Customer application note | 001903

Pharma

Acarbose impurity analysis: method migration from UV detection to universal charged aerosol detection

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Keywords

Acarbose, glucosidase inhibitor, impurity analysis, method transfer, Vanquish Charged Aerosol Detector H, HILIC, Amide-HILIC column, graphite column

Application benefits

Impurity analysis of acarbose using a Thermo Scientific™ Vanquish™ Flex UHPLC system with a Thermo Scientific™ Vanquish™ Charged Aerosol Detector (CAD) as an alternative to pharmacopoeial UV detection to extend the range of impurities to include those that cannot be determined due to a lack of a strong chromophore.

Goal

This application note examines the suitability of a HPLC-CAD system for impurity analysis of acarbose as an alternative/supplement to the UV detection used in the European Pharmacopoeia (Ph. Eur.) monograph 2089.

Introduction

Acarbose is an α-glucosidase inhibitor used for the treatment of diabetes mellitus type 2. Typical doses range from 150 mg to 300 mg a day.[1]

Acarbose is a competitive inhibitor of glucosidase as its chemical structure is similar to the enzyme's natural substrate (e.g., starch or sucrose). As presented in Figure 1, acarbose consists of an acarviosin moiety (aglycon cyclitol bonded to an amino sugar) coupled to two D-glucose units. Acarbose is commonly produced by a fermentation process,[2,3]

which can result in an impurity profile discussed in the transparency list of the pharmacopoeial monograph of acarbose.[4] The structures of the acarbose impurities arise from differences in the sugar present, while the acarviosin part remains constant (Figure 1).

All compounds have no pronounced chromophore and thus, up to now, low wavelength detection at 210 nm is applied in the Ph. Eur. related substances test of acarbose.[4]

Charged aerosol detection is a universal technique ideally suited for the detection of this class of compounds. In this application note, we examine the impurity analysis of acarbose (according to the Ph. Eur.) and present two alternative methods for the impurity analysis by means of charged aerosol detection. The suitability of these two methods for analysis of acarbose is also discussed.

