MEMBER THE TEXAS STATE UNIVERSITY SYSTEM

Identification of Desomorphine in Urine

Jessica Winborn, BS; Stephanie Basiliere, BS; Sarah Kerrigan, PhD*
Department of Forensic Science, Sam Houston State University, Huntsville, TX 77340

SOFT 2018 MINNEAPOLIS, MN

ABSTRACT

Desomorphine, a semi-synthetic opioid that is structurally similar to morphine, is a major component of the drug Krokodil, the use of which has been associated with severe side effects, including necrotic ulcers and osteonecrosis. In this study we describe a sensitive method for the identification of desomorphine in urine utilizing solid phase extraction and liquid chromatography-quadrupole/time of flight-mass spectrometry (LC-Q/TOF-MS). The extraction efficiency was 90% and the limits of detection and quantification were 0.5 ng/mL. Bias ranged from -4-0% and intra- and inter-assay CVs were 3-5% (n=3) and 4-7% (n=15) respectively. Ion suppression averaged -1% at 20 ng/mL and -7% at 400 ng/mL. The calibration ranged was 0.5-500 ng/mL using a weighted (1/x) quadratic model. No qualitative or quantitative interferences were observed using sixty-six common drugs, including twenty-four structurally related opioids. This is the first LC-Q/TOF-MS method capable of detecting desomorphine at forensically relevant concentrations.

INTRODUCTION

Desomorphine is a semi-synthetic opioid that is structurally related to morphine. Its analgesic potency is reported to be ten-fold greater than morphine, with a faster onset of action but a shorter duration of action (1). It is the major component of Krokodil, which has been used as a substitute for heroin since the early 2000s (2). Krokodil is commonly synthesized from codeine using red phosphorus and hydroiodic acid and as it is abused intravenously. Residual precursor chemicals can cause severe side effects, including skin necrosis, osteonecrosis and limb amputation. Published case reports indicate that there is often a significant delay between actual drug use and medical treatment associated with its necrotic side effects. Due to these delays, and the absence of toxicological tests to detect its use, desomorphine is rarely identified in suspected Krokodil users.

An evaluation of commercially available enzyme-linked immunosorbent assays (ELISAs) targeting opioids indicated that cross-reactivity towards desomorphine was highly variable (<2.5-77%) (3). Immunoassays with low cross-reactivity towards the drug may not be effective for identification purposes. The metabolism of desomorphine has been studied and is known to involve glucuronidation, sulfation, *N*-demethylation, hydroxylation, and *N*-oxide formation (4,5). Due to the absence of commercially available metabolite standards, however, analytical methods in urine must target the parent drug. Su *et al* developed a quantitative method to identify desomorphine in urine using gas chromatography-mass spectrometry (GC-MS) but the limit of quantitation (250 ng/g) was not sufficient for forensic needs (6). We previously described quantitative methods for desomorphine in urine using both GC-MS (7) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) (8). We now describe a high resolution mass spectrometry (HRMS) assay using LC-Q/TOF-MS.

MATERIALS & METHODS

Extraction

Calibrators and controls were prepared at 0.5, 1, 2, 5, 25, 75, 150, 250, 350 and 500 ng/mL by fortifying 0.5 mL of urine with desomorphine and desomorphine-D_3 (25 ng/mL). After the addition of 1 mL of 0.1 M HCl, samples were transferred to PolyChrom Clin II SPE columns (3 cc, 35 mg) and washed successively with 1 mL of deionized water, 0.1 M HCl, methanol, and ethyl acetate. After drying under vacuum for 5 minutes, desomorphine was eluted with 2 volumes (0.5 mL) of 4% ammonium hydroxide in ethyl acetate. Extracts were evaporated under nitrogen and reconstituted with 30 µL of mobile phase A and B (92:8). An aliquot (2 µL) was injected into the LC-Q/TOF-MS for analysis.

Instrumentation

An Agilent 1290 Infinity Binary LC System and a 6530 Accurate-Mass Q/TOF-MS equipped with an electrospray ionization (ESI) source was operated in positive mode. Separation was achieved using a Poroshell EC-18 column (2.1 x 100 mm, 2.7 µm) with a matching guard column (2.1 x 5 mm, 2.7 µm) maintained at 35°C. The mobile phase consisted of 0.1% formic acid in deionized water (A) and 0.1% formic acid in acetonitrile with the elution profile: 8% B, 0-2 min (0.3 mL/min); 20% B, 2-6 min (0.3 mL/min); 90% B, 6-6.5 min (0.4 ml/min); 8% B, 6.5-7 min (0.4 mL/min). Data was acquired in targeted mode with the following parameters: gas temperature, 350°C (10 L/min); sheath gas temperature, 400°C (10 L/min); nebulizer, 20 psi; capillary voltage, 2500 V; nozzle voltage, 0 V; fragmentor, 150 V; skimmer, 65 V. The MS and MS/MS scan rates were 5 spectra/sec and the ion transitions are detailed in **Table 1**.

