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A NEW POLARIMETERIC METHOD FOR THE ANALYSIS OF SUCROSE - IN IRON SUCROSE RAW MATERIAL, IRON SUCROSE INJECTION AND INPROCESS BULK FORMULATIONS

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ABSTRACT: A simple, sensitive, rapid, accurate and economical polarimeteric method has been developed and subsequently validated for simultaneous determination of Sucrose - in Iron Sucrose Raw material, Iron Sucrose Injection and Inprocess Bulk sample of Iron Sucrose formulations in pharmaceuticals. The usual measure of purity of sucrose is by polarimetry - the measurement of the rotation of plane-polarized light by a solution of sucrose. The specific rotation at 20°C using yellow "sodium-D" light (589 nm) is +66.47°. Commercial samples of sucrose are assayed using this parameter. Readings are not affected by the molecular weight of the Iron Sucrose, the entire procedure takes much less time to perform. The developed method was compared with RP-HPLC method. The described method was linear over a concentration range of 10-70 mg per ml for the assay of Sucrose. Results of analysis were validated statistically and by recovery studies. The limit of detection (LOD) and the limit of quantification (LOQ) for Sucrose were found to be 0.076 and 0.231mg per ml. The results of the study showed that the proposed polarimeteric method is simple, rapid, precise, accurate and economical (as compared to conventional RP-HPLC methods) which is useful for the routine determination of Sucrose.

INTRODUCTION: Sucrose is the organic compound commonly known as table sugar and sometimes called saccharose. Chemically, Sucrose is α–D–glucopyranosyl-(1→2)–β–D-fructo-furanoside; β–D-fructofuranosyl-(2→1)–α–D-glucopyranoside; β-(2S, 3S, 4S, 5R)–fructo-furanosyl–α-(1R, 2R, 3S, 4S, 5R)-glucopyranoside; α-(1R, 2R, 3S, 4S, 5R)–glucopyranosyl-β-(2S, 3S, 4S, 5R)-fructofuranoside (**figure 1 and 2**).

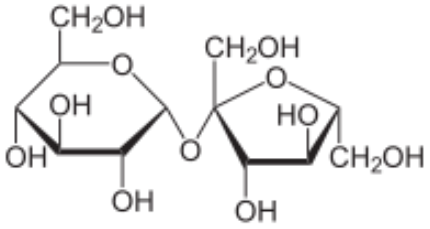

FIGURE 1: SKELETAL FORMULA OF SUCROSE

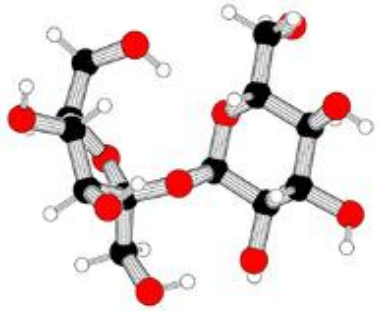

FIGURE 2: BALL-AND-STICK MODEL OF SUCROSE

QUICK RESPONSE CODE

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Iron Sucrose has been used for iron replacement therapy because of its lower rate of adverse events and Sucrose has been used as sweetening agent, it is best known for its nutritional role. Sucrose is registered as official additive and excipient in Indian pharmacopeia [1], British Pharmacopeia [2] and United States Pharmacopeia [3]. Literature surveys reveal Spectrophotometric methods [4, 5], HPLC methods [6, 7] and LCMS method [8] for its determination.

This paper presents simple, accurate and reproducible polarimeteric method for simultaneous determination of Sucrose - in Iron Sucrose Raw material, Iron Sucrose Injection and Inprocess Bulk Formulation. So far, no method has been reported for estimation of Sucrose in Iron Sucrose by polarimeter, hence we attempted to develop a simple, accurate, and economical analytical method.

This paper describes validated Polarimeteric method for simultaneous estimation of Sucrose in Iron Sucrose.

