VALIDATION OF HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF PSEUDOEPHEDRINE HCL, GUAIFENESIN, CHLORPHENIRAMINE MALEATE AND DEXTROMETHORPHAN HBR

Abstract

Pseudoephedrine HCL, Guaifenesin, Chlorpheniramine Maleate and Dextromethorphan HBr combination is a common combination cough syrup. The chemical analysis of each individual component is efforts and time consuming. HPLC method had been develop for simultaneous determination of the four compounds in one HPLC injection of 20 µl using detector at 210 nm, column C18 4.6 mm × 250 mm, 3µm and mobile phase of Potassium dihydrogen orthophosphate, acetonitrile, orthophosphoric acid , triethanolamine and water . At column oven temperature of 40 ℃, flow rate 0.8 ml/min and 60 minutesrun time. The method had been evaluated for system suitability and validated according to the ICH guidelines with respect to method specificity, linearity and range, precision, accuracy and robustness. Limit of detection quantitation limit and solution stability had been assessed. The results showed that the method fulfilled all acceptable criteria for all validation parameters. It had been recommended that the method can be used for routine analysis of products containing the four components.

Key words:

Chemical method validation, chromatographic system validation, four in one method of analysis, pseudoephedrine, guaifenesin, chlorpheniramine and dextromethorphan combination method of analysis.

Introduction

Validation of an analytical procedure is the process by which it isestablished, by laboratory studies, that the performance characteristics of the procedure meet the requirements for the intended analytical application[1].As per the ICH guidelines, the validation process of the method includes the specificity, linearity and range, precision, accuracy, solution stability, assay of pharmaceutical product and robustness[2].

Compounds structural formula[3]:

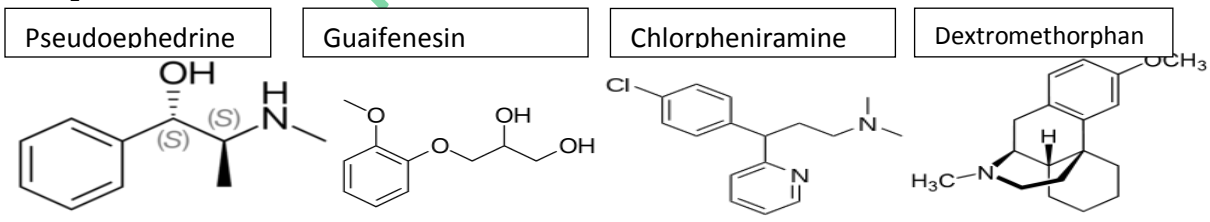

Figure 1: the structural formulas of the compounds

Pseudoephedrine is a systemic decongestant, Quiafenesin $C_{10}H_4O_4$is used as expectorant and to liquefy the bronchial secretion, chlorpheniramine is used for symptomatic relief of allergy, and dextromethorphan is a cough suppressant[4]. The USP HPLC method for its individual assay uses water/ methanol/glacial acetic acid as mobile phase, 4.6 mm×250 mm column packed with L1 10µm, 276 nm detector and 2ml/min rate flow. The retention time is 7 mins.[1]

The USP method for assay of solution three or more of Acetaminophen, Chlorpheniramine Maleate, Dextromethorphan HBr and Pseudoephedrine HCL uses menthol/ water, monobasic potassium phosphate, triethylamine, sodium lauryl sulphate and phosphoric acid as mobile phase. Column 4.6 mm×150 mm,L11, 214 nm detector and 2m/min flow rate [1].Many studies to

asaayGuaifenesin alone and in combination of other drugs had been done using Spectrophotometric methods and HPLC methods [5].

The objective is to validate a method for quantitative determination of Pseudephedrine HCL, Guaifensin, Chlorpheniramine Maleate and Dextromethorphan HBr simultaneously in one single HPLC injection.

Materials and Methods

Materials

Purified water, Blue Nile research Centre, Sudan. Potassium DihydrogenOrthophophate, ScharlauChemie, Spain.Acetonitrile HPLC grade, SharlauChemie, Spain.Triethanolamine 99.8% AR, Chem lab NV; Belgium.Orthophosphoric acid 88% LubaChemie, India.

