Journal of Analytical Toxicology, 2022, **46**, 757–764
DOI: https://doi.org/10.1093/jat/bkab113
Advance Access Publication Date: 26 October 2021
Article

Norbuprenorphine Interferences in Urine Drug Testing LC–MS-MS Confirmation Methods from Quetiapine Metabolites

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Abstract

Norbuprenorphine interferences were observed in urine drug testing LC–MS-MS confirmation methods used to assess patient compliance with prescribed buprenorphine for chronic pain and opioid use disorder. The interferences were observed in the norbuprenorphine MS-MS transitions, *m/z* 414.4/83.1 and 414.4/187.2, at and near the norbuprenorphine retention time at multiple laboratories using different sample preparation procedures and chromatographic conditions. When the interferences were present, a norbuprenorphine result could not be reported. Upon investigation, the interferences were correlated with prescribed quetiapine (Seroquel, Seroquel XR), a second-generation antipsychotic medication approved for the treatment of schizophrenia, bipolar disorder and more recently as an adjunct treatment for major depressive disorder. In addition to the approved indications, quetiapine is prescribed off-label for other conditions including insomnia and anxiety disorders. Off-label prescribing has increased in recent years, thereby exacerbating this analytical issue. Here, we present the study of four quetiapine metabolites found to have significant direct or potential interferences in norbuprenorphine quantitation. The four metabolites were putatively identified as two hydroxyquetiapine acids differing in the site of hydroxylation and a quetiapine sulfoxide acid diastereomer pair. As a result of this study, interference-free norbuprenorphine MS-MS transitions, *m/z* 414.4/340.2 and 414.4/326.1, were found that were selective for norbuprenorphine while maintaining an acceptable 10 ng/mL lower limit of quantitation.

Introduction

Buprenorphine is a semisynthetic derivative of thebaine first introduced in Europe as an opioid analgesic in the early 1980s and as a treatment for opioid addiction in 1996 (1). In the USA, it is currently designated a Schedule III narcotic. Although structurally similar to other opioids, buprenorphine's pharmacology is quite different. As a potent but partial μ-opioid receptor agonist, buprenorphine provides analgesia at therapeutic doses, displaces other opioids (e.g., methadone, morphine) and prevents cravings and withdrawal symptoms. Compared to full opioid agonists, buprenorphine has a favorable safety profile. It has a "ceiling effect" or plateau for mood alteration and respiratory depression and fewer drug–drug interactions than oxycodone and hydrocodone. Additionally, its long half-life allows daily or less-than-daily dosing (1, 2).

Buprenorphine is approved for the treatment of moderate to severe chronic pain and opioid use disorder (OUD). Current marketed buprenorphine products for the management of pain include Butrans transdermal patch and Belbuca buccal film. For OUD, several buprenorphine–naloxone (abuse deterrent) combination products are available in various dosage forms including Suboxone sublingual film, Bunavail buccal film and Zubsolv sublingual tablets; generic versions are also available. In recent years, extended-release formulations for the treatment of OUD have been approved, including Probuphine implant and Sublocade and Brixadi injections (3). Buprenorphine products for pain indications are typically formulated for lower-dose delivery than those for OUD indications; this difference has important analytical implications when establishing the lower limit of quantitation (LLOQ) and/or cut-off concentration for a buprenorphine urine drug testing method.

Buprenorphine is metabolized via CYP3A4-mediated N-dealkylation to form the active metabolite norbuprenorphine (Figure 1). Both buprenorphine and norbuprenorphine readily undergo conjugation to form corresponding 3-glucuronides. Although glucuronidation is typically a detoxification and inactivation pathway, both glucuronides have biological activity at relevant dose levels and may contribute to buprenorphine pharmacology (1, 4). It has been reported that the relative abundance of buprenorphine and metabolites in urine is dependent on the formulation. Buprenorphine glucuronide was the most abundant metabolite detected from transdermal delivery (e.g., Butrans patch); norbuprenorphine glucuronide was the most abundant

