

COMPARATIVE QUANTITATIVE DETERMINATION OF PARACETAMOL BY RP-HPLC AND UV-SPECTROPHOTOMETRY FROM ITS FORMULATED TABLETS

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ABSTRACT

These both methods have been described for the quantitatively determination of paracetamol from the single formulated drug tablets by reverse phase high performance liquid chromatography (RP-HPLC) and UV-Spectrophotometry techniques. These both methods were reported in terms of linearity, accuracy, precision and limit of detection and limit of quantification. In both methods the linearity were computed from regression analysis. These both methods showed good linearity over the concentration range of 5–50 µg/mL. The linearity was obtained for paracetamol by reverse phase high performance liquid chromatography (RP-HPLC) $R^2=0.995$ and by UV-Spectrophotometry $R^2=0.988$. The accuracy was checked by recovery method. The recovery coefficient variation (CV %) was obtained 2.90 for RP-HPLC and 0.87 for UV-Spectrophotometry. In both cases coefficient variation was less than 10; therefore proposed methods were successfully applied for the analysis of in its commercial tablets. Any one of the validated method can be used for the analysis of formulated paracetamol tablets.

Keywords: Paracetamol, RP-HPLC, UV-Spectrophotometry.

INTRODUCTION

Paracetamol, N-(4hydroxy phenyl) acetamide has analgesic and antipyretic. It is commonly used for the relief of headaches, relief of fever, and minor aches and pains as well as for the management of more severe pain, where it allow lower dosage of additional nonsteroidal anti-inflammatory drug to be used their by minimizing over all side effect [1-3]. The main mechanism of action of paracetamol is considered to be the inhibition of cyclooxygenase (COX) and recent finding suggest that it is highly selective for cox-z [4]. Numerous methods have been reported for the analysis of paracetamol and its combination in pharmaceuticals or in biological fluids. Paracetamol has been determined in combination with other drugs using U.V spectrophotometry [5-8] and reverse phase high performance liquid chromatography [9-15] in pharmaceutical preparation. The main purpose of this study was developed a cheaply and validated suitable method for quantitative determination of paracetamol form its formulated tablets.

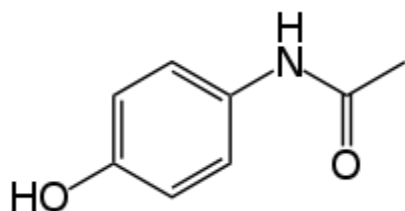


Fig. 1: It shows structure of paracetamol.

MATERIALS AND METHODS

Chemicals and reagents

Paracetamol reference standard was provided by Intas Pharmaceutical Ltd. Tablets of, paracetamol 500 mg manufactured by Paracip Pharmaceutical Ltd. HPLC grade methanol and water were obtained from Merck India Limited and 0.45µm nylon membrane filter was obtained from Pall life Sciences, Mumbai.

Instrumentation

The method development was performed with a cyber lab reverse phase high performance liquid chromatography separating system and thermo scientific UV-Spectrophotometry. Chromatographic analysis was performed on C_{18} column. Separation was achieved using a mobile phase methanol and water in the ratio of (65:35) at flow rate 1.0 ml/min. The eluent was monitored with a UV-Detector

at a wavelength 243 nm. The column was maintained at ambient temperature and injection volume of 20 µl was used. The mobile phase was filtered through 0.45µm nylon membrane filter. The absorbance have been taken with same solvents methanol and water (65:35) and same wave length 243 nm. The absorbance was measured with 3 mL capacity quartz cuvette.

Preparation of Stock solution

The stock solution of paracetamol 100 ppm was prepared in mobile phase methanol and water (65:35). The solutions were filtered through a 0.45 µm nylon membrane. The mobile phase was degassed with sonication instrument. The mobile phase was sonicated to five min.

Preparation of sample solution

To preparation of sample solution, twenty tablets were weighed accurately. These tablets were powdered and mixed well. Taken an equivalent quantity of the powder was transferred into a small conical flask and extracted with mobile phase the extract was filtered into a 100 ml volumetric flask. The volume was maintained with same solvent.

Method validation study

The describe method was validated according to International conference on harmonized guidelines [16] with respect to linearity, specificity, precision, limit of detection (LOD) and limit of quantification (LOQ).

Linearity

Accurately pipette volumes of 0.25, 0.5, 0.75, 1.0, 1.25 and 2.50 ml of paracetamol stock solution was placed in 5 mL volumetric flasks and diluted to 5 mL with mobile phase. These different serial dilutions were filtered through a 0.45µm nylon membrane and sonicate. The each solution of 20 µl was injected into the column in thrice and 3 mL each solution was used to absorbance. The calibration curves were obtained by plotting peak area and absorbance versus concentration.

Specificity

To determine the specificity was taken excipients of the tablets in equivalent to the sample weight. The solution was prepared similarly to the sample solution. The solution was analyzed as per the proposed method. There was no interference was observed from added excipients. Therefore, it is concluded that the both methods was specific.

