www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
TPI 2024; 13(2): 200-208

www.thepharmajournal.com
Received: 25-12-2023
Accepted: 30-01-2024

Ravshankhon Makhmudov
Tashkent State Technical University, State Unitary Enterprise Science end Progress, Tashkent, Uzbekistan

Furkat Yunusov
Tashkent State Technical University, State Unitary Enterprise Science end Progress, Tashkent, Uzbekistan

Corresponding Author:
Ravshankhon Makhmudov
Tashkent State Technical University, State Unitary Enterprise Science end Progress, Tashkent, Uzbekistan

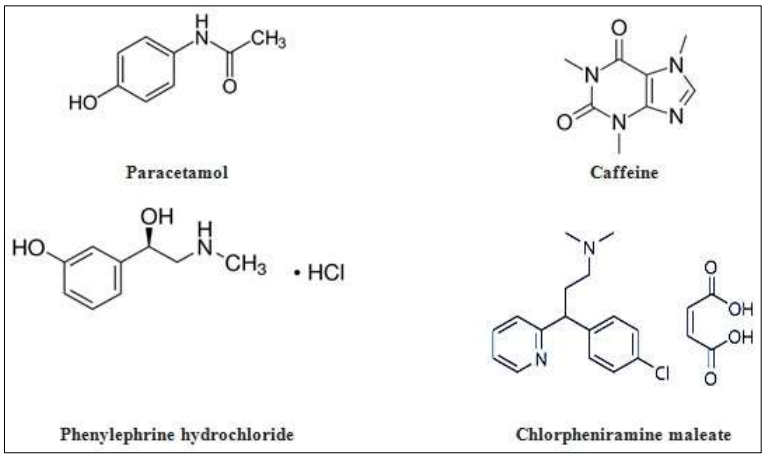
Development and validation of RP-HPLC method for quantitative determination and estimation of paracetamol, caffeine, phenylephrine hydrochloride and chlorpheniramine maleate in pharmaceutical dosage form

Ravshankhon Makhmudov and Furkat Yunusov

Abstract
A reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for determination and quantification the pharmaceutically active ingredients Phenylephrine hydrochloride, Paracetamol, Caffeine and Chlorpheniramine maleate in tablets. The developed method was validated according to the ICH guidelines. Isocratic elution was performed with a mobile phase water: methanol:glacial acetic acid (70:25:5 v/v/v) mixture. The flow rate was 1.0 mL min^{-1} and UV detection was at 275 nm. The internal standards Phenylephrine hydrochloride, Paracetamol, Caffeine and Chlorpheniramine maleate retention times were within 4.07, 6.80, 9.70 and 26.46 minutes, respectively. The developed method was accurate, precise, robust and rapid enough. So it was applied successfully for the quality control assay of Paracetamol, Caffeine, Phenylephrine hydrochloride and Chlorpheniramine maleate in tablet dosage form.

Keywords: RP-HPLC, Paracetamol, Caffeine, Phenylephrine hydrochloride, Chlorpheniramine maleate, validation, pharmaceutical dosage form

Introduction
Paracetamol (PAR), Phenylephrine hydrochloride (PE) and Chlorpheniramine maleate (CPM) are commonly used in clinical practice as antipyretic and analgesic drugs to ameliorate pain and fever in cold and flu conditions. Paracetamol, also known as acetaminophen, is a medication used to treat fever and mild to moderate pain [1-4]. At a standard dose paracetamol only slightly decreases body temperature. Paracetamol significantly relieves pain in acute migraine but only slightly in episodic tension headache. However, the paracetamol/caffeine combination helps with both conditions and is recommended as a first-line treatment for them. Phenylephrine is a medication primarily used as a decongestant, to dilate the pupil, to increase blood pressure, and to relieve hemorrhoids [5]. Chlorpheniramine maleate, being a first-generation alkyl amine antihistamine, H_1 - receptor antagonist, is used to treat runny nose, sneezing, itching, and watery eyes caused by allergies, the common cold, or the flu by reducing the effects of natural chemical histamine in the body [6]. Caffeine (CAF) is a central nervous system (CNS) stimulant of the methylxanthine class. It is the world's most widely consumed psychoactive drug [7].
HPLC provides a sensitive and precise technique to separate and to identify active compounds in medical products. Reverse phase-high performance chromatography (RP-HPLC) is a common analytical method widely used for the development and characterization of pharmaceutical and natural substances [8-10].



