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# Determination of N-(3-nitro-4-quinoline)morpholino-4-carboxamidine in plasma by high-performance liquid chromatography

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## ABSTRACT

A high-performance liquid chromatographic (HPLC) method was developed for the determination of the radiosensitizing agent N-(3-nitro-4-quinoline)morpholino-4-carboxamidine (EGIS-4136) in plasma using an internal standard. HPLC separation was achieved on a LiChrosorb $C_{18}$ column using acetonitrile–sodium acetate buffer (pH 7.2) (40:60) as the mobile phase and UV detection at 330 nm. The plasma samples were prepared for measurement by protein precipitation with methanol and centrifugation. The assay was validated with respect to linearity, sensitivity, accuracy, precision, stability and recovery in plasma. The limit of detection for EGIS-4136 in plasma was 0.05 μg/ml. A straight line was obtained on plotting the peak-area ratio of EGIS-4136 to the internal standard against concentration in plasma in the range 0.1–20 μg/ml. The method was applied to study the pharmacokinetics of EGIS-4136 in six male rats after a single oral dose of 50 mg/kg, and allowed the compound to be monitored in the concentration range 5–10 μg/ml occurring in rats 24 h post-administration.

## INTRODUCTION

N-(3-Nitro-4-quinoline)morpholino-4-carboxamidine (EGIS-4136) (Fig. 1) is a nitroquinoline derivative [1] which enhances the radiosensitivity of hamster's ovarian cells in both normoxia and hypoxia. The reference compound misonidazole [2–4] at a concentration of 5 m*M* exerted a radiosensitizing effect as strong as 0.5 m*M* EGIS-4136. The radiosensitizing activity of the drug can be detected also on HeLa cells (uterus cell culture). The irradiation-evoked immunosuppression was not influenced by EGIS-4136.

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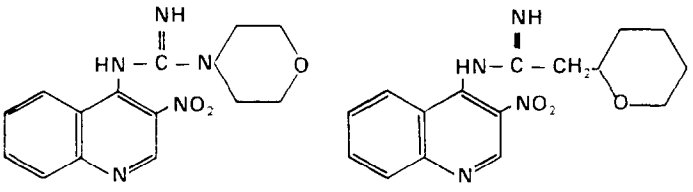


Fig. 1. Structures of (I) EGIS-4136 and (II) Z-3421 (internal standard).

## EXPERIMENTAL

### *Chemicals*

EGIS-4136 and the internal standard 4-tetrahydrofurfurylquanidino-3-nitroquinoline (Z-3421) (Fig. 1) were supplied by EGIS Pharmaceuticals (Budapest, Hungary). Acetonitrile and methanol were purchased from E. Merck (Darmstadt, F.R.G.) and anhydrous sodium acetate of analytical-reagent grade from Reanal (Budapest, Hungary). Water was purified and deionized using an ion-exchange filtration system (Simax, Prague, Czechoslovakia).

### *Chromatography*

A Model 1330 analytical liquid chromatograph (Bio-Rad Labs., Vienna, Austria) equipped with a 250 mm × 4.0 mm I.D. LiChrosorb $C_{18}$ (10 $\mu$m) column (Bioseparation GMK, Budapest, Hungary) was fitted with a Bio-Rad Labs. Model 1306 variable-wavelength UV detector and coupled to a Model 3390A computing integrator (Hewlett-Packard, Avondale, PA, U.S.A.). The mobile phase consisted of 0.08 *M* sodium acetate in acetonitrile–water (40:60) and was used after de-gassing and filtration. The flow-rate was 1.2 ml/min and the eluate was monitored at 330 nm. The retention times of EGIS-4136 and the internal standard Z-3421 were 8.2 and 9.8 min, respectively.

Samples could be automatically injected at 15-min intervals using an AS-48 autosampler (Bio-Rad Labs.) equipped with a 50-$\mu$l sample loop. The mobile phase was changed every 3 days (*ca.* 200 samples).

### *Standard solutions*

Stock solutions of 1 mg/ml EGIS-4136 and the internal standard were prepared in methanol. Further dilutions were made with distilled water to give concentrations ranging from 0.1 to 20 $\mu$g/ml for EGIS-4136 and 2 $\mu$g/ml for the internal standard.

### *Sample preparation*

Methanol (0.3 ml) was added to 0.2 ml of rat plasma and 10 $\mu$l of the internal standard solution. The mixture was shaken for 30 s with a vortex mixer and after equilibration for 10 min it was shaken again for 30 s. After centrifugation at 3000 *g* for 10 min, the supernatants were transferred into conical Eppendorf vials and 50-$\mu$l aliquots were injected.

*Pharmacokinetic study*

Male RG-Wistar rats were kept under standardized conditions in rat cages and fed with standard rat-food (LATI, Gödöllő, Hungary). Water was allowed *ad libitum*. After an overnight fast, six rats received EGIS-4136 in a single oral dose of 50 mg/kg. The drug was emulsified with 0.5% carboxymethyl-cellulose—Na in distilled water and the animals were treated with this emulsion through a gastric gavage. Blood samples were collected prior to and 0.25, 0.5, 1.0, 1.5, 2, 3, 4, 6, 10, 18, 22 and 24 h after oral administration. The blood samples (0.2 ml) were taken under diethyl ether narcosis from the periorbital plexus of the rats by using heparinized glass capillary test-tubes.

