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Note

Analysis of riboxamide in urine by high-performance liquid chromatography

N.M. MELTZER and L.A. STERNSON*

Department of Pharmaceutical Chemistry, The University of Kansas, Lawrence, KS 66045 (U.S.A.)

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We have recently described an analytical method for the determination of the experimental antineoplastic agent riboxamide (TCAR, see Fig. 1) in plasma [1]. The procedure, based on high-performance liquid chromatography (HPLC), utilizes a column-switching configuration consisting of two different solvent generated anion-exchange columns which exhibit different retention characteristics toward the analyte. Initial pharmacokinetic evaluation of the drug has revealed it to be primarily excreted in urine [2]. Unfortunately, the procedure reported through our earlier communication [1] is not directly applicable to urine analysis of riboxamide. In this note, a modification of that method is described which permits routine urinary monitoring of the drug in clinical settings.

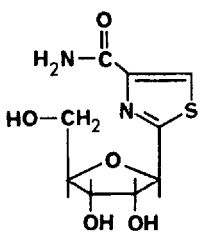

Fig. 1. Chemical structure of riboxamide (TCAR).

MATERIALS AND METHODS

Riboxamide (TCAR) was obtained from the National Cancer Institute. μBondapak C_{18} columns (10-μm particles, 300 × 4.6 mm) were purchased from Waters Assoc. (Milford, MA, U.S.A.) and ODS Hypersil bulk packing (5-μm particles) obtained from HETP (Macclesfield, U.K.) was slurry packed [3]

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into 150 × 4.6 mm I.D. columns. The chromatographic apparatus was configured as described by Riley et al. [1] except that a μBondapak C_{18} column was used as column 1 and an ODS Hypersil column was used as column 2. Urine samples were filtered through 3-μm Millipore filters and the filtrate applied directly to the chromatographic system. Column 1 was eluted with 0.1 *M* phosphate buffer (pH 2.1) and column 2 was eluted with 0.1 *M* phosphate buffer (pH 7.0). Following elution of TCAR from column 1, the eluent strength (for column 1) was increased to methanol—phosphate buffer (pH 2.1) (20:80) and maintained there for 10 min to purge the column of strongly retained urinary contaminants. The column was then allowed to re-equilibrate for 10 min with the purely aqueous mobile phase prior to the application of the next sample. Eluent was monitored spectrophotometrically at 254 nm.

RESULTS AND DISCUSSION

Urine samples were prepared for HPLC analysis by simply subjecting them to a single filtration of the biological fluid through a 3-μm Millipore filter and direct injection of the filtrate onto the HPLC system. This procedure was also applicable to plasma and represents a significant simplification of the clean-up step reported previously [1] for plasma analysis of TCAR. This modification saves ca. 20 min per sample in total analysis time. The recovery of TCAR from urine was 98 ± 0.6% and 97 ± 0.8% when spiked at the 10 and 1 μg/ml levels, respectively. This indicates that TCAR is not retained to a significant extent by the filter apparatus. Similar recoveries were noted with plasma samples.

Whereas plasma analysis involved separation on a dynamic anion-exchange system [1], an ion-exchange system was not required to chromatographically resolve TCAR from urinary components. Resolution was achieved using a column-switching configuration involving two columns packed with different reversed-phase (C_{18}) materials which in turn provided different selectivities toward the analyte. This selectivity difference was amplified by eluting the components with buffers differing only in pH. Optimal resolution was achieved using the basic dual-column hardware employed earlier [1] but achieving initial separation on a μBondapak C_{18} column eluted with 0.1 *M* phosphate buffer (pH 2.1) followed by transfer of the eluent slice containing TCAR ($k' = 4.11$) onto an ODS Hypersil column and elution of the analyte with 0.1 *M* phosphate buffer (pH 7.0). The overall retention time for TCAR in this system was 21.5 min (Fig. 2).

The peak height of TCAR was linearly related to the amount of solute injected, q, over the range 1—200 μg/ml according to the equation $P = 1.01q - 0.54$ ($r = 0.999$). Peak heights (P in mm) were corrected for changes in detector attenuation, using 0.02 a.u.f.s. as the reference. The response factor was independent of detector attenuation (0.005 to 0.160) at constant injection volumes [although a slight decrease in response factor was observed with large ($\geq$ 200 μl) injection volumes].

The day-to-day reproducibility (expressed as coefficient of variation, $n \geq 5$) of the response factor was 7%. To maximize precision, the response factor was recalculated after analysis of every fourth urine sample using an external standard. Within a single day, the coefficient of variation of the peak heights of

