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Novel RP-HPLC method for estimation of paracetamol and promethazine simultaneously in syrup formulation

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ABSTRACT: Paracetamol with Promethazine HCl is most popular over the counter drug which is used as antipyretic and antiemetic. A novel, simple, precise, less time consuming, economical and accurate reverse phase HPLC method has been developed for their estimation using water, methanol and acetic acid in 79:20:1 v/v/v ratio as eluting phase on Kromasil Silica column keeping flow rate at 1ml/min and detection at 249 nm. Both drugs observed linearity between 10-50µg/ml and successfully resolved within 6 minutes (3.565 and 5.641 minutes for paracetamol and promethazine respectively) with percent recovery between 98-101%. Tailing factor is within the range and number of theoretical plates is more than 2500. Method was validated as per ICH guidelines and the results indicate that these drugs could be quantified simultaneously without excipient interference and thus suitable for routine analysis of drugs in combination.

KEYWORDS: Paracetamol; promethazine; HPLC; validation; simultaneous estimation.

1. INTRODUCTION

Paracetamol (PCM) (Figure 1a), a para-aminophenol derivative and chemically N-(4-hydroxyphenyl) acetamide is a white odourless crystalline powder, sparingly soluble in water. It is centrally and peripherally acting non-opioid analgesic having antipyretic and anti-inflammatory properties [1-8].

Promethazine (PMZ) (Figure 1b), a phenothiazine derivative and chemically N-(2'-dimethylamino-2'-methyl) ethyl phenothiazine is white or faintly yellow crystalline powder, soluble in water and ethanol but insoluble in ether and acetone. It is H_1 antagonist used as an antiemetic in motion sickness, an antipsychotic drug for treating mental disorders and also for enhancing analgesic, anesthetic and sedative effect of other medicines [19-20]. Various analytical methods like UV, HPLC etc. have been used for estimation for paracetamol [1-3, 5-18] and promethazine [19-27] individually or in combination with each other [28-30] as well as other drugs.

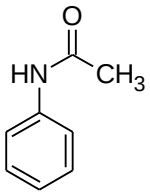

Figure 1(a). Paracetamol

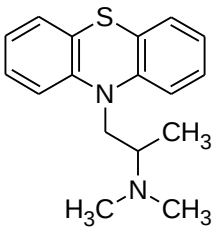

Figure 1(b). Promethazine

So the present study has been designed to develop a better cost effective, accurate, rapid and precise RP-HPLC method which can be used for the estimation of PCM and PMZ in combination.

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2. RESULTS AND DISCUSSION

2.1. Method development

Both paracetamol and promethazine were satisfactorily resolved at 249 nm using mobile phase comprising of mixture of water, methanol and acetic acid (79: 20: 1% v/v/v) flowing at 1ml/min on Kromasil Silica column (250 mm × 4.6 mm, 5SL-II), with Prominence Diode Array Detector maintained at a temperature between 25-30°C. Under optimized chromatographic parameters, the retention time of 3.565 min and 5.641 min was obtained for PCM and PMZ, respectively. Drug content as found was approximately 98% which shows accuracy of the method.

2.2. Method validation

The developed method was validated as prescribed by the ICH guidelines with respect to specificity, selectivity, system suitability, linearity, accuracy, robustness, precision [31-32].

2.2.1. Specificity and selectivity

Specificity and selectivity of the method was done to check for the interference of impurities. It was assessed by comparing the chromatograms obtained from mobile phase (blank), syrup solution with standard solution. No interfering peak was observed in chromatogram of blank (mobile phase) at retention time of both drugs. Moreover, the retention times of both drugs from standard as well as syrup solution were found almost identical, and no co-eluting peaks were observed, thus the method was specific as well as selective for quantitative estimation of both drugs in the commercial formulation [33].

2.2.2. System suitability

The system suitability parameters viz. retention time, peak area, resolution, theoretical plates and tailing factor) were calculated from the obtained chromatograms of the standard drugs. The obtained results are tabulated in Table 1 which are in acceptable criteria.

