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A comparision study for estimation of gymnemic acids by HPLC and gravimetry method for various extracts of *Gymnema sylvestres*

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Abstract

Objective: To compare the method of estimation of Gymnemic acids by HPLC and Gravimetry. Material and methods: *Gymnema sylvestres* leaves, Glass ware for Gravimetry analysis, Vacuum Tray driers (VTD), an Isocratic, Reversed phase (RP) HPLC procedure has been adopted using a mixture of Acetonitrile (23%) and 0.1% Orthophosphoric acid as mobile phase, C_{18} column as stationary phase and UV detector. Results: The comparative method shows high resolution, accuracy and reproducibility. Conclusion: The Comparative method shows that HPLC method of analysis is better than Gravimetry estimation of Gymnemic acids in *Gymnema sylvestres*.

Key words: Gymnemic acids, *Gymnema sylvestres*, HPLC, Gravimetry.

1. Introduction

Gymnema sylvestres R. Br. (Family: Asclepiadaceae) a climber plant, the leaves of which have the property of neutralizing temporarily the taste for sensation of sugars [1] and hence the name "Gurmar" attributed to it. A lot of work has been done on Gymnemic acids, a group of incompletely categorized triterpene saponins found in the leaves of *Gymnema sylvestres*. The plant has been long used in this country as antidiabetic [2]. On the contrary, medicinal plants offer a great potential to cure diabetes, *Gymnema sylvestres* is one such plant [3-5]. Various authors have reported the estimation of Gymnemic acids by gravimetry, which is a rather crude method and does not give the exact gymnemic acids content. Deacyl Gymnemic acid, the basic frame work of various Gymnemic acids [6] is reported as the constituent of *Gymnema sylvestres* [7]. Suzuki et. al., 1993 have reported the estimation of gymnemic acids content as Deacyl gymnemic acid. Hence this plant can be standardized with reference to the marker. A comparison study of gravimetry and HPLC method is attempted. The study was carried out at Natural Remedies Private Limited, Bangalore.

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2. Materials and methods

2.1 Plant Material

Gymnema sylvestres leaves were collected from Natural Remedies Private Ltd. in March 2007.

2.2 Chemicals

Acetonitrile (ACN) and methanol were obtained from Ranbaxy (HPLC grade), potassium hydroxide, potassium di-hydrogen phosphate, sodium hydroxide, ortho phospharic acid (AR grade) and hydrochloric acid was obtained from Ranbaxy (AR grade).

2.3 Extraction of Plant Materials

100g of dried and powdered *Gymnema sylvestres* leaves was accurately weighed into three 250 ml round bottom flask. The contents were extracted with 100 ml of 25 % methanol, 50 % methanol and 75 % methanol separately for 30 minutes on a boiling water bath, after cooling the methanolic extracts were filtered through suitable filter paper (Whatman 41 grade) and the filtrate concentrated to obtain 25 %, 50 % and 75 % Gymnemic acids methanolic extract.

All the extracts were completely dried using VTD, to obtain dried powder of various extract of *Gymnema sylvestres* containing 25 %, 50 % and 75 % Gymnemic acids methanolic extract.

2.4 Gravimetry estimation

Total Gymnemic acids can be estimated by gravimetry method [8]. 5gms, 7.5gms and 10gms of the above extracts is dispersed completely in 100 ml of 0.1N Sodium hydroxide followed by acidification with 2 N Hydrochloric acid, the precipitate obtained was centrifuged at 10000 RPM, the supernatant was discarded, followed by washing of the precipitate with distilled water, the precipitate was completely dissolved in alcohol and filtered, the filtrate is concentrated completely followed by drying at 80°C. The residue obtained was weighed and the percentage of Gymnemic acids was calculated.

2.5 HPLC Equipments

Shimadzu integrated liquid chromatographic system LC/2010 comprising of system controller unit, degassing unit, low pressure isocratic unit, two solvents pump unit, mixer, Auto sampler, Column oven, UV - Vis detector and class VP ver 6.0 work station was used for analysis.

Column: ODS – C 18 (Phenomenex) Luna 5 µ C 18, 250 X 4.6 mm column was used. Wavelength: 210 nm

2.6 Experimental conditions

The Isocratic analysis was performed at a flow rate 2.0 ml/minutes using mobile phase 23% ACN and 0.1% Orthophosphoric acid.

2.7 Reference standard

Deacyl gymnemic acid was isolated from *Gymnema sylvestres* in the Phytochemistry laboratory, R & D, Natural Remedies Pvt. Ltd and the identity was confirmed by comparing the IR, 1H NMR and 13C NMR with those reported in the literature [7].

2.8 Identification of Deacyl gymnemic acid peak

Accurately weighed 10 mg and transferred to 10 ml volumetric flask to prepare 1mg/ml solution. 10 µl was injected to identify the retention time.

