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Original Article

Spectrophotometric Method for Quantitative Determination of Inulin in Naturolax–A Powder

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ABSTRACT

Objective : The aim of the current experimental work is to test spectrophotometric method based on resorcinol assay for analysis of inulin in one of the powder formulations made for relieving constipation viz. "Naturolax–A powder".To evaluate the application of this method for routine analysis, the precision and accuracy have to be defined. **Experimental approach:** Trials were carried out on "Placebo"," Plain" formulation with 80:20 mixture of only Isabgol:Inulin, and "Actual"- complete formula with 15%inulin – the product Naturolax A powder. The spectrophotometric method based on hydrolysis of inulin to fructose was used. Resorcinol in alcohol was used in specific acidic medium to form a complex.The color was developed at 80^{0}C for 25minutes. Then absorbance at 490nm was measured after cooling under tap water and diluting the complex to appropriate volume. Concentration of inulin was calculated by applying the formula. **Findings and Discussion :** Content was found to be in minor quantity in Placebo, whereas, around 19% and 14.50% in Plain, and Actual formulation – "Naturolax A" powder respectively. All these values are more than 90% of expected value in respective formulations.The proposed method was validated for its analytical performance parameters. The validated method is found to be precise and accurate for assay of inulin. Estimated values of inulin in three different lots of Naturolax–A powder were found to be in the range of95% to 105% (percentage of theoretical value).**Conclusion:** Hence, this method can be introduced into the routine quality analysis of Inulin in the mix powder formulation.

Keywords : Inulin, Spectrophotometry, Resorcinol, Naturolax – A powder.

1. INTRODUCTION

Now a day, people are suffering from various diseases. Imbalanced intake of food, increasing stress level, insufficient rest, irregularity in food intake, intake of unhealthy diet- especially one without enough fibers etc. ;are such factors that lead to "Constipation". Many medicines are available to overcome this complaint. However, people

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prefer medicines with natural origin to avoid side effects and the habit forming tendency. Popularly known and commonly used ingredients having purgative property are senna leaves, harda, isabgol husk, inulin and so on. Naturolax A is one such formulation in powder form, which mainly contains very popular Isabgol husk and Inulin as active ingredients. Isabgol swells up in water and swelling index gives desired medicinal effect. The safety [1]of inulin and oligofructose for use in foods was evaluated by many legal authorities worldwide. As a result, both inulin and oligofructose are accepted in most countries as food ingredients that can be used without restrictions in food formulations. Inulin is used in foods to improve organoleptic characteristics, to balance the nutritional value, to replace fats and to enrich products with dietary fibers. Lourencetti et al[2] carried out experiments on cookies. The partial replacement of fat by inulin in the production of cookies was effective in reducing the percentage of total lipids in the final product. Hence, inulin ingredient can be considered as a fat mimic, efficient and feasible for formulations of biscuits type cookies with good technological and nutraceutical properties. Similarly, Zomara et al[3] also proved in their study that fat can be replaced with inulin and pectin in frankfurter sausages to produce healthy and functional products. Inulin can be used in different food formulations other than pharmaceutical products too. Mansouripour et al [4] have developed a formulation and produced an acceptable prebiotic ketchup using inulin and GOS – Galactooligosaccharides. Konar et al[5] studied the effects of inulin as a prebiotic substance at various levels 0, 60, 90 and 120 g/kg in milk chocolates and found that the brightness remained unaffected in inulin containing milk chocolate.

Inulin is a starchy substance found in many fruits, vegetables and herbs. It is soluble fiber and fructo-oligo saccharide (FOS) that function as a prebiotic to promote digestive health.In the taxonomy of fibers, inulin is classified [6] as both “soluble” and “prebiotic”. It is a carbohydrate made up of 25 -30 D-fructofuranoseunits.[7]Interestingly, inulin is not digested or absorbed in the stomach. Instead it goes to the bowel where bacteria are able to use it to grow supporting bowel function and general health. It acts as “food” for the friendly bacteria that live in our large intestine and it activates their growth allowing them to flourish and multiply. Thus creating a healthy intestinal eco-system. The “friendly” bacteria break the inulin down into beneficial gases such as butyrate in our bowels which sometimes we pass out as “wind”.

