World Journal of Advanced Research and Reviews, 2020, 08(02), 213–223

World Journal of Advanced Research and Reviews

e-ISSN: 2581-9615, Cross Ref DOI: 10.30574/wjarr

Journal homepage: https://www.wjarr.com

(RESEARCH ARTICLE)

Development and validation of stability indicating method for determination of sodium citrate in pediatric cough syrup

Abdrhman Mahmoud Gamil [1,*] and Mohamed Awad al-lkareem Hamad [2]

[1] *Associate Professor of Pharmaceutics, Al-Neelain University, Sudan.*
[2] *Department of Chemistry, Blue Nile Research Centre, Sudan.*

Publication history**:** Received on 12 November 2020; revised on 20 November 2020; accepted on 21 November 2020

Article DOI: https://doi.org/10.30574/wjarr.2020.8.2.0427

Abstract

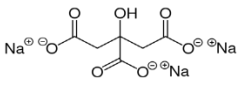
Sodium Citrate is widely used in analytical and food processes. It is used in various medical applications as anticoagulant in blood transfusion process and bladder washout. It is used in cough preparation as mucolytic. There are many methods for determination of Sodium Citrate in different preparations. Some of which use spectrophotometric methods, others used HPLC methods. The USP method uses titration and determining the end point potentionometrically. Many arguments take place between the Drug Regulatory Affairs and manufacturers regarding the appearance of the colour at the end point. The objective of this study is to develop and validate a simple, easy and precise HPLC stability indicating method for routine work and to be submitted to the Drug Regulatory Affairs. Chromatographic system with detector PDA at 210nm is utilized, Reprosil-XR C18, 250 mm×4 mm, 5 µm column is used. Column temperature 30^{0}C and flow rate of 1 ml/min. A clear peak of acceptable purity at 3.38 min retention time appears. The method is subjected to thermal stress, acid and base hydrolysis at extreme pH and forced oxidation. The result is that there is no interference of degradation products with the substance peak. The method is then subjected to validation study according to the ICH guidelines. The method comply the specificity, precision, linearity, accuracy and robustness acceptable criteria. Thus, the method satisfies the stability indicating method and validation requirements that it can be submitted to the Drug Regulatory Affairs and used in QC routine activities and stability studies.

Keywords: Sodium Citrate HPLC Determination; Stability Indicating Method; Validation of Analytical Method; Forced Degradation of Sodium Citrate.

1. Introduction

Sodium Citrate Sodium Citrate is anhydrous or contains two molecules of water of hydration. It contains NLT 99.0o/o and NMT 1 00.5% of $C_6H_5Na_3O_7$, calculated on the anhydrous basis having molecular weight of 258.07 [1].

Sodium Citrate structural formula [2]:



1.1. Structural Formula of Sodium Citrate

Sodium Citrate is widely used as chemical reagent and in buffers preparations. It is used as anticoagulant [1]. Sodium Citrate is used as bladder washout, to relief comfort in mild urinary tract infection, as enema in constipation, as irrigation solution and in oral rehydration salt [3]. Sodium Citrate is used in cough preparation as a mucolytic agent which thins and loosens the mucus (phlegm) making it easier to be coughed out [4].

* Corresponding author: Abdrhman Mahmoud Gamil
Associate Professor of Pharmaceutics, Al-Neelain University, Sudan.

Many methods are used to determine Sodium Citrate in a mixture or solutions. Titration methods are used. The USP method is potentiometric titration [1]. Spectrophotometric methods are also used [5]. Many studies uses ion chromatography of which is the determination of citric acid and sodium Citrate in an aqueous ophthalmic preparation which uses titration for Citric acid and chromatographic method for Sodium Citrate [6]. Also Sodium Citrate was determines by RP-HPLC method using Ammonium Sodium Phosphate and Methanol as mobile phase and detected at 210 nm [7]. Stability – indicating method is an analytical, quantitative and validated method that detects the drug substance accurately and specifically in the presence of its degradation products without interference [8]. Degradation takes place by time but it can be forced by stress testing by inducing challenging conditions that are more drastic than those used in the accelerated stability testing. This can be executed by exposing the drug to extreme pH value to accelerate acid and base hydrolysis, by using elevated thermal conditions and by subjecting the product to H_2O_2 to induce oxidation [9]. The adequacy of the stability indicating method is obligated to ensure specificity, precision, reproducibility, and sensitivity. The method should separate and quantify the drug in the presence of its decomposition products and other dosage form components [10].

