Application Note

Pharmaceuticals

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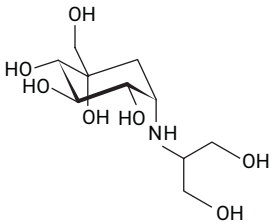
Analysis of Voglibose Using an Agilent LC with Mass Selective Detection

A cost-effective, efficient, and sensitive alternative to traditional fluorescence detection



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Introduction

This Application Note describes the advantage of routine mass detection for the rapid analysis of active pharmaceutical ingredients (APIs) in pharmaceutical tablets. It uses an Agilent liquid chromatography single quadrupole (SQ) mass spectrometer (LC/MS) and describes the benefits of the LC/MS method compared to the standard UHPLC-fluorescence detection (FLD) method.

Pharmaceutical manufacturers are required to monitor and quantify the composition of drug formulations. LC/MS technology is a selective and sensitive technique for the quantification of pharmaceutical compounds. Agilent single quadrupole MS systems deliver high sensitivity, stable results, reliable performance, and are combined with sophisticated data processing capabilities. This Application Note presents the rapid characterization of an API, Voglibose, using the Agilent 1260 Infinity II LC coupled to an Agilent single quadrupole mass spectrometer.

Single quadrupole mass spectrometry offers significant advantages over fluorescence detection.

- First, MS provides direct mass information, eliminating the reliance on retention time for compound confirmation.
- Second, the LC/MS-based method eliminates the tedious and multistage chemical derivatization process. Typical FLD methods require post column derivatization[2] using reagents taurine and sodium periodate.
- Finally, the run times for LC/MS-based methods are quite short compared to lengthy fluorescence-based methods.

An LC/MS method for the quantitative analysis of Voglibose in drug formulations at low concentration levels in the presence of two other compounds, Metformin and Glimepiride, was developed. Commercially available Voglibose tablets contain Metformin and Glimepiride as major components. The standard methodology for the quantification of Voglibose uses HPLC-FLD with post column derivatization, and requires sample preparation, costly reagents, and valuable hands-on time. The MSD overcomes the analytical challenges of fluorescence, including reproducibility and sensitivity.

Diabetes mellitus is a worldwide morbid disease, with increasing incidence. α-Glucosidase inhibitors, namely Acarbose, Voglibose, and miglitol, are used for therapy. Voglibose is an α-glucosidase inhibitor used for lowering postprandial blood glucose levels in people with diabetes mellitus. Voglibose delays the absorption of glucose, reducing the risk of macrovascular complications[1]. Voglibose is well tolerated and effective in comparable doses among these drugs[3].

Experimental

Sample preparation

To facilitate dissolution, three drug tablets were dissolved in 80 mL of water followed by sonication for 20 minutes. After sonication, the solution was equilibrated to room temperature. The final volume of the solution was increased to 200 mL with acetonitrile. The final solution was vortexed rigorously for three minutes, then filtered using a 0.45 µm nylon syringe filter. Further dilutions were prepared in acetonitrile:water (20:80) and analyzed by LC/MS.

LC/MS Analysis

Instrumentation

All experiments were performed using an Agilent 1260 Infinity II LC, comprising a binary pump, vacuum degasser, thermostatted well plate autosampler, and column oven, coupled to an Agilent 6120B Single Quadrupole LC/MS System with an Agilent ESI ion source. Comparable analysis can be achieved with the latest model single quad, the Agilent InfinityLab LC/MSD Series. Ten microliters of sample were injected. Data were acquired and analyzed with OpenLab CDS ChemStation Edition.

HPLC conditions

Parameter	Value
Mobile Phase	A) 5 mM Ammonium formate B) Acetonitrile
The HPLC analysis was carried out under isocratic separation conditions with:	
Mobile Phase A	50 %
Mobile Phase B	50 %
Flow Rate	0.5 mL/min
HPLC Column	Agilent ZORBAX HILIC Plus Rapid Resolution, 4.6 × 50 mm, 3.5 µm

MSD conditions

Parameter	Value
Ion Mode	Positive
Nebulizer	25 psig
Drying Gas	11 L/min at 350 °C
VCap	4,000 V
Fragmentor	120 V

SIM ions

Analyte	*m/z*
Voglibose	268.1
Glimepiride	491.2
Metformin	130.1

Results and discussion

Chromatographic and mass spectral analysis

The compounds were well separated by LC using the Agilent HILIC column.

