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Method development and validation of paracetamol drug by RP-HPLC

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Abstract

A simple and reproducible method was developed for paracetamol by Reverse Phase High Performance Liquid Chromatography (RP-HPLC). Paracetamol was separated on C18 column [4.6x250mm, particle size 5µm], using ortho phosphoric acid buffer with pH of 3.5 at the UV detection of 207nm. Isocratic elution of acetonitrile (ACN) and water was used as a mobile phase with various ratios and flow rates, eventually 25:75 v/v ACN and water was being set with the flow rate of 1mL/min. The statistical validation parameters such as linearity, accuracy, precision, inter-day and intra-day variation were checked, further the limit of detection and limit of quantification of paracetamol concentrations were found to be 120ng/mL and 360ng/mL. Recovery and assay studies of paracetamol were within 99 to 102% indicating that the proposed method can be adoptable for quality control analysis of paracetamol.

Key words: RP-HPLC, Validation, paracetamol, acetonitrile

Paracetamol is an acetanilide derivative chemically 4-hydroxy acetanilide having analgesic, antipyretic and weak antiinflammatory action[1,2] and also administered in the management of more severe pains in advanced cancers[3]. In literature several analytical techniques like colorimetric[4], spectroflurimetric[5] methods have been reported on assay of paracetamol in combination with other drugs. However pure drug analysis was studied by spectrometry[6] and scanty literature is available on paracetamol pure drug analysis with HPLC. Several HPLC methods using a variety of columns and detection techniques have been reported on paracetamol in combination with other drugs[7,8,9] and physical and chemical stability studies[10]. In the present study, novel method was developed with acetonitrile (ACN) as solvent in HPLC; it is a simple method to study, detect and separate the paracetamol from mixture of compounds and can be adopted for regular quality assessment in pharmaceutical industry and scientific laboratories.

Materials and Methods

All reagents used were of analytical-reagent grade. Water purification systems, reverse osmosis and

ultra pure water (Nanopure Human Corporation, Korea), sonicator (Digital citizen ultra sonic cleaner) for degassing of HPLC grade ACN and ortho phosphoric acid 88% (S.D. FineChem Limited, Mumbai, India) and pure paracetamol drug.

Chromatographic system

The RP-HPLC system composed of Agilent 1200 series instant pilot software: ChemStation Plus, EZChrom Elite, and certified for pharmaceutical QA/QC. It constitutes micro vacuum degasser G1379B, binary pump G1312B, diode array detector SL G1315C, thermostatted column compartment G1316B with C18 column [4.6x250mm, pore size 5µm], high performance autosampler G1367C, thermostat for high performance autosampler G1330B. It is most flexible configuration for the maximum in gradient and low flow rate accuracy and precision, high-speed, multi-wavelength and full spectral UV-visible detection for peak purity analysis and spectral confirmation. The chromatographic and integrated data were recorded in computer system.

Chromatographic conditions

Two solvents were used, solvent-A containing ACN filtered through 0.2µm filter paper and solvent-B containing ultra pure water filtered through 0.25µm PTFE (Poly Tetra Fluoro Thylene) filter [pH adjusted to 3.5 with 88% ortho phosphoric acid], degassed with sonication. Various flow rates and solvent compositions were provided at room temperature [28°C] to develop a method. The detection was performed at 207nm using diode array detector SL G1315C.

Method

Solution 10mg/mL of pure paracetamol was prepared in the mobile phase. The solution was adequately diluted to 50µg/mL and it was taken to develop a method by applying various solvent ratios and flow rates as shown in table 1 and chromatograms in figure 1.