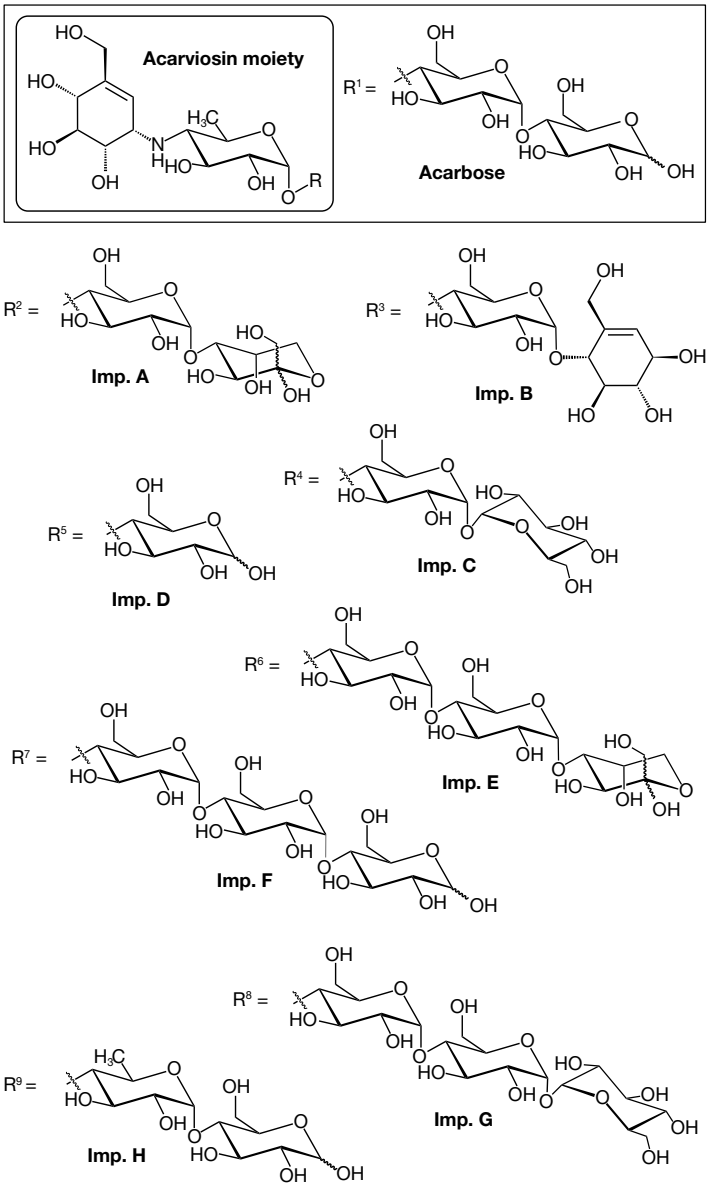

Figure 1. Impurity profile of acarbose according to the Ph. Eur. 11.0[4]

Experimental

Chemicals

Chemical name	Part number
Deionized water, 18.2 MΩ·cm resistivity or higher	N/A
Acetonitrile, Fisher Chemical™ Optima™ LC/MS grade	A955-212
Potassium dihydrogen phosphate, ACS, 99.0% min, Thermo Scientific™	011594.A1
Disodium hydrogen phosphate dihydrate, 99+%, Thermo Scientific™	343810250
Ammonium acetate, Fisher Chemical™ Optima™ LC/MS grade	A114-50
Acetic acid, Fisher Chemical™ Optima™ LC/MS grade	A113-50
Trifluoroacetic acid, Fisher Chemical™ Optima™ LC/MS grade	A116-50
Acarbose Certified Reference Standard (CRS)	Y0000500
Acarbose for peak identification CRS	Y0000427
Acarbose, 95%, Thermo Scientific™ Acros™	15492252

Sample handling

Item name	Part number
Fisher Scientific™ Fisherbrand™ Mini Vortex Mixer	14-955-152
Vials (amber, 2 mL), Fisher Scientific™ Fisherbrand™	03-391-6
Cap with Septum (Silicone/PTFE), Thermo Scientific™	13-622-292

Sample preparation

All test and reference solutions were prepared in accordance with the Ph. Eur. monograph of acarbose.[4] The test solution was prepared by accurately weighing and dissolving 200 mg of the substance to be examined in 10.0 mL of water. For reference solution (1) a vial of acarbose for peak identification CRS (acarbose containing impurities A, B, C, D, E, F, and G) was dissolved in 1.0 mL of water. A 1.0 mL volume of the test solutions was again diluted to 100.0 mL with water to obtain the reference solution (2).

Instrumentation

The current Ph. Eur. method for related substances was performed for comparison, using an aminopropylsilyl silica column (Thermo Scientific™ Hypersil™ APS-2) on an Agilent™ 1100 HPLC system consisting of an online vacuum degasser, a binary pump G1312A, an autosampler G1313A, and a thermostatted column compartment G1316A (Agilent Technologies Deutschland GmbH, Waldbronn, Germany). Agilent™ OpenLab™ CDS Rev. C.01.10 [201] software was used for data processing.

All other experiments were performed on a Thermo Scientific™ Vanquish™ Flex UHPLC system consisting of:

- Vanquish System Base Horizon/Flex (P/N VF-S01-A)
- Vanquish Dual Pump F (P/N VF-P32-A-01)
- Vanquish Split Sampler FT (P/N VF-A10-A-02)
- Vanquish Column Compartment H (P/N VH-C10-A-02)
- Vanquish Charged Aerosol Detector H (P/N VH-D20-A)
- Thermo Scientific™ Corona™ Nitrogen 1010 nitrogen generator (P/N 6295.0200)

Chromatography Data System

The Thermo Scientific™ Chromeleon Chromatography Data system (CDS), version 7.3.0 was used for data acquisition and analysis.

