Development and validation of high performance liquid chromatography (HPLC) modified method for dexketoprofen trometamol

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Abstract

In this study, an economic, easy, basic, quick, sensitive and error-free reverse phase high performance liquid chromatography (RP-HPLC) modified method was developed for the determination of dexketoprofen trometamol. NUCLEODUR C_{18} (5.0 µ, 250 mm × 4.6 mm) was used as the stationary phase while acetonitrile-methanol (25:75 v/v) mixture was the mobile phase. Flow rate was 1 mL.min^{-1} and the detection wavelength was selected to be258 nm. The method developed was validated for precision, accuracy, specificity and linearity. Linearity was determined to be at a concentration range of 10-80 µg·mL^{-1}.The method developed for dexketoprofen trometamol was decided to be precise due to RSD values of <2 %for repeatability and intermediate precision. Recovery of the method was satisfactory owing to <2 % RSD value. Conclusively, procedure proposed in this study can be used for routine, simultaneous and concurrent dexketoprofen trometamol determination.

Key words: Dexketoprofen Trometamol, HPLC

1. Introduction

IUPAC name of dexketoprofen trometamol (DT)is (2S)-2-[3-(benzoyl) phenyl] propanoic acid (**Figure 1**) (Mulla*et al.* 2011; Matsui *et al.* 2011).It is an active optical isomer of ketoprofen (Bhusari and Dhaneshwar, 2011;Hawkey, 2001).DT belongs to the nonsteroidal antiinflammatory drug (NSAID) group which is a rapidly acting analgesic ingredient for the treatment of painful musculoskeletal disorders such as back pain and osteo-arthritis (Chaudhari and Trivedi, 2012). In addition, it is used as an agent for the treatment of dental or post-operative pains and dysmenorrhea.

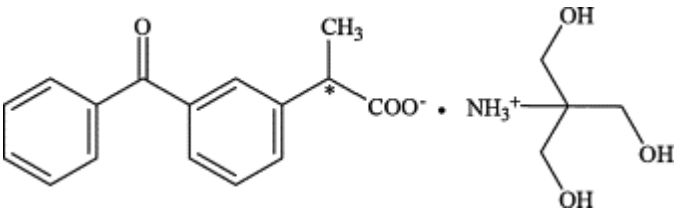

Figure 1. Chemical structure of DT(Blanco et al. 2006)

DT is a relatively new NSAID with analgesic, anti-inflammatory and antipyretic properties. This enantiomer is considered as one of the very powerful direct inhibitors of prostaglandin synthesis (Matsui*et al.* 2011; ChaudhariandTrivedi, 2012). Cyclo-oxygenase being responsible for prostaglandin formation, prostaglandins are produced responding to damage or disease which result in blain, pain and inflammation (ChaudhariandTrivedi, 2012; BhusariandDhaneshwar, 2011). DT obviates the production of prostaglandins and consequently decreases pain and inflammation. Along with peripheral analgesic action, it possesses central analgesic activity (Matsui*et al.* 2011; ChaudhariandTrivedi, 2012; Hawkey, 2001).

High performance liquid chromatography (HPLC) classified under liquid chromatography is a method used to isolate and quantify compounds dissolved in a mixture or solution (Snyder*et al*. 2012).Both in HPLC and liquid chromatographic methods, different solutes in the sample solution interact with the stationary phase when the sample solution comes into contact with a second solid or liquid phase. Therefore, differences in interaction with the column lead to separation of different sample components from each other (Yong *et al.* 2014; Kupiec, 2004).

HPLC is currently the most widely used method of quantitative analysis in pharmaceutical industry and pharmaceutical analysis laboratories (Kupiec, 2004). DT quantification is not featured in pharmacopoeia (TrivediandChaudhari, 2012), however various methods such as UV (Trivedi ve Chaudhari, 2012; Pandya, 2011), HPLC (Mulla et al. 2011;TrivediandChaudhari, 2012;ArchanaandVikas, 2013)were reported in the literature.

This study was focused on developing an accurate, error-free and precise reverse-phase HPLC method for the determination of DT.