Validation

The extraction efficiency, calibration model, bias, precision, limit of detection (LOD), limit of quantitation (LOQ), interferences and carryover were assessed in accordance with published guidelines (9). Common drugs (n=66) including 24 structurally related opioids were included in the interference study. Interferences from other drugs were evaluated at 10 and 100-fold excess concentrations (relative to desomorphine). Interferences from matrix and internal standard were also evaluated in addition to processed sample stability. The limits of detection and quantitation were determined using three independent urine sources fortified with desomorphine and IS analyzed in duplicate over three days. Bias and precision were evaluated in urine at 2, 250 and 400 ng/mL, using three independent sources of pooled fortified matrix analyzed in triplicate over five days. The calibration model was established using five independent runs using ten non-zero calibrators and carryover was evaluated in both blank matrix and negative controls analyzed after the highest calibrator. Matrix effects were evaluated at 20 and 400 ng/mL using ten independent urine sources extracted in the absence of desomorphine and fortified post extraction. Ion suppression was calculated by comparison with the equivalent concentration in mobile phase. Processed sample stability was evaluated at 24 hr after storage in the autosampler (4°C) at 2, 250 and 400 ng/mL (n=3).

ACKNOWLEGEMENTS

This work was supported by Award No. 2015-R2-CX-0031 awarded by the National Institute of Justice, Office of Justice Programs, U.S. Department of Justice. The opinions, findings, and conclusions or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect those of the Department of Justice.

RESULTS & DISCUSSION

The SPE extraction was previously optimized (7) and the extraction efficiency found to be 90%. Three ions transitions were selected for both desomorphine and the IS (**Table 1**). An extracted ion chromatogram (EIC) at the LOQ (0.5 ng/mL) is shown in **Figure 1** and **Table 2** summarizes the results of the method validation. A weighted (1/x) calibration model was chosen for quantification (**Figure 2**). *F*-tests indicated no significant difference between unweighted and weighted (1/x) calibration models but standardized residuals indicated weighted models (1/x) were superior at low concentrations and a quadratic weighted (1/x) model was superior over the linear model at elevated concentrations (**Figure 3**). No interferences or carryover were present, a processed samples were stable for 24 hr at 4°C.

Table 1. Ion transitions, collision energies and retention times for desomorphine and desomorphine-D_3.

Compound	MS Transition (m/z)	CE (eV)	RT (min)
Desomorphine	272.1645 > 167.0837 272.1645 > 195.0784 272.1645 > 152.0604	45	4.4
Desomorphine-D_3	275.1833 > 167.0837 275.1833 > 195.0784 275.1833 > 152.0604	45	4.4

