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HPLC method validation and Forced degradation study of Paracetamol in bulk and formulation

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Abstract

To validate HPLC method for determination of Paracetamol in Bulk and formulation.

The chromatograms were developed using a mobile phase of MeOH: OPA (35:65) with a flow rate of 1 ml/min. C18 Column of 4.6 x 250 mm dimension was used as a stationary phase, particle size 10µm. The detection was carried out at 286 nm wavelength.

The method was validated according to ICH guidelines for linearity, accuracy, precision, LOD, LOQ and robustness. The response was found to be linear in concentration range of 62.5-312.5 mcg/ml for Paracetamol. The LOD and LOQ were found to be respectively 3.3625 and 10.1896 respectively for Paracetamol.

The developed method was simple, precise, accurate and therefore suitable for routine analysis of drugs in tablet dosage form.

Key words : HPLC, Paracetamol, Development, Validation.

Paracetamol (PARA) [*N*-(4-hydroxyphenyl) ethanamide, *N*-(4-hydroxyphenyl) acetamide] is analgesic & Anti-pyretic drug. Paracetamol reduce pain and fever by inhibiting some chemical messengers that cause pain and fever. HPLC is new and advance technique for qualitative as well as quantitative estimation of pharmaceuticals. In current study, HPLC method has been validated for the estimation of Paracetamol in bulk and dosage form. For the preparation of the manuscript relevant references[1-18] have been consulted.

Chromatographic equipments :

HPLC (Yunglin) was used to develop and validate this method. The chromatographic separation was carried out at ambient temperature by using C_{18} column (4.6 x 250 mm). Class 'A' Borosilicate glassware was employed for volumetric and general purpose in the study.

Reagents and Chemicals :

Ondansetron reference standard were supplied by J.B Chemicals, Ankleshwar, India. Pharmaceutical dosage form (Ondem-P) was obtained commercially. Methyl alcohol and O-Phosphoric as HPLC grade were used as solvents.

Preparation of Mobile phase :

A mixture of o-phosphoric acid (0.05% in water) and methanol in the ratio of 65:35 was prepared (filtered and degassed).

Preparation of standard solution :

Standard stock solution of Paracetamol was prepared by dissolving 125 mg of Paracetamol in 10 ml MeOH, to get solution containing 1250 µgm/ml Paracetamol (STOCK- I). Then, this stock solution is diluted to get solutions containing 62.5-312.5 µgm/ml Paracetamol.

Preparation of sample solution (Tablet solution preparation) :

Brand Name: - ONDEM-P (125 mg Paracetamol + 2 mg Ondansetron)

The twenty tablets were weighed, crushed and mixed (label claim 125 mg Paracetamol + 2 mg Ondansetron). Average weight of tablet (mg) was transferred into 10 ml volumetric flask and dissolved in methanol and then diluted up to the mark. Now, the solution contains 12500 µgm/ml Paracetamol

(Stock Solution-II). 0.1 ml sample was taken from this solution and diluted up to 10 ml. Now it contains 125 µgm/ml Paracetamol.

Forced degradation study :

This Study was carried out to check the effective separation of Paracetamol and its degradation product. Forced degradation study was performed to evaluate the stability indicating properties of the method. Forced degradation study was carried out by treating sample with Acid, alkali, Hydrogen peroxide (oxidative degradation), Neutral (Water). These are discussed below-

1) *Acidic degradation :*
   0.2 ml sample was taken from stock solution-I and transferred in 10 ml volumetric flask. To this, 5 ml 1 N Hydrochloric acid (HCL) was added, solution was made up to the mark with mobile phase and solution was heated at 60° C for 30 minutes. After 30 minutes flask were removed and cooled to room temperature. The Chromatogram was recorded for this solution.
2) *Alkaline degradation :*
   Sample (0.2 ml) was taken from stock solution-I and transferred in 10 ml volumetric flask. To this, 5 ml 1 N Sodium hydroxide (NaOH) was added, solution was made up to the mark with mobile phase and solution was heated at 60° C for 30 minutes. After 30 minutes flask were removed and cooled to room temperature. The Chromatogram was recorded for this solution.
3) *Oxidative degradation :*
   Sample of 0.2 ml was taken from stock solution-I and transferred in 10 ml volumetric flask. To this, 5 ml 10 % Hydrogen peroxide (H_2O_2) was added, solution was made up to the mark with mobile phase and solution was heated at 60° C for 30 minutes. After 30 minutes flask were removed and cooled to room temperature. The Chromatogram was recorded for this solution.
4) *Hydrolysis :*
   0.2 ml sample was taken from stock solution-I and transferred in 10 ml volumetric flask. To this, 5 ml water was added, solution was made up to the mark with mobile phase and solution was heated at 60° C for 30 minutes. After 30 minutes flask were removed and cooled to room temperature. The Chromatogram was recorded for this solution.