Validation differs from testing, that testing is for the identification of errors and validation is documented evidence that a system performance is as expected [11]. It is an analytical procedure or process by which it is established, by laboratory studies, that the performance characteristics of the procedure meet the requirements for the intended analytical application [1]. The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose [12].

The objectives of this study is to establish an easy, precise and simple method for the ease of routine laboratory work by developing a simple validated stability indicating method for determination of Sodium Citrate in pediatric cough syrup by using HPLC chromatography.

2. Material and methods

2.1. Instruments and equipment

Two HPLC-Prominence (PDA), Schimadzu, Japan, Analytical Balance RO water system HPLC Column ReproSil-XR 120 C18, 5µm (250 mm x 4mm), HPLC Column, Rotatory shaker pH meter Sonicator, Laboratory Oven.

2.2. Materials

Purified Water RO grade, BNP, Acetonitrile HPLC grade Orthophosphoric acid AR grade Glass wares Class 'A' grade. USP Sodium Citrate RS Lot: R086 N0% Potency87.12% reference standard. Sample PD Batch No012B16 Mfg. Date10/2019

2.3. METHODS

2.3.1. Chromatographic conditions

Column: ReproSil-XR 120C18, 5µm (250 mm x 4mm) or equivalent, Detector: PDA Detector, Wavelength: 210 nm, Flow rate: 1.0 ml/min, Injection Volume: 50µl, Column Temperature: 30^0C, Run time: 35 minutes

2.3.2. Gradient Program

Table 1 Gradient Program

Time (minutes)	Mobile phase - A	Mobile phase - B
0	100	0
10	100	0
15	20	80
20	20	80
25	100	0
35	100	0

2.3.3. Preparation of mobile phase A

Adjust the pH 2.0 ± 0.05 with orthophosphoric acid. Filter through 0.45µ-nylon membrane filter.

2.3.4. Preparation of Mobile phase B

Mix Acetonitrile and mobile phase A in the ratio of 90:10 v/v. sonicate to degas and filter through 0.45µ-nylon membrane filter. Diluent: Use water as diluent/blank

2.3.5. Preparation of Standard solution

Accurately weigh and transfer 55.0mg of Sodium citrate working standard into 100 volumetric flask; add about 70 ml of diluent and sonicate to 5 minutes to dissolve the content, markup the volume with diluent and mix well. Further dilute 5 ml of the solution to 25 ml with diluents and mix (Concentration of Sodium citrate is about 0.11 mg/ml).

2.3.6. Preparation of Test solution

Determine the density of sample. accurately weigh and transfer quantity equivalent 5.0 mL of syrup into 100 mL volumetric flask , add about 70 mL of diluent and shake for 10 minutes on a rotary shaker and sonicate for 5 minutes, make up to volume with diluent and mix well. Further dilute 10 ml of the solution to 25 ml with diluent and mix. Filter the solution through membrane 0.45 µm filter, discard few mL of filtrate and collect the remaining for analysis. (Concentration of Sodium citrate is about 0.114 mg/ml)

2.3.7. Protected Placebo preparation

Accurately transfer 5.0 mL placebo of syrup into 100 mL volumetric flask , add about 70 mL of diluent and shake for 10 minutes on a rotary shaker and sonicate for 5 minutes, make up to volume with diluent and mix well. Further dilute 10 ml of the solution to 25 ml with diluent and mix. Filter the solution through membrane 0.45 µm filter, discard few mL of filtrate and collect the remaining for analysis.

