Journal of Chromatography B, 925 (2013) 40–45

Contents lists available at SciVerse ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Short communication

Quantitative method for analysis of hydrocodone, hydromorphone and norhydrocodone in human plasma by liquid chromatography–tandem mass spectrometry☆

Sandra Valtier[a,*], Robert L. Mueck[a], Vikhyat S. Bebarta[b]

[a] Clinical Research Division, 59th Medical Wing, Lackland AFB, TX, United States
[b] Medical Toxicology, Department of Emergency Medicine, San Antonio Military Medical Center, San Antonio, TX, United States

ARTICLE INFO

Article history:
Received 25 September 2012
Accepted 25 February 2013
Available online 1 March 2013

Keywords:
Hydrocodone
Hydromorphone
Norhydrocodone
LC–MS–MS
Plasma

ABSTRACT

A selective, sensitive and accurate high-performance liquid chromatography–tandem mass spectrometry (LC–MS–MS) method for the quantitation of hydrocodone, hydromorphone and norhydrocodone in human plasma was developed. The internal standard stock solution comprised of hydrocodone-d6, hydromorphone-d6 and norhydrocodone-d3 was added to 0.5 mL plasma samples. Samples were extracted using a copolymeric sorbent (mixed mode) solid phase extraction (SPE) column. Chromatographic separation was carried out using a reversed-phase C18 analytical column with a gradient mobile phase consisting of solvent A = 5% acetonitrile with 0.1% formic acid and solvent B = 100% acetonitrile. MS analysis was performed using positive electrospray ionization (ESI) in multiple reaction monitoring (MRM) mode. Linearity was established over the range 1–100 ng/mL with correlation coefficients ≥0.998 for all three analytes. The coefficient of variation (CV) of intra-day samples was ≤5.6% at 10 ng/mL. The precision of inter-day (6 days) samples resulted in CVs ≤8.1% at concentrations tested at 2.5, 10 and 25 ng/mL for all three analytes. The lower limit of quantification (LOQ) was 1.0 ng/mL with signal-to-noise (S/N) ratio >10, the limit of detection (LOD) was 0.25 ng/mL with S/N ratio >3 for the drug and its metabolites. Dilution effects, extraction recovery, stability, interference, carryover and ion suppression were also evaluated. This method was successfully applied to human subject plasma samples in support of a hydrocodone pharmacokinetic study.

Published by Elsevier B.V.

1. Introduction

There has been a substantial increase in prescribing and misuse of the opioid pain medication, hydrocodone. Hydrocodone is a semi-synthetic opioid that has been used for decades as an analgesic [1–3] and is prescribed frequently for patients suffering from acute and chronic pain. It is highly addictive and has a high potential for abuse. Hydrocodone is metabolized by the body to hydromorphone, norhydrocodone and other minor metabolites. Although hydrocodone has been shown to have some activity, the more active compound is hydromorphone [4]. The cytochrome P450 (CYP) isoforms involved in the oxidative metabolism of hydrocodone have been well characterized. Hydrocodone is O-demethylated by cytochrome P450 2D6 (CYP2D6) to its active metabolite, hydromorphone and N-demethylated by cytochrome P450 3A4 to form norhydrocodone [4–9]. Because hydrocodone is biotransformed into another commercially available prescription opiate, hydromorphone, detecting the active metabolite in biological matrices may not be the best indicator for hydrocodone use. On the other hand, norhydrocodone is not available as a prescription drug thus may be the more useful metabolite in monitoring and understanding hydrocodone use.

Several quantitative methods for hydrocodone and/or hydromorphone in blood or plasma have been reported [5,10–17]; however, detection or quantitation of the nor-metabolite was not accomplished. In other studies, description of quantitative methods for hydrocodone, hydromorphone and norhydrocodone have been published; however, the matrix in those studies was human liver microsomes [18] or urine [19]. In the current study, a quantitative method for analysis of hydrocodone, hydromorphone and norhydrocodone in plasma using SPE and LC–MS–MS was validated and

☆ The views expressed in this article are those of the authors and do not reflect the official policy of the Department of Defense or other Departments of the US Government.

* Corresponding author at: Clinical Research Division/SGVUL, 59th Medical Wing, 1255 Wilford Hall Loop, Lackland AFB, TX 78236-5319, United States.
Tel.: +1 210 292 6172; fax: +1 210 292 6053.
E-mail address: sandra.valtier@us.af.mil (S. Valtier).

