# 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,
Seattle, WA 98109

Anne E. Mack
Agilent Technologies, Inc.



## Abstract

Oxycodone and its oxidative metabolites (noroxycodone, oxymorphone, and noroxymorphone) were 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 used an ammonium acetate/acetonitrile gradient with detection by a mass spectrometer in electrospray mode with positive polarity. Spiked human plasma samples underwent solid phase extraction (SPE) prior to LC/MS analysis. This method provided 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]. There is, therefore, a need to qualify and quantify oxycodone and its metabolites in a variety of matrices.

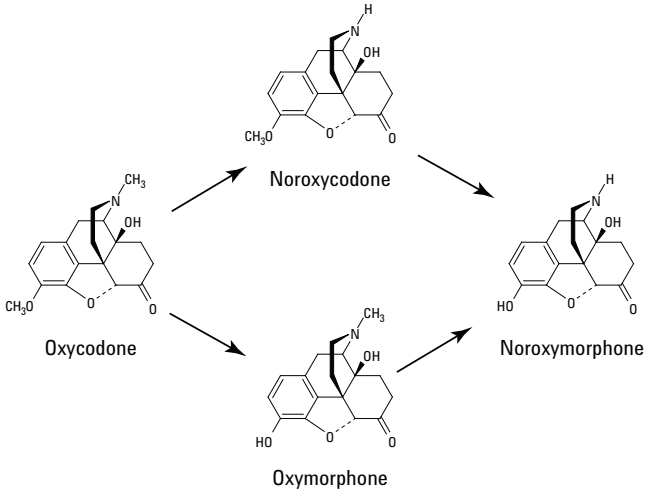


Figure 1. Metabolic scheme of oxycodone to noroxycodone, oxymorphone, and noroxymorphone.

Liquid chromatography coupled with mass spectrometry (LC/MS) is ideal for the detection of oxycodone and its metabolites. These alkaloid compounds can be analyzed through electrospray mass spectrometry without derivatization. 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 LC/MS was used for this work:

- Agilent 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.
- Agilent G1367A Wellplate Autosampler (ALS). Injection volume was 5.0 µL, with needle wash in flushport for 5 seconds with water/acetonitrile (50:50).
- Agilent G1316A Thermostated Column Compartment (TCC). Temperature was 30 °C.
- Agilent 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.
- Agilent ChemStation version B.01.01 was used to control the LC/MS and process the data.

An Agilent ZORBAX Narrow Bore RRHT StableBond SB-C18, 2.1 mm × 50 mm, 1.8 µm column (p/n 827700-902) was used for the 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 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) demonstrated excellent selectivity with a well buffered mobile phase. The non-end capped bonded phase provided 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 allowed for superior resolution and efficiency over 3.5 or 5 µm particles. Additional benefits of this column were the short 50 mm length and the small internal diameter (id), 2.1 mm. The short column allowed for increased productivity with faster analysis times, while the small id allowed 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 were well resolved for each of the five

