Confirmation of Pain Medications in Oral Fluid Using Inert Source GC/MS

Application

Forensics

Authors

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Abstract

Oral fluid is being considered as an alternative to urine in many forensic and clinical arenas for the detection of prescription medications, which may be diverted and abused by medical professionals. Since "Med-Pro" testing panels are now widespread, the analysis of these medications in the less-invasive oral fluid is an attractive alternative for routine monitoring. In general, the concentration of drugs in oral fluids is much lower than in urine, so sensitive extraction and analytical procedures are required. There are no specific "cut-off" concentrations recommended for oral fluid analysis for these drugs, so procedures were developed to the limit of quantitation. The Agilent 5975 GC/MS with an inert source achieves the required sensitivity for the detection of meperidine, tramadol, propoxyphene, and oxycodone in oral fluid. While these drugs have been detected in other matrices, the increasing utility of saliva for drug analysis makes development of laboratory procedures necessary and timely.

Introduction

Various laboratories currently offer "Medical Professional" drug test panels, which as the name implies, are targeted at the detection of prescription medications as well as the more common drugs of abuse. Standard prescription medication drug test panels include meperidine (Pethidine, Demerol), tramadol (Ultram), propoxyphene (Co-proxamol) and oxycodone (Percocet, Oxycontin).

While blood and urine are more commonly used for these test profiles, oral fluid is increasing in popularity as an alternative matrix due to its ease of collection, difficulty of adulteration, and improving sensitivity of analytical techniques. One of the main issues with the quantitation of drugs in oral fluid is the difficulty of collection in terms of specimen volume. Many of the currently available devices do not give an indication of how much oral fluid is collected, thereby rendering any quantitative results meaningless without further manipulation in the laboratory. Further, devices incorporating a pad or material for the saliva collection do not always indicate how much of each drug is recovered from the pad before analysis, again calling into question any quantitative result. The drug concentration reported is dependent on the collection procedure used.

This work employed Immunalysis Corporation's QUANTISAL oral fluid collection device, which collects a known amount of neat oral fluid. The efficiency of recovery of the drugs from the collection pad into the transportation buffer was determined in order to increase confidence in the quantitative value. The extracts were analyzed using a standard single quadrupole Agilent GC/MS 6890-5975 instrument.

Experimental

Oral Fluid Collection Devices

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 one milliliter 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 1:3 when using Quantisal collection devices, and drug concentrations detected were adjusted accordingly.

Standards and Reagents

- Deuterated internal standards: Cis-tramadol d4, meperidine-d4, oxycodone-d6, propoxyphene-d5, and unlabeled drug standards: meperidine, tramadol, oxycodone, and propoxyphene were purchased from Cerilliant (Round Rock, TX).
- Clin II solid phase extraction columns (Part #691-0353T) were purchased from SPEWare (San Pedro, CA).
- Derivatizing agents: N,O-Bis (trimethylsilyl) trifluoroacetamide + 1% trimethylchlorosilane (BSTFA + 1% TMCS), and N-methyl-N-trimethylsilyltrifluoroacetamide +1% trimethylchlorosilane (MSTFA +1% TMCS) were from Pierce (Rockford, IL).

Internal Standard Concentration

Meperidine, propoxyphene, and tramadol 250 ng/mL; oxycodone 200 ng/mL

Sample Preparation for Chromatographic Analysis

- 1 mL Quantisal specimen (equivalent to 0.25 mL of oral fluid)
- Add internal standard
- For meperidine, tramadol, and oxycodone, add 0.1 M sodium phosphate buffer (pH 6.0; 1 mL)
- For propoxyphene, add 0.05 M sodium hydrogen carbonate buffer (pH 8.0; 1 mL)
- Condition SPE columns: methanol (2 mL), 0.1 M phosphate buffer (pH 6.0; 2 mL)
- Add samples
- Wash columns:
  - deionized water (1 mL)
  - 0.1 M acetate buffer (pH 4; 1 mL)
  - methanol (1 mL)
  - ethyl acetate (1 mL)
- Dry columns under nitrogen (30 psi; 2 min)
- Elute: freshly prepared ethyl acetate: ammonium hydroxide (98:2 v,v; 2 mL)
- Evaporate to dryness under nitrogen

No Derivatization

Propoxyphene: Reconstitute in ethyl acetate (50 µL); transfer to autosampler vials

Meperidine: Meperidine does not derivatize since there are no active hydrogen sites available for reaction; however, the extract was reconstituted in ethyl acetate (20 µL). BSTFA + 1% TMCS (20 µL) were added, capped, and heated (50 °C/20 min). The addition of a silanizing reagent to the extract improved stability for the extract and produced markedly better chromatography of meperidine.

Derivatization

Tramadol: Reconstitute in ethyl acetate (25 µL); add BSTFA +1% TMCS (25 µL); transfer to autosampler vials, cap, and incubate (70 °C/20 min).

Oxycodone: Reconstitute in 1% hydroxylamine HCl in pyridine solution (50 µL) and incubate (45 °C/30 min). Add MSFTA + 1% TMCS (50 µL), cap, and incubate (65 °C/20 min).

