Asian Journal of Pharmaceutical and Clinical Research
Vol. 4, Issue 2, 2011 ISSN - 0974-2441

Research Article

ANALYSIS OF MULTICOMPONENT DRUG FORMULATIONS (DICLOFENAC AND PARACETAMOL)

KALLOL JANA, LOPAMUDRA ADHIKARI, S.K MOITRA AND ANINIDITA BEHERA

Department of Pharmaceutical Analysis and Quality Assurance, School of Pharmaceutical Sciences (SPS), Siksha 'O' Anusandhan University, Ghatikia, Kalinga Nagar, Bhubaneswar, India mail: kalloljana@yahoo.co.in

ABSTRACT

A simple, specific, accurate, cost effective & time efficient reversed phase high performance liquid chromatographic method was developed for the simultaneous determination of paracetamol and diclofenac, using RP-18 (4.6 mm X 2.5 cm) column and a mobile phase composed of acetonitrile : methanol (90:10 v/v) at flow rate 1 ml/min. The retention time of paracetamol and diclofenac were found to be 2.8 min and 3.9 min respectively which is much less than the other method used for the estimation of paracetamol and diclofenac . Linearity was established for paracetamol and diclofenac in the range 20 – 200 µg / ml and 300- 500 µg / ml respectively. The percentage recoveries of paracetamol and diclofenac were found to be in the range 99.56% and 103.05% respectively.The proposed method is precise, accurate, selective and rapid for the simultaneous determination of paracetamol and diclofenac for QC level.

Key words: Paracetamol , Diclofenace, RP-HPLC.

INTRODUCTION

Paracetamol and Diclofenac in combined tablet dosage form is widely marketed along all the NSAIDS till this date. Quality evaluation of this drug through the application of various analytical methods is attracting much attention. HPLC methods employing buffered mobile phases are extensively used [1-13]. The use of buffer itself adds to the cost of analysis as it involves extensive washing of the columns using considerable amount of HPLC grade solvents. The time required for washing the column also adds to the cost of analysis. This additional cost is also reflected in the cost of the drug. The present method has been developed excluding the use of buffer which makes it highly cost effective with respect to use of solvents as well as time of analysis. A RP – 18 column (4.6 mm X 2.5 cm,) and a mobile phase composed of acetonitrile-methanol (90:10v/v) at a flow rate of 1 ml/ min was used. The retention time of Paracetamol and Diclofenac were found to be 2.8 min and 3.9 min respectively.

EXPERIMENTAL

Reagents and materials

Reference standard of Diclofenac sodium and Paracetamol were procured from M/s C.I Laboratories, Kolkata. The tablet formulations DICLOGESIC, DYNAPAR, Diclomol were procured from Torrent, troika and Win-Medicare respectively. Acetonitrile, methanol, were of HPLC grade and purchased from Merck, India. Membrane Filters P/N – M47N45, 47 mm, 0.45µ were obtained from MZ ANALYSENTECHNIK.

Preparation of standard stock and sample solution:

The standard stock solution 20 mg/ml of paracetamol and diclofenac were prepared separately by dissolving 1 gm of each drug in 50 ml of acetonitrile.The solution were further diluted with the same solvent to obtain final concentration of 1000 µg / ml. Twenty tablet were powdered and made a solution of concentration of 0.35 mg/ml of diclofenac. Further dilution were made using acetonitrile to get the final concentration of 100 µg / ml of paracetamol.

Table 1: Calibration Curve of standard Drugs

Paracetamol		Diclofenac	
Concentration (µg / ml)	Peak area(µv)	Concentration (mg / ml)	Peak area (µv)
0	0	0	0
40	0.2754	0.3	0.165
60	0.44	0.4	0.23
80	0.541	0.45	0.25
100	0.6910	0.5	0.27

Assay

20 µl of standard and sample were injected into an injector of liquid chromatograph, from the peak area of paracetamol and diclofenac amount drug in samples were computed. The values are given in Table 2.