1570-0232/$ – see front matter Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.jchromb.2013.02.030

Report Documentation Page

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
01 APR 2013	**N/A**	**-**

4. TITLE AND SUBTITLE	5a. CONTRACT NUMBER
Quantitative method for analysis of hydrocodone, hydromorphone and norhydrocodone in human plasma by liquid chromatography-tandem mass spectrometry	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)	5d. PROJECT NUMBER
Valtier S., Mueck R. L., Bebarta V. S.,	5e. TASK NUMBER
	5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)	8. PERFORMING ORGANIZATION REPORT NUMBER
United States Army Institute of Surgical Research, JBSA Fort Sam Houston, TX	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)
	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release, distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			
unclassified	**unclassified**	**unclassified**	**UU**	**6**	

applied to a pharmacokinetic study in plasma of human subjects administered hydrocodone.

2. Experimental

2.1. Chemicals and reagents

Hydrocodone, hydromorphone, norhydrocodone, hydrocodone d-6, hydromorphone-d6 and norhydrocodone-d3 were obtained from Cerilliant (Round Rock, TX). β-Glucuronidase from *Helix pomatia*, Type HP-2 (aqueous solution, activity ≥100,000 U/mL) was obtained from Sigma Chemical Company (St. Louis, MO). Acetonitrile (Optima® LC/MS), water (Optima® LC/MS), methanol (HPLC grade), methylene chloride (HPLC grade), acetic acid (HPLC grade) isopropyl alcohol (A.C.S.), and sodium acetate (HPLC grade) were purchased from Fisher Scientific (Fair Lawn, NJ). Ammonium hydroxide, 28–30% (A.C.S.) was obtained from J.T. Baker Inc. (Phillipsburg, NJ). The Kinetex 2.6μ, C18, 50 mm × 2.10 mm analytical column was purchased from Phenomenex (Torrance, CA) and the Clean Screen XCEL I, 130 mg/3 mL extraction column from United Chemical Technology ([UCT], Bristol, PA). Formic acid was from Michrom Bioresources, Inc. ([Ultra Pure], Auburn, CA).

2.2. Sample preparation

A stock solution was prepared by adding 1000 ng/mL hydrocodone, hydromorphone and norhydrocodone to opioid negative human pooled plasma. Further dilutions were made at the following concentrations: 0.1, 0.25, 0.5, 1, 2.5, 5, 10, 25, 50, and 100 ng/mL. Enzyme hydrolysis was used. Twenty-five microliters of 1 μg/mL d6-hydrocodone/d6-hydromorphone/d3-norhydrocodone, 500 μL 0.1 M sodium acetate buffer, pH 5 and 20 μL 10,000 U/mL β-glucuronidase were added to 500 μL of plasma. Samples were mixed, heated to 60 °C for 2 h in a water bath, cooled to room temperature, centrifuged at 2500 rpm for 15 min then transferred to a clean glass tube and then extracted. A 500 μL aliquot of hydrolyzed plasma was extracted with UCT Clean Screen XCEL I 130 mg/3 mL column using a Zymark RapidTrace robotic system. The extraction protocol was as follows: the sample was loaded onto the column at 1 mL/min, dried with nitrogen for 1 min, rinsed with 2 mL 2% acetic acid in methanol, then dried with nitrogen for 5 min. The analytes were eluted with 1 mL freshly prepared solution of methylene chloride/isopropyl alcohol/ammonium hydroxide (78:20:2, v/v/v) at 1 mL/min. The extracts were evaporated to dryness under a stream of nitrogen in a 45 °C water bath. The dry residues were reconstituted in 100 μL mobile phase (5% acetonitrile aqueous solution with 0.1% formic acid) and injected onto the LC–MS–MS system.

