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Development and validation of liquid chromatography and capillary electrophoresis methods for acarbose determination in pharmaceutical tablets

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Abstract

Liquid chromatography and capillary zone electrophoresis, respectively coupled to an evaporative light scattering detector and a UV detector have been developed for the analysis of acarbose without any derivatization procedure. The electrophoretic separation of acarbose anomers was achieved through the manipulation of the working temperature. Both methods were validated and showed good validation data in terms of precision, accuracy and linearity. The validated methods were successfully applied to the dosage of acarbose in commercially available Glucobay® tablets. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Acarbose; Liquid chromatography; Capillary electrophoresis; Evaporative light scattering detector; Validation

1. Introduction

Carbohydrate analysis is of primordial importance in the pharmaceutical and food industry. Since most carbohydrates lack chromophore and/or fluorophore groups, their analysis by liquid chromatography (LC) often requires derivatization procedures [1]. However, liquid chromatography coupled to an evaporative light scattering detector (ELSD) has shown to be a well suited technique for the analysis of non-UV absorbing solutes without derivatization, and several applications have been published for the determination of carbohydrates [2–5].

Recently, capillary electrophoresis (CE) using UV detection has proven to be an interesting alternative for the analysis of pharmaceutical compounds because of its efficiency, flexibility, accuracy and very high resolution [6]. Furthermore, the application range of the technique has been extended to carbohydrates and glycoconjugates by using several procedures such as direct UV detection at low wavelength, complexation with borate [7], indirect UV detection [8,9] or by performing direct UV or fluorescence detection after derivatization with suitable chromophore or fluorophore reagents [10].

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Acarbose (Fig. 1) is an oral α-glycosidase inhibitor used in the management of non-insulin-dependent diabetes mellitus (NIDDM). It is obtained from fermentation processes of a microorganism, *Actinoplanes utahensis*. Acarbose is structurally similar to an oligosaccharide derived from starch digestion. Due to the presence of the intramolecular nitrogen, acarbose binds to the carbohydrate site of the α-glycosidase enzyme with an affinity exceeding that of the normal substrate. Hence, the enzymatic reaction stops because the C–N linkage cannot be cleaved. Therefore, glucose absorption is delayed and consequently the postprandial rise in blood glucose is decreased [11–13]. Recently, capillary zone electrophoresis (CZE) was used to determine acarbose and its main metabolite in human urine using laser induced fluorescence detection after derivatization with 7-aminonaphthalene-1,3-disulfonic acid [14]. To our knowledge, no LC method has been published for the analysis of acarbose.

In the course of our continuing investigations in the development of straightforward approaches for the analysis of drugs of pharmaceutical interest [15,16], the present study was aimed to evaluate the abilities of LC-ELSD and CZE-UV methods for the analysis of acarbose. Both analytical methods were validated and successfully applied to the determination of acarbose in commercially available tablets.

2. Experimental

2.1. Chemicals

Acarbose and Glucobay® tablets were kindly donated by Bayer Pharma (Wuppertal, Germany). Internal standards, sucrose and oxitropium, were from Fluka (Buchs, Switzerland) and Boehringer (Ingelheim, Germany), respectively. Tris (hydroxymethyl)-aminomethane (Tris) and phosphoric acid were purchased from Fluka. LC grade methanol and dichloromethane were obtained from Romil (Kölliken, Switzerland). Ultrapure water, obtained by a Milli-Q RG unit from Millipore (Bedford, MA), was used for standard and sample preparation. Electrolyte solutions were filtered through a 0.20 μm microfilter (Supelco, Bellefonte, PA) before use.

2.2. Instrumentation

2.2.1. LC procedure

The liquid chromatograph consisted of a Waters 600E multisolvent delivery system (Milford, MA) and a Waters 717 plus autosampler. The measurements were carried out on a Nucleosil-NH2 column (250 × 4.6 mm, 5 μm) from Macherey–Nagel (Oensingen, Switzerland). The flow rate was 1 ml min^{-1} and the injection volume 20 μl. A mixture of methanol and dichloromethane (65:35, v/v) was used as mobile phase.

