Available online at www.pharmacie-globale.info ISSN 0976-8157

Research Article

PHARMACIE GLOBALE

INTERNATIONAL JOURNAL OF COMPREHENSIVE PHARMACY

SIMULTANEOUS DETERMINATION OF PARACETAMOL AND LORNOXICAM BY RP-HPLC IN BULK AND TABLET FORMULATION

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Received: 22 June 2011; Revised: 3 August 2011; Accepted: 30 August 2011; Available online: 5 September 2011

ABSTRACT

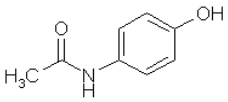
A simple, specific, accurate and precise reverse phase high pressure liquid chromatographic method has been developed for the simultaneous determination of paracetamol and lornoxicam from tablets by reverse phase C18 column ODS HyperSil (250 mm, 4.6 mm, 5 µm). The sample was analyzed using methanol: water (85:15, v/v), as a mobile phase at a flow rate of 1 mL/min. and detection at 259 nm. The retention time for paracetamol and lornoxicam was found to be 3.30 min and 2.14 min, respectively. The method can be used for estimation of combination of these drugs in tablets. The method was validated as per ICH guidelines. The linearity of developed method was achieved in the range of 0.4-1.4 µg/mL for paracetamol and 0.6-1.6 µg/ mL for lornoxicam and recoveries from tablets were between 100.36% and 100.17%. Due to these attributes, the proposed method could be used for routine quality control analysis of these drugs in combined dosage forms.

Keywords: HPLC, lornoxicam, paracetamol, simultaneous determination, validation.

INTRODUCTION

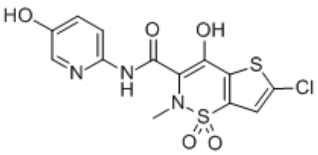
Paracetamol and lornoxicam are available in tablet dosage form. Chemically, paracetamol (PAR) is N acetyl-p-aminophenol (figure **1**).

Figure 1. Structure of Paracetamol



It has antipyretic and analgesic activity. Paracetamol is official in IP[1], BP[2] and USP[3]. Literature survey reveals many analytical methods for determination of paracetamol such as UV spectrophotometry[4], HPLC[5-10], and Capillary electrophoresis[11] methods from pharmaceutical preparations. Lornoxicam (LOR) is (3E)-6-chloro-3- [hydroxyl (pyridine-2-ylamino) methylene] -2-methyl- 2,3-dihydro-4H-thieno [2,3-e][1,2] thiazin-4-one- 1,1-dioxide (figure **2**).

Figure 2. Structure of Lornoxicam



It has non-steriodial anti-inflammatory activity. Lornoxicam is not official in any pharmacopoeia, but listed in the Merck Index.[12] On detailed literature survey, it was found that only few selected polarigraphic, spectrophotometric, RP-HPLC, LC-MS and LC/MS/MS methods were reported for estimation of Lornoxicam.[13-21] However, there is no reported method for simultaneous estimation of both drugs in combination. The current Research presents simple, rapid and economical method for the simultaneous analysis estimation of both the drugs from pharmaceutical dosage form.

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MATERIALS AND METHODS

Materials

Paracetamol and Lornoxicam were obtained as a gift sample by Itros Pharmaceutical Ltd. Pune. Methanol (HPLC grade) was purchased from Merck Chemicals Limited, Mumbai. In-house double distilled water was used throughout the study. Fixed dose combination tablet (Lornsafe-plus) containing 500mg paracetamol and 8mg lornoxicam were procured from local market.

Instrumentation

The HPLC system consisted of Intelligent HPLC pump model (Jasco PU 2080 Plus) with sampler programmed at 20 µL capacity per injection was used. The detector consisted of a UV/ VIS (Jasco UV 2075 Plus). Data was integrated using Jasco Borwin version 1.5, LC-Net II/ADC system. ODS HyperSil C18 (250 mm, 4.6 mm, 5 µm) column was used, Japan.

Preparation of standard and sample solutions

The standard stock solution was prepared by transferring 10 mg of paracetamol and 10mg lornoxicam in a 10 mL volumetric flask. Add about 10 mL of mobile phase and sonicate to dissolve. Dilute 0.1mL of this solution to 10mL with Mobile phase and mix. Final standard concentration of paracetamol and lornoxicam is 10 µg/mL. Lornsafe-plus 20 intact tablets were accurately weighed to determine average weight of tablets. Then tablets were finely crushed and tablet powder equivalent to about 500mg of paracetamol and 8mg of lornoxicam was transferred into 100 mL volumetric flask. Then 60 mL methanol was added to flask and sonicated for 30 minutes with intermittent shaking. Make the volume up to mark with mobile phase

and mix. Solution was filtered through 0.45 µm millipore filter; collect the filtrate by discarding first few mL of the filtrate. Dilute 0.1mL of this solution to 10 mL with mobile phase and mix to obtain final concentration of 50µg/mL for Paracetamol and 0.8µg/mL for Lornoxicam.

