A Simple and Sensitive HPLC Method for Simultaneous Analysis of Nabumetone and Paracetamol in Pharmaceutical Formulations

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Abstract: This paper describes a high-performance liquid chromatographic method for simultaneous estimation of nabumetone and paracetamol in binary mixture. The method was based on RP-HPLC separation and quantitation of the two drugs on hypersil C-18 column (250 mm × 4.6 mm) using a mobile phase consisting of acetonitrile and 0.05 % aqueous acetic acid (70:30v/v) at flow rate of 1 mL min⁻¹. Quantitation was achieved with PDA detector at 238 nm based on peak area with linear calibration curves at concentration ranges 5-25 µg mL⁻¹ for both the drugs. Naproxen sodium was used as internal standard. The method has been successively applied to pharmaceutical formulation. No chromatographic interference from the tablet excipients was found. The method was validated in terms of precision, robustness, recovery and limits of detection and quantitation. The intra and inter-day precision and accuracy values were in the acceptance range as per ICH guidelines.

Keywords: Nabumetone, Paracetamol, RP-HPLC, Method validation, Pharmaceutical Formulations

Introduction

Nabumetone (NAB), chemically, 4-(6-methoxy-2-naphthyl)-2-butanone is a non-steroidal anti-inflammatory drug of the arylalkanoic acid family used to treat pain or inflammation caused by arthritis¹. It is official in B. P.² and U. S. P³ paracetamol (PAR), 4-hydroxyacetanilide is a widely-used analgesic and antipyretic drug^{4,5}.

The literature review reveals that colorimetric⁶ and several chromatographic methods have been reported for the analysis of nabumetone in biological fluids⁷⁻¹² and in pharmaceutical formulations¹³⁻¹⁴ either alone or in combination with other drugs. Also methods have been published for analysis of paracetamol which include HPLC¹⁵⁻¹⁶, non-suppressed ion chromatography¹⁷, spectrophotometry¹⁸⁻²², either as single or in combinations with other drugs, but no HPLC method has yet been reported for simultaneous estimation of nabumetone and paracetamol. This paper describes simple, accurate, precise and sensitive HPLC method for simultaneous determination of nabumetone and paracetamol in combined tablet dosage form. The proposed method was optimized and validated as per the International Conference on Harmonization (ICH) guidelines²³⁻²⁴.

Experimental

Gifts of NAB and PAR pure samples were obtained from Divi's Laboratories Ltd. (Hyderabad) and Cipla Ltd. (Pune, India), respectively used as such without further purification. Fixed dose combination used in this study was Niltis P tablets (Ipca laboratories Ltd., India) labeled to contain 500 mg of NAB and 500 mg of PAR were procured from the local market. Chemicals and reagents of analytical-grade were purchased from Merck Chemicals, Mumbai, India.

HPLC instrumentation and chromatographic conditions

Jasco HPLC system, consisting of Jasco PU-2080 plus HPLC pump, Jasco MD-2010 plus PDA detector and JASCO Borwin Ver 1.50 software was used for analysis. Separation was carried out on hypersil C-18 (250 mm × 4.6 mm, 5 μ) column using Acetonitrile and 0.05 % aqueous acetic acid (70:30v/v) as mobile phase at flow rate of 1 mL min⁻¹. Samples were injected using Rheodyne injector with 20 μL loop. Naproxen (NAP) was used as internal standard and detection was carried out at 238 nm. All weighing were done on Shimadzu balance (Model AY-120).

Preparation of standard stock solutions

Standard stock solution of NAB, PAR and NAP were prepared by dissolving 10 mg of each drug in 10 mL of mobile phase in separate volumetric flasks to get concentration of 1 mg mL⁻¹. One mL of each stock solution was further diluted to 10 mL with mobile phase to get a working standard solution of concentration 100 μg/mL.

Procedure for analysis of tablet formulation

Twenty tablets were weighed accurately and a quantity of tablet powder equivalent to 10 mg of each NAB and PAR was weighed and transferred to a 10 mL volumetric flask containing about 7 mL of mobile phase, ultrasonicated for 5 min and volume was made up to the mark with the mobile phase. The solution was filtered through Whatman filter paper No. 41 and 1 mL of filtrate was further diluted to 10 mL with mobile phase. One mL of this solution was transferred to 10 mL calibrated volumetric flask and 0.5 mL stock solution of NAP was added and the volume was made up to the mark with the mobile phase. After setting the chromatographic conditions and stabilizing the instrument to obtain a steady baseline, the tablet sample solution was injected, chromatogram was obtained and the peak areas were recorded. The peak area ratios of each of the drugs to the internal standard were calculated and the amount of each drug in sample was estimated from the respective calibration curves.

Method Validation

Linearity

Aliquots 0.5, 1, 1.5, 2 and 2.5 mL of working standard solution of NAB and PAR were transferred in a series of 10 mL calibrated volumetric flasks separately. To each flask, 0.5 mL stock solution of NAP was added and the volume was made up to the mark with the mobile phase. Five replicates per concentration were injected and chromatograms were recorded. The peak area ratios of NAB to NAP and PAR to NAP were calculated and respective calibration curves were plotted of response factor against concentration of each drug.