Chlorpheniramine Maleate, Guiafensin, Dextromethorphan hydrobromide and Pseudoephedrine working standards.AD test samples.

Instruments

High Performance Liquid Chromatography, Prominece – LC 2030, Shimatsu, Japan.Software Lab solution, Shimatsu, Japan.Column ; insert Sustain C18; 4.6 mm× 250 mm; 3 µm. Electronic Balance AY 220, Schimatsu. pH meter Mi 150; Hanna instruments, Romania. Rocking Shaker SK-330-pro, USA.Sonicator 621.05.003 IsolabograreGmpH instruments, Germany.

Chromatographic System

Column: insert Sustain C18; 4.6 mm× 250 mm; 3 µm. Flow rate: 0.8 ml/min. wave length 210 nm. Detector: PDA/UV. Oven temperature: 40℃. Injection volume: 20 µL. Run time: 60 min.

Preparation of 0.2 M Potassium dihydrogen orthophosphate: dissolve 27.218 gram in 700 ml water and complete to 1000 ml.

Preparation of mobile phase: to 550 ml of 0.2 M Potassium dihydrogen Orthophosphate in a 1 litre volumetric flask add 200 ml of Acetoniltrile, 30 ml of 10% Orthophosphoric acid and 1 ml Triethanolamine 99.8%. Dilute to volume by water and adjust the pH to 3 with orthophosphoric acid or Sodium hydroxide.

Preparation pf diluent: use the mobile phase as a diluent.

Preparation of the Standard: 100 mg Guiafenesin, 30 mg Pseudoephedrine HCL, 10 mg Dextromethorphan and 2 mg chlorpheniramine Maleate working standards into 100 ml volumetric flask, add 60 diluent,shake and sonicate for 5 minutes, cool and make up to volume with diluent. Mix well, transfer to 10 ml to 50 ml volumetric flask make up to volume with the dilueny, mix and filter using 0.45 µL nylon syringe filter.

Preparation of the Sample: Transfer 2 ml of the sample of specific gravity 1.2779 g/cm^3= 2.5558 grams to 100 m volumetric flask, add 60 ml diluent, shake well for 10 minutes, make up to volume with diluent, filter using0.45 µL nylon syringe filter.

Procedure

Equilibrate the column with mobile phase for sufficient time until stable baseline is obtained. Separately inject equal volumes 20µL of the standard preparation and the assay preparation into the chromatographic system, record the chromatogram and measure the areas of the major peaks. Inject the blank once, the standard solution for 6 replicates and the sample preparation in triplicates.

The tailing factor for each peak should not be more than 2 and the RSD should not be more than 2. Calculate the quantity in percentage by the formula:

$R_u/R_s \times C \times (100/W_u) \times D \times P/100 \times 1/L \times 100$where, D is the density in mg/ml, W_u is the weight in mg of the sample taken, R_u and R_s are the peak areas responses from the assay preparation and

the standard preparation respectively, *P* is the potency of tested API in % and *L* is the labeled quantity.

Steps on Method Validation [6,7]

1. Develop a validation protocol or operating procedure for the validation.
2. Define the application, purpose, and scope of the method.
3. Define the performance parameters and acceptance criteria.
4. Define validation experiments.
5. Verify relevant peformance characteristics of equipment.
6. Qualify materials (e.g., standards and reagents).
7. Perform prevalidation experiments.
8. Adjust method parameters or/and acceptance criteria if necessary.
9. Perform full internal (and external) validation experiments.
10. Develop SOPs for executing the method in the routine.
11. Define criteria for revalidation.
12. Define type and frequency of system suitability tests and/or analytical quality control (AQC) checks for the routine.
13. Document validation experiments and results in the validation report.

