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Reversed-Phase-HPLC Assay Method for Simultaneous Estimation of Sorbitol, Sodium Lactate, and Sodium Chlorides in Pharmaceutical Formulations and Drug Solution for Infusion

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Abstract

A rapid, straightforward, sensitive, efficient, and cost-effective reverse-phase high-performance liquid chromatographic method was employed for the simultaneous determination of Sorbitol, Sodium Lactate, and Chlorides in a drug solution for infusion. Sorbitol, Sodium lactate, and Chloride are all officially recognized in the USP monograph. Assay methods are provided through various techniques, with titrations being ineffective for trace-level quantification. Alternatively, IC, AAS, and ICP-MS, though highly accurate, are costly and often unavailable to most testing facilities. When considering methods, it's important to prioritize both quality control requirements and user-friendly techniques. A simple HPLC simultaneous method was developed for the quantification of Chlorides, Sorbitol, and Sodium Lactate with a shorter run time. The separation utilized a Shim-pack SCR-102(H) ion exclusion analytical column (7.9 mm × 300 mm, 7 μm), with a flow rate of 0.6 mL per min. The column compartment temperature was maintained at 40˚C, and the injection volume was set at 10 μL, with detection at 200 nm. All measurements were conducted in a 0.1% solution of phosphoric acid. The analytical curves demonstrated linearity (r > 0.9999) in the concentration range of 0.79 to 3.8 mg per mL for Sodium Lactate (SL), 0.16 to 0.79 mg per mL for Sodium Chloride (SC), and 1.5 to 7.2 mg per mL for Sorbitol. Validation of

the developed method followed the guidelines of the International Conference on Harmonization (ICH Q2B) and USP<1225>. The method exhibited precision, robustness, accuracy, and selectivity. In accelerated stability testing over 6 months, no significant variations were observed in organoleptic analysis and pH. Consequently, the developed method is deemed suitable for routine quality control analyses, enabling the simultaneous determination of Sodium Lactate, Sodium Chloride, and Sorbitol in pharmaceutical formulations and infusions.

Keywords

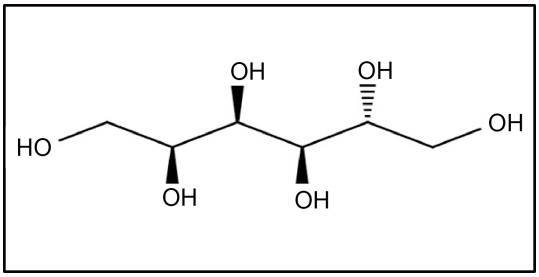
Sorbitol, Sodium Lactate and Chloride, Assay, Analytical Validation, HPLC

1. Introduction

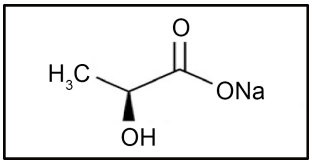
Sorbitol (depicted in **Figure 1(a)**) is a colorless, odorless solid with a molecular weight of 182.17 g/mol. Classified as a sugar alcohol or polyol, sorbitol is a type of carbohydrate. It contains approximately one-third fewer calories than sugar and possesses 60 percent of the sweetness. Naturally occurring in various berries and fruits such as apples and blackberries, sorbitol finds application as a laxative for constipation relief and as a urologic irrigating fluid in certain surgical procedures. In commercial settings, sorbitol serves multiple purposes, including moisture preservation, sweetness enhancement, texture improvement in products, and potential contributions to digestive and oral health.

Sodium Lactate (**Figure 1(b)**) is an organic sodium salt with lactate as the counterion, having a molecular weight of 112.06 g/mol. It serves as both a food preservative and a regulator of food acidity. This compound, an organic sodium salt and lactate salt, contains lactate. In medical applications, Compound Sodium Lactate (Hartmann's) is employed to replenish body fluids and mineral salts lost for various medical reasons. Particularly suitable when losses lead to an excessive presence of acid in the blood, it acts as an electrolyte replenisher and systemic alkalizer. Sodium Lactate Injection serves as a source of bicarbonate, aiding in the prevention or control of mild to moderate metabolic acidosis in patients with restricted oral intake, whose oxidative processes are not severely impaired.

