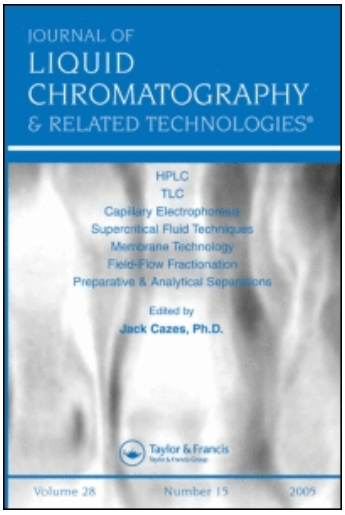

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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To cite this Article Aboul-Enein, Hassan Y. and Islam, M. Rafiqul(1992) 'Enantiomeric Separation of Ketamine Hydrochloride in Pharmaceutical Formulation and Human Serum by Chiral Liquid Chromatography', Journal of Liquid Chromatography & Related Technologies, 15: 18, 3285 — 3293
To link to this Article: DOI: 10.1080/10826079208020884
URL: http://dx.doi.org/10.1080/10826079208020884

JOURNAL OF LIQUID CHROMATOGRAPHY, 15(18), 3285-3293 (1992)

ENANTIOMERIC SEPARATION OF KETAMINE HYDROCHLORIDE IN PHARMACEUTICAL FORMULATION AND HUMAN SERUM BY CHIRAL LIQUID CHROMATOGRAPHY

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ABSTRACT

A commercially available microcrystalline cellulose triacetate (CA-1) chiral stationary phase was used for the enantiomeric resolution of ketamine [(±)-2-(2-chlorophenyl)-2-methylaminocyclohexanone]. A simple isocratic and direct liquid chromatographic resolution of racemic ketamine was accomplished without derivatization, using pure ethanol as eluent with flow rate of 1 ml/min and at 25°C. The enantiomeric elution order was determined by chromatographing the racemic ketamine and the separate enantiomers under the similar conditions. The capacity factor (k) for the first eluted peak was 2.2, stereochemical separation factor (α) and stereochemical resolution factor (R) were 2.32 and 3.38, respectively. This method has been applied to determine and identify ketamine enantiomers in human serum and a pharmaceutical dosage form.

INTRODUCTION

Over the past few years, there has been widespread interest in the implications of stereochemistry on the pharmacokinetics of racemic drugs. (1, 2, 3). This interest stems in part from the realization that individual drug enantiomers may exhibit different

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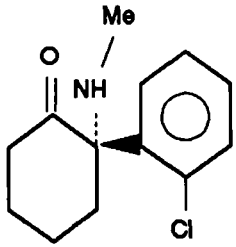
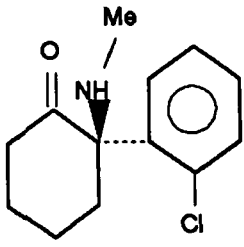


(+)-S-Ketamine



(-)-R-Ketamine

Scheme 1. The absolute configuration of (+)-S-ketamine and (-)-R-ketamine.

pharmacokinetics. Ketamine, chemically known as (±)-2-(2-chlorophenyl)-2-methylaminocyclohexanone (Scheme 1) is an easily administered parenteral anesthetic that produces profound analgesia at subanesthetic doses and lacks the cardiorespiratory depression seen with most other general anesthetic agents. (4). Ketamine molecule contains a chiral center so that it exists as two optical isomers, or enantiomers. Yet all published clinical studies to date have utilized only the racemic mixture. Based on studies in animals, the (+)-S-isomer of ketamine was assumed to be approximately three times more potent as an anesthetic than (-)-R-ketamine (5, 6, 7).

Evaluation of emergence phenomena and the side effects of ketamine and its isomers in the immediate postanesthetic period by analysis of the verbal responses suggests that significantly more psychic emergence reactions (e.g., vivid illusions, "weird trips", sensations of drunkenness or delirium) occurred after (-)-R-ketamine. Also, a lower incidence of amnesia for the operation was recorded following (-)-R-ketamine anesthesia. With respect to observed motor activity in the recovery room, emergence from (-)-R-ketamine was associated with significantly more restlessness, thrashing or combative behavior. Disorientation, agitation and pain also occurred more commonly in

the racemic and (-)-R-ketamine groups. A further assessment of the analgesic properties of the ketamine enantiomers revealed that four times as many patients in the (-)-R-isomer group reported experiencing pain as compared with the (+)-S-ketamine group. Although medication was rarely needed to control pain or any of the other side effects following ketamine anesthesia, narcotics were more commonly administered to patients in the (-)-R-ketamine group (4). Although the overall frequency of unpleasant dreams was low, the (-)-R-ketamine group reported the highest incidence by White et al. (4). On the basis of reports in the human medical literature and experiences in avian species, it seems likely that purified preparations of the (+)-S-isomer would be clinically advantageous over either the racemic mixture or the (-)-R-enantiomer which are the main source for these unwanted side effects (6, 7, 8, 9).

