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Short Communication

High-performance liquid chromatographic method for the determination of mangiferin, likviritin and dihydroquercetin in rat plasma and urine

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ABSTRACT

The use of reversed-phase high-performance liquid chromatography for the determination of the biologically active plant phenolic compounds mangiferin, likviritin and dihydroquercetin is described. Perchloric acid (35%) was used for deproteinization in the case of mangiferin and likviritin, and acidified methanol for dihydroquercetin. Detection was performed at 254, 275 and 290 nm for mangiferin, likviritin and dihydroquercetin in plasma, and 365, 312 and 290 nm in urine, respectively. The limit of detection was 0.2 μg/ml for plasma and 0.5 μg/ml for urine.

INTRODUCTION

Mangiferin (2-C-D-glucopyranosyl-1,3,6,7-tetrahydroxyxanthon, Fig. 1a), is a main component (not less than 98% of the composition) of the antiviral drug Alpizarin [1]. The compound is derived from the herb *Hedysarum alpinum* L. or *H. flavescens Regel et Schmalh* (the overground portion). Alpizarin shows an antiviral activity for herpes DNA viruses. High-performance liquid chromatography (HPLC) of xanthone glycosides on amino and cyano sorbents has been described. The results were unsatisfactory. Better results were achieved on a reversed-phase C_{18} column with methanol–water [2–4].

Likviritin [2,3-dihydro-2-(4′-glucosyloxyphenyl)-7-hydroxy-4*H*-1-benzopyran-4-one, Fig. 1b] is a glycoside of flavanone nature, derived from

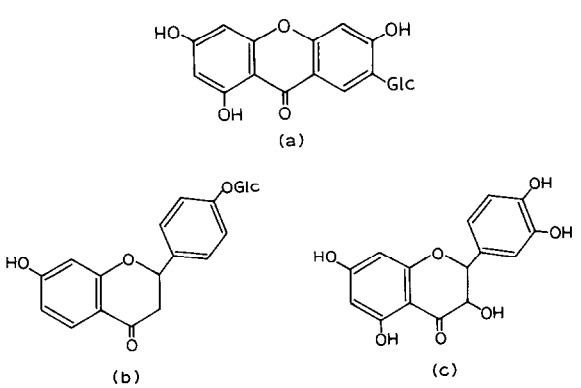

Fig. 1. Structures of mangiferin (a), likviritin (b), and dihydroquercetin (c).

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Glycyrrhiza glabra roots. Likviritin has anti-inflammatory, antianemic, and immunopotentiative effects. It is a component of Likviriton and Khalkorin, two drugs manufactured in Russia [5]. Reversed-phase HPLC has been used for component analysis of Likviriton and Khalkorin [6]. An HPLC method, with an ODS column and a gradient solvent system, has also been described for the separation of liquorice extracts [7,8].

Dihydroquercetin (3,5,7,3′,4′-pentahydroxyflavanone, Fig. 1c), is also a glycoside of flavanone nature, derived from *Larix daurica* and *L. sibirica* [9].

The pharmacokinetics of these three compounds, and methods for their analysis in biological fluids, have not been described. The aim of the present study was to develop simple and sensitive methods of analysis of these compounds in rat plasma and urine.

EXPERIMENTAL

Chemicals

Alpizarin was supplied by the Institute of Plant Substances (Moscow, Russia). Likviritin was from the Institute of Chemistry and Technology of Drugs (Kharkov, Ukraine). Dihydroquercetin was from the Institute of Organic Chemistry (Irkutsk, Russia). Acetonitrile was HPLC grade (Reachim, Kharkov, Ukraine). Acetic acid (chemical grade) (Reachim) was frozen, and the liquid fraction was eliminated. The remainder after thawing was distilled, and the fraction with the boiling point of 116.5–117.5°C was used. Methanol, acetonitrile and perchloric acid (70%) were from Merck (Darmstadt, Germany). A 0.1-ml volume of 35% perchloric acid was added to 10 ml of methanol to prepare the acidified methanol used for deproteinization. Glass-distilled water was used throughout.

Equipment

All separations were performed with an SP-8700 liquid chromatograph equipped with an SP-4100 integrator (Spectra-Physics, Darmstadt, Germany). The sample injector was a Rheodyne syringe-loading injector (Model 7125, Rheodyne, Berkeley, CA, USA). The precolumn (75 mm × 2.1 mm I.D.; Chrompack, Middelburg, Netherlands) was dry-filled with Chrompack RP sorbent (particle size 30–40 μm). The analytical columns were Spherisorb 10 ODS (250 mm × 3.0 mm I.D., particle size 10 μm; Chrompack) for mangiferin and likviritin, and Spheri-5 RP-8 (100 mm × 4.6 mm I.D., particle size 5 μm; Brownlee Labs., Santa Clara, CA, USA) for dihydroquercetin. Detection was performed with a Spectra-Physics SP-8400 absorbance detector at 254, 275 and 290 nm for mangiferin, likviritin and dihydroquercetin in plasma, and 365, 312 and 290 nm in urine, respectively.

