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A VALIDATED RP- HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF PARACETAMOL AND NAPROXEN IN TABLET FORMULATION

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ABSTRACT

The study describes a validated reverse-phase HPLC method for the simultaneous estimation of paracetamol and naproxen in bulk and in tablet formulation. The proposed RP-HPLC method utilizes Eclipse XDB C18 column (150 ×4.6 mm i.d., 5 µm), optimum mobile phase consisted of gradient run of initial ratio of water (pH-2.5 adjusted with orthophosphoric acid): acetonitrile (87:13) with the effluent flow rate of 1.0 ml/min, and UV detection wavelength 263 nm. The developed method was statistically validated for linearity, precision, robustness, ruggedness and specificity. The method was linear over the range of 5-80 µg/ml for paracetamol and 3-48µg/ml for naproxen. The mean recovery was 99.72% and 100.88% for paracetamol and naproxen respectively. The intermediate precision data obtained under different experimental setup was quite concurrent with less critical % RSD. The proposed method can be useful in the quality control of paracetamol and naproxen in bulk drug and drug products.

INTRODUCTION: Paracetamol (PARA) chemically *N*- (4-hydroxyphenyl) acetamide is a well-known analgesic drug. It is an effective analgesic and antipyretic for the treatment of minor, non-inflammatory conditions in patients who are prone to gastric symptoms [1]. Naproxen (NAP), chemically 2-(6-methoxy-naphthalen-2-yl) propanoic acid is a nonsteroidal anti-inflammatory drug.

It works by inhibition of cyclooxgenase, an enzyme involved in the arachidonic acid conversion pathway, resulting in a decrease of prostaglandin synthesis.

It is used in the treatment of rheumatoid arthritis and other musculoskeletal disorders [2, 3]. The structures of these two drugs are shown in figure 1.

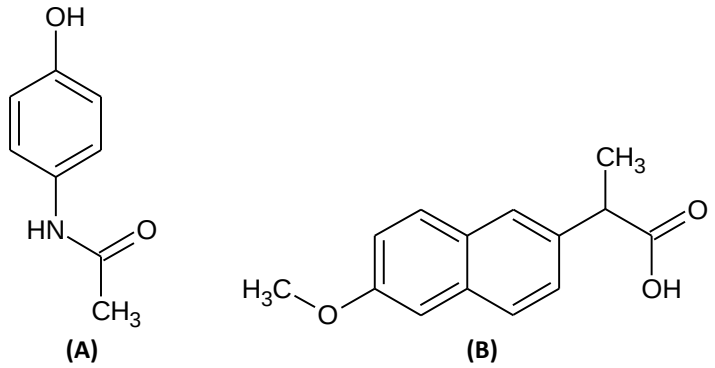

FIG. 1: STRUCTURE OF [A] PARACETAMOL AND [B] NAPROXEN

Literature survey has revealed that several methods have been reported for estimation of paracetamol and naproxen individually, in bulk drug, formulations and biological samples by various analytical methods. UV-spectrophotometric assay of paracetamol in tablets and urine samples had been reported [4]. Several UV spectroscopic methods have been reported for estimation of paracetamol in combination with other

drugs in various formulations [5-9]. HPLC methods of paracetamol in combination with other drugs in various formulations [10-13] and in various biological samples [14, 15] have been reported. UV-spectroscopic method for estimation of naproxen from tablet formulation is available [16]. HPLC methods of naproxen in combination with other drugs in pharmaceutical formulation [17-19] and in human urine [20] have been reported. HPTLC method for simultaneous estimation of paracetamol and naproxen in tablet formulation exists but the method does not define validation of method [21]. Hitherto, there are no HPLC methods available for estimation of paracetamol and naproxen in fixed dose combination formulation. The present study describes simple, precise and accurate reverse phase HPLC method for simultaneous determination of paracetamol and naproxen in tablet formulation.

