Simultaneous Estimation of Nimesulide and Paracetamol in Solid Dosage Form by Rp-Hplc Method

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ABSTRACT:A simple, specific, accurate and precise reverse phase high pressure liquid chromatographic method has been developed for the simultaneous determination of nimesulide and paracetamol from tablets by reverse phase C18 column (HiQsil C18, 250 mm x 4.6 mm). The sample was analyzed using Methanol: Ammonium acetate buffer in the ratio of 80:20, (pH adjusted to 7.0 with triethylamine) as a mobile phase at a flow rate of 1.0 ml/min and detection at 276.0 nm. The retention time for paracetamol and nimesulide was found to be 3.00 and 4.245 min respectively, and recoveries from tablet were between 95 and 105 %. The method can be used for estimation of combination of these drugs in tablets.
Key words: Nimesulide, Paracetamol, RP-HPLC.

INTRODUCTION
Nimesulide is an anti-inflammatory drug. Chemically, nimesulide is N-(4-nitro-2-phenoxyphenyl) methane sulphonamide. It is approved for used in treatment of musculoskeletal disorder, dyemenorrhoea, thrombophlebitis and dental pain, inflammation. Some HPLC [1, 2] and spectrophotometric [3, 4], methods have been reported in the literature for its estimation. Paracetamol is chemically N-(4-hydroxyphenyl) acetamide. It is a centrally and peripherally acting non-opioid analgesic and antipyretic. Many methods have been described in the literature for the determination of paracetamol with other drugs individually and in combination [5-13].

EXPERIMENTAL
Chemicals and Reagents
Nimesulide and paracetamol were obtained from, Aristo Pharma. Ltd. Mumbai. Water (HPLC grade), methanol (HPLC grade), triethylamine and ortho-phosphoric acid were of reagent grade. The pharmaceutical preparations of combination of nimesulide and paracetamol that is Nimucet Gold tablet (Intas Pharma., Ahmedabad).

Instrumentation
A Gradient HPLC PU 2080 Plus (JASCO) with UV-2075 Plus detector and RP-C18 column was used. A Rheodyne injector with a 20 μl loop was used for the injection of sample. The HPLC system was equipped with Borwin software for data processing.

Chromatographic Condition
The mobile phase containing methanol: ammonium acetate buffer (80:20), pH adjusted to 7.0 with triethylamine was found to resolve nimesulide and paracetamol. Triethylamine was used for pH adjustment of buffer. The mobile phase was filtered on a 0.45 micron membrane filter and then ultrasonicated for 15 min. The flow rate was set to 1.0 ml/min. The 276.0 nm wavelength was selected for analysis. All determinations were performed at constant column temperature (25 ± 2^0C).

Preparation of Ammonium acetate buffer:
3.85gm of ammonium acetate dissolved in 50ml water then volume made upto 1000ml with water. From this solution 50ml diluted to 1000ml with 0.05M acetic acid and pH adjusted to 7.0 with triethylamine.

Preparation of Stock Solutions
Standard stock solutions containing nimesulide and paracetamol was prepared by dissolving 10 mg of nimesulide and 10 mg of paracetamol in 80ml of methanol in two separate 100ml volumetric flasks. It was then sonicated for 15 minutes and the final volume of the solution made up to 100 ml with ammonium

acetate buffer to get stock solutions containing 100 μg/ mL of nimesulide and paracetamol each.

Calibration curve

Calibration curves were prepared by taking appropriate aliquots of standard nimesulide and paracetamol stock solutions in different 10 ml volumetric flask and diluted up to the mark with mobile phase to obtain final concentrations of 5-25 μg/ml of nimesulide and 5-25 μg/ml paracetamol. Standard solutions were injected through 20 μl loop system and chromatograms were obtained using 1.0 ml/min. flow rate. The effluent was monitored at 276.0nm. Calibration curve was constructed by plotting average peak area against concentration and regression equation was computed.

Validation of the method

The developed method was validated in terms of linearity, accuracy, specificity, limit of detection, limit of quantification, intra-day and inter-day precision and repeatability of measurement.

Analysis of marketed tablet formulation:

A total of 20 tablets were accurately weighted and triturated with glass mortar and pestle. An amount equivalent to one tablet (containing 100 mg of nimesulide and 500mg of paracetamol) was transferred to a 100ml volumetric flask; 400mg of pure nimesulide added to it for making 1:1 ratio nimesulide and paracetamol, 50 ml of mobile phase was added and the flask was kept in an ultrasonic bath for 15 min. The volume was made up to mark with mobile phase and the solution was filtered through 0.45 micron membrane filter. The final volume of the solution was made up to 100 ml with mobile phase to get stock solutions containing 5000 μg/ mL nimesulide and 5000 μg/ mL paracetamol. The diluted solution (20 μg/ mL) was analyzed under optimized chromatographic conditions and chromatogram is depicted in Fig. No.1.