2.3.8. Procedure

Equilibrate the column with mobile phase for sufficient time until stable baseline is obtained. Inject 50 µl of Blank and Standard preparation as per the sequence of injections. If the system suitability criteria meet the requirements then inject the Sample preparation as per the sequence and record the chromatograms

2.3.9. Sequence of Injection

Blank 1 Injections, Standard solution 6 replicates injections, Test solution triplicate injections.

2.3.10. Evaluation of System suitability

1. The relative standard deviation of peak area responses of Sodium Citrate for six replicate injections of the standard solution should not be more than 2.0%

2. Theoretical plates for the peak due to Sodium Citrate obtained from standard solution should not be less than 2000

3. Tailing factor for the peak due to Sodium Citrate obtained from standard solution should not be more than 2.0

2.3.11. Calculations

Calculate the amount of sodium citrate present in percentage of solution using following formula: (R_U/R_S)*(Ws/100)*(5/25)*(100/W_U)*(25/10)*D*P/100*1/L*F*5*100. Where,

R_U = Average area of Sodium citrate peak in the chromatogram obtained for standard solution

R_S = Average area of Sodium citrate peak in the chromatogram obtained for sample solution

W_S = Weight of Sodium Citrate standard in mg.

W_U = Actual quantity in mg of sample

D = Density of sample in mg

L = Label claim of Sodium Citrate in mg (28.5mg/5ml)

F= 28.5/28.5+X. Where,

X = Actual quantity added of citric acid in the in-process manufacturing of the batch.

For batch (012B16); citric acid quantity = 2.6 mg per 5 ml of syrup

F= (28.5)/(28.5+2.6) =0.9164.

2.4. Forced Degradation Parameters

Parameters are temperature stress, acid Stress, base Stress and oxidation stress.

2.5. Validation Parameters [12, 13, 14]

Validation parameters are System Suitability, Specificity, Accuracy study (Recovery study), Linearity, Range and Precision i) System Precision ii) Repeatability (Method Precision) iii) Intermediate Precision.

Robustness by changing the flow rate from 1.0 ml/min. to 1.1 ml/min, changing the flow rate from 1.0 ml/min. to 0.9 ml/min, changing the column temperature from 30^0C to 28^0C, changing the column temperature from 30^0C to 32^0C, changing the wavelength from 210 nm to 208 and change the wavelength from 210 nm to 212 nm.

3. Results and discussion

3.1. System Suitability

6 injections for the standard and 3 for the test sample, the following results were obtained:

Table 2 Results of system suitability

Standard solution			**Test sample solution**			
	Area	RT	Area	Assay %	Peak purity	Purity threshold
Mean	233945	3.845	298602	98.56	0.013902	1.0000
STD	315.494	0.003				
RSD %	0.13	0.08				

