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Determination of acarbose by capillary zone electrophoresis

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Dedicated to Professor Dr. Theo Dingermann, Frankfurt, on the occasion of his 65th birthday.

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Acarbose (Glucobay®, Bayer AG) acts as a potent α-glucosidase-inhibitor, which delays the intestinal starch digestion resulting in a reduction of postprandial blood glucose and insulin levels. Acarbose is a pseudo-tetrasaccharide, with two D-glucose units linked via an α 1→4 glycosidic bond to acarviosin, which is a N-glycoside composed of an unsaturated cyclitol and 4-amino-4,6-dideoxy-α-D-glucopyranose. Several methods for the determination of acarbose by capillary electrophoresis can be found in literature. They are based either on the derivatisation with 7-aminonaphthalene-1,3-disulfonic acid (ANDS) or on the detection of the unsaturated cyclitol at wavelengths below 200 nm. The aim of our work was the determination of acarbose making use of a previously developed method based on reductive amination with *S*-phenylethylamine. The aminoalditols generated in the reaction formed differently charged borate-complexes depending on the configuration of the sugar. After successful method optimisation we were able to separate two potential impurities of acarbose, D-maltose und D-glucose. For the quantitation of acarbose in Glucobay® tablets an additional borate-buffer system was established, reducing the total time of analysis to less than 10 min.

1. Introduction

Acarbose (Glucobay®, Bayer AG) acts as a potent α-glucosidase-inhibitor (Puls et al. 1977), which leads to a delay in the intestinal starch digestion. Due to the slower resorption of glucose, the postprandial blood glucose and insulin levels are significantly reduced (Hildebrandt et al. 1979; Bischoff 1991). It has been shown in several clinical trials that acarbose has a positive therapeutic impact both with overweight and non-overweight persons. Because of its specific mode of action, acarbose can be used either together with insulin in the case of diabetes type I patients or in the case of type II patients alone and in combination with other oral anti-diabetic drugs, respectively. Acarbose is a pseudotetrasaccharide with a molecular weight of 645,6 g/mol, in which two D-glucose units are linked via an α 1→4 glycosidic bond to acarviosin, which is a N-glycoside composed of an unsaturated cyclitol and 4-amino-4,6-dideoxy-α-d-glucopyranose. The acarviosin moiety is crucial for the inhibitory effect of the drug. Acarbose is obtained by fermentation from *Actinoplanes* strains.

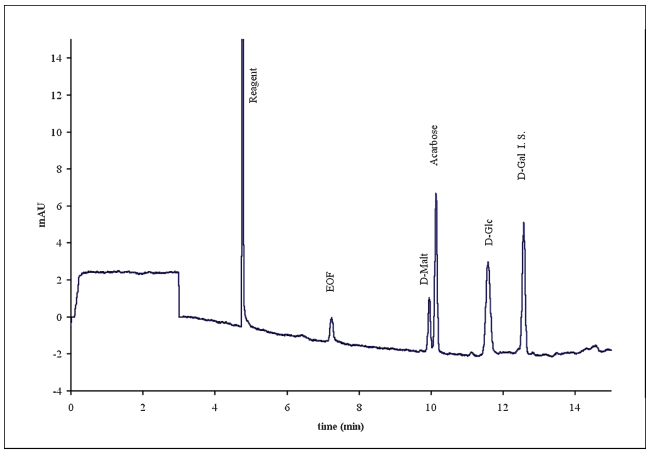
For the quantitation of acarbose and its related substances a HPLC method with UV detection at 210 nm has been published in the European Pharmacopoeia (European Pharmacopoeia 2011). A review of several other analytical techniques for the determination of acarbose is given by Wang et al. (2011). Methods for the determination of this drug and related impurities by capillary zone electrophoresis are also available in literature. Cherkaoui et al. (1988) described the determination of the acarbose-anomers and the quantitation in pharmaceutical formulations without any derivatisation procedure utilising the absorbance of the acarviosin moiety at 191 nm. Based on the derivatisation with 7-aminonaphthalene-1,3-disulfonic acid (ANDS) Rethfeld and Blaschke (1997) developed a method for the detection of acarbose and its main metabolite in urine. In both publications, the authors used buffers at low pH for the separation of the compounds. In case of the derivatisation with ANDS in combination with LIF-detection, quite low detection limits were reached. A drawback of this method is that the migration of the analyte is depending on the negative charge of the reagent only. Very recently, the determination of acarbose during the evaluation of a chemical process for the production of valienamine has been published (Wei et al. 2010).

