

**Research Article**
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## A Novel Reversed Phase HPLC Assay Method for Simultaneous Estimation of Glucose, Sodium Citrate and Chlorides in Pharmaceutical Formulations and Drug Solution for Oral Rehydration

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### ABSTRACT

A rapid, straightforward, sensitive, efficient, and cost-effective reverse-phase high-performance liquid chromatographic method was employed to simultaneously determine Glucose, Sodium Citrate, and chloride in a drug solution for oral solution. Glucose, Sodium Citrate, and Chlorides are all listed in the USP monograph. While various assay methods are available, titrimetric methods are ineffective for trace-level quantification. Although IC, AAS, and ICP-MS offer high accuracy, they are expensive and often inaccessible to many testing facilities. When selecting methods, it's crucial to consider quality control requirements and user-friendly techniques. A simple proprietary HPLC method has been developed to simultaneously quantify Chlorides, Glucose, and Sodium Citrate, with a shorter run time. The separation was carried out using a Shim-pack SCR-102(H) ion exclusion analytical column (7.9 mm x 300 mm, 7  $\mu$ m) with a flow rate of 0.6 mL/min. The column compartment was kept at 40°C, and the injection volume was 10  $\mu$ L, with detection at 200 nm. All measurements were performed in a 0.1% phosphoric acid solution. The analytical curves showed excellent linearity ( $r > 0.9999$ ) within the concentration ranges of 0.74 to 1.74 mg/mL for Sodium Citrate (SC), 1.0 to 2.5 mg/mL for Sodium Chloride (SC), and 3.0 to 7.3 mg/mL for Glucose. The method was validated according to the guidelines of the International Conference on Harmonization (ICH Q2B) and USP<1225>. The method demonstrated precision, robustness, accuracy, and selectivity. During accelerated stability testing over six months, no significant variations were observed in organoleptic analysis and pH. Consequently, the developed method is considered suitable for routine quality control analyses, enabling the simultaneous determination of Sodium Citrate, Chloride, and Glucose in pharmaceutical formulations and solutions for oral rehydration.

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### Abbreviations

**HPLC:** High-Performance Liquid Chromatography

**RP:** Reverse Phase

**USP:** United States Pharmacopeia

**NF:** National Formulary

**PF:** Pharmacopeial Forum

**ICH:** International Conference on Harmonization

**QC:** Quality Control

**$\mu$ g/mL:** microgram/milliliter

**mg/mL:** milligram/milliliter

**% :** Percentage

**%RSD:** Percent Related Standard Deviation

**LOD:** Limit of Detection

**LOQ:** Limit of Quantification

**SC:** Sodium Citrate

**CL:** Chlorides

### Key Highlights of the Study

- A highly selective RP-HPLC method has been developed for the efficient estimation of sugars (glucose), sodium citrate, and chlorides, all achieved within a shorter run time using HPLC with a UV detector.
- This study is unique as it represents the progressive development of an HPLC method specifically designed for the analysis of three components in both liquid pharmaceutical

formulations and solutions for oral rehydration.

- The established method was effectively employed for the estimation of glucose, sodium citrate, and chlorides in solutions for oral rehydration.

### Introduction

Dextrose or glucose (depicted in Figure 1a) is a colorless, odorless solid with a molecular weight of 180.16 g/mol. Dextrose, a type of sugar typically derived from corn or wheat, is nearly identical to glucose, the sugar present in the bloodstream. Due to this similarity, dextrose can be rapidly utilized as an energy source by the human body. It is frequently used in foods as an artificial sweetener or preservative and can also neutralize overly spicy or salty dishes. With a high glycemic index, dextrose quickly raises blood sugar levels, making it an effective energy source. Doctors sometimes prescribe dextrose alone or in combination with other medications, administering it either intravenously or orally. It is commonly used to treat low blood sugar and dehydration. Additionally, people with diabetes are often advised to carry dextrose tablets that dissolve in the mouth to quickly counteract episodes of low blood sugar and restore blood sugar levels to normal.