Table 1. Comparative method parameters for the impurity profiling of acarbose

	Ph. Eur. method[4]	Amide-HILIC method		Hypercarb method	
Column	Thermo Scientific™ Hypersil™ APS-2 (250 × 4.0 mm, 5 µm, P/N 30705-254030)	Thermo Scientific™ Accucore™, 150 Amide HILIC (100 × 2.1 mm, 2.6 µm, P/N 16726-102130)		Thermo Scientific™ Hypercarb™ (150 × 4.6 mm, 3 µm, P/N 35003-154630)	
Mobile phase	A: aqueous solution of 0.60 g/L potassium dihydrogen phosphate and 0.35 g/L sodium hydrogen phosphate dihydrate B: acetonitrile	A: 50 mM ammonium acetate pH 5.8 in water B: acetonitrile		A: aqueous solution of 0.1% trifluoroacetic acid B: acetonitrile with 0.1% trifluoroacetic acid	
Gradient	Isocratic: 25% mobile phase A 75% mobile phase B	min	%B	min	%B
		0–35	87	0–2	8
		35–45	87–84	2–26	8–13
		45–80	84	26–31	13
		80–110	87	31–33	13–8
				33–38	8
Runtime	2.5 times the runtime of acarbose; here around 50 min	100 min		38 min	
Flow rate	2.0 mL/min	0.6 mL/min		1.0 mL/min	
Column temperature	35 °C	45 °C		90 °C	
Autosampler temperature	n.a.	8 °C		8 °C	
Injection volume	20 µL	2.5 µL		2.5 µL	
Detection wavelength	210 nm	n.a.		n.a.	
Detector settings (CAD)	n.a.	Evaporation temperature: 50 °C; Power function value: 1.0; Filter constant: 5.0 s; Data collection rate: 10 Hz		Evaporation temperature: 50 °C; Power function value: 1.0; Filter constant: 1.0 s; Data collection rate: 10 Hz	

Results and discussion

With regards to the impurity profile of acarbose (Figure 1), impurity A, B, C, D, E, F and impurity G are specified in the Ph. Eur. monograph. Acceptance limits for impurities in an acarbose drug substance are not more than: 0.6% imp. A, 0.5% imp. B, 1.5% imp. C, 1.0% imp. D, and 0.2% imp. E, while for the impurities F and G a maximum content of 0.3% is allowed, respectively.[4]

As per the Ph. Eur. monograph, percent impurity levels are calculated by comparing individual peak areas in the test solution to the principal peak obtained in reference solution (2), which is a 100-fold dilution of that test solution. As described in the monograph, peak areas for several impurities must be multiplied by correction factors, presumably to account for differences in UV detector response factors. For CAD, correction factors were not required, which is based on the analytical assumption of equivalent detector response for these non-volatile analytes.[5]

Experiments in accordance with the pharmacopoeial related substances test of acarbose were intended to serve as a benchmark for all further experiments with the CAD. An example chromatogram of reference solution (1) obtained with the Ph. Eur. method is displayed in Figure 2.

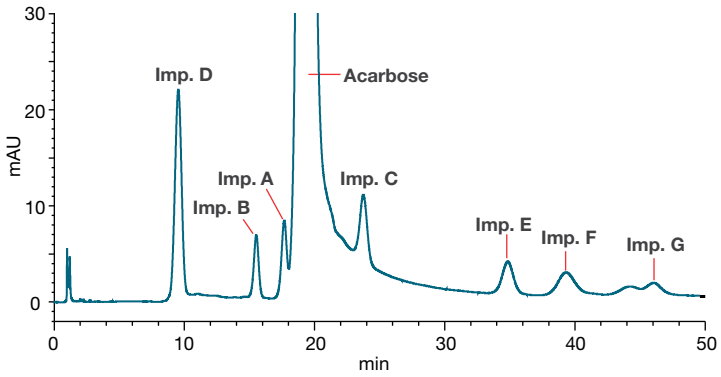

Figure 2. Impurity analysis of acarbose reference solution (1) by means of the current Ph. Eur. related substances test for acarbose. Hypersil APS-2 column (250 × 4 mm, 5 µm), UV-detection at 210 nm.

The current Ph. Eur method for the related substances test of acarbose uses an aminopropyl-silyl (APS) column with phosphate buffer. Instability of these columns applied with this monograph has been observed,[6] resulting in the motivation to look for alternative stationary phases run with volatile mobile phases compatible with charged aerosol detection to establish a more stable and sensitive method.