Inulin is a generic term that covers all linear fructans with beta (2 – 1) fructosyl – fructose glycoside bonds. Because of the beta configuration of the bonds between fructose monomers, inulin – type fructans resist enzymatic hydrolysis by human salivary and small intestinal digestive enzymes-specific for alpha – glycosidic bonds. As a result, inulin type fructans are indigestible and are fermented in the colon[8].

In large concentrations, inulin occurs naturally in chicory roots. It is present inside the roots of plants as a means of storing energy and regulating the plants internal temperature. It has osmotically active properties. Inulin is predominantly isolated from chicory root.Mensinket al[9] have given three steps process of isolation of inulin from chicory in their review article.

Inulin has special role in the formulation meant for constipation. Hence quantification of inulin in formulation like“Naturolax A” is necessary.

PetkovaandDenev,[10] in their review article, have compared different analytical methods for determination of inulin in food products. They concluded the spectrophotometric, enzyme and HPLC methods as an excellent choice for answer to the rapid and specific method for analysis.

In the present paper, authors have worked on spectrophotometric resorcinol method for quantification of inulinin the formulation viz. Naturolax A powder. Reagents used for testing purpose are easily available. Inulin in acid medium is hydrolyzed to fructose, which transfers into the 5-hydroxymethyl furfural. Resorcinol reacts with it to give a condensed colored compound whose absorption is measured at the appropriate wavelength.

Proposed spectrophotometric method is validated for determining and maintaining the quality of the product Naturolax A.

2. MATERIALS AND METHODS

Samples: Plain sample with 80:20 Isabgol : Inulin, Actual – complete formulation with 15% Inulin and Placebo without inulin–Two different formulations with different concentrations of inulin and one sample of blend without Inulin were taken initially for developing the method. After developing the method, it was confirmed for routine testing by applying on three more final batches of the formulation.

Chemicals and Reagents: All the chemicals and reagents used in different processes were procured from M/s Qualigens.

Equipments and Instruments used : All the glasswares used were well calibrated and were procured from M/s Borosil Glass Works Ltd., Instruments used were Weighing Balance (M/s Schimadzu Corporation), Water Bath with temperature controller – Buchi 461 waterbath (M/s. Buchi Laboratortechnik),UV-Visible spectrophotometer (M/s Shimadzu). UV visible spectrophotometer was calibrated as per the pharmacopoeial[11] method to assure the precision and accuracy in the absorbance readings through out the exercise.

Methods: Content of Inulin was determined by spectrophotometric method.

Reagents :

1. 0.1% solution of Resorcinol in 95% alcohol.
2. Hydrochloric acid solution : This solution is prepared by adding 22.4cc of water to 100cc conc. Hydrochloric acid (36% pure)

Standard preparation: Weighed accurately about100mg of standard powder with more than90% purity, in 100ml volumetric flask, dissolved it in water and diluted to 100ml with water. After shaking well, diluted 5ml of the aliquot to 50ml, again from this well mixed stock, accurately pulled out 1.0ml of the aliquot in 20ml volumetric flasks for color development.

Sample preparation :Final formulation / Placebo : Weighed accurately about 500mg of homogenous, uniform sample of final formulation / 425mg of uniform placebo in individual 100ml volumetric flask, dissolveditin about 50ml distilled water, made the volume to 100ml with distilled water. Performed the occasional shaking for about 1 hour.Kept aside for another 1 hour to ensure complete dissolution.Filtered through ordinary filter paper.Diluted 5ml of the filtrate to 50ml with distilled water.Shakenwell.Removedaccurately1.00ml of the aliquot from corresponding diluted filtrate stock in two different 20ml volumetric flasks for color development.

METHOD: Added accurately about 2ml of 0.1% Resorcinol in 95% alcohol followed by 5ml of hydrochloric acid solution in all flasks containing respective aliquot of standard solution , sample solution, and placebo solution. Mixed well by shaking thoroughly and placed them in a water bath at 80^0C for exactly about 25 minutes, after which they were cooled in tap water for 3 minutes and diluted to the mark with water. Similarly carried out the blank, omitting the sample. The corresponding absorbance reading were recorded at 490nm on a previously calibrated suitable spectrophotometer against the blank solution.