Results and Discussion

Precision

System suitability

The following table presents the average of 6 injection of the standard

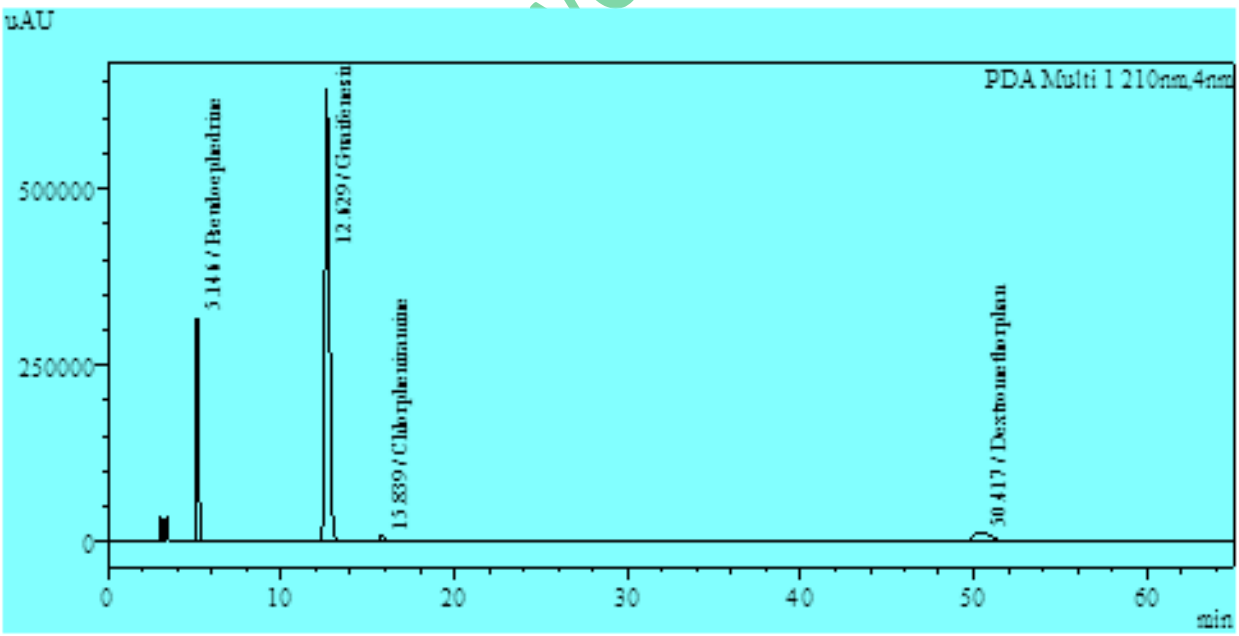

Figure 2: Chromatogram for System Suitability

Table (1) Results of the Method Precision

6 replicates	Pseudoephedrine	Guaifenesin	Chlorpheniramine	Dextromethorphan
Average RT	5.5 mins	12.63 mins	15.85	50.44
RSD%	0.07	0.05	0.08	0.07
Average Area	2850535.33	11585256.33	201544.17	936327
RSD%	0.04	0.04	0.19	0.05
Plates	46780	72286.83	79354	81109.17
Tailing factor	1.38	1.27	1.28	1.23
Peaks resolution	-	20.47	5.6	28.65

The RSD% for the retention timesand he peaks areas of all substances is less than 1%, the theoretical plates is more than 2000, the tailing factors are more than 2 and the resolution between the peaks is more than 2. Thus complying the precision acceptance criteria.

Specificity

Using placebo suspension in the same weight and way of the sample test, following the same procedure, no interference from the placebo was observed at the retention time of the drugs peaks.

