J. Indian Chem. Soc.,
Vol. 86, February 2009, pp. 192-194

Standardisation of Fenugreek extract using 4-hydroxyisoleucine as a marker compound

Atul N. Bedekar*, Abhinay Pise, Adesh Naik and Paresh Bhosale

Department of Chemistry, B. N. Bandodkar College of Science, Thane, (Maharashtr), India

E-mail : atulbedekar@gmail.com

Manuscript received 20 December 2005, revised 7 August 2006. accepted 23 October 2008

ABSTRACT :

Abstract : 4-Hydroxyisoleucine is reported as bioactive compound, which is responsibl antidiabetic activity of Fenugreek. Our present work Includes standardization and preparat different extracts, which are enrich with 4- hydroxyisoleucine. The paper includes some unexp observation in extraction pattern of 4-hydroxyisoleucine with different solvents. The quanti estimation of 4-hydroxyisoleucine is done by spectrophotometry on the basis of its reaction ninhydrin.

Keywords : 4-Hydroxylsoleucine, antidiabetic, herbal, extraction, HPTLC, spectrophotometry.

Introduction :

Herbal products are emerging as promising antidiabetic drugs due to lower cost, lesser side effects and renewable natural source. Fenugreek is a proven antidiabetic[1] properties of 4-hydroxyisoleucine present in Fenugreek are already established[2]. In 1973 Fowden et al[3], isolated 4-hydroxyisoleucine from Fenugreek seeds and its stereochemistry was further established by Alcock et al. Therefore in our laboratory work was carried out on sandardization of Fenugreek extract using 4- hydroxyisoleucine as marker compound.

Result and Discussion :

Fig. 1 shows results for absorbance values with different extracting solvents and Fig. 2 depicts results for change in absorbance with change in solid to liquid ratio during extraction. the value Of absorbance for saturated amino acid extract in methanol was found to be 0.38.

The absorbance values for calibration graph of glycine are given in Table 1 and the absorbance values for Fenugreek extracts are given in Table 2.

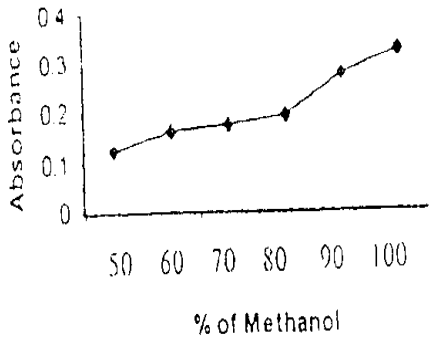


Fig. 1

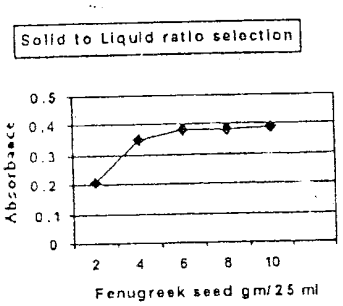


Fig. 2

Table 1

Sample No.	Molarconc. Of glycine	Absorbanoeat 570 nm
1	2×10^{-5}	0.195
2	4×10^{-5}	0.417
3	6×10^{-5}	0.600
4	8×10^{-5}	0.790

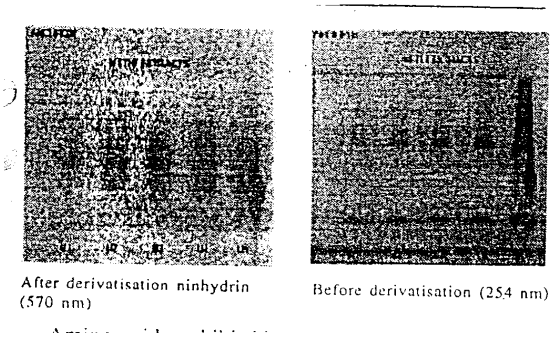
Table 2

Sample solution	**Absorbance**
Methanol-water extract (1 : 1)	0.13
Methanol-water extract (6 : 4)	0.16
Methanol-water extract (7 : 3)	0.17
Melthanol-water extract (8 : 2)	0.19
Methanol-water extract (9 : 1)	0.27
Methanol extract (2 g / 25 ml)	0.21
Methanol extract (4 g/ 25 ml)	0.35
Methanol extract (5 g/ 25 ml)	0.38
Methanol extract (6 g/ 25 ml)	0.38
Methanol extract (8 g/25 ml)	0.38
Methanol extract concentrate	0.168
Trigo syrup	0.59

The HPTLC results are tabulated in Table 3.

Table 3

Sample No.	**Area under 570 nm at R_f 0.43**	**% of 4-hydroxyisoleucine (at R_f 0.43)**
ul (1g/25 ml)	15924	31.43
u2(2g/25 ml)	22764	32.90
u3(3g/25 ml)	26183	31.77
u4(4g 25 ml)	29951	30.18



After derivatisation ninhydrin (570 nm)

Before derivatisation (254 nm)

Amino acids exhibit higher solubility in water than methanol since absorbance increases with the % increases of methanol in extraction solvent. However during our study we found that methanol is better solvent than water for extraction of amino acids from Fenugreek seeds. It may be due to tendency of Fenugreek to form mucilage with water.