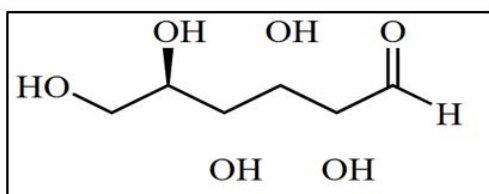


Figure 1a: Glucose Structure

Sodium Citrate (Figure 1b) is an organic sodium salt of citric acid as the counterion, having a molecular weight of 258.07 g/mol. It appears as white, crystalline powder or white, granular crystals, slightly deliquescent in moist air. It readily dissolves in water but is practically insoluble in alcohol. Similar to citric acid, it possesses a sour taste. Medically, it is utilized as an alkalizing agent. Sodium citrate prevents and treats high acid levels in your body. It may also be used to help prevent gout or kidney stones, conditions caused by high uric acid levels. It works by decreasing the amount of acid in the human body.

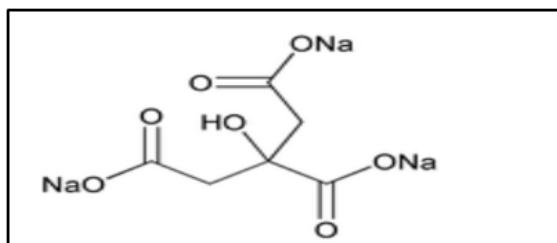


Figure 1b: Sodium Citrate Structure

Sodium Chloride (depicted in Figure 1c), commonly known as table salt, has a molecular weight of 58.44 g/mol. It is an ionic compound with the chemical formula NaCl, indicating a 1:1 ratio of sodium to chloride ions. Sodium chloride is crucial in determining the salinity of seawater and the extracellular fluid in many multicellular organisms. A 23.4% sodium chloride injection is used to replenish lost water and salt in the body under conditions such as hyponatremia or low salt syndrome. Additionally, it serves as an additive in total parenteral nutrition (TPN) and intravenous fluids containing carbohydrates. Furthermore, sodium chloride

functions as a tonicity agent in injectable formulations and solutions for oral rehydration.

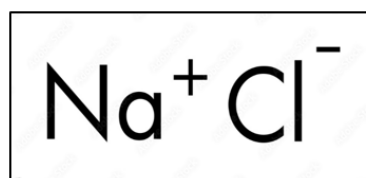


Figure 1c: Sodium Chloride

The literature review highlights the lack of a developed method for the simultaneous estimation of glucose, sodium citrate, and chlorides in pharmaceutical formulations and solutions for oral rehydration. Although methods such as titrations, ion chromatography, AAS, and ICP-MS exist, each has limitations. Titrations, for example, are ineffective for trace levels of chlorides, and expensive techniques like ICP-MS and AAS are often unavailable in many testing facilities.

This study addresses these challenges by employing HPLC with an ion exclusion column, specifically an H-type sulfonated styrene polymer as the stationary phase. This column is well-suited for analyzing organic acids using an acid-aqueous solution, such as phosphoric acid, as the mobile phase. The study successfully assesses all three components (glucose, sodium citrate, and chlorides) in various pharmaceutical formulations and solutions for oral rehydration using a UV detector, with a chromatographic run time of 25 minutes.

The primary objective of this study is to achieve the simultaneous estimation of these three components in a single analysis. This approach not only saves time but also proves cost-effective for routine, stability, and commercial sample analyses in quality control labs.

Potentiometric titration is used to determine the assay of chlorides atomic absorption spectroscopy is employed for assaying sodium, cadmium, and potassium and a high-performance liquid chromatography with a refractive index detector is applied for dextrose or glucose assay [1-3]. It is important to note that these three different techniques are explicitly mentioned in the USP monograph for the independent quantification of chloride, glucose, and sodium citrate.

Sodium Citrate, and Glucose. Other articles have focused solely on the estimation of sugar alcohols for Chlorides', and for Sodium Citrate [4-23]. An article was published for the Simultaneous estimation of sorbitol, sodium lactate, and sodium chloride in pharmaceutical formulations and drug solutions for infusion by spippalla [24]. From the published article, the chromatographic parameters adopted for this current research for the quantification of glucose, sodium citrate, and sodium chloride estimation.

