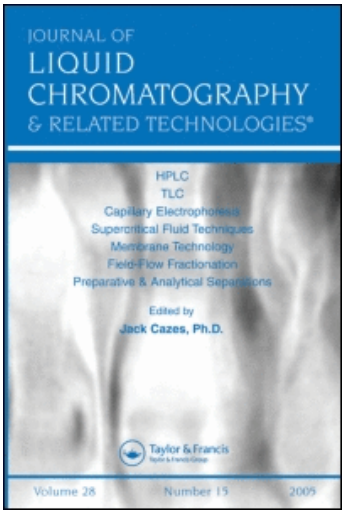

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Analysis of Dezocine in Serum and Urine by High Performance Liquid Chromatography and Pre-Column Derivatizationxs

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ANALYSIS OF DEZOCINE IN SERUM AND URINE BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY AND PRE-COLUMN DERIVATIZATION

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ABSTRACT

We present a reverse phase liquid chromatographic assay involving pre-column o-phthalaldehyde derivatization and fluorescence detection with the capability of measuring dezocine in human serum and urine for pharmacokinetic or forensic purposes. It features a simple liquid-liquid extraction scheme using hexane, conservative detection limits of 1 ng/ml, linearity to 1000 ng/ml, precision of 10% (CV%) in the low ng/ml region, approximate chromatography time of 25 minutes and the use of a new internal standard, WY-19083.

INTRODUCTION

Dezocine is a recently developed, parenterally administered, opioid analgesic for use in the management of postoperative pain. The pharmacology of the drug has been recently reviewed[1,2]. It has a clearly distinct receptor-binding profile compared to other narcotic agents, binding preferentially to mu_1 receptors over mu_2 receptors and weakly to kappa and delta receptors[3]. While dezocine is as potent as morphine when administered for clinical effect it is a significantly less potent respiratory depressant.[4,5]

Pharmacokinetically, dezocine is characterized by rapid distribution, rapid elimination and very low circulating blood levels[6,7]. There is an indication that dezocine may exhibit capacity limited elimination[6] and dosages, particularly in repeated administrations, should be monitored carefully.

Analyses for dezocine have been reported using gas chomatography with electron capture detection[7] and by liquid chromatography using electrochemical detection[8]. Both of these systems are highly sensitive owing to the need for measurement of dezocine levels down to concentrations of 1 ng/ml for pharmacokinetic studies. Our laboratory did not possess the cited equipment and to validate the blood concentration data in the literature it was necessary to develop and evaluate an additional method with comparable sensitivity. The analytical potential offered by the production and separation of fluorescent derivatives has been well reviewed[9] and the fact that dezocine possesses at least two functionalities which could be immediately exploited for that purpose suggested to us that a means existed offering the possibility of an assay with high sensitivity and precision. o-Phthalaldehyde has proved to be a particularly useful derivatizing agent due to the prevalence of biochemically important amines and the fact that, while its derivatization products are intensely fluorescent, the reagents are not fluorescent at all[10,11].

MATERIALS

Instrumentation.

Our chromatographic system utilized a model 6000A solvent delivery system, a model U6K injector with a 2 ml sample loop (Waters Assoc., Millipore Corp., Milford, MA) and a model FS 970 Kratos Fluorescence detector (Applied Biosystems Inc., Ramsey , NJ). The chromatographic column was a micro-Bondapak C18, 300 mm in length by 4.6 mm id., and packed with 10 micron particles (Waters Assoc., Millipore Corp.,Milford, MA). Two columns were placed in series to provide protection from contamination for the analytical column and additional retention to separate the internal standard peak from poorly retained products of derivatization. The mobile phase was 76% methanol in pH 4.0 sodium acetate buffer to which was added 10 ml of concentrated acetic acid for each 1000 ml of mobile phase. The typical flow rate for this system was 1.5 ml/min. The detector excitation wavelength was 260 nm and the emission filter was 489 nm as provided by the manufacturer. Data was output to a 10 mV Houston Instrument Co., model 500 strip chart recorder(Houston Inst. Co, Austin, TX).