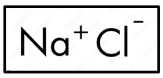
Sodium Chloride (depicted in **Figure 1(c)**), commonly recognized as table salt, has a molecular weight of 58.44 g/mol. It is an ionic compound denoted by the chemical formula NaCl, showcasing a 1:1 ratio of sodium to chloride ions. This salt, sodium chloride, plays a pivotal role in determining the salinity of seawater and the extracellular fluid in numerous multicellular organisms. Sodium chloride 23.4% injection is employed to restore lost water and salt in the body under specific 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



(a)



(b)



(c)

Figure 1. (a): Sorbitol structure; (b): Sodium Lactate structure; (c): Sodium Chloride.

as a tonicity agent in injectable formulations and infusion solutions.

The literature review highlights the absence of a developed method for the simultaneous estimation of Sorbitol, Sodium Lactate, and Sodium Chloride in pharmaceutical formulations and infusion solutions. While various approaches such as titrations, ion chromatography, AAS, and ICP-MS exist, each method has its limitations. Titrations, for instance, prove ineffective for trace levels of chlorides, and expensive techniques like ICP-MS and AAS face constraints due to their unavailability 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 a stationary phase. This column is well-suited for analyzing organic acids using an acid-aqueous solution (such as a phosphoric acid solution) as the mobile phase. The study successfully assesses all three components (sorbitol, Sodium lactate, and sodium chloride) in various pharmaceutical formulations and infusion solutions 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 in the quality control lab for routine, stability, and commercial sample analyses.

Potentiometric titration is employed to determine the assay of sodium chloride [1], Atomic Absorption Spectroscopy is used for the assay of sodium, cadmium, and potassium [2], and high-performance liquid chromatography with a refractive index detector is applied for sorbitol assay [3]. It's important to highlight that these three different techniques are explicitly mentioned in the USP monograph for independently quantifying sodium chloride, sodium lactate, and

sorbitol. Other articles have focused solely on the estimation of sugar alcohols [4]-[17] for chlorides, and [18] [19] for sodium lactate. The use of composite sodium lactate and sorbitol composition in fluid resuscitation for shock in patients with major burn [20], the Published article explained the feasibility of the use of sodium lactate and sorbitol (CISS) in fluid resuscitation for shock in patients with major burns. 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 [21]-[26]. It's worth noting that this marks the first-ever development of an RP-HPLC method for quantifying the assay of sorbitol, sodium lactate, and sodium chloride in pharmaceutical formulations and infusion solutions through HPLC.

2. Materials and Methods

2.1. Standard Reference Substances and Reagents

Reference standards for sorbitol (Potency 97.8%), sodium lactate (Potency 60.4%), and sodium chloride (Potency 99.9%) were provided by Sigma. The commercial sample used in the study was RHEOPAR-S® infusion solution sourced from the market. Analytical-grade water and phosphoric acid were utilized in the research work.

2.2. Equipment and Chromatographic Conditions

The methodology was established 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 achieved isocratically with a flow rate of 0.6 mL·min^{-1} on a Shim-pack SRC-102(H) ion exclusion analytical column (300 mm × 7.9 mm, 8 µm). The mobile phase comprised 0.1% phosphoric acid in water, and detection took place at 200 nm. A 10 µL injection volume and a running time of less than 30 minutes were employed. The analyses were conducted at a room temperature of 25.0˚C ± 1.0˚C).

2.3. Preparation of Standard Solutions and Commercial Samples

The reference standards for Sodium lactate (SL), Sodium chloride (SC), and Sorbitol were created by preparing standard solutions with concentrations of 3.10 mg per mL, 0.65 mg per mL, and 6.0 mg per mL, respectively, in water. The procedure involved weighing 310 mg of SL, 65 mg of SC, and 600 mg of sorbitol, transferring them to 100 mL volumetric flasks, and completing the volume with water. This resulted in solutions at concentrations of 3.10 mg·mL^{-1}, 0.65 mg·mL^{-1}, and 6.0 mg·mL^{-1} for each respective substance. Subsequently, these solutions underwent a 15-minute ultrasonic bath treatment.

2.4. Sample Preparation

Precisely transfer 10 ml of the commercial sample solution to a 100 ml volumetric flask, fill it to the mark with water, and thoroughly mix. Filter the solution through a 0.22 μm nylon filter, discarding the initial 5 ml of filtrate.