Table 1: Acetonitrile and paracetamol ratios in mobile phase to resolve the paracetamol in HPLC column in method development

Percentage of solvents		**Flow rate mL/min**	**Retention time**	**Area under curve**	**Recovery %**
Solvent-A (ACN)	**Solvent-B (paracetamol)**				
80	**20**	1.00	2.4	17757574	68
75	**25**	1.00	2.5	18151357	70
70	**30**	1.00	2.5	18107124	69
65	**35**	1.00	2.5	18181693	70
60	**40**	1.00	2.6	18361606	70
50	**50**	1.00	2.7	17990111	69
45	**55**	1.00	2.9	18024387	69
40	**60**	1.00	3.0	17233765	66
25	**75**	0.75	4.8	31606577	121
25	**75**	1.00	3.6	26115405	100
25	**75**	1.50	2.6	16444903	62
25	**75**	2.00	2.1	13922704	53

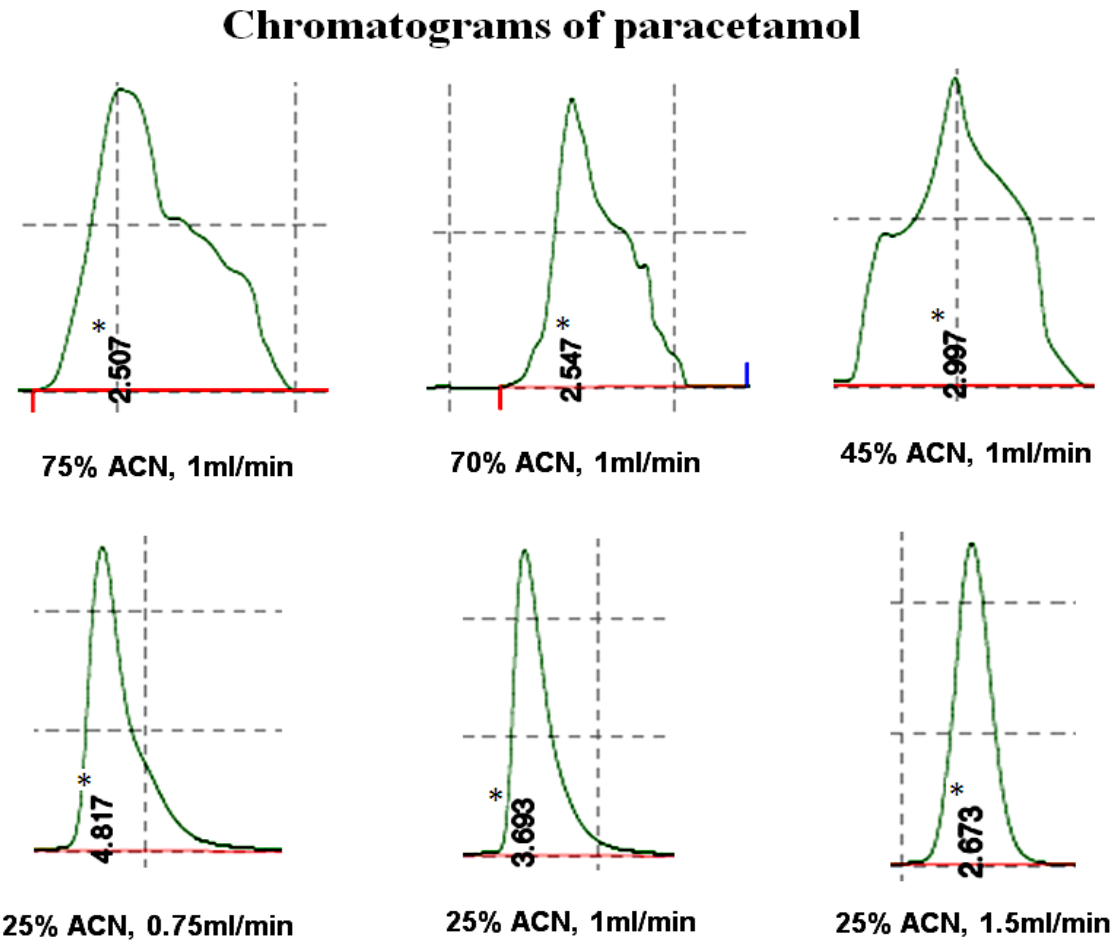

Fig 1. Chromatograms of paracetamol in varied ACN% and flow rates while method development. *Retention time

Validation of the method

Validation of the optimized HPLC method was carried out with the following parameters.

