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Spectrophotometric and Reversed-Phase High-Performance Liquid Chromatographic Methods for Simultaneous Determination of Paracetamol and Nabumetone in Combined Tablet Dosage Form

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ABSTRACT

Simple, accurate, precise and sensitive ultraviolet spectrophotometric and reversed-phase high-performance liquid chromatographic (RP-HPLC) methods for simultaneous estimation of paracetamol (PCM) and nabumetone (NAB) in combined tablet dosage form have been developed and validated. Linearity of PCM and NAB was found in concentration range of 2-30 and 1-12 µg/mL in methanol at 249 and 230 nm, respectively for the spectroscopic method. The detection was carried out using a diode array detector set at 239 nm in Shimadzu LC 10 AT VP system and methanol: acetonitrile: water (55: 30: 15, v/v/v) as the mobile phase. Linearity of the LC method was in the concentration range of 5.0-100.0 and 5-60 µg/mL for PCM and NAB, respectively. The recoveries were in the range of 99.70 ± 0.38 and 99.38 ± 0.36 for PCM and 99.85 ± 0.09 and 99.31 ± 0.23 for NAB in simultaneous equation method and HPLC method, respectively. Both methods have been successfully applied for the analysis of PCM and NAB in a pharmaceutical formulation. Results of analysis were validated statistically.

Keywords: absorptivity, robustness, simultaneous equation, validation

Abbreviations: NAB, nabumetone; **PCM**, paracetamol; **RP-HPLC**, reversed phase high performance liquid chromatography

INTRODUCTION

Paracetamol (PCM) is chemically known as *N*-(4-hydroxyphenyl)acetamide and belongs to the class of compounds known as Non-steroidal anti-inflammatory and also having antipyretic action. It is official in Indian Pharmacopoeia, British Pharmacopoeia and United States Pharmacopoeia (Anonymous 1998, 2004, 2007). Its action mediates through cyclo-oxygenase-3 inhibition and modulation of central serotonergic pathways.

Nabumetone (NAB) is chemically known as 4-(6-methoxy-2-naphthalenyl)-2-butanone and is official in British Pharmacopoeia (2004), United state pharmacopoeia 30-National formulary 25(USP30-NF25) NAB is NSAID with prominent analgesic and has been most effective for the treatment of rheumatoid arthritis (Anonymous 2004, 2007). Chemical structures of PCM and NAB are shown in **Fig. 1**.

A literature survey revealed that the assay of the PCM in pure and dosage forms is official in Indian Pharmacopoeia, British Pharmacopoeia and USP30-NF25 (Anonymous 1998, 2004, 2007) apart from Pharmacopeias several analytical methods have been reported for the determination of PCM in biological fluids and urine (Hart *et al*. 1984; Nicholls *et al*. 1997; Hewavitharana *et al*. 2008), including column high-performance liquid chromatography (HPLC), HPLC/MS, and HPLC-NMR.

HPLC for determination of NAB from tablet formulation is official in USP30-NF25 and BP (2004). Several analytical methods that have been reported for the determination of NAB in biological fluids and in bulk as well as pharmaceutical formulations include HPLC, UV absorption spectrophotometry, fluorometry, gas chromatography/MS (GC/MS), and Fourier transform Raman and infrared spectrophotometry (Kobylinska *et al.* 2003; Margarita *et al.* 2003; Nobilis *et al*. 2004; Sahu and Annapurna 2009).

Fig. 1 Chemical structures of PCM and NAB.

This paper describes simple, accurate, precise, and sensitive UV-spectrophotometric and reversed-phase RP-HPLC methods for simultaneous determination of PCM and NAB in a combined tablet dosage form. The proposed methods were optimized and validated according to International Conference on Harmonization (ICH) guidelines (ICH Harmonized Tripartite Guideline 2005).

MATERIALS AND METHODS

Drugs and chemicals

Acetonitrile (HPLC grade) and methanol (HPLC and AR grade) were purchased from Merck (Mumbai, India) and water (HPLC grade and AR grade) was prepared in institute. All other reagents used were of analytical grade for the spectrophotometric determination and of HPLC grade for the HPLC method. Standard bulk drug samples of PCM (99.60% pure) and NAB (99.80% pure) were provided by Ipca laboratories Ltd. (Ratlam, India) as gratis sample. The pharmaceutical dosage form used in this study was Nilitis-P tablets labeled to contain PCM 500 mg and NAB 500 mg/tablets (Ipca lab, Mumbai, India).