Table 1. System suitability parameters.

Parameter	PCM	PMZ
Linear Range (µg/ml)	10.0-50.0	10.0-50.0
Slope	13553x	15994x
Intercept	20724	13349
Regression coefficient (r^2)	0.999	0.998
Limit of Detection (µg/ml)	1.2	0.51
Limit of Quantitation (µg/ml)	3.65	1.55
Retention time (min)	3.565	5.641
Tailing factor	1.17	1.11
Resolution factor	3.703	
Capacity factor	0.237	0.456
Theoretical plate	27574.444	33077.879

2.2.3. Linearity

Linearity was studied by plotting graph between peak area (Y-axis) and concentration (X-axis). Both the drugs were found to be linear in concentration range of 10.0-50.0 µg/ml (Figure 2). Regression analysis was made for the slope (m), intercept (c) and coefficient of determination (r^2) as shown in Table I. Higher values of r^2 indicate good linearity of the calibration curve for both the drugs. Sensitivity of the method was confirmed by low value of limit of detection (LOD) and limit of quantitation (LOQ) calculated using linear regression method.

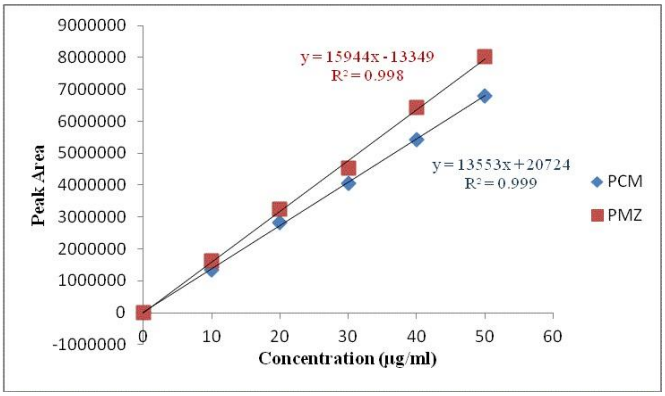


Figure 2. Linearity studies for PCM and PMZ at 249 nm.

2.2.4. Accuracy

The accuracy of the developed method was evaluated by taking 80%, 100%, 120% of the sample solution and assessed by calculating % RSD (Table 2). Recovery values greater than 98% with % RSD less than 2% indicated that these drugs could be quantified simultaneously and that there is no interference of the excipients present in the formulation which indicates that these drugs could be quantified simultaneously.

Table 2. Accuracy

Level of % recovery	Amount present (mg/tab)		Total amount recovered (mg)		% Mean Recovery± SD		% RSD	
	PCM	PMZ	PCM	PMZ	PCM	PMZ	PCM	PMZ
80%	100	40	97.78	39.38	97.78 ± 0.13	98.46 ± 0.23	0.132	0.23
100%	125	50	123.06	50.36	98.44 ± 0.24	100.73 ± 0.16	0.244	0.15
120%	150	60	146.57	59.6345	97.71 ± 0.18	99.39 ± 0.35	0.184	0.22

2.2.5. Precision

Repeatability (Intra-day precision)

It was checked by analyzing sample solution three times on the same day. The results were found to be within the specified range (Table 3).

Ruggedness (Interday precision)

It was done by performing the same procedure by two different analysts on two different days. The results were found to be within the specified range (Table 3).

% RSD was found to be less than equal to 2 which indicate that the proposed method has good repeatability as well as reproducibility.

