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Short communication

Development and validation of LC methods with visible detection using pre-column derivatization and mass detection for the assay of voglibose

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ABSTRACT

Two sensitive and selective liquid chromatographic methods were developed for the assay of voglibose (VB) and validated as per International Conference on Harmonization (ICH) guidelines. First method is based on the pre-column derivatization of VB followed by visible detection (LC–VD) and second method involves mass spectrometric detection (LC–MS). In LC–VD method, VB was derivatized with sodium metaperiodate and 3-methyl-2-benzothiazolinone hydrazone hydrochloride monohydrate (MBTH). The derivatized color product of VB (DCPVB) was run through Novapak C18 (300 × 3.9 mm, 4 μm) column using the mobile phase containing buffer (0.01 M mixture of sodium di hydrogen orthophosphate and disodium hydrogen orthophosphate, pH 6.0) and acetonitrile in 35:65 v/v ratio. The eluted DCPVB was monitored at 667 nm. The fixation of optimum conditions in LC–VD method is described. DCPVB structure was confirmed by mass spectral analysis. In LC–MS method, VB was passed through Venusil XBPPH (150 × 4.6 mm, 5 μm) column using a 95:5 v/v mixture of 0.01% formic acid and methanol as mobile phase. The assay concentrations of VB in pure form and in tablets for LC–VD and LC–MS methods are 25 and 5 ng ml^{-1}, respectively.

1. Introduction

Voglibose (3,4-Dideoxy-4-[[2-hydroxy-1-(hydroxymethyl)ethyl]amino]-2-C-(hydroxy methyl)-D-epiinositol, VB) [1,2], is an alpha-glucosidase inhibitor used for lowering post-prandial blood glucose levels in people with diabetes mellitus. In literature only one article for the determination of VB was found [3]. This article describes two liquid chromatographic methods one with fluorescence detection (LC–FD) using post-column derivatization and other with mass detection (LC–MS). Since VB does not have chromophoric groups, post-column derivatization technique was opted in LC–FD method. But this method requires high temperature and elutes VB at longer retention time. Hence, we have opted pre-column derivatization technique with visible detection (LC–VD) [4,5] because for pre-column derivatization, no special equipment is required, no restrictions on the reaction conditions like reaction time, reaction temperature, number of reagents etc. In addition, small volumes of reagents are sufficient for derivatization and the derivatized products often permits easier selection of stationary and mobile phases than the original compounds. We have also developed a highly sensitive LC–MS method for the assay of VB. Both the methods were validated as per ICH guidelines [6].

2. Experimental

2.1. Chemicals and reagents

Sodium di hydrogen orthophosphate, disodium hydrogen orthophosphate, formic acid, HPLC grade acetonitrile and methanol were procured from Merck, India. 1% solution of sodium metaperiodate (Sigma–Aldrich Corporation, Bangalore, India) and 1% solution of 3-methyl-2-benzothiazolinone hydrazone hydrochloride monohydrate (MBTH, Sigma–Aldrich Corporation, Bangalore, India) were prepared in Milli Q water (Millipore, Bangalore, India).

2.2. Sample preparation

One milligram per millilitre stock solution of VB was prepared by dissolving 100 mg in 100 ml of Milli Q water. This solution is further diluted with Milli Q water to get the solutions with desired concentrations for validation. Tablet powder equivalent to 100 mg of VB was dissolved in 50 ml of water and sonicated for 30 min. This solution is filtered into a 100 ml volumetric flask and diluted up to the mark with 100 ml of Milli Q water to get 1 mg ml^{-1} tablet

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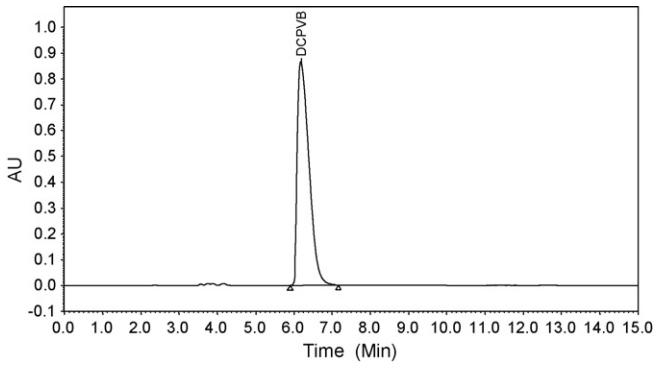


Fig. 1. LC–VD chromatogram of DCPVB.

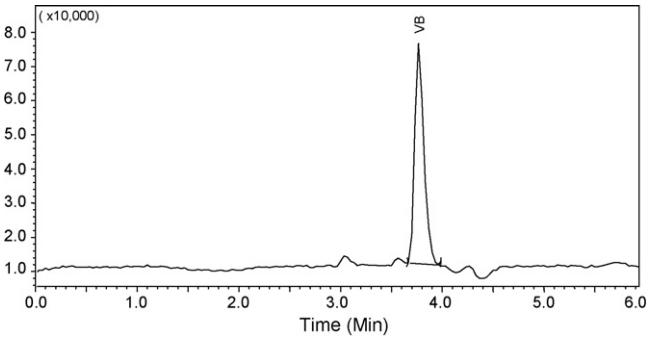


Fig. 2. Total ion chromatogram of VB in SIM mode.