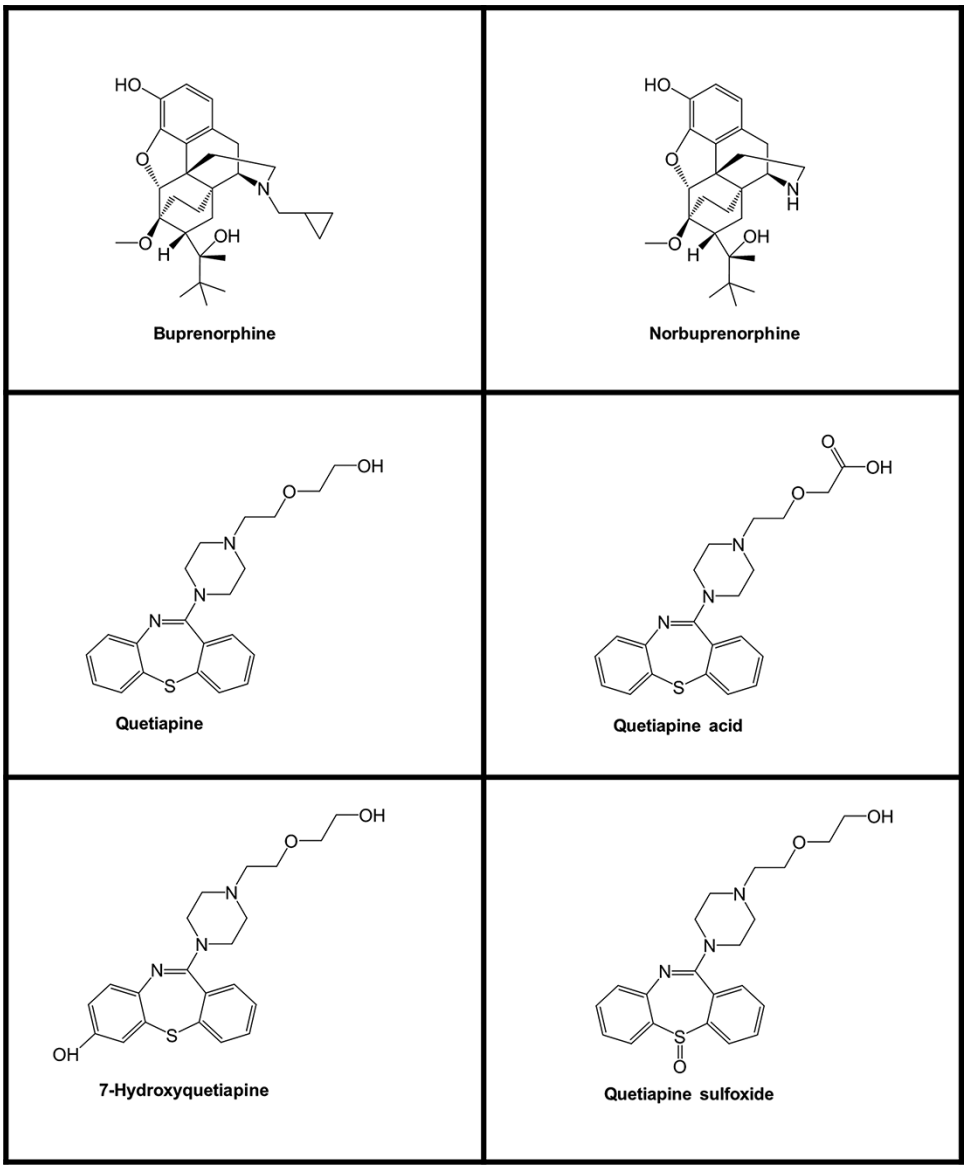

Figure 1. Structures of commercially available standards for buprenorphine, quetiapine and their respective urinary metabolites.

metabolite detected from sublingual delivery (5). A lower norbuprenorphine level from transdermal delivery is consistent with the reduced hepatic exposure associated with this dosage form.

The benefits of monitoring both buprenorphine and norbuprenorphine for medication compliance have been well-established (6, 7). In random urine specimens, expected ratios of norbuprenorphine-to-buprenorphine span a large range but are still useful for detecting outliers. For patients prescribed sublingual buprenorphine for OUD, a norbuprenorphine-to-buprenorphine ratio of <0.02 has been recommended as a guide for detecting specimen adulteration, whereby medication has been added directly to the specimen (7). Because this ratio has been reported as having high specificity but low sensitivity, other ratios have been proposed (6). In our laboratories, the buprenorphine confirmation methods detect and report concentrations of buprenorphine, norbuprenorphine and naloxone.

Quetiapine, initially approved by the FDA in 1997 and marketed under the brands Seroquel and Seroquel XR, is a second-generation antipsychotic approved for the treatment of schizophrenia, bipolar disorder and more recently as an adjunct therapy with antidepressants for major depressive disorder. Typical quetiapine dosage is 300–400 mg/day up to a maximum of 800 mg/day (8, 9). In addition to the approved indications, quetiapine is prescribed off-label for insomnia, various anxiety disorders and other conditions (10, 11). Its use among patients for OUD has been primarily off-label as a sedative agent; it has been speculated that clinicians perceive quetiapine as a safer alternative to controlled substances (12–14). A recent in-house survey showed that approximately 4% of patient specimens coming from OUD treatment centers are positive for quetiapine, confirming significant quetiapine use in this population. Quetiapine can also be misused, including misuse to potentiate the effects of buprenorphine–naloxone therapy (15, 16). Because quetiapine may have

serious side effects, the increased use and misuse in recent years have raised concerns (10, 11, 13–16).

Quetiapine is extensively metabolized by CYP3A4. The main metabolic pathways are sulfoxidation, oxidation of the terminal alcohol group to the carboxylic acid, N-dealkylation, O-dealkylation, hydroxylation of the dibenzothiazepine ring system, phase II conjugation and combinations thereof. The quetiapine parent drug sulfide moiety is prochiral; consequently, the sulfoxidation pathway gives rise to diastereomers. The quetiapine sulfoxide and carboxylic acid metabolites are inactive *in vitro* and in animal models. The main active metabolite is N-desalkylquetiapine, and it has been suggested that this metabolite is responsible for quetiapine's antidepressant effect. CYP2D6 makes a small contribution to the formation of the 7-hydroxy metabolites, 7-hydroxyquetiapine and 7-hydroxy-N-desalkyl-quetiapine. These metabolites are also active, but circulation levels are low and the pharmacological contribution is not significant. In radio-labeled studies, 73 and 21% of quetiapine dose was recovered in urine and feces, respectively. Less than 1% of the excreted radioactivity was unchanged quetiapine (17–21).