Experimental

Materials and reagents: Paracetamol was obtained from Changshu Huagang Pharmaceuticals Co. Ltd (China), Phenylephrine hydrochloride was received from Shenzhen Oriental Pharmaceuticals Co. Ltd (China), Chlorpheniramine maleate was obtained from Supriya Life science Ltd. (India) and Caffeine was supplied by Siegfried Pharma Chemikalien Minden GmbH (Germany).

HPLC grade acetonitrile was purchased from VWR Chemical (France). Water was purified by a Milli-Q water purification system (Millipore, Bedford, MA, USA).

Equipment

The modular Shimadzu Nexera X2 HPLC system is consisted of the following parts, each of them is produced by Shimadzu USA Manufacturing, Inc.: An autosampler SIL-30AC, a liquid chromatograph LC-30AD, a column oven CTO-30A; a binary pump; a prominence UV–Vis detector SPD-20AV; a degassing unit DGU-20A5R and a communications bus module CMB-20A. The chromatographic parameters (peak areas, retention times, theoretical plates etc.) were recorded and calculated by Shimadzu LabSolutions v.5.73 software running under MS Windows 7. HPLC experiments were performed isocratically at room temperature on a stainless-steel column filled with octadecylsilane chemically bonded to porous silica particles (InertSustain C18, 5 µ, 250 mm x 4.6 mm, Japan). All pH measurements were performed on «Metrohm» 827 pH Lab (Switzerland). Weighing was carried out on an electronic analytical scales QUINTIX 224-10RU, Sartorius Lab Instrument GmbH (Germany).

Chromatographic conditions

Instrument: HPLC Shimadzu Nexera X2, Japan;
Equipment: Liquid Chromatograph with UV detector with variable wavelength and column-thermostat;
Column: InertSustain C18 5 µm 4.6 x 250 mm or equivalent.
Flow rate: 0.5 ml/min;
Detection wavelength: 275 nm;
Injection volume: 5 µl;
Column temperature: Room temperature;
Degassing: Ultrasonic;
Filter: 0.22 µm syringe filter;
Mobile phase: Purified water, methanol, and glacial acetic acid in the ratios of 70:25:5.

Method validation

The suggested method was subjected to validation for the several parameters such as the system suitability, specificity, range and linearity, accuracy and precision in accordance with the International Conference on Harmonization guidelines (ICH) [11]. Then the system suitability, repeatability, peaks symmetry (symmetry factor), theoretical plates of the column, resolution between the peaks, tailing factor and relative retention time were assessed. The concentration of each active compound in solution was as follows: 20 µg/ml CPM, 100 µg/ml PE, 5000 µg/ml PAR and 300 µg/ml CAF in placebo. The chromatograms of the test sample solutions were compared with that of the reference standard. The linearity of an analytical method is of high importance for the confirmation of the method's sensitivity for the analysis of the analyte's concentration within a defined range, which can be explained as its capability to show that the results are directly proportional to the concentration of the analyte in the sample. As per the method validation ICH Q2(R1) guideline, linearity of a given response must be evaluated using at least a minimum of 5 concentrations of the analyte (multi-point calibration) and the data must be statistically analyzed, e.g. by performing regression analysis using the method of the least squares. So the calibration curve was graphically plotted using increasing amounts of a standard solution (80, 90, 100, 110 and 120%) of all four active ingredients according to the ICH guidelines and this proposed method was evaluated by its correlation coefficient and intercept value which were found within the limit.

The next step of the validation the accuracy was studied as mean % recovery. In this step the recoveries of the model samples composed of the active components CPM, PE, PAR and CAF in placebo at the concentration range 80, 100 and 120% were investigated.

The experiments on the repeatability of the method have been performed twice on different days. On each day we studied six solutions of 100% concentration of those four active components of the drug. The% RSD (the relative standard deviation) of each component of the drug is determined to be less than 2%.