2.9 Calibration curve

10 mg of Deacyl gymnemic acid was accurately weighed and added to a 10 ml volumetric flask, dissolved in HPLC grade methanol and the volume was made upto 10 ml with the same to 1000 mcg/ml solution. Finally, appropriate dilutions were made to get 10 mcg/ml, 20 mcg/ml and 30 mcg/ml solutions. 20 µl of each of these solutions was

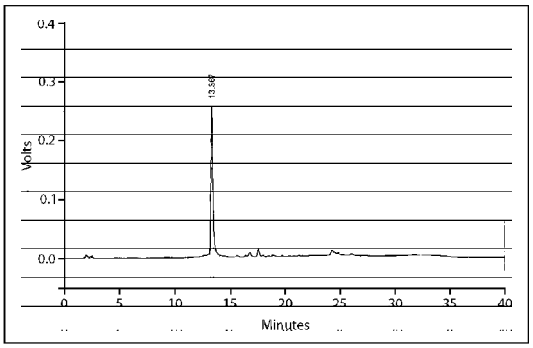

Fig. 1.
HPLC Chromatogram of Deacyl Gymnemic Acid

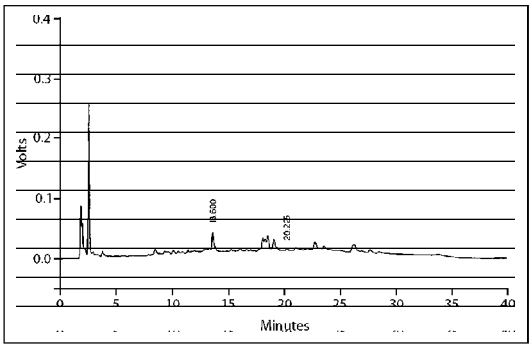

Fig. 2.
HPLC Chromatogram of 25% Gymnema Extract

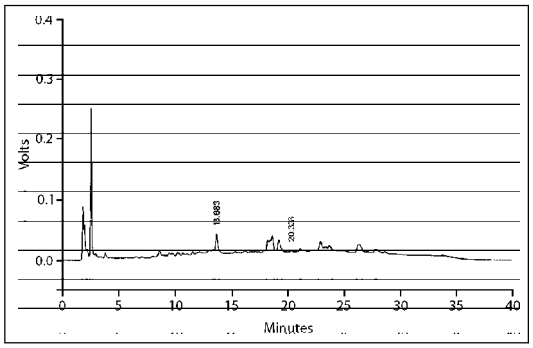

Fig. 3.
HPLC Chromatogram of 50% Gymnema Extract

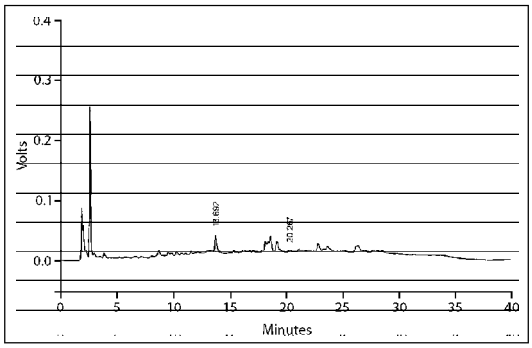

Fig. 4.
HPLC Chromatogram of 75% Gymnema Extract.

Table No. 1

Extracts	By gravimetry estimation	By HPLC estimation
1. 25 % Gymnema extract	22.8 %	8.71 %
2. 50 % Gymnema extract	48.4 %	16.2 %
3. 75 % Gymnema extract	72.0 %	19.31 %

injected in triplicate and the average area for Deacyl gymnemic acid was plotted and the regression coefficients were calculated (>0.99).

2.10 Estimation of Gymnemic acids as Deacyl gymnemic acid in various extracts of Gymnema sylvestres

Accurately weighed quantity of various extracts of Gymnema was transferred to round bottom flasks. The content was saponified using 3.0% potassium hydroxide in methanol. The saponified mixture was concentrated. The residue was dissolved in 1:1 mixture of methanol and HPLC grade water, followed by acidifying with concentrated hydrochloric acid. The acidified sample was transferred to a 10 ml volumetric flask and the volume was made up to 10 ml with methanol (50%) and filtered through Whatman No.1 filter paper and used for further HPLC analysis. The HPLC estimation

was carried out by injecting 20 µl of the sample solution. Percentage of Deacyl gymnemic acid was estimated using the area under the curve obtained from the sample by comparing the same with standard. The accuracy of estimation is validated using spike recovery studies.

3. Results and discussion

A comparison study by Gravimetry and HPLC method of estimation of Gymnemic acids has been attempted. The gravimetry estimation was performed by precipitating the Gymnemic acids, drying and weighing the residue.

For the HPLC method of estimation, the basic frame work of Gymnemic acids: Deacyl gymnemic acid is used as marker. The calibration curve for Deacyl gymnemic acid was found to be linear over the range of 10 mcg/ml to 30 mcg/ml. The respective regression co – efficient for Deacyl gymnemic acid was found to be >0.99.

In conclusion, the comparative study carried out, suggests that HPLC method of estimation is better then the gravimetry method of estimation of Gymnemic acids.

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