In our laboratories, confirmation methods go through rigorous specificity evaluations to ensure they are free of interferences prior to implementation. However, newly marketed drugs and supplements and changes in usage patterns and/or dose for existing products make the evaluation of specificity an on-going task. For interferences due to minor metabolites, finding the source of the interference, frequent absence of literature references and commercially available materials add challenges to the effort.

In this paper, we present the study of significant norbuprenorphine interferences from quetiapine metabolites. These interferences have been observed in multiple laboratories using different sample preparation procedures and chromatographic conditions.

When the interferences are observed, a norbuprenorphine result cannot be reported. As a result of this study, we found interference-free MS-MS transitions for norbuprenorphine to improve our methods for buprenorphine urine drug testing.

Materials and Methods

Although norbuprenorphine interferences were observed in multiple laboratories, we present herein the experimental conditions for the laboratory with the more rigorous enzymatic hydrolysis/solid-phase extraction (SPE) sample preparation procedure (compared to a simpler enzymatic hydrolysis/dilution procedure), demonstrating that the norbuprenorphine interferences persist even with SPE sample clean-up.

Chemicals and reagents

Surine DOA (synthetic urine) was purchased from Dyna-Tek Industries (Lenexa, KS). Buprenorphine, norbuprenorphine, naloxone and their respective deuterated internal standards were obtained from Cerilliant (Austin, TX). For quetiapine metabolite characterization experiments, quetiapine, 7-hydroxyquetiapine and quetiapine acid were also acquired from Cerilliant, and quetiapine sulfoxide was purchased from Toronto Research Chemicals (Toronto, Ontario, Canada). HPLC-grade acetonitrile, methanol and ammonium hydroxide (ACS grade) were obtained from Thermo Fisher (Waltham, MA). Formic acid (98%, ACS grade) was purchased from EMD Millipore (Billerica, MA), and phosphoric acid (ACS grade) was purchased from Sigma-Aldrich (St. Louis, MO). Purified water was supplied by an in-house MilliQ system (EMD Millipore). IMCSzyme recombinant β-glucuronidase and Rapid Hydrolysis Buffer (pH 7.2–7.8), used for enzymatic hydrolysis in all laboratories, were purchased from IMCS (Integrated Micro-Chromatography Systems, Irmo, SC).

Stock and working solution preparation

Stock solutions of buprenorphine, norbuprenorphine and naloxone (200 µg/mL) were prepared in methanol and stored at −20°C. Working calibrators (10, 50, 600, 2,000 and 5,000 ng/mL) and QCs (0, 6, 12 and 4,000 ng/mL) were prepared in Surine DOA by dilution of the stock solution and stored at 2–8°C.

Sample preparation

The patient specimens used in this investigation were originally collected as random urines and among those routinely submitted to Dominion Diagnostics for confirmation testing. Specimens retained for investigation were stored at −20°C. Calibrators, QCs and patient specimens were prepared for analysis in the 96-well plate format using Freedom EVO liquid handling systems (Tecan US, Morrisville, NC). Samples and specimens (220 µL) were combined with internal standards (29 µL) and β-glucuronidase in Rapid Hydrolysis Buffer (56 µL). The mixture was incubated for 2 h at 65°C. After incubation, 305 µL of 4% phosphoric acid in water was added to each well in preparation for SPE.

Waters Oasis MCX µElution plates were conditioned with 200 µL of methanol, followed by 200 µL of water. Samples (550 µL) were then loaded onto the SPE plate. The SPE plate was washed successively with 200 µL of 2% formic acid in water and 200 µL of methanol. Samples were then eluted with 100 µL of 57:38:5 acetonitrile:methanol:ammonium hydroxide solution. The eluates were evaporated to dryness and reconstituted with 200 µL of 0.05% formic acid in water. Finally, the sample plate was vortexed for 30 s and transferred to the LC–MS-MS system for analysis.

LC–MS-MS conditions

The buprenorphine confirmation method was run on an Acquity UPLC I-Class system coupled to a Xevo TQD triple quadrupole mass spectrometer (Waters, Milford, MA). Chromatographic separation was performed using a CORTECS Phenyl column (2.1 mm × 50 mm; 1.6 µm particle size) (Waters, Milford, MA) at a temperature of 40°C with 0.05% formic acid in water as mobile phase A and 0.1% formic acid in acetonitrile as mobile phase B. The flow rate was 0.5 mL/min, and the gradient conditions were as follows: 5% B (initial conditions), 5–25% B (0.01–1.50 min), 25–100% B (1.50–2.50 min), 100% B (2.50–3.50 min) and 100–5% B (3.50–3.60 min), followed by re-equilibration at initial conditions. The injection volume was 7.5 µL.

The Xevo TQD source was equipped with an ESI probe and operated in positive ion mode. Data was acquired by multiple reaction monitoring (MRM). For the buprenorphine confirmation method, three MRM transitions were used for norbuprenorphine monitoring, one quantitative transition (*m/z*

414.4/83.1) and two qualitative transitions (*m/z* 414.4/101.1 and *m/z* 414.4/187.2). Typically, only one qualitative transition is used, but in this case, a second qualitative transition was included for improved norbuprenorphine selectivity unrelated to the issue discussed herein. Although norbuprenorphine interferences were observed for all transitions, we have limited the scope of this paper to the *m/z* 414.4/83.1 and *m/z* 414.4/187.2 transitions used in all laboratories. The MRM transitions for buprenorphine, norbuprenorphine, naloxone and internal standards, along with the cone voltage and collision energy used for each, are shown in Supplementary Data, Table SI. Other source parameters were as follows: capillary voltage 1 kV, extractor voltage 2 V, source temperature 125°C and desolvation heater temperature 400°C. The desolvation and cone gas flow rates were 800 L/h and 25 L/h, respectively. Masslynx 4.1 was used for data acquisition and processing.