Reagents.

Dezocine and the internal standard(WY-19083), depicted in Figure 1. were provided by the Astra USA (Westborough, MA) and by Wyeth-Ayerst Research (Princeton, NJ), respectively, and were stored in methanol solution at a concentration of 100 mcg/ml at -4 deg. C. Working standards were prepared in 60 gm/l bovine serum albumin Factor V(Sigma Chemical Co, St. Louis, MO) at concentrations of 1, 2, 5, 10, 20, 50, 100, 500 and 1000 ng/ml and stored at -4 deg C until use. Standards and patient serum and urine samples were extracted with 2% isoamyl alcohol in hexane. The o-phthalaldehyde/mercaptoethanol derivatizing reagent was purchased from the Pierce Chemical Co (Rockford, IL).

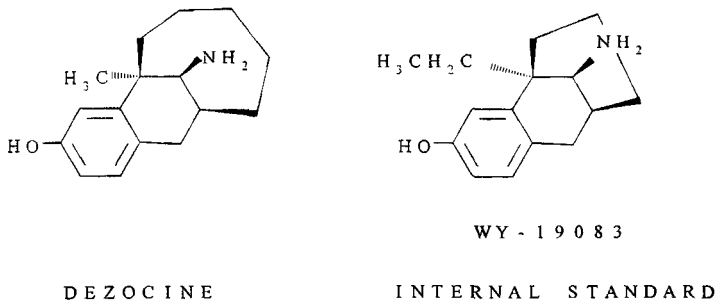

Figure 1. Molecular structure of dezocine and the internal standard(WY-19083).

Mercaptoethanol obtained from the Aldrich Chemical Co. (Milwaukee, WI) was used occasionally to restore the activity of the reagent.

METHODS

Procedure.

Two ml of standard, control or patient sample is added with a volumetric pipet to a 15 ml screwtop glass test tube containing 1 ml of internal standard (WY-19083, 50 ng/ml in 0.5 N NaOH). Ten ml of the extraction solvent(98% hexane/isoamyl alcohol) is added to each tube and rotated for ten minutes on a rotary mixer. The extraction tubes are centrifuged for 5 minutes to separate the liquid fractions and the hexane layer is removed to a 12 ml glass conical screw top centrifuge tube. The organic solvent was evaporated under nitrogen in a water bath at 45 deg C. When dry, 0.1 ml of o-phthalaldehyde derivatizing reagent is added to each tube, vortexed to mix, capped, and allowed to sit overnight. On the next day the tubes are uncapped and the derivatizing reagent is removed by evaporation to dryness in a water bath under nitrogen, again at 45 deg C. Each tube is reconstituted with 0.1 ml of HPLC grade methanol just prior to injection of 20 microliters into the liquid chromatograph.

RESULTS

Derivatization.

o-Phthalaldehyde has provided the basis for a variety of analytical procedures, notably for amino acids, drugs and tumor markers[9]. The presence of a primary amine is the necessary structural feature. The likely derivative is shown in Figure 2 for dezocine and analogously for the internal standard. The rate of the reaction and the stability of the products can be limiting features in the use of the reagents[11]. An informal evaluation showed that product formation increased over the first four hours after addition of the o-phthalaldehyde reagent and decreased only slightly over the next three days. As a result, it was convenient to perform extraction of twenty or more samples over approximately six hours, derivatize our samples overnight and perform the chromatography on the next day. Occasionally, a noticable decrease in product formation would occur. If so, 20 microliters of mercaptoethanol was added to the o-phthalaldehyde working bottle (20 ml) and the derivatization efficiency would return.

Extraction.

Several solvents were evaluated for their ability to extract dezocine: ethyl acetate, hexane and methylene chloride. In our view hexane provided the cleanest extracts and adequate recovery.

Chromatography.