However, there is a notable absence of articles or studies published on the simultaneous estimation of all three components using HPLC with a shorter run time. Additionally, the developed method underwent validation following established guidelines, and the method validation sections in the current research refer to articles [25-30].

It's worth noting that this marks the first-ever development of an RP-HPLC method for quantifying the assay of Glucose, Sodium Citrate, and chloride in pharmaceutical formulations and oral

rehydration solutions through HPLC.

## Materials and Methods

### Standard Reference Substances and Reagents

Reference standards for Glucose (Potency 91.0%), Sodium Citrate (Potency 100%), and Sodium chloride (Potency 60.4%) were provided by Sigma. The commercial sample used in the study was ORS® Oral Rehydration Solution sourced from the market. Analytical-grade water and phosphoric acid were utilized in the research work.

### Equipment and Chromatographic Conditions

The methodology was developed and validated using a Shimadzu Prominence high-performance liquid chromatograph, equipped with a Diode Array Detector (DAD) covering the 200 to 400 nm range, and a quaternary pump. Chromatographic separation was performed isocratically with a 0.6 mL/min flow rate on a Shim-pack SRC-102(H) ion exclusion analytical column (300 mm x 7.9 mm, 8 µm). The mobile phase consisted of 0.1% phosphoric acid in water, and detection occurred at 200 nm. A 10 µL injection volume and a runtime of less than 30 minutes were used. All analyses were conducted at a room temperature of 25.0 ± 1.0 °C

### Preparation of Standard Solutions and Commercial Samples

The reference standards for Sodium Citrate (SC), Chlorides (Sodium Chloride), and Glucose were prepared by making standard solutions with concentrations of 1.45 mg/mL, 2.2 mg/mL, and 6.75 mg/mL, respectively, in water. This process involved weighing 145 mg of sodium citrate, 220 mg of sodium chloride, and 675 mg of glucose, transferring each to separate 100 mL volumetric flasks, and then filling the flasks with water to reach the final volume. As a result, the solutions had concentrations of 1.45 mg/mL, 2.2 mg/mL, and 6.75 mg/mL for the respective substances. These solutions were then subjected to a 15-minute ultrasonic bath treatment.

### Sample Preparation

Transfer precisely 50 mL of the commercial sample solution to a 100 mL volumetric flask. Fill the flask to the mark with water and mix thoroughly. Filter the solution through a 0.22 µm nylon filter, discarding the initial 5 mL of filtrate.

### Analytical Parameters

The HPLC technique proposed in this study was validated as per ICH-Q2(R1) guidelines. Key validation parameters, such as selectivity, precision, linearity, accuracy, robustness, LOD, and LOQ, were meticulously selected to evaluate the method's validity.

### Selectivity

The method's selectivity was evaluated by examining the potential interference of adjuvants at the wavelength used for the quantitative determination of the combined drugs in the commercial sample. Since the list of adjuvants in the commercial sample was confidential, a placebo solution was prepared, containing a mixture of sodium citrate (14%), glucose (66%), sodium chloride (12.7%), and potassium chloride (7.3%). Both the sample and placebo solutions were prepared in water (q.s.p. 100 mL) using a procedure similar to the one described earlier for sample solution preparation. The chromatograms of the commercial sample and the placebo solution were then compared.

### Linearity

The evaluation involved constructing three analytical curves for each drug, based on dilutions of their respective standard stock

solutions. The analytical curve for Sodium Citrate (SC) was established within a concentration range of 0.72 to 1.75 mg/mL, for Chlorides (CL) from 1.1 to 2.65 mg/mL, and for Glucose from 3.3 to 8.1 mg/mL. Each determination was performed in triplicate. The resulting data were analyzed using linear regression to obtain the analytical curves, line equations, and correlation coefficients for each drug.

