

CASE STUDY

USP Diazoxide Assay and Organic Impurities

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Introduction

Diazoxide is a member of the thiazide family of drugs and prevents insulin release from the pancreas. The development of a quick and efficient analysis of Diazoxide and its related organic impurities is of interest for generic drug manufacturers. The USP monograph for Diazoxide Assay and Organic Impurities, which became official on November 1st, 2022, uses the Kinetex™ 2.6 µm, 50 x 4.6 mm Biphenyl LC column. It is worth noting that, while the official USP monograph does not specify a column by name (simply indicated as “L11, 2.6 µm, 50 x 4.6 mm”), the draft which was published in USP-PF 45(1) does indicate that the Kinetex 2.6 µm, 50 x 4.6 mm Biphenyl column was the column that was used in the elaboration of the monograph.

Since the USP does not publish chromatograms in the official monograph methods, we wanted to demonstrate in this case study that the Kinetex Biphenyl column gave the desired results and to share the chromatographic results with those interested in this USP monograph method for Diazoxide.

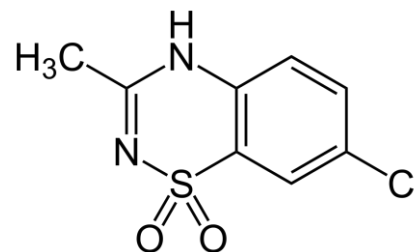
System suitability per USP Monograph for the Diazoxide Assay is a symmetry factor no more than (NMT) 2.0 and a percent relative standard deviation (%RSD) of NMT 0.73 % for Diazoxide. As expected, system suitability requirements for Diazoxide Assay were met by the Kinetex 2.6 µm Biphenyl column (**Figure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

Key Concepts:

- Applicability of Core-shell Biphenyl for USP Diazoxide Assay and Organic Impurities
- Chromophore and concentration differences between sample/solvent can lead to severe baseline shifts

Figure 1. Diazoxide



Application Methods

LC-UV Conditions - Assay

Column: Kinetex™ 2.6 µm Biphenyl

Dimensions: 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

Dimensions: 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

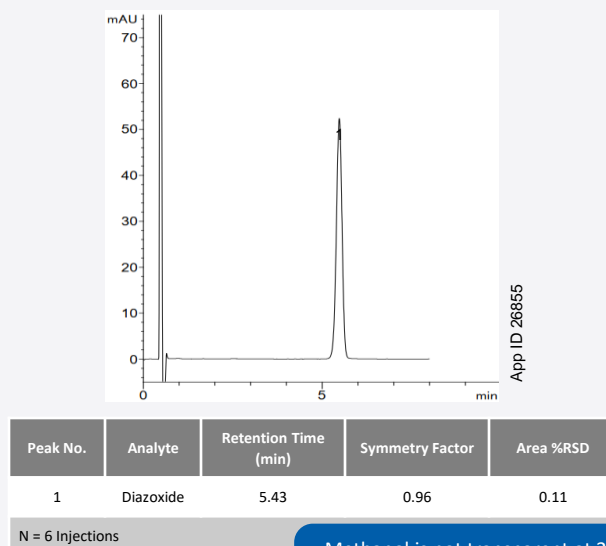
System: Agilent 1260 Binary UHPLC

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

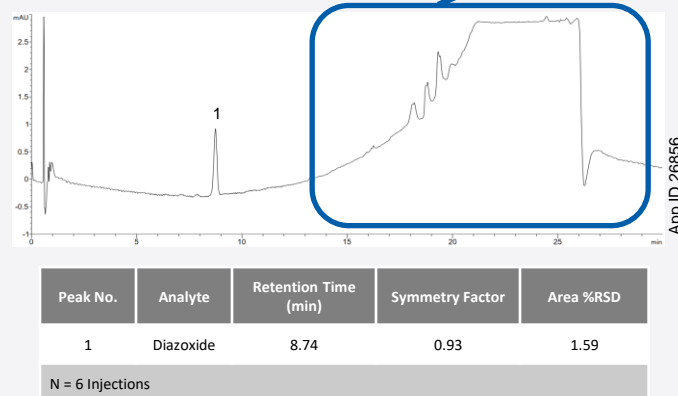
Results and Discussion

Figure 2. Standard Solution - Assay



Methanol is not transparent at 220 nm so the rising baseline is due to the increasing percent of Methanol during the gradient.

Figure 3. Standard Solution – Organic Impurities



In both **Figure 3** and **4**, there was an abrupt rise in the baseline after 10 minutes that returns after 25 minutes when the mobile phase composition is returned to the initial conditions. This rise was also seen in the solvent blank (not shown), so it was not due to the Standard Solution or Sensitivity Solution composition. The rising baseline is due to the increasing % of Methanol in the mobile phase during the gradient. Methanol has some absorbance (is not as UV transparent as Acetonitrile) at 220 nm, so as the % Methanol increases the UV absorbance also increases, resulting in the rising baseline. This is especially noticeable at lower analyte concentrations or when the analytes of interest contain weak chromophores. Note that a reference wavelength of 360 nm was used here to minimize this issue.

