Validation of liver oxymorphone analysis using LC/MS/MS: Comparison with associated blood concentrations in fatal intoxications

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Abstract

The objective of this study was to develop and validate a reliable method for the quantification of oxymorphone in liver using LC/MS/MS. Oxymorphone calibrators ranging from 0.5 – 500 µg/kg in liver and four control samples ranging from 6 – 300 µg/kg were prepared in drug and ethanol negative matrix. Solid phase extraction was employed to process the calibrators, controls and samples. One quantification and two target transitions were monitored for both oxymorphone and the internal standard.

Validation studies:

- Selectivity
- Calibration model
- Accuracy
- Limit of quantification
- Carryover
- Interference
- Linear range
- Precision
- Limit of detection
- Ion suppression/enhancement

Case studies:

Liver and blood specimens from cases were analyzed by this validated method, and the values were compared.

ER 5 mg tablet **ER 7.5 mg tablet** **ER 10 mg tablet** **ER 15 mg tablet**

ER 20 mg tablet **ER 30 mg tablet** **ER 40 mg tablet**

Figure 1. Oxymorphone extended-release tablets.
http://www.justice.gov/dea/pubs/states/phila_opana.pdf

Introduction

In 2006, Endo Pharmaceuticals released oxymorphone (OM) as Opana® in both immediate-release (5 and 10 mg) and extended-release (5, 10, 20 and 40 mg) tablets for the treatment of moderate to severe pain (1,2). Although only on the market as an oral formulation for a short time, OM is currently the most common opioid present in fatal accidental overdose deaths in West Virginia. The majority of these deaths also involve other central nervous system depressants, most often benzodiazepines, muscle relaxants, anti-histamines and alcohol.

Oxymorphone is a semi-synthetic mu-opioid receptor agonist which has 6-8 times the analgesic potency of morphine. OM is also often present in postmortem samples as a minor, but active metabolite of oxycodone, an *O*-demethylation reaction catalyzed by CYP3A4 and CYP2D6 (1,2,3,4). Oral bioavailability of OM has been determined to be low at about 10% (1). However, in cases of fatal overdose, insufflation (snorting) of crushed tablets is often the route of administration.

Oxymorphone is not highly bound to serum proteins. The median oral clearance of OM is 18 to 26 L/min, and it exhibits a volume of distribution of approximately 3 L/kg. OM is metabolized in the liver to form two primary metabolites, oxymorphone-3-glucuronide and 6-hydroxy-oxymorphone (3,5). Less than 1% of the administered dose is excreted unchanged in urine. Approximately 35% of the dose is excreted in urine as oxymorphone-3-glucuronide, and 0.5% is excreted as 6-hydroxy-oxymorphone (3,5).

Oxymorphone's ability to generate feelings of euphoria and relaxation make it a target for abuse (2). The West Virginia Office of the Chief Medical Examiner has seen a dramatic increase in incidence of accidental deaths related to Opana® since 2007. As a result, it was determined that a rigorously validated method for the analysis of OM was needed. Liver is a commonly analyzed alternate specimen when blood is not available and thus was chosen as the matrix for this validation.

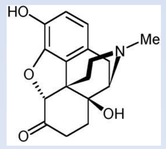

Figure 2. Structure of oxymorphone
http://opioids.com/oxymorphone/structure.html

Materials

- Methanolic standards of OM (1.0 mg/mL) and d3-OM (0.1 mg/mL) – Cerilliant (Round Rock, TX)
- All solvents and reagents were of ACS grade or better.
- Trace-B SPE columns - SPE Ware (Baldwin Park, CA)
- Negative liver - autopsy specimens, 1:4 dil. with deionized water
- Blank whole blood - UTAK Laboratories, Inc. (Valencia, CA)
- Acquity® UPLC HSS T3 2.1 x 100 mm,1.8 µm, column - Waters (Milford, MA)

