Available online at www.sciencedirect.com
ScienceDirect
Journal of Pharmaceutical and Biomedical Analysis 42 (2006) 328–333
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Quantitative determination of voglibose in pharmaceutical tablets using high-performance liquid chromatography–fluorescence detection with post-column derivatization and mass spectrometric detection

Jong Soo Woo [a,*], Jae Kuk Ryu [b]

[a] *Pharm. R&D Institute, Hanmi Pharm. Co. Ltd., Hwasung 445-913, Republic of Korea*
[b] *College of Pharmacy, Sungkyunkwan University, Suwon 440-746, Republic of Korea*

Received 3 February 2006; received in revised form 3 April 2006; accepted 6 April 2006
Available online 26 May 2006

Abstract

A highly sensitive liquid chromatographic procedure with post-column derivatization using fluorescence detection (LC–FD) was developed and validated for the determination of voglibose in pharmaceutical tablets. Sample pre-treatment included a simple extraction and centrifugation without pre-column derivatization. Taurine and sodium periodate dissolved in water was used as a post-column reagent. Detection was performed at an excitation wavelength of 350 nm and an emission wavelength of 430 nm. LC separation was carried out in less than 25 min. In addition to the LC procedure with post-column derivatization, an LC–MS assay procedure was also investigated for the analysis of voglibose without derivatization. Voglibose was detected in an electrospray ionization (ESI) mode with single ion recording (SIR, *m/z* 268.1). After selection of the optimum conditions, both assay methods were validated, providing good performances with respect to precision, linearity and accuracy. Linearities of both methods were obtained with an average $r^2 > 0.999$. The lower limits of detection (LLOD) were 9.4 and 18 ng/ml for LC–FD and LC–MS, respectively. Both methods could be successfully applied to the quantification of voglibose in commercially available tablets.

Keywords: Voglibose; Post-column derivatization; LC–MS

1. Introduction

Voglibose (Fig. 1), 3,4-dideoxy-4-[[2-hydroxy-1-(hydroxymethyl)ethyl]amino]-2-*C*-(hydroxymethyl)-D-epiinositol, has attracted considerable interests due to its wide range of therapeutic and pharmacological properties, including its excellent inhibitory activity against α-glucosidase and its action against hyperglycemia and various disorders caused by hyperglycemia. Voglibose, a new potent glucosidase inhibitor used for type 2 diabetes, has shown strong anti-obesity and anti-diabetic activity. As a glucosidase inhibitor, the compound exerts its activity within the gastrointestinal tract of humans. The drug delays glucose absorption and thus, reduces the post-prandial blood glucose peaks [1–3]. Voglibose obtained from organic synthesis processes is similar to structurally related carbohydrates found naturally [4,5].

Carbohydrate analysis is of 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 [6]. However, LC coupled to an evaporative light scattering detector or refractive index detector 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 [7–10]. These methods may be applied to raw materials or acarbose tablets which have high contents of active ingredient (50–100 mg of acarbose per tablet). They, however, are not appropriate for the analysis of voglibose products that contain small amounts of voglibose, e.g. 0.2 or 0.3 mg tablets. Therefore, a novel analytical method for dissolution and content uniformity tests need to be developed for the voglibose products with low strengths.

The purpose of this study was to develop and validate LC–MS and LC with post-column derivatization using fluorescence detection (LC–FD) after derivatization with taurine for the analysis of voglibose in pharmaceutical tablets. No LC method with

* Corresponding author. Tel.: +82 31 356 3311; fax: +82 31 356 7139.
E-mail address: jswoo@hanmi.co.kr (J.S. Woo).

