Journal of Pharmaceutical & Biomedical Analysis
Vol. 5, No. 3, pp. 289–293, 1987
Printed in Great Britain

0731–7085/87 $3.00 + 0.00

Short Communication

Determination of glycyrrhizinic and glycyrrhetinic acids in pharmaceuticals by high-performance liquid chromatography

NINA SADLEJ-SOSNOWSKA

Institute for Drug Research and Control, Chelmska 30/34, 00-725 Warsaw, Poland

Keywords: *Reversed-phase HPLC; ion-pair; glycyrrhizinic acid; glycyrrhetinic acid.*

Introduction

Glycyrrhizinic acid (GZ) occurs as its potassium–calcium salt in liquorice root, and is a component of a number of pharmaceutical formulations and food products. During acidic hydrolysis the GZ molecule releases two molecules of glucuronic acid to form the aglycone, 18 β-glycyrrhetinic acid (18 β-GT). It has not been established whether this aglycone normally occurs in liquorice root.

Many analytical methods have been employed for the determination of GZ. Gravimetric, volumetric and colorimetric methods have lacked sensitivity and accuracy, whilst HPLC methods in general are superior [1–5]. Authors have used both ion-exchange columns with gradient [6–8] or isocratic elution [4, 9, 10] and reversed-phase columns with gradient [11–13] or isocratic elution [1, 5, 14–26]. In one method an ion-pairing reagent, tetrabutylammonium hydroxide was used as a component of the mobile phase.

Most of the papers cited above, however, do not describe the simultaneous determination of GZ and 18 β-GT, and for the determination of both compounds two different mobile phases [17, 23, 25] or even two different columns [19] have been used. In those papers where both compounds were determined in one chromatographic run, gradient elution was employed and retention times of GZ and GT were large, e.g. 44 and 68 min [11]. Considering it is more difficult to obtain the reproducible results employing gradient elution [27] and that the need for column re-equilibration increases the time required for analysis, this study was undertaken to obtain isocratic conditions suitable for the routine simultaneous analysis of GZ and 18 β-GT in pharmaceutical preparations, in a reasonable time.

Experimental

Reagents and materials

Cetyltrimethylammonium bromide (CTAB) pure, from International Enzymes Limited, was used as the ion-pairing agent. Solutions were filtered through a Millipore sintered-glass filter prior to chromatography. The 18 β-glycyrrhetinic acid was obtained from the German Institute of Drug Research (G.F.R.). The glycyrrhizinic acid (assay 84.1%), and the dried plant extract "Blasen und Nieren Tee Aufgußpulver" (BN) were produced by Natterman (Köln, G.F.R.). Extractum Glycyrrhizae Siccum (Extr. Gly. Sicc.) and the coated tablets Tussipect and Tussilinar were from Herbapol (Poland).

Standard solutions

Standard solutions of GZ (4–150 μg ml^{-1}) in methanol or in the mixture methanol–water (50:50, v/v) were prepared by weighing about one to several milligrams of the standard and dissolving it directly in an appropriate volume of solvent. Solutions of 18 β-GT (1.3–5 μg ml^{-1}) were prepared in the same way but incorporated a two-step dilution.

Apparatus and chromatographic conditions

A Pye Unicam liquid chromatograph consisting of an LC-XPD pump, a variable wavelength detector LC-UV and a PM 8251 single pen recorder, was used. It was equipped with a Rheodyne 7125 injection valve equipped with a 20 μl loop. A 10-μm C_{18} Nucleosil column (250 × 4.6 mm) was used with a mobile phase of water–ethanol (23:77, v/v), containing 0.5 or 1% CTAB. Other operating conditions were: wavelength 256 nm, flow rate 1 ml min^{-1}, and ambient temperature.

Calibration data

A standard calibration curve for GZ was established over the range 3.93–144.4 μg ml^{-1}. For each solution the ratio of the peak height at 0.01 a.u.f.s. to its concentration was calculated, and their mean value was 17.61 mm/(μg ml^{-1}) (n = 6, R.S.D. = 0.017). The constant value of this ratio indicated that the calibration plot was linear over the concentration range studied.

Extraction procedure

A sample of the formulation BN was extracted by a method employed by the manufacturer, Natterman, using a mixture of water and methanol (50:50, v/v) at 60°C. About 300 mg of powder was sonicated in 40 ml of solvent, cooled, filtered and made up to 50 ml. Other formulations were extracted with the same solvent mixture at room temperature. All powdered samples were mechanically shaken for 30 min, then sonicated for 10 min, and passed through filter paper. The amounts used for the extraction were *ca* 100 mg of Extr. Gly. Sicc. or 120–140 mg of powdered tablets (Tussipect and Tussilinar).