2. Materials and methods

Pure DT sample was supplied from Abdi İbrahim (İstanbul, Turkey). All reagents and chemicals of HPLC grade were procured from Merck (Germany).

HPLC device (Shimadzu- 20A, Japan) used was mounted with reversed-phase (RP) NUCLEODUR C_{18} gravity column (column length: 250 mm, column diameter: 4.6 mm, particle diameter: 5 μm) (ArchanaandVikas, 2013).25:75 (v/v) acetonitrile-methanol was used as the mobile phase for perfect declaration of DT. Flow rate of the mobile phase was set at 1 mL/min^{-1} and 25 μL invariable volume of specimen were injected by an automatic injector (Shimadzu, Japan).Temperature of the column was set to 30°C while a fluorescent detector (Shimadzu, Japan) was used at 258 nm.

Validation studies were performed according to the ICH guidelines for the reliability of data obtained (Q2(R1), 2014). Parameters detailed below were determined for validation procedure.

2.1 Method validation

2.1.1. Linearity

It is common practice to check the linearity of a calibration curve by examining the correlation coefficient (ShrivastavaandGupta, 2012). Aliquots from a standard stock solution (1000 $\mu g \cdot mL^{-1}$) of DT were used to prepare different sets of dilutions. A series of dilutions consisted of different concentrations of DT in the range of 10-80 $\mu g \cdot mL^{-1}$. Absorbance values were measured and calculations were made to determine DT concentration.

2.1.2. Precision

Precision is the criterion that exhibits "the closeness of agreement between a series of measurements" and is of utmost importance for any analysis (Ermer*et al.* 2005).Intermediate precision and repeatability values when using the device in this study was verified by repeated scanning and measurement of absorbances (n=6) for DT (25 $\mu g \cdot mL^{-1}$, 50 $\mu g \cdot mL^{-1}$, 75 $\mu g \cdot mL^{-1}$). Repeatability studies were performed six times on the same day by analyzing three different concentrations of 25 $\mu g \cdot mL^{-1}$, 50 $\mu g \cdot mL^{-1}$, 75 $\mu g \cdot mL^{-1}$ for DT. Repeating tests on three consecutive days verified intermediate precision of the method. Results were expressed as RSD % of the measurements obtained.

2.1.3. Limit of detection and limit of quantitation (sensitivity)

Detection and quantification limits are the two principal components of method validation (VialandJardy, 1999). Limit of Detection (LOD) and Limit of Quantitation (LOQ) were separately determined based on the calibration curve obtained according to ICH Q2 (R1) recommendations (Eq. 1, Eq. 2)(Q2(R1), 2014). Standard deviation of y-intercept and slope of the calibration curve were used to calculate LOD and LOQ, respectively.

$$LOD = 3.3 \times \sigma/S \qquad \text{Equation 1}$$

$$LOQ = 10 \times \sigma/S \qquad \text{Equation 2}$$

where, σ = the standard deviation of the response and S = slope of the calibration curve.

2.1.4. Accuracy

Accuracy was calculated as deviation of mean from nominal concentration (Rouini*et al.* 2006).Accuracy of the method used was determined by calculating recoveries of DT by standard addition method. Standard solutions containing specific amount of DT (20 $\mu g \cdot mL^{-1}$, 40 $\mu g \cdot mL^{-1}$, 60 $\mu g \cdot mL^{-1}$) were used and percentage of recoveries were calculated.

3. Results and discussion

Different proportions of acetonitril and methanol and flow rates were tested for method optimization and it was found that acetonitrile-methanol in the proportion of 25:75 v/v and a flow rate of 1 $mL.min^{-1}$ give admissible retention time (RT) and good resolution for both the mobile phase and DT (**Figure 2** and **Figure 3**).

