INTERNATIONAL JOURNAL OF DRUG DISCOVERY AND HERBAL RESEARCH (IJDDHR)
1(4): October -December: (2011), 106-110
ISSN: 2231-6078

Research Article

Comparative Study For The Determination Of Reducing Sugars By High-Performance Liquid Chromatography and Titration Method in 'Chyawanprash' : A Traditional Polyherbal Formulation

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Abstract

Chyawanprash' is one of the most popular Ayurvedic preparations placed under 'Rasayana' group of drugs, used widely as a health promotive and disease preventive avaleha. Sugar and honey play an important role in Chywanprash, where they work together as carrier of herbs (anupan). In the present study comparison of reducing sugar content by High-Performance Liquid Chromatography (HPLC) and Titration method in 'Chyawanprash' has been conducted for the first time. Our result indicates that there is a difference in content of reducing sugars in Chyawanprash when determined by HPLC and Titration method. Titration method gives a higher value of total reducing sugar content than HPLC method.

Key Words: High-performance liquid chromatographic (HPLC), Total sugar, Titration method, Polyherbal Formulation

Introduction

Chyawanprash, a time-tested, age-old formulation is popular for health & vigor. Chyawanprash is a complex mixture of more than 40 herbal ingredients (Govindrajan et al., 2005).All the ingredients in Chyawanprash have been scientifically studied individually for their health benefits. The combination of these nutrients used in Chyawanprash in a specific quantity and manner of blending creates a powerful synergy for optimum health benefits. Chyawanprash is helpful in clearing the accumulated excreta by promoting digestion and excretion (Parle et al., 2006 and Manjunatha et al., 2001). It is not only hepatoprotective but it also streamlines the metabolism of fats and proteins (Jeena et al., 2000 and Handa et al., 1986). It relieves cough, asthma, bronchospasm, respiratory tract infections and tuberculosis (Ojha et al., 1975).

Being a well-known Ayurvedic formulation, 'Chyawanprash' has been the subject of study by several researchers. As sugar is one of the important ingredient of Chyawanprash, the present study emphasizes on the comparison of reducing sugar content as determined by High-performance liquid chromatography (HPLC) and Titration method in 'Chyawanprash'

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Material and Methods

Standards and chemicals:

All the chemicals used in the experiments were of HPLC and analytical grade. Glucose, Fructose and Sucrose were from Sigma, USA.

Instrumentation:

The HPLC system (Agilent 1200 series, USA) consisted of a refractive index detector (RID/G1362A), solvent delivery module (LC-10ATVP), online degasser (G332A), an auto-injector (ALSG1329A), flow channel system (FCV-14AH), system controller (SCL- 10AVP), and a HPLC column (Hyper REZ XP carbohydrate column 300 mm × 4.6 mm, 8µm particle size, Sigma, USA). Data analysis was carried out using chemstation software (Agilent, USA).

Chromatographic conditions:

The chromatographic elution was carried out in isocratic mode using water (pH adjusted to 2) as mobile phase. The analysis was performed at 30 ^{0}C at a flow rate of 0.7 mL min-1 with a run time of 20 min.

Samples:

Three sample of 'Chyawanprash' were obtained from Dabur India Limited. About 1 gram each of sample was weighed accurately and made up to 100ml with mobile phase. Samples were filtered through 0.45 micron nylon filters, before injecting into the liquid chromatograph.

Preparation of reference solution:

About 10 milligram of standard (Glucose, Fructose and Sucrose) were weighed, dissolved and diluted to 100 ml using mobile phase. This was treated as stock solution and the subsequent dilutions were prepared in mobile phase.

Statistics

Calculations were done by % (w/w) basis with the given formula.

$$\frac{\text{Area of sample} * \text{Weight of std} * \text{sample dilution}}{\text{Area of standard} * \text{Weight of sample} * \text{Std dilution}} * 100$$

Results and discussion

The result indicates that there is a difference in content of reducing sugars in HPLC and Titration method. For estimating total sugars by HPLC, a summation of Glucose, Fructose & Sucrose was done (Table 1). The value obtained was then compared with the value obtained from titration (Table 2). The peaks in the sample were identified basis the retention time of the standard glucose, fructose and sucrose as obtained from reference chromatogram (Fig 2).