MATERIAL AND METHODS:

1. **Reagents and equipments:** A gratuitous sample of pure paracetamol was obtained from Zim Laboratories, (Kalmeshwar, Nagpur) and naproxen from Ranbaxy Laboratories Ltd. (Gurgaon). HPLC grade acetonitrile, orthophosphoric acid and water were procured from Merck (Mumbai, India). Analytical reagent (AR) grade sodium hydroxide (NaOH) was purchased from Ranbaxy Laboratories, hydrochloric acid (HCl) from LOBA Chemie Pvt. Ltd (Mumbai, India) and hydrogen peroxide (H_2O_2) from S.D. Fine-Chem Ltd. (Boisar, India). Milipore 0.45µ Nylon filters for solvent filtration and 0.22 µ Nylon filters for sample filtration were used. Fixed dose combination tablet formulation of paracetamol and naproxen (Naprosyn-P, containing 500mg of paracetamol and 300mg of naproxen; RPG Life Sciences Ltd.) was purchased from the local market. The HPLC system consisted of Agilent HPLC 1200 System, Quaternary gradient pump G1311A with On Line Degasser G1322A, Variable wavelength UV-VIS detector aceG1314B, Eclipse XDB C_{18} column (150×4.6mm i.d., 5µm), Rheodyne injector 7725 I with 20 µl loop were used. Other equipments used were Digital pH meter (Labtronics LT-11, Mumbai), Balance Shimadzu AUY 220 (Japan), Sonicator (PCI Services, Mumbai) and Milipore filter assembly.

2. **HPLC analysis of samples**: HPLC analysis was performed on both the drugs, and then on a mixture of drug solution. The mixture of water (adjusted to pH 2.5 with orthophosphoric acid) and acetonitrile (87:13) was used as diluent for the preparation of samples. The preliminary chromate-graphic conditions were set by injecting 20 µl standard solution of paracetamol and naproxen (20µg/ml) prepared in diluent and the chromatograms were recorded for the drug. The mobile phase containing mixture of water (adjusted to pH 2.5 with orthophosphoric acid) (A): acetonitrile (B) was used with run initially 13% of solvent B upto 2.8 min gradually increased to 50% upto 5.5 min. This ratio is maintained upto 6 min and finally solvent B was decreased gradually from 6-10 min to 13%. The flow rate was kept at 1.0 ml/min during gradient run with column temperature at 25°C.

The chromatographic conditions were found to yield good separation with satisfactory retention time of about 3.005 min for paracetamol and 7.402 min for naproxen with sharp symmetrical peak (**Figure 2**).

