the corresponding parent drug **1**. At the LC_{50} level **1** exhibited an about 1 log unit stronger cytotoxic effect (MGM $-\log LC_{50}$ (**1**) = 5.25/5.10 vs. MGM$-$ log LC_{50} (**2**) = 4.02) and at the GI_{50} and TGI level it even led to an increase in growth inhibitory effects of about 1.5 log units (MGM$-$ log GI_{50} (**1**) = 6.42/6.45 vs. MGM$-$ log GI_{50} (**2**) = 4.95 and MGM$-$ log TGI (**1**) = 5.88/5.87 vs. MGM$-$ log TGI (**2**) = 4.31).

Furthermore, formation of the 2-hydroxyl compound **1** was observed upon incubation of prodrug **2** at a concentration of 10^{-5} M with 2 μg/ml enzyme and 5×10^{-5} M NADH in phosphate buffer at 37 °C which is a clear evidence that prodrug **2** is a substrate for for *E. coli* nitroreductase (data not shown).

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Determination of valdecoxib in serum using a HPLC-diode array detector and its application in a pharmacokinetic study

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A simple, sensitive, isocratic and reproducible reversed phase HPLC method for the determination of valdecoxib, a novel specific COX-2 inhibitor in human serum was developed using a diode array detector and celecoxib as internal standard. The system consisted of a C18 column and a detector set at 240 nm. The mobile phase was a mixture of acetonitrile : water acidified to pH 3.2 with orthophosphoric acid (OPA) (60 : 40) pumped at room temperature and a flow rate of 1 ml/min. The mean absolute recovery value was about 90%, while the intra ($n = 5$) and inter ($n = 5$) assay variations were $<18\%$. The calibration was linear over a concentration range of 20 ng/ml to 200 μg/ml with $r^2 > 0.999$. The limit of detection was ≤ 10 ng/ml. The method was used to study the pharmacokinetics of valdecoxib after a single dose oral administration to human volunteers.

Valdecoxib is a potent and specific COX-2 inhibitor approved by FDA for the treatment of rheumatoid arthritis, osteoarthritis and in primary dysmenorrhea. Valdecoxib has shown to be a highly selective and potent inhibitor of COX-2 in human whole blood and against the recombinant human enzyme. Valdecoxib has a mean absolute bioavailability of 83% (Bextra Valdecoxib Package Insert 2001) and is primarily metabolized by CYP 2C9 and CYP 3A4 enzymes (Yuan et al. 2002).

To date, two SPE-LC-MS-MS methods were developed and validated for the determination of valdecoxib and its metabolites in human urine (Zhang et al. 2003a) and plasma (Zhang et al. 2003b). An HPLC method using an UV-VIS detector was reported for the quantitation of valdecoxib in human plasma (Ramakrishna et al. 2004). Rao et al. (2005) reported the estimation of COX-2 inhibitors in pharmaceutical dosage forms and estimations in human plasma. Pavan Kumar et al. (2005) reported the estimation of valdecoxib and few other NSAIDS in human plasma. So far no simple HPLC method was reported for the determination of valdecoxib in human serum using UV-VIS or diode array detection. Here we report a simple, sensitive, reproducible and fast HPLC assay method for the determination of valdecoxib in human serum using UV detection. This method is applied to estimate the pharmacokinetics of valdecoxib after a single dose oral administration of valdecoxib tablet to human volunteers.

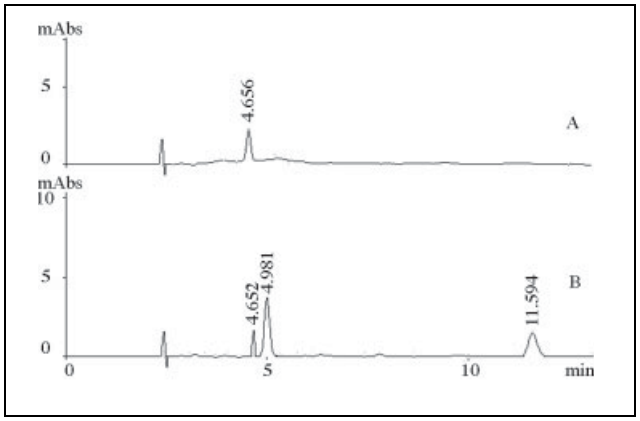

Fig. 1: HPLC profiles. A) Blank serum and B) Serum with Drug (100 ng/ml) and I.S. (100 ng/ml). The value above the peak indicates retention time in min

Diode array detector of HPLC has shown λ_{max} of 238 nm where a negligible baseline drift was observed at 240 nm and thus the assay was developed at this wavelength. Good resolution and a sharp peak were obtained at pH 3.2. The mobile phase used allowed the separation of the valdecoxib (R_t 5.0 min) and I.S. (R_t 11.6 min) from an endogenous serum eluted at 4.6 min (Fig. 1).