Each tablet weighed 1 g and contained 0.2 mg of Voglibose, 2 mg of Glimepiride, and 500 mg of Metformin. To avoid injecting high concentrations of Metformin and Glimepiride, and to focus the analysis on the compound of interest (Voglibose), the acquisition method was optimized such that only compounds eluting after four minutes were injected into the 6120B Single Quadrupole LC/MS System. This eliminated the introduction of 500 mg of Metformin into the SQ and ensured system robustness even with a very high concentration of Metformin. Figure 1 shows the total ion chromatogram (TIC) separation of the standard comprised of the three analytes with a total LC/MS run-time of eight minutes. The components were well separated.

To assess the performance of Voglibose detection using the LC/MS single quadrupole, seven concentrations of Voglibose were prepared with a concentration range of 10–900 ng/mL, and analyzed by single quadrupole LC/MS. Excellent chromatographic linearity, reproducibility, and relative response were observed across the concentration range.

Figure 3 shows the extracted ion chromatogram (EIC) for Voglibose, and demonstrates good reproducibility. The good peak shapes and response helped achieve sensitivity at very low levels.

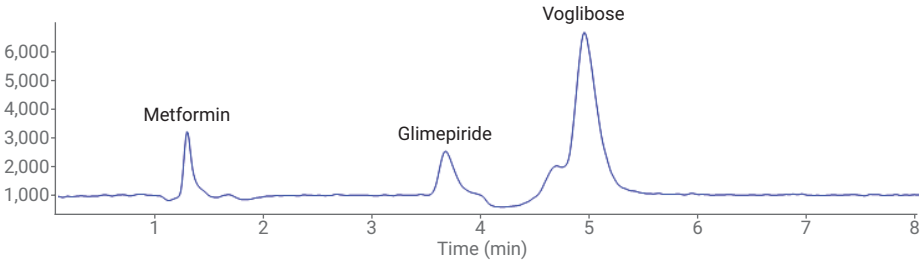

Figure 1. The TIC from the LC/MS analysis of the standard. Voglibose (100 ng/mL), Metformin (250 µg/mL), and Glimepiride (1,000 ng/mL) are well separated chromatographically.

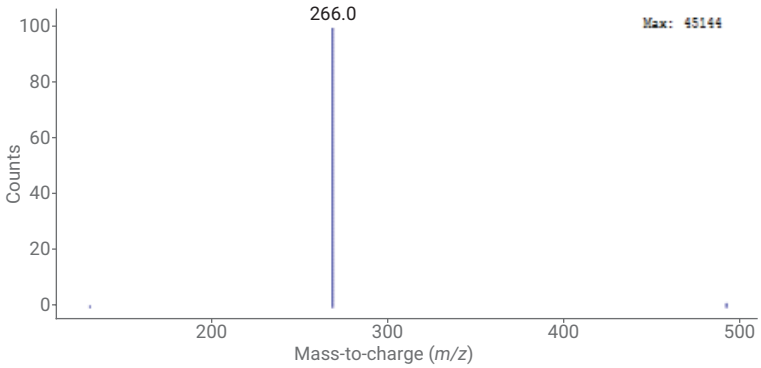

Figure 2. Mass spectrum of Voglibose.