The LC column was coupled to an evaporative light scattering detector (Sedex 55 model, Sedere, Alfortville, France). Nebulization of the eluent in the ELSD was provided by a stream of pressurized air (2.5 bar) with a flow rate of approximately 4 l min^{-1} at room temperature. The nebulized solvent was evaporated at 40°C. The detection output was interfaced to a software program Chrom-Card (Fisons instruments, Milan, Italy) on a AST Bravo LC 4/33 computer for data handling and chromatogram generation.

2.2.2. Electrophoretic procedure

CE data was generated by a HP ^{3D}Capillary Electrophoresis system (Hewlett-Packard, Waldbronn, Germany) equipped with an on-column diode-array detector, an autosampler and a power supply able to deliver up to 30 kV. The extended light path capillary (Hewlett-Packard) was 48.5 cm long (40 cm effective length) with a 50 μm

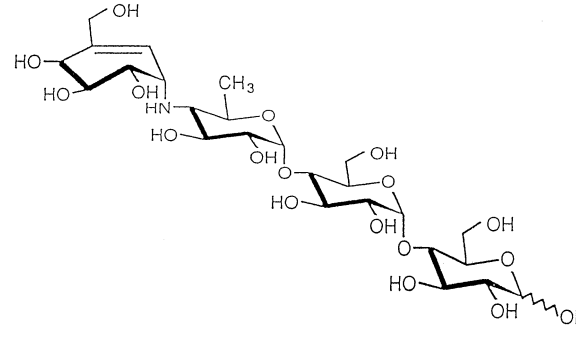

Fig. 1. Structure of acarbose.

internal diameter (bubble factor 3). An alignment interface, containing an optical slit matched to the internal diameter, was used and the detection wavelength was set at 191 nm with a bandwidth of 2 nm. A CE Chemstation (Hewlett-Packard) was used for instrument control, data acquisition and data handling.

All experiments were carried out in cationic mode (anode at the inlet and cathode at the outlet). The capillary was thermostated at 40°C. A constant voltage of 30 kV, with an initial ramping of 500 Vs^{-1}, was applied during analysis. Sample injections (16 nl injection volume) were achieved using the pressure mode for 10 s at 50 mbar.

Before use, the capillary was washed with 0.1 M sodium hydroxide for 20 min, followed by water for 10 min. To achieve high migration time reproducibility and to avoid solute adsorption on the capillary wall, the capillary was washed between analyses with 0.1 M sodium hydroxide for 2 min, followed by water for 2 min, then equilibrated with the running buffer for 3.5 min.

As electrolysis can alter the running buffer and subsequently change the electroosmotic flow (EOF), a replenishment system was also used to maintain a high reproducibility. Prior to each sequence, two blank injections were performed to stabilize the capillary wall surface, and allowing buffer and sample solutions to reach a constant temperature on the autosampler tray.

A mixture of Tris and phosphoric acid (pH 2.5) was used as running buffer.

2.3. Sample preparation

2.3.1. Standard solutions

Stock standard solution of acarbose (5 mg ml^{-1}) was prepared in water. Working standard solutions were achieved by diluting the stock standard solution with water for CZE analysis or with methanol for LC determinations. The use of water as a dissolving solvent for electrophoretic experiments allowed the sample stacking effect to occur. Thus, the sensitivity of the method was improved. A calibration curve of peak areas as a function of acarbose concentration was established in the range of 0.15–0.35 mg ml^{-1}, in the presence of sucrose and oxitropium as internal standards for LC and CZE experiments, respectively.

2.3.2. Tablet preparation

Five tablets were finely powdered and the equivalent of one tablet was accurately weighed and quantitatively extracted three times with 5 ml of water, with sonication for 15 min and vortex mixing at 5 min intervals to avoid aggregation of the powdered sample. After centrifugation ($2750 \times g$ for 5 min), supernatants were collected and diluted in a 20 ml volumetric flask with water. Fifty μl of this solution was diluted to 1 ml with water for CZE, and methanol for LC, in order to obtain a final concentration of 250 μg ml^{-1}. The internal standard was added at a concentration of 100 μg ml^{-1}. Both solutions were then filtered through a 0.2 μm filter and injected.

3. Results and discussion

3.1. LC-ELSD

LC experiments were performed in normal phase mode at ambient temperature. Aminopropyl bonded phase was selected since it is known to be an appropriate chromatographic support for the analysis of carbohydrates [17]. Several solvents including water, methanol, acetonitrile and dichloromethane were investigated as mobile phase constituents. Nevertheless, the use of water has to be discarded in order to avoid the hydrolysis of the column phase and to reduce the baseline noise. Thus, after optimization of the eluent composition, a mixture of methanol-dichloromethane (65:35, v/v) was selected for subsequent studies.

