T2282

# Determination of Morpholine in Linezolid by Ion Chromatography

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## Overview

**Purpose:** To use cation-exchange ion chromatography (IC) with suppressed conductivity detection for the determination of morpholine in linezolid.

**Method:** To prevent column contamination by linezolid, matrix elimination is performed by concentrating trace morpholine on a cation concentrator column, followed by removing linezolid with DI water using an autosampler. Morpholine is then is separated from common cations on a high-performance cation-exchange column using a Reagent-Free™ IC (RFIC™) system and detected with suppressed conductivity detection.

**Results:** A sensitive and reproducible method to quantify trace morpholine in linezolid was developed.

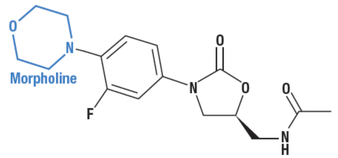
## Introduction

Morpholine is a six-membered heterocyclic compound that features amine and ether functionalities, making it a commonly used compound in organic synthesis. Morpholine has been used in the production of many types of therapeutic agents such as antibacterials, antimicrobials, anticancers, antitussives, antimalarials, anticonvulsants, and analgesics.

Morpholine is used as a building block in a multistep synthesis to produce linezolid, which is a synthetic antibiotic of the oxazolidinone class.[1] As a member of this drug class, linezolid is effective against most Gram-positive bacteria, such as those causing infections of the skin, pneumonia, and infections caused by *Enterococcus facecium*, a resistant bacterium. Because morpholine is used in the preparation of linezolid, it can remain as an impurity in the final product. The level of impurities in a drug substance or product must be carefully controlled and monitored because impurities can diminish the pharmacological efficacy of the active pharmaceutical ingredient (API) or cause unwanted side effects. Therefore, a sensitive method is needed to determine morpholine in synthetic drugs, such as linezolid.

Although linezolid and its degradation products are usually assayed by high-performance liquid chromatography (HPLC) with UV detection, morpholine lacks a suitable chromophore for detection. Therefore, cation-exchange chromatography with suppressed conductivity detection is considered the best alternative to selectively determine morpholine in linezolid.

**FIGURE 1. Structure of linezolid.**




## Methods

### Sample Preparation

0.1 mg/mL (w/v) linezolid: weigh approximately 5 ± 2.0 mg of linezolid on an analytical balance and dissolve the solid in 50 mL of 10% $CH_3OH$ in a 50 mL volumetric flask.

### Chromatographic Conditions

Thermo Scientific Dionex ICS-5000⁺ system including:

- SP Single Pump
- EG Eluent Generator
- DC Detector/Chromatography Compartment
- AS-AP Autosampler with:
  - 5.0 mL Sample Syringe
  - 8.5 mL Buffer Line

| | |
|---|---|
| Columns: | Thermo Scientific™ Dionex™ IonPac™ CG19 Guard, 2 × 50 mm (P/N 076029) and Dionex IonPac CS19 Analytical, 2 × 250 mm (P/N 076028) |
| Eluent Source: | Thermo Scientific Dionex EGC 500 MSA Eluent Generation Cartridge with Thermo Scientific Dionex CR-CTC 500 Continuously Regenerated Cation Trap Column |
| Eluent: | 7.5 mM MSA |
| Flow Rate: | 0.25 mL/min |
| Inj. Volume: | 100 µL |
| Matrix Elim. Volume: | 1 mL DI water |
| Concentrator Column: | Dionex IonPac TCC-ULP1 Ultralow Pressure Trace Cation Concentrator, 5 × 23 mm |
| Detection: | Suppressed conductivity, Thermo Scientific™ Dionex™ CSRS™ 300 Cation Self-Regenerating Suppressor, 2 mm, recycle mode, 7 mA current* |
| Background: | 0.3 µS |
| Noise: | 0.4 nS/min peak-to-peak |
| System Backpressure: | ~ 2400 psi |
| Run Time: | 15 min |

* Equivalent or improved results may be achieved with the Thermo Scientific™ Dionex™ ERS™ 500 Electrolytically Regenerated Suppressor.

### Data Analysis

Thermo Scientific™ Dionex™ Chromeleon™ 7.1 Chromatography Data System software was used for chromatographic data collection and processing.