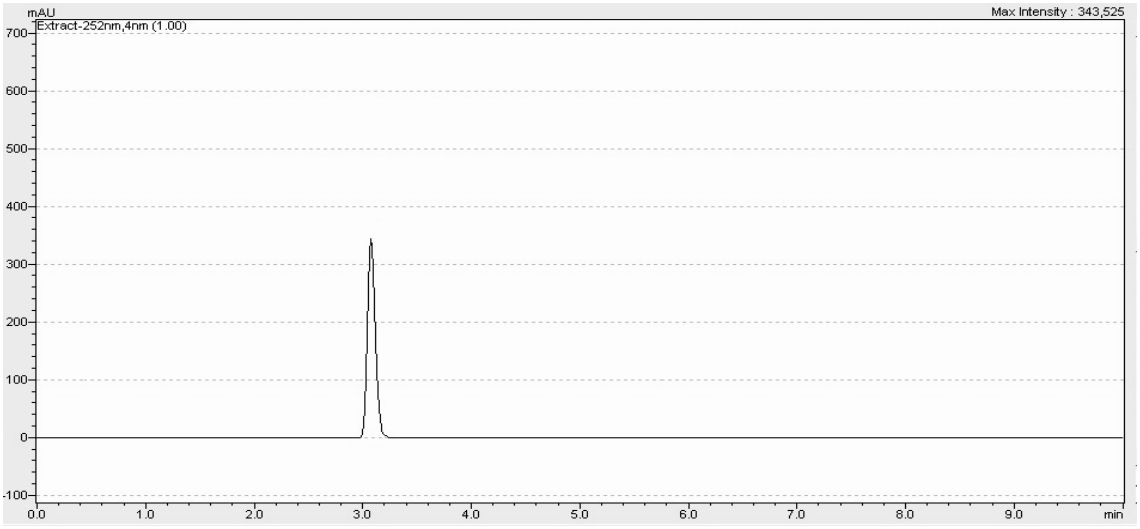

Figure 2. Chromatogram of DT

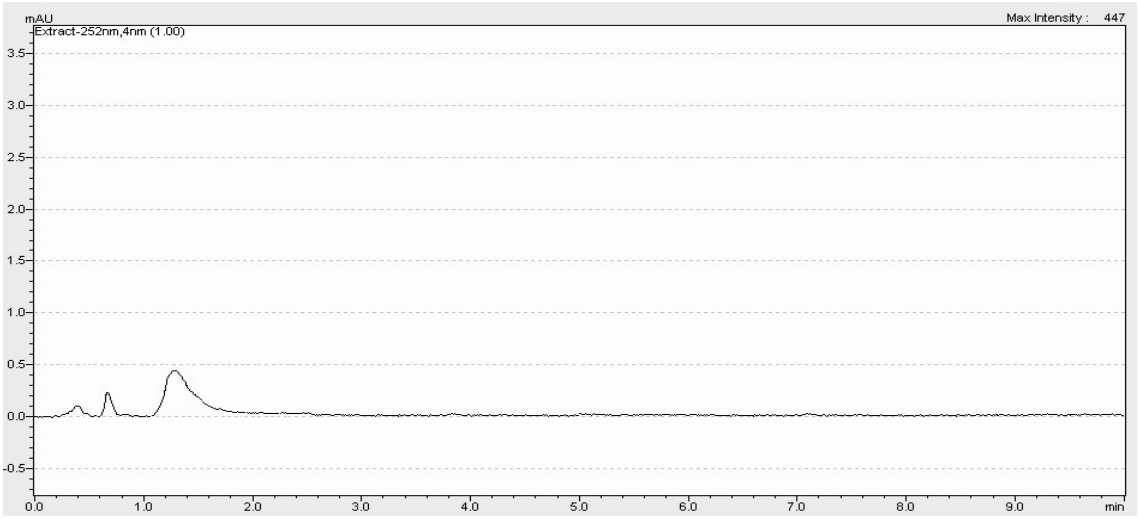

Figure 3. Chromatogram of mobile phase

3.1. Specificity

Characteristic HPLC chromatogram of DTis given at **Figure 2**. It can be seen that chromatogram recorded for the combination of non-functioning components exposed no peaks at retention time of 3.1 minutes.

3.2. Linearity

Linearity range of DT for the method used was found to be 10-80 $\mu g \cdot mL^{-1}$ while regression equation was determined to be y = 67363x - 243811 by plotting concentration (x) versus peak area (y).Correlation coefficient (r^2) of 0.9999 was highly significant. Linearity test results are shown in **Table 1** and regression curve is presented in **Figure 4**.

