ISSN 2582 – 6050

Available Online at www.ijpba.info
International Journal of Pharmaceutical & Biological Archives 2021; 12(4):178-189

RESEARCH ARTICLE

QbD-based Simultaneous Estimation of Chlorpheniramine Maleate, Dextromethorphan Hydrobromide and Guaifenesin in its Combined Dosage Form Using High-Performance Thin-Layer Chromatographic Method

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Received: 10 October 2021; Revised: 28 November 2021; Accepted: 11 December 2021

ABSTRACT

Current research work entails systematic analytical quality by design-based development of high-performance thin-layer chromatographic method for simultaneous estimation of chlorpheniramine maleate (CPM), dextromethorphan hydrobromide (DEX), and guaifenesin (GUA) in their combined dosage form. Analytical target profile was defined and critical method attributes and potential method parameters were screened by preliminary trials and scientific knowledge. Critical method parameters were identified using Taguchi OA screening design. 3^2 full factorial design was used for optimization of analytical method taking volume of water and migration distance as critical method parameters and by evaluating resolution between consecutive peaks of three drugs. Response surface model was validated by comparing predicted response with actual responses. Chromatographic separation was accomplished using alumina backed silica gel $60F_{254}$ as stationary phase and n-butanol-water-glacial acetic acid (7:2.5:0.5, % v/v) as mobile phase. Validation of developed method was performed as per ICH guidelines with linearity ranging between 50 and 2500 ng/band for CPM and 1000 and 5000 ng/band for DEX and GUA. Marketed syrup formulation was analyzed using developed analytical method. The results demonstrated utilization of analytical quality by design approach for screening and optimization of factors contributing development of high-performance thin-layer chromatographic method for better separation and quantification of CPM, DEX and GUA.

Keywords: High-performance thin-layer chromatography, analytical quality by design, Taguchi OA, 3^2 full factorial design, chlorpheniramine maleate, dextromethorphan hydrobromide, and guaifenesin

INTRODUCTION

Combinations of decongestant and antihistaminic preparations are widely used for cough and cold treatment. Chlorpheniramine maleate (CPM) is chemically (Z)-but-2-enedioic acid;3-(4-chlorophenyl)-N,N-dimethyl-3-pyridin-2-ylpropan-1-amine [Figure 1] is a powerful H_1 receptor antagonist and widely used for symptomatic relief of common cold and rhinitis. Dextromethorphan hydrobromide (DEX) which is chemically 4-methoxy-17-methyl-17-azatetracyclo[7.5.3.$0^{1,10}$.$0^{2,7}$]heptadeca-2(7),3,5-triene;hydrate;hydrobromide [Figure 2] is a cough suppressant used for the relief of non-productive cough, it has a central action on cough centers. Guaifenesin (GUA) which is chemically 3-(2-methoxyphenoxy)propane-1,2-diol [Figure 3] is used as expectorant, it acts by increasing the volume and reducing the viscosity of sputum.[1-5]

Quality-by-design (QbD) has become an important paradigm in the pharmaceutical industry since

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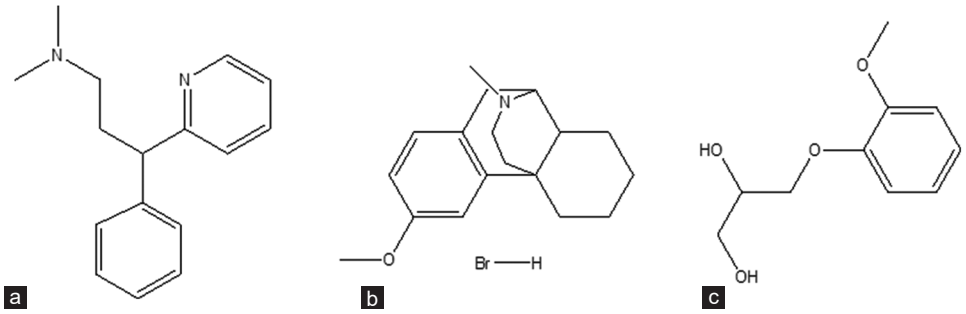

Figure 1: Chemical structure of (a) Chlorpheniramine maleate (b) Dextromethorphan hydrobromide (c) Guaifenesin

