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Method Development and Process Validation of Glucose Estimation in Chewable Tablets by RP-HPLC

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ABSTRACT

Several analytical procedures use the HPLC method to test glucose in other dosage forms, but there isn't yet for tablet dosage forms. To determine glucose in chewable tablet dosage form using RP-HPLC, we thus concentrate on creating a single efficient approach. This study aims to develop and validate a simple, accurate, rapid, and economical method for glucose chewable tablets by RP-HPLC. The validated method is used to validate the process at each stage of tablet production. Chromatographic runs were carried out on a Bondapak NH2 (10 µm, 3.9 mm x 300 mm) column with a mobile phase of water and acetonitrile (70:30) at a flow rate of 1 mL/min and detected using an RI detector as per ICH guidelines. The method is shown to be linear in the range of 80% (1.6 mg/mL) to 120% (2.4 mg/mL) of operating concentration with a correlation coefficient of 0.999, accurate at a recovery rate of 98.0% and 102.0%, and robust to changes in mobile phase ratio and flow rate. It is a simple, accurate, economical, fast, and precise method for glucose.

1. Introduction

One of the most important analytical methods for research and laboratory studies is high-performance liquid chromatography (HPLC)[1.] Its intrinsic capacity to analyze, separate, and purify various chemical samples, including but not limited to acidic, basic, and neutral analytes, is extensively utilized in chemical, pharmaceutical, and biological analysis and drug treatment monitoring[2.] pharmaceutical residues are a field of particular interest due to the adverse effects to either human health or aquatic and soil environment. Because of the diversity of these compounds, at least 3000 substances were identified and categorized into 49 different therapeutic classes, and several actions are urgently required at multiple steps, the main ones: (i In reversed-phase liquid chromatography (RP-HPLC), the analysis is performed using a polar hydro-organic mobile phase and a nonpolar stationary phase that may or may not be spiked with a buffer salt[3]. The solutes' hydrophobicity, the surface's hydrophobicity, and the mobile phase's polarity all increase retention as they become more hydrophobic. Two phenomena–partitioning (where analyte molecules fully submerge themselves in the bonded phase) and adsorption (which takes place at the bonded-phase/solvent interface)–are used to accomplish separation.[4,5.]

Glucose is an aldose monosaccharide integral to photosynthesis and respiration, functioning as an energy store and metabolic fuel in most organisms. As a monomer and component of more complex compounds such as polysaccharides and glucosides, glucose also plays a significant role in contemporary food items, notably in taste and structure6. Chewable glucose tablets are a flexible dosage form with several benefits, including patient-centered medication administration, simple swallowing, the stability benefits of solid dosage forms, and oral drug delivery without the need for water. They provide a practical way to give pediatric medications and dietary supplements like chewable multivitamins. Chewable pills are used in veterinary medicine as well.[7,8]

Before conducting a quantitative analysis, a qualitative analysis is required. Separation's part is often necessary for qualitative analysis. The results of conventional quantitative analysis can be computed from the amount of analyte in the sample and the volume or mass of the sample. Analyzing pharmaceutical chemistry quantitatively relies on instruments. New drugs are constantly being developed, and to control their quality, new methods are needed. A modern pharmaceutical analysis must meet the following requirements: The analysis should be finished as soon as feasible, adhere to pharmacopeia standards, be economical, and be exact and discerning. as opposed to letting a solvent drop down the column naturally. The variety of detection techniques available is one of the most significant advancements over column chromatography. These techniques are highly automated and incredibly delicate.[9-11]

Diana et al. developed and validated a liquid chromatographic method to quantify glucose, fructose, and sucrose in raw tubers of Solanum tuberosum. Group Phureja and AMINEX HPX 87H columns were used. This extraction method achieved 94.14 to 99.77% recovery. The three sugars' detection limits were 3.0 mg/L[12.] Wilson et al.[13] found that using an HPLC with a refractive index (RI) detector could determine glucose, sucrose, and fructose in potatoes in a simple, reproducible manner. The method recovered 93% or more of all sugars using the HPLC system, which comprised a Bondapak/carbohydrate column and an acetonitrile/water solvent system (75:25). A range of 1.39-13.31% was observed in the coefficients of variation for the experiment.[13] There have been reports on several analytical methods for determining sugars and sugar alcohols, including gas chromatography[14,] spectrophotometry methods, high-performance liquid chromatography[15], and capillary electrophoresis.[16] A major drawback of gas chromatography is the time-consuming process of derivatizing samples to trimethyl silane or alditol acetates. Calorimetric procedures do not directly distinguish monosaccharides, glucose, and fructose. Due to these drawbacks, HPLC methods are a better choice. It is becoming increasingly popular to use HPLC to separate sugars according to their quantitative composition. HPLC combined with a UV-VIS

detector,[15] a diode-array detector,[17] a refractive-index detector18, a pulse-amperometric detector,[19] an evaporative light scattering detector,[20] and a charged aerosol detector[21] have been used. As sugars and sugar alcohols lack any visible chromophore, the specific UV method is unreliable or impossible. HPLC methods for detecting these compounds rely on refractive index detectors.