0731-7085/$ – see front matter © 2006 Published by Elsevier B.V.
doi:10.1016/j.jpba.2006.04.018

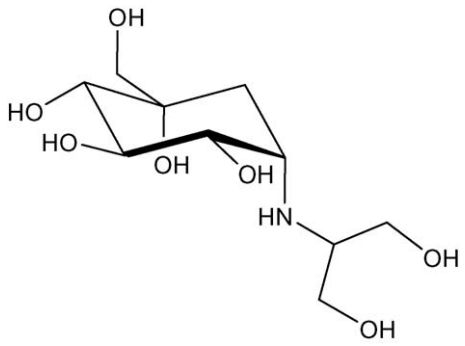

Fig. 1. Structure of voglibose.

post-column derivatization using taurine and mass detector has been published for the analysis of voglibose. Both analytical methods could be successfully applied to the analysis (content, content uniformity, dissolution test) of voglibose in commercially available tablets.

2. Experimental

2.1. Materials

Voglibose, fructose and B tablets (commercially available voglibose tablets in Korea) were kindly provided by Hanmi Pharmaceutical Company (Seoul, Korea). Sigma–Aldrich, Inc. (St. Louis, MO, USA) was the supplier of taurine and sodium periodate. Millex-GV 0.22 μm filters (Millipore Corp., Bedford, MA, USA) were used for filtration of injected solutions. Water used for LC–MS was from Merck (Darmstadt, Germany). Other chemicals were of reagent or HPLC grades.

2.2. Instrumentation

2.2.1. LC procedure with post-column derivatization

All experiments were carried out on a semi-micro HPLC system from Shiseido (Nanospace SI-1, Tokyo, Japan) consisting of two pumps, an autosampler, a column oven and a degasser. The post-column reagent was delivered by a Nanospace SI-1 pump from Shiseido (Fig. 2). A Hitachi Model L-7485 fluorescence detector (Tokyo, Japan) was used in the assay. All data were obtained and analyzed using the dsCHROM99 software from Donam Instrument Inc. (Seongnam, Korea). The measurements were carried out on a Cosmosil® 5NH2-MS column (150 mm × 4.6 mm, 5 μm) from Nacalai Tesque Inc. (Kyoto, Japan). The flow rate was 0.6 ml/min and the injection volume was 50 μl. A mixture of acetonitrile and 30 mM NaH_2PO_4 (pH 6.5) (2:1, v/v) was used as a mobile phase. The post-column reagents, 6.25 g of taurine and 2.56 g of sodium periodate, were dissolved in 1000 ml water. The post-column reagents were applied at the same flow rate of the mobile phase. A heating process for derivatization was performed in a CRX-400 from Pickering Lab (Mountain View, CA, USA). A cooling process was conducted in a VTRC-620 cooling bath from Jeio Tech (Kimpo, Korea). The detection was performed at an excitation wavelength of 350 nm and an emission wavelength of 430 nm [11].

The effects of the length of a reaction coil on the derivatization of voglibose were investigated from 5 to 20 m. The temperature of the reaction coil was varied from 80 to 110 °C with the length of the coil fixed at 5–20 m. The peak area of the reaction product of voglibose was determined after post-column fluorogenic derivatization using taurine.

2.2.2. LC–MS procedure

No post-column derivatization was required. The Waters Alliance HT 2795 Chromatography System (Waters Corp., Milford, MA, USA) was utilized. The system control and data processing was performed by MassLynx 3.5 (Waters Corp., Milford, MA, USA). For separation, Cosmosil® 5NH2-MS column (150 mm × 4.6 mm, 5 μm, Nacalai Tesque Inc., Kyoto, Japan) was used. The column temperature was held at 40 °C. The injection volume was 10 μl. A mixture of 10 mM aqueous NH_4OAc and acetonitrile (3:7, v/v) was used as a mobile phase at a flow rate of 0.5 ml/min. The quantitative determination of voglibose was performed with a Waters ZQ 4000 mass spectrometer (Waters Corp., Milford, MA, USA). Data were acquired in an electrospray ionization (ESI) mode with positive ion detection applying single ion recording (SIR, *m/z* 268.1). Cone voltage, capillary voltage and desolvation temperature were 29 V, 3.00 kV and 150 °C, respectively.