For drug screening by LC–MS-MS and quetiapine metabolite characterization, the LC–MS-MS system comprised an Acquity UPLC I-Class system (Waters Corp., Milford, MA) coupled to a Q-Exactive hybrid quadrupole-Orbitrap high-resolution accurate mass (HRAM) mass spectrometer (Thermo Fisher Scientific, Waltham, MA). Chromatographic separation was performed with a Kinetex Biphenyl column (3.0 × 50 mm, 2.6 µm particle size, Phenomenex, Torrance, CA) at a temperature of 40°C with 0.1% formic acid in water as mobile phase A and 0.1% formic acid in methanol as mobile phase B. The flow rate was 0.5 mL/min, and gradient conditions were as follows: 10% B (initial conditions), 10–30% B (0.01–2.50 min), 30–90% B (2.50–9.50 min), 90% B (9.50–10.51 min), 90–10% B (10.50–10.51 min), followed by re-equilibration at initial conditions. The Q-Exactive HESI source parameters were as follows: spray voltage 3500, sheath gas flow rate 65, auxiliary gas flow rate 20, sweep gas flow rate 2, capillary temperature 320°C, S-lens RF level 55 and auxiliary gas heater temperature 420°C. The acquisition method comprised a full scan (*m/z* 130–500 at 70,000 resolution) followed by MS-MS scans (17,500 resolution) triggered from a Data-Independent Analysis inclusion list. Analyte identification parameters were the same as those reported in Muñoz- Muñoz et al. (22). For quetiapine metabolite identification, MS-MS spectra were acquired at multiple collision energies in both positive and negative mode. TraceFinder 3.2 was used for data acquisition and processing. Thermo Xcalibur Qual Browser 3.0 was also used for data processing.

Results and Discussion

Multiple norbuprenorphine interferences were observed in a small but significant fraction of patient specimens submitted for buprenorphine confirmation testing by LC–MS-MS. The interfering peaks eluted at and near the retention time of norbuprenorphine (Figure 2C). When present, and depending on the chromatographic conditions, either a recognizable multiple-peak pattern and/or ion ratio failure signaled interference and a norbuprenorphine result could not be reported. The ion ratio parameter (transition 2 peak area/transition 1 peak area) is used to confirm the identity of the detected compound; the acceptable range is calculated from the average ion ratio of the calibrators of ± 20%.

For investigation, specimens exhibiting the interferences were further examined using a Q-Exactive HRAM research method designed to screen 162 drugs, including quetiapine. Of the 25 specimens analyzed, 23 were positive for quetiapine. The two specimens that were not positive for quetiapine had unrelated norbuprenorphine interferences and were not investigated further for this study. These results agreed with the medication lists submitted with the specimens. Additionally, 22 patient specimens that tested positive for quetiapine but had no buprenorphine medication history were analyzed by the buprenorphine confirmation method. As expected, buprenorphine and naloxone were negative for all 22 samples, but norbuprenorphine-like peaks similar to those shown in Figure 2C were observed in all cases (Figure 2D).

Quetiapine has a molecular formula of $C_{21}H_{25}N_3O_2S$ and a *m/z* of 384.1740 forprotonated molecule $[M+H]^+$. The observed norbuprenorphine interferences have a precursor mass of *m/z* 414, suggesting quetiapine metabolites as the source of the interference. Using the Q-Exactive HRAM method, which has a longer gradient and different LC column than the buprenorphine confirmation assay, the quetiapine metabolites (*m/z* 414.1482) were resolved from norbuprenorphine (*m/z* 414.2639) by *m/z* and retention time (Figure 3). The mass difference between quetiapine and the metabolites is 29.9742 mass units, which corresponds to the addition of two oxygens with concomitant loss of two hydrogens.

The metabolite profile for quetiapine in urine has been reported and includes metabolites that are products of multiple oxidative biotransformations (17–20). Of the reported metabolites, only the quetiapine sulfoxide acid diastereomers ($C_{21}H_{23}N_3O_4S$) have a precursor mass of *m/z* 414.1482. When the quetiapine sulfoxide acid accurate mass (*m/z* 414.1482, ±5 ppm) was extracted from the full-scan spectra of patient samples positive for quetiapine, four peaks were consistently present (Figure 3A). All four peaks showed an isotope pattern consistent with the presence of one sulfur atom in the molecular formula. All four peaks were also detected in negative-ion mode (data not shown). Using the Q-Exactive HRAM method, it was possible to obtain an isolated accurate-mass MS-MS spectrum for each of the four peaks without interference from norbuprenorphine (Figure 3). Spectra were acquired in both positive- and negative-ion modes at multiple collision energies; spectra presented herein were acquired in positive-ion mode with a collision energy of 40 unless otherwise indicated (Supplementary Data, Figure S1).

Quetiapine metabolites with accurate mass of *m/z* 414.1482 were not commercially available; therefore, to characterize the unknown metabolites, quetiapine, 7-hydroxyquetiapine, quetiapine acid and quetiapine sulfoxide standards were used (Figure 1). As such, identification of the unknown metabolites could be proposed but not confirmed. The MS-MS spectra of quetiapine and the metabolite standards were acquired and are presented as Supplementary Data, Figure S2.