Linearity study :

The various concentrations ranging from 62.5 to 312.5 µgm/ml of Paracetamol were injected and peaks were recorded. In these concentrations baseline were obtained for the drugs. The correlation coefficient (r^2) value was 0.999 for the drug. The graph was plotted as a concentration of drug versus peak area is depicted in figure 1.

Table-1 displays the linearity study of Paracetamol. Paracetamol used in a concentration range of 62.5 to 312.5 µgm/ml. The mean areas of different concentrations obtained were 743.93, 1535.77, 2223.05, 2994.39 and 3807.95. The %RSD for these concentrations was 0.96, 0.37, 0.20, 0.17, and 1.03 respectively.

Table-1. Results of Linearity

Paracetamol						
Sr No.	Concμg/ml	Area I	Area II	Mean	SD	%RSD
1	62.5	749	738.86	743.93	7.17	0.96
2	125	1531.76	1539.77	1535.77	5.66	0.37
3	187.5	2226.18	2219.92	2223.05	4.43	0.20
4	250	2998.05	2990.73	2994.39	5.18	0.17
5	312.5	3780.11	3835.79	3807.95	39.37	1.03

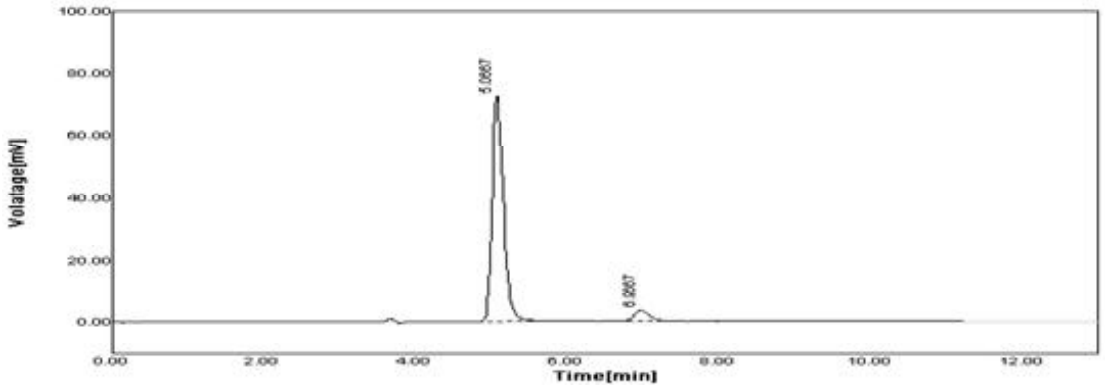

Figure 1: chromatogram of Linearity (1:62.5 μg/ml)

iv) Accuracy (Recovery study) :

Figure 2 displayed the Accuracy (% Recovery) study of Paracetamol. For this, Paracetamol was used in a concentration of 80%, 100% and 120%. The mean of % amount recovered of these concentrations was 99.30, 102.26 and 100.07 respectively.

