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Journal of Chromatography B, 819 (2005) 91–96
JOURNAL OF CHROMATOGRAPHY B
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High performance liquid chromatographic determination of *N*-butyryl glucosamine in rat plasma

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Received 30 August 2004; accepted 31 January 2005
Available online 19 February 2005

Abstract

Purpose: A high performance liquid chromatography (HPLC) method for determination in plasma of *N*-butyryl glucosamine (GLBU), a highly water-soluble compound with no chromophore was developed. *Method:* To 100 μL of plasma containing GLBU was added fucose as internal standard. GLBU and fucose were derivatized using 1-phenyl-3-methyl-5-pyrazolone in the presence of sodium hydroxide at 70 °C for 30 min. The solution was neutralized with hydrochloric acid and the excess derivatizing reagent was extracted with chloroform. The aqueous layer was injected into an isocratic HPLC system consisting of an autoinjector, a single pump and a UV detector set at 245 nm. Two different 25 cm reversed phase columns were used, a 4 and a 10 μm C_{18} columns. The mobile phase was a mixture of phosphate buffer (pH 7) and acetonitrile (80:20), which was run through a pump at a flow rate of 1.0 mL/min at ambient temperature. *Results:* Derivatized fucose and GLBU appeared 24 and 28 min, and at 34 and 37 min using 4 and 10 μm columns, respectively. The assay was linear over the range of 0.2–200 μg/mL with a limit of quantification of 0.2 and 1 μg/mL for the 4 and 10 μm columns, respectively. The method was applied to the determination of GLBU in rat plasma after oral administration of 233 mg/kg of GLBU. *Conclusion:* The present assay is precise, and accurate with sufficient sensitivity for pharmacokinetic studies following therapeutically relevant doses.

Keywords: *N*-Butyryl glucosamine; 1-Phenyl-3-methyl-5-pyrazolone; Rat plasma; High performance liquid chromatography; Glucosamine; Fucose; Post-column derivatization; C_{18} columns

1. Introduction

N-Butyryl glucosamine (GLBU) is an acylated derivative of glucosamine with a substituted butyryl group at the N atom of the amino group (Fig. 1) [1]. In a rabbit model of osteoarthritis and the rat model of streptococcal cell wall antigen, the compound has shown beneficial effects following oral doses of 20 and 200 mg/kg, respectively. The compound, therefore, is under development for therapeutic use. There is no reported analytical method for the evaluation of GLBU. The drug is highly hydrophilic hence its extraction from plasma with organic solvents is unfeasible. This renders the removal of many endogenous compounds with chemical structure similar to GLBU challenging. In addition, the drug has no chromophore hence has no light absorption at the UV range. For a similar compound, derivatization with a chromophore-containing reagent has resolved the latter problem [2]. Fortunately, similar to glucosamine, GLBU is administered in large doses. The assay sensitivity, therefore, is not a limiting step and the assay may be carried out without the need for extraction and subsequent condensation.

We have previously reported an assay for glucosamine that involves derivatization for the purpose of adding a strong UV chromophore to the compound [2]. The assay, however, was found unsuitable for GLBU as the method necessitates

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doi:10.1016/j.jchromb.2005.01.034

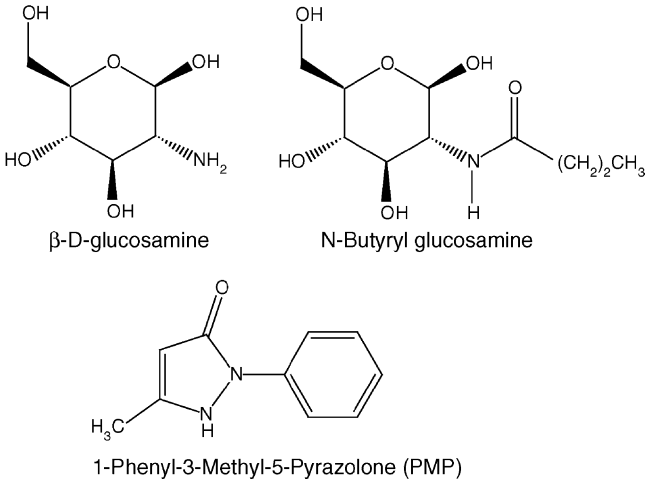

Fig. 1. Structure of β-D-glucosamine, *N*-butyryl glucosamine, 1-phenyl-3-Methyl-5-Pyrazolone.

derivatization at the N atom of an amine group. Such a group is not present in GLBU. Alternatively, hydroxyl groups, abundant in the structure of GLBU, were targeted. Such an approach is expected to result in anomeric derivatives of the carbohydrate [3–6]. Honda et al. [7] have successfully used 1-phenyl-3-methyl-5-pyrazolone (PMP) for derivatization of carbohydrates. In the present study we have described a rapid and simple HPLC method suitable for determination of GLBU in rat plasma following derivatization with PMP.

