

Simple and sensitive LC-ESI-MS method for estimation of dexketoprofen in plasma samples

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Abstract

A simple and sensitive LC-ESI-MS assay was developed to determine the concentration of dexketoprofen in human plasma. Dexketoprofen and ketoprofen D3 (internal standard) were extracted from the plasma using solid phase extraction technique and injected onto Phenomenex Luna C18 100mm*4.6 mm, 3 μ m. The mobile phase consisted of Acetonitrile: 0.2% Formic Acid (65:35, v/v) and was delivered isocratically at a flow rate of 1.0 mL/min (Split 50:50 v/v). The MRM mode for dexketoprofen and internal standard ketoprofen D3 were detected at m/z 255.1 \rightarrow 209.2 and 258.1 \rightarrow 212.2 and the run time was 3 min. The detection limit of dexketoprofen was 50.0ng/mL, and the calibration curve was linear between 50.0 and 6000.0ng/ml. Concentrations of drugs were determined by using a validated LC-MS/MS method.

Keywords: LC-MS/MS, Plasma, Validated method, Dexketoprofen, SAS, Statistical analysis.

Introduction

Clinical Pharmacology Pharmacodynamic Properties and Mechanism of action:

The suppression of the cyclooxygenase pathway, which lowers prostaglandin synthesis, is the mechanism of action of non-steroidal anti-inflammatory medicines. The process that converts arachidonic acid into cyclic endoperoxides, PGG₂ and PGH₂, which generate prostaglandins PGE₁, PGE₂, PGF_{2a}, and PGD₂, as well as prostacyclin PGI₂ and thromboxanes (TxA₂ and TxB₂), is specifically inhibited.

In addition to the direct effects, the suppression of prostaglandin synthesis may also have an indirect effect on other mediators of inflammation, such as kinins¹⁻².

Pharmacodynamic effects: It has been shown that dexketoprofen inhibits the activity of COX-1 and COX-2 in both people and experimental animals.

Clinical efficacy and safety: Dexketoprofen has been shown in clinical trials to have excellent analgesic efficacy on a number of pain models. In certain tests, the analgesic effect started to take effect 30 minutes after the medication was administered. The pain-relieving action lasts for four to six hours.

Pharmacokinetics Properties: Absorption: Humans receive dexketoprofen trometamol orally, and the C_{max} is attained in 30 minutes (range: 15 to 60 minutes). The AUC of dexketoprofen does not alter when given with food concurrently; nevertheless, its C_{max} lowers and its absorption rate is delayed (increased t_{max}).

Distribution: For dexketoprofen, the values for the distribution and elimination half-lives are 0.35 and 1.65 hours, respectively. Its typical value for volume of distribution is less than 0.25 l/kg, in line with other medications that have a high plasma protein binding (99%). It was found in multiple-dose pharmacokinetic studies that there is no drug accumulation because the AUC recorded following the last injection is the same as that obtained following a single dose³⁻⁵.

Biotransformation and elimination: The S-(+) enantiomer is the only one seen in urine after dexketoprofen and trometamol are given, indicating no conversion to the R-(-) enantiomer.

Therapeutic Dosage: The recommended dosage for pain is generally 12.5mg every 4-6 hours or 25mg every 8 hours, depending on the severity of the pain⁶.

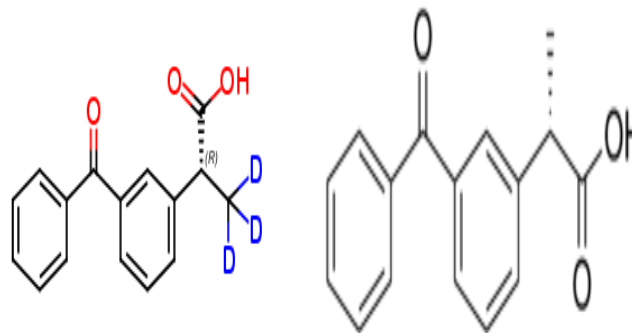


Figure-1: Chemical structures of Dexketoprofen (A) and ketoprofen D3.

Materials and Methods

Reagents and chemicals: Dexketoprofen was from Clearsynth labs ltd and the internal standard (IS, ketoprofen D3) was obtained from Clearsynth labs ltd. Acetonitrile and methanol of HPLC grade were obtained from J.T. Baker Chemical Company and Fisher ltd respectively.