Methods

Solid-Phase Extraction Procedure

- 0.5 mL aliquot of liver homogenate + 5 µL of 10 µg/mL (50 ng) internal standard, d3-oxymorphone,
- Add 2.0 mL of 0.1 M phosphate buffer (pH 6.0)
- Vortex and centrifuge 10 minutes at 2400 x g
- Condition Trace-B, mixed mode, SPE columns sequentially with
  - 2.0 mL ethyl acetate, 2.0 mL methanol, 1.0 mL deionized water
- Add sample and pass through utilizing positive pressure
- Wash cartridges sequentially with
  - 1.0 mL deionized water, 1.0 mL 0.1 M HCl, 1.0 mL methanol, 1.0 mL ethyl acetate
- Fully dry column
- Elute OM and d3-OM with 2.0 mL of ethyl acetate/isopropanol/ammonium hydroxide (70:20:5)
- Evaporate to dryness under nitrogen at 40°C
- Reconstitute in 100 µL water/acetonitrile/formic acid (99:1:0.1)
- Transfer to high recovery autosampler vial for LC/MS/MS analysis.

Liquid Chromatography-Tandem Mass Spectrometry

All chromatography was performed using a Waters Acquity® ultra-performance liquid chromatography (UPLC) system with a Tandem Quadrupole Detector (TQD). Instrument parameters are found in Table 1. Table 2 contains the inlet method and linear gradient used.

Table 1. Instrument parameters

Mobile Phase A	Water + 0.1% Formic acid
Mobile Phase B	Acetonitrile + 0.1% Formic acid
Column Temp	40°C
Mode	Pos ESI
Source Temp	150°C
Desolvation Temp	400°C
Desolvation Gas flow	800 L/h
Cone gas	11 L/h

Table 2. Inlet method parameters

Time (min)	Flow rate (mL/min)	%A	%B	Curve
Initial	0.450	99	1	Initial
0.50	0.450	99	1	6
2.00	0.450	70	30	6
3.00	0.450	0	100	6
4.00	0.450	0	100	6
4.10	0.450	99	1	6
5.00	0.450	99	1	6

One quantification transition and two target transitions were monitored for both OM and the deuterated internal standard. Only the quantification transition area was used for d3-OM in the data analysis. The details of the MRM transitions are shown in Table 3.

Table 3. MS/MS method parameters

	Compound	Precursor	Product	Cone (V)	Collision (V)
Quant	**OM**	**302.25**	**284.195**	**40**	**24**
Qual	OM	302.25	227.083	40	32
Qual	OM	302.25	242.175	40	32
Quant	**OM d3**	**305.25**	**230.085**	**40**	**34**
Qual	OM d3	305.25	245.175	40	30
Qual	OM d3	305.25	287.197	40	20

Results

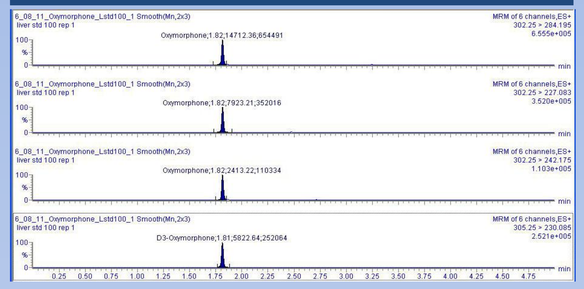

Figure 3. Chromatograms of 100 µg/kg oxymorphone calibrator.

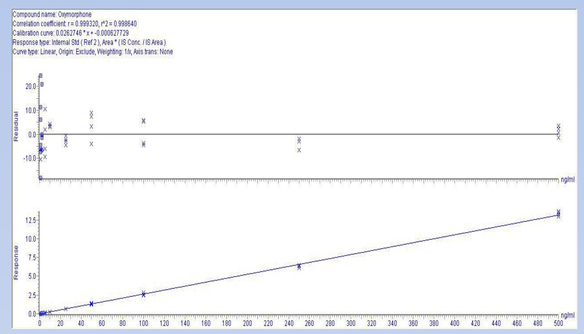

Figure 4. Calibration curve of Oxymorphone in Liver homogenate.