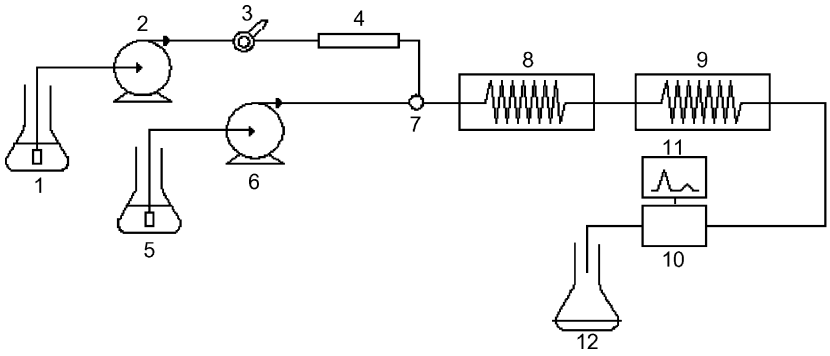

Fig. 2. Diagram for post-column derivatization using fluorescence detection. (1) Mobile phase; (2) pump for mobile phase; (3) injector; (4) column; (5) post-column reagent; (6) pump for post-column reagent; (7) T-shaped union; (8) reaction coil (0.5 mm ID, 5–20 m length) and heating system; (9) cooling coil (0.3 mm ID, 2 m length) and cooling system; (10) detector; (11) computer; (12) waste.

2.3. Sample preparation

2.3.1. Standard solutions

Stock standard solution of voglibose (0.1 mg/ml) was prepared in water, and working standard solutions were prepared by diluting the stock standard solution with the mobile phase. A calibration curve of peak area ratio as a function of voglibose concentration was constructed in the range of 50–1000 ng/ml, using fructose (10 μg/ml) as an internal standard for LC experiments.

2.3.2. Preparation of tablet sample

Twenty tablets were finely powdered and the equivalent of one tablet (0.3 mg as voglibose) was accurately weighed and extracted with 100 ml of the mobile phase. It was sonicated for 30 min with vortex mixing at 10 min intervals to avoid aggregation of the powdered samples. After centrifugation (2000 × *g* for 10 min), 10 ml of supernatant was collected and diluted in a 50 ml volumetric flask with the mobile phase in order to obtain a final concentration of 0.6 μg/ml. The internal standard solution was added at a concentration of 10 μg/ml. This solution was then filtered through a 0.22 μm filter and injected.

2.4. Application of the methods

2.4.1. Content uniformity test

To ensure the consistency of dosage unit, each unit in a batch should have a drug substance content within a narrow range around the label claim. The term, uniformity of dosage units is defined as the degree of uniformity in the amount of drug substance among dosage units (USP 28). The uniformity of dosage units can be demonstrated by either content uniformity test or weight variation test. The test for content uniformity is based on the assay of the individual content of the drug substance in a number of individual dosage units to determine whether the individual content is within the limits set. For the determination of dosage-unit uniformity by assay of individual tablets, 30 tablets were selected randomly.

One hundred millilitres of the mobile phase was used to extract each of 10 tablets. It was sonicated for 30 min and vortex-mixed at 10 min intervals to avoid aggregation of the samples. After centrifugation (2000 × *g* for 10 min), 10 ml of supernatant was collected and diluted in a 50 ml volumetric flask with the mobile phase in order to obtain a final concentration of 0.6 μg/ml of voglibose. The internal standard solution was added at a concentration of 10 μg/ml. This solution was then filtered through a 0.22 μm filter and injected.

2.4.2. In vitro dissolution study

A dissolution study was carried out according to the USP 28 method (Apparatus 2, [12]), employing an Erweka DT800 dissolution apparatus (Heusenstamm, Germany). Five hundred millilitres of water was used as the test medium. The stirring rate was 50 rpm and the temperature of the medium was maintained 37 ± 0.5 °C. The dissolution was carried out on six tablets. At each sampling time (10 and 30 min) within a tolerance of ±2%, 3 ml of the dissolution medium was withdrawn, filtered through a 0.22 μm filter and analyzed by both LC–FD and LC–MS.