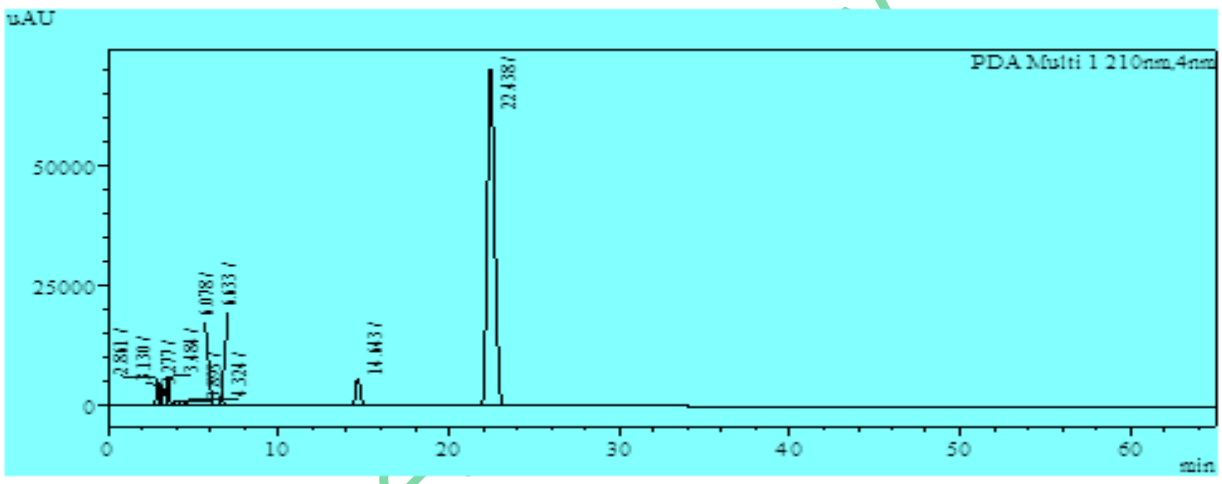

Figure 3: The peak purity without interference of Placebo and excipients

Peak purity demonstrates that the observed chromatographic peak is attributed to a single component that the excipients were not interfering with the component peaks at the specific retention time.The acceptance criteria for the peak purity are to be attributed to 90 -100% purity.

The impurities for pseudoephedrine are detected at 4.97 mins, 13.85 min for Guaiphenesin, 16.73 mins for chlopheniramine and 52.38 mins for dextromethprphan giving rise to peak purity 99.16%, 92.2%, 94.95% and 96.28% respectively.

Thus, this demonstrates the method specificity.

Linearity

Table (2) Levels of concentration of Standard µg/ml

ConcLevel	Pseudoephedrine	Guaifenesin	Chlorpheniramine	Dextromethorphan
1-5%	3 µg/ml	10 µg/ml	0.2 µg/ml	1 µg/ml
2- 10%	6 µg/ml	20 µg/ml	0.4 µg/ml	2 µg/ml
3- 25%	15 µg/ml	50 µg/ml	1 µg/ml	5 µg/ml
4- 50%	30 µg/ml	100 µg/ml	2 µg/ml	10 µg/ml
5- 75%	45 µg/ml	150 µg/ml	3 µg/ml	15 µg/ml
6- 100%	60 µg/ml	200 µg/ml	4 µg/ml	20 µg/ml
7- 125%	75 µg/ml	250 µg/ml	5 µg/ml	25 µg/ml
8- 150%	90 µg/ml	300 µg/ml	6 µg/ml	30 µg/ml
9- 175%	105 µg/ml	350 µg/ml	7 µg/ml	35 µg/ml
10- 200%	120 µg/ml	400 µg/ml	8 µg/ml	40 µg/ml

The Area versus concentration of the four compounds were tabulated as follows:

Table (3) Peak area and RSD% for linearity

	Pseudoephedrine		Guaifenesin		Chlorpheniramine		Dextromethorphan	
Level	Area	RSD%	Area	RSD%	Area	RSD%	Area	RSD%
1	164023.3	0.08	709131	0.24	15970.67	0.4	52153.33	0.89
2	302652.3	0.08	1305768	0.06	22415.67	0.54	88761.67	0.38
3	729054.7	0.04	3096022	0.03	54007	0.42	228668.7	0.25
4	1488262	0.15	6191429	0.10	106605	0.38	480969.3	0.77
5	2153761	0.19	8860252	0.31	162422	0.46	704803.7	0.53
6	2853314	0.57	11555520	0.64	218128	0.92	938008	0.82
7	3512556	0.03	14304351	0.02	267495.3	0.33	1159430	0.2
8	4250768	0.88	17240602	0.35	324816	1.0	1402909	0.83
9	4882828	0.04	19679804	0.04	371301	0.16	1613694.7	0.13
10	5535872	0.24	22624204	0.26	427313	0.33	1888020	0.6

