Analysis of Glucuronolactone and Glucuronic Acid in Drug Formulations by High-Performance Liquid Chromatography

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Abstract

Glucuronolactone and glucuronic acid in drug formulations and beverages are determined as 1-phenyl-3-methyl-5-pyrazolone (PMP) derivatives using high-performance liquid chromatography. Intra-ester linkage (i.e., the lactone of glucuronolactone) is spontaneously hydrolyzed to free-acid form and is analyzed as the PMP derivative of glucuronic acid. By omitting three evaporation steps in the original derivatization procedure, the total analysis time is shortened to approximately 40 min. Reproducibility of determination is within 4.0% for all drug formulations. The present method satisfies the requirements for routine analysis in the quality control of drug formulations containing glucuronolactone or glucuronic acid.

Introduction

Vitamin preparations and beverages are available at drug stores throughout Japan as over-the-counter (OTC) drugs. The market for these pharmaceutical products is worth approximately 2600 billion yen (approximately $22 billion in U.S. currency) on an annual basis and occupies one-third of the OTC drug market (1). Many of these pharmaceutical preparations contain glucuronolactone (GlcLA) or glucuronic acid (GlcA), which are used in the treatment of bilirubinemia because they improve the liver condition (2). Carbazole–sulfuric acid assay is usually used for their photometric assay in pharmaceutical preparations (3), but this method can be dangerous because it employs a corrosive acid, such as concentrated sulfuric acid, at a high temperature. Chromatographic methods using an anion-exchange column are often used for GlcLA and GlcA determination (4) because many vitamin tablets and beverages contain complex mixtures from biological sources. However, the methods require gradient elution and a post-column derivatization device. Furthermore, when GlcA and GlcLA are analyzed by chromatographic methods, the equilibrium between GlcA and its lactone form (i.e., GlcLA in an aqueous solution) should be considered (2).

We have developed a method for the derivatization of reducing carbohydrates with 1-phenyl-3-methyl-5-pyrazolone (PMP) using high-performance liquid chromatography (HPLC) (5). The derivatization reaction is performed under slightly alkaline conditions as shown in Figure 1. In the first step of the derivatization reaction, a reducing carbohydrate is dissolved in an aqueous solution of sodium hydroxide. Because GlcLA is easily converted to free acid form (i.e., GlcA) by spontaneous hydrolysis of an intramolecular ester linkage at basic condition, the PMP method is easily applied to determine GlcLA as the PMP derivative of GlcA in pharmaceutical preparations. In the present paper, we describe an easy method for determining GlcA and GlcLA in pharmaceutical preparations by the PMP labeling method.

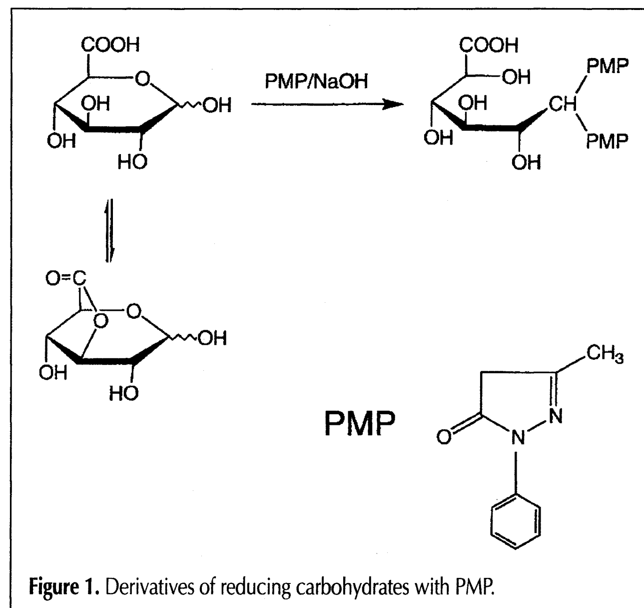

Figure 1. Derivatives of reducing carbohydrates with PMP.