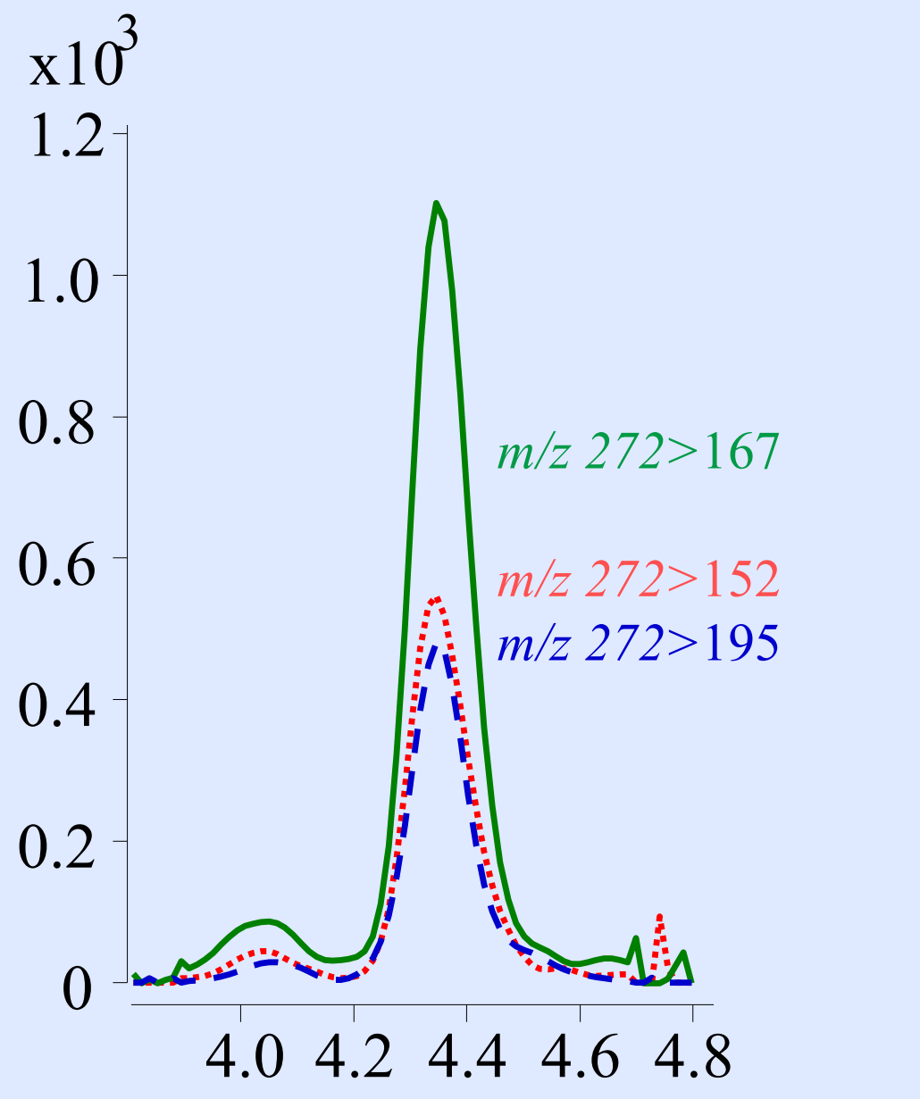

Figure 1. EICs of desomorphine in urine at 0.5 ng/mL (LOQ).