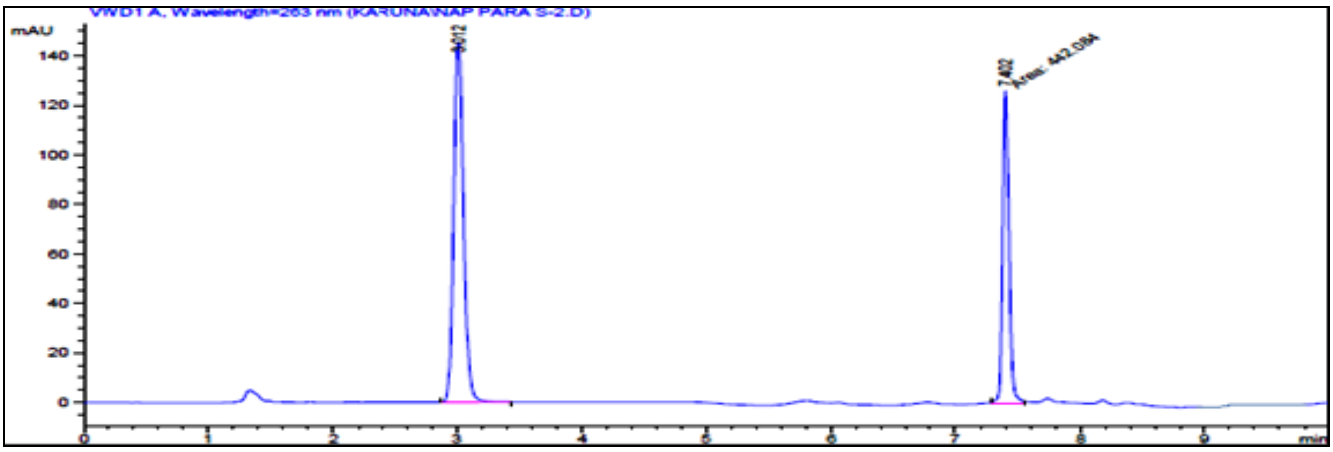

FIG. 2: CHROMATOGRAM OF STANDARD SOLUTION OF PARACETAMOL AND NAPROXEN

3. **Estimation of paracetamol and naproxen in Tablet Formulation**: Twenty tablets were weighed and average weight was calculated. The tablets were crushed to fine powder. An accurately weighed quantity of tablet powder equivalent to 25 mg was sonicated with 15 ml methanol for 15 minutes and the volume was made to 25.0 ml with methanol. The solution was filtered and 1.0 ml of clear filtrate was diluted to 10.0 ml with diluent. The resultant solution (2.0ml) was further diluted to 10.0 ml with diluent, so that final concentration of 20µg/ml for paracetamol and 12µg/ml for naproxen on the basis of labeled claim was obtained. Five replicate sample solutions were prepared in similar manner.

4. **Validation of the Developed Method**: Validation was done with respect to various parameters, as required under ICH guideline Q2 (R1) [22]. A standard solution of 20µg/ml for paracetamol and 12µg/ml of naproxen was prepared for comparison of results. Accuracy of the proposed method was ascertained on the basis of recovery studies performed by standard addition method over the range of 70 to 130% of labeled claim.

   Accurately weighed quantities of preanalysed tablet powder equivalent to about 17.4 mg of paracetamol and 10.4 mg of naproxen was transferred to five different 25 ml volumetric flask and accurately known amount of standard paracetamol and naproxen were added in about 15 ml methanol. The flasks were sonicated for 15 min and volumes were made up to the mark with methanol. The drug contents in different flasks represent 70%-130% of the labeled claim with fixed amount of excipients. The solutions were filtered and 1.0 ml of clear filtrate was diluted to 10.0 ml with diluent. The resultant solutions (2.0 ml) were further diluted to 10.0 ml with diluent.

   Precision of method was ascertained by replicate analysis of homogeneous samples of tablet powder. To study linearity of method, a stock solution of mixed standard solution of both the drugs were diluted appropriately to yield solutions in the concentration of 5-80 µg/ml for paracetamol and 3-48 µg/ml for naproxen. The intermediate precision was established by injecting drug solutions on the same day and on consecutive three days, and also by two different analysts on the same day. The robustness of the method was established by deliberate change in detection wavelength by ±2 nm in the estimation of tablet.

   Specificity of the method was established to ascertain how accurately and specifically the analyte of interest are estimated in presence of other components (e.g. impurities, degradation products and excipients) by exposing the tablet powder to different stress conditions such as normal, acid, alkali, light, heat and oxidation. Accurately weighed pre-analysed tablet powder equivalent to about of 25 mg paracetamol was exposed to different stress conditions like normal (at room temperature), for hydrolytic stress in 1.0 ml each of 0.1N HCL and 0.1N NaOH at 50^{o}C, for oxidation stress in 3% H_2O_2 at 50^{o}C, for photolytic stress in sunlight and for thermal stress by heating at 60^{o}C in different 25 ml volumetric flasks. After 24 hrs, all samples were cooled to room temperature, sonicated with 15 ml methanol for 15 min and volumes were made up to 25.0 ml with methanol. The solutions were then analyzed in a similar manner as described under estimation of tablets.

RESULTS AND DISCUSSION: Paracetamol was eluted at t_R 3.005 and naproxen at t_R 7.042 with capacity factor values 1.01 and 3.95 respectively indicated good retention of both the drugs and high number of plates 7773.4 and 111068.4 per meter for paracetamol and naproxen respectively indicates substantially high efficiency of method, as shown in system suitability parameters (**Table 1 and 2**).

Validation of the Developed Method: The detector response was found to be linear over the range 5-80µg/ml for paracetamol and 3-48µg/ml for naproxen with correlation coefficient values well about 0.999 for paracetamol with the regression equation of y=38.62x+5.233 and 0.997 for naproxen with the regression equation of y=16.62x+114.6. Recoveries of the drugs were observed to be very close to 100% representing the accuracy of the method and also non-interference of excipients (**Table 3**).

