*International Journal of Pharmaceutics*, 84 (1992) 279–281



IJP 02819

# Determination and UV spectral identification of 18α-glycyrrhetinic acid and 18β-glycyrrhetinic acid for stability studies

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(Received 1 November 1991)
(Accepted 3 February 1992)

*Key words*: 18α-Glycyrrhetinic acid; 18β-Glycyrrhetinic acid; Stability study; Photodiode array detection; HPLC; Shelf-life

## Summary

The stability of 18α-glycyrrhetinic acid (αGA) and 18β-glycyrrhetinic acid (βGA) was studied at 37, 45 and 55°C. The change in the rate of decomposition was followed by an HPLC method coupled with detection on a photodiode array. This procedure is useful for the determination and UV spectral identification of αGA and βGA. Both compounds appeared to be thermally stable on storage under 37–55°C for 3 months. We deduced that the shelf-lives ($t_{90}$) of αGA and βGA at room temperature (25°C) were 9.2 and 8.6 years, respectively.

18α-Glycyrrhetinic acid (αGA) and 18β-glycyrrhetinic acid (βGA) are active principles obtained from licorice. The yields obtained from an aqueous extract of 1 g licorice were reported to be 5.9 μg of αGA and 95.3 μg of βGA (Tsai and Chen, 1991a). The two chemicals demonstrate different pharmacokinetic behavior after intravenous administration (Tsai and Chen, 1991b). In carrageenan-induced edema in mice, αGA was found to be more active than βGA (Amagaya et al., 1984). In the present paper, we report an HPLC method coupled with spectral identification for the determination of αGA and βGA in an acceleration study.

αGA and βGA (Fig. 1) were purchased from Sigma Chemicals (St. Louis, MO, U.S.A.). Perchloric acid (70%), ammonia solution (32%) and methanol were obtained from E. Merck (Darmstadt, Germany).

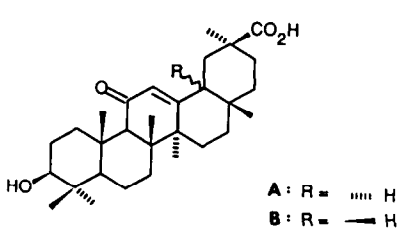


Fig. 1. Structure of αGA and βGA.

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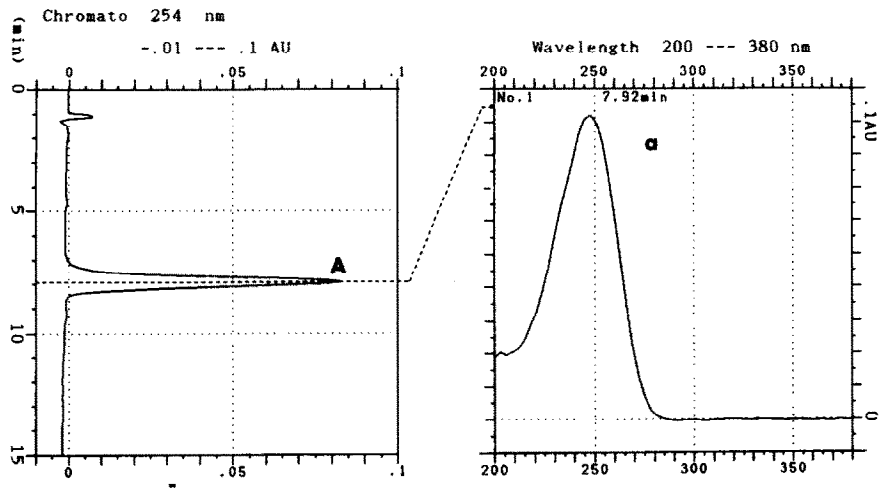


Fig. 2. Chromatogram and UV spectrum of $\alpha$GA.

The decomposition experiments were carried out in a temperature oven. The HPLC system consisted of a Rheodyne 7125 injector (Cotati, CA, U.S.A.), a Waters M990 photodiode array detector, which permits the scanning of chromatographic and spectral data, and two Waters 510 chromatographic pumps (Milford, MA, U.S.A.). Separation was achieved on a reversed-phase column (LiChrospher RP-18, 125 × 4 mm, i.d.) fitted with a guard column (LiChrospher RP-18, 4 × 4 mm, i.d.) (E. Merck, Darmstadt, Germany). The mobile phase was methanol-water-ammonia solution-perchloric acid (80:20 : 0.4:0.4, v/v, pH 7.5–7.5), at a flow rate of 1.0 ml/min. Detection was monitored at 254 nm and the wavelengths scanned between 200 and 380 nm for the photodiode array detector.

$\alpha$GA or $\beta$GA was put into glass vials. The vials were then tightly sealed and covered with parafilm. Subsequently, the vials were placed in a preheated temperature oven (75% relative humidity) at 37, 45 or 55°C (±2°C).