The mobile phase composition was varied slightly for the different compounds investigated. It was acetonitrile–3% acetic acid (16:84, v/v, flow-rate 0.5 ml/min) for mangiferin; acetonitrile–3% acetic acid (20:80, v/v, flow-rate 0.5 ml/min) for likviritin; and acetonitrile–5% acetic acid (20:80, v/v, flow-rate 1.0 ml/min) for dihydroquercetin. The water and the mobile phase were passed through a 0.45-μm filter (Millipore, Bedford, MA, USA) and degassed *in vacuo* prior to use.

Standard solutions

Stock solutions (2 mg/ml) were prepared in water–acetone (1:1) for mangiferin, in methanol for likviritin and in acetonitrile for dihydroquercetin. Stock solutions were further diluted from 10 to 1000 times in water. Plasma or urine standards were prepared by diluting appropriate volumes of the stock solutions in drug-free plasma or urine, to give final concentrations of the compounds of 0.2, 1, 5, 10 and 20 μg/ml.

Sample preparation

A 10-μl volume of 35% perchloric acid in the case of mangiferin and likviritin, and 200 μl of acidified methanol in the case of dihydroquercetin, was added to 0.1 ml of plasma and urine. All samples were vortex-mixed for 5 min and centrifuged (8800 *g*) for 5 min. Clear supernatant (20 μl) was injected into the column.

