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Development and Validation of analytical method for parenteral drug acetaminophen by using HPLC

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ABSTRACT:

Acetaminophen is an effective antipyretic and analgesic agent. The study was conducted to develop and validate a precise, simple and rapid RP-HPLC method for the estimation of Acetaminophen in bulk and parenteral formulation. Study was performed with column (Symmetry C18 (4.6 x 250mm, 5µm). A Methanol: Phosphate Buffer (pH6.8) in the ratio of 75:25 was used to create an isocratic mobile elution phase. A UV detector was used for 245 nm detection, which had an 20 µL injection volume, a flow rate of 1.0 mL/min, and an 35°C column temperature. Acetaminophen was found to have retention time of 4.963 minute. The approach was validated for accuracy, specificity, selectivity, precision, linearity, sensitivity and robustness. Plots of linear calibration were made for concentrations between 50- 250 µg/mL. The correlation coefficient (r^2) for the proposed approach was determined to be 0.999. Evaluation of system and method accuracy revealed that the technique is accurate within the acceptable range. In particular, the number of theoretical plates, tailing factor, and RSD were calculated for both solutions. It was evident from the results that the suggested method works well for the quick, precise, and accurate measurement of Acetaminophen.

Key words- Acetaminophen, RP-HPLC, Parenteral formulation.

INTRODUCTION:

High Performance Liquid Chromatography (HPLC) is now one of the most formidable instruments in analytical chemistry. It is essential to the discovery, development, and production of pharmaceutical goods. It has the capability to separate, identify, and quantify the chemicals contained in any material that is soluble in a liquid (1,2). The advancements in HPLC analytical methodologies, influenced by several factors, provide substantial data throughout analytical measures. Despite HPLC being a flexible separation technology with several applications, the procedure may be challenging owing to the multitude of variables that must be meticulously calibrated prior to each run. The optimization of HPLC procedures is a difficult process, since several factors (such as buffer concentration, mobile phase pH, flow rate, detector wavelength and column temperature,) must be simultaneously regulated to achieve the required separations (3). Currently, reversed-phase chromatography is the predominant separation technology used in HPLC owing to its extensive variety of applications. It is believed that more than 65% (perhaps up to 90%) of all HPLC separations are conducted in reversed phase mode. The rationale for this encompasses the simplicity, adaptability, and breadth of the reversed-phase approach, which can accommodate molecules with varying polarity and molecular weight (4).

Acetaminophen (Figure 1) is an efficacious mild analgesic and antipyretic agent(5). By activating descending serotonergic pathways it shows central analgesic effect. There is contention over its principal site of action, which may include the active metabolite affecting cannabinoid receptors or an suppression of prostaglandin (PG) production (6).

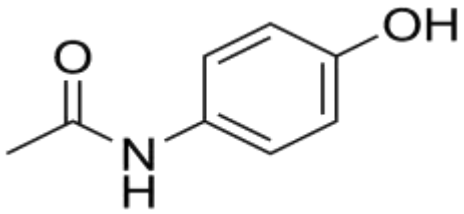

Figure 1: Structure of Acetaminophen

There are different HPLC analytical methods in formulations reported in the literature for acetaminophen alone (7–9), acetaminophen with other agents (10–17). Literature also shows the use of UPLC technique (16) and UV-Visible method acetaminophen alone and in combination with other agents (18–20) for the determination of from different dosage form. The present study

is aiming to develop and validate a sensitive, simple, rapid, and economic RP-HPLC method for the determination of Acetaminophen by using chromatographic method.

METHODS:

Instrumentation:

For the Analytical study of Acetaminophen, Shimadzu UV-1900i UV-VIS spectrophotometer, HPLC system with Empower software was used. The Waters alliance system with 2695 quaternary pump, auto sampler, column (Symmetry C18 (4.6 x 250mm, 5µm) and PDA 2996 were used.

Chemicals and Reagents:

The Pure API of Acetaminophen was obtained as a gift sample from Wockhardt Research Centre, Chhatrapati Sambajinagar. The Formulation was procured from the local market. Acetonitrile, Methanol, sodium dihydrogen phosphate monohydrate, potassium dihydrogen phosphate, disodium hydrogen phosphate anhydrous used were of HPLC grade.

Preparation of standard stock solution

25mg of Acetaminophen were precisely weighed and put into 25 mL volumetric flasks. 15ml of diluent is added to the flask, sonicated, and the volume is adjusted to 25ml using diluent to achieve a 1000 µg/mL solution of Acetaminophen. 1 ml of this solution was withdrawn and diluted it to a total volume of 10 ml with the diluent. Additional dilutions were made for the investigation according to requirements from the stock solution of 1000 ppm or 100 ppm.