The detection principle of ELSD [18,19] is based on column effluent nebulization into droplets which are carried by a nebulizing gas in a drift tube and then directed towards a light beam. Light is scattered by residual particles of non volatile material and measured by a photomultiplier. The signal intensity is related to the mass of the analyte in the eluent.

Operating conditions for the ELSD were optimized by controlling both the gas flow rate into

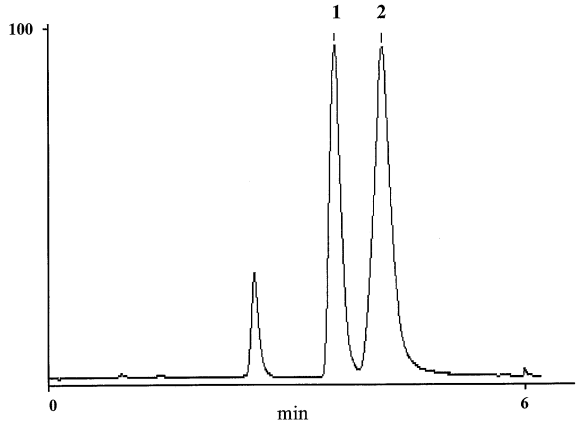

Fig. 2. Typical chromatogram of sucrose (1) and acarbose (2) obtained by LC-ELSD using the following conditions: column, Nucleosil-NH2 (250 × 4.6 mm, 5 μm); isocratic elution at 1 ml min^{-1} using a mixture of methanol and dichloromethane (65:35, v/v). For detection conditions, see experimental.

the nebulizer and the temperature of the drift tube. The nebulization temperature has no significant effect in detector response, and therefore, the nebulization was performed at room temperature.

Working at high gas pressure results in response decrease due to smaller droplets formation during nebulization. On the other hand, increasing the drift tube temperature results in both response and baseline noise decrease. Thus, as a compromise, temperature and gas pressure were fixed at 40°C and 2.5 bar, respectively. Under these conditions, acarbose and sucrose were separated in less than 5 min, as shown in Fig. 2.

3.2. CZE-UV

Since acarbose possesses a secondary amino group, an acidic pH was selected to obtain this compound in its cationic form. Furthermore, an acidic pH results in resolution improvement due to the reduction of the negative charge on the fused silica capillary wall. Several parameters were considered for the optimization of the electrophoretic analysis, including the running buffer concentration, the pH, the applied voltage, the sample volume, the capillary and the temperature. The effect of the latter was the most important since it is known that temperature plays a role in the mutarotation of sugars in aqueous phase. Indeed, the analysis of carbohydrates and glycoconjugates is quite difficult due to the large number of possible isomeric forms. As reported in Fig. 3, decreasing the working temperature results in the mutarotation rate decrease and consequently the separation of α and β anomers. Thus, the separation temperature was fixed at 40°C. Finally, a 50 mM Tris-phosphate buffer at pH 2.5 was chosen for the separation of acarbose and oxitropium in less than 8 min, as shown in Fig. 4.

3.3. Method validation

The optimized methods were validated for acarbose determination, using sucrose and oxitropium as internal standard for LC and CE experiments, respectively. The validation requires the assessment of retention (migration) time and peak area reproducibility, detector response linearity with sample concentration, sensitivity and accuracy.

3.3.1. Linearity

Detector response linearity was determined by preparing five calibration samples covering the concentration range of 0.15–0.35 mg ml^{-1}. Each sample was injected in triplicate. Because ELSD gives a non linear relationship between the peak area and the concentration of the analyte, a plot of peak area versus sample concentration in double logarithmic coordinates was used [5]: $\log A = \log a + b \log C$, where A is the peak area, C is the concentration and a and b are two constants determined principally by the nature of the mobile phase [5]. Correlation coefficients and calibration curves for both methods are shown in Table 1. In the case of CE, the y intercept was not significantly different from zero.

The limit of detection (LOD), defined as the lowest concentration of analyte that can be clearly detected, is estimated as three times the signal to noise ratio. The LOD was determined by injecting standard solutions of various concentrations. For both methods, the estimated limit of detection (Table 1) was determined as less than 5 μg ml^{-1}, giving a limit of quantitation (LOQ) value of less than 15 μg ml^{-1}.