Figures S1A and S2A–C show the MS-MS spectra for the unknown metabolite with a retention time of 3.96 min and the quetiapine acid, 7-hydroxyquetiapine and quetiapine sulfoxide standards, respectively. In comparing the MS-MS spectra for the unknown metabolite and quetiapine acid (Figure S2A), both show a fragment at *m/z* 103.0388 ($C_4H_7O_3$) corresponding to the full alkyl chain. The presence of three oxygen atoms in this fragment is consistent with the oxidation of the alkyl chain, presumably the oxidation of the terminal alcohol to the carboxylic acid as this transformation

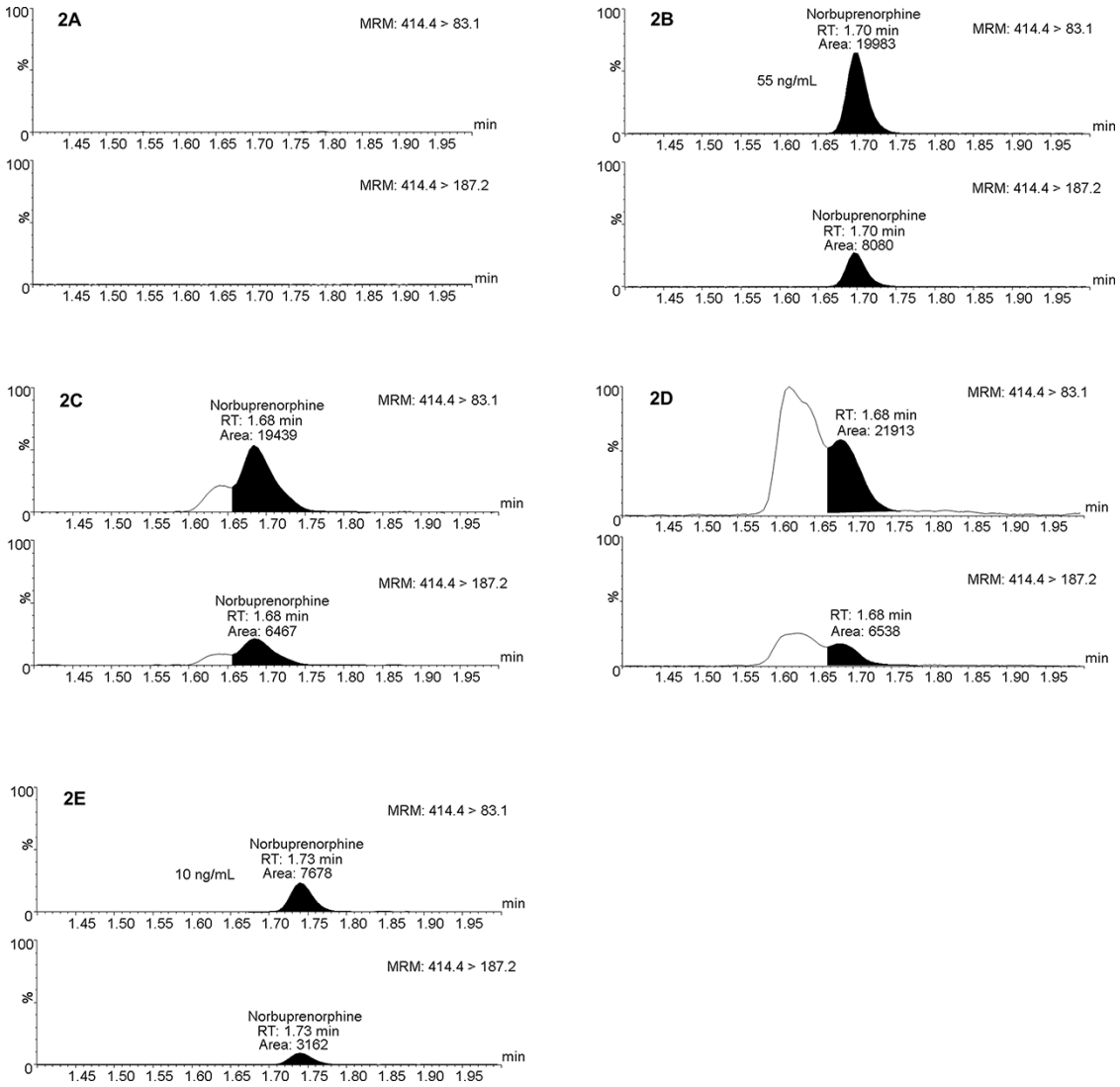

Figure 2. Chromatograms (with same *y*-axis scaling) showing norbuprenorphine interferences observed in patient specimens. (2A) Patient negative for norbuprenorphine, (2B) patient positive for norbuprenorphine, (2C) patient positive for norbuprenorphine and quetiapine, (2D) patient positive for quetiapine and (2E) norbuprenorphine at the LLOQ (10 ng/mL).