In this work, an RP-HPLC method has been developed for the quantitation of glucose in chewable tablets. According to a literature review, the HPLC method was employed by various analysts to measure glucose in other dosage forms. Still, no such techniques have been developed for the analysis of glucose in tablet dosage forms.

2. Experimental

2.1. Reagents and chemicals

Glucose Standard was a generous gift from Twenty-first-century Pharmaceuticals Pvt Ltd., Ambattur, Chennai (India). HPLC-grade acetonitrile and water were purchased from Merck, India. All dilutions were performed in standard class-A volumetric glassware. All other chemicals used were of analytical grade. Triple distilled water was used in the entire study. Glucose sample tablets (NUVIT) were obtained from the local market.

2.2. Instrumentation

Chromatographic separation was performed on Agilent Technologies (1220 Infinity II LC) series HPLC have a Bondapak NH2, Column (10 µm, 3.9 mm x 300 mm). The column temperature was maintained at 40 °C. To facilitate the chemicals' dissolution, an Analytical Technologies Ltd. Sonicator was used.

2.3. Chromatographic conditions

The high-performance liquid chromatographic (HPLC) system used was operated isocratically with the column temperature maintained at 40°C, using a mobile phase composition of water: acetonitrile (70:30), the mobile phase solvents were filtered through 0.45 µm filter paper to remove particulate matter and degassed by sonication. The flow rate employed for analysis was 1.0 mL/min with run time 30min and the refractive index (RI) detector is the choice of detector for the determination of sugars.

2.4. Preparation of stock standard solution and working standard solution

a) **Preparation of 4mg/ml standard stock solution (Solution A)**

Accurately weighed and transferred 200mg of Glucose WRS into a 50ml volumetric flask. Added 30ml of mobile phase and sonicated to dissolve. Diluted to volume with mobile phase and mixed well.

b) **Preparation of 1.6 mg/mL solution: (80% solution)**

Pipetted out 4.0 mL of the standard stock solution (Solution A) into a 10 mL volumetric flask. Diluted to volume with diluent and mixed well.

c) **Preparation of 1.8 mg/mL solution: (90% solution)**

Pipetted out 4.5ml of the standard stock solution (Solution A) into a 10 mL volumetric flask. Diluted to volume with diluent and mixed well.

d) **Preparation of 2.0 mg/mL solution: (100% solution)**

Pipetted out 5.0ml of the standard stock solution (Solution A) into a 10 mL volumetric flask. Diluted to volume with diluent and mixed well.

e) **Preparation of 2.2 mg/mL solution: (110% solution)**

Pipetted out 5.5ml of the standard stock solution (Solution A) into a 10 mLvolumetric flask. Diluted to volume with diluent and mixed well.

f) **Preparation of 2.4 mg/mL solution: (120% solution):** Pipetted out 6.0 mL of the standard stock

solution (Solution A) into a 10 mL volumetric flask. Diluted to volume with diluent and mixed well.

Each of the standard solutions was injected 3 times, and the mean peak area of the drug was calculated and plotted against the concentration of the drug. The regression equation was found by using a standard curve.

2.5. Preparation of sample solution:

Calculate the average weight of 20 tablets. Mix the content thoroughly and weigh accurately about 140 mg (Equivalent to 50 mg of glucose) of the mixed contents into a 25 mL standard flask. Add about 20 mL of the mobile phase and sonicate for 30 min with occasional shaking, cool and dilute to 25 mL with the mobile phase. Filter through a 0.45 µm membrane filter.

a) Preparation of 4.8 mg/mL of sample solution (80% solution):

Accurately weighed and transferred 120.02 mg of sample into a 25 mL volumetric flask. Added 10 mL of mobile phase and sonicated to dissolve. Diluted to volume with mobile phase and mixed well.

b) Preparation of 6 mg/mL of sample solution (100% solution):

Accurately weighed and transferred 150.40 mg of sample into a 25 mL volumetric flask. Added 10 mL of mobile phase and sonicated to dissolve. Diluted to volume with mobile phase and mixed well.

c) Preparation of 7.1 mg/mL of sample solution (120% solution):

Accurately weighed and transferred 179.68 mg of sample into a 25 mL volumetric flask. Added 10 mL of mobile phase and sonicated to dissolve. Diluted to volume with mobile phase and mixed well.