Preparation of standard solutions

1. Preparation of a standard solution of Chlorpheniramine maleate (CMP)

A working standard solution containing CPM 1.0 mg/ml was prepared by dissolving 25 mg (accurately weighed) of CPM (BP, USP) in a 25 ml volumetric flask, dissolved in 15 ml of 0.1 M hydrochloric acid solution, sonicated for 15 minutes,

then the volume of the resulting solution brought with the mobile phase to the mark and mixed.

2. Preparation of a mixed standard solution of Paracetamol, Caffeine, Phenylephrine hydrochloride and Chlorpheniramine maleate

About 500 mg (accurately weighed) paracetamol (PAR) (BP, USP), about 10 mg (accurately weighed) phenylephrine hydrochloride (PE), about 30 mg (accurately weighed) caffeine (CAF) (BP, USP) were placed in a 100 ml volumetric flask, dissolved in 60 ml 0.1 M hydrochloric acid solution, sonicated until dissolved for 5 min, 2 ml of a standard solution of chlorpheniramine maleate (CPM) was added, brought the volume of the resulting solution to the mark with the mobile phase and mixed.

Preparation of sample solutions

Finely powdered and accurately weighed sample containing 20 µg/ml CPM, 100 µg/ml PE, 5000 µg/ml PAR and 300 µg/ml CAF was transferred into a 100 ml volumetric flask. About 50 ml of 0.1 M hydrochloric acid was added and solution was ultrasonicated for 15 min, finally its volume adjusted to the mark by 0.1 M hydrochloric acid, mixed and filtered.

Results and Discussion

Quality control of drug products is of highest practical importance. Of instrumental methods of analysis HPLC method is most often used for the quantitation of drug substances. The purpose of this investigation was to develop a rapid, sensitive, accurate, precise and reliable HPLC method for the analysis of the drug containing PAR, CPM, PE and CAF.

We developed the validation method for the determination active compounds PAR, CPM, PE and CAF in an isocratic mode using the reverse phase C_{18} column, which separated the drug active components efficiently. All studies have been carried out according to the ICH guideline.

The mobile phase consisting of purified water, methanol and glacial acetic acid in the ratios of 70:25:5 is found to be optimal.

Method Validation

System suitability check: To determine the suitability and effectiveness of this chromatographic system the system suitability check for this method has been done. The characteristic chromatographic parameters, such as the number of effective theoretical plates, resolution, asymmetry, detection limit and selectivity have been established and the obtained results are summarized in Table 1.

Table 1: System suitability check

Parameter	PAR	CAF	PE	CPM
Retention time (Rt/min)	6.80	9.70	4.07	26.46
Resolution (Rs)	11.3	9.1	-	23.8
Theoretical plates (N)	9287	11650	6592	10202
Tailing factor (T)	1.11	1.02	1.28	1.08

Range and linearity

The common guideline used for method validation, the ICH Q2 (R1), defines range as an interval from the upper to the lower concentration of the analyte in the sample e.g. drugs for which the analytical method has been demonstrated to work with acceptable level of trueness, precision, and linearity. The linearity studies for a method usually define the range for it.

Range is a parameter that needs to be evaluated during the validation of QC laboratory purity tests and assay methods. The method validation guideline ICH Q2(R1) recommends for assay tests the range should be 80 to 120% of the test concentration.

The suitability of the analytical method is proved by the linearity in the defined concentration range (Figure 2). The regression statistics are shown in Tables 2.1. - 2.4. The correlation coefficients (r) for PAR, CAF, CPM and PE were found to be 0.996, 0.997, 0.997 and 0.997 respectively. It indicated functional linear relationship between the analyte concentration and the peak area.

Accuracy

The accuracy was evaluated by the recovery of PAR, CAF, CPM and PE at three different percentage concentrations (80, 100 and 120%). The results of accuracy studies are shown in Tables 3.1 - 3.4. The recoveries of PAR, CAF, CPM, PE were calculated to be 99.06 - 99.94%, 99.08 - 100.71%, 99.50 - 101.16% and 97.42 - 100.88%, respectively, with the RSD 0.30%, 0.71%, 0.51% and 1.08%, respectively. As the RSD < 2, the method can be concluded to be accurate within the desired range.

