DEXTROAMPHETAMINE: A PHARMACOLOGIC COUNTERMEASURE FOR SPACE MOTION SICKNESS AND ORTHOSTATIC DYSFUNCTION

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ABSTRACT

Dextroamphetamine has potential as a pharmacologic agent for the alleviation of two common health effects associated with microgravity. As an adjuvant to space motion sickness (SMS) medication, dextroamphetamine can enhance treatment efficacy by reducing undesirable central nervous system (CNS) side effects of SMS medications. Secondly, dextroamphetamine may be useful for the prevention of symptoms of post-mission orthostatic intolerance caused by cardiovascular deconditioning during spaceflight.

There is interest in developing an intranasal delivery form of dextroamphetamine for use as a countermeasure in microgravity conditions. Development of this dosage form will require an analytical detection method with sensitivity in the low ng range (1 to 100 ng/mL). During the 1995 Summer Faculty Fellowship Program, two analytical methods were developed and evaluated for their suitability as quantitative procedures for dextroamphetamine in studies of product stability, bioavailability assessment, and pharmacokinetic evaluation. In developing some of the analytical methods, β-phenylethylamine, a primary amine structurally similar to dextroamphetamine, was used.

The first analytical procedure to be evaluated involved hexane extraction and subsequent fluorescamine labeling of β-phenylethylamine. Assay standard curves were linear from 0.025 to 10 μg/mL. The lower limit of detection was 0.1 μg/mL. When serum was spiked with drug and extracted with hexane, the standard curve was linear in the 1.0 to 10.0 μg/mL range. The lower limit of detection was 1.0 μg/mL. The recovery of drug was 55 percent. The fluorescamine assay had insufficient sensitivity for quantitation of β-phenylethylamine at the levels which would occur in serum or saliva. However, this method would be amenable to measuring β-phenylethylamine or dextroamphetamine in urine where higher drug concentrations are observed.

The second analytical procedure to be evaluated involved quantitation of dextroamphetamine by an enzyme-linked immunosorbent assay (ELISA). The primary antibody was a purified, monoclonal antibody (mouse) to dextroamphetamine. The secondary antibody was sheep anti-mouse IgG conjugated with horse radish peroxidase (HRP). The HRP activity was determined by measuring A490 with a microplate reader.

For spiked human serum, the ELISA assay standard curves were linear from 50 to 1000 ng/mL. The lower limit of detection was 100 ng/mL. The sensitivity of the ELISA procedure was comparable to the combined extraction and fluorescamine-labeling procedures. The ELISA assay had insufficient sensitivity for quantitation of dextroamphetamine at the levels which would occur in serum or saliva. However, if the ELISA procedure is coupled with an organic solvent extraction procedure or with a membrane ultrafiltration concentration procedure, the combined procedures may prove adequate for measurement of dextroamphetamine in serum or saliva. This possibility will be investigated in future experiments at Louisiana Tech University.

INTRODUCTION

Dextroamphetamine as a countermeasure

A common health effect of space flight is motion sickness which presents severe operational decrements during early flight days (1,2). The choice treatment for this flight specific illness is administration of promethazine intramuscularly (3). Unfortunately, promethazine has side effects that can affect alertness and performance. Dextroamphetamine (Dexdrine) given in conjunction with an antimotion sickness drug such as promethazine or scopolamine has been shown to enhance the effectiveness and reduce intensity of side effects (4). Furthermore, dextroamphetamine has been shown to reduce the risk and be beneficial for treatment of vasodepressor syncope (5).

Physiologic changes of the GI function, symptoms of motion sickness, and other related GI disturbances render oral administration of medications a undesirable option for the effective treatment of these disturbances during space flight (6). There is interest in examining the bioavailability and effectiveness of an intranasal dosage form of dextroamphetamine in conjunction with promethazine. The goal is to develop noninvasive methods of pharmacologic treatment to provide safe, effective medications in space, as well as to offer these methods for the general benefit of clinical populations.

During the 1995 Summer Faculty Fellowship Program, the feasibility of developing an intranasal formulation of dextroamphetamine was examined. Development of this dosage form will require an analytical method suitable for conducting product stability studies, bioavailability assessment, and pharmacokinetic evaluation. Two existing assays for dextroamphetamine analysis, fluorometric assay and enzyme-linked immunosorbent assay (ELISA), were modified and compared.