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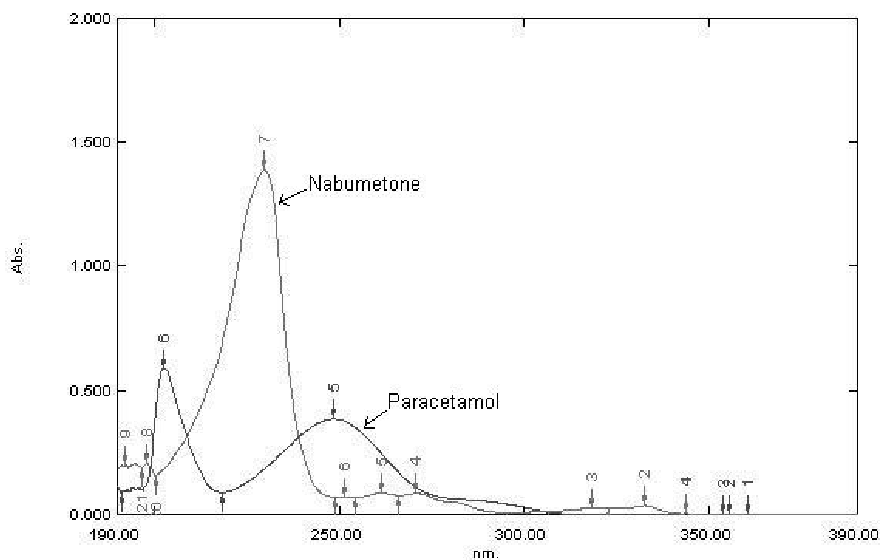

Fig. 2 Overlain spectra of PCM (4 µg/mL) and NAB (4 µg/mL).

Instrumental

A UV-visible double beam spectrophotometer (Model 1601; Shimadzu, Japan) with 1 cm matched quartz cells and UV probe software version 2.10 was used for the spectrophotometric method. For the HPLC method, an HPLC system consisting of LC 10 AT VP pump equipped with diode array detector (Shimadzu, Japan) and Luna C18 (4.6 mm id) column and class M10A software version 1.6 was used. A Rheodyne (Rohnert Park, CA) injector with a 20-µL loop was used for injecting the sample.

Method I: Spectroscopic method (Simultaneous equation method)

A stock solution of each drug having a concentration of 1 mg/mL (i.e. 1000 µg/mL) was prepared by dissolving PCM and NAB separately in methanol. Aliquots of the stock solutions were further diluted in distilled water and were scanned in the wavelength range of 400–200 nm. Zero order overlain spectra are presented in **Fig. 2**. The determinations were carried out at 249 and 230 nm, the maximum absorbance wavelength (λ_{max}) of PCM and NAB, respectively. Appropriate dilution were prepared using water from the stock solution of 1000 µg/mL of PCM and NAB to get aliquots of 2, 4, 6, 8 and 10 µg/mL. The calibration curves were plotted from mean absorbance values from six replicates. Absorptivity values for both drugs were determined at their respective λ_{max} by measuring absorbance values for working standards of PCM and NAB (Gandhi *et al*. 2008).

Procedure for analysis of tablet formulation: Twenty tablets were weighed accurately, and a quantity of tablet powder equivalent to 100 mg PCM and 100 mg NAB was transferred to a 100 mL volumetric flask, 70 mL methanol was added, and the flask was shaken vigorously for 5 min. The volume was made up to the mark with methanol. The solution was filtered and further diluted with distilled water to obtain a concentration within the Beer's law range. The absorbance of sample was measured at 249 and 230 nm. The contents of PCM and NAB were calculated by solving the following equations (Davidson *et al*. 2004).

$$A_1 = a_{x1}.b.C_x + a_{y1}.b.C_y$$

$$A_2 = a_{x2}.b.C_x + a_{y2}.b.C_y$$

where, a_{y1} and a_{y2} are the absorptivity of drug Y at λ_1 and λ_2, A_1 and A_2 are the absorbencies of the diluted sample at λ_1 and λ_2, b is the path length and C_x and C_y is the concentration of PCM and NAB, respectively in the diluted sample.

Method II: Chromatographic method (RP-HPLC method)

In the RP-HPLC method, separation and analysis of PCM and NAB were carried out on a Luna C18 column (4.6 mm id) with the diode array detector set at 239 nm. Mobile phase consisting of methanol: acetonitrile: water (55: 30: 15, v/v/v; filtered through a 0.2 µm membrane filter, degassed and sonicated) was used with a flow rate of 0.6 mL/min.

(a) Standard stock solutions: Standard stock solutions containing 100 µg/mL PCM, 10 µg/mL NAB were prepared by dissolving the pure drugs separately in the mobile phase (Gandhi *et al*. 2008; Sabnis *et al*. 2008).

