

Development and Validation of Reverse phase high performance liquid chromatography method for simultaneous estimation of Paracetamol and Nabumetone in tablet dosage form

K. Anand Babu* and B. Jaykar

Department of Pharmaceutical Analysis, Vinayaka Mission's College of Pharmacy, Salem, Tamilnadu, India

ABSTRACT

In this study, reverse phase high performance liquid chromatographic method have been developed and validated for the simultaneous determination of Paracetamol and Nabumetone in combined pharmaceutical formulation. HPLC separation was achieved with a Phenomenex – Luna, C18 (250 x 4.6 mm i.d., 5 μ) as stationary phase and Methanol:Acetonitrile:Water (55:30:15 v/v/v) as eluent, at a flow rate of 0.6 ml/min. UV detection was performed at 239 nm. The retention time of Paracetamol and Nabumetone was found to be 4.2 and 7.2min respectively. Results of the analysis were validated statistically and by recovery studies. Linearity, accuracy, and precision were acceptable in the ranges of Paracetamol and Nabumetone (5-25 μ g/ml) respectively. The calibration curves were linear ($r^2 > 0.999$) in the range for each analyte. The % recovery for Paracetamol and Nabumetone is 100.49 and 99.42 respectively. No spectral or chromatographic interferences from the tablet excipients were found. The result of the studies showed that the proposed RP-HPLC method is simple, rapid, precise and accurate, which can be used for the routine determination of Paracetamol and Nabumetone in bulk and in its pharmaceutical dosage forms.

Key words: Paracetamol, Nabumetone, RP-HPLC, Validation.

INTRODUCTION

Paracetamol (acetaminophen) is one of the most popular over-the-counter analgesic and antipyretic drugs [1-4]. Paracetamol is available in different dosage forms: tablet, capsules, drops, elixirs, suspensions and suppositories. Paracetamol is chemically N - (4-hydroxyphenyl) acetamide and is used as analgesic and anti-pyretic agent. 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 [5]. Paracetamol[6-13] individually and in combination with other drugs like Valdecoxib, Aceclofenac, Chlorpheniramine maleate, Dipyrrone, Caffeine and Cetrizine in human plasma and pharmaceuticals were reported to be estimated by UV Spectroscopy and RP-HPLC. Nabumetone

belongs to a new class of NSAID with a low potential for causing gastrointestinal mucosal irritancy and inhibition of platelet function and has little effect on renal prostaglandin secretion and had less of an association with CHF (congestive heart failure) than other traditional drugs of the class [14]. The literature survey reveals that several chromatographic methods have been used for the analysis of Nabumetone in biological fluids [15-19] and in pharmaceutical formulations [20-21].

No reports were found for simultaneous determination of Paracetamol and Nabumetone in combined tablet dosage form by high performance liquid chromatographic method (HPLC).

MATERIALS AND METHODS

Standard gift samples of Paracetamol were provided by Divi's Laboratories Ltd. (Hyderabad, India) and Nabumetone was obtained as gift sample from Cipla Ltd. (Pune, India). Combined dose capsule formulation the tablet dosage form, Niltis P (Ipca laboratories Ltd., India) labeled to contain 500 mg of Nabumetone and 500 mg of Paracetamol were purchased from local market. All chemicals and reagents used were of HPLC-AR grade of S.D. Fine chemicals were used for the study.

Instrumentation

The HPLC system consisted of a Pump Shimadzu LC 10AT VP. Universal loop injector Rheodyne 7725 (injection capacity 20 μ L). Detector consists of photodiode array detector (PDA) SPD-10 AVP UV-Visible detector. CLASS-VP software.

Chromatographic conditions

Phenomenex – Luna, C18 (250 x 4.6 mm i.d., 5 μ) was used as stationary phase. Methanol:Acetonitrile:Water (55:30:15 v/v/v) was used as mobile phase and was filtered before use through 0.45 μ membrane filter. A constant flow of 0.6 ml/min was maintained throughout the analysis. Detection was carried out using UV detector at 239 nm. To ascertain the suitability of the proposed chromatographic conditions, system suitability tests were carried out and the results are shown in Table 1. Chromatogram of standard solution containing Paracetamol and Nabumetone is shown in Fig. 1.

Table 1: System Suitability Parameters

System suitability Parameter	Paracetamol			Nabumetone		
	RT	AUC	No. of theoretical plates	RT	AUC	No. of theoretical plates
Rep-1	4.226	1520948	7317	7.209	2352937	13810
Rep-2	4.228	1521166	7220	7.210	2349800	13927
Rep-3	4.231	1522644	7412	7.212	2350428	13856
Mean	4.22833	1521586	7316	7.210333	2351055	13864.36
S.D.	0.002517	922.7156		0.001528	1659	
% R.S.D.	0.0595%	0.0606 %		0.0211%	0.0701%	