Results and Discussion

Extraction

It had previously been reported [7] that 0.05 M ammonia was the most suitable medium for the extraction of GZ from pharmaceutical formulations. However, when GZ

and 18 β-GT were dissolved in this medium the retention times and heights of the chromatographic peaks were irreproducible, and additional unidentified peaks appeared. Due to these complications the extraction with the ammonia solution was given up.

Chromatography

Initially, mobile phases were selected from the literature. They consisted of methanol–water mixtures without [13, 16] or with [1, 5, 14–16] acetic acid. The following mixtures were investigated: water–methanol (15:85, 30:70 and 60:40, v/v), water–methanol–acetic acid (60:34:3 and 60:34:6, v/v), water–ethanol (23:77, v/v) and water–propan-1-ol (40:60, v/v). These mobile phases produced short (4 min) and similar retention times for both compounds. Under these conditions peaks of the two acids could not be separated from each other or from the other peaks present in a plant extract. An ion-pairing agent, CTAB, was therefore added to the mobile phase to increase the retention times of the acidic components.

The chromatogram of a Tussipect extract (Fig. 1) shows that the retention times of 18 β-GT and GZ have been increased to 6.8 and 10 min, respectively. Peaks of both compounds are sufficiently separated and well resolved from the background. The dependence of the GZ peak height on the concentration of the injected solution was linear over a wide concentration range. For 18 β-GT this dependence was not investigated because in the preparations studied its concentration was too low.

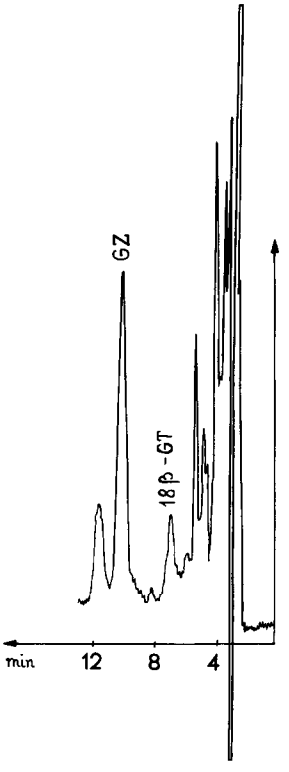

Figure 1
Chromatogram of the extract of Tussipect coated tablets. A 10-μm C_{18} Nucleosil was employed using water–ethanol (23:77, v/v) containing 0.5% w/v CTAB as eluent. Detection wavelength was 256 nm.

Recovery of GZ was determined by spiking five samples of Tussipect with about 1.5 mg GZ and by analysis of the fortified samples using the proposed method. Recovery was 93.6 ± 6%.

The results of quantitative determinations are presented in Table 1. It can be seen that although the precision of the method is sufficient for GZ, for 18 β-GT the precision is less in formulation BN because of its very low concentration. Precision was not evaluated in Tussilinar, because only traces of 18 β-GT were found. It is worth noting that 18 β-GT was found in all the preparations studied. Its presence in, e.g. Extr. Gly. Sicc. and in the pharmaceuticals containing it may be due to its presence in liquorice root or to its formation during the process of extraction.

Table 1
Results of GZ and 18 β-GT determination in pharmaceutical preparations

Formulation	GZ assay	R.S.D.	18 β-GT assay	R.S.D.
Extractum Glycyrrhizae Siccum	8.4%	0.02 (n = 7)	0.89%	0.02 (n = 7)
Blasen and Nieren Tee, containing aqueous extracts of: Folium Mate, Herba Virgaureae, Folium Ortosiphonis, Radix Glycyrrhizae, Folium Melissae and Oleum Melissae	0.5%	0.004 (n = 4)	0.048%	0.08 (n = 4)
Tussipect, containing ephedrine hydrochloride, extract of liquorice root, powdered liquorice root, saponin, thyme extract, sodium benzoate, ammonium chloride	8.5 mg/tablet	0.05 (n = 7)	0.5 mg/tablet	0.03 (n = 7)
Tussilinar, containing ammonium glycyrrhizinate, noscapine, lactose, potato starch, saponin, talc, gum arabic, glycerin and magnesium stearate	24 mg/tablet	0.04 (n = 8)	traces 0.03 mg/tablet	— (n = 8)

The addition of the ion-pairing reagent to the mobile phase permitted the simultaneous determination of GZ and 18 β-GT under isocratic conditions. The method is simple with relatively short analysis times, and should be suitable as a routine method for the determination of liquorice root extracts in herbal medicines.