Precision

The method repeatability was determined by inter-day precision. The experimental values gotten for the repeatability of PAR, CAF, CPM and PE in the samples are given in Table 4. The variability was calculated by performing the assay on two days, and the average RSD was < 2% (Tables 4.1 - 4.4). All the data received were within the acceptance criteria.

Specificity: The specificity of the drug formulation composed of PAR, CAF, CPM and PE with the excipient compounds was investigated. The resolution between the peaks of components was good.

Table 2.1: Linearity of Paracetamol

Sample	Paracetamol model sample	
	Concentration (mg/ml) from the declared content	Peak area, mAU
1	0,4030	808615
2	0,4580	943058
3	0,5010	999052
4	0,5560	1127519
5	0,6000	1189511
Unweighted linear regression: y=a+b·x		
Slope, b		1930411,257
Segment on the ordinate axis, a		41395,89115
Standard deviation, RSD		0,077854351
Correlation coefficient, r		0,995714251

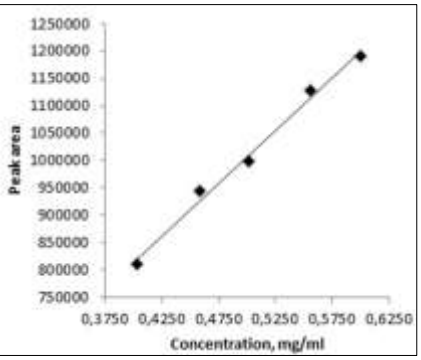


Table 2.2: Linearity of Caffeine

Sample	Caffeine model sample	
	Concentration [mg/ml] from the declared content	Peak area, mAU
1	0,24080	1395604
2	0,27590	1653809
3	0,30100	1744160
4	0,33110	1950414
5	0,36120	2113448
Unweighted linear regression: y=a+bx		
Slope, b		5872018,692
Segment on the ordinate axis, a		-1846,643021
Standard deviation, RSD		0,046848426
Correlation coefficient, r		0,996542718

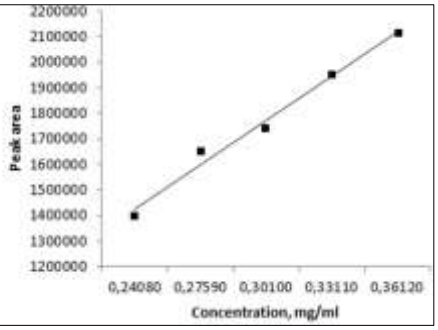


Table 2.3: Linearity of Chlorpheniramine maleate

Sample	Chlorpheniramine maleate model sample	
	Concentration [mg/ml] from the declared content	Peak area, mAU
1	0,01608	136738
2	0,01839	161965
3	0,02010	171298
4	0,02211	190584
5	0,02412	206963
Unweighted linear regression: y=a+bx		
Slope, b		8566657,415
Segment on the ordinate axis, a		806,6028212
Standard deviation, RSD		0,003133169
Correlation coefficient, r		0,996821364

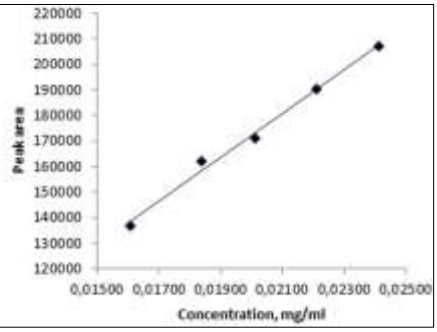


Table 2.4: Linearity of Phenylephrine hydrochloride

Sample	Phenylephrine hydrochloride model sample	
	Concentration [mg/ml] from the declared content	Peak area, mAU
1	0,08	440509
2	0,09	520401
3	0,10	550800
4	0,11	619068
5	0,12	672042
Unweighted linear regression: y=a+bx		
Slope, b		5748830,731
Segment on the ordinate axis, a		-18588,88538
Standard deviation, RSD		0,015517732
Correlation coefficient, r		0,997778263