## Results

### Sample Preparation

The solubility of the API in water or other aqueous solutions is a primary consideration in the development of a suitable IC method for pharmaceuticals. The limited solubility of drugs and intermediates in aqueous solutions poses a potential challenge because this can lead to precipitation of the API in the chromatography system, causing excessive backpressure and column contamination. Linezolid is marginally soluble in water with a solubility of approximately 3 mg/mL,[2] but its solubility increases at pH values below 3 and at higher temperatures.[3]

Because this method provides sufficient sensitivity for morpholine, linezolid was prepared with a final concentration of 0.1 mg/mL (w/v). Therefore, no organic solvent was needed to achieve a complete dissolution at room temperature. In this method, a 10% $CH_3OH$ solution was used to expedite linezolid dissolution.

### Matrix Elimination

In the development of an IC method for pharmaceuticals, the interaction between the API and the ion-exchange column needs to be considered. Some APIs are charged with certain degrees of hydrophobicity and can be strongly retained on the ion-exchange column.

Although linezolid is not ionized in aqueous media above pH 4 ($pK_a$ = 1.7),[4] in the acidic eluent (pH ~2), linezolid is positively charged and retained on the cation-exchange column. In addition to the electrostatic interaction, the retention likely includes some hydrophobic interaction with the column. Therefore, matrix elimination is necessary for this analysis to increase the lifetime of the separation column.

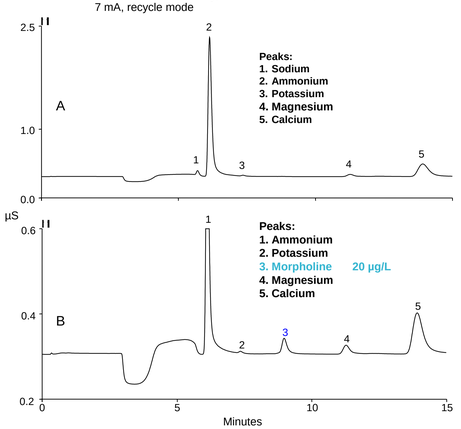
In this work, a Dionex IonPac TCC-ULP1 concentrator column was used to trap morpholine from the sample, while the uncharged linezolid was removed by matrix elimination using 1 mL of DI water. This configuration enables the determination of trace concentrations of morpholine in linezolid without extensive and laborious sample pretreatment.

### Separation

The Dionex IonPac CS19 column set offers unique selectivity for small polar amines; therefore, morpholine was separated from other common cations with excellent peak shape using simple isocratic MSA elution with no need to add organic solvent to the eluent.

**FIGURE 2. A) 10% $CH_3OH$ matrix blank and B) 20 µg/L morpholine in 10% $CH_3OH$. Please note that the following conditions apply to Figures 2–4.**

| | |
|---|---|
| Columns: | Dionex IonPac CG19/CS19 2 mm column set |
| Eluent Source: | Dionex EGC 500 MSA with Dionex CR-CTC 500 |
| Eluent: | 7.5 mM MSA |
| Flow Rate: | 0.25 mL/min |
| Inj. Volume: | 100 µL |
| Concentrator Column: | Dionex IonPac TCC-ULP1 |
| Detection: | Suppressed conductivity, Dionex CSRS 300, 2 mm, 7 mA, recycle mode |




### Linearity, Limit of Detection (LOD), and Limit of Quantitation (LOQ)

**TABLE 1. Calibration data and method detection limits of morpholine.**

| Analyte | Range (µg/L) | Coefficient of Determination ($r^2$)[a] | LOD[b] (µg/L) | LOQ[c] (µg/L) |
|---|---|---|---|---|
| **Morpholine** | 5–200 | 0.9995 | 0.86 | 2.9 |

[a] Linear Fit
[b] LOD = 3 × S/N
[c] LOQ = 10 × S/N

### Sample Analysis

The U.S. Pharmacopeia monograph for linezolid requires that the total impurities and any individual unspecified impurity be <0.5% and <0.08%, respectively.[5] In this study, the detection limit of morpholine in 0.1 mg/mL linezolid was 0.7 µg/L (0.0007% of linezolid), which easily meets these requirements.

