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Original Article

Quantitative analysis of α-glucosidase inhibitors by ECD with a column of the ion exchange resin of core-shell type filler

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ABSTRACT

The α-glucosidase inhibitors, voglibose (VB), miglitol (MT), and acarbose (AB), are used as postprandial hyperglycemia inhibitors in the treatment of type 2 diabetes. The aim of this study was to develop a quantitative analytical method for α-glucosidase inhibitors using electrochemical detection (ECD) and column of the ion exchange resin of core–shell type filler. The retention times of VB, MT, and AB were determined as 3.8, 4.0, and 14.9 min, respectively, under measurement conditions of an ECD, column of core–shell type filler, column temperature of 25 °C, flow rate of 0.5 mL/min, injection volume of 20 μL, and a mobile phase consisting of 0.1 mol/L NaOH:0.1 mol/L CH_3COONa in 1:1 ratio. The calibration curves of VB, MT, and AB presented good linearity (R^2 > 0.99). The repeatability, precision and accuracy were considered good. The limit of detection and quantification of VB were determined to be 0.281 and 0.851 μg/mL, those of MT 0.163 and 0.493 μg/mL, and those of AB 0.161 and 0.489 μg/mL, respectively. In conclusion, the analysis of α-glucosidase inhibitors could be carried out in a simple way using ECD and a column of core–shell type filler.

Keywords: electrochemical detection, core–shell, α-glucosidase inhibitor, limit of quantification

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INTRODUCTION

The prevalence of type 2 diabetes, a chronic metabolic disease, is increasing worldwide due to aging [1]. According to the International Diabetes Federation, the prevalence of diabetes will increase worldwide to about 400 million by 2015 and 650 million by 2040. Type 2 diabetes is characterized by hyperglycemia, insulin resistance, and relative insulin deficiency, increasing the risk of complications such as arteriosclerosis and cardiovascular disease by inducing continuous postprandial hyperglycemia [2][3]. Therefore, the suppression of postprandial hyperglycemia is effective in preventing cardiovascular diseases in type 2 diabetes patients [4].

One of the type 2 diabetes treatments is α-glucosidase inhibitors. Voglibose, miglitol, and acarbose are known as α-glucosidase inhibitors [5]. α-Glucosidase inhibitors work by blocking complex carbohydrates to simple sugar of due to glucosidase. The pharmacological effect of α-glucosidase inhibitors is to inhibit the hydrolysis of disaccharides, such as maltase and sucrose, in the brush border membrane of the small intestine, thereby delaying the absorption of glucose and suppressing the increase in blood glucose levels after meals. In addition, acarbose reduces the risk of cardiovascular disease [6]. However, the quantitative analysis of α-glucosidase inhibitors in formulations and biological samples has been scarcely reported. The quantitative methods already reported include reverse phase high-performance chromatography (RP-HPLC) [7], high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) [8], and capillary zone electrophoresis [9]. However, these quantitative methods are not convenient because they are complicated techniques.

In recent years, electrochemical detection (ECD) has been widely used as a simple quantitative detection method in high-performance liquid chromatography. For example, the quantification of ascorbic acid [10] and nitroglycerine [11] has been reported using electrochemical detectors. The principle underlying ECD is the measurement of the amount of electricity flowing during oxidation–reduction reactions of the drug with an electrochemical detector. Therefore, compounds prone to oxidation–reduction reactions are the target of this technique. In particular, saccharides can be detected by dissociation of an alcoholic hydroxyl group under strong alkaline conditions, affording an ionic molecule. Since it is possible to use strong alkali as the mobile phase in ECD, the ionized sugars can be analyzed by anion exchange chromatography. The advantages of ECD are its high sensitivity, the possibility of using it with samples with poor absorption in the ultraviolet region, and its simplicity, not requiring procedures such as derivatization for the quantification of the target analyte.

In recent years, columns of various fillers have been used in liquid chromatography for the quantification of drugs. One example is columns of core–shell type filler, whose filler structure comprises a hard core wrapped by a porous layer. For example, columns of core–shell type filler have been utilized for the quantitative determination of tea catechins [12] and sugars [13]. The mobile phase and sample do not pass through the hard-core part of the column. This type of filler is advantageous compared to those comprising a general porous layer as the diffusion path becomes shorter, the elution time of the sample becomes faster, and sharper peaks are obtained.