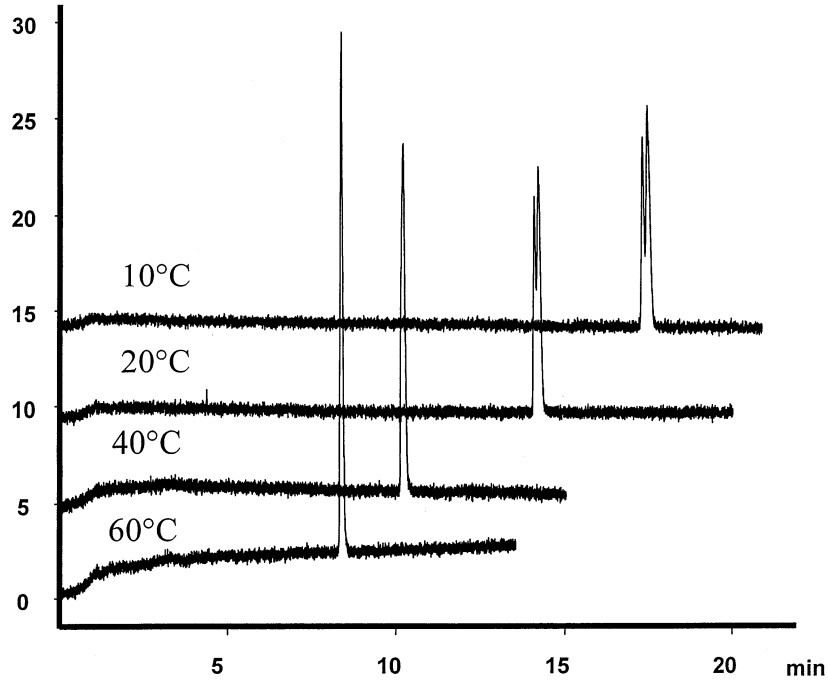

Fig. 3. Influence of the temperature on the separation of acarbose anomers. Conditions: 50 mM Tris-phosphate pH 2.5, applied voltage 30 kV. Other conditions are given in Section 2.2.

3.3.2. Reproducibility

Method reproducibility was determined by measuring repeatability and intermediate precision (between-day precision) of retention (migration) times and peak areas for acarbose.

In order to evaluate the repeatability of the method, replicate injections ($n = 6$) of a 250 μg ml^{-1} solution containing acarbose and internal standard were carried out. In Table 2, relative standard deviation (RSD) values are given for retention (migration) times and peak area ratios. In both cases, repeatability was better than 1% for the retention (migration) times and 2% for the peak area ratio, respectively. In the case of CE experiments, the use of an internal standard is necessary in order to compensate the low precision of the hydrodynamic injection, and hence to achieve a good method precision.

The intermediate precision was also evaluated over 3 days by performing daily six successive injections. Results (Table 2) show that RSD values were satisfactory for both techniques.

3.3.3. Accuracy

The accuracy of the proposed methods was evaluated by recovery experiments, using the standard addition technique. Three different concentrations of standard acarbose were added to diluted Glucobay® tablets, as shown in Table 3. Each experiment was repeated thrice. The mean recovery of 100 and 98.1% were observed on the CE and LC methods, respectively. Thus, quantitative recoveries were achieved for both methods.

The two methods were used for the assay of acarbose in commercially available tablets containing 100 mg of acarbose. After extraction and dilution in the appropriate solvent, tablets were analyzed in the presence of the internal standard. As shown in Table 4, determination of acarbose content by the CE method was in good agreement with the labeled content, which confirms the good accuracy of this technique. In the case of LC-ELSD, the obtained value is surprisingly higher, which may be explained by the

