

# Determination of an Acceptable Preservative for Use in Minocycline Solution Extracts from Antibiotic Impregnated Medical Devices

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## Abstract

This method uses a 50:50 THF:water extraction solvent for the preparation of standards and samples. Preservative at a level of 0.1 weight percent in solution was added to stabilize the preparations. Analysis was accomplished by HPLC carried out on a Phenomenex Luna Phenyl Hexyl (150 x 4.6 mm) column at a flow rate of 1.0 mL/min. The separation was completed with a mobile phase of 35 mM KH<sub>2</sub>PO<sub>4</sub>:Triethylamine:CH<sub>3</sub>CN: (74.5:0.5:25 v:v:v), pH 7.5. The detector wavelength was set at 280 nm and the column temperature adjusted to 50 °C. A run time of 12 minutes was sufficient to elute the peaks of interest for isocratic separation and 23 minutes for gradient separation.

Standard solutions made with sodium sulfite preservative were found to be stable, showing only nominal amounts of epiminocycline. However, atypical degradation patterns were sometimes observed with this solvent combination. Chromatograms of degraded solution showed the main band splitting into two peaks. Detergent carryover from labware cleaning was found to facilitate the degradation. The chromatographic profile of degraded solution was very similar to that observed with forced thermal degradation. Conversely, using sodium citrate as a preservative in place of sulfite did not show this atypical degradation pattern, even after aging for more than seven days in the presence of detergent. Chromatographic interference prevented the use of ascorbic acid as an antioxidant for this assay.

Both sodium sulfite and sodium citrate demonstrated no chromatographic limitations when used as a preservative in the extraction solvent, although sodium sulfite was the better preservative. However, unlike solutions made with sodium sulfite, mixtures using sodium citrate were not prone to the degradation caused by the inadvertent combination of THF and detergent.

## Introduction

Minocycline has been found to help prevent catheter colonization and intravascular device-related bloodstream infections when impregnated in medical devices, such as catheters.

Extraction and quantitation of the antibiotic from the medical device is needed for quality control purposes.

Minocycline is readily degraded in solution. Consequently, the use of a preservative is necessary to increase the solution stability of the standard or extracted sample beyond one day.

## Extraction Procedure

The extraction procedure involves serial extractions using 50:50 THF:water.

Each cycle uses ~7 mL of extraction solvent. Four cycles are needed to attain >95% extraction efficiency.

Extracts are combined into a 50-mL volumetric flask and preservative is added. Solutions are then brought to volume with water. Solutions are stored ambient, protected from light. Minocycline recovery is determined using HPLC with UV detection. Quantitation is accomplished using external calibration standards.

## Preservatives Tested

Preliminary experiments evaluated a variety of potential preservatives including ascorbic acid, BHA, BHT, sodium nitrite, sodium citrate, and sodium sulfite.

Chromatographic interference or matrix incompatibility eliminated ascorbic acid, BHA, BHT, and sodium nitrite as viable candidates. Sodium sulfite and sodium citrate showed some promise and were studied further.

0.1 weight percent preservative in sample and standard solutions was found to give acceptable capacity without adversely impacting chromatography

## Chromatographic Conditions

Instrument: Agilent 1100 stack

Column: Phenomenex Luna Phenyl-Hexyl analytical column (150x4.6 mm) with guard column

Detection Mode: UV detection at 280 nm

Flow rate: 1.0 mL/minute

Column temperature: 50 °C

Injection volume: 5 µL

Quantitation: peak area; external standards

Mobile phase A: 35 mM KH<sub>2</sub>PO<sub>4</sub>, pH7.5:TEA:ACN (74.5:0.5:25)

