six times and CV was calculated (Table No.6-9).

Specificity and Selectivity

Specificity is a procedure to detect quantitatively the analyte in presence of component that may be expected to be present in the sample matrix, while selectivity is the procedure to detect qualitatively the analyte in presence of components that may be expected to be present in the sample matrix.

Specificity of an analytical method is ability to measure specifically the analyte of interest without interferences from blank and placebo. It was checked for interference from blank.

Preparation of Blank syrup

Accurately weigh 66.7 gm Sucrose was taken and dissolve it in 100 ml distilled water (Aqueous Solution 1) [Syrup I.P.-66.67%]. 5 ml blank syrup was measured and transferred to 25 ml volumetric flask and dissolved in 10 ml methanol. The flask was sonicated for 10 min. The flask was shaken and volume was made up to the mark with methanol. From this solution the 0.2 ml aliquots were transferred to volumetric flask of 10 ml capacity. The volume was made up to the mark with mobile phase. This solution was used for taking chromatogram of blank syrup (Figure No.5).

Preparation of Propyl paraben solution

A 0.05%w/v of Propyl paraben is used as preservative in syrup. 5 ml syrup contains 2.5 mg of Propyl paraben. Accurately weigh 2.5 mg of Propyl paraben was taken in 25 ml volumetric flask and dissolve it in 15 ml HPLC grade Methanol. The flask was sonicated for 10 min. The flask was shaken and volume was made up to the mark with methanol. The above solution was filtered through whatmann filter paper (0.45µ). The 0.2 ml of aliquot was transferred to volumetric flask of 10 ml capacity. Volume was made up to the mark with the methanol to give a solution containing 2 µg/ml of Propyl Paraben (Figure No.6).

Preparation of Methyl Paraben solution

The concentration of Methyl paraben 0.15 %w/v is used as preservative in syrup. 5.0 ml syrup contains 7.5 mg of Methyl paraben. Accurately weigh 7.5 mg of Methyl paraben was taken in 25 ml volumetric

flask and dissolve it in 15 ml HPLC grade Methanol. The flask was sonicated for 10 min. The flask was shaken and volume was made up to the mark with methanol. The above solution was filtered through whatmann filter paper (0.45µ). The 0.2 ml of aliquot was transferred to volumetric flask of 10 ml capacity. Volume was made up to the mark with the methanol to give a solution containing 6 µg/ml of Methyl Paraben (Figure No.7).

Preparation of Caramel I.P. solution

The concentration of Caramel I.P. 0.04 %w/v is used as colouring agent in syrup. 5.0 ml syrup contains 2 mg of Caramel I.P. Accurately weigh 2 mg of Caramel I.P. was taken in 25 ml volumetric flask and dissolve it in 15 ml HPLC grade Methanol. The flask was sonicated for 10 min. The flask was shaken and volume was made up to the mark with methanol. The above solution was filtered through whatmann filter paper (0.45µ). The 0.2 ml of aliquot was transferred to volumetric flask of 10 ml capacity. Volume was made up to the mark with the methanol to give a solution containing 1.6 µg/ml of Caramel. Accurately weigh 66.7 gm Sucrose was taken and dissolve it in 100 ml distilled Water (Aqueous Solution 1) [Syrup I.P.-66.67%]. From this 75.0 ml of syrup was transferred in 100 ml volumetric flask and dissolve 0.15 gm of Methyl paraben, 0.05 gm of Propyl paraben and 0.04 gm of Caramel in the flask. The flask was sonicated for 10 min. The flask was shaken and volume was made up to the mark with methanol. The above solution was filtered through whatmann filter paper (0.45µ). The 0.2 ml of aliquot was transferred to volumetric flask of 10 ml capacity. Volume was made up to the mark with the methanol (Figure No.8).

Robustness

The sample solution was prepared and then analyzed with change in the analytical conditions like different concentration of TEA (-0.2), different flow rate (+0.1 and -0.1) and different mobile phase composition (+2 and -2).

Limit of Detection and Limit of Quantitation (LOD and LOQ)

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by using the following equations as per International Conference on Harmonization (ICH) guidelines which is based on the calibration curve.

$LOD = 3.3 \times \sigma / S$

$LOQ = 10 \times \sigma / S$

Where σ = the standard deviation of y-intercepts of regression lines

S = Slope of calibration curve.

RESULTS AND DISCUSSION

For the selection of mobile phase, various mobile phase systems were tried for chromatographic separation. Calibration data for PMZ and PCM are shown in (Table No.1 and 2) respectively (Figure No.3 and 4).

Finally, the system containing Methanol: Water with 1% TEA (50:50:0.2 %v/v) gave well resolved peaks of all the three drugs. The average retention time of PMZ and PCM were found to be 3.10 min and 1.72 min respectively.

For the selection of analytical wavelength, the overlain spectra of 10 µg/ml PMZ and 50 µg/ml PCM revealed that at 250 nm the two drugs possess absorbance.

Various system suitability parameters were calculated and are shown in Table No.10. The calibration curves for PMZ and PCM were prepared by plotting area and concentration. The following equations for straight line were obtained for PMZ and PCM:

Linear equation for PMZ, y = 690.88x - 32.902

Linear equation for PCM, y = 60.683x - 882.3

The developed RP-HPLC method was validated. The linear range, correlation coefficient, detection limit and standard deviation for PMZ and PCM by HPLC method are shown in Table No.14.

The LOD for PMZ and PCM was found to be 0.32 µg/ml and 12.62 µg/ml respectively. The LOQ for PMZ and PCM was found to be 0.98 µg/ml and 38.26 µg/ml respectively Accuracy was determined by calculating the recovery (Table No.13). The method was found to be accurate with % recovery 99.34 -100.33 % for PMZ and 99.57 - 101.95 % for PCM (Table No.14).

Precision was calculated as repeatability and intra and interday variation for both the drugs. The method was found to be precise with C.V. 1.58 - 1.71 for intraday (n=3) and C.V. 1.55 – 1.71 for interday (n=3) for PMZ and C.V. 1.18 – 1.61 for intraday (n=3) and C.V. 1.01 – 1.58 for interday (n=3) for PCM (Table No.14).