### Limit of Detection (LD) and Limit of Quantification (LQ)

The LD and LQ of SC, CL, and Glucose were determined from three analytical curves obtained for each drug, using the standard deviation of the intercept (SD) and the mean slope (a). Equations (1) were utilized to compute LD and LQ.

$$(1) LD = 3.3 x \frac{SD}{a} \quad LQ = 10 x \frac{SD}{a}$$

### Precision

The method's precision was assessed through intra-day (repeatability) and inter-day (intermediate precision) tests. Repeatability involved analyzing six determinations of the stock solution of the commercial sample at concentrations of 1.45, 2.2, and 6.75 mg/mL for SC, CL, and Glucose, respectively. This analysis was conducted under identical chromatographic conditions and by the same analyst. Intermediate precision was evaluated by preparing new sample-stock solutions, with two analysts performing the analysis on three different days, again at the aforementioned concentrations. Relative standard deviations (%RSD) were then calculated based on the test results.

### Accuracy

The accuracy of the method was determined through recovery tests, which involved adding known amounts from standard solutions to commercial sample solutions. Separate assays were conducted for each drug to cover the linear concentration range of the method. In the recovery tests, standard solutions and the previously described stock samples were subjected to recovery assays at three concentration levels (50% to 150% of the target concentration) for each drug, performed in triplicate. Quantities of the solution prepared with the raw materials of the drugs were added to the stock solutions to obtain concentration solutions of 0.7, 1.45, and 2.2 mg/mL for SC; 1.1, 2.2, and 3.3 mg/mL for CL; and 3.4, 6.75, and 10.1 mg/mL for Glucose.

The recovery percentages of each drug were calculated using equation 3 (AOAC, 2005): (3) %R = [(Ca-Cna)/Ctp] x 100 Where: R: recovery, Ca: drug concentration found in the standard added sample (mg mL<sup>-1</sup>), Cna: drug concentration found in the standard non-added sample (mg mL<sup>-1</sup>) and Ctp: theoretical standard concentration added to the sample (mg mL<sup>-1</sup>).

### Robustness

The method's robustness was intentionally assessed by introducing alterations to the chromatographic conditions. Variations were made in the column temperature (±5°C), specifically at 35°C and 45°C, and the flow rate of the mobile phase was adjusted (±0.1 mL/minute) within the range of 0.5 to 0.7 mL/min. For each condition, only one parameter was modified, while all other factors remained constant. The robustness evaluation was conducted in triplicate by injecting sample solutions containing 1.45, 2.2, and 6.75 mg/mL of SC, CL, and Glucose, respectively.

The impact of variations in each parameter on the final results was evaluated by calculating the average of the results obtained with normal parameters and comparing it with the average

corresponding to altered parameters. The effect generated by each variable was determined as the difference between the results obtained under normal conditions and those obtained with changed parameters.

### Solution Stability

To evaluate solution stability, both test and standard preparations were conducted following the specified procedure and stored under two conditions: at ambient temperature (20-25°C) and in the refrigerator (2-8°C). On the initial day, day 1, day 2, and day 3, these solutions were injected into the HPLC system. The assay percentage for the test preparation was calculated by comparing it to a freshly prepared standard solution. The calculated % limits were found to be within acceptable ranges for solutions stored for 3 days in the refrigerator and 24 hours at ambient temperature (20-25 degrees Celsius), indicating that the assay of SC, CL, and Glucose remained stable under both ambient and refrigerated conditions.

### Method Applicability

The commercial sample of ORS Oral Rehydration Salts (manufactured by Cipla Pharmaceuticals), consisting of sodium citrate (14%), sodium chloride (12.7%), potassium chloride (7.3%), and glucose (65.0%), underwent analysis using the proposed analytical method. Sample-stock solutions were prepared as outlined earlier, resulting in theoretical concentrations of SC 1.45 mg/mL, CL 2.2 mg/mL, and Glucose 6.5 mg/mL. The content of each drug in the commercial sample was then calculated using the established analytical curves.

The HPLC technique presented in this study is applicable for the analysis of pharmaceutical product formulations, drug substances, routine and in-process samples, as well as the measurement of pharmaceutical formulations and solutions for oral rehydration. Notably, this technique offers simplicity, user-friendliness, and cost-effectiveness, making it suitable for routine quality control (QC) analysis.

