

IN-VIVO PHARMACOKINETIC EVALUATION OF DEVELOPED TRANSDERMAL PATCH OF ACECLOFENAC AND THIOLCHICOSIDE IN TREATMENT OF GOUT

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ABSTRACT

In present work, transdermal drug delivery systems (TDDS) was developed to overcome the drawback of oral formulation comprised of Aceclofenac and Thiocolchicoside in the symptomatic treatment of gout. Aceclofenac has major side effect on stomach walls which is peptic ulcer, while thiocolchicoside has lower bioavailability in oral route due to extensive first pass metabolism, therefore, it was thought of interest to developed long acting transdermal patch of Aceclofenac and Thiocolchicoside. The bioanalytical method was developed using Phenomenex Luna-C18 (250 x 4.60 mm, 5 μ m) column as stationary phase. The mobile phase used for HPLC method was 15 mM potassium di-hydrogen orthophosphate buffer (pH=3.11) and acetonitrile in gradient mode. Sample preparation was done using protein precipitation technique Bioanalytical method was validated according to Guidance for Industry: Bioanalytical Method Validation of U.S. Department of Health and Human Services. The optimized mobile phase gave peak of Aceclofenac, Thiocolchicoside and ISTD at $R_t = 5.17 \pm 0.04$, 14.60 ± 0.07 and 9.2 ± 0.03 min respectively at 260 nm. Pharmacokinetic data of developed transdermal patch showed better Area under curve (AUC), elimination half-life ($t_{1/2}$), Mean residence time (MRT) as compared to marketed formulation, therefore, the developed transdermal patch is able to surmounting drawbacks of oral marketed formulation of title drugs.

Keywords: Bioanalytical method, transdermal drug delivery system, aceclofenac, thiocolchicoside.

INTRODUCTION:

Gout is an inflammatory disorder which caused by the deposition of monosodium urate (MSU) crystals in articular and periarticular tissues. In gout hyperuricemia occurs because of increased level of serum uric acid/urate above 6.8 mg/dl, approximately the saturation point for Urate solubility at physiologic temperature and pH¹. Many people with high level of serum urate never develop gout, the risk depending on the degree of hyperuricemia². It is characterized by deposition of sodium urate crystals in synovial tissues of joints and elsewhere and produces inflammatory responses.

The range for prevalence was from 0.03% (Nigerian men) to 15.2% (Taiwanese aboriginal men) ³. So overall, it was affecting about 1–2% adult men in Western countries. In black Africans, it was relatively rare, whereas the prevalence rates were very high in Aboriginal populations in Asia and Australasia ³.

A considerable number of drugs are available for treatment of gout. Because of intensity of inflammatory reaction characterizing the acute attack, oral colchicine and/or NSAID are appropriate first line agents for this phase including thiocolchicoside, allopurinol and others NSAIDs like Aceclofenac. Thiocolchicoside (TCS) (2-demthoxy-2- glucosidoxythio-colchicine) (Figure 1A) is a semi synthetic sulphur derivative of colchicoside and give activities like muscle relaxant, anti-inflammatory, and analgesic. It interacts with g-amino butyric acid (GABA) type A receptor that induces depression of the central nervous system and, in turn, muscle relaxation ⁴. Aceclofenac (ACE) [2-[(2, 6-Dichlorophenyl) amino] phenylacetoxy acetic acid] (Figure 1B) inhibits prostaglandin synthesis. It is a potent inhibitor of the enzyme cyclooxygenase (COX), which is involved in the production of prostaglandins and it is responsible for producing inflammation ⁴.

It has been found that Thiocolchicoside has lower bioavailability in oral solid dosage form due to first pass hepatic metabolism. It has been seen that Aceclofenac has major side effect on stomach walls which is peptic ulcer ⁵. The solution of stated problem is to minimize or avoid first pass metabolism of drugs and minimize side effect of Aceclofenac on stomach walls, which can be done by formulating such a dosage form that will introduce the drugs in body by other than the oral route ⁵.

In line with this notion, the above mentioned bioavailability and side effect problem can be resolved by formulating a Transdermal Drug Delivery System (TDDS) of Aceclofenac and Thiocolchicoside in the form of patch that will release drug through dermis into the body at the site of application.

The skin as a route for systemic drug delivery has become very attractive, potentially noninvasive, continuous, and controllable alternative especially for pediatric population and premature neonates ⁶. Hence, transdermal delivery approach would be an appropriate strategy to overcome oral limitations. The poor stability, high cost of nanovesicular systems with expensive and complex technique may limit their use on a large scale. Therefore, our attention was directed towards the transdermal patches as drug delivery system by avoiding first pass metabolism and ultimately increased bioavailability can be obtained. This will also lead to decrease in frequency of dose administration and thus will increase patient's compliance.

For many drugs, transdermal patches could prolong the pharmacological effect and remove fluctuations of oral administered drug levels ⁷. Transdermal patches used currently are either reservoir or matrix patches. Matrix-type patches are very commonly used being thinner, less expensive to manufacture, more flexible, conformable, and comfortable than reservoir-type ^{8,9}.

Moreover, the objective of the pharmacokinetic study aims to describe time course of drug concentration in blood in mathematical terms so that performance of the pharmaceutical dosage form can be evaluated in terms of rate and amount of drug they deliver to the blood and the dosage regimen of the drug can be adjusted to produce and maintain therapeutically effective blood concentration with little or no toxicity¹⁰⁻¹². In context to this, the research study also included evaluating the pharmacokinetic profile of TDDS in rat plasma.