2.6. Assay for RP-HPLC method

Inject 20 µL of standard and sample solutions into the HPLC system and record the chromatograms.

2.7. Method validation

As the International Conference on Harmonization (ICH) and the Association of Official Analytical Chemists International suggested, the RP-HPLC technique was validated for linearity, accuracy, detection limit, quantification, precision, specificity, and robustness.

2.8. Linearity

The linearity of the method was determined by analyzing several aliquots of a standard glucose solution. For the RP-HPLC method, linear correlations were obtained between peak area and concentration for glucose in the ranges of 1.6 mg/mL, 1.8 mg/mL, 2.0 mg/mL, 2.2 mg/mL, and 2.4 mg/mL, respectively.

2.9. Accuracy

Three samples, each of 80% (4.8 mg/mL), 100% (6 mg/mL), and 120% (7.1 mg/mL) of the actual quantities present, were prepared for NUVIT tablets and the recoveries studied. Accuracy was assessed using the nine determinations of recoveries for each tablet.

2.10. Precision

Precision was assessed using the determination of a homogeneous sample of NUVIT at 100% test concentration. The method, system, and intermediate precisions were determined using relative standard deviation percentages to determine intermediate precision. Precision studies were repeated on different days.

2.11. Robustness

As per ICH guidelines, small but deliberate variations in the mobile phase concentration were made