Mobile Phase B: 90:10 ACN:H<sub>2</sub>O

Isocratic (mobile phase A): standards

Gradient: samples

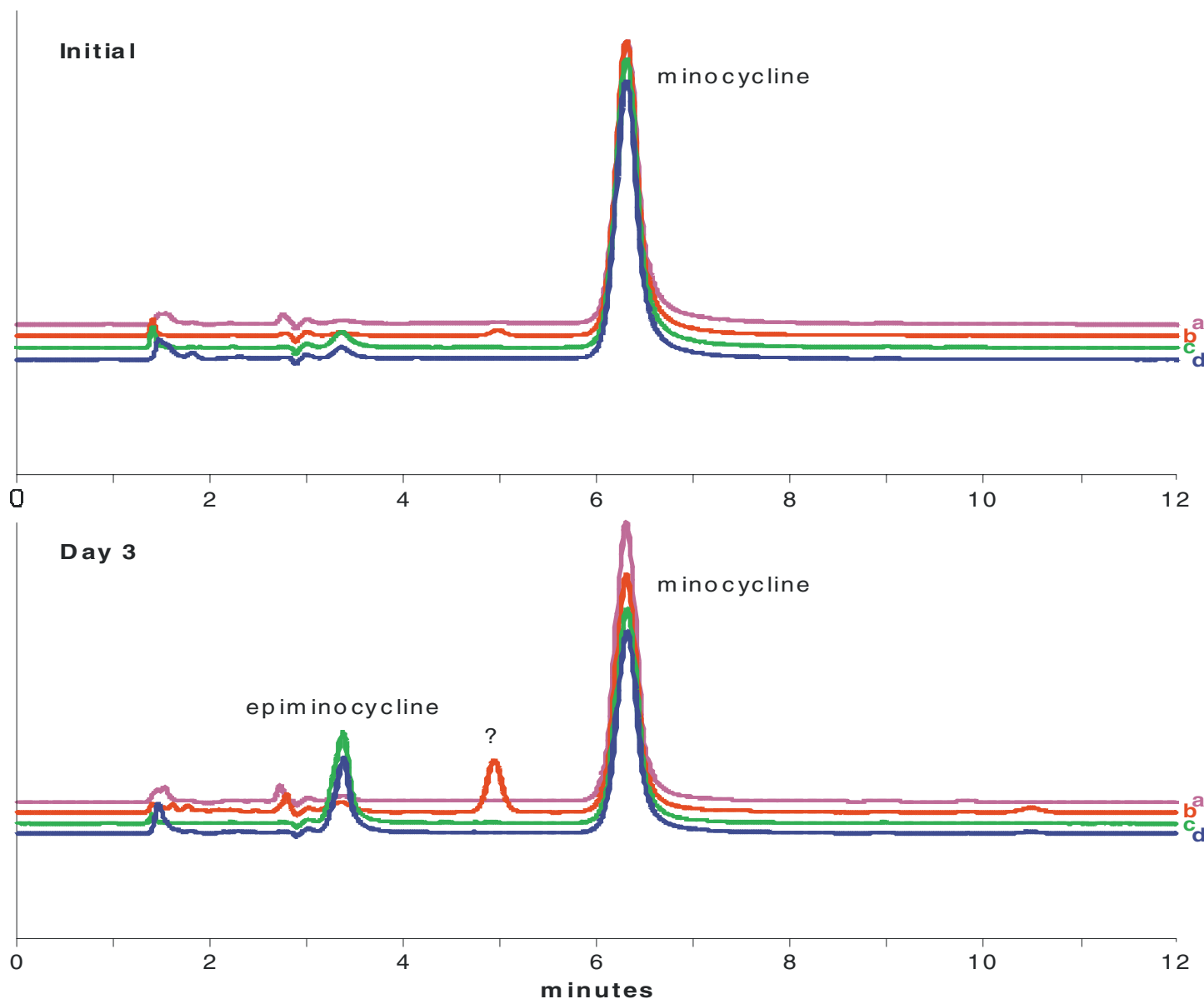
Time (min.)	%B
0.0	0
8.0	0
11.0	100
14.0	0
23.0	0

## Standard Solution Stability

Minocycline standard solutions were prepared in 29:71 THF:aqueous. The individual preservatives were present at a concentration of 0.1 weight percent salt. The pH given below represents the pH of the preservative solution. Solution stability was measured over seven days. Solutions made with sodium sulfite (pH 9.5) were the most stable under these conditions. Lower pH was found to facilitate the degradation of the main band to epiminocycline. The growth of an unknown peak was observed with sodium citrate preservative at the higher pH.