2. Experimental

2.1. Materials and reagents

D-(+)-Glucosamine butyrate was synthesized by the Department of Medicine, Queen's University (Kingston, Ont., Canada), D-(+)-fucose and 1-phenyl-3-methyl-5-pyrazolone (>95%) were purchased from Sigma Chemical Company (St. Louis, MO, USA). Methanol and acetonitrile were purchased from Caledon Laboratory Ltd (Georgetown, Ont., Canada). All chemicals and solvents were of analytical or HPLC grade. All standard solutions and mobile phase were prepared in double distilled water.

2.2. Sample preparation and derivatization

Due to hydrophilic nature of GLBU, the conventional liquid-liquid extraction of the intact drug from plasma proved unfeasible.

A stock solution of GLBU was freshly prepared by dissolving 50 mg of GLBU in 100 mL of double distilled water. Aliquots of 0.1 mL of blank rat plasma were spiked with 20 μL of fucose solution (50 μg/mL water) as internal standard and various GLBU standard solutions in 1.5 mL Eppendorf tubes to obtain concentrations of 0.2–200 μg/mL of the drug. To this solution was added 100 μL of a methanolic 0.5 M solution of PMP. The latter was premixed with an equal volume of sodium hydroxide 0.3 M. The resultant solution was heated at 70 °C for 30 min. Subsequently the solution was cooled down to room temperature and neutralized with 0.3 M hydrochloric acid. Phosphate buffer (0.2 mL, pH 2.5) was added, vortex mixed for 30 s and centrifuged at 19,000 × *g* for 1 min. The liquid part of the sample was separated from precipitated plasma residual and transferred into a 1.5 mL Eppendorf tube. To remove the excess derivatizing reagent and its degradation products as well as other unwanted compounds, the resultant aqueous solution was subjected to three times of extraction with 0.3 mL chloroform followed by vortex mixing for one min and centrifuging at 19,000 × *g* for 30 s. The upper aqueous layer, which contained GLBU was separated and 0.1 mL of it was injected into the HPLC system.

To assess the effect of addition of plasma on the recovery of GLBU, two series of triplicate samples containing 1, 10, and 20 μg/mL of the drug were prepared as described above, one with and another without plasma. To the set without plasma, water was added instead of plasma. The percent recovery was determined from

$$\%\ \text{Recovery} = \frac{\text{Peak response (plasma standard)}}{\text{Peak response (water standard)}}$$

For the assessment of precision and intra- and inter-day variation, triplicates of spiked plasma samples at concentration range of 0.2–200 μg/mL were used (Table 1).

To test the stability of derivatized GLBU and internal standard during the assay period, samples were placed a pH range of 2–8 at room temperature.

2.3. Interference with glucosamine and glucose

To determine the possibility of interference with glucose and glucosamine, aqueous solutions containing the latter compounds were subjected to the method described for GLBU. The resulting solutions were injected into the HPLC under identical conditions described for GLBU. For glucosamine, this experiment was repeated after spiking of blank plasma samples as well.

2.4. HPLC system

The HPLC system consisted of a single model M 45 HPLC pump (Waters, Mississauga, Ont., Canada), model SIL-9A autoinjector, a variable UV detector model SPD-10A UV–vis (Shimadzu, Japan) set at 245 nm and an integrator model 3390A (Hewlett Packard, USA).

Two different HPLC columns both from Phenomenex (Torrance, CA, USA) were tested, a 250 mm × 4.6 mm i.d. 10 μm of reversed-phase C_{18} and a 250 mm × 4.6 mm i.d. 4 μm of Synergi Hydreo-RP 80 Å polar endcapped C_{18} analytical columns. To both columns was attached an HPLC precolumn insert packed with C_{18} (Waters, Mississauga, Ont., Canada).