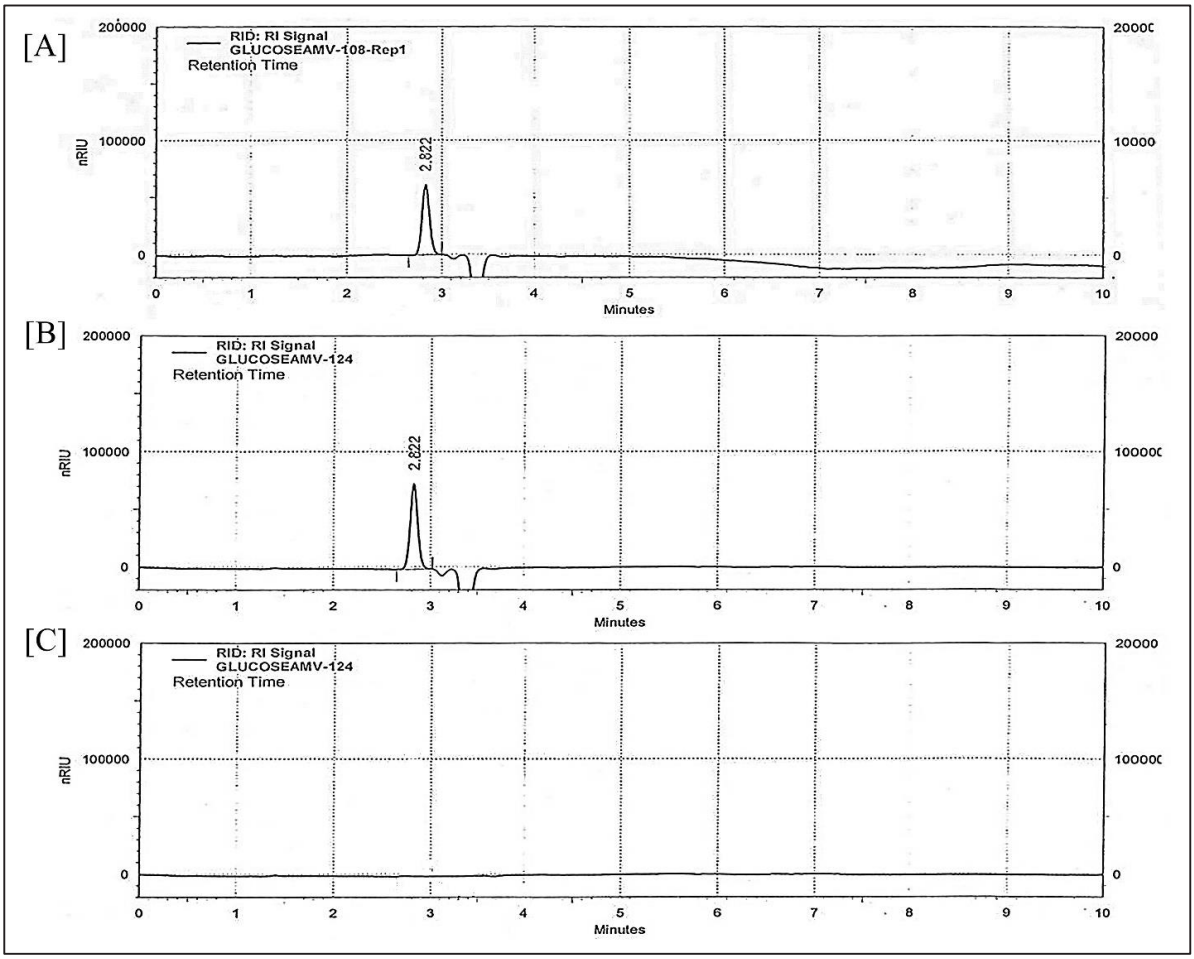

Figure 1. *HPLC chromatogram of [A]. Glucose Standard WRS [B]. Glucose Sample [C]. Blank*

to check the method's robustness. All samples were injected two times and analyzed.

3. Results and Discussion

3.1 Method development and optimization

3.1.1. Mobile phase selection

The mobile phase was selected based on best separation, peak purity index, point symmetry, theoretical plate, etc. The different solvent system was selected for analysis of glucose with varying concentrations of water and acetonitrile, Water: Acetonitrile (25:75), Water: Acetonitrile (50:50), Water: Acetonitrile (75:25), Water: Acetonitrile (70:30). The diluent is selected based on the nature of the drug, such as pKa, and solubility. The solvent in which the sample drug has the maximum solubility is selected as the diluent. Glucose is freely soluble in water, so water is used as a diluent. Many trials were made to determine the mobile phase for eluting the sample drug. All the mobile phase solvents were filtered through 0.45 µm filter paper to remove particulate matter and degassed by sonication. The flow rate employed for analysis was 1.0 mL/min. The mobile phase found to be most suitable for analysis was

Table 1. The results of linearity parameters for glucose.

Parameters	Results
Concentration (mg/mL)	1.6 to 2.4
Regression equation	177996x + 547707
Correlation coefficient R^2	0.9998
LOD (mg/mL)	0.1015
LOQ (mg/mL)	0.3077

Table 2. Results of Intra-day and Inter-day precision.

Concentration (mg)	Intra-day precision		Inter-day precision	
	Amount found (mg)	% Recovery	Amount found (mg)	% Recovery
143.2	150.7089	100.4726	150.7089	100.4726
140.4	150.4335	100.289	150.4335	100.289
142.6	150.5945	100.3963	150.5945	100.3963
142.6	151.4083	100.9389	151.4083	100.9389
139.8	150.7071	100.4714	150.7071	100.4714
141.9	151.2171	100.8114	151.2171	100.8114
Mean	**150.8449**	**100.5633**	**150.8449**	**100.5633**
Std. Dev	**0.38**	**0,25**	**0.38**	**0,25**
RSD	**0.252498**	**0.252498**	**0.252498**	**0.252498**

Water: Acetonitrile (70:30) and the refractive index (RI) detector is the choice of detector for the determination of sugars.

3.1.2. System suitability parameter

After optimizing separation settings and allowing the mobile phase to saturate the column at 1mL/min, glucose working standard replicates were individually injected into the column. The peak areas, retention duration, theoretical plates, tailing factor, resolution, capacity factor, and AUC was assessed to determine the appropriateness of the system.

3.1.3. Optimization

Several parameters, including the composition of the mobile phase, the flow rate, the column type, and the detectors used, were varied to optimize the operating conditions for RP-HPLC. The glucose analysis method showed the best resolution with Bondapak NH2, 10 µm, 3.9 mm x 300 mm column and mobile phase consisting of Water: Acetonitrile (70:30) with a flow rate of 1.0 mL/min. The mean retention time for glucose was 2.82min with good peak resolution and shape (Figure 1).

3.2 Method validation

3.2.1 Linearity and Range

The operating concentration of glucose was 2 mg/mL. Concentrations between 80% to 120% of operating concentration were prepared and injected into the HPLC system, and the peak areas were noted (Table 1).