2.5. Analytical Parameters

The HPLC technique proposed in this study underwent validation following the guidelines outlined in ICH-Q2(R1). The validation parameters, including selectivity, precision, linearity, accuracy, robustness, LOD, and LOQ, were carefully chosen to assess the method's validation.

2.5.1. Selectivity

The method's selectivity was determined by assessing the interference of adjuvants at the wavelength employed for quantitatively determining the combined drugs in the commercial sample. As the list of adjuvants in the commercial sample was confidential, a placebo solution was created, comprising a mixture of adjuvants: Sodium lactate (19%), sorbitol (60%), sodium chloride (6%), calcium chloride (0.1%), magnesium chloride (0.2%), and potassium chloride (0.3%). Both the sample and placebo solution were prepared in water (q.s.p. 100 mL), following a procedure similar to the one described earlier for sample solution preparation. The chromatogram of the commercial sample was then compared with that of the placebo solution.

2.5.2. Linearity

The evaluation involved constructing three analytical curves for each drug, derived from dilutions of the respective standard stock solutions. Specifically, the analytical curve for Sodium Lactate (SL) was established within the concentration range of 0.79 to 3.8 $mg{\cdot}mL^{-1}$, for Sodium chloride (SC) in the range of 0.16 to 0.79 $mg{\cdot}mL^{-1}$, and for Sorbitol in the range of 1.5 to 7.2 $mg{\cdot}mL^{-1}$. These determinations were performed in triplicate, and the obtained results underwent linear regression analysis to derive analytical curves, line equations, and correlation coefficients for each drug.

2.5.3. Limit of Detection (LD) and Limit of Quantification (LQ)

The LD and LQ of SL, SC, and Sorbitol 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 used to calculate LD and LQ:

$$LD = 3.3 \times \frac{SD}{a} \qquad LQ = 10 \times \frac{SD}{a} \tag{1}$$

2.5.4. Precision

The precision of the method was evaluated 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 3.15, 0.65, and 6.0 $mg{\cdot}mL^{-1}$ for SL, SC, and Sorbitol, respectively. This analysis

was conducted under the same chromatographic conditions and by the same analyst. Intermediate precision was assessed by preparing new sample-stock solutions, with two analysts performing the analysis on three different days, again at the concentrations mentioned above. Relative standard deviations (%RSD) were then calculated based on the test results.

2.5.5. Accuracy

The accuracy of the method was determined through recovery tests, involving the addition of 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 (80% to 120% 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 150, 190, and 230 mg·mL^{-1} for SL; 32, 36, and 46 mg·mL^{-1} for SC; and 480, 600, and 720 mg·mL^{-1} for Sorbitol.

The recovery percentages of each drug were calculated using equation 3 (AOAC, 2005): (3) %R = [(*C*a-*C*na)/*C*tp] × 100 Where: *R*: recovery, *Ca*: drug concentration found in the standard added sample (mg·mL^{-1}), *C*na: drug concentration found in the standard non-added sample (mg·mL^{-1}) and *Ct*p: theoretical standard concentration added to the sample (µg·mL^{-1}).

2.5.6. Robustness

The method's robustness was deliberately evaluated by introducing changes to the chromatographic conditions. Variations were made in the column temperature (±5˚C), specifically at 35˚C and 45˚C, and the flow 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, keeping all other factors constant. The robustness assessment was conducted in triplicate by injecting sample solutions containing 3.1, 0.65, and 6.0 mg·mL^{-1} of SL, SC, and Sorbitol, respectively.

The impact of variations in each parameter on the final results was assessed 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.

2.5.7. Solution Stability

To assess solution stability, both test and standard preparations were carried out following the specified procedure and stored under two conditions: at ambient temperature (20˚C - 25˚C) and in the refrigerator (2˚C - 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 in comparison 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˚C - 25˚C), indicating that the assay of SL, SC, and sorbitol remained stable under both ambient and refrigerated conditions.