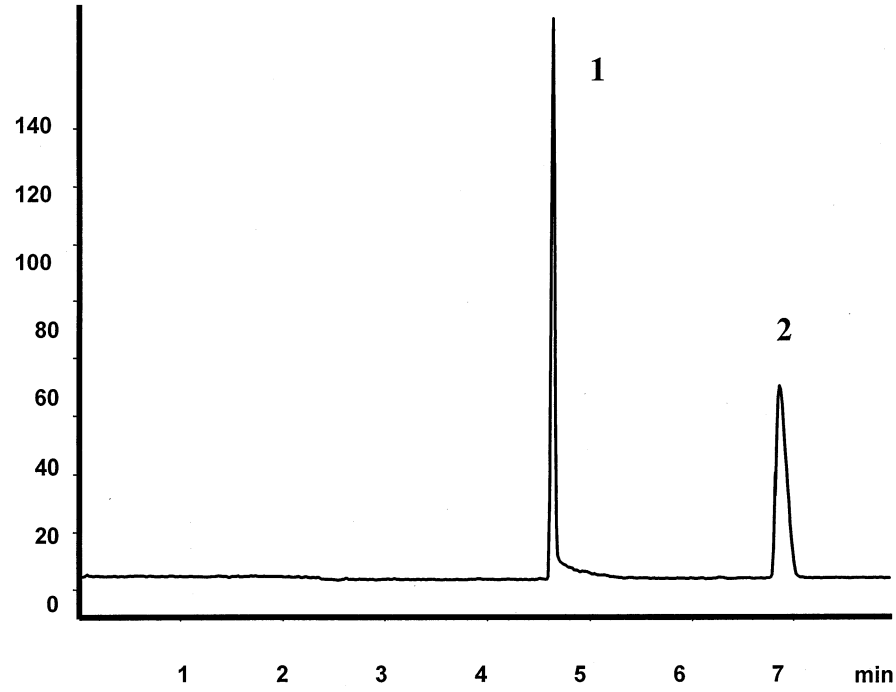

Fig. 4. Typical electropherogram of oxitropium (1) and acarbose (2) obtained by CZE-UV using 50 mM Tris-phosphate pH 2.5, applied voltage 30 kV ($i = 60$ μA), temperature 40°C. Other operating conditions are given in Section 2.2.

presence of interfering excipients in tablet formulation. It is noteworthy that, in all cases, RSD values were low, attesting the good precision of the methods.

4. Conclusion

LC-ELSD and CZE-UV methods were investigated for the analysis of acarbose without any derivatization procedure. The separation of acarbose anomers was possible by CZE through the manipulation of the working temperature. Once the optimized conditions selected, both methods were validated and showed good performances with respect to precision, linearity and accuracy. Results of this study demonstrate that LC-ELSD and CZE-UV methods can be used for the deter-

Table 1
Regression data for the calibration curves

	LC	CE
Range (mg ml^{-1})	0.15–0.35	0.15–0.35
Line	$\log y = 2.65 + 1.45 \log x$	$y = -0.0024 + 0.0050x$
Correlation coefficient	0.9996	0.9915
LOD (μg ml^{-1})	5	5
LOQ (μg ml^{-1})	15	15

Table 2
Method precision given as RDS values in%

	LC	CE
Repeatability		
Retention (Migration) time	0.42	0.97
Peak area ratio[a]	2.06	1.86
Intermediate precision		
Retention (Migration) time	1.69	1.44
Peak area ratio[a]	3.35	2.68

[a] For LC-ELSD experiments, the precision is based on the logarithm of peak area ratio.

Table 3
Accuracy determination of LC-ELSD and CE-UV methods

Dosage	Component	Labeled claim (μg ml^{-1})	Amount added (μg ml^{-1})	Amount recovered (μg ml^{-1})		% Recovery		RSD (%)	
				LC	CE	LC	CE	LC	CE
Tablet	Acarbose	200	50.0	48.0	50.2	96.0	100.4	1.74	0.65
			100.0	100.7	98.7	100.7	98.7	0.93	0.74
			150.0	146.3	151.1	97.5	100.7	1.46	0.42

Table 4
Results of acarbose assay in Glucobay® tablets ($n = 3$)

	LC-ELSD	CZE-UV
Labeled claim (mg)	100.00	100.00
Amount found (mg)	104.62	99.42
RSD (%)	1.11	3.28

mination of acarbose in commercially available GLUCOBAY® tablets.

Owing to its simplicity, rapidity and low cost, the coupling of LC with ELSD can be an effective alternative to conventional detectors for the dosage of low volatile drug substances in pharmaceutical preparations. The CZE method is more selective in the case of charged compounds dosage since most excipients present in pharmaceutical preparations are neutral. Thus, these compounds will migrate with the electroosmotic flow and will never interfere with the investigated compound.

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