HPLC grade water was purified by means of a Milli-Q, HPLC and Spectroscopy water was manufactured by Finar. HPLC grade formic acid was obtained from Biosolve.

Analytical system: The plasma Dexketoprofen levels were quantified using a LC-MS/MS TRIPLE QUAD 4500 (Sciex) and HPLC (Exion LC TM) equipped with an ESI probe and a quadrupole mass analyzer. The control of the LC-MS system and data acquisition was performed using Analyst 1.7.1 and Analyst 1.7.2 (Sciex). The compounds were separated on a Thermo, Phenomenex Luna C18 100 mm*4.6 mm, 3µm. The mobile phase used was Acetonitrile: 0.2% Formic Acid (65:35, v/v), which was delivered isocratically at a flow rate of 1.0 mL/min (Split 50:50 v/v). The mobile phase was filtered and degassed using 0.22 mm filters before use. The MRM mode for Dexketoprofen and internal standard ketoprofen D3 were detected at m/z 255.1→209.2 and 258.1→212.2. The optimized MS conditions for the ESI operating in the positive mode were set as follows: CUR: 35, CAD: 6, IS: 5000, TEM: 500, GS1: 40 and GS2: 60⁷⁻¹⁰.

Preparation of Calibration Standards and Quality Control Samples: A stock solution of Dexketoprofen were prepared in methanol at a concentration of 5.00 mg/ml. Standard solutions for Dexketoprofen (1.251, 2.501, 13.440, 26.880, 67.200, 96.000, 120.000 and 150.000ng/ml) were prepared by serial dilution of the stock solution with Diluent Solution (Methanol : Water: :50:50,v/v). Low, medium-1, medium, and high concentration quality control for Dexketoprofen (QC) solutions (3.378, 22.518, 73.589 and 118.500ng/ml) were prepared in the similar way. The stock solution of IS ketoprofen D3 (1.0mg/ml) was also prepared in dimethyl sulphoxide and methanol and then diluted with Diluent Solution (Methanol:Water: 50:50, v/v to a final concentration of 3000.0ng/ml. All solutions were stored at 2-8°C until use.

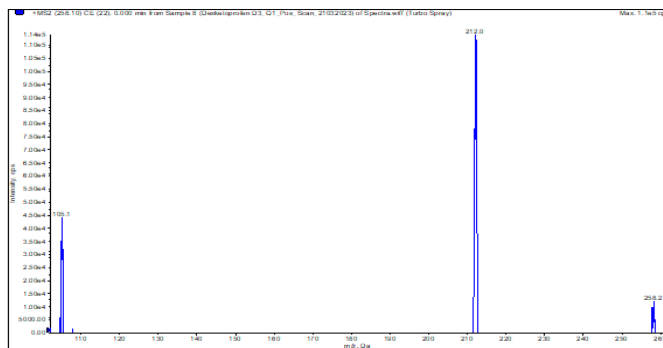


Figure-2: (I) Dexketoprofen and (II) ketoprofen D3 MSMS Positive Scan.

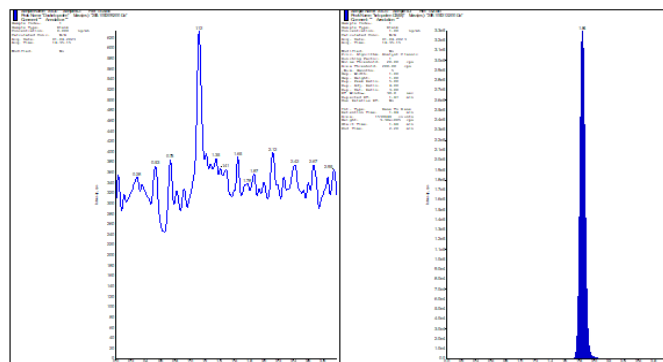
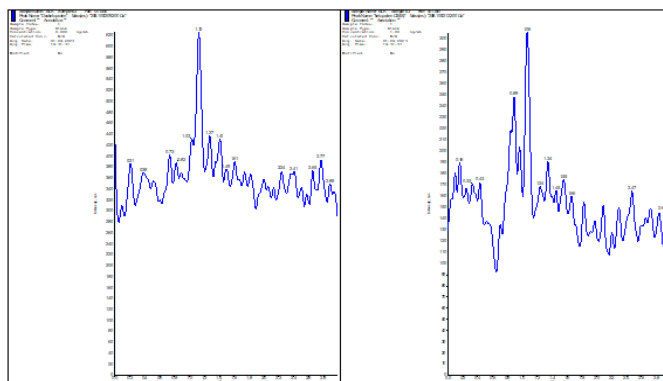


Figure-3: Chromatograms of (I) Blank plasma and (II) IS ketoprofen D3.