Table 3. Precision studies

			Inter day (RUGGEDNESS)							
			DAY-I				DAY-II			
	Intra day		ANAL - I	ANAL -II	ANAL- I	ANAL - II	ANAL - I	ANAL - II	ANAL -I	ANAL- II
	PCM	PMZ	PCM		PMZ		PCM		PMZ	
Amount Found (mg)	122.96	4.917	125.5	123.85	5.03	4.906	125.15	125.77	5.031	4.91
% Amount found	98.37 %	98.34%	100.4	99.08	100.06	98.12	101.72	100.61	100.62	98.20
Mean % ± S.D	98.37% ± 0.117	98.34% ± 0.002	99.74 ± 0.933		99.09 ± 1.371		101.16 ± 0.784		99.41 ± 1.711	
Precision, % RSD	0.095	0.556	0.935		1.384		0.775		1.721	

2.2.6. Robustness

No marked change was observed on slightly modifying the chromatographic parameters like wavelength, flow rate, temperature etc. which indicates the robustness of developed method. Results are indicated in Table 4.

Table 4. Robustness studies

Method Parameter	Level	Retention Time		Tailing factor	
		PCM	PMZ	PCM	PMZ
Wavelength					
249	– 1	3.558	5.369	1.157	1.758
250	0	3.558	5.447	1.163	1.789
251	+ 1	3.559	5.359	1.16	1.756
Flow Rate (mL/min)					
0.8	– 0.2	3.957	5.971	1.789	1.789
1.0	0	3.558	5.447	1.163	1.789
1.2	+ 0.2	3.225	4.876	1.164	1.765
Temperature (°C)					
20	-5	3.467	5.561	1.123	1.772
25	0	3.558	5.447	1.163	1.789
30	+5	3.523	5..654	1.145	1.758

2.2.7. Content uniformity (Assay)

The commercially available syrup was analyzed using developed method and the drug content was found to be 98.38% ± 0.863 and 98.31% ± 0.117 for PCM and PMZ respectively (Table 5).

Table 5. Assay Results for Syrup Formulation

Drug	Label Claim	Peak area		Amount found (mg)	% drug found ± SD	%RSD
		Standard	Sample			
PCM	125 mg/5ml	29583707	29564277	122.97	98.37 ± 0.108	0.087
PMZ	5 mg/ml	2647377	2583984	4.91	98.3 ± 0.005	0.118

3. CONCLUSION

The developed isocratic RP-HPLC method has been found economic, simple, rapid, accurate, and precise which can be routinely used for simultaneous estimation of PCM and PMZ in syrup formulation without the interference of excipients. All the parameters viz. accuracy, specificity, robustness, precision

were in acceptance criteria as per ICH guidelines with retention time less than 6 minutes for both the drugs. The developed method was also found to be better and precise than the reported methods in terms of better accuracy, more efficiency as indicated by high number of theoretical plates, low tailing factor, and simple readily available column has been used.

4. MATERIALS AND METHODS

4.1. Materials

Pure samples of Paracetamol and Promethazine were obtained as gift samples. Syrup formulation containing PCM 125.0 mg/5ml and PMZ HCl 5.0 mg/ml was procured from the local market. HPLC grade methanol, water and acetic acid were purchased from SD Fine Chemicals Ltd., India which were used after filtration under vacuum from 0.45 membrane filter.

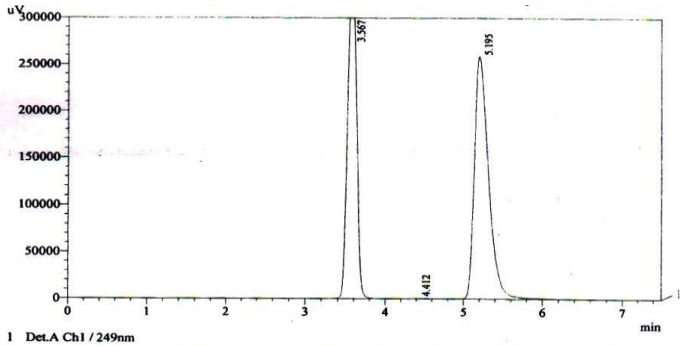
4.2. Instrumentation

Chromatographic separation was performed on Shimadzu LC- 20 AT HPLC (Double pump) with Rheodyne 7725i type injector (20µl loop capacity), Kromasil Silica column (250mm × 4.6mm, 5SL-II), SPD M20A, Prominence Diode Array Detector. Various mobile phases were tried from which mobile phase comprising of mixture of water, methanol and acetic acid (79: 20: 1% v/v/v) flowing at 1ml/min was found to be most suitable. Mobile phase was filtered (0.45 membrane filter) under vacuum and degassed in ultrasonic bath for 30 minutes before passing through the instrument. Chromatographic separations were carried out at room temperature (25-30°C) with detection at 249nm.