The aim of our work was the determination of acarbose after derivatisation with S-1-phenylethylamine, similar to determination of different monosaccharides published previously by our group (Noe and Freissmuth 1995). After reductive amination with the reagent, the resulting aminoalditols form differently charged borate-complexes in a borate buffer system, depending on the configuration of the sugar. This effect could be also used for the determination of the pseudosaccharide acarbose. After successful validation and calibration, the method was expanded to the quantitation of acarbose in the pharmaceutical formulation Glucobay®.

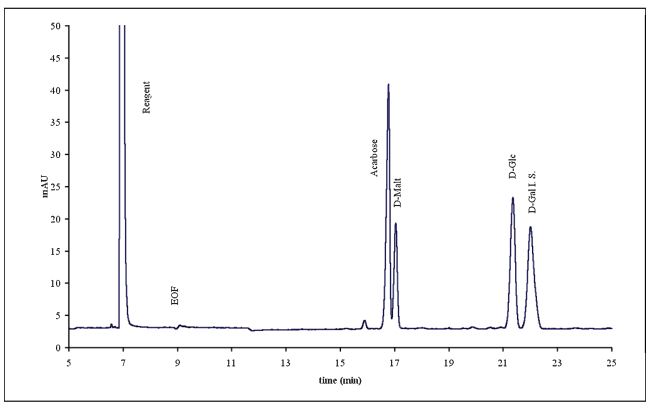
2. Investigations, results and discussion

2.1. Method development

Based on an assay published previously for the determination of several reducing disaccharides (Noe et al. 1999) a method optimisation for acarbose has been undertaken. In all cases, D-galactose was added to the sample solutions as an internal standard. A mixture of acarbose, D-maltose und D-glucose was used as a test-solution for the evaluation of the different electrophoretic parameters. The first parameter to be modified was



(a)



(b)

Fig. 1: (a) Separation of acarbose, D-maltose, D-gucose and D-galactose (I. S.); 100 mM sodium tetraborate, pH 9.4; capillary: 50 µm I. D, 30/37 cm length; 10 kV, 20 °C. (b) Separation of acarbose, D-maltose, D-glucose and D-galactose (I. S.); 100 mM sodium tetraborate, pH 10.2; capillary: 50 µm, 30/37 cm length; 10 kV, 20 °C

the sodium tetraborate concentration. The best results could be obtained with 100 mM buffers (e. g. separation of acarbose and D-maltose). After further optimisation of buffer pH and voltage applied, a standard system could be established for the separation of the test-mixture containing the above mentioned four substances. Utilizing a 100 mM sodium tetraborate buffer pH 9.4 and a capillary with a total length of 37 or 40 cm respectively, we were able to separate acarbose, D-maltose, D-glucose and D-galactose in less than 15 min, applying a voltage of 10 kV (Fig. 1a). To prove these results, an additional buffer system with a higher pH-value was evaluated. Although this system leads to increased separation times, it has the positive impact that D-maltose and acarbose change their migration time and order (Fig. 1b). Application of both methods allows detection of potential impurities which co-migrate in one of the systems.