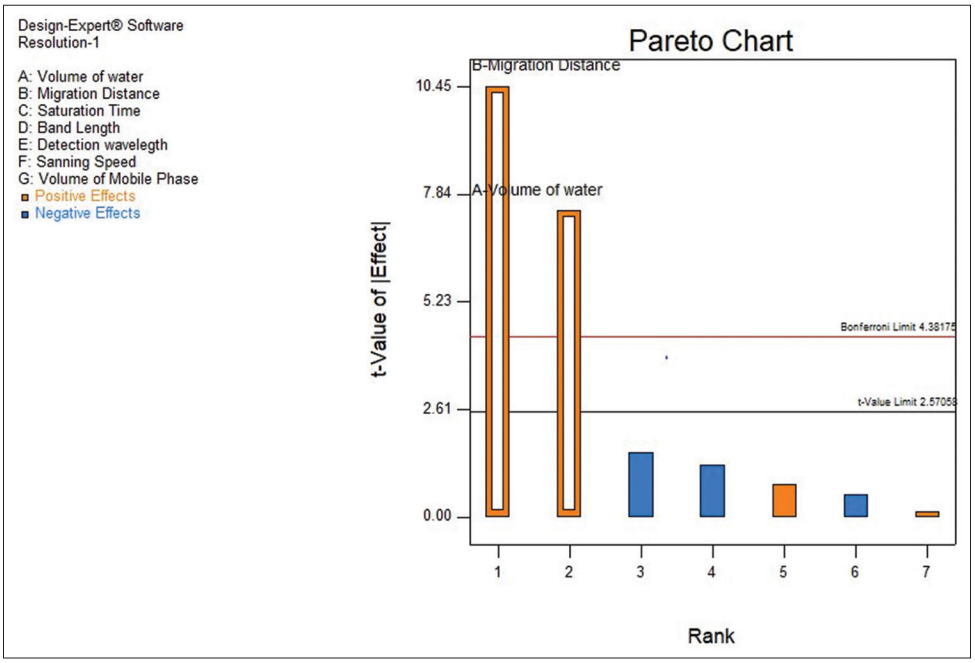

Figure 2: Pareto chart for screening of factors affecting resolution-1

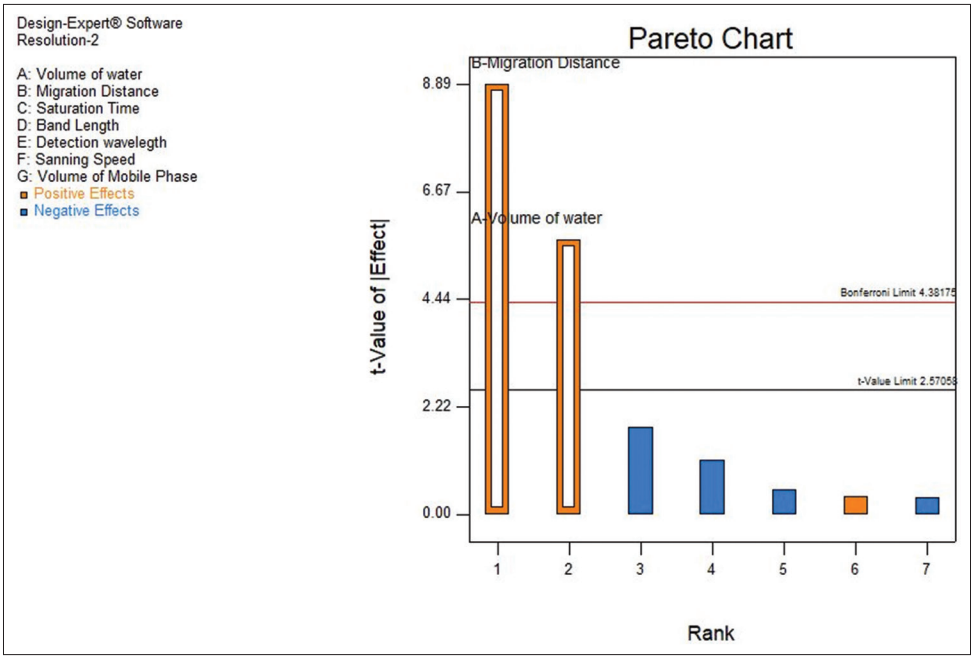

Figure 3: Pareto chart for screening of factors affecting resolution-2

its introduction by the US Food and Drug Administration. The concept of QbD can be extended to analytical methods. QbD mandates the definition of a goal for the method and emphasizes thorough evaluation and scouting of alternative methods in a systematic way to obtain optimal method performance. Using AQbD, the method performance can be understood and improved if necessary and a control strategy can be defined to manage risk and ensure the method performs as desired when validated and deployed.[6]