### Example Chromatograms of 50 $\mu\text{g}/\text{mL}$ Standard as a Function of Preservative - Initial and Day 3

- a: Sodium Sulfite, pH 9.5
- b: Sodium Citrate, pH 9.0
- c: Sodium Citrate, pH 4.3
- d: Sodium Sulfite, pH 4.3



# Standard Solution Stability versus Preservative and pH

Standard Concentration: 50 µg/mL

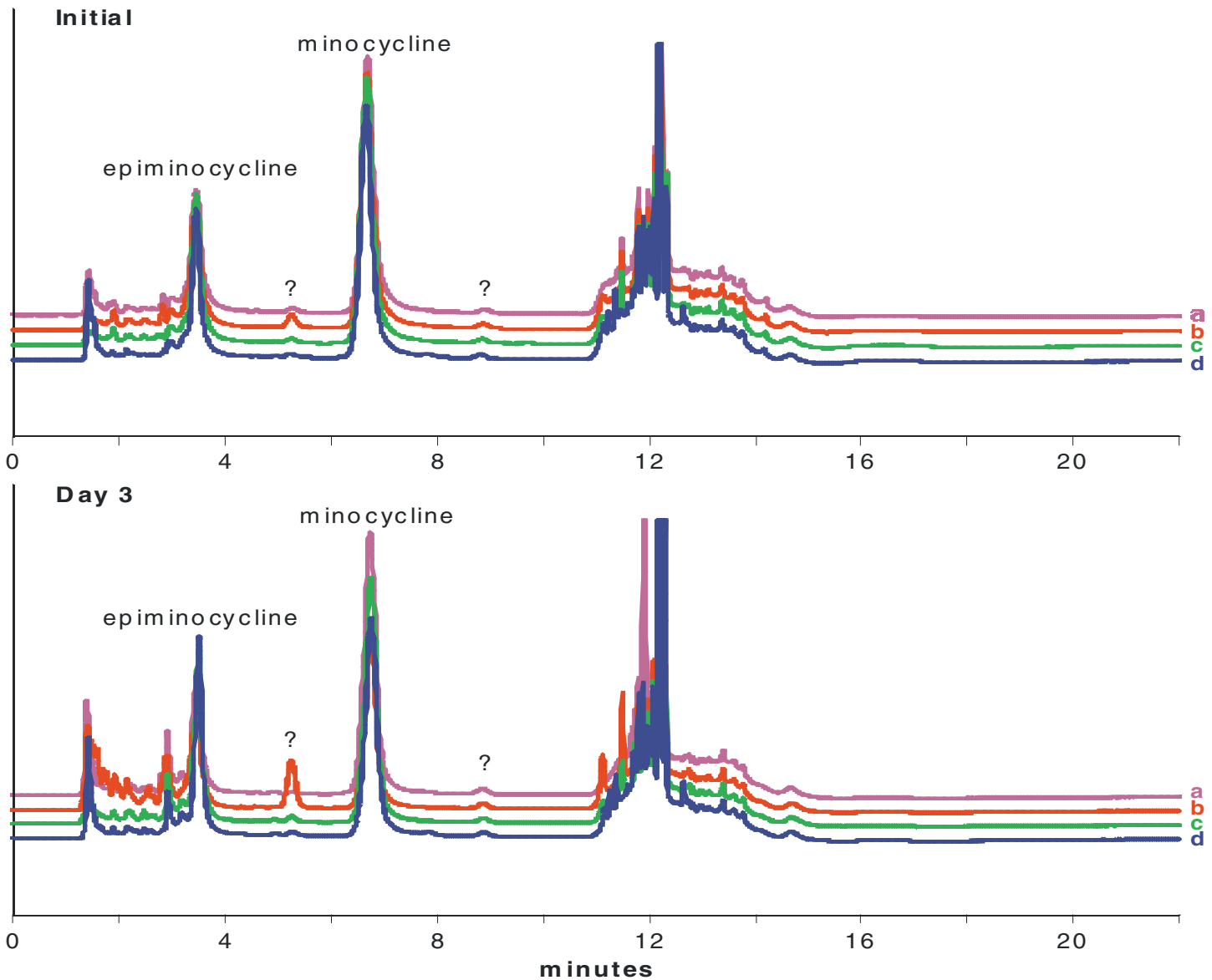
Preservative	pH	Percent of Initial				
		Initial	Day 1	Day 2	Day 3	Day 7
sodium sulfite	9.5	100.0	99.8	99.6	100.4	96.3
sodium sulfite	4.3	100.0	88.6	81.5	76.6	58.4
sodium citrate	9.0	100.0	95.4	89.4	84.3	60.5
sodium citrate	4.3	100.0	88.7	81.0	77.1	67.8
sodium phosphate (no preservative effect)	4.3	100.0	95.0	90.2	86.1	73.9