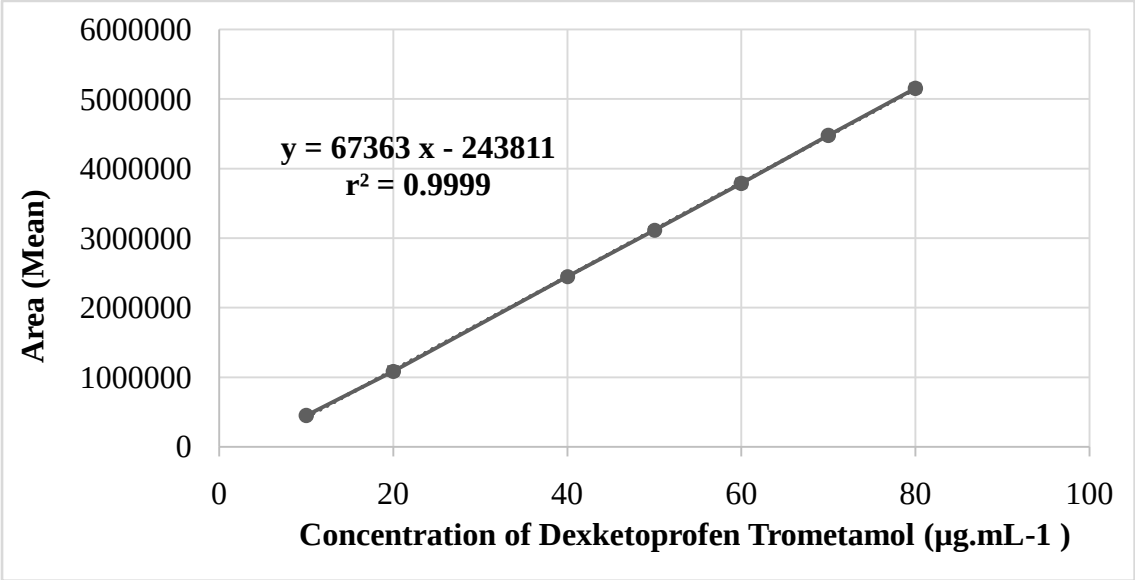

Figure 4. Regression profile of DT

Table 1. Series and area values prepared for linearity study (n = 6)

DT	Area(mAU)						
(µg/mL)	1st set	2nd set	3rd set	4th set	5th set	6th set	Mean±SE
10	473564	458835	400954	449403	458226	456137	449536.50±10235.77
20	1157463	1158086	1026067	1118133	1048344	1016929	1087503.67±26522.35
40	2445455	2514408	2455184	2408264	2413220	2457160	2448948.50±15636.73
50	3097585	3187891	3089947	3121867	3122932	3084470	3117448.67±15545.86
60	3746606	3781373	3757593	3814872	3723403	3899937	3787297.33±25886.79
70	4419778	4500729	4456986	4529531	4503362	4458910	4478266.00±16340.97
80	5139575	5068814	5186356	5207974	5221657	5100180	5154092.67±25099.04

3.3. Precision

Results of intermediate precision and repeatability tests on different concentrations are given in Table 2, Table 3 and Table 4. RSD values for both intermediate precision and repeatability were <2 %.Therefore, the method developed for DT was found to be precise according to the suggestions in ICH Q2(R1) guidelines and also in literature (Q2(R1), 2014;Bhadra et al. 2011).

Table 2. Precision results for 25 µg.mL^{-1} of DT

1st day (area)	2nd day (area)	3rd day (area)	1st day (concentration)	2nd day (concentration)	3rd day (concentration)
1533030	1530736	1546271	26.3771	26.3431	26.5737
1517832	1543920	1578250	26.1515	26.5388	27.0484
1553144	1588217	1563561	26.6757	27.1964	26.8303
1547768	1585254	1574465	26.5959	27.1524	26.9922
1513513	1538741	1505341	26.0874	26.4619	25.9661
1519463	1560049	1529350	26.1757	26.7782	26.3225
Mean			26.3439	26.7451	26.6222
Standard error			0.1009	0.1477	0.1717
Coefficient of variation (RSD)			0.9388	1.3533	1.5799
95% confidence interval			0.2595	0.3798	0.4414