The method was found to be reproducible (Table No.11-12). The method was also found to be specific as no interference observed when the drugs were estimated in presence of excipients (Table No.9).

The method was also rugged as there was no change in area up to 24 hours of preparation of solution in mobile phase and no significant change in area was found by changing various parameters such as flow rate, concentration of TEA and Mobile phase ratio. Summary of validation parameters is shown in Table No.14.

Formulation was analyzed by the proposed method and assay result of formulation is shown in Table No.15. Optimized chromatographic condition for the estimation of PMZ and PCM by RP-HPLC is shown in Table No.14.

Table No.1: Result of calibration readings for PMZ by HPLC method

S.No	Concentration (µg/ml)	Area Mean ± S.D.	C.V
1	2	1332.91 ± 17.9467	1.35
2	4	2597.28± 20.2738	0.78
3	6	4312.17 ± 53.1621	1.23
4	8	5558.20 ± 102.6740	1.85
5	10	6761.22 ± 107.1873	1.58

Table No.2: Result of calibration readings for PCM by HPLC method

S.No	Concentration (µg/ml)	Area Mean ± S.D.	C.V
1	50	2279.81 ± 30.4914	1.33
2	100	4876.13 ± 58.0489	1.19
3	150	8266.08 ± 131.7724	1.59
4	200	11579.95 ± 107.9071	0.93
5	250	14098 ± 195.2902	1.39

Table No.3: Accuracy studies for PMZ and PCM

S.No	Conc. (µg/ml)	Intraday (Area ± S.D)	C.V.	Interday (Area ± S.D)	C.V.
1	50	2280.50 ±34.5742	1.51	2288.37 ± 23.3183	1.02
2	150	8251.50 ± 132.7967	1.61	8251.70 ± 130.409	1.58
3	250	14126.57 ± 166.83	1.18	14365.97 ± 214.40	1.49

Table No.4: Precision data for PMZ by HPLC method (n = 3 determination)

S.No	Conc. (µg/ml)	Intraday (Area ± S.D)	C.V.	Interday (Area ± S.D)	C.V.
1	50	2280.50 ±34.5742	1.51	2288.37 ± 23.3183	1.02
2	150	8251.50 ± 132.7967	1.61	8251.70 ± 130.409	1.58
3	250	14126.57 ± 166.83	1.18	14365.97 ± 214.40	1.49

Table No.5: Precision data for PCM by HPLC method (n = 3 determination)

S.No	% Level of Recovery	Amount of drug in sample (µg/ml)	Amount of standard added (µg/ml)	Total amount of Drug (µg/ml)	Amount of drug recovered (µg/ml) ± SD	% Recovery ± SD
		PMZ (µg/ml)	PMZ (µg/ml)	PMZ (µg/ml)	PMZ (µg/ml)	% PMZ
1	Unspiked	4	0	4	4.0018 ± 0.0527	-
2	50 %	4	2	6	5.94 ± 0.0528	99.00 ± 0.8803
3	100 %	4	4	8	8.029 ± 0.1135	100.33 ± 1.4183
4	150 %	4	6	10	10.10 ± 0.1184	99.74 ± 0.3349
S.No		PCM (µg/ml)	PCM (µg/ml)	PCM (µg/ml)	PCM (µg/ml)	% PCM
1	Unspiked	100	0	100	98.19 ± 1.8516	-
2	50 %	100	50	150	151.87 ± 0.9605	101.28 ± 0.5674
3	100 %	100	100	200	202.56 ± 1.1348	99.57 ± 1.3810
4	150 %	100	150	250	248.92 ± 3.4553	99.56 ± 3.4526

Table No.6: Repeatability data for PMZ by HPLC method (n = 6 determination)

S.No	Concentration (µg/ml)	2	4	6	8	10
1	Area	1334.57	2556.3	4403.6	5579.6	6738.6
		1312.4	2578.3	4315.5	5689.6	6871.6
		1355.7	2618.1	4244.9	5474.6	6608.8
		1351.4	2607.7	4277.8	5468.7	6674.3
		1327.8	2628.6	4319.5	5667.8	6872.2
		1315.6	2594.7	4311.7	5468.9	6801.8
2	Mean	1332.91	2597.28	4312.16	5558.2	6761.22
3	S.D.	17.9467	26.7180	53.1621	102.6740	107.1874
4	C.V	1.3464	1.0287	1.2328	1.8473	1.5853

Table No.7: Repeatability data for PCM by HPLC method (n = 6 determination)

S.No	Concentration (µg/ml)	50	100	150	200	250
1	Area	2279.1	4867.3	8265.4	11686.7	14115.4
		2312.5	4807.6	8125.8	11419.6	14344.6
		2249.7	4935.9	8110.51	11798.6	13896.7
		2257.6	4807.3	8398.6	11405.7	14314.9
		2321.4	4923.1	8425.3	11550.5	13915.6
		2258.6	4915.6	8270.9	11618.6	14004.5
2	Mean	2279.82	4876.133	8266.08	11579.95	14098.617
3	S.D.	30.4914	58.0489	131.7724	153.3882	195.2902
4	C.V	1.3374	1.1904	1.5941	1.3246	1.3851

Table No.8: Repeatability data of sample application (40 µg/ml PMZ and PCM µg/ml)

S.No	Concentration (µg/ml)	PMZ	PCM
1	Peak Area	2586.3	4867.3
		2598.3	4886.3
		2608.1	4935.3
		2599.7	4757.3
		2578.6	4913.1
		2599.7	4885.6
2	Mean	2595.1167	4874.4667
3	S.D.	10.6887	62.1971
4	C.V.	0.4119	1.2760

Table No.9: Specificity and Selectivity study

S.No	Study	PMZ	PCM
1	**Specificity**	Specific	Specific
2	**Selectivity**	Selective	Selective

Table No.10: System suitability parameters for PMZ and PCM by HPLC method (n=3 determination)