While it was possible to elute dezocine more rapidly using a single analytical column, the application of this arrangement to the larger scale pharmacokinetic studies involving runs of twenty to fifty serum extracts per day proved damaging to column performance. Our best results were obtained after placing a previously used column of the same type at the head to act as a guard column. Though retention times were extended, better separation was obtained for the internal standard relative to polar serum derivatization products and for dezocine from

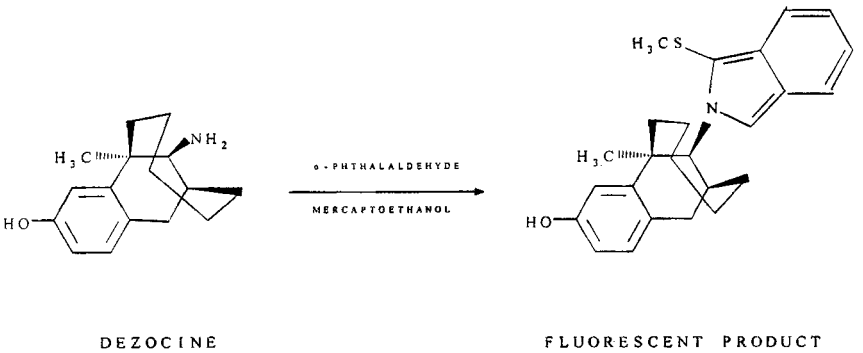

Figure 2. The expected derivative formed from dezocine by o-phthalaldehyde.

later baseline disruptions. Figure 3 represents the appearance of standards in bovine serum albumin and a pre- and post-dose subject sample. Wy-19083 elutes in approximately 16 minutes and dezocine elutes in 24 minutes. On rare occasions a late eluting peak from a previous injection would interfere with the calculation of the internal standard peak height. It was necessary to allow the late peak to elute before reinjecting.

Linearity.

Linearity was evaluated by assaying bovine serum albumin solutions containing dezocine in concentrations ranging from zero to 1000 ng/ml. Standard curves, such as depicted in Figure 4, were found to be satisfactory.

Detection Limits.

A 1 ng/ml standard was run with each standard curve. It was always possible to detect a dezocine peak originating in this sample. Injection of quantities larger than 20 microliters is possible, but our experience was that, if done consistently, column performance would be degraded and either cleaning or replacement would become necessary.

