IJBPAS, June, 2025, 14(6): 3514-3530

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

METHOD DEVELOPMENT AND VALIDATION BY UV SPECTROSCOPY FOR ESTIMATION OF ARTIFICIAL SWEETENERS IN LOCALLY AVAILABLE MARKETED SOFT DRINKS & PACKED FRUIT JUICES

POOJITHA G[1], HARSHITHA REDDY B[2], SWETHA SRI R[3*] AND T SIRI[4]

[1]Research Student, Sarojini Naidu Vanita Pharmacy Maha Vidyalaya, Osmania University, Tarnaka-500017, Secunderabad, Telangana, India

[2]Research Student, Sarojini Naidu Vanita Pharmacy Maha Vidyalaya, Osmania University, Tarnaka-500017, Secunderabad, Telangana, India

[3]Assistant Professor, Department of Pharmaceutical Analysis Sarojini Naidu Vanita Pharmacy Maha Vidyalaya, Osmania University, Tarnaka- 500017, Secunderabad, Telangana, India

[4]Research Student, Sarojini Naidu Vanita Pharmacy Maha Vidyalaya, Osmania University, Tarnaka-500017, Secunderabad, Telangana, India

***Corresponding Author: R. Swetha Sri: E Mail: sweety.rss@gmail.com**

Received 15th March 2024; Revised 20th April 2024; Accepted 11th Aug. 2024; Available online 1st June 2025

https://doi.org/10.31032/IJBPAS/2025/14.6.9139

ABSTRACT

Low-calorie sweeteners, commonly found globally, are blended together to balance negative flavors. International regulations demand constant monitoring by regulatory authorities and food safety labs of products allowed under different permitted sweetener lists across countries. Identification and quantification of sweeteners require analytical methods capable of affirming analyte identity. These are some examples: Saccharin, acesulfame potassium, aspartame, neotame, and sucralose are various types of sweeteners, which are determined by UV and FTIR as well as by the linearity (Saccharin 50-400 µg / ml at 266 nm; Aspartame 60-720 µg / ml at 256 nm;

Sucralose 50 to 400 µg /ml at 260 nm), LOD (Saccharin 5.58 ppm; Aspartame 33.95 ppm; Sucralose 13.5 ppm), LOQ (Saccharin 16.9 ppm; Aspartame 102.8 ppm; Sucralose 41.04 ppm), Range (Saccharin 0.495; Aspartame 1.34; Sucralose 2.87) Precision (Saccharin (0.60 & 0.26); Aspartame (1.96 & 1.911); Sucralose (1.87 & 1.95)), Robustness (Saccharin (265 at 0.71 & 267 at 0.43); Aspartame (at 255 nm is 1.65 & 257 nm is 1.62); Sucralose (259 nm is 0.97 & 261 nm is 0.97), Ruggedness (Saccharin 150µg / ml is 0.43; Aspartame 360 µg / ml is 1.895; Sucralose 350 µg / ml is 0.65). And the quantification of sweeteners was done by Beer Lamberts Law, which identified that they were within the limits for marketed products.

Keywords: non-nutritive sweeteners, foods, UV-spectroscopy

INTRODUCTION ON NON-NUTRITIVE SWEETENERS

To add sweetness without the calories, many foods replace natural sugars with artificial sweeteners **[1]**. This category encompasses numerous food additives crucial for imparting technological advantages, such as sweetening **[2]**. These two categories serve as the foundation for comprehending sweeteners as a whole. Texture and preservation qualities come with added benefits in these reduced-calorie options through natural ingredients. Quantum satis certification indicates zero limits on sugar alcohol safety. Intensely sweet products boast greater sugar- reduction capabilities than traditional sucrose. From completely manufactured to partly derived through biological means, these categories represent various sources **[3]**.

Such fake sugars comprise ASP, SCL, SAC, CYC, ACS-K, ALI, NEO, and DUL. While STV and REB A exhibit inherent nature, NHDC holds distinctive semi-artificial traits **[4]**. From one continent to another, each land has a singular compilation of permitted sweets **[5]**. An illustration here lies in CYC and NHDC, which are allowed per the EU but rejected by the US Food and Drug Administration (FDA) **[6]**. Yang, D.J., and Chen, B., described Simultaneous determination of nonnutritive sweeteners in foods by HPLC/ESI-MS method **[5]**, whereas work reported by Wasik, A ***et al*** was simultaneous determination of 9 intense sweeteners in food by using HPLC and Evaporative light scattering detection **[2]**. The Analysis of 9 artificial sweeteners by HPLC-MS/MS method was enumerated in an article published by Lim, H.S *et al* **[6]**. A few more HPLC/MS methods were reported for Analysis of nonnutritive sweeteners **[7–9]**. Pól, J., Hohnová, B., and Hyötyläinen, T., reported comprehensive Two- dimensional liquid chromatography time- of-flight mass

spectroscopy for Characterisation of *Stevia rebaudiana* **[10]**. From the literature, it has been studied that HPLC/ MS method was used for the determination of artificial sweeteners in foodstuffs. So, this article enumerates a UV spectroscopy method for the determination of artificial sweeteners in locally available, marketed soft drinks and packed fruit juices.

MATERIALS AND METHOD

Chemicals

Methanol, Acetic acid, and Ethanol were purchased from Avantor Performance Materials Private Limited; Ethyl acetate was purchased from Moly Chem and P-Nitro Benzoyl Chloride was purchased from Sigma Aldrich.

Instruments

UV-visible spectrophotometer (UV 1800 240 v) Shimadzu and (UV 3200) Lab India, FTIR (Alpha) Bruker, Cyclomixer (Cm 101) Remi Equipment, Sonicator (2.2517h) Bio Technics India was used for sonication, and the Digital Weighing Balance (Infra Digi) Roy instrument.