Linearity Chromatograms

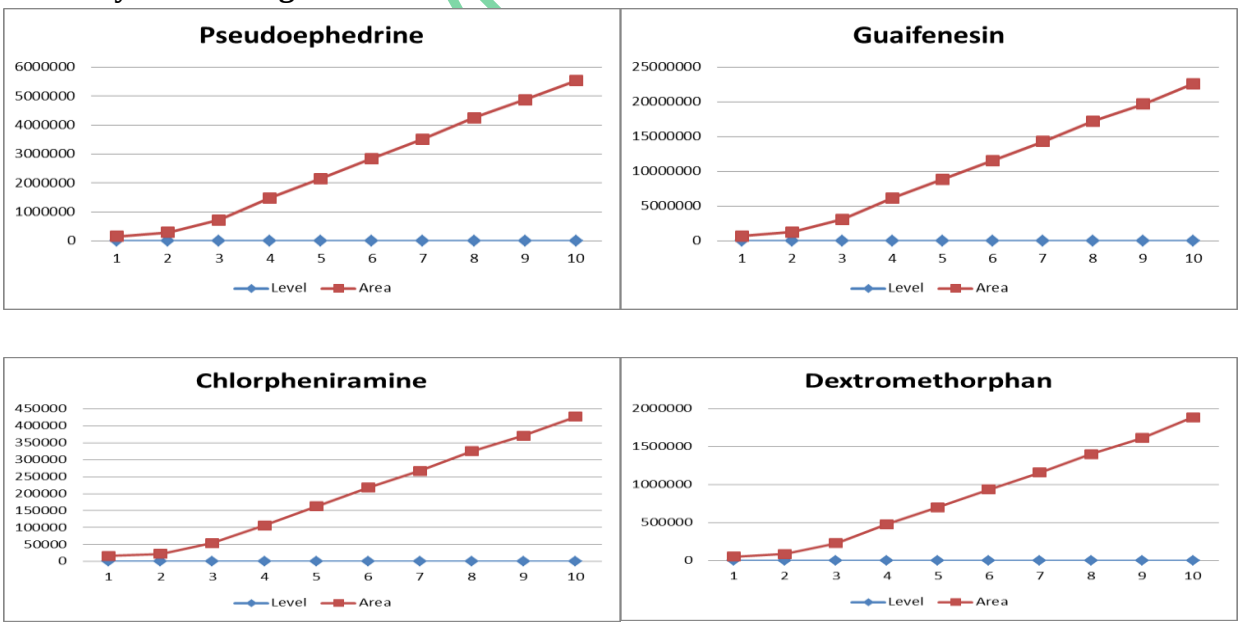

Figure 4: Linearity Chromatograms

Table (4) Linearity Results

Parameter	Pseudoephedrine	Guaifenesin	Chlorpheniramine	Dextromethorphan
Correlation	0.9998	0.9996	0.9997	0.9997

Coefficient r^2				
Slope	46098.9590	55897.1449	53117.76	46687.1513
y- intercept	56476.2818	337530.7956	2636.4341	1366.97
Regression line equation	Y= 46098.959 x +56476.2818	Y= 55897.1449 x + 337530.7956	Y= 53117.76 x + 2636.4341	Y= 46687.15 x + 1366.97

The acceptance criteria for the correlation Coefficient r^2should be ≥ 0.999 for the range of concentration 75 – 125% of the target concentration. Thus, the method comply the requirement for linearity.

Range

The data obtained from the accuracy studies may be used to assess the range of the method. 50% to 150% of the target concentration is utilized.

Limit of detection DL and limit of quantitation QL

DL = 3.3 × The mean root square error MRSE / slope, QL = 10 ×MRSE / slope.

DL µg/ml: 2.67, 10, 0.15, 0.86 for Pseudoephedrine, Guaifenesin, Chlorpheniramine, Dextromethorphan respectively.QL µg/ml: 8.08, 31.14, 0.47, 2.6.

