



Determination of meperidine, tramadol and oxycodone in human oral fluid using solid phase extraction and gas chromatography–mass spectrometry

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Abstract

Analytical procedures for the determination of meperidine, tramadol and oxycodone in oral fluid have been developed and validated using gas chromatography–mass spectrometry (GC/MS) following initial screening with enzyme linked immunosorbent assay (ELISA). The oral fluid samples were collected using the Quantisal™ device, and any drugs present were quantified using mixed mode solid-phase extraction and electron impact GC/MS. For confirmation, three ions were monitored and two ion ratios determined, which were within 20% of those of the known calibration standards. The limits of quantitation were 10 ng/mL; the intra-day precision of the assays ($n = 5$) was 2.33%, 1.00% and 7.61%; inter-day precision 2.48%, 2.44% and 5.8% ($n = 10$) for meperidine, tramadol and oxycodone, respectively. The percentage recovery of the drugs from the collection pads was 86.7%, 87.7% and 96.6%, respectively ($n = 6$). The methods were applied to specimens obtained during research studies in the USA. © 2006 Published by Elsevier B.V.

Keywords: Oral fluid; Meperidine; Tramadol; Oxycodone

1. 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. Gunnar et al. reported on the analysis of 30 drugs of abuse in oral fluid using long column fast GC procedure, but none of these drugs were included in the profile [1]. Wylie et al. reported the analysis of 49 different drugs in oral fluid collected using the Omni-Sal® device, and a combination of LC/MS/MS and GC/MS. Tramadol was one of the drugs included, with a reported limit of quantitation of 4.9 ng/mL [2]. The determination of propoxyphene in

oral fluid using immunoassay and GC/MS has been previously reported by our research group [3], so this publication focuses on validated immunoassay and gas chromatography–mass spectrometric methods for the determination of meperidine, tramadol and oxycodone.

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 [4,5]. 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 [6].

This work employed the Quantisal™ oral fluid collection device, which collects a known amount of neat oral fluid. The efficiency of recovery of the three drugs from the collection pad into the transportation buffer was determined, in order to increase confidence in the quantitative value. The stability of the drugs in the buffer at room temperature and at 4 °C was studied, as well as the stability of extracted oral fluid specimens. The procedures were applied to specimens received into our laboratory from research studies.

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2. Experimental

2.1. Oral fluid collection devices

QuantisalTM 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 ($\pm 10\%$) 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 QuantisalTM collection devices, and drug concentrations detected were adjusted accordingly.

2.2. Standards and reagents

The following kits were obtained from Immunalysis Corporation (Pomona, CA) and used for screening the oral fluid samples: Meperidine Direct ELISA Kit (Catalog #220); Oxycodone Direct ELISA Kit (Catalog #221B); Tramadol Direct ELISA Kit (Catalog #225). For confirmatory procedures, deuterated internal standards (*cis*-tramadol d4, meperidine-d4 and oxycodone-d6) as well as unlabelled drug standards for each of the drugs were obtained from Cerilliant (Round Rock, TX). Solid phase extraction columns (Clin II, 691-0353T) were obtained from SPEWare (San Pedro, CA). The derivatizing agents *N,O*-bis (trimethylsilyl) trifluoroacetamide + 1% trimethylchlorosilane (BSTFA + 1% TMCS), and *N*-methyl-*N*-trimethylsilyltrifluoroacetamide + 1% trimethylchlorosilane (MSTFA + 1% TMCS) were purchased from Pierce (Rockford, IL). All solvents were HPLC grade or better, and all chemicals were ACS grade.

2.3. Calibrators

For the chromatographic calibration standards, three working solutions for the deuterated internal standards were prepared in methanol at concentrations of 250 ng/mL for meperidine and tramadol; 200 ng/mL for oxycodone. Unlabelled drug standards were prepared in methanol at the same concentrations. All the working solutions were stored at -20°C when not in use. For each batch, four calibration standards were prepared in synthetic oral fluid (1 mL) then transportation buffer from the QuantisalTM collection device was added (3 mL). For tramadol and meperidine, drug concentrations of 10, 25, 50 and 100 ng/mL of neat oral fluid equivalents were prepared (internal standard concentration: 50 ng/mL); for oxycodone, 10, 20, 40 and 80 ng/mL (internal standard concentration: 80 ng/mL).