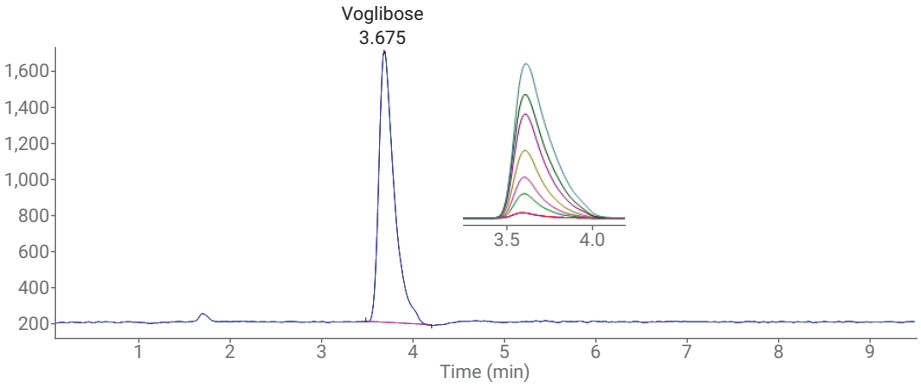

Figure 3. EIC of Voglibose. The inset shows the overlaid EIC of seven concentrations from 10 to 900 ng/mL.

A seven-point linearity curve for concentrations 10, 50, 100, 200, 400, 600, and 900 ng/mL was achieved with an excellent coefficient of linearity (R^2) of 0.99, as shown in Figure 4. Excellent reproducibility of the signal response was observed with the lowest concentration level at 10 ng/mL. Area % RSD was much lower than 1 %, at 0.26 % (n = 7), as shown in Figure 5.

This method was used to determine the percent of label claim of the pill formulation. The measured amount of Voglibose was 0.1983 mg/g of a pill. The amount listed on the label was 0.2 mg. This demonstrated that the method result was within >99 % of the listed amount confirming the capabilities of this method for the analysis of Voglibose in pill formulations.

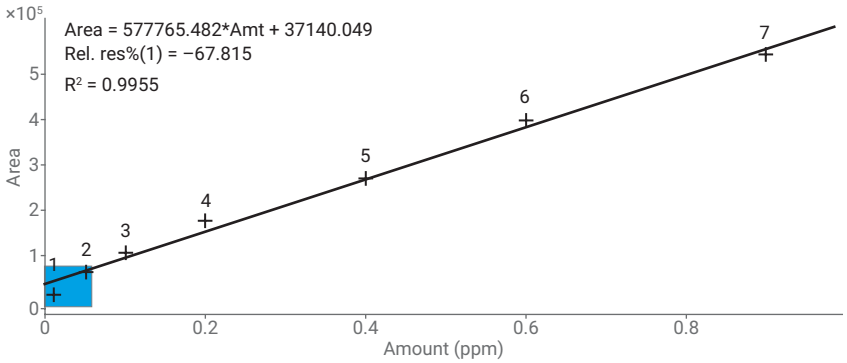

Figure 4. Linearity curve with a coefficient of linearity R^2 >0.99.

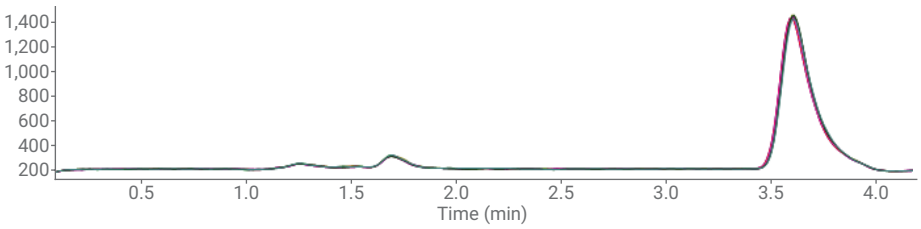

Figure 5. Overlaid chromatogram of seven injections of Voglibose at 10 ng/mL, demonstrating excellent reproducibility at low concentrations.

Conclusion

Rapid and sensitive quantification of the API Voglibose was demonstrated using the 1260 Infinity LC coupled to the 6120 Single Quadrupole MS system. This approach is ideal for the routine and fast analysis of valuable pharmaceutical drugs and does not require the time-consuming process of chemical derivatization required for fluorescence detection. The method is robust, with excellent reproducibility and sensitivity with an analysis time of fewer than eight minutes, versus the more conventional 30-minute fluorescence methods. In addition to Voglibose, this method is capable of chromatographically separating the other two major components of the formulation, Metformin and Glimepiride.

The determination of Voglibose content using the present LC/MS method is in agreement with the labeled content of the Voglibose, confirming the accuracy of this method.

References

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