Ultimately, the analytical methodology developed during this Summer Faculty Program will be used to compare the bioavailability and pharmacodynamics of two dosage forms of dextroamphetamine. Concentrations of parent drug and metabolites will be measured in blood, saliva, and urine after intranasal (IN) and oral (IM) administration. Pharmacokinetics and bioavailability will be estimated, and pharmacodynamics will be assessed. The analytical methodology developed during the Summer Faculty Program in conjunction with the ongoing research at the Johnson Space Center may ultimately provide a new dosage form for dextroamphetamine and may contribute to applications of the drug dextroamphetamine in space flight.

Pharmacologic background

Dextroamphetamine is a non-catecholamine, sympathomimetic amine with CNS stimulant activity and is used chiefly for its CNS effects. D-Amphetamine exerts most of its CNS effects by releasing biogenic amines from their storage sites in the nerve terminals. The alerting effect of amphetamine, its anorectic effect, and at least

a component of its locomotor-stimulating action are presumably mediated by release of norepinephrine from central noradrenergic neurons (7).

Following an oral dose, dextroamphetamine is completely absorbed within 3 hours. It is widely distributed in the body with high concentrations in the brain. Therapeutic blood levels range from 50 to 10 ng/mL (7). In a pharmacokinetic study in humans, ingestion of 10 mg dextroamphetamine produced an average peak blood level of 29.2 ng/mL at 2 hours post-administration (8). The average half-life was 10.25 hours. The average urinary recovery was 45% in 48 hours. Ingestion of a sustained release capsule containing 15 mg produced a peak blood level at 8 to 10 hours post-administration with peak urinary recovery at 12 to 24 hours. The dextroamphetamine volume of distribution was 270 liters/70 kg. The plasma protein binding of dextroamphetamine at concentrations of 10 and 100 ng/mL was 16% bound (9).

Urinary excretion of the unchanged drug is pH dependent (10). Urinary acidification to pH < 5.6 yields a plasma half-life of 7 to 8 hrs. Urinary alkalinization increases half-life ranging from 18.6 to 33.6 hours. For every one unit increase in urinary pH, there is an average 7 hour increase in plasma half-life. Proportionality between blood and urine data allowed an estimate of 120 mL/min for the apparent renal clearance of amphetamine.

METHODS

Materials

A purified monoclonal antibody against dextroamphetamine was purchased from Biodesign International. Dextroamphetamine standard (1 mg/mL in methanol) was purchased from Sigma Chemical Company. For fluorescamine labeling experiments, β-phenylethylamine, a drug structurally similar to dextroamphetamine, was used as a drug standard for assay development.

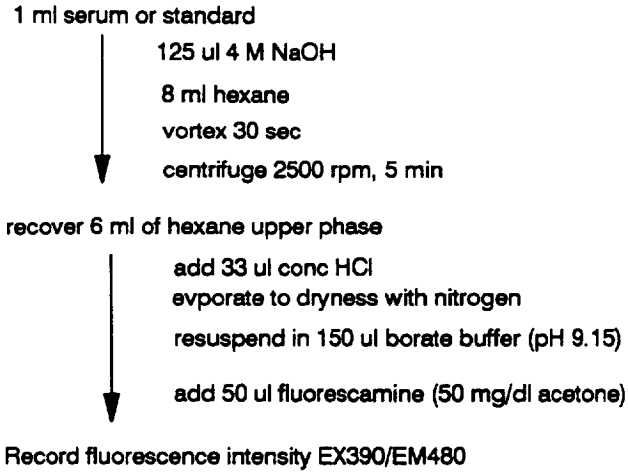

Figure 1.- Steps in the extraction and labeling procedure for fluorescamine assay of beta-phenylethylamine.

Extraction of β-phenylethylamine

Aliquots of β-phenylethylamine standard or serum spiked with β-phenylethylamine were prepared with a final volume of 1.0 mL in 4 inch culture tubes. Assay mixtures received 0.125 mL of 4 N NaOH and were extracted with 8 mL hexane by vortexing for 30 sec. After centrifugation at 2,500 rpm for 5 min, the upper organic phase was recovered and evaporated to dryness by passing a stream of nitrogen over the sample. The dry residue was used for fluorescamine labeling.