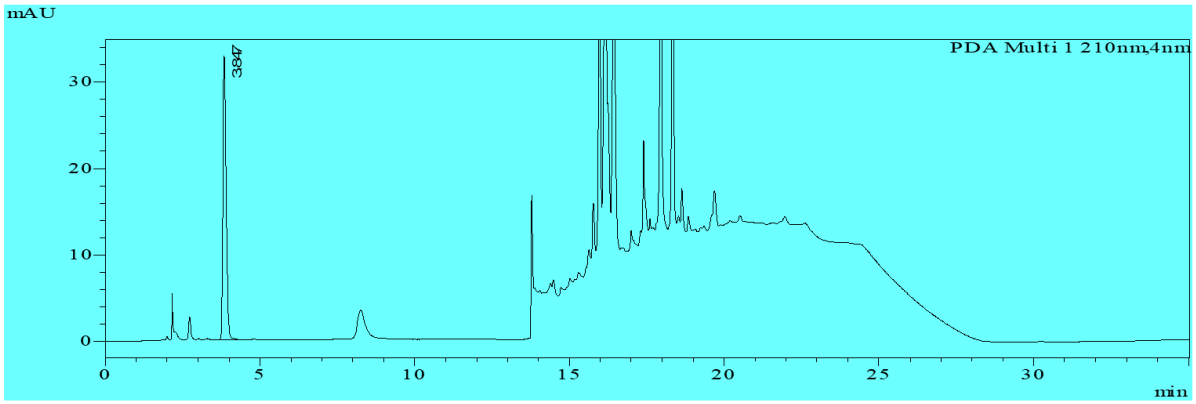


Figure 1 Peak of system suitability

3.1.1. Acceptance Criteria

In the chromatogram of the placebo solution, there should be no interference at the retention time corresponding to the peak of Sodium Citrate. The % relative standard deviation for the peak area of Sodium Citrate for six replicate injections of standard solution should not be more than 2.0 %.

3.1.2. Inference for System suitability

In the chromatogram of the placebo solution, there is no interference at the retention time corresponding to the peak of Sodium Citrate. The % relative standard deviation for the peak area of Sodium Citrate for six replicate injections of standard solution is 0.13%

3.2. Result of stress testing

Temperature stress testing at 60^{0}C by exposure the sample and placebo for 1 hrs. Temperature stress was performed by analyzing samples by injecting triplicates of 50 µL of placebo solution and 50 µL of test solution under stress of temperature of 60ºC. Acid stress was performed by analyzing samples by injecting 50 µL of placebo solution prepared using 0.01N HCl and injecting 50 µL of test solution degraded with 0.01N HCl in triplicates. Base Stress was performed by analyzing samples by injecting 50 µL of placebo solution prepared using 0.01N NaOH in single and injecting 50 µL of test solution degraded with 0.01N NaOH in triplicates. Oxidation Stress was performed by analyzing samples by injecting 50 µL of placebo solution prepared using 3% v/v hydrogen peroxide in single and injecting 50 µL of test solution degraded with 3% v/v hydrogen peroxide in triplicates.

After reflux with 0.01N HCl for 2 hours also with 0.01 N NaOH and with 3% v/v H_2O_2, the Pink colour of sample solution remained same. Acceptance Criteria in the chromatogram of the placebo solution, there should be no interference at the retention time corresponding to the peak of Sodium Citrate and the peak purity should be less than purity threshold.

Table 3 Results of Forced Degradation Testing

Parameter	Area	% Assay	Peak Purity	Purity Threshold
Thermal stress by 60ºC	297397	97.51	0.009447	1.0000
Acid stress by 0.01 N HCl	299756	98.67%	0.027568	1.0000
Base stress by 0.01 N NaOH	297244	98.06	0.078130	1.0000
Oxidation stress by 3% H_2O_2	292989	96.72	0.044457	1.0000