2.4. Screening assay

Enzyme linked immunosorbent assays (ELISA) technology is based upon the competitive binding to antibody of enzyme labeled antigen and unlabeled antigen in proportion to their

concentration in the reaction well. The oral fluid specimens were screened according to the manufacturer's instructions, which recommended cut-off concentrations of 25 ng/mL for oxycodone; 50 ng/mL for meperidine and tramadol of neat oral fluid equivalents. A standard curve consisting of a drug free negative oral fluid specimen and drug free oral fluid specimens spiked at 50 and 200% of the recommended cut-off concentrations was analyzed with every batch. The optimal sample sizes as suggested by the manufacturer were: meperidine and tramadol (40 μL); oxycodone (25 μL). The sample volume was pipetted directly from the collection device into the microplate. Specimens screening positively using ELISA, were carried forward to confirmation using the described procedures.

2.5. Sample preparation for chromatographic analysis

An aliquot (1 mL) from the QuantisalTM collection device, equivalent to 0.25 mL of oral fluid was removed and internal standard was added (50 μL for meperidine and tramadol; 100 μL for oxycodone). 0.1 M sodium phosphate buffer (pH 6.0; 1 mL) was added to each calibrator, control or oral fluid specimen. 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 0.1 M 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), 0.1 M 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, v:v; 2 mL). The extracts were evaporated to dryness under nitrogen and reconstituted in ethyl acetate.

2.5.1. Derivatization

2.5.1.1. 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) was added, the vial was capped and heated at 50°C for 20 min. The reason for this was that the addition of a silanizing reagent to the extract improved stability for the extract and produced markedly better chromatography of meperidine.

2.5.1.2. Tramadol. The trimethylsilyl derivative of tramadol was prepared by reconstituting the dried extract in ethyl acetate (25 μL); BSTFA + 1% TMCS (25 μL) was added, transferred to auto-sampler vials, capped and incubated at 70°C for 20 min. Since silylation reagents are moisture sensitive, and easily hydrolyzed, they cannot be used in aqueous solutions. Excess derivatization reagent is incorporated into the procedure in order to eliminate water and ensure efficient derivatization. Further, BSTFA derivatives generally form under mild conditions, so addition of heat forces the reaction to completion.

2.5.1.3. Oxycodone. The oxime derivative of oxycodone was prepared by reconstituting the dried extract in 1% hydroxylamine hydrochloride in pyridine solution (50 μL) and

162 incubating at 45 °C for 30 min. MSFTA + 1% TMCS (50 µL)
163 was added; the vial was capped and incubated at 65 °C for
164 20 min.

165 2.6. Gas chromatography–mass spectrometry
166 (GC/MS)

167 For all assays, an Agilent Technologies 6890 gas chromato-
168 graph coupled to a 5975 mass selective detector (MSD) with
169 an inert source, operating in electron impact mode was used
170 for analysis (GC/MS). The gas chromatographic column was
171 a DB-5 MS, 0.25 mm ID, 0.25 µm film thickness, 15 m length
172 (J & W Scientific), and the injection temperature was 250 °C.
173 The purge flow was 50 mL/min for 1 min and the carrier gas
174 was helium. The injection mode was splitless, injection volume
175 2 µL and the operation mode was constant flow at 1.5 mL/min.
176 The transfer line was held at 280 °C, the quadropole at 150 °C,
177 the ion source at 230 °C and the dwell time for all ions was
178 50 ms.

179 Three ions were selected for each drug from the full scan
180 spectra. Fig. 1 a–c show the full scan spectrum of the trimethylsilyl
181 derivative of tramadol, the full scan spectra of meperidine
182 and the full scan spectra of the oxime derivative of oxycodone,
183 respectively. For tramadol, the abundant ions 335.3, 320.2 and
184 245.2 were selected, and each subsequent analysis required the
185 ratio between the quantitative ion (335.3) and the two quali-
186 fier ions to be within ±20% in order to meet the criterion for
187 a positive result. The ion ratios were determined at drug con-
188 centrations of 25 ng/mL for tramadol and meperidine; 20 ng/mL
189 for oxycodone. The ions monitored, ion ratio criteria and the
190 chromatographic oven program for each of the drugs is detailed
191 in Table 1.

192 2.7. Data analysis

193 Calibration using deuterated internal standards was cal-
194 culated using linear regression analysis over a concentration
195 range of 10–100 ng/mL for meperidine and tramadol; and
196 10–80 ng/mL for oxycodone. Peak area ratios of target analytes
197 and their respective deuterated standards were calculated using
198 Agilent DrugQuant ChemStation software. The data were fit to
199 a linear least-squares regression curve forced through the origin.

