Separation of Diazoxide and its Organic Impurities per USP Monograph

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Overview

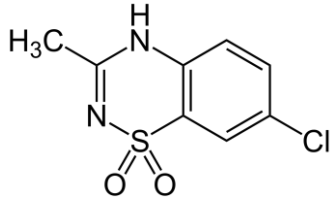
Diazoxide is a member of the thiazide family of drugs and prevents insulin release from the pancreas, helping to return blood sugar to normal levels. The development of a quick and efficient analysis of Diazoxide and its related organic impurities is of interest for generic drug manufacturers. In this application note, we report the separation of Diazoxide and its related organic impurities using a Kinetex™ 2.6 µm Biphenyl column according to the USP monograph for Diazoxide.

System suitability per USP Monograph for the Diazoxide Assay is a symmetry factor no more than (NMT) 2.0 and a percent relative standard deviation (%RSD) of NMT 0.73 % for Diazoxide. System suitability requirements for Diazoxide Assay were met by the Kinetex 2.6 µm Biphenyl column (**Figure 2**).

System suitability per USP Monograph for the Diazoxide Organic Impurities is a symmetry factor NMT 2.0, a %RSD of NMT 5.0 %, and a signal-to-noise (S/N) ratio no less than 10 for Diazoxide. All requirements for System Suitability for Diazoxide Organic Impurities were met by the Kinetex 2.6 µm Biphenyl column (**Figures 3** and **4**).

All solutions were prepared as indicated in the USP Monograph for Diazoxide. USP Diazoxide RS (Catalog No. 1186000) was purchased from USP.

Figure 1. Diazoxide



LC-UV Conditions - Assay

Column: Kinetex 2.6 µm Biphenyl
Dimension: 50 x 4.6 mm
Part No.: 00B-4622-E0
Mobile Phase: Acetonitrile/Methanol/Buffer (10:10:80, v/v/v)

Buffer: Dissolve 1.56 g of Sodium Phosphate Monobasic in 1 L of water. Adjust with Phosphoric Acid to a pH of 2.5.

Flow Rate: 1 mL/min (Isocratic)
Injection Volume: 20 µL
Temperature: 30 °C
Detector: UV @ 254 nm
System: Agilent® 1260 Binary UHPLC

LC-UV Conditions - Organic Impurities

Column: Kinetex 2.6 µm Biphenyl
Dimension: 50 x 4.6 mm
Part No.: 00B-4622-E0
Mobile Phase: A: Methanol/Buffer (15:85, v/v)
B: Acetonitrile/Methanol (70:30, v/v)

Buffer: Dissolve 1.56 g of Sodium Phosphate Monobasic in 1 L of water. Adjust with Phosphoric Acid to a pH of 2.5.

Gradient:

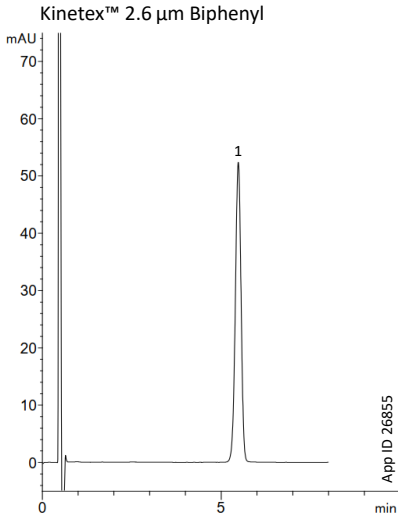
Time (min)	%B
0	5
10	15
20	65
25	65
25.1	5
30	5

Flow Rate: 0.8 mL/min
Injection Volume: 20 µL
Temperature: 30 °C
Detector: UV @ 220 nm
System: Agilent 1260 Binary UHPLC