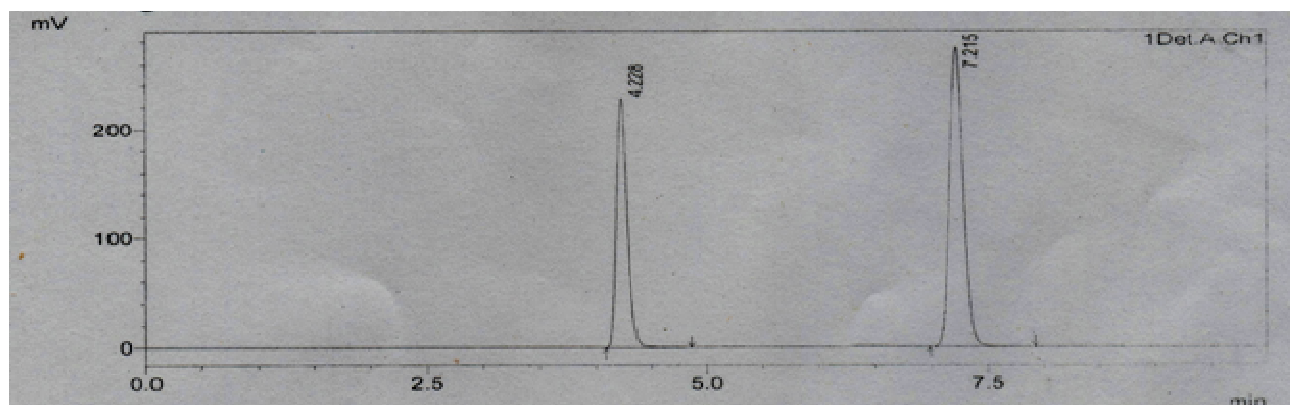


Figure 1: Typical chromatogram of Paracetamol and Nabumetone

Preparation of standard stock solution

Fifty mg each of Paracetamol and Nabumetone were accurately weighted and dissolved in few ml of methanol then volume was made up to 50 ml with the mobile phase to get solution of 1000 $\mu\text{g/ml}$. From the standard stock solutions of 1000 $\mu\text{g/ml}$ different dilutions were prepared using mobile phase for each drug having concentration in the linearity range with solvent. Then 20 μL of these solutions were injected into the LC system with the help of Hamilton syringe. Then the chromatograms were recorded at 239 nm., from the chromatogram it was cleared that Paracetamol retention time is 4.226 min and Nabumetone retention time is 7.209 min from which their area was noted and calibration curve was plotted between the peak area against their respective concentrations. From the calibration curve it was cleared that Paracetamol and Nabumetone has linearity range between 5-70 $\mu\text{g/ml}$ respectively.

Analysis of commercial preparation

For the preparation of the stock solution of tablet dosage form, 20 tablets of commercial tablets were taken and their average weight was determined, they were crushed to fine powder. Then powder equivalent to 10 mg of Paracetamol (respective quantity of Nabumetone) was taken in 50 ml volumetric flask and dissolved in 30 ml of methanol with vigorous shaking for 5-10 minutes. The supernatant liquid was transferred to 100 ml of volumetric flask through a whatman filter paper. The residue was washed twice with solvent and the combined filtrate was made up to 100ml mark. After that 10 ml of the above solution was diluted up to 100 ml with mobile phase. Six replicates of sample solutions were prepared of required concentrations of the three drugs. Then 20 μL of each replicates were injected into the system and their chromatograms were recorded. From the chromatograms it was observed that Paracetamol and Nabumetone were eluted at 4.225 and 7.209 minute respectively. The concentrations of these drugs were extrapolated from their respective calibration curves by using the area. Results of analysis of tablet formulation are shown in Table No. 2.

Table 2: Results of analysis of Tablet Formulation

Component	Label Claim*	Amount found*	Percentage label claim*
Paracetamol	500	499.93	99.98
Nabumetone	500	500.10	100.02

**Average of three determinations*

Method Validation

Accuracy

To ascertain the accuracy of the proposed method recovery studies were carried out by standard addition method, adding known amount of each drug to the pre analysed tablet powder, at three levels 80 %, 100 % and 120 % of the label claim. Recovery studies were carried out in triplicate at each level. The results of recovery studies were expressed as percent recovery and are shown in Table No. 3

Table 3: Result of recovery studies

Spike level	Amt. Taken(mg)		Amt. of pure drug added (mg)		% recovery	
	Paracetamol	Nabumetone	Paracetamol	Nabumetone	Paracetamol	Nabumetone
80	500	500	400	400	99.11	99.42
100	500	500	500	500	99.28	99.08
120	500	500	600	600	100.49	98.86

Precision

Intra-day precision was determined by analyzing the tablet samples at three different time intervals on the same day and for inter-day precision tablet samples was analyzed on three different days. Standard deviation for intra-day and inter-day assay precision was calculated. Results of precision studies are shown in Table No. 4.

Table 4: Result of Precision studies

Parameters	Component	% Estimation
Intra-day	Paracetamol	99.97
	Nabumetone	100.02
Inter-day	Paracetamol	99.99
	Nabumetone	100

Limit of detection (LOD) and Limit of quantitation (LOQ)

LOD and LOQ for both the drugs were calculated by using the values of slopes and intercepts of the calibration curves.