EXPERIMENTAL:

Chemicals, reagents and Instrumental Conditions: Standard bulk drug sample Sucrose and Iron Sucrose were provided by Nirlife Healthcare (Healthcare Division of Nirma), Ahmedabad, Gujarat, India. Iron Sucrose Injection and its Inprocess Bulk Formulations were procured from the production department of Nirlife Healthcare. All other reagents (Finar Reagent, Ahmedabad, India) used were of HPLC grade or AR grade. Whatman filter paper no. 41 (Whatman International Ltd, England) were used in the study. Sucrose solution is measured on AUTOPOL V (Rudolph) Polarimeter- the measurement of the rotation of plane-polarized light by a solution of sucrose. The specific rotation at 20 °C using yellow "sodium-D" light (589 nm) is +66.47°.

- The AUTOPOL V Automatic Polarimeter is designed for today's FDA regulated analytical laboratories. Whether you are a Pharmaceutical, Nutraceutical or University Lab, the AUTOPOL V has the standard features you must have, including:
  - TempTrol™ - Electronic cooling and heating from 15°-30°C
  - 21CFR Part 11 Compliance: Electronic signature and secure local data storage
  - Six Standard Wavelengths: 365nm, 405nm, 436nm, 546nm, 589nm, 633nm
  - Standard Accessories: TempTrol™ NIST Traceable Quartz Standard, TempTrol™ 100mm Polarimeter Cell, TempTrol™ Temperature Validation Cell and Built-In Sample Measurement Probe.
  - 3-year domestic warranty and 20-year support guarantee

Statistics of Polarimeter: AUTOPOL V Polarimeter calculates the mean, the standard deviation and records the minimum and maximum readings for up to 999 measurements. Measured data for statistical calculation can be printed in either of two ways: automatically, at predetermined time intervals, or manually, by pressing the print key. Statistics for a batch of samples can be printed by pressing the page feed key. Statistics on time varying optically active samples can be compiled by measuring a sample at a predetermined rate and for a predetermined number of measurements (**figure 3**).

FIGURE 3: POLARIMETER AUTOPOL V DISPLAY

Sample Specific Programs of Polarimeter: In factory or laboratory environments where the same types of samples are repeatedly measured, sample specific programs can be generated. These programs allow parameters such as tube length, concentration, specific rotation, wavelength, measurement mode, and temperature control and correction to be specified.

The programs can be labeled with alpha-numeric names. The factory or laboratory operator simply selects the sample program from the sample program menu and the AUTOPOL V Polarimeter is properly configured to make measurements of the user's samples. Sample range limits can also be specified in sample specific programs, (e.g. 24.8° Arc - 25.7° Arc). When a range is specified and the measurement falls outside this specification the display will read "out of range". Two TTL level signals are available for out of range conditions. These signals are useful for process control or alarms.

- Mettler Toledo XP 205 Analytical balance (Switzerland) was used for weighing: Mettler Toledo analytical balances have earned their place as the world's best. This analytical balances have unparalleled weighing performance, ease-of-use with touch screen technology, and comprehensive connectivity options: essentials for today's regulated environment, but also invaluable for increasing productivity and quality in every lab.

- R-8C BL Bench Top Centrifuge (REMI) was used for centrifuge a sample solution: The new generation of microprocessor based tabletop centrifuge model use brushless induction motor with frequency drive enabling the user to pre-set speed & time with a high degree of accuracy. Display of set parameters like speed, time etc., make the unit an ideal choice for repetitive sample analysis.

Methodology (Analysis of Formulation): Transfer a volume or accurately weighed sample of Iron Sucrose raw material, Iron Sucrose injection, Iron Sucrose inprocess bulk sample equivalent to 2.5 grams of Sucrose, in to a 50-ml volumetric flask, add 1.25 ml of water, and mix. Add 1.25 ml of a monobasic sodium phosphate solution [(sodium dihydrogen phosphate monohydrate; $NaH_2PO_4.H_2O$; M.W. 137.99), (sodium dihydrogen orthophosphate monohydrate)], prepared by dissolving 6.09 gram in 50 ml, and mix. Allow the resulting solution to stand for 10 minutes to precipitate the colloidal ferric hydroxide. Dilute with water to volume, and mix. Centrifuge this solution at 3000 rpm for 15 minutes. Pass the resulting solution through a filter, discarding the first two ml of the filtrate.

