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Rapid FTIR Method for Estimation of Sucrose in a Traditional Indian Polyherbal Formulation

Kartik Chandra Patra[a*], K.Jayaram Kumar[b], Suvendu Kumar Sahoo[a], Podilama Suresh[a]

[a]GITAM Institute of Pharmacy, GITAM University, Visakhapatnam-530045, A.P, India
[b]Department of Pharmaceutical Sciences, Birla Institute of Technology, Mesra, Ranchi-835215, Jharkhand, India

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Abstract

A simple, fast and reliable method has been developed for the quantification of sucrose in an Indian traditional polyherbal formulation samples (*Amukkara choornam*) through Fourier Transform Infrared absorbance measurements (FTIR) at 1051 cm^{-1} with a base line established between 750 cm^{-1} to 1500 cm^{-1}. This method was validated following the united state pharmacopoeia guidelines for method validation. Sensitivity, Linearty, Limit of detection (LOD), Limit of quantification (LOQ), Accuracy and Specificity were considered. Sucrose was used as standard.

Keywords:

Sucrose, FTIR, Polyherbal formulation, *Amukkara choornam*

1. Introduction

Amukkara choornam is a polyherbal siddha formulation having herbs, spices and sucrose as the major ingredients used in Gastric troubles, Spleen enlargement, leucorrhoea, Hiccup, Anemia, tuberculosis and Kappa disease. [1]. According to traditional system of medicine sucrose play important roles like maintaining balance between usna (hot) drabya and sita (cold) drabya in a formulation, development of semen, beneficial for eyes, eleminates excessive humors of wind and bile in the body and cheaks vomiting [2]. Traditional methods for determination of sucrose include High performance liquid chromatography (HPLC), Gas chromatography (GC), Flow injection spectrophotometry, sequential injection analysis, voltametry, colorimetry, flame atomic absorption spectrophotometry, Amperometry, Enzymetic spectrophotometry etc [3] Neverthess some above technique have more or less suffered from diverse disadvantages with regard to the expensive equipments, insufficient selectivity, complicated derivitization for sensitive detection and time consuming process of sample clear-up to prevent deteriorating chromatographic columns. To ensure the quality for routine analysis and also for testing by regulatory bodies it is essential for developing a method which is fast, reliable and also interference of other ingredients is minimum. The aim of this paper is to develop a direct and simple procedure for the determination of sucrose in polyherbal formulation which is easy to handle, speed enough and cheaper than those existing methods.

[*] **Corresponding Author**
E-mail: herbalkartik@gmail.com
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2. Experimental

2.1. Samples and Reagents

Various samples were collected from the physicians and manufacturers of Aravindh Herbal laboratories (P) Ltd (SAMPLE-A), Rajapalayam (south India) (SAMPLE-B), SKM Siddha and Ayurvedic Medicines India (P) Ltd. Erode. (SAMPLE-C), In house preparation as per Siddha formulary (SAMPLE-D), which is being used for the treatment of Gastric troubles, spleen enlargement, leucorrhoea, hiccup, anemia, tuberculosis and kappa diseases. Sucrose, analytical grade reagent purchased from Sigma Aldrich was employed for standard preparation. Other reagents such as glucose, fructose and lactose employed for the study of interferences, were of analytical reagent grade. Solutions were prepared using MilliQ deionised water. Potassium bromide (KBr) from sigma Aldrich, India, was used as back ground correction.

2.2. Apparatus and software

A Mattson Model Research1 FTIR Spectrophotometer (Shimazdu WI) was employed to do infrared measurements using a Specaclamp IN-Compartment Contact Sampler horizontal DRS from Graseby Specac (Orpington, UK) with a 45° crystal Zn Se through top-plate. The FTIR spectra obtained were processed using the IR Solution software, from Shimazdu.

2.3. FTIR Method

Different concentrations of sucrose solution were prepared in MilliQ deionised water and mixed with KBr to prepare 1%,2%,3%,4% and 5% mixture, for standard plot. Similarly 5% of the each formulation was taken for determination of sucrose. The spectra obtained in the absorbance mode from 2000 to 400 cm^{-1} by accumulating 20 scans, working with a spectral resolution of 4 cm^{-1}. Absorbance spectra were corrected, versus a spectrum of KBr, obtained in the same instrumental conditions. The band at 1051 cm^{-1} was selected for analytical measurements [4] using a base line correction established between 1500 and 750 cm^{-1}. The experiment was done in triplicate.