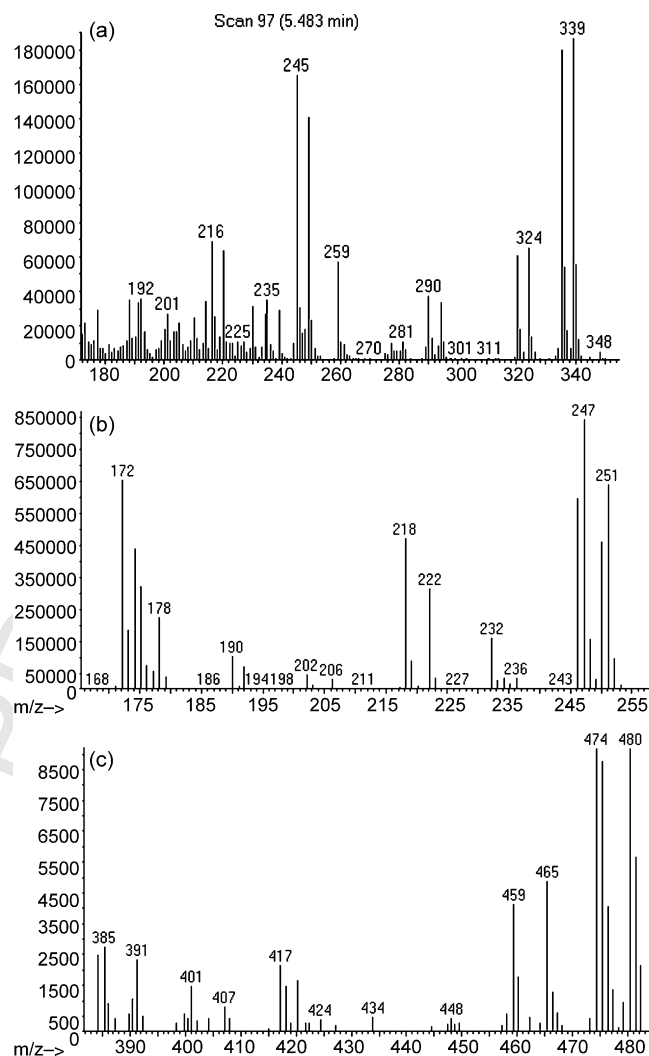


Fig. 1. (a) Full scan spectrum of trimethylsilyl derivative of tramadol. (b) Full scan spectrum of meperidine. (c) Full scan spectrum of oxime derivative of oxycodone.

200 2.8. Selectivity

201 Drug free oral fluid specimens were obtained from volunteers
202 and extracted and analyzed according to the described proce-
203 dures in order to assess interference from the collection buffer
204 with the assays.

Table 1
Chromatographic oven program, selected ions and ion ratio criteria (±20%)

Drug	Oven program	Ions monitored	Ion ratio acceptable range (±20%)
Meperidine	50 °C	d4 251.2^a , 222.2	218/247: 38.7–58.1%
	Ramp at 30 °C/min to 280 °C	247.2^a , 218.2, 172.2	172/247: 54.4–81.7%
Oxycodone	100 °C for 0.5 min;	d6 480.3^a , 391.3	385/474: 8.9–13.3%
	Ramp 10 °C/min to 270 °C	474.3^a , 385.3, 459.3	459/474: 18.1–27.1%
Tramadol	65 °C for 1 min;	d4 339.3^a , 324.2	245/335: 61.7–92.5%
	Ramp 40 °C/min to 200 °C;	335.3^a , 245.2, 320.2	320/335: 33.0–49.6%
	Ramp 15 °C to 230 °C; Ramp 100 °C/min to 290 °C		

^a Quantitative ion in bold type.

In addition, potential interferences from commonly encountered drugs were added to the drug free oral fluid specimens and subjected to the same extraction and analysis procedures. The following drugs were analyzed using the described procedures at a concentration of 200 ng/mL: morphine, 6-acetylmorphine, codeine, hydrocodone, hydromorphone, cocaine, norcocaine, cocaethylene, benzoylecgonine, tetrahydrocannabinol (THC), 9-carboxy-THC, amphetamine, methamphetamine, methylenedioxyamphetamine (MDMA), methylenedioxyamphetamine (MDA), methylenedioxyethylamphetamine (MDEA), pseudoephedrine, phentermine, fluoxetine, sertraline, zolpidem, carisoprodol, methylphenidate, norbuprenorphine, cotinine, methadone, phencyclidine, diazepam, nordiazepam, oxazepam, alprazolam, chlordiazepoxide, bromazepam, temazepam, lorazepam, flurazepam, 7-aminoflunitrazepam, α -hydroxyalprazolam, nitrazepam, triazolam, α -hydroxytriazolam, secobarbital, pentobarbital, butalbital, amobarbital, butabarbital, and phenobarbital.