The optical rotation of resulting solution was measured in 1 dm tube at 20°C on polarimeter and percentage of sucrose was calculated by this equation.

$$\% = (\alpha \times 50 \times 100) / (L \times M \times 66.47)$$

Were, α = observed rotation; L= length of polarimeter tube; M= volume in ml of sample or weight in gram of sample

Preparation of Calibration Curve (Linearity): Accurately weighed Sucrose standard were transferred to a volumetric flask to make a standard working solutions equivalent to 10, 20, 30, 40, 50, 60, and 70 mg per ml with water. Each solution was measured under the operating polarimeteric condition as described above and rotations were recorded. Calibration curves were constructed by plotting the rotation versus the concentration, and the regression equations were calculated. Each response was average of three determinations (**fig. 4**).

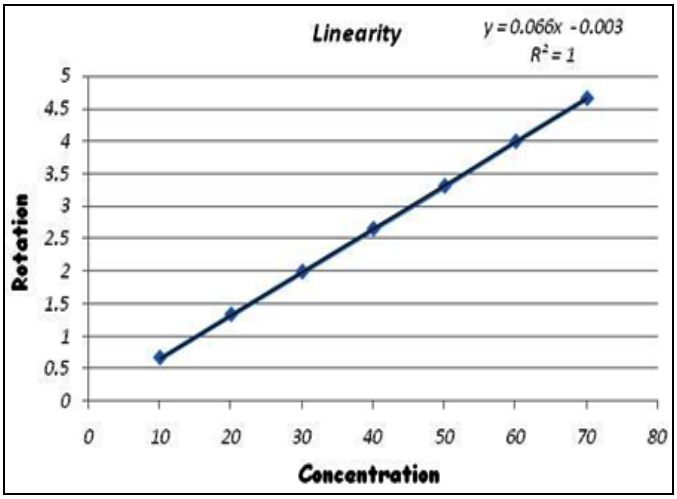

FIGURE 4: A GRAPH OF LINEARITY

Analysis of Marketed Formulation: SUCRONIR injection (Iron Sucrose Injection) (20 mg of elemental iron and 260-340 mg of Sucrose per ml) was taken in 50 ml volumetric flask. After treatment of sample as described above, the volume was made up to the mark with water to get final concentration. The optical rotation of resulting solution was measured in 1 dm tube at 20°C on polarimeter and percentage of sucrose was calculated by the equation. The analysis of procedure was repeated three times with SUCRONIR injection formulation.

Method Validation: The method was validated in compliance with ICH guidelines: Q2 (R1) [9] (**tables 1- 5**).

1. **Accuracy (Recovery study):** The accuracy of the method was determined by calculating the recoveries of Sucrose in Iron Sucrose by the standard addition method. Known amounts of standard solutions of Sucrose were at added at 80, 100 and 120 percent level to prequantified sample solutions of Sucrose. The amounts of Sucrose were estimated by applying obtained values to the respective regression line equations. At each level, three determinations were performed and results obtained.

2. **Method precision (Repeatability):** The precision of the instrument was checked by repeatedly observed rotation (n=6) of 300 mg per ml Sucrose solution in 1 dm tube at 20°C without changing the parameters.

3. **Intermediate precision (Reproducibility):** The Intraday and Interday precisions of the proposed method was determined by estimating the corresponding responses seven times on the same day and on seven different days over a period of one week for three different concentrations of standard solutions of Sucrose. The results were reported in terms of relative standard deviation (% RSD).