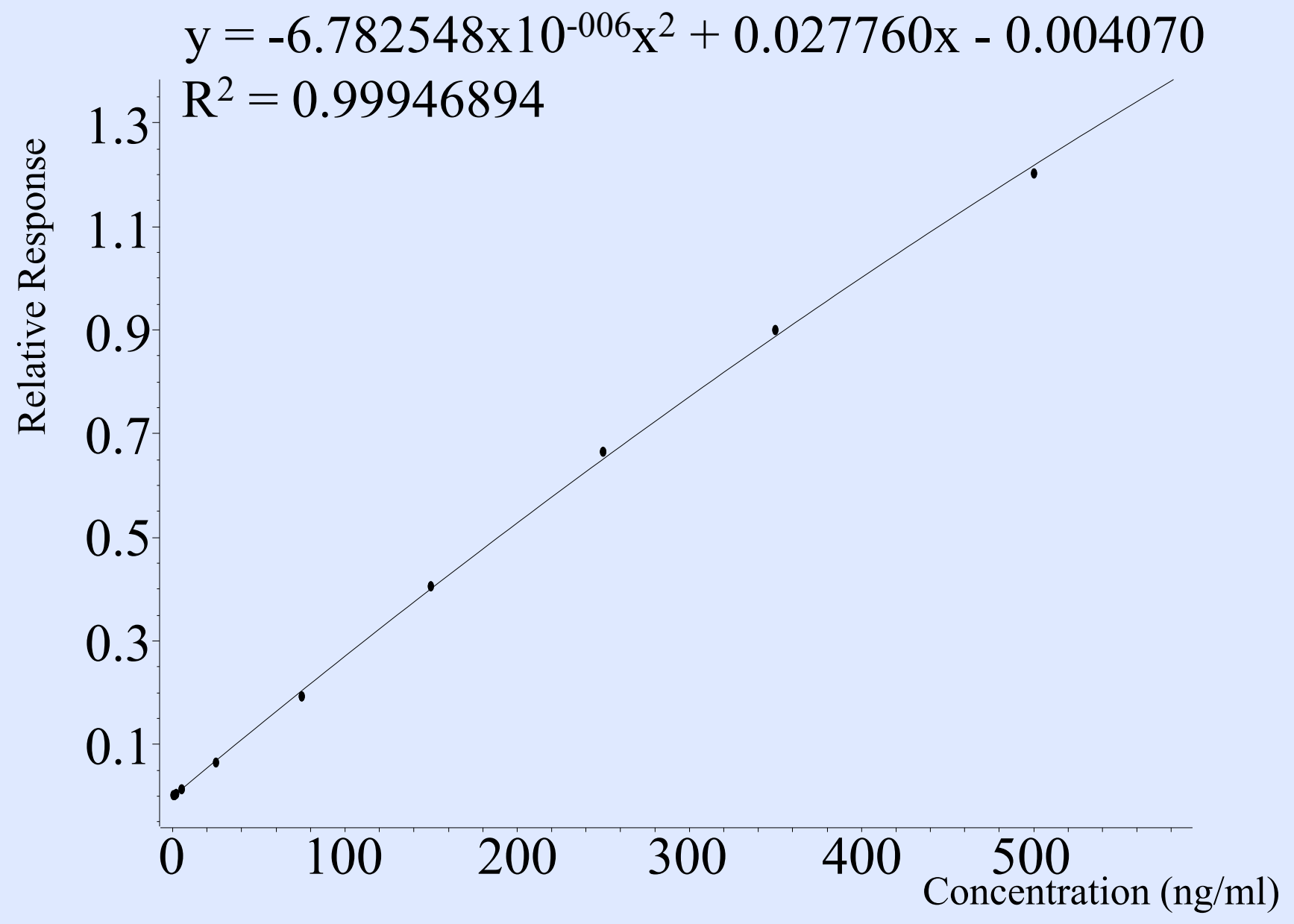

Figure 2. A weighted (1/x) quadratic calibration model used for the quantification of desomorphine.

Table 2. Validation parameters for the identification and quantification of desomorphine in urine using LC-Q/TOF-MS.

Validation Parameters	Results
LOD	0.5 ng/mL
LOQ	0.5 ng/mL
Calibration Model	0.5 to 500 ng/mL (Quadratic, weighted 1/x)
Carryover	No carryover at 500 ng/mL
Precision	3 to 5% (Intra-assay CV; n=3) 4 to 7% (Inter-assay CV; n=15)
Bias	-4 to 0% (n=15)
Interferences	None detected
Matrix Effect Range (Mean) (n=10)	-22 to 11% (-1%) at 20 ng/mL -22 to 2% (-7%) at 400 ng/mL