2.5. Data analysis

Calibration curve was constructed over a concentration range from 50 to 1000 ng/ml. For each standard curve, five different concentrations were used. These data were fit to a linear least-squares regression curve with no weighting factor.

2.6. Validation

The following criteria were used to validate the methods: sensitivity, linearity, intra- and inter-day precision, accuracy, stability and selectivity. The sensitivity of the method was evaluated by determining the limit of detection (LOD) and quantitation (LOQ). According to ICH guidelines (ICH Topic Q2B, [13]), LOD was defined as $3.3\sigma/S$ and LOQ was $10\sigma/S$ based on standard deviation of the response and slope of the calibration curves. The standard deviation of *y*-intercepts of regression lines was used as σ (the standard deviation of the response) and S is the slope of the calibration curve. The linearity of the method was evaluated by calculation of the regression line by the method of least squares and expressed by the coefficient of determination (r^2). Linearity of each method was determined with at least five concentration levels not including the blank. Precision was evaluated over the linear dynamic range at five different concentration levels. Intra-day precision was assessed by three determinations per concentration in 1 day. Inter-day precision was assessed by three determinations per concentration on five separate days. Precision was expressed as relative standard deviation (R.S.D.). Accuracy was evaluated with separately prepared individual and mixed stock and working solutions of all standards over the linear dynamic range at five different concentration levels. Accuracy was expressed as (mean back − calculated concentrations)/(nominal concentrations) × 100. Stability of the stock solutions was tested on a daily basis by injection of freshly prepared working solutions at room temperature.

3. Results and discussion

3.1. LC procedure with post-column derivatization.

Voglibose was derivatized in order to increase the sensitivity of detection. The derivatization was based on the imination (–C=N–) of carbonyl group (–C=O) of voglibose, which was produced by oxidation of the hydroxy methyl group ($–CH_2–OH$) by sodium periodate, with the primary amino group of a fluorophore such as taurine. The main advantage of taurine as a labeling agent is its capability of facilitating highly sensitive fluorescence detection of trace amounts. Fig. 3 shows an LC separation of a taurine-derivatized monosaccharides and oligosaccharides, such as fructose, lactose and voglibose.

To optimize the derivatization procedure for sensitivity, the peak area of voglibose after post-column fluorogenic derivatization with taurine was investigated by varying the temperature of the coil from 80 to 110 °C with the coil length fixed at 5, 10 and

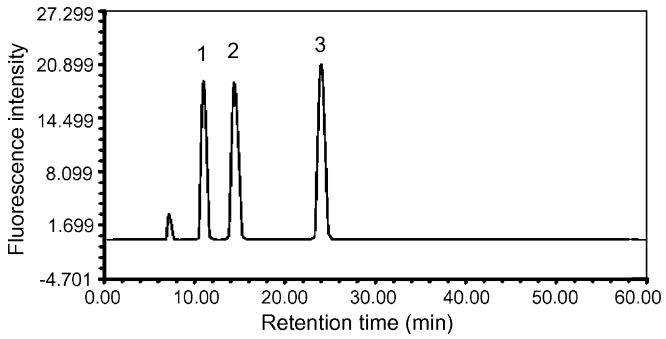

Fig. 3. Chromatogram of taurine-derivatized carbohydrates. (1) Fructose (10 μg/ml); (2) lactose (30 μg/ml); (3) voglibose (1 μg/ml).

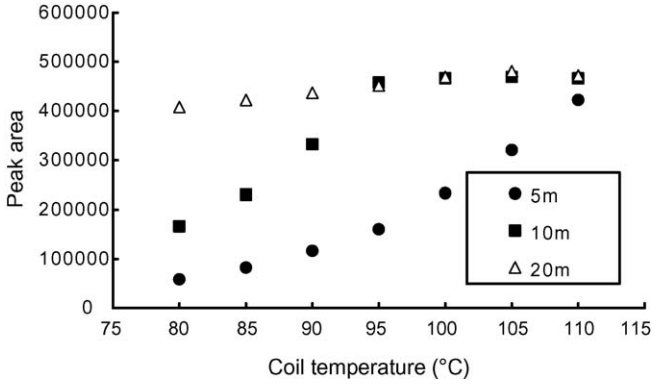

Fig. 4. Effect of the length of the reaction coil and temperature on the fluorescence intensity (peak area).

