Analysis Of Oxycodone And Its Metabolites-Noroxycodone, Oxymorphone and Noroxymorphone In Plasma By LC/MS With An Agilent ZORBAX StableBond SB-C18 LC Column

Application Note

Pharmaceutical

Authors

Linda L. Risler,
Fred Hutchinson Cancer Research Center,
1100 Fairview Ave. N., PO Box 19024,
Seattle, WA 98109

Anne E. Brooks
Agilent Technologies, Inc.
2850 Centerville Road
Wilmington, DE 19808
USA

Abstract

Oxycodone and its oxidative metabolites (noroxycodone, oxymorphone and noroxymorphone) are analyzed by high performance liquid chromatography/mass spectrometry (HPLC/MS), coupled with chromatographic separation by an Agilent ZORBAX Rapid Resolution High Throughput (RRHT) StableBond SB-C18 column. The method utilizes an ammonium acetate/acetonitrile gradient, with detection by mass spectrometer in electrospray mode with positive polarity. Spiked human plasma samples undergo solid phase extraction prior to LC/MS analysis. This method provides good linearity ($R^2 > 0.9900$) and reproducibility (< 10% difference between duplicates) for all compounds, while increasing productivity with a fast, efficient analysis and minimal solvent usage.

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Introduction

Oxycodone was developed in 1916 as an opioid analgesic medication intended to replace the far too addictive analgesic at the time, heroin. Today, oxycodone is a Schedule II drug in the US, which means, while it has proven medical uses, it is still considered highly addictive with the possibility of both physical and psychological dependencies. Figure 1 shows oxycodone and its metabolic scheme, yielding noroxycodone, oxymorphone and noroxymorphone (a secondary metabolite) [1]. Because pain is subjective and metabolic rates differ from person to person, it can be difficult to determine appropriate dosages of oxycodone. One must find the balance between alleviating pain and causing adverse side effects, such as constipation, dizziness, drowsiness, headache, nausea, sleeplessness, vomiting and weakness [2]. The key to achieving

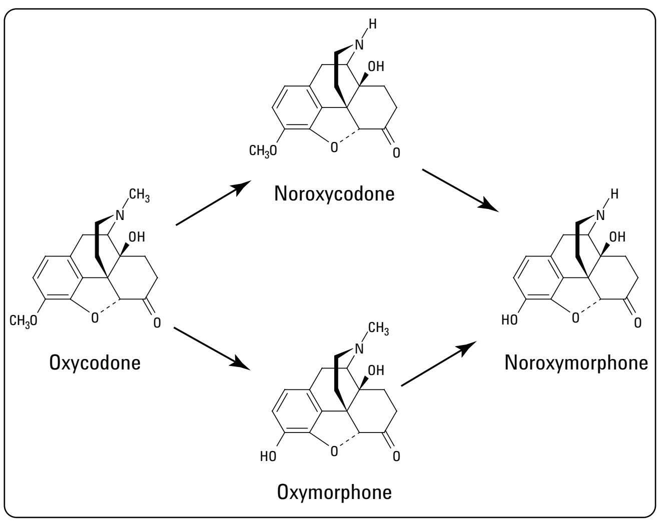

Figure 1. Metabolic scheme of oxycodone to noroxycodone, oxymorphone and noroxymorphone.

this balance is by monitoring the rate of metabolism of oxycodone to its metabolites. Extensive metabolisers require higher concentrations of oxycodone in plasma to achieve the therapeutic effects, while poor metabolisers may experience toxicity due to slow drug clearance and excessive plasma concentration. Due to the nature of this drug, it is no surprise that there is a need to qualify and quantify oxycodone and its metabolites in a variety of matrices.

Liquid chromatography coupled with mass spectrometry (LC/MS) is ideal for the detection of oxycodone and its metabolites. These alkaloid compounds can be analyzed via electrospray mass spectrometry without derivatization [3]. Additionally, mass spectrometry allows for a sensitive analysis, especially in a complex matrix such as urine, blood, hair or anywhere else one might look for drug residues.