Detection of wavelength:

The standard solution was examined between 200 and 400 nm in the UV spectrophotometer, with the diluents acting as a blank. The λ_{max} of acetaminophen is 245 nm. Thus, the detection wavelength of the RP-HPLC study was set at 245 nm as shown in figure 2.

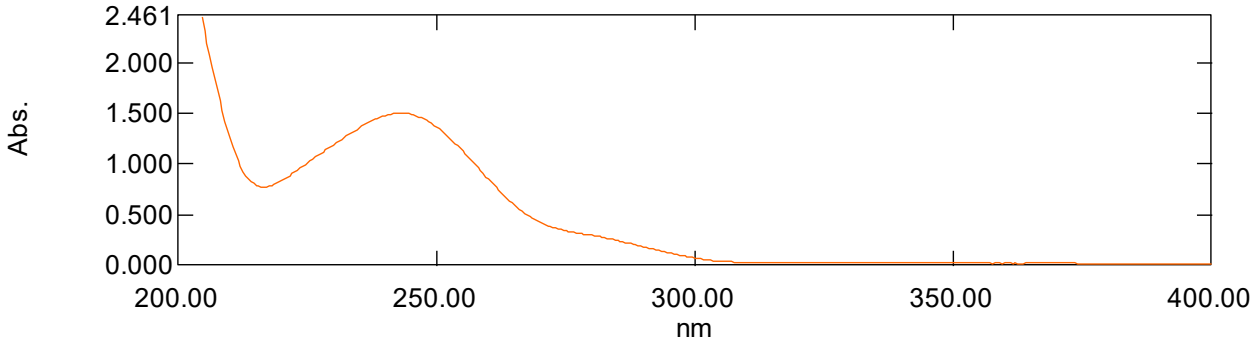

Figure 2: Absorbance maxima of Acetaminophen

METHOD DEVELOPMENT

Preparation of Mobile Phase:

Phosphate Buffer (pH6.8) and methanol (25:75 v/v) was prepared and sonicated it for 15 minutes and used for the analysis of Acetaminophen.

Optimization of Chromatographic Conditions and Method Development

Various mobile phase compositions were used to obtain the optimum resolution and separation in order to design an appropriate and reliable RP-HPLC technique for the estimation of Acetaminophen. Using a mobile phase including water and an organic modifier such as methanol or acetonitrile, the method development process was started using a symmetry C18 column. Peaks were not clearly separated with this mobile phase. To improve the peak shapes, phosphate buffer was used in place of water. It resulted in sharp peaks. Finally the best results were observed using a column Symmetry C18 (4.6 x 250mm, 5µm), mobile phase consisting of Methanol: Phosphate Buffer (pH6.8) (75:25 v/v) at a flow rate of 1.0 ml/min. In this selected parameters of chromatographic conditions it gives all results within acceptable limit for Acetaminophen.

RESULT AND DISCUSSION:

Optimization of Chromatographic Conditions:

Utilizing the physiochemical features of Acetaminophen and existing literature on analytical procedures for similar drugs, several solvent systems were used to achieve enhanced separation, specifically in terms of resolution and selectivity. The various mobile phases used to attain optimized chromatographic conditions for the separation and quantification of Acetaminophen. Inadequate resolution, suboptimal peak forms, and baseline disturbances were among the primary reasons for the rejection of the trials. Figure 3 illustrates the optimized trail chromatogram.

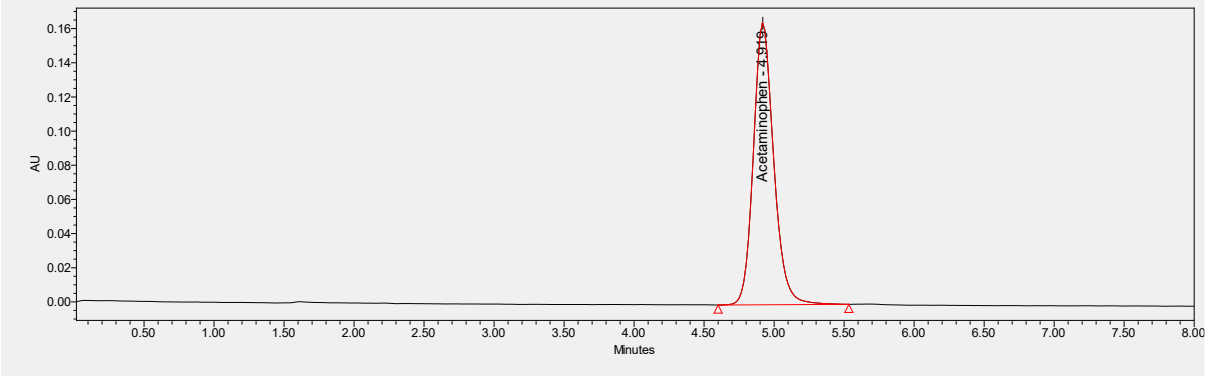

Figure 3: Optimized trial for Method development of Acetaminophen

Chromatographic Conditions

Based upon system suitability results following are the parameters finalized to run RP-HPLC system for estimation of acetaminophen:

Column : Symmetry C18 (4.6 x 250mm, 5µm).