Chromatographic condition

Analytical conditions were standardized through the HPLC system using ODS HyperSil C18 column (250 X 4.6 mm, 5 µm). The mobile phase used was methanol: water (85:15, v/v), at a flow rate of 1 ml/ min. UV detection was made at 259 nm. The volume of injection was fixed at 20 µL. All analyses were done at temperature 30°C. The mobile phase was prepared fresh each day, vacuum-filtered through 0.45µm Millipore nylon filters.

Validation of the method

Validation was done as per ICH guideline Q2 (R1)[22]. The developed method was validated with respect to parameters such as linearity, LOD and LOQ, precision, accuracy and specificity

System suitability: The system suitability of the HPLC method was determined by making six replicate injections from freshly prepared standard solutions and analyzing each solute for their peak area, theoretical plates (N), resolution (R), and tailing factors (T).

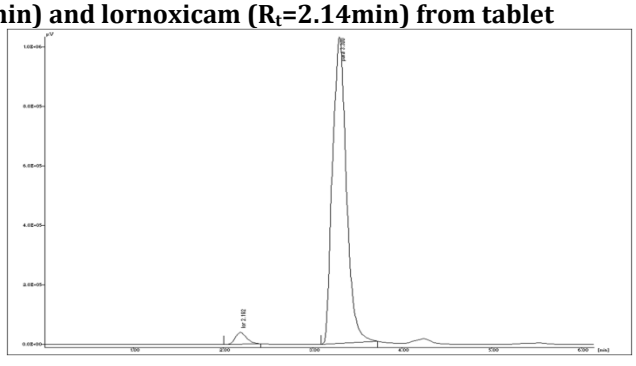
Linearity and range: Stock solution was prepared by dissolving 10 mg each of paracetamol and lornoxicam in 10 mL volumetric flask with methanol. From the above stock solutions, dilutions were made to get the concentration in the range of 0.6-1.6 µg/mL for paracetamol and 0.4-1.4 µg/mL for lornoxicam. A volume of 20 µL of each sample was injected into column. All measurements were repeated three times for each concentration and calibration curve was constructed by plotting the peak areas of analyte versus the corresponding drug concentration.

Limit of detection and limit of quantitation: The LOD and LOQ were calculated according to the 3.3 σ/s and 10 σ/s criteria, respectively; where σ is the standard deviation of the peak area and s is the slope of the corresponding calibration curve.

Precision: The precision of the proposed method was assessed as repeatability and intermediate precision by preparing three different sample solutions at low, medium and high concentrations, which were freshly prepared and analyzed daily. These experiments were repeated over a 3-day period to evaluate day-to-day variability (intermediate precision).

Specificity: The specificity of the method was ascertained by analysis of drug standards and samples. The mobile phase resolved both the drugs very efficiently, as shown in Figure **3**.

Figure 3. Chromatogram of paracetamol (R_t=3.30 min) and lornoxicam (R_t=2.14min) from tablet



Accuracy: To check the accuracy of the developed method and to study the interference of formulation additives, analytical recovery experiments were carried out by standard addition method, at 80, 100 and 120% level. The experiment was conducted in triplicate. Percentage recovery and relative standard deviation were calculated.

Robustness: The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

RESULTS AND DISCUSSION

Method development

Initially, the mobile phase used was methanol: water 70: 30 v/v spliting of peaks was observed. Then ratio of the solvents was varied, at 80:20v/v broad peak was observed. Good resolution of both the components was obtained with methanol: water at ratio 85:15 v/v. The flow rate of 1.0 mL/min was optimum. At 259 nm paracetamol is having low UV absorbance whereas lornoxicam has high UV absorbance with good Beer's law range. Also at this wavelength both paracetamol and lornoxicam can be quantified at tablet concentration ratio. Hence, 259 nm determined empirically has been found to be optimum. The average retention times for paraceatmol and lornoxicam was found to be 3.30 and 2.14 min, respectively.

System suitability

To ascertain its effectiveness, system suitability tests were carried out on freshly prepared stock solutions. The parameters obtained are shown in Table **1**.