Experimental

An Agilent 1100 Series HPLC/MS was used for this work:

- G1312A Binary Pump. Mobile phase A: 20 mM ammonium acetate, pH 4.0 and B: acetonitrile. Flow rate was 0.300 mL/min. Hold 5% B for 2.33 minutes, then increase B from 5% to 20% from 2.33 to 4.33 minutes, stop time is 6 minutes, and post time is 4 minutes.
- G1367A Wellplate Autosampler (ALS). Injection volume was 5.0 µL, with needle wash in flushport for 5 seconds with water/acetonitrile (50:50).
- G1316A Thermostated Column Compartment (TCC). Temperature was 30 °C.
- G1956B Mass Spectrometer (MS) was operated in atmospheric pressure ionization electrospray mode with positive polarity. Ion 288 *m/z* was monitored for noroxymorphone, 302 *m/z* for oxymorphone and noroxycodone, 316 *m/z* for oxycodone, and 322 *m/z* for d6-oxycodone (internal standard). Spray chamber gas temperature was 350 °C at 12 L/min.
- ChemStation version B.01.01 was used to control the HPLC/MS and process the data.

An Agilent ZORBAX Narrow Bore Rapid Resolution High Throughput (RRHT) StableBond SB-C18, 2.1 mm × 50 mm, 1.8-µm column (Agilent p/n 827700-902) was used for this chromatographic separation.

Acetonitrile, ammonium acetate, methanol, methylene chloride, isopropanol and ammonium hydroxide were purchased from Fisher. Boric acid was purchased from Baker. Standard solutions of oxycodone, noroxycodone, oxymorphone and noroxymorphone in methanol were purchased from Cerilliant, concentrations were 1 mg/mL for oxycodone, noroxycodone and oxymorphone, and 0.1 mg/mL for noroxymorphone. A composite sample was then made by combining 25 µL aliquots of oxycodone, noroxycodone and noroxymorphone, 2.5 µL of oxymorphone and 25 mL of methanol.

Matrix samples were prepared by spiking 1 mL of clean human plasma with various concentrations of the composite sample. Metabolites were extracted from plasma by solid phase extraction (SPE); SPE bonded phase was a non-end capped mixed-mode sorbent: octyl (C8) and benzenesulfonic acid (SCX). Cartridges were conditioned with 2 mL methanol, followed by 2 mL deionized water. Each spiked plasma sample was diluted with 1.5 mL borate buffer, pH 8.9, loaded into the SPE cartridge, then washed with 2 mL deionized water, 1 mL 10 mM ammonium acetate, pH 4 and 2 mL methanol, and finally eluted with 3 mL methylene chloride/isopropanol/ammonium hydroxide (80:20:2). Samples were dried under air at 60 °C, and then reconstituted in 60 µL of 10 mM ammonium acetate, pH 4/acetonitrile (95:5).

Results and Discussion

At pH 4, the StableBond SB-C18 stationary phase (a non-end capped type B silica) demonstrates excellent selectivity with a well buffered mobile phase. The non-end capped bonded phase provides more varied selectivity for polar compounds, like oxycodone and its metabolites (bases), than end capped phases due to additional interactions with exposed silanol groups. These interactions can be controlled and optimized by altering mobile phase conditions. The small 1.8-µm particle size allows for superior resolution and efficiency over 3.5 or 5 µm particles. Additional benefits of this column are the short 50-mm length and the small internal diameter (id), 2.1 mm. The short column allows for increased productivity with faster analysis times, while the small ID allows for prudent solvent usage.