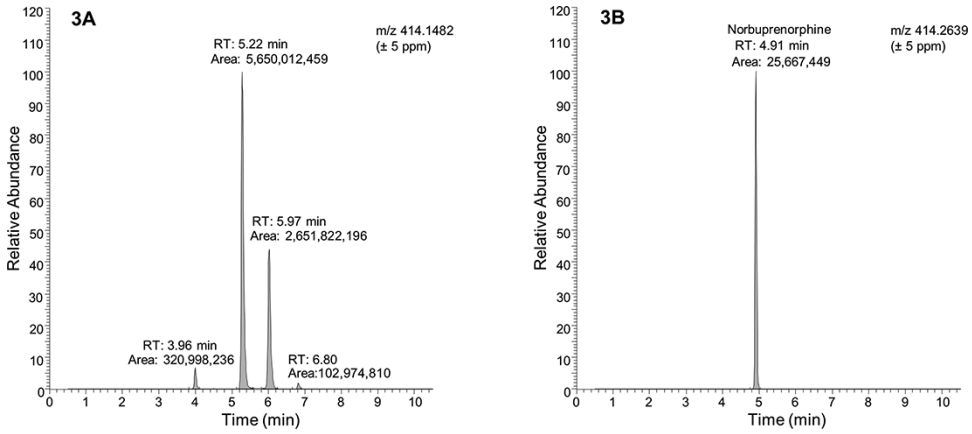

Figure 3. Q-Exactive HRAM extracted mass chromatograms from a patient positive for norbuprenorphine and quetiapine showing resolution by *m*/*z* and retention time of each of the quetiapine metabolites (3A) from norbuprenorphine (3B).

is well-documented (17–20). The presence of fragments at *m/z* 146.0812 ($C_6H_{12}O_3N$) and 172.0966 ($C_8H_{14}O_3N$) in the unknown metabolite spectrum, consistent with the quetiapine acid spectrum and corresponding to the full alkyl chain and portions of the piperazine ring, supports this assignment and also indicate the absence of further oxidation to this portion of the molecule. The fragments observed at *m/z* 210.0369 ($C_{13}H_8NS$), 253.0792 ($C_{15}H_{13}N_2S$) and 279.0951 ($C_{17}H_{15}N_2S$) in the quetiapine acid spectrum (Figure S2A) correlate with *m/z* 226.0318 ($C_{13}H_8NOS$), 269.0739 ($C_{15}H_{13}ON_2S$) and 295.0899 ($C_{17}H_{15}ON_2S$) observed in both the unknown metabolite spectrum and the 7-hydroxyquetiapine spectrum (Figure S2B), with the two sets of fragments differing in mass by one oxygen atom. These fragments correspond to the dibenzothiazepine ring system and portions of the piperazine ring. The *m/z* 226.0318 ($C_{13}H_8NOS$) fragment confirms the dibenzothiazepine moiety as the site of oxidation. Because hydroxylation and sulfoxidation cannot be differentiated by mass, additional information was required to propose a structure.

Quetiapine sulfoxide ($C_{21}H_{25}N_3O_3S$) has a *m/z* of 400.1689 for the protonated molecule $[M+H]^+$ and produces two chromatographic peaks (diastereomers) from sulfoxide-induced stereochemistry. The MS-MS spectra for the quetiapine sulfoxide diastereomers (Figure S2C) show a fragment at *m/z* 352.2021 ($C_{21}H_{26}O_2N_3$) corresponding to the loss of the sulfoxide moiety. The presence of the (–SO) fragment in quetiapine metabolite spectra is reasonably diagnostic as this fragment has been observed for all sulfoxide metabolites reported to date (17). For the unknown metabolite, the absence of an *m/z* 366.1809 (–SO) fragment and second peak corresponding to a diastereomer support hydroxylation. Additionally, the *m/z* 237.1020 ($C_{15}H_{13}ON_2$) fragment observed in the 7-hydroxyquetiapine spectrum (Figure S2B) and the *m/z* 221.1066 ($C_{15}H_{13}N_2$) fragment observed in the quetiapine sulfoxide spectrum (Figure S2C) correspond to an oxidized and unoxidized dibenzothiazepine moiety without sulfur and have been used as a differentiator between 7-hydroxyquetiapine and quetiapine sulfoxide (20). The presence of an *m/z* 237.1020 ($C_{15}H_{13}ON_2$) fragment in the unknown metabolite spectrum further supports a proposed identification for the retention time 3.96 min metabolite as hydroxyquetiapine acid, with site of hydroxylation generally assigned to one of the aromatic rings. To our knowledge, this metabolite has not been previously reported.

Figures S1B and S2C show the MS-MS spectra for the unknown metabolites with retention times 5.22 and 5.97 min and the quetiapine sulfoxide standard, respectively. The MS-MS spectra for the unknown metabolites are identical suggesting diastereomers. The *m/z* 366.1812 ($C_{21}H_{24}N_3O_3$) fragment corresponding to the loss of the sulfoxide moiety is present as the fragment at *m/z* 103.0390 ($C_4H_7O_3$) corresponding to the oxidation of the alkyl chain. These observations led to proposed identifications for the retention time 5.22 and 5.97 metabolites as quetiapine sulfoxide acid diastereomers; these metabolites have been previously reported (17, 19). In our experience with multiple production methods, these metabolites are consistently the most abundant of the four *m/z* 414.1482 metabolites and most likely to interfere with norbuprenorphine based on retention time. The presence of the diastereomers, particularly in methods where they are resolved and present as two distinct peaks, is a useful visual indicator of interference in addition to the ion ratio issues that accompany this interference.