The HPLC method gives sugar content before inversion as indicated by the presence of peaks for Glucose, Fructose & Sucrose (Fig1-Fig5). Absence of any other peak in the chromatogram rules out presence of any other sugar in Chyawanprash. A sum of all these individual sugars in the sample gives the content for total sugars in Chyawanprash by HPLC; reducing & non-reducing sugars can be determined by calculations using the data obtained from reference standard & sample.

The sample processing in titration method involves inversion of non-reducing sugar to reducing sugars. Titration of the reducing sugars thus obtained gives a value for total sugars. Principally, the reducing sugars reduce the Fehling's solution which by calculation gives the total sugars present in the sample subtraction of reducing sugars obtained before inversion from the total sugar gives the non-reducing sugar content.

Plants contain diverse mixture of starch, carbohydrates, phenolics and many other components which may either undergo inversion or have an added reducing effect on Fehling's solution. Chyawanprash is reported to be containing high content of phenolics which itself is reducing in nature (Govindrajan et al., 2007). Thus, it can be concluded that it is the presence of these reducing components which amounts to a higher values of total sugars by titration when compared to the values obtained from analysis by HPLC.

Table 1. Sugar Profile in Chyawanprash Sample by HPLC

Sample	Total sugar	% Total Sugar
A	%Glucose	7.71
	%Fructose	7.19
	%Sucrose	50.5
B	%Glucose	10.81
	%Fructose	10.42
	%Sucrose	39.47
C	%Glucose	13.49
	%Fructose	13.43
	%Sucrose	29.42

Table 2. Comparative Sugar Profile in Chyawanprash by HPLC & Titration.

Sample No.	Total sugar by HPLC	Total sugar by Titration	Reducing sugars by HPLC	Reducing sugars by Titration
A	66.12	66.52	14.9	17.95
B	60.7	62.9	21.23	25.21
C	56.34	60.84	26.92	30.01

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Fig no. 1: HPLC chromatogram of Blank

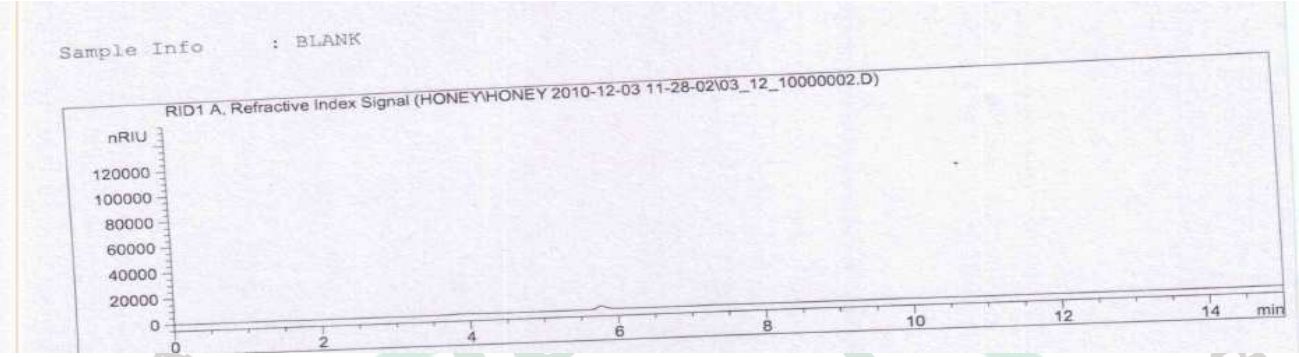

Fig no. 2: HPLC Chromatogram of Standard mix (Glucose, Fructose & Sucrose)

