Abstract

The determination of propoxyphene in oral fluid using solid-phase extraction and gas chromatography–mass spectrometry is described for the first time. The method employs collection of oral fluid with the Quantisal™ device, immunoassay screening of the specimen, confirmation of the positive screened samples after extraction using cation exchange/hydrophobic solid-phase extraction columns, optimized derivative formation, and gas chromatography–mass spectrometry in electron impact mode. Validated parameters including selectivity, linearity, accuracy, intra- and interday precision, extraction efficiency, and limit of quantitation were all within acceptable limits. The method was applied to authentic specimens taken from an individual prescribed propoxyphene following surgery.

Introduction

Dextropropoxyphene (Darvon®), Eli Lilly, Indianapolis, IN), or Darvocet® or Coproxamol® (propoxyphene and acetaminophen), is a narcotic analgesic, structurally similar to methadone. It is intended for the treatment of moderate to severe pain, has high abuse potential because of its addictive properties and has been associated with a number of overdose accidents and deaths in the United States. At therapeutic levels it causes sedation, drowsiness, and fatigue in addition to its intended use of pain relief. Side effects include dry mouth, constipation, dizziness, nausea, sweating, and decreased appetite (1). Propoxyphene is highly toxic when combined with alcohol or other drugs, and it has been strongly linked to suicides and accidental poisonings in the United Kingdom, where it was withdrawn from the market approximately one year ago. According to a study conducted in Sweden, the frequency of fatal poisoning by dextropropoxyphene ingestion is high. The ratio between the number of fatal poisonings and prescription of defined daily dose/1000 inhabitants during a 12-month period in Sweden indicated that the highest ratio was attributed to unmixed preparations of dextropropoxyphene (2,3). This further indicates the highly toxic nature of the drug not only in combination with alcohol or other drugs but by itself also and gives reason for concern, especially in cases involving driving under the influence. Various publications describe determining propoxyphene in urine (4,5), blood, and hair (6), but no one has reported a method for determining propoxyphene from oral fluid. Our study showed the viability of the addition of propoxyphene to the oral fluid testing profile when Quantisal is used. Keeping in mind the advantages of oral fluid as a sample matrix over conventional biological matrices (i.e., blood and urine), specifically the ease of collection, this study was conducted to develop a sensitive and specific quantitative method for detection of propoxyphene in oral fluid.

Materials and Methods

Experimental

Quantisal devices for the collection of oral fluid specimens were obtained from Immunalysis Corporation (Pomona, CA). The devices contain a collection pad with a volume adequacy indicator, which turns blue when 1 mL of oral fluid (± 10%) has been collected. The pad is then placed into transport buffer (3 mL), allowing a total specimen volume available for analysis of 4 mL (3 mL buffer + 1 mL oral fluid). This is specifically advantageous in cases where the specimen is positive for more than one drug and the volume of specimen available for analysis may be an issue. The oral fluid concentration is diluted 1:4 when using Quantisal collection devices, and drug concentrations detected were adjusted to neat oral fluid equivalents accordingly. Oral fluid screening kits were also obtained from Immunalysis. The Propoxyphene Direct ELISA Kit (catalog #237) was used for screening the specimens according to the manufacturer's instructions. For confirmatory procedures, penta-deuterated propoxyphene-d5 (internal standard) and the unlabelled propoxyphene drug standards were obtained from Cerrilliant (Round Rock, TX). Solid-phase extraction columns (Clin II, 691-0353T) were obtained from SPEWare (San Pedro, CA). All solvents were high-performance liquid chromatography (HPLC) grade or better, and all chemicals were ACS grade.