2.4. Calibration plot

A series of standard curves were prepared over a concentration range of 1%-5% of sucrose (n=3). The data of Concentration versus absorbance was treated by linear least square regression analysis.

2.5. Analysis of sample

The samples were analyzed by the proposed method taking 5% of each sample in KBr. The amount of sucrose was determined by extrapolation in the standard curve.

2.6. Validation of method

2.6.1. Sensitivity, Linearity, limit of detection and limit of quantification

These parameters were calculated from the data set obtained from a linear calibration curve in the range 1–5 mg (two replicates for each standard). For this purpose, a 1 % (w/w) of sucrose was studied for its absorbance. For linearity study different concentration (1-5%age) was observed for its absorbance. Slope and regression standard deviation (SY/X) values were used to establish sensitivity (SY/X/b) [5].

LOD was calculated with the following equation: $LOD = 3\left(\frac{SY/X}{b}\right)\sqrt{\frac{n-2}{n-1}}$

The calibration curves were plotted using the response factors versus concentration of standard solution.

2.6.2. Accuracy

To the pre-analyzed sample, 1mg of sucrose was added and the mixture was analyzed by the proposed method. The experiment was conducted in triplicate to check, recovery and accuracy of the system.

2.6.3. Specificity

In the present study, the specificity of the analytical method was determined in samples containing sucrose, galactose, lactose and mannose analyzed according to the proposed method.

3. Result and discussion

FTIR Method: In this study the quantitative FTIR method was developed for estimation of sucrose. Fig-1 shows the FTIR spectra of Standard which is well resolved between 750-1500 cm^{-1} . The region 1051 $cm^{-1}$was selected for analysis. It is seen that there is a good agreement between the spectra of both natural samples and standards, when appropriate base line is defined.