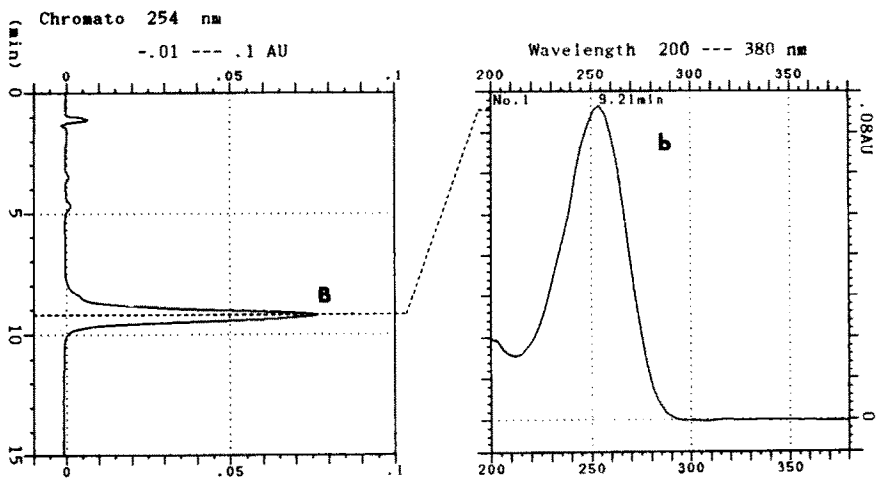


Fig. 3. Chromatogram and UV spectrum of $\beta$GA.

Samples (at least duplicate) were removed from the oven and determined in combination with spectral identification at each 15 day interval.

The retention time as evaluated from the chromatogram (Fig. 2A) of αGA was 7.9 min and the characteristic feature of the spectrum in the mobile phase was the absorption maximum at 248 nm (Fig. 2a). The retention time of βGA in the chromatogram (Fig. 3B) was found to be 9.2 min and the spectrum in the mobile phase displayed a characteristic absorption maximum at 254 nm (Fig. 3b).

The contents of both compounds at the different temperatures were determined from the linear regression equation of the calibration graph for each compound. The respective equations for αGA and βGA are $y = 0.0232x - 0.0001$ ($r = 0.999$) and $y = 0.0277x - 0.0001$ ($r = 0.999$), where $x$ is the amount of compound analyzed and $y$ the response in peak area.

The kinetics were investigated at elevated temperatures (37–55°C), since the rates of decomposition of αGA and βGA at lower temperatures were too slow to obtain reliable kinetic data.

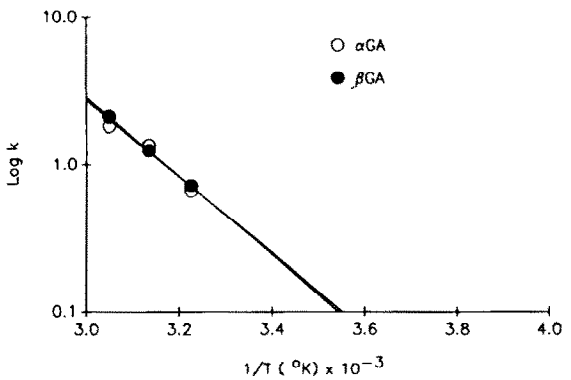


Fig. 4. Typical Arrhenius plot of log $k$ vs $1/T$.

An Arrhenius plot (Connors et al., 1979) of the log of the observed rate constant as a function of the reciprocal of the absolute temperature was linear. The correlation coefficient of αGA and βGA was 0.998 and 0.990, respectively (Fig. 4). The activation energy ($E_a$) for the decomposition of αGA and βGA over the temperature range 37–55°C was evaluated as 12.28 and 11.51 kcal/mol, respectively. If the reaction mechanism remains unaltered, the rate constant and shelf-life ($t_{90\%}$) of αGA and βGA at room temperature (25°C) should be 9.2 and 8.6 years, respectively.

Based upon the above results, the method described herein was found to be suitable for determining the stability of αGA and βGA and their spectral identification.

## Acknowledgment

This work was supported by Research Grant DOC-CD16 from the Department of Health, Executive Yuan, R.O.C.

## References

Amagaya, S., Sugishita, E., Ogihara, Y., Ogawa, S., Okada, K. and Aizawa, T., Comparative studies of the stereoisomers of glycyrrhetinic acid on anti-inflammatory activities. *J. Pharm. Dyn.*, 7 (1984) 923–928.

Connors, K.A., Amidon, G.L. and Kennon, L., *Chemical Stability of Pharmaceuticals*, Wiley, New York, 1979, pp. 8–32.

Tsai, T.H. and Chen, C.F., Determination of three active principles in licorice extract by reversed-phase high-performance liquid chromatography. *J. Chromatogr.*, 542 (1991a) 521–525.

Tsai, T.H. and Chen, C.F., High-performance liquid chromatographic determination of 18α-glycyrrhetinic acid and 18β-glycyrrhetinic acid in rat plasma: application to pharmacokinetic study. *J. Chromatogr.*, 567 (1991b) 405–414.