Therefore, the aims of this work were to develop a simple quantitative method for α-glucosidase inhibitors using ECD and a column of the ion exchange resin of core–shell type filler and to identify the optimal measurement conditions.

MATERIALS AND METHODS

Materials: Voglibose (VB) and miglitol (MT) were prepared by the Sawai Pharmaceutical Corporation and Sanwa Kagaku Kenkyusho Corporation. Acarbose (AB) was purchased from Wako Pure Chemical Industries. The structure of each drug is shown in Fig. 1. The other reagents were of special commercial grade from Wako Pure Chemical Industries.

Methods

Preparation of the samples: VB, MT, and AB (25 mg) were used to prepare 500 μg/mL solutions with distilled water. From this solution, further samples were prepared at 1, 5, 12.5, 25, and 50 μg/mL.

Measurement of samples: The measurement of samples was performed using an electrochemical detection (ECD: SU-300, DKK-TOA). The measurement conditions were a column temperature of 25 °C, flow rate of 0.5 mL/min, and an injection volume of 20 μL. The measurements were carried out using two different solutions as the mobile phase: 0.1 mol/L NaOH and the mixture 0.1 mol/L NaOH:0.1 mol/L CH_3COONa = 1:1. A column of the ion exchange resin of core–shell type filler used prototyped by reaction with an amine (S-30/70 (Styrene/Divinylbenzene)-5 (Tetramethyl diaminohexane), φ4.6 mm × 150 mm) was used by the method. The structure of the column of core–shell filler is shown in Scheme 1.

Validation of the proposed method: The calibration curves were prepared using the results of measurement in 0.1 mol/L NaOH:0.1 mol/L CH_3COONa = 1:1 for mobile phase. The linearity was evaluated by the correlation coefficients of prepared calibration curves. The repeatability, precision and accuracy were evaluated by calculated the relative standard deviation (RSD). The limit of detection (LOD) and limit of quantification (LOQ) were calculated using the obtained calibration curves from the following equations:

$LOD = 3.3 \times (s/a)$

$LOQ = 10 \times (s/a)$

s: SD of the blank sample absorbance.

a: slope of the calibration curve near the detection limit.

RESULTS AND DISCUSSION

Measurement of samples: The results of the VB, MT, and AB analysis using a mobile phase of 0.1 mol/mL NaOH are shown in Fig. 2. The specific absorption peaks for VB and MT were confirmed at retention times of 4.5 and 4.7 min (Fig. 2a and b). However, no peak for AB was observed (Fig. 2c). These observations were considered that are attributed to AB being adsorbed on the porous layer and not eluting from the column of core–shell type filler. To suppress the AB adsorption on the filler, the mobile phase was changed to 0.1 mol/L NaOH:0.1 mol/L CH_3COONa = 1:1 and measured (Fig. 3). The specific absorption peaks for VB, MT and AB were confirmed at retention times of 3.8, 4.0 and 14.9 min (Fig. 3a-c). The above results were considered because adding sodium acetate reduced the pH of the mobile phase and the elution time of AB from the column was faster. Therefore, ECD and the column of core–shell type filler were considered suitable to analyze VB, MT, and AB with a mobile phase of 0.1 mol/L NaOH:0.1 mol/L CH_3COONa＝1:1.

Validation of the proposed method

Linearity: To evaluate for the linearity of VB, MT and AB, the calibration curves of each concentration solution (1.0, 5.0, 12.5, 25 and 50 μg/mL) were prepared and the correlation coefficients (R^2) were calculated (Fig. 4). The correlation coefficients of the calibration curves of VB, MT, and AB were considered that were all above 0.99 and indicated good linearity in the range of 1.0 to 50 μg/mL.

Repeatability and precision: To evaluate for repeatability and precision, the relative standard deviations (RSD) of the slope, intercept and correlation coefficient at the calibration curves (n=10) of VB, MT and AB were calculated (Table 1). The slope, intercept and correlation coefficient at the calibration curves of VB, MT and AB were considered that were all above 5 %. As the results this measurement method was considered good repeatability and satisfactory precision.

Accuracy: To evaluate for accuracy, the recoveries of VB, MT and AB were calculated (Table 2-4). The recoveries of VB, MT and AB were 100±5 %. As the results, this measurement method was considered good accuracy.