GC/MS Conditions

Instrument:	Agilent 6890 GC 5975 MSD; inert source; 220/240V oven
Detection mode:	Electron impact
Column:	DB-5 MS, 0.25 mm id, 0.25-µm film thickness, 15-m length
Injection temperature:	250 °C
Purge flow:	50 mL/min for 1 min
Carrier gas:	Helium
Injection mode:	Splitless
Injection volume:	2 µL
Mode of operation:	Constant flow at 1.5 mL/min
Transfer line:	280 °C
Quadrupole:	150 °C
Ion source:	230 °C
Dwell time:	50 ms

Oven Programs

Propoxyphene:	60 °C for 1 min; ramp 30 °C/min to 200 °C; hold 0.2 min; ramp 80 °C/min to 250 °C
Meperidine:	50 °C; ramp at 30 °C/min to 280 °C
Tramadol:	65 °C for 1 min; ramp 40 °C/min to 200 °C; ramp 15 °C/min to 230 °C; ramp 100 °C/min to 290 °C
Oxycodone:	100 °C for 0.5 min; ramp 10 °C/min to 270 °C

Ions Monitored

Drug	Ions monitored					
Propoxyphene	Deuterated (d5)	**213.2**,	198.1;	**208.2**,	193.1,	179.1
Meperidine	Deuterated (d4)	**251.2**,	222.2;	**247.2**,	218.2,	172.2
Tramadol	Deuterated (d4	**339.3**,	324.2;	**335.3**,	245.2,	320.2
Oxycodone	Deuterated (d6)	**480.3**,	391.3;	**474.3**,	385.3,	459.3

Quantitative ions in bold type

Retention Times

	Minutes
Propoxyphene:	5.5
Meperidine:	5.1
Tramadol:	5.4
Oxycodone:	16.0

Results and Discussions

One of the issues associated with oral fluid analysis is recovery of drug from a collection pad if a device is used. Extraction efficiency of the collection system for these drugs was determined. Oral fluid was fortified with all three drugs at the concentration of 25 ng/mL for tramadol and meperidine, 20 ng/mL for oxycodone, and 10 ng/mL propoxyphene.

A collection pad was placed into the fluid until the volume adequacy indicator turned blue, showing that 1 mL (±10%) of oral fluid had been absorbed. The pads were placed into the Quantisal buffer (3 mL), capped, and allowed to remain at room temperature overnight to simulate transportation to the laboratory. The following day, the pads were removed and an aliquot (1 mL) of the specimens was analyzed according to the described procedures (n = 6).

	Meperidine	Oxycodone	Tramadol	Propoxyphene
Mean drug recovery (%)	86.7	96.6	87.7	92.0

GC/MS Method Validation

The analytical methods were validated according to standard protocols, whereby the limit of quantitation, linearity range, correlation, and intra- and inter-day precision were determined via multiple replicates over a period of five days. The slope of the calibration curve was forced through the origin.

Analyte	Limit of quantitation (ng/mL)	Equation (mean SD)	Correlation (r^2)	Linearity range (ng/mL)
Meperidine	10	y = 0.0196x	0.999	10–100
Propoxyphene	5	y = 0.0392x	0.999	5–300
Oxycodone	10	y = 0.0132x	0.997	10–80
Tramadol	10	y = 0.0190x	0.999	10–100

Inter-day Precision (n = 10)

Drug	Expected concentration (ng/mL)	Observed concentration (mean ±SD) (ng/mL)	Precision (%)
Meperidine	25	25.32 ± 0.628	2.48
Propoxyphene	10	10.12 ± 0.36	3.59
Oxycodone	20	20.74 ± 1.202	5.80
Tramadol	25	25.65 ± 0.627	2.44

Inter-day Precision (n = 5)

Drug	Expected concentration (ng/mL)	Observed concentration (mean +-SD) (ng/mL)	Precision (%)
Meperidine	25	25.72 ± 0.60	2.33
Propoxyphene	10	9.8 ± 0.23	2.39
Oxycodone	20	23.08 ± 1.75	7.61
Tramadol	25	25.88 ± 0.25	1.00

Commonly encountered drugs were extracted and analyzed at high concentrations and found not to interfere with the assays. Figure 1 shows tramadol (1175 ng/mL) in oral fluid from an authentic specimen.

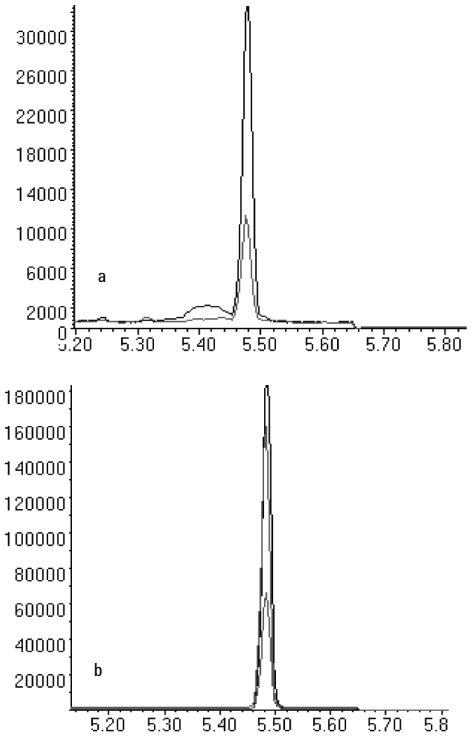

Figure 1. Oral fluid specimen collected using the Quantisal device and analyzed using the described procedure.

a) D4-tramadol: Ions 339.3; 324.3

b) Tramadol 335.3, 320.3, 245.2

Conclusions

The procedures described are suitable for the detection of these pain medications in oral fluid using an Agilent Technologies single quadrupole GC/MS system with an inert source. The methods are in routine use for the measurement of drug concentrations in oral fluid.

Reference

S. Rana, C. Moore, A. Agrawal, C. Coulter, M. Vincent, and J. Soares. Determination of propoxyphene in oral fluid. *J Anal Toxicol* 2006; 30(8): 516-518.

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