TABLE 1: SYSTEM SUITABILITY PARAMETERS OF PARACETAMOL

Sr. No	Retention Time (min)	Capacity Factor	Symmetry	No. of plates	Peak Area
1	3.006	1.01	0.84	7964	777.78
2	3.012	1.01	0.83	7607	789.74
3	3.014	1.02	0.84	7849	784.03
4	3.002	1.00	0.85	7892	783.71
5	3.002	1.01	0.84	7755	792.72
Mean	3.0072	1.01	0.84	7813.4	785.59
±SD	0.0049	0.0063	0.0063	123.39	5.1966
%RSD	0.1629	0.6237	0.7500	1.5792	0.6614

TABLE 2: SYSTEM SUITABILITY PARAMETERS OF NAPROXEN

Sr. No	Retention Time (min)	Capacity Factor	Symmetry	No. of plates	Peak Area
1	7.400	3.95	0.83	112180	435.42
2	7.406	3.96	0.84	112369	435.26
3	7.402	3.95	0.83	112239	442.08
4	7.399	3.94	0.83	109220	438.91
5	7.403	3.95	0.82	109334	443.73
Mean	7.402	3.95	0.83	111068.4	439.08
±SD	0.0024	0.0063	0.0063	1464.39	3.4245
%RSD	0.0324	0.1594	0.7590	1.3184	0.7799

TABLE 3: RESULT OF RECOVERY STUDIES OF PARACETAMOL AND NAPROXEN

Sr. No.	Amt of std added (mg)		Area		Amt of std recovered (mg)		% Recovery	
	PARA	NAP	PARA	NAP	PARA	NAP	PARA	NAP
1	-	-	544.63	319.09	-	-	-	-
2	3.75	2.24	633.30	354.69	3.77	2.28	100.53	101.78
3	7.5	4.49	754.16	424.86	7.36	4.53	98.13	100.89
4	11.25	6.74	869.08	483.18	11.27	6.67	100.17	98.96
5	15.00	8.98	1001.46	563.95	15.01	9.15	100.06	101.89
						Mean	99.72	100.88
						± SD	0.9357	1.1743
						%RSD	0.9380	1.1640

Replicate estimations (n=5) of paracetamol and naproxen in tablet by proposed method have yielded 98.81% labeled claim with % RSD of 0.7027 for paracetamol and 100.33% of labeled claim with %RSD of 1.1957 for naproxen, indicating good precision of method.

The results of estimation by proposed method on different days and by different analysts were very much reproducible with maximum %RSD of 0.5517 for paracetamol and 0.6305 for naproxen (**Table 4**). This indicates the ruggedness of the method in the hands of different analyst and on different time intervals. The variations in detection wavelength by ±2 nm have shown significant change in the area of paracetamol with deviation of ±15% of standard area. Whereas naproxen does not show any change in its area. In cases of specificity studies, paracetamol and naproxen were unaffected by acid, alkali, thermal and photolytic stress conditions.

TABLE 4: RESULTS OF RUGGEDNESS STUDIES

Observations	% Drug estimation					
	Intra- day		Inter-day		Different Analyst	
	PARA	NAP	PARA	NAP	PARA	NAP
I	99.58	100.24	100.69	99.87	99.58	100.87
II	99.88	100.95	99.86	100.25	99.22	99.81
III	100.52	99.58	99.36	99.23	100.25	99.33
Mean	99.99	100.25	99.97	99.78	99.68	100.03
± SD	0.3920	0.5594	0.5485	0.4208	0.4267	0.6433
%RSD	0.3920	0.5580	0.5486	0.4217	0.4280	0.6433

However, oxidative stress condition showed significant degradation of paracetamol to 28.8% and 89.16% for naproxen.

DISCUSSION: The present study highlights the benefit of the use of the method for simultaneous estimation of both the drugs. The results of assay of paracetamol and naproxen tablet obtained by proposed HPLC method are quite concurrent and reproducible. The recovery of the drugs from matrix was about 100% with %RSD <2 indicating accuracy and reliability of method with non-interference of excipients.

Paracetamol and naproxen were found to be unstable in oxide stress conditions, whereas it was much stable in acidic, alkali, thermal stress conditions and in sunlight. At the same time method is simple, rapid, reasonably specific and rugged. Thus, the proposed HPLC method can be successfully applied for the routine quality control analysis of paracetamol and naproxen from their fixed dose combination tablet formulation.

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