### Accelerated Stability

The research adhered to ICH Q1 stability guidelines, subjecting the commercial ORS sample from Cipla to a six-month exposure at 40 ± 2°C using a drying oven (NI 1521, NOVA Instruments®) equipped with a temperature controller. Organoleptic assessment occurred at 0, 3, and 6 months. The assay utilized high-performance liquid chromatography (HPLC) with a previously validated method. Chromatographic conditions included a Shimadzu Shim-pack SRC-102(H) ion exclusion analytical column (300 mm x 7.9 mm, 8µm), a mobile phase of 0.1% phosphoric acid in isocratic mode, a flow rate of 0.6 mL/min, an injection volume of 10 µL, and a diode array detector set at 200 nm. Sample solutions, prepared in water, aimed for theoretical concentrations of 1.45 mg/mL for SC, 2.2 mg/mL for CL, and 6.5 mg/mL for Glucose. Triplicate recordings of areas were made, and drug contents were calculated using equations derived from the analytical curves.

## Results and Discussion

### Chromatographic Conditions and Method Development

After extensive testing of various chromatographic conditions, including different columns and mobile phases, a method for the simultaneous determination of SL, SC, and Glucose was developed. The selected conditions yielded optimal results regarding parameters such as resolution, asymmetry, plates, and

maximum purity. This ensured system compliance, data quality, and indicated system selectivity, column accuracy, and efficiency. Table 1 outlines the chromatographic conditions chosen for the development and validation of the analytical method, including a Shimadzu Shim-pack SRC-102(H) ion exclusion analytical column (300 mm x 7.9 mm, 8µm), a flow rate of 0.6 mL/min, and a column temperature set at 40°C, with 0.1% phosphoric acid as the mobile phase. Specific wavelengths for drug quantification were selected after determining the chromatographic conditions for drug analysis and developing the analytical method. Spectral scans, conducted using a Diode Array Detector (DAD) in the 200-400 nm range, identified the wavelengths for the quantification of SC, CL, and Glucose, with 200 nm being selected for this purpose.

**Table 1: Chromatographic Conditions of RP-HPLC Method**

HPLC Column	Shimadzu Shim-pack SRC-102(H), 300mm x 7.9 mm, 8µm
Mobile Phase	0.1% Phosphoric acid
Flow Rate	0.6 mL/min
Column Temperature	50°C
Autosampler Temperature	25°C
Injection Volume	10 µL
Detector	200 nm
Run Time	20 minutes

### System Suitability

The system suitability parameters were assessed and confirmed to meet the prescribed criteria.

Please consult Table 2 for a comprehensive breakdown of the findings.

**Table 2: System Suitability Results**

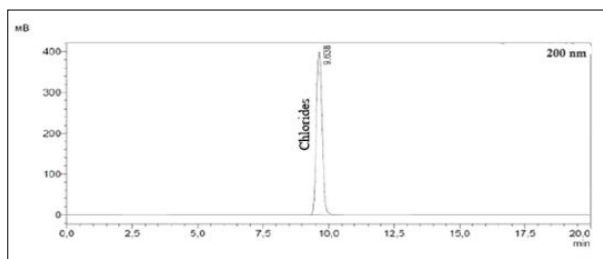
Parameter	Glucose	Sodium Citrate	Chlorides
Percent RSD (≤2.0) (n =5)	1.0	1.1	1.2
Tailing factor (≤2.0) (n =5)	1.2	1.3	0.9

### Selectivity/ Specificity

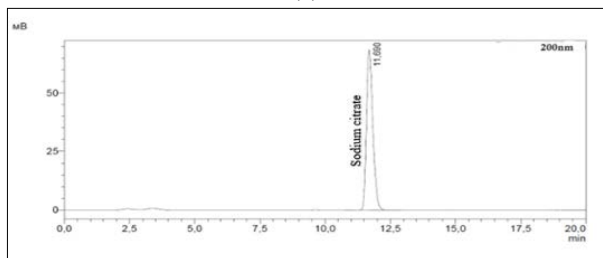
Specificity, defined as the capability to accurately determine an analyte in the presence of expected components, was assessed in the method. This involved analyzing solutions of blank, placebo, and control samples in the HPLC system and documenting chromatograms. Further information is provided in Figure 2, Figure 3 and Table 3.