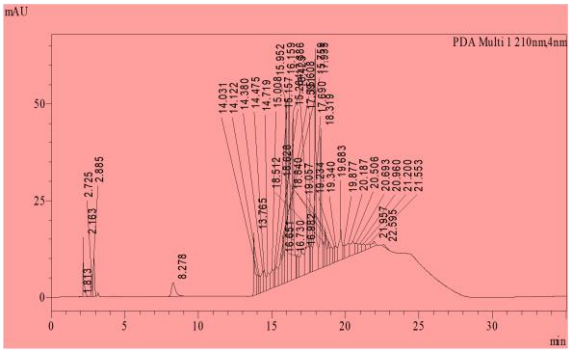


Figure 2 Placebo Thermal stress

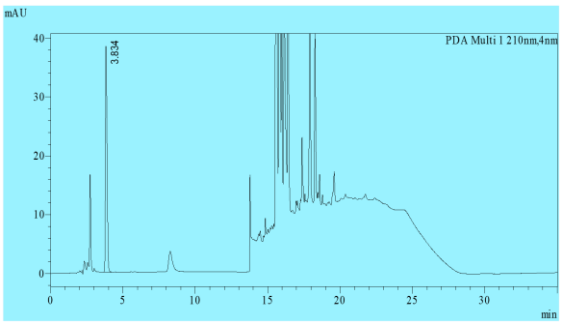


Figure 3 Sodium citrate solution thermal stress

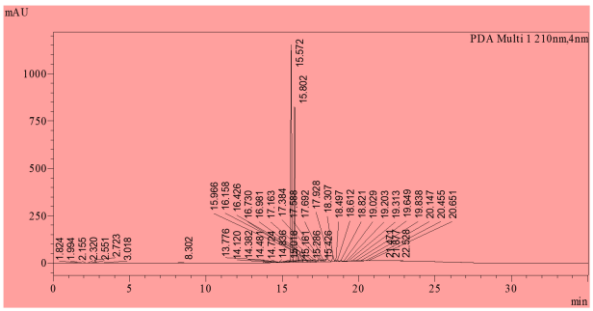


Figure 4 Placebo Acid& base hydrolysis

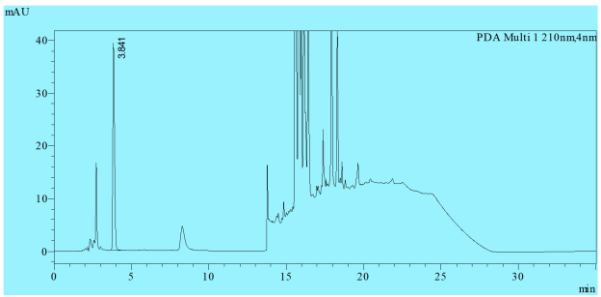


Figure 5 Solution Acid& base hydrolysis

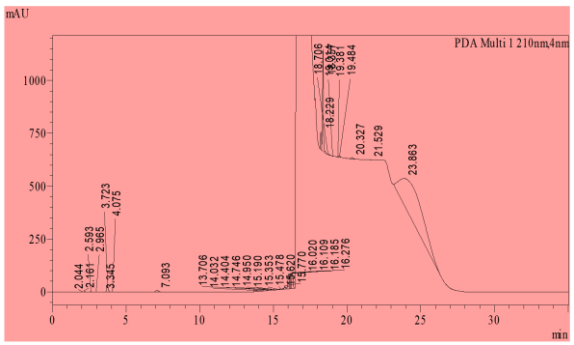


Figure 6 Placebo Oxidation stress

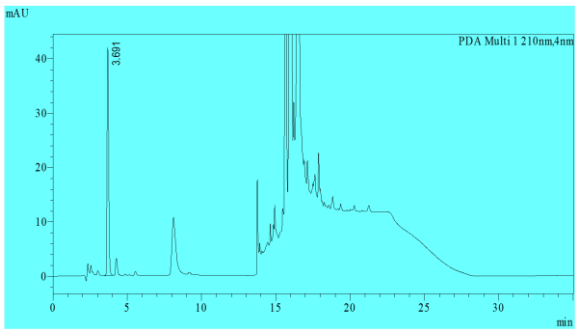


Figure 7 Test solution oxidation stress

3.2.1. Inference for stress testing

There is no significant change observed in the appearance of the pink colour of the sample which is treated with elevated temperature at 60ºC for 1 hour, treated with 0.01 N Hydrochloric acid, with 0.01N Sodium hydroxide and with 3% v/v Hydrogen peroxide. The sample has undergone no degradation; the % assay value decreases from 98.56% to 97.51% on thermal stress, from 98.56% to 98.67% on acid stress, from 98.56% to 98.06% on base stress and from 98.56% to

96.72% on induced oxidation stress. The peak purity is less than purity threshold. It proves that no impurity peaks are co-eluting with peak of Sodium citrate. Hence the method is stability indicating.