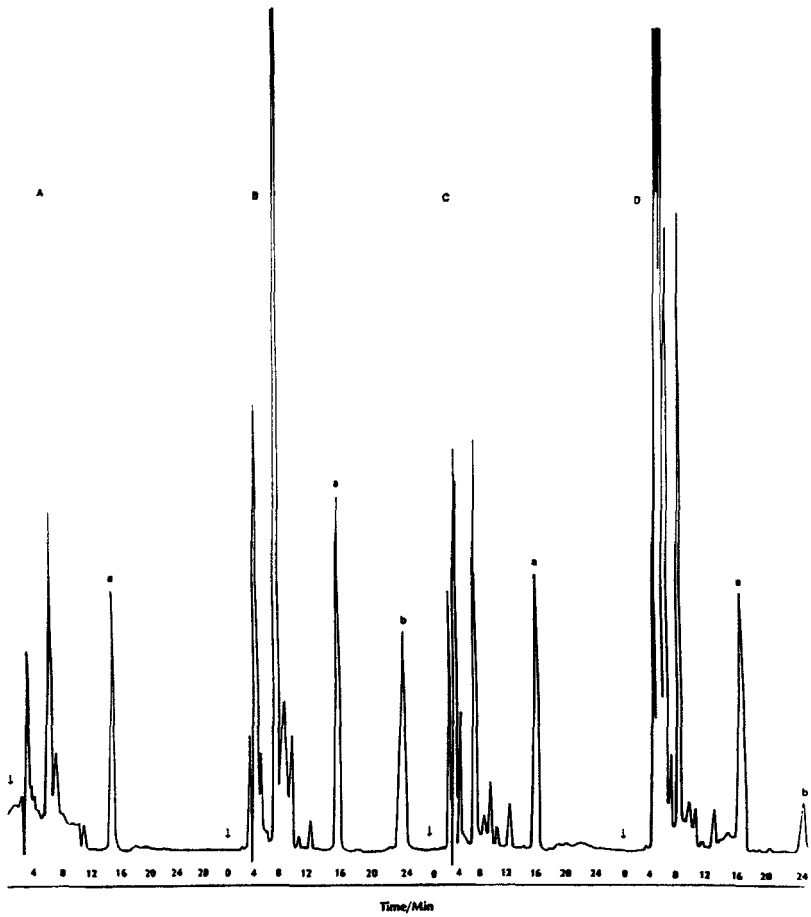

Figure 3. LC chromatograms showing A) a blank standard from bovine serum albumin, B) a 50 ng/ml standard from bovine serum albumin, C) a serum sample drawn prior to receiving dezocine and D) a serum sample drawn 20 minutes after a 20 mg IV injection of dezocine.

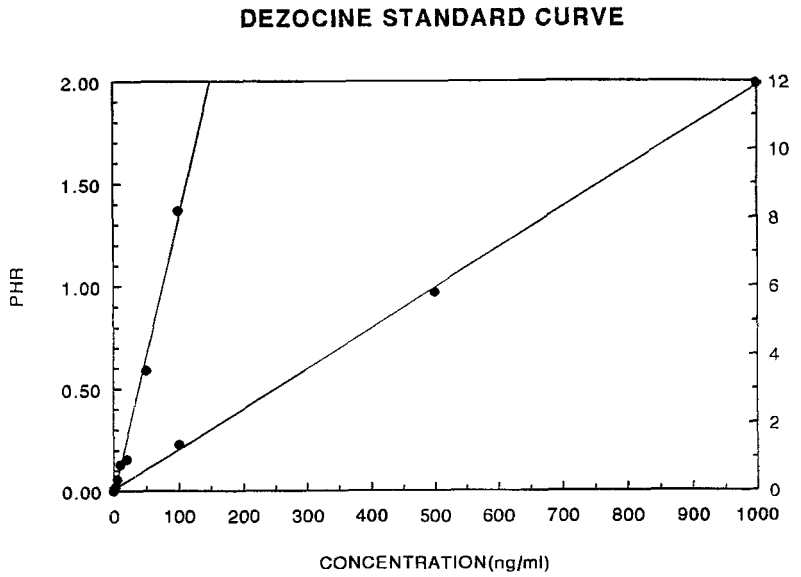

Figure 4. A standard curve demonstrating the linearity of the LC assay from 1 to 1000 ng/ml.

Precision.

Within-run precision was evaluated at approximately 27 ng/ml by combining equal amounts of the 1, 2, 5, 10, 20, 50 and 100 ng/ml standards. Fourteen out of sixteen samples exhibited satisfactory chromatographic performance with a mean value of 27.92 ng/ml and a standard deviation of 3.05 for a coefficient of variation of 10.9%. Day-to-day precision obtained over a ten week period gave a mean of 27.55 ng/ml with a standard deviation of 4.79 for a coefficient of variation of 17.4%(Table 1). The range of these values was 20.0 ng/ml to 36.8 ng/ml with 6 of 10 values within 3 ng/ml of the mean value.

Application.

The assay has been used for the determination of dezocine pharmacokinetics. Figure 5 is a concentration versus time plot of serum levels of dezocine in a