Table 3. Accuracy

S. No	Recovery Level		Peak area	Amount added (mg)	Amount obtained (mg)	Recovery
Accuracy 80%						
1	80%	Sample 1	28628881	120.1	119.16	99.28
2		Sample 2	28857295	120.5	119.71	99.74
3		Sample 3	28514716	119.4	119.38	99.47
				Average	119.42	99.50
				Std. Dev	0.27	0.23
				%RSD	0.23	0.23
Accuracy 100%						
1	100%	Sample 1	36165035	120.5	150.03	99.75
2		Sample 2	36296512	121.1	149.83	99.62
3		Sample 3	36177027	120.6	149.70	99.54
				Average	119.42	99.50
				Std. Dev	0.27	0.23
				%RSD	0.23	0.23
Accuracy 120%						
1	120%	Sample 1	43136873	119.5	180.45	100.42
2		Sample 2	43139496	119.2	180.91	100.68
3		Sample 3	42864302	119.1	179.91	100.13
				Average	180.42	100.41
				Std. Dev	0.50	0.27
				%RSD	0.27	0.27

Limit: *Between 98.0% and 102.0% of added value,* ***RSD*** *Limit not more than 2.0%*

Concentration was plotted against area response for glucose, and a straight-line graph was obtained. The method is linear in 80% and 120% of operating concentration. The coefficient of correlation for linearity was found to be 0.999, typically specified in method validation protocols. The limit for the coefficient of correlation is not less than 0.995.

1.1.2 Precision

3.2.2.1 System precision:

In HPLC, the peak areas of each of the six injections of the working standard solution were measured. The Chromatogram of the system precision is shown in Figure S1.

3.2.2.2 Method precision: (Intra-day precision)

The working sample (test) solutions were injected six times, and the area was measured for all six samples in HPLC. The %RSD for the area of six replicate injections should be within the limit (Table 2). Chromatogram of the method precision is shown in Figure S2.

3.2.2.3 Intermediate precision: (Inter-day precision / Ruggedness)

Six individual samples were prepared by a second analyst using a different column and injected in

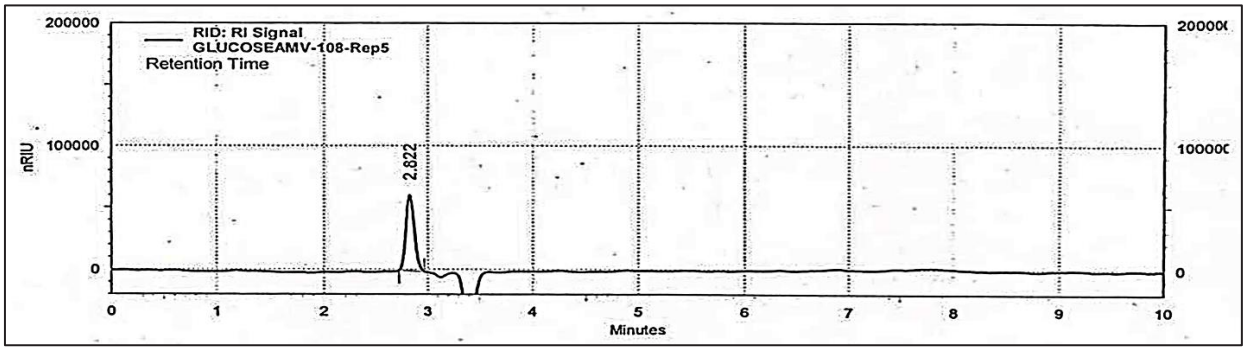

Figure 2. *Chromatogram of water: acetonitrile (68:32).*

Table 4. Variation in ratio 68:32.

S. No	Sample ID	Peak Area	Amount added (mg)	Amount obtained (mg)	Recovery
1	Sample 1	29367473	144.7	150.66	100.44
2	Sample 2	29192439	144.2	150.28	100.19
			Mean	150.47	100.31
			Std. Dev	0.26	0.17
			RSD	0.17	0.17

a different HPLC system on intraday. as per the assay method. The proposed method for glucose was verified by method precision, system precision, and intermediate precision (Ruggedness). The results were as tabulated (Table 3). The deviations among the results from the average value are +0.47% and -0.56%. Limit: 2% RSD values are well within limits, and the deviation among the results was also within the limit. Hence the method has good precision. Chromatogram of the Intermediate precision as shown in Figures S3 and S4.

