International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2013): 6.14 | Impact Factor (2014): 5.611

RP-HPLC Method Development and Validation for the Analysis of Pharmaceutical Drugs – Paracetamol

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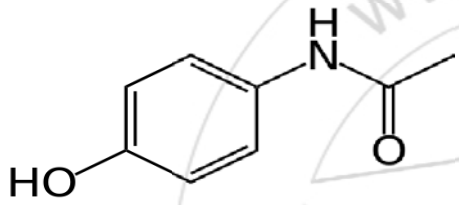
Abstract: ***A simple, selective, linear, precise and accurate RP-HPLC method was developed and validated for rapid assay of PARACETAMOL. Isocratic elution at a flow rate of 1.0 ml /min was employed on a symmetry C18 column at ambient temperature. The mobile phase consisted of Acetonitrile: 0.1M Acetic Acid 50:50 (v/v). The UV detection wavelength was at 210 nm. Linearity was observed in concentration range of 100-140 mg/ml. The retention time for Paracetamol was 3.0 min. The method was validated as per the ICH guidelines. The proposed method can be successfully applied for the estimation of Paracetamol.***

Keywords: Paracetamol, Method Development, Validation, 210nm

1. Introduction

DRUGS: Paracetamol

Structure:



IUPAC NAME	*N*-(4-hydroxyphenyl)ethanamide
FORMULA	$C_8H_9NO_2$
MOLACULAR WEIGHT	151.163 g/mol
DENSITY	1.263 g/cm³
MELTING POINT	169 °C (336 °F) [2][3]
SOLUBILITY	12.78[1] mg/mL (20 °C)

PHARMACOKINETIC DATA

BIOAVAILABILITY	~100% (oral)
PROTEIN BINDING	Low (31%)
METABOLISM	90 tae 95% Hepatic
HALF-LIFE	1–4 h
EXCRETION	Renal

Paracetamol, also known as **acetaminophen** or **APAP**, is a widely used over-the-counter pain medication and medication to reduce fever.[3][4] It is commonly used to help with headaches, other minor aches and pains, and is a major ingredient in many cold medications. In combination with opioid analgesics, paracetamol is used in the management of more severe pain such as post-surgical and cancer pain.[5] Though paracetamol is used to treat inflammatory pain, it is not classified as an NSAID because it exhibits only weak anti-inflammatory activity. While generally safe for use at recommended doses, even small overdoses can be fatal. Compared to other over-the-counter pain relievers, paracetamol is significantly more toxic in overdose but may be less toxic when used chronically at recommended doses.[6] Paracetamol is classified as a mild analgesic.

2. Experimental

Chemicals and reagents

All HPLC SOLVENTS used like Acetonitrile, Acetic Acid which are of HPLC grade were purchased from E.Merck,

Instrumentation and analytical conditions

The analysis of the drug was carried out on Shimadzu HPLC model (VP series) containing LC-20AT (VP series) pump, variable wave length programmable UV/visible detector SPD-20A and rheodyne injector (7725i) with 20µl fixed loop. Chromatographic analysis was performed using phenolex C-18 column with 250 x 4.6mm internal diameter and 5µm particle size. Shimadzu electronic balance (AX-200) was used for weighing. Isocratic elution with, Acetonitrile, 0.1MAcetic Acid 50:50(v/v) was selected with a flow rate of 1.2 ml /min .The detection wavelength was set at 210 nm with a run time of 10 min. The mobile phase was prepared freshly and it was degassed by sonicating for 5 min before use. The column was equilibrated for at least 30min with the mobile phase flowing through the system. The column and the HPLC system were kept at ambient temperature.

Preparation of Stock, working standard solutions and Sample solutions

10 mg of Paracetamol was weighted and transferred into a 10 ml volumetric flask. Water was added and sonicated to dissolve it completely and made up to the mark with the same solvent. Further 10 ml of the above stock solution was pipette into a 100ml volumetric flask and diluted up to the mark with water. The contents were mixed well and filtered through Ultipor N66Nylon 6, 6 membrane sample filter paper. The calibration curve was plotted with the concentrations of the 100 to 140 ppm solutions. Calibration solutions were prepared and analyzed immediately after preparation.

Table 1 chromatographic condition for Paracetamol

S.NO	*Test*	*Result*
	H.P.L.C CONDITIONS	
1	Elution	ISOCRATIC
2	A.P.I Conc.	100ppm
3	Mobile Phase	Acetonitrile:0.1M Acetic Acid(50:50)
4	pH	3.0
5	Column	C18
6	Wavelength	210 nm
7	Flow Rate	1.2ml/min
8	Runtime	10 Min
9	Retention Time	2.850
10	Area	1525.843
11	Th.Plates	9950
12	Tailing Factor	1.089
13	Pump Presure	75 kgf

Method Validation procedure

The objective of the method validation is to demonstrate that the method is suitable for its
Intended purpose as it is stated in ICH guidelines. The method was validated for linearity, precision, accuracy, specificity, and limit of detection, limit of quantification, robustness and system suitability.

