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Note

Determination of nefopam in equine plasma by gas chromatography—mass spectrometry with chemical ionization

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Nefopam (Acupan®) is a non-narcotic analgesic [1] that has been used for relieving pain associated with musculoskeletal disorders in man. The drug has also been abused as a doping substance in horses. To correlate the plasma concentration of nefopam with possible effects on performance in the horse [2], determination of the drug in equine plasma was required.

The determination of nefopam in human plasma has been performed earlier by gas chromatography with flame-ionization [3, 4] and nitrogen-selective [5] detection. Owing to the low plasma concentrations expected in equine plasma, a gas chromatographic—mass spectrometric (GC—MS) method was developed using deuterium-labelled nefopam as an internal standard.

EXPERIMENTAL

Apparatus

The analysis was performed on a Model 4000 gas chromatograph—mass spectrometer (Finnigan, Sunnyvale, CA, U.S.A.) equipped with an INCOS data system. The separations were performed in a 25 m × 0.32 mm I.D. fused-silica capillary column coated with OV-1701 (OY, Separation Research, Turku, Finland). Helium was used as the carrier gas at a flow-rate of 1.5 ml/min. The tem-

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perature of the column was 240°C and the samples were introduced by the falling-needle technique.

For electron-impact (EI) mass spectrometry the ion source temperature was 270°C and the electron energy was 70 eV. Positive-ion chemical ionization (CI) mass spectrometry was performed, using ammonia as the reagent gas at a source pressure of 0.4 Torr. The temperature of the source was 210°C and the electron energy was held at 150 eV.

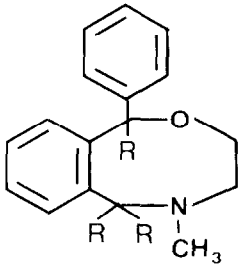
In the analysis of plasma samples, the instrument was adjusted to record the protonated positive molecular ion at m/z 254 and 257 for nefopam and the internal standard, respectively.

Chemicals

Nefopam (3,4,5,6-tetrahydro-5-methyl-1-phenyl-1*H*-2,5-benzoxazocine hydrochloride) was kindly supplied by Riker Labs. (Northridge, CA, U.S.A.).

[$^2H_3$]Nefopam (see Fig. 1) was prepared by a minor modification of the method described for the synthesis of nefopam [6, 7]. In the reduction of N-(2-hydroxyethyl)-N-methyl-O-benzoylbenzamide, lithium aluminium deuteride was used instead of lithium aluminium hydride. The prepared hydrochloride salt of [$^2H_3$]nefopam had a melting point of 237—241°C. The overall yield of the compound was 30% with an isotopic purity of 99% as determined by mass spectrometry.

Analytical-reagent grade solvents were used without further purification.



Nefopam R=H

[$^2H_3$] Nefopam R=^{2}H

Fig. 1. Structure of nefopam and the internal standard.

Determination of nefopam in plasma

To 1.0 ml of plasma, 100 µl of internal standard (200 ng/ml [$^2H_3$]nefopam) were added together with 0.5 ml of 0.5 *M* sodium hydroxide solution. The mixture was extracted with 4.0 ml of toluene—2-butanol (9:1) for 15 min and centrifuged at 700 *g* for 10 min. The organic phase was transferred into another tube containing 1.0 ml of 0.05 *M* sulfuric acid. The tube was rotated for 10 min and centrifuged at 700 *g* for 10 min. The organic phase was discarded and 1.0 ml of 0.5 *M* sodium hydroxide solution and 4.0 ml of dichloromethane were added. The mixture was shaken for 15 min and