Accuracy

To determine the accuracy of the both methods, the recovery was checked at the three theoretical concentrations level 25, 50 and 75 µg. The chromatograms were recorded and the percentage recovery was calculated using the following equation [17]:

$$\% \text{ Recovery} = [A] \times 100 / [B]$$

Where [A] is the net peak area of the drug in sample, [B] is the peak area of the drug in standard mixture.

System suitability test

Repeatability of sample application and measurement of peak area were carried out using three replicates of same concentration of standard and sample, respectively.

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

Limits of detection (LOD) and limits of quantification (LOQ) represent the concentration of the analyte that would yield

signal to noise ratios of 3 for LOD and 10 for LOQ respectively. To determine the limit of detection (LOD) and limit of quantification (LOQ) serial dilutions of mixed standard solution of paracetamol was made from the standard stock solution and prepared in replicates of three. The samples were injected in HPLC system and measured signal from the samples was compared with those of blank samples. Same method was also used for UV-Spectrophotometry.

RESULTS AND DISCUSSION

Chromatographic conditions

In this method a mobile phase methanol and water in the ratio of (65:35) was used. The flow rate was 1.0 mL/min. The separation was performed at the wave length 243 nm. The ambient temperature during the analysis was 25 °C. The paracetamol was depicted a well defined chromatographic separation within a run time of 8 min. The retention times of paracetamol 4.98 min. ± 0.08 with standard error.

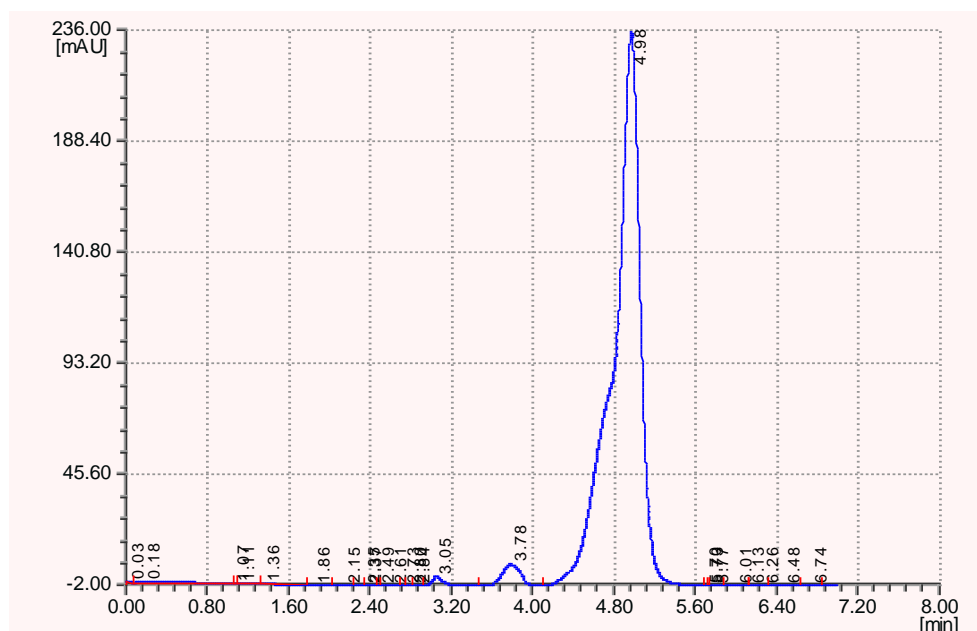


Fig. 2: Chromatogram of paracetamol.

System suitability test

System suitability test (SST) parameters were performed during the development and selection of the method. System suitability test parameters were checked to ensure that, the system is working correctly during the analysis. The system suitability test was performed by injecting the standard mixture in triplicate and the parameters were calculated as reported by USP [18] and international conference harmonized guidelines. System suitability parameters including retention time, tailing factor, resolution factor and theoretical plates were shown.

Table 1: It shows summary of validation system suitability parameters

S. No.	Parameters	Mean	Standard error
1	Retention time	4.98 min	± 0.08
2	Tailing factor	0.77	± 0.01
3	Resolution factor	2.74	± 0.10
4	Theoretical plates	1472.452	± 0.18

Linearity

To determine linearity a calibration graph was obtained by plotting paracetamol concentration against peak area and absorbance. Both

methods were linear in the range of 5-50 µg/ml for paracetamol concentration with a correlation co-efficient 0.995-0.988.

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

The limit of detection (LOD) is defined as the lowest active substance concentration which can be determined by the method, however not calculated precise and accurately [19, 20]. An estimation of the limits can be achieved by the determination of the signal/noise ratio of 3:1 (LOD) and 10:1 (LOQ). The limit of detection (LOD) and limit of quantification (LOQ) values were found for RP-HPLC method 0.03-0.1 µg/mL and for UV-Spectrophotometry method 0.04-0.11 µg/ml, respectively.

Specificity

The specificity of the method was determined by checking the interference with the components from placebo. No interference was observed for any of the components like excipients of both drugs.