Table 1. System suitability data of paracetamol and lornoxicam analysis

Parameters	Paracetamol	Lornoxicam
Retention time	3.30 min	2.14 min
Resolution	4.55	0.00
Asymmetry	1.41	1.11
Slope	10394	88427
Intercept	18842	17322
Theoretical plates	4346	3805
Peak area	154068	134246
Correlation coefficient (r^2)	0.998	0.997

Method validation

Linearity: Linear regression data for the calibration plots revealed good linear relationships between response and concentration over the ranges 0.6-1.6 µg/mLfor paracetamol and 0.4-1.4 µg/mL for lornoxicam. The linear regression equations were Y =10394X + 188842 (r^2= 0.998) for paracetamol and Y = 88427X - 17322 (r^2= 0.997) for lornoxicam.

Limits of Detection and Quantitation: The limits of detection and quantitation, calculated as described above, were 0.1µg/mL and 0.2µg/mL respectively, for paracetamol and 0.2µg/mL and 0.4µg/mL for lornoxicam. This indicates the method is sufficiently sensitive.

Precision: The precision of the method was expressed as relative standard deviation (RSD, %). The results listed in Table **2** reveal the high precision of the method.

Accuracy: The difference between theoretical added amount and practically achieved amount is called accuracy of analytical method. Accuracy was determined at three levels 80%, 100% and 120% of the target concentration in triplicate. The results are presented in Table **3**.

Robustness: The relative standard deviation of peak areas

was less than 2%. The RSD shown in Table **4** indicate the robustness of the method.

Table 2. Intra-day and inter-day precision of the method

Drug	Conc µg/ml	Intra-day precision (n=6)		Inter-day precision (n=6)	
		Measured Conc (µg/ml)	%RSD	Measured Conc (µg/ml)	%RSD
Paracetamol	0.6	0.58	0.18	0.55	0.12
	1	0.99	0.72	0.98	0.72
	1.4	1.3	0.63	1.4	0.64
Lornoxicam	0.8	0.79	0.14	0.81	0.15
	1.2	1.1	0.39	1.4	0.42
	1.6	1.3	0.71	1.5	0.75

Table 3. Results from recovery studies

Drug	Label claim mg/tab	Amount added %	Total amount mg	Amount recoverd mg	% Recovery[a]	(%RSD)
Lornoxicam	8	80%	14.4	14.2	98.61	0.69
		100%	16.0	16.1	100.62	0.92
		120%	17.6	17.5	99.43	0.57
Paracetamol	500	80%	900	896	99.55	0.063
		100%	1000	997	99.7	0.05
		120%	1100	1098	99.81	0.051

Table 4. Robustness of the method[a]

Chromatographic factors	Level	Chromatographic changes in R_t	
		Paracetamol	Lornoxicam
Flow rate ml/min			
0.90	-0.1	3.38	2.23
1.00	0	3.30	2.14
1.10	+0.1	3.21	2.08
Mean± SD		3.296 ± 0.085	2.15 ± 0.076
Methanol (± 5%)			
80	- 5%	3.34	2.18
85	0	3.30	2.14
90	+ 5%	3.31	2.13
Mean± SD		3.316 ± 0.021	2.15 ± 0.0264

[a]*Mean from six estimates*

Sample Analysis

When the Lornsafe-plus tablets were analysed, sharp and well defined peaks for paracetamol and lornoxicam were obtained at R_t 3.30 and 2.14, respectively, when scanned at 259 nm. The amount of the label claim measured were 99.67±1.52% for paracetamol and 99.33±0.5% for lornoxicam.

CONCLUSION

The proposed method is accurate, simple, economical, rapid and selective for the simultaneous estimation of paracetamol and lornoxicam in bulk and in tablet dosage form without prior separation. The excipients of the commercial sample analyzed did not interfere in the analysis, which proved the specificity of the method for these drugs. The proposed method involves direct quantification of both the components. By HPLC method analysis can be done within 10 min with the use of simple solvents. Hence, developed HPLC method can be conveniently adopted for the routine quality control analysis in the combination formulations.

ACKNOWLEDGMENT

The authors are thankful to Poona College of Pharmacy, Pune (M.S.), India, for providing necessary facilities and author also thankful to Itros Pharmaceutical Ltd. Pune, for providing drug samples.

