

- Pasch, J.H., vonElbe, J.H. and Dinesen, N., **Food Product Development**, 1975, 9, 38.
- Pasch, J.H. vonElbe, J.H., **J. Food Sci.**, 1975, 40, 1145.
- Dreiding, A.S., In; Mabry, T.J., Eds., *Recent Advances in Phytochemistry*, Vol. 1, Appleton-Century-Crofts, New York, 1968, 147.
- Ansel, H.C., Allen, L.V., Popovich, N.G., In; *Pharmaceutical Dosage Forms and Drug Delivery systems*, 17th Edn., Lippincott Williams and Wilkins, New York, 1999, 318.
7. United States Pharmacopoeia 25, National Formulary 20, U.S. Pharmacopoeia Convention Inc., 12601, Twinbrook Parkway, Rockville, MD 20852, 2002, 2340.
8. VonElbe, J.H., Maing, Y. and Amundson, C.H., **J. Food Sci.**, 1974, 39, 335.
9. Jackman, R.L. and Smith, J.H., In; Hendry, G.A. F., Eds., *Natural Food Colourants*, Blackie and Son Ltd., London, 1992, 222.
10. Kibbe, A.H., Eds., In; *Handbook of Pharmaceutical Excipients*, 3rd Edn., American Pharmaceutical Association, 2000, 283.

RP-HPLC Estimation of Rofecoxib and Tizanidine in Combination Tablets

G. SUBRAMANIAN, S. PANDEY AND N. UDUPA*

Department of Quality Assurance and Dr. T. M. A. Pai Pharmaceutical Research Centre,
College of Pharmaceutical Sciences, MAHE, Manipal-576104

Accepted 8 September 2004

Revised 19 May 2004

Received 28 November 2003

A reverse phase high performance liquid chromatography method was developed for the simultaneous estimation of rofecoxib and tizanidine in tablet formulations. The separation was achieved by Luna C₁₈ column and methanol : phosphate buffer pH 3.5 (55:45 v/v) as eluent, at a flow rate of 1 ml/min. Detection was carried out at 240 nm. Valdecoxib was used as an internal standard. The retention time of rofecoxib and tizanidine was found to be 4.53 and 5.92 min, respectively. The method has been validated for linearity accuracy and precision. Linearity for tizanidine and rofecoxib were in the range of 0.6-1.4 µg/ml and 7.5-17.5 µg/ml, respectively. The mean recoveries obtained for tizanidine and rofecoxib were 98.73% and 99.70%, respectively. The developed method was found to be accurate, precise, selective and rapid for simultaneous estimation of rofecoxib and tizanidine in tablets.

Tizanidine HCl, 5-chloro-4-[2 imidazolin-2-yl amino]-2,1,3-benzothiadiazole, which is used as a centrally acting muscle relaxant¹. Rofecoxib is 4-[4-(methylsulfonyl)-phenyl]-3-phenyl-2 (5H)-furanone, a specific cyclooxygenase-2 inhibitor². It is indicated for the treatment of spasm and pain associated with musculoskeletal disorders. A tablet formulation containing 2 mg of tizanidine and 25 mg of rofecoxib is available (Siox-MR, Sysmed Laboratories). A survey of literature revealed that HPLC methods are reported for the determination of rofecoxib in tablets^{3,4} and biological fluids⁵⁻⁶. A HPTLC method for estimation of rofecoxib has also been reported⁷. RP-HPLC method was reported for the simultaneous estimation of tizanidine and

nimesulide in tablets⁸. Methods based on electroanalytical techniques⁹⁻¹⁰ and supercritical fluid chromatography¹¹ have also described in the literature for the estimation of tizanidine. However, no HPLC method for the simultaneous estimation of rofecoxib and tizanidine in combined dosage forms has so far been reported. The present work describes the development of a simple, precise and accurate reverse phase HPLC method for simultaneous estimation of rofecoxib and tizanidine in tablets.

The drug samples, rofecoxib and tizanidine were obtained as gift samples from the Sun Pharmaceutical Industries, Vadodara. HPLC grade methanol was supplied by Merck Co. Mumbai. Water of HPLC grade was collected from a Milli-Q system. Sodium dihydrogenorthophosphate AR and phosphoric acid AR were purchased from S. D.

***For correspondence**

E-mail: n.udupa@cops.manipal.edu

Fine Chemicals, Mumbai.

