Simultaneous RP-HPLC Estimation of Tizanidine, Diclofenac Potassium and Paracetamo in Tablets

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A simple, fast, precise and accurate liquid chromatographic method was developed for the simultaneous estimation of tizanidine, diclofenac potassium and paracetamol in tablets. This combination is used for spasm and pain associated with musculoskeletal disorders. Drugs are chromatographed on a reverse phase Luna C_{18} column using a mobile phase, 25 mM phosphate buffer (pH 7.0) and acetonitrile in the ratio of 40:60 v/v. Carbamazepine was used as an internal standard. The retention time of Tizanidine, Diclofenac potassium, Paracetamol and carbamazepine was 5.00, 8.61, 3.43 and 11.68 min respectively. The validation of the proposed method was also carried out. The method was found to be linear (correlation co-efficient r>0.999), precise (residual standard deviation: 0.51 % for paracetamol, 0.42 % for diclofenac potassium and 0.81 % for tizanidine), accurate (overall average recovery yields: 99.0 % for tizanidine, 99.3 % for diclofenac potassium and 98.6 % for paracetamol) and selective. Due to its simplicity and accuracy the proposed method can be used for routine quality control analysis of these drugs in combination tablets.

Tizanidine HCl (TIZ), 5-Chloro-4-[2-imidazolin-2-yl-amino]-2,1,3-benzothiadiazole, is used as a skeletal muscle relaxant. Diclofenac potassium (DCL) is used as a non-steroidal antiinflammatory drug and it is chemically potassium [o-(2,6-dichloroanilino)phenyl] acetate. Paracetamol (PAR), chemically 4-hydroxy acetanilide, is a centrally and peripherally acting non-opioid analgesic and antipyretic. A combination of these drugs, TIZ (2 mg), DCL (50 mg) and PAR (325 mg) is available commercially as Jusgo MR, Indi Pharma, Mumbai. This combination is used for spasm and pain associated with musculoskeletal disorders. Many methods[1-12] have been described in the literature for the determination of TIZ, DCL and PAR, individually. However, there is no HPLC method reported for the simultaneous determination of these drugs either as active pharmaceutical ingredient or from dosage forms. The present work describes a simple, precise and accurate reverse phase HPLC method for simultaneous estimation of TIZ, DCL and PAR in combined dosage forms.

The drug samples, tizanidine, diclofenac potassium and carbamazepine were obtained as gift samples from the Sun Pharmaceutical Industries, Mumbai. Paracetamol was obtained as gift sample from Karnataka Antibiotics and Pharmaceuticals, Bangalore. Sodium dihydrogen orthophosphate AR, orthophosphoric acid AR and acetonitrile of HPLC grade were supplied by S.D. Fine Chemicals, Mumbai. Water of HPLC grade was obtained from a Milli-Q RO water purification system.

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 for the analysis.

The method was carried out on a Luna C-18 (250 mm x 4.6 mm i.d., 5 µ) column as a stationary phase and acetonitrile:25 mM phosphate buffer (pH adjusted to 7.0 with orthophosphoric acid) in the ratio of 40:60 v/v as the mobile phase at the flow rate of 1 ml/min. The mobile phase was filtered through a 0.45 μ membrane filter and degassed before analysis. A Rheodyne 7725 injector with a 20 μl loop was used for the injection of samples. Detection was

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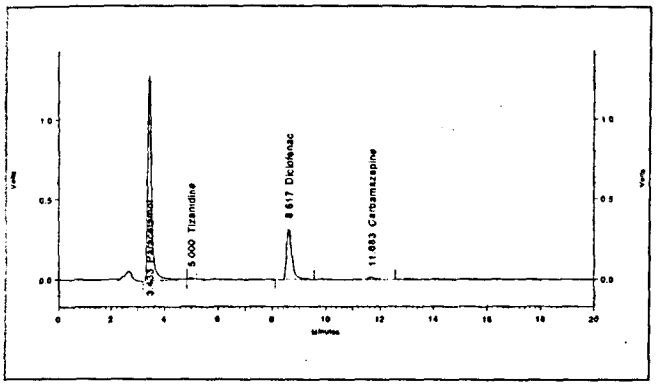

Fig.1: Typical Chromatogram of the Sample Solution

Chromatogram showing sample solution of commercially marketed tablets along with carbamazepine as an internal standard

done at 220 nm and separation was carried out at the room temperature of about 20°.

Standard stock solution of TIZ, DCL, PAR and carbamazepine (100 μg/ml) were prepared separately in a mixture of acetonitrile and water (1:1 v/v). From the standard stock solutions, mixed standard solution was prepared containing 0.1 μg/ml of TIZ, 2.5 μg/ml of DCL, 16.25 μg/ml of PAR and 10 μg/ml of carbamazepine as internal standard.