## RESULTS

*Chromatography*

The chromatogram of blank plasma is shown in Fig. 2A. All the endogenous compounds visible at 330 nm are eluted in the front peak and no further peaks appeared on the chromatogram. EGIS-4136 and the internal standard are well separated from the peaks of contaminants (Fig. 2B and C).

*Linearity*

A calibration graph for plasma was obtained by plotting the peak-area ratio against the concentration of EGIS-4136. The relationship was linear in the range 0.1–20 μg/ml. The correlation coefficient was typically higher than 0.999. The determination limit was 0.05 μg/ml (signal-to-noise ratio = 3:1).

*Reproducibility and recovery*

The within-day precision for the plasma was assessed by using five samples spiked with EGIS-4136 at two different concentrations. These samples were prepared

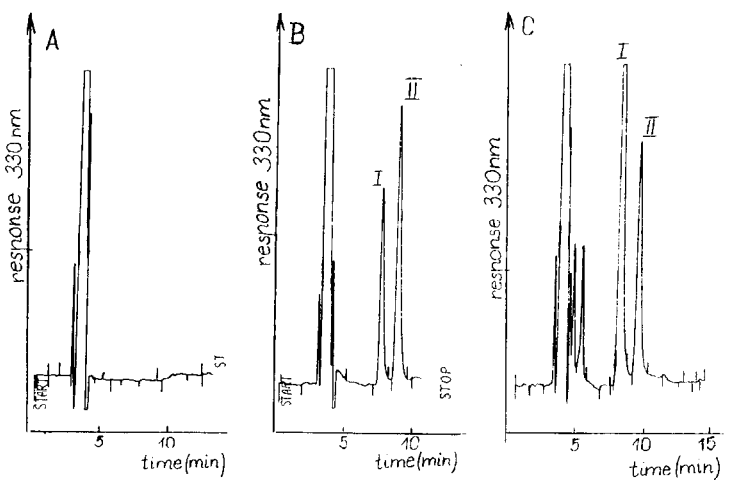


Fig. 2. Representative chromatograms of (A) blank plasma, (B) blank plasma spiked with 800 ng/ml of E-4136 and (C) rat plasma 1.5 h after receiving a single 50 mg/kg oral dose of EGIS-4136, Z-3421 being used as internal standard. Peaks: I = EGIS-4136; II = Z-3421.

TABLE I

WITHIN-DAY AND BETWEEN-DAY REPRODUCIBILITY OF THE ASSAY OF EGIS-4136 IN PLASMA

| Spiked concentration (ng per 200 μl) | Mean concentration found ± S.D. (ng per 200 μl) | Relative standard deviation (%) | Recovery (%) |
|---|---|---|---|
| *Within-day (n=5)* | | | |
| 50 | 46.58 ± 2.9 | 6.2 | 93.2 |
| 400 | 389.09 ± 13.7 | 3.5 | 97.3 |
| *Between-day (n=6)* | | | |
| 20 | 19.29 ± 2.6 | 13.4 | 96.45 |
| 50 | 47.60 ± 3.7 | 7.7 | 95.20 |
| 100 | 91.52 ± 5.7 | 6.2 | 91.52 |
| 200 | 191.37 ± 8.1 | 4.2 | 95.69 |
| 400 | 373.72 ± 13.5 | 3.6 | 94.43 |
| 1000 | 928.40 ± 59.0 | 6.5 | 92.84 |
| 2000 | 1868.40 ± 124.7 | 7.1 | 93.42 |

and analysed on the same day. The between-day precision was assessed by using six samples spiked with EGIS-4136 at seven different concentrations. These samples were analysed on six different days. The precision and accuracy were characterized by relative standard deviations (R.S.D.) generally less than 10%. However, at the lowest concentration examined (0.1 μg/ml) the R.S.D. value was 13.4% (Table I).

*Stability*

The stability of different plasma samples spiked with 0.25 and 0.50 μg/ml of EGIS-4136 was analysed weekly for 5 weeks. The samples were stored frozen at −18°C. The drug is stable in plasma during this storage period.

*Pharmacokinetic applications*

The procedure was applied to measure plasma concentrations following a single oral dose of 50 mg/kg to six rats. The pharmacokinetic curve showed an absorption phase followed by a distribution phase and an elimination phase with an apparent elimination half-life of several hours. After a single dose of 50 mg/kg p.o. to six rats, plasma levels reached a maximum of 3041.6 ± 1357.2 ng/ml of EGIS-4136 eliminated from plasma with a biological half-life of 5.2 h The value of the area under the curves (AUC) was found to be 9293.8 ng/ml · h.

## CONCLUSION

The method described appears to be suitable for determining EGIS-4136 in plasma samples from pharmacokinetic studies in the rat.

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