2.5.8. Method Applicability

The commercial sample RHEOPAR-S® (consisting of Sodium Lactate 19%, Sodium Chloride 6.0%, and Sorbitol 60.0%) underwent analysis using the proposed analytical method. Sample-stock solutions were prepared as outlined earlier, resulting in theoretical concentrations of SL 3.1 mg·mL^{-1}, SC 0.65 mg·mL^{-1}, and Sorbitol 6.0 mg·mL^{-1}. 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 infusion solutions. Notably, this technique offers simplicity, user-friendliness, and cost-effectiveness, making it suitable for routine quality control (QC) analysis.

2.5.9. Accelerated Stability

The study followed ICH Q1 stability studies, subjecting the commercial sample RHEOPAR-S® to exposure at 40˚C ± 2˚C for six months using a drying oven (NI 1521, NOVA Instruments®) equipped with a temperature controller. Organoleptic evaluation was conducted at 0, 3, and 6 months. The assay was performed using high-performance liquid chromatography (HPLC) with the previously validated method. The chromatographic conditions included a Shimadzu Shim-pack SRC-102(H) ion exclusion analytical column (300 mm × 7.9 mm, 8μm), a mobile phase of 0.1% phosphoric acid in isocratic mode, a flow rate of 0.6 mL·min^{-1}, an injection volume of 10 μL, and a diode array detector set at 200 nm. Sample solutions were prepared in water, yielding theoretical concentrations of 0.31 mg·mL^{-1} for SL, 0.65 mg·mL^{-1} for SC, and 6.0 mg·mL^{-1} for Sorbitol. Areas were recorded in triplicate, and drug contents were calculated using the equations of the straight lines obtained from analytical curves.

3. Results and Discussion

3.1. Chromatographic Conditions and Method Development

Following extensive testing of various chromatographic conditions, involving different columns and mobile phases, the method for the simultaneous determination of SL, SC, and Sorbitol was developed. The selected conditions yielded optimal results regarding parameters such as resolution, asymmetry, plates, and maximum purity. This ensured system compliance, and 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 × 7.9 mm, 8 μm), a flow rate of 0.6 mL·min^{-1}, 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

Table 1. Chromatographic conditions of RP-HPLC method.

HPLC Column	Shimadzu Shim-pack SRC-102(H), 300 mm × 7.9 mm, 8 µm
Mobile Phase	0.1% Phosphoric acid
Flow Rate	0.6mL/min
Column Temperature	40˚C
Autosampler Temperature	25˚C
Injection Volume	10 µL
Detector	200 nm
Run Time	20 minutes

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 SL, SC, and Sorbitol, with 200 nm being selected for this purpose.

3.1.1. System Suitability

The system suitability parameters were examined and validated to meet the acceptance criteria. Refer to **Table 2** for a detailed presentation of the results.

3.1.2. Selectivity/Specificity

Specificity, defined as the ability to accurately assess an analyte in the presence of anticipated components, was evaluated in the method. This was accomplished by analyzing solutions of blank, placebo, and control samples in the HPLC system and recording chromatograms. Additional details can be found in **Figure 2**, **Figure 3**, and **Table 3**.

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

In the investigation of linearity, preparations were made for five different concentrations of Sodium Lactate (SL), Sodium Chloride (SC), and Sorbitol, 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 Sorbitol. 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 Sorbitol were. The regressioncoefficient values (r) were all greater than 0.999 for each component, indicating linearity. The linearity of the area response versus concentration met the criteriareferred to in **Table 4**, and the figures are depicted in **Figure 4**.

3.1.4. Precision and Intermediate Precision

The precision and intermediate precision tests for the analyzed drugs using the developed method are summarized in **Table 5**. The results, expressed as Percent Relative Standard Deviations (%RSD) for Sodium Lactate (SL), Sodium Chloride (SC), and Sorbitol, were observed to be below the maximum recommended limit of 5.0% (ICH, 2005). This outcome affirms the accuracy of the developed analytical method.

Table 2. System suitability results.

Parameter	Sorbitol	Sodium Lactate	Chlorides
Percent RSD (≤2.0) (n = 5)	0.8	1.2	0.5
Tailing factor (≤2.0) (n = 5)	1.3	1.2	1.3

Table 3. Specificity results.