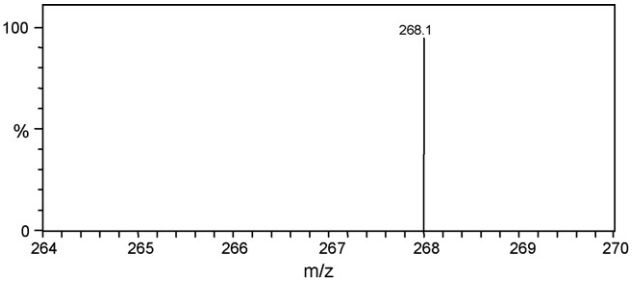


Fig. 3. Mass spectrum of VB.

solution. This solution is further diluted with Milli Q water to get the solutions with desired concentrations for specificity.

2.3. LC–VD conditions

LC–VD analysis was carried out on Waters Alliance 2695 separation module equipped with 2996 PDA detector (Waters Corporation, Milford, USA) and empower software. Novapak C18 (300 × 3.9 mm, 4 μm, Waters Corporation, Milford, USA) column and 35:65 v/v mixture of buffer (0.01 M mixture of sodium di hydrogen orthophosphate and disodium hydrogen orthophosphate, pH 6.0) and acetonitrile were used as stationary and mobile phases, respectively. The flow rate of the mobile phase was kept at 1.0 ml min^{-1}.

Aliquots of VB solutions (200, 225, 250, 275 and 300 ng ml^{-1}) were prepared by diluting 1 mg ml^{-1} VB stock solution with water and taken into a series of 10 ml volumetric flasks. To these flaks, 1.5 ml each of $NaIO_4$ and MBTH solutions were added and kept for 10 min. Then the solutions were made up to the mark with water and cyclomixed to get DCPVB solutions. The final concentration of VB in the DCPVB solutions were 20.0, 22.5, 25.0, 27.5 and 30.0 ng ml^{-1}. 100 μl of each DCPVB solution was injected and the eluted DCPVB at retention time ~6.4 min was monitored at 667 nm (Fig. 1).

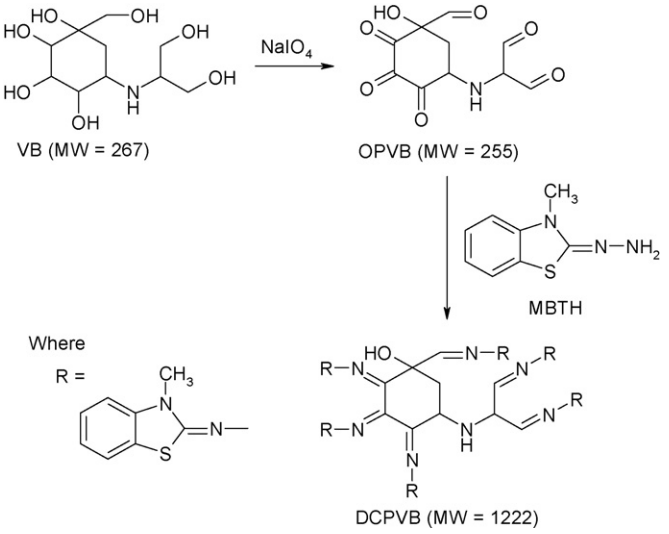
2.4. LC–MS conditions

LC–MS analysis was carried out on Shimadzu LCMS-2010 EV system (Shimadzu Corporation, Japan) having LCMS solution software in electro spray ionization (positive) mode. Selective ion monitoring of VB was done. Interface, curve dissolvation line and detector voltages are 4.4 kV, 0.0 V and 1.5 kV, respectively. Nebulization gas flow is 1.5 l min^{-1}. Interface, curve dissolvation line and heat block temperatures are 250, 230 and 200 °C, respectively. Venusil XBPPH (150 × 4.6 mm, 5 μm) column and a 95:5 v/v mixture of 0.01% formic acid and methanol were used as stationary and mobile phases, respectively. The flow rate of the mobile phase was 0.4 ml min^{-1}. VB solutions (4.0, 4.5, 5.0, 5.5 and 6.0 ng ml^{-1}) were prepared in a 50:50 v/v mixture of 0.01% formic acid and methanol. The total ion chromatogram of VB in SIM mode and its mass spectrum are presented in Figs. 2 and 3, respectively.

3. Results and discussion

3.1. LC–VD method development

Initially, 250 ng ml^{-1} of VB was taken into a 10 ml volumetric flask and 1 ml each of $NaIO_4$ and MBTH solutions were added. DCPVB was developed within 2 min., then the total volume was made up to the mark with water and the resulting solution was used for LC–VD method development. DCPVB was run through literature conditions [2] viz. Cosmosil 5NH_2–MS column (150 × 4.6 mm, 5 μm) as stationary phase and a 2:1 v/v mixture of acetonitrile and 30 mM NaH_2PO_4 (pH 6.5) as mobile phase. DCPVB was not eluted in this condition. Hence, the above column was replaced with Novapak C18 (300 mm × 3.9 mm × 4 μm) column and the same mobile phase was maintained with a flow rate of 1.0 ml min^{-1}. DCPVB was eluted in this condition, but blank interference and peak tailing were observed. Then, it was planned to modify mobile phase and hence, buffer (0.01 M mixture of sodium di hydrogen orthophosphate and disodium hydrogen orthophosphate, pH 6.0) and acetonitrile in 30:70 ratio was opted. In this condition, peak tailing is reduced, but DCPVB eluted too early. Finally by altering the



Scheme 1. Formation of derivatized colored product of VB through oxidation product.