Method Saccharin

Preparation of Standard Curve

In a 100ml volumetric flask, the weighed quantity of 0.0269mg of saccharin was taken and diluted with water; further, it was sonicated for 15 minutes, and the volume was made up to the mark with water. Thus, 1000 ppm of stock solution was prepared. In 10 ml of a volumetric flask, a 0.5 ml sample was taken from a starting concentration of 50 ppm. The greatest absorption was seen at 266 nm when water at 1000 ppm was scanned between 200-400 nm. Saccharin-containing water solutions of 50, 75, 100, 125, 150, 175, and 200 ppm were created. These solutions absorbance was measured at 266nm. Concentration data and absorbance data were plotted accordingly on the X-axis and Y-axis to obtain a calibration curve.

Qualitative Analysis by FTIR

FTIR was activated and waited for initialization to finish. After initialization, the opus software was launched, and measurement parameters were set up. Methanol was used to clear the FTIR disc and tip of the bulb. 16 scans were done in order to assess the background measurement. After placing the sample on the disc, the scan measurement for 16 scans was initiated. The sample needs to be clear and thin enough to let infrared light travel through it. By measuring the amount of infrared light absorbed by various molecular vibrations inside the sample, the scan measurement captures the spectrum of the sample. Utilising the opus programme, acquire the spectrum and assess it for peak picking. Based on the distinctive peaks, the spectrum was identified as

saccharin.

Aspartame

Preparation of the Standard Curve

In a 100 ml volumetric flask, the weighed quantity of 72mg of aspartame was taken and diluted with methanol and acetic acid; further, it was sonicated for 15 minutes, and the volume was made up to the mark with water. Thus, 720 ppm of stock solution was prepared. [720ppm]. In 10 ml of a volumetric flask, a 0.83 ml sample was taken from a starting concentration of 60 ppm. The greatest absorption was seen at 256 nm when the methanol and acetic acid mixture at 720 ppm was scanned between 200-400nm. Aspartame containing methanol and acetic acid mixture solutions 60, 120, 180, 240, 300, 360, 420, 480, 560, 600, 660, and 720 ppm were created. These solutions absorbance was measured at 256nm. Concentration data and absorbance data were plotted accordingly on the X-axis and Y-axis to obtain a calibration curve.

Qualitative Analysis by FTIR

FTIR was activated and waited for initialization to finish. After initialization, the opus software was launched, and measurement parameters were set up. Methanol was used to clear the FTIR disc and tip of the bulb. 16 scans were done in order to assess the background measurement. After placing the sample on the disc, the scan measurement for 16 scans was initiated. The sample needs to be clear and thin enough to let infrared light travel through it. By measuring the amount of infrared light absorbed by various molecular vibrations inside the sample, the scan measurement captures the spectrum of the sample. Utilising the opus programme, acquire the spectrum and assess it for peak picking. Based on the distinctive peaks, the spectrum was identified as aspartame.

Sucralose

Preparation of Standard Curve

By being treated with p-nitro benzoyl chloride, sucralose was transformed into a significantly ultraviolet (UV)-absorbing derivative with strong absorption at 260 nm. Samples that had been homogenised were diluted and cleaned, and before the residue was derivatized, the eluate was evaporated to dryness. The sucralose derivative was then purified using hexane-ethyl acetate and eluted with acetone, and UV was performed. These solutions absorbance was measured at 260nm. Concentration data and absorbance data were plotted accordingly on the X-axis and Y-axis to obtain a calibration curve. The linear curve was plotted with a concentration range of 50 ppm to 400 ppm.

Qualitative Analysis by FTIR

FTIR was activated and waited for

initialization to finish. After initialization, the opus software was launched, and measurement parameters were set up. Methanol was used to clear the FTIR disc and tip of the bulb. 16 scans are done in order to assess the background measurement. After placing the sample on the disc, the scan measurement for 16 scans was initiated. The sample needs to be clear and thin enough to let infrared light travel through it. By measuring the amount of infrared light absorbed by various molecular vibrations inside the sample, the scan measurement captures the spectrum of the sample.

Extraction Procedures for Working Samples

Drink-1: In a separating funnel of 250 ml, 100 ml of drink-1 was mixed with 100 ml of ethanol and acetic acid in an 80:20 ratio. Then the mixture was blended for 20 minutes to mix well. After shaking, the separating funnel was kept aside for 24 hours to let the layers separate. As time passes, the less dense organic layer will get separated from the water-based layer. Once 24 hours had elapsed, the aqueous layer formed in the separating funnel was gently decanted away without disturbing the organic layer. Then the aqueous layer obtained was kept in an airy place for 24 hours to evaporate residual solvents (acetone, ethanol, and acetic acid). The dried, extracted layer was then scraped off and placed in an evaporating dish using a spatula. Methanol and acetic acid were added to the collected material. As no reference was given about how much alcohol and acid to add in, this mixture was undecided about which proportion or amount it should be added to. From

the prepared sample, 1 ml was pipetted out and transferred into a 10 ml volumetric flask, and the volume was made up by adding ethyl alcohol and acetic acid, which diluted the 1 ml prepared sample. The contents of the volumetric flask were well mixed by shaking.

Drink-2: In a separating funnel of 250 ml, 100 ml of drink-2 was mixed with 100 ml of ethanol and acetic acid in an 80:20 ratio. Then the mixture was blended for 20 minutes to mix well. Then the separating funnel was kept aside for 24 hours to let the layers separate. As time passes, the less dense organic layer will be separated from the denser aqueous phase. After 24 hours, the aqueous layer formed in the separating funnel was decanted without disturbing the organic layer. The obtained aqueous layer was later dried at room temperature for 24 hours to separate the residual solvents (ethyl alcohol and acetic acid). The dried, extracted layer was then scraped off and placed in an evaporating dish using a spatula. The sample was then treated

with ethyl alcohol and acetic acid. As no reference was given about how much alcohol and acid to add in, this mixture was undecided about which proportion or amount it should be added to. From the prepared sample, 1 ml was pipetted out and transferred into a 10 ml volumetric flask, and the volume was made up by adding ethyl alcohol and acetic acid, which diluted the 1 ml prepared sample. 1 ml of this diluted solution was pipetted out and transferred into a 10 ml volumetric flask, and its volume was made up to the mark. The contents of the volumetric flask were well mixed by shaking.