Linearity

Paracetamol standard stock solution of 10mg/mL was used for preparation of subsequent aliquots; aliquots of 100, 50, 25, 12.5 and 6.25 µg/mL concentrations were prepared by serial dilution. The solution of 200 µL was loaded in autosamplar tray and 20 µL was being injected into column. All measurements were repeated three times for each concentration. The calibration curves of the area under curve versus concentration were recorded.

Accuracy

Paracetamol standard stock solution of 10mg/mL was used to prepare 10, 35, 55 µg/mL concentrations and injected for the accuracy studies. The area under curve obtained was checked and analyzed for the recovery percentage.

Precision

The precision of method was checked and verified by repeatability, inter-day and intra-day precision. Repeatability was checked by injecting 80µg/mL concentration of paracetamol for 6 times on the same day and for intraday precision one concentration was injected and analyzed at different time intervals on the same day. Similarly, for the inter-day precision another concentration was analyzed on different days.

Limit of detection (LOD) and limit of quantification (LOQ)

The LOD and LOQ of paracetamol were separately determined on the basis of signal (S) and noise (N) ratio, LOD and LOQ concentrations of paracetamol

were confirmed and recorded by the S/N ratio where 3 and 9 were shown.

Robustness of the method

To determine the robustness of the developed method, minute changes were made in the flow rate, percentage of ACN and the pH of the mobile phase and is studied for the deviations from optimized method.

System suitability parameters

To perform the system suitability tests the standard solution was freshly prepared and injected under the condition of optimized method to study the following parameters.

Analysis of the marketed drug

Ten tablets of paracetamol (Crocin) were weighed and crushed into fine powder. The average weight for one tablet was calculated and weighed. The weighed fine powder of paracetamol tablet was dissolved in 100 mL of mobile phase in volumetric flask by shaking it for 30 minutes. Then, the solution was kept for ultrasonication for 20 min and filtered through 0.2 µm filter paper. Further dilutions were made for the analysis of the drug content in the tablet.

Results and discussion

The developed and validated method of paracetamol was aimed to establish chromatographic conditions, capable of qualitative and quantitative determination of paracetamol in pharmaceutical preparations. Paracetamol was completely separated on C18 column by RP-HPLC using the isocratic elution of ACN and water as mobile phase. When the ACN percentage was reduced starting from 80% by a decrement of every 5%, broadening, fronting and tailing of peaks were observed (Fig 1). As a result of higher concentrations of ACN in mobile phase and decrease in the percentage of ACN the peak was sharp pointed and well separated. The unusual peaks could be the result of improper dissolution of paracetamol in higher concentration of ACN, therefore the chromatographic column was not able to resolve the paracetamol properly and even there was decrease in the recovery percentage of paracetamol (Table 1). As ACN concentration gradually decreases the peak broadening, fronting and tailing were remarkably reduced. It is evident that the flow rate of mobile phase in chromatography plays an important role in resolving the paracetamol, as the flow rate increases from 0.75 mL/min to 1.50 mL/min the retention time also decreased with fluctuation in paracetamol recovery (Table 1), eventually proper resolution was achieved at flow rate of 1mL/min and retention time of 3.6 minutes.

Linearity

The method gave a linear response to paracetamol drug within the concentration range of 6.25 - 100 µg/mL with r^2= 0.999 as shown in figure 2.

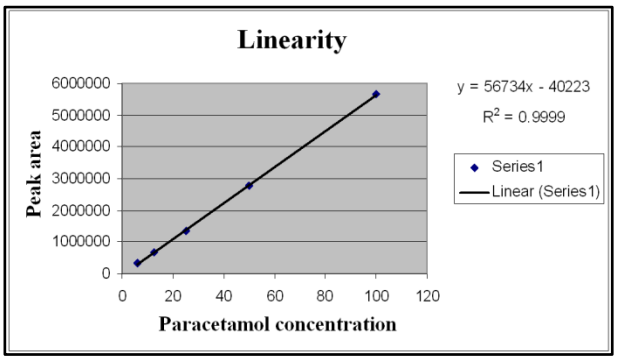

Fig 2. Linear response of peak area against paracetamol concentration

Accuracy

The paracetamol was recovered in the range of 98.8 to 102.0 % for various concentrations as shown in the table 2.