4.3. Method

4.3.1. Preparation of standard solution

Pure promethazine (50 mg) was dissolved in methanol (50 ml) to get standard solution (1000 µg/ml). 5ml of this solution was added to another solution prepared by dissolving pure paracetamol (125 mg) in 50ml methanol to get solution of 100 µg/ml and 500 µg/ml of Promethazine and Paracetamol respectively. The resulting solution after filtration (5.0 µl) was injected into the chromatographic system and the chromatogram was recorded (Figure. 3).



PeakTable

Detector A Ch1 249nm

Peak#	Name	Ret. Time	Area	Height	Area %	Height %
1	PARACETAMOL	3.567	2713186	345286	45.550	57.047
2		4.412	5260	404	0.088	0.067
3	PROMETHAZINE	5.195	3238068	259577	54.362	42.886
Total			5956513	605268	100.000	100.000

k'	Tailing Factor	heoretical Plates/met	Resolution
0.000	1.123	29144.631	0.000
0.237	1.309	16566.754	2.966
0.456	1.664	26504.283	2.292

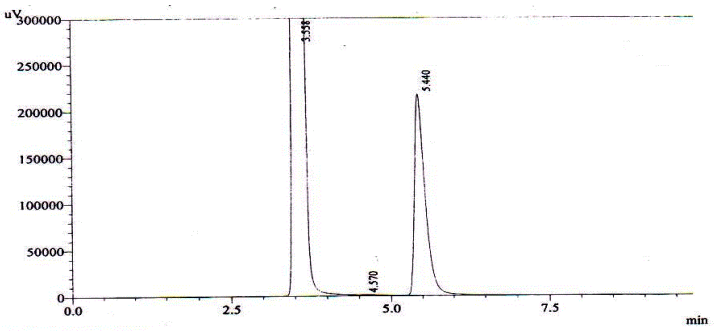
Figure 3. Chromatogram for standard paracetamol and promethazine.

4.3.2. Preparation of calibration curve

Appropriate aliquots were pipetted out from the standard stock solution to get set of solutions having the concentration range, ranging from 10.0-50.0µg/ml of each drug. These solutions (5µl) were chromatographed and the peak areas were measured. Peak areas were then plotted against the respective concentrations for both the drugs (Fig. 2). From the plots the linear range for both PCM and PMZ was found to be between 10.0-50.0 µg/ml.

4.3.3. Preparation of sample solution

Syrup formulation labeled 125mg/5ml PCM and 5mg/ml PMZ was added to 50ml of HPLC grade methanol. The resulting solution (500.0 µg/ml and 100.0 µg/ml of PCM and PMZ respectively) after filtration was injected into the chromatographic system and the chromatogram was recorded (Figure. 4). Results of the analysis are shown in Table 5.



PeakTable

Detector A Ch1 249nm

Peak#	Name	Ret. Time	Area	Height	Area %	Height %
1	PARACETAMOL	3.558	29484092	3368114	91.936	93.935
2		4.570	2962	210	0.009	0.006
3	PROMETHAZINE	5.440	2583061	217271	8.054	6.060
Total			32070116	3585595	100.000	100.000

k'	Tailing Factor	heoretical Plates/met	Resolution
0.000	1.162	27357.900	0.000
0.284	3.078	16251.804	3.417
0.529	1.795	32979.731	2.560

Figure 4. Chromatogram of syrup formulation.

