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Note

Determination of tolazoline in plasma by gas chromatography—mass spectrometry

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Tolazoline [4,5-dihydro-2-(phenylmethyl)-1H-imidazole] has been used extensively since 1939 for its histaminergic, alpha-adrenergic antagonist and vasodilator activities [1]. Tolazoline is administered frequently to newborn infants as a pulmonary vasodilator [2]. Since repeated administration to neonates has been associated with undesirable side effects, careful monitoring of the dosage regimen may be necessary [3]. Few methods have been reported for the determination of tolazoline. Farkas [4], Boon and Sudds [5] and Mollica et al. [6] reported methods for the assay of tolazoline in pharmaceutical preparations, while Brodie et al. [7] described a colorimetric method for its determination in biological fluids and tissues. This colorimetric technique requires 1—5 ml of plasma, detects 5—25 μg of tolazoline per sample and has been used to study tolazoline pharmacokinetics in a limited number of adult humans [7] and animals [7,8]. None of the reported assays for tolazoline is suitable for small sample volumes and low concentrations. Consequently, pharmacokinetics of tolazoline have not been reported in young animals or infants.

We report a new procedure for the determination of tolazoline in plasma which is chemically specific for tolazoline and applicable to small sample

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volumes. Tetrahydrozoline [4,5-dihydro-2-(1,2,3,4-tetrahydro-1-naphthalenyl)-1H-imidazole] is added to the plasma sample as an internal standard. After solvent extraction, tolazoline and the internal standard are converted to trifluoroacetyl (TFA) derivatives and quantitated by gas chromatography—mass spectrometry with selected ion monitoring. The clinical applicability of the method is shown by the determination of tolazoline concentrations in a few neonatal patients.

EXPERIMENTAL

Standards and reagents

Tolazoline and tetrahydrozoline were kindly furnished by CIBA Pharmaceutical Co. and Pfizer Labs., respectively. Standard solutions were prepared in methanol and only freshly prepared solutions were used. Trifluoroacetic anhydride (TFAA) and triethylamine (TEA) were obtained from Sigma. All solvents were spectrophotometric grade. Water was glass distilled.

Instrument

A Hewlett-Packard 5992b gas chromatograph—mass spectrometer with a jet orifice separator was used. The 91.4 cm × 2 mm I.D. glass column was packed with SP-2100 (Supelco). The injector temperature was maintained at 240°C, while the column temperature was programmed to increase from 190°C to 230°C at 15°C/min beginning 1.0 min after injection. The helium carrier gas flow-rate was 20 ml/min. The electron energy was 70 eV with an electron multiplier voltage of 2.2—2.6 kV according to the parameters established each day by the Hewlett-Packard autotune program using perfluorotributylamine as a standard.

The Hewlett-Packard peakfinder program was used to obtain spectra of the derivatives of tolazoline and the internal standard by scanning in 0.1 a.m.u. increments at 330 a.m.u./sec with recording of the chromatogram of the total ion current and of the spectra of ions comprising the peaks in the chromatogram. A background spectrum can be selected manually or automatically from the minimum total ion current during the analysis and later subtracted by computer from the spectra being analyzed. Although the scanner is computer-focused to 0.1 a.m.u., small day-to-day variations in the instrument result in fluctuations of ± 0.1 a.m.u. in the ions recorded in the spectrum of a given compound. Due to this variation, the ions in the spectra of Fig. 1C and D are labeled to the nearest a.m.u. With the Hewlett-Packard single ion monitoring program, the ion currents of as many as six ions, focused to 0.1 a.m.u., are recorded individually and in summation (Fig. 2). Ratios of the peak areas of selected ions can be obtained.

Derivatization

Toluene (200 μl), TEA (5 μl) and TFAA (25 μl) were added to 2—8 μg of tolazoline and 10 μg of tetrahydrozoline in conical centrifuge tubes. The tubes were stoppered, shaken on a Vortex mixer and heated for 40 min at 60°C in a shaking water bath. The tubes were cooled immediately and 200 μl water

added to each. The tubes were shaken on a Vortex mixer, centrifuged and an aliquot of the toluene layer injected onto the column.

