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Short communication

Solid-phase extraction of 18β-glycyrrhetinic acid from plasma and subsequent analysis by high-performance liquid chromatography

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Abstract

A new method is described for the solid-phase extraction of 18β-glycyrrhetinic acid from plasma or serum, with subsequent analysis by HPLC. New aspects of the method include the use of commercially available 18α-glycyrrhetinic acid as the internal standard and the use of a Bond Elut C_2 (ethyl) extraction column, to avoid the need to use large volumes of organic solvent to elute the isolates from the columns. Separation was achieved on a Shandon Hypersil BDS C_{18} analytical column, with a mobile phase consisting of acetonitrile–0.02 *M* phosphate buffer, pH 5.7 (55:45, v/v). The column effluent was monitored at 248 nm. Compared with previous methods, the procedure is much easier to carry out, whereas the sensitivity (limit of detection, 10 ng/ml, and limit of quantitation, 50 ng/ml), the precision (0.3–6.2%) and the accuracy (97.2–101.9%) are of the same order of magnitude.

Keywords: Glycyrrhetinic acid

1. Introduction

Intake of licorice in large doses may cause sodium retention and potassium loss, which are associated with hypertension. One of the main constituents of licorice is 18β-glycyrrhetinic acid, which impairs the metabolism of cortisol through inhibition of the activity of the isozyme 11β-hydroxysteroid dehydrogenase type 2 [1,2]. As a result, an increased stimulation of mineralocorticoid receptors by cortisol may occur. The adverse effects of 18β-glycyrrhetinic acid seems to be dose-dependent, however, a wide interindividual variation has been reported [3,4]. This variability may be explained by differences in pharmacodynamics and/or pharmacokinetics, and it is therefore necessary to have a reliable method for measuring 18β-glycyrrhetinic acid concentrations in plasma.

A number of high-performance liquid chromatographic methods for the quantitation of 18β-glycyrrhetinic acid in biological fluids have been published. For the sample clean-up of plasma or serum, some of the methods used methanol [5–7], methanol together with urea [8], acetonitrile [9], or acetonitrile together with sodium bisulfate and sodium chloride [10], to precipitate the protein. One study used a sophisticated column switching procedure [11], while others employed a multiple liq-

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uid–liquid extraction procedure [12] or solid-phase extraction [13,14]. Of the two studies that employed solid-phase extraction, one used Bond Elut PH (phenyl) cartridge columns [13], while the other [14] used C_{18} Sep-Pak cartridges. Both of them used internal standards that are not commercially available.

Takeda et al. [13] did not get a clean eluate with their solid-phase extraction procedure as they needed a liquid–liquid extraction step to further clean up their sample. Brown-Thomas et al. [14] needed 2 ml of methanol to elute their isolates from the cartridge columns, because the isolates were undesirably strongly bound to the sorbent when C_{18} columns were used in conjunction with an ion-pairing reagent. They had to concentrate their eluate by evaporation before measurement. To avoid the above-mentioned difficulties, we developed a new solid-phase extraction procedure using a Bond Elut C_2 (ethyl) cartridge column. Like Abe et al. [12], we used the commercially available 18α-glycyrrhetinic acid as the internal standard. Our eluate could be measured without further treatment and was found to be stable for more than one week at room temperature.

2. Experimental

2.1. Reagents and materials

18β-Glycyrrhetinic acid and 18α-glycyrrhetinic acid (Fig. 1) were purchased from Sigma (St. Louis, MO, USA). Potassium dihydrogen phosphate, disodium hydrogen phosphate 2-hydrate, citric acid monohydrate, potassium hydroxide and urea were all of analytical grade (Merck, Darmstadt, Germany). A Vac Elut manifold and Bond Elut C_2 (ethyl) columns (1 ml capacity, to which a 4-ml Bond Elut reservoir was attached using a Bond Elut adaptor) were manufactured by Analytichem International (Harbor City, CA, USA). Methanol and acetonitrile were of HPLC grade (Merck). Human blood plasma, stored at −20°C, was obtained from the local blood bank.