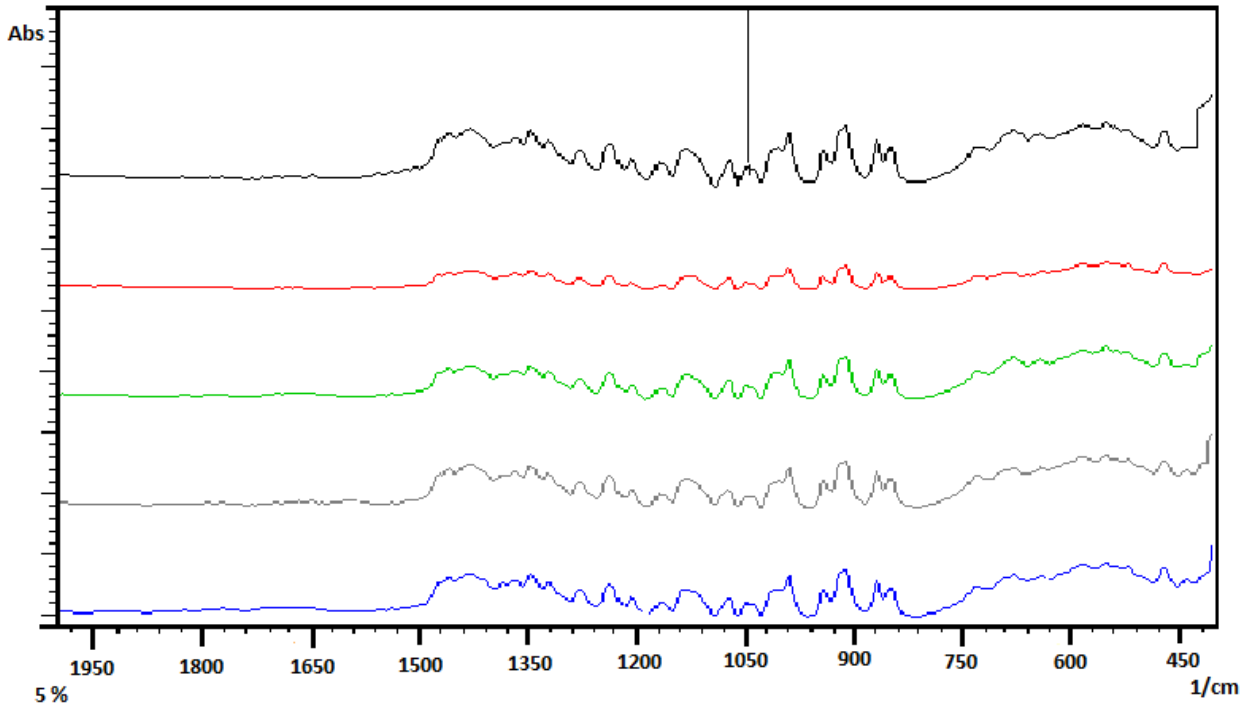

Fig.1. Overlapping FTIR Spectra of different concentration of Standard (Sucrose)

3.1. Calibration plot

The polynomial regression data (Table 1 and Fig.2) for the calibration plots (n=3) showed a good linear relation ship over concentration range 1%-5%. No significant difference was observed in the slopes.

Table 1. Polynomial regression data for standard curves

Amount of sucrose	r^2±SEM	Slope×10^2±SEM
1%-5%	0.9933±0.002	12.8±0.210

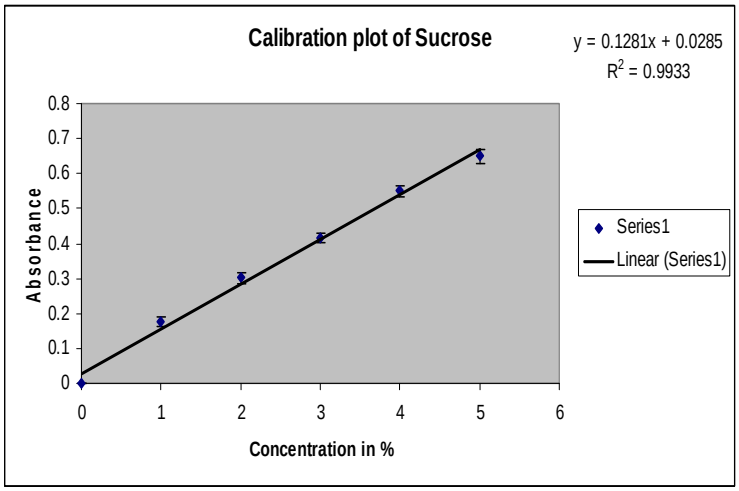

Fig.2. Calibration plot of Standard (Sucrose)

3.2. Analysis of samples

The FTIR spectra of various *Amukkara choornam* samples shown in Fig.3, 4, 5 and 6. The results of the sucrose concentration in the various samples are depicted in Table 2.

Table 2. Absorbance and % of sucrose in different samples of *Amukkara Choornam*

Sample No	Sample name	Absorbance±SEM	% of sucrose(Mean±SEM)
1	A	0.350±0.00057	50.53±0.335
2	B	0.391±0.00120	56.38±0.286
3	C	0.384±0.00120	55.65±0.326
4	D	0.377±0.00120	55.18±0.5343