Note: It is best to pour the reaction mixture in the cuvette at a distance from the spectrophotometer and to keep the cuvettes stoppered because the strong acid fumes may attack the spectrophotometer.

Percentage of Inulin in respective sample was calculated by applying dilution factors of standard and sample.

Calculation :

Content of Inulin in %w/w

=(Spl abs/Std abs) x(Stdwt g/100)x5/50x1/20x(100/Splwt g) x50/5x20/1x%purity/100 x 100

NOTE : Deduction of the % quantity obtained in "PLACEBO" from final sample calculation lead to the removal of all possible interferences and can give exact concentration of inulin in the final formulation. Again, application of moisture factor to the final calculation imparts accuracy and consistency to the final estimation.

Precautions :

- Since the determination of inulin by the Resorcinol reaction depends upon the hydrolysis of inulin to fructose, the strength of acid employed in the hydrolysis and the time and temperature of heating will all be critically important.
- Although the red color follows the Beer Lambert's law rather closely in concentration up to 2mg/100cc, the slope of the curve decreases somewhat variably with greater concentrations. (Hence, 0.50, 1.00, 2.00 cc /100cc dilutions are recommended)

Validation Parameters: Developed method was validated by considering the parameters such as Precision, Linearity, Range and Accuracy.

3. RESULTS AND DISCUSSIONS

For initial development of method,"Plain" formulation with extra inulin content i.e. mixure of 80:20 - Isabgol husk: Inulin excluding other ingredients, "Placebo" – excluding inulin and the actual product"Naturolax A"with complete formula having active as well as inactive ingredients were considered as listed in table 1.

Table1 :Composition of Plain, Placebo, and Naturolax A-formulations

Sr. No.	Ingredients	Plain formula	Placebo	Naturolax A
		Each 100g contains	Each 100g contains	Each 100g contains
1	Isabgol Husk 85%	80.00g	67.50g + 9% overages	52.50g + 9% overages
2	Inulin powder	20.00g	Nil	15.00g
3	Other ingredients	Nil	Saccharin Sodium, Sucrose, Nimbukamlam, Orange DC 116PH, Sodium Bicarbonate, Orange oil 700	Saccharin Sodium, Sucrose, Nimbukamlam, Orange DC 116PH, Sodium Bicarbonate, Orange oil 700

The spectrophotometric method was applied to estimate the content of inulin in all these three trial formulations. API used in the formulation with 93% of inulin was used as a working standard. The reference standard received from Sigma Aldrich was also tried on three regular final lots. Results of the duplicate trial formulations were recorded in table 2.

Table 2: Findings of two experimental trials on three trial formulations.

Sr. No.		Placebo		Plain formulation (80:20-Isabgol:Inulin)		Naturolax A (Actual – complete formulation)	
		Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
1	Loss On drying at 105^0C in %w/w	5.02	4.97	6.35	6.42	7.18	7.00
2	Actual as is content of Inulin in %w/w	2.49	2.50	20.89	20.53	16.16	16.15
3	Corrected content of Inulin in %w/w	NA	NA	18.40	18.03	13.67	13.65
				92% of actual value	90.15% of actual value	91.13% of actual value	91.00% of actual value
4	Actual content of Inulin on dry basis in %w/w	2.62	2.63	19.64	19.26	14.72	14.67
				98.20% on dry basis	96.30% of on dry basis	98.10% on dry basis	97.80% of actual value

Corrected value of inulin content was calculated by deducting content of percentage inulinin placebo from the percentage inulin content obtained in corresponding actual samples on as is basis. For more perfect and consistent results, corrected content was calculated on dry basis by applying respective loss on drying in percentage w/w. Resultant inulin content was found to be around 91%on as is basis and around 97%on dry basis of expected value.

This method was then validated for its analytical performance parameters.

Validation of Method:

The method was validated by considering the parameters such as Precision, Linearity, Range and Accuracy.