Quantitation of plasma samples

For extraction of tolazoline from plasma, toluene was selected over ethyl acetate due to variation in the ethyl acetate volume separating from the aqueous layer both after extraction and after derivatization. Water-saturated ethyl acetate was avoided because of potential inactivation of TFAA by hydrolysis during derivatization. Although the volume of 5 *N* sodium hydroxide solution was varied from 25 to 150 μl/ml plasma, extraction was maximum with 50 μl. Addition of salt sufficient to saturate the plasma layer improved the linearity of standard curves. Vigorous vortex mixing for 1 min frequently produced a gel-like emulsion of the plasma proteins. To maintain alkaline conditions, freezing was selected over perchloric acid precipitation to denature the proteins and improve separation of the plasma proteins from toluene during later centrifugation. If the gel-like layer remained after centrifugation, it was disrupted with a glass rod and the sample recentrifuged. Vortex mixing for 60 sec was found to be equivalent to extraction by mechanical shaking (Dubnoff shaker) for 5—10 min. No difference in extent of extraction was noted when mechanical shaking was varied from 5 to 20 min.

Thus, the following procedure was utilized for quantitation of tolazoline in plasma. To 1.0 ml plasma containing tolazoline were added 2 μg of internal standard, 1.0 g sodium chloride, 5.0 ml toluene and 50 μl 5 *N* sodium hydroxide solution. The samples were mixed for 60 sec on a Vortex mixer, frozen at 0°C for 10 min and centrifuged for 10 min at 5000 *g* to separate the layers. If necessary the organic layer was stirred with a glass rod and the sample was recentrifuged. The organic layer was transferred to another tube and evaporated to dryness at 50°C in vacuo. The sample was then derivatized as described earlier and an aliquot injected onto the column. Quantitation of plasma samples was achieved with selected ion monitoring (SIM) by comparison of the peak area *m/e* 352.1 for tolazoline to the peak area of *m/e* 392.1 for tetrahydrozoline. Secondary ions of *m/e* 283.1 and 295.2 for tolazoline and tetrahydrozoline, respectively, were also monitored to insure that the primary ions monitored were unique to these compounds. For calculation of recoveries of tolazoline and tetrahydrozoline, the peak areas at the appropriate *m/e* of the extracted plasma samples were compared to the peak areas of samples to which an equal amount of standard and internal standard were added prior to derivatization.

RESULTS AND DISCUSSION

To insure completeness of reaction, the conditions for derivatization were varied and optimum conditions were established as follows: 25 μl TFAA, 5 μl TEA, 200 μl toluene, and incubation at 60°C for 40 min.

Fig. 1A shows the structure of tolazoline and the mass spectra of the TFAA derivatives of tolazoline and the internal standard obtained with the Hewlett-Packard peakfinder program when *m/e* 84 to 505 were scanned (Fig. 1B, C and D). Although the scan was initially extended to *m/e* 800, only the di-TFA