Table 4. Validation parameters

Parameter	Result
Accuracy	Intra day > 90%; Interday > 91%
Calibration Range	5 - 500 µg/kg
Calibration Model	Linear with 1/X weighting
Carryover	< 1% of signal of lowest calibrator
Interference Studies	1. None detected from matrix 2. No cross contribution detected for OM or d3-OM to each other 3. All quant and primary ion ratios for replicates of 24 cmpds, but secondary ion ratios failed for 1 rep of 4 cmpds
Matrix Effects	Ion suppression 68% at 5 µg/kg, 62% at 100 µg/kg
Limit of Detection	5 µg/kg
Lower Limit of Quantification	5 µg/kg
Precision	Intraday CV < 7%; Interday CV < 10%

Table 5. Accuracy and Precision validation data.

		Intraday (n=4)		Interday (n=20)	
	Conc. (µg/kg or µg/L)	**CV (%)**	**Bias (%)**	**CV (%)**	**Bias (%)**
Liver	6	6.58	-9.97	9.41	-3.51
	15	2.72	-7.40	5.79	-7.91
	60	1.78	-7.85	6.94	-8.70
	300	2.92	-3.60	6.68	-6.09
Blood curve Liver QC	6	6.93	-11.03	5.54	-7.73
	15	1.64	-7.79	4.78	-8.35
	60	2.67	-8.28	5.47	-7.30
	300	2.92	-0.81	4.73	-3.68

Table 6. Case data for Oxymorphone only intoxication fatalities.

Case	Subject	OM script	OM Conc. in Peripheral blood (µg/L)	OM Conc. in Liver (µg/kg)	History/Scene Findings
1	21m	N	108	1430	Known to snort pills.
2	32m	N	55.4	288	Reported to have been crushing Opana® and snorting it
3	38f	N	142	350	Hx of chronic back and neck pain. Hx of snorting/injecting behavior. Previous OM overdose..
4	22m	N	22.4	122	Found with nasal and mouth pluming. Half of an Opana® was found in his pants pocket.
5	48m	Y	122	1740	Hx of drug abuse
6	42f	N	64.2	260	Hx of crushing and snorting pills. Brown mucous coming from mouth.
7	28f	N	155	140	Found with Opana® 40mg tablet on person.
8	37m	N	23.9	59.7	
9	25m	N	33.0	77.7	In preceding 24 hours, had obtained unknown yellow pills
10	35m	N	24.4	57.7	
11	27m	N	35.3	48.4	Given 1/4 tablet of OM by friend and ingested orally.

Discussion and Conclusions

Negative matrix samples, negative matrix spiked with OM and negative matrix spiked with d3-OM were analyzed for interference and cross-contribution, but none was found. Forensic toxicology cases often contain substances that may interfere with analysis of the analyte of interest. Twenty-four commonly encountered substances were examined using replicate analyses. The quantification ion and primary ion ratio were all within the acceptable range. Secondary ion ratios failed on a single replicate for 4 of the 24 compounds. It is uncertain if the failed ratios can be attributed to interference or matrix effects at the OM concentration tested (6 µg/kg).

Calibration model and linearity were determined experimentally, as shown in Figure 4 and Table 4. The 1/X weighting was selected to compensate for the heteroscedasticity associated with a calibration range of this magnitude. The lower limits of quantification (5 µg/kg) and detection (5 µg/kg) were determined experimentally with acceptable accuracy and precision across all replicates. The upper limit of quantification was set administratively (500 µg/kg). Accuracy and precision determinations are shown in Table 5. Carryover was tested experimentally at 500 µg/kg, and no peak area was found to be greater than 1% of the peak area of the lowest calibrator.

Significant ion suppression of OM was observed in liver homogenate at 5 µg/kg (68%) and 100 µg/kg (62%) using the approach of Matuszewski, et. al. (6). Because the LLOQ and LOD met the current needs of the laboratory, the matrix effects were not a hindrance to the analysis of OM in liver homogenate.

Thirty-three case samples were successfully analyzed using this validated method. In 11 deaths attributed solely to oxymorphone acute intoxication, blood concentrations ranged from 22.4 to 155 ng/mL. Liver concentrations in these deaths ranged from 48.4 to 1430 µg/kg. In all but one death (Case 7) liver concentration exceeded the blood concentration. Plotting these data, no correlation was observed between oxymorphone blood and liver concentrations.

Acknowledgements

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