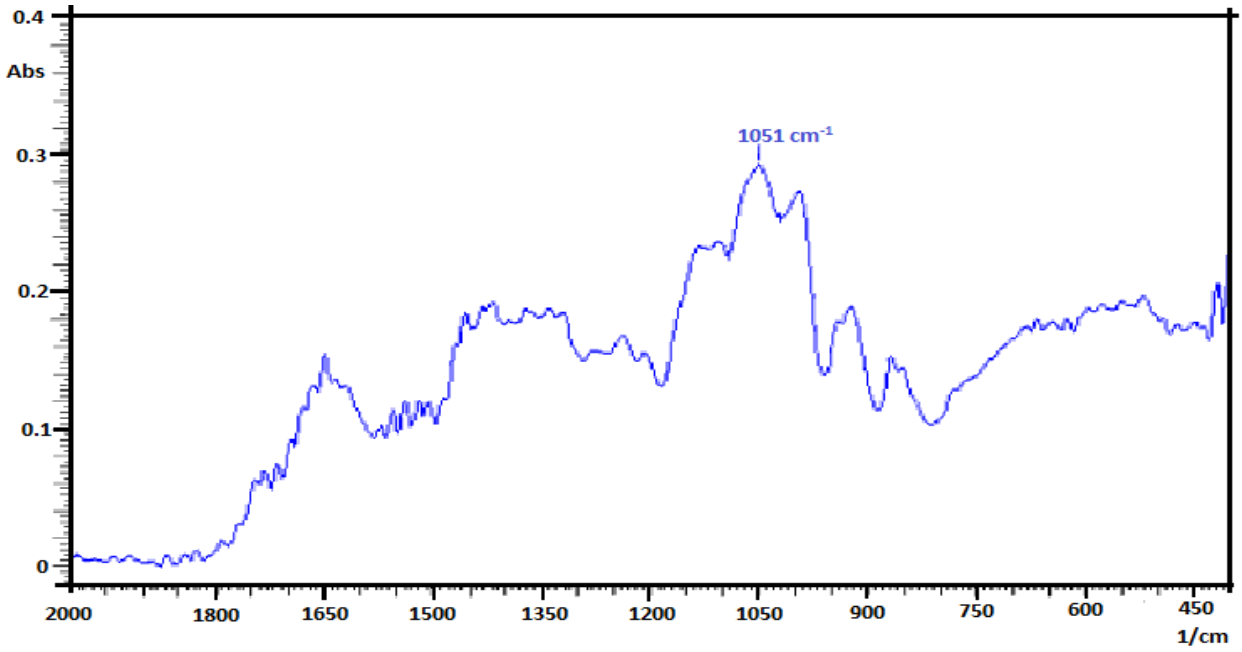

Fig. 3. FTIR Spectra of Sample-A

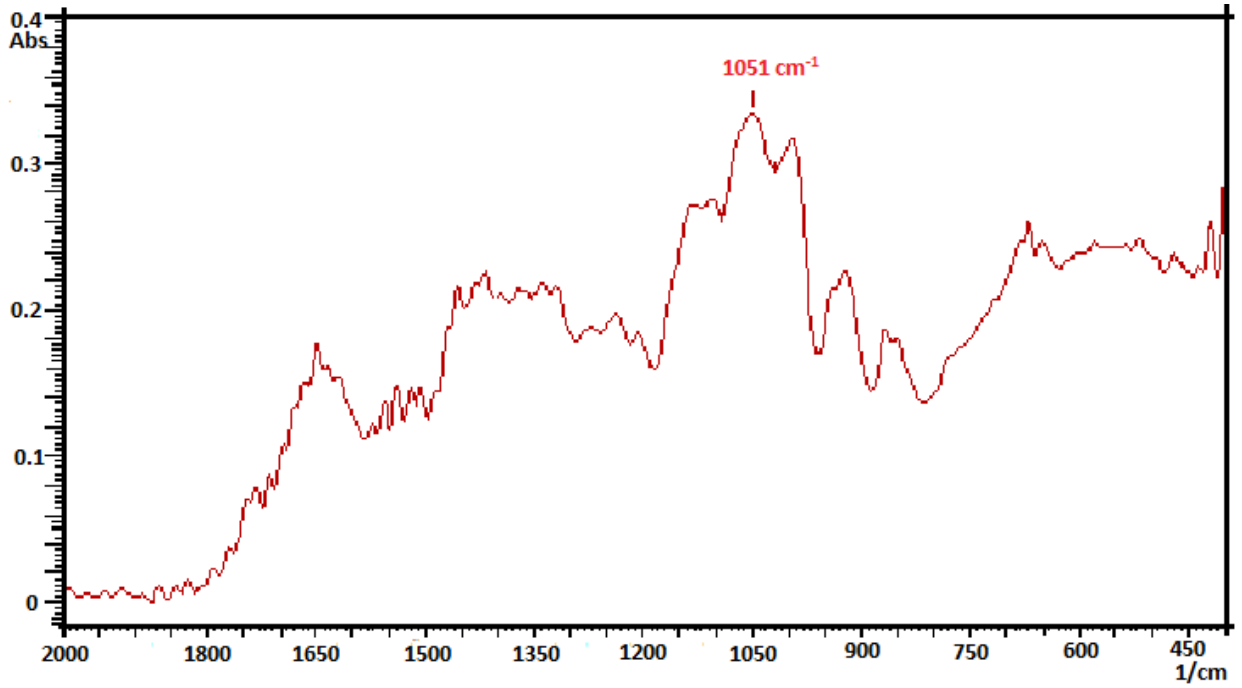

Fig.4. FTIR spectra of Sample-B

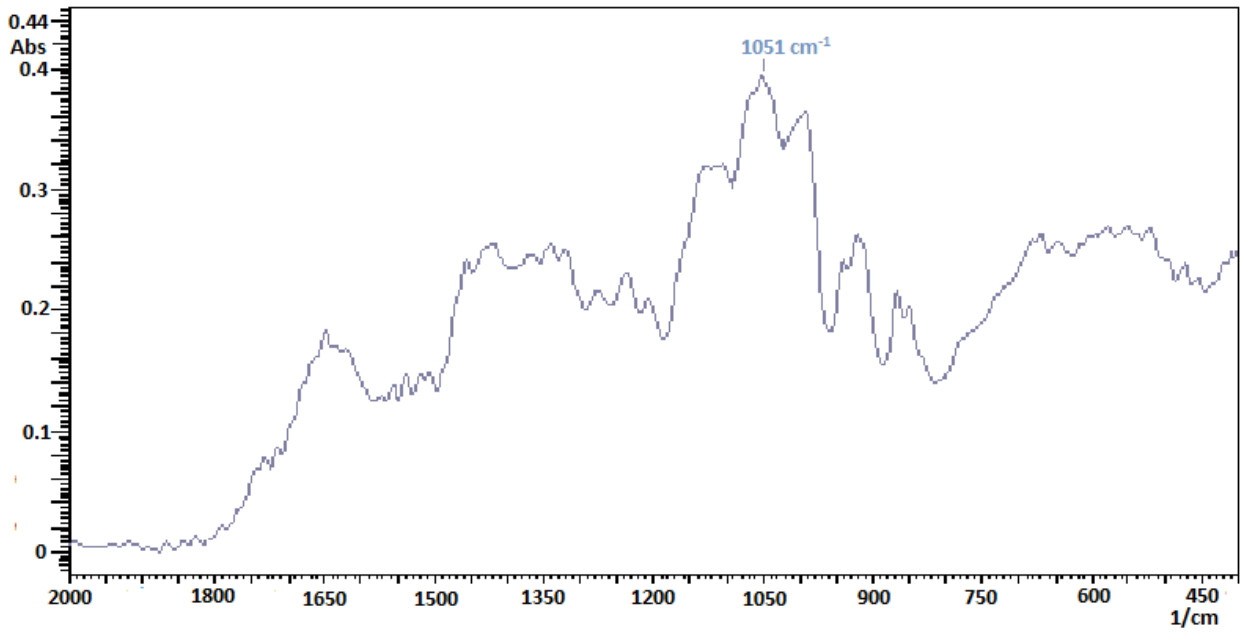

Fig.5. FTIR Spectra of Sample-C

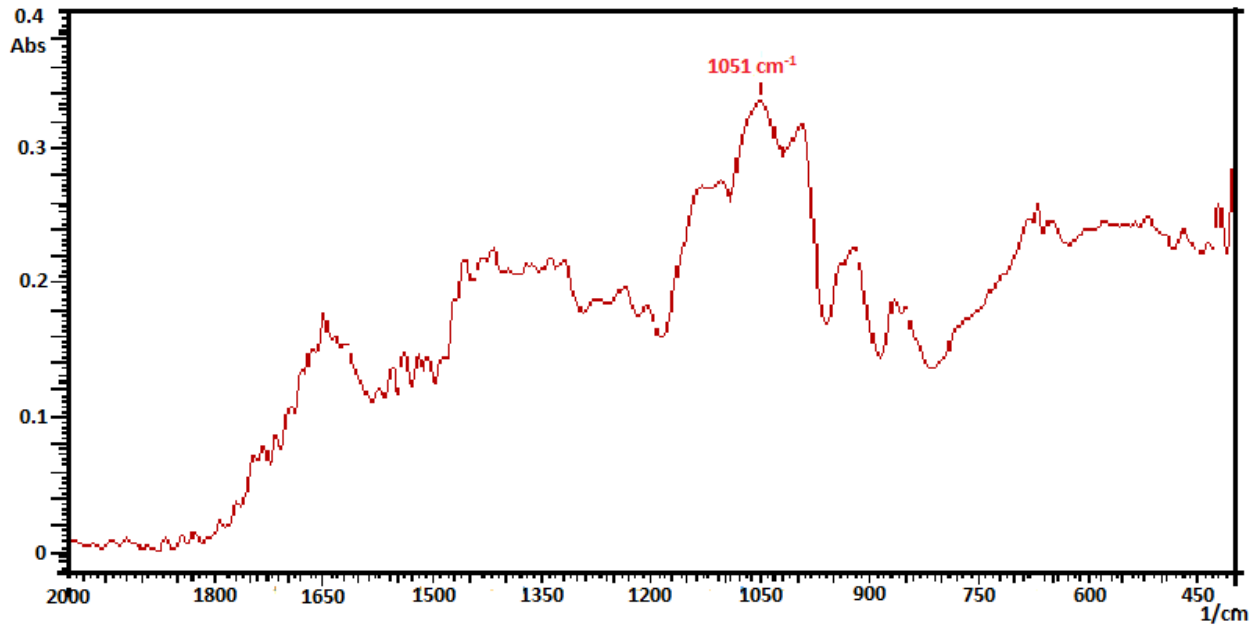

Fig.6. FTIR Spectra of Sample-D

3.3. Validation of method

3.3.1. Sensitivity, Linearity, limit of detection and limit of quantification

The calibration curves were plotted using the response factors versus concentration of standard solution. These data demonstrate that the methods have adequate sensitivity confirmed from the recovery study data.(Table 3) and linearity (Fig.2) to the concentrations of the analytes. The regression equation Y=0.1281X+0.0285, R^2=0.9933shows the linearity of the method. The LOD and LOQ of the developed method were determined by analyzing low concentrations of the standard solutions using the proposed methods. The LOD is the smallest concentration of the analyte that gives a measurable response. The LOD and LOQ were found