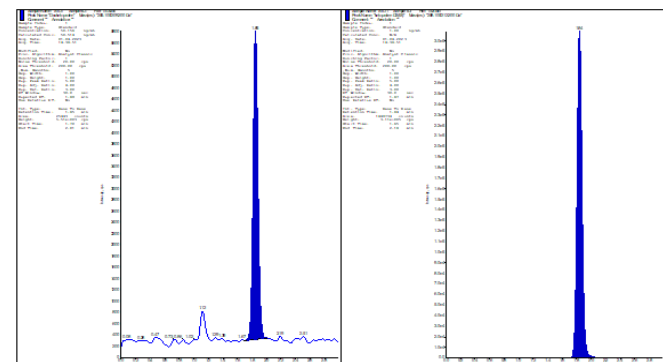
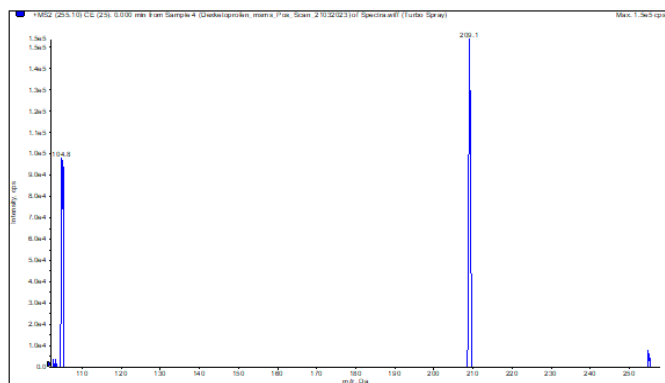


Figure-4: Chromatograms of LLOQ Dexketoprofen and its internal standard ketoprofen D3.

Sample preparation: Retrieve the required set of CC and QC samples along with subject samples (in case of project sample analysis) from the deep freezer and allow them to thaw at room temperature and arrange the sample as per batch sequence. Vortex the thawed samples to ensure complete mixing. Aliquot 100 μ l of Plasma sample in to pre-labelled Ria vial. Add 50 μ l of internal standard dilution solution~ 3000.000ng/mL for ketoprofen D3 to each pre-labelled Ria vial except blank sample and add 50 μ l of diluent solution in blank sample vortex for few second. Add 200 μ l of 0.1% formic Acid Solution into all samples and vortex for few seconds. Use SPE Method, Orochempanthra Deluxe Cartridges. Conditioning: 1ml of Methanol. Equilibration: 1 ml of water. Load the entire samples on Cartridges. Wash-I: 1 ml of water. Wash-II:1 ml of 5% Methanol in water. Elute the Samples with 1.000ml of Mobile Phase and vortex for few seconds. Transfer the samples into pre-labelled autosampler vials. Note: All activity shall be carried out at room temperature & under monochromatic light.

Calibration Curves: The analytical curves of Dexketoprofen were constructed in the concentrations ranging from 50.156 ng/mL to 6051.671ng/mL in human plasma. The calibration curve was made by using an instrument response (ratio of Dexketoprofen peak area to IS peak area) against the Dexketoprofen concentration (ng/mL) for 3 consecutive days by weighted 1/x² quadratic regression model. The fitness of the calibration curve was confirmed by back-calculating the concentrations of calibration standards.

Bioanalytical method validation: Selectivity: The 10 evaluated blank plasma extracts did not exhibit any peaks from endogenous compounds during the drug retention time. The Interfering peak area response at Analyte RT in Blank 0.0% of the signal at the LLOQ of 50.156 ng/mL for Dexketoprofen and 0.0% for IS. Figure-3 shows a representative ion chromatogram for the blank plasma (a) and LLOQ (b) with 5 μ l injection volume.

Carry over: To assess carryover, three blank samples were injected into the same sample one before and two immediately after the injection of a sample processed ULOQ. The peak of Dexketoprofen and IS corresponded to 0.00% and 0.00% of the respective peaks in the sample of LLOQ. Carry-over was therefore considered acceptable.

