Reverse Phase HPLC Method for Determination of Aceclofenac and Paracetamol in Tablet Dosage Form

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A simple, precise, accurate, and validated reverse phase HPLC method has been developed for the simultaneous estimation of aceclofenac and paracetamol in tablet by reverse phase C-18 column (Intersile 4.6 mm×25 cm, 10 µm) using acetonitrile: 50 mM NaH_2PO_4 in a ratio of 65:35 (pH adjusted to 3.0 with orthophosphoric acid) as a mobile phase at a flow rate of 1.5 ml/min and detection at 276 nm. The retention time for aceclofenac and paracetamol was found to be 1.58 and 4.01 min, respectively, and recoveries from tablet were between 99 and 101%. The method can be used for estimation of combination of these drugs in tablets.

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Aceclofenac (ACF), {[2-(2', 6'-dichlorophenyl) amino] phenyl acetoxyacetic acid} is a new phenyl acetic acid derivative with potent analgesic and antiinflammatory properties and improved gastric tolerance. Paracetamol (PCM), chemically 4-hydroxy acetanilide, is a centrally and peripherally acting analgesic and antipyretic agent. Several combination dosage forms of these two drugs containing ACF (100 mg) and PCM (500 mg) are available commercially. This combination is used for pain relief and management of rheumatoid arthritis. Only a few methods[1-4] have been reported for determination of ACF individually, whereas many methods[8-10] have been described in literature for determination of PCM alone or in combination with other drugs. However, there is no HPLC method reported for the simultaneous estimation of these drugs in pharmaceutical dosage forms. The present work describes a simple, precise, and accurate reverse phase HPLC method for simultaneous estimation of ACF and PCM in combined dosage form.

An isocratic HPLC system (Jasco HPLC) consisting of Jasco PU-980 pump, UV visible detector (Jasco UV 1580), a ODS C-18 RP C-18 column (Intersile 4.6 mm×25 cm, 10 µm), Rheondyne injection syringe and Windows-based Browin software (version 1.21) was used for analysis. Pure samples of aceclofenac and paracetamol were obtained from IPCA Laboratories Ltd., Mumbai; and Torrent Pharmaceutical Ltd., Ahmedabad. Acetonitrile and water used were of HPLC grade and obtained from E. Merck (India) Ltd., Mumbai. All other chemicals used were of AR grade. Optimized chromatographic conditions are listed in Table 1.

Standard stock solution (1 mg/ml) of ACF and PCM were prepared by dissolving 25 mg of drug in 25 ml of acetonitrile, separately. The solutions were suitably diluted with mobile phase to get mixed standard solution containing 3 µg/ml of ACF and 15 µg/ml of PCM.

Twenty tablets (Zerodol-P, IPCA Laboratories Ltd., Mumbai) each containing 100 mg of ACF and 500 mg of PCM were weighed, and powder equivalent to 25 mg of PCM was weighed accurately and taken into 25 ml volumetric flask. The drugs were extracted into acetonitrile, volume was adjusted to 25 ml, vortexed and then filtered through 0.45 µ membrane filter. From this solution, further dilutions were made using mobile phase to get a final concentration of 3 µg/ml of ACF and 15 µg/ml of PCM. Twenty microlitres of solution was injected into HPLC system to obtain chromatogram for standard drug solution (five replicates) and sample solution (five replicates). Concentrations of ACF and PCM in the formulation were calculated by comparing AUC of sample with that of standard.

Linearity and range of method was determined on standard solution by analysing 70 to 130% of test concentration, and the calibration curve was plotted using AUC versus concentration of standard solution. Accuracy of method was ascertained by recovery study by adding a known amount of standard drug (±20% of test

TABLE 1: OPTIMIZED CHROMATOGRAPHIC CONDITIONS

Parameter	Optimized condition
Chromatograph	Jasco HPLC
Column	ODS C-18 (Intersile 4.6 mm x 25 cm, 10 µm)
Mobile phase*	Acetonitrile: 50 mM NaH_2PO_4 (65:35% v/v), pH3 (dil orthophosphoric acid)
Flow rate	1.5 ml/min
Detection	UV at 276 nm
Injection volume	20 µl
Temperature	Ambient
Retention time- ACF	4.01 min
Retention time- PCM	1.58 min

*Filtered through a 0.45 µ membrane filter (Millipore), degassed and sonicated

TABLE 2: SYSTEM SUITABILITY PARAMETERS

Parameter	Aceclofenac	Paracetamol
Calibration range (µg/ml)	1.8 - 4.2	9.0 - 21
Theoretical plates	11434.73	6241.22
Resolution	-	4.83
Tailing factor	1.17	0.94
LOD (µg/ml)	0.045	0.030
LOQ (µg/ml)	0.160	0.100