2.3. LC–MS–MS conditions

The LC–MS–MS configuration was comprised of an Agilent LC system coupled to an Applied Biosystems 4000 QTrap mass spectrometer. The samples were analyzed using a Phenomenex Kinetex analytical column (2.6μ, 50 mm × 2.1 mm). The LC mobile phase consisted of solvent A = 5% acetonitrile with 0.1% formic acid and solvent B = 100% acetonitrile; flow rate was set at 0.5 mL/min. The column oven temperature was maintained at 25 °C and the injection volume was 10 μL. The gradient flow method consisted of an opening condition of 5% solvent B, with a linear increase to 60% solvent B over 2.2 min, then a linear increase to 95% solvent B at 2.23 min, 0.27 min at 95% solvent B, and then a return to the opening condition (5% solvent B) via a linear gradient over 0.15 min, followed by 2.35 min re-equilibration at opening conditions. The total run time was 5 min for each sample. Analysis was performed using positive ion electrospray MS–MS in multiple reaction monitoring (MRM) mode. Two MRM transitions (transition 1: quantifier; transition 2: qualifier) per analyte were used, and the declustering potential (DP), collision energy (CE) and collision cell exit potential (CXP) were optimized as shown in Table 1. The detector conditions were as follows: polarity mode, ESI positive; Scan type, MRM; Curtain gas 15 psi, Collision–activated dissociation (CAD) gas = high; ion source gas 1 = 40; ion source gas 2 = 40; ion spray voltage 5000 V; temperature 600 °C; entrance potential 10.

2.4. Validation

Quantitation and detection were based on a six-point calibration using calibration standards containing 1, 2.5, 5, 10, 50 and 100 ng/mL of hydrocodone, hydromorphone and norhydrocodone prepared in drug free plasma with each standard containing 50 ng/mL of deuterium-labeled isotopomer internal standards (hydrocodone-d6, hydromorphone-d6, norhydrocodone-d3). To test dilution effects and linearity range, concentrations at 1, 2.5, 25, 50, and 100 prepared from a 1000 ng/mL pooled plasma stock of the three analytes were evaluated on six consecutive runs. Transition ions monitored (Table 1) for the analytes and their respective internal standards were as follows: hydrocodone m/z 300.2 > 199.1, 300.2 > 171.0; hydrocodone-d6, 306.2 > 174.0; hydromorphone 286.1 > 185.0, 286.1 > 157.0; hydromorphone-d6, 292.2 > 185.2; norhydrocodone, 286.2 > 199.1, 286.2 > 241.1; and norhydrocodone-d3, 289.0 > 202.0. The mass spectra of the three opioids and deuterated internal standards are shown in Fig. 1. Acceptance criteria were: quantitative values within ±20% of the target concentration with proper qualitative identification as determined by transition ion area ratios within ±20% and retention time within ±2% of the calibration standards while exhibiting acceptable chromatography. The calculated ion area ratio range was based on the average of ratio ranges obtained for the six standards.

Twelve samples were analyzed to demonstrate the efficiency of the extraction procedure. Drug and internal standards were added to 6 samples prior to extraction. To the remaining 6 samples, internal standard was added after the samples had been extracted. The mean and standard deviation were calculated for each set of samples and extraction efficiency determined. The linear range was established for this procedure by the analysis of at least seven different runs with concentrations ranging from 1 to 100 ng/mL hydrocodone, hydromorphone and norhydrocodone. Within run precision was measured by testing replicates (n = 6) of the three analytes at 10 ng/mL and respective internal standards at 50 ng/mL. Between run precision was measured by testing concentrations at 2.5, 10, and 25 ng/mL of the three analytes and respective internal standards on six separate runs. The LOD was assessed by testing analyte concentrations at 0.25 and 0.5 ng/mL on at least six runs. Six random plasma specimens collected from human volunteers were analyzed to check for potential endogenous interferences with the analytes of interest. Stability of the drug and metabolites in plasma was also evaluated. Plasma stock solutions at concentrations of 25 and 50 ng/mL of the analytes were stored refrigerated (2–8 °C) or frozen (−70 °C). Refrigerated samples were tested after 2 months of storage; frozen samples were tested for up to 1 year from storage date. The freeze–thaw stability was evaluated by analyzing three subject samples with known drug concentrations and two quality control samples at concentrations of 7.5 and 25 ng/mL after undergoing three freeze–thaw cycles. Samples remained unfrozen for a minimum of 2 h per cycle with at least 20 h between cycles. An autosampler stability (room temperature) determination of six quality control sets was performed at a 24 h period.

Since matrix effect can influence the extent of analyte ionization, an ion suppression experiment was conducted. Drug negative

Table 1
MRM transitions.