Literature reviewed reveals a few quantitative bioanalytical methods available for the estimation of ACE and TCS, in biological fluids. For ACE, several methods were available like HPLC in rat plasma using protein precipitation extraction¹³, HPLC in human plasma using liquid-liquid extraction¹⁴, HPLC-MS/MS in human plasma using protein precipitation¹⁵, LC-TMS in human plasma using protein precipitation¹⁶, HPTLC in human plasma using protein precipitation¹⁷ and for TCS, LC-TMS in human plasma using liquid-liquid extraction¹⁸, HPLC in human plasma using protein precipitation¹⁹, LC-MS/MS in human plasma using liquid-liquid extraction²⁰. But there was no bioanalytical method available for combination of ACE and TCS in human and rat plasma using simple HPLC with UV detection.

In line with this notion, the present manuscript focuses on the development and validation of RP-HPLC-UV bioanalytical method for ACE and TCS and its pharmacokinetic application in rat plasma.

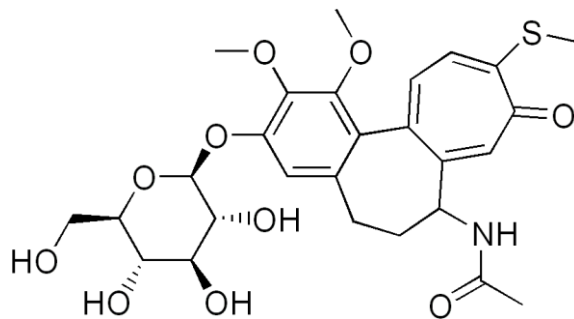


Figure 1A

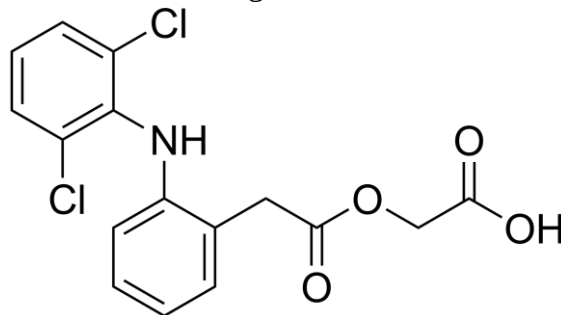


Figure 1B

Figure 1: A - Structure of aceclofenac and B - Structure of thiocolchicoside.

MATERIALS AND METHODS

Materials

Aceclofenac, Thiocolchicoside and Domperidone (ISTD) were supplied as gratis sample by Aum Research Laboratory, Ahmedabad, Gujarat, India, Intas Pharmaceuticals Pvt. Ltd., Ahmedabad, Gujarat, India and Dwarkesh Pharmaceuticals Pvt. Ltd., Ahmedabad, Gujarat, India respectively. Methanol (Lichrosolv grade), acetonitrile (Lichrosolv grade) and orthophosphoric acid (HPLC-grade) were obtained from Merck, Mumbai, Maharashtra, India. Potassium dihydrogen orthophosphate (HPLC-grade), triethylamine (HPLC grade) and were obtained from SD Fine Chemicals Pvt. Ltd., Mumbai, Maharashtra, India. Double distilled water was prepared in-situ. Drug-free human plasma was supplied as gratis sample from Red Cross Society, Anand and stored in polypropylene bottle at -20°C until analysis. All other chemicals and solvents were of analytical grade or equivalent.

Instruments

A High Performance Liquid Chromatography system, with LC solutions data handling system (Shimadzu-LC2010-CHT), with PDA detector (SPD-M20A) and an auto sampler was used for analysis. The data was recorded using LC 2010 solutions software version 1.25. Shimadzu AUW220 balance, Japan, Eltek centrifuge TC 450 D, Remi motors Cyclo Mixer CM101, Vacuum filtration assembly TID 15, and AXIVA Nylon membrane filters 0.2 µm were used.

DEVELOPMENT AND VALIDATION OF RP-HPLC-UV BIOANALYTICAL METHOD

Chromatographic conditions

Chromatographic separations were performed on column (250 mm x 4.6 mm) filled with ODS chemically bonded to porous silica particles of 5µm, the mobile phase containing 15 mM potassium di-hydrogen orthophosphate buffer (pH=3.11) and acetonitrile in gradient mode. The mobile phase was filtered using 0.45 µm membrane filter and degassed in sonicator for 10 min. Flow rate was 1.0 ml/min and the elution was monitored at 260 nm. The injection volume was 20 µl and column temperature was maintained at 25°C.

Sample preparation

Extraction of ACE, TCS and ISTD were carried out by protein precipitation technique. In 2 ml ependroff tube, samples for extraction were added in following sequence. Step 1: 100 µL of Spiking Solution of drugs was added in Ependroff, Step 2: 100 µL of ISTD dilution was added and Step 3: 200 µL of blood plasma was added and vortex to mix properly. After adding blood plasma to the mixture of spiking solution and ISTD, it was vortexed for about 5 min to get homogenous mixture of all the components

and Step 4: 600 μ L of extracting solvents (400 μ L Acetonitrile + 200 μ L Methanol) were added. After addition of extracting solvents to ependroff tube it was centrifuged for 15 min at 6000 rpm. It was then filled in a pre-labeled auto sampler vials which was injected to RP- HPLC.

Preparation of calibration standards and quality control samples

The combined stock solution of Aceclofenac and Thiocolchicoside was prepared by dissolving requisite amount of drugs in methanol to make final concentration 50 μ g/ml ACE and 100 μ g/ml TCS. The 100 μ g/ml of Domperidone (ISTD) solution was prepared separately. Calibration curve was prepared by spiking known amounts of ACE, TCS and Domperidone (ISTD) to plasma. The peak area ratio of ACE and TCS to Domperidone (ISTD) was plotted against the concentration of ACE and TCS and corresponding regression equation was derived. The linearity of response between concentration and peak area ratio was obtained from 12.5 to 1000 ng/ml for ACE and 25 to 2000 ng/ml of TCS. For each calibration curve, blank plasma was also analyzed in order to check for the interference from plasma. All the responses were measured at wavelength 260 nm. From mentioned concentrations, three concentrations were selected: 25 ng/ml (LQC), 250 ng/ml (MQC) and 1000 ng/ml (HQC) for ACE and 50 ng/ml (LQC), 500 ng/ml (MQC) and 2000 ng/ml (HQC) for TCS as QC samples. Calibration standards and QC samples were prepared in bulk and stored in a deep freezer at $-20 \pm 2^\circ\text{C}$ until analysis.