The ratio of peak area of valdecoxib to that of I.S. was used for the quantification of valdecoxib in serum samples. The calibration curves were linear in the concentration range 20 ng/ml to 200 μg/ml with $r^2 > 0.999$. The limit of quantification (LOQ) and limit of detection (LOD), having a reproducibility with a relative standard deviation (RSD) less than 18%, were found to be 20 and 10 ng/ml, respectively. The improved optical system in the diode array detector has made excellent S/N performance resulting in lower limit of detection. Intra and inter assay variations (both $n = 5$) were <18% using the range 20 ng/ml to 200 μg/ml. The coefficient of variation (%CV) of slopes and intercepts of each standard curve from ten independent HPLC assay run over 2 months were less than 0.9 and 8.2% respectively. The absolute recovery ranged from 89 to 92%. The accuracy was verified by comparing the concentration of valdecoxib measured in extract with the actual concentrations added.

The method was applied to a pharmacokinetic study after single dose oral administration of valdecoxib tablets to human volunteers. Fig. 2 shows the mean serum concentration-time profiles after oral administration of valdecoxib tablets (20 mg). Peak concentration (C_{max}) of valdecoxib

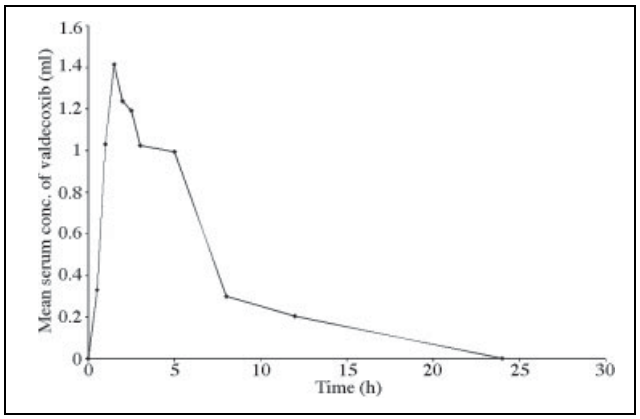

Fig. 2: Mean serum concentration – time profile of valdecoxib after administration of valdecoxib tablet (20 mg) ($n = 12$).

(1385 ± 82 ng/ml) was reached after 1.5 h (T_{max}). Area under the curve ($AUC_{0-\infty}$) was 9062 ± 484 ng/ml/hr with a half life of 3.7 ± 0.2 h. Volume of distribution (Vd/F) and clearance (Cl/F) were 12.1 ± 1.3 l/kg and 2.2 ± 0.1 l/h/kg respectively.

Experimental

Primary stock solutions of 1 mg/ml of valdecoxib and celecoxib were prepared in acetonitrile. Working stock solutions of 0.01, 0.02, 0.03, 0.04, 0.05, 0.1, 0.2, 0.4, 0.8, 4, 20, 50, 100 and 200 μg/ml of valdecoxib and I.S. of 100 ng/ml were prepared in acetonitrile. All the solutions were stored at −20 °C in a deep freezer. Appropriate volumes of working stock solutions were added to serum in the preparation of calibration curves (0.02, 0.03, 0.04, 0.05, 0.1, 0.2, 0.4, 0.8, 4, 20, 50, 100 and 200 μg/ml). Serum samples containing 100, 400, 800 and 4000 ng/ml of valdecoxib were taken to study the recovery.

All the serum samples, after addition of drug and I.S. (2 ml final volume each) were extracted with 3 × 2 ml of ethyl acetate. The organic phase was evaporated and reconstituted to 1 ml with acetonitrile. The samples were centrifuged at 13,000 rpm and 15 °C for 10 min and the supernatants were used for analysis.

The HPLC system consisted of Shimadzu LC-10AT pump, a Rheodyne 7725i sample injector with a 20 μl loop and a Shimadzu SPD-M10Avp diode array detector set at 240 nm, coupled to a Shimadzu LC10 computing integrator. The analytical column used was Phenomenex Luna C18, 250 × 4.6 mm I.D. and 5 μm. The mobile phase was a mixture of acetonitrile : water acidified to pH 3.2 with OPA (60 : 40) pumped isocratically at room temperature and a flow rate of 1 ml/min.

Peak area ratios were recorded and the standard curves were constructed using simple linear regression. Intra and inter day assay variations were calculated with $n = 5$. The recoveries were calculated at concentrations of 100, 400, 800 and 4000 ng/ml with $n = 6$. The absolute recovery from the serum samples was determined by calculating the ratio of peak area of valdecoxib from serum sample to that from direct injection using standard sample. The accuracy of the method was estimated by expressing mean calculated concentration as a percentage of the spiked/nominal concentration.

Pharmacokinetics of valdecoxib were studied in 12 male healthy 22–25 years old human volunteers after a single dose oral administration of valdecoxib 20 mg tablet. Blood samples (5 ml) were collected from the ante cubital vein at the intervals of 0, 0.5, 1, 1.5, 2, 2.5, 3, 5, 8, 12 and 24 h. The blood samples were allowed to clot and were centrifuged at 3000 rpm for 20 min. The serum was separated and stored at −20 °C. Pharmacokinetic parameters viz., C_{max}, T_{max}, $AUC_{0-\infty}$, $t^1/_2$, Vd/f and CL/f for valdecoxib were obtained by extravascular non-compartment model using WinNonlin 1.1 software.

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