Compound transition 1/2[a]	MRM transition (*m*/*z*)	Dwell time (ms)	Declustering potential (DP)	Collision energy (CE)	Collision cell exit potential (CXP)
Hydrocodone 1	300.2 → 199.1	100	90	42	13
Hydrocodone 2	300.2 → 171.0	100	90	54	13
Hydrocodone-D6	306.2 → 174.0	100	97	42	13
Hydromorphone 1	286.1 → 185.0	100	94	42	11
Hydromorphone 2	286.1 → 157.0	100	94	57	11
Hydromorphone-d6	292.2 → 185.2	100	100	44	12
Norhydrocodone 1	286.2 → 199.1	100	85	39	14
Norhydrocodone 2	286.2 → 241.1	100	85	34	14
Norhydrocodone-d3	289.0 → 202.0	100	80	40	10

[a] Transition 1: quantifier and transition 2: qualifier.

plasma was hydrolyzed and extracted in the same manner as test samples to best mimic matrix complexity. A 200 ng/mL solution of the analytes in acetonitrile was prepared and was loaded into the infusion pump syringe. The infusion pump was connected post-column via a tee connector; the solution was infused at10 μL/min. Once the baseline was stable, injections of drug-free plasma extract were made in the same manner as standard acquisitions.

3. Results and discussion

3.1. LC–MS–MS

One of the advantages of LC–MS–MS is the ability to spectrally separate these opioids. An LC gradient with run time of 5 min was instituted to achieve better separation of the compounds. Retention time for hydrocodone was 2.49 min, 1.48 min for hydromorphone and 2.42 min for norhydrocodone. Hydromorphone and norhydrocodone share the same precursor ion but were separated chromatographically in time. Hydrocodone and norhydrocodone were not totally separated but were spectrally distinguishable due to the difference in precursor ion between the two analytes.

The hydroxy metabolite exists in both the free and glucuronide-conjugated forms. The samples were subjected to enzyme hydrolysis prior to extraction to provide measurement of total (free and conjugated) hydromorphone. Evaluation of conjugated versus unconjugated metabolite in plasma samples from subjects administered hydrocodone was assessed. Results showed that total hydromorphone was clearly generated by hydrolysis. Specimens were hydrolyzed by a 2 h enzymatic treatment prior to assay to provide measurement of total drug.