Running the APS column by switching the non-volatile mobile phase from phosphate buffer to the volatile mobile phase 10 mM aqueous ammonium acetate was not successful, as the CAD signal showed a relative high background current of 75 pA. This is likely due to memory effects on the column and other issues like column bleeding.[7] The decreased sensitivity due to increased noise level made it necessary to investigate other stationary phase options.

Since the analytes differ only in the sugar chain, the column for acarbose analysis must be highly selective. Another challenge of this application is that most of the analytes show epimerization, which can lead to peak splitting or distortion. The epimerization speed can be increased by a higher column temperature or a higher pH of the mobile phase.[8] The APS stationary phase of the Ph. Eur. method generates a locally high pH (due to the amino functionalities) and thus results in single peaks. However, higher pH values of the mobile phase may cause silica-based stationary phases to degrade.[6,9]

A compromise of acceptable peak shape and stability of the stationary phase was found in the Accucore 150 Amide HILIC column. The column oven temperature was set to 45 °C, and ammonium acetate buffer pH 5.8 was used as the aqueous phase in HILIC separation. Narrow columns (100 × 2.1 mm, 2.6 µm) were chosen for the charged aerosol detection to improve the analytical performance by higher signal intensities due to less peak dispersion.[7] For these column geometries used with CAD, the injection volume had to be reduced, otherwise loss of chromatographic resolution would occur by overloading. A gradient, as shown in Table 1, was applied to separate acarbose from its impurities. Of note, the mobile phase composition changes only by 3% within 10 minutes. These small changes are necessary for a sufficient separation.[6] Additionally, the re-equilibration step could be shortened to 20 minutes. An example chromatogram of reference solution (1) obtained with the Amide-HILIC method is displayed in Figure 3.

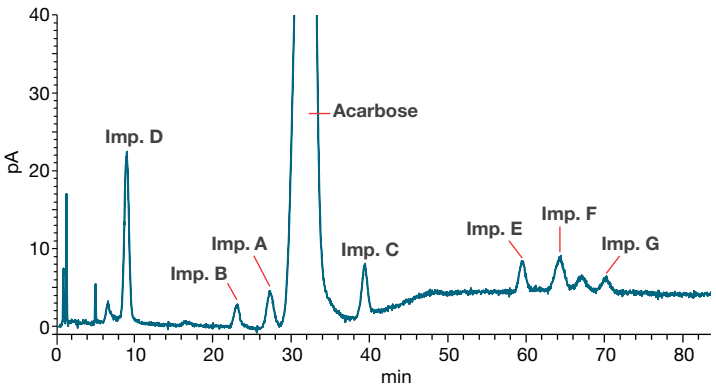

Figure 3. Chromatogram obtained with acarbose reference solution (1) and the Amide-HILIC method. Accucore 150 Amide HILIC column (100 × 2.1 mm, 2.6 µm), flow rate 0.6 mL/min, gradient elution, and CAD detection. Further details are displayed in Table 1.

Method validation was performed according to the ICH Q2(R1) guideline.[10] Specificity could be demonstrated by the analysis of acarbose reference solution (1), which contains acarbose and its impurities A, B, C, D, E, F, and G. All compounds were separated (Figure 3). The LOQ of this method is quite high as the acceptable signal-to-noise ratio of 10:1 was only met for a solution with a concentration level of 0.20%, which had also an impact to the repeatability and accuracy data. For more validation details, refer to Reference 6. Although the validation results were in an acceptable range, for improvement of sensitivity further stationary phases were evaluated.

Polar compounds, like sugars can also be separated using graphite columns, such as the Hypercarb columns. Polar analytes have a high affinity to the graphite surface and thus show retention. Furthermore, Hypercarb columns, unlike those based on bonded silica, can be used at high temperatures and high acidic or alkalinic conditions.[11,12]

Although higher pH values are advantageous for the described epimerization, an acidic pH proved to be beneficial for good separation on the Hypercarb column. Best results were obtained by a gradient with acetonitrile/water containing 0.1% TFA. To avoid double peaks of the epimers, the column oven was set to 90 °C.[6] Good separation of the components was achieved by the method described in Table 1. An exemplary chromatogram obtained with reference solution (1) is displayed in Figure 4A.