As seen from Fig. 2 the absorbance value increases from sample 2 g/25 ml to 5 g / 25 ml, it can be concluded that 1:5 is the optimum solid to liquid ratio for extraction of amino acids.

To determine concentration of 4-hydroxyisoleucine first total amino acid content of the extract was found out by using spectrophotometric method. Then the relative concentration of 4-hydroxyisoleucine in the mixtrue of amino acid was found out by HPTLC. Thus by using combination of spectrophotometry and HPTLC it was possible to determine concentration of 4-hydroxyisoleucine in different extracts. The molar amino acid concentration (expressed as glycine equivalent) in concentrated methanol extract was found to be 1.58×10^{-5} M. Based on this the 4-hydroxyisoleucine content in the concentrated methanol extract was estimated as 0.7%. Similarly the molar amino acid concentration (expressed as glycine equivalent) in Trigo, was found to be 5.97×10^{-5} M and 4 -

hydroxyisoleucine content was estimated as 0.026%.

Experimental

Materials and methods :

Apparatus : Absorbance measurements are carried out on Systronics spectrophotometer 104 and HPTLC was carried out using CAMAG HPTLC setup.

Reagents : The ninhydrin spray reagent is prepared by dissolving 0.4 g of ninihydrin (A.R. grade, Merck) in 100 ml ethanol. Fenugreek seeds are purchased from local market. Methanol and hexane used for extraction are of L.R. grade.

General procedure for preparation of Fenugreek extract : Fenugreek seeds were air dried and ground in a grinder so that the powder could pass through a 0.8 mm mesh sieve. Fenugreek powder is then defatted with hexane at 40°C for 2 h under stirring and filtered through a Whatman filter paper and dried. Defatted Fenugreek seeds are stirred with extracting solvent at 55-60 °C for 1 h and filtered over Whatman filter paper

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Selection of solvent for defatted Fenugreek powder : With water Fenugreek seeds swells and it is difficult to filter. Therefore extraction was tried with increasing methanol concentration in water. The extracts were subjected for colour development with ninhydrin and absorbance was measured. A graph (Fig. 1) was plotted for absorbance vs % of methanol

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Optimization of solid to liquid ratio for methanol extraction of defatted Fenugreek seeds : In this experiment the volume of methanol is kept same (25 ml) and quantity of Fenugreek seeds was increased i.e. (2, 4, 6, 8, 10 g). The extracts obtained were subjected to colour development with ninhydrin and absorbance is checked. The graph of absobance vs Fenugreek seeds g/25 ml is plotted (Fig. 2).

Quantitative estimation of amino acid by spectorphotometry : A stock solution of 0.2 M glycine is prepared by dissolving 0.150 g of glycine in 100 ml of water. Using stock solution samples of 2, 4, 6, 8, (x 10^{-5} M) are prepared. Each sample is treated with ninhydrin to produce a purple colour and measured absorbance at 570 nm. A calibration graph is plotted. The absorbance values are given in Table 1.

1 g of conventrated methanol extract was diluted with 50 ml of methanol. 5 ml of the diluted methanol solution was used for the colour development with ninhydrin solution (2 ml) and diluted to 100 ml. 1 g of commercial sample, Trigo was diluted to 50 ml with methanol and 5 ml was used for colour development with ninhydrin solution (2 ml) and diluted to 10 ml to measure the absorbance at 570 nm for determination of its amino acid content.

Determination of 4-hydroxyi-soleucine concentration by HPTLC : HPTLC was run using n-butanol : acetic acid : water (4 : 1 : 1) as a mobile phase and spot visualization was carried out at 254 nm. Then derivatisation was done by spraying with ninhydrin solution and spot visualization was carried out at 570 nm. The different methanol extracts prepared were checked by HPTLC. R_f value for 4-hydroxyisoleucine4 is reported as 0.43. The HPTLC results are tabulated in Table 3.

Conclusion :

This analytical method can be applied to the Fenugreek extract samples to check the potency of the extract by ascertaining the percentage of 4-hydroxyisoleucine which one of the established constituents of Fenugreek extract responsible for antidiabetic activity. One such commercial formulation, Trigo based on Fenugreek extract was analysed for its 4-hydroxyisoleucine content by this method. Therefore in conclusion 4-hydroxyisoleucine can be used as a bioactive marker compound for standardization of Fenugreek extracts.

Acknowledgement

Authors are thankful to the Principal, B. N. Bandodkar College of Science for providing the facilities for conducting the research work. Authors are also thankful to Anchrom Labs, Mulund, Mumbai for providing us with HPTLC support.

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