Figure 2 shows extracted ion chromatograms (EIC) of a human plasma sample, previously determined to be free of oxycodone and its metabolites, that has been spiked with 50 ng/mL oxycodone, 50 ng/mL noroxycodone, 5 ng/mL oxymorphone, 5 ng/mL noroxymorphone and 40 ng/mL d6-oxycodone (an internal standard), and then extracted by SPE. Despite being in a complex sample matrix (plasma), the chromatograms are well resolved for each of the five

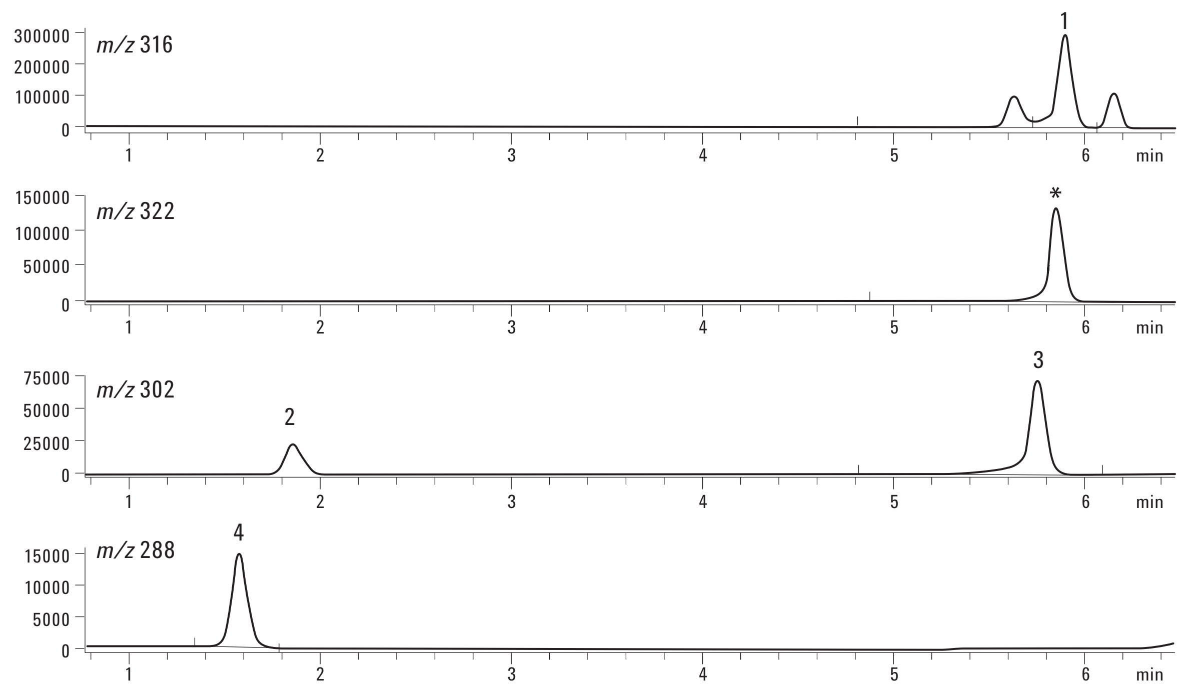

Figure 2. Human plasma sample spiked with 50 ng/mL oxycodone (1) and noroxycodone (3), 5 ng/mL oxymorphone (2) and noroxymorphone (4), and 40 ng/mL internal standard, d6-oxycodone (*). Sample was extracted by SPE, then analyzed by LC/MS with an Agilent ZORBAX StableBond SB-C18 column. The extracted ion chromatograms are shown.