RESULT AND DISCUSSION

To develop a precise, accurate and suitable RP- HPLC method for the estimation of nimesulide and paracetamol, different mobile phases were tried and the proposed chromatographic conditions were found to be appropriate for the quantitative determination. System suitability tests were carried out as per USP XXIV and parameters are summarized in Table.1. The results obtained by the analysis of marketed formulation are summarized in Table.2**.**

Method Validation [14]

The proposed HPLC method was validated as per ICH guidelines.
Specificity

The peak purity of nimesulide and paracetamol were assessed by comparing the retention time (Rt) of standard nimesulide and paracetamol. Good correlation was obtained between the retention time of standard and sample of nimesulide and paracetamol.

Linearity

Linearity was studied by preparing standard solutions at different concentration levels. The linearity range for nimesulide was found to be 5- 25 μg/ml and for paracetamol was found to be 5- 25 μg/ml. The regression equation for nimesulide was found to be $y = 19578x - 2666.822$ and for paracetamol was found to be $y = 19534x + 20395.413$ with coefficient of correlation 0.9997 and 0.9992 respectively.

Precision

Precision was evaluated by carrying out six independent sample preparation of a single lot of formulation. The sample solution was prepared in the same manner as described in sample preparation. Percentage relative standard deviation (%RSD) was found to be less than 2% for within a day and day to day variations, which proves that method is precise.

Accuracy (Recovery studies)

To check the degree of accuracy of the method, recovery studies were performed in triplicate by standard addition method at 80%, 100% and 120%. Known amounts of standard mixture of nimesulide and paracetamol was added to pre-analyzed samples and was subjected to the proposed HPLC method. Results of recovery studies are shown in Table 3.

Robustness of method

To evaluate the robustness of the developed RP-HPLC method, small deliberate variations in the optimized method parameters were done. The effect of change in flow rate, pH and mobile phase ratio on the retention time and tailing factor were studied. The method was found to be unaffected by small changes like ± 0.1 change in pH, ± 0.1 change in flow rate and ± 1 change in mobile phase.

CONCLUSION

The most striking feature of this method is its simplicity and rapidity, non- requiring- consuming sample preparations such as extraction of solvents, heating, degassing which are needed for HPLC procedure. These methods can be employed for routine quality control analysis. The described methods give accurate and precise results for determination of nimesulide and paracetamol mixture in marketed formulation.

Table 1: System suitability parameters

Parameter	Paracetamol	Nimesulide
Linearity range (µg/ml)	5-25	5-25
Correlation coefficient	0.9992	0.9997
Slope	19534	19578
Retention time (min.)	3.00	4.245
Resolution factor	1.491	2.412
Tailing factor	1.229	1.238

**Table 2: Results of analysis of marketed tablet formulation.**

Tablet	Nimucet Gold	
Analyte	**Paracetamol**	**Nimesulide**
% Estimated	103.17	97.15
S.D.	0.041833	0.6328
%R.S.D.	0.040054	0.6514
Amount found in mg	515.85	97.15
%LOD	0.0007	0.01066
%LOQ	0.00214	0.03232

S.D.= Standard Deviation, R.S.D.= Relative Standard Deviation

Table 3: Recovery study data

% Standard Addition	% Estimated		S. D.		% R.S.D.	
	P	N	P	N	P	N
80	100.79	101.69	0.7253	1.9079	0.71961	1.8762
100	100.55	98.40	0.7702	1.3551	0.76598	1.3771
120	98.19	95.01	2.0012	1.4802	2.0381	2.1451