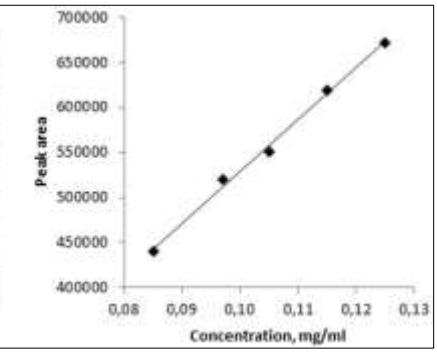


Table 3.1: Accuracy of Paracetamol

Active substance	#	Quantity entered [mg] and stated theoretical percentage		Amount recovery, mg	Amount recovery, %
Paracetamol	1	400	80%	398,039	99,51
	2	400	80%	396,667	99,17
	3	400	80%	398,165	99,54
	4	500	100%	496,083	99,22
	5	500	100%	499,710	99,94
	6	500	100%	495,288	99,06
	7	600	120%	598,518	99,75
	8	600	120%	597,387	99,56
	9	600	120%	595,401	99,23
Amount recovery [%]					99,44
95%-confidence interval					99,44 ±0,19
Standard deviation					0,29
RSD [%]					0,30

Table 3.2: Accuracy of Phenylephrine hydrochloride

Active substance	#	Quantity entered [mg] and stated theoretical percentage		Amount recovery, mg	Amount recovery, %
Phenylephrine hydrochloride	1	8	80%	7,934	99,18
	2	8	80%	7,946	99,33
	3	8	80%	7,994	99,93
	4	10	100%	10,071	100,71
	5	10	100%	10,040	100,40
	6	10	100%	10,006	100,06
	7	12	120%	12,120	101,00
	8	12	120%	11,917	99,31
	9	12	120%	11,890	99,08
Amount recovery [%]					99,89
95%-confidence interval					99,89 ±0,45
Standard deviation					0,71
RSD [%]					0,71

Table 3.3: Accuracy of Caffeine

Active substance	#	Quantity entered [mg] and stated theoretical percentage		Amount recovery, mg	Amount recovery, %
Caffeine	1	24	80%	23,892	99,55
	2	24	80%	23,895	99,56
	3	24	80%	24,021	100,09
	4	30	100%	30,017	100,06
	5	30	100%	29,964	99,88
	6	30	100%	29,910	99,70
	7	36	120%	36,418	101,16
	8	36	120%	35,952	99,87
	9	36	120%	35,819	99,50
Amount recovery [%]					99,93
95%-confidence interval					99,93 ±0,32
Standard deviation					0,51
RSD [%]					0,51

Table 3.4: Accuracy of Chlorpheniramine maleate

Active substance	#	Quantity entered [mg] and stated theoretical percentage		Amount recovery, mg	Amount recovery, %
Chlorpheniramine maleate	1	1,6	80%	1,584	99,00
	2	1,6	80%	1,604	100,25
	3	1,6	80%	1,614	100,88
	4	2	100%	1,977	98,85
	5	2	100%	1,973	98,65
	6	2	100%	1,966	98,30
	7	2,4	120%	2,374	98,92
	8	2,4	120%	2,353	98,04
	9	2,4	120%	2,338	97,42
Amount recovery [%]					98,92
95%-confidence interval					98,92 ±0,68
Standard deviation					1,07
RSD [%]					1,08

Precision

Table 4.1: Precision of the Method for Paracetamol

Active substance	#	The quantitative content of the model drug from the 100% theoretical content	
		Day 1	Day 2
Paracetamol	1	99,67	99,15
	2	99,41	99,75
	3	99,74	99,98
	4	99,22	99,58
	5	99,05	99,11
	6	99,42	99,36
Mean percentage		99,42	99,49
Standard deviation		0,26	0,34
RSD [%]		0,263	0,346
95%-confidence interval		99,42 ±0,21	99,49 ±0,28

Table 4.2: Precision of the Method for Phenylephrine hydrochloride

Active substance	#	The quantitative content of the model drug from the 100% theoretical content	
		Day 1	Day 2
Phenylephrine hydrochloride	1	99,48	98,75
	2	99,41	98,98
	3	99,21	99,03
	4	99,38	99,23
	5	99,19	99,25
	6	99,43	99,05
Mean percentage		99,35	99,05
Standard deviation		0,12	0,18
RSD [%]		0,122	0,185
95%-confidence interval		99,35 ±0,099	99,05 ±0,15