Drink-3: To a separating funnel of 250 ml, 100 ml of drink-3 and 100 ml of ethyl acetate were added. Then the mixture was blended for 20 minutes to mix well. After shaking, the separatory funnel was kept aside for 24 hours, allowing the layers to settle. As time passes, the less dense organic layer will separate from the aqueous layer. After 24 hours, the aqueous layer formed in the separatory funnel was carefully drained off without disturbing the aqueous layer. From the above- extracted sample, 1 ml was pipetted out and poured into a 10 ml volumetric flask. Ethyl acetate was added to the volumetric flask to bring the volume to 10 ml and dilute the 1 ml sample. The contents of the volumetric flask were well mixed by shaking.

Drink-4: To a separating funnel of 250 ml, 100 ml of drink-3 and 100 ml of ethyl acetate were added. Then the mixture was blended for 20 minutes to mix well. After shaking, the separatory funnel was kept aside for 24 hours, allowing the layers to settle. As time passes, the less dense organic layer will separate from the aqueous layer. After 24 hours, the aqueous layer formed in the

separatory funnel was carefully drained off without disturbing the aqueous layer. From the above- extracted sample, 1 ml was pipetted out and poured into a 10 ml volumetric flask. Ethyl acetate was added to the volumetric flask to bring the volume to 10 ml and dilute the 1 ml sample. The contents of the volumetric flask were well mixed by shaking.

Drink-5: To a separating funnel of 250 ml, 100 ml of drink-3 and 100 ml of ethyl acetate were added. Then the mixture was blended for 20 minutes to mix well. After shaking, the separatory funnel was kept aside for 24 hours, allowing the layers to settle. As time passes, the less dense organic layer will separate from the aqueous layer. After 24 hours, the aqueous layer formed in the separatory funnel was carefully drained off without disturbing the aqueous layer. From the above- extracted sample, 1 ml was pipetted out and poured into a 10 ml volumetric flask. Ethyl acetate was

added to the volumetric flask to bring the volume to 10 ml and dilute the 1 ml sample. The contents of the volumetric flask were well mixed by shaking.

Drink-6: To a separating funnel of 250 ml, 100 ml of drink-6 and 100 ml of ethanol were added. Then the mixture was blended for 20 minutes to mix well. After shaking, the separatory funnel was kept aside for 24 hours, allowing the layers to settle. As time passes, the less dense organic layer will separate from the aqueous layer. After 24 hours, the aqueous layer formed in the separatory funnel was carefully drained off without disturbing the aqueous layer. From the above- extracted sample, 1 ml was pipetted out and poured into a 10 ml volumetric flask. Ethanol was added to the volumetric flask to bring the volume to 10 ml and dilute the 1 ml sample. The contents of the volumetric flask were well mixed by shaking.

Validation Parameters of UV Linearity

The linearity was determined by using the diluted concentrations of saccharin (50ppm, 75ppm, 100ppm, 125ppm, 150ppm, 175ppm, 200ppm), aspartame (60ppm, 120ppm, 180ppm, 240ppm, 300ppm, 360ppm, 420ppm, 480ppm, 540ppm, 600ppm, 660ppm, 720ppm), and sucrose (50ppm, 100ppm, 125ppm, 150ppm, 200ppm, 250ppm, 300ppm, 350ppm, 400ppm).

LOD (Limit of Detection) and LOQ (Limit of Quantification)

Response of standard deviation and is determined by the linearity slope. The detection limit (DL) can be written as: **DL = 3.3 σ/S**, where σ is the response's standard deviation and S is the calibration curve's slope. The LOQ can be written as **LOQ = 10 σ/S**, where σ is the response's standard deviation and S is the calibration curve's slope. The slope is calculated by the analyte calibration curve.

Precision

The precision was performed by measuring the absorbance of the samples: saccharin (100 ppm) in 6 replicates, aspartame (240 ppm) in 6 replicates, and sucralose (100 ppm) in 6 replicates.

Robustness

Robustness was calculated by making slight changes in wavelength for saccharin at 265 nm & 267 nm, aspartame at 255 nm & 257 nm, and sucrose at 259 nm & 261 nm.

Ruggedness

Ruggedness of saccharin, aspartame and sucralose was estimated by using different analysts and different instruments.

RESULTS AND DISCUSSIONS

After preparing standard solutions of saccharin, aspartame, and sucralose, the λ_{max} of the corresponding standard solutions from

which the values are derived is created. The FTIR was used for performing a qualitative examination of the samples and standard solutions by obtaining spectra from the solutions, interpreting the spectra of various functional groups, and analysing the samples. The linearity, precision, robustness, and ruggedness of the quantitative analysis were assessed once the maximum of the standard solutions had been obtained. Following sample extraction, sample solutions were made, diluted, and the UV absorbance was measured. and determined to what extent the sample's sugar content complies with the limits.

Linearity

For saccharin 50, 75, 100, 125, 150, 175, 200 ppm solution in water was prepared, and the absorbance of these solutions was measured at 266 nm, for aspartame 60, 120, 180, 240, 300, 360, 420, 480, 540, 600, 660, 720 ppm solution in methanol and acetic acid (80:20) was prepared, and the absorbance of the solutions was measured at 256nm and for sucralose 50 ppm to 400 ppm of a solution in a para-nitro benzoyl chloride derivative was prepared, and the absorbance of the solutions was measured at 260nm. Concentration data and absorbance data were plotted accordingly on the X-axis and Y-axis to obtain a calibration curve. The linearity value is mentioned in

Table 1.