Method validation

Measurements for each analyte in the biological matrix should be validated according to relevant guidelines ²¹. Method development and validation included calibration curve, sensitivity, selectivity, accuracy and precision, recovery, stability of analyte in spiked samples. The calibration for ACE and TCS was obtained in the range of 12.5-1000 ng/ml and 25-2000 ng/ml respectively by taking five replicates. The linearity obtained by plotting graph of area ratio v/s concentration of ACE and TCS and was subjected to regression analysis to establish the calibration equation and a correlation coefficient. Moreover, Bartlett's test was applied for test of homoscedasticity or heteroscedasticity of data ^{22, 23}. The LOD and LOQ were calculated from the 5 set of the calibration curve used to determine the linearity. LOD was calculated from, $\text{LOD} = 3.3 \sigma/s$ and LOQ was calculated from, $\text{LOQ} = 10 \sigma/s$. Where, s = Slope of calibration curve, σ = Standard deviation of response. For selectivity, analysis of blank samples of different lots of plasma was done. Each blank sample should be tested for interference, and selectivity should be ensured at the lower limit of quantification (LLOQ) (12.5 ng/ml and 25 ng/ml) concentration of ACE and TCS. The precision study was performed in 5 replicates on 3 QC samples (1000, 250 and 25 ng/ml for ACE and 2000, 500 and 50 ng/ml for TCS) and area ratio were measured. Intraday precision was performed on 3 QC samples on the same day and interday precision was performed on different day on 3 QC samples. Each concentration level should not exceed 15% of the

%CV except for the LLOQ, where it should not exceed 20% of the %CV. In accuracy study back calculated concentrations of all QC samples (HQC, MQC, and LQC) must be within 85.00-115.00% of their nominal concentration except at LLOQ sample where it should not deviate by more than 80.00-120.00% of its nominal concentration. Recovery of an analyte was determined at 3 QC samples (HQC, MQC, and LQC) and recovery of an internal standard is determined at concentration to be used during method validation. The %mean recovery will be determined by comparing the mean peak area of the 3 replicates of HQC, MQC and LQC (1000, 250 and 25 ng/ml for ACE, 2000, 500 and 50 ng/ml for TCS and 1000 ng/ml DOMP) against respective mean peak area of the 3 replicates of Un-extracted quality control samples. % Extraction recovery of analyte = (Mean peak area of extracted analyte /Mean peak area Unextracted analyte)*100. Stock solution stability study was performed on the stock solution of drugs i.e. ACE and TCS 500 µg/ml and 1000 µg/ml respectively and stock solution of ISTD (DOMP) i.e. 100 µg/ml. The stability of test sample was compared with the freshly prepared stock solution at the end of 6 h. In short term stability study three aliquots of each of the frozen samples of HQC and LQC (at -20 °C for 12 h) concentrations should be thawed at room temperature and kept at this temperature from 6 hour. It should be evaluated by comparing mean response of stability sample against mean response for freshly prepared comparison samples. Analyte stability should be determined after three freeze thaw cycles. Three aliquots at each of the HQC and LQC should be stored at -20 °C storage temperature for 24 h and thawed unassisted at room temperature for 1 h. When thawed completely, the samples should be refrozen for 12 h under the same conditions. The freeze thaw cycle should be repeated twice more, then at the end of the third cycle analyzed samples and evaluated by comparing mean response of stability sample against mean response for freshly prepared comparison samples.

In-vivo pharmacokinetic study

In-vivo studies were carried out using either sex of Wistar albino rats (150-350 g). The animals were housed under standard laboratory conditions of temperature ($21 \pm 1^\circ\text{C}$), relative humidity ($55 \pm 5\%$) with fixed 12 h light/dark cycles. Animals were fed with a standard pellet diet and were provided with water. The experimental protocols (Protocol no. 1332, dated on 23/11/2013) were approved by Institutional Animal Ethical Committee of animal house of Anand Pharmacy College as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India. The rats were randomly divided into two groups. Each group had 3 animals. Group 1 was administered oral marketed formulation of ACE and TCS (20.55 mg/kg ACE and 1.62 mg/kg TCS). Group 2: rats were applied newly formulated optimized transdermal patch (2.052 mg/2 cm² ACE and 0.41 mg/ 2 cm² TCS). The animals of Group 2 were selected after superficial examination of skin surface for abnormalities. About

10 cm² of skin was shaved on the dorsal side of Wistar albino rats. Before application of patches rats of Group 2 were observed for 24 h for any untoward effect of shaving, they were fasted over this period. Area of patch sample was 2 cm². Patch attachment was sustained for 24 h and patch samples were detached after 24 h. Blood samples were withdrawn by retro-orbital route from rat at 0, 0.5, 1, 2, 3, 4 and 6 h for oral marketed formulation and 0, 1, 2, 3, 4, 6, 10, 18, 24 h for newly formulated transdermal patch. Blood samples were centrifuged to yield blood plasma which was stored at -20 °C until analysis. Collected samples were extracted and analyzed for various pharmacokinetic parameters like C_{max}, T_{max}, elimination half-life, elimination rate constant, Mean residence time and AUC.