Table 2: Recovery percentage of paracetamol in accuracy studies

Concentration (µg/mL)	Area	Amount Recovered	Recovery (%)
10	520308	09.88	98.8
35	1963621	35.32	100.9
55	3143689	56.12	102.0

Precision

The repeatability, intra-day and inter-day precision results are shown in the table 3. The RSD values were below 3%, indicating a good precision. The t-test value for inter-day precision was less than 0.1%, indicating the significant precision.

Table 3: Developed method was checked for precision with different intervals

Repeatability					
Injection No.	**Area**	**Amount Recovered**		**Recovery %**	
1	4498501	80.00		100.00	
2	4517340	80.33		100.40	
3	4527683	80.69		100.86	
4	4575290	81.35		101.68	
5	4602753	81.83		102.28	
6	4624520	82.22		102.77	
Mean: 4559348, SD: 49642.59, RSD: 1.088					
Intra-day precision					
Time (hrs)	**Concentration (µg/mL)**	**Mean of area**	**SD**	**RSD**	
0	25	1352231	15349	1.13	
3	25	1357626	7423.9	0.54	
0	50	27796225	44542	1.60	
3	50	2767559	27159.2	0.98	
Inter-day precision					
Day	**Concentration (µg/mL)**	**Mean of area**	**SD**	**RSD**	**t-test**
1	100	5645864	17302.9	0.30	0.009
4	100	6526371	169296	2.59	
1	6.25	338998	335.16	0.09	0.0003
4	6.25	378366	1371.78	0.36	

LOD and LOQ

The LOD and LOQ concentrations of paracetamol were found to be 120ng/mL and 360ng/mL.

Robustness of the method

The robustness of the method gave the mean, standard deviation (SD) and RSD within the limits. Results are shown in table 4.

Analysis of marketed drug

The labeled amount of paracetamol in two different tablet strips of 250 and 500 mg/tablet recovered 99.56% and 99.75% respectively. The RSD value is below 2%. The retention time was found to be 3.6 min and the results are shown in table 5.

System suitability studies

The system suitability parameters such as retention time, capacity factor, theoretical plate number, peak purity and resolution factor of optimized method were associated with confined values as shown in the table 6.

Conclusion

The optimized reverse phase HPLC method for paracetamol is linear, accurate, precise, robust, simple, rapid and selective. It can be adopted apparently for routine quality control analysis of raw materials, formulations and testing.

Table 4: Slight deviation from the optimized parameters to check the robustness of the method

Factor	Level	Retention time	Theoretical plates	Area	% content
Flow rate (mL/min)					
0.9	-1	3.98	9308	1341377	97.4
1.0	0	3.69	9272	1363085	98.9
1.1	+1	3.21	9231	1330170	96.6
Mean		3.62	9270.3	1344877	97.6
SD		0.38	38.52	16734.35	1.16
RSD		1.07	0.41	1.24	1.19
% of ACN in the mobile phase (v/v)					
24	-1	3.62	9227	1341608	97.4
25	0	3.69	9272	1363085	98.9
26	+1	3.71	9381	1338798	97.2
Mean		3.67	9293.3	1347830	97.8
SD		0.04	79.18	13285.43	0.92
RSD		1.28	0.85	0.98	0.94
pH of mobile phase					
3.4	-1	3.73	9260	1329658	96.5
3.5	0	3.69	9272	1363085	98.9
3.6	+1	3.62	9189	1332413	96.7
Mean		3.68	9240.3	1341719	97.3
SD		0.05	44.85	18554.9	1.33
RSD		1.51	0.48	1.38	1.36

Table 5: Recovery studies of paracetamol in marketed drug

Labelled amount (mg/tablet)	Amount found	Recovery (%)	SD	RSD
250	248.92	99.56	0.76	0.30
500	498.72	99.74	0.9	0.18

Table 6: System suitability parameters of optimized method

S. No.	System suitability parameters	Associated values
1	Retention time	3.69
2	Capacity factor	17.4
3	Theoretical plate number	3631
4	Resolution factor	0.0

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Conflict of interest: None

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