**Table 3: Specificity Results**

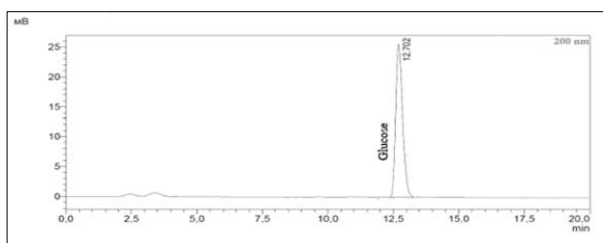
Parameter	Glucose	Sodium Citrate	Chlorides
Retention Time (min)	~12.7	~11.6	~9.6
Placebo Interference (Yes/No)	No	No	No



(a)

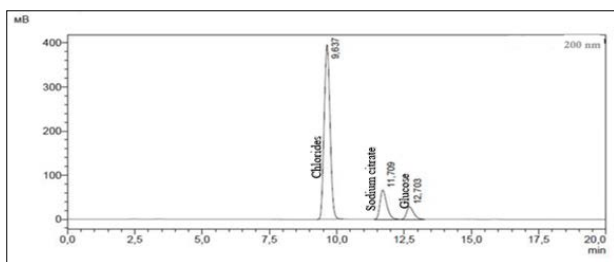


(b)

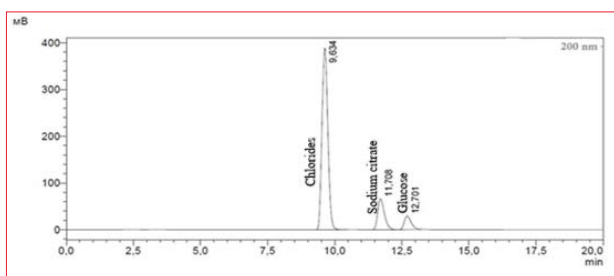


(c)

**Figure 2:** Individual Standard Chromatograms a) Chlorides, b) Sodium Citrate and c) Glucose



(a)



(b)

**Figure 3:** Specimen Chromatograms a) Standard Solution and b) Sample Solution

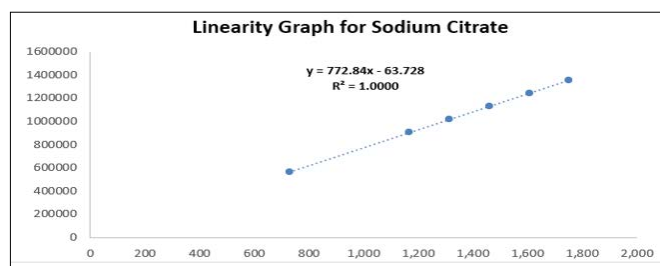
Linearity, Limit of Quantification (LQ) and Limit of Detection (LD)

In the investigation of linearity, preparations were made for five different concentrations of Sodium Citrate (SC), Chlorides (CL), and Glucose, covering a range from 25 to 120% of the specification level. The correlation coefficient was determined by

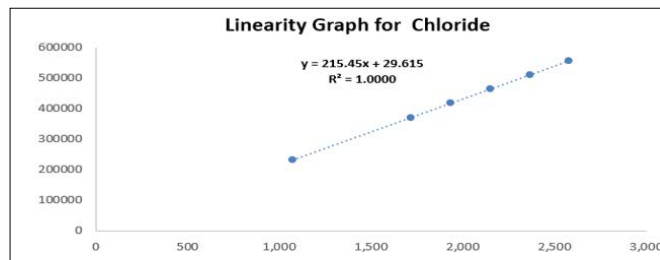
plotting concentration (X-axis) against peak area (Y-axis) for SL, SC, and Glucose. The concentration ranges for linearity solutions were 0.9 mg/mL to 2.1 mg/mL, 0.2 mg/mL to 0.45 mg/mL, and 3.0 mg/mL to 7.2 mg/mL, respectively. Regression equations of SL, SC, and Glucose were. The regression coefficient values (r) were all greater than 0.999 for each component, indicating linearity. The linearity of the area response versus concentration met the criteria referred to in Table 4, and the figures are depicted in Figure 4.