An extensive literature survey reveals various spectrophotometric, high-performance liquid chromatography and ultra-high performance liquid chromatographic techniques for simultaneous estimation of CPM, DEX, and GUA in its combined dosage form[7-21] but no high-performance thin-layer chromatography (HPTLC) method is reported for estimation of CPM, DEX, and GUA in its combined dosage form using analytical quality by design approach. Hence, this paper describes QbD based simultaneous estimation of CPM, DEX and GUA in its combined dosage form using HPTLC method. The method was validated as per ICH guidelines.[22]

MATERIALS AND METHODS

Instrumentation

HPTLC system (CAMAG, Muttenz, Switzerland) with Linomat V semi-automatic spotting device, TLC Scanner IV, twin-trough development chamber (10 × 10 cm), UV cabinet with dual wavelength UV lamps, winCATS software, syringe (100 μL capacity, Hamilton, Bonaduz, Switzerland) was used for chromatographic study. Weighing was performed on electro-analytical balance (Shimadzu AUX-220, Kyoto, Japan). Design expert software version 10.0.7 (trial version) was used for screening and optimization designs.

Chemicals and Reagents

DEX, CPM, and GUA were obtained from Balaji Drugs, Surat, Gujarat, as a gift sample. Sample syrup formulation, Grilinctus (Franco-Indian Pharmaceuticals Pvt. Ltd., Mumbai, India, Content per 5 mL: 2.5 mg CPM + 5 mg DEX + 50 mg GUA) was purchased from a local pharmacy. All chemicals and reagents used during the study were of analytical grade and purchased from S. D. Fine-Chem Limited, Mumbai, India.

Preparation of Standard Solutions

To prepare standard stock solution, accurately weighed aliquot of CPM (10 mg), DEX (20 mg), and GUA (20 mg) was transferred to 10 ml volumetric flask and diluted to the mark with methanol to get the standard stock solution of 1000 μg/ml of CPM + 2000 μg/ml of DEX + 2000 μg/ml of GUA. To prepare working standard solution, 1 ml aliquot from the standard stock solution was diluted up to 10 ml with methanol to obtain concentration of 100 μg/ml of CPM + 200 μg/ml of DEX + 200 μg/ml of GUA.

Analytical Quality by Design

Selection of analytical target profile, potential method attributes, and potential method variables

As the purpose of present research work is simultaneous estimation of CPM, DEX, and GUA, to serve the purpose, method must be able to quantify CPM, DEX, and GUA accurately in its combined pharmaceutical dosage form without any interferences. To achieve analytical target profile and to reduce interferences in estimation, drugs must be completely separated and peaks of all three drugs must be well resolved; hence, resolution between CPM and DEX (Resolution-1) and resolution between DEX and GUA (Resolution-2) was selected as critical method attributes (CMA). From preliminary trials and scientific knowledge, seven potential method parameters were identified, which can affect CMA as mentioned in Table 1.

Screening of critical risk factors

Screening of critical method parameters was accomplished using Taguchi OA screening design as it gives good results with minimum experimental runs. After entering seven potential method parameters at two levels, Taguchi OA suggests eight experimental runs. These runs were

performed in laboratory in three replicates and the resolution obtained by each experimental condition was entered against respective experimental runs and analyzed for their effect on resolution-1 and resolution-2 as shown in Table 2.

Response surface modeling

After screening, response surface modeling was performed using 3^2 full factorial design as it gives maximum information with minimum runs. Two critical method variables identified from Taguchi OA screening design which were further taken to response surface modeling at three different levels to identify its relationship with critical method attributes, resolution-1 and resolution-2. Suggested thirteen experimental runs by the design was performed in laboratory in three replicates and results of each run was entered against respective experimental runs in the software and relationships between critical method parameters and critical method attributes were established. The design metrics of 3^2 full factorial optimization design is depicted in Table 3.

Table 1: Potential method parameters employed for screening in Taguchi OA design

Potential method parameters	Levels	
	−1	+1
Factor A: Volume of water	1 mL	4 mL
Factor B: Migration Distance	70 mm	80 mm
Factor C: Saturation time	15 min	45 min
Factor D: Band length	4 mm	8 mm
Factor E: Detection wavelength	272 nm	276 nm
Factor F: Scanning speed	10 mm/s	20 mm/s
Factor G: Volume of mobile phase	8 mL	10 mL

Response surface model validation

Many solutions were suggested by the software with desirability one, from which five solutions were performed experimentally in laboratory and actual resolutions obtained were compared with predicted resolutions to validate 3^2 response surface model.