(b) Preparation of the calibration curves: Aliquots of 1, 2, 3, 4, 5 and 6 mL stock solution of PCM and 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mL stock solution of NAB were transferred into a series of 10 mL volumetric flasks and the volume was made up to the mark with the mobile phase. Each solution was injected, and chromatogram was recorded. Mean retention times for PCM and NAB were found to be 4.608 and 7.057 min, respectively. The peak area of PCM and NAB were noted, and respective calibration curves were plotted as peak area against concentration of each drug (Gandhi *et al*. 2008; Sabnis *et al*. 2008).

(c) Procedure for analysis of tablet formulation: Twenty tablets were weighed accurately and a quantity of tablet powder equivalent to 50 mg PCM and 50 mg NAB weighed and transferred to a 50 mL volumetric flask containing about 35 mL mobile phase, ultrasonicated for 5 min, and the volume was made up to the mark with the mobile phase. The solution was filtered through Whatman (Florham Park, NJ) No. 41 paper, 0.2 mL filtrate was transferred to a 10 mL volumetric flask and the volume was made up to the mark with the mobile phase. The tablet sample solution was injected, the chromatogram was obtained and the peak areas were recorded. A representative chromatogram is given in **Fig. 3**. Form the peak area the both the drugs concentration of each drug/tablet was estimated from the respective calibration curves (Gandhi *et al*. 2008; Sabnis *et al*. 2008).

(d) Robustness studies: The influence of small, deliberate variations of the analytical parameters on the retention time of the drugs was examined. The following factors were selected for change: the wavelength at which the drugs were recorded (239 ± 1 nm) and the flow rate of the mobile phase (0.6 ± 0.02 mL/min). One factor at the time was changed to estimate the effect. The solutions containing 20 µg/mL PCM and 20 µg/mL NAB were applied onto the column. Six replicate analyses (n = 6) were conducted at 3 levels of the factor (–, 0, +) (Gandhi *et al*. 2008; Sabnis *et al*. 2008).

(e) Recovery studies: Accuracy of the method were analyzed by recovery studies carried out by addition of standard drug solution to pre-analyzed sample at 3 different levels: 80, 100, and

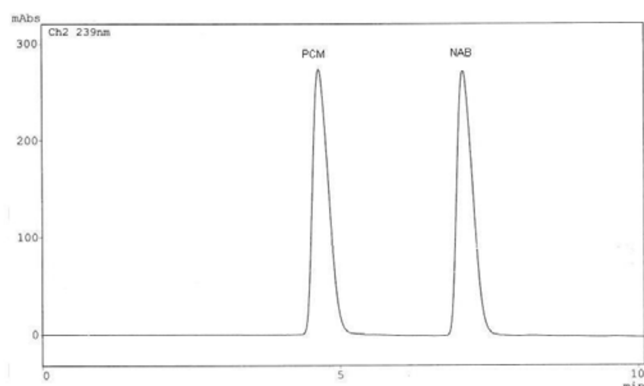

Fig. 3 Chromatogram of PCM and NAB in tablet dosage form.

120% (Gandhi *et al*. 2008; Sabnis *et al*. 2008).

(f) Precision: Precision of the method was checked by 3 replicate readings at 3 concentration levels of within range expressed as RSD values (Gandhi *et al*. 2008; Sabnis *et al*. 2008).

Statistical analysis

Means, standard deviation (SD), relative standard deviation (RSD), and linear regression analyses were calculated using Microsoft Excel 2003.

RESULTS AND DISCUSSION

For the spectroscopic method (Method I) methanol was used as common solvent for both the drugs. The linearity range of PCM and NAB was in the concentration range of 2-30 and 1-12 μg/mL at 249 and 230 nm, respectively.

For the RP-HPLC method (Method II), chromatogramphic conditions were optimized to achieve the best resolution and peak shape for PCM and NAB. Different mobile phases containing methanol, acetonitrile and water were examined (data not shown), and the mobile phase methanol: acetonitrile: water (55: 30: 15, v/v/v) was selected as optimal for obtaining well-defined and resolved peaks. The optimum wavelength for detection and quantitation was 239 nm, at which the best detector response for both the substances was obtained.

Straight line calibration curves were obtained for PCM and NAB in methods I and II. **Table 1** summarizes Beer's law limit, linear regression equation, correlation coefficient, standard deviations (SD), and limit of detection (LOD) and limit of quantitation (LOQ) values for both methods all the statistical validation parameters were found to be satisfactory and as per ICH Guideline. System suitability parameters for the RP-HPLC method are listed in **Table 2**.

Although spectroscopic and chromatographic methods (Anonymous 2007, Anonymous 2004, Anonymous 1998; Hart *et al.* 1984; Hewavitharana *et al.* 2008; Kobylinska *et al.* 2003; Sahu and Annapurna 2009) have been reported for analysis of PCM and NAB individually in bulk drug, biological fluid and urine but no method has been reported for simultaneous analysis of PCM and NAB.