## Sample Solution Stability

The preservatives used for standards were also studied with sample preparations. Preservatives were added after the final extraction step to a concentration of 0.1 weight percent salt in solution. Solutions were stored in the dark at ambient conditions. A gradient separation was needed to prevent chromatographic interference from extractables. As was observed with standards, solutions preserved with sodium sulfite (pH 9.5) were the most stable of those studied, exhibiting only nominal amounts of degradation after seven days.

# Example Chromatograms of ~40 µg/mL Minocycline in Sample Solutions as a Function of Preservative - Initial and Day 3

- a: Sodium Sulfite, pH 9.5
- b: Sodium Citrate, pH 9.0
- c: Sodium Citrate, pH 4.3
- d: Sodium Sulfite, pH 4.3



# Sample Solution Stability versus Preservative

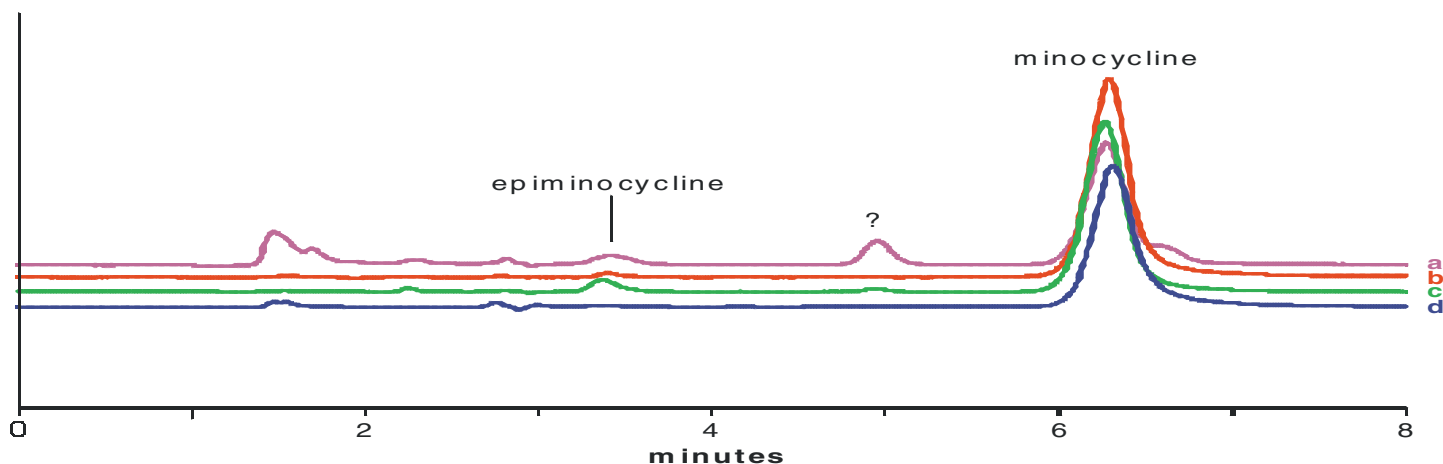
Minocycline Concentration in Sample Solutions: ~40 µg/mL

Preservative	pH	Percent of Initial				
		Initial	Day 1	Day 2	Day 3	Day 7
sodium sulfite	9.5	100.0	102.0	101.7	101.9	99.3
sodium sulfite	4.3	100.0	95.1	92.7	88.3	80.9
sodium citrate	9.0	100.0	87.5	77.0	65.6	37.6
sodium citrate	4.3	100.0	97.4	95.0	93.0	90.0
sodium phosphate (no preservative effect)	4.3	100.0	99.0	95.4	91.7	77.7