Chromatographic Conditions

Chromatographic separation was performed on 10 × 10 cm aluminum plates pre-coated with 250 µm layer of silica gel 60 F_{254}. The TLC plate was pre-washed with methanol and activated at 60°C for 5 min prior to spotting. The samples were spotted on TLC plate 15 mm from the bottom edge by Linomat V semiautomatic spotter using following parameters: band width 6mm; track distance 11.6 mm; application rate 100 nL/s. The TLC plate was developed in twin-trough chamber using n-butanol-water-glacial acetic acid (7:2.5:0.5, v/v) as mobile phase at temperature, 27 ± 2°C, chamber saturation time 30 min; migration distance, 75 mm. The TLC plate was dried, scanned and analyzed by TLC scanner and winCATS software using following parameters: slit dimension 4 × 0.30 mm; scanning speed 20 mm/s; detection wavelength, 274 nm.

Method Validation

Calibration curve and linearity

Linearity of the method was ascertained by plotting graph of peak area versus concentration

Table 2: Taguchi OA screening design matrix with responses

Run	Factors[a]							Responses	
	A	B	C	D	E	F	G	Resolution-1	Resolution-2
	mL	mm	min	Mm	nm	mm/s	mL		
1	1	80	45	4	272	20	10	2.184	2.074
2	1	70	15	4	272	10	8	1.407	1.426
3	4	80	15	4	276	20	8	2.956	2.763
4	1	80	45	8	276	10	8	2.189	2.061
5	4	70	45	8	272	20	8	1.782	1.656
6	4	80	15	8	272	10	10	3.082	2.898
7	1	70	15	8	276	20	10	1.326	1.262
8	4	70	45	4	276	10	10	2.034	1.82

[a]Factors: A-Volume of water, B-Migration distance, C-Saturation time, D-Band length, E-Detection wavelength, F-Scanning speed, G-Volume of mobile phase

Table 3: 3^2 full factorial optimization design metrics with responses

Run	Factor A Migration Distance (mm)	Factor B Volume of water (mL)	Response 1 Resolution-1	Response 2 Resolution-2
1	80	1	2.336	2.197
2	75	1	2.016	1.986
3	70	4	2.42	2.313
4	70	2.5	2.335	2.121
5	75	2.5	2.639	2.538
6	75	2.5	2.731	2.591
7	70	1	1.563	1.383
8	75	2.5	2.833	2.541
9	75	4	2.932	2.673
10	75	2.5	2.684	2.582
11	75	2.5	2.831	2.613
12	80	2.5	2.783	2.731
13	80	4	3.128	2.873

and determining correlation coefficient of linear regression analysis, in addition residual plot was also constructed to determine relationship between two variables. From working standard solution aliquots of 5, 10, 15, 20, and 25 µl were applied on the TLC plate and the plate was developed and analyzed 5 times as per chromatographic conditions.

Specificity

Specificity of the method was ascertained by analyzing standard drug and sample. The band for CPM, DEX and GUA in individual samples was confirmed by comparing the R_f and UV spectra of the band with those obtained from standard. The peak purity of CPM, DEX, and GUA was assessed by correlating the spectra acquired at peak start, peak apex, and peak end.

Precision

Method precision was performed by carrying out repeatability of sample application, interday, and intraday precisions. Repeatability of sample application was performed by application of 15 µl of combined working standard solution for 7 times on same TLC plate and the plate was developed and analyzed as per the chromatographic conditions. Peak areas of seven replicate spots were measured and %RSD was calculated. The variation of results within same day (intraday precision) was determined by repeating calibration curve of CPM, DEX and GUA 3 times on same day and %RSD of peak area was calculated for all three drugs and the variation of results among different days (interday precision) was determined by repeating the calibration curve for 3 consecutive days and %RSD of peak area was calculated for all three drugs.

Accuracy

The accuracy of the method was determined by standard addition method. Accurately measured 10 ml of syrup (containing 5 mg of CPM, 10 mg of DEX, and 100 mg of GUA) was transferred to each four individual separators. Standard CPM, DEX, and GUA in the quantity of 4 mg, 8 mg, and 80 mg for 80% recovery level; 5 mg, 10 mg, and 100 mg for 100% recovery level; and 6 mg, 12 mg, and 120 mg for 120% recovery were spiked in first, second and third separator, respectively. Then, the content was mixed and 10 ml, 5M NaOH was added to each separator. The contents of all four separators were extracted with 20 ml chloroform for 5 times individually. Then the mixture was shaken and allowed to stand for separation. Organic portion of each separator was collected and allowed to dry completely. The residue obtained was dissolved up to 50 ml methanol individually (solution-1). From the above solutions, 1 ml aliquot was diluted up to 10 ml with methanol (solution-2). Eight microliters of above prepared solutions were applied individually on same TLC plate and analyzed using optimized chromatographic conditions. Here, solution 1 was used for analysis of CPM and DEX while the solution 2 was used for analysis of GUA. The %recovery was determined for all three drugs.