20 m [14]. As shown in Fig. 4 the highest fluorescence intensity was achieved at a coil length of 20 m and a coil temperature of 105 °C. The coil length of 10 m and the coil temperature of 100 °C was selected for this study to shorten the analysis time, measuring one sample in less than 25 min.

3.2. LC–MS procedure

LC–MS provides molecular weight as well as structural information. Several advantages can be exploited using LC–MS: there is no need to derivatize analytes, impurities are easily detected using mass spectral data, which is of most importance for quantification work, and fully resolved peaks are not required for mass spectral analysis. The analytical method presented in this study is based on electrospray ionization (ESI). Three instrument parameters of the LC/MS's ESI can be tuned on a particular compound (class): (a) cone voltage which can influence the fragmentation behavior and ion transmission, (b) capillary voltage (mass dependent with little impact on sensitivity) and (c) desolvation temperature (heat for drying aerosol). The voglibose sensitivity was maximized at a cone voltage setting of 29 V and capillary voltage of 3.00 kV. At 150 °C desolvation temperature, the mobile phase was evaporated sufficiently (250 l/h) without thermal degradation of the analyte. Fig. 5 represents a chromatogram of a standard solution (100 μg/ml) with the total ion chromatogram (TIC) on the top and the mass spectrum on the bottom. Fig. 6 shows a SIR chromatogram (SIR, *m/z* 268.1) of tablet sample.

3.3. Method validation

The optimized methods were validated for the determination of voglibose, using fructose as an internal standard for LC–FD and LC–MS experiments. The validation requires the assessment of 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 50–1000 ng/ml. Each sample was injected in triplicates. Coefficients of determination and calibration curves for both methods are shown in Table 1.

Linearity was obtained with $r^2 > 0.999$, over a calibration range of the analyte tested. The limit of detection (LOD) was estimated as $3.3\sigma/S$. The LOD was determined by injecting standard solutions of various concentrations. For both methods, the estimated limit of detection (Table 1) were 9.4 and 18 ng/ml for

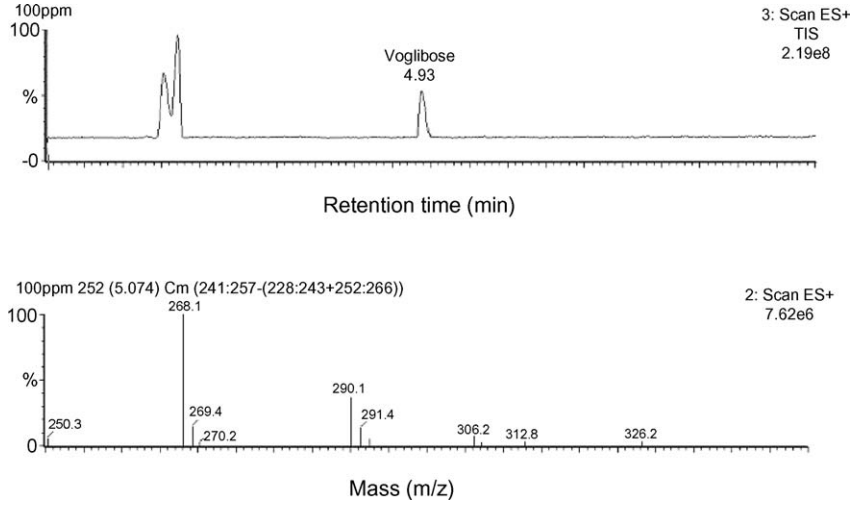

Fig. 5. Total ion chromatogram (top) and mass spectrum (bottom) of voglibose.