Accuracy

According to the ICH guide lines Q2 the accuracy is assessed using three replicates of each of the concentrations 50%, 100% and 150% were analyzed for theoretical values, RSD and percent recovery. The following results were obtained:

Table (5) Results for Accuracy

Conc	**Pseudoephedrine**		**Guaifenesin**		**Chlorpheniramine**		**Dextromethorphan**	
	% Mean recovery	RSD%	% Mean recovery	RSD%	% Mean recovery	RSD%	% Mean recovery	RSD%
50%	100.85%	0.11%	100.94	0.01	100.74	0.07	99.71	0.56
100%	100.85%	0.11%	99.43	0.16	100.41	0.16	100.21	0.18
150%	100.83%	0.06%	99.39	0.06	100.73	0.48	100.12	0.19

Since the acceptance criteria is that the measured recovery should be 95% - 105%, so the method comply the requirement for accuracy.

Precision

Repeatability

10 replicates of the sample were used and the mean, stand deviation and relative standard deviation were obtained.

Table (6) Repeatability Results

	Pseudoephedrine		**Guaifenesin**		**Chlorpheniramine**		**Dextromethorphan**	
	RT	Area	RT	Area	RT	Area	RT	Area
Mean	5.22	2890773	12.77	11780051	16.23	190932	51.71	179522
RSD%	0.23%	0.2%	0.24%	0.25%	0.44%	0.27%	0.4%	0.19%

The FDA and ICH stated that the RSD should be ± 1% for the drug substance and ± 2% for the drug product. Thus, the method fulfilled the repeatability criterion.

Intermediate Precision

Intermediate precision within laboratory variations had been demonstrated by two analysts, using two HPLC systems on different days and evaluating the relative percent purity data across the two HPLC systems at three concentration levels; 50%, 100% and 150%. The following results were obtained:

S_1A and S_1B is the RSD% of concentration 50% for analysts A and B. S_2A and S_2B is the RDS% of concentration 100% for analysts A and B. S_3A and S_3B is the RSD% of concentration 150% for analysts A and B. Two diffident systems at two different days technique were used.
S2a + S2b are 0.52, 0.27, 0.09, and 0.17 for the four compounds respectively.
S3a +S3b are 0.97, 1.0, 0.34, and 0.21 for the four compounds respectively.
Since the acceptance criterion for intermediate precision is that the results obtained by two analysts using two instruments at different days should have statistical RSD ≤ 2%, thus the method comply the acceptable criteria.

Robustness

Effect of change in column temperature

Table (7) Results of robustness on change in column temperature

Variable	**Pseudoephedrine**				**Guaifenesin**			
	Mean RT min	Mean area	Theoretical plates	Tailing factor	Mean RT min	Mean area	Theoretical plates	Tailing factor
35℃	5.27	2899252	50442	1.32	13.18	11790008	79212	1.25
RSD%	00	0.08	0.2	0.4	0.03	0.08	0.19	0.14
40℃	5.19	2910793	50395	1.36	12.71	11790008	79086	1.26
RSD%	0.25	0.39	0.55	0.19	0.22	0.8	0.49	0.08
45℃	5.1	2897807	49702	1.42	12.26	11790008	78530	1.26
RSD%	00	0.08	0.2	0.04	0.03	0.08	0.19	0.14
Variable	**Chlorpheniramine**				**Dextromethorphan**			
	Mean RT min	Mean area	Theoretical plates	Tailing factor	Mean RT min	Mean area	Theoretical plates	Tailing factor
35℃	17	188365	88614	1.25	55.1	937097	85090	1.22
RSD%	0.04	0.62	0.13	0.23	0.02	0.04	0.23	0.12
40℃	17.51	190058	88982	1.25	51.2	945921	87644	1.22
RSD%	0.4	1.02	0.42	0.14	0.22	0.78	1.11	0.25
45℃	17.88	189603	88894	1.24	47.4	928239	91395	1.21
RSD%	0.14	0.49	0.43	0.12	0.06	0.52	0.28	0.46