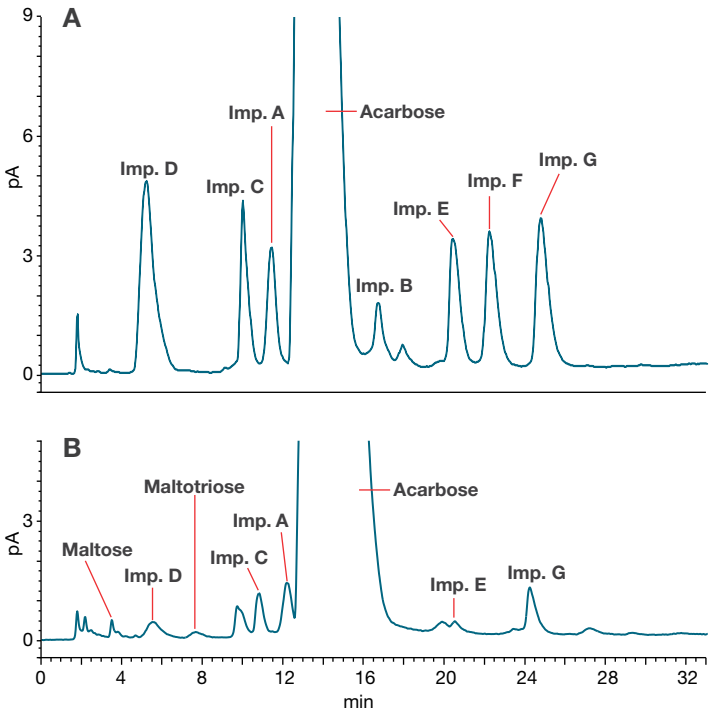

Figure 4. (A) Chromatogram obtained with acarbose reference solution (1) and the Hypercarb method; (B) Chromatogram obtained with acarbose test solution and the Hypercarb method. Hypercarb graphite column: (150 × 4.6 mm, 3 µm.), flow rate 1 mL/min, gradient elution, and CAD detection. Further details are displayed in Table 1. Impurity mix was used for peak assignment.

Furthermore, the CAD has the potential to determine additional impurities in the acarbose batches that are not detectable with a UV detector used in the compendial monograph. At the beginning of the chromatogram some additional peaks occurred (Figure 4B). Two of them were identified through spiking experiments as the sugar fragments maltose and maltotriose.

Again, validation experiments were performed following the ICH Q2(R1) guideline.[10] Specificity, repeatability, LOQ, linearity, range, accuracy, and robustness were investigated. As shown in Figure 4, all components were separated. However, since only an impurity mix and no individual impurities were available, which are necessary for a full validation, only a suggestion for the peak assignment could be made. The %RSD of three different concentration levels and six replicates were investigated to demonstrate repeatability. Values of 0.35–1.28% indicate a precise method. Also the LOQ is sufficient: The required signal-to-noise ratio 10:1 for the LOQ was obtained for a solution of 0.10%. All other validation parameters are in an acceptable order of magnitude. More details are displayed in Reference 6.

Conclusion

- Impurity profiling of acarbose is generally challenging.
- Successful method migration from UV detection to the Vanquish CAD system was demonstrated. Both CAD methods met the requirements of the Ph. Eur. “related substances” test for the impurity analysis of acarbose.
- Both the Accucore 150 Amide HILIC column and the Hypercarb column are suitable for the impurity profiling of acarbose, while being more stable than the APS column used in the monograph method and operating under MS compatible conditions.
- Detection of additional impurities without a chromophore at low level is enabled by CAD.
- The Vanquish UHPLC system with the Vanquish Charged Aerosol Detector is versatile. For example, the ability to adjust evaporation temperature enables improved performance.
- Usage of LC-MS grade chemicals is recommended.

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