Pharmacokinetic data analysis

The rat blood plasma samples were analyzed by newly developed and validated method along with QC samples. The plasma samples at different time interval were subjected to pharmacokinetic analysis to calculate various parameters: maximum plasma concentration (C_{max}), time to reach maximum concentration (T_{max}) Area under curve (AUC), Mean residence time. The C_{max} and T_{max} were obtained directly from the arithmetic plot of time versus plasma concentration of ACE and TCS. The AUC was calculated from trapezoidal rule. The elimination rate constant was calculated from slope of the line by regression analysis and half-life was obtained from elimination rate constant.

RESULTS AND DISCUSSION

Development and validation of RP-HPLC-UV bioanalytical method

Optimization of chromatographic condition.

For RP-HPLC method optimization different ratios and different pH of methanol, acetonitrile and potassium di-hydrogen orthophosphate buffer were tried. Here as one drug is polar (TCS) and another one is non-polar (ACE), for the elution of non-polar drug the run time had to be 45 min and more. Thus, the gradient method for development of mobile phase was also tried using different ratios of acetonitrile, water and potassium di-hydrogen orthophosphate buffer for the separation of both the drugs with minimum run time. The optimized chromatographic condition for HPLC given in Table 1.

Table 1: Optimized chromatographic conditions for RP-HPLC.

Column	Phenomenex C ₁₈ (250 mm × 4.6 mm, 5μm)		
Column temperature	Room temperature		
Mobile phase	Mobile phase A) 15 mM potassium di-hydrogen orthophosphate buffer (pH 3.11) set with orthophosphoric acid and Mobile phase B) Acetonitrile		
Flow rate	1.1 ml/min		
Injection volume	20 μl		
Wavelength	260 nm		
Run time	21 min		
Solvent	Methanol		
Gradient	Time	Mobile phase A	Mobile phase B
R _t = TCS	(min)	(ml)	(ml)
=5.2 min			
R _t = DOMP	0.01	80	20
= 9.2 min	3	80	20
R _t = ACE	10	45	55
=14.6 min	11	35	65
	20	45	55
	21	80	20

Optimized sample preparation

The final optimized extracting solvent mixture included Acetonitrile: Methanol (2:1). The samples along with drug solutions, ISTD solution, plasma and extracting solvents were vortexed for 5 min and were centrifuged for 15 min at 6000 rpm.

Method Validation

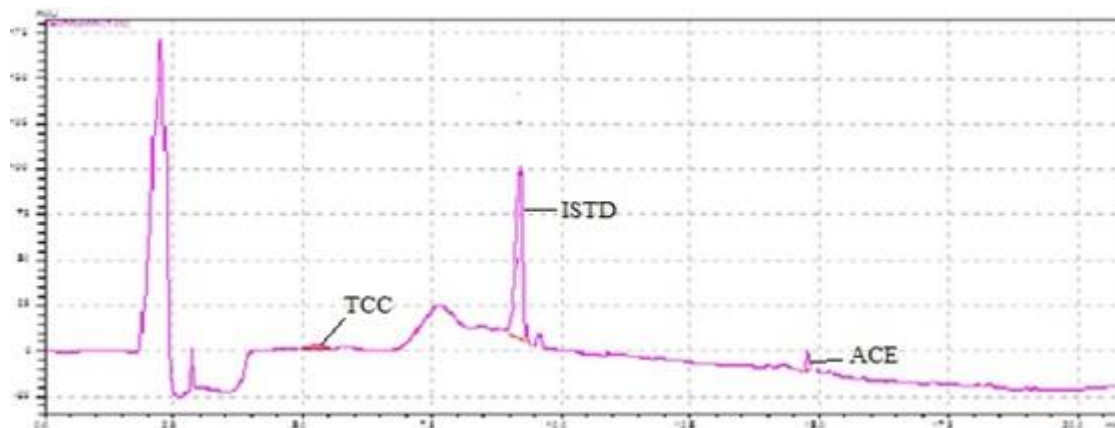
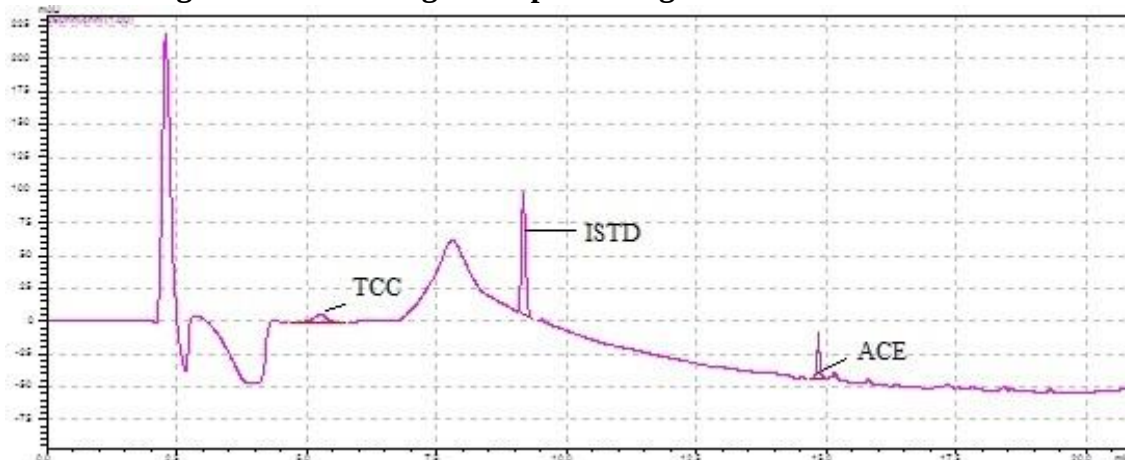
The linearity was found over the concentration range of 12.5 to 1000 ng/ml for ACE and 25 to 2000 ng/ml for TCS when the ratio of ACE and TCS with DOMP (ISTD) was plotted against the concentration range. The adherence to the Beer's law was observed by regression coefficient (r^2) of 0.9996 and 0.9986 respectively. Bartlett's test showed. Test for homoscedasticity was confirmed by Bartlett's test and response of peak area ratio for ACE and TCS to ISTD showed homogenous variance that was exemplified by the χ^2 value less than the tabulated value χ^2 (0.05, 6) = 12.592 at 95% confidence interval level. The linear regression parameters of ACE and TCS are depicted in Table 2.