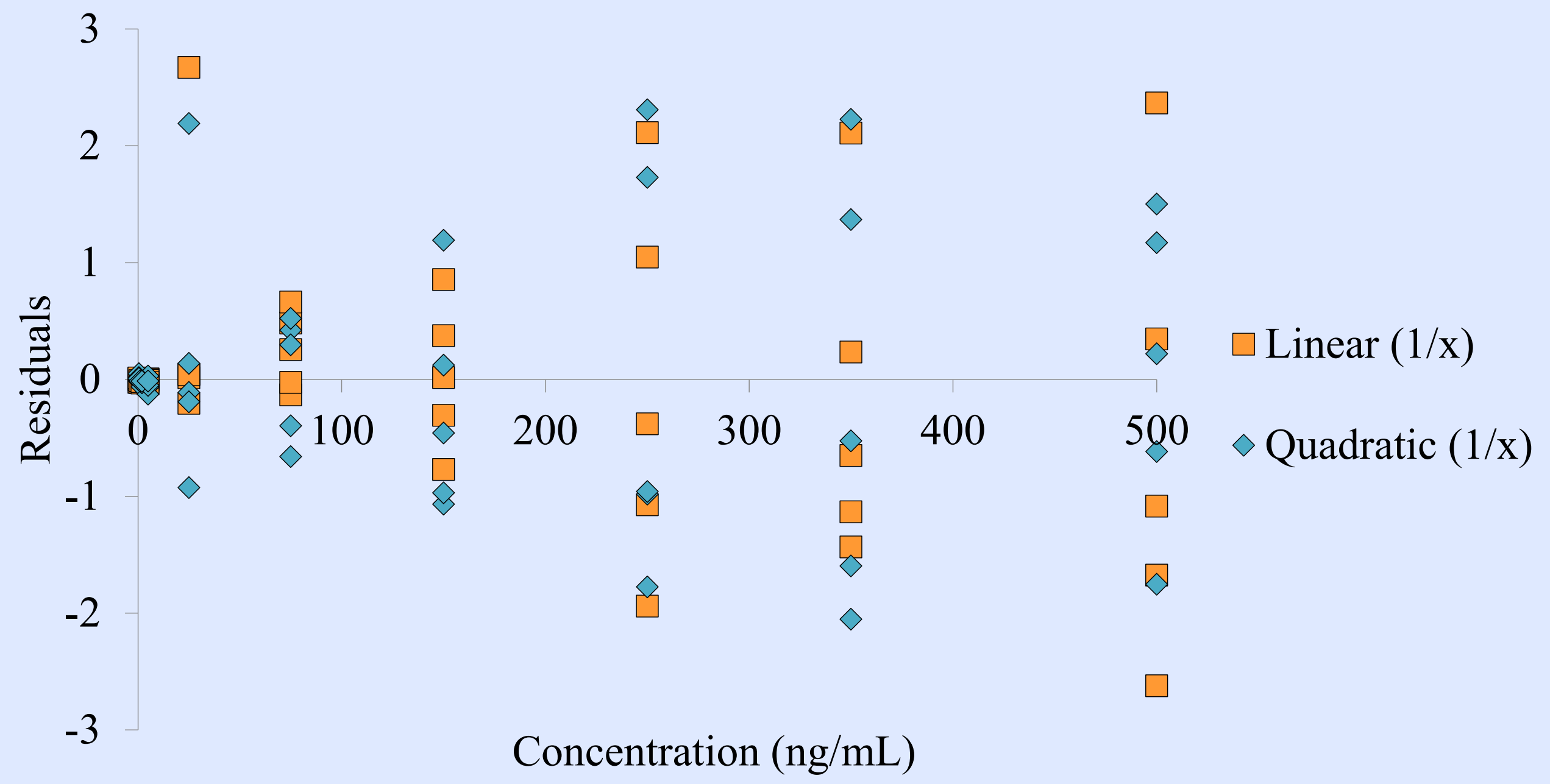

Figure 3. Standardized residual plots of weighted (1/x) linear and quadratic models.

CONCLUSIONS

Krokodil use continues to be self-reported by drug users but analytically confirmed case reports in the literature are scarce. LC-Q/TOF-MS was used to quantitate desomorphine in urine at forensically relevant concentrations to facilitate its identification. This is the first method to utilize LC-Q/TOF-MS to analyze desomorphine in a biological matrix.

REFERENCES

1. Eddy, N.B., Halback, H., Braenden, O.J. (1957) Synthetic substances with morphine-like effect: Clinical experience: potency, side-effects, addiction liability. *Bulletin of the World Health Organization*, 17, 569-863.
2. Gahr, M., Freudenmann, R.W., Hiemke, C., Gunst, I.M., Connemann, B.J., Schöndfeldt-Lecuona, C. (2012) Desomorphine goes “Crocodile”. *Journal of Addictive Diseases*, 31, 407-412.
3. Winborn, J., Kerrigan, S. (2017) Desomorphine screening using commercial enzyme-linked immunosorbent assays. *Journal of Analytical Toxicology*, 41, 455-460.
4. Winborn, J., Haines, D., Kerrigan, S. (2018) In vitro metabolism of desomorphine. *Forensic Science International*, 289, 140-149.
5. Richter, L.H.J., Kaminski, Y.R., Noor, F., Meyer, M.R., Maurer, H.H. (2016) Metabolic fate of desomorphine elucidated using rat urine, pooled human liver preparations, and human hepatocyte cultures as well as its detectability using standard urine screening approaches. *Analytical and Bioanalytical Chemistry*, 408, 6283-6294.
6. Su, C.J., Srimurugan, S., Chen, C., Shu, H.C. (2011) Sol-gel titania-coated needles for solid phase dynamic extraction-GC/MS analysis of desomorphine and desocodeine. *Analytical Sciences*, 27, 1107-1107.
7. Winborn, J., Kerrigan, S. (2018) Quantitative analysis of desomorphine in blood and urine using solid phase extraction and gas chromatography-mass spectrometry. *Journal of Chromatography B* (In press).
8. Winborn, J., Kerrigan, S. (2018) Analysis of desomorphine in urine using liquid chromatography-tandem mass spectrometry. *Journal of Analytical Toxicology* (Submitted).
9. Scientific Working Group for Forensic Toxicology. Scientific Working Group for Forensic Toxicology (SWGTOX) standard practices for method validation in forensic toxicology. *Journal of Analytical Toxicology*, 37, 452-474.