Table 1. Preparation of Solutions

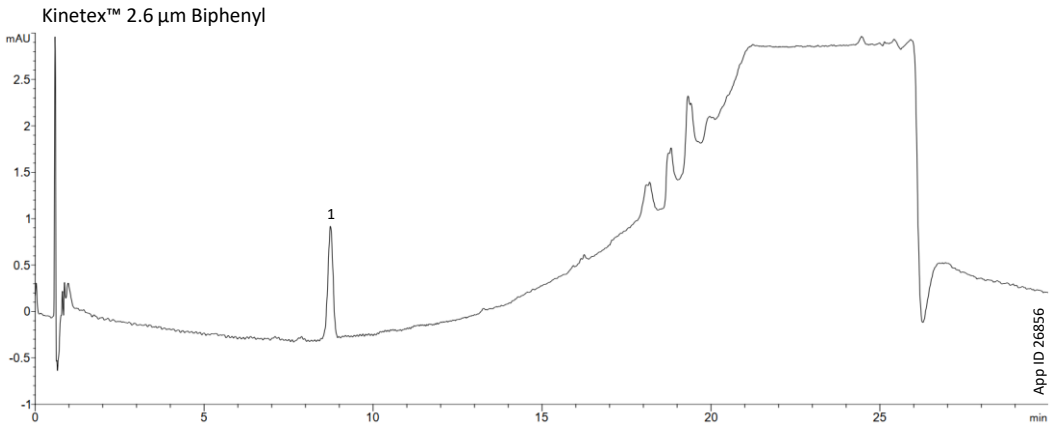
Solution	Composition
Diluent	Methanol/Buffer (50:50, v/v)
Standard Solution – Assay	0.05 mg/mL of USP Diazoxide RS in Diluent Sonicate to dissolve if needed
Standard Solution – Organic Impurities	0.5 µg/mL of USP Diazoxide RS in Diluent
Sensitivity Solution – Organic Impurities	0.25 µg/mL of USP Diazoxide RS in Diluent

Figure 2. Standard Solution – Assay



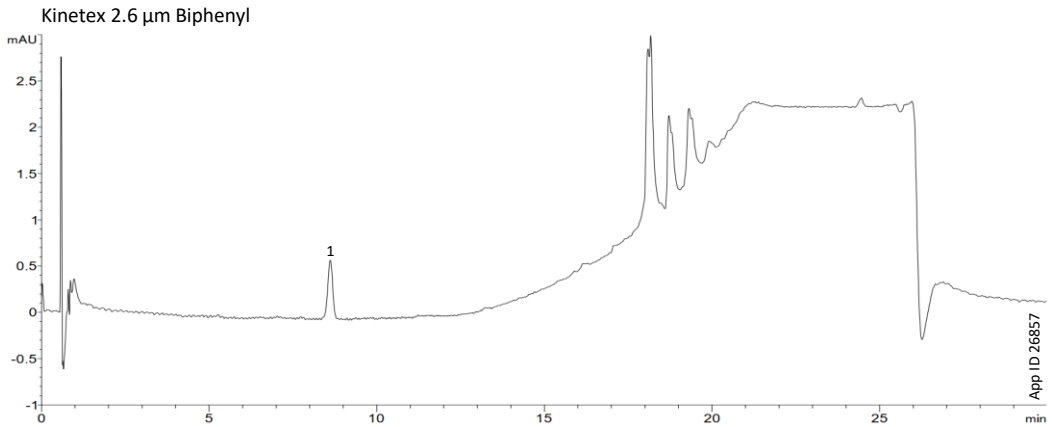
Peak No.	Analyte	Retention Time (min)	Symmetry Factor	Area %RSD
1	Diazoxide	5.43	0.96	0.11
N = 6 Injections				

Figure 3. Standard Solution – Organic Impurities



Peak No.	Analyte	Retention Time (min)	Symmetry Factor	Area %RSD
1	Diazoxide	8.74	0.93	1.59
N = 6 Injections				

Figure 4. Sensitivity Solution – Organic Impurities



Peak No.	Analyte	Retention Time (min)	S/N Ratio
1	Diazoxide	8.62	25.87
N = 3 Injections			

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