Table 3. Precision results for 50 µg.mL^{-1} of DT

1st day (area)	2nd day (area)	3rd day (area)	1st day (concentration)	2nd day (concentration)	3rd day (concentration)
3266219	3318870	3306404	52.1062	52.8878	52.7027
3148650	3315985	3268826	50.3609	52.8450	52.1449
3316408	3234179	3332771	52.8513	51.6306	53.0942
3166293	3381829	3290351	50.6228	53.8224	52.4644
3238394	3252933	3342994	51.6931	51.9090	53.2459
3212493	3317101	3288808	51.3086	52.8615	52.4415
Mean			51.4905	52.6594	52.6823
Standard error			0.3801	0.3215	0.1715
Coefficient of variation (RSD)			1.8081	1.4957	0.7972
95% confidence interval			0.9770	0.8266	0.4407

Table 4. Precision results for 75 µg.mL^{-1} of DT

1st day (area)	2nd day (area)	3rd day (area)	1st day (concentration)	2nd day (concentration)	3rd day (concentration)
4749992	4730109	4727311	74.1327	73.8376	73.7960
4857276	4806928	4787966	75.7254	74.9779	74.6965
4840482	4729851	4610718	75.4760	73.8337	72.0652
4800131	4712138	4774587	74.8770	73.5708	74.4978
4721966	4709191	4762852	73.7167	73.5270	74.3236
4746458	4799769	4770032	74.0803	74.8717	74.4302
Mean			74.6680	74.1031	73.9682
Standard error			0.3342	0.2655	0.4001
Coefficient of variation (RSD)			1.0965	0.8775	1.3248
95% confidence interval			0.8592	0.6824	1.0284

3.4. Limit of detection and limit of quantitation

LOD of an analytical procedure is the minimum amount of analyte which can be detected in a sample (BhusariandDhaneshwar, 2011).LOD and LOQ for DT were found to be 0.5613 µg.mL^{-1} and 1.7010 µg.mL^{-1}respectively.

3.5. Accuracy

As shown in **Table 5** perfect recoveries of DT at various concentrations were obtained between 99.5332 - 100.9968 % .**Table 5** indicates good accuracy of the HPLC method developed in this study.

Table 5. Accuracy test results

Area(mUA)			**Concentration**		
20 µg.mL^{-1}	**40 µg.mL^{-1}**	**60 µg.mL^{-1}**	**20 µg.mL^{-1}**	**40 µg.mL^{-1}**	**60 µg.mL^{-1}**
1097256	2451155	3757601	19.9081	40.0066	59.4007
1128104	2429998	3759998	20.3660	39.6925	59.4363
1125049	2436078	3790698	20.3207	39.7828	59.8921
1117033	2417396	3825631	20.2017	39.5055	60.4106
1098454	2494770	3743029	19.9258	40.6541	59.1844
1116879	2469071	3797652	20.1994	40.2726	59.9953
			Recovery %		
			20 µg.mL^{-1}	**40 µg.mL^{-1}**	**60 µg.mL^{-1}**
			99.5403	100.0166	99.0012
			101.8300	99.2314	99.0605
			101.6033	99.4570	99.8201
			101.0083	98.7637	100.6844
			99.6292	101.6352	98.6407
			100.9968	100.6815	99.9922
Recovery % (mean)			100.7680	99.9642	99.5332
Standard error			0.3975	0.4299	0.3120
Coefficient of variation (RSD)			0.9662	1.0535	0.7679
95% confidence interval			1.0217	1.1052	0.8021

4. Conclusion

HPLC method developed in this study was found to be economic, simple, quick, sensitive, accurate/error-free and conventional for routine quantification of DT. Perfect precision and accuracy was assessed through statistical analysis of linearity, precision, accuracy *etc*. Validation tests based on literature and ICH Q2(R1) guidelines indicated the potential use of the method developed for routine analysis of DT in pure and pharmaceutical preparations and formulations.

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