Screening assay

The Immunalysis Propoxyphene Direct ELISA kit is based upon the competitive binding to antibody of enzyme labeled antigen and unlabeled antigen in proportion to their concentration in the reaction well. A standard curve consisting of a drug-free negative oral fluid specimen and drug-free oral fluid specimens spiked at 20, 40, and 80 ng/mL of neat oral fluid.
equivalents of propoxyphene was prepared. An aliquot of the diluted oral fluid (10 μL) was then added to the individual microplate well. The assay performance was optimal when 50–200 pg was placed into the microplate well. A sample size of 10 μL of oral fluid gave good separation at the screening cut-off concentration of 40 ng/mL used for this study. The percentage binding (B/Bo displacement value) at 20, 40, and 80 ng/mL was 75%, 52%, and 26%, respectively.

Confirmation assay
The internal standard solution contained penta-deuterated propoxyphene-d₅ at a concentration of 250 ng/mL. A calibration curve was prepared in the transport buffer associated with the Quantisal device. The concentrations were equivalent to neat oral fluid concentrations of 5, 10, 25, 50, 100, and 200 ng/mL of propoxyphene.

Internal standard (25 μL) and 0.05M sodium hydrogen carbonate buffer (pH 8.0, 1 mL) was added to each calibrator, control, or oral fluid specimen (1 mL of buffer = 0.25 mL of neat oral fluid). Solid-phase mixed mode extraction columns (Clin II, 691-0353T) were placed into a positive pressure manifold. Each column was conditioned with methanol (2 mL), and 0.1M phosphate buffer (pH 6.0, 2 mL). The samples were allowed to flow through the columns, and then the columns were washed with deionized water (1 mL), 0.1M acetate buffer (pH 4, 1 mL), methanol (1 mL), and ethyl acetate (1 mL). The columns were allowed to dry under nitrogen pressure (30 psi, 2 min). The drugs were finally eluted using freshly prepared ethyl acetate/ammonium hydroxide (98:2, 2 mL). The extracts were evaporated to dryness under nitrogen, reconstituted in ethyl acetate (50 μL) and transferred to autosampler vials for analysis by gas chromatography–mass spectrometry (GC–MS).

Analytical procedure (GC–MS)
An Agilent Technologies 6890 GC coupled to a 5975 mass selective detector (MSD) with an inert source, operating in electron impact mode was used for analysis (GC–MS). The GC column was a 15-m DB-5 MS (0.25-mm i.d., 0.25-μm film thickness, J&W Scientific, Folsom, CA), and the injection temperature was 250°C. The purge flow was 50 mL/min for 1 min and the carrier gas was helium. The injection mode was splitless, injection volume 2 μL, and the operation mode was constant flow at 1.5 mL/min. The oven was programmed as follows: 60°C for 1 min, ramped at 30°C/min to 200°C, held for 0.2 min, then ramped at 80°C/min to 250°C. The transfer line was held at 280°C, the quadrupole at 150°C, and the ion source at 230°C, and the dwell time was 50 ms. The ions monitored were 213.2 and 198.1 for deuterated propoxyphene and 208.2, 193.1, and 179.1 for propoxyphene. The retention time for propoxyphene was 5.53 min.

Results and Discussion
Method validation
Screening assay. The precision of the Immunalysis Propoxyphene Direct ELISA KIT was verified by assessment of the mean, standard deviation (SD), and coefficients of variation (CV) in data resulting from repetitive assays. Intra- and interassay precision were determined using spiked controls. Negative oral fluid was spiked at 0, 20, 40, and 80 ng/mL of propoxyphene. A 10-μL aliquot of the diluted control (1:4 dilution) was then added to individual microplate well, resulting in a concentration of 0, 50, 100, and 200 pg/well. The intra-assay precision (n = 16) at 0, 50, 100, and 200 pg/well was 2.86%, 4.76%, 7.15%, and 8.02%, respectively. The interassay precision (n = 48) at 0, 50, 100, and 200 pg/well was 1.48%, 3.37%, 3.56%, and 3.91%, respectively.