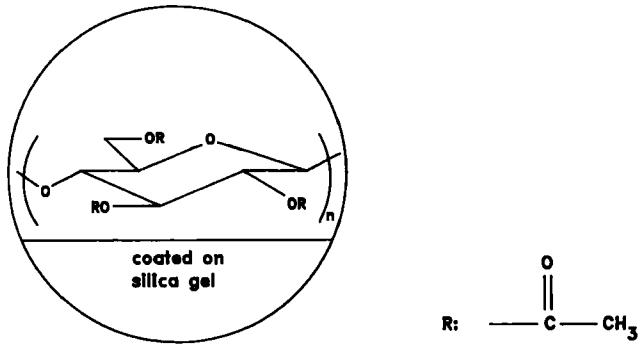
Hermansson(10) previously reported the resolution of racemic ketamine on α_1-glycoprotein column using phosphate buffer pH 7.0 with addition of 1.95 mM N,N-dimethyloctylamine as a mobile phase. However, the enantiomeric elution order were not determined due to the lack of the individual ketamine enantiomers.

The present study reports the direct separation and identification of ketamine enantiomers in human serum and pharmaceutical formulation (Ketalar®) using microcrystalline cellulose triacetate (Scheme 2) column.

EXPERIMENTAL

Apparatus

The Water (Waters Associates, Millford, MA, U.S.A.) LC system consisted of a Model 6000A pump, a U6K injector, and a Lambda-Max Model 481 LC spectrophotometer UV detector operated at 269 nm. A microcrystalline cellulose triacetate (CA-1) column (Daicel Chemical Industries, Ltd., Tokyo, Japan, 250 mm x 4.6 mm, I.D.) with a particle size 10 μm was used.



Scheme 2. The structure of the chiral stationary phase (CA-1-CSP) used in this study.

Chemicals

Ketamine (+)-S-isomer (PD 055309-0002, Lt T 0-010) and ketamine (-)-R-isomer (PD 074657-0002, Lot P 0-010) were supplied by Warner-Lambert Company, Pharmaceutical Research Division, Ann Arbor, MI, U.S.A. Ketalar® (50 mg/ml ketamine hydrochloride, (stock 35-582-1-119A) was obtained from Parke, Davis and Company, Pontypool, Gwent, U.K. HPLC-grade ethanol was obtained from BDH Chemicals Ltd., Poole, England.

Sample pretreatment

Ketamine hydrochloride (150 mgs) was added to 10 ml human serum. It was diluted up to 100 ml with water, basified with $NaHCO_3$, extracted with dichloromethane (3 x 25 ml), dried over anhydrous sodium sulfate, filtered and dried under vacuum. It was again acidified using ethanolic hydrogen chloride solution, dried with nitrogen flow, dissolved in methanol and injected onto the column. Ketalar® (50 mg/ml) was diluted to 416 times with water and injected without further treatment.

Chromatographic conditions

The maximum stereochemical resolution of ketamine was obtained using ethanol as a mobile phase on CA-1 (micro crystalline cellulose triacetate) column (250 x 4.6 mm,

I.D.). Flow rate was 1 ml/min, chart speed was 0.5 cm/min. Temperature was maintained at 25°C. Detection was obtained at UV 269 nm with sensitivity range 0.005 AUFS. Sample amount injected was 20nmole for racemate ketamine, and 10 nmole for (+)-S-ketamine and 10 nmole for (-)-R-ketamine.

Determination of Enantiomeric Elution Order

The enantiomeric elution order was determined by chromatographing the separate enantiomers under similar conditions. Thus the peak that eluted with lower capacity factor was identified as (+)-S-ketamine, and the peak that eluted with a higher capacity factor was identified as (-)-R-ketamine.

RESULTS AND DISCUSSION

Reported here is an HPLC method where ketamine enantiomers could be resolved in human serum and a pharmaceutical preparation using microcrystalline cellulose triacetate (CA-1) column and one isocratic solvent system, thus making it more rapid and convenient. Ethanol, methanol and various concentrations of water in ethanol were tried as a mobile phase to optimize the separation at room temperature. Chromatogram of enantiomer separation of ketamine on microcrystalline cellulose triacetate (CA-1) column using ethanol as a solvent is shown in Figure 1. Compared with the chromatogram of (+)-S-ketamine (Fig. 2a) and (-)-R-ketamine (Fig. 2b), the peak that eluted with a lower capacity factor was identified as (+)-S-ketamine and the peak that eluted with a higher capacity factor was identified as (-)-R-ketamine. The capacity factor (k) for the first eluted peak was 2.2, the stereochemical separation factor (α) was 2.32 and the resolution factor (R) was 3.38. Enantiomeric separation of methylene chloride extract of racemic ketamine in a serum sample is shown in Figure 3 and enantiomeric separation of racemic ketamine in Ketalar sample is shown in Figure 4. Preservatives present in formulation do not interfere with the described chromatographic conditions. All the peaks were easily identified from retention times comparing them with Figure 2.

