LC-MS/MS Separation of Doxepin and its Metabolite N-Desmethyldoxepin Using the Kinetex® 2.6 µm Biphenyl and Luna® Omega 1.6 µm C18 Columns

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Overview

Doxepin hydrochloride belongs to a class of drugs referred to as tricyclic antidepressants (TCA) which are widely used to treat a broad range of psychotic depressive disorders. N-Desmethyldoxepin is the primary metabolite of Doxepin (**Figure 1**). This drives the interest in developing a reversed phase HPLC method able to separate and analyze Doxepin and its metabolites successfully and in a short amount of time. In this application note, we present an LC-MS/MS method to separate Doxepin and its metabolite by utilizing Kinetex 2.6 µm Biphenyl and Luna Omega 1.6 µm C18 columns.

The Kinetex Biphenyl column provided separation for Doxepin and its primary metabolite through a mixture of pi-pi and polar interactions; and the higher efficiency provided by the core-shell particle morphology also resulted 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. Depending on the mobile phase used, there was a shift in elution order observed. Using Acetonitrile instead of Methanol in the mobile phase on the Luna Omega 1.6 µm C18 column gave narrower peaks and the same elution order observed with the Kinetex Biphenyl column (**Figure 2b, 2c**). There was a slightly shorter run time when using Methanol in the mobile phase on the Luna Omega 1.6 µm C18 column, which is attributed to the weaker elution strength of methanol vs. acetonitrile. Regardless of mobile phase, the run time was much shorter on the Luna Omega 1.6 µm C18 column compared to the Kinetex 2.6 µm Biphenyl column, which is likely due to the multiple interactions between the analytes and the Biphenyl stationary phase.

LC-MS/MS Conditions

Column: Kinetex 2.6 µm Biphenyl (00B-4622-AN)
Luna Omega 1.6 µm C18 (00B-4742-AN)
Dimension: 50 x 2.1 mm
Mobile Phase: A: 0.1 % Formic Acid in Water
B: See Chromatogram

Gradient:

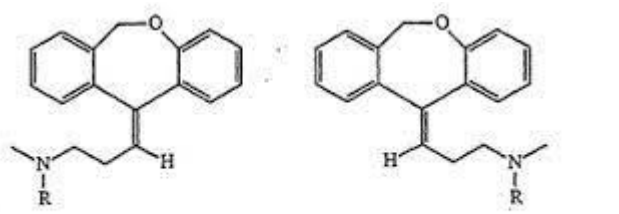
Time (min)	%B
0	40
6	70
6.1	40
8	40

Flow Rate: 500 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)
Doxepin hydrochloride-1	280	107
Doxepin hydrochloride-2	280	115
N-Desmethyldoxepin-1	266.1	107
N-Desmethyldoxepin-2	266.1	235.1

Figure 1. Doxepin and N-Desmethyldoxepin



Interestingly, when using methanol both the Luna Omega C18 and Kinetex Biphenyl columns provided separation of the (E) and (Z) isomers of Doxepin, which are present at an approximate 85:15 ratio, with the Biphenyl providing complete separation. This was confirmed as the mass transitions for the (E) and (Z) isomers are the same. This separation could be due to the increased retention observed when using methanol, which is a weaker elution solvent than acetonitrile, with the increased interaction allowing for separation of the isomers.