Accuracy

The accuracy of the method was analyzed by determination of recovery for three concentrations covering the range of the method. The amount of paracetamol recovered and calculated. The mean recovery of paracetamol and was found with less than 10 % coefficient variation.

Table 2: It shows linearity result, limit of detection (LOD) and limit of quantification (LOQ)

S. No.	Parameters/matrix	HPLC	UV-Spectrophotometry
1	Correlation range	5-50 µg/mL	5-50 µg/mL
2	Regression equation	Y=10066x+1006	Y=0.068x-0.195
3	Regression coefficient (R ²)	0.995	0.988
4	Limit of detection (µg/mL)	0.03 µg/mL	0.04 µg/mL
5	Limit of quantification(µg/mL)	0.1 µg/mL	0.11 µg/mL

Table 3: A: It shows recovery experiment results for paracetamol by RP-HPLC

S. No.	Added in µg	Found	Recovery in %
1	25	24.13	96.52374
2	50	51.74	103.4751
3	75	74.14	98.8409
			Mean= 99.61
			CV%= 2.90

Table 3: B: It shows recovery experiment results for paracetamol by UV-Spectrophotometry

S. No.	Added in µg	Found	Recovery in %
1	25	24.90	96.58621
2	50	50.66	101.3103
3	75	75.12	100.1609
			Mean= 100.35
			CV%= 0.87

CONCLUSION

In this study, described both techniques reverse phase high performance liquid chromatography (RP-HPLC) and UV-Spectrophotometry were successfully applied in control laboratories for their determination in single dosage form. The results of validation show both reverse phase high performance liquid chromatography (RP-HPLC) and UV-Spectrophotometry techniques were simple, linear, precise, accurate and selective. Hence the above any one method can be recommended for simultaneous determination of paracetamol from formulated products.

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REFERENCES

- Goyal RS, Voltametric determination of paracetamol of 60-modified glassy carbon electrode Electrochim. Acta, 2006; 51:3008-3012.
- Singh DV, Bayes S, Phadke G, Jadhav M. Simultaneous determination of etodolac and acetaminophen in tablet dosage form by RP-LC. Chromatographia. 2009; 69: 1019-1023.
- Graham G, Scott K. Mechanism of action of paracetamol. Am. J. Ther. 2005; 12:46-55.
- [Http://en.wikipedia .org/wiki/paracetamol](http://en.wikipedia.org/wiki/paracetamol) (accessed on 9th jan 2011).
- Reynolds JEF. Mantindale the extra pharmacopoeia 31stad, 1996; pp. 27-28, pharmaceutical press, London.
- Chand HK, Grant DJW. Int. J. pharma, 1989; 57: 117-124.
- Erk N, Onour F, Anal. Lett. 1997; 30 (6),1201-1210.
- Sharma SK, Barot GB, Multani PJ. Development and validation of vierdot's and q-ratio method for the estimation of paracetamol, domperidone and flunarizine in solid oral dosage form. Int J Pharm Pharm Sci. 2013; 5(2), 347-351.
- Dogan HN, pharmazie. 1996; 51, (10): 773-774.
- Kahela P, Laine E, Anttila M. Drug Dev. Ind. Pharma. 1987; 13, (2):213-224.
- Suzen S, Akay C, Tartilmis S, Erdol RS, Onal A, Cevheroglu S. J. faculty of pharma. Ankara Univ. 1998; 27 (2): 93-100.
- Sisco WR, Rittenhuse C, Everhart T LA, McLaughlin AM. J. Chromatography. 1986; 355-366.
- Hussain M, Ayres J W. Int. J. Pharm. 1996, 133: 223-235.
- Ali HM, Homeida MM, Ford A J, Truman CA, Roberts CJC, Badwan AA. Int. J. Pharm. 1988, 42: 155-159.
- Yen K H, Peh KK, Quah YL, Chan KL. Drug Dev. Ind. Pharm. 1997; 23(2): 225-228.
- International Conference on Harmonization (ICH), Q2A . 2005; Validation of Analytical Procedures: Text and Methodology.
- Entidhar JAA, Lalaa AAR , Adnan AB, Nawzat DAJ, Nidal AQ. Development and validation of a sensitive and accurate method for determination of atorvastatin and rosuvastatin in rat plasma by reversed- phase high performance liquid chromatography with uv detection. Int J Pharm Pharm Sci. 2013; 5 (2), 211-219.
- Orsi DD, Gagliardi L, Bolasco A, Tonelli D. J Chromatographia. 1996; 43 (9/10): 496-500.
- Islam SMA, Abuzar SM, Paul PK. Validation of UV-Spectrophotometric and RP-HPLC methods for the simultaneous analysis of Pracetamol and Acelofenac in marketed tablets. I J of Pharmacy and Life Science. 2011; 12: 1267-1275.
- Bergh JJ, Lotter AP. Drug Dev. Ind. Pharma. 1984; 10 (1): 127-136.