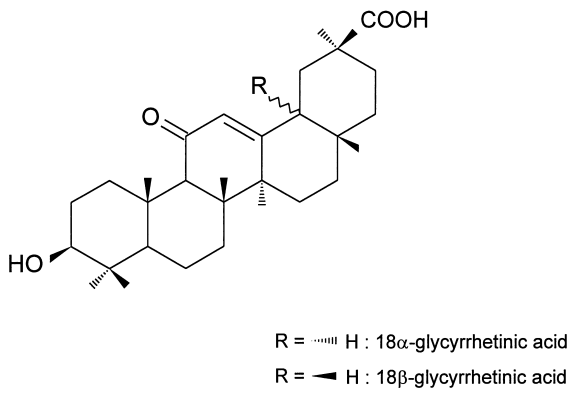

Fig. 1. Structures of 18β-glycyrrhetinic acid and the internal standard 18α-glycyrrhetinic acid.

2.2. Standard solutions

Stock solutions of 18β-glycyrrhetinic acid (1 mg/10 ml) and 18α-glycyrrhetinic acid (1 mg/10 ml) were prepared in methanol. Working standard solutions were prepared by diluting the stock solutions with methanol. Stock solutions of both compounds were stable for at least six months when stored at 4°C.

2.3. Apparatus and chromatographic conditions

The chromatographic system consisted of a Spectra Physics (Breda, The Netherlands) P2000 binary gradient pump, a Hewlett-Packard (Amsterdam, The Netherlands) 5 μm ODS Hypersil guard column (20×2.1 mm I.D.), a Shandon (Applied Science Group, Emmen, The Netherlands) 5 μm Hypersil BDS C_{18} analytical column (200×4.6 mm I.D.) and a Spectra Physics AS 3000 autosampler with a built-in column heater. The mobile phase, acetonitrile–0.02 *M* phosphate buffer, pH 5.7 (55:45, v/v), maintained under a helium sparge during use, was delivered at a flow-rate of 1 ml/min, the resulting pressure being 11.5 MPa. The column effluent was monitored with a Spectra Physics UV1000 variable wavelength detector set at 248 nm. The signal was processed by a Spectra Physics SP4400 integrator. The column heater was set at 40°C and the injection volume was 10 μl (30 μl at concentrations lower than 0.05 μg/ml).

2.4. Sample pretreatment

Into a test tube, 100 μl of internal standard (2 mg/100 ml) were pipetted and evaporated to dryness

under a gentle stream of nitrogen at room temperature. Aliquots containing 0.5 ml of plasma and 1.5 ml of a 50% (w/w) aqueous solution of urea were successively pipetted into the test tube. The tube was vortex-mixed gently for 1 s. After standing for 10 min, 2.0 ml of a 0.01 *M* potassium citrate buffer (pH 3.0) were added to the test tube. The tube was again vortex-mixed gently for 1 s. The prepared sample was now ready to be loaded onto a C_2 solid-phase extraction column.

2.5. Extraction procedure

Extraction of 18β-glycyrrhetinic acid and 18α-glycyrrhetinic acid from plasma was achieved by the use of a bonded-silica solid-phase extraction column (Bond Elut C_2, 1 ml capacity). The column was conditioned prior to use by drawing three column volumes (approx. 3 ml) of methanol followed by a similar volume of 0.01 *M* potassium citrate buffer (pH 5.0) through the column. The prepared sample was loaded onto and drawn through the column. The column was then washed with 10 ml of 0.01 *M* potassium citrate buffer (pH 5.0). The cover of the manifold was then removed and the blunt-nose stainless-steel needle of the Vac Elut cover was wiped with a tissue, to remove drops of washing solution. The Vac Elut rack, holding a 2-ml glass sampling tube, was placed under the column. 18β-Glycyrrhetinic acid and 18α-glycyrrhetinic acid were eluted from the column with 1 ml of acetonitrile–0.02 *M* phosphate buffer, pH 7.0 (55:45, v/v). The eluate collected was measured as described.

3. Results and discussion

3.1. Chromatography

Fig. 2 shows typical chromatograms for blank plasma and for blank plasma spiked with 18β-glycyrrhetinic acid and the internal standard (I.S.), 18α-glycyrrhetinic acid. The retention times of 18β-glycyrrhetinic acid and 18α-glycyrrhetinic acid were 10.8 and 9.6 min, respectively, while a peak of an endogenous substance appeared at 13.2 min.