Robustness studies of the HPLC method, carried out after deliberate alterations of the analytical wavelength and flow rate of mobile phase, showed that small changes of these operational parameters did not lead to changes of retention times for the peaks of interest. The effect of a single factor at two levels indicated that the selected factors remained unaffected by small variations of these parameters. Therefore, this method is suitable for routine analysis (**Table 3**).

The proposed methods were also evaluated in the assay of commercially available tablets containing PCM and NAB. Six replicate determinations were performed on the accurately weighed amounts of tablets. For PCM, recovery (mean%, ± SD, n = 6) was 99.71 ± 0.31 and 99.88 ± 0.29 for Methods I and II, respectively. For NAB, recovery was 99.05 ± 0.50 and 99.31 ± 0.51 for Methods I and II, respectively (**Table 4**).

For PCM, the recovery study results ranged from 98.86 to 100.20% and 98.54 to 99.89% for Methods I and II, respectively, with relative standard deviation (RSD) values ranging from 0.1 to 0.5% and 0.4 to 0.5%, respectively. For NAB, the recovery results ranged from 98.93 to 100.23% and 98.67 to 99.89% for methods I and II, respectively, with RSD values ranging from 0.03 to 0.7% and 0.4 to 0.5%, respectively. Results of recovery studies are reported in **Table 5**.

Table 1 Regression analysis of calibration curves of methods I and II.

Parameters	Method I		Method II	
	PCM	NAB	PCM	NAB
λ_{max}	249	230	239 [a]	239 [a]
Beer's law limit, μg/mL	2-30	1-12	5-100	5-60
Correlation coefficient	0.9994	0.999	0.9992	0.9994
Molar absorptivity	0.094	0.038	-	-
Linear regression equation [b]				
Intercept	0.0041	0.0728	- 22548	21685
Slope	0.919	0.337	204846	236829
SD [c]	0.014	0.027	10053.6	13074.9
Detection limit, μg/mL	0.51	0.27	0.16	0.18
Quantitation limit, μg/mL	1.56	0.82	0.49	0.55

[a] Detection wavelength for HPLC method.
[b] y = mx + c, where y is the absorbance and x is the concentration (μg/mL).
[c] SD = standard deviation.

Table 2 System suitability parameters for RP-HPLC method.

Parameters	PCM	NAB
Calibration range, μg/mL	5-100	5-60
Theoretical plate number	2974	3808
HETP [a]	0.0084	0.0066
Asymmetric factor	1.14	0.92
Tailing factor	1.29	1.03
Capacity factor (k')	1.26	3.92
Resolution	-	6.74

[a] HETP = Height equivalent to theoretical plate, cm

Table 3 Robustness data in terms of retention time for PCM and NAB [a].

Level	Wavelength [b]		Flow rate [c]	
	PCM	NAB	PCM	NAB
-	4.608 ± 0.085	7.057 ± 0.152	4.608 ± 0.084	7.057 ± 0.097
0	4.608 ± 0.093	7.057 ± 0.124	4.608 ± 0.082	7.057 ± 0.015
+	4.609 ± 0.110	7.057 ± 0.101	4.608 ± 0.134	7.057 ± 0.059

[a] Mean ± SD, n = 6.
[b] 230 ± 1 nm.
[c] 0.6 ± 0.02 mL/min.

Table 4 Results of analysis of commercial formulation.

Method	Label claim, mg/tablet		% claim, estimated [a]	
	PCM	NAB	PCM	NAB
I	500	500	99.48 ± 1.1	99.89 ± 0.15
II	500	500	100.1 ± 0.616	99.96 ± 0.351

[a] Mean ± Relative standard deviation, n = 6.

CONCLUSION

The validated UV-spectrophotometric and RP-HPLC methods developed here proved to be simple, fast, accurate, precise and sensitive. Thus, they may be used for routine analysis of PCM and NAB in combined tablet dosage form without prior separation.

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Table 5 Recovery studies of PCM and NAB by methods I and II.

Drug	Concentration taken, µg/ml for methods	Concentration added, µg/ml for methods	Total concentration found µg/ml		Recovery, % [a]	
			Method I	Method II	Method I	Method II
PCM	5	4	8.94	8.92	99.34 ± 0.0052	99.11 ± 0.0046
	5	5	9.96	9.92	99.68 ± 0.0029	99.25 ± 0.0054
	5	6	11.01	10.86	100.1 ± 0.0011	99.80 ± 0.0050
NAB	5	4	8.99	8.96	99.92 ± 0.0003	99.55 ± 0.0050
	5	5	9.99	9.90	99.98 ± 0.0013	99.29 ± 0.0056
	5	6	10.97	10.90	99.75 ± 0.0071	99.09 ± 0.0049

[a] mean ± relative standard deviation (n = 3).

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