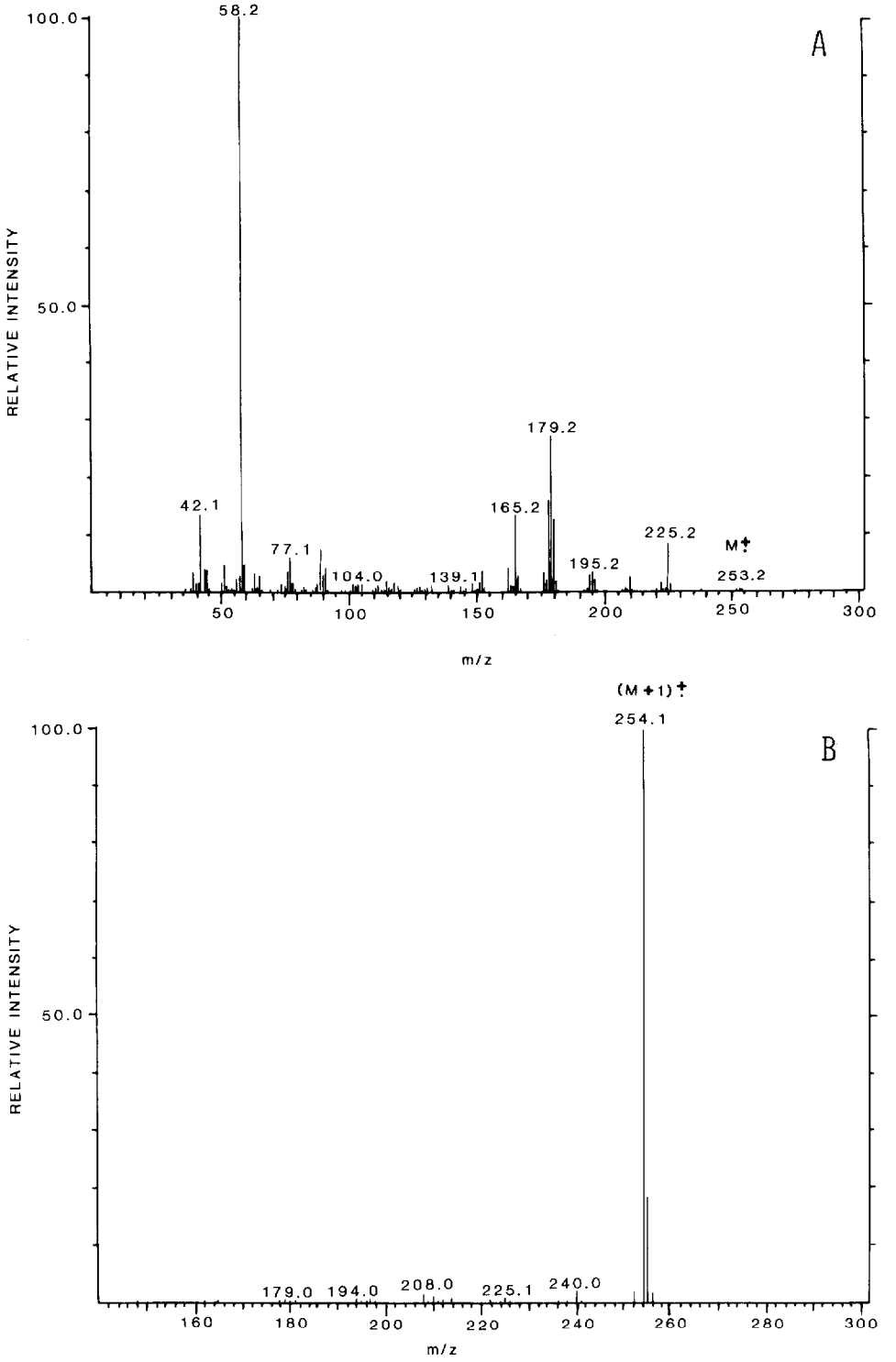


Fig. 2. Mass spectra of an authentic nefopam sample. (A) Electron impact at 70 eV; (B) chemical ionization using ammonia as reagent gas.

centrifuged as given above. The organic phase was transferred into another tube and the solvent was evaporated to dryness at 50° C in a stream of nitrogen. The residue was dissolved in 50 μl of chloroform before analysis by GC—MS.

A calibration graph (0—500 ng/ml) was prepared by adding known amounts of nefopam to drug-free plasma and the samples were analysed as described above. The peak-height ratio of drug to internal standard was plotted against the corresponding concentration. Peak-height ratios of unknown samples were similarly determined and concentrations calculated from the calibration graph.

