

Photometric and HPLC-ELSD analytical methods for *Tribulus terrestris*

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INTRODUCTION

Tribulus terrestris is a summer growing prostrate herb, with long stems, pinnate leaves, small yellow flowers and large spined fruit. The spined fruit give the plant its common names of Caltrop and Puncture Vine. It has almost world wide distribution and is used by many different ethnic communities for medicinal value. Ayurvedic and Traditional Chinese Medicine use the fruit whereas the aerial parts have been used to manufacture product in Bulgaria. The aerial parts of *Tribulus terrestris* have been used to manufacture the Tribestan® product which has been used to treat male and female sexual disfunctions, this action is attributed to the steroidal saponins protodioscin and protogracillin. A photometric method of analysis(1) has been used by this manufacturer and adopted by other manufacturers in Bulgaria. The saponins have very poor UV absorption and are unable to be determined by HPLC-DAD, a recent report has outlined the determination of steroidal saponins of *Tribulus terrestris* by RP-HPLC with ELSD detection(2). A comparison of the photometric method and the HPLC-ELSD has been undertaken to compare the accuracy and specificity of the two analytical methodologies.

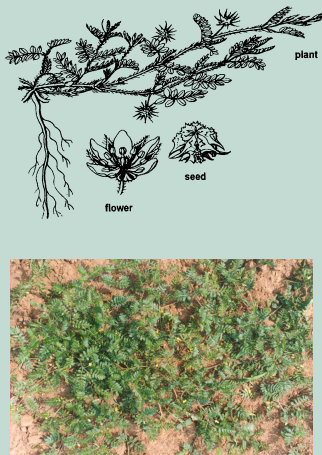
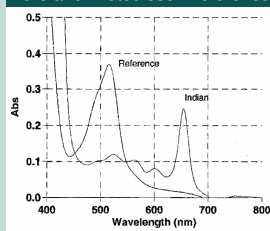


Figure 2: Scan of the absorption spectrum for Indian herb and Protodioscin reference



PHOTOMETRIC METHOD OF ANALYSIS

The photometric method(1) relies upon the reaction of the steroidal saponins with a modified Ehrlich reagent (34 mL concentrated hydrochloric acid, 100 mL methanol and 1 gram of p-dimethylaminobenzaldehyde p-DMAB). The methanolic samples are added to Ehrlich reagent and heated for 2 hours at 58 °C. A linear response has been shown between the amount of p-DMAB and the amount of protodioscin used; however, a non-linear response has been shown for the strength of the concentrated hydrochloric acid utilised. A maximum absorption is found when acid of 25% strength is used. Heating of reagent prior to color development also increased the result obtained.

HPLC-ELSD METHOD OF ANALYSIS

HPLC methods have been proposed by several concentrate manufacturers using DAD detection either at 254 or 205 nm. Protodioscin does not exhibit any absorption at these wavelengths and even at 190 nm does not show any significant absorption. The recent publication(2) of a HPLC-ELSD method has highlighted the usefulness of this technique in the analysis of non volatile materials which do not exhibit a UV chromophore, steroidal saponins fall very readily into this category. The calibration curve for protodioscin is of the form:

$$ax^2 + bx + c \quad \text{where: } a = -1.60e-012, b = 4.52e-005 \text{ and } c = 0$$

This quadratic fit is preferred over the log plot which is an estimate of the true line of best fit.

Sample Preparation:

500 mg of tribulus preparation is ultrasonicated in 100 mL of 50% water : acetonitrile. Filter through a 0.45 mm, PVDF membrane filter prior to HPLC analysis.