Precision (repeatability) :

The precision of the instruments was checked by repeatedly analyzing (n=6) standard solution 5 ppm. The results are reported in terms of relative standard deviation (RSD). Six individual samples were prepared and injected. The results are reported in terms of relative standard deviation (RSD).Refer table (3)

Linearity and Range :

Five solutions of different concentrations of Inulin (2.5, 3.75, 5.0, 6.25, 7.5 ppm) were analysed. First and last level were calculated for Range . Regression equation and co-efficient of correlation (r2) was derived.Refer table (3)

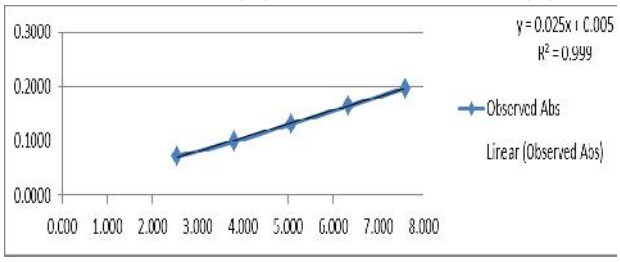

Fig 1: Linearity graph for Inulin

Accuracy :

The method was found accurate over the range of 50% to 150% of test concentration of Assay for Inulin. For detailed results,refertable (3)

Table3: Validation parameters

Parameters	Descripton	Acceptance Criteria	Results
Linearity	Coefficient of correlation	Not less than 0.995	0.999
	% y-intercept	Not more than 3.0%	0.005%
Precision	RSD for Assay values of precision study	Not more than 2.00%	1.48%
Accuracy	% Individual recovery for each accuracy level	90.0% to 110.0%.	50% Level: 106.97%
			100% Level: 109.27%
			150% Level: 106.90%
	% Average Recovery	90.0% to 110.0%.	107.71%
	% RSD for recovery at each level and overall % RSD for recovery	NMT 2.0%.	1.57%

The developed method was applied to three more lots of final formulation - Naturolax A powder for confirming the accuracy and precision.

Results of three lots were systematically reported in table 4.

Table 4: Content of Inulin in Naturolax A powder

Name of the sample	Lot No	LOD at 105^0 C %w/w	Weight taken (g)	1st Dilution	2nd Dilution	Purity In %	Std. abs.	Placebo abs.	Placebo % interference
Inulin standard	Ref Std of Inulin from Sigma		Std0.1011 Placebo 0.425	5ml → 50ml	1ml → 20ml	99.98	0.195	0.0210	2.560
Name of the sample	Lot No		Weight taken (g)	1st Dilution	2nd Dilution	Abs	Actual As is Assay (%)	Corrected Assay (%)	Assay % of actual value
Naturolax A	Lot I	7.02	0.5011	5ml → 50ml	1ml → 20ml	0.1720	17.79	15.23 16.38*	101.53 109.20*
Naturolax A	Lot II	7.10	0.4997	5ml → 50ml	1ml → 20ml	0.1600	16.59	14.03 15.10*	93.56 100.66*
Naturolax A	Lot III	6.98	0.4997	5ml → 50ml	1ml → 20ml	0.1550	16.08	13.52 14.53*	90.11 96.86*
								Mean	95.07
								STDV	5.86
								%RSD	6.16

NOTE :* Percentage values on dry basis.

Findings of all the three batches were found to be satisfactory. Results were in the range of 13.52%w/w to 15.23%w/w on as is basis, whereas, in the range of 14.53%w/w to 16.38%w/w on dry basis. Since the formula was set for 15% inulin the range of percentage value with respect to actual value was found to vary from 90.11% to 101.53% and 96.86 % to 109.20% on as is basis and on dry basis respectively.

Mensinket al[12] have given different pharmaceutical applications of inulin. Insoluble isoforms of inulin have an adjuvant effect on the immune response achieved with several vaccines. Inulin could therefore serve two purposes as an excipient for vaccines, achieving both stabilization and an increased effectivity.

Kathy Niness [13] had reported the content of inulin and oligofructose about 15 – 20% and 5-10% respectively in the root of the Cinhoriumintybus plant. Recommended its wide use in functional foods throughout the world for their health promoting and technological properties.

4. CONCLUSIONS

Inulin used in the formulation can be quantified accurately by adopting spectrophotometric technique. Spectrophotometric method based on Resorcinol assay is found to be easy to carry out, less expensive, accurate and precise. Hence can be recommended for its application to estimate the inulin content in Naturolax A powder to control the quality of the product.

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