RESULTS AND DISCUSSION

The procedure for the extraction of nefopam from plasma was a modification of the method described by Ehrsson and Eksborg [3]. The calculated recovery was over 99%.

The EI and ammonia CI mass spectra of nefopam are shown in Fig. 2. The EI mass spectrum is dominated by peaks at low m/z. These fragment ions are unsuitable for selected-ion monitoring (SIM) because they occur at m/z values where interfering ions are likely to occur. None of the higher mass ions in the EI mass spectrum were sufficiently abundant to be satisfactory for SIM in horse plasma.

The CI mass spectrum of nefopam obtained using ammonia as the reagent gas shows only one abundant ion, the protonated molecular ion (m/z 254). The corresponding mass spectrum of [$^2H_3$]nefopam is similar except that the abundant ion in the CI mass spectrum is shifted 3 daltons higher. A typical chromatogram is shown in Fig. 3. The chromatogram showed no interfering compounds, which made it possible to inject samples every second minute.

Nefopam added to drug-free plasma to a final concentration of 5 ng/ml plasma gave a relative standard deviation of 3.7% (n=10). The determination limit was 0.5 ng/ml nefopam in plasma.

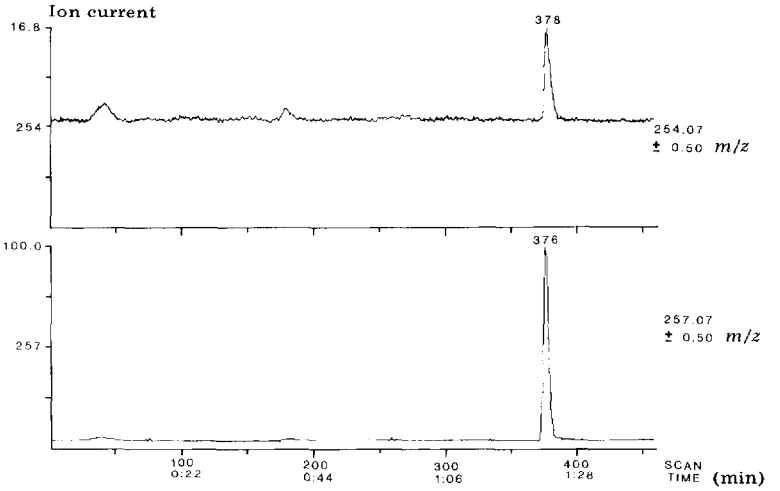


Fig. 3. Mass chromatogram focusing at m/z 254 and 257 of a plasma sample taken 5 h after oral administration of 2.0 mg/kg body weight of nefopam hydrochloride to a horse. The sample contained 1.5 ng/ml of nefopam.

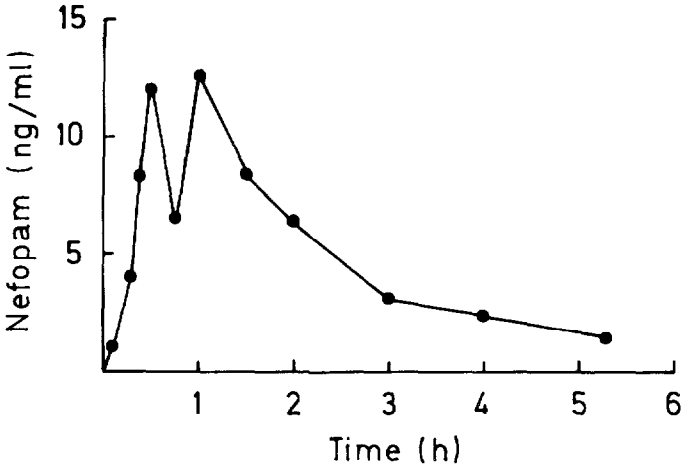


Fig. 4. Plasma time versus concentration curve of nefopam following oral administration of nefopam hydrochloride (2.0 mg/kg body weight) to a horse.

With the developed analytical method, plasma samples could be analysed up to 5 h after an oral administration of 2.0 mg/kg nefopam hydrochloride to a horse. The plasma concentration versus time curve is shown in Fig. 4.

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