3.2.2.4 Accuracy

Three samples of 80% (4.8 mg/mL), 100% (6 mg/mL), and 120% (7.1 mg/mL) of the actual quantities were prepared for NUVIT tablets and the recoveries studied. Accuracy was assessed using the nine determinations of recoveries for each tablet. The details are tabulated below (Table 3). The chromatogram of the accuracy is shown in Figure S5.

Recovery studies verified the proposed method's accuracy.

The recovery percentage range was between 98.0%, and 102.0% it is a good index of the accuracy and repeatability of the method. The results were tabulated in the Table 3. All parameters, including temperature, wavelength, detection, sensitivity, and flow rate, were maintained constant throughout the procedure.

3.2.2.5 Robustness

The method's robustness was checked by chang-

Table 5. Summary of system suitability.

Parameter	Glucose	Limits
%RSD	35559672	NMT 2
%RSD Retention Time	2.822	NLT 2
Theoretical plates	5436	NLT 1000
Tailing Factor	1.17	NMT 2

Table 6. Blend sample batch.

S.No	Sample id	Injection 1	Injection 2	Average	Amount added (mg)	Amount obtained (mg)	Recovery
Blend Sample Batch-1							
1	Front top	35046419	34913531	34979975	118.0	149.8002	99.86682
2	Back left top	35527238	35468963	35498101	120.1	149.361	99.57397
3	Back right top	34770233	34763857	34767045	118.2	148.6364	99.09096
4	Front middle	35512040	35587495	35549768	119.8	149.9529	99.96862
5	Back left middle	35214864	35320471	35267668	119.5	149.1365	99.42431
6	Back right middle	35127341	35228918	35178130	118.9	149.5085	99.67233
7	Front bottom	35523769	35597190	35560480	118.5	151.6437	101.0958
8	Back right bottom	34312741	34441755	34377248	118.3	146.8457	97.89716
9	Back left bottom	3469563	34748429	34721846	118.2	148.4432	98.96214
					Mean	149.2587	99.50579
					Std dev	1.295455	0.863637
					RSD	0.867926	0.867926
	Pooled sample	35503988	35548102	35526045	119.8	149.8529	99.90191
Blend Sample Batch-2							
1	Front top	32928792	32978989	32953891	118.2	149.0791	99.38606
2	Back left top	33476887	33438014	33457451	119.4	149.836	99.89064
3	Back right top	33958564	34032850	33995707	120.6	150.7316	100.4877
4	Front middle	33371303	33331452	33351378	118.6	150.3684	100.2456
5	Back left middle	33504953	33459732	33482343	119.1	150.3251	100.2168
6	Back right middle	33346643	33213548	33280096	120.2	148.0497	98.69982
7	Front bottom	33699432	33662815	33681124	119.5	150.7114	100.4743
8	Back right bottom	33095486	33061712	33078599	119.0	148.6373	99.0915
9	Back left bottom	33014482	32960240	32987361	118.6	148.7272	99.15147
					Mean	149.6073	99.73821
					Std dev	1.003157	0.668771
					RSD	0.670527	0.670527
	Pooled sample	33740652	33747868	33744260	121.0	149.1221	99.41475

Table 6 continued

Blend Sample Batch-3							
1	Front top	34132107	34131538	34131823	120.1	150.8767	100.5845
2	Back left top	33336282	33449506	33392894	119.5	148.3515	98.90099
3	Back right top	34086686	34126401	34106544	120.5	150.2645	100.1763
4	Front middle	33742561	33724844	33733703	119.8	149.4903	99.66018
5	Back left middle	33946499	33939680	33943090	119.5	150.7958	100.5305
6	Back right middle	33730125	33692495	33730125	119.5	149.8497	99.89978
7	Front bottom	33245771	33243247	33244509	118.5	148.9386	99.29241
8	Back right bottom	33213682	33261525	33237604	118.3	149.1594	99.43961
9	Back left bottom	34022602	34086686	34054644	121.1	149.2925	99.52832
					Mean	149.6688	99.77918
					Std dev	0.852802	0.568534
					RSD	0.569793	0.569793
	Pooled sample	35503988	35548102	35526045	121.9	149.511	99.67402

Table 7. Core tablet batch.