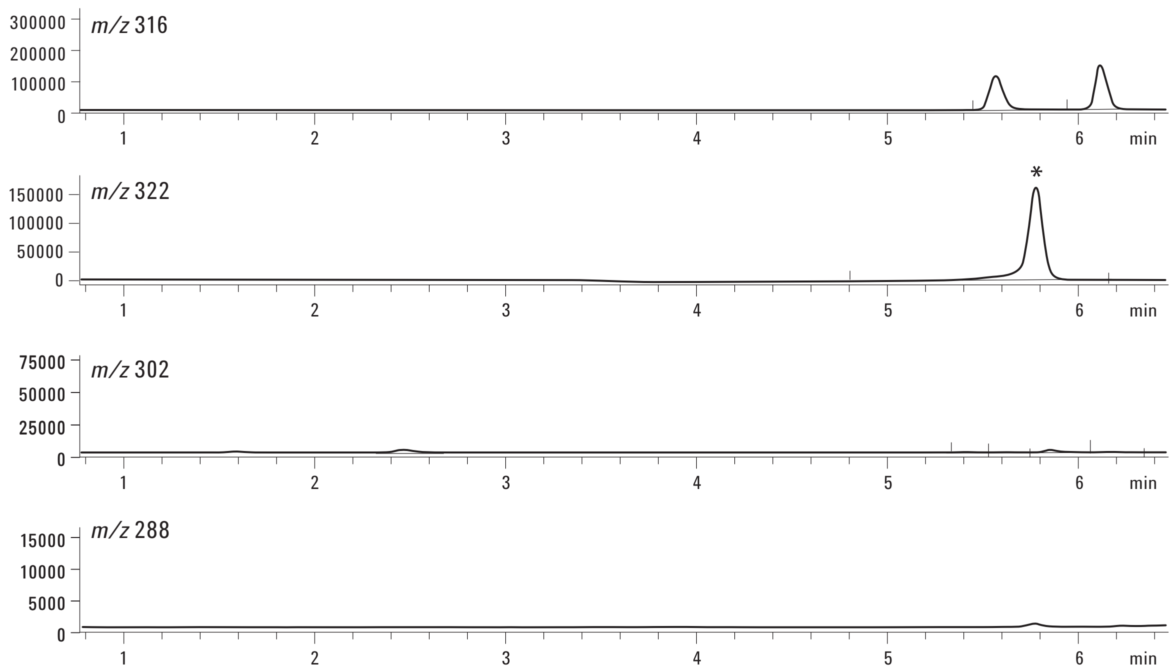

Figure 3. Human plasma sample, free from oxycodone and its metabolites, spiked with 40 ng/mL internal standard, d6-oxycodone (*). Sample was extracted by SPE, then analyzed by LC/MS with an Agilent ZORBAX StableBond SB-C18 column. The extracted ion chromatograms are shown.

compounds. In the extracted ion chromatogram for *m/z* 316, two additional peaks elute. As shown in Figure 3, an EIC for a blank plasma sample, these two peaks appear to be part of the plasma matrix.

Good linearity is found for all compounds with R^2 >0.9900 over the concentration range of 2 to 50 ng/mL for oxycodone and noroxycodone, and 0.2 to 5 ng/mL for oxymorphone and noroxymorphone. The limit of detection/quantification is 0.5 ng/mL for oxycodone, 1 ng/mL for noroxycodone, and 0.2 ng/mL for both oxymorphone and noroxymorphone with an Agilent 1100 Series LC/MS. Reproducibility is good with less than a 10% difference between each duplicate sample set over the aforementioned concentration range.

Conclusion

Oxycodone and its metabolites are successfully analyzed by LC/MS with an Agilent ZORBAX RRHT StableBond SB-C18 column over a range that is suitable for clinical use. This column selection provides an efficient, rapid analysis for increased productivity, while keeping solvent usage to a minimum. For all compounds, calibration curves show good linearity, with sensitive and reproducible results in a complex or dirty matrix, such as plasma.

References

1. B. Lalovic, et al., "Quantitative Contribution of CYP2D6 and CY3PA to Oxycodone Metabolism in Human Liver and Intestinal Microsomes," *Drug Metabolism and Disposition.* 32, (2004): 447–454.
2. A. Furlan, et al., "Opioids for Chronic Noncancer Pain: A Meta-analysis of Effectiveness and Side Effects," *Canadian Medical Association Journal*. 174(11). (2006): 1589–1594.
3. S. Schlueter, et al., "Determination of Opiates and Metabolites in Blood Using Electrospray LC/MS," *Agilent Technologies Publication 5988-4805.* (2005).

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Printed in the USA
May 27, 2009
5990-3815EN

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