2.2. Determination of the acarbose content in the formulation Glucobay®

To quantify the acarbose content in the formulation Glucobay® a calibration curve was generated. Before derivatisation D-galactose was added as internal standard to freshly prepared acarbose solutions in the concentration range of 9.256 to 0.05785 mg per ml. Each solution was measured five times. The calibration curve and the amounts of acarbose in each solution together with the standard deviation and the relative standard deviation of the resulting peak areas are given in Fig. 2. We found a limit of quantitation of approximately 0.04 mg per ml and a limit of detection of less than 0.01 mg/ml. The equation of the calibration function was calculated to y = 0.4312x - 0.01. It had a coefficient of determination (R^2) of 0.9987. These data indicate the linearity of the method. For the generation of the data of the calibration curve, a 100 mM sodium tetraborate buffer was used (for additional parameters see Fig. 3). The same system was used for the determination of the acarbose content in Glucobay® tablets. By applying a constant voltage of 18 kV at a temperature of 22 °C we were able to achieve run times of less than 10 min, including the complete rinsing procedure. To avoid invalid results due to co migrating substances, the obtained results were checked with a second electrophoretic system (100 mM sodium tetraborate, pH 9.4, 15 kV).

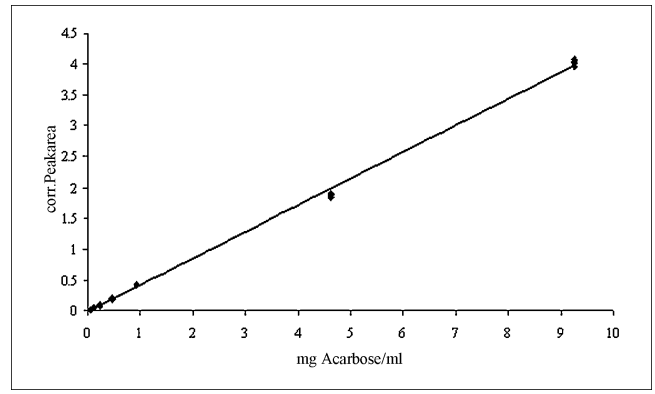


Fig. 2: Linearity of the calibration function

For the determination of the acarbose content in the Glucobay® tablets, several 50 mg tablets of Glucobay® were weighed and pestled. Aliquots were transferred into a 100 ml flask and dissolved in water. Samples were centrifuged to avoid technical problems during the analysis due to undissolved components of the tablet matrix. 20 µl of internal standard (D-galactose) were added to an aliquot of 50 µl sample solution prior to derivatisation with *S*-phenylethylamine. Each of the samples was measured five times. The mean value of these five measurements was used for the calculation of the acarbose content using the calibration equation (Table 1). To test the accuracy of the method, sample solutions of acarbose-substance were prepared and handled accordingly. A mixture of acarbose and D-galactose was used to verify the reproducibility of migration times and peak areas, as well as the impact of the capillary electrophoresis system used. After the derivatisation step with *S*-phenylethylamine, the mixture was measured seven times on each of the two capillary electrophoresis units. The mean values and the standard deviation (SD/RSD) of the migration times of the seven measurements were calculated together with the mean values and standard deviation of the peak areas (based

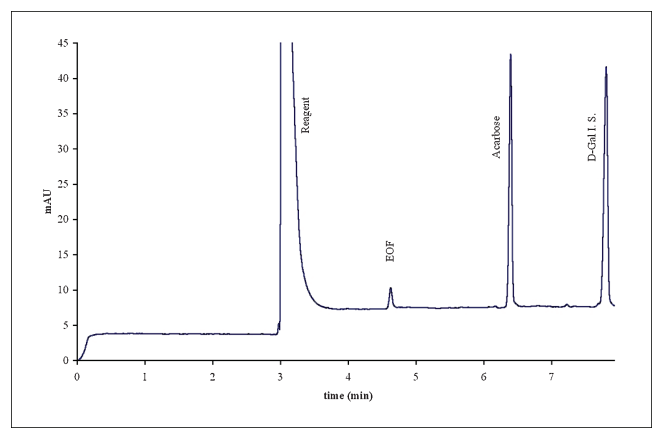


Fig. 3: Electropherogram of acarbose; 100 mM sodium tetraborate, pH 9.4; capillary: 50 µm, 30/40 cm length; 18 kV, 22 °C

