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Short Communication

Separation of 18α- and 18β-glycyrrhetinic acid by high-performance thin-layer chromatographic densitometry

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ABSTRACT

A high-performance thin-layer chromatographic method with scanning densitometry was developed for the simultaneous separation and determination of 18α- and 18β-glycyrrhetinic acid.

INTRODUCTION

Glycyrrhetinic acid (GA), the aglycone of glycyrrhizin (GL), the main component of the root of ***Glycyrrhiza glabra*** L., has anti-inflammatory [1–3], antiulcerous [4], antihepatotoxic [5–7], antiallergic [8], anticarcinogenic [9] and hypertensive activity [7].

Glycyrrhetinic acid exists as two stereoisomers, 18α-GA *(tram)* and 18β-GA (cis) (Fig. 1). Their specific activities have not yet been ascertained: the anti-inflamma-

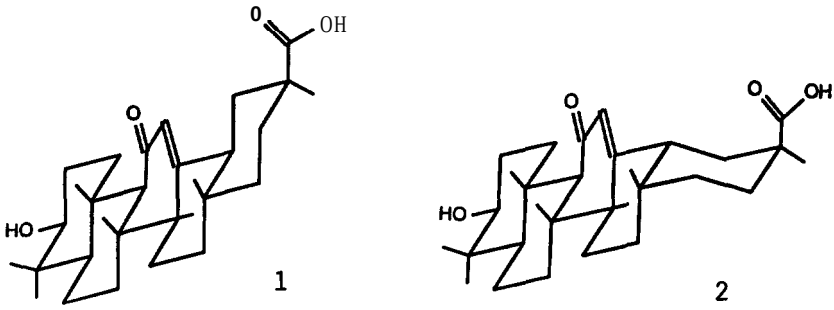

Fig. 1. Structures of (1) 18α-GA and (2) 18β-GA.

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tory action, one of the most important properties of glycyrrhetinic acid, has been attributed, for example, either to the 18β-form [2] or to the 18α-form, or to a combination of the two [10].

Qualitative and quantitative analyses of 18β-GA have been conducted by means of various chromatographic techniques, such as high-performance liquid chromatography (HPLC) [11–15] and, as the silyl ether [16] or methyl derivative [17], by gas chromatography (GC). Only Amagaya et al. [18] have determined both isomers simultaneously by CC, after derivatization with diazomethane.

As the two stereoisomers seem to have different pharmacological properties, it is clearly important to test each isomer separately, and therefore the aim of this study was to establish a method to separate 18α-GA and 18β-GA using a high-performance thin-layer chromatographic (HPTLC) approach.

EXPERIMENTAL

18α- and 18β-GA were purchased from Sigma (St. Louis, MO, U.S.A.). All solvents were of analytical-reagent grade and were obtained from BDH (Milan, Italy).

Pure samples of 18α- and 18β-GA (10^{-4} *M*) were dissolved in chloroform–methanol (1:1, v/v) and spotted, separately and in combination, on 10 × 20 cm precoated silica gel F_{254} plates (Merck, Darmstadt, Germany) using a Linomat IV instrument (Camag, Muttenz, Switzerland).

The solvent used for development was butanol-ethanol-2 *M* ammonia solution (6:2: 1, v/v/v). All chromatograms were developed at room temperature using the ascending technique in a chromatographic tank previously saturated with eluent mixture. The layers were dried in a forced current of air, then analysed at 254 nm by the fluorescence quenching method using a Camag TLC Scanner II (Camag) equipped with an M 280 Olivetti PC operating the Cats 3.04 scanning program. The scanner was set up as follows: band with, 10 nm; span, 25; slit, 5 × 0.2 mm; and scanning speed, 5 mm/s.

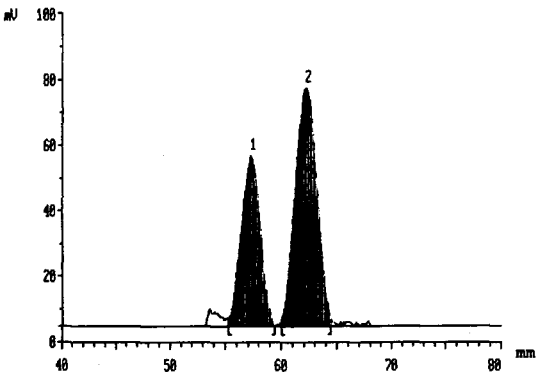

Fig. 2. Separation of (1) 18α-GA (157 ng) and (2) 18β-GA (302 ng) by HPTLC with the solvent sysfem butanol–ethanol–2 *M* ammonia solution (6:2:1, v/v/v).

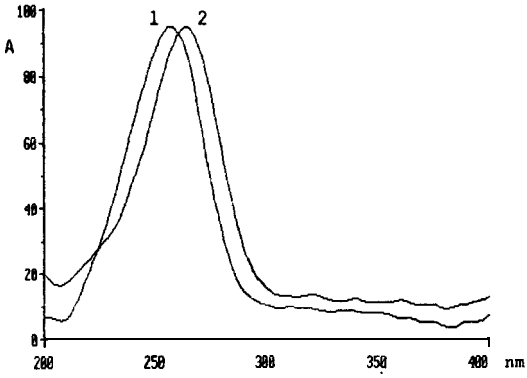

Fig. 3. UV absorption spectra of (1) 18α-GA and (2) 18β-GA determined by scanning the HPTLC plate in the reflectance mode.

RESULTS AND DISCUSSION

Fig. 2 shows the chromatographic resolution of the mixture of the two isomers; the spots were baseline separated with this development system. Fig. 3 shows the UV absorption spectrum of 18α- and 18β-GA obtained by scanning the HPTLC plate in the reflectance mode; 18α-GA shows maximum absorption at 258 nm and 18β-GA at 264 nm.

Table I shows the experimental analyses of a series of isomer mixtures containing various amounts of the two components. The experimental values were calculated by comparing the areas under the peaks with a calibration graph. The summarized data represent the means of six determinations.

The detection limit (at a signal-to-noise ratio of 2) was 1.93 ng for 18α-GA and 2.09 ng for 1 8β-GA. Although the determination of the a-isomer is the more accurate and reproducible of the two, the HPTLC method may be said to be accurate, reproducible and selective and to allow the rapid, simultaneous determination of the two isomers.

TABLE I

ANALYTICAL RECOVERY OF A MIXTURE OF 18α- AND 18β-GLYCYRRHETINIC ACID

18α-GA				18β-GA			
Amount applied (ng)	Amount found (ng)	Recovery (%) (mean, n = 6)	S.D. (ng)	Amount applied (ng)	Amount found (ng)	Recovery (%) (mean, n = 6)	S.D. (ng)
117.10	113.62	97.02	1.39	171.45	172.77	100.70	5.78
175.65	177.93	101.30	5.23	228.60	225.22	98.52	8.50
234.20	233.27	99.60	9.71	171.45	171.75	100.30	8.72
292.75	299.75	102.33	6.14	114.30	107.30	93.90	5.40
117.10	116.89	99.80	2.95	285.75	284.25	99.47	10.21
292.75	293.46	100.20	6.46	228.60	226.51	99.08	7.95

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