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## Note

# Quantitative determination of alizapride in human plasma by high-performance liquid chromatography

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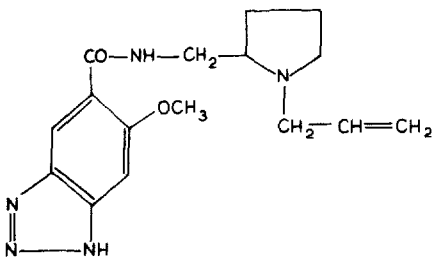
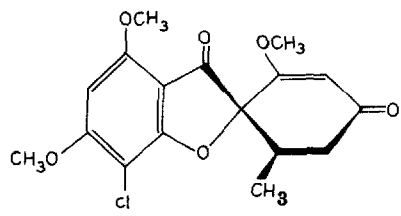
Alizapride (Plitican®), N-[(1-allyl-2-pyrrolidinyl)methyl]-6-methoxy-1H-benzatriazole-5-carboxamide (I), is a new potent compound with anti-emetic properties [1, 2]. It is currently used in cancer treatment to avoid the side-effects commonly encountered. Pharmacokinetic studies were previously performed using a high-performance liquid chromatographic (HPLC) [3] method, which suffers from lack of internal standard, leading to variability. We purpose a modification of the previously described method, which was applied to a pharmacokinetic study of a renal-insufficient patient.




(I)




(II)

Fig. 1. Chemical structures of alizapride (I) and griseofulvin (II).

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## EXPERIMENTAL

### *Reagents*

Alizapride was obtained from Delagrange Labs. and griseofulvin (II) from Clin Midy Labs. They showed no impurities in two different thin-layer chromatographic systems.

All reagents were of analytical grade. Tris(hydroxymethyl)aminomethane and triethylamine were obtained from Merck (Darmstadt, F.R.G.), and methanol and chloroform from Prolabo (Paris, France). All were used without further purification.

### *Equipment*

A liquid chromatograph (Waters Assoc., Paris, France: M45 pump and U6K injector) equipped with a fluorescence detector (Schoeffel FS 970) and a continuous flow cell of 8 $\mu$l capacity was used. According to the fluorescence spectrum of alizapride, excitation and emission wavelengths were 323 and 370 nm, respectively (7-54-370 filter). A 250-mm steel column (Hibar prepacked RT-250-4, Merck) was used at room temperature, packed with LiChrosorb RP-18 with an average particle size of 10 $\mu$m. The chromatographic solvent [methanol—0.05 *M* monopotassium phosphate—triethylamine (77:20:1) with 2 ml of 2.6 *M* hydrochloric acid (pH 7.6)] was delivered at a flow-rate of 1.5 ml/min.

### *Preparation of standard solutions*

Standard solutions of 1 mg/ml alizapride and griseofulvin were prepared in methanol for each series of analyses. The standard solutions of alizapride were then dissolved in drug-free plasma to give final concentrations of 25—2000 ng/ml. The internal standard solutions were diluted to a final concentration of 10 $\mu$g/ml.

### *Assay procedure*

Plasma (1 ml) and 50 $\mu$l of the solution of internal standard were pipetted into a 20-ml glass-stoppered centrifuged tube. After gentle shaking, 1 ml of 0.2 *M* Tris buffer (pH 8.1) was added and mixed carefully. Chloroform (5 ml) was added and the tube shaken for 1 min using a multitube vortexer (Corning, Paris, France). After centrifugation (20 min at 1500 *g*), the organic phase was transferred into a 5-ml glass tube and carefully evaporated to dryness under a stream of nitrogen. The residue was dissolved in the chromatographic solvent (100 $\mu$l) with vigorous shaking (vortex mixer). A 20-$\mu$l volume of this solution is then injected into the column.

## RESULTS AND DISCUSSION

A typical chromatogram of a plasma extract containing alizapride and the internal standard is shown in Fig. 2. The retention times are 3.0 and 3.5 min, respectively. Calibration graphs were fitted by the least-squares method for the peak-height ratio of the sample substance and the internal standard ($y$) versus the amount of substance added ($x$). The equation of this regression line is:

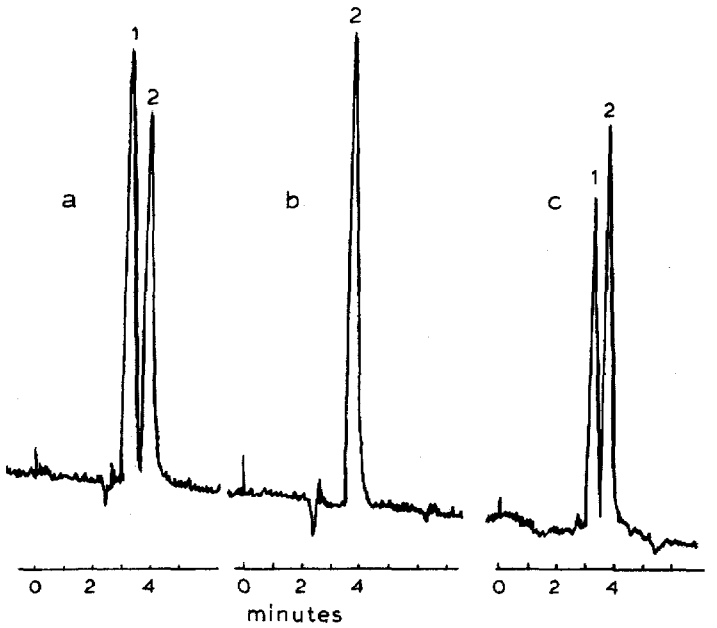


Fig. 2. Chromatograms of alizapride (1) and internal standard (2). (a) Blank plasma extract for standard calibration curve; (b) plasma extract for standard calibration curve containing 1000 ng/ml alizapride; (c) patient sample obtained 1 h after drug administration containing 620 ng/ml alizapride.

TABLE I

APPARENT RECOVERY OF ALIZAPRIDE

| Assay procedure | Peak-height ratio alizapride/internal standard (mean ± S.D.) | Coefficient of variation (%) |
|---|---|---|
| Without extraction | 0.755 ± 0.007 | 0.9 |
| After extraction | 0.541 ± 0.028 | 5.1 |

$y = 0.00124x + 0.048$, with a correlation coefficient of 0.9992. This indicates reasonable linearity between the detector response and amounts added to plasma in the range of tested concentrations, i.e. 25—2000 μg/l.

Recovery of internal standard, griseofulvin, is 94.1% with a coefficient of variation of 4.7%. Apparent recovery of alizapride is indicated in Table I. Comparison of the peak-height ratio after and without extraction for samples containing 500 ng/ml alizapride gives an apparent recovery of 71.7%.

The detector sensitivity for alizapride is 2 ng. Taking into account the apparent recovery, the overall sensitivity is 15 ng/ml alizapride when 20 μl of the solution of extracted sample are injected.

Replicate analyses of plasma samples, to which known amounts of alizapride were added, demonstrated that the method has acceptable accuracy and precision (Table II). The reproducibility of this method is better than that

TABLE II

PRECISION AND ACCURACY OF ANALYSIS OF ALIZAPRIDE

The mean ± S.D. in ng/ml for ten determinations is given, followed by the C.V.

| Concentration added (ng/ml) | Concentration found* (mean ± S.D., $n$ = 10) (ng/ml) | Coefficient of variation (%) | Accuracy (%) |
|---|---|---|---|
| 25 | 25.72 ± 4.67 | 18.2 | 102.9 |
| 50 | 49.64 ± 2.77 | 5.6 | 99.3 |
| 100 | 99.90 ± 6.24 | 6.2 | 99.9 |
| 250 | 256.80 ± 14.40 | 5.6 | 102.7 |
| 500 | 495.89 ± 25.48 | 5.1 | 99.2 |
| 1000 | 1004.58 ± 55.08 | 5.48 | 100.5 |
| 2000 | 2014.50 ± 62.79 | 3.12 | 100.7 |

*Concentration found using internal standard.

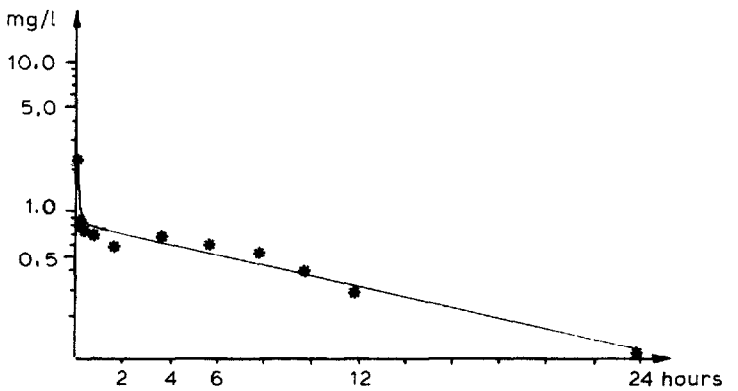


Fig. 3. Plasma profile of alizapride in a patient following a 100-mg intravenous administration.

previously developed [3], with the advantage of the presence of an internal standard which allows more flexibility and reliability.

The method was applied to the determination of alizapride in plasma and urine using fifteen patients suffering from renal insufficiency. None of them showed any contaminant in the zero-time plasma sample corresponding to the retention times of the tested drug (Fig. 2).

A plasma profile of alizapride over 24 h for one of the subjects after a single intravenous dose of 100 mg is shown on Fig. 3. It shows the typical consequence of such disease on the pharmacokinetics of a renal-extracted drug, i.e. an increase of the elimination half-life owing to the decrease of the renal clearance of the drug. With this drug, protein-binding alterations and the presence of an oedema lead to slight modifications of the apparent distribution volume of the drug, which contribute also to modifications of the terminal half-life. This shows that the described technique is sufficiently sensitive for the determination of plasma levels of alizapride, allowing pharmacokinetic or drug monitoring studies in patients after therapeutic doses.

ACKNOWLEDGEMENT

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