Table 1: Results obtained for the analysed commercial samples

Sample	Balance weigth (mg)	mg / volume	Acarbose mg/ml	Found value (mg/ml) acarbose	% recovery
Tabl. 1	137.84	123.9 / 20 ml	2.25 mg	2.36 mg	105.0%
Tabl. 2	138.4	82.4 / 20 ml	1.49 mg	1.50 mg	100.9%
Tabl. 3	138.65	61.1 / 25 ml	0.88 mg	0.87 mg	99.2%
Standard			3.70 mg	3.71 mg	100.3%

Table 2: Migration times

	Relative peak areas	SD	RSD	Migration time acarbose (min)	SD (min)	RSD
P/ACE 5510	1.037	0.011	1.0%	5.59	0.034	0.6%
P/ACE MDQ	1.040	0.006	0.54%	6.17	0.01	0.16%

Mean values of 7 independent determinations

on the corrected peak areas according to the internal standard). Results are presented in Table 2. The obtained data show, that in both cases satisfactory results could be obtained. Both with the P/ACE MDQ or with the older system P/ACE 5510 similar standard deviations of approximately 1 % were obtained in all cases.

2.3. Conclusion

Using acarbose as an analyte, we demonstrated that our method for the determination of saccharides can be also applied to compounds of pharmaceutical relevance. After the optimisation of different parameters and calibration of our capillary zone electrophoresis method, a fast and precise determination of acarbose in pharmaceutical formulations, e. g. tablets, without any sample clean-up has become feasible. The precision and accuracy of the method was shown utilizing two different types of equipment. The developed method can be useful in the analysis of acarbose-containing pharmaceuticals.

3. Experimental

3.1. Materials

Acarbose (Lot no. 311409A) was kindly supported by Bayer AG, Leverkusen. Glucobay® tablets (Lot no. CAFNV1 and CAFNW1) were purchased in a pharmacy. D-Galactose, D-glucose, D-maltose, S-phenylethylamine and sodium cyanoborohydride were purchased from Merck, Darmstadt. Water was used in in-house HPLC quality. All buffers were prepared using a 100 mM sodium tetraborate stock solution, the pH-value was adjusted with either 10 M sodium hydroxide solution or 10 M hydrochloric acid. All of the solutions were freshly prepared each day. All other solvents were used in HPLC quality.

3.2. Equipment

Beckman P/ACE 5510 with diode array-detector. System Gold® 8.0 software (Beckman Instruments, Munich) was applied for instrument control, data acquisition and analysis. Beckman P/ACE MDQ with UV-detector. 32 Karat® software (Beckman Instruments, Munich) was applied for instrument control, data acquisition and analysis. Capillary: Uncoated fused-silica capillaries (Polymicron) of 50 μm I. D. (375 μm O. D.) were used for all separations. Effective and total length of the capillaries used are mentioned in the electropherograms. The separations were performed at constant temperature (Beckman capillary cartridge coolant). Detection: Photometric on-column detection was carried out at 200 nm, the detector was situated 7 cm (P/ACE 5510) or 10 cm (P/ACE MDQ) from the cathodic end (normal polarity). Samples were introduced into the capillary with pressure (0.5 psi).

3.3. Sample and standard preparation

Standard solutions:
An accurately weighed amount of acarbose was transferred into a 100 ml flask. The solution was filled to the appropriate volume with water.
Sample solutions:
The tablets were weighed, pestled and aliquots afterwards transferred into a 100 ml flask and filled up to volume with water.

3.4. Derivatisation with S-1-phenylethylamine

To 50 μl of either the standard or the sample solution 20 μl of the internal standard solution (D-galactose) were added and subsequently mixed with 15 μl of an 1 M solution of S-1-phenylethylamine (pH 6.8). After 10 min incubation at 90 °C additional 4.5 μl of a 5 M solution of $NaBH_3CN$ were added and the resulting mixture was incubated additional 60 min. at 90 °C.

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