Matrix Effect: The mean normalized matrix factor for Dexketoprofen was 1.02577 and % CV was 0.82. The MRM channel used for quantification of Dexketoprofen is not subjected to any major ion suppression or enhancement as indicated by this method.

Linearity: The eight-point calibration curve was linear in the concentration range of 50.156ng/mL to 6051.671ng/mL. The calibration model was selected based on the data using quadratic regression with a weighting factor of 1/x². In the concentration

range considered, the regression coefficient r² of the calibration curves was always greater than 0.99 with at $\pm 15\%$ ($\pm 20\%$ at LLOQ) newly calculated calibration test.

Accuracy and precision: Accuracy and precision determined with LLOQ, LQC, MQC and HQC samples are given in Table-2. Between-run Precision and Accuracy: QC Coefficient of variation 3.62 to 8.18% and QC % Nominal value 97.235 to 99.810%, respectively. Quantification of the Dexketoprofen in plasma that was subjected to 5 freeze-thaw cycles (-70^oC to room temperature) showed the stability of the analyte. No significant degradation was observed even after a 49 hours 05 minutes storage period in the autosampler tray. The room temperature stability of Dexketoprofen in QC samples after 18 hours 47 minutes was also evaluated. These results confirmed the stability of Dexketoprofen, as shown in Table-2.

Pharmacokinetics (PK) and Statistical Analysis: Pharmacokinetics (PK) parameters from human plasma samples were calculated by using SAS (SAS Institute Inc., U.S.A.). Plasma Dexketoprofen concentration-time profiles were visually inspected and C_{max} and T_{max} values were determined. C_{max} AUC_{0-t} and AUC_{0-∞} was Primary Pharmacokinetic parameters for Dexketoprofen. The AUC_{0-t} was obtained by linear trapezoidal method. AUC_{0-∞} was calculated up to the last measurable concentration and extrapolations were obtained using the last measurable concentration and the terminal elimination rate constant (K_{el}), K_{el} was estimated from the slope of the terminal exponential phase of the plasma of Dexketoprofen concentration-time curve (by means of the linear regression method). The terminal elimination half-life t_{1/2} was then calculated as 0.693/K_{el}. Statistical analysis of the pharmacokinetic parameters will be performed using SAS. Descriptive statistics was computed and reported for pharmacokinetic parameters. Consistent with two one-sided tests procedure for bioequivalence, ANOVA (Analysis of Variance) will be performed on natural log-transformed Primary Pharmacokinetic parameters: C_{max}, AUC_{0-t} and AUC_{0-∞} for Dexketoprofen.

Results and Discussion

Validation of LC-MS/MS has been used as one of the most powerful analytical tools in clinical pharmacokinetics for its selectivity, sensitivity and reproducibility. The goal of this work is to quantify the drug Dexketoprofen in human plasma. This procedure incorporates solid phase extraction LCMS/MS with ESI triple quad 4500 MS. chromatographic conditions, especially the composition and nature of the mobile phase were optimized through several trials to achieve best resolution and increase the signal of analytes and internal standard. Pharmacokinetics (PK) and Statistical analysis was using SAS performed on data obtained from 28 subjects who completed the study. Graphical representation of Mean Plasma Concentrations versus Time is gives in Figure-5 and Figure-6.

Table-1: Within and Between-Run Precision and Accuracy.

