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Short Communication

Determination of temazepam in plasma and urine by high-performance liquid chromatography using disposable solid-phase extraction columns

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Introduction

Temazepam (Fig. 1) is a 1,4-benzodiazepine used clinically as an hypnotic agent [1]. Its metabolism and pharmacokinetics have been described previously [2–3]. Assay methods for temazepam have been described using polarography [4–5], gas chromatography [6–8] and high-performance liquid chromatography (HPLC) [9]. All of the methods described utilize organic extraction for sample preparation prior to analysis. This report describes an HPLC method using inexpensive disposable solid-phase extraction columns which represents a significant improvement over previous techniques in speed of sample preparation and analysis time.

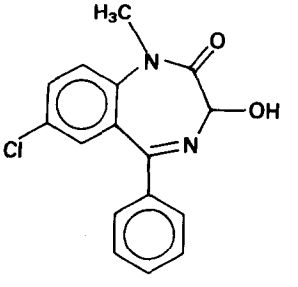

Figure 1
Structure of temazepam.

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Experimental

Chemicals and equipment

Temazepam was supplied by the Analytical Research Department of Abbott Laboratories (Abbott Park, IL, USA). Diazepam, the internal standard, was obtained from the chemical stores of Abbott Laboratories. Baker C_{18} disposable 1 ml extraction columns were obtained from American Scientific Products (McGaw Park, IL, USA). All solvents were HPLC grade from J. T. Baker Chemical Co. (Phillipsburg, NJ, USA).

Stock solutions

Stock solutions of 1000 μg temazepam ml^{-1} were prepared in absolute methanol and 100, 10 and 1 μg temazepam ml^{-1} in methanol/water by serial 1:10 dilutions of the 1000 μg ml^{-1} stock solution with distilled water. A stock solution of diazepam, the internal standard, was prepared at a concentration of 100 μg ml^{-1} in absolute methanol. HPLC eluents were filtered through a Nuclepore (Pleasanton, CA, USA) 400 nm polycarbonate membrane after preparation, and degassed by vacuum sonication just before use.

Chromatographic systems and conditions

HPLC separations were performed using a Waters Assoc. (Milford, MA, USA) Model M-6000 or M-6000A or Beckman (Fullerton, CA, USA) Model 110A reciprocating pump at 1.5 ml min^{-1} for solvent delivery. A Waters C_{18} μ-Bondapak (300 × 3.9 mm i.d.) reverse-phase HPLC column was used in this study. The eluent was composed of 62% (v/v) methanol and 6% (v/v) tetrahydrofuran in redistilled water. A Waters WISP 710B or Perkin–Elmer (Norwalk, CT, USA) Model ISS-100 automatic sampler was used for sample processing. The HPLC system was operated at ambient temperature, and the effluent was monitored for UV absorption with a Laboratory Data Control (Riviera Beach, FL, USA) SpectroMonitor III variable wavelength liquid chromatography detector. Peak areas were determined using a Spectra-Physics (Santa Clara, CA, USA) Model SP4100 integrator.

Sample preparation procedure

The disposable extraction columns were conditioned with two 1 ml washes of absolute methanol, then two 1 ml washes of distilled water. A suitable volume of plasma or urine up to 1 ml was passed through the columns. The columns were washed with 1 ml of 50% (v/v) methanol in water to elute background material from the samples. The eluates were discarded. Temazepam was then eluted from the columns with 400 μl of absolute methanol. The eluates were spiked with 50 μl of 100 μg diazepam ml^{-1} (20 μl for urine samples) in absolute methanol and evaporated to dryness under a gentle stream of air in a water bath at 30–50°C. The residues were redissolved in 200 μl of HPLC eluent, mixed well, and 10–100 μl of each sample was injected into the chromatograph.

Calibration curves for plasma and urine

Sets of standard plasma and urine samples were prepared by the addition of known amounts of temazepam to blank plasma or urine. The chromatographic peak area ratios of temazepam/diazepam were subjected to linear regression versus the corresponding temazepam concentrations. The resulting equation was used to calculate the concentration of temazepam in the test samples. The temazepam concentrations, sample size

and the diazepam concentrations added to the samples may be varied to suit the concentration ranges of the intended analyses.

Recovery

Recovery of temazepam from the extraction procedures was determined by comparing the peak area ratios of test samples to blank samples which were spiked with temazepam at the same concentration following extraction.

Results and Discussion

Figure 2 shows typical chromatograms for extracted samples of blank human plasma (a), blank human plasma spiked with 0.2 μg temazepam ml^{-1} and 10 μg ml^{-1} of diazepam, the internal standard (b), blank human urine (c), and blank human urine spiked with 1 μg temazepam ml^{-1} and 10 μg diazepam ml^{-1} (d).

To determine the precision and accuracy of the assay methods, replicate samples (3–4) were analysed at seven concentrations of the plasma assay and six concentrations of the urine assay. The results of these analyses are summarized in Table 1.

