Thiocolchicoside (TCS) is a muscle relaxant agent with anti-inflammatory and analgesic actions and is absorbed rapidly from the gastrointestinal tract, after oral administration and peak plasma concentrations are observed within approximately 1 h. Analytical methods published for the determination of TCS to date, are either non specific radioimmunoassay techniques or use enzymatic hydrolysis of the TCS to 3-desmethyliothiocolchicine (3DMT). TCS is metabolized so rapidly after oral administration that it becomes impractical to determine its concentration in plasma over the periods generally used for bioequivalence studies. A sensitive, reliable and reproducible method has been developed for the determination of 3-desmethyliothiocolchicine (3DMT) in plasma, using high performance liquid chromatographic separation with tandem mass spectrometric detection (LC-MS/MS).

The plasma samples were extracted with ethyl acetate and separated on a Phenomenex Gemini column with a mobile phase consisting of methanol: water containing 0.5% formic acid (95.5:4.5 v/v) at a flow rate of 0.45 ml/min. Detection was achieved by an Applied Biosystem, API 2000 mass spectrometer (LC-MS-MS) set at unit resolution in the multiple reaction monitoring mode. Turbo Ion Spray ionization was used for ion production. The MRM transition of m/z 402.10 - 360.10 and m/z 354.00 - 214.00 were used to measure 3DMT and Aceclofenac (IS) respectively. The method was validated over the concentration range of 2 to 50ng/ml for 3DMT in human plasma. The mean recovery for 3DMT was 79%, with a lower limit of quantification set at 1ng/ml. The increased selectivity of mass spectrometric (MS-MS) detection allowed us to distinguish between TCS and its major metabolite 3DMT in human plasma, thereby giving more insight about the pharmacokinetics of the drug in human.