Fluorescamine labeling of β-phenylethylamine

All samples to be labeled with fluorescamine were brought to a final volume of 0.15 mL with 0.1 M sodium borate buffer (pH 9.15). Each sample received 0.05 mL of fluorescamine reagent (50 mg fluorescamine/100 mL of acetone). In preliminary experiments, an additional 2.3 mL of 0.1 M borate buffer (pH 9.15) was added and fluorescence intensity was measured in a 3 mL cuvette using a fluorimeter. Excitation was at 390 nm with a bandwidth of 10 nm, and emission was measured at 480 nm with a bandwidth of 20 nm. Alternatively, the undiluted fluorescamine-

labeled samples were drawn into Hoefer TKO capillary tubes, and the fluorescence intensity was measured in a Hoefer TKO capillary cuvette.

Immunoassay of dextroamphetamine

Dextroamphetamine standards with concentrations of 0, 10, 50, 100, 500, and 1000 ng/mL were prepared in either 20 mM potassium phosphate, 150 mM NaCl, pH 7.2 (PBS) or pooled human serum. Triplicate aliquots (50 μL) of standards were dispensed into Immulon 96-well microtiter plates and incubated over night at room temperature. The plates were washed with five changes of Dulbecco' phosphate-buffered saline (D-PBS) containing 1 mM magnesium chloride, 1 mM calcium chloride, 20 mM potassium phosphate, and 150 mM NaCl (pH 7.2) over a 20-minute period. For 1 hr at 37°C, wells of plates were blocked with 150 μL of Blocking Buffer containing 4% bovine serum albumin (BSA) and 4% heat-inactivated sheep serum in calcium- and magnesium-free PBS, pH 7.2 (CMF-PBS). Blocking Buffer was removed, and wells were washed five times with Wash Buffer containing 0.05% Tween 20 in D-PBS (pH 7.2). Wash Buffer was removed, and primary antibody (mouse monoclonal anti-dextroamphetamine) was added in 50 μL of Antibody Blocking Buffer containing 1% BSA and 1% HISS in calcium-magnesium-free PBS (pH 7.2). Plates containing primary antibody were incubated for 2 hr at 37°C, and wells were washed five times with Wash Buffer. Wash Buffer was removed, and secondary antibody (horse radish peroxidase conjugated sheep anti-mouse antibody) was added in 50 μL of Antibody Blocking Buffer. Plates containing secondary antibody were incubated for 1 hr at 37°C, and wells were washed five times with Wash Buffer. Wash Buffer was removed, and each well received 150 μL of Chromagen Reaction Buffer containing 9.25 mM o-phenylenediamine, 0.2 mg% hydrogen peroxide in 70 mM sodium citrate (pH 6.0). Reaction time was 1 hr, and the reaction was stopped by addition of 150 μL of 2 M sulfuric acid. Absorbance was measured at 490 nm with a plate reader.

RESULTS AND DISCUSSION

Calibration of spectrofluorimeter with riboflavin

To calibrate the spectrofluorimeter for use in fluorescence measurements, a riboflavin standard curve was prepared, and native fluorescence was measured. Concentrations of standards ranged from 0.4 to 40 ng/mL. Samples volumes were 2.5 mL, and fluorescence intensity was determined in a 3 mL cuvette with 1 cm light path. By performing excitation and emission scans, the optimum wavelengths for detection of native fluorescence were excitation at 425 nm and emission at 530 nm. The standard curve was linear from 0.4 to 30 ng/mL (data not shown).

Labeling of β-phenylethylamine with fluorescamine

Ten β-phenylethylamine standards with 0.15 mL volume in 0.1 M borate buffer (pH 9.15) were prepared. The standard concentrations ranged from 0.01 μg to 100 μg per tube. A 0.05 mL solution of fluorescamine reagent was added to each tube. The mixtures were further diluted with 2.3 mL of borate buffer, and fluorescence intensity was measured in a 3 mL cuvette with 1 cm light path. By performing excitation and emission scans, the optimum wavelengths for detection of fluorescamine-labeled β-phenylethylamine were excitation at 390 nm with a bandwidth of 10 nm and emission at 480 nm with a band width of 20 nm. The standard curve was linear from 0.05 to 10 μg per tube (data not shown).

Percent recovery of β-phenylethylamine from serum

Water or serum was spiked with 0, 1, 5, or 10 μg of β-phenylethylamine. The samples were extracted and labeled with fluorescamine. The fluorescence intensity was determined using a 3 mL cuvette. A set of standards which was not extracted was also assayed so that recovery could be estimated (Fig. 2). The recovery of β-phenylethylamine from water was 80%, while the recovery from serum was 55%.