A gradient high pressure liquid chromatograph (Shimadzu HPLC Class VP series) with two LC-10AT VP pumps, variable wavelength programmable UV/Vis detector SPD-10AVP, SCL-10AVP system controller (Shimadzu) and operating software Shimadzu Class VP version 6.12 SP2 data station was used. The chromatography column used was a reverse phase Luna C-18 column (250 mm X 4.6 mm i.d., particle size 5 μ).

A mixture of methanol and 20 mM phosphate buffer (adjusted to pH 3.5 using orthophosphoric acid) in the ratio of 55:45 v/v was used as mobile phase and was filtered before use through 0.45 μ membrane filter. The flow rate of the mobile phase was maintained at 1 ml/min. Detection was carried out at 220 nm at the room temperature of 20°.

Standard stock solution of, rofecoxib and tizanidine (100 μ g/ml) was prepared in a mixture of warm methanol and water (1:1 v/v). The standard solutions were further diluted in mobile phase to contain a mixture of rofecoxib 12.5 μ g/ml and tizanidine 1 μ g/ml. Twenty tablets of Sioxx-MR (Sysmed Laboratories, Baroda), each containing 25 mg of rofecoxib and 2 mg of tizanidine were weighed and finely powdered. A quantity of powder equivalent to 12.5 mg of rofecoxib and 1 mg of tizanidine (half of an average weight) was weighed and transferred to a sintered glass crucible. The drugs were extracted with three 20 ml quantities of a mixture of methanol and water (1:1 v/v). The combined extracts were made upto 100 ml with mobile phase and further dilutions were made to get a concentration of 12.5 μ g/ml of rofecoxib and 1 μ g/ml of tizanidine and (theoretical value) as internal standard (test solution). The con-

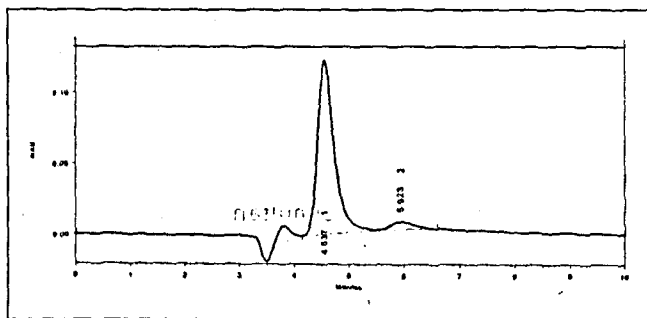


Fig.1: Typical Chromatogram of the Sample Solution
Chromatogram showing sample solution of rofecoxib and tizanidine at the approximate concentrations of 12.5 μ g/ml and 1 μ g/ml respectively. The excipient peak does not have interference with analytes.

TABLE 1: RECOVERY STUDIES

Drug	Amount added (μ g/ml)	Amount recovered (μ g/ml) (n=3)	Recovery (%)	Average recovery (%)
Tizanidine	0.1000	0.0984	98.40	98.73
	0.2000	0.1973	98.65	
	0.3000	0.2975	99.15	
Rofecoxib	1.2500	1.2467	99.74	99.70
	2.5000	2.4896	99.58	
	3.7500	3.7423	98.79	

Recovery studies data showing amount of drug recovered from sample solution and average percent recovery.

tents were mixed thoroughly and filtered through a 0.45 μ filter. Twenty microlitres of the test and standard solutions were injected separately and chromatogram was recorded upto 10 min.

The present investigation was aimed at developing a simple, precise and accurate HPLC method to estimate rofecoxib and tizanidine in tablets using the widely used RP-HPLC C₁₈ column (Luna). The mobile phase was optimized with 20 mM sodium phosphate buffer (pH 3.5) and methanol in the proportions of 45:55 v/v. With the above mentioned composition of mobile phase a good resolution between rofecoxib and tizanidine was achieved with a reasonably short runtime of 10 min. The criteria employed for assessing the suitability of above said solvent system were cost, time required for analysis, solvent noise, preparatory steps involved and the use of same solvent system for the extraction of the drug from the formulation excipient matrix for the estimation of the drug content. UV detection was carried out at 240 nm as tizanidine and rofecoxib showed good absorbance at this wavelength. The UV absorption of tizanidine was more than rofecoxib at this wavelength and because of which it was possible to have appreciable response for tizanidine (1 μ g/ml) and also the detector response was not saturated for rofecoxib (12.5 μ g/ml) peak. Thus it was possible to analyze both the drugs in a single chromatogram.