2.9. Linearity and sensitivity

The linearity of the assays was established with four calibration points, excluding the drug free matrix. The sensitivity of the method was determined by establishing the limit of quantitation (LOQ) defined as the lowest concentration detectable with a signal to noise (S:N) ratio of at least 5 and retention time within 0.2 min of the calibration standard. Since all values are quantitated, the limit of detection was not determined. Any specimens found to be beyond the linear range of the assay were diluted so as to be accurately quantitated within the linear portion of the curve.

2.10. Precision

Inter and intra-day assay precision of the assays were determined at the calibration point of 25 ng/mL for meperidine and tramadol; 20 ng/mL for oxycodone. Intra-day data were obtained from 5 analyses performed on 1 day; inter-day data were obtained by analyzing a total of 10 specimens over 5 days (2 samples per day for 5 days; $n = 10$).

2.11. Extraction efficiency from the pad

One of the issues associated with oral fluid analysis is recovery of drug from a collection pad if a device is used. Extraction efficiency 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. A collection pad was placed into the fluid until the volume adequacy indicator turned blue showing that 1 mL ($\pm 10\%$) of oral fluid had been absorbed. The pads were then placed into the QuantisalTM 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 using serum separators, and an aliquot (1 mL) of the specimens was analyzed according to the described procedures. The procedure was repeated six times for each drug.

2.12. Stability

The stability of the drugs in collection devices was determined in duplicate. Meperidine, tramadol and oxycodone were added to the QuantisalTM buffer, and allowed to remain at room temperature, and in the refrigerator (4 °C) for ten days. Aliquots of the buffer were analyzed after 1, 3, 6, 8 and 10 days. Stability of analytes after derivatization was also examined. Autosampler vials containing oral fluid extracts were analyzed, then stored at room temperature for 24 and 48 h after which time they were re-analyzed. The concentrations after 24 and 48 h were compared to same day analysis ($n = 3$).

2.13. Application to authentic specimens

As part of various on-going research studies, our laboratory receives oral fluid specimens collected using the QuantisalTM device, as well as paired blood specimens. All the oral fluid samples are screened for a panel of drugs, including the pain medications described, and if positive, are confirmed using the procedures validated in this report.

3. Results and discussion

3.1. Method development

Meperidine, tramadol and oxycodone are prescription medications often abused by medical professionals, and are therefore included in drug test panels by many laboratories [7]. The development of simple chromatographic assays for their detection in oral fluid, to support screening techniques is reported. While these drugs have been detected in other matrices [8,9], the increasing utility of saliva for drug analysis makes development of laboratory procedures necessary and timely.

3.2. Method validation

The chromatographic procedures developed for meperidine, tramadol and oxycodone were validated according to accepted protocols. The limit of quantitation for each drug, and calibration curve data were determined as described in the Section 2. Linearity was obtained with an average correlation coefficient for all the drugs of >0.99 over the dynamic range from 10 to 100 ng/mL of oral fluid (Table 2).

Oral fluid specimens collected from drug free individuals showed no interference with any of the assays, which was not unexpected, since it is unlikely these drugs are similar to endogenous substances in oral fluid. For exogenous interferences, commonly encountered drugs of abuse were studied as described in the Section 2. No chromatographic interference was observed in the channels of these SIM assays.