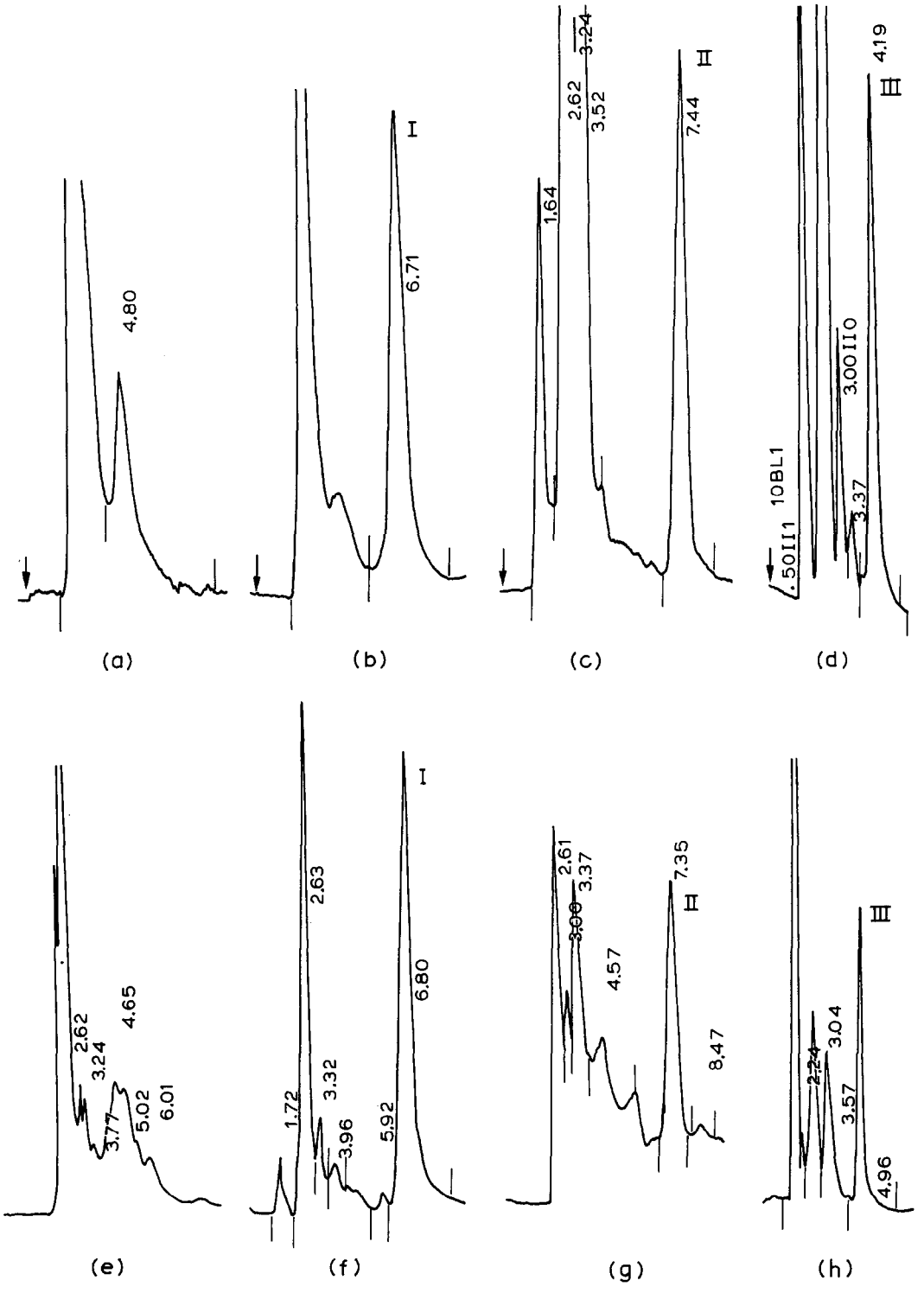

Fig. 2. Chromatograms of mangiferin (I), likviritin (II), and dihydroquercetin (III) after intravenous administration to rats (10 mg/kg): (a) and (e) 20 μl of rat plasma and urine before drug administration; (b), (c) and (d) 20 μl of plasma containing 30.1 μg/ml mangiferin, 40 μg/ml likviritin and 18.3 μg/ml dihydroquercetin, respectively; (f), (g) and (h) 20 μl of urine containing 25.7 μg/ml mangiferin, 17.0 μg/ml likviritin and 9.2 μg/ml dihydroquercetin, respectively. Separation conditions: (a), (b), (e) and (f) detection, UV at 254 nm for plasma and at 365 nm for urine, 0.08 a.u.f.s.; (c) and (g) detection, UV at 275 nm for plasma and at 312 nm for urine, 0.04 a.u.f.s.; (d) and (h) detection, UV at 290 nm for plasma and urine, 0.04 a.u.f.s.

TABLE I

REGRESSION DATA AND THE RECOVERY OF MANGIFERIN, LIKVIRITIN AND DIHYDROQUERCETIN FROM PLASMA AND URINE

Substance	Matrix	Slope[a] (*b*)	Intercept[a] (*a*)	*r*	Recovery[b] (%)	C.V. (%)	
						Within-day	Day-to-day[c]
Mangiferin	Plasma	0.58	0.14	0.997	92	1.3	2.1
	Urine	0.91	1.62	0.982	98	1.5	2.6
Likviritin	Plasma	1.14	0.36	0.994	75	1.9	2.9
	Urine	0.85	−0.58	0.995	90	2.5	3.8
Dihydroquercetin	Plasma	2.30	0.22	0.999	90	1.7	2.3
	Urine	1.77	0.02	0.993	94	1.9	2.9

[a] Regression equation is $C = a + b(S \cdot 10^{-4})$ where C is the concentration of the compound in μg/ml and S is the peak area in relative units.

[b] Average absolute recoveries were determined based on ten injections (at 10 μg/ml).

[c] For the concentrations of 5 and 20 μg/ml ($n = 5$).

RESULTS AND DISCUSSION

The methods described above allowed an evaluation in rat plasma and urine of mangiferin, likviritin and dihydroquercetin levels (Fig. 2): their retention times were 6.5, 7.3 and 5.0 min, respectively. Chromatograms of blank plasma and urine are shown for mangiferin conditions; for other separation conditions they were similar. Perchloric acid deproteinization in the case of mangiferin and likviritin gave good peak shapes and high percentage recoveries (Table I). The dilution of the samples was minimal. For dihydroquercetin, a high percentage recovery was achieved only after simultaneous deproteinization and extraction with acidified methanol. It was necessary to set the analytical wavelength for urine samples containing mangiferin and likviritin to 365 and 312 nm, respectively. The sensitivity of the method was slightly diminished, but the absorbance of the impurities preventing mangiferin and likviritin determination was greatly decreased.

Column lifetime

In a series of *ca.* 1000 analyses of mangiferin and likviritin, with an injection volume of 20 μl of plasma and urine, there was no need to change the analytical column. The results showed the selectivity and resolution to be constant.

Precision, linearity and sensitivity

For the determination of the investigated compounds the external standard method was used. The slope and intercept of the calibration curves, the recovery of the compounds and the coefficients of variation (C.V., within-day and day-to-day) are presented in Table I. The calibration curves were linear over the 0.5–20 μg/ml concentration range. The limit of detection was 0.2 μg/ml for plasma and 0.5 μg/ml for urine.

CONCLUSION

The HPLC method described for the determination of the biologically active plant phenolic compounds mangiferin, likviritin and dihydroquercetin is simple, rapid and sensitive. It may be used for pharmacokinetic studies of these compounds.

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