TABLE 1

Precision Studies

	Within-run	Run-to-run
N	14	10
MEAN	27.92	27.55
STD DEV	3.05	4.79
CV(%)	10.9	17.4

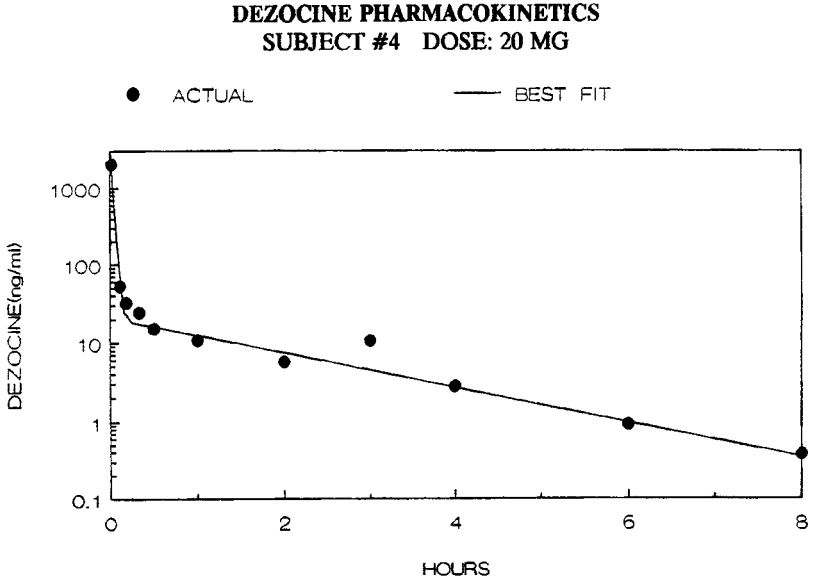

Figure 5. A plot of serum dezocine concentration of serum from a human subject with time after a 20 mg IV injection.

subject that received 20 mg of drug intravenously over thirty seconds into the right arm. Blood was first drawn from the left arm thirty seconds after administration. The serum concentration at this time was 2008 ng/ml. Seven minutes after administration the serum level was 53 ng/ml. Sixty minutes after administration the serum level was 10.6 ng/ml. Clearly, dezocine is rapidly distributed after iv administration. The data was fit to a two compartment open model and the value obtained for the distribution phase half-life was 1 minute. The half-life in the elimination phase was 1.4 hours.

Urine Testing.

The same assay has been applied to urine testing. The same subject whose data is presented in Figure 5 also had urine collected. Concentrations found in urine exceeded that of serum over the eight hour blood sampling period (Table 2). A total of 0.2612 mg(1.3 % of the dose) was excreted unchanged in eight hours.

DISCUSSION

Analysis of dezocine requires careful sample preparation and special detection systems. Analytical approaches to this time have involved gas chromatography with electron capture detection[7] and liquid chromatography with electrochemical detection[8]. We describe a straight forward approach that involves a single hexane extraction at a basic pH, overnight derivatization with o-phthalaldehyde and mercaptoethanol and liquid chromatography with fluourescence detection. We have found detection limits to 1 ng/ml and linearity over 4 orders of magnitude. The assay has been used for serum and urine pharmacokinetic studies[12] and has recently been effective in an *in vitro* evaluation of dezocine plasma protein binding[12]. Because dezocine has a very rapid distribution, concentrations of drug in the serum quickly reach levels of 10 ng/ml

TABLE 2

Dezocine Urine Excretion

Time of Collection (minutes)	Urine Volume (mls)	Dezocine Conc (ng/ml)	Amount (mg)
0-125	144	1126	0.1621
126-180	65	336	0.0218
181-245	80	243	0.0194
246-360	280	116	0.0325
361-495	300	85	0.0254
496-715	250	20	0.0050
716-955	730	5	0.0037
956-1435	355	2	0.0007

and place considerable demands on the analytical system. We have demonstrated precision on the order of 10% within-run and 17% from run to run. These standard deviations of 3 and 5 ng/ml are acceptable considering the levels measured. Since dezocine is a narcotic substance and has the potential for abuse, the capability of measuring very low serum levels expands the window of detectability. It would appear that dezocine could be detected in serum samples as long as 12 hours after a single dose and potentially longer given repeat administrations. Dezocine can be detected unchanged in the urine for 24 hours by this method and expands the window further. It has been reported that dezocine is metabolized principally via conjugation routes in monkeys and rats[13] and, since these are likely to also be important metabolic routes in man, it is probable that

hydrolysis of the urine would aid both in screening and confirmation tests and permit even greater possibility of detection.

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