The inter-day (between day) and intra-day (same day) precision of the assays was determined using replicate analyses as described. The intra-day precision for all assays was less than 8%; inter-day less than 6%. The p -variance in the mean values from the 2 days was determined using a two-tailed T -test with an assumed two sample equal variance (homoscedastic). The

Table 2
Limits of quantitation, linearity, and calibration curve equations, forced through the origin, for meperidine, oxycodone, and tramadol in oral fluid

Analyte	Internal standard	Limit of quantitation (ng/mL)	Equation (mean SD)	Correlation (r^2)	Linearity range (ng/mL)
Meperidine	Meperidine- <i>d4</i>	10	$y = 0.0196x$	0.999	10-100
Oxycodone	Oxycodone- <i>d6</i>	10	$y = 0.0132x$	0.997	10-80
Tramadol	Tramadol- <i>d4</i>	10	$y = 0.0190x$	0.999	10-100

Table 3
Inter-day and intra-day precision for the determination of meperidine, oxycodone and tramadol in oral fluid

Inter-day ($n = 10$)					
Drug	Expected concentration (ng/mL)	Observed concentration (mean \pm SD) (ng/mL)	Precision (%)	p -variance (two-tailed T -test)	
Meperidine	25	25.32 \pm 0.628	2.48	0.577	
Oxycodone	20	20.74 \pm 1.202	5.80	0.663	
Tramadol	25	25.65 \pm 0.627	2.44	0.817	
Intra-day ($n = 5$)					
Drug	Expected concentration (ng/mL)	Observed concentration (mean \pm SD) (ng/mL)	Precision (%)		
Meperidine	25	25.72 \pm 0.60	2.33		
Oxycodone	20	23.08 \pm 1.75	7.61		
Tramadol	25	25.88 \pm 0.25	1.00		

probability of the quantitative value being true was assessed by the determined statistical value. If the p -value is less than 0.05, the probability that the observed value is true is low; if the p -value is higher than 0.05, then the probability that the observed concentration is true is likely (Table 3).

The recovery of the drugs from the collection pad has been a source of error in oral fluid testing. Several studies have addressed this issue, and all have focused on the major drugs of abuse such as cocaine and marijuana [10,11]. Table 4 shows the recovery of these prescription medications from the collection pad into the transportation buffer. The overall efficiency

of the collection system was determined to be 86.7, 87.7 and 96.6% for meperidine, tramadol and oxycodone, respectively ($n = 6$).

Finally, the stability of the drugs in the collection system and the stability of the extracts were assessed and the results are given in Fig. 2 (drug stability in QuantisalTM) and Table 5 (extract stability). The drugs were extremely stable over a period of 10 days, both in refrigerated conditions and at room temperature.

Table 4
Recovery of meperidine, oxycodone and tramadol from the QuantisalTM collection device ($n = 6$), overnight at room temperature (RT)

	Meperidine	Oxycodone	Tramadol
Concentration of drug in buffer: no pad (ng/mL)	25.9	21.1	24.3
Concentration of drug in buffer collected with pad; placed in Quantisal TM device overnight (RT)			
Replicate 1	22.2	23.9	21.1
2	22.1	19.9	21.1
3	22.6	19.9	21.2
4	22.2	19.3	21.0
5	23.0	20.8	22.4
6	22.7	18.6	21.1
Mean	22.46	20.4	21.31
SD	0.356	1.86	0.53
CV (%)	1.58	9.13	2.50
Drug recovery (%)	86.7	96.6	87.7

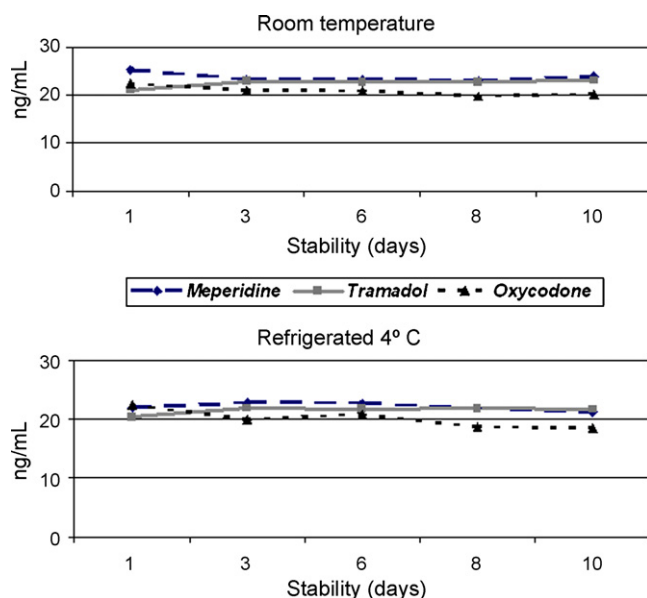


Fig. 2. Stability of drugs in QuantisalTM collection device at room temperature and refrigerated for 10 days (4 °C).