Linearity

Table 2: Linearity of Paracetamol

S. No	*Conc*	*Area*
1	100 ppm	1525.843
2	110 ppm	1678.427
3	120 ppm	1831.011
4	130 ppm	1985.595
5	140 ppm	2156.180

The developed method has been validated as per ICH guidelines.Solutions of Paracetamol in the mass concentration range of 100 ppm to 140 ppm was injected into the chromatographic system. The chromatograms were developed and the peak area was determined for each concentration of the drug solution. Calibration curve of Paracetamol was obtained by plotting the peak area ratio versus the applied concentrations of Paracetamol. The linear correlation coefficient was found to be 0.9997

Table.3 Linear Regression Data for Calibration curve

Drug	Paracetamol
Concentration range	100-140ppm
Slope (m)	15.25
Intercept (b)	0.843
Correlation coefficient	0.9997

Precision

Repeatability of the method was checked by injecting replicate injections of 100 ppm of the solution for five times on the same day as intraday precision study of Paracetamol and the RSD was found to be 0.1238 for intraday and 0.1162 for interday

Table 4: Precision parameters of Paracetamol

Injection	*Concentration*	*Intra Day*	*Inter Day*
1	100 ppm	1529.144	1679.315
2	100 ppm	1526.182	1678.418
3	100 ppm	1530.277	1678.678
4	100 ppm	1525.917	1675.889
5	100 ppm	1527.340	1674.834
	RSD	0.1238	0.1162

Accuracy

The accuracy of the method was determined by calculating recovery of Paracetamol by the method of standard addition. Known amount of Paracetamol (100 ppm) was added to a pre-quantified sample solution and the amount of Paracetamol was estimated by measuring the peak area ratios and by fitting these values to the straight line equation of calibration curve. The recovery studies were carried out three times over the specified concentration range and amount of Paracetamol was estimated by measuring the peak area ratios by fitting these values to the straight line equation of calibration curve.

Specificity

The specificity of the method was determined by comparing test results obtained from analysis of sample solution containing excipients with that of test results those obtained from standard drug.

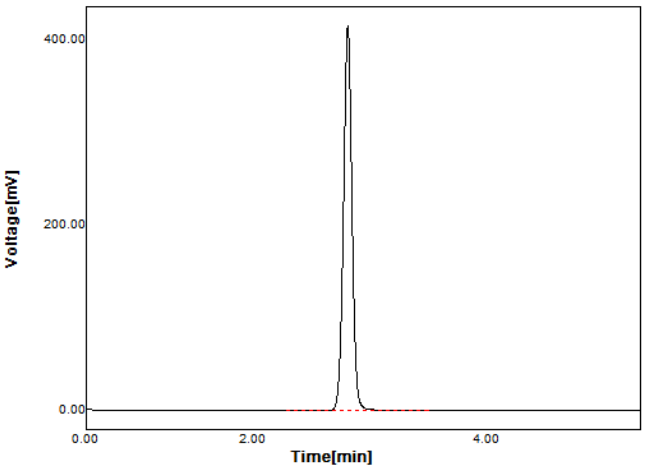


Figure: Typical chromatogram of Paracetamol

LOD and LOQ

Limit of detection (LOD) and limit of quantification (LOQ) were calculated as 1ppm and 10 ppm respectively as per ICH guide-lines. Results are shown in table 5.

Table 5: Results of LOD and LOQ.

Parameter	*Measured*
LOD	1ppm
LOQ	10ppm

Robustness

To determine the robustness of the method, two parameters from the optimized chromatographic conditions were varied. First, Instrument and place were changed and second pH was changed 3.0 to 2.8. Results of Robustness are shown in table 6& 7.

Table 6: Robustness parameters

Parameter	*Modification*
M.PHASE	Acetonitrile:0.1M Acetic Acid(50:50)
PH	2.8
WAVELENGTH	210 nm
R.T	2.503 Min

Table 7: Robustness results

Accuracy	*Precision*
1606.903	1587.266
1606.503	1586.729
1606.511	1589.328
	1584.277
	1584.566
RSD: 0.014	RSD: 0.131

System Suitability Parameter

System suitability tests were carried out on freshly prepared standard stock solutions of Paracetamol and it was calculated by determining the standard deviation of Paracetamol standards by injecting standards in five replicates at 5 minutes interval and the values were recorded in Table 8.

Table 8: System suitability parameters of Paracetamol

Parameters	*Values*
λ max (nm)	210 nm
Correlation coefficient	0.9997
Retention time	2.850min
Theoretical plates	9950
Tailing factor	1.089
Limit of detection	1ppm
Limit of quantification	10 ppm

3. Result and Discussion

Optimization of the chromatographic conditions

The nature of the sample, its molecular weight and solubility decides the proper selection of the stationary phase. The drug Paracetamol being non-polar is preferably analyzed by reverse phase columns and accordingly C18 column was selected. So the elution of the compound from the column was influenced by polar mobile phase. Different mobile phases were tried but satisfactory separation, well resolved and good symmetrical peaks were obtained with the mobile phase Acetonitrile: 0.1M Acetic Acid (50:50).The retention time of Paracetamol was found to be 2.850 min, which indicates a good base line. The RSD values for accuracy and precision studies obtained were less than 2% which revealed that developed method was accurate and precise.

4. Conclusion

A validated RP-HPLC method has been developed for the determination of Paracetamol in bulk form. The proposed method is simple, rapid, accurate, precise and specific. Its chromatographic run time of 2.850 min allows the analysis of a large number of samples in short period of time. Therefore, it is suitable for the routine analysis of Paracetamol in pharmaceutical analysis.

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