Chromatography

Chromatographic separation was performed on a Jasco HPLC system consisting of Jasco PU – 2089 pump, Jasco UV 2010 plus photo diode arrary detector. Rheodyne injection syringe with 20 µl loop volume and windows based chrompass software. An ODS C-18 RP- column (Intersite 4.6 mm X 2.5 cm,) was used for separation . The elution was carried out isocratically at flow rate of 1 ml / min using acetonitrile : methanol (90: 10 v/v) as mobile phase.The run time was 10 min. Before analysis both mobile phase and sample solutions were degassed by sonication and filtered through 0.2-µm filter . The analytes were monitored at 254 nm. The analytes were identified by comparison of retention times obtained from sample and standard solutions. The work was performed in an air-conditioned room maintained at 25 ± 2°C.

Table 2: Analysis of formulation:

Tablet formulation	Label claim	Amount found	Drug content %	S.D	COV%	S.E.
Formulation - I	Paracetamol -500mg	494.0 mg	98.8	0.368	0.371	0.212
	Diclofenac – 50 mg	51.55 mg	103.101	0.496	0.478	0.286
Formulation- II	Paracetamol -500mg	501.05mg	100.211	0.773	0.771	0.446
	Diclofenac – 50 mg		102.366	0.651	0.630	0.375

*Average of six determinations.

RESULTS AND DISCUSSION

HPLC Method Development and Optimization

Column chemistry, solvent type, solvent strength (volume fraction of organic solvent(s) in the mobile phase and pH of the solution), detection wavelength, and flow rate were varied to determine the chromatographic conditions giving the best separation.

The mobile phase conditions were optimized so the tablet components were free from interference from the solvent and from excipients. Other criteria, for example time required for analysis, appropriate *k* range for eluted peaks, assay sensitivity, solvent noise, and use of the solvent system for extraction of the drug from formulation matrices during drug analysis were also considered. Columns containing different stationary phases, the final choice giving satisfactory resolution and run time was the 25 cm × 4.6 mm,C18 reversed-phase column. A series of aqueous mobile phases containing methanol Acetonitrile and water solutions of different volume fractions were also tested. The best results were obtained by use of 90:10 (*v*/*v*) acetonitrile– methanol. The flow rate was determined by testing the effect of different flow rates on peak area and resolution; 1.0 mL min^{-1} was found to be optimum. All experiments were performed at ambient temperature. The appropriate wavelength for simultaneous determination of Paracetamol and Diclofenac is 254nm in 90:10 (*v*/*v*) acetonitrile–methanol.

Under the optimum chromatographic conditions, the retention times obtained for Paracetamol and Diclofenac were found to be 2.8 min and 3.9 min respectively. (Fig.1). Resolution (*RS*) between paracetamol and Diclofenac is 1.5680. Capacity factors, tailing factors, and number of theoretical plates are reported in Table 3.The values obtained for *k* and *RS* (1 < *k* < 10, *RS* > 1) show these chromatographic conditions are appropriate for separation and quantification of two compounds. The number of plates (*N*) is a measure of column efficiency; which shows the high separation efficiency of the column used.

Table 3

Property	Paracetamol	Diclofenac
R_t	2.853	3.973
T_f	1.92	1.39
κ'	1.013	2.133
N	3314.73	833.04
Rs	1.5680	
α	2.1056	

*R*t,retention time; *T*f, tailing factor; *k*′, capacity factor; *N*, number of theoretical plates; *R*S, resolution, α separation factor.

Validation of the Method

The method was validated for linearity, accuracy, precision, repeatability, selectivity, and specificity. All validation studies were performed by replicate injection of sample and standard solutions.

Linearity

Several aliquots of standard of paracetamol and diclofenace were taken in different 10 ml volumetric flasks and diluted up to the mark with acetonitrile such that the final concentration of paracetamol and diclofenace is 20- 200 µg / ml and 0.3 – 0.5 mg / ml respectively. Evaluation of two drug were performed with PDA detector at 254 nm, peak area recorded for all the peaks and are given in the table 1. Slope and intercept value for calibration curve was Y = 0.006X + 0.004(R^2 = 0.997)for paracetamol & Y = 0.550X + 0.001 (R^2 = 0.997)for diclofenac.