REFERENCES

1. The Indian Pharmacopoeia, 2nd Ed, Vol II. Controller publications, New Delhi. 1996; 554.
2. The British Pharmacopoeia. Vol. II, 2007; 1575.
3. United States Pharmacopeia and National Formulary (USA NF) Asian edition vol 2. 2007; 1269.
4. Wadher S J, Pathankar P R, Puranik M et al. Simultaneous spectrophotometric estimation of Paracetamol and Metoclopromide HCl in solid dosage form. *Indian J of Pharm Sci*.2008; 70(3):393-395.
5. Ghada M, Hadad, Samy Emara, Waleed, Mahmand M M; Stability indicating RP-HPLC method for determination of Paracetamol with dantrolene and Cetrizine and Pseudoephedrine in two pharmaceutical dosage forms. *Talanta*. 2009; 79:1360-1367.
6. Udupa N, Karthik A, Subramanian G, Ranjith Kumar A; Simultaneous estimation of Paracetamol and Domperidone by HPLC method. *Indian J Pharm Sci*. 2007; 69(1):140-144.
7. Subramanian G, Vasudevan M, Ravishankar S, Suresh B; Validation of RP-HPLC method for simultaneous determination of Paracetamol, Methocarbamol, Diclofenac potassium in tablets. *Indian J Pharm Sci*. 2005; 67(2):260-263.
8. Fijalek Z, Wyszecka-Kaszuba E, Warowna-Grzeskiewicz M; HPLC with amperometric detection for the determination of 4- aminophenol, the main impurity of Paracetamol in multicomponent analgesic preparation. *J Pharm Boimed Anal*. 2003; 32:1081-1086.
9. Lotfi Monser, Frida Darghouth; Simultaneous LC determination of Paracetamol and related compound in pharmaceutical formulation using carbon based column. *J of Pharm Biomed Anal*. 2002; 27:851-860.
10. Vasudevan M, Ravisankar S, Ravibabu T, Nagarajan; Estimation of acetaminophen, dextropropoxyphene and oxyphenbutazone in combined dosage form by HPLC method. *Indian J Pharm Sci*. 2000; 62(2):122-125.
11. Shulin Zahao, Dan Xiao, Wenling Bai, Hongyan Yuan; Capillary electrophoresis with chemiluminescence detection of Paracetamol. *Anal Chim Acta*. 2006; 559: 195-199.
12. Merck Index - an encyclopedia of chemicals and drugs and biologicals, 13th edition, 5612.

13. Ibrahim Çetin, Nisa Koçak, Sule Aycan; Polarographic determination of lornoxicam in pharmaceutical formulations. *C B U J Sci*. 2009; 5(1):11-18.
14. Patel A, Sahoo U, Patel N et al. Development and Validation of Analytical Methods for the Simultaneous Estimation of Lornoxicam and Paracetamol from their Pharmaceutical Dosage Forms. *Current pharma research.* 2011; 1(2):140-144.
15. Nemutlu E, Demircan S and Kir S; Determination of lornoxicam in pharmaceutical preparations by zero and first order derivative UV Spectrophotometric methods. *Pharmazie*. 2005; 60(6):421-425.
16. Patil K, Devanand S, Shinde B et al. Stability indicating LC method for analysis of Lornoxicam in dosage form. *Chromatographia*. 2009; 69:1001-1005.
17. Shekhar M. Bhavsar, Dasharath M. Patel et al. Validated RP-HPLC method for simultaneous estimation of Lornoxicam and Thiocolchicoside in solid dosage form. *J Chem Pharm. Res*. 2010; 2(2):563-572.
18. Radhofer-Welte S, Dittrich P; Determination of the novel non-steroidal anti-inflammatory drug lornoxicam and its main metabolite in plasma and synovial fluid. *J Chromatography B: Biomedical Sciences and Applications.* 1998; 707(1-2): 151-159.
19. Mahesh Attimarad; Rapid RP-HPLC Method for Quantitative Determination of Lornoxicam in Tablets, *Journal of Basic and Clinical Pharmacy*, 2010; 001(002): 115-118.
20. Young Hoon Kim, Hye Young Ji, Eun -Seok Park et al. Liquid chromatography- electrospray lonization tandem mass spectrometric determination of lornoxicam in human plasma. Arch Pharmacal Res. 2007; 30(7):905-910.
21. Zeng Y L, Chen X Y, Zhang Y F et al. Determination of lornoxicam in human plasma by LC/MS/MS. Yao Xue Xue Bao. 2004; 39(2):132-5.
22. .ICH, Q2B. Validation of Analytical Procedure: Methodology. International Conference on Harmonisation, IFPMA, Geneva, 2005.