Qualitative Analysis by FTIR:

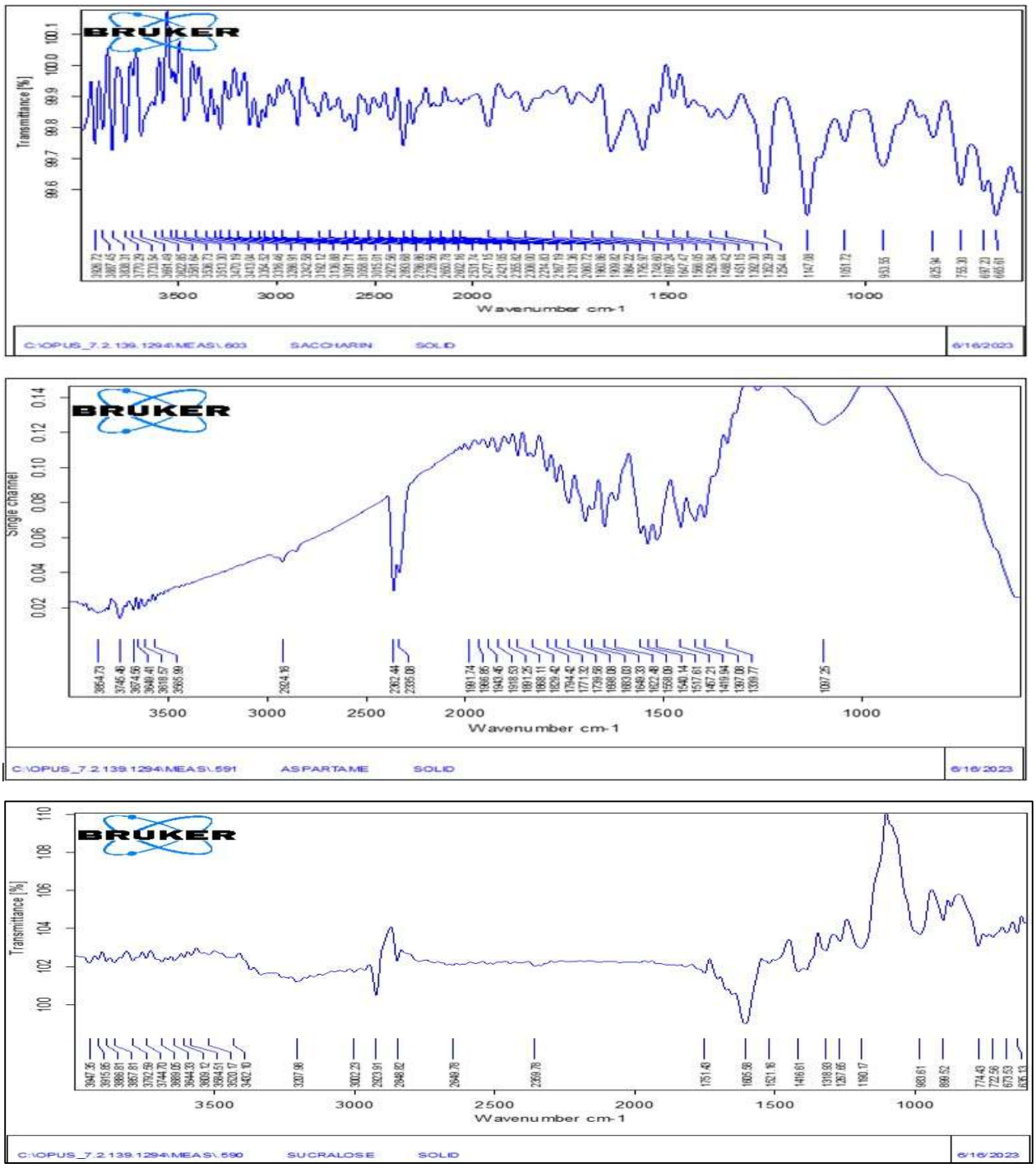

Figure 1: FTIR of Standard samples

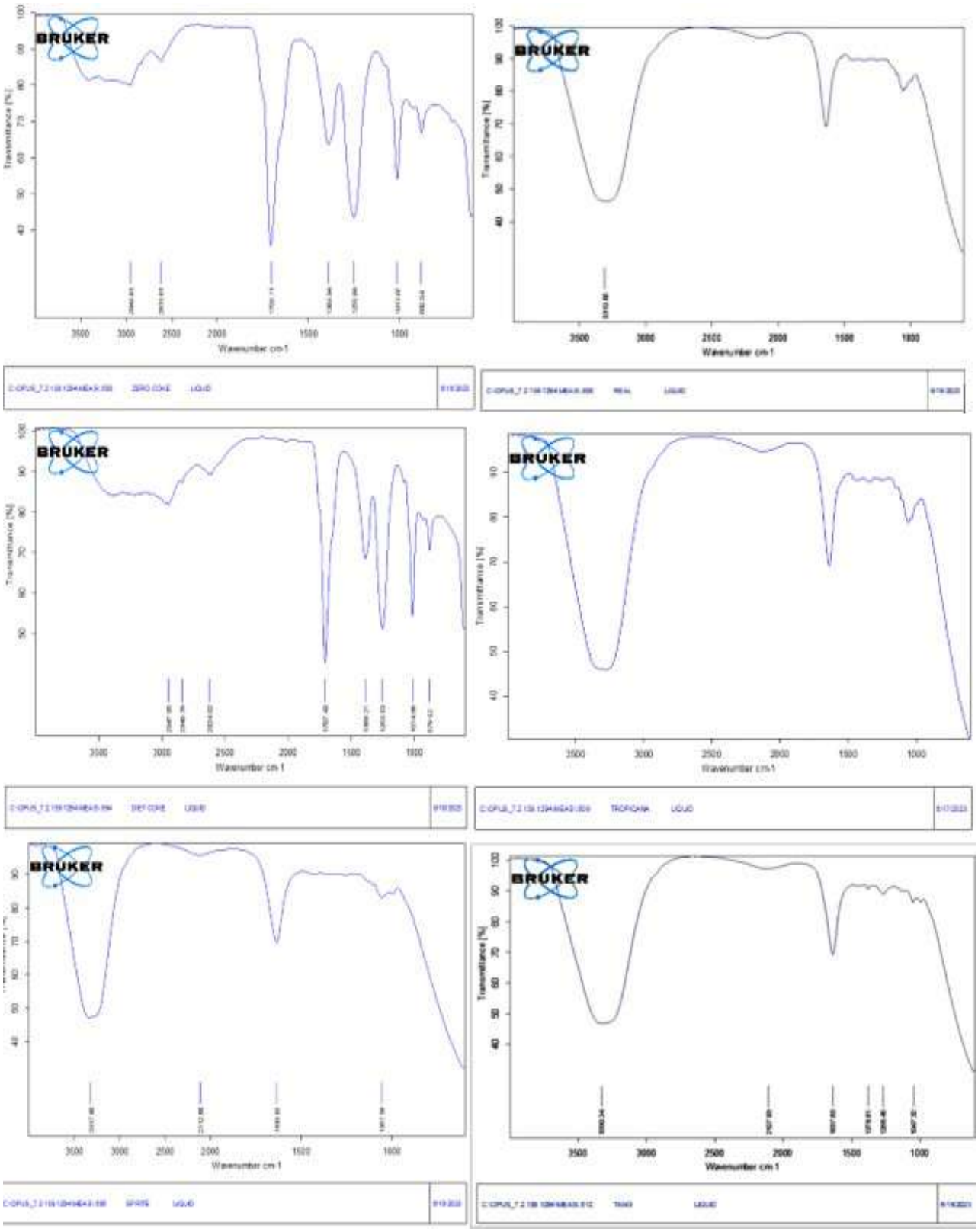

Figure 2: FTIR spectra of the products

Quantitative Analysis by UV

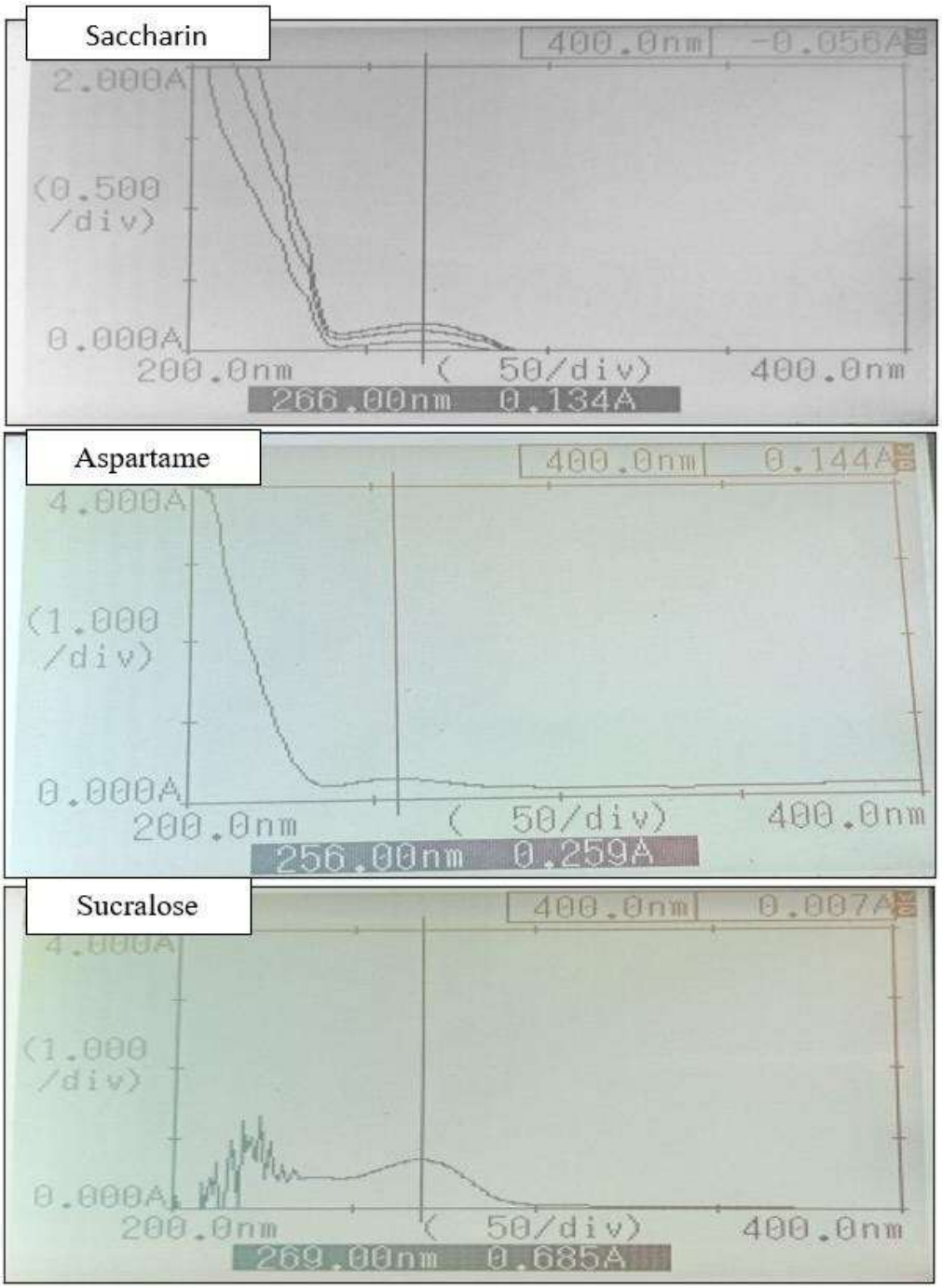

Figure 3: UV spectrum of Standard samples

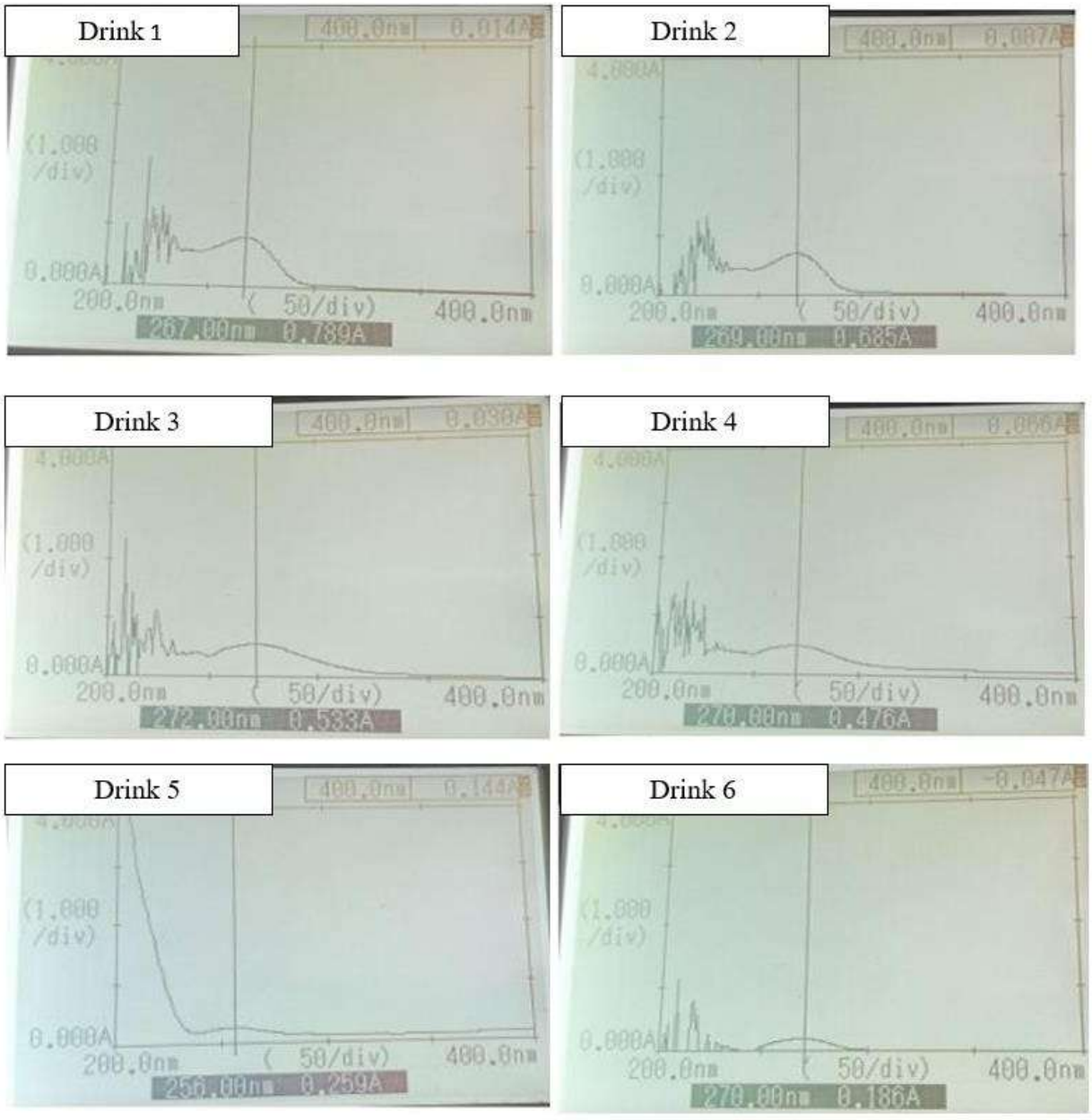

Figure 4: UV Spectrum of Products

Table 1: Linearity of Saccharin, Aspartame & Sucralose

SAC[a]		ASP[b]		SCL[c]	
Concentration	**Absorbance**	**Concentration**	**Absorbance**	**Concentration**	**Absorbance**
50 ppm	**0.06**	**60 ppm**	**0.06**	**50 ppm**	**0.13**
75 ppm	**0.134**	**120 ppm**	**0.134**	**100 ppm**	**0.48**
100 ppm	**0.214**	**180 ppm**	**0.214**	**125 ppm**	**0.68**
125 ppm	**0.303**	**240 ppm**	**0.303**	**150 ppm**	**0.89**
150 ppm	**0.395**	**300 ppm**	**0.395**	**200 ppm**	**1.3**
175 ppm	**0.468**	**360 ppm**	**0.468**	**250 ppm**	**1.75**
200 ppm	**0.555**	**420 ppm**	**0.555**	**300 ppm**	**2.2**

480 ppm	**0.06**	**350 ppm**	**2.63**
540 ppm	**0.134**	**400 ppm**	**3**
600 ppm	**0.214**		
660 ppm	**0.303**		
720 ppm	**0.395**		