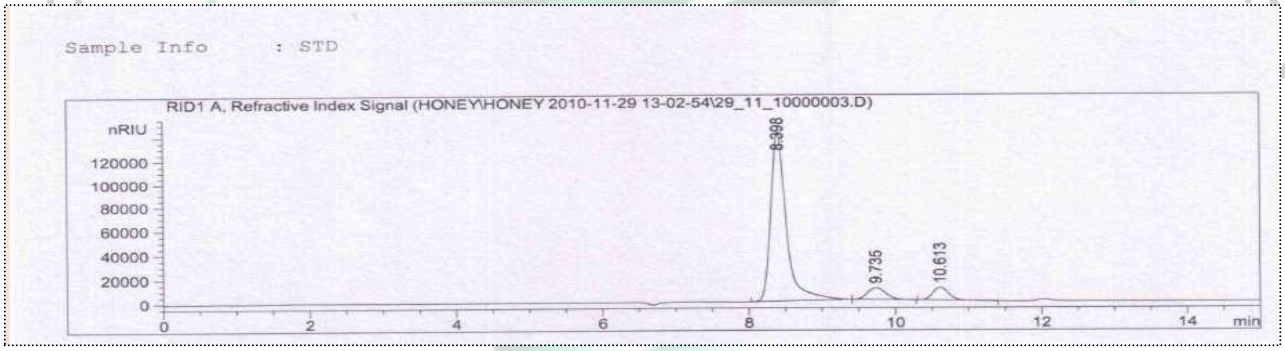

Fig no. 3: HPLC chromatogram of Sample A

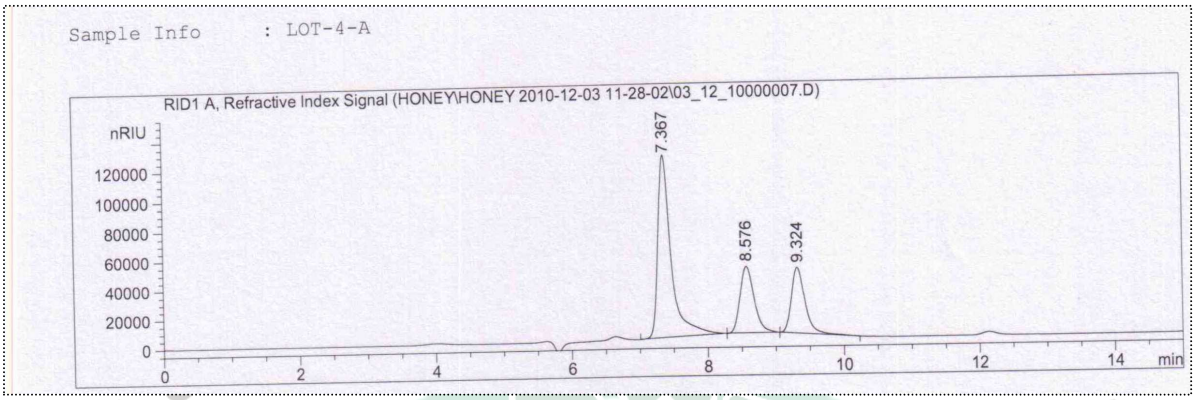

Fig no. 4 HPLC Chromatogram of sample B

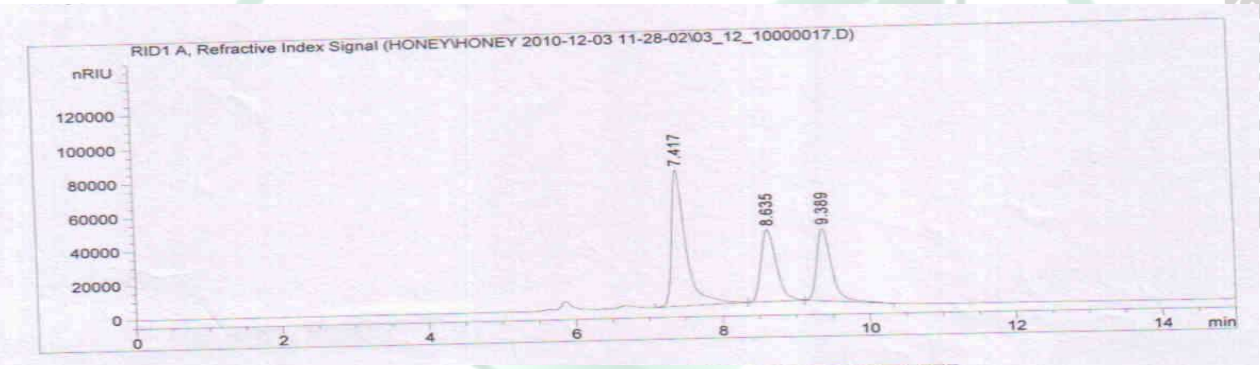

Fig no. 5 HPLC profile of Sample C