Parameter	Sorbitol	Sodium Lactate	Chlorides
Retention Time (min)	14.0	16.0	9.6
Placebo Interference (Yes/No)	No	No	No

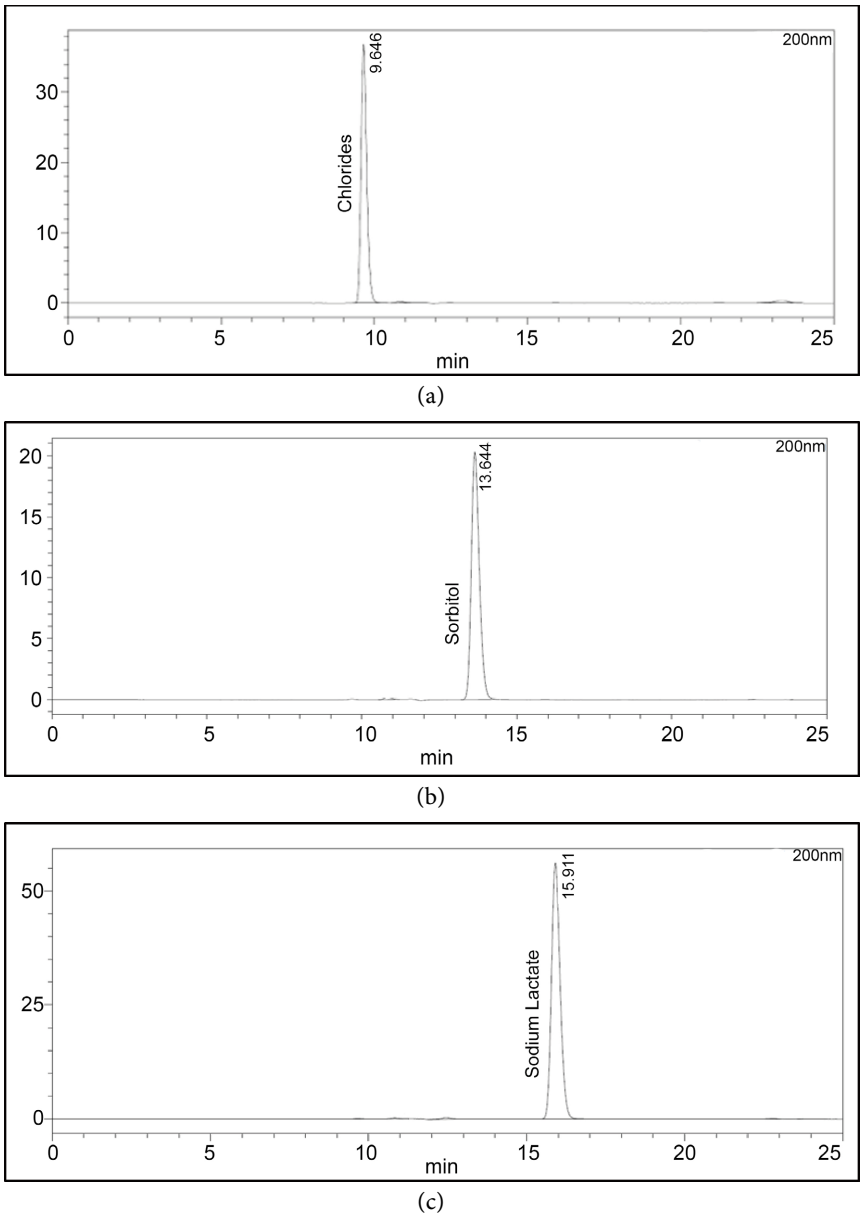
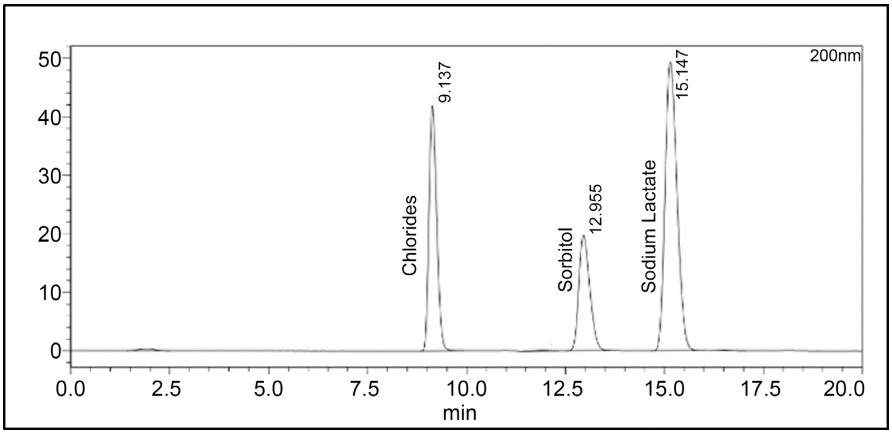


Figure 2. Individual Standard chromatograms (a) Sodium Chloride, (b) Sodium Lactate and (c) Sorbitol.