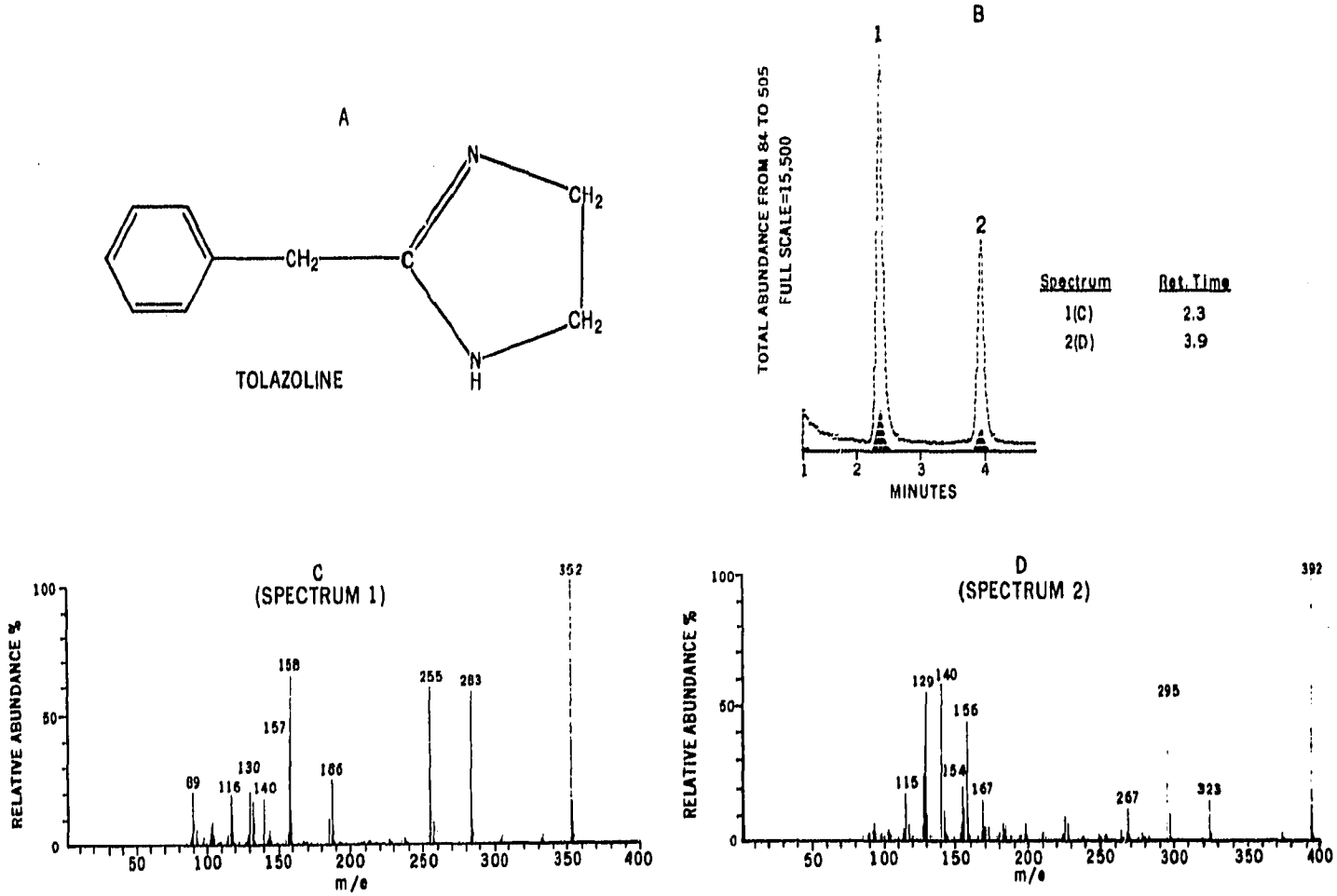

Fig. 1. (A) Tolazoline structure, mol.wt. 160.2; (B) chromatogram of the TFAA derivatives of tolazoline (1) and internal standard, tetrahydrozoline (2) after extraction from plasma; (C) electron impact mass spectrum of peak 1, tolazoline di-TFA; (D) electron impact mass spectrum of peak 2, tetrahydrozoline di-TFA.

derivative of both compounds was obtained. Representative selected ion chromatograms, monitoring the ions *m/e* 352.1 and 283.1 for tolazoline di-TFA and *m/e* 392.1 and 295.2 for tetrahydrozoline di-TFA, are shown for a standard (Fig. 2A) and a patient sample (Fig. 2B). An unidentified compound containing *m/e* 295.2 (a secondary ion of tetrahydrozoline di-TFA) with a retention time of 2 min was observed occasionally in patient samples, but did not interfere with quantitation.

The stability of the derivatives was tested by keeping the samples at 0°C for one month and reinjecting the toluene layer; no deterioration was observed after this period.

A recovery of 59.2 ± 5.5% (mean ± S.D., $n = 26$) was obtained when tolazoline was added to plasma in the range of 2—8 μg/ml. The recovery of the internal standard (concentration = 10 μg/ml) was 67.9 ± 9.6% (mean ± S.D., $n = 33$).

Ten plasma standard curves with 1-ml samples (peak area 352.1/peak area 392.1 × 100% vs. [tolazoline]) including 54 points over the concentration