Requirement of acid addition before evaporation to dryness

The fluorescamine labeling reaction is strongly pH dependent with a pH maximum of 8.0 to 9.5. The extraction procedure includes the addition of 33 μl of concentrated HCl to the hexane aliquot before evaporation to dryness. To determine if the addition of HCl to the hexane extract before evaporation was essential to the labeling reaction, water and serum standards of β-phenylethylamine were prepared and extracted. The organic phase was evaporated to dryness either with or without the 33 μl concentrated HCl addition. After labeling, fluorescence intensity was determined using a 3 mL cuvette. When acid was added before evaporation, the recovery of β-phenylethylamine was 80% from water and 55% from serum (Fig. 3). When acid addition was omitted, recoveries from water and serum were 20% and 15%, respectively (Fig. 3). Addition of concentrated HCl was required for successful labeling of β-phenylethylamine.

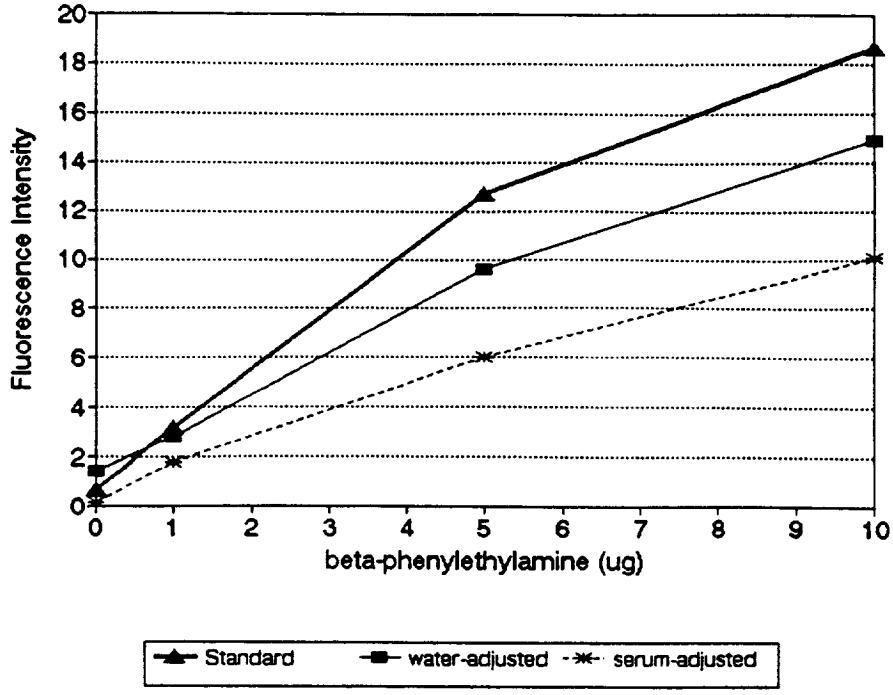

Figure 2.- Fluorescamine labeling of beta-phenylethylamine after extraction from water or serum