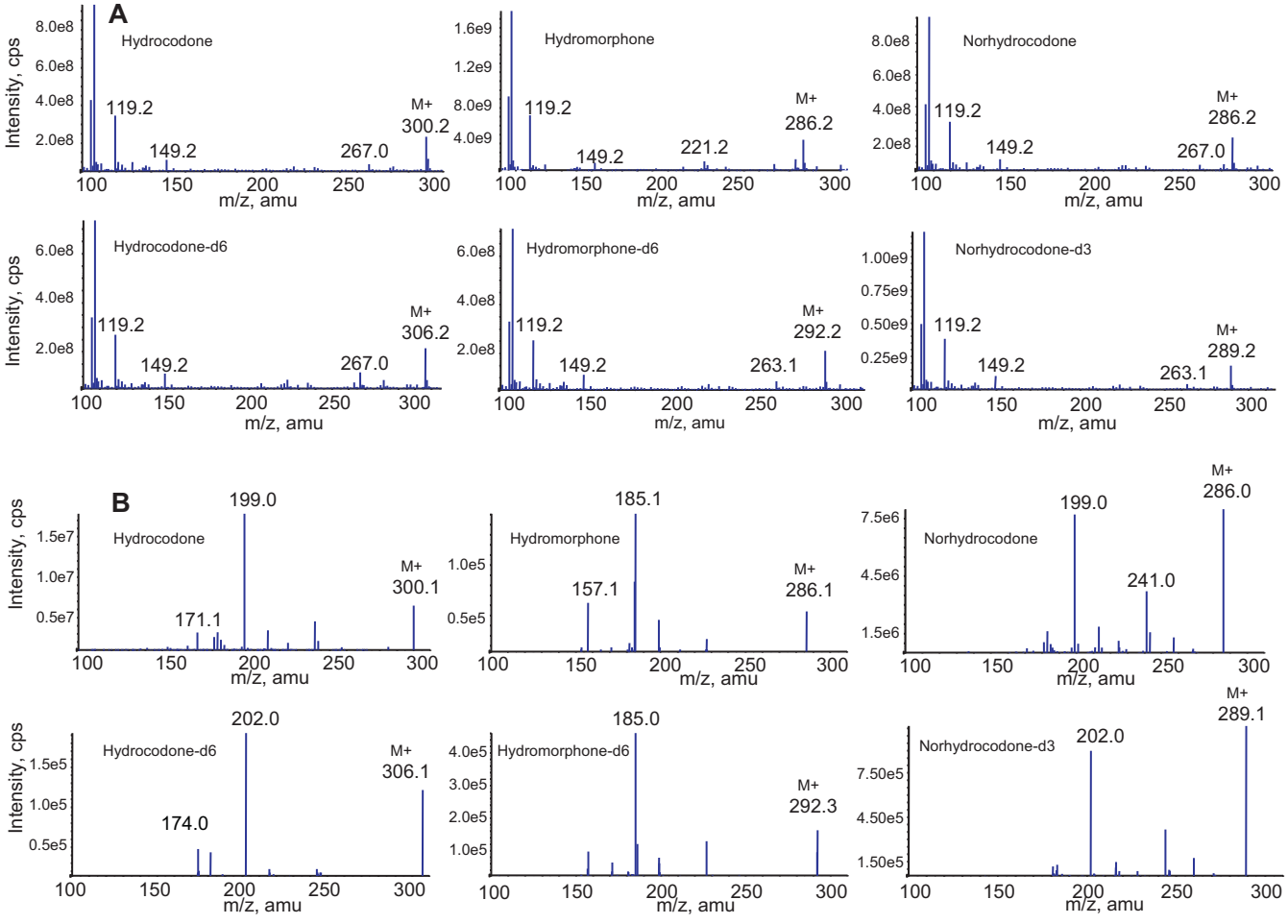

Fig. 1. (A) ESI-mass spectra and (B) MS–MS spectra of the opioids, and their deuterated internal standards.

3.2. Dilution integrity

Samples diluted from a stock prepared in opioid negative human plasma were analyzed to study dilution effects. The 100 ng/mL concentration was diluted directly from the 1000 ng/mL stock. Concentrations diluted directly from the 100 ng/mL stock were the 75, 50, and 10 ng/mL; the 7.5, 5, and 1 ng/mL were diluted from the 10 ng/mL stock. The 25 ng/mL was diluted from the 50 ng/mL stock; 2.5 ng/mL from 5 ng/mL; 0.5 from 1 ng/mL; 0.25 ng/mL from 0.5 ng/mL and 0.1 ng/mL from 1 ng/mL. Concentrations were analyzed on 6 different runs. The assay yielded good reproducible results (±20% of target concentration) and were within the linear range of the standard curve with exception of the 0.1 and 0.25 ng/mL concentrations. In addition, 1:40 and 1:20 dilutions of the 1000 ng/mL concentration were prepared in opioid negative human plasma, results were within acceptable range of target value. At dilution factor of 40×, quantitative results were 1024, 1020 and 976 ng/mL; at 20× dilution factor, quantitative results were 986, 996 and 1004 ng/mL for hydrocodone, hydromorphone and norhydrocodone, respectively.

3.3. Linearity and recovery

A multi-level calibration was used; the curve was constructed by calculating the amount ratio and the response ratio for the calibrator levels using the instrument's data system software. The average correlation coefficient and standard deviation for the curves was calculated to determine the minimum acceptable value for the calibration curve (1–100 ng/mL). Based on these data, the minimum allowable value for a calibration curve for all analytes is 0.995. Acceptable linearity was defined as a set of concentration values with a linear-regression correlation coefficient (r) of ≥0.995 and ion ratio values within ±20% of average standard values; all standard curves over the range from 1 to 100 ng mL hydrocodone, hydromorphone, and norhydrocodone were within acceptable calibration criteria. Quantitation was based on a six-point calibration curve: 1, 2.5, 5, 10, 50, 100 ng/mL with final internal standard concentrations of 50 ng/mL. The linear range for hydrocodone, hydromorphone and norhydrocodone is 1–100 ng/mL. The linear range was established for this procedure by analysis on 6 different runs on concentrations ranging from 0.1 to 100 ng/mL of the analytes prepared in opioid negative plasma. Values were considered within acceptable range if the measured amount was within ±20% of target concentration and ±20% of ion ratio calculation. Originally, 12 concentration points (0.1, 0.25, 0.5, 1, 2.5, 5, 7.5, 10, 25, 50, 75 and 100 ng/mL hydrocodone, hydromorphone and norhydrocodone) were tested; however, the measured amount for 0.1, 0.25 and 0.5 ng/mL of each analyte was not always within acceptable limits.

