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# DETERMINATION OF CLOZAPINE IN HUMAN SERUM BY CAPILLARY GAS CHROMATOGRAPHY

KLAUS RICHTER

*Institut für Klinische Pharmakologie der Medizinischen Akademie "Carl Gustav Carus", Fiedlerstrasse 27, DDR-8019 Dresden (G.D.R.)*

## SUMMARY

A gas chromatographic method using a fused-silica wide-bore capillary column and a nitrogen-specific detector for the determination of the antipsychotic agent clozapine in human serum is described. This method was found to be suitable for the determination of serum levels down to 1–2 ng/ml. The sensitivity, precision and accuracy of this method are adequate for studies on pharmacokinetics and bioavailability.

## INTRODUCTION

The very potent antipsychotic agent clozapine was introduced into clinical practice in 1972–1975 [1,2], but there are only a few publications concerning its determination in patients' blood [3–6] and no pharmacokinetic studies could be found in the literature. For this reason, a method for the determination of clozapine in human serum suitable for both pharmacokinetic investigations and drug monitoring was developed.

## EXPERIMENTAL

### *Chemicals*

Diethyl ether, methanol and sodium carbonate were of analytical-reagent grade. Clozapine was obtained from GERMED (Stammbetrieb AWD, Radebeul, G.D.R.) and maprotiline hydrochloride was available as a USP Reference Standard (U.S. Pharmacopeial Convention, Rockville, MD, U.S.A.).

### *Internal standard*

The acetyl derivative of the antidepressive agent maprotiline was found to be useful as an internal standard. It was prepared from 1 mg of the free base of

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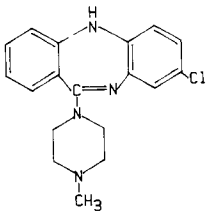

Fig. 1. Structure of clozapine.

maprotilin extracted twice with 3 ml of diethyl ether from a solution of its hydrochloride in 1 ml of 0.1 *M* sodium hydroxide. A volume of 0.2 ml of acetic anhydride was added to the combined ether phases. After reducing the volume to about 0.2 ml in a stream of air at 50°C (water-bath), the temperature was increased to 80°C and the solution was evaporated to dryness. The residue was dissolved in 2 ml of methanol, of which 0.1 ml was diluted to 5 ml to give a concentration of 10 ng/$\mu$l.

*Extraction*

Clozapine (Fig. 1) is a basic compound possessing a p$K_a$ value of 7.6. The partition coefficient in octanol–water is 0.4 at pH 2, 600 at pH 7, 1000 at pH 7.4 and 1500 at pH 8 [7]. As expected, diethyl ether was found to be suitable for the extraction of clozapine from serum or plasma.

A 1-ml volume of serum after addition of 200 ng of internal standard in 20 $\mu$l of methanol and 0.1 ml of saturated sodium carbonate solution was extracted with 5 ml of freshly redistilled diethyl ether. The 10-ml glass-stoppered tube was vortex-mixed for 10 s and the separated ether phase was transferred into another tube and evaporated to dryness in a stream of air at 50°C (water-bath). The residue was dissolved in 25–50 $\mu$l of methanol and 1 or 2 $\mu$l were injected for gas chromatographic (GC) determination.

*Gas chromatography*

GC was performed on a Varian (Zug, Switzerland) 3400 instrument equipped with a nitrogen-specific detector (Varian thermionic specific detector) operated with hydrogen at 4.5 ml/min, air at 175 ml/min and nitrogen as make-up gas at 24 ml/min. A 15 m×0.53 mm I.D. Megabore DB5 column with a 1.5-$\mu$m film thickness (J&W Scientific, Folsom, CA, U.S.A.) was used with helium as the carrier gas at a flow-rate of 6 ml/min. The operating temperatures were: injector 250°C, column oven 250°C and detector 300°C. The peak heights or areas were integrated with a Varian 4270 computing integrator.