S.No	Parameter	PMZ	PCM	Range	Inference
1	Retention time (Rt) (in minutes)	3.1020	1.7235	-	-
2	Resolution (Rs)	4.6466 ± 0.4438		>2	Criteria met
3	Peak asymmetry factor (AF)	1.22± 0.1648	1.284 ± 0.1038	Between 0.95-1.15	Criteria met
4	Theoretical Plates (Plates/Meter)	28317.25 ± 3097.52	8677.201± 1101.20	Above 2000	Criteria met

Table No.11: Reproducibility data for PMZ (4 µg/ml)

S.No	Analyst 1 Area ± S.D. (n=3)	Analyst 2 Area ± S.D. (n=3)	Result of t test*	Inference
1	2557.16 ± 20.81	2551.633 ± 25.402	0.7442	Not significant difference

* At 95% confidence interval, (t-Tabulated = 4.30)

Table No.12: Reproducibility data for PCM (100 µg/ml)

S.No	Analyst 1 Area ± S.D. (n=3)	Analyst 2 Area ± S.D. (n=3)	Result of t test*	Inference
1	4862.46 ± 44.448	4876.5333 ± 46.057	0.8016	Not significant difference

* At 95% confidence interval, (t-Tabulated = 4.30)

Table No.13: Determination of LOD and LOQ

S.No	PMZ (µg/ml)		PCM (µg/ml)	
1	LOD based on mathematical equation	0.32	LOD based on mathematical equation	12.63
	PMZ (µg/ml)		**PCM (µg/ml)**	
2	LOQ based on mathematical equation	0.97	LOQ based on mathematical equation	38.26

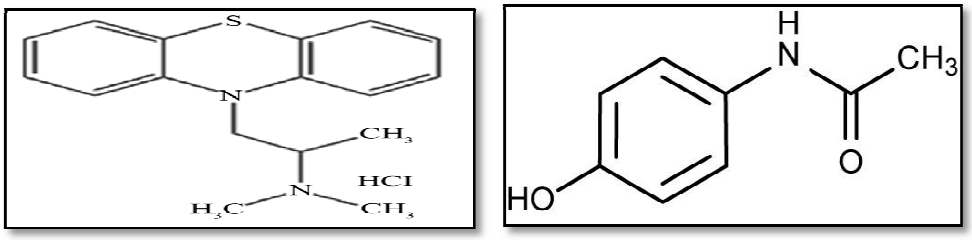
Table No.14: Summary of Validation Parameters of RP-HPLC method

S.No	Parameters	PMZ	PCM
1	Linearity and Range (µg/ml)	2-10	50-250
2	Recovery %	99.0 - 100.33 %	99.58 - 101.28 %
3	Repeatability (C.V.) (n=6)	1.58 - 1.71	1.07 - 1.55
4	Precision Intra-day (n=3) Inter-day (n=3)	1.18 - 1.61 0.77 - 0.97 1.07 - 1.55	1.02 - 1.58 0.69 - 0.97 0.97 - 1.31
5	Limit of Detection (µg/ml)	0.32	12.63
6	Limit of Quantitation (µg/ml)	0.97	38.26
7	Specificity	Specific	Specific
8	Robustness	Robust	Robust
9	Solvent suitability	Suitable for 24 hrs	Suitable for 24 hrs

Table No.15: Assay result of marketed formulation

S.No	Formulation	Drug	Amount Taken (µg/ml)	Amount Found (µg/ml) (n=3)	Labeled claim (mg/5 ml)	Amount found (mg/5 ml)	% Label claim ± SD
1	Phena P syrup	PMZ	4	4.01	5	5.01	100.25 ± 0.7460
		PCM	100	99.73	125	124.66	99.73 ± 0.9609

Each 5 ml contains 5 mg of PMZ and 125 mg PCM.



(a) ***(b)***

Figure No.1 (a and b): Chemical structure of (a) PMZ and (b) PCM

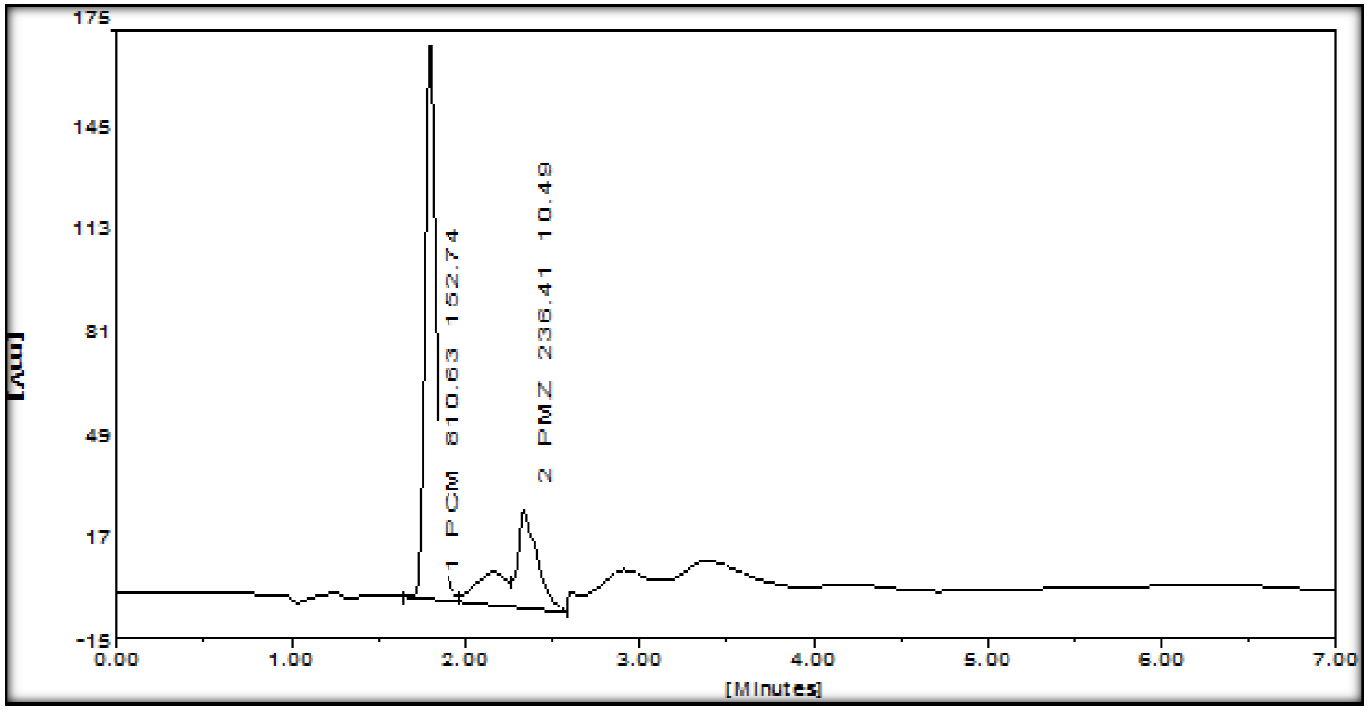


Figure No.2: Chromatogram of mixed standard solution containing 10 µg/ml PMZ and 100 µg/ml PCM, using mobile phase Methanol: Water with 1%TEA (30:70 v/v) (Proposed Method)