The limits of detection and the limits of quantification: Each detection and quantification limit of VB, MT and AB was calculated from prepared the calibration curves (Table 5). The LOD and LOQ of VB were determined to be 0.281 and 0.851 μg/mL, those of MT 0.163 and 0.493 μg/mL, and those of AB 0.161 and 0.489 μg/mL, respectively. According to a medical package insert, the maximum blood concentration of MT in a male adult is about 1.3 μg/mL upon administration of 50 mg MT in a single dose. This value is way over the LOQ of MT calculated from the calibration curve. Therefore, our method based on ECD and a column of ion exchange resin of the core–shell type filler can be considered promising for the analysis of VB, MT, and AB. Also, quantitation of levodopa, carbidopa and 3-oxymethyldopa in rat plasma using ECD was already reported [14]. Therefore, using ECD as a detector was considered enable to be applied to the quantitation of α-glucosidase inhibitors in rat plasma. As a result, the analysis of VB, MT, and AB could be performed using ECD and a column of the ion exchange resin of the core–shell type filler, a simpler method than the previously reported methods for the quantitation of α-glucosidase inhibitors [8][9].

CONCLUSION

It became possible to carry out the measurement by using sodium hydroxide solution as mobile phase which could not be used with conventional ODS column by using the column of core-shell type filler reacted with the amine. Upon adding 0.1 mol/L CH_3COONa to the mobile phase, the peak for AB could finally be observed owing to the faster elution of the analyte. Next, we will aim to apply the developed method in the analysis of medicinal and biological samples.

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Conflicts of interest: The authors declare no conflicts of interest.

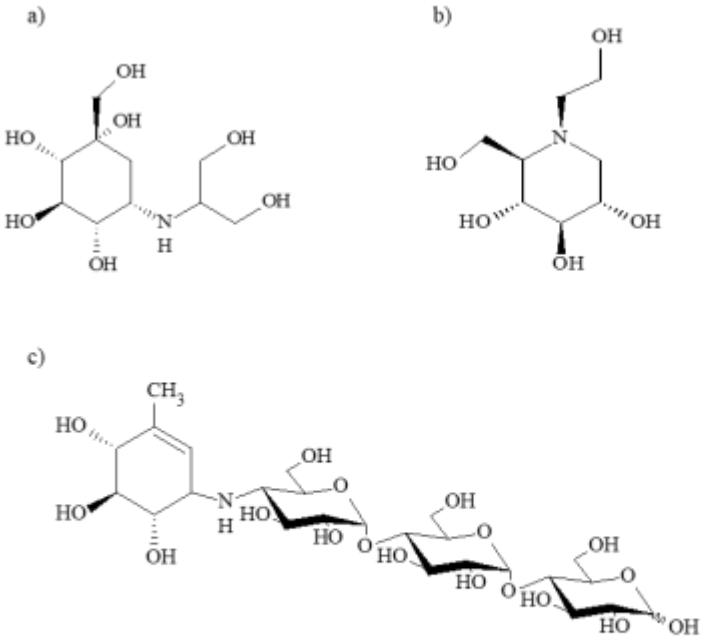
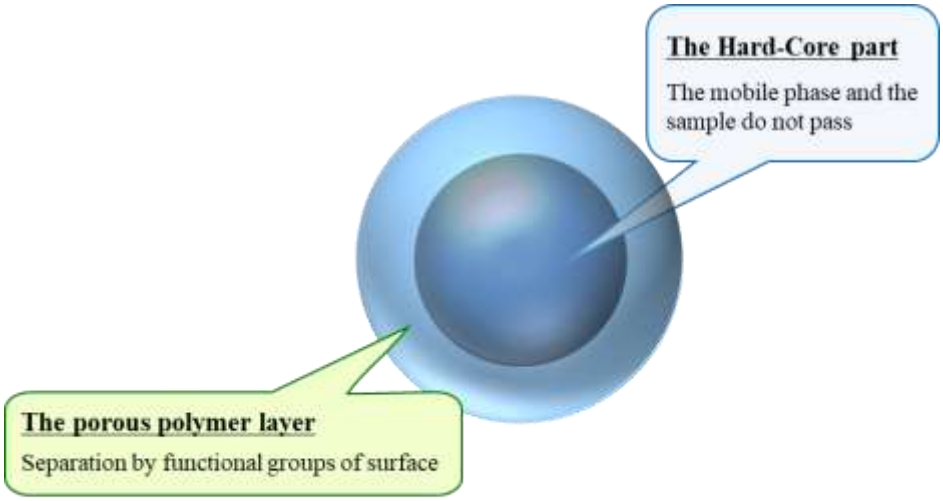


