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ANALYTICAL METHODS TO ESTIMATE THE AMOUNT OF ADDITIVES IN CONFECTIONERIES

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ABSTRACT The objective of the study is to develop simple, accurate and precise analytical method for the estimation of sucralose, which is used as an artificial sweetener. The method was developed using UV-Visible spectrophotometer. UV-Visible spectrophotometric method using calibration curve, was established for the estimation. The developed method were validated according to ICH guidelines. The purity of drug was checked by using FTIR spectroscopy. Sucralose show better solubility in methanol. Sucralose is estimated using Romini's reagent which is a mixture of sodium nitroprusside, ZnCl2 and acetone. Romini's reagent in reaction with sucralose produces a compound which has max at 291.5nm. This method showed linearity within the range of 2-24μg/ml. Correlation coefficient was found to be 0.9995.The developed method were validated for linearity, accuracy, precision, intra-day and inter- day precision, limit of detection and limit of quantification. The method is simple, linear, precise, accurate and suitable for the estimation of sucralose. In conclusion, using these developed analytical method, analysis of the sucralose can be done accurately in a short time with low cost and without prior extraction.

KEYWORDS : Sucralose, FTIR, UV-Visible spectrophotometer, Method development, validation.

INTRODUCTION

Food additives are substances that are added to preserve or enhance it's freshness, safety, flavor, texture, or appearance[1]. Some food additives, including salt (in meals like bacon or dried fish), sugar (in marmalade), or sulfur dioxide (in wine), have been used for food preservation for hundreds of years. The use of food additives is only acceptable where there is a technological requirement, there is no consumer misinformation, and the additives serve a clearly defined technological purpose, such as maintaining the product's nutritional value or improving it's stability.

Anti-Caking Agents

Anticaking agents are additives added to powdered or granulated products, like table salt or confections, to stop the formation of lumps (caking) and to improve flow ability, packing, shipping, and consumer convenience[2].

Preservatives

With the goal to stop things from decomposing due to microbial growth or unfavorable chemical changes, preservatives are substances or chemicals that are added to products including food, drinks, pharmaceutical medications, paints, biological samples, cosmetics, wood, and many more products[3].

Example: sorbic acid, sodium sorbate, sorbate

Emulsifiers

Emulsifiers are food additives that the Food and Drug Administration has allowed. They aid in the integrating of goods that contain immiscible food ingredients, such as oil and water[5].

Artificial Sweeteners

Artificial sweeteners are synthetic, calorie-free sweeteners that have a potent sweetening flavour. They are primarily present in dairy products, sugar-free chocolates, soft drinks, and snack items[11]. They are frequently referred to as "intense sweeteners" because they offer a flavour that is comparable to table sugar but up to a thousand times sweeter. Despite the fact that some sweeteners have calories, the quantity required to sweeten items means that you end up eating essentially no calories. Artificial sweeteners are used by the food industry as an alternative to added sugars, which are now known to have harmful effects on a number of chronic conditions. Most commonly used artificial sweeteners are aspartame, acesulfame-K and sucralose. Our objective was to develop an analytical method estimate the amount of sucralose in confectioneries.

Chemical Structure Of Sucralose

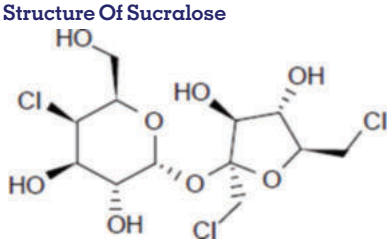

Fig 1: Chemical Structure Of Sucralose

Reagents and Chemicals
Sucralose from KanBo chemicals in Delhi, Sodium nitroprusside, Zinc chloride and Acetone
From Classic chemicals in Hyderabad

Instrumentation
For analytical method development and validation of Sucralose, a SHIMADZ model Pharma spec- 1700UV- VIIBLE Spectrophotometer (double beam) with software system (UV probe) was utilized. Electronic Balance by Tandem TJ series. FTIR by Bruker ATR with Alpha interferometer attached OPUS Software.