Accuracy

The accuracy of the method was confirmed by studying recovery at three different concentrations, 80, 100, and 120% of those expected, in accordance with ICH guidelines, by replicate analysis (*n* = 6). Standard drug solutions were added to a pre analyzed sample solution and percentage drug content was measured. The results from study of accuracy are reported in Table. 4. From these results it was clear that the method enables very accurate quantitative estimation of Paracetamol and Diclofenac in tablet dosage form, because all the results were within acceptable limits, i.e. COV < 2.0% and S.D. < 1.0.

Table 4

Drug	Amount Taken	Amount %	Added µg mL^{-1}	Recovery (%±SD)	COV (%)
Paracetamol	50	80	40	99.560 ±0.412	0.413
		100	50	100.583 ±0.470	0.467
		120	60	99.006 ±0.281	0.283
Diclofenac	400	80	320	103.056 ±0.342	0.332
		100	400	103.782 ±0.544	0.524
		120	480	103.94 ±0.606	0.583

Precision, LOD and LOQ

Precision was studied both intra-day and inter-day. Six replicate sample solutions were prepared from the stock solution. For study of intra-day precision the concentrations of the two drugs were measured three times on the same day at intervals of 1 hr. In the inter-day study the drug concentrations were measured on three different days. The limits of detection and quantitation, LOD and LOQ, were calculated by use of the equations LOD = $3.3\sigma/S$ and LOQ = $10\sigma/S$, where σ is the standard deviation of the blank and *S* is the slope of the calibration plot. The results are reported in **Table. 5.6.7**

Table 5

Drug	LOD µg mL^{-1}	LOQ µg mL^{-1}
Paracetamol	0.952	2.886
Diclofenac	0.0028	0.0085

Table 6

Drug	Slop	Intercept	Correlation coefficient
Paracetamol	0.006	0.003± 0.001732	≥0.997
Diclofenac	0.5556± 0.005477	0.0006± 0.001732	≥0.997

Table 7

Drug	Intra-Day Presision (COV%)	Inter-Day Presision (COV%) Day [1a]	Day [2a]	Day [3a]
Paracetamol	0.523	0.718	0.130	0.817
Diclofenac	0.5688	0.5424	0.584	0.623

Selectivity and Specificity

The selectivity of the method was checked by injecting solutions of Paracetamol and Diclofenac. It was observed that two sharp peaks for Paracetamol and Diclofenac were obtained at retention times 2.8 and 3.9 min, respectively. The specificity of the method was assessed by comparing chromatograms obtained from drug standards with that obtained from tablet solutions. The retention times of the drug standards and the drugs from sample solutions were same, so the method was specific. The method was also specific and selective because there was no interference from excipients in the tablets.

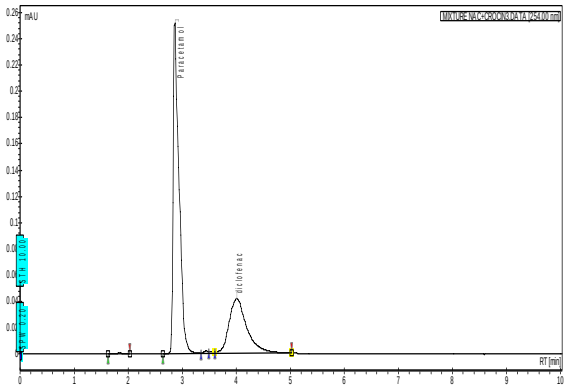

Fig. 1:

CONCLUSION

A new, rapid reversed-phase HPLC method has been developed for simultaneous analysis of Paracetamol and Diclofenac in a tablet formulation. It is shown above that the method was accurate, reproducible, repeatable, linear, precise, and selective, proving the reliability of the method. The run time is relatively short, i.e. 10 min, which enables rapid quantitation of many samples in routine and quality-control analysis of tablet formulations. The same solvent was used throughout the experimental work and no interference from any excipient was observed. These results show the method could find practical application as a quality-control tool for simultaneous analysis of Paracetamol and Diclofenac from their combined dosage forms in quality-control laboratories.