[a] Saccharin, [b] Aspartame, [c] Sucralose

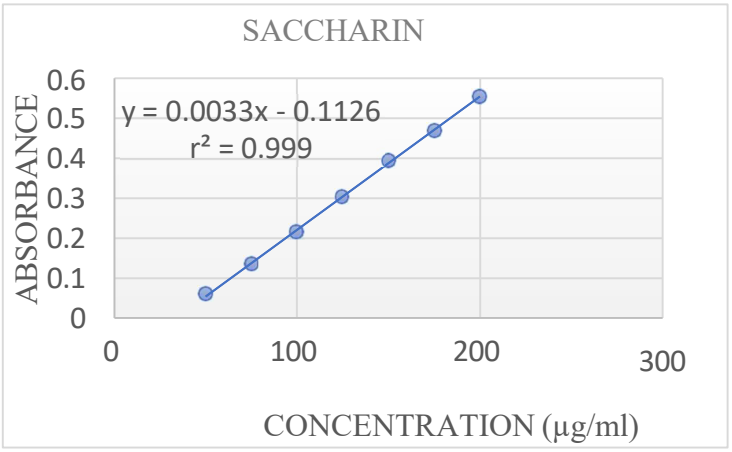

Figure 5: Calibration graph of Saccharin

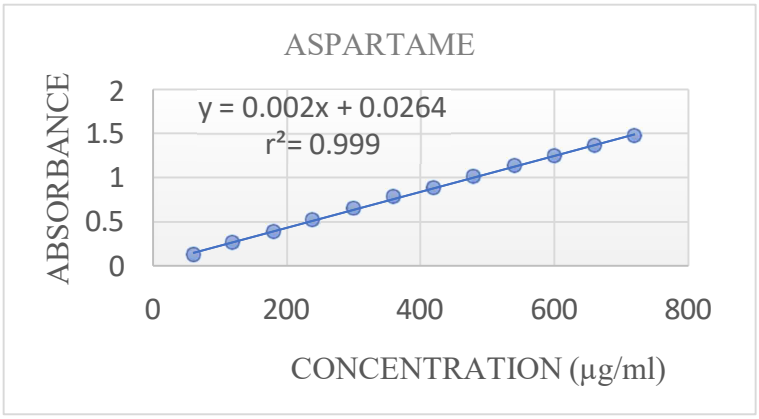

Figure 6: Calibration graph of Aspartame

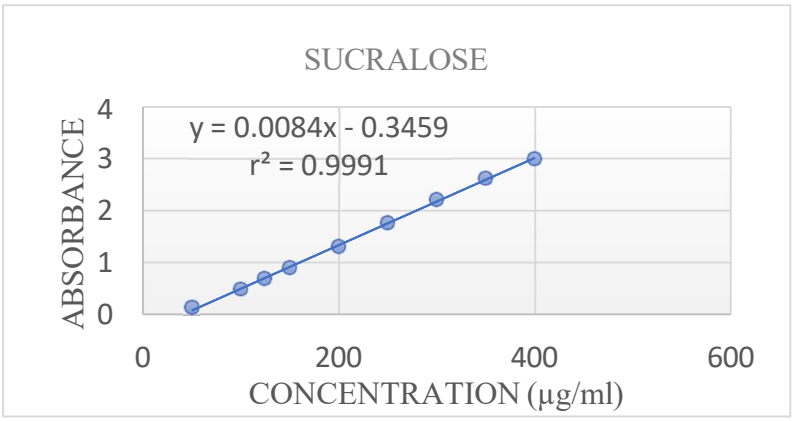

Figure 7: Calibration graph of Sucralose

LOD and LOQ values

The calculated LOQ & LOD values of saccharin, aspartame & sucrose are mentioned in **Table 2**.

Precision

Precision of was carried out by measuring the response for a single concentration for saccharin of 100 ppm, aspartame of 240 ppm and sucralose of 100 ppm for 6 replicates, [acceptance criteria: %RSD<2%]. The calculated precision value is mentioned in **Table 3**.

Robustness

The robustness results of saccharin, aspartame, and sucralose are mentioned in **Tables 4**.

Ruggedness

The ruggedness results of saccharin, aspartame, and sucrose are mentioned in **Tables 5**.

Table 2: LOD and LOQ

	Saccharin	Aspartame	Sucralose
LOD	5.58ppm	33.95ppm.	13.5ppm
LOQ	16.9ppm	102.88ppm	41.104ppm
Range	0.495	1.34	2.87

Table 3: Precision of Saccharin, Aspartame & Sucralose

Intraday (Absorbance)				Interday (Absorbance)			
	SAC[a]	ASP[b]	SCL[c]		SAC[a]	ASP[b]	SCL[c]
	0.214	0.57	0.47		0.213	0.54	041
	0.215	0.54	0.49		0.214	0.56	0.4
	0.214	0.57	0.47		0.211	0.55	0.42
	0.213	0.52	0.47		0.213	0.56	0.4
	0.214	0.57	0.48		0.215	0.57	0.42
	0.214	0.54	0.49		0.212	0.54	0.41
Mean	0.214	0.5633	0.47833	Mean	0.213	0.558333	0.41
SD	0.00058	0.011055	0.08975	SD	0.001291	0.010672	0.008165
%RSD	0.26979	1.9625	1.876364	%RSD	0.606101	1.91138	1.991455

Absorbance obtained for [a] Saccharin of 100ppm, [b] Aspartame of 240ppm, [c] Sucralose of 100ppm

Table 4: Robustness of Saccharin, Aspartame & Sucralose

	SAC[a] Wavelength		ASP[b] Wavelength		SCL[c] Wavelength	
	265 nm	267nm	255nm	265 nm	267nm	255nm
	0.131	0.132	0.42	0.131	0.132	0.42
	0.132	0.134	0.43	0.132	0.134	0.43
	0.133	0.132	0.42	0.133	0.132	0.42
	0.132	0.132	0.42	0.132	0.132	0.42
	0.134	0.133	0.43	0.134	0.133	0.43
	0.132	0.131	0.41	0.132	0.131	0.41
Mean	0.132333	0.132333	0.421667	0.132333	0.132333	0.421667
SD	0.000943	0.000943	0.006872	0.000943	0.000943	0.006872
%RSD	0.71425	0.432909	1.629686	0.71425	0.432909	1.629686

Absorbance obtained for [a] Saccharin of 75 ppm, [b] Aspartame of 180 ppm, [c] Sucralose of 250 ppm

Table 5: Ruggedness of Saccharin, Aspartame & Sucralose

	SAC[a]		ASP[b]		SCL[c]	
	Analyst 1	Analyst 2	Analyst 1	Analyst 2	Analyst 1	Analyst 2
	0.397	0.395	0.77	0.79	2.63	2.6
	0.396	0.393	0.75	0.79	2.65	2.61
	0.394	0.394	0.76	0.77	2.64	2.62
	0.392	0.392	0.77	0.76	2.62	2.64
	0.393	0.396	0.79	0.75	2.61	2.65
	0.395	0.397	0.79	0.77	2.6	2.63
Mean	0.3945	0.3945	0.771667	0.771667	2.625	2.625
SD	0.001708	0.00178	0.014625	0.014625	0.01708	0.01708
%RSD	0.432909	0.432909	1.895241	1.895241	0.6506	0.6506