Figure 2a. Kinetex® 2.6 µm Biphenyl

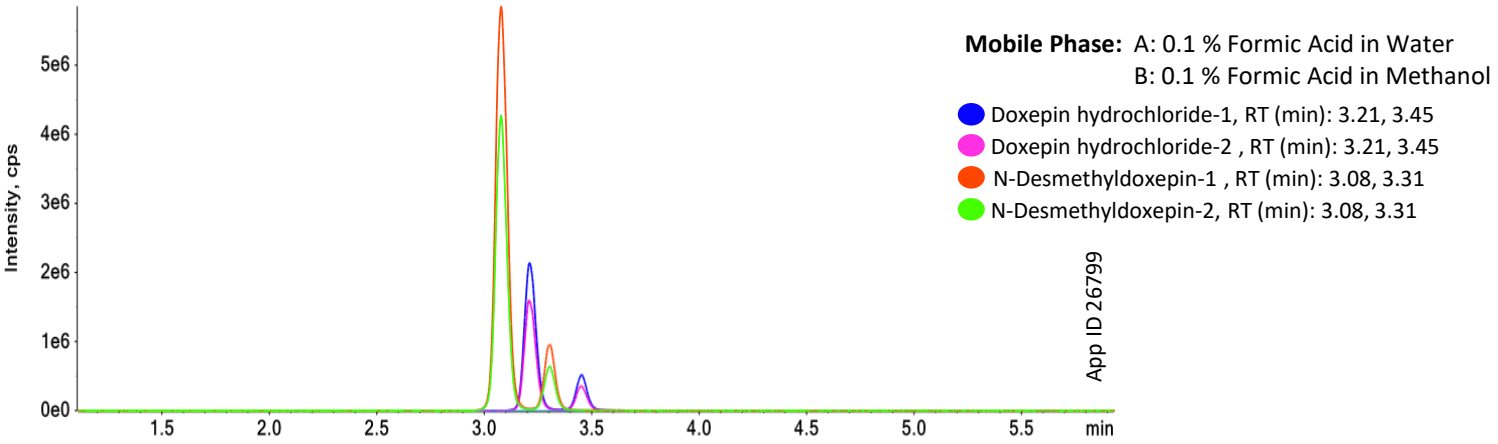


Figure 2b. Luna Omega 1.6 µm C18

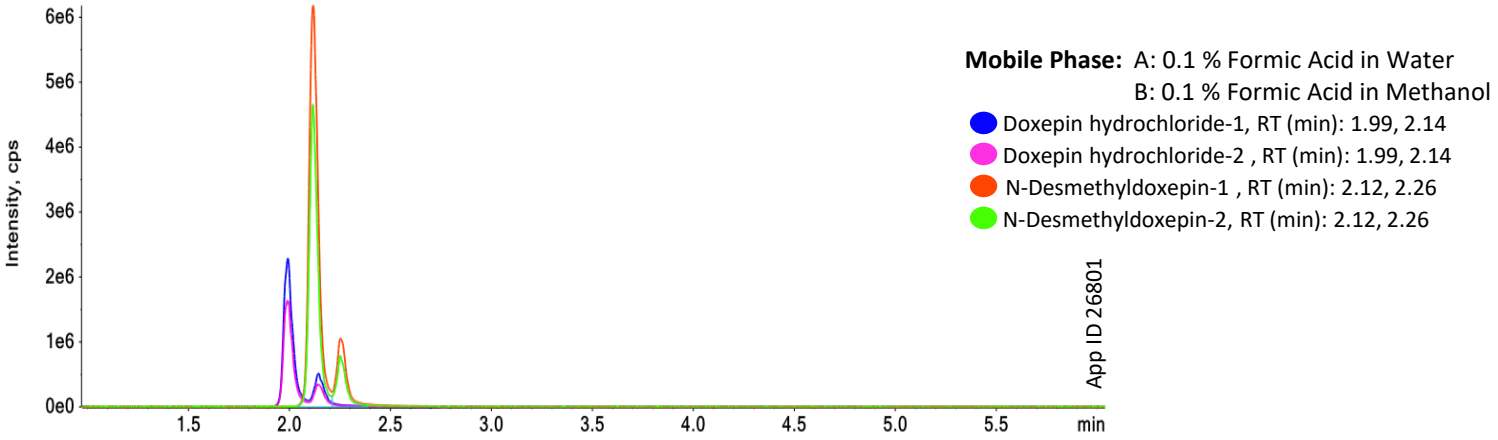
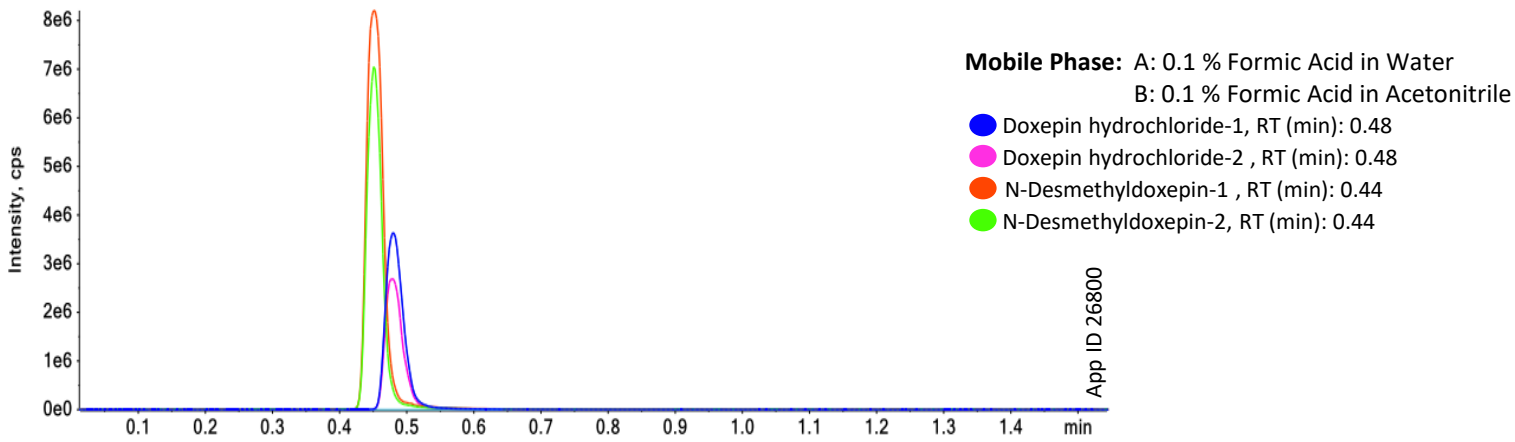


Figure 2c. Luna® Omega 1.6 µm C18



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