For extraction efficiency evaluation, twelve samples were either subjected to addition of internal standard pre-SPE or to addition of internal standard post-extraction. Drug (10 ng/mL hydrocodone, hydromorphone, norhydrocodone) and 50 ng/mL internal standard (hydrocodone-d6, hydromophone-d6, norhydrocodone-d3) were added to 6 samples prior to extraction. To the remaining 6 samples, internal standard was added after the samples had been extracted. The mean and standard deviation were calculated for each set of samples and extraction efficiency determined. The extraction efficiency for hydrocodone, hydromorphone, and norhydrocodone was 74, 72, and 73%, respectively.

Table 2
Between run precision.

Compound	Concentration (ng/mL)	Mean (n = 6)	Std. dev.	% CV
Hydrocodone	2.5	2.43	0.10	4.2
	10.0	9.84	0.79	8.1
	25.0	24.18	1.23	5.1
Hydromorphone	2.5	2.52	0.16	6.3
	10.0	10.0	0.72	7.2
	25.0	23.88	0.81	3.4
Norhydrocodone	2.5	2.61	0.11	4.1
	10.0	10.18	0.35	3.4
	25.0	24.15	1.27	5.3

3.4. Precision

Intra-assay (within run) precision was assessed by testing replicates (n = 6) of the analytes and internal standard in a single assay. Inter-assay (between run) precision was determined by testing replicates (n = 6) of the analytes and internal standard in at least six consecutive runs. The mean, standard deviation and CV were calculated. Within-run precision CV was ≤5.6% for all analytes. Between run precision CVs were ≤6.3% at 2.5 ng/mL, ≤8.1% at 10 ng/mL and ≤5.3% at 25 ng/mL for all three analytes (Table 2).

3.5. Sensitivity

The limit of detection (LOD) is the minimum concentration at which the analyte can be identified (signal-to-noise [S/N] ratio >3). The lower limit of quantitation (LLOQ) by this procedure was defined as the lowest concentration of the analyte that can be quantified with an acceptable level of precision and accuracy and for which ion ratios fall within 20% of the established range and S/N ratios are at least >10. To determine LOD and LOQ, concentrations at 0.1, 0.25 and 0.5 ng/mL hydrocodone, hydromorphone, and norhydrocodone were assayed on six runs. Under these conditions, the LOD (acceptable ion ratios, presence of all MRM transitions and signal-to-noise [S/N] ratio ≥3) was 0.25 ng/mL. Representative chromatograms of the LOD sample for the analytes are shown in

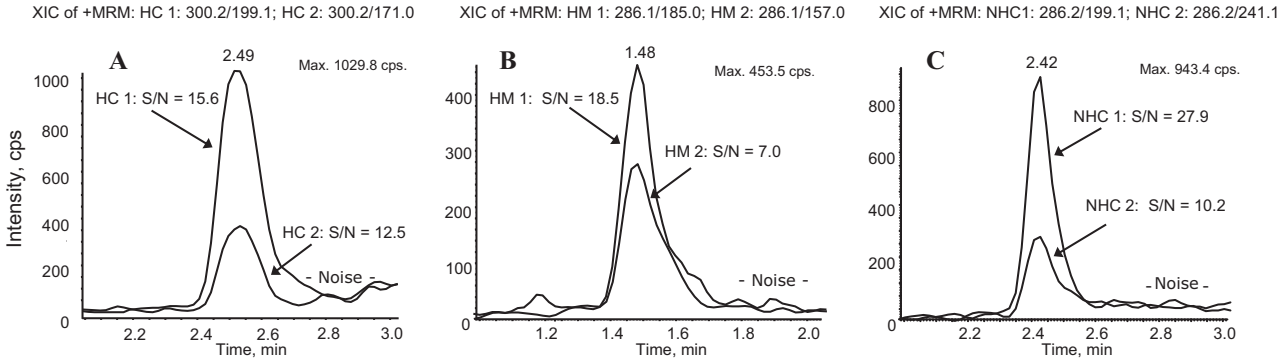

Fig. 2. Signal-to-noise ratio (S/N) for 0.25 ng/mL: (A) HC, (B) HM and (C) NHC MRM transitions 1 and 2.