Table (8) resolution of peaks at different Temperature

Column temp	**Pseudoephedrine**		**Guaifenesin**		**Chlorpheniramine**		**Dextromethorphan**	
	RT	Resolution	RT	Resolution	RT	Resolution	RT	Resolution
35℃	5.27	-	13.18	21.8	17	7.1	55.1	30
40℃	5.19	-	12.7	21.3	17.5	8.9	51.2	28
55℃	5.1	-	12.3	20.8	17.9	10.5	47.4	26.4

Effect of change in the detective wavelength

Table (9) Results of Change in the Wavelength

Variable	**Pseudoephedrine**				**Guaifenesin**			
	Mean RT min	Mean area	Theoretical plates	Tailing factor	Mean RT min	Mean area	Theoretical plates	Tailing factor
208 nm	5.22	3165558	51109	1.35	12.78	13490113	75159	1.26
RSD%	0.07	0.3	0.55	00	0.08	0.24	0.2	0.05
210 nm	5.22	2818701	50945	1.35	12.78	11838864	78557	1.25
RSD%	0.07	0.29	0.55	0.04	0.08	0.26	0.02	0.05
112 nm	5.22	2614346	50723	1.35	12.78	10609336	81459	1.25
RSD%	0.07	0.27	0.56	0.04	0.08	0.29	0.03	0.05
Variable	**Chlorpheniramine**				**Dextromethorphan**			
	Mean RT min	Mean area	Theoretical plates	Tailing factor	Mean RT min	Mean area	Theoretical plates	Tailing factor
208 nm	17.66	208296	88796	1.25	51.5	1208256	85090	1.22
RSD%	0.13	0.53	0.19	0.09	0.11	0.38	0.23	0.12
210 nm	17.66	189909	88911	1.25	51.5	947748	86680	1.2
RSD%	0.13	0.29	0.2	0.09	0.11	0.37	0.07	0.25
112 nm	17.66	175248	89032	1.25	51.5	748245	86879	1.2
RSD%	0.13	0.4	0.19	0.05	0.1	0.38	0.16	0.13

Table () resolution of peaks at different Wavelengths

Column temp	**Pseudoephedrine**		**Guaifenesin**		**Chlorpheniramine**		**Dextromethorphan**	
	RT	Resolution	RT	Resolution	RT	Resolution	RT	Resolution
208 nm	5.2	-	12.8	21	17.7	8.9	51.5	28
210 nm	5.22	-	12.78	21.3	17.66	9	51.5	28
212 nm	5.2	-	12.8	21.5	17.7	9.1	51.5	28

Effect of change in the flow rate

Table (10) Results of robustness on change of flow rate

Variable	**Pseudoephedrine**				**Guaifenesin**			
	Mean RT min	Mean area	Theoretical plates	Tailing factor	Mean RT min	Mean area	Theoretical plates	Tailing factor
0.7 ml/min	5.89	3294494	55188	1.32	14.43	13380124	84056	1.24
RSD%	00	0.07	0.17	0.08	0.01	0.02	0.07	0.05
0.8 ml/min	5.23	2908409	51315	1.35	12.8	11810239	78508	1.25
RSD%	0.05	0.19	0.36	0.13	0.05	0.12	0.1	0.09
0.9	4.66	258257	45811	1.42	11.42	1047853	73876	1.26

ml/min		1				4		
RSD%	0.26	0.24	1.1	0.19	0.24	0.26	1.3	0.23
Variable	**Chlorpheniramine**				**Dextromethorphan**			
	Mean RT min	Mean area	Theoretical plates	Tailing factor	Mean RT min	Mean area	Theoretical plates	Tailing factor
0.7 ml/min	19.86	213070	93639	1.24	57.9	1067617	90142	1.2
RSD%	0.01	0.11	0.02	0.08	0.02	0.01	0.05	0.22
0.8 ml/min	17.71	189127	89044	1.25	51.6	939955	86973	1.2
RSD%	0.07	0.37	0.07	0.12	0.06	0.37	0.05	0.25
0.9 ml/min	15.75	167279	85353	1.24	4.2	832535	86286	1.22
RSD%	0.45	0.31	0.71	0.08	0.21	0.05	0.86	0.28