Table 2: Linear Regression parameters for ACE and TCS by proposed method.

Parameters	Aceclofenac	Thiocolchicoside
Linearity range (ng/ml) ^a	12.5-1000	25-2000
Correlation coefficient	0.9996	0.9986
Slope $\pm$ SD	0.019 $\pm$ 0.0005	0.010 $\pm$ 0.0002
Confidence limit of slope	0.0285 - 0.0095	0.0105 - 0.0095
Intercept $\pm$ SD	0.011 $\pm$ 0.0217	0.020 $\pm$ 0.0244
Confidence limit of intercept	0.0115 - 0.0104	0.0201 - 0.0199
Bartlett's Test	5.113	5.074

^a Five replicates, SD= standard deviation

The limit of detection (LOD) Figure 2, and limit of quantification (LOQ) Figure 3, for ACE and TCS were found to be 3.774 ng/ml and 7.645 ng/ml and 11.436 ng/ml and 23.169 ng/ml respectively which showed good sensitivity of method in the presence of the endogenous substance present in blood plasma.

**Figure 2: Chromatogram representing LOD of ACE and TCS.****Figure 3: Chromatogram representing LOQ of ACE and TCS.**

The selectivity of method was assured in presence of endogenous substance present in different plasma samples at LLOQ sample (12.5 ng/ml for ACE and 25 ng/ml for TCS). The study was done in triplicate. % CV has to be less than 20% for LLOQ (Table 3). From Figure 4 & 5 it is seen that there is no interference observed between plasma and the spiked drugs.

Table 3: Selectivity study for different blood plasma samples.

Plasma samples	Drugs	Spiked amount (ng/ml)	% Mean recovered ^a	SD	%CV
Plasma sample 1	ACE	250	95.123	3.5846	3.713
	TCS	500	96.178	2.7188	2.878
Plasma sample 2	ACE	250	96.002	4.0142	4.615
	TCS	500	96.541	1.8074	1.897
Plasma sample 3	ACE	250	96.930	2.5083	2.645
	TCS	500	91.034	1.7378	1.927

^a Mean of three replicates, %CV= % coefficient of variance, SD= standard deviation

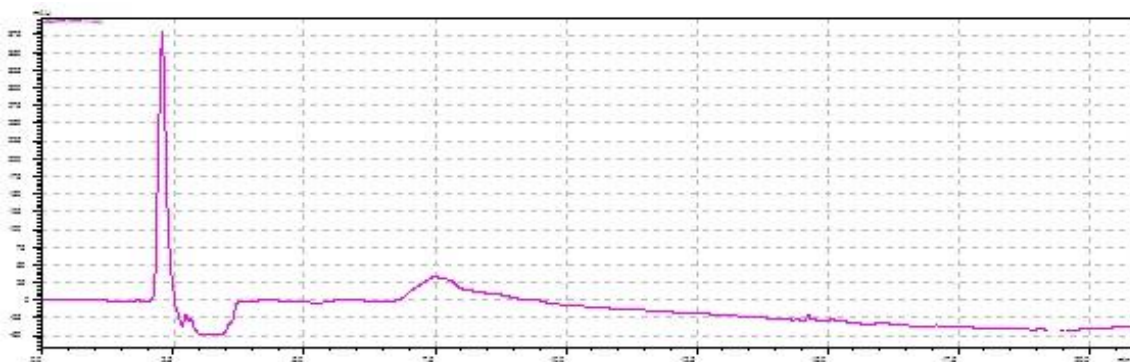


Figure 4: Representative chromatogram of blank plasma

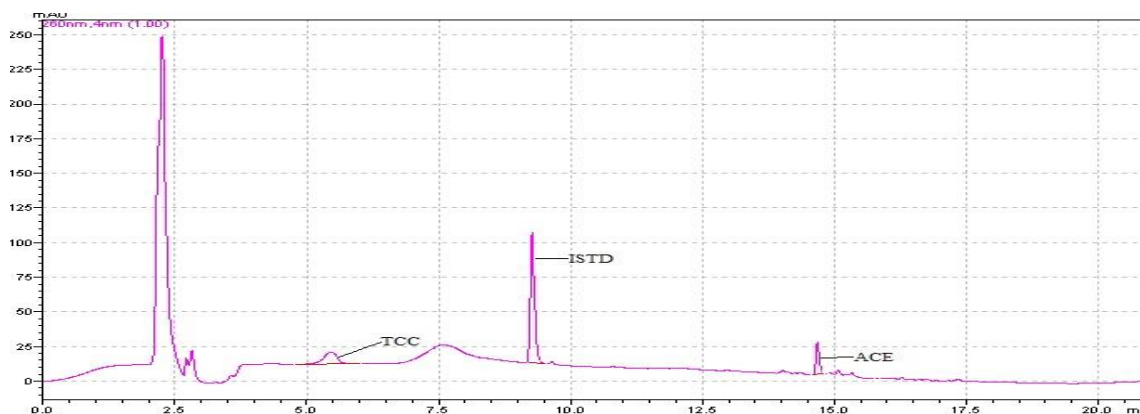


Figure 5: Representative Chromatogram of Plasma spiked with ACE and TCS at LLOQ

Intraday and Interday precision and accuracy studies performed on 3 QC samples (1000, 250 and 25 ng/ml for ACE and 2000, 500 and 50 ng/ml for TCS) showed % CV < 15 % (Table 4). Thus, it will indicate proposed method was precise and accurate in nature.