Identification Using FTIR Spectroscopy
FTIR was scanned from 400-4000 cm^{-1}. Spectrum was used for the identification of drugs.

Method Development
Preparation of Standard Stock Solution (1000μg/ml)
An accurately weighed quantity of sucralose (50mg) were transferred to a separate 50 ml standard flask. Methanol is used as the dissolving agent for sucralose. Then the volume was made up to the mark with methanol to get the solution having a concentration of 1000μg/ml. And the solution is used as the first stock. From that further dilutions are carried out.

Preparation of Working Standard Solutions
From the above prepared stock solutions of sucralose 1ml were transferred separately to 10 ml volumetric flask to obtain working standard solutions having a concentration of 100μg/ml.

Selection of Wavelength Range for Estimation
Appropriate amount of sucralose were dissolved in methanol and suitable dilutions of sucralose were prepared by taking aliquots from the stock solution. To this, 110μl of sodium nitroprusside, 16μL of 0.1% ZnCl2, 1 ml of acetone were added, and the volume was made up to the mark using methanol[23].The solution were scanned from 200-400nm and from that wavelength ranges are selected for the estimation of sucralose.

Preparation of Calibration Curve
From the above working standard solution of sucralose (0.2, 0.6, 1, 1.4, 1.8, 2, 2.4 ml) aliquots were transferred separately in a series of 10 ml volumetric flask. To each flask, 110μL of sodium nitroprusside, 16μL of 0.1% ZnCl2, 1 ml of acetone were added, and the volume was made up to the mark using methanol to get the working sample of 2-24μg/ml. The absorbance of all the solutions were calculated by scanning from 200-400nm, against methanol as the blank.

Preparation of Reagent
Sodium nitroprusside solution- 1g of sodium nitroprusside was dissolved in 10 ml of distilled water. Zinc chloride solution- 0.1g of zinc chloride was dissolved in 100 ml of distilled water.

Methodology
The working sample solutions of sucralose were scanned in UV from the range of 200- 400nm were it shows 291.5 nm as the wavelength having maximum absorbance. And this wavelength is selected for the quantitative estimation of sucralose.

Method Validation
As per ICH Q2 (R1) guidelines the method was validated for different parameters: accuracy, precision, linearity, range.

Linearity
The linearity of the method was checked in the concentration range of 2-24 μg/mL for sucralose. The calibration curves were constructed by plotting the graph of absorbance versus concentration. The linear Regression equation was obtained over the concentration range (y=mx+c).

Range
The range is the interval between the upper and lower concentration of the analyte for which it has been demonstrated that the analytical method has a suitable level of precision, accuracy, and linearity. The range for the method was observed in a concentration of sucralose (2-24μg/mL). For the evaluation of the range accurately, measured standard working solutions of sucralose were prepared.

Precision
The precision of the instrument was checked by repeated scanning and measuring the absorbance of the solution of (n = 6), sucralose (6 μg/mL) without changing the parameters of developed methods.

Reproducibility
The intraday and interday precision was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days over a period of 1 week for 3 different concentrations of standard solutions of sucralose (4.8,6,7 μg/mL). Relative standard deviation (% RSD) was used to report the results.

Accuracy (% Recovery)
Accuracy can be reported in terms of % recovery. The percentage spiking levels are 80,100 and 120%, About 8 μg/mL of CLOMI and 3 μg/mL of MELA were used for the study.

Limit of Detection and Limit of Quantification (LOD & LOQ)
The limit of detection (LOD) and the limit of quantification (LOQ) of the drug was derived by calculating the signal-to-noise ratio (i.e.,3.3 for LOD and 10 for LOQ)using the following equation designated by the International Conference on Harmonization(ICH) guidelines.