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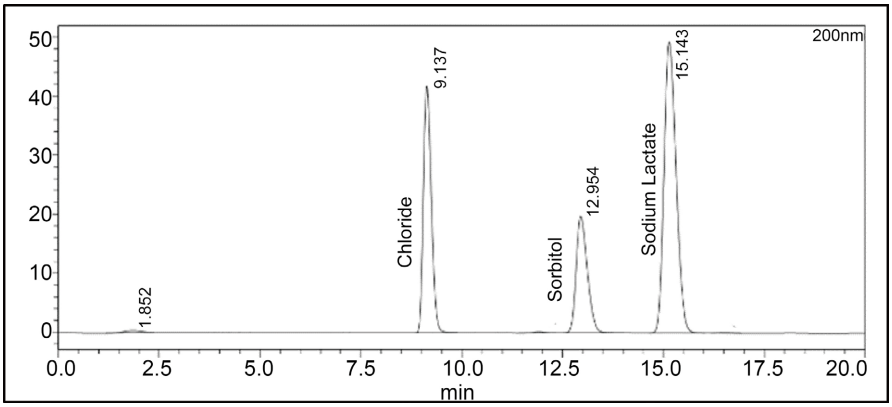
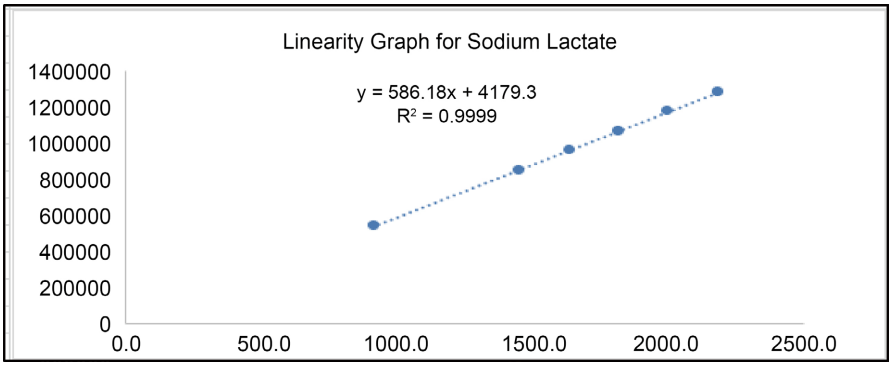


(b)

Figure 3. Specimen Chromatograms (a) Standard Solution, (b) Sample Solution.

Table 4. Results of linearity study.

Name of the component	Linearity (mg/mL)	Intercept	Slope	Correlation coefficient
Sodium Lactate	0.9 - 2.1	4179.332	586.183	0.9999
Sodium Chloride	0.2 - 0.45	42576.748	2817.534	0.9988
Sorbitol	3.0 - 7.2	3300.0541	178.4699	0.9999



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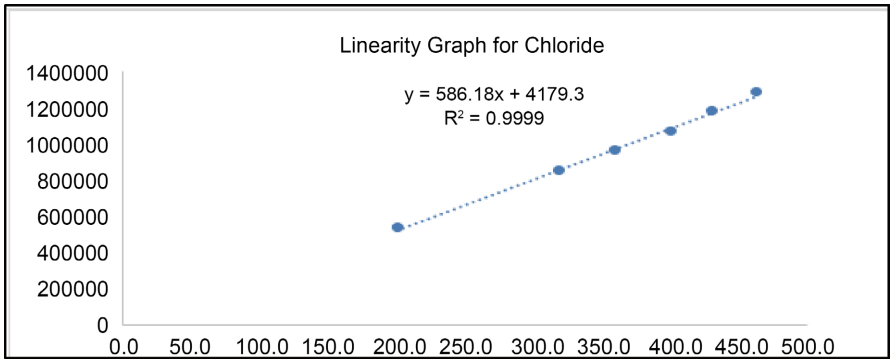

