Original Article

Development and Validation of Reversed-Phase High Performance Liquid Chromatographic Method for Estimation of Dexketoprofen Trometamol in Bulk and Tablet Dosage Form

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ABSTRACT

The Literature survey indicates several methods for the determination of Dexketoprofen trometamol. So an attempt was made to develop and validate a simple, precise, accurate, and economical RP-HPLC method as per ICH guidelines for the estimation of Dexketoprofen trometamol in bulk and pharmaceutical dosage forms. A simple reverse phase HPLC method was developed for the determination of Dexketoprofen trometamol present in pharmaceutical dosage forms. C18 (4.6ID x 250mm) in an gradient mode with mobile phase Acetonitrile: Methanol (25:75) was used. The flow rate was 0.9ml/ min and effluent was monitored at 262nm. The retention time was 2.9min for Dexketoprofen trometamol. The linearity ranges were found to be 30-70µg/ml

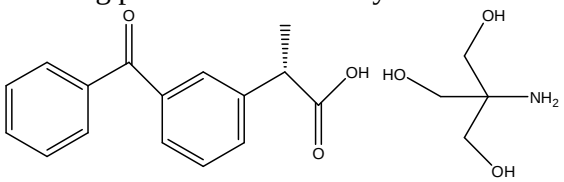
Keywords: Dexketoprofen trometamol**,** HPLC, Method Validation

INTRODUCTION

Dexketoprofen trometamol (DKP) chemically, 2-amino-2-(hydroxymethyl) propane-1,3-diol; 2-(3-benzoylphenyl propanoic acid is a water-soluble salt of the dextrorotatory enantiomer or (S)-(+)-enantiomer of the nonsteroidal anti-inflammatory drug (NSAID) ketoprofen[1]. The enantiomer is a relatively new oral NSAID with analgesic, anti-inflammatory and anti-pyretic properties and is one of the most potent in vitro inhibitors of prostaglandin synthesis[2].

Dexketoprofen trometamol is a new, quick acting analgesic for the treatment of painful musculoskeletal conditions such as osteoarthritis and low back pain. It is also used as a treatment for post-operative pain, toothache and dysmenorrhoea[3]. It is the active optical isomer (eutomer) of ketoprofen, a propionic acid non-steroidal anti-inflammatory drug (NSAID). The eutomer has been separated to halve the dosage required and halve the metabolic load. The inactive isomer (distomer) has

been discarded in the hope of eliminating or reducing potential unnecessary side effects[4].



Dexketoprofen trometamol

MATERIALS AND METHODS

Chemicals and Reagent

Acetonitrile of HPLC grade and Methanol of HPLC grade is purchased of company Hexon chemical.

Conditions

Instrument : HPLC
Binary Gradient System
Detector : UV
Column : C18 (4.6ID x 250mm)
Temparature : 25^0 C
Flow Rate : 0.9ml/min
Wavelength : 262 nm
Run Time : 6 min.
Injection volume: 20µl
Mobile Phase: Acetonitrile:Methanol (25:75)
Diluent : Mobile phase
Retention Time: 2.9

Stock Solution and Standard

Selection of Mobile phase

A number of trials were made to find out the ideal solvent system (mobile phase) for eluting the drug. The mobile phase containing Methanol: Water (50:50), was tried. Better peak resolution and adequate retention time were obtained with the ratio of Acetonitrile (HPLC grade) : Methanol (HPLC grade) (25:75).

Preparation of Mobile phas

The mobile phase was prepared by mixing Acetonitrile (HPLC grade) : Methanol (HPLC grade) in the ratio of 25:75. The mobile phase is then sonicated using Ultra-Sonicator to remove the impurities and dissolved gases, as they may lead to unwanted peaks in the chromatogram.

Preparation of standard stock solution

Stock solution of Dexketoprofen trometamol was prepared by dissolving 100mg of Dexketoprofen trometamol in 100ml of volumetric flask containing mobile phase. The solution was sonicated for about 10min and then made up to volume with mobile phase.

Preparation of sample solution

20 tablets of were weighed and powdered in glass mortar. The powder equivalent to 25mg of active ingredient present was transferred into a 100ml volumetric flask, 70ml of diluent was added to it and was shaken and sonicated for about 20minutes and diluted up to the mark with diluent and the solution was filtered through 0.45µm filter before injecting into the HPLC system.

METHOD VALIDATION

Accuracy, Precision, linearity, Limit of detection and limit of quantification were studied to validate the RP-HPLC method for determination of Dexketoprofen trometamol.

RESULTS & DISCUSSION

Linearity

The linearity of an analytical procedure is its ability to obtain test results which are directly proportional to the concentration of analyte in the sample. Calibration curve was constructed by plotting absorbance versus concentration which showed linearity over the concentration range of 30-70µg/ml.

Accuracy

The accuracy of the method was established by using recovery experiments i.e.

by external dilution method. The known amount of standard was added at three different levels of 50%, 100% and 150% of sample. The percentage recoveries were calculated from calibration curve. The data is summarised in table 2.