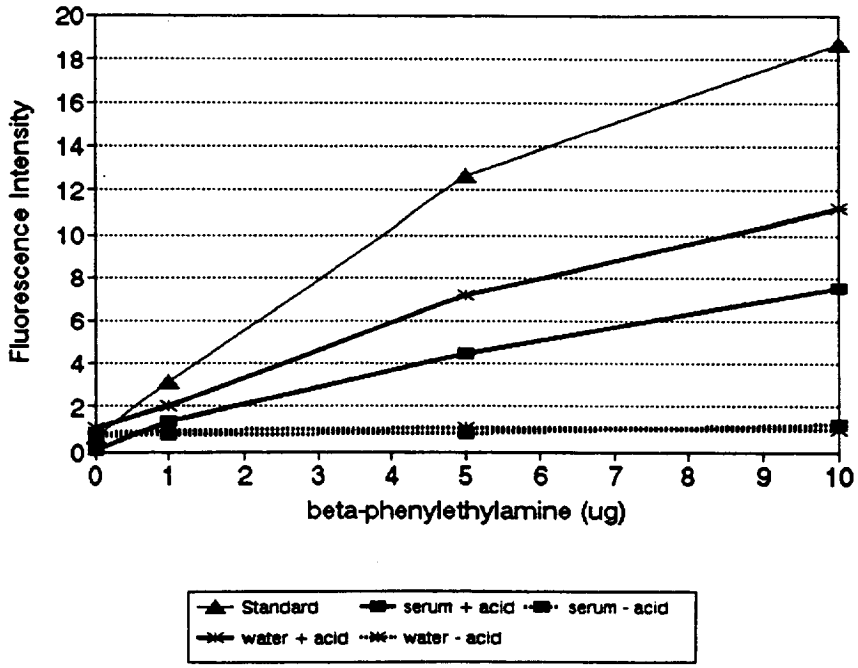

Figure 3.- Effect HCl addtion on recovery and labeling of beta-phenylethylamine in the fluorescamine assay.

In another experiment, the amount of concentrated HCl added to the hexane extract before evaporation was doubled to 66 μl. The percent recovery for both water and serum samples was unchanged by doubling the HCl addition (data not shown). The results suggest that HCl addition is required and that 33 to 66 μl of concentrated HCl is an adequate addition to support subsequent fluorescamine labeling.

Effect of mechanical mixing on drug extraction

The effect of prolonged mechanical mixing on drug recovery was examined. Spiked water and serum samples were extracted by either 30 min rotation on a clinical mixing table or by 30 sec vortexing by hand. The percent recovery for both water and serum samples was unchanged by 30 min agitation versus simple vortexing. This demonstrates that the more elaborate extraction procedure is not required (Data not shown).

Use of capillary cuvette to measure fluorescence intensity

The use of a 3 mL quartz cuvette for measuring fluorescence intensity necessitated the dilution of the 0.2 mL labeling mixture to 2.5 mL. To avoid this dilution, a Hoefer TKO capillary cuvette and TKO quartz capillary tubes were employed to measure the fluorescence intensity of undiluted 0.2 mL samples after labeling. For a 10 μg/mL standard, the fluorescence intensity for the diluted 2.5 mL samples and undiluted 0.2 mL samples were 21 and 98, respectively (Fig. 4). For all subsequent assays, the capillary cuvette was employed, and dilution of labeled samples was avoided.

To further examine the utility of the capillary cuvette, serum samples were spiked with β-pheynylethylamine to create a standard curve ranging from 0.01 to 1 μg/mL. Samples were extracted with hexane. After labeling with fluorescamine, the fluorescence intensity was determined using the capillary cuvette. A standard curve of β-phenylethylamine in water was also prepared and labeled without extraction. The standard curve for unextracted samples was linear from 0.025 to 1 μg. The lower limit of detection was 0.025 to 0.1 μg (Fig. 5). Although unextracted aqueous samples could readily be assayed in the 0.1 to 1 μg range, recoveries for extracted serum samples were low, and quantitation was not possible from serum. The fluorescence intensity of extracted serum samples was only slightly higher than blanks. The capillary cuvette method increased the fluorescence intensity of samples, but the sensitivity of the method was not improved by this technique.

Time dependence of fluorescamine labeling

The labeling of primary amines with fluorescamine is both pH and time dependent. Several 10 μg samples of β-phenylethylamine were prepared and labeled for different times ranging from 5 to 30 min. The extent of labeling was unchanged throughout the time interval examined and did not increase for incubations as long as either 2 or 3 hr (data not shown).