A comparison of weighting factors including 1 (unweighted), 1/concentration, 1/concentration^2, 1/response, and 1/response^2 showed that the 1/response^2 ($1/R^2$) weighting was most similar to 1/variance weighting for the plasma standard curve, while the 1/response ($1/R$) weighting was most similar to 1/variance weighting for the urine standard curve (Table 1). Using $1/R^2$ weighting (Table 1), the mean predicted plasma concentrations ranged from 94 to 109% of the calculated concentrations. The relative

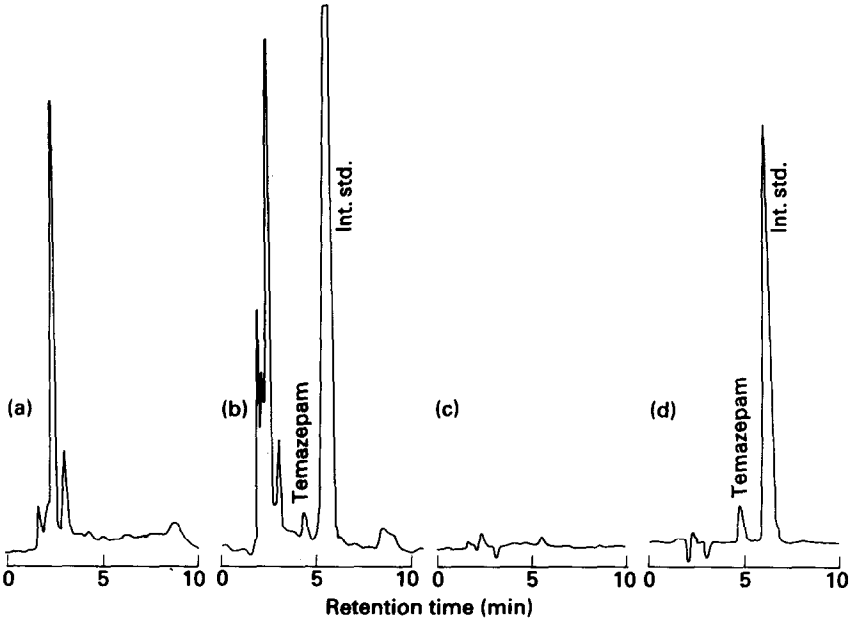

Figure 2
Chromatograms from human plasma (0.5 ml) and human urine (0.2 ml) extracts: (a) blank plasma; (b) blank plasma spiked with 0.2 μg temazepam ml^{-1} and 10 μg diazepam ml^{-1}; (c) blank urine; (d) blank urine spiked with 1 μg temazepam ml^{-1} and 10 μg diazepam ml^{-1}. Detector sensitivity was 0.1 a.u.f.s. (a, b) or 0.5 a.u.f.s. (c, d). Injection volume was 100 μl.

Table 1
Precision and accuracy data. Plasma and urine standard curves

Calculated concentration of temazepam ($\mu g\ ml^{-1}$)	Observed mean peak area ratio	Predicted mean concentration of temazepam [% of theory] ($\mu g\ ml^{-1}$)	Relative standard deviation (%)
Plasma			
0.05	0.047	0.052 [104%]	6.9
0.10	0.078	0.094 [94%]	4.5
0.50	0.357	0.471 [94%]	1.6
1.00	0.787	1.051 [105%]	1.5
5.00	3.500	4.713 [94%]	6.9
10.00	7.633	10.290 [103%]	0.8
30.00	24.250	32.716 [109%]	4.2
			Mean = 3.8
Urine			
5.00	0.327	4.687 [94%]	1.8
10.00	0.670	9.840 [98%]	2.6
30.00	2.320	34.628 [115%]	9.0
50.00	3.240	48.449 [97%]	4.8
70.00	4.640	69.481 [99%]	4.0
100.00	6.580	98.625 [99%]	3.0
			Mean = 4.2

standard deviation (RSD) of the peak height ratios ranged from 0.8 to 6.9% (mean = 3.8%). The mean predicted urine concentrations ranged from 94 to 115% ($1/R$ weighting). The urine assay had an RSD range of 1.8–9.0% (mean = 4.2%). The data from Table 1 were subjected to linear regression analysis, and standard curves constructed which were linear from 0.05 to 30 μg temazepam ml^{-1} in plasma ($r = 0.998$) or from 5 to 100 μg temazepam ml^{-1} in urine ($r = 0.998$). The lower limit of detection was about 20 ng temazepam ml^{-1} using a 0.5 ml plasma sample and about 50 ng temazepam ml^{-1} using a 0.2 ml urine sample.

Mean recoveries of temazepam from the extraction procedure were as follows: plasma, 77, 85 and 82% at 0.1, 1 and 10 $\mu g\ ml^{-1}$, respectively; urine, 86 and 97% at 10 and 100 $\mu g\ ml^{-1}$, respectively. The linearity of this assay over such a broad range of concentrations may extend its clinical utility to toxicological levels for cases of overdose or to animal toxicology studies.

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