Table (11)Resolution of peaks in changing the rate flow:

Flow rate	**Pseudoephedrine**		**Guaifenesin**		**Chlorpheniramine**		**Dextromethorphan**	
	RT	Resolution	RT	Resolution	RT	Resolution	RT	Resolution
0.7 ml/min	5.9	-	14.4	22.1	19.9	9.2	57.9	28.6
0.8 ml/min	5.2	-	12.8	21.4	17.7	9	51.6	28
0.9 ml/min	5.2	-	12.8	20.5	17.7	8.8	51.5	27.9

Acceptance Criteria for Robustness

1- The number of the theoretical plates should be less than 2000.
2- The tailing factor for compounds should not be more than 2.0.
3- The RSD% of the peaks areas of the replicates of either the standard solution or the compounds should not be more than 2.0%.
4- The resolution between the peaks of the compounds should be ≥ 2.0.

The method fulfilled the acceptance criteria as the number of the theoretical plates in all variables is more than 2000, the RSD% of the retention time and peaks area are less than 2.0%, the tailing factor for all peaks of the different variables are less than 2.0 and the resolution between the peaks is more than 2.0.

Thus, the method satisfied the requirements for robustness on changing the column temperature, on changing the detective wavelength and on changing the flow rate.

Solution Stability

The test had been carried out by initial testing then after preservation of the test solution for 6 hours, 12 hours, 18 hours, 24 hours and 48 hours.

Table (12). The average and RSD% of peak areas for solution stability

Paramete	**Pseudoephedrine**	**Guaifenesin**	**Chlorpheniramine**	**Dextromethorphan**
Mean peaks	2879033	11675642	187949	98897

areas				
RSD%	0.12	0.19	0.15	0.48

The RSD% for the peaks areas of all compounds is less than 2%, therefore, the standard preparation is stable for 48 hours at room temperature.

Conclusion

The analytical method used for determination of Pseudoephedrine HCL, Guaifenesin, Chlorpheniramine Maleate and Dextromethorphan HBr in AD- solution as four-in-one was found to be consistent and precise and in conformance with the acceptable criteria of validation parameters of specificity, system suitability, linearity and range, precision, accuracy, reproducibility and robustness. The method is fully validated and can be used in routine testing for simultaneous determination of such combination products.

Acknowledgement

The team and manager of Blue Nile Research Centre were greatly acknowledged for their great support and encouragement.

References

1. United States Pharmacopeia2013 ,USP 39 - The National Formulary, 1/5/2016, 12601 Twinbrook Parkway, Rockville, MD 2052, USP Volume 1 p 1641, volume 2, p 2310, 4164 volume 2.
   (tablet containing at least three of the following – acetaminophen chlorpheniramine, dextromethorphan and pseudoephedrine.
2. ICH Q2 validation of analytical procedures Part 2, 6/1995, ICH, Guidance for Industry Q1A(R2) Stability Testingof New Drug Substances and Products,2003., www.emea.eu.int
3. Wikipedia, www.en.wikipedia.org 25/10/2020, 13:00
4. . British National Formulary 80, September 2020, Royal Pharmaceutical Society, published by BMI Group and Pharmaceutical Press, p 221, www.pharmapress.com
5. PrayasAcharya, T Prasanth Kumar, Immanuel Agasteen, SreeramaRajasekhar and G Neelima, 2017, A review on Analytical Methods for Determination of Guaifenesin Alone and in Combination with other Drugs in Pharmaceutical Formulation, Saudi Journal of Medical and Pharmaceutical Sciences, DIO: 10.21276/sjmps.2017.3.3.7, http://scholarsmepub.com.
6. Validation of Analytical Methods and Processes- Ludwig Huber, Agilent Technologies GmbH, Waldbronn, Germanyp 544
7. FDA Guidance for Industry, Analytical Procedures and Method Validation for Drugs and Biologics, July 2015.