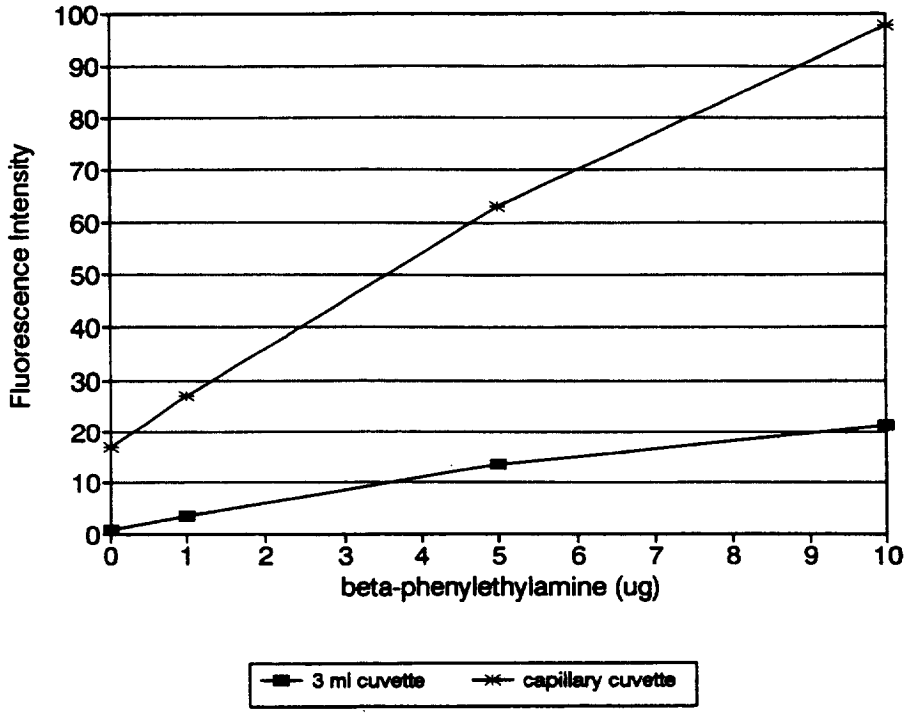

Figure 4.- Quantitation of fluorescamine labeled beta-phenylethylamine using a capillary cuvette and a 3 ml cuvette.

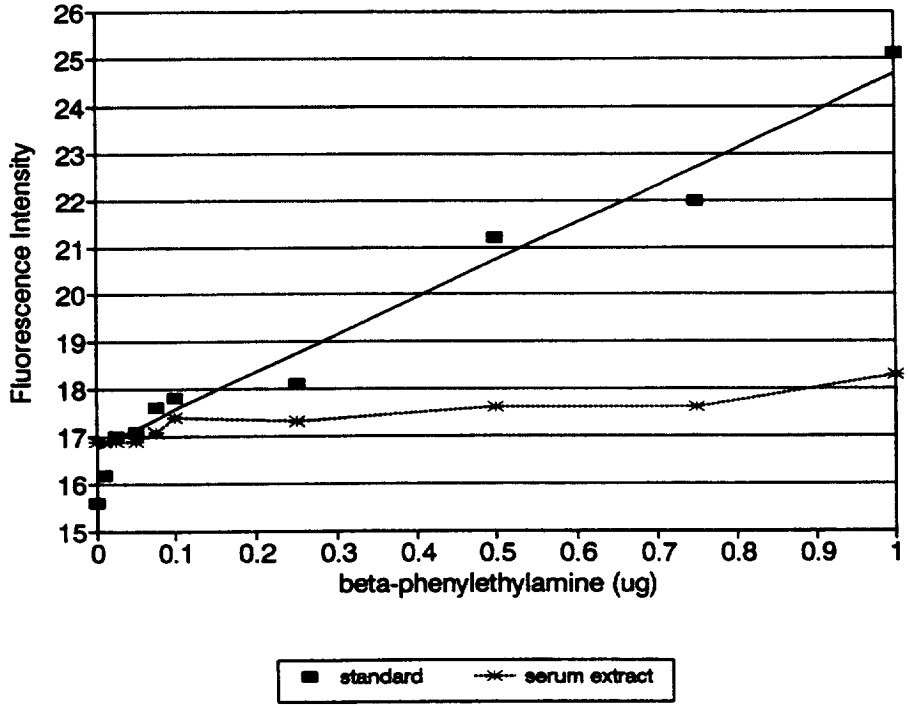

Figure 5.- Capillary cuvette measurement of fluorescamine-labeled beta-phenylethylamine in standards or in serum extract.