Absorbance obtained for [a] Saccharin of 150 ppm, [b] Aspartame of 360 ppm, [c] Sucralose of 350 ppm

Estimation of Identified Artificial Sweeteners in Locally Available Products- Soft drinks and Packed Fruit juices

The concentration of artificial sweeteners in locally available products was estimated by applying Beer-Lamber's law. $A = \log (I_o/I_t) = a*b*c$

(A: absorbance; I_o: intensity of incident light; I_t: intensity of transmitted light; a: molar absorptivity; b: length of path length; c: concentration of sample)

Concentration of Standard/Concentration of sample = Absorbance of Sample/Absorbance of Standard

Table 6: Comparison of Concentrations of Artificial Sweeteners in Drinks

S. No	Products	Artificial Sweeteners	Concentration According to FSSAI	Calculated Concentration by Beer's Lamber's Law
1	Drink 1	Saccharin	100 ppm	75.09 ppm
		Aspartame	700 ppm	614.44 ppm
		Sucralose	300 ppm	169.20 ppm
2	Drink 2	Saccharin	100 ppm	86.49 ppm
		Aspartame	700 ppm	545.69 ppm
		Sucralose	300 ppm	194.89 ppm
3	Drink 3	Saccharin	100 ppm	71.06ppm
		Aspartame	700 ppm	526.82ppm
		Sucralose	300 ppm	250.46ppm
4	Drink 4	Saccharin	100 ppm	79.56ppm
		Aspartame	700 ppm	139.9ppm
		Sucralose	300 ppm	280.46ppm
5	Drink 5	Saccharin	100 ppm	82.62 ppm
		Aspartame	700 ppm	481.85ppm
		Sucralose	300 ppm	185.32ppm
6	Drink 6	Saccharin	100 ppm	54.03 ppm
		Aspartame	700 ppm	670.96 ppm
		Sucralose	300 ppm	258.06 ppm

CONCLUSION

The UV spectrophotometric method and FTIR were developed for the standard solutions of saccharin, aspartame, and sucrose. The method was validated for

Linearity (Saccharin 50-200 µg/ml; Aspartame 60-720µg/ml; Sucralose 50-400 µg/ml), LOD (Saccharin 5.58 ppm; Aspartame 33.95 ppm; Sucralose 13.5 ppm),

LOQ (Saccharin 16.9 ppm; Aspartame 102.8 ppm; Sucralose 41.04 ppm), Range (Saccharin 0.495; Aspartame 1.34; Sucralose 2.87)

Precision (Saccharin 100ppm; Aspartame 240 ppm; Sucralose 100 ppm),

Robustness (Saccharin 265 nm&268 nm; Aspartame 255 nm&256 nm; Sucralose 259 nm&261 nm),

Ruggedness (Saccharin 150 µg/ml; Aspartame 360 µg/ml; Sucralose 350 µg/ml).

Following ICHQ2 (R1) standards, all validation parameters were evaluated, and it was found that each parameter fell within permissible limits. Artificial sweeteners were isolated from the samples of Zero Coke, Diet Coke, Real, Tang, Sprite, and Tropicana, and their presence in those was determined by FTIR analysis. Artificial sweetener concentrations present in the samples were accounted for by measuring UV absorbance, and the results were within permissible limits.

ACKNOWLEDGEMENT

The authors thank the management of Sarojini Naidu Vanita Pharmacy Maha Vidyalaya, Tarnaka, Secunderabad, for supporting this study.

CONFLICTS OF INTEREST

There is no conflict of interest.

REFERENCES

[1] Zygler, A., Wasik, A., Kot-Wasik A, Namieśnik J., The content of high-intensity sweeteners in different categories of foods available on the Polish market. Food Addit. Contam. Part A Chem. Anal. Control Expo. Risk Assess., 29(9), 1391–1401, 2012.

[2] Wasik, A., J. McCourt, J., Buchgraber M., Simultaneous determination of nine intense sweeteners in foodstuff s by high performance liquid chromatography and evaporative light scattering detection—Development and single-laboratory validation. J. Chromatogr., 1157(1–2): 187–196, 2007.

[3] Mortensen, A., Sweeteners permitted in the European Union: Safety aspects. Scand. J. Food Nutr., 50(3), 104–116, 2006.

[4] Pawar, R.S., Krynitsky, A.J., Rader, J.I., Sweeteners from plants—with emphasis on *Stevia rebaudiana* (Bertoni) and Siraitia grosvenorii (Swingle). Anal. Bioanal. Chem., 405(13), 4397–4407,

2013.

[5] Yang, D.J. and Chen, B., Simultaneous determination of nonnutritive sweeteners in foods by HPLC/ESI-MS. J. Agric. Food Chem., 57(8), 3022–3027, 2009.

[6] Lim, H.S., Park, S.K., Kwak, K.I.S., Kim, H., Sung, J.H., Jang, S.J., Byun, M.Y., Kim,
S.H., HPLC-MS/MS analysis of 9 artificial sweeteners in imported foods. Food Sci. Biotechnol., 22(1), 233–240, 2013.

[7] Ni, F., Ammann, J., Mabud, A., Monitoring stevioside in soju by high-performance liquid chromatography and liquid chromatography/mass spectrometry. J. AOAC Int., 90(5), 1365–1372, 2007. 30 Food Safety: Innovative Analytical Tools.

[8] Kolb, N., Herrera, J.L., Ferreyra, D.J., Uliana, R.F., Analysis of sweet diterpene glycosides from *Stevia rebaudiana* : Improved HPLC method. J. Agric. Food Chem., 49(10), 4538–4541, 2001.

[9] Romina Shah, Lowri S. de Jager. "Recent Analytical Methods for the Analysis of Sweeteners in Food: A Regulatory Perspective" Wiley, 2016

[10] Pól, J., Hohnová, B., Hyötyläinen, T., Characterisation of *Stevia rebaudiana* by comprehensive two-dimensional liquid chromatography time-of-flight mass spectrometry. J. Chromatogr., 1150(1–2), 85–92, 2007.