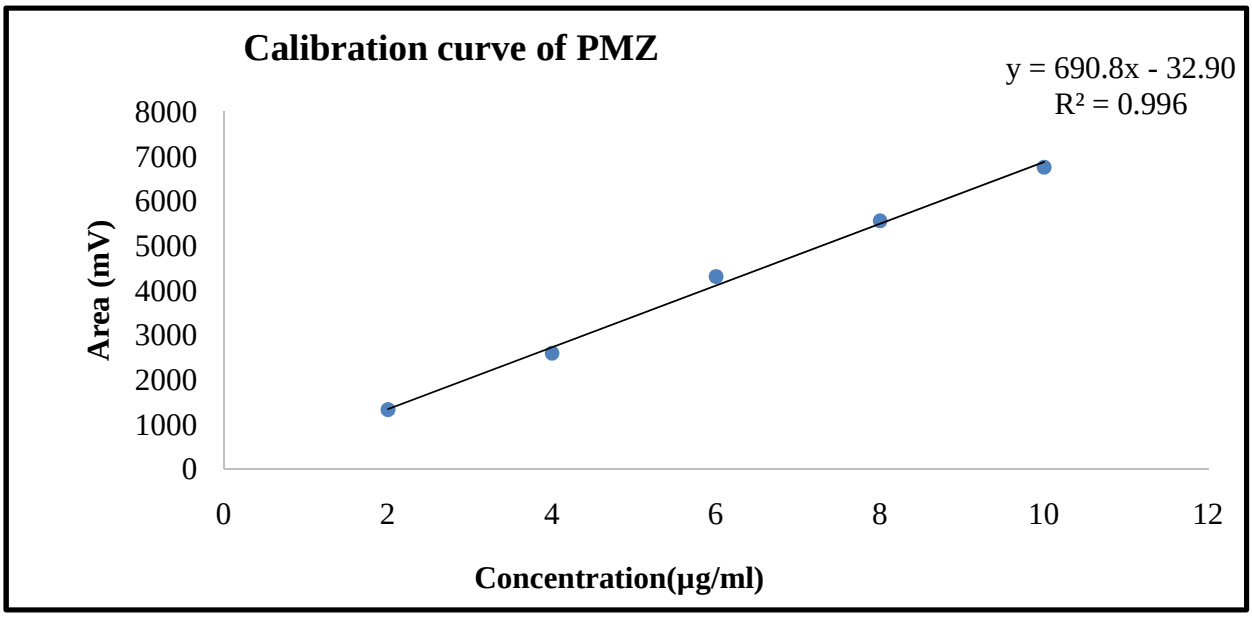


Figure No.3: Calibration curve of PMZ by HPLC method

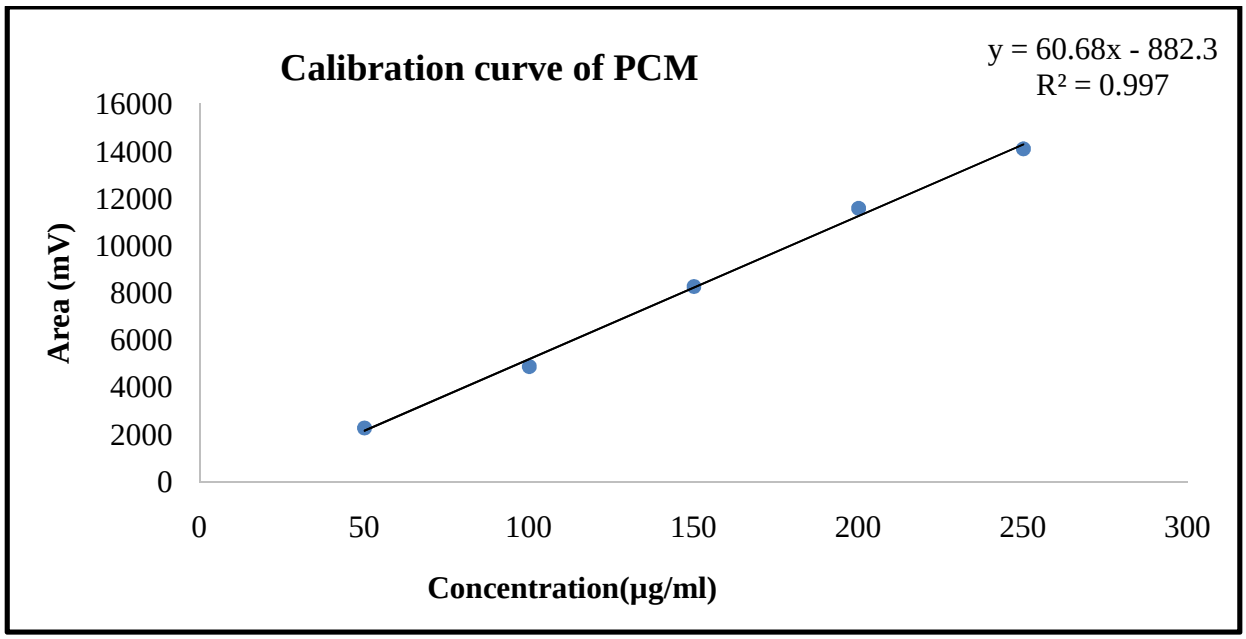


Figure No.4: Calibration curve of PCM by HPLC method

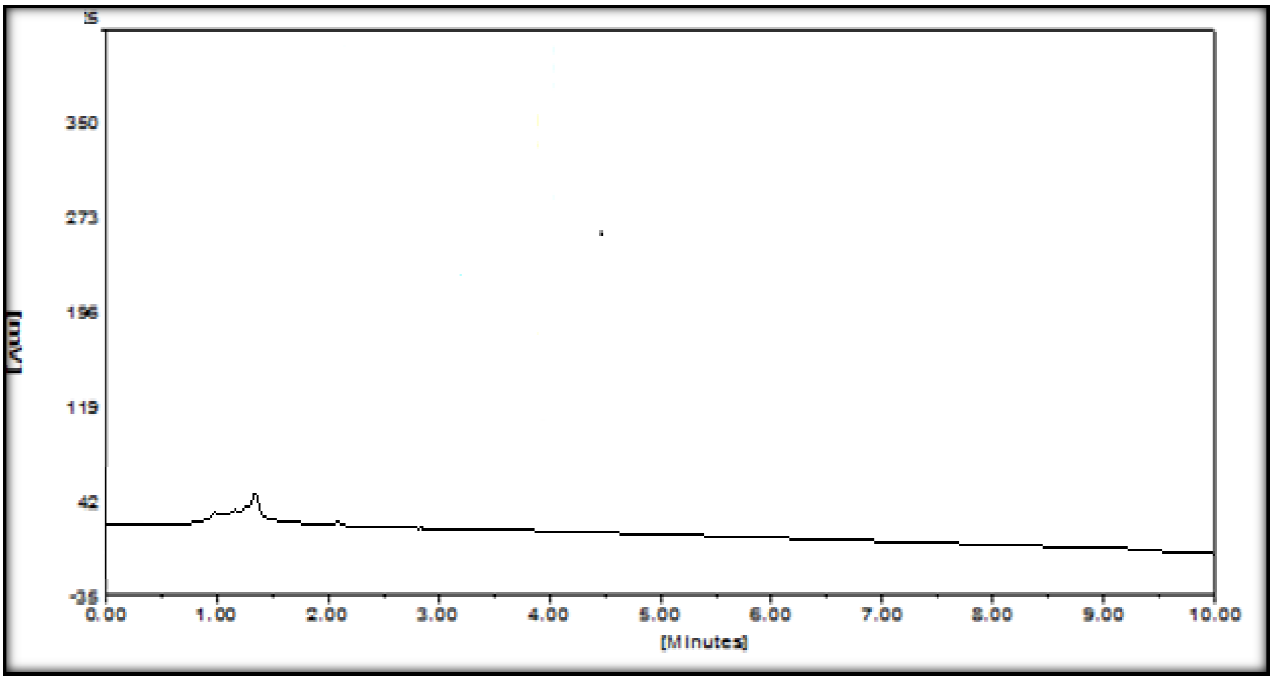