Table 4: Intraday and interday precision and accuracy study for ACE and TCS.

Nominal Concentration (ng/ml)	Precision				Accuracy	
	Intraday		Interday		Mean Concentration Found (ng/ml)	%CV
	Mean ^a	%CV	Mean ^a	%CV		
	Concentration Found (ng/ml)		Concentration Found (ng/ml)			
ACE						
25	23.694	7.334	23.272	4.309	24.141	10.273
250	241.190	2.988	247.387	2.592	248.742	3.812
1000	990.708	1.766	993.738	1.867	992.382	4.661
TCS						
50	48.867	5.149	48.043	3.216	46.629	7.858
500	493.015	2.069	493.093	1.387	492.052	3.009
2000	1993.167	1.761	1987.831	1.049	1992.513	1.050

^a Mean of three replicates, %CV= % coefficient of variance,

Extraction recovery was performed at three QC samples in blood plasma (1000 ng/ml, 250 ng/ml and 25 ng/ml for ACE and 2000 ng/ml, 500 ng/ml and 50 ng/ml for TCS). This study shows 96.68- 97.084 % recovery for ACE and 95.150- 97.992 % for TCS and also % CV was less than 15% (Table 5). Hence, the present method shows high extraction efficiency and sensitivity.

Table 5: Extraction Recovery of ACE and TCS after spiking in blood plasma.

Samples	Mean Concentration found (µg/ml) ^a		Recovery (%) (Mean ± SD)	%CV
	Extracted samples	Unextracted samples		
ACE				
25	24.072	24.91	96.689 ± 3.58	3.70
250	241.19	248.52	97.08 ± 3.37	3.47
1000	986.375	999.216	97.47 ± 2.55	2.61
TCS				
50	48.86	51.45	95.15 ± 4.11	4.32
500	493.172	505.51	97.62 ± 2.84	2.91
2000	1993.16	2034.01	97.99 ± 1.42	1.45

^a Average of three determinations; %CV, Coefficient of variance; SD, Standard deviation.

The stability of ACE and TCS in human plasma was investigated under a variety of storage and process conditions. The results of the stability studies (Table 6 & 7) did not reveal any significant degradation under the conditions of the experiment.

Table 6: Stock solution stability study of ACE and TCS

Sample Name	Mean Peak Area ^a / %CV		% Stability
	Test Sample (mV)	Freshly Prepared sample (mV)	
ACE	90783956 / 1.62	91786006 / 1.27	98.90
TCS	101236987 / 1.09	102847598 / 1.36	98.43
ISTD	56610823 / 1.81	57984096 / 1.47	97.63

^a Average of three determinations; %CV, Coefficient of variance**Table 7: Stability data for ACE and TCS in human plasma under different storage conditions**

Drug	Stability study	Nominal Concentration (ng/ml)	Mean concentration of Test sample (ng/ml) / %CV	Mean concentration of Freshly prepared sample (ng/ml) / %CV	% Stability
ACE	Short term	25	24.77 / 2.20	24.86 / 3.69	99.63
		1000	999.68 / 1.84	999.93 / 2.54	99.97
	Freeze/Thaw	25	23.85 / 3.14	24.78 / 1.23	96.24
		1000	996.14 / 3.45	998.19 / 2.17	99.79
TCS	Short term	50	49.8 / 6.9	49.85 / 4.86	99.89
		2000	1998.31 / 2.18	1999 / 2.67	99.96
	Freeze/Thaw	50	49.05 / 3.19	49.19 / 4.06	99.71
		2000	1998.98 / 2.14	1999.01 / 2.92	99.99

a, Average of three determinations; %CV, Coefficient of variance

Applicability of the Method

After validation, the RP-HPLC method was used to determine ACE and TCS concentrations in plasma samples after applying transdermal patch and oral administration of marketed formulation to rats. Pharmacokinetic parameters were calculated from the equation of linearity obtained from the graph of concentration of the drug in blood plasma vs. time. The maximum drug concentration (C_{max}) after oral administration and T_{max} was 555.42 ng/ml and 6 h respectively for ACE while it was 298.62 ng/ml and 6 h respectively for TCS whereas, the maximum drug concentration and T_{max} , after transdermal patch were 545.42 ng/ml and 8 for ACE and 264.30 ng/ml and 8 h for TCS. The results indicated that the elimination half-life ($t_{1/2}$) and AUC of oral ACE were 5 hours and 3273 ng*h/ml and for patch it was 12 hours and 5753 ng*h/ml. Similarly, for TCS elimination half-life ($t_{1/2}$) and AUC were 4.5 hours and 550.52 ng*h/ml for oral and 12 hours and 2112.6 ng*h/ml for patch, which gives idea that drug remained in the body for a longer period of time and gave more sustained action than the oral formulation. The area under curve (AUC) values observed were higher with transdermal patch that indicates increased

bioavailability of both the drugs when given in patch as compared to oral administration. All the pharmacokinetic parameters obtained with ACE and TCS transdermal patch was different from those obtained with oral administration of both the drugs as evident from Table 8.

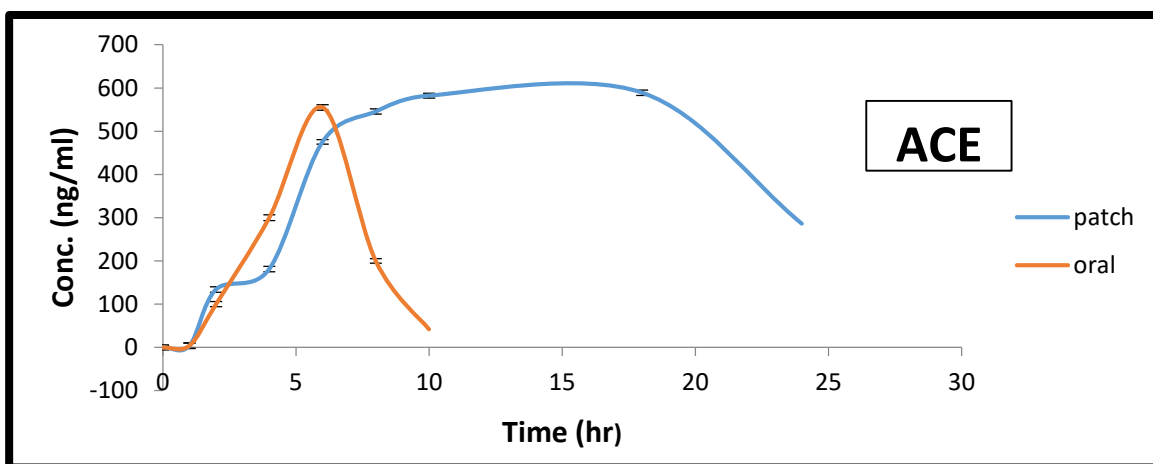


Figure 6: Graph of plasma concentration-time profile of ACE in rat plasma.

Figure 6 shows the ACE concentration in rat blood plasma is as a function of time. The concentration of ACE in newly formulated transdermal patch was higher than those of marketed oral formulation almost at each time point. This indicates that in the initial time period, the newly formulated transdermal patch has lesser elimination owing to a sustained action of the drug.

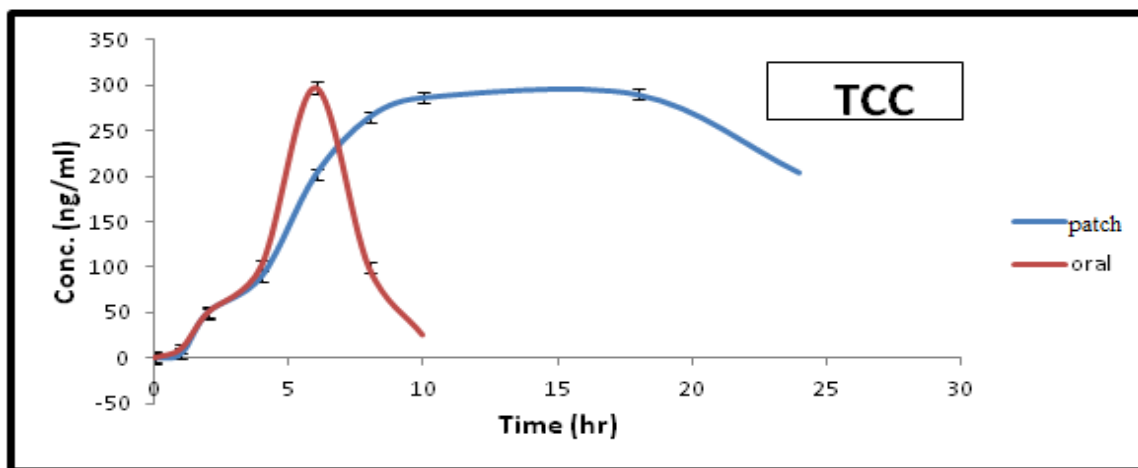


Figure 7: Graph of plasma concentration-time profile of TCS in rat plasma

Figure 7 shows the TCS concentration in blood plasma is a function of time. The concentration of TCS in newly formulated transdermal patch was higher than of marketed oral formulation at each time point. This indicates that at the initial time period, the newly formulated transdermal patch has sustained effect in the body due to lesser elimination of the drug from body.

The chromatograms (Figure 8 & 9) reveal the release of ACE and TCS after application of oral marketed formulation and newly developed transdermal patch to rats. The released drugs do not show any interference after application to rats.

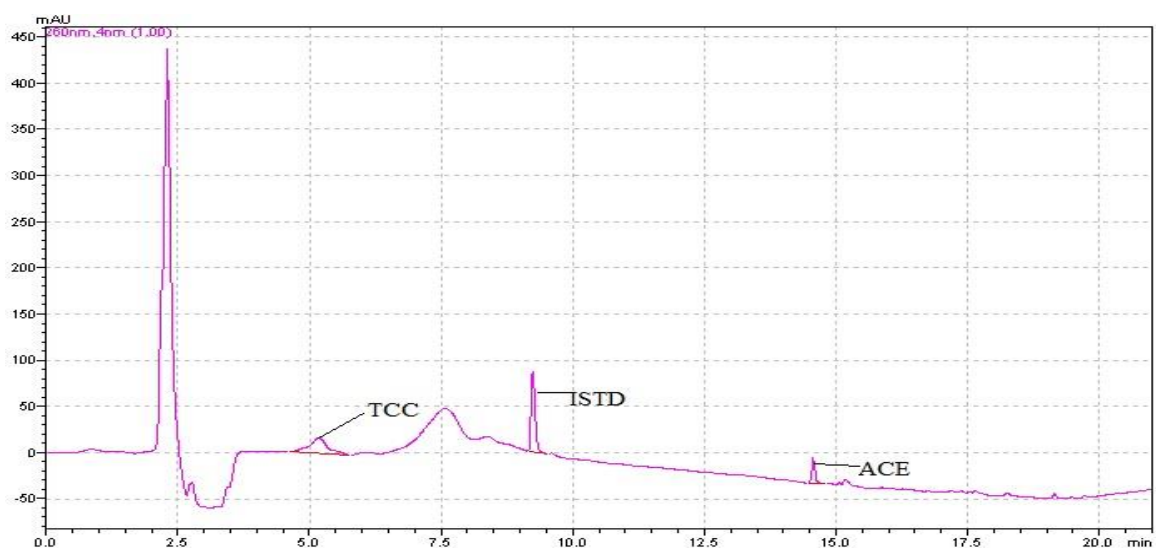


Figure 8: Chromatogram representing release of ACE and TCS from marketed formulation

