LC-MS/MS Separation of Furosemide and its Metabolite Using the Kinetex® 2.6 µm Biphenyl, Luna® Omega 1.6 µm C18, and Luna Omega 1.6 µm Polar C18 Columns

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Overview

Furosemide belongs to the diuretic drugs and can be administered either orally or by injection. It is mainly used for the treatment of edema. One of the metabolites formed from Furosemide is 4-chloro-5-sulfamoyl anthranilic acid (Saluamine) whose pharmacological activity remains unknown (**Figure 1**). This drives the interest in developing a reversed phase HPLC method able to separate and analyze Furosemide and its metabolites successfully and in a short amount of time. In this application note, we present an LC-MS/MS method to separate Furosemide and its metabolite by utilizing three different columns: Kinetex 2.6 µm Biphenyl, Luna Omega 1.6 µm C18, and Luna Omega 1.6 µm Polar C18.

The Kinetex Biphenyl column provided separation for Furosemide and its metabolite through a mixture of pi-pi and polar interactions; and the higher efficiency provided by the core-shell particle morphology also results in narrow peaks and increased MS sensitivity (**Figure 2a**).

The Luna Omega C18 column is a conventional C18 column with TMS endcapping, making this column a good choice for retention of analytes through hydrophobic interactions. The Luna Omega Polar C18 incorporates a polar surface treatment to offer increased retention for polar analytes, in addition to hydrophobic retention via the C18 bonded phase. The Luna Omega Polar C18 did provide a slight increase in retention for the more polar Saluamine (**Figure 2b, 2c**), while both Luna Omega columns showed an overall reduction in retention compared to the Kinetex Biphenyl column, which is likely due to the multiple interactions between the analytes and the Biphenyl stationary phase (**Figure 2a**).

LC-MS/MS Conditions

Column: Kinetex 2.6 µm Biphenyl (00B-4622-AN)
Luna Omega 1.6 µm C18 (00B-4742-AN)
Luna Omega 1.6 µm Polar C18 (00B-4748-AN)

Dimension: 50 x 2.1 mm

Mobile Phase: A: 0.1 % Formic Acid in Water
B: See Chromatogram

Gradient:

Time (min)	%B
0	5
4	95
5	95
5.1	5
7	5

Flow Rate: 400 µL/min

Injection Volume: 1 µL

Temperature: 40 °C

LC System: Agilent® 1200 Series

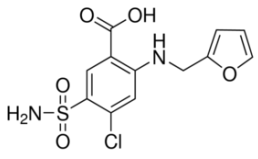
Detection: MS/MS

Detector: SCIEX® 4500

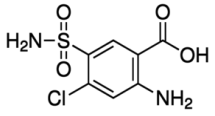
MRM Transitions

Analyte	Q1 (m/z)	Q3 (m/z)
Furosemide-1	329	205
Furosemide-2	329	285
Saluamine-1	248.98	205
Saluamine-2	248.98	126