Figure No.5: Chromatogram of Sucrose (Blank syrup) using mobile phase Methanol: Water with 1%TEA (30:70 v/v)

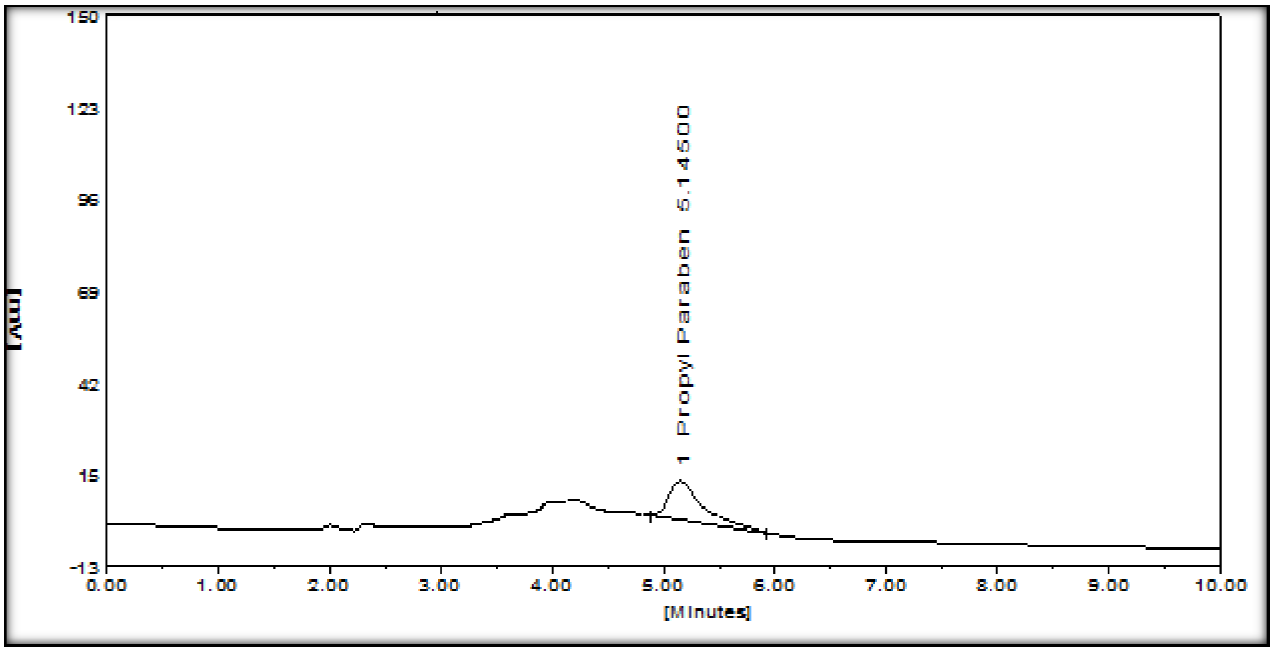


Figure No.6: Chromatogram of Propyl Paraben using mobile phase Methanol: Water with 1%TEA (30:70 v/v)