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Conflict of interest statement: The authors declared no conflict of interest

REFERENCES

[1] Arayne MS, Sultanab N, Siddiquia FA. Simultaneous determination of paracetamol and orphenadrine citrate in dosage formulations and in human serum by RP-HPLC. J Chin Chem Soc. 2009; 56: 169-174. [CrossRef]

[2] Mahaparale S, Telekone RS, Raut RP, Damle SS, Kasture PV. Simultaneous spectrophotometric determination of drotaverine hydrochloride and paracetamol in tablet. Indian J Pharm Sci. 2010; 72(1): 133-136. [CrossRef]

[3] Kapil K, Naik S, Garima J, Mishra N. Spectrophotometric method for simultaneous estimation of paracetamol and domperidone in tablet formulation. Asian J Chem. 2009; 2(2): 50-54.

[4] Baheti KG, Shaikh S, Shah N, Dehgan MH. Validated simultaneous estimation of paracetamol and etoricoxib in bulk and tablet by HPTLC method. Inter J Res Pharma Biomed Sci. 2011; 2(2): 672-675.

[5] Pattan SR, Jamdar SG, Godgel RK, Dighel NS, Daithankar AV, Nirmal SA, Pai MG. RP- HPLC method for simultaneous estimation of paracetamol and etoricoxib from bulk and tablets. J Chem Pharm Res. 2009; 1(1): 329-335.

[6] Battu PR, Ready MS. RP-HPLC method for simultaneous estimation of paracetamol and ibuprofen in tablets. Asian J Res Chem. 2009; 2(1): 70-72.

[7] Issa YM, Zayed SIM, Habib IHI. Simultaneous determination of ibuprofen and paracetamol using derivatives of the ratio spectra method. Arab J Chem. 2011; 4(3): 259–263. [CrossRef]

[8] Kirtawade R, Salve P, Seervi C, Kulkarni A, Dhabale P. Simultaneous UV spectrophotometric method for estimation of paracetamol and nimesulide in tablet dosage form. Int J Chemtech Res. 2010; 2(2): 818-821.

[9] Behera S, Ghanty S, Ahmad F, Santra S, Banerjee S. UV-Visible spectrophotometric method development and validation of assay of paracetamol tablet formulation. J Anal Bioanal Tech. 2012; 3(6): 151-156. [CrossRef]

[10] Nagendra P. Spectrophotometric estimation of paracetamol in bulk and pharmaceutical formulations. EJ Chem. 2011; 8(1): 149-152. [CrossRef]

[11] Giriraj P, Sivakkumar T. New simple spectrophotometric method for the simultaneous estimation of paracetamol and flupirtine maleate in pure and pharmaceutical dosage form. Int J Spectrosc. 2014: 1-6. [CrossRef]

[12] Oza CK, Nijhawan R, Pandya MK, Vyas AJ, Patel AI. Q–Analysis spectrophotometric method for the simultaneous determination of nabumetone and paracetamol in API and in tablet dosage form. Asian J Pharm Anal. 2013; 3(1): 9-16.

[13] Rote AR, Kumbhoje PA and Bhambar RS. UV-visible spectrophotometric simultaneous estimation of paracetamol and nabumetone by AUC method in combined tablet dosage form. Pharm Method. 2012; 3(1): 40-43.[CrossRef]

[14] Likhar AD, Gupta KR and Wadodkar SG. Spectrophotometric methods for the simultaneous estimation of paracetamol and etoricoxib in tablet dosage forms. Int J Pharm Pharm Sci. 2010; 2(1): 156-161.

[15] Thippeswamy M, Somanna P, Krishnan P, Bhandare B, Sahajanand H. A new method development and validation for estimation of paracetamol in pharmaceutical dosage form by reverse phase-high performance liquid chromatography. Int J Pharm Pharm Sci. 2015; 7(8): 190-194.

[16] Devi TA, Setti A, Srikanth S, Nallapeta S, Pawar SC, Rao JV. Method development and validation of paracetamol drug by RP-HPLC. J Med Allied Sci. 2013; 3(1): 8-14.

[17] Maslarska VN, Tsvetkova BG and Peikova LP. RP-HPLC method for simultaneous determination of paracetamol, diclofenac sodium and famotidine in tablet dosage form. Int J Pharm Res Scholars. 2015; 4(2): 55-61.