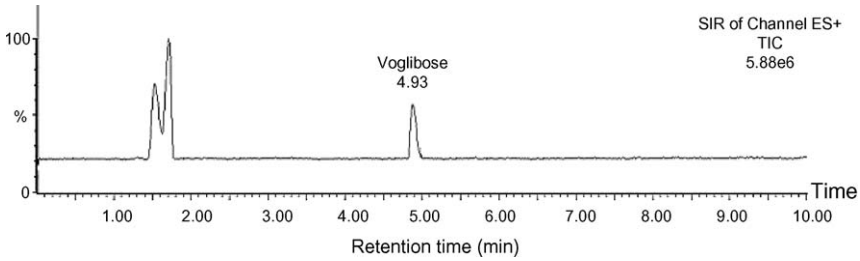

Fig. 6. SIR chromatogram (SIR, *m/z* 268.1) of tablet sample.

Table 1
Regression data for the calibration curves

	LC–FD	LC–MS
Range (ng/ml)	50–1000	50–1000
Line	$y = 0.001972x + 0.010606$	$y = 0.002782x + 0.001477$
r^2	0.9997	0.9994
LOD (ng/ml)	9.4	18
LOQ (ng/ml)	29	52

LC–FD and LC–MS, respectively. The lower limits of quantitation (LLOQ) were 29 and 52 ng/ml for LC–FD and LC–MS, respectively.

3.3.2. Precision and accuracy

In Table 2, the results for accuracy and intra-day and inter-day precisions are presented. The intra-day precision showed R.S.D.s of 0.7–3.7% for LC–FD procedure and 6.1–11.3% for LC–MS procedure. The inter-day precision R.S.D.s varied from 0.5 to 3.2% for LC–FD procedure and 0.6–12.7% for LC–MS procedure (Table 2). The intra-day accuracy ranged from 97.5 to 106.7% for LC–FD procedure and from 81.5 to 107.8% for LC–MS procedure. The inter-day accuracy ranged from 97.7 to 107.7% for LC–FD procedure and from 90.7 to 104.7% for LC–MS procedure.

Table 2
Assessment of the accuracy and precision

Nominal concentration (ng/ml)	Concentration found (ng/ml)	Precision (%)	Accuracy (%)
LC–FD			
Intra-day ($n = 3$)			
50	53.4	3.7	106.7
100	101.1	1.9	101.1
250	252.6	1.6	101.0
500	487.7	1.3	97.5
1000	1005.2	0.7	100.5
Inter-day ($n = 3$)			
50	53.8	3.2	107.7
100	104.2	1.7	104.2
250	251.8	1.3	100.7
500	488.4	1.9	97.7
1000	1003.9	0.5	100.4
LC–MS			
Intra-day ($n = 3$)			
50	40.7	11.3	81.5
100	107.8	3.5	107.8
250	259.5	2.7	103.8
500	489.3	6.4	97.9
1000	1002.6	6.1	100.3
Inter-day ($n = 3$)			
50	45.3	12.7	90.7
100	104.7	8.5	104.7
250	258.8	2.7	103.5
500	493.5	3.5	98.7
1000	1001.5	0.6	100.2

n: number of replicates.

The two methods were employed for the assay of voglibose in commercially available tablets containing 0.3 mg of voglibose. The selectivity of the method was investigated by screening three different batches of commercially available tablets to check if interference components co-eluted with voglibose. There were no interferences co-eluting with the peaks of voglibose and the internal standard. After extraction in appropriate solvent, tablets were analyzed in the presence of the internal standard. The stability of test solutions was assessed immediately and 24 h after preparation at room temperature (25 °C).

As shown in Table 3, determinations of voglibose content by both methods were in good agreement with the labeled content, which confirms the good accuracy of these techniques. Especially, in LC–FD procedure with post-column derivatization, R.S.D. values were low, implying the better precision of the method. Both voglibose and the internal standard were stable when kept at room temperature for at least 24 h, with no changes in concentration.