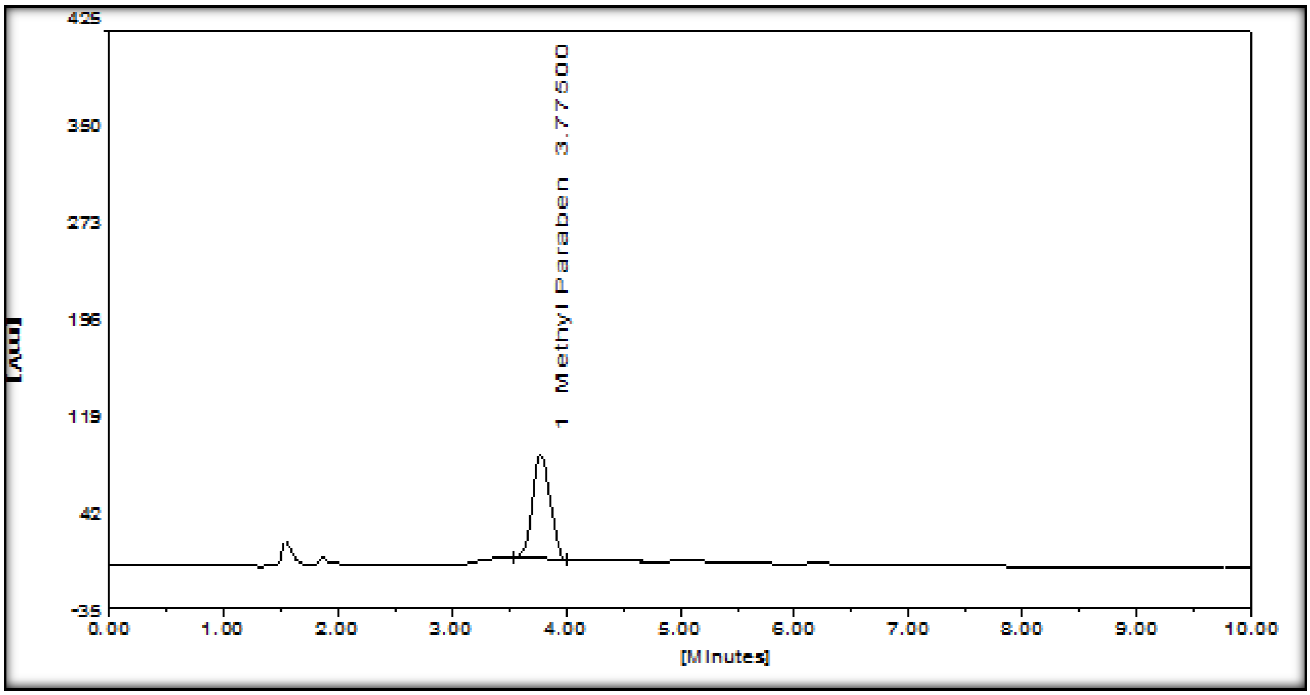


Figure No.7: Chromatogram of Methyl Paraben using mobile phase Methanol: Water with 1%TEA (30:70 v/v)

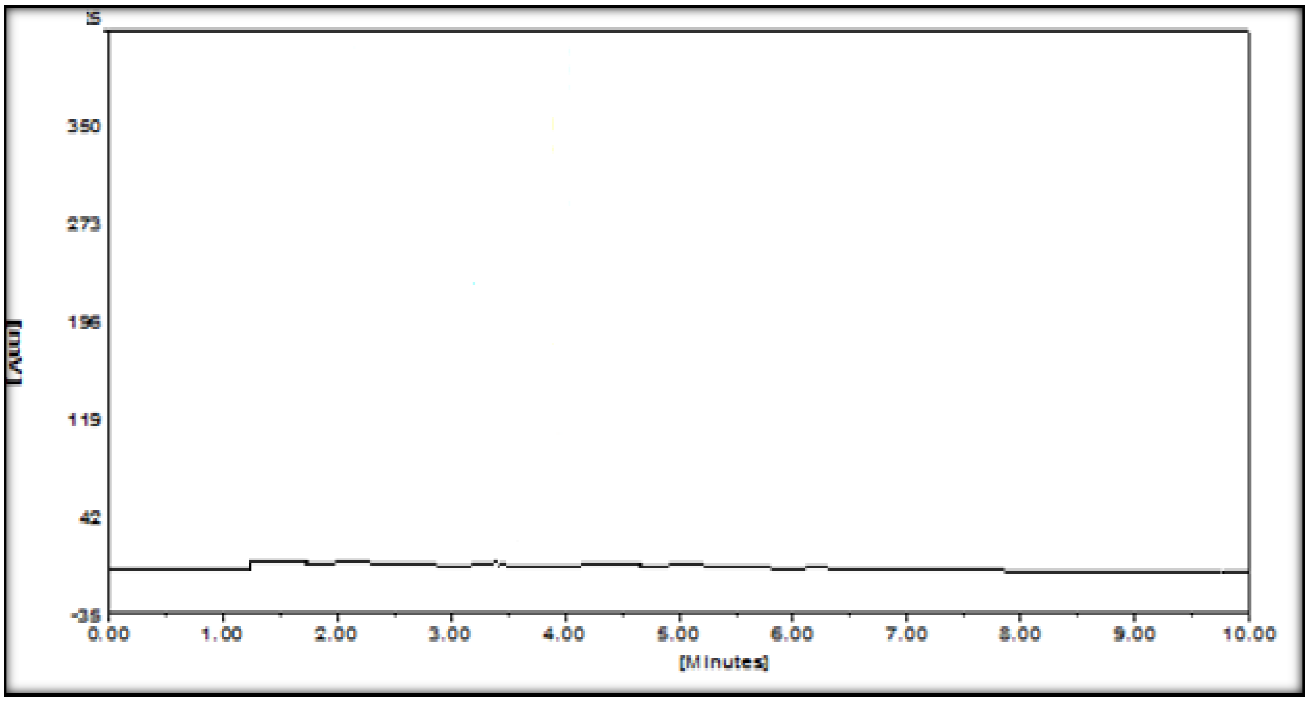


Figure No.8: Chromatogram of Coloring agent Caramel using mobile phase Methanol: Water with 1%TEA (30:70 v/v)

CONCLUSION

The proposed HPLC method provide simple, specific, precise, accurate and reproducible quantitative analysis for simultaneous determination of PMZ and PCM in combined dosage form. The methods were validated as per ICH guidelines in terms of specificity, linearity, accuracy, precision, limits of detection (LOD) and quantification (LOQ), robustness and reproducibility. The proposed methods can be used for routine analysis and quality control assay of PMZ and PCM in combined dosage form.

