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# HPLC Method for Simultaneous Estimation of Rofecoxib and Tizanidine hydrochloride in Tablets

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**A reverse phase high performance liquid chromatography method for the simultaneous estimation of rofecoxib and tizanidine hydrochloride in tablets is developed. The determination was carried out on a Wakosil C18 (250x4.6 mm, 5 µm) column using a mobile phase of acetonitrile and phosphate buffer pH 5.0 (50:50%v/v). The flow rate was 0.5 ml/min with detection at 240 nm. The retention time for tizanidine hydrochloride was 4.9 min and rofecoxib 12.2 min. Rofecoxib showed a linear response in the concentration range of 50-200 µg/ml and tizanidine hydrochloride 10-80 µg /ml. The results of analysis have been validated statistically and by recovery studies. The recoveries obtained for standard rofecoxib and tizanidine hydrochloride from the formulation ranged from were 98.20 to 101.6%.**

Rofecoxib is widely used as an analgesic and antiinflammatory drug. Tizanidine hydrochloride is a centrally acting muscle relaxant. A tablet formulation containing rofecoxib (50 mg) and tizanidine hydrochloride (6 mg) in each tablet is marketed in India. Literature survey revealed that rofecoxib and tizanidine Hydrochloride can be estimated independently by a few spectrophotometric[1-3] and HPLC[4-6] methods. There is no official method published for simultaneous estimation of rofecoxib and tizanidine hydrochloride. Hence attempts were made to develop a simple, accurate and rapid HPLC method for simultaneous estimation of these two drugs in marketed tablet dosage forms.

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A Shimadzu HPLC SPD 10-AT Chromatograph equipped with UV/Vis detector and a Rheodyne injector with 100 ml external loop was used. A Wakosil C18 (250x4.6 mm, 5 µm), was the column employed. Elution was carried out using a mobile phase of composition potassium dihydrogen phosphate (0.02 M), pH 5.0 and acetonitrile in ratio of 50:50 v/v and a flow rate of 0.5 ml/min. The detector was set at 240 nm. Response of the peak areas were recorded and integrated using Winchrom oracle software. Standard samples of rofecoxib and tizanidine hydrochloride were gift sampled by Sun Pharmaceutical Industries Ltd., Mumbai. Acetonitrile of HPLC grade (Ranchem) and potassium dihydrogen phosphate of AR grade (Qualigens) were used.

Standard stock solutions of rofecoxib (1000 µg/ml) and tizanidine hydrochloride (120 µg/ml) were prepared in the

TABLE 1: RECOVERY STUDIES FROM TABLET FORMULATION

| Drug | Amount of standard (µg) | Amount of sample (µg) | Total standard and sample (µg)* | Recovery of Standard (µg) | % Recovery |
|---|---|---|---|---|---|
| Rofecoxib | 50 | 50 | 100.82 | 50.82 | 101.6 |
| | 100 | 50 | 148.92 | 98.92 | 98.92 |
| | 150 | 50 | 199.93 | 149.93 | 99.95 |
| Tizanidine | 10 | 12 | 21.82 | 9.82 | 98.20 |
| | 20 | 12 | 31.72 | 19.72 | 98.60 |
| | 30 | 12 | 41.98 | 29.98 | 99.93 |

*Average of six determinations

mobile phase. The stock solutions were suitably diluted with mobile phase and mixed to obtain mixed standard solution of rofecoxib (200 µg/ml) and tizanidine hydrochloride (24 µg/ml). Test solution was prepared by grinding 20 tablets (Rofact-MR by Sun Pharmaceutical Industries Ltd., Batch No. SK 30904) and powder equivalent to 50 mg rofecoxib and 6 mg tizanidine hydrochloride was transferred to a 50 ml volumetric flask. The active constituents of the powder were first dissolved in about 20 ml of mobile phase. The volume was then made up to 50 ml with mobile phase. The solution was filtered through Whatman No. 1 paper. A volume of 2 ml of this solution was diluted to 10 ml with mobile phase in a volumetric flask. The resulting solution was sonicated for 5 min. A volume of 100 µl of the standard and the test solution were injected separately and the chromatogram recorded. The retention time for tizanidine hydrochloride was 4.9 min and for rofecoxib 12.2 min.

The proposed method was validated as per standard analytical procedures. System precision and method precision experiments yielded results that were precise with percentage relative standard deviation of less than 2%. The %RSD for system precision was 1.81% for rofecoxib and 1.99% for tizanidine. Similarly %RSD for precision of the method was 1.44% for rofecoxib and 1.88% for tizanidine. Linearity response was found in the concentration range of 50-200 µg/ml for rofecoxib and 10-80 µg/ml for tizanidine hydrochloride with percentage curve fitting of 99.8% for rofecoxib and 99.9% for tizanidine hydrochloride. The correlation co-efficient 'r' value for rofecoxib was 0.998 and for tizanidine hydrochloride 0.999. The method was specific as no other peaks were visible up to 15 min. Robustness of the method was determined by carrying out the assay during which the mobile phase ratio and pH of mobile phase were altered. Percentage recovery was found to be in the range of 98.3% to 99.2% which was well within the acceptance limits of 98 to 102%. Ruggedness was determined by performing the same assay by different analysts and by performing the assay on different days. The recovery was 99.3% to 103% for rofecoxib and 98.8 to 103% for tizanidine hydrochloride. The test results were thus found to be well within the acceptance limit of 95% to 105%.

The accuracy of method was determined by adding known amount of standard to the previously analysed marketed sample at three different levels. The recovery was well within the acceptance limits and found to be within 98.9% to 101.6% for rofecoxib and 98.20% to 99.93% for tizanidine hydrochloride as shown in the Table 1. The system suitability parameters were calculated. The number of theoretical plates per meter of the column for rofecoxib and tizanidine hydrochloride were calculated as 19946 and 12180, with tailing factor of 1.11 and 1.19, respectively. The resolution between the two peaks was 0.974. Hence the proposed HPLC method was simple, accurate, robust, rugged and can be useful for simultaneous estimation of rofecoxib and tizanidine hydrochloride in tablet formulations.

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