Limit of detection (LOD) and limit of quantification (LOQ)

The LOD and LOQ of the developed method were calculated from the standard deviation of the intercept (σ) and mean slope of the calibration curves (S) using the given formula:

LOD = 3.3 σ/S and LOQ = 10 σ/S

Where, σ = standard deviation of intercepts of five calibration curves and S = mean slope of five calibration curves.

Assay of Marketed Formulation

Accurately measured 5 ml of syrup was transferred to a separator and 5 ml of 5 M sodium hydroxide solution was added and mixed. Content was extracted with 10 ml portions of chloroform 3 times. This aqueous-organic solvent mixture was vigorously shaken and was allowed to stand for separation. All three organic extract portions were collected in porcelain vessel and evaporated to dryness. Residues were collected and dissolved in 10 ml methanol (solution 1). One millileter aliquot from solution 1 was diluted to 10 ml with methanol (solution 2). From above solution 1 and solution 2, 6 µl was applied individually on same TLC plate and analyzed using optimized chromatographic conditions.

RESULTS AND DISCUSSION

Analytical Quality by Design

Screening of risk factors

Taguchi OA design model was found significant as ANOVA analysis shows model F values 82.28 and 55.64 for resolution-1 and resolution-2, respectively. Two risk factors - volume of water and migration distance were found critical as P values were <0.05. P values for scanning speed, saturation time, detection wavelength, band width, and volume of mobile phase were found more than 0.05 which indicates, these risk factors are showing insignificant effects on resolution-1 and resolution-2. Pareto chart also shows bars of volume of water and migration distance above the line of significance. Hence form ANOVA [Table 4] and Pareto chart analysis [Figures 4 and 5], it can be said that volume of water and migration distance are critical method variables that affects resolution-1 and resolution-2.

Optimization using response surface methodology

3^2 full factorial design model F values were found 44.11 and 244.74 for resolution-1 and resolution-2, respectively, as per ANOVA table [Table 5], which indicates the suggested quadratic model is significant. The model terms: Volume of methanol, migration distance, volume of methanol^2 and migration distance^2 were found significant for resolution-1 while, volume of methanol, migration distance, volume of methanol^2, migration distance^2 and volume of methanol × migration distance were found significant for resolution-2 as P values for all mentioned factors were below 0.05. Following mathematical equation shows relationship between critical method parameters and critical method attributes, which can be used for response optimization.

Resolution-1 = −37.535 + 0.967 * migration distance + 0.968 * volume of water −2.166 × 10^{-3} * migration distance * volume of water −5.986× 10^{-3} * migration distance^2 −0.104 * volume of water^2.

Resolution-2 = −37.374 + 0.943 * migration distance + 1.421 * volume of water −8.466 × 10^{-3} * migration distance * volume of water −5.706 × 10^{-3} * migration distance^2 −0.106 * volume of water^2.

There is only 33.50% and 25.05% chance that a lack of fit f-value this large could occur due to noise for resolution-1 and resolution-2, respectively.

The lack of fit F-value of 1.54 and 2.04 for resolution-1 and resolution-2 implies that the lack of fit is not significant relative to the pure error.

The actual response was found in good agreement with predicted response as predicted Rsquared 0.817 and 0.961 is in reasonable agreement with adjusted Rsquared 0.947 and 0.990 for resolution-1 and resolution-2, respectively.

Response surface model validation

When five experimental runs from the solution found by software were repeated in laboratory. It shows better correlation between predicted and experimental values. Small variations in the factors within the design space reflect that results are similar to those of predicted values. Hence, it can be said that the suggested model is robust [Table 6].