It is important to note that this rise in baseline was not seen in **Figure 2** due simply to the scaling difference. This can be explained due to the much higher concentration of Diazoxide in the *Standard Solution – Assay*. With the Diazoxide peak at full scale, the rise in baseline was not visible.



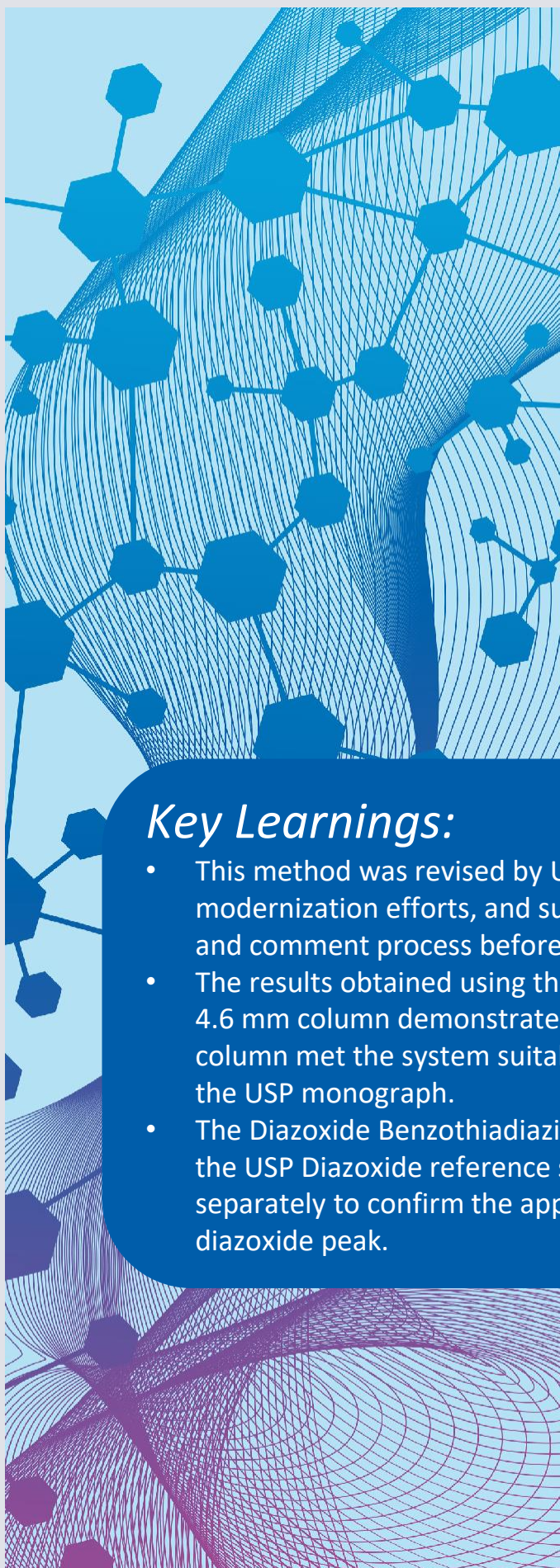
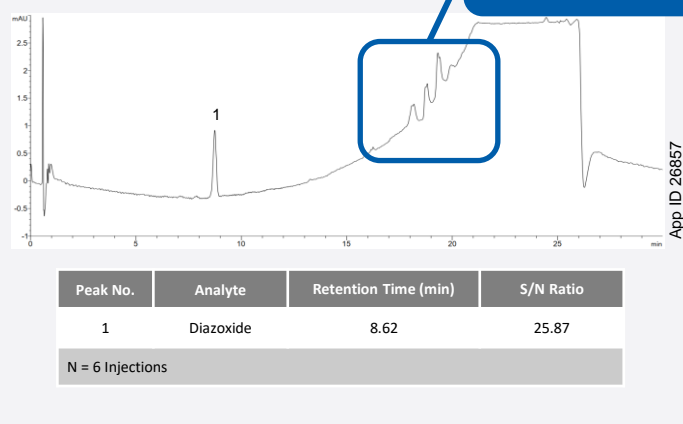


Figure 4. Sensitivity Solution – Organic Impurities



Both **Figure 3** and **4** contain unidentified impurity peaks between 15- and 20-minutes in the chromatograms. These peaks were also observed in the solvent blank (not shown), so were not due to the Standard Solution or Sensitivity Solution composition. It is important to note that these impurity peaks were not seen in **Figure 2** due simply to the scaling difference. This can be explained due to the much higher concentration of Diazoxide in the *Standard Solution – Assay*. With the Diazoxide peak at full scale, these impurity peaks were not visible due to their very low absorbance.

Key Learnings:

- This method was revised by USP as part of the USP monograph modernization efforts, and subject to the typical USP review and comment process before being becoming official.
- The results obtained using the Kinetex™ 2.6 μm Biphenyl 50 x 4.6 mm column demonstrate that the Kinetex 2.6 μm Biphenyl column met the system suitability requirements as published in the USP monograph.
- The Diazoxide Benzothiadiazinone analog was not observed in the USP Diazoxide reference standard and was not obtained separately to confirm the approximate RT of 0.45 relative to the diazoxide peak.

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