REFERENCES

1. **The Merck Index,** 10th Ed., Merck sharp and Dohme research Labs., USA, 1983, 378.
2. **Martinadale - The Extra Pharmacopoeia,** 29th Ed., The Pharmaceutical press, London, 1989, 905.
3. Indrayanto, G. and Handajani, R., **Drug Dev. Ind. Pharm.,** 1994, 20, 1639.
4. Mikami, E. Ieoh, Y. Ohno, T and Hayakawa, J., **Iyakuhin Ken Kiju.,** 1996, 27, 626.
5. Fernandez, O. German, C. Lucangioli, D. E. and Cavolucci, C., **J. chromatogr.,** 1993, 654, 87.
6. Perez-Ruiz, T. Martinez-Lozano, C. Sanz, A. and San migucl, M. T. **Talanta.,** 1996, 43, 1029.
7. Brizzi, V. and Pasetti, U., **J. Pharm. Biomed Anal.,** 1990, 107.

Determination of Glycyrrhizin in *Glycyrrhiza glabra* and its extract by HPTLC

S. K. CHAUHAN*, B. P. SINGH, G. P. KIMOTHI AND S. AGRAWAL
R&D Laboratory, INDIAN HERBS, Saharanpur (UP)

Accepted 14 April 1998
Received 21 November 1997

A simple reproducible HPTLC method for the determination of glycyrrhizin in *Glycyrrhiza glabra* and its extract was developed and is described. The sensitivity was found to be linear in the range of 0.2 to 1.0 μg. The proposed method being precise, sensitive and reproducible can be used for detection, monitoring and quantification of glycyrrhizin in *g. glabra* and its extract.

GLYCYRRHIZA glabra Linn, commonly known as Mulethi, is a highly reputed ayurvedic plant and is used in herbal preparations as a tonic, expectorant, demulcent, mild laxative and for allaying cough and catarrhal affections[1,2].

Not many methods for quantitative estimation of glycyrrhizin have been reported in the literature. Some of these methods are gravimetric and colorimetric[3,4] which are not very precise. A HPLC method[5,6] has also been reported for the estimation of glycyrrhetenic acids, aglycone of glycyrrhizin which involves critical steps such as hydrolysis. The method presented in this paper is quick, simple, accurate and provides a clear resolution and separation of peaks.

Dried and powdered roots (1 g) were extracted with water (35 ml x 3). The extracts were filtered, pooled and dried over a steam water bath to make the final volume to 100 ml. In case of *G. glabra* extract, around 400 mg of dried powder extract was accurately weighed and dissolved in 100 ml distilled water. Two and 5 ul of these test samples were applied on a aluminium TLC plate precoated with Silica gel 60 F 254 (E. Merck) alongwith 2, 5, 7 and 10 ul of standard glycyrrhizin (concentration 0.10 mg/ml) from about 1 cm edge of TLC plate using a band width of 6 mm and 5 mm distance between tracks using a sample applicator Linomat IV (M/s Camag, Switzerland).

The chromatogram was developed in n-Butanol:Acetic acid:Water 5:1:4, (upper layer) upto 80 mm. The plate was

Table-1 : Estimation of Glycyrrhizin in *G. glabra* and its extract

Name of Sample	% of Glycyrrhizin
1. Crude *G. glabra*	9.054
2. *G. glabra* extract DP	17.48

DP = dried powder
Each value is the average of three replicates

*For correspondence

Table-2 - Method Validation and Recovery

Sl. No.	Sample	Amount of sample taken (mg) [A]	Amount of Glycyrrhizin present in A (mg) [B]	Amount of Glycyrrhizin added to A (mg) [C]	Total Glycyrhizin taken B+C (mg) [D]	Total Glycyrhizin found (mg) [E]	% recovery
1.	Crude *G. glabra*	1010	91.04	4.37	95.41	95.23	99.81
2.	Crude *G. glabra*	1050	95.08	8.74	103.82	102.88	99.08
3.	Crude *G. Glabra*	1020	92.74	17.48	110.22	109.72	99.55
4.	*G. glabra* Extract	395	69.10	8.50	77.66	77.00	99.22
5.	*G. glabra* Extract	420	71.40	17.00	88.40	87.06	98.48
6.	*G. glabra* Extract	400	71.88	20.00	91.88	91.04	99.08

Note : Average percent recovery = 99.20

air dried and scanned at 260 nm in absorbance mode using M/s Camag TLC Scanner II. The amount of glycyrrhizin was determined using the calibration curve plotted between concentration and area of standard glycyrrhizin which is reported in Table-1.

For method validation and to know the percent recovery, a known amount of standard glycyrrhizin was added to the crude drug as well as to its extract. The samples were processed and analysed as per the procedure mentioned above. The results are mentioned in Table-2.

Using the proposed method, the Rf of glycyrrhizin was about 0.25. The calibration curve was linear in the range of 0.2 to 1.0 ug. The method allows reliable quantification of glycyrrhizin from other constituents of *G. glabra*. Further, recovery values were also found satisfactory which showed the reliability and suitability of the method. The proposed HPTLC method is rapid, simple and accurate for quantitative monitoring of glycyrrhizin in *G. glabra* and its extract.

REFERENCES

1. Tangri K. K. Sethi P. K., Parmar S. S. and Bhargava K. **P. Biochem. Pharmacol**, 1965, 14, 1277.
2. **The Wealth of India,** Vol.IV, Council of Scientific & Industrial Research, New Delhi, 1956, 151.
3. Dumazert, C. and Luu V. V.**Bull. Soc. Pharma Marseille** 1963, 12, 50.
4. Mahran G. H., Balhaa S. I., El-Hossary G. A. and Selin M. A. **Bull. Fac. Pharma,** 1973, 12, 71.
5. Tsai, T. H. and Chen, C. F. **J. Chromatogr.** 1991, 567, 405.
6. Tsai, T. H., Shen, C. C. and Chen, C. F. **International J. Pharmaceutics** 1992, 84, 278.