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Experimental

Reagents

PMP and standardized solutions of 1.0M sodium hydroxide and 1.0M hydrochloric acid were obtained from Kishida Chemical (Chuo-ku, Osaka, Japan). Solutions of 0.3M sodium hydroxide and 0.3M hydrochloric acid were prepared by dilution of the standardized solution with water. PMP was used after recrystallization with methanol–water. GlcA and GlcLA were reagent-grade and were obtained from Nacalai Tesque (Nakagyo-ku, Kyoto, Japan) and Wako Pure Chemical (Chuo-ku, Osaka, Japan), respectively. The internal standard D-galactose (Gal) was also obtained from Wako Pure Chemical. Phosphate buffer (30mM, pH 7.0) was prepared by mixing solutions of 30mM sodium monohydrogen phosphate and 30mM sodium dihydrogen phosphate to adjust the pH to 7.0. All aqueous solutions were prepared using water purified with a Milli-Q purifying system (Millipore Japan, Shinagawa-ku, Tokyo, Japan). All other reagents and solvents used in the present study were of the highest grade commercially available or HPLC-grade. Evaporation of the aqueous solutions was performed on a centrifugal evaporator (Tomy Kogyo, Nerima-ku, Tokyo, Japan).

HPLC

The HPLC apparatus consisted of a Hitachi 655A-12 double-plunger pump (Minato-ku, Japan), a Rheodyne 7125 injector (Cotati, CA) with a 20-µL loop, a Shimadzu SPD-6A UV spectromonitor (Nakagyo-ku, Kyoto, Japan) equipped with an 8-µL quartz cell, and a Shimadzu CR-6A Chromatopak data processor. A column (150 mm × 6-mm i.d.) packed with Cosmosil 5C18AR (octadecyl-silica, Nacalai Tesque) was eluted with a mixture of 30mM phosphate buffer (pH 7.0) and acetonitrile (82:18, v/v) at a flow rate of 1.0 mL/min. The elution buffer was prepared by mixing 30mM sodium dihydrogen phosphate and disodium hydrogen phosphate to adujst the pH to 7.0. The PMP derivative of GlcA was detected at 245 nm, the wavelength at which the PMP group showed maximum absorption.

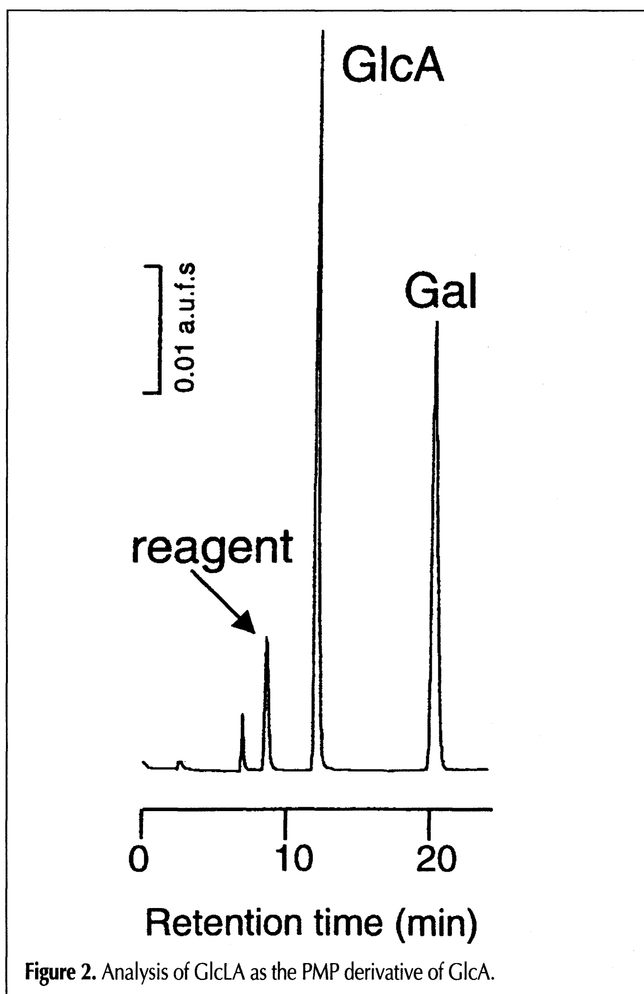

Figure 2. Analysis of GlcLA as the PMP derivative of GlcA.

Preparation of sample solution

Tablet samples

Twenty tablets (each tablet containing 50 mg of GlcLA) were weighed accurately and dissolved in 200 mL of water with sonication. The solution was diluted to 1000 mL in a volumetric flask. A portion (50 µL) was used for derivatization with PMP for the determination of GlcLA.

Beverages

The beverages obtained for the present study contained GlcLA or GlcA within a range of 3 to 30 mg/mL. A 5.0-mL portion of beverage was diluted with water to 100.0 mL. A 50-µL portion was derivatized with PMP for the determination of GlcLA or GlcA.