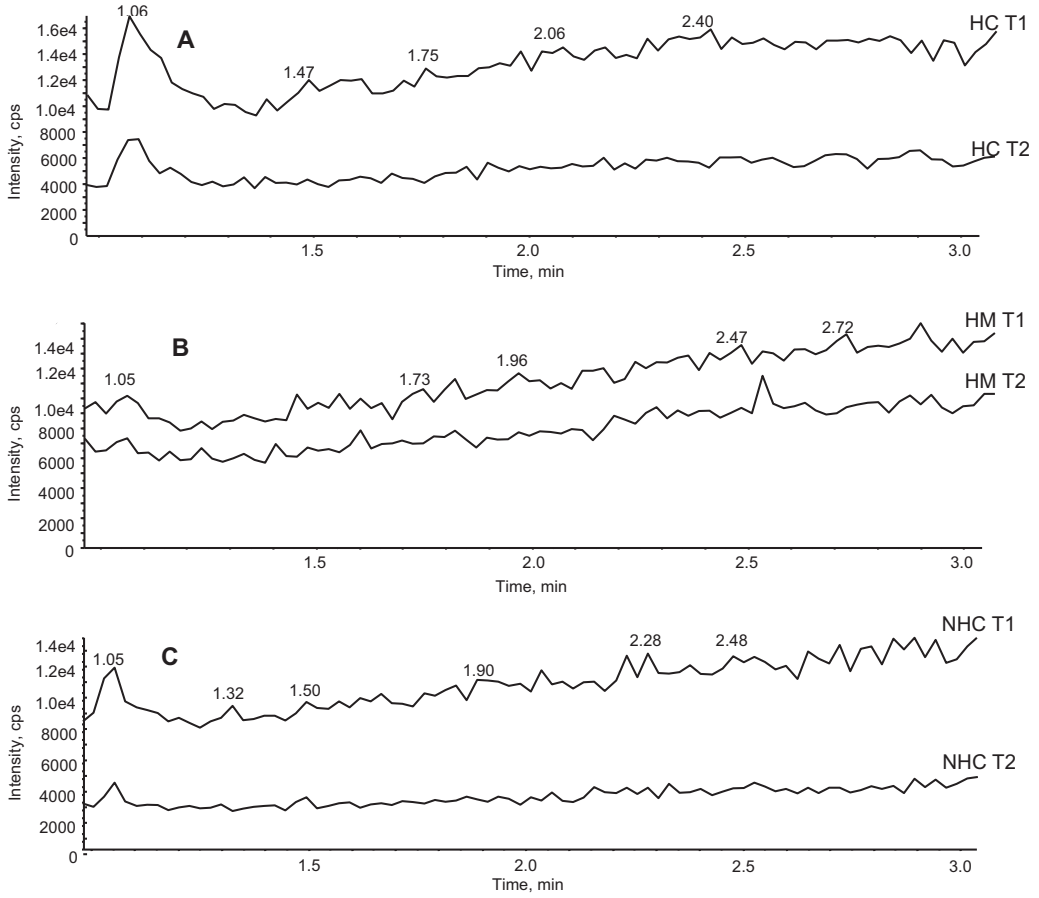

Fig. 3. Ion suppression evaluation. (A) HC MRM transition 1 (300.2/199.1) and 2 (300.2/171.0), (B) HM MRM transitions 1 (286.1/185.0) and 2 (286.1/157.0) and (C) NHC MRM transitions 1 (286.2/199.1) and 2 (286.2/241.1).

Fig. 2. The LOQ was 1.0 ng/mL for all three analytes with S/N ratios greater than 12:1.

3.6. Stability.

Hydrocodone, hydromorphone and norhydrocodone plasma spiked samples and samples from human subjects administered hydrocodone were aliquoted into several sets and stored either at 2–8 °C for 2 months or −70 °C for up to 12 months. No statistically significant difference ($p > 0.05$) was seen between results for first run vs. refrigerated or freezer stored samples, therefore, under these conditions, the analytes are stable for at least 2 months at 2–8 °C and up to 12 months at −70 °C. The analytes were found to be stable in plasma through three freeze (−70 °C)–thaw (4 °C) cycles, and for at least 24 h in the reconstitution solution on the autosampler tray (room temperature).