Table 4.3: Precision of the Method for Caffeine

Active substance	#	The quantitative content of the model drug from the 100% theoretical content	
		Day 1	Day 2
Caffeine	1	99,30	98,23
	2	98,38	98,83
	3	98,27	98,98
	4	98,76	98,92
	5	99,43	99,28
	6	98,47	99,14
Mean percentage		98,77	98,90
Standard deviation		0,49	0,36
RSD [%]		0,498	0,368
95%-confidence interval		98,77 ±0,4	98,9 ±0,3

Table 4.4: Precision of the Method for Chlorpheniramine maleate

Active substance	#	The quantitative content of the model drug from the 100% theoretical content	
		Day 1	Day 2
Chlorpheniramine maleate	1	99,00	99,59
	2	98,07	98,58
	3	98,50	98,27
	4	98,46	98,52
	5	98,17	98,24
	6	98,61	98,05
Mean percentage		98,47	98,54
Standard deviation		0,33	0,55
RSD [%]		0,337	0,557
95%-confidence interval		98,47 ±0,28	98,54 ±0,46

Conclusion

The simultaneous determination of PAR, CAF, CPM and PE in pharmaceutical dosage form by the reverse-phase HPLC was developed and by validation proved to be suitable for the quality control.

The method is simple, accurate selective and linear over the concentration range (80 - 120%) with a correlation coefficient of 0.998. All components of the drug including the excipients show no interference in the determination. The RSD was calculated to be less than 2% proving high precision of the proposed method. We hope chemists will find the method useful in a quality control of pharmaceuticals.

References

1. Dongre VG, Shah SB, Bayes GS, Phadke M, Jadhav VK. Simultaneous determination of chlorpheniramine maleate, phenylephrine hydrochloride, paracetamol and caffeine in pharmaceutical preparation by RP-HPLC. Chromatographica. 2009;69:1019-1023.
2. Warwick C. Paracetamol and fever management. Journal of the Royal Society for the Promotion of Health. 2008 Nov;128(6):320-323.
3. Saragiotto BT, Abdel Shaheed C, Maher CG. Paracetamol for pain in adults. BMJ. 2019 Dec;367:l6693.
4. Chiumello D, Gotti M, Vergani G. Paracetamol in fever in critically ill patients-an update. Journal of Critical Care. 2017 Apr;38:245-252.
5. Savic I, Nikolic G, Bankovic V. Synthesis and spectroscopic characterizatSion of some new (E)-3-(4-phenylthiazol-2-yl)-2-styrylquinazolin-4(3H)-ones. Macedonian Journal of Chemistry and Chemical Engineering. 2008;27(2):149-156.
6. Paton DM, Webster DR. Clinical Pharmacokinetics. 1983;10:477.
7. Burchfield G. What's your poison: caffeine. Australian Broadcasting Corporation; c1997. Available at: https://www.abc.net.au/health/library/stories/2008/02/14/2151848.htm. Accessed 2014 Jan 15.
8. Sahu PK, Ramisetti NR, Cecchi T, Swain S, Patro CS, Panda J. An overview of experimental designs in HPLC method development and validation. Journal of Pharmaceutical and Biomedical Analysis. 2018;147:590-611.
9. Assali M, Shawahna R, Dayyeh S, Shareef M, Alhimony IA. Dexamethasone-diclofenac loaded polylactide nanoparticles: preparation, release and anti-inflammatory activity. European Journal of Pharmaceutical Sciences. 2018;122:179-184.
10. Redasani VK, *et al*. Simultaneous determination of chlorpheniramine maleate, phenylephrine hydrochloride, paracetamol and caffeine in pharmaceutical preparation by RP-HPLC. Chemical Industry & Chemical Engineering Quarterly. 2013;19(1):57-65.
11. International Conference on Harmonization (ICH). Validation of analytical procedures: Text and Methodology. Geneva, Switzerland; c2005.