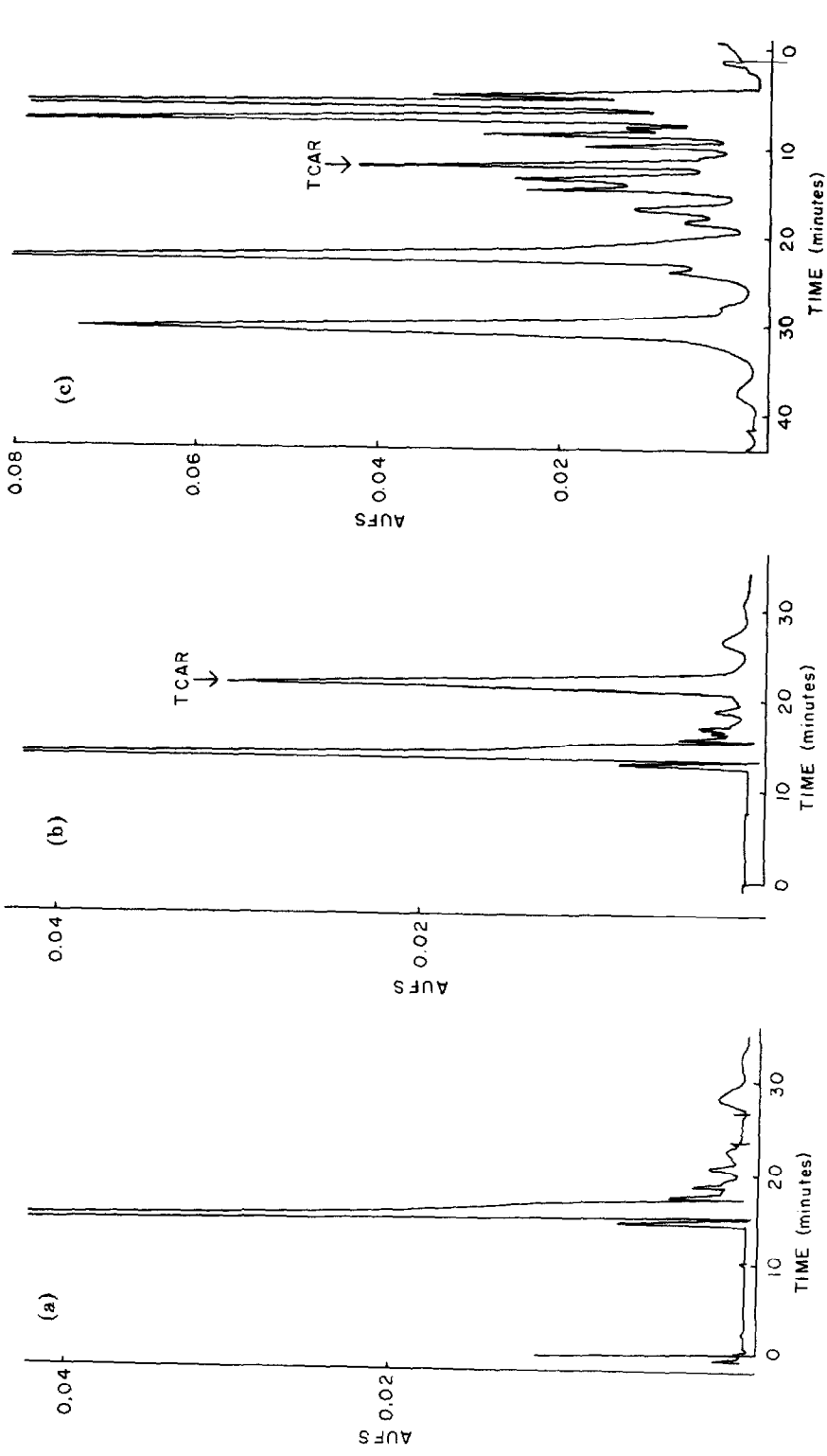

Fig. 2. Chromatograms obtained during analysis of patient urine for TCAR. (a) Urine blank; and (b) urine sample containing TCAR (500 ng/ml) carried through the column-switching procedure described in this report; (c) TCAR in urine obtained on a single μBondapak C_{18} column eluted with 0.1 *M* phosphate buffer (pH 2.1).

the external standards was $\leqslant$ 3% (n = 8) for 20-μl injections. The limit of detection for TCAR in urine was 200 ng/ml which gave a signal-to-noise ratio of 3:1 with an injection volume of 20 μl.

APPLICATION

The urinary excretion of TCAR was monitored in a patient receiving a daily intravenous dose of 550 mg/m^2 for five consecutive days. Urine collections (24 h) were analyzed for drug (Table I). Over a 24-h period, approximately 30% of the administered dose is excreted in the urine as unchanged drug, which can be expressed as an average excretion rate of 13.65 ± 2.17 μg/ml/h. Thus, a facile, rapid assay for riboxamide in urine is presented and, through a modification of the initial clean-up step, the plasma assay [1] is greatly simplified.

TABLE I

DAILY URINARY EXCRETION OF RIBOXAMIDE IN A MALE PATIENT RECEIVING DRUG ON FIVE CONSECUTIVE DAYS

TCAR administered as a single daily intravenous dose of 550 mg/m^2 on days 1—5.

Day	Total urinary excretion* (μg TCAR per ml)	Average hourly excretion rate (μg/ml h)
1	286.21	11.92
2	285.86	13.14
3	379.31	14.54
4	393.10	17.75
5	272.41	11.35
6	332.76	14.47
7	310.34	12.41

*Values represent the average of three determinations of each 24-h pooled urine specimen.

ACKNOWLEDGEMENT

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