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A simple and sensitive RP-HPLC method for simultaneous analysis of nabumetone and paracetamol in pharmaceutical formulations

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ABSTRACT

Nabumetone is a non-steroidal anti-inflammatory drug used to treat pain or inflammation caused by arthritis and Paracetamol is analgesic and antipyretic drug. This paper describes a simple, accurate and sensitive high-performance liquid chromatographic method for simultaneous quantification of these compounds as the bulk drug and in tablet dosage forms. RP-HPLC separation and quantitation of drugs was carried out on Jasco HPLC system with Hypersil C₁₈ column (250 mm×4.6 mm) using a mobile phase of acetonitrile and 0.05 % aqueous acetic acid (70:30, v/v) at flow rate of 1 ml/min using Naproxen sodium as internal standard and detection using PDA detector was carried out at 238 nm. The method was validated for specificity, linearity, precision, accuracy, limit of detection, limit of quantification. The method has been successfully applied for the analysis of drugs in pharmaceutical formulation. The accuracy of the method was assessed by evaluation of linearity (5-25 µg/ml for both Nabumetone and Paracetamol), precision (intra-day RSD 0.1097 % and inter-day RSD 0.9899 % for Nabumetone and intra-day RSD 0.0550 % and inter-day RSD 1.1511 % for Paracetamol), and accuracy (99.07-100.12 ± 0.977 % for Nabumetone and 100.82 ± 0.660 % for Paracetamol) in accordance with ICH guidelines.

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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 arthritis^[1]. It is official in British Pharmacopoeia^[2] and United States Pharmacopoeia^[3]. Paracetamol (PAR), 4-hydroxyacetanilide is a widely-used analgesic and antipyretic drug^[4,5].

Literature review reveals that several chromatographic methods have been reported for the analysis of Nabumetone in biological fluids^[6-11] and in pharmaceutical formulations^[12] either alone or in combination with other drugs. Also methods have been published for analysis of Paracetamol which include HPLC^[13,14], non-suppressed ion chromatography^[15], spectrophotometry^[16-19] 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,

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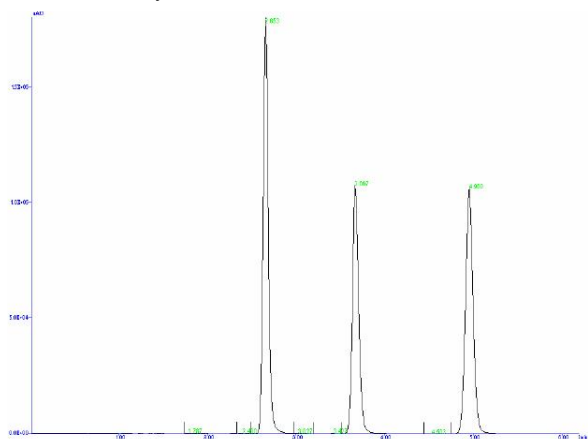


Figure 1 : Representative chromatogram obtained for standard mixture of Paracetamol (10 µg/ ml, 2.657 min), Naproxen sodium (5 µg/ ml, 3.667 min) and Nabumetone (10 µg/ ml, 4.960 min)

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) guidelines^[20].

EXPERIMENTAL

Chemicals and reagents

Analytically pure samples of NAB and PAR 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.

Instrumentation and chromatographic conditions

Jasco HPLC system consisting of Jasco PU-2080 plus HPLC pump, MD 2010 PDA detector and BORWIN-PDA (Version 1.50) software was used for analysis. Separation was carried out on Hypersil C₁₈ (250 mm×4.6 mm) column using Acetonitrile and 0.05 % aqueous acetic acid in the ratio (70:30, v/v) as mobile phase at flow rate of 1 ml/ min. 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

TABLE 1: System suitability parameters for RP-HPLC method

Sr. 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

* with respect to previous peak

done on Shimadzu balance (Model AY-120).

Preparation of standard stock solution

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. One millilitre 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 millilitre 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.

System suitability

The system suitability was assessed by six replicate injections of the mixture containing 10 µg/ ml of NAB and 10 µg/ ml of PAR and 5 µg/ ml of NAP were applied onto the column. The resolution, peak asymmetry, number of theoretical plates and HETP were calculated as represented in TABLE 1. The values obtained

TABLE 2 : Regression analysis of calibration curves for RP-HPLC method

Parameter	NAB	PAR
Linearity range ($\mu\text{g}/\text{ml}$)	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 ($\mu\text{g}/\text{ml}$)	0.233	0.260
LOQ ^b ($\mu\text{g}/\text{ml}$)	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

demonstrated the suitability of the system for the analysis of these drugs in combination. Mean retention times were found to be 2.657 min, 3.667 min and 4.960 min, for PAR, NAP and NAB respectively. The representative chromatogram of the standard solution of mixture is shown in figure 1.

Method validation

The method was validated for linearity, accuracy, intra-day and inter-day precision and robustness, in accordance with ICH guidelines^[20].

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.

Precision

One set of three different concentrations of mixed standard solutions of NAB and PAR were prepared and NAP (5 $\mu\text{g}/\text{ml}$) 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

TABLE 3 : 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

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 %.

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 ($1.0 \pm 0.02 \text{ ml}/\text{min}$), 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 a time was changed to estimate the effect. The solutions containing 10 $\mu\text{g}/\text{ml}$ of NAB and 10 $\mu\text{g}/\text{ml}$ of PAR and 5 $\mu\text{g}/\text{ml}$ 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:

For RP-HPLC method different mobile phases were tried and the mobile phase containing Acetonitrile and 0.05 % aqueous acetic acid in ratio (70:30, v/v) was

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found to be optimal for obtaining well defined and resolved peaks with mean retention times 2.657 min, 3.667 min and 4.960 min, for PAR, NAP and NAB respectively.

Straight-line calibration graphs were obtained for NAB and PAR with RP-HPLC method with high correlation coefficient. TABLE 2 summarizes linearity range, correlation coefficient, standard deviation, LOD and LOQ for the method. 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 % RSD values were satisfactorily low indicating reproducibility of the method. Results of recovery studies were found to be satisfactory (close to 100 %) and are reported in TABLE 3 for proposed RP-HPLC method.

CONCLUSIONS

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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