Figure S1C (collision energy 40) shows the MS-MS spectrum for the unknown metabolite with retention time 6.80 min. The presence of fragments at *m/z* 103.0388 ($C_4H_7O_3$), 146.0812 ($C_6H_{12}O_3N$) and 172.0966 ($C_8H_{14}O_3N$) support oxidation to the carboxylic acid with no additional oxidation to the alkyl chain and majority of the piperazine ring. However, significant fragments at *m/z* 162.0758 ($C_6H_{12}O_4N$) and 188.0913 ($C_8H_{14}O_4N$), analogous to *m/z* 146.0812 and 172.0966 but with an additional oxygen (+O), are also present and challenge the preceding statement. The *m/z* 188.0913 ($C_8H_{14}O_4N$) fragment represents the full alkyl chain and majority of the piperazine ring. The *m/z* 312.1159 ($C_{17}H_{18}ON_3S$) fragment represents the dibenzothiazepine moiety and piperazine ring. Comparing these two fragments, the only structural overlap is the piperazine ring; consequently, we assigned the piperazine ring as the site of hydroxylation and attribute the two sets of seemingly contradictory fragments to pathways both with and without a preceding loss of the piperazine oxygen through water loss or other mechanism. Indeed, the metabolite at retention time 6.80 is the only *m/z* 414.1482 metabolite that shows a water-loss fragment at *m/z* 396.1372 ($C_{21}H_{22}O_3N_3S$) and a fragment at *m/z* 370.1218 ($C_{19}H_{20}O_3N_3S$), corresponding to loss of C_2H_4O, presumably a portion of the piperazine ring. These fragments are present in Figure S1C but have higher relative abundance at lower collision energies (Figure S1D, collision energy 20). Likewise, the fragment at *m/z* 70.0652 (C_4H_8N), which is present for all four *m/z* 414.1482 metabolites and represents a piperazine nitrogen and four neighboring carbons, is accompanied by the analogous (+O) fragment, *m/z* 86.0599 (C_4H_8ON) and *m/z* 253.0792 ($C_{15}H_{13}N_2S$) and 279.0944 ($C_{17}H_{15}N_2S$), corresponding to the dibenzothiazepine ring system and portions of the piperazine ring, are present with the (+O) fragments, *m/z* 269.0739 ($C_{15}H_{13}ON_2S$) and 295.0899 ($C_{17}H_{15}ON_2S$) (Figure S1D).

Interestingly, the retention time 6.80 min unknown metabolite spectrum also shows a fragment at *m/z* 366.1814 ($C_{21}H_{24}O_3N_3$) corresponding to loss of SO. This fragment was described as diagnostic for the sulfoxide moiety; however, in this case, it is thought to be a concerted loss of both the sulfur and piperazine oxygen. Of the quetiapine metabolites included in this study, this fragmentation pathway is unique to the retention time 6.80 min metabolite as it was not observed for the non-sulfoxide standards or retention time 3.96 min metabolite. It is also noteworthy that a second diastereomer was not observed for the retention time 6.80 min metabolite. The preceding observations led to a proposed identification for the retention time 6.80 min metabolite as a hydroxyquetiapine acid, with site of hydroxylation generally assigned to the piperazine ring. Piperazine hydroxylation would afford intra-molecular hydrogen bonding opportunities with nitrogen and other oxygen atoms, perhaps explaining the longer retention time. To our knowledge, this metabolite has not been previously reported.

All the aforementioned quetiapine metabolites generate fragment (product) ions with the same nominal mass as those used for norbuprenorphine in the buprenorphine confirmation method (Table SI). Although the interfering

quetiapine metabolite product ions are not the most abundant, their signal was high enough to contribute significantly to the norbuprenorphine peak area, particularly for methods designed to monitor low-dose buprenorphine pain medications (e.g., Butrans). For these methods, the norbuprenorphine LLOQ is typically 5 ng/mL or lower to provide meaningful monitoring. The putative interfering quetiapine metabolite product ions are *m/z* 83.0604 ($C_4H_7N_2$) and 187.1077 ($C_8H_{15}N_2O_3$), corresponding to the norbuprenorphine product ions *m/z* 83.0855 (C_6H_{11}) and 187.0754 ($C_{12}H_{11}O_2$), respectively.

Two approaches are proposed to avoid norbuprenorphine interferences from the quetiapine metabolites. The first is to separate norbuprenorphine from the quetiapine metabolites chromatographically by using a longer LC gradient like the one described here for the Q-Exactive HRAM method. The second, more production-friendly option is to maintain a shorter chromatographic run time but select norbuprenorphine MS-MS transitions that are free of interference. Both norbuprenorphine and the quetiapine metabolites are extensively fragmented; consequently, they share numerous nominal mass fragments, especially below *m/z* 300. Multiple attempts were made to find MS-MS transitions that were selective for norbuprenorphine while maintaining an acceptable LLOQ. We found that the norbuprenorphine transitions *m/z* 414.4/340.2 and 414.4/326.1 were selective and provided a reasonable LLOQ (10 ng/mL) for monitoring patients prescribed buprenorphine for OUD. The two product ions, *m/z* 340.2 and 326.1 are separated by only 14 mass units (-CH_2); unfortunately, a more specific second transition could not be found. Because these transitions have lower intensity than other norbuprenorphine transitions commonly used, it is anticipated that additional method development (e.g., sample preconcentration) may be required to meet the LLOQ requirement for monitoring Butrans medication compliance. Figure 4A–D shows repeat analysis of the Figure 2 samples with the *m/z* 414.4/340.2 and 414/326.1 norbuprenorphine transitions and also a 10 ng/mL standard illustrating the signal intensity at the LLOQ (Figure 4E).