Fig. 1 Structure of α-glucosidase inhibitors: (a) voglibose (VB), (b) miglitol (MT), and (c) acarbose (AB)



Scheme1 Structure of the column of core-shell filler

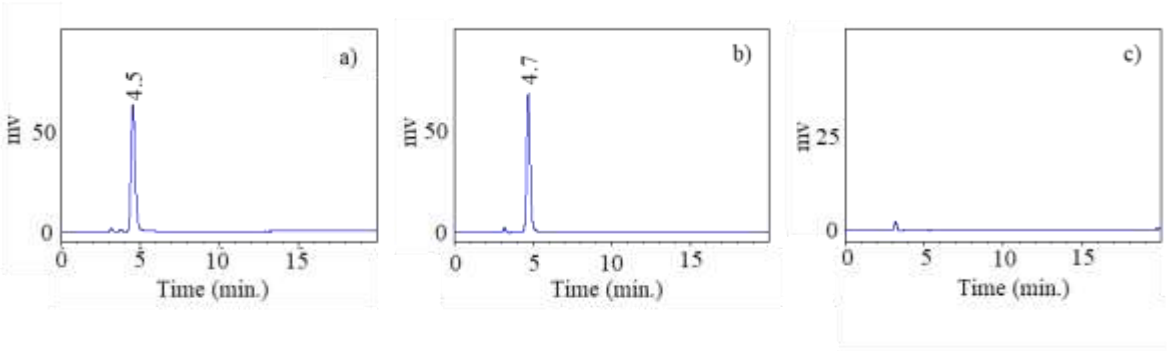


Fig. 2 The chromatograms of (a) VB, (b) MT, and (c) AB
The mobile phase: 0.1 mol/L NaOH

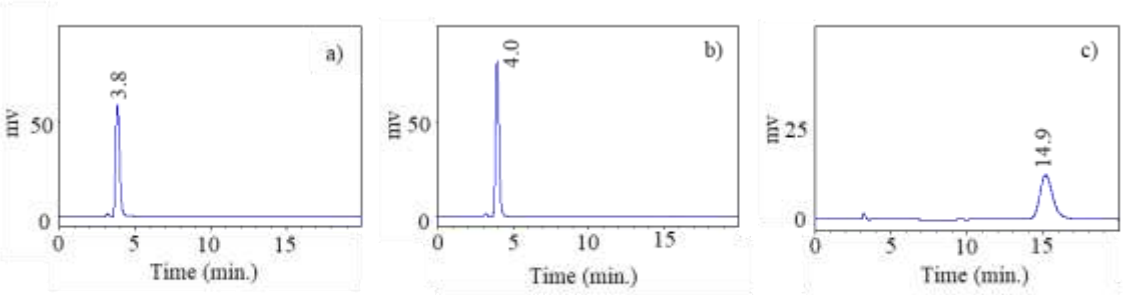


Fig. 3 The chromatograms of (a) VB, (b) MT, and (c) AB
The mobile phase: 0.1 mol/L NaOH:0.1 mol/L CH_3COONa = 1:1

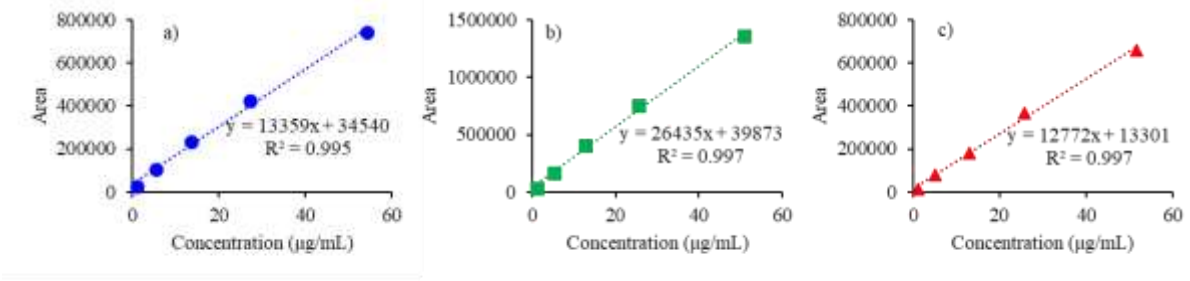


Fig. 4 The calibration curves of (a) VB, (b) MT, and (c) AB
The mobile phase: 0.1 mol/L NaOH:0.1 mol/L CH_3COONa = 1:1