Column Temp : 35°C.

Wave length : 245 nm.

Run Time : 08 min.

Pump Mode : Isocratic.

Flow Rate : 1.0 ml/min.

Retention time : About 4.9 minutes (Acetaminophen).

Injection Volume: 20 µl.

Method Validation by RP-HPLC

a. System Suitability Study;

An HPLC technique has been established for quantifying the percentage assay of Acetaminophen. The retention time (RT) for acetaminophen was determined to be 4.986 minutes, and other characteristics such as tailing factor, resolution, and theoretical plates (see Table 1) were within acceptable limits.

Table 1: System Suitability Parameters for Acetaminophen

Sr. No.	Name	RT (min)	Area	Tailing Factor	Theoretical PlateCount	Selectivity	Resolution
1	Acetaminophen	4.963	3114180	1.1280	7995.863	2.603	10.784

b. Specificity

The lack of supplementary peaks in the chromatogram indicates the absence of excipient interference. The blank exhibited no interference during the retention period of the analyte peaks.

c. Linearity and Range

Linearity was established by creating standard solutions of Acetaminophen within the range of 50-250 µg/ml. Twenty microlitres of each concentration were put into the HPLC. The response

was measured at 245 nm, and the associated chromatograms were documented. The mean peak areas were derived from the chromatograms, and individual linearity plots of concentration against mean peak areas were generated. The regressions of the plots were calculated using the least squares regression technique (Table2 and Figure 4).

Table 2: Linearity and Range for Acetaminophen

Level	Concentration(µg/ml)	PeakArea
Level 1	50	1619110
Level 2	100	3138439
Level 3	150	4740722
Level 4	200	6447563
Level 5	250	8096812
Slope		32529.0560
Intercept		-70829.2000
Correlation coefficient		0.999

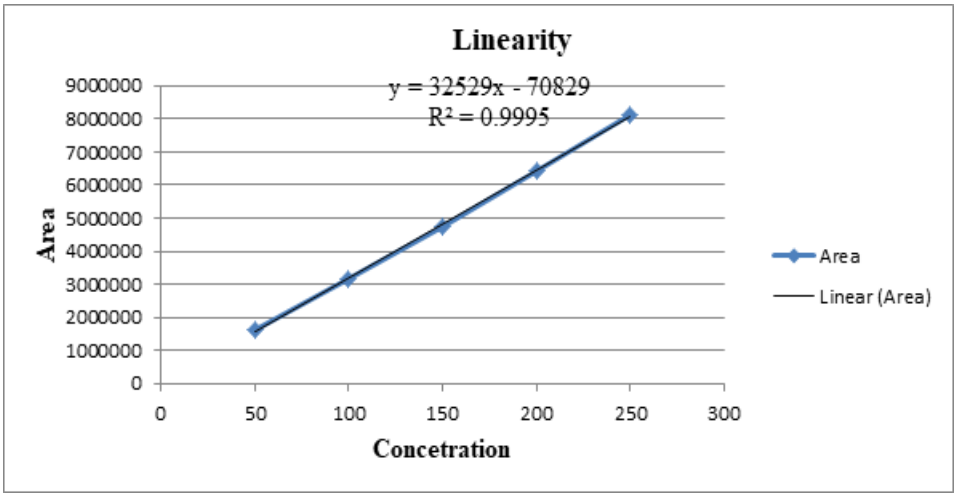

Figure 4: Standard Calibration Curve for Acetaminophen

d. Precision

i. System Precision

It was performed by taking of peak area for standard drugs solutions in five replicates. % RSD for Acetaminophen was found to be 1.15% (Table 3).

Table 3: System Precision Data of Acetaminophen

Sr. No.	Peak areas of Acetaminophen
1.	1673761
2.	1666587
3.	1663328
4.	1629170
5.	1640216
Mean	**1654612.4**
SD (±)	**18977.57**
RSD (%)	**1.15**

ii. Method Precision

It was performed by taking the peak area for sample solutions in five replicates. The % assay for Acetaminophen five samples was calculated (Table 4).