Design space and control strategy

Design space was generated after validating the model to get values of resolution above 1.5. From

Table 4: ANOVA table for Taguchi OA screening design

Source	Sum of Squares	df	Mean Square	F Value	*P*-value Prob>F	
ANOVA for resolution-1						
Model	2.81	2	1.40	82.28	0.0001	Significant
A-Volume of water	0.94	1	0.94	55.32	0.0007	
B-Migration Distance	1.86	1	1.86	109.25	0.0001	
Residual	0.085	5	0.017			
Cor Total	2.89	7				
ANOVA for resolution-2						
Model	2.32	2	1.16	55.64	0.0004	Significant
A-Volume of water	0.67	1	0.67	32.29	0.0024	
B-Migration Distance	1.64	1	1.64	78.99	0.0003	
Residual	0.10	5	0.021			
Cor Total	2.42	7				

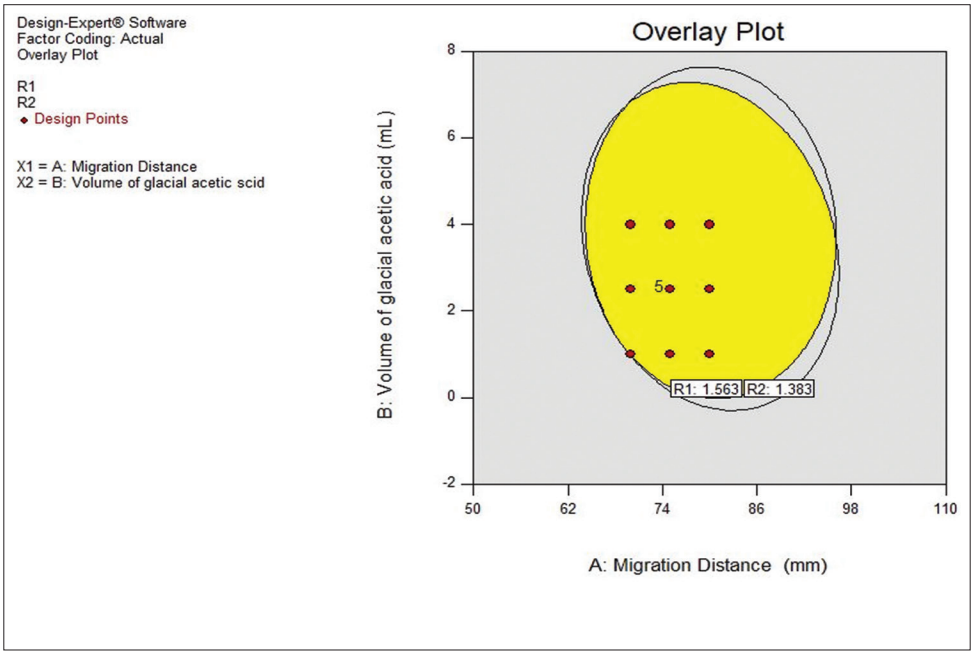

Figure 4: Method operable design region

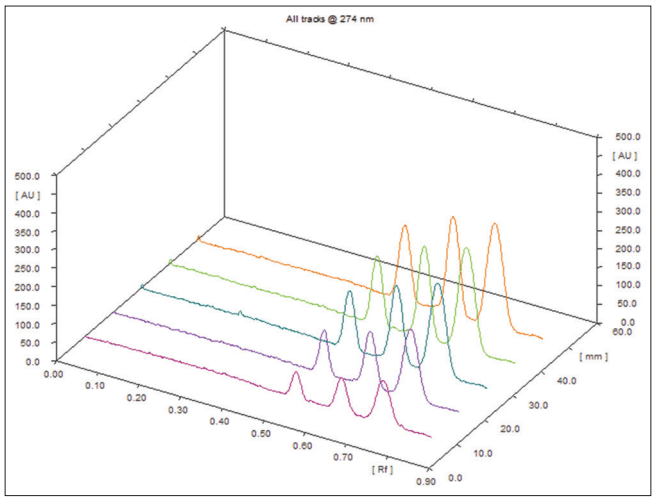

Figure 5: Calibration 3D chromatogram of standard CPM (500–2500 ng/band), DEX (1000–5000 ng/band), and GUA (1000–5000 ng/band)

design space [Figure 6], control strategy was implemented for development of analytical method with resolution more than 1.5 as shown in Table 7

Method Validation

Calibration curve and linearity

The linear relation was observed over the concentration range of 500–2500 ng/spot, 1000–5000 ng/spot, and 1000–5000 ng/spot with the correlation coefficient of 0.9984, 0.9973, and 0.9964 for CPM, DEX, and GUA, respectively. The regression line equations for CPM, DEX and GUA were found to be y = 1.9081x + 27.74, y = 1.4914x + 533.6, and y = 2.3603x +