Nominal Conc.	LLOQQC (ng/mL)		LQC (ng/mL)		M1QC (ng/mL)		MQC (ng/mL)		HQC (ng/mL)	
	50.245		139.568		930.454		3001.465		4802.345	
Range	40.196	60.294	118.633	160.503	790.886	1070.022	2551.245	3451.685	4081.993	5522.697
Result Table ID	Conc. Found	% Nominal	Conc. Found	% Nominal	Conc. Found	% Nominal	Conc. Found	% Nominal	Conc. Found	% Nominal
1.	43.576	86.727	131.011	93.869	896.074	96.305	2511.930	83.690	4362.542	90.842
	47.125	93.791	119.618	85.706	787.379	84.623	2560.408	85.305	4522.425	94.171
	48.320	96.168	114.439	81.995	805.041	86.521	2602.504	86.708	4733.226	98.561
	46.906	93.354	114.223	81.841	738.523	79.372	2681.122	89.327	4455.732	92.782
	49.364	98.247	123.698	88.629	797.041	85.662	2822.285	94.030	4435.726	92.366
	43.184	85.947	124.338	89.088	805.317	86.551	2790.661	92.977	4966.930	103.427
N	6		6		6		6		6	
Mean	46.4125		121.2212		804.8958		2661.4850		4579.4302	
S.D.	2.51277		6.46889		51.16160		125.69066		228.05765	
% C.V.	5.41		5.34		6.36		4.72		4.98	
% Nominal	92.372		86.855		86.506		88.673		95.358	
2.	47.077	93.694	140.967	101.002	930.837	100.041	2977.666	99.207	4881.231	101.643
	52.352	104.193	142.157	101.855	961.573	103.344	3066.766	102.176	4979.901	103.697
	51.270	102.041	139.638	100.050	993.037	106.726	2994.494	99.768	4838.809	100.759
	48.687	96.899	142.054	101.781	1019.402	109.560	2991.005	99.652	4896.194	101.954
	50.387	100.283	144.891	103.814	937.006	100.704	3121.706	104.006	4856.279	101.123
	47.373	94.285	141.978	101.727	959.885	103.163	3026.633	100.839	4776.898	99.470
N	6		6		6		6		6	
Mean	49.5243		141.9475		966.9567		3029.7117		4871.5520	
S.D.	2.14970		1.73295		33.79752		55.30407		67.38719	
%C.V.	4.34		1.22		3.50		1.83		1.38	
% Nominal	98.566		101.705		103.923		100.941		101.441	
3.	47.923	95.378	140.062	100.354	935.706	100.564	2984.564	99.437	4918.387	102.416
	49.359	98.237	144.131	103.269	934.023	100.384	2980.476	99.301	4810.336	100.166
	51.311	102.122	139.888	100.229	985.845	105.953	2978.234	99.226	4813.370	100.230
	48.768	97.061	148.668	106.520	955.835	102.728	2976.430	99.166	4797.630	99.902
	51.533	102.563	141.056	101.066	960.295	103.207	3048.448	101.565	4956.980	103.220
	47.750	95.035	139.765	100.141	964.524	103.662	3091.652	103.005	4898.894	102.010
N	6		6		6		6		6	
Mean	49.4407		142.2617		956.0380		3009.9673		4865.9328	
S.D.	1.64297		3.54105		19.37361		48.57977		67.30022	
%C.V.	3.32		2.49		2.03		1.61		1.38	
% Nominal	98.399		101.930		102.750		100.283		101.324	

Nominal Conc.	LLOQQC (ng/mL)		LQC (ng/mL)		M1QC (ng/mL)		MQC (ng/mL)		HQC (ng/mL)	
	50.245		139.568		930.454		3001.465		4802.345	
Range	40.196	60.294	118.633	160.503	790.886	1070.022	2551.245	3451.685	4081.993	5522.697
Result Table ID	Conc. Found	% Nominal	Conc. Found	% Nominal	Conc. Found	% Nominal	Conc. Found	% Nominal	Conc. Found	% Nominal
4.	48.819	97.161	141.205	101.173	926.530	99.578	3077.868	102.546	4753.965	98.993
	51.485	102.468	139.739	100.122	951.805	102.295	2980.623	99.306	4835.404	100.688
	50.562	100.630	141.592	101.450	929.813	99.931	2984.716	99.442	4880.849	101.635
	49.517	98.551	143.476	102.800	974.619	104.747	2935.071	97.788	4875.418	101.522
	49.298	98.115	144.846	103.782	976.110	104.907	2927.409	97.533	4838.361	100.750
50.591	100.688	141.314	101.251	965.214	103.736	3049.703	101.607	4951.583	103.108	
N	6		6		6		6		6	
Mean	50.0453		142.0287		954.0152		2992.5650		4855.9300	
S.D.	0.99789		1.82508		21.83656		60.49204		65.23355	
%C.V.	1.99		1.29		2.29		2.02		1.34	
% Nominal	99.603		101.763		102.532		99.703		101.116	
Global Statistics-Between Batch Precision and Accuracy										
N	24		24		24		24		24	
Mean	48.8557		136.8648		920.4764		2923.4323		4793.2113	
S.D.	2.30495		9.91637		75.32894		171.62343		173.58753	
%C.V.	4.72		7.25		8.18		5.87		3.62	
% Nominal	97.235		98.063		98.928		97.400		99.810	

Table-2: Matrix Stability Details.