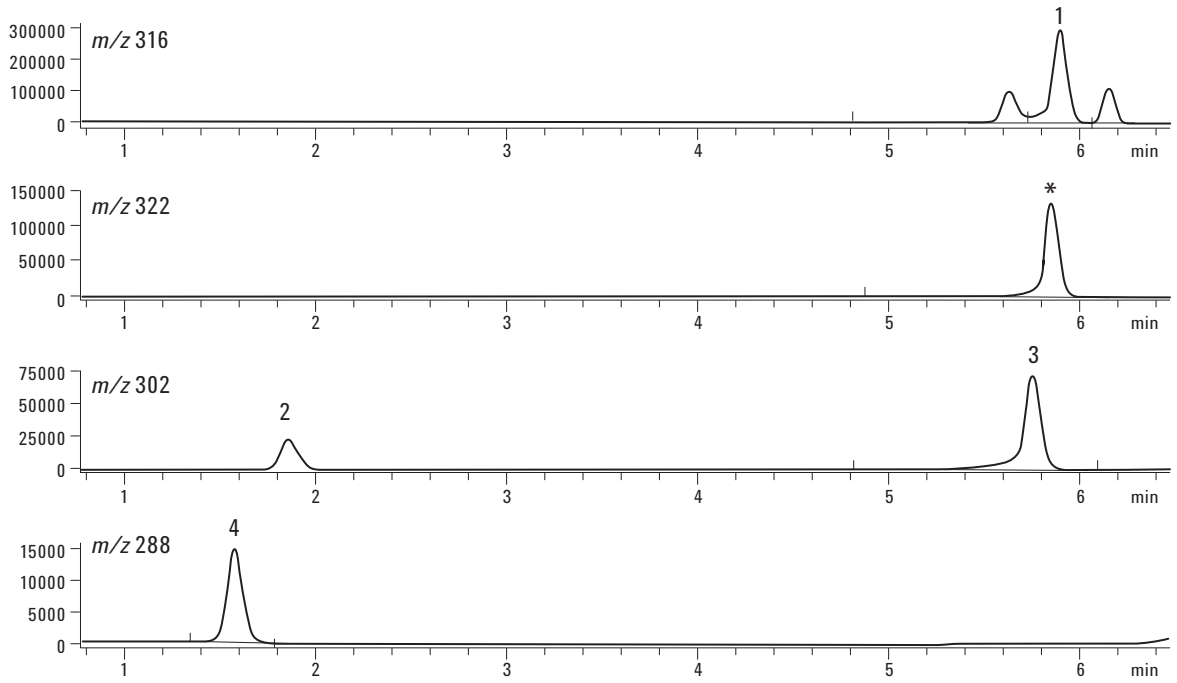


Figure 2. Human plasma sample spiked with 50 ng/mL oxycodone (1) and noroxycodone (3), 5 ng/mL oxymorphone (2) and noroxymorphone (4), and 40 ng/mL internal standard, d6-oxycodone (*). Sample was extracted by SPE, then analyzed by LC/MS with an Agilent ZORBAX StableBond SB-C18 column. The extracted ion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

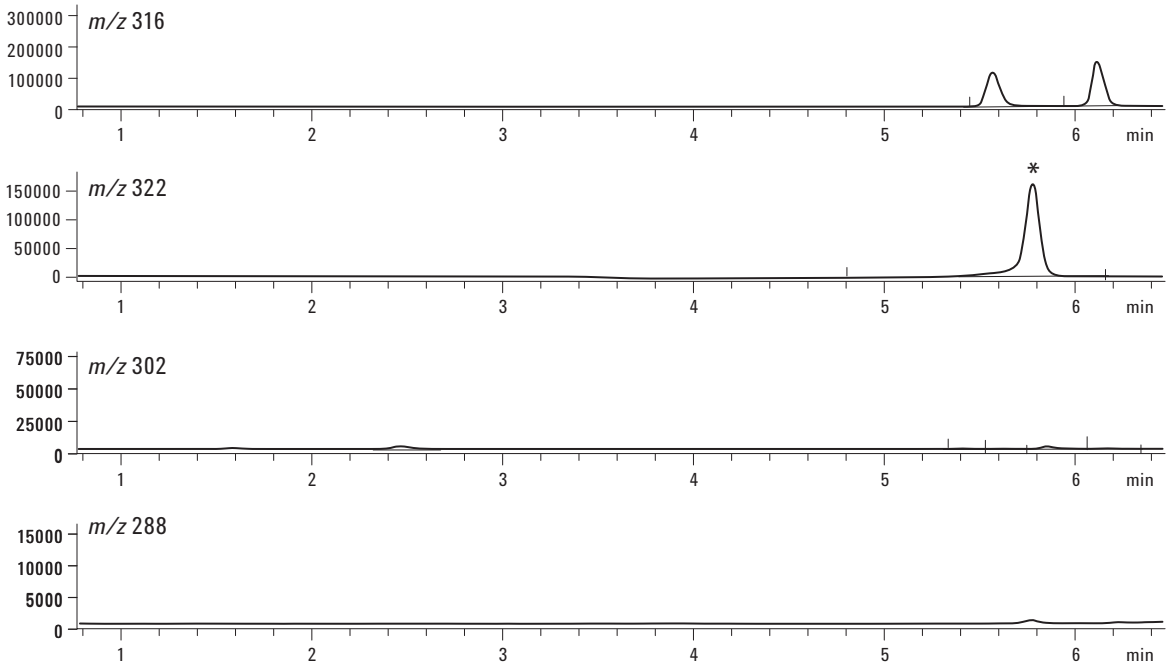


Figure 3. Human plasma sample, free from oxycodone and its metabolites, spiked with 40 ng/mL internal standard, d6-oxycodone (*). Sample was extracted by SPE, then analyzed by LC/MS with an Agilent ZORBAX StableBond SB-C18 column. The extracted ion chromatograms are shown.

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

Good linearity was 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 was 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 was good with less than a 10% difference between each duplicate sample set over the aforementioned concentration range.

## Conclusion

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

## Reference

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.

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