Ten marketed tablets (Jusgo MR Indi Pharma, Mumbai), each containing 2 mg of TIZ, 50 mg of DCL and 325 mg of PAR were weighed and finely powdered. A quantity of powder equivalent to 1 mg of TIZ, 25 mg of DCL and 162.5 mg of PAR (half of an average weight) was weighed accurately and transferred to a sintered glass crucible. To this 10 ml of carbamazepine (10 mg/ml) was added and the drugs were extracted with three quantities, each of 20 ml of mixture of acetonitrile and water (1:1 v/v). The combined extracts were made up to 100 ml with mobile phase and further dilutions (100 times) were made to get a concentration of 0.1 μg/ml of TIZ, 2.5 μg/ml of DCL, 16.25 μg/ml of PAR and 10 μg/ml carbamazepine (theoretical value). The content was vortexed, filtered through a 0.45 μ membrane filter and injected in triplicate. The ratio of peak area of drug to that of internal standard was calculated. The mixed standard solution was subjected to proposed HPLC method of analysis for finding out intra and interday variations. Linearity and range of the method was also determined by analyzing mixed standard solutions. The calibration curve was plotted using response factor versus concentration of the standard solutions. Recovery studies were carried out

TABLE 1: RECOVERY STUDIES

Drug	Amount added (μg/ml)	Amount recovered (μg/ml) n=3	Recovery (%)	Average Recovery (%)
TIZ	0.07	0.069	98.6	99.04
	0.10	0.099	99.1	
	0.013	0.13	99.5	
DCL	1.75	1.74	99.5	99.26
	2.50	2.49	99.6	
	3.25	3.24	98.9	
PAR	11.38	11.36	99.8	98.62
	16.25	15.90	97.8	
	21.13	20.87	98.2	

Recovery studies data showing amount of drug recovered from sample solution and average percentage recovery. TIZ stands for tizanidine hydrochloride, DCL for diclofenac potassium and PAR for paracetamol.

by adding known amount of standard drug to the pre-analyzed samples and reanalyzing them using the HPLC method of analysis which is being developed.

The present study was carried out to develop a simple and rapid HPLC method for the simultaneous estimation of TIZ, DCL and PAR using most widely used Luna C-18 column. The typical chromatogram of TIZ, DCL, and PAR with the internal standard carbamazepine in the formulation is presented in fig. 1. The retention time of TIZ, DCL, PAR and carbamazepine was found to be 5.00, 8.61, 3.43 and 11.68 min, respectively. The assay concentration of 0.1 μg/ml TIZ, 2.5 μg/ml of DCL and 16.25 μg/ml of paracetamol was selected according to the labeled claim. The peaks were well resolved and the capacity factor (k') between PAR and TIZ was found to be 4.88 whereas between TIZ and DCL was 9.14. The drug peaks were symmetrical in shape and asymmetry factor for all the peaks were found to be less than 1.20.There was good repeatability of the proposed method as the precision of the method was less than 2% for all the three drugs. The coefficient of variance for TIZ, DCL and PAR where found to be 0.81%, 0.42% and 0.51%, respectively that shows the method is highly precise.

Linearity experiment was performed thrice for all the three components and response was found to be linear in the concentration range of 0.06-1.14 μg/ml for TIZ, 1.5-3.5 μg/ml for DCL and 9.75-22.75 μg/ml for PAR. Regression

lines were obtained at 95% confidence interval using least square method. Correlation coefficient 'r' values (n=3) for all three drugs were ≥0.999. Accuracy of method was determined by recovery studies (n=3). The concentration of standard spiked to the sample was 0.07-0.13 μg/ml for TIZ, 1.75-3.25 μg/ml for DCL and 11.4-21.1 μg/ml for PAR. Recovery data from the study are reported in Table 1. The mean % recovery was found to be 99.0 % for TIZ, 99.3 % for DCL and 98.6 % for PAR. The content of the drugs in the commercial dosage form was found to be 99.1 % of TIZ, 99.5 % of DCL and 99.6 % of PAR per tablet by this method. The estimated amount was within the acceptable limits of the labeled claim of the formulation.

The developed RP-HPLC method provides a convenient and efficient method for the separation and estimation of TIZ, DCL and PAR in combined dosage form. There was no interference from the excipients used in the tablet formulation and hence the method is suitable for analysis of tablets. The results of validation showed that the proposed method is simple, linear, precise, accurate and selective and employed in routine assay of TIZ, DCL and PAR in tablets.

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Stability Evaluation of Beetroot Colour in Various Pharmaceutical Matrices

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A red pigment was obtained from dried juice of *Beta vulgaris* Linn, which exhibited a λmax of 533 nm and 484 nm indicative of the presence of betacyanins (red) and betaxanthins (yellow), respectively. The influence of temperature and pH on the stability of the red pigment in pharmaceutical liquid oral bases was investigated. The colour was also evaluated for its stability in its adsorbed form on microcrystalline cellulose, lactose and dextrose. The colour was found to exhibit highest stability in adsorbed form with microcrystalline cellulose.

Colours form an integral part of pharmaceutical additives to enhance the organoleptic properties and patient acceptance. The use of certain synthetic colours has been banned, as they are well known to produce toxicity in animals, e. g. auramine found to inhibit growth and lead to liver and kidney dysfunction and so many others known for hypersensitivity and carcinogenic reactions[1]. Hence a lot of attention has been given to develop natural pigments as colourants with higher safety margins. In caryophyllales the

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