Acknowledgements: The author wishes to thank Dr Irena Wilczyńska-Wojtulewicz for her interest and valuable discussions of this work, and her critical reading of the manuscript. This work was supported by a grant from the Ministry of Health and Social Welfare (Poland) MZ-XII, 329.

References

[1] J. Martin and M. C. Garcia Rumbao, *An. R. Acad. Farm.* **46**, 183–201 (1980), ref. *CA* **94**, 53030 g.
[2] K. Okada, J. Tanaka, A. Miyashita and K. Imoto, *Yakugaku Zasshi* **101**, 822–828 (1981).
[3] P. S. Vora, *J. Assoc. Off. Anal. Chem.* **65**, 572–574 (1982), ref. *CA* **97**, 4758 d.
[4] S. Ogawa, A. Yoshida and Y. Mitani, *Yakugaku Zasshi* **96**, 122–124 (1976).
[5] W. J. Hurst, J. M. McKim and R. A. Martin, *J. Agric. Food Chem.* **31**, 387–389 (1983).
[6] Y. Akada and Y. Tanase, *Yakugaku Zasshi* **96**, 1035–1037 (1976).
[7] Y. Akada, Y. Sakiya, S. Kawano and S. Adachi, *Chem. Pharm. Bull.* **26**, 1240–1246 (1978).
[8] Y. Sakiya, Y. Akada, S. Kawano and Y. Miyauchi, *Chem. Pharm. Bull.* **27**, 1125–1129 (1979).

[9] S. Ogawa, A. Yoshida and Y. Mitani, *Yakugaku Zasshi* **96**, 1488–1491 (1976).
[10] F. Salto and J. Maria Arin, *An. Fac. Vet. Leon* **25**, 43–47 (1979), ref. *CA* **95**, 116 d.
[11] T. H. Beasley, H. W. Ziegler and A. D. Bell, *J. Chromatogr.* **175**, 350–355 (1979).
[12] K. Yasuda, T. Shibuya, M. Nozaki, K. Tsurumi, H. Fujimura and F. Kaneuchi, *Yakugaku Zasshi* **98**, 1545–1547 (1978).
[13] Y. Hiraga, H. Endo, K. Takahashi and S. Shibata, *J. Chromatogr.* **292**, 451–453 (1984).
[14] J. Killacky, M. S. F. Ross and T. D. Turner, *Planta Med.* **30**, 310–316 (1976).
[15] O. Sticher and F. Soldati, *Pharm. Acta Helv.* **53**, 46–52 (1978).
[16] C. Tisse, J. Artaud, M. C. Iatrides, J. P. Zahra and J. Estienne, *Ann. Falsif. Expert. Chim.* **72**, 565–570 (1979), ref. *CA* **92**, 157272 n.
[17] Nam Ho Paik, Man Ki Park, Jeong Hill Park, Chung Sun Kim and Jung Jin Such, *Hoe Chi Yakhak* **25**, 1–7 (1981), ref. *CA* **95**, 209736 a.
[18] T. L. Lunder and C. Nielson, *Mitt. Geb. Lebensmittelunters. Hyg.* **71**, 236–241 (1980), ref. *CA* **93**, 192086 x.
[19] J. Bricaut, *Ann. Nutr. Aliment.* **32**, 947–955 (1978).
[20] M. Okada, T. Yamada, Y. Mori and A. Fujio, *Mie-ken Eisei Kenkyusho Nenpo* **28**, 29–34 (1982), ref. *CA* **101**, 12281 j.
[21] K. Shimada, T. Sakaguchi, Y. Sato, H. Moridaira and K. Omata, *Yakugaku Zasshi* **104**, 347–350 (1984).
[22] D. Petitbois, Y. Hamann, G. Lesgards and R. Bozzi, *Ann. Falsif. Expert. Chim. Toxicol.* **78**, 19–31 (1985).
[23] T. Ichikawa, S. Ishida, Y. Sakiya and Y. Akada, *Chem. Pharm. Bull.* **32**, 3734–3738 (1984).
[24] G. Benetti and M. Luise, *Boll. Chim. Ig., Parte Sci.* **36**, (S2) 81–85 (1985), ref. *CA* **103**, 177037 p.
[25] A. Collinge, B. Hermesse and A. Noirfalisse, *Belg. J. Food Chem. Biotechnol.* **40**, 143–145 (1985), ref. *CA* **105**, 5268 q.
[26] K. Sagara, Y. Ito, T. Oshima, M. Kawaura and T. Misaki, *Chem. Pharm. Bull.* **33**, 5364–5368 (1985).
[27] L. R. Snyder, in *High-Performance Advances and Perspectives* (Cs. Horvath, Ed.), Vol. 1. Academic Press, New York (1980).

[*First received for review 27 February 1986; revised manuscript received 11 September 1986*]