Table 5: ANOVA table for 3^2 full factorial design

Source	Sum of Squares	df	Mean Square	F Value	*P*-value Prob>F	Sum of Squares
ANOVA table for Resolution-1						
Model	2.05	5	0.41	44.11	< 0.0001	Significant
A-Migration Distance	0.62	1	0.62	66.58	< 0.0001	
B-Volume of water	1.10	1	1.10	117.72	< 0.0001	
AB	1.056×10^{-3}	1	1.056×10^{-3}	0.11	0.7462	
A^2	0.062	1	0.062	6.64	0.0366	
B^2	0.15	1	0.15	16.33	0.0049	
Residual	0.065	7	9.315×10^{-3}			
Lack of Fit	0.035	3	0.012	1.54	0.3350	Not significant
Pure Error	0.030	4	7.571×10^{-3}			
ANOVA for Resolution-2						
Model	1.88	5	0.38	244.74	<0.0001	Significant
A-Migration Distance	066	1	0.66	426.34	<0.0001	
B-Volume of water	088	1	0.88	569.48	<0.0001	
AB	0.016	1	0.016	10.48	0.0143	
A^2	0.056	1	0.056	36.53	0.0005	
B^2	0.16	1	0.16	102.66	<0.0001	
Residual	0.011	7	1.539×10^{-3}		0.2505	
Lack of Fit	6.518×10^{-3}	3	2.173×10^{-3}	2.04		Not significant
Pure Error	4.254×10^{-3}	4	1.064×10^{-3}			

Table 6: Validation of response surface model

Migration distance (mm)	Volume of water (mL)	Resolution-1		Difference	Resolution-2		Difference
		Predicted	Observed		Predicted	Observed	
80	2.5	2.905	2.806	0.099	2.760	2.882	0.122
75	2.5	2.734	2.889	0.155	2.572	2.671	0.099
80	4	3.082	2.862	0.22	2.839	2.802	0.037
80	1	2.260	2.390	0.13	2.202	2.087	0.115
75	4	2.926	2.831	0.095	2.715	2.841	0.126

Table 7: Control strategy for method development

Method variables	Operating range	Condition selected for estimation of drugs
Volume of water (mL)	1–4	2.5
Migration distance (mm)	70–80	75
Saturation time (min)	15–45	30
Band length (mm)	4–8	6
Detection wavelength (nm)	272–276	274
Scanning speed (mm/s)	10–20	20
Volume of mobile phase (mL)	8–10	10

1464.3, respectively. The residual plot shows random point dispersion, which indicates the linear regression model is appropriate [Figures 6 and 7]. The linearity data are depicted in Table 8.

Specificity

Results of specificity study shows only three peaks of drugs having R_f values of 0.53 ± 0.02, 0.62 ± 0.02, and 0.74 ± 0.02 for CPM, DEX, and GUA, respectively. Peak purity check of all three drugs from marketed formulation and standard drug showed high degree of correlation (>0.996) between spectra scanned at peak start, peak apex, and peak end position. The good correlation between absorbance reflectance spectrum of all three standard drugs and sample drugs from combined marketed formulation confirms the purity of all three drugs.

Precision

The method was found repeatable as %RSD for repeatability of sample application is 0.76, 0.86,