[18] Chandra R, Verma D, Sharma KD, Kumar S, Alam N, Singh S. Comparative quantitative determination of paracetamol by RP-HPLC and UV-spectrophotometry from its formulated tablets. Int J Pharm Pharm Sci. 2013; 5(3): 863-865.

[19] Vanapalli SR, Kambhampatil SP, Putcha L, Bourne DWA. A liquid chromatographic method for the simultaneous determination of promethazine and three of its metabolites in plasma using electrochemical and UV detectors. J Chromatogr Sci. 2001; 39: 170-172. [CrossRef]

[20] Xiao P, Wu W, Yu J, Zhao F. Voltammetric sensing of promethazine on a multi-walled carbon nanotubes coated gold electrode. Int J Electrochem Sci. 2007; 2: 149-157.

[21] Lin SX and Huo CG. Determination of promethazine hydrochloride in promethazine hydrochloride syrup by HPLC. Chin J Mod Appl Pharm. 2008: 03.

[22] Nurahmanto D. Development and validation of UV spectrophotometric method for quantitative estimation of Promethazine HCl in phosphate buffer saline pH 7.4. Int Curr Pharm J. 2013; 2(8): 141-142. [CrossRef]

[23] Khaleda HS and Rana AH. Spectrophotometric determination of promethazine hydrochloride and paracetamol in pharmaceutical tablets. J Al-Nahrain Univ. 2014; 17(1): 14-23.

[24] Sharma A, Upadhyay N, Sapra S, Dhar KL, Sharma M. Method development and validation for simultaneous estimation of promethazine hydrochloride and pholcodine in bulk and marketed formulation (Linctus) by UV spectrophotometery. Develop Drugs. 2013; 2(4): 73. [CrossRef]

[25] Balammal G, Sagari NSM, Kumar BSM and Reddy PJ. Spectrophotometric estimation of promethazine hydrochloride in bulk and pharmaceutical formulation. Int J Pharm Res Anal. 2012; 2(16): 6-8.

[26] Qader HA and Fakhre NA. Spectrophotometric determination of promethazine hydrochloride in pure and pharmaceutical dosage forms. ZJPAS. 2017; 29: s107-s114.

[27] Al-kahdimy AS. Flame emission and molecular absorption spectrophotometric determination of promethazine hydrochloride via potassium dichromate as oxidant reagent. World J Pharm Sci. 2016; 4(3): 323-329.

[28] Sawant RL, Ahmed R, Supriya RS, Sheetal DR. Spectrophotometric estimation of paracetamol and promethazine in tablet dosage forms. Der Pharma Chemica. 2012; 4(2): 714-719.

[29] Borkar DD, Godse VP, Bafana YS, Bhosale AV. Simultaneous estimation of paracetamol and promethazine hydrochloride in pharmaceutical formulations by RP-HPLC method. Int J Chemtech Res. 2009; 1(3): 667-670.

[30] Kakadiya J, Parmar N, Shah N. Development and validation of RP-HPLC method for simultaneous estimation of promethazine hydrochloride and paracetamol in combined liquid formulation. Asian J Res in Biol Pharm Sci . 2014; 2(1): 11-26.

[31] International Conference on Harmonization Q1B, Stability testing: Photostability testing of new drug substances and products, International Conference on Harmonization, Geneva, 1996.

https://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q1B/Step4/Q1B_Guideline.pdf

[32] International Conference on Harmonization, ICH Guidelines, Validation of analytical procedures technical requirements for registration of pharmaceuticals for human use: Text and Methodology Q2(R1), International Conference on Harmonization, Geneva, Switzerland, November 2005. http://www.gmp-compliance.org/guidemgr/files/Q2(R1).PDF

[33] Yenduri G, Navuluri S. Analytical high performance liquid chromatography method for estimating the combination of aspirin and omeprazole in bulk and tablet dosage form. Marmara Pharm J. 2018; 22 (4): 502-510. [CrossRef]