Core Tablets Batch-1							
S1. No	**Batch**	**Injection 1**	**Injection 2**	**Average**	**Average Wt of tablet (mg)**	**Amount added (mg)**	**Amount obtained (mg)**
1	Initial	33961294	33794584	33877939	359.6	120.1	149.9696
2	Middle	33669975	33756890	33713433	360.7	119.8	150.3264
3	Final	33752343	3399662	33874503	361.2	118.0	149.4966
						Mean	149.9309
						Std. Dev	0.416219
						RSD	0.277607
Core Tablets Batch-2							
1	Initial	33606757	33570040	33588399	361.0	120.1	149.755
2	Middle	33951195	34017682	33984439	358.6	120.5	150.0138
3	Final	33657553	33688305	33672929	360.8	119.1	151.3086
						Mean	150.3591
						Std. Dev	0.832362
						RSD	0.553583
Core Tablets Batch-3							
1	Initial	35411654	35442186	35426920	360.5	120.1	149.3118
2	Middle	35615888	35647667	35631778	359.5	119.8	150.1337
3	Final	34890401	34857520	34873961	362.2	118.0	150.3025
						Mean	149.916
						Std. dev	0.530002
						RSD	0.353533

Table 8. Coated tablets batch.

S.No	Tests	Results
Coated Tablets Batch-1		
1	Description	Red/Blue/Green/Purple colored; Circular; Biconvex-shaped sugarcoated tablets.
2	Identification	Positive for glucose
3	Average weight (Between 399.0 mg and 441.0 mg)	435.3 mg
4	Assay Each sugarcoated Chewable Tablet contains glucose between 142.50 mg and 157.50 mg.	149.80 mg
Coated Tablets Batch-2		
1	Description	Red/Blue/Green/Purple colored circular biconvex-shaped sugarcoated tablets.
2	Identification	Positive for glucose
3	Average weight (Between 399.0 mg and 441.0 mg)	430.5 mg
4	Assay Each sugarcoated Chewable Tablet contains glucose between 142.50 mg and 157.50 mg.	149.25 mg

ing some chromatographic conditions slightly, like changing the mobile phase flow rate and ratio. There were no drastic changes in chromatograms observed (Figure 2). The details are tabulated below (Table 4).

3.2.2.6 System Suitability

The parameters checked for system suitability as shown in Table 5

Relative standard deviation from standard injections - Acceptance criteria – (NMT) not more than 2.0%.

Column efficiency: Acceptance criteria **-** the limit is not less than (NLT) 1000 theoretical plates.

Tailing factor: Acceptance criteria - not more than 3.0%.

4. Process Validation

The validated method was used for process validation in each stage, such as blending, core tablets, and coated tablets of the tablet dosage form production (Table S1). In the blending stage, each 360.0 mg of the blend contains glucose 150 mg of samples were collected from various places like back left top, front top, front middle, back right top, back right middle, back left middle, front bottom, back right bottom, and back left bottom are validated for 3 batches (Table 6). Chromatogram of the Blend samples are as shown in Figures S6 to S8. Validation results for core and coated tablets are given in Tables 7 and 8, respectively. Chromatogram as shown in Figures S9 to S13.

5. Conclusion

The RP-HPLC method developed for the analysis of glucose chewable tablets is rapid, accurate, precise, and requires a very short run time, as retention time was 2.822 min. The developed method was validated successfully, showing satisfactory results for all method validation parameters. The percent recovery was 99.64%. An accurate, rapid, simple, and precise RP-HPLC method was developed to es-

timate glucose. The proposed method can be used to estimate glucose in chewable tablets. The developed and validated method was used for process validation in each stage, and the report shows excellent results. The method was developed and validated according to the ICH guidelines. Hence, the developed method can be used for routine analysis and process validation. □

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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For supplementary Material click here

References:

1. Sahu P.K., Ramisetti N.R,.; Cec,chi T.; Swain S., Patro C.S.;, Panda J. An Overview of Experimental Designs in HPLC Method Development and Validation. *J. Pharm. Biomed. Anal.147*, 590–611, 2018.
2. Almeida C.M.M. Overview of Sample Preparation and Chromatographic Methods to Analysis Pharmaceutical Active Compounds in Waters Matrices. *Separations*. *8*, 1–50, 2021.
3. Chapter 4. Reversed Phase Chromatography of Peptides. Editor(s): Dominic M. Desiderio. *Tech. Instrum. Anal. Chem. 6*, 51–73, 1984**.**
4. Gritti F., Guiochon G. Adsorption Mechanism in Reversed-Phase Liquid Chromatography. Effect of the Surface Coverage of a Monomeric C18-Silica Stationary Phase. *J. Chromatogr. A*. *1115*, 142–163, 2006.
5. De Luca C.,; Buratti A., Krauke Y., Stephan S.,Monks K., Brighenti V., Pellati F., Cavazzini A., Catani M., Felletti S. Investigating the Effect of Polarity of Stationary and Mobile Phases on Retention of Cannabinoids in Normal Phase Liquid Chromatography. *Anal. Bioanal. Chem. 414*, 5385–5395, 2022.
6. Galant A.L., Kaufman R.C., Wilson J.D. Glucose: Detection and Analysis. *Food Chem. 188*, 149–160, 2015.
7. Musuc A.M., Anuta V., Atkinson I., Sarbu I., Popa V.T., Munteanu C., Mircioiu C., Ozon E.A., Nitulescu G.M., Mitu M.A. Formulation of Chewable Tablets Containing Carbamazepine-β-Cyclodextrin Inclusion Complex and f-Melt Disintegration Excipient. The Mathematical Modeling of the Release Kinetics of Carbamazepine. *Pharmaceutics*. *13*, 915, 2021.
8. Sawatdee S., Atipairin A., Yoon A.S., Srichana T., Changsan N., Suwandecha T. Formulation Development of Albendazole-Loaded Self-Microemulsifying Chewable Tablets to Enhance Dissolution and Bioavailability. *Pharmaceutics*. *11*, 134, 2019.
9. Sahu P.K., Ramisetti N.R., Cecchi T., Swain S., Patro C.S., Panda J. An Overview of Experimental Designs in HPLC Method Development and Validation. *J. Pharm. Biomed. Anal. 147*, 590–611, 2018
10. Tian M., Row K.H. Separation of Glucose and Bioethanol in Biomass with Current Methods and Sorbents. *J. Chromatogr. Sci. 51*, 819–824, 2013.
11. Bortolotti F., Sorio D., Bertaso A., Tagliaro F. Analytical and Diagnostic Aspects of Carbohydrate

Deficient Transferrin (CDT): A Critical Review over Years 2007–2017. *J. Pharm. Biomed. Anal*. *147*, 2–12, 2018.
12. Duarte-Delgado D., Narváez-Cuenca C.E., Restrepo-Sánchez L.P., Kushalappa A., Mosquera-Vásquez T. Development and Validation of a Liquid Chromatographic Method to Quantify Sucrose, Glucose, and Fructose in Tubers of Solanum Tuberosum Group Phureja. *J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 975*, 18–23, 2015.
13. Wilson A.M., Work T.M., Bushway A.A., Bushway R.J. HPLC Determination of Fructose, Glucose, and Sucrose in Potatoes. *J. Food Sci. 46*, 300–301, 1981.
14. Wahjudi P.N., Patterson M.E., Lim S., Yee J.K., Mao C.S., Lee W.N.P. Measurement of Glucose and Fructose in Clinical Samples Using Gas Chromatography/Mass Spectrometry. *Clin. Biochem. 43*, 198–207, 2010.
15. Parpinello G.P., Versari A. A Simple High-Performance Liquid Chromatography Method for the Analysis of Glucose, Glycerol, and Methanol in a Bioprocess. *J. Chromatogr. Sci*. *38*, 259–261, 2000.
16. Wang J. Electrochemical Detection for Capillary Electrophoresis Microchips: A Review. *Electroanalysis*. *17*, 1133–1140, 2005.
17. Mattila P., Kumpulainen J. Determination of Free and Total Phenolic Acids in Plant-Derived Foods by HPLC with Diode-Array Detection. *J. Agric. Food Chem. 50*, 3660–3667, 2002.
18. Al-Sanea M.M., Gamal M. Critical Analytical Review: Rare and Recent Applications of Refractive Index Detector in HPLC Chromatographic Drug Analysis. *Microchem. J. 178*, 107339, 2022
19. Bao Y., Silva T.M.J., Guerrant R.L., Lima A.A.M., Fox J.W. Direct Analysis of Mannitol, Lactulose and Glucose in Urine Samples by High-Performance Anion-Exchange Chromatography with Pulse Amperometric Detection. Clinical Evaluation of Intestinal Permeability in Human Immunodeficiency Virus Infection. *J. Chromatogr. B Biomed. Appl. 685*, 105–112, 1996.
20. Mourey T.H., Oppenheimer L.E. Principles of Operation of an Evaporative Light-Scattering Detector for Liquid Chromatography. *Anal. Chem.56*, 2427–2434, 1984.
21. Moreau R.A. The Analysis of Lipids via HPLC with a Charged Aerosol Detector. *Lipids*. *41*, 727–734, 2006.