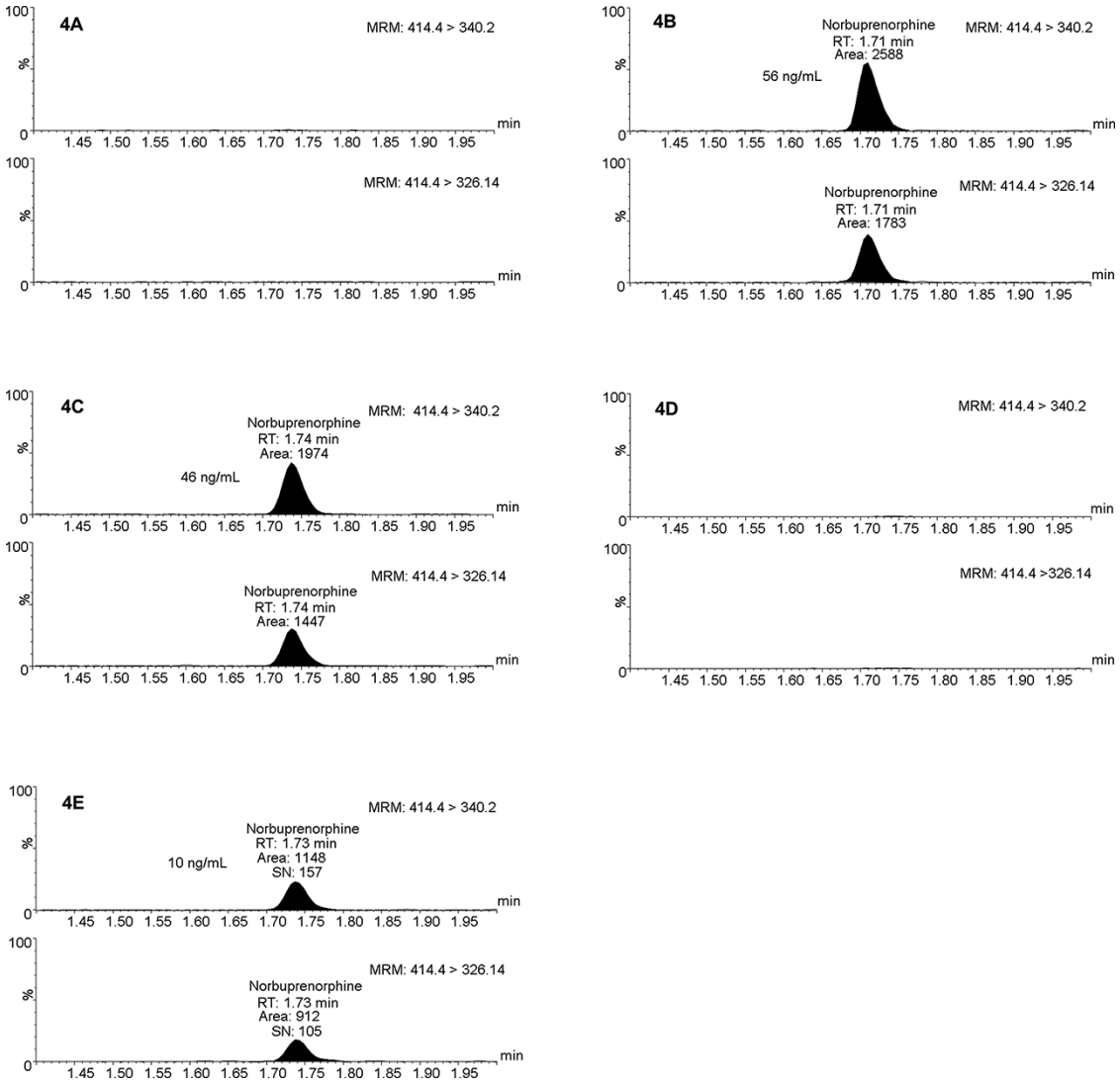

Figure 4. Chromatograms (with same y-axis scaling) showing the elimination of norbuprenorphine interferences using alternative MRM transitions. (4A) Patient negative for norbuprenorphine, (4B) patient positive for norbuprenorphine, (4C) patient positive for norbuprenorphine and quetiapine, (4D) patient positive for quetiapine and (4E) norbuprenorphine at the LLOQ (10 ng/mL).

Conclusions

In this study, we investigated and proposed identifications for four quetiapine metabolites that can potentially interfere with norbuprenorphine urine drug testing by LC–MS-MS. The metabolites are the products of multiple biotransformations involving oxidation of the quetiapine terminal alcohol to the carboxylic acid and either hydroxylation or sulfoxidation. Two of the metabolites investigated and putatively identified have not been previously reported. As the major buprenorphine oxidative metabolite, norbuprenorphine is an important analyte for pain management and OUD medication compliance monitoring; non-reportable results due to interference affect patient care. To improve norbuprenorphine LC–MS-MS testing, alternative MS-MS transitions were investigated, and the transitions *m/z* 414.4/340.2 and 414.4/326.1 were found to be free of quetiapine metabolite interference. The signal levels for these transitions were lower when compared to the current production levels; however, signal and signal-to-noise were both acceptable for monitoring patients prescribed buprenorphine for OUD.

Supplementary Data

Supplementary data is available at *Journal of Analytical Toxicology* online.

Funding

No funding is declared for this study.

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