Table 4: Method Precision Data of Acetaminophen

Sample No.	% Assay of Acetaminophen(w/w)
1.	98.55
2.	98.11
3.	97.91
4.	95.81
5.	96.49
Mean	**97.38**
SD (±)	**1.167**
RSD (%)	**1.12**

iii. Intraday and Inter-day Precision

The % RSD in intraday precision for Acetaminophen (50, 150, 250 µg/ml) was found to be 1.4269, 0.6289, 0.2602%. In inter-day precision % RSD for Acetaminophen (50, 150, 250 µg/ml) was found to be 0.934, 0.686, 0.392 %. % RSD in intraday and inter-day studies were found well within the acceptable limits (Table 5 and 6).

Table 5: Intraday Precision data of Acetaminophen

Sr. no.	Conc. (µg/ml)	Area	Mean Peak Area	SD(±)	%RSD
1	50	1601143	1625642.667	23196.853	1.4269
		1647269			
		1628516			
2	150	4720677	4744646.333	29840.988	0.6289
		4778069			
		4735193			
3	250	8004859	8028819.667	20887.044	0.2602
		8043184			
		8038416			

Table 6: Inter-day Precision data of Acetaminophen

Sr. no.	Day	Conc. (µg/ml)	Peak Area	Mean Peak Area	SD(±)	%RSD
1	1	50	1670968	1661427.33	15520.16	0.934
	2		1643519			
	3		1669795			
2	1	150	4773607	4736651.667	32487.06	0.686
	2		4723754			
	3		4712594			
3	1	250	8010163	8045714	31549.93	0.392
	2		8056598			
	3		8070381			

e. Accuracy (Recovery Study)

The standard addition procedure was conducted at 80%, 100%, and 120% levels. The solutions were examined in triplicate at each level according to the specified methodology. The percentage recovery and % RSD were determined, and the findings are reported in Table 7. The suggested

approach yielded satisfactory recoveries between 98.36 and 99.20. This signifies that the recommended procedure was accurate.

Table 7: Recovery study for Acetaminophen

Level	Set	Amount added(µg/ml)	Amount found(µg/ml)	Mean Conc.	SD	%RSD	%Recovery
80%	1	160	178.613	178.561	0.148	0.083	99.20
	2	160	178.394				
	3	160	178.676				
100%	1	200	195.7724	196.563	0.702	0.357	98.28
	2	200	196.8060				
	3	200	197.1134				
120%	1	240	7154499	216.402	1.210	0.559	98.36
	2	240	7079222				
	3	240	7096812				

f. Sensitivity:

The LOD and LOQ for Acetaminophen were determined to be 0.72547 µg/ml and 2.19840 µg/ml, respectively. These figures demonstrate that the approach is appropriate for ascertaining lower concentrations and affirm that the suggested method is sensitive for this conclusion.

g. Robustness

The robustness investigation was conducted by making minor adjustments to the flow rate of the mobile phase. No significant alterations were noticed in the chromatograms, indicating that the new approach exhibited robustness. The findings of the robustness study are shown in Table 8.

Table 8: Robustness data of Acetaminophen

Flow Rate	Parameters			
0.8 ml/min				
	RT	**Area**	**Theoretical Plates**	**Tailingfactor**

Average	5.053	1648731	7536.67	1.24
S. D	0.0270	21423.70	68.595	0.0153
% RSD	0.5348	1.2994	0.910	1.234
1.2 ml/min				
Average	4.826	1644238	6681.00	1.10
S. D	0.0053	17430.57	100.000	0.0100
% RSD	0.1096	1.0601	1.497	0.909

h. Analysis of Marketed Formulation

The concentrations in the samples were determined using a multilevel calibration method that was established on the same HPLC equipment and under the same circumstances. This calibration method used a linear regression equation. The findings are shown in Table 9.

Table 9: Analysis of marketed formulations for Acetaminophen

Injection	**Formulation 1**		**Formulation 2**	
	Peak Areas	**%Assay**	**Peak Areas**	**%Assay**
1	3118036	98.031	3154499	99.15
2	3154499	99.152	3149222	98.99
3	3140365	98.718	3149982	99.01
4	3137419	98.627	3155338	99.18
5	3125882	98.272	3153664	99.13
Mean	3135240.2	98.560	3152541	99.09
SD	14013.141	0.431	2760.541	0.085
% RSD	0.447	0.437	0.088	0.086

Conclusion:

The proposed method has been validated to evaluate its robustness, ruggedness, linearity, LOD, and LOQ. The validation findings indicate that these parameters conform to the permissible

limits set by ICH standards. The proposed HPLC method for quantifying Acetaminophen in pharmaceutical formulations was found to be user-friendly, accurate, reliable, and sensitive. This method may efficiently conduct regular quality control assessments of both pure and therapeutic dose forms of Acetaminophen. The newly developed and validated method for Acetaminophen, in both parenteral and bulk forms, may be advantageous for researchers engaged in the development of advanced drug delivery systems and subsequent investigations.

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