Precision

The precision of analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurement obtained from multiple sampling of the same homogenous sample under the prescribed condition. Precision was determined by repeatability (intra-day) and intermediate precision (inter-day). Repeatability was evaluated by 3 determinations of 3 different concentrations during the same day. Intermediate precision was determined during 3 different days. Precision (intra-day and inter-day) were expressed as relative standard deviation.

Detection and quantification limits

Limit of detection

The limit of detection of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected it was calculated using the following formula

$$LOD = 3.3\sigma/S$$

Where, σ = the standard deviation of the response,

S = the slope of the calibration curve (of the analyte)

Limit of quantification

The limit of quantification of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined. It was calculated using the following formula

$$LOQ = 10\sigma/S$$

Where σ, =the standard deviation of the response,

S=the slope of the calibration curve (of the analyte).

CONCLUSION

The developed method was found to be simple, sensitive, and accurate and can be used for routine quality analysis of Dexketoprofen trometamol in bulk and Tablet dosage form.

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Table 1. Calibration curve data for Dexketoprofen trometamol

Concentration (µg/ml)	Area
30	1392028
40	1839568
50	2239252
60	2699032
70	3261520

Table 2. Determination of accuracy by percentage recovery method

Drug	Label claim	Amount of drug taken (µg/ml)	Amount added (%)	% mean recovery ±S.D (n=3)
Dexketoprofen trometamol	25mg	50	50	99.97 ± 0.3055
		50	100	99.94 ± 0.3511
		50	150	100.0 ± 0.4303

Table 3. Summary of validation parameters of Dexketoprofen trometamol

Parameters	Results
Linearity Range (µg/mL)	30-70µg/ml
Slope (m)	45984
Intercept (c)	12944
Correlation Coefficient	0.996
Limit of Detection (µg/mL)	0.01298µg/mL
Limit of Quantitation (µg/mL)	0.05544µg/mL
Precision (%RSD)	
Intra-day precision	0.2449
Inter-day precision	0.04785
Recovery (%) (n=3)	
50% 100% 150%	99.97% 99.94% 100%
Robustness	Robust
Assay	101.63

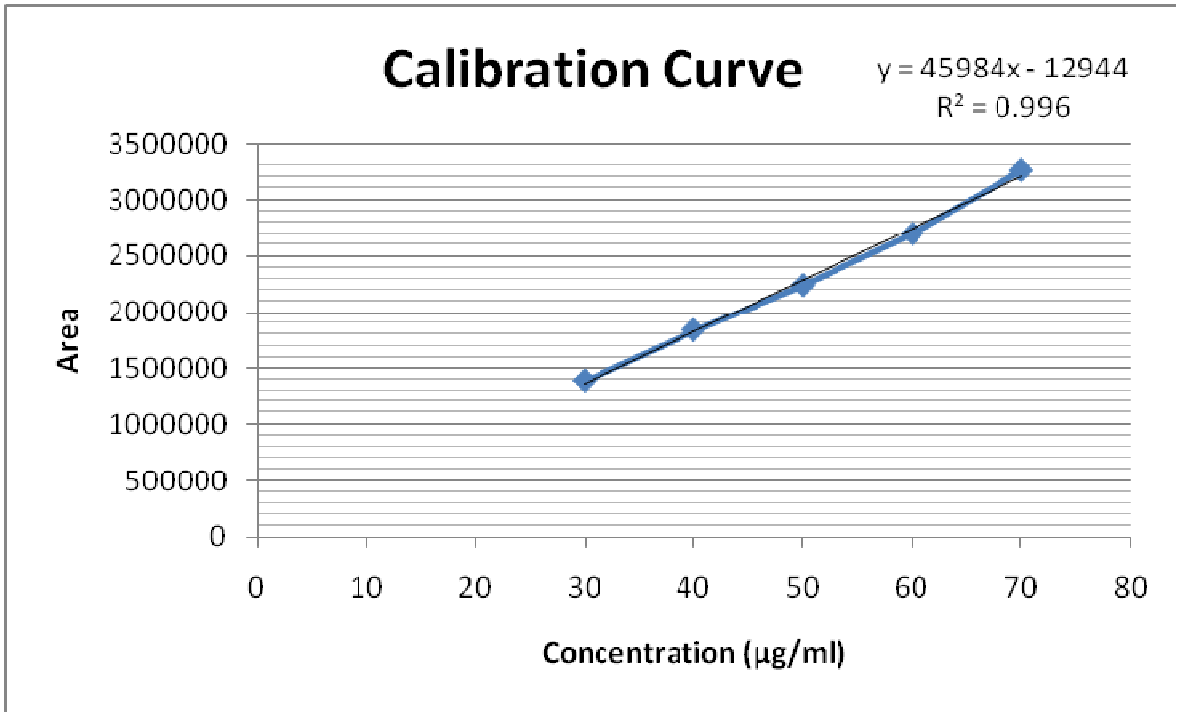

Figure.1. Calibration curve for linearity of Dexketoprofen trometamol