The specificity of the Immunalysis ELISA for propoxyphene was determined by generating inhibition curves for propoxyphene and nor-propoxyphene. The assay cross-reactivity with nor-propoxyphene was 49% at a concentration of 100 pg/well. Aliquots of human oral fluid were also spiked at 10,000 pg/well with unrelated drugs: Acetaminophen, acetylsalicylic acid, amphetamine, amitriptyline, ampicillin, amobarbital, ascorbic acid, atropine, barbital, benzoylcoคอนnine, bромazepam, butabarbital, caffeine, carbamazepine, cocaine, codeine, chloroquine, chlorpromazine, desipramine, dextromethorphan, 5,5-diphenylhydantoin, 10-11-dihydrocarbamazepine, diacetylmorphine, diazepam, doxepine, ethosuximide, ethotoin, ethylmorphine, flurazepam, glutethimide, hexobarbital, hydrocodone, hydromorphone, ibuprofen, imipramine, lidocaine, lorazepam, LSD, medazepam, methadone, methadone metabolite, methaqualone, mephenytoin, meprobartal, methyl PEMA, methylphenidate, morphine, meperidine, nalorphine, nicamidamine, nortoxin, norethidrone, normethsuximide, nortryptiline, oxazepam, oxycodone, phenobarbital, phenxusimide, PEMA, primidone, phencyclidide, pentobarbital, propoxyphene, propylated, propyliyline, quinine, secobarbital, temazepam, trimipramine, and THC triocoh were tested.

None of these compounds gave values in the assay that were equal to or greater than the assay sensitivity level (1 ng/mL).

Confirmation assay. The linearity of the assay was determined by using oral fluid specimens from drug-free individuals fortified with 5 to 300 ng/mL of propoxyphene and 25 ng/mL of deuterated propoxyphene (d₅). The assay was linear over the range tested with a correlation coefficient of r² = 1.00. The limit of quantitation of the procedure (LOQ) was 5 ng/mL, and the limit of detection (LOD) was 2 ng/mL. The precision of the assay at a spiked concentration of 10 ng/mL was 2.13% and 3.59% intra- (n = 5) and interday (n = 5), respectively. The extraction efficiency was calculated by soaking the collector pad in oral fluid (1 mL) spiked with propoxyphene at a concentration of 10 ng/mL and then placing the pads in the tubes containing the extraction buffer. The devices were stored in the refrigerator (2–8°C) for two days. The pads were separated using a serum separator provided with the kit and 1mL of the sample was then removed for analysis. Propoxyphene extracted from the pad and buffer with an average efficiency over 92%. The mean extracted value was 9.2 ng/mL (n = 5). The standard deviation and C.V. were 0.5% and 5.6%, respectively. No interfering peaks affected the performance of the method. In general, oral fluid is a much cleaner biological matrix than conventionally used specimens in toxicological analyses (e.g.,
whole blood/plasma/serum and urine), which typically leads to a lower level of background interference in chromatographic measurements.

Application to authentic specimens

Oral fluid specimens were collected from an individual prescribed propoxyphene following in-patient surgery. Sample collection was approved under Immunalysis Institutional Review Board IRB# 2006-01-001. The subject, a 32-year-old female (140 lbs), willingly consented to sample collection and reported no use of prescription medications (including propoxyphene) prior to surgery. Two oral fluid samples were collected using the Quantisal device 1 h after ingestion of propoxyphene (100 mg), and one sample was collected 8 h after ingestion. The percentage binding (B/Bo displacements values) for the samples collected after 1 h of ingestion was 6.4% and 3.9%, and for the sample collected after 8 h, the value was 33.83%. The two oral fluid samples taken after 1 h were confirmed for the presence of propoxyphene at levels of 223 and 215 ng/mL. A significant amount of the drug was still remaining in the oral fluid collected after 8 h (37 ng/mL). Chromatograms of the negative and positive oral fluid are shown in Figure 1.

The determination of propoxyphene in oral fluid is described for the first time. The method was precise, accurate, sensitive, and easily allowed the detection of propoxyphene in authentic samples taken from an individual prescribed the painkiller following surgery. The detection of measurable amounts of propoxyphene at least 8 h following ingestion indicate potential applications of the method, which may include therapeutic and medical professional drug monitoring, and the detection of drivers under the influence of propoxyphene.

References