



## Comparative Pharmacokinetic and Relative Bioavailability Study of Ursodeoxycholic Acid Tablets in Healthy Indian Volunteers

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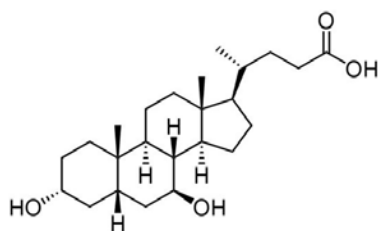
### ABSTRACT

The best bile acid therapy currently available is ursodeoxycholic acid (UDCA), which is a bile salt that both reduces the secretion of cholesterol into bile and increases cholesterol solubility. The aim of this study is to compare pharmacokinetic parameters as well as relative bioavailability of the developed enteric coated tablet of UDCA 300 mg prepared from micronized drug (MCUDCA) with respect to film coated tablet of UDCA 300 mg prepared from non-micronized drug (NMUDCA) available in the market on healthy human volunteers. A simple and sensitive LC-MS/MS was developed to detect and quantify UDCA with a LLOQ of 50ng/ml and using Valsartan as internal standard in human plasma. The method was validated as per the guidelines of US FDA bioanalytical method validation. The linearity of the method (concentration ranging from 50-10000 ng/ml), extraction recovery and accuracy were 0.9988, 82.39 - 91.34 % and 99.60-103.63 % respectively. Comparative pharmacokinetic study revealed that  $t_{max}$  was same for the two types of the formulations and it came around three and half hour. The in vivo pharmacokinetic performances of micronized formulation were much better than non-micronized one. The mean  $C_{max}$  of UDCA from MCUDCA 300 mg enteric coated tablet was 5091.806ng/ml whereas  $C_{max}$  of the drug in the NMUDCA formulation was 4848.66ng/ml. So it can be said that this improvement of plasma drug concentration was due to particle size reduction or micronization. The relative bioavailability study revealed that the enteric coated tablets prepared with micronized UDCA were 112% when compared with marketed UDCA tablets prepared with non-micronized drug. So, it can be concluded that enteric coated tablets 300 mg prepared with micronized UDCA had generated better promising result when compared with the non-micronized type with respect to the pharmacokinetic parameters.

**Keywords:** Bioequivalence study, Comparative bioavailability, Liquid chromatography-mass spectrometry, Ursodeoxycholic acid.

### INTRODUCTION

Ursodeoxycholic acid (UDCA) is a white, odorless, crystalline powder with a bitter taste. Chemically it is  $3\alpha, 7\beta$ -dihydroxy-5-cholan-24-oic acid (Figure 1). It is a water insoluble drug used as a drug for the dissolution of cholesterol gallstones<sup>1-3</sup> because it reduces the cholesterol saturation of bile.<sup>4</sup> The use of UDCA for the treatment of other liver diseases, such as primary biliary cirrhosis, chronic hepatitis and biliary pains has been demonstrated.<sup>5-7</sup> However in vivo studies have shown that intestinal absorption and consequently the bioavailability of the drug are generally poor and erratic both among different subjects, and within the same subject.<sup>8</sup> More than 50% is lost in the stool<sup>9</sup> after a single oral dose of 300 mg.



**Figure 1:** Ursodeoxycholic acid (CAS number 128-13-2)

Pharmacokinetic study of a drug deals with its absorption, distribution, metabolism and excretion by the body. The pharmacokinetic study is done by evaluating some pharmacokinetic parameters such maximum plasma

concentration ( $C_{max}$ ), time taken to reach maximum plasma concentration ( $t_{max}$ ), area under the curve (AUC), elimination rate constant ( $K_{el}$ ), elimination half life ( $t_{1/2}$ ) etc. Pharmacokinetic study<sup>9</sup> as well as the bioavailability study<sup>10,11</sup> of ursodeoxycholic acid was reported in various literatures.

In the present study, the bioavailability of test containing enteric coated tablet of ursodeoxycholic acid 300mg was evaluated against ursodeoxycholic acid 300 mg film coated tablets as reference. An attempt was made to study the pharmacokinetic profile of reference and test tablets in the local population of Indian origin.

### MATERIALS AND METHODS

#### Materials and Reagents

Ursodeoxycholic acid (99%) was supplied by Albert David Ltd., Kolkata, India. Valsartan as internal standard (IS) was obtained from Torrent, Ahmadabad, India. Methanol (HPLC grade), formic acid (98%), ethyl acetate and isopropyl alcohol of analytical grade were purchased from Merck Pvt., Mumbai, India. Water (resistivity of 18 MΩ) was collected from a Milli-Q A10 academic). The blank human plasma with EDTA-K<sub>3</sub> anticoagulant was collected from Clinical Pharmacological Unit (CPU) of Bioequivalence Study Centre, Jadavpur University, Kolkata, India.