to be 0.5 µg and 5µg respectively, which indicate adequate sensitivity of the method. The LOD and LOQ values determined are affected by the proposed method.

Table 3. Recovery study of sucrose by FTIR method

Sample No	Amount of sucrose in powder (mg)	Amount of sucrose Added (mg)	Amount found In mixture (mg)±SEM	Recovery (%)±SEM
1	55.755	5	58.601±0.505	95.143±0.01

3.3.2. Accuracy

Table 4 depicts the accuracy of the quantification of sucrose in the various samples in triplicate, in terms of the mean values and the % CV values of sucrose.

Table 4. Accuracy with respect to Mean and standard deviation

Sample No	Sample	% of Sucrose in triplicate.			Mean ± SEM	CV(%)
1	A	50.45	51.98	50.41	50.53±0.335	1.445
2	B	54.76	55.94	56.41	56.38±0.286	2.813
3	C	55.91	55.54	61.62	55.65±0.326	4.864
4	D	55.25	54.340	55.55	55.18±0.534	1.006

3.3.3. Specificity

This method does not show any interference with others i.e. galactose, lactose, and mannose etc. FTIR method developed for the quantification of sucrose in various samples was found to be simple, sensitive, precise, accurate and specific for estimation and can be conveniently employed for studies on *Amukkara choornam*.

4. Conclusion

The method developed through this study shows that, based on direct FTIR measurement at 1051cm^{-1} with a base line established between 750-1500 cm^{-1}, it can determine sucrose In powder polyherbal samples. The method developed is sensitive, accurate, linear and specific. On the other hand, the method offers an appropriate alternative to time consuming chromatographc procedures and reagent consuming enzymic procedures.

Acknowledgement

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