3.3. Results of Validation Parameters

3.4. System suitability

System suitability was demonstrated by making six replicate injections of standard solution as per the specification method. The peak area of Sodium Citrate for six replicates injections was recorded. The precision was evaluated by computing the relative standard deviation for the peak area of these replicate injections.

Table 4 System suitability results

Standard solution	RT (min)	Area
Average of six replicates	3.794	221915
SD	0.0030	406.4
%RSD	0.0782	0.1832
Theoretical plate	38227	
Tailing factor	1.325	

3.4.1. Acceptance Criteria

1. There should be no interference at the retention time of Sodium Citrate in the chromatogram of blank, the theoretical plates for the peak due to Sodium Citrate obtained from standard solution should not be less than 2000 and the tailing factor for the peak due to Sodium Citrate obtained from standard solution should not be more than 2.0. The relative standard deviation of peak area responses of Sodium Citrate for six replicate injections of the standard solution should not be more than 2.0%

3.4.2. Inference

In the chromatogram of blank, there is no interference at the retention time of Sodium Citrate. The theoretical plates for the peak of Sodium Citrate obtained from standard solution 38227 which is more than 2000. The tailing factor for the peak due to Sodium Citrate obtained from standard solution is 1.325 and the relative standard deviation of peak area responses of Sodium Citrate for six replicate injections of the standard solution is 0.0782% which is less than 2.

3.5. Specificity

Prepare a representative of standard solution, and sample solution as per the methodology. Inject diluent and placebo. Inject the standard solution and sample solution of product by using the chromatographic system described in the methodology.

The results of the blank and placebo 1 &2, indicates that there is no interference neither in the retention time nor in the peak area. The average of the retention time of the six replicates of the standard solution is 3.797 mins and that of the test solution is 3.795 mins.

3.5.1. Acceptance Criteria

The system should meet the system suitability criteria, there should be no potentially interfering peaks in blank and placebo which can adversely affect the quantitation of Sodium Citrate peak in the sample.

3.5.2. Inference

The system meets the system suitability criteria, There are no potentially interfering peaks in blank and placebo which can adversely affect the quantitation of Sodium Citrate peak.

3.6. Accuracy

A placebo was spiked with Sodium Citrate standard from levels corresponding the approximately 80%, 100%, and 120% of the sample solution concentration.

Prepare the recovery samples in triplicate for each level.

Table 5 Accuracy results

Level	Concentration %	Average Recovery% of triplicates
1	80	99.7
2	100	99.2
3	120	99.2

Calculations: $\%\ Recovery = amount\ added/amount\ found \times 100$

3.6.1. Acceptance Criteria

The mean percentage recovery of Sodium Citrate peak should be 98.0 – 102.0% at each level.

3.6.2. Inference

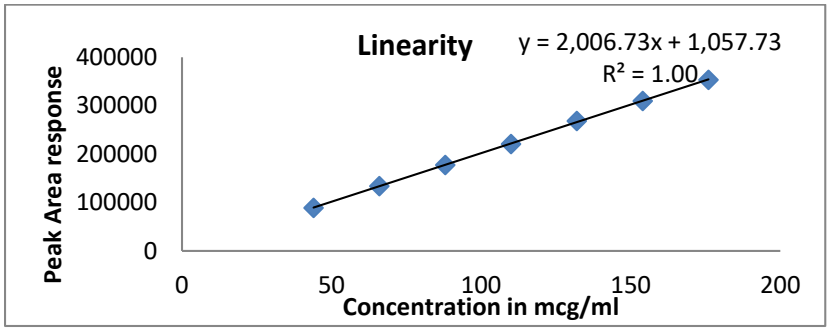
The mean percentage recovery of Sodium Citrate peak is 99.7 – 99.2 % which is within 98.0 – 102.0% at each level, thus the method complies the acceptable criteria.