S.D.= Standard Deviation, R.S.D.= Relative Standard Deviation

Fig.1. Typical chromatogram of Paracetamol (P) and Nimesulide (N)
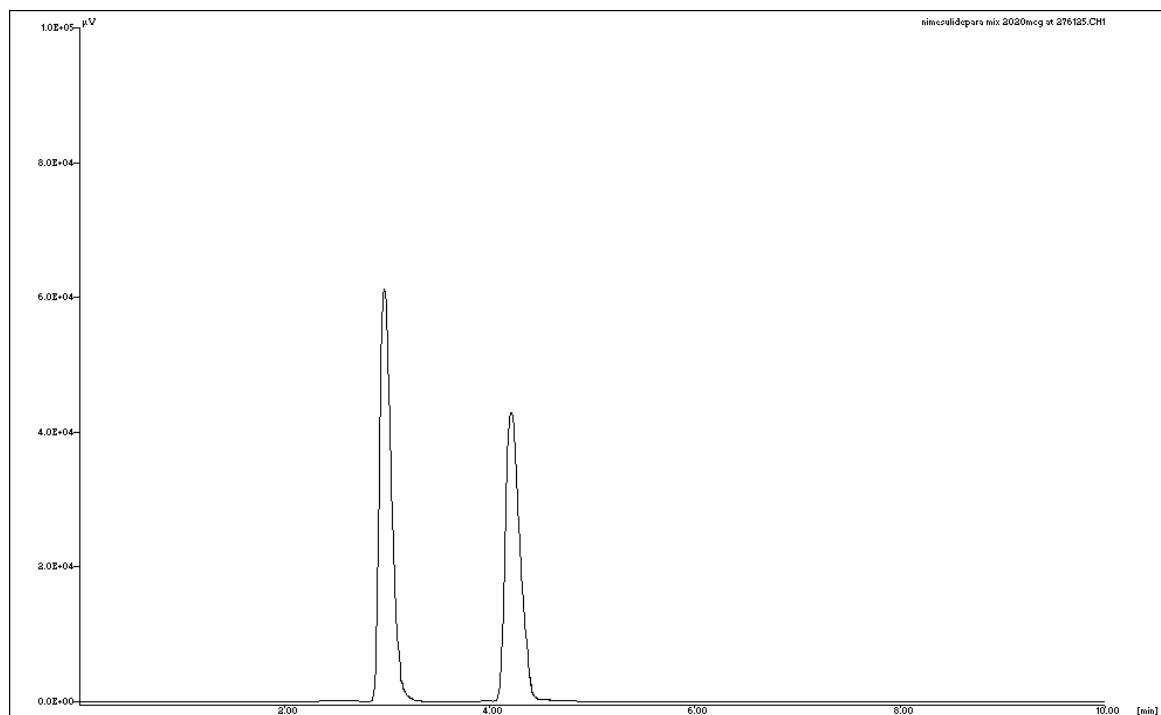
REFERENCES
1. Zarapkar SS, Bhandari NP, Halkar UP. Simultaneous determination of nimesulide and chlorzoxazone in pharmaceutical dosage by RPHPLC. Indian Drugs 2000; 37:467.
2. Nagoji KE, Vijaysrinivas S, Kumar KM, Mathivanan N, Kumar SM, Rao ME. Simultaneous RP-HPLC estimation of nimesulide and diclofenac sodium. Indian J Pharma Sci 2003; 65:407.
3. Rajput SJ, Randive G Assay of nimesulide in pharmaceutical dosage formulation. Eastern Pharmacist 1997; 475:113
4. Nagoji KE, Rao SS, Rao ME, Rao KV. New Spectroscopic method for the estimation of Nimesulide in Pharmaceutical Dosage formulation. Eastern Pharmacist 1999; 496:117
5. British pharmacopoeia, Vol II, London: Her mejesty's Stationary office 1998, p. 1854.
6. Indian pharmacopoeis Vol II, New Delhi, The controller of Publications. Govt of India, 1996, p. 554.
7. Hasan, N.Y., Abdel-Elkawy, M., Elzeany, B.E. and Wagieh, N.E., Farmaco., 2003, 58, 91.
8. EI-Saharty, Y.S., Refaat, M. and EI-Khateeb, S.Z., Drug. Develop. Ind. Pharm., 2002, 28, 571.
9. Zawilla, N.H., Mohammad, M.A., EI-Kousy, N.M. and EI-Moghazy Aly, S.M., J.Pharma. Biomed. Anal., 2002, 27, 243.
10. Hinz, B., Auge, D., Rau, T., Rietbrock, S., Brune, K. And Werner, U., Biomed. Chromatogr., 2003, 17, 268.
11. Liu, XQ., Chen, X.J., Zhao, L. H. And Peng, J.H., Yao Xue Ba., 1997, 32, 546.
12. Lee, H.S., Jeong, C.K., Choi, S.J. Kim, S.B., Lee, M.H., Ko, G.I, and Sohn, D.H., J. Pharm. Biomed. Anal., 2000, 23, 775.
13. EI-Kousy, N.M., J. Pharm. Biomed. Anal., 1999, 20, 185.
14. ICH, Validation of Analytical procedure: Methodology (Q2B), International Conference on Harmonization, IFPMA, Geneva, 1996.

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