Table 1 Each summary of different linearity parameters for VB, MT and AB (n=10)

	VB			MT			AB		
No.	Slope	Intercept	R^2	Slope	Intercept	R^2	Slope	Intercept	R^2
1	13418	34894	0.994	24374	38367	0.996	13124	13267	0.997
2	13484	33729	0.995	24274	40964	0.996	13162	12241	0.998
3	13256	36010	0.993	26999	39787	0.997	12994	14105	0.996
4	13554	32443	0.996	27068	38721	0.997	13030	14306	0.995
5	13431	33691	0.995	27111	37838	0.997	12957	13754	0.996
6	13394	34042	0.995	26952	39319	0.997	12907	13417	0.996
7	13326	34002	0.995	26945	40473	0.997	12950	13699	0.997
8	13245	35326	0.994	26827	41637	0.996	12871	12613	0.996
9	13264	35528	0.994	26867	41438	0.996	12815	13724	0.997
10	13222	35736	0.995	26937	40189	0.996	12772	13301	0.997
Average	13359	34540	0.995	26435	39873	0.997	12958	13443	0.997
S.D.	113.6	1136.4	0.001	1116.2	1302.3	0.001	125.1	633.2	0.001
RSD (%)	0.850	3.290	0.085	4.222	3.266	0.053	0.966	4.710	0.085

Table 2 Recovery of VB (n=10)

Injection Number	Retention Time (min.)	Area	Measurement Concentration (μg/mL)	Recovery (%)
1	3.8	181700	11.02	103.2
2	3.9	179855	10.88	101.9
3	3.8	179341	10.84	101.5
4	3.8	182940	11.11	104.0
5	3.8	180228	10.91	102.1
6	3.9	182977	11.11	104.1
7	3.8	182484	11.07	103.7
8	3.8	182269	11.06	103.6
9	3.9	182886	11.10	104.0
10	3.8	180326	10.91	102.2
Average	3.8	181501	11.00	103.0
S.D.	0.048	1420	0.11	0.995
RSD (%)	1.261	0.782	0.966	0.966

The prepared concentration of VB: 10.68 μg/mL

Table 3 Recovery of MT (n=10)

Injection Number	Retention Time (min.)	Area	Measurement Concentration (μg/mL)	Recovery (%)
1	3.9	310686	10.24	99.6
2	3.9	313749	10.36	100.7
3	4.0	319501	10.58	102.9
4	4.0	322652	10.70	104.0
5	4.0	320445	10.61	103.2
6	4.0	318546	10.54	102.5
7	3.9	314876	10.40	101.2
8	3.9	313254	10.34	100.6
9	4.0	308490	10.16	98.8
10	4.0	306691	10.09	98.1
Average	4.0	314889	10.40	101.2
S.D.	0.052	5329	0.20	1.970
RSD (%)	1.304	1.692	1.951	1.948

The prepared concentration of MT: 10.28 μg/mL

Table 4 Recovery of AB (n=10)

Injection Number	Retention Time (min.)	Area	Measurement Concentration (μg/mL)	Recovery (%)
1	14.9	152966	10.77	104.4
2	14.9	151702	10.67	103.5
3	14.9	152132	10.70	103.8
4	14.9	147206	10.32	100.1
5	14.5	141812	9.90	96.1
6	14.9	143847	10.06	97.6
7	14.8	146365	10.26	99.5
8	14.8	148917	10.45	101.4
9	14.8	152522	10.73	104.1
10	14.9	144953	10.15	98.4
Average	14.8	148242	10.40	100.9
S.D.	0.125	4004	0.31	2.994
RSD (%)	0.844	2.701	2.981	2.968

The prepared concentration of AB: 10.31 μg/mL

Table 5 The limits of detection and limits of quantification

	LOD (μg/mL)	LOQ (μg/mL)
α-GIs agents	3.3s/a	10s/a
VB	0.281	0.851
MT	0.163	0.493
AB	0.161	0.489

s: SD of absorbance of blank sample

a: The slope of the calibration curve near the detection limit

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