Derivatization procedure

A sample solution (50 µL) was mixed with a solution (50 µL)

Table I. Recovery Studies of GlcLA and GlcA in Spiked Samples of Beverage B

Compound	Concentration (mg/100 mL)	Recovery* (mg/100 mL)	RSD (%)
GlcLA	100	103 ± 2	1.9
	500	505 ± 19	3.0
	1000	1010 ± 20	2.0
GlcA	100	98 ± 1	1.0
	500	505 ± 6	1.2
	1000	998 ± 20	2.0

* Mean ± SD (*n* = 5).

Table II. Analysis of GlcLA and GlcA in Pharmaceutical Preparations as PMP Derivatives of GlcA

Pharmaceutical preparation	Number of determinations*	Amount of GlcA found (g)	Amount described in the prescription sheet
A (tablet)	27	1.03 ± 0.04	1.0 g/20 tablets
B (beverage)	20	1.02 ± 0.04	1.0 g/100 mL
C (beverage)	19	1.10 ± 0.02	1.0 g/30 mL
D (beverage)	18	0.0793 ± 0.04	0.100 g/30 mL

* The samples obtained are products from different lots.

of the internal standard (100 nmol/μL) in a polypropylene tube (1.5-mL volume). A solution (20 μL) of 0.3M NaOH was added, and the mixture was agitated by a vortex mixer for 20 s. After the addition of 0.5M PMP in methanol (20 μL), the mixture was incubated for 20 min at 70°C. A solution of 0.3M hydrochloric acid (20 μL) was added to the mixture to neutralize the solution, and the mixture was agitated vigorously by a vortex mixer for 20 s. Water (100 μL) and chloroform (200 μL) were added to the mixture, and the mixture was shaken vigorously with a vortex mixer for 20 s. After centrifugation for 30 s at 1000 × g on a microcentrifuge, the chloroform layer (lower layer) was carefully removed using a syringe. The chloroform (200 μL) extraction procedure was repeated twice. A portion (20 μL) of the aqueous layer was injected into the HPLC column. An example chromatogram is shown in Figure 2.

Recovery studies

The recoveries of GlcLA and GlcA were obtained using spiked samples of beverage B (Table I). Spiked samples were prepared by adding a known amount of GlcLA or GlcA to beverage B (pharmaceutical preparation B in Table II) and analyzing the mixture using same the derivatization procedures. The concentrations of the added GlcLA or GlcA were 100, 500, and 1000 mg/100 mL. The peak areas obtained were compared with those observed in the analysis of beverage B.

Results and Discussion

GlcA and GlcLA are present in the equilibrium state in an aqueous solution. For the determination of GlcA or GlcLA in pharmaceutical preparations, conversion to either form is necessary for easy determination. When reducing carbohydrates are derivatized with PMP, the sample should be dissolved in an alkaline solution at the initial step. An intramolecular ester linkage (i.e., the 3,6-lactone bond of GlcLA) is considered to be easily and spontaneously hydrolyzed to a free acid form. Therefore, derivatization of GlcLA and GlcA with PMP yields the PMP derivative of GlcA exclusively. In the original derivatization procedure (5), the derivatization reaction was started immediately after dissolution of the sample in 0.3M NaOH by the addition of 0.5M PMP in methanol. To confirm the complete hydrolysis of lactone to free acid form, an alkaline solution of glucuronolactone was incubated for a specified period at 70°C prior to addition of the PMP solution. The results are shown in Figure 3.

Recovery of GlcA from GlcLA at 0 min was the highest. This indicated that preincubation of GlcLA in an alkaline solution was not necessary. Furthermore, prolonged preincubation of GlcLA in an alkaline solution caused a decrease in recovery of GlcA, probably because of the degradation of GlcA by β-elimination reaction of the hydroxyl group at C-4.

The original derivatization procedure required three evaporation steps (5). For the first evaporation step, the aqueous sample solution was evaporated to dryness. For the second step, the reaction mixture was evaporated to dryness after neutralization of the mixture with hydrochloric acid. For the third step, the aqueous layer was evaporated after extraction of the excess amount of the PMP reagent with chloroform. It has been recommended that dried samples be stored in the refrigerator for several weeks (6). However, for the analysis of GlcLA and GlcA in pharmaceutical preparations for routine analytical purposes, easy operation and short-time analysis are prerequisite, so we developed the simple procedures shown in the Experimental section. The response of relative peak area to that of galactose remained unchanged, even when the evaporation steps were omitted (data not shown).