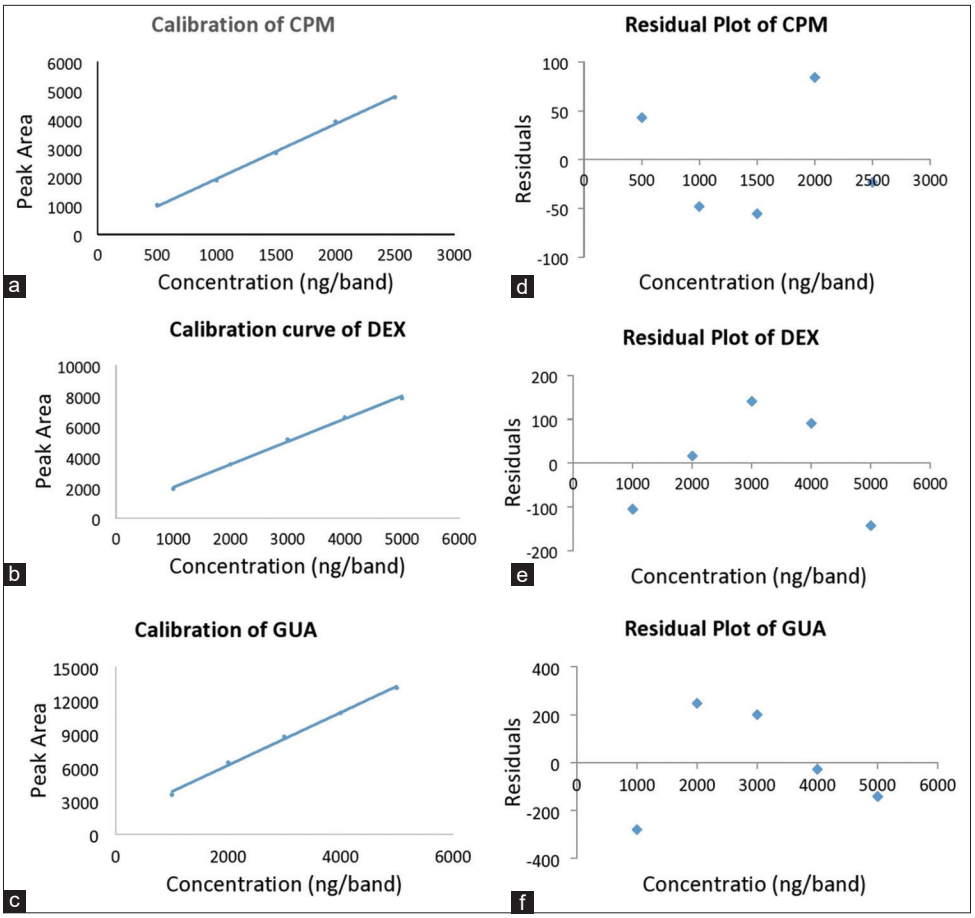

Figure 6: Calibration graph of (a) CPM, (b) DEX, (c) GUA and residual plot of (d) CPM, (e) DEX, and (f) GUA

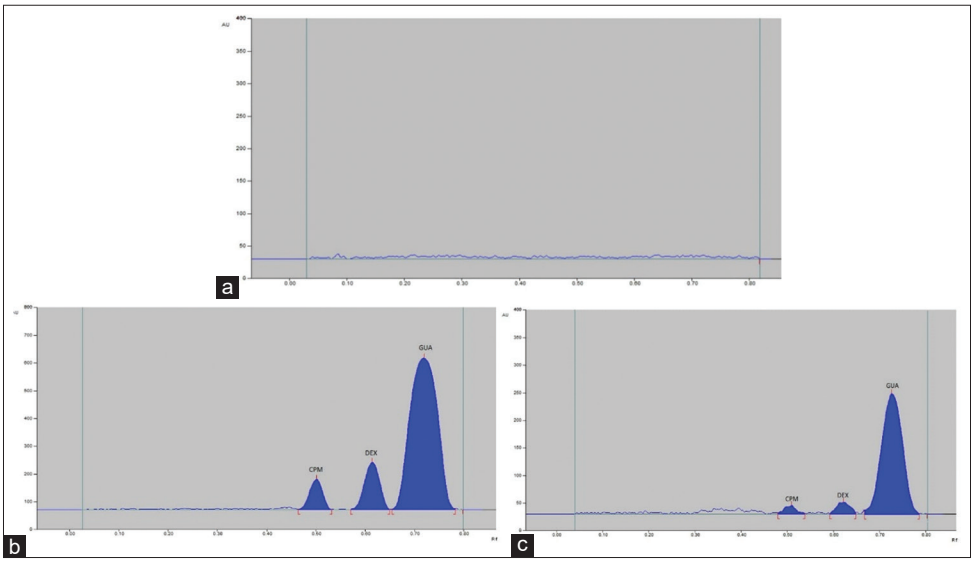

Figure 7: Chromatogram of (a) placebo and (b) marketed formulation for estimation of CPM and DEX (c) marketed formulation for estimation of GUA

and 0.65 for CPM, DEX, and GUA, respectively. %RSD for intraday precision was found to be 0.97–1.35, 0.97–1.24, and 0.74–0.85 and %RSD for interday precision was 1.23–1.41, 1.21–1.44, and 0.95–1.04 for CPM, DEX, and GUA, respectively.

Table 8: Linearity data of CPM, DEX and GUA

CPM			DEX			GUA		%RSD
Concentration (ng/band)	**Peak Area Mean±SD**	**%RSD**	**Concentration (ng/band)**	**Peak Area Mean±SD**	**%RSD**	**Concentration (ng/band)**	**Peak Area Mean±SD**	
500	1024.68±13.18	1.29	1000	1919.82±21.28	1.11