REFERENCES

1. Ravisankar S,Vasudevan M and Najan MJ. Reverse phase HPLC method for the estimation of paracetamol, chlorzoxazone, and diclofenace sodium in formulation. Indian Drugs. 1997;34(11)663-665.
2. Bhatia MS, Kashedikar SG and Chaturvedi SC. Comparative evaluation of Different Spectrophotometric methods for Simultanious estimation of paracetamol, chlorzoxazone and diclofenace sodium in combined dosage form, Indian Drugs.1997;34(3)49-153.
3. Jensen, L.S., Valentine, J., Milne, R.W. and Shimada, K.,J. Pharm. Biomed. Anal.,2004,34,585.
4. Godse VP. _Deodhar MN, Bhosale AV, Sonawane RA, Sakpal PS, Borkar DD and Bafana YS, Reverse Phase HPLC Method for Determination of Aceclofenac and Paracetamol in Tablet Dosage Form, *Asian J. Research Chem.. 2(1): Jan..-Mar. 2009.*
5. R. Bhushan, Deepak Gupta and Aparna Mukherjee, Liquid chromatographic analysis of certain commercial formulations for non-opioid analgesics, *Biomed. Chromatogr*. 21: 1284–1290 (2007).
6. M. Levent ALTUN, HPLC Method for the Analysis of Paracetamol Caffeine and Dipyrone, Turk J Chem, 26 (2002) , 521 { 528.
7. B.Gowramma, S. Rajan, S. Muralidharan, S. N. Meyyanathan and B. Suresh, A validated RP-HPLC method for simultaneous estimation of Paracetamol and Diclofenac Potassium in Pharmaceutical formulation, International Journal of ChemTech Research, Vol.2, No.1, pp 676-680, Jan-Mar 2010.
8. Prasanna Reddy.Battu, Simultaneous RP-HPLC Determination of Nimesulide and Paracetamol in Tablets, International Journal of PharmTech Research, Vol.1, No.3, pp 514-516,july-sept 2009.
9. Prasanna Reddy Battu1 and MS Reddy, RP-HPLC Method for Simultaneous Estimation of Paracetamol and Ibuprofen inTablets, *Asian J. Research Chem. 2(1): Jan.-March, 2009.*
10. V. G. Nayak, V. R. Bhate, S. M. Purandare, P. M. Dikshit, S. N. Dhumal and C. D. Gaitonde, Rapid liquid chromatographic determination of paracetamol and diclofenac sodium from a combined pharmaceutical dosage,Drug Development and Industrial Pharmacy, 1992, Vol. 18, No. 3, Pages 369-374.
11. G Subramanian, P Musmade, S Agarwal, N Udupa, Simultaneous RP HPLC estimation of tizanidine, diclofenac potassium and paracetamol in tablets, Indian Journal ofPharmaceutical sciences Year : 2004 ,Volume : 66 Issue : 5 Page : 694-696.
12. A Karthik, G Subramanian, A Ranjith Kumar, N Udupa, Simultaneous estimation of paracetamol and domperidone in tablets by reverse phase HPLC method,Indian Journal ofPharmaceutical sciences. Year : 2007. Volume : 69 , Issue : 1 , Page : 142-144.
13. YANG Fang , YUE Yong-Bo*(Dept.of Technology, Hebei Yuanzheng Pharmaceutical Co. Ltd., Shijiazhuang 050041) ,Determination of Diclofenac Sodium and Paracetamol in Compound Diclofenac sodium Injection by HPLC,Chinese Journal of Pharmaceutical 2006-01.
14. ICH, Q2A Validation of Analytical Procedures: Consensus Guidelines; ICH Harmonized Tripartite Guidelines,1994.
15.] ICH, Q2B Validation of Analytical Procedures: Methodology, Consensus, Consensus Guidelines;ICH Harmonized Tripartite Guidelines, 1996.
16. M. Ribani, C.H. Collins, and C.B.G. Bottoli, J. Chromatogr. A,1156,201 (2007)