**Table 4: Results of Linearity Study**

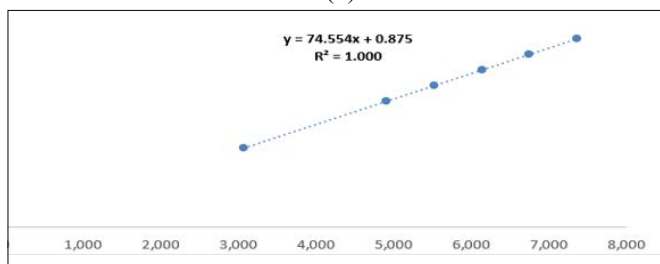
Name of the Component	Linearity (mg/mL)	Intercept	Slope	Correlation Coefficient
Sodium Citrate	0.7 – 1.7	63.72813	772.8358	1.0000
Chlorides	1.0 – 2.5	29.61529	215.4497	1.0000
Glucose	3.3 – 7.3	0.8750487	74.55382	1.0000



(a)



(b)



(c)

**Figure 4:** Linearity Plot (a) Sodium Citrate, (b) Chlorides and (c) Glucose

**Precision and Intermediate Precision**

The precision and intermediate precision evaluations of the analyzed drugs using the devised method are summarized in Table 5. The results, expressed as Percent Relative Standard Deviations (%RSD) for Sodium Citrate (SL), Chlorides (SC), and Glucose, consistently remain below the maximum recommended threshold of 5.0% (ICH, 2005). This confirms the accuracy of the developed analytical approach.

**Table 5: Precision and Intermediate Precision Results**

Sample	Sodium Citrate		Chlorides		Glucose	
	Analyst-1	Analyst-2	Analyst-1	Analyst-2	Analyst-1	Analyst-2
01	100.4	100.0	100.9	101.2	101.6	101.1
02	100.1	99.9	100.1	100.9	101.5	100.2
03	100.3	99.7	100.2	101.9	101.3	100.9
04	99.7	100.9	101.1	100.8	101.2	100.8
05	99.9	100.1	100.7	101.2	101.5	101.2
06	100.0	100.3	100.2	99.9	101.1	100.5
Average	100.1		100.8		101.1	
%RSD	0.3		0.6		0.4	

### Accuracy

The accuracy of the method was assessed through recovery tests, and the results for the recovery of standard solution amounts for each component are detailed in Table 6. The recovered amounts of Sodium Citrate (SC), Chlorides (CL), and Glucose fell within the acceptable limits of 98-102% for all three concentration levels analyzed. The %RSD of the average of these levels was below 2.0%. The mean recovery percentages for SC, CL, and Glucose were 100.4%, 100.8%, and 100.9%, respectively. Consequently, the developed method demonstrates satisfactory accuracy for the simultaneous determination of SC, CL, and Glucose.

**Table 6: Results for Recovery**

Amount added	Sodium Citrate	Chlorides	Glucose
50 %	101.6	100.9	101.2
100 %	99.7	101.1	100.9
150 %	100.0	100.5	100.7
%Avg	100.4	100.8	100.9

%RSD-Percentage Relative standard deviation

### Robustness

The System suitability parameters were evaluated, and it was determined that the results were within acceptable limits.

### Conclusion

A high-performance liquid chromatography (HPLC) method was devised and validated for the simultaneous determination of Sodium Citrate, Chlorides, and Glucose in pharmaceuticals, and solutions for oral rehydration for human use. This method exhibited characteristics of being rapid, selective, linear, precise, accurate, robust, and cost-effective. It is suitable for quality analyses in the pharmaceutical sector, ensuring safety and efficacy, and for detecting these compounds in environmental and biological samples intended for human use. The accelerated stability study indicated no significant alterations in the organoleptic properties of the commercial sample. Thus, these findings emphasize the importance of establishing dependable, swift, cost-effective, and user-friendly analytical methods for routine quality control in industries to guarantee the safety and effectiveness of medications.

**Conflicts of Interest:** The authors have declared no conflicts of interest to the publication of this research.

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