Figure 1. Furosemide and Saluamine

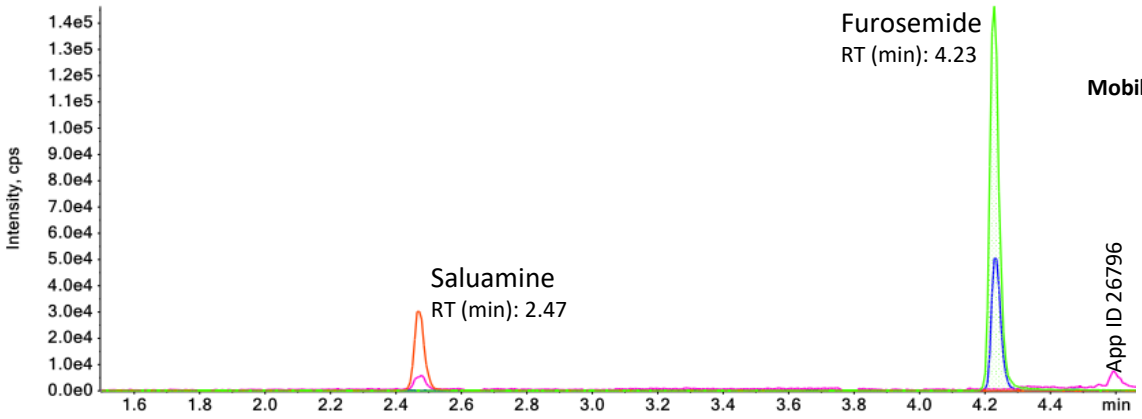


Furosemide



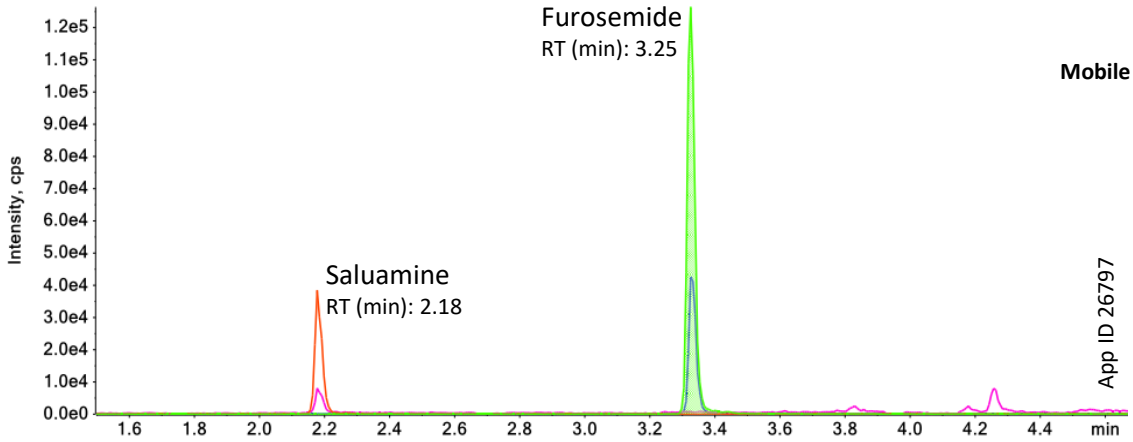
Saluamine

Figure 2a. Kinetex® 2.6 µm Biphenyl



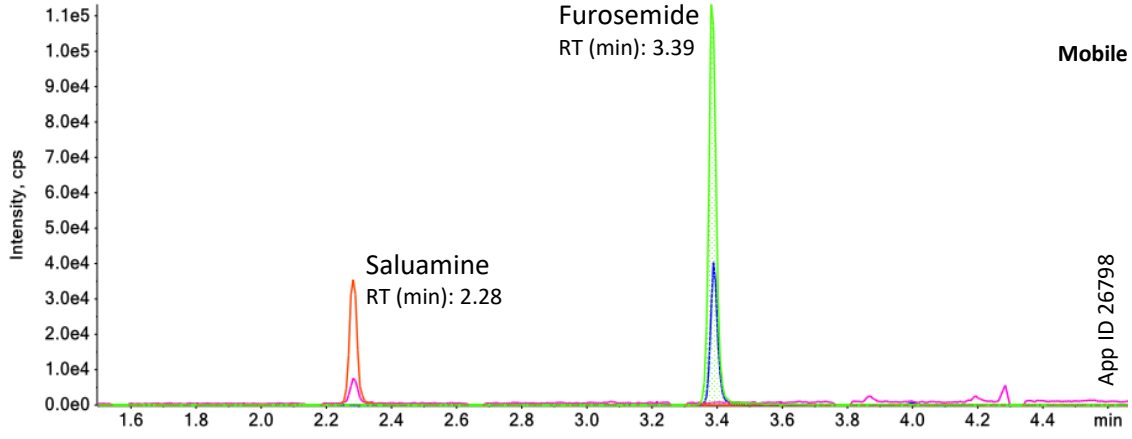
Mobile Phase: A: 0.1 % Formic Acid in Water
B: 0.1 % Formic Acid in Methanol

Figure 2b. Luna® Omega 1.6 µm C18



Mobile Phase: A: 0.1 % Formic Acid in Water
B: 0.1 % Formic Acid in Acetonitrile

Figure 2c. Luna Omega 1.6 µm Polar C18



Mobile Phase: A: 0.1 % Formic Acid in Water
B: 0.1 % Formic Acid in Acetonitrile

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