## Atypical Degradation

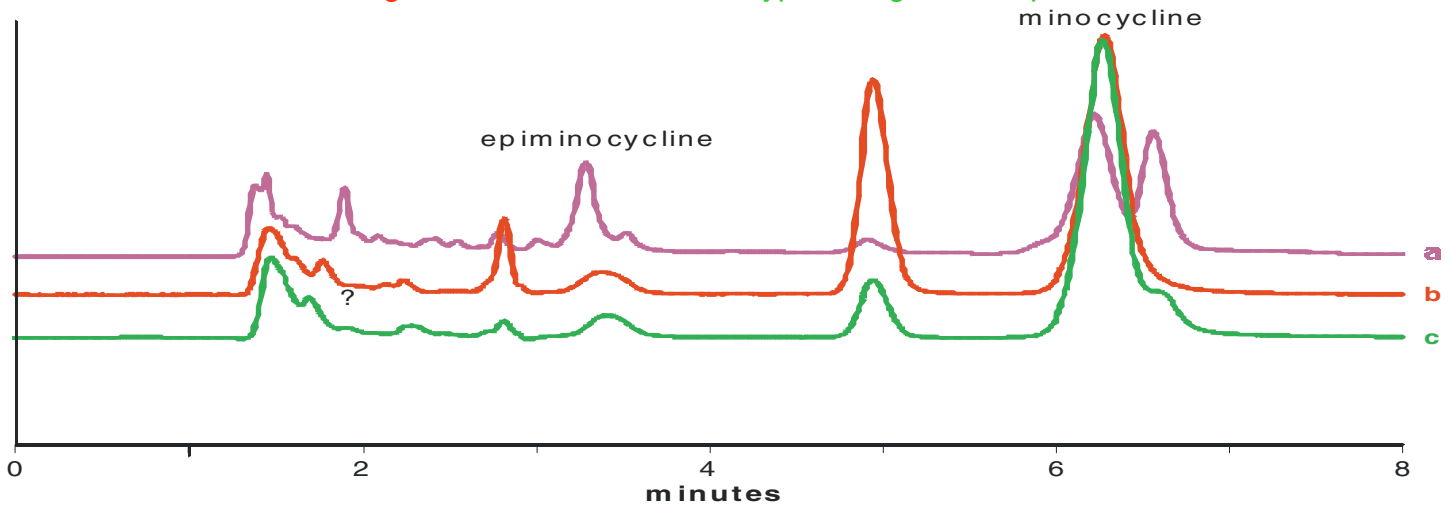
Although sodium sulfite (pH 9.5) was found to effectively stabilize standard and sample extraction solutions, unusual degradation patterns were sometimes observed for this preservative. The degradation involved splitting of the main band and the growth of additional peaks. Component identification was not performed, but peaks are thought to be minocycline related substances (e.g. 9-minocycline, 6-deoxy-6-demethyltetracycline, 7-didemethylminocycline, 7-monodemethylminocycline). The degradation is only observed when both extraction components (i.e. THF and sodium sulfite) are present in solution with minocycline.

a: THF+Na<sub>2</sub>SO<sub>3</sub>   b: Na<sub>2</sub>SO<sub>3</sub> only   c: THF only   d: water only



The degradation pattern, which is similar to that observed with forced thermal degradation, was dependent on the particular solution of sodium sulfite (pH 9.5) used. Most sodium sulfite solutions did not produce this degradation effect. For those solutions that did generate the atypical degradation results, the age of solution was not a factor. The random occurrence would suggest contamination of the labware. Dishwasher detergent was suspected. Addition of small amounts of detergent to representative standard solutions did result in chromatograms similar to the atypical degradation given above.

a: forced thermal   b: detergent added   c: observed atypical degradation pattern



## Conclusions

Sodium sulfite (pH 9.5; 0.1 wt % in solution) is a viable preservative for use in extraction solutions of medical devices containing minocycline, with demonstrated expiration out to at least 3 days. Solutions containing the preservative, however, are prone to atypical degradation, which is facilitated by glassware contamination.