Robustness

Robustness of the proposed method was ascertained by deliberately changing the chromatographic conditions such as change in flow rate of the mobile phase (± 0.1 mL/min), change in composition of the mobile phase (± 1 ml) and change in pH of the buffer solution used in mobile phase. Effect of change in chromatographic parameters on resolution and tailing factor of peak was studied.

RESULTS AND DISCUSSION

The proposed chromatographic system was found suitable for effective separation and quantitation of Paracetamol (RT-4.2min) and Nabumetone (RT-7.2min) with good resolution, peak shapes and minimal tailing. The peak areas of the drugs were reproducible as indicated by low coefficient of variance indicating the repeatability of the proposed method. Both the drugs were found to give linear detector response in the concentration range under study with correlation coefficient of 0.9999 for both Paracetamol and Nabumetone, respectively. The sample recoveries from the formulation were in good agreement with their respective label claim indicating that there is no interference from the tablet excipients. The method exhibited good selectivity and sensitivity. Percent recoveries for Paracetamol and Nabumetone were 100.49 %

and 99.42% respectively indicating accuracy of the proposed method. %RSD for capsule analysis, recovery studies and intra-day & inter-day precision studies is less than 2. LOD and LOQ were found to be 8.4µg/ml for Paracetamol and 6.27µg/ml for Nabumetone, respectively. The results of robustness study also indicated that the method is robust and is unaffected by small deliberate variations in the method parameters.

CONCLUSION

The proposed method was validated as per ICH guidelines. The standard deviation and % RSD calculated for the proposed method is low, indicating high degree of precision of the method. The results of the recovery studies performed show the high degree of accuracy of the proposed method. Hence, it can be concluded that the developed RP-HPLC method is accurate, precise and selective and can be employed successfully for the estimation of Paracetamol and Nabumetone in bulk and marketed formulation.

REFERENCES

- [1] Current Index of Medical Specialties, Update Prescribers Handbook published by CMP Medica India Pvt. Ltd., Bangalore, Jul-Oct **2007**, 117-119,263.
- [2] Merck Index, Merck Research Laboratories, division of Merck and company NJ, USA, 13th ed., **2001**, 1088.
- [3] United State Pharmacopoeia 26, NF 21, **2003**, and 1259.
- [4] Indian Pharmacopoeia, Govt. of India, Ministry of Health and Family Welfare, Controller of Publications, New Delhi, 4th ed., vol.-2, **1996**, 554.
- [5] <http://en.wikipedia.org/wiki/nabumetone> accessed in April **2008**.
- [6] Celema C, Allue J.A., Prunonosa J., Peraire C and Obach R., *Journal of Chromatography Analysis*. **2000**, 870(1-2), 77-86.
- [7] Nagaralli B.S., Seetharamappa J., Gowda B.G. and Melwanki M.B., *Journal of Chromatography B*. **2003**, 798 (1), 49–54.
- [8] Eswaraselvam A., *I.P.C. Abstracts*. **2004**, 83.
- [9] Subramanian, G., *Indian J. Pharm. Sci.*, **2004**, 66(5), 694-696.
- [10] Momin M.Y., Yeole P.G., Puranik M.P and Wadher S.J., *Indian J. Pharm. Sci.*, **2006**, 68 (3), 387-389.
- [11] Bhavsar A.S., Talele G.S., Fursule R.A. and Surana S.J., *Indian J. Pharm. Sci.*, **2006**, 68(5), 675-677.
- [12] Nagulwar, V., Dhurvey, Y., Despande S., Upadhye K., Bakhle, S. and Wadetwar R., *Indian J. Pharm. Sci.*, **2006**, 68 (5), p. 639-640.
- [13] Mahaparale P.R., Sangshetti J.N. and Kuchekar B.S., *Indian J. Pharm. Sci.*, **2006**, 69 (2), 289-292.
- [14] Kristensen Jrgen, Viklund Andreas, Feltelius Nils and Larsson Anders, *Platelets*.**1996**, **7**, 149 - 152
- [15] AL-Momani I. F., *Anal. Lett.*, **1997**, 30, 2485.
- [16] Kobylinska K., Barlinska M., Kobylinska M., *J. Pharm. Biomed. Anal.*, **2003**, 32, 323. 17.
- [17] Nobilis M., Kopecky J., Kvetina J., Svoboda Z., Pour M., Kunes J., Holcapek M., *J. Pharm. Biomed. Anal.* **2003**, 32, 641.
- [18] Nobilis M., Holcapek M., Kolárová L., Kopecký J., Kunes M., Svoboda Z., Kvetina J., *J. Chromatogr. A*. **2004**, 1031, 229.
- [19] Rao R. N., Meena S., Nagaraju D., Rao A. R. R., *Biomed. Chromatogr.*, **2004**, 19, 362.
- [20] Mikami E., Goto T., Ohno T., Matsumoto H., Nishida M., *J. Pharm. Biomed. Anal*, **2000**, 23, 917.

[21] Can N. O., Tuncel M., Aboul-Enein H. Y., *Pharmazie*, **2003**, 58, 22.