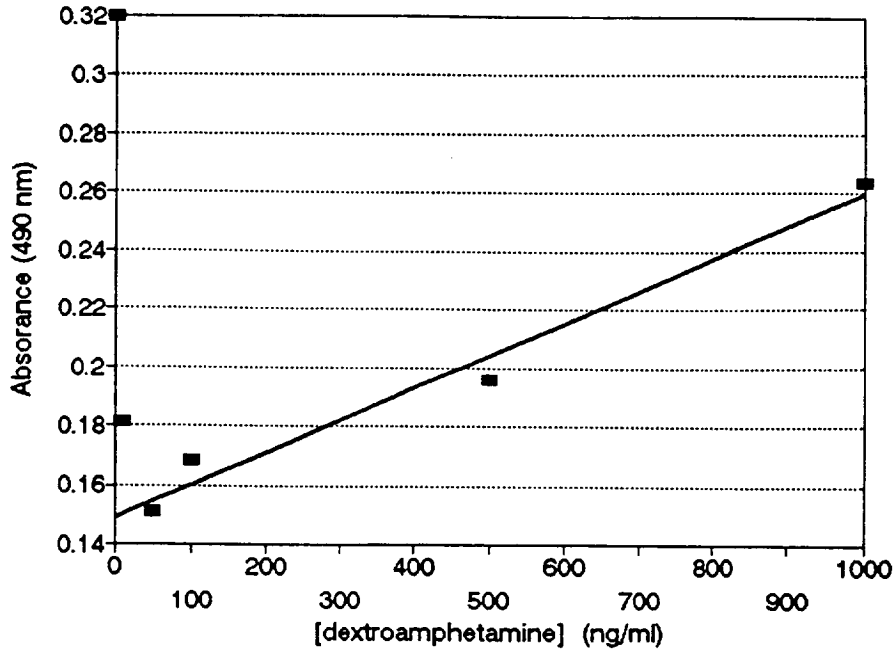

Figure 6.- ELISA standard curve of dextroamphetamine in human serum.

Enzyme-linked immunosorbent assay of dextroamphetamine

Enzyme-linked immunsorbent assays (ELISA) have been used to measure ng and pg quantities of proteins, peptides, and drugs in biological specimens. A purified, monoclonal antibody to dextroamphetamine was purchased and used to measure dextroamphetamine in spiked human serum. As shown in Fig. 6, a linear standard curve was obtained in the range of 50 to 1000 ng/mL. The lower limit for dextroamphetamine detection in spiked human serum was 100 ng/mL.

The lower limit for detection of dextroamphetamine by ELISA was similar to the lower limit for dextroamphetamine detection by the fluorescamine labeling procedure. However, the ELISA procedure involved the detection of dextroamphetamine in only 50 μl of serum, whereas the fluorescamine procedure involved extraction and assay of dextroamphetamine from 1 mL of serum.

It is anticipated that much lower limits of detection could be obtained by the ELISA procedure if serum dextroamphetamine samples could be concentrated before analysis. Concentration of serum dextroamphetamine samples could be accomplished by either extraction with organic solvents or by membrane ultrafiltration coupled with Speed-Vac solvent evaporation. Studies of the utility of these concentration procedures for enhancing ELISA measurement of dextroamphetamine will be continued in the Biochemistry Program at Louisiana Tech University.

CONCLUSIONS

Primary amines such as β-phenylethylamine were readily extracted from alkalinized serum by hexane. Recoveries ranged from as high as 80% for aqueous solutions of the amine to about 50% for spiked serum samples.

Hexane extraction of β-phenylethylamine followed by covalent labeling of the primary amine with fluorescamine was a viable analytical procedure for quantitation. Assay standard curves were linear from 0.025 to 10 μg/mL. The lower limit of detection was 0.1 μg/mL for aqueous solutions of the amine and 1.0 μg/mL for spiked serum.

The fluorescamine assay had insufficient sensitivity for quantitation of β-phenylethylamine at the levels which would occur in serum or saliva. However, this method would be amenable to measuring β-phenylethylamine or dextroamphetamine in urine where higher drug concentrations are observed.

The enzyme-linked immunosorbent assay (ELISA) procedure proved to be a viable analytical method for determination of dextroamphetamine. The primary antibody was a purified, monoclonal antibody (mouse) to dextroamphetamine, and the secondary antibody was sheep anti-mouse IgG conjugated with horse radish peroxidase (HRP). For spiked human serum, the ELISA assay standard curves were linear from 50 to 1000 ng/mL. The lower limit of detection was 100 ng/mL.

The sensitivity of the ELISA procedure was comparable to the sensitivity of the combined hexane extraction/fluorescamine-labeling procedures. The ELISA assay had insufficient sensitivity for quantitation of dextroamphetamine at the levels which would occur in serum or saliva. However, if the ELISA procedure is coupled with an organic solvent extraction procedure or with a membrane ultrafiltration concentration procedure, the combined procedures may prove adequate for measurement of dextroamphetamine in serum or saliva. This possibility will be investigated in future future experiments at Louisiana Tech University.

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