By using the standard procedure described in the Experimental section, calibration curves for GlcLA exhibited good linearity as shown in Figure 4, at least in the range of 18–570 nmol. Linearity was expressed as $y = 0.0166x + 0.00706$

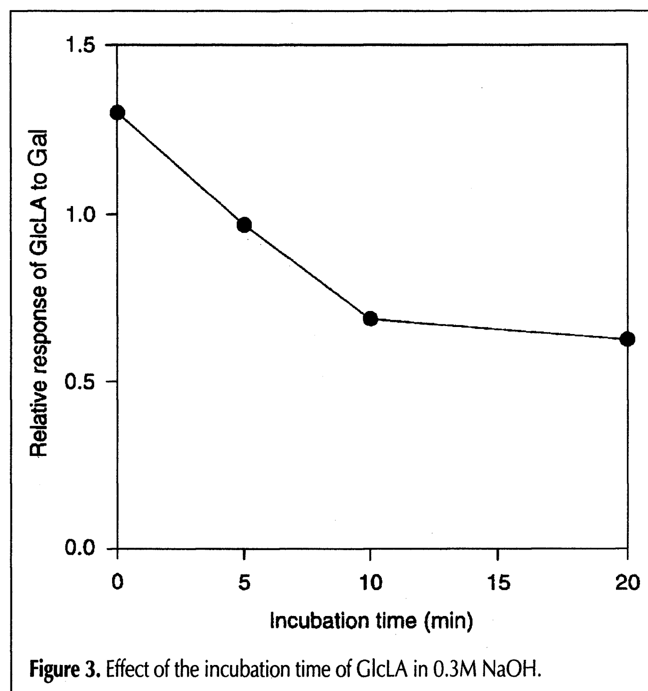

Figure 3. Effect of the incubation time of GlcLA in 0.3M NaOH.

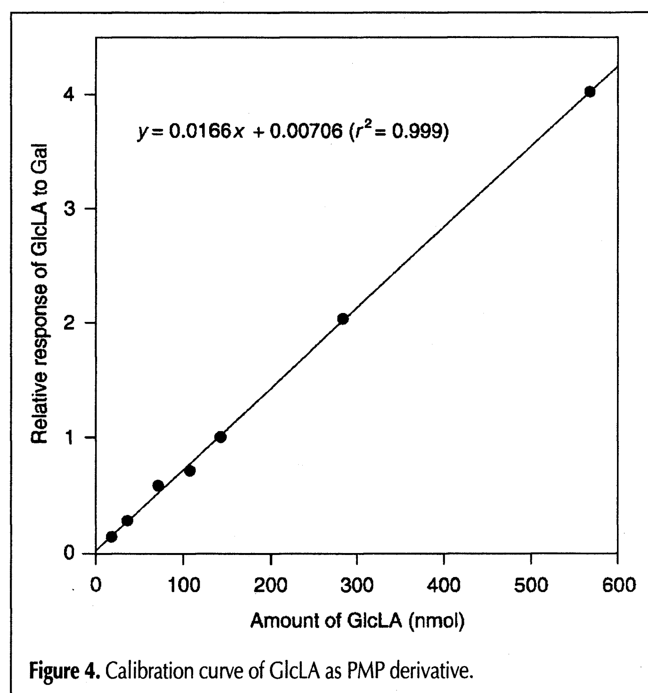

Figure 4. Calibration curve of GlcLA as PMP derivative.

(correlation coefficient $r^2 = 0.999$), where x is the amount of GlcLA (nmol) and y is relative response to the internal standard. The relative standard deviations in repeated analyses ($n = 7$) of GlcLA are 2.8%, 2.8%, and 2.2% for 18 nmol, 71 nmol, and 284 nmol of GlcLA, respectively. Similar results were observed for the calibration curves of GlcA (data not shown).

The recoveries of GlcLA and GlcA from spiked samples of beverage B are shown in Table I. Although the present method includes an extraction step during derivatization, the recoveries were quite high and reproducibility was satisfactory for both GlcLA and GlcA. The high recovery and good reproducibility were probably caused by the presence of a hydrophilic carboxyl group of GlcA. Hydrophilicity of the derivatized product minimized the loss in the extraction step.