Experiments			Results Summary		
Freeze and Thaw Stability at -70°C ±10°C after 5 th Cycles					
Calculated with Nominal concentration			QC Coefficient of variation 2.85% for LQC and 1.49% for HQC		
			QC % Change 0.241% for LQC and -1.709% for HQC		
Calculated with Fresh QC samples			QC Coefficient of variation 3.24% for LQC and 1.63% for HQC		
			QC % Change 2.764% for LQC and -0.032% for HQC		
Auto sampler Stability at 10°C after 49 Hours 05 Minutes					
Calculated with Nominal concentration			QC Coefficient of variation 2.97% for LQC and 2.08% for HQC		
			QC % Change value 1.904% for LQC and -1.789% for HQC		
Calculated with Fresh QC samples			QC Coefficient of variation 2.97% for LQC and 1.49% for HQC		
			QC % Change value 2.271% for LQC and 0.301% for HQC		
Wet Extract Stability at room temperature after 02 Hours 51 Minutes					
Calculated with Nominal concentration			QC Coefficient of variation 3.81% for LQC and 1.87% for HQC		
			QC % Change value 1.193% for LQC and -2.139% for HQC		
Calculated with Fresh QC samples			QC Coefficient of variation 2.97% for LQC and 1.49% for HQC		
			QC % Change value 1.557% for LQC and -0.057% for HQC		
Bench Top Stability of Analyte in Matrix at Room Temperature after 18 Hours 47 Minutes					
Calculated with Nominal concentration			QC Coefficient of 2.87% for LQC and 2.12% for HQC		
			QC % Change value 0.246% for LQC and -0.181% for HQC		
Calculated with Fresh QC samples			QC Coefficient of variation 3.24% for LQC and 1.63% for HQC		
			QC % Change value 2.769% for LQC and 1.522% for HQC		

Statistical Result of analyzed formulations: The ratios (T/R) of geometric least squares means (and confidence intervals) of the test product (T) and reference product (R) for the Ln-transformed pharmacokinetic parameters C_{max} and AUC_{0-t} of Dexketoprofen were found to be 114.49 (99.41% - 131.87%) and 104.61 (101.92% - 107.36%) and 104.49 (101.76% - 107.29%) respectively.

The Power (and Intra-subject CV%) of the test product (T) and reference product (R) for the Ln-transformed pharmacokinetic parameters C_{max} and AUC_{0-t} of Dexketoprofen were found to be 73.56 (31.75%), 100.00 (5.71) and 100.00 (5.81%) respectively.

Bioequivalence Conclusion: The 90% confidence intervals of the ratio of geometric least squares mean for the Ln-transformed pharmacokinetic parameters C_{max} and AUC_{0-t} of Dexketoprofen are within the bioequivalence acceptance limits of 80.00 - 125.00%.

Hence, it is concluded that the test product (T) and reference product (R) are bioequivalent with respect to rate and extent of absorption.

Application to Biological Samples: The validated method was used in the determination of Dexketoprofen in plasma samples for establishing the pharmacokinetics parameters of a single 25 mg dose (one 25 mg tablet) in 28 healthy volunteers. Plasma concentrations versus time profiles are shown in Figure-5 and Figure-6.

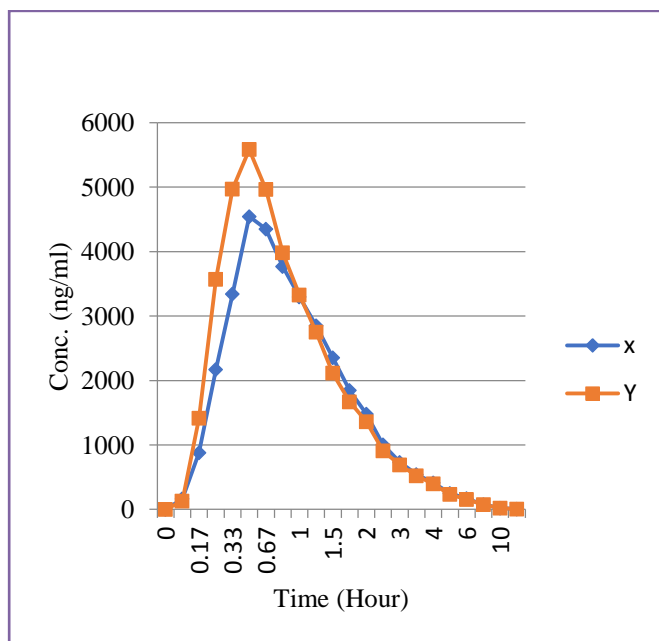


Figure-5: Mean Linear Plot of Mean Plasma Concentrations (ng/mL) of Dexketoprofen vs. Time (hr).