TABLE 3: ANALYSIS OF FORMULATION AND RECOVERY STUDIES

Drug	Label claim (mg/ml)	*Estimation		**Recovery	
		mg/tablet	% label claim	Amount added µg/ml	% Recovery
ACF	100	99.98	99.98 (0.82)	2.4	99.80 (0.18)
				3.0	99.70 (0.45)
				3.6	99.90 (0.39)
PCM	500	501.2	100.2 (0.61)	12	100.03 (0.55)
				15	100.15 (0.84)
				18	99.90 (0.73)

*mean (% RSD) of five observations, **mean (% RSD) of three determinations

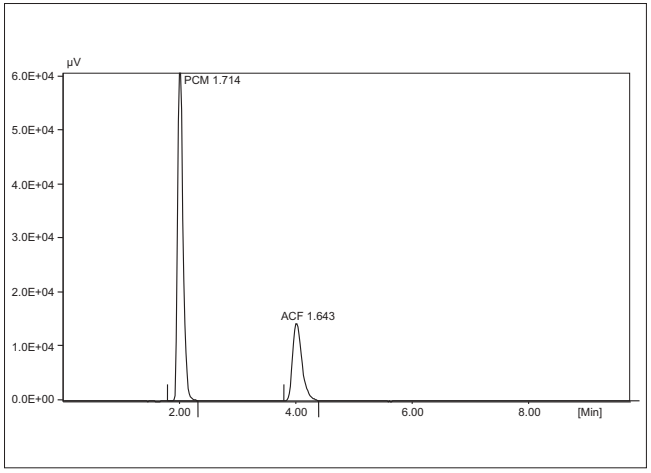

Fig. 1: Typical chromatogram of the sample solution.

concentration) to preanalysed sample and reanalysing the samples by proposed method. Precision was studied by analysing five replicates of sample solution. Specificity was carried out by exposing the sample to different stress conditions for 24 h, such as acidic (0.1 N HCL, 1 ml, 40°), basic (0.1 N NaOH, 1 ml, 40°), heat (60°), UV light (260 nm, 40°) and humidity (75% RH, 40°), before analysis by proposed method. Ruggedness[11] of method was evaluated by performing the assay with different analysts and on different days.

The chromatographic parameters were also validated by system suitability studies (Table 2), which were carried out on freshly prepared standard stock solutions. The typical chromatogram obtained from the formulation is presented in fig.1. The retention time for PCM and ACF was found to be 1.58 and 4.01 min, respectively. Peaks were well resolved with resolution of 4.83 between the two drugs and were symmetrical in shape with asymmetry factor less than 1.20. Linearity was observed in the concentration range of 1.8-4.2 µg/ml for ACF and 9-21 µg/ml for PCM, with the correlation coefficient of 0.9995 for ACF and 0.9999 for PCM, respectively. Accuracy of the method was ascertained by recovery study (n=3). The concentration of standard spiked to the sample was 2.4-3.6 µg/ml for ACF and 12-18 µg/ml for PCM. Recovery data from the study are reported in Table 3. The method was found to be accurate with percent recoveries between 99 and 101%. There was good repeatability of proposed method with coefficient of variance of 0.82% for ACF and 0.61% for PCM. The results of specificity studies indicated no interference from excipients, impurities, and degradation products under various stress conditions and assured that the peak response was due to a single component only. Hence, the present method is cost-effective, faster, and can be used for the routine analysis of these drugs from tablet formulations.

REFERENCES

1. Zawilla, N.H. and Mohammad, M.A., **J. Pharm. Biomed. Anal.,** 2000, 27, 243.
2. Saharty, Y.S. and el-Khateeb, S.Z., **Drug Develop. Ind. Pharm.,** 2002, 28, 571.
3. Hasan, N.Y. and Elkaway, M., **Farmaco.,** 2003, 58, 91.
4. Lee, H.S., Jeong, C.K. and Choi, S.T., **J. Pharm. Biomed. Anal.,** 2000, 23, 775.
5. Harish, L., Arora, A.R. and Gundu Rao, P., **Indian Drugs,** 1991, 28, 285.
6. Subramanian, G., Musmade, P. and Udupa, N., **Indian J. Pharm. Sci.,** 2004, 66, 694.
7. Patil, D. and Raman, B., **Indian Drugs**, 2001, 38, 36.
8. Marin, A., Garcia, E., and Barbas, C., **J. Pharm. Biomed. Anal.,** 2002, 29, 701.
9. Garcia, A., Ruperez, F. J. and Maza, A., **J. Chromatogr.,** 2003, 785, 237.
10. Hinz, B., Auge, D., Rietbrock, S. and Weren, U., **Biomed. Chromatogr.,** 2003, 17, 263.
11. ICH, Validation of Analytical Procedure: Methodology (Q2B), International Conference on Harmonization, IFPMA, Geneva, 1996.

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