3.7. Interference and carry-over

Six different random opioid-negative plasma samples were evaluated to check for any indication of interference with the monitored ions for the analytes or internal standards under the conditions used in this study. No interference was seen; endogenous components in plasma did not interfere with any of the analytes at the retention time of peaks for hydrocodone, hydromorphone, norhydrocodone and respective internal standards in negative plasma samples.

Carry-over was evaluated by injection of blank sample (mobile phase) or known opioid negative plasma following high concentration (100 and 1000 ng/mL) samples. Solvent and negative plasma injections following the high concentration injections showed no significant carryover. Although no significant carryover was seen following high concentration samples, one solvent injection was placed between each sample injection.

Ion suppression was tested by infusing a 200 ng/mL solution of the analytes in acetonitrile. Once the baseline became stable, injections of drug-free plasma ($n = 3$) were then made as normal acquisitions. No ion suppression was seen at the retention times of interest (see Fig. 3).

4. Conclusion

This study provides a validated quantitative method for the analysis of hydrocodone, hydromorphone and norhydrocodone in plasma by LC–MS–MS. Extraction was performed using mixed mode SPE cartridges for sample preparation and a C18 column LC column for separation of the analytes. The six-point calibration curve consisted of 1, 2.5, 5, 10, 50 and 100 ng/mL of the compounds; however use of a 4 or 5 point calibration curve was found to be acceptable. The limit of quantitation is 1 ng/mL and LOD is 0.25 ng/mL for all three analytes. The method provided a reliable and sensitive procedure for the quantitation of hydrocodone, hydromorphone and norhydrocodone in human plasma samples for a hydrocodone pharmacokinetic study.

Acknowledgements

This work was supported by the DoD Psychological Health/Traumatic Brain Injury (PH/TBI) Research Program of

the Office of the Congressionally Directed Medical Research Programs (CDMRP), Award Number: W81XWH-11-2-0126.

References

[1] J.A. Forbes, et al., J. Oral Surg. 39 (2) (1981) 108.
[2] J.A. Forbes, et al., Pharmacotherapy 14 (2) (1994) 139.
[3] D.R. Morse, Alpha Omegan 83 (4) (1990) 26.
[4] K. Parfitt, in: Martindale (Ed.), The Complete Drug Reference, 32nd ed., Pharmaceutical Press, Taunton, MA, 1999, 2315 pp.
[5] S.V. Otton, et al., Clin. Pharmacol. Ther. 54 (5) (1993) 463.
[6] E.J. Cone, et al., Drug Metab. Dispos. 6 (4) (1978) 488.
[7] E.J. Cone, W.D. Darwin, Biomed. Mass Spectrom. 5 (4) (1978) 291.
[8] B. Lalovic, et al., Clin. Pharmacol. Therapeut. 79 (5) (2006) 461.
[9] M.R. Hutchinson, et al., Br. J. Clin. Pharmacol. 57 (3) (2004) 287.
[10] Y.L. Chen, et al., J. Chromatogr. B: Analyt. Technol. Biomed. Life Sci. 769 (1) (2002) 55.
[11] B.A. Goldberger, C.W. Chronister, M.L. Merves, Methods Mol. Biol. 603 (2010) 399.
[12] T. Dahn, et al., Methods Mol. Biol. (2010) 411.
[13] R. Coles, et al., J. Anal. Toxicol. 31 (1) (2007) 1.
[14] R. Zhang, et al., Artif. Cells Blood Substit. Immobil. Biotechnol. 37 (5) (2009) 203.
[15] J.D. Ropero-Miller, M.K. Lambing, R.E. Winecker, J. Anal. Toxicol. 26 (7) (2002) 524.
[16] R. Meatherall, J. Anal. Toxicol. 29 (5) (2005) 301.
[17] N.B. Tiscione, et al., J. Anal. Toxicol. 35 (2) (2011) 99.
[18] A. Menelaou, et al., J. Chromatogr. B: Analyt. Technol. Biomed. Life Sci. 785 (1) (2003) 81.
[19] S. Valtier, V.S. Bebarta, J. Anal. Toxicol. 36 (7) (2012) 507.