$LOD = 3.3 \times \sigma/S$

$LOQ = 10 \times \sigma/S$

Where, σ = the standard deviation of the response S = slope of the calibration curve

RESULTS
Identification of Drugs by IR Spectroscopy
FTIR was scanned from 400-4000 cm^{-1}.Spectrum was used for the identification of drugs

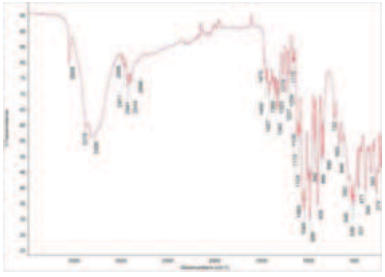

Fig 2: FT-IR Spectrum of Sucralose

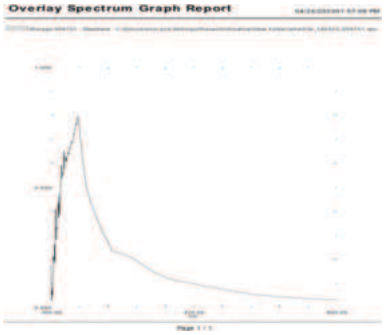

Fig 3: Spectrum of Reagents in Methanol

Method Validation

Linearity

Different concentrations were made. 2,6,10,14,18,20,24 μg/ml of sucralose were used to collect the absorbances of each solution at their respective λmax, then a calibration curve was generated from the collected data.

Table 1: Calibration Data of Sucralose at 291.5 nm

SL.NO	Concentration μg/ml	Absorbance at 291.5 nm
1	2	0.126
2	6	0.126
3	10	0.201
4	14	0.244
5	18	0.285
6	20	0.306
7	24	0.346

Fig 4: Linearity of Sucralose

Accuracy

Here the recovery results indicate the accuracy of the proposed method. The accuracy was calculated by recovery studies in various levels.

Table 2: Data of Accuracy

Accuracy Level %	Actual Amount (μg/ml)	Amount Added (μg/ml)	Amount Found (μg/ml)	% Re-covery	Mean ±SD	% RSD
80%	3	2.4	5.32	98.5	99.5133 ± 0.8167	0.8207
100%	3	3	6.03	100.5		
120%	3	3.6	6.57	99.54		

Table 3: Repeatability

Concentration (n=6)	Absorbance (291.5 nm) (Sucralose 6μg/ml)
1	0.161
2	0.162
3	0.163
4	0.163
5	0.162
6	0.165
MEAN	0.1626
SD	0.001247
% RSD	0.7667

Table 4: Reproducibility

Concen-tration (μg/ml)	Intraday (n=3)		Interday (n=3)	
	Absorbance Mean± SD	%RSD	Absorbance Mean± SD	%RSD
4.8	0.152±0.000471	0.30945	0.153±0.000523	0.06199
6	0.164±0.000481	0.28802	0.163±0.000141	0.08628
7	0.175±0.000816	0.4692	0.174±0.000408	0.23462

Limit Of Detection and Limit Of Detection

According to ICH guidelines, there are several methods for the determination of LOD and LOQ. In the present study, the LOD and LOQ were calculated by the equation.

The LOD and LOQ of sucralose were found to be 1.4μg/ml and 4.24μg/ml

CONCLUSION

Simple as well as precise analytical method was developed for the estimation of sucralose in confectioneries. The method was developed using UV-Visible spectrophotometer. Methanol was the diluting solvent. The absorption maxima of sucralose was found to be 291.5nm. The method developed for the estimation of sucralose shows linearity from 2-24μg/ml and a correlation coefficient of 0.9995. The developed spectroscopic method was validated for linearity, accuracy, method precision, intra-day and inter-day precision, limit of detection and limit of quantification. The method is simple, linear, precise, accurate and suitable for estimation of sucralose in confectioneries. In conclusion, using this developed analytical methods, analysis of sucralose can be run fast with low cost and without prior extraction or losing accuracy.

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