3.7. Linearity and Range

Prepare a standard stock solution of Sodium Citrate. From this solution diluted to level approximately 40% of the nominal sample solution concentration to approximately 160% of the nominal sample solution concentration at 7 levels from 40%, 60%, 80%, 100%, 120%, 140% and 160% respectively. Plot a graph of concentration (at X-axis) versus average peak area of analyte (at Y-axis). Evaluate the correlation coefficient (r)

Table 6 Linearity Results

No of 6 inj	40%	60%	80%	100%	120%	140%	160%
Average	88991	133803	177432	220760	268516	309622	353466
SD	146.4	89.8	136.5	26.2	91.2	1344.9	246.8
RSD	0.16	0.07	0.08	0.01	0.03	0.43	0.07



Graph 1 Linearity Curve

Correlation Co-efficient is 0.99992, Y intercept is1057.73214and slope is 2006.73295

3.7.1. Acceptance Criteria

The correlation coefficient should be equal or greater than 0.999 (i.e. $r^2 \geq 0.999$ and the RSD% of peak area of Sodium Citrate for Linearity Level 1 and Level 7 should not be more than 2.0.

3.7.2. Inference

The correlation coefficient $r^2$is 0.99992 which is greater than 0.999, the % RSD of peak area of Sodium Citrate for Linearity Level 1 and Level 7 is 0.01 -0.43 which is less than 2.0.

3.8. System Precision

System suitability was demonstrated by making six replicate injections of standard solution as per the specification method. The peak area of Sodium Citrate for six replicates injections was recorded. The precision was evaluated by computing the relative standard deviation for the peak area of these replicate. The acceptance criteria as mentioned in the system suitability.

3.8.1. Inference

The relative standard deviation of peak area responses of Sodium Citrate for six replicate injections of the standard solution is 0.101%. The theoretical plates for the peak due to Sodium Citrate obtained from standard solution is 37548 which is more than 2000, the tailing factor for the peak due to Sodium Citrate obtained from standard solution is 1.351 which is less than 2.

3.8.2. Method Precision (Repeatability)

Prepare and inject six replicates of sample solution as per sample preparation procedure described in the methodology, inject triplicate for each of the 6 test solutions. The resulted average concentration was 100.1% with standard deviation 0.35 and RSD% 0.349.

3.8.3. Acceptance Criteria

The relative standard deviation of the %assay should be ≤ 2.0%, and in the chromatogram obtained with the test solutions, % assay should be within the specification limit**.**

3.8.4. Inference

%RSD for six replicate samples were found within the acceptance criteria**.**

3.9. Intermediate Precision

To demonstrate the intermediate precision, prepare six samples in the same way as per sample preparation in repeatability. Analysis was performed by different analyst on different days and in different instrument.

Table 7 Comparison between Method Precision and Ruggedness Method Precision

No of 6 samples	Analyst 1	Analyst 2	Overall results
Mean of assay%	100.15%	99.72%	99.93%
STD	0.33	0.773	0.62
RSD%	0.330	0.775	0.62

3.9.1. Acceptance Criteria

%RSD of each analyst should not be more than 2.0%, %RSD of mean data of two analysts should not be more than 3.0%.

3.9.2. Inference

The %RSD for analyst-1 & analyst-2 was found within 0.349%& 0.775% respectively and the overall % RSD of both analysts is 0.62%. Thus meet the acceptance criteria.

3.10. Robustness

Three parameters were deliberately changed to evaluate the robustness of the method. In each parameter sample were prepared and the mean assay of these three replicate injections was compared with the mean assay of samples where all parameters were normal in precision study. 6 replicates were used for system precision and triplicates for method precision.

Table 8 Results for Robustness

Robustness variable	Limits	Mean Assay %	Variation %
Flow rate	1 ml/min	100.15	00
	1.1 ml/min	98.4	-1.7
	0.9 ml/min	98.9	-1.25
Column temperature	30^0C	100.15	00
	32^0C	100.1	- 0.05
	28^0C	100.6	0.45
Wavelength	210 nm	100.15	00
	212 nm	100.2	0.05
	208 nm	100.03	-.0.12

3.10.1. Acceptance criteria

The % assay variation should not be more than 3.0% for changed parameters when compared to normal parameter

3.10.2. Inference

System suitability criteria are met. There is no significant variation in the % Assay of the sample.