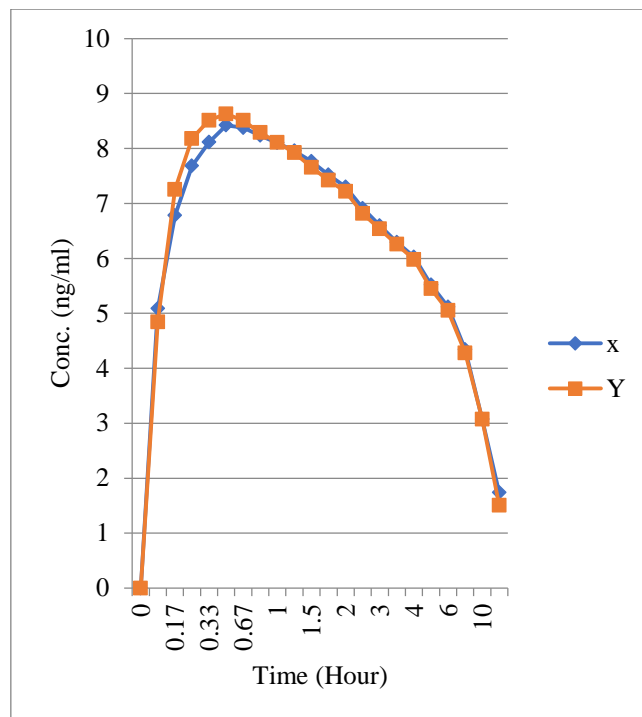


Figure-6: Mean Log-Linear Plot of Mean Plasma Concentrations (ng/mL) of Dexketoprofen vs. Time (hr).

All the plasma concentrations of Dexketoprofen were in the standard curve region and retained above LLOQ for the entire sampling period.

The observed values were reported in Table-3 for Dexketoprofen. Therefore, it can be concluded that the two analyzed formulations (reference and test) are bioequivalent.

Conclusion

In this paper we have reported the use of LCMS/MS for the precise and reliable quantification of Dexketoprofen concentrations in human plasma after oral administration of 25 mg to healthy volunteers.

The method described here is accurate, robust and fast. Each sample requires less than 3.0 min run time.

The sensitivity of the assay is sufficient to follow accurately the pharmacokinetics of Dexketoprofen following oral administration.

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Table-3: Pharmacokinetic parameters for Dexketoprofen: Descriptive Statistics of Pharmacokinetic (PK) Parameters of Test Product (T) and Reference Product (R) for Dexketoprofen.

Form	Variable	Mean	SD	Minimum	Median	Maximum	CV%
R	Cmax (ng/mL)	5890.2003	1819.1474	3173.4520	6021.8780	9694.8080	30.8843
	Tmax (hr)	0.7071	0.3755	0.1700	0.5950	1.5000	53.1077
	AUC0-t (ng.hr/mL)	7891.9852	1634.8788	5488.1340	7918.9405	13306.5020	20.7157
	AUC0-∞ (ng.hr/mL)	8085.5589	1682.2778	5636.7980	8111.2520	13831.0110	20.8060
	Kel (hr-1)	0.3869	0.1101	0.1690	0.3765	0.7220	28.4587
	t1/2 (hr)	1.9407	0.6089	0.9600	1.8400	4.1000	31.3742
	AUC_% Extrapol_obs	2.3996	0.6690	1.3800	2.3650	3.7900	27.8798
T	Cmax (ng/mL)	6732.3938	1978.5875	3209.2630	7096.4910	10423.6930	29.3891
	Tmax (hr)	0.5721	0.2771	0.2500	0.5000	1.2500	48.4400
	AUC0-t (ng.hr/mL)	8259.0831	1663.5106	5560.6060	8443.2655	12419.4610	20.1416
	AUC0-∞ (ng.hr/mL)	8448.3161	1680.5988	5675.9840	8588.8960	12665.6570	19.8927
	Kel (hr-1)	0.4027	0.1073	0.2100	0.4090	0.6440	26.6440
	t1/2 (hr)	1.8521	0.5382	1.0800	1.6950	3.3100	29.0569
	AUC_% Extrapol_obs	2.2857	0.5542	1.4100	2.1400	4.0400	24.2465

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