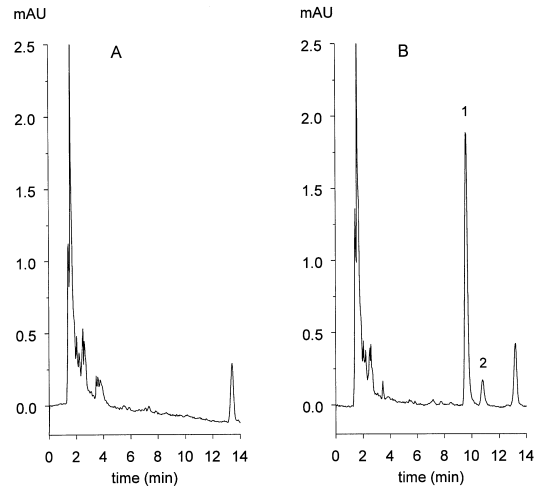

Fig. 2. Chromatograms obtained for (A) blank plasma and (B) blank plasma spiked with 0.52 μg/ml 18β-glycyrrhetinic acid (2) and 4.4 μg/ml 18α-glycyrrhetinic acid (1). Injection volume, 10 μl.

3.2. Calibration curves

The 18β-glycyrrhetinic acid concentration in a sample was determined by comparing peak-height ratios of 18β-glycyrrhetinic acid to the I.S. with a standard curve. Whenever samples containing 18β-glycyrrhetinic acid were measured, a standard curve was generated by adding different amounts of 18β-glycyrrhetinic acid to blank plasma. A linear relationship was found between the peak height ratio of 18β-glycyrrhetinic acid to I.S. (y) and the plasma 18β-glycyrrhetinic acid concentration (x), as given by the equations $y=2.1310x-0.0028$ ($r=0.9986$, $n=5$) for the plasma concentration range 0.052–0.515 μg/ml (I.S.=0.44 μg/ml) and $y=0.1978x-0.0080$ ($r=0.9997$, $n=5$) for the range 0.515–4.12 μg/ml (I.S.=4.4 μg/ml).

3.3. Recovery

The overall recovery obtained with the extraction procedure was determined by comparing the peak heights of 18β-glycyrrhetinic acid and 18α-glycyrrhetinic acid, obtained after injection of non-extracted standard solutions with peak heights obtained after injection of extracted plasma containing equal concentrations of both compounds. The abso-

Table 1
Recovery of 18β-glycyrrhetinic acid and 18α-glycyrrhetinic acid from plasma

Compound	Concentration (μg/ml)	Recovery (%)	C.V. (%)
18β-Glycyrrhetinic acid	0.52	97.9	4.3
	1.03	93.2	2.1
	2.06	94.3	3.2
	4.12	97.4	6.0
18α-Glycyrrhetinic acid	0.44	98.1	4.4
	4.4	95.7	5.8

For all concentrations, $n=5$; C.V.=coefficient of variation.

lute recovery of 18β-glycyrrhetinic acid and of 18α-glycyrrhetinic acid is complete and independent of the concentration (Table 1).

3.4. Sensitivity, precision and applicability

The limit of detection (signal-to-noise ratio of two) of the method described was 10 ng/ml and the limit of quantitation was 50 ng/ml. Table 2 shows the precision and accuracy of 18β-glycyrrhetinic acid measurement in plasma. The results of Abe et al. [12] and of Takeda et al. [13] indicate that the sensitivity of the present method is sufficient for pharmacokinetic studies. Both studies showed that after oral administration of approx. 25 mg of glycyrrhizin, the plasma concentration of 18β-glycyrrhetinic acid was higher than 40 ng/ml during the first 24 h.

Table 2
Precision and accuracy of 18β-glycyrrhetinic acid in spiked plasma samples

Spiked concentration (μg/ml)	Observed concentration (μg/ml)	Precision (C.V. %)	Accuracy (%)
0.052	0.052	3.0	100.0
0.103	0.105	6.2	101.9
0.206	0.200	6.1	97.2
0.515	0.515	2.4	100.0
1.030	1.010	2.0	98.1
2.060	2.010	0.9	97.6
4.120	4.150	0.3	100.7

For all concentrations, $n=5$; C.V.=coefficient of variation.

3.5. Conclusion

The extraction method described here for the quantitation of 18β-glycyrrhetinic acid in plasma or serum is a significant improvement over previously published solid-phase extraction methods in terms of simplicity and general applicability. It should prove to be valuable for the clinical monitoring of plasma or serum levels and for detailed pharmacokinetic studies.

Acknowledgements

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