## RESULTS

Typical chromatograms obtained with extracts from human serum are shown in Fig. 2. Analysis of blank human serum samples showed no substances that interfered with the internal standard at 3.7 min or clozapine at 4.6 min. Known amounts of clozapine were added to blank serum and carried through the assay.

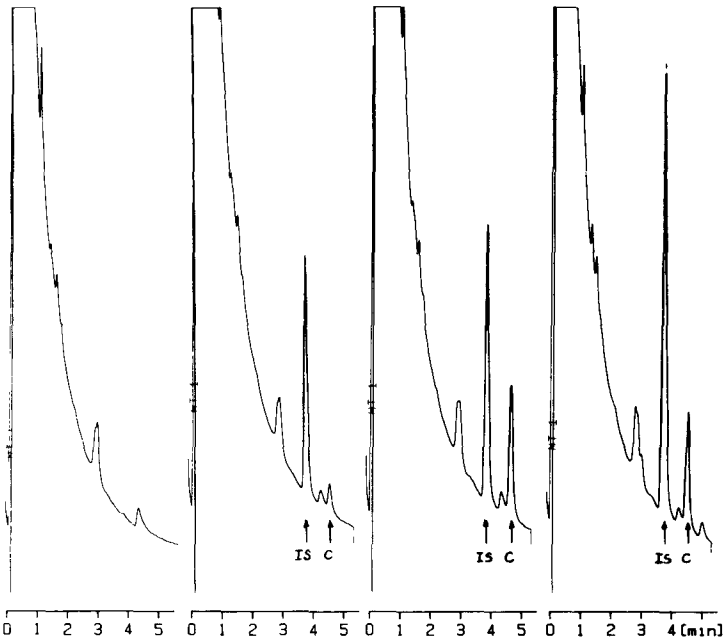


Fig. 2. Typical chromatograms of (from left to right) blank serum, standard samples containing 10 and 50 ng/ml clozapine, respectively, and serum from a patient who had received a dose of 3×25 mg daily (concentration 27 ng/ml). Peaks: C = clozapine; IS = internal standard.

TABLE I

PRECISION OF THE METHOD

| Concentration added (ng/ml) | Concentration found (mean ± S.D., $n=5$) (ng/ml) | Coefficient of variation (%) |
|---|---|---|
| 1.5 | 1.5 ± 0.27 | 18.2 |
| 2.5 | 2.7 ± 0.48 | 18.1 |
| 5.0 | 5.0 ± 0.39 | 7.9 |
| 10.0 | 9.98 ± 0.27 | 2.7 |
| 20.0 | 19.1 ± 1.38 | 7.2 |
| 50.0 | 50.6 ± 1.92 | 3.8 |
| 100.0 | 99.9 ± 1.04 | 1.0 |

Measuring the peak heights, calculating peak-height ratios and linear regression of calibration graphs were performed by the integrator. The equation of the calibration graph for a peak-height ratio $y$ and a concentration $x$ was found to be $y=0.001+0.0067x$ ($r=0.999$). The calibration graph was linear up to at least 300 ng/ml and clozapine levels down to 1–2 ng/ml could be determined. The precision of the method was assessed from the determination of seven concentrations in five independent series of samples, and the results are given in Table I. A wide-bore fused-silica column of I.D. 0.53 mm is of advantage because it permits the splitless injection of the sample directly into the column.

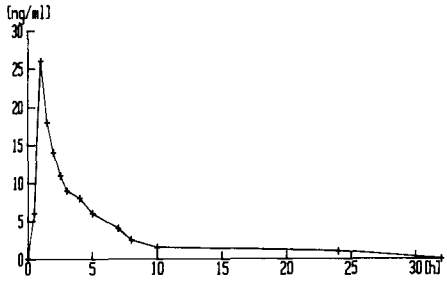


Fig. 3. Clozapine serum levels after a single dose of 25 mg.

Fig. 3 shows the clozapine serum level for a patient who had received a single dose of 25 mg.

The yield of the extraction step could not be determined exactly owing to the lack of pure free base, but is was estimated to be more than 80%. Problems arising from adsorption of clozapine by the tube walls were not observed.

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