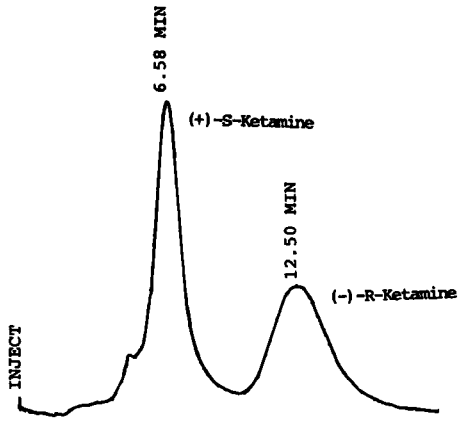


Figure 1. Enantiomeric separation of racemic ketamine. Column: Chiralcel CA-1 (250 x 4.6 mm, I.D.); mobile phase: ethanol; flow rate: 1 ml/min; chart speed: 0.5 cm/min.; temperature: 25°C; detector: UV 269 nm, sensitivity: 0.005 AUFS; Sample amount: 20 nmole.

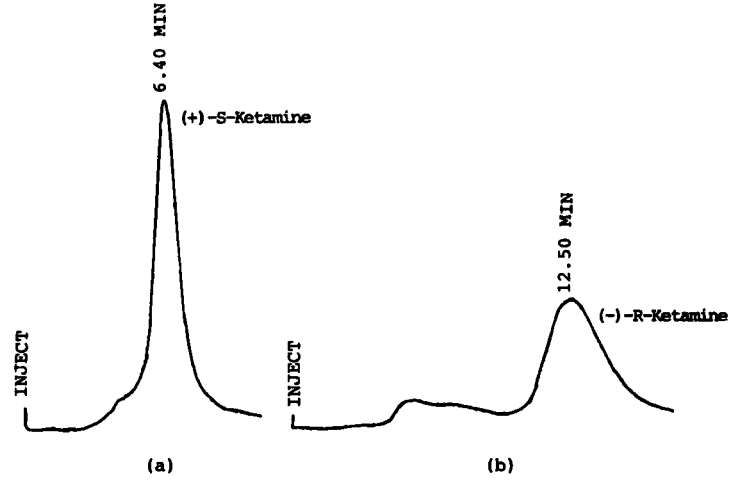


Figure 2. Chromatograms of (a) (+)-S-ketamine and (b) (-)-R-ketamine. Conditions were same as in Figure 1, except the sample amount injected was 10 nmole for each of the samples.

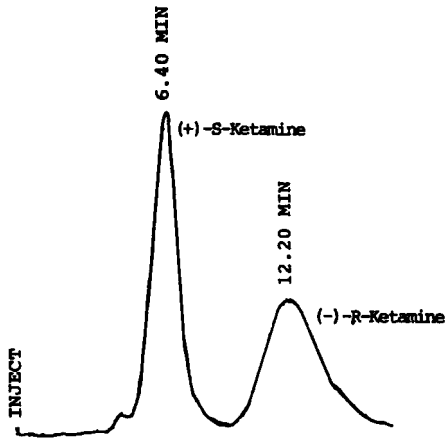


Figure 3. Chromatogram of racemic ketamine obtained from the methylene chloride extract of human serum sample. Conditions were same as in Figure 1.

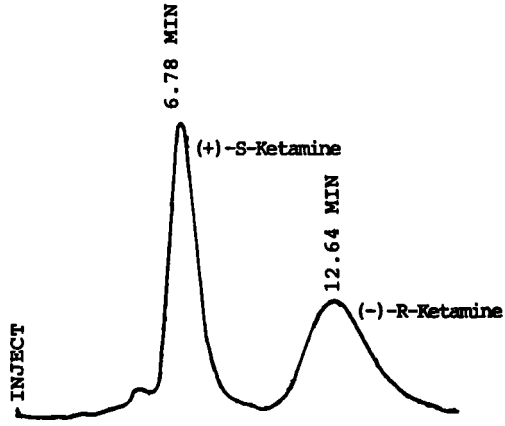


Figure 4. Chromatogram of racemic ketamine in Ketalar . Conditions were same as in Figure 1.

CONCLUSIONS

Direct stereochemical separation of racemic ketamine was achieved on commercially available micro crystalline cellulose triacetate (CA-1) column using single isocratic solvent system ethanol with flow rate 1 ml/min at 25°C. The method was applied to determine and identify the enantiomers of ketamine in the serum and pharmaceutical formulation Ketalar® (50 mg/ml ketamine hydrochloride). This method could be used for the optical purity determination of the drug in bulk and formulation dosage forms. Furthermore, this method has the advantage of being simple and fast as it requires about 20 minutes to perform so it can be adopted to quantitate ketamine enantiomers in serum for further pharmacokinetic studies.

ACKNOWLEDGMENTS

The authors thank the Administration of King Faisal Specialist Hospital and Research Centre for their continuous support to the Drug Development Research Program.

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Received: March 9, 1992
Accepted: June 30, 1992