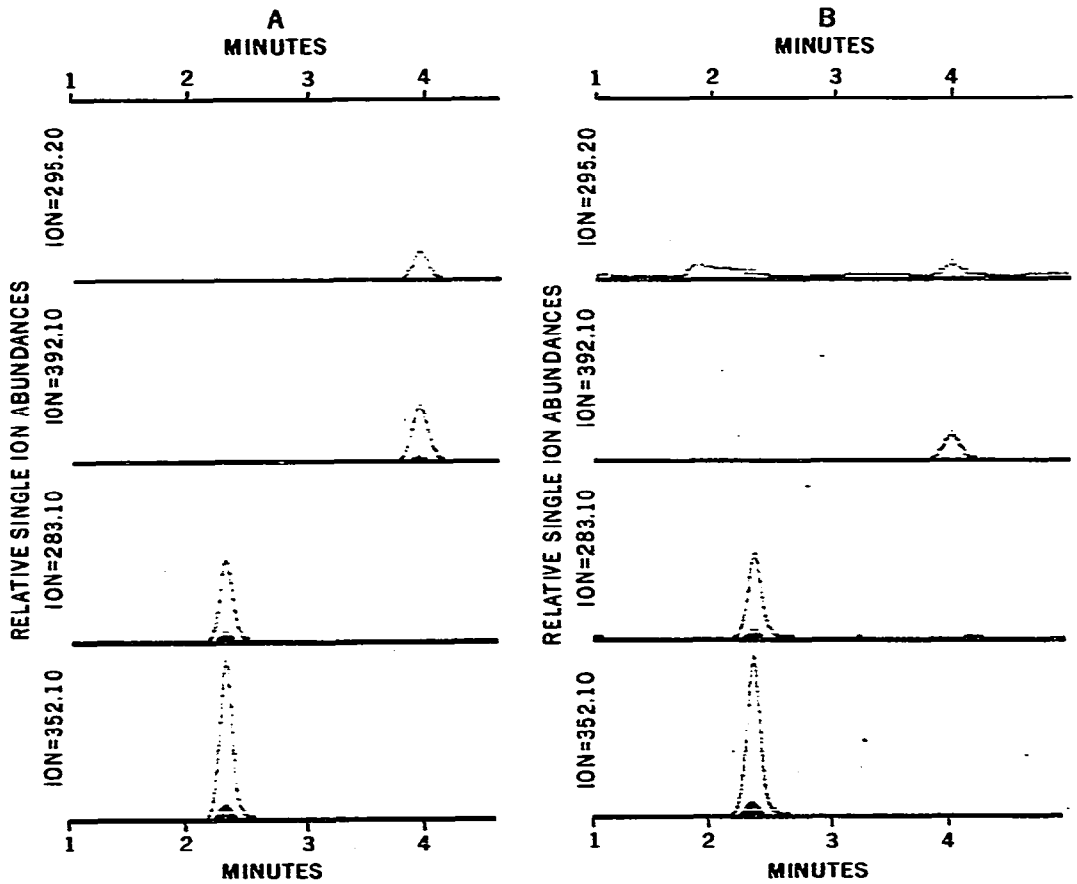

Fig. 2. GC—MS SIM chromatograms of TFAA derivatives of tolazoline, ions *m/e* 352.1 and 283.1 and tetrahydrozoline, ions *m/e* 392.1 and 295.2. (A) Blank plasma and (B) patient plasma sample.

range of 0.01—50.00 μg/ml were linear (correlation coefficient of 0.996 ± 0.006, mean ± S.D.) with a slope of 0.0121 ± 0.0017 and passed close to the origin. When 100-μl plasma samples were analyzed, 400 μl blank ovine plasma were added to facilitate extraction. Using the micro-method with 100-μl plasma samples and 0.5 μg internal standard, five standard curves from 1.0—30.0 μg/ml (40 points) had a slope of 0.0876 ± 0.0016 (mean ± S.D.) and linear correlation coefficients of 0.996 ± 0.003. Precision of this method was examined by five analyses of 100-μl aliquots of a lamb plasma sample and produced a coefficient of variation of 5.6%. Accuracy of analysis was examined by repeated injections (n = 8 per sample) of low (0.5 μg/ml) and high (10.0 μg/ml) concentration samples of tolazoline and produced coefficients of variation of 4.9% and 2.3%, respectively.

Using 200-μl samples, six plasma tolazoline concentrations were determined in four infants receiving the drug at different dosages and were found to range from 2.7—9.8 μg/ml.

The following drugs which are often used by clinicians concomitantly with tolazoline were found not to interfere with the assay: ampicillin, gentamicin, pancuronium bromide, heparin, dopamine, morphine and furosemide.

The present extraction and derivatization procedure has been performed with 1-ml volumes, but when sample size is limited the entire procedure can be successfully performed with plasma volumes as low as 100 μl. This makes the method particularly attractive for application in the neonatal field.

This method is currently being used to study the pharmacokinetics and renal excretion of tolazoline in lambs and newborn infants.

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