System suitability

The system suitability was assessed by six replicate injections of the mixture containing $10 \mu\text{g mL}^{-1}$ of NAB, $10 \mu\text{g mL}^{-1}$ of PAR and $5 \mu\text{g mL}^{-1}$ of NAP as internal standard. The resolution, peak asymmetry, number of theoretical plates and HETP were calculated and represented in Table 1.

Table 1. System suitability parameters for RP-HPLC method

S. No.	Parameters	PAR	NAP	NAB
1	Theoretical plates	10761	13039	16784
2	HETP, cm	0.0023	0.0019	0.0015
3	Resolution	---	1.936	1.509
4	Asymmetry factor	1.24	1.16	1.01

The values obtained demonstrated the suitability of the system for the analysis of these drugs in combination. Mean retention times for PAR, NAP and NAB were found to be 2.665 min, 3.669 min and 4.984 min, respectively. The typical chromatogram of the standard solution of mixture is shown in Figure 1.

Figure 1. Representative chromatogram for paracetamol, naproxen (IS) and nabumetone

Precision

One set of three different concentrations of mixed standard solutions of NAB and PAR were prepared and NAP ($5 \mu\text{g mL}^{-1}$) was added in each solution. All the solutions were analyzed three times, in order to record any intra day variations in the results. Response factors of each drug to internal standard were calculated. For Inter day variations study three different concentrations of the mixed standard solutions in linearity range were analyzed on three consecutive days.

Accuracy

To check the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at three different levels of 50%, 100% and 150%. The percentages of recoveries were calculated, results of which are represented in Table 2.

Table 2. Recovery studies of NAB and PAR

Level of % Recovery	% Mean Recovery*		Standard Deviation		% R.S.D.*	
	NAB	PAR	NAB	PAR	NAB	PAR
50	101.90	99.67	0.4386	0.5232	0.4304	0.5249
100	99.44	101.61	1.4283	0.5734	1.4363	0.5643
150	99.07	101.32	1.0642	0.8845	1.0741	0.8729

*Avg. of three determinations, R.S.D. is relative standard deviation

LOD and LOQ

LOD and LOQ were calculated as $3.3 \sigma / S$ and $10 \sigma / S$ respectively; where σ is the standard deviation of the response (y-intercept) and S is the slope of the calibration plot.

Robustness

The influence of small, deliberate variations of the analytical parameters on peak area of the drugs was examined. The factors varied were flow rate of the mobile phase ($1.0 \pm 0.02 \text{ mL min}^{-1}$), a wavelength at which the drugs were recorded ($238 \pm 2 \text{ nm}$) and mobile phase percentage with respect to acetonitrile ($\pm 2\%$). One factor at the time was changed to estimate the effect. The solutions containing $10 \mu\text{g mL}^{-1}$ of NAB and $10 \mu\text{g mL}^{-1}$ of PAR and $5 \mu\text{g mL}^{-1}$ of NAP were applied onto the column. A number of replicate analyses ($n = 3$) were conducted at 3 levels of the factor (-, 0, +). It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed is robust.

Results and Discussion

Results were found to be linear in the concentration range of $5\text{--}25 \mu\text{g mL}^{-1}$ for both NAB and PAR with high correlation coefficient. The proposed method was also evaluated by the assay of commercially available tablets containing NAB and PAR. The % assay was found to be 100.60 ± 1.0068 for NAB and 100.21 ± 0.9545 for PAR (mean $\pm$ S.D., $n = 6$). The method was found to be accurate and precise, as indicated by recovery studies and % RSD not more than 1.5. The summary of validation parameters of proposed HPLC method is given in Table 3.

Table 3. Summary of validation parameters of proposed RP-HPLC method

Parameter	NAB	PAR
Linearity range, $\mu\text{g mL}^{-1}$	5-25	5-25
Correlation co-efficient	0.9999	0.9999
Slope (m)	0.1133	0.1355
Intercept (c)	-0.0600	0.0279
LOD ^a , $\mu\text{g mL}^{-1}$	0.233	0.260
LOQ ^b , $\mu\text{g mL}^{-1}$	0.698	0.789
Accuracy (% Recovery)	99.07-101.90	99.67-101.61
Precision (% RSD) ^c		
Intra day (n ^d = 3)	0.1097	0.0550
Inter day (n = 3)	0.9899	1.1511

^aLOD = Limit of detection. ^bLOQ = Limit of quantitation. ^cRSD = Relative standard deviation.
^dn = Number of determination

Conclusion

The simple, accurate and sensitive validated RP-HPLC method for simultaneous determination of two-component drug mixture of NAB and PAR has been developed. The method may be recommended for routine and quality control analysis of the investigated drugs in pharmaceutical formulations.

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