Table 5
Stability of extracted samples at room temperature: meperidine fortified at 25 ng/mL; tramadol 25 ng/mL; oxycodone 20 ng/mL

Extract	24 h			48 h		
	Meperidine	Tramadol	Oxycodone	Meperidine	Tramadol	Oxycodone
1	26.3	27.3	21.3	25.8	24.2	20.5
2	25.2	27.5	21.2	25.3	24.4	20.3
3	25.5	28.1	20.3	25.6	24.7	19.7

Table 6
Concentration of tramadol detected from paired blood-oral fluid specimens received into the laboratory

Specimen type	Blood (ng/mL)	Oral fluid (ng/mL)
Drug (s) detected	Tramadol 19 (Desmethyltramadol 28) Tramadol 130 (Desmethyltramadol 22)	Tramadol 160 Tramadol 1175

The extracts were stable for at least 2 days when kept at room temperature in the instrument rack.

3.3. Authentic specimens

For tramadol, blood samples received into the laboratory had corresponding oral fluid collections. These were analyzed according to the described procedures. The concentration in both matrices is shown in Table 6 and a chromatogram of one of the oral fluid specimens is shown in Fig. 3. Tramadol appears to have a much higher concentration in oral fluid than in the corresponding blood, making it potentially viable for therapeutic drug monitoring using oral fluid. However, the blood:oral fluid partition may be affected by the hydration status of an individual. We were unable to acquire authentic specimens for oxycodone or meperidine analysis from drug users; however, our method successfully allows their detection at the concentrations reported.

4. Conclusions

The determination of prescription medications: meperidine, tramadol and oxycodone, in oral fluid samples collected using the Quantisal™ device is described. The recovery from the oral fluid collection device was greater than 85% for all drugs; the GC/MS procedures are reproducible, robust and precise. The methods are useful for the analysis of prescription pain medications in saliva, and are easily incorporated into routine testing for a laboratory.

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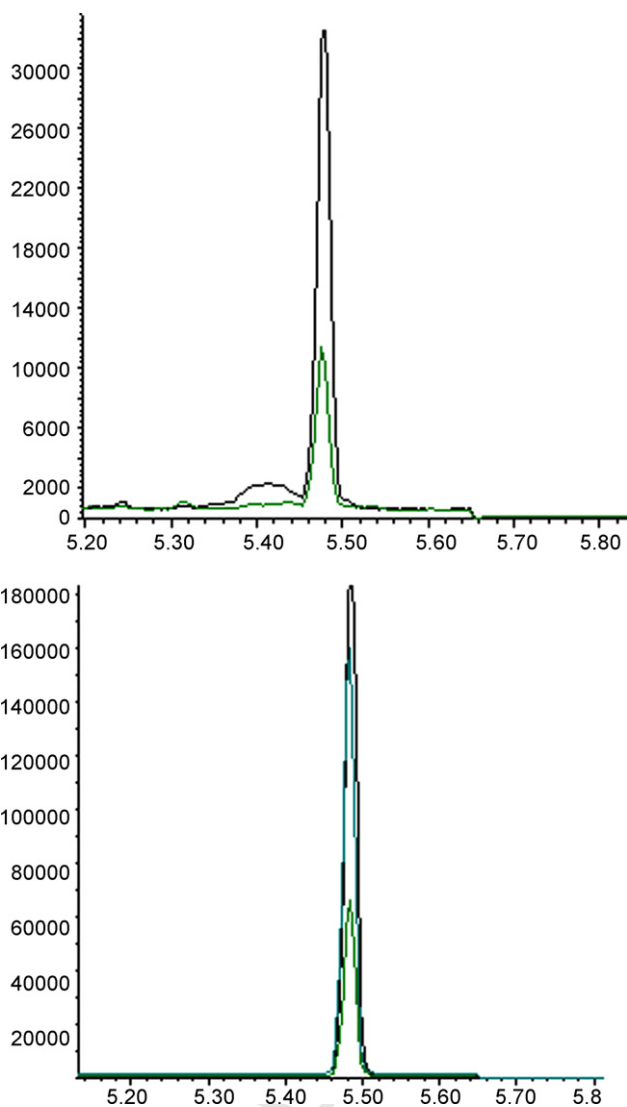


Fig. 3. (a) Selected ion chromatogram of internal standard (d4-tramadol) following solid phase extraction and derivatization: ions 339.3, 324.3. (b) Selected ion chromatogram of oral fluid specimen containing tramadol (1175 ng/mL) following solid phase extraction and derivatization: ions 335.3, 320.3, 245.2.