The retention time of rofecoxib and tizanidine was found to be 4.53 and 5.92 min, respectively. A typical chromatogram of the test solution was shown in the fig. 1. The

TABLE 2: SYSTEM SUITABILITY PARAMETERS

Parameter	Rofecoxib	Tizanidine
Tailing factor	1.12	1.93
Theoretical plates	4693	4473
Capacity factor	1.35	1.46
Resolution factor	-	2.25
Calibration range	7.5 – 17.5 $\mu\text{g/ml}$	0.6 – 1.4 $\mu\text{g/ml}$

System suitability parameters which were observed for rofecoxib and tizanidine

capacity factor (k') of tizanidine and rofecoxib was found to be 4.34 and 5.97, respectively. The peak shapes of both the drugs were symmetrical and the asymmetry factor was lesser than 2.0. The response factor (peak area ratio of standard peak area and internal standard peak area) of the standard and test solutions was calculated. The proposed method was validated as per the standard analytical procedures¹². Each of the samples was injected 6 times and the retention time was observed in all the cases. Precision of proposed HPLC method was found to be 0.36% for rofecoxib and 0.81% (relative standard deviation) for tizanidine. The low RSD values indicated that the proposed method had good precision. Linearity experiments were performed thrice for both the components and the response was found to be linear in the range of 7.5 to 17.5 $\mu\text{g/ml}$ for rofecoxib and 0.6 to 1.4 $\mu\text{g/ml}$ for tizanidine. Linearity of rofecoxib and tizanidine was plotted by a graph of response factor versus concentration. The correlation coefficient 'r' values ($n=3$) for both tizanidine and rofecoxib were ≥ 0.999 . Accuracy of the method was calculated by recovery studies ($n=3$) at three levels. Standard drug solutions containing drugs in the range 10-30% of nominal concentration (12.5 $\mu\text{g/ml}$ of rofecoxib and 1 $\mu\text{g/ml}$ of tizanidine) was added to previously analysed test solution. Amount of drug recovered at

each level ($n=3$) was determined. Percent recovery at each level was calculated. Table 1 shows the data from the recovery study data for tizanidine and rofecoxib. The average recovery of rofecoxib and tizanidine were 99.7% and 98.7%, respectively. The sample recovery in the formulation was in good agreement with the label claim. High percentage recovery showed that the method was free from interferences of the excipients used in formulations. System suitability parameters of rofecoxib and tizanidine were given in the Table 2. Assay of the combination in tablet dosage form was found to be 99.0% of rofecoxib and 99.2% of tizanidine. The method was simple and had short runtime of 10 min, which make the method rapid. The results of the study indicate that the proposed HPLC method was simple, precise, highly accurate specific, and less time consuming.

REFERENCES

- Reddy, K.S.S.R.K., Babu, M.J., Dubey, P.K., Shekar, B.C., Reddy, O.G. and Vyas, K., *J. Pharm. Biomed. Anal.*, 2002, 29, 355.
- Reynolds, J.E.F. Eds., In; Martindale, The Extra Pharmacopoeia, 29th Edn., Pharmaceutical Press, London, 1989, 31
- Radhakrishna, T., Rao, S.D. and Reddy, G.O., *J. Pharm. Biomed. Anal.*, 2001, 26, 617
- Aruna, A., Anusuya, P., Balamarriappan, C., Lakshmanan, M.K., Nanjappan, K and Kumarpavan, A.R., *Indian Drugs*, 2001, 38, 523
- Woolf, E., Fu I and Matuszewski, B., *J. Chromatogr. B. Biomed. Sci. Appl.*, 1999, 730, 221
- Chevez-Eng, C.M., Constanzer, M.L., and Matuszewski, B., *J. Chromatogr. B. Biomed. Sci. Appl.*, 2000, 748, 38
- Shingare, M.S., Naidu, K.R. and Kale, U.N., *Indian J. Pharm. Sci.*, 2003, 65, 315
- Tuncel, M. and Dogrulol, D., *Anal. Lett.*, 1992, 25, 1087
- Bouklouge, A.A., El Jammal, A., Vire, J-C and Patriache, G.J., *Anal. Chim., Acta.*, 1992, 257, 41
- Bhoir, I.C., Raman, B., Sundaresan, M. and Bhagawat, A.M., *Anal. Lett.*, 1998, 31, 1533
- International Conference on Harmonization, Guidance for Industry In; Q2B Validation on Analytical Procedures: Methodology, 1996, 2.