Table II summarizes the results obtained from several commercially available pharmaceutical preparations. The tablet preparations contained 1.03 ± 0.04 g of GlcLA in 20 tablets, and the relative standard deviation was within 3.8%. We also determined the amount of GlcLA or GlcA in three commercially available vitamin drink products (beverage samples B, C, and D) from different companies. According to their prescription sheets, beverages B and C contained GlcLA at concentrations of 1.0 g/100 mL and 1.0 g/30 mL, respectively. The results obtained by the present method were in good accordance with those described in their respective prescription sheets. An example of the analysis of beverage B is shown in Figure 5.

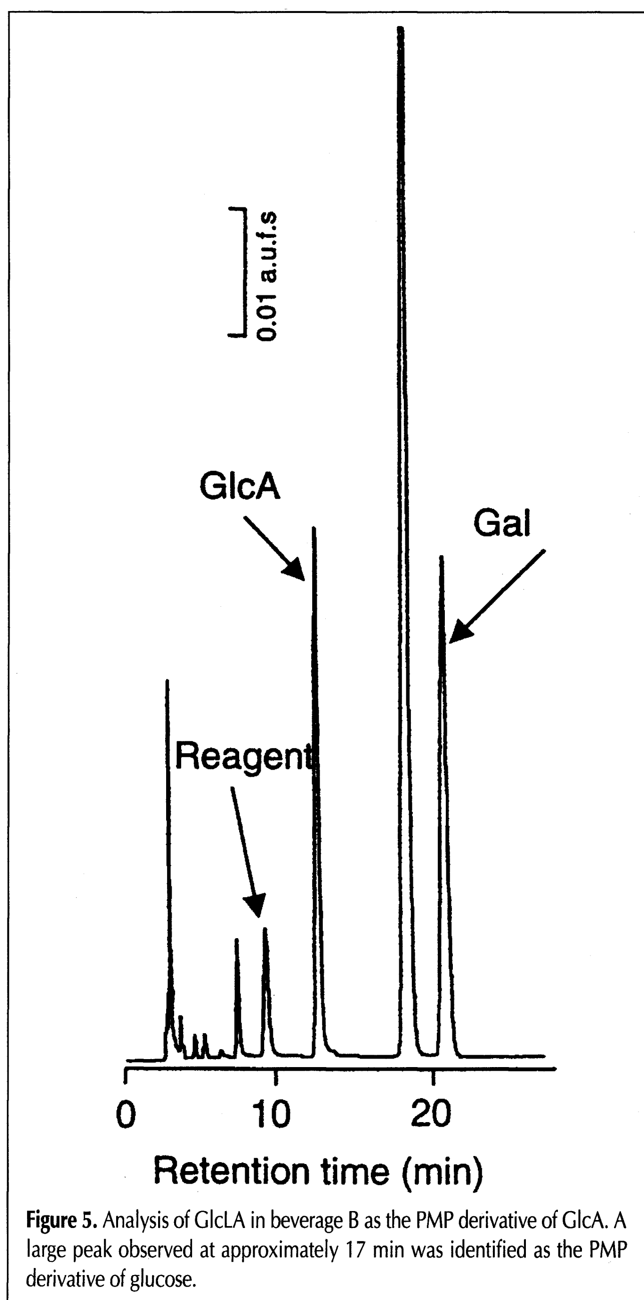

Figure 5. Analysis of GlcLA in beverage B as the PMP derivative of GlcA. A large peak observed at approximately 17 min was identified as the PMP derivative of glucose.

All the samples examined in the present study showed similar elution patterns after derivatization with PMP. Any interference due to the presence of excipients was not observed. A large peak that was not GlcA was observed at approximately 17 min. It was identified as the peak of the PMP derivative of glucose using co-injection of the standard sample. The product sheet of beverage D claimed that it contained GlcA at a concentration of 100 mg/30 mL. However, the results showed that the preparation contained less than that amount (79.3 mg/30 mL). Addition of the known amount of GlcA or GlcLA to beverage D showed quantitative recovery of both GlcA and GlcLA (data not shown). Assuming that beverage D contained GlcA as a salt form of sodium or calcium, we calculated the concentration of GlcA in beverage D. Nevertheless, beverage D contained less GlcA than was described in the sheet from the manufacturer. Based on these results, we propose that manufacturers should have to indicate a more accurate amount of GlcA in their product when GlcA is used in product formulation.

Conclusion

GlcA and GlcLA in drug formulations were easily determined as their PMP derivatives using HPLC. The present method allows short-time analysis within 40 min and is suitable for routine analyses such as quality control of drug formulations.

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