HPLC Conditions for Analysis:

HPLC System: Shimadzu VP-Series HPLC System equipped with Column Oven, Diode Array Detector and S.E.D.E.R.E Sedex 55 Evaporative Light Scattering Detector. (Tube temp 52 °C, Pressure 2.3 Bar)
Column: Phenomenex Luna C-18 column (150 x 4 mm, 5µm)
Solvent A water; Solvent B: Acetonitrile
Flowrate: 0.8 mL/Min; Oven Temp: 35°C
Gradient: 0 Min-20 Min: 10%B to 100%B (linear); 20min-30min: 100%B;

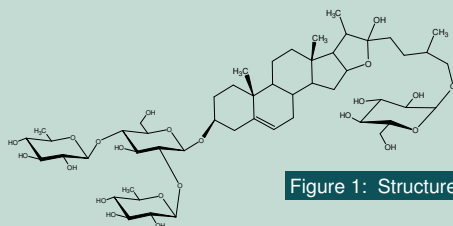


Figure 1: Structure of Protodioscin

Reference Standards:

Protodioscin was purchased as a primary standard from Chromadex of California, USA. Reference samples of Tribestan® product manufactured by SOPHARMA AD of Sofia - Bulgaria were used to compare the analytical methodology.

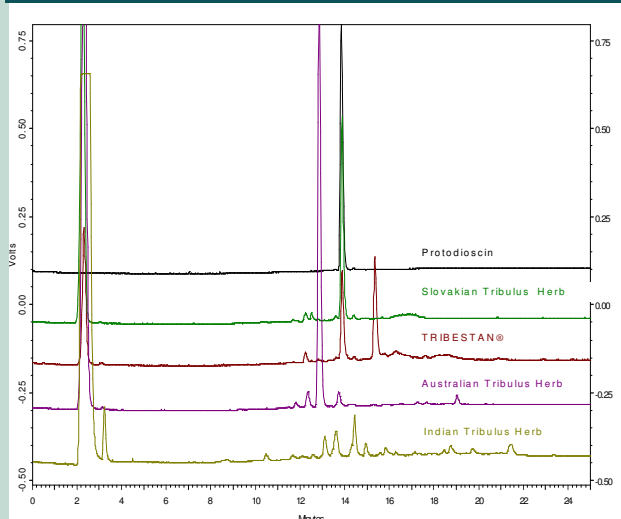
COMPARISON OF METHODS

The photometric method is a simple method of analysis but is non-selective in its response and can give misleading results. Even with the aid of a photometric scan the results are not conclusive for the presence of protodioscin, as illustrated by the results obtained for Australian Tribulus herb which contains no protodioscin. The scan is however able to distinguish the very different Indian herb from the protodioscin containing herb samples. (Refer Figure 2)

The HPLC-ELSD method is much more selective but must be used with caution due to the degradation(2) that occurs when protodioscin containing herbs are extracted with methanol. Acetonitrile is the preferred extraction solvent. This degradation is evident in HPLC-ELSD in samples extracted by methanol as a bulge in the baseline at similar retention time to protodioscin. The HPLC-ELSD traces for various samples are given in Figure 3 and illustrate the wide phytochemical variation evident in the Tribulus of different geographical origin.

Sample	Photometric	Protodioscin (HPLC-ELSD)	Total Saponins (HPLC-ELSD)
Tribestan® Tablets	16.18%	3.41%	7.51%
Slovakian Herb	4.27%	1.98%	1.98%
Slovakian Fruit	1.21%	0	1.17%
Indian Herb	1.23%	0	0.54%
Australian Herb	3.32%	0	3.96%

Figure 3: HPLC-ELSD Chromatograms of Protodioscin, Slovakian Herb, Tribestan® Tablets, Australian Herb and Indian Herb



SUMMARY

The photometric method is unable to accurately measure the level of protodioscin and related compounds in even an ideal Tribulus preparation. The poor specificity of the photometric method leads to increasingly more inaccurate results once the sample constituents vary. A wide variation in the saponin distribution of drug samples of different geographical and plant part has been found. It is shown that the photometric method responds to a range of related saponin compounds and samples containing no protodioscin or related saponins still respond to this method. In some samples with no protodioscin the absorption spectrum is markedly different to that obtained with protodioscin.

With the ready availability of reference standard materials of high purity and confidence, adoption of the HPLC-ELSD as the preferred method of analysis of *Tribulus terrestris* products is strongly recommended.

References:

- [1] Gjulemetowa, R., Tomova, M. et al, (1982) *Pharmazie*, **37**, 296
- [2] Ganzera, M., Bedir, E. et al, (2001) *Journal of Pharmaceutical Sciences*, **90**, 1752-1758.