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BIBLIOGRAPHY

1. Discoverysciences.com: Promethazine, http:// www.discoverysciences.com/uploadedFiles/Library/Technical_Literature/Brochures/B505A_Drug_Catalog_LR_04_17_09, 2013.
2. Wikipedia.com Promethazine, http://en. wikipedia.org /wiki/Promethazine, 2013.
3. Theiaa Najim, Sabha A, Nief Rahman Ahmad and Mona, Ismael, Ibrahim. Spectrophotometric determination of promethazine hydrochloride via oxidative coupling reaction with sulfanilic acid, *University of Sharjah Journal of Pure and Applied Sciences,* 3, 2006, 1-12.
4. Dwi Nurahmanto. Development and validation of UV spectrophotometric method for quantitative estimation of Promethazine HCl in phosphate buffer saline pH 7.4. *International Current Pharmaceutical Journal,* 2, 2013, 141-142.
5. Balammal G, Midhuna Sagari NS, Manoj Kumar B S, Reddy P Jayachandra. Spectrophotometric Estimation of Promethazine Hydrochloride in Bulk and Pharmaceutical Formulations, *International Journal of Pharmaceutical Research and Analysis,* 2, 2012, 6-8.
6. Divani M B, Suhagia B N and Shah S A. Spectrophotometric determination of promethazine hydrochloride in bulk powder and its dosage form, *Indian J. Pharm. Sci,* 61(2), 1999, 110-112.
7. Mallapu E Rani, Cerimetric determination of promethazine bulk drug form and in tablet, *World Journal of Pharmaceutical research,* 2(1), 2012, 69-75.
8. Mohamed M El-Kerdawy, Sayed M Hassan, Saadia M, El-Ashry. Spectrophotometric determination of some N-substituted phenothiazine derivatives using N-bromophthalimide, *Microchimica Acta,* 108(36), 1992, 323-328.
9. HPLC Analysis of Promethazine Enantiomers on Astec® CHIROBIOTIC® application for HPLC, Sigma-Aldrich.htmhttp://www.sigmaaldrich.com /catalog/ product/supelco/g004404? Lang=en and region=in.
10. Einosuke Tanaka, Takako Nakamura, Masaru Terada, Tatsuo Shinozuka, *et al*. Simple and simultaneous determination for twelve phenothiazines in human serum by reversed-phase high-performance liquid chromatography, *Journal of Chromatography B,* 854(1-2), 2007, 116-120.
11. Thumma S, Zhang S Q, Repka M A. Development and validation of a HPLC method for the analysis of promethazine hydrochloride in hot-melt extruded dosage forms, *Pharmazie,* 63(8), 2008, 562-7.
12. Vanapalli1Sreenivasa R, KambhampatiSivarama P, Putcha Lakshmi and David W A Bourne. A Liquid Chromatographic Method for the Simultaneous Determination of Promethazine and Three of its Metabolites in Plasma Using Electrochemical and UV Detectors, *Journal of Chromatographic Science,* 39, 2001, 70-72.
13. Zuzana Bosakova, Iveta Klouckova, Eva Tesarova. Study of the stability of promethazine

enantiomers by liquid chromatography using a vancomycin-bonded chiral stationary phase, *Journal of Chromatography B,* 770(1-2), 2002, 63-69.

14. Toochinda T, Shapiro B, Kral A, Lynch K UCSF, San Francisco C A. Detecting Promethazine Use in the Methadone Maintenance Population by Liquid Chromatography Tandem Mass Spectrometry, *Clinical Chemistry*, 57(10), 2011, A57-A58.
15. Udo Huber. Analysis of Antihistaminic drugs by HPLC, Agilent Technologies Innovating the HP Way, 5968-2889E http://www.chem.agilent.com /Library /applications/59682889.pdf.
16. Wang Xin, Zhao Zhe. Determination of Reserpine and Promethazine Hydrochloride in Tabellae Reserpini Compositae by HPLC. *Drug Stanoaros of China,* (0)4, 1999, 264-266.
17. Chen Jian LI, Yun Zhang Jia-ming, Wang Li-qin. HPLC determination of reserpine and promethazine hydrochloride in Compound Capsules of Antihypertension, *Chinese journal of pharmaceutical analysis*, 2008, 2(3) 78-80.
18. Martin Wachira. Development and validation of a liquid chromatographic method for the simultaneous determination of diphenhydramine, promethazine, chlorpheniramine and ephedrine in cold-cough syrup, *Published on Board of Postgraduate Studies*, February 2013; 1: 1-2.
19. Danlami Uzama. High-Performance Liquid Chromatographic Determination of Paracetamol, Chloroquine and Promethazine in some pharmaceutical preparation. Postgraduate School, Ahmadu Bello University, Zaria, Nigeria, http://kubanni.abu.edu.ng:8080/jspui/bitstream/123456789/3785/1, 2012.
20. Elobieta Wyszomirska, Krystyna Czerwi-Ska, Elobieta Kublin and Aleksander P Mazurek. Identification and Determination of Ketotifen Hydrogen, Fumarate, Azelastine Hydrochloride, Dimetindene Maleate and Promethazine Hydrochloride by densitometric method, *Acta Poloniae Pharmaceutica - Drug Research*, 70(6), 2013, 951-959.
21. www.drugs.com. Paracetamol, http://www. drugs.com/paracetamol.htm, August 2013.
22. www.wikipedia.com. Paracetamol, http://en. wikipedia. org/wiki/Paracetamol, August 2013.
23. Dudhane N P, Vidhate S S, Borkar B H, Lohiya R T, Umekar M J. Simultaneous UV Spectrophotometric estimation of Metoclopramide hydrochloride and Paracetamol in solid dosage form, *J. Pharm. Sci. and Res.*, 2(1), 2010, 48-52.
24. Kalra Kapil, Naik S, Jarmal Garima and Mishra N. Spectrophotometric Method for Simultaneous Estimation of Paracetamol and Domperidone in Tablet Formulation, *Asian J. Research Chem.*, 2(2), 2009, 112-114.
25. Kothapalli Lata P, Karape Archana K, Thomas A B. Simultaneous spectrophotometric estimation of Paracetamol and Dexketoprofen Trometamol in pharmaceutical dosage form, *Der Pharma Chemica*, 3(1), 2011, 365-371.
26. Narajji Chandrashekhar, Hardik R Patel, Manohar D Karvekar. Simultaneous Estimation of Aceclofenac, Paracetamol and Tizanidine in their Combined Dosage forms by Spectrophotometric and RP- HPLC Method, *J Anal Bioanal Techniques*, 2(4), 2011, 1-5.
27. Mahaparale Sonali, Telekone R S, Raut R P. Simultaneous spectrophotometric determination of drotaverine HCl and Paracetamol in tablet, *Indian Journal of Pharmaceutical sciences*, 72(1), 2010, 133-136.
28. Shukla Rajesh, R. Shivkumar, Kumar Nalla Shivan, Development of a UV-Spectrophotometric method for the simultaneous determination of tramadol hydrochloride and paracetamol in bulk and marketed product, *Bulletin of Pharmaceutical Research*, 1(1), 2011, 62-63.
29. Sawant Ramesh, Bhangale Lokesh, Joshi Rupali and Lanke Prashant. Validated spectrophotometric methods for simultaneous estimation of Paracetamol, Domperidone and Tramadol HCl in pure and tablet dosage form, *Journal of chemical metrology*, 4(1), 2010, 21-27.