4. **Specificity:** The specificity of the method was checked for the interference of monobasic sodium phosphate solution in the analysis of a blank solution (without any sample) and then a drug solution of 300 mg per ml was observed on polarimeter, under optimized polarimeteric conditions. As there was no interference of monobasic sodium phosphate solution and also no change in the observed rotation at 20°C on polarimeter, the method was found to be specific and also confirmed with the results of analysis of formulation.

5. **Robustness:** To determine the robustness of the method, experimental conditions such as the concentration of the monobasic sodium phosphate, Temperature of sample solution (20^o & 25°C) and Laboratory change (QC 1 and QC 2 of Nirlife Healthcare) and the purity of sucrose were evaluated. No significant change was observed.

6. **Limit of detection (LOD) and Limit of quantification (LOQ):** The limit of detection (LOD) and Limit of quantification (LOQ) of the method were determined by standard deviation of response and slope method. Limit of detection (LOD) and limit of quantification (LOQ) were calculated as 3.3 ∂/S and 10 ∂/S, respectively as per ICH guidelines, where ∂ is the standard deviation of the response (y-intercept) and S is the slope of the calibration plot.

TABLE 1: THE RESULTS OF VALIDATION PARAMETERS AND SYSTEM SUITABILITY PARAMETERS

Validation Parameters	Results
Linearity range (n=5)	10-70 mg per ml
Equation	Y= 0.066x-0.003
R^2	1
Limit of detection (LOD)	0.076 mg per ml
Limit of quantification (LOQ)	0.231 mg per ml
Intraday precision (% RSD) (n=3)	0.033
Interday precision (% RSD) (n=3)	0.038
Repeatability (% RSD) (n=6)	0.017
Accuracy	100.01-100.04

RSD=Relative Standard Deviation

TABLE 2: RECOVERY STUDIES OF SUCROSE (N=6)

Level	Amount of sample taken (mg per ml of sucrose)	Amount standard spiked (%)	Mean % Recovery
I	50	80	100.04
II	50	100	100.02
III	50	120	100.01

TABLE 3: LINEARITY

Concentration	Observed Rotation
10	0.666
20	1.333
30	1.993
40	2.653
50	3.311
60	3.999
70	4.662

TABLE 4: ANALYSIS OF MARKETED FORMULATION

Brand name	Label claim	Amount found	% Assay ± SD (n=3)
SUCRONIR	260-340 mg of Sucrose per ml	334.1 mg of Sucrose per ml	100.02 ± 0.56

TABLE 5: COMPARISON OF ASSAY RESULTS BY DEVELOPED AND REPORTED METHOD

Brand name	% Assay ± SD by developed method	% Assay ± SD by reported method
SUCRONIR	100.02 ± 0.56	99.48 ± 0.85

Comparison with Reported RP-HPLC Method: The developed method was compared with reported RP-HPLC method by applying student-t Test (paired-t-test) to the assay result. The results of t-test revel that t-table value is more than t-cal value. This shows that there is no significant difference between two methods.

So both the methods, Polarimeteric and RP-HPLC, could be used for estimation of Sucrose in Iron Sucrose from Raw material, Inprocess Bulk formulation and Pharmaceuticals formulations.

CONCLUSIONS: The developed method was validated in terms of accuracy, repeatability, and precision. A good linear relationship was observed for Sucrose in the concentration ranges of 10–70 mg per ml. The correlation coefficient for Sucrose was found to be 1.0000. The inter-day and intra-day precision results were good enough to indicate that the proposed method was precise and reproducible.

The assay experiment showed that the contents of Sucrose estimated in the Raw material, Iron Sucrose Injection and Inprocess Bulk of pharmaceutical formulations were free from the interference of excipients. This demonstrated that the developed Polarimeteric method was simple, linear, precise, and accurate, and could be conveniently adopted for the routine quality control analysis of Sucrose in Raw material, Iron Sucrose Injection and Inprocess Bulk of pharmaceutical formulations simultaneously.

Simplicity, ease of operation and short time required for analysis makes the developed Polarimeteric method a useful tool for routine analysis of Sucrose in Bulk, Raw material as well as in pharmaceutical formulation.

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