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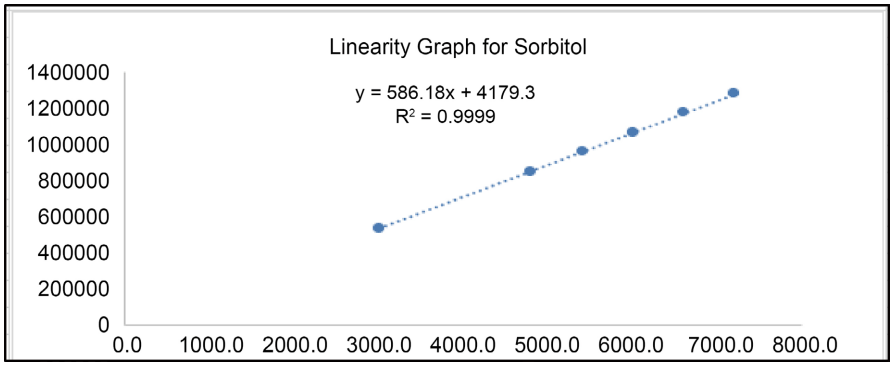

(c)

Figure 4. Linearity plot (a) Sodium Lactate, (b) Chloride, and (c) Sorbitol.

Table 5. Precision and intermediate precision results.

Sample	Sodium Lactate		Chlorides		Sorbitol	
	Analyst-1	Analyst-2	Analyst-1	Analyst-2	Analyst-1	Analyst-2
01	99.3	100.5	101.5	102.4	102.9	103.5
02	99.5	100.9	100.2	102.8	103.0	102.9
03	98.4	100.7	101.2	101.9	102.9	103.9
04	97.3	101.1	100.3	102.0	102.8	102.9
05	99.2	100.2	100.9	101.6	103.5	103.8
06	99.4	100.5	101.2	103.6	102.7	103.8
Average	99.8		101.6		103.2	
%RSD	1.1		1.0		0.4	

3.1.5. 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 Lactate (SL), Sodium Chloride (SC), and Sorbitol 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 SL, SC, and Sorbitol were 99.3%, 99.1%, and 99.8%, respectively. Consequently, the developed method demonstrates satisfactory accuracy for the simultaneous determination of SL, SC, and Sorbitol.

Table 6. Results for recovery.

Amount added	Sodium Lactate	Sodium Chloride	Sorbitol
50%	99.3	98.8	100.7
100%	99.1	98.9	100.3
150%	99.0	99.1	100.4

%RSD-Percentage Relative standard deviation.

3.1.6. Robustness

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

4. Conclusion

A high-performance liquid chromatography (HPLC) analytical method was developed and validated for the simultaneous determination of Sodium Lactate in combination with Sodium Chloride and Sorbitol in pharmaceutical and infusion solutions for human use. The method demonstrated attributes of being fast, selective, linear, precise, accurate, robust, and cost-effective. It is applicable for quality analyses in the pharmaceutical industry to ensure safety and efficacy, as well as for determining these drugs in environmental and biological matrices for human use. The accelerated stability study revealed no major changes in the organoleptic analysis of the commercial sample. Therefore, the findings underscore the significance of developing reliable, rapid, cost-effective, and easy-to-perform analytical methods for routine quality control in industries to ensure the safety and efficacy of medicines.

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
µg/mL: microgram/milliliter
%RSD: Percent Related Standard Deviation
LOD: Limit of Detection
LOQ: Limit of Quantification
SL: Sodium Lactate
SC: Sodium Chloride

Highlights of the Work

- An RP-HPLC method, characterized by high selectivity, has been developed for the efficient estimation of Sugars (Sorbitol), Sodium Lactate, and Sodium

Chloride, all achieved within a shorter run time by HPLC with UV detector.

- This study is unique as it represents the pioneering development of an HPLC method specifically designed for the analysis of three components in both liquid pharmaceutical formulations and infusion solutions.
- The established method was effectively utilized to estimate Sorbitol, Sodium Lactate, and Sodium Chloride in infusion solutions.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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