30. Lotfi Monser A, Frida Darghouth, Simultaneous LC. Determination of paracetamol and related compounds in pharmaceutical formulations using a carbon-based column, *Journal of Pharmaceutical And Biomedical Analysis*, 27(6), 2002, 851-860.
31. Prasanna Reddy, Battu and Reddy M S. RP-HPLC Method for Simultaneous Estimation of Paracetamol and Ibuprofen in Tablets, *Asian J. Research Chem.*, 2(1), 2009, 70-72.
32. Prasanna Reddy, Battu. Simultaneous RP-HPLC Determination of Nimesulide and Paracetamol in Tablets, *International Journal of Pharm Tech Research*, 1, 2009, 514-516.
33. Dudhane NP, Umekara MJ, Lohiya RT. Validated RP-HPLC Method for Estimation of Metoclopramide Hydrochloride and Paracetamol in solid dosage form, *Journal of Pharmacy Research*, 2(12), 2010, 3064-3066.
34. Narajji Chandrashekhar, Hardik R. Patel, Manohar D. Karvekar. Simultaneous Estimation of Aceclofenac, Paracetamol and Tizanidine in their Combined Dosage forms by Spectrophotometric and RP- HPLC Method, *J Anal Bioanal Techniques*, 2(4), 2011, 1-5.
35. Momin M Y, Yeole P G, Puranik M P, Wadher S J. Reverse phase HPLC method for determination of aceclofenac and paracetamol in tablet dosage form, *Indian J Pharm Sci*, 68, 2006, 387-389.
36. Pawar Uttam D, Naik Abhijit V, Sulebhavikar Aruna V, Datar Tirumal A and Mangaonkar Kiran V. Simultaneous Determination of Aceclofenac, Paracetamol and Chlorzoxazone by HPLC in Tablet Dose Form, *E-Journal of Chemistry*, 6(1), 2009, 289-294.
37. Suryan A L, Bhusari V K, Rasal K S, Dhaneshwar S R. Simultaneous Quantitation of Paracetamol, Phenyl propanolamine Hydrochloride and Cetrizine Hydrochloride RP-HPLC in Bulk Drug and Formulation, *International Journal of Pharmaceutical Sciences and Drug Research*, 3(4), 2011, 303-308.
38. Jane Fatima Mary Titus, Thenmozhi A, Sridharan D and Palanivelu M. HPTLC Method For Simultaneous Estimation of Tramadol Hydrochloride and Paracetamol in Combined Tablet Dosage Form, *International Journal of Pharma Recent Research*, 1, 2009, 22-26.
39. Yadav Alok, Singh M. Raman, Mathur C. Satish, Saini K Pawan and Singh N. Gyanendra. A Simple and Sensitive HPTLC Method for Simultaneous Analysis of Domperidone and Paracetamol in Tablet Dosage Forms, *Journal of Planar Chromatography*, 22(4), 2009, 421- 424.
40. Rao Janhavi R, Toufik S. Mulla, Bharekar Vishal V. Simultaneous HPTLC Determination of Paracetamol and Dexketoprofen trometamol in pharmaceutical dosage form, Der *Pharma Chemica*, 3(3), 2011, 32-38.
41. Deshpanday P B, Gandhi V. Santosh, Bhangale S, Yogeshwari S. HPTLC Method for Simultaneous estimation of Etoricoxib and Paracetamol in Combined Tablet Dosage, *Journal of Pharmaceutical and Biomedical Analysis*, 3, 2010, 1-5.
42. Prafulla P, Mahadev, Apshingekar, Mahadik V, Dhaneshwar Sunil R. Validated HPTLC Method for simultaneous quantitation of Paracetamol, Tramadol and Aceclofenac in tablet formulation, *Scholar Research Library*, 2(6), 2010, 28-36.
43. Sawant Ramesh L, Raihan Ahmed, Ramdin S. Supriya, Darade R. Sheetal. Spectrophotometric estimation of paracetamol and promethazine in tablet dosage forms, *Der Pharma Chemica*, 4(2), 2012, 714-719.
44. Borker D D, Godse V P, Bafana Y S, Bhosale A V. Simultaneous estimation of paracetamol Promethazine Hydrochloride in pharmaceutical formulations by RP-HPLC method, *International Journal of ChemTech Research*, 3, 2009, 667-670.
45. ICH Harmonized Tripartite Guidelines. Validation of Analytical Procedures: Text and Methodology; Q2 (R1), Geneva, Nov 2005, 6-13.