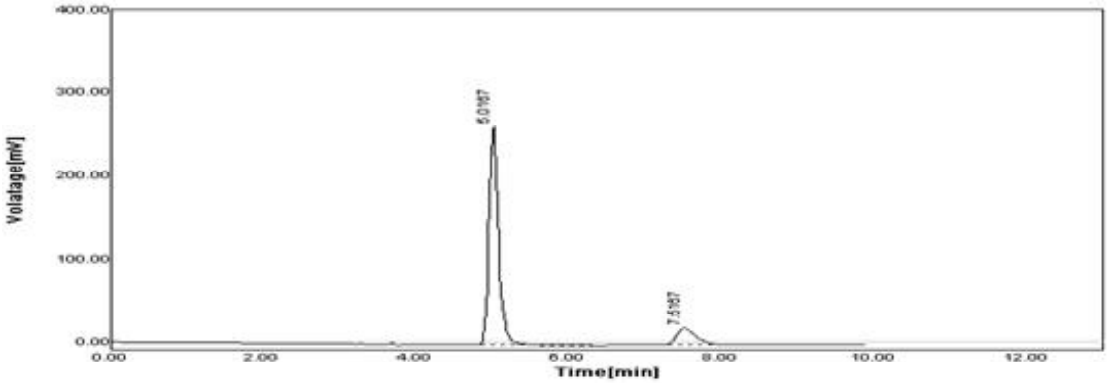

Figure 2: chromatogram of Accuracy (80%)

LOD

Table-2 shows minimum detection limit of Ondansetron and Paracetamol. LOD of Ondansetron and Paracetamol was found to be 0.3026 and 3.3625 respectively. These LOD values indicate that the method is suitable to determine lower concentration of Ondansetron and Paracetamol and confirms that the developed method is sensitive for determination.

Table-2. Limit of Detection (LOD)

Ondansetron	Paracetamol
Formula LOD = 3.3×avg S.D/Slope Avg.SD = 6.36 Slope = 69.34 LOD = 3.3×6.36/69.34 = **0.3026**	Formula LOD = 3.3×avg S.D/Slope Avg.SD = 12.36 Slope = 12.13 LOD = 3.3×12.36/12.13 = **3.3625**

LOQ

Table-3 shows minimum quantitation limit of Ondansetron and Paracetamol. LOQ of Ondansetron and Paracetamol was found to be 0.9172 and 10.1896 respectively. These LOQ values indicate that the method is suitable to determine lower concentration of Ondansetron and Paracetamol and confirms that the developed method is sensitive for determination.

Table-3. Limit of Quantitation (LOQ)

Ondansetron	Paracetamol
Formula LOQ = 10×avg S.D/Slope Avg.SD = 6.36 Slope = 69.34 LOQ = 10×6.36/69.34 = **0.9172**	Formula LOQ = 10×avg S.D/Slope Avg.SD = 12.36 Slope = 12.13 LOQ = 10×12.36/12.13 = **10.1896**

Forced degradation study :

Ondansetron and Paracetamol standard sample was undergone acidic, alkaline, Oxidative and Hydrolytic degradation. The sample shows 10%, 16%, 14% and 16.5% degradation in acidic, alkaline, Oxidative and Hydrolysis conditions respectively. The degradation was under acceptance criteria. It shows stability indicating properties of the method. The chromatograms of sample are shown in Figure 3.

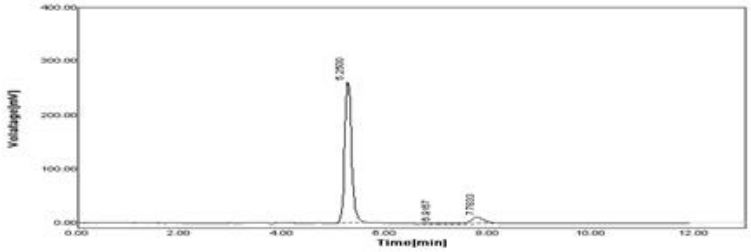

Figure 3: Chromatogram of Acidic degradation (1 N HCL)

The aim of current chromatographic study was to validate HPLC method for the estimation of Paracetamol in bulk and dosage form. Hence, RP-HPLC method has been validated for LOD and LOQ. Forced degradation study was also performed under four different stress conditions. From the chromatographic study, we concluded that developed method is more linear, accurate, precise, reliable and reproducible for routine analysis of Paracetamol in bulk and Pharmaceutical dosage form. So one can perform validation and forced degradation study.

Conflict of interest statement :

"The authors declared no conflict of interest" in the manuscript.

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