The difference in the percent assay of changed parameter was not more than 3.0% when compared with the normal parameter.

4. Conclusion

The HPLC chromatographic method for determination of Sodium Citrate in pediatric cough syrup complies the acceptance criteria for the forced degradation stress test of acid and base hydrolysis. It passes the thermal and oxidation challenging conditions. The method also complies the acceptance criteria of the validation parameters in accordance with the ICH guidelines with respect to specificity, precision, linearity, accuracy and robustness. Thus, the method is simple, easy and can be considered as validated stability indication method. It can be submitted to the Drug Regulatory Affairs and can be used in route QC activities and stability studies.

Compliance with ethical standards

Acknowledgments

Acknowledgement to the Blue Nile Research Centre team and Manager to their helpful support.

Disclosure of conflict of interest

Authors declare that there is no conflict of interest in publishing this work.

References

[1] United States Pharmacopeial Convention, USP 41, 1/5/2018, .12601 Twinbrook Parkway, Rockville, MD 20852, USP Volume 1<1225>Validation of Compendial Procedures, 7665-7671, volume 3, Sodium Citrate, P 329, 3787, 5870.

[2] Wikipedia, www.en.wikipedia.org. 5/11/2020; 12:50

[3] British National Formulary 80, September 2020, Royal Pharmaceutical Society, Published by BMI Group and Pharmaceutical Press, p 832, www.pharmapress.com

[4] Dr. Love Sharma, www.1mg.com/generics/ammoniumchloride-sodium citrate-diphenhydramin-menthol, 400245

[5] Krukowski S, Karaiewicz M, Kolodziejski W, Convient UV-Spectrophotometric Determination of Citrate in Aqueous Solution with pharmaceutical Electrolyte Formulation, Journal of Food and Drug Analysis, July 2017; volume 25, issue 3:717-722.

[6] Hotha Kishore, Development of Better-Quality Assay Method for the Citric acid and Sodium Citrate in Opthalmic/Oral Solutions and their Application to Deformulation Studies, American Journal of Analytical Chemistry, 12/2014; 5 (17):1249-1260.

[7] Luo L, Luo Y, Wang C, Determination of Potasium Citrate and Sodium Citrate Chewable Tablets Content and Related Substances by RP-HPLC, Latin American Journal of Pharmacy, January 2014; 32 (1):37-42.

[8] Bakshi M., Singh S. Development of validated stability-indicating assay methods—critical review. Journal of Pharmaceutical and Biomedical Analysis. 2002; 28(6):1011–1040. doi:10.1016/s0731-7085(02)00 047-x. [PubMed] [CrossRef] [Google Scholar]

[9] Singh S., Bakshi M. Guidance on Conduct of Stress Testing to Determine Inherent Stability of Drugs. Pharmaceutical Technology; 2000. [Google Scholar]

[10] Quanyun A. Xu, Lawrence A Trissel, 2003, Stability Indicating HPLC Methods for Drug Analysis, second edition, published by American Pharmacist Association and the Pharmaceutical Press, London SE1, 7JN, UK. www.pharmpress.com

[11] Savant D. A, Pharma Pathway industrial and Applied Pharmacy, Validations page 2.20 – 2.39, published by Nirali Prakashan, Abhyudaya Pragati, 1312, Shivaji Nagar, 2012, niralipune@pragationline.com

[12] ICH Q2 (R1), 1997, Validation of Analytical Procedures, Pages 2, 4, EMEA 226, www.emea.eu.int

[13] Ludwig Huber, Validation of Analytical Methods and Processes, Agilent Technologies GmbH, Waldbronn, Germany p 544

[14] FDA Guidance for Industry, Analytical Procedures and Method Validation for Drugs and Biologics, July 2015, page 7.