3.4. Application of the methods

After validation, these methods were applied in evaluating voglibose tablets. Content uniformity test and dissolution test

Table 3
Results of voglibose analysis in commercially available tablet ($n = 3$)

	LC–FD	LC–MS
Content ($n = 3$)		
Labeled claim (mg)	0.300	0.300
Amount found (mg)	0.299	0.295
R.S.D. (%)	1.51	3.19
Content uniformity ($n = 10$)		
Mean (mg)	0.298	0.297
S.D. (mg)	0.011	0.016
Dissolution ($n = 6$)		
Dissolved at 10 min (mg)[a]	0.104 ± 0.008	0.105 ± 0.010
Dissolved at 30 min (mg)	0.296 ± 0.005	0.297 ± 0.011

[a] Mean of six determinations ± S.D.

were conducted using both LC–FD and LC–MS. Determination of the content of the active compound in each tablet is important because voglibose is a very potent drug. The uniformity of voglibose content in 10 tablets was investigated. The results showed that voglibose tablets had good content uniformity (Table 3). Voglibose is known to have a very high water-solubility and, therefore, a fast dissolution of this drug is expected. The dissolution of voglibose was almost complete 30 min after initiation of the dissolution test at a paddle speed of 50 rpm in water. The results of the dissolution test are summarized in Table 3.

4. Conclusion

The LC–FD procedure with post-column derivatization was developed and validated for the determination of voglibose in commercially available tablets. The LC–MS in the positive mode was also developed and investigated for the analysis of voglibose without any derivatization procedure. Once the optimized conditions were selected, both methods were validated, providing good performances with respect to precision, linearity and accuracy.

On the basis of these results, both methods could be used for the analysis of voglibose in commercially available tablets. The LC–FD procedure with post-column derivatization could be applied to pharmaceutical preparations containing voglibose owing to its simplicity, rapidity, low cost and good validation results.

References

[1] Y. Yamasaki, N. Katakami, R. Hayaishi-Okano, M. Matsuhisa, Y. Kajimoto, K. Kosugi, M. Hatano, M. Hori, Diabetes Res. Clin. Pract. 67 (2005) 204–210.
[2] K. Watanabe, H. Uchino, C. Ohmura, Y. Tanaka, T. Onuma, R. Kawamori, J. Diabetes Complicat. 18 (2004) 183–186.
[3] A. Vichayanrat, S. Ploybutr, M. Tunlakit, P. Watanakejorn, Diabetes Res. Clin. Pract. 55 (2002) 99–103.
[4] H. Zhang, C.R. Sun, O. Ishurd, Y.J. Pan, L.S. Ding, Carbohydr. Res. 339 (2004) 2027–2030.
[5] X. Chen, Y. Zheng, Y. Shen, Curr. Med. Chem. 13 (2006) 109–116.
[6] I. Rethfeld, G. Blaschke, J. Chromatogr. B 700 (1997) 249–253.
[7] S. Cherkaoui, Y. Daali, P. Christen, J.-L. Veuthey, J. Pharm. Biomed. Anal. 18 (1998) 729–735.
[8] M. Lafosse, B. Herbreteau, M. Dreux, L. Morin-Allory, J. Chromatogr. 472 (1989) 209–218.
[9] Y. Wei, M.-Y. Ding, J. Chromatogr. A 904 (2000) 113–117.
[10] M. Kohler, W. Haerdi, P. Christen, J.L. Veuthey, Trends Anal. Chem. 16 (1997) 475–484.
[11] K.E. Maudens, G.F. Zhang, W.E. Lambert, J. Chromatogr. A 1047 (2004) 85–92.
[12] United States Pharmacopoeia 28, Section 711, Dissolution, United States Pharmacopoeia Convention Inc., Rockville, MD, 2005.
[13] International, Conference on Harmonization, Note for Guidance on Validation of Analytical Procedures: Methodology, Committee for Proprietary Medical Products, CPMP/ICH/281/95, approval 18 December 1996.
[14] Y. Hirano, M. Tsunoda, T. Funatsu, K. Imai, J. Chromatogr. B 819 (2005) 41–46.