### Sample Accuracy and Precision

**TABLE 2. Intraday recoveries of morpholine in linezolid.**

| Amount Found (µg/L) | Amount Spiked (µg/L) | Total Found (µg/L) | Peak Area RSD (n = 3) | Recovery (%) |
|---|---|---|---|---|
| <LOD | 10 | 9.99 | 2.38 | 99.9 |
| <LOD | 20 | 19.8 | 0.85 | 98.8 |
| <LOD | 30 | 30.3 | 0.64 | 101 |

**FIGURE 3. A) 0.1 mg/mL linezolid in 10% $CH_3OH$ and B) 0.1 mg/mL linezolid in 10% $CH_3OH$ spiked with 30 µg/L morpholine. A 10% signal offset has been applied.**

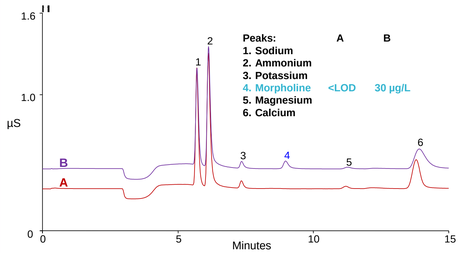


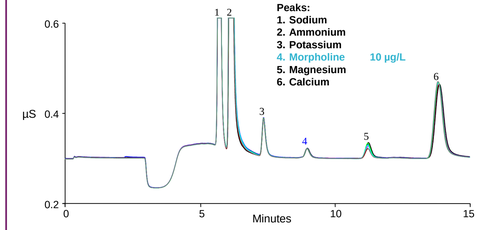

**TABLE 3. Between-day recoveries of morpholine in linezolid.**

| | Amount Found (µg/L) | Amount Spiked (µg/L) | Total Found (µg/L) | Peak Area RSD (n = 3) | Recovery (%) |
|---|---|---|---|---|---|
| **Day 1** | <LOD | 10 | 9.99 | 2.38 | 99.9 |
| **Day 2** | <LOD | 10 | 10.3 | 2.70 | 103 |
| **Day 3** | <LOD | 10 | 10.1 | 0.78 | 101 |

**TABLE 4. Retention time and peak area precisions of morpholine determination.**

| Sample | Retention Time RSD | Peak Area RSD |
|---|---|---|
| **10 µg/L Morpholine Standard** | 0.07 | 1.30 |
| **0.1 mg/mL Linezolid Spiked with 10 µg/L Morpholine** | 0.09 | 1.56 |

**FIGURE 4. Overlay of seven chromatograms of 0.1 mg/mL linezolid spiked with 10 µg/L morpholine.**




## Conclusion

- To prevent column contamination by linezolid, in-line matrix elimination was performed to concentrate trace morpholine on a concentrator column, followed by removal of the uncharged linezolid with DI water using the autosampler. The matrix elimination increases the lifetime of the separation column without laborious sample pretreatment.
- Efficient separation was achieved using the Dionex IonPac CG19/CS19 column set with electrolytically generated high-purity MSA eluent, and trace morpholine was detected using suppressed conductivity.
- The RFIC system enhances the level of automation and ease of use, and ensures excellent reproducibility.
- This IC method also allows simultaneous determination of sodium, ammonium, potassium, and other cations present in the sample.

## References

1. Pearlman, B. A. Phthalamide Compounds. U.S. Patent 6,362,334, March 26, 2002.
2. Takhi, M.; Munikumar, C.M.M.; Bhaskarreddy, K.M.; Singh, G.; Sreenivas, K.; Sitaramkujmar, M.; Selvakumar, N.; Das, J. Trehan, S.; Iqbal, J. Synthesis and Antibacterial Activity of Novel Exazolidinones Bearing *N*-hydroxyacetamidine Substituent. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2391–2395.
3. Plock, N. Target Site Pharmacokinetics of Antiinfectives in the Treatment of Serious Gram-positive Infections. Ph.D. Thesis, Martin Luther University of Halle-Wittenberg, February 2007.
4. U.S. Food and Drug Administration (FDA) Nonclinical Pharmacology, ADME, and Toxicology Summary. Appendix A. Preclinical Summary. www.fda.gov/ohrms/dockets/ac/00/backgrd/3597b1bb.pdf (accessed Oct 23, 2013).
5. U.S. Pharmacopeial Convention (USP) Pending Monograph Draft 1 for Linezolid: www.usp.org/sites/default/files/usp_pdf/EN/USPNF/pendingStandards/m6189.pdf (accessed Oct 23, 2013).



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