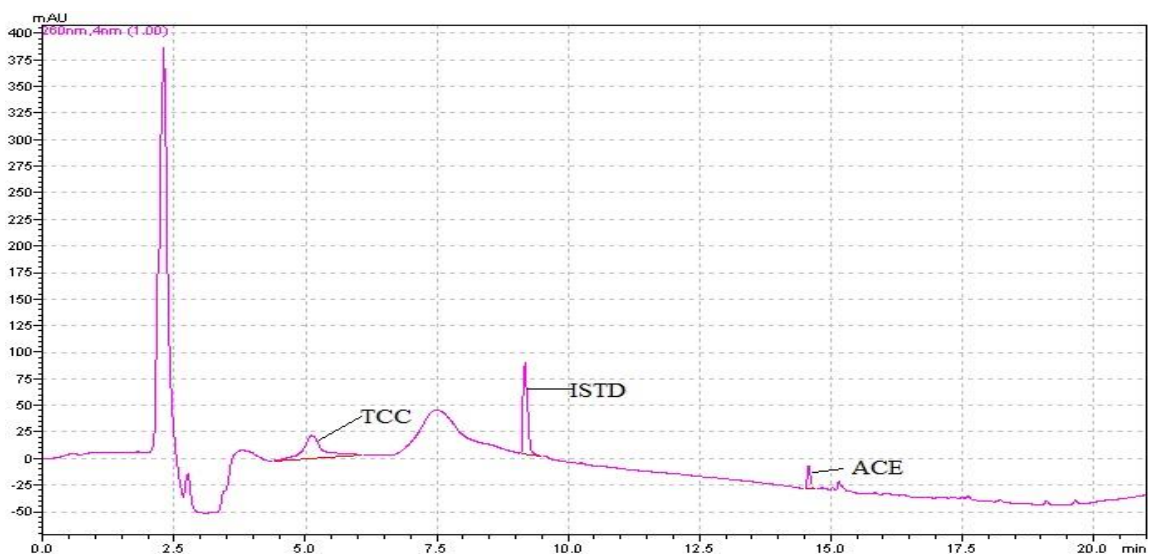


Figure 9: Chromatogram representing release of ACE and TCS from Transdermal patch

Table 8: Pharmacokinetic parameters comparison of ACE and TCS.

Pharmacokinetic parameters	Oral marketed formulation (Mean $\pm$ SD)	Transdermal patch (Mean $\pm$ SD)
ACE		
C _{max} (ng/ml)	555.42 $\pm$ 50	545.42 $\pm$ 6.82
T _{max} (h)	6 $\pm$ 0.6	8 $\pm$ 0.4
AUC (ng*h/ml)	3273 $\pm$ 10.7	5753 $\pm$ 8.6
AUMC (ng*h/ml)	19597 $\pm$ 12.36	99833 $\pm$ 10.44
t _{1/2} (h)	5 $\pm$ 0.12	12 $\pm$ 0.6
Ke (elimination rate constant) (1/h)	1.68 $\pm$ 0.03	0.025 $\pm$ 0.05
MRT (Mean residence time) (h)	5.986 $\pm$ 0.4	17.35 $\pm$ 2
TCS		
C _{max} (ng/ml)	298 $\pm$ 6.20	264.3 $\pm$ 5.03
T _{max} (h)	6 $\pm$ 0.30	8 $\pm$ 0.50
AUC (ng*h/ml)	550.52 $\pm$ 8.36	2112.6 $\pm$ 9.13
AUMC (ng*h/ml)	2226.96 $\pm$ 10.33	26130.3 $\pm$ 12.93
t _{1/2} (h)	4.5 $\pm$ 0.16	12 $\pm$ 0.12
Ke (elimination rate constant) (1/h)	0.25 $\pm$ 0.06	0.041 $\pm$ 0.08
MRT (Mean residence time) (h)	3.092 $\pm$ 0.5	12.36 $\pm$ 1.0

n=3 wistar albino rats were used for each group, SD= standard deviation

From Table 8, it is seen that mean residence time (MRT) of both drugs is higher for transdermal patch than in oral formulation which suggests that the drug resides more in body if they are given in form of transdermal patch rather than in oral formulation.

Conclusion

A sensitive, specific, precise, accurate, rapid, simple and economical HPLC method for estimation of Aceclofenac and Thiocolchicoside in plasma was developed and validated according to USFDA guidelines. The developed method utilizes “protein precipitation technique” for sample extraction; hence, it is simple and economic. Developed method could effectively detect drugs in human plasma with good recovery value (> 90%) for titled analyte in the study, suggesting high sensitivity of method. Pharmacokinetic parameters demonstrated that as compared to oral administration, C_{max} value after transdermal administration is significantly decreased while their AUC and MRT values are evidently increased over extended period of time. Prepared transdermal patch is capable of surmounting the shortcomings of oral administration of ACE and TCS such as low bioavailability, short half-life. The present study demonstrated that Transdermal Delivery System has given good and acceptable result in rat model with respect to C_{max}, AUC, t_(1/2) and MRT and therefore the developed TDDS can be applied for In-vivo study in human for exploration of further findings.

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