## Instrument

The chromatographic system used for LC-MS/MS analysis consisted of a LC-20AD pump, SIL-20AC auto sampler and CTO-10ASvp column oven (Shimadzu, Kyoto, Japan). The LC-MS/MS system consisting of turbo spray and atmospheric pressure ionization source (API-2000) with triple quadrupole tandem mass spectrometer (AB Sciex Instruments, Foster, CA) was used for quantitation of ursodeoxycholic acid in human plasma. Data integration was performed with Analyst 1.5 software (AB Sciex Instruments, Foster, CA).

## Sample preparation and extraction

Ursodeoxycholic acid was dissolved in methanol to prepare a stock solution of 1mg/ml. The internal standard (IS) i.e. valsartan was also dissolved in methanol to prepare a 5 µg/ml of stock solution. Stock solutions were stored at -20° C until they were used to prepare working solutions. Eight different working solutions of ursodeoxycholic acid of 50, 100, 250, 500, 1000, 2500, 5000 and 10000 ng/ml were prepared by diluting the stock solution appropriately with the mobile phase. These working solutions were used to spike heparinized plasma to make calibration standards of 50, 100, 250, 500, 1000, 2500, 5000 and 10000 ng/ml and quality control samples of 150 ng/ml (Low Quality Control Sample; LQC), 4000 ng/ml (Middle Quality Control Sample; MQC) and 8000 (High Quality Control Sample; HQC) ng/ml in replicate of six respectively. Working solution of valsartan was also prepared at a concentration of 5µg/ml by proper dilution with the mobile phase. Final concentration of internal standard was 5µg/ml in all working solutions.

A simple liquid-liquid extraction method was followed to prepare plasma samples of ursodeoxycholic acid and IS. For calibration standards, an aliquot quantity of 0.5 ml plasma sample was mixed with 0.1 ml of internal standard in a 10 ml stopper test tube. To it 6 ml of organic solvent ethyl acetate:IPA::9:1 V/V was added and after mixing for 15 min the solution was centrifuged at 5000 rpm for 10 min. The organic layer was separated and evaporated to dryness at 35°C under N<sub>2</sub> atmosphere. The residue was reconstituted with 250 µl of mobile phase by vortex mixing and filtered through 0.22 µm membrane filter. Finally 20 µl of the prepared sample was injected into the HPLC system associated with tandem mass spectrometry.

## Dosing procedure and blood sampling

The in vivo study was carried out as per guidelines of DCGI (Drugs Control General of India, India) and in compliance with the U.S. Code of Federal Regulations (Title 21, Part 56) and the Declarations of Helsinki. Before inclusion of study, informed consents were obtained from volunteers after making all aspects of the study clear to them. These informed consents and the protocol was approved through the "Institutional Ethical Committee" of Jadavpur University, Kolkata, India. After approval of concerned ethical committee the pharmacokinetic study was carried out in an open level, fasting, single dose and

single phase manner on healthy male human volunteers. Total twelve volunteers were enrolled for the study based on their pathological tests (serum chemistry, hematology and urine analysis), medical history, physical examination and HIV screening. Blood sampling for measurement of plasma ursodeoxycholic acid concentrations was conducted for each subject using an intravenous canula inserted into an arterial vein. For the reference tablet blood was collected at predose and at 0, 1, 2, 2.5, 3, 3.5, 4, 6, 8 and 12 hour after the morning administration of drug. The test tablet samples were collected as per same schedule of reference. Collected blood samples were centrifuged immediately; plasma was separated and stored in polypropylene tubes at -20°C after properly labeled until assayed.

## Pharmacokinetic parameters studied

The pharmacokinetic parameters were determined from plasma concentrations of ursodeoxycholic acid using zero-moment non compartmental method. The maximum plasma concentration (C<sub>max</sub>) and time to reach maximum plasma concentration (T<sub>max</sub>) were directly obtained from the plasma concentration vs. time profile of the drug. Area under the plasma concentration – time curve from time zero to last concentration time point (AUC<sub>0-t</sub>) was determined by trapezoidal rule. Area under the plasma concentration – time curve from time zero to infinity (AUC<sub>0-∞</sub>) was determined by the following equation:

$$AUC_{0-\infty} = AUC_{0-t} + \frac{C_t}{K_e}$$

Where, K<sub>e</sub> is the elimination rate constant, estimated as a slope of the straight line by plotting the concentration (C<sub>max</sub> to last concentration) versus corresponding time on a semi-logarithm graph paper and C<sub>t</sub> is the last quantifiable concentration. The elimination half life (t<sub>1/2</sub>) was calculated as 0.693/K<sub>e</sub>.

## Comparative bioavailability study

The relative bioavailability of MCUDCA and the reference film coated ursodeoxycholic acid tablet (URSOCOL 300) was calculated as follows:

$$\text{Relative Bioavailability (F\%)} = \frac{AUC_{\text{Test}}}{AUC_{\text{Ref}}} \times \frac{\text{Dose}_{\text{Ref}}}{\text{Dose}_{\text{Test}}} \times 100$$

The comparative bioavailability of MCUDCA was calculated by considering the reference product with 100% bioavailability and the equations are as follows:

$$\text{Relative Bioavailability of MCUDCA (F}_{\text{MCUDCA}} \%) = \frac{AUC_{\text{MCUDCA}}}{AUC_{\text{Ref}}} \times 100$$

[Dose equivalent]

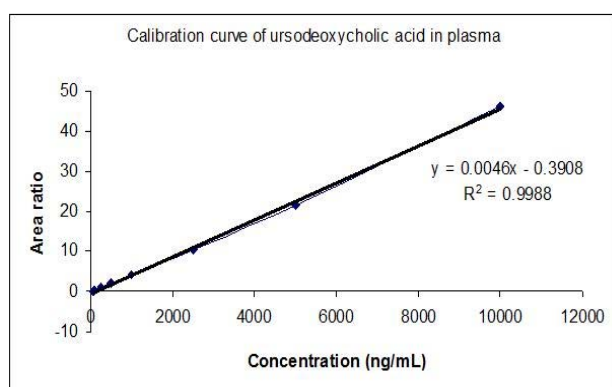
## RESULTS AND DISCUSSION

A simple and sensitive LC-MS/MS method was developed to quantify ursodeoxycholic acid with a LLOQ of 50ng/ml and using valsartan as internal standard. The method was validated as per the guidelines of US FDA bioanalytical

method validation. The linearity of the method (concentration ranging from 50-10000 ng/ml) (Figure 2), extraction recovery and accuracy were 0.9988, 82.39 - 91.34 % and 99.60-103.63 % respectively. So it can be concluded that ursodeoxycholic acid can be successfully quantified in human plasma by the developed LC MS/MS method.

Comparative pharmacokinetic study was carried out between micronized enteric coated tablets of ursodeoxycholic acid and marketed film coated tablet of ursodeoxycholic acid prepared with non-micronized drug as reference. The study revealed that  $t_{max}$  was same for the two types and it came around 3 and half hour. The elimination rate of micronized type has been slightly lesser with respect to non-micronized reference product. From the in vivo kinetic study on human subjects it can be shown that the in vivo pharmacokinetic performances of micronized formulation were much better than non-micronized one (Figure 3). The mean  $C_{max}$  of ursodeoxycholic acid from micronized ursodeoxycholic acid 300 mg enteric coated tablet was 5091.806ng/ml whereas  $C_{max}$  of the drug in the non-micronized formulation was 4848.66ng/ml (Table 1). So it can be said that this improvement of plasma drug concentration was due to particle size reduction or micronization.

The relative bioavailability study revealed that the enteric coated tablets prepared with micronized ursodeoxycholic acid were 112% when compared with marketed ursodeoxycholic acid tablet prepared with non-micronized drug. So, it can be concluded that enteric coated tablets of 300 mg prepared with micronized ursodeoxycholic acid had generated better promising result when compared with the non-micronized type with respect to the pharmacokinetic parameters.

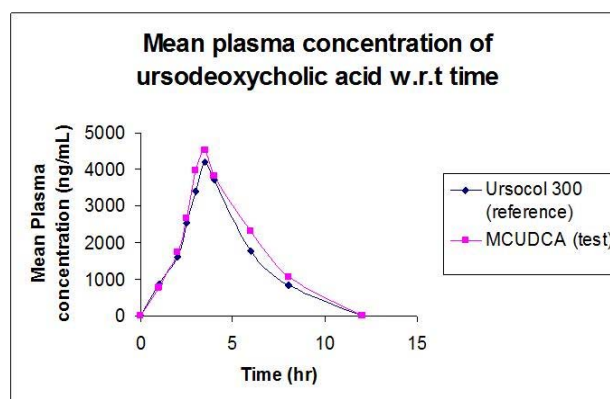


**Figure 2:** Calibration curve of ursodeoxycholic acid for plasma analysis

## CONCLUSION

The outcome of this research can be summarized and concluded like this that micronization has proved to be an efficient method to enhance the in vivo performance of poorly water soluble but highly permeable drug like ursodeoxycholic acid. An enteric coated tablet developed with micronized ursodeoxycholic acid drug particle showed improved in vivo performances and this approach

would overcome the bioavailability problem of such BCS class II drugs like ursodeoxycholic acid.



**Figure 3:** Mean plasma concentration of ursodeoxycholic acid with respect to time for Ursocol 300 (reference) and MCUDCA (test)

**Table 1:** Main pharmacokinetic parameters studied for MCUDCA and Reference product (NMUDCA)

Parameters	NMUDCA	MCUDCA
$C_{max}$ (ng/ml)	4848.669 ± 198.685	5091.806 ± 611.855
$T_{max}$ (hr)	3.583 ± 0.376	3.417 ± 0.376
$AUC_{0-t}$ (ng*hr/ml)	16115.289 ± 772.675	18092.422 ± 2618.107
$AUC_{0-\infty}$ (ng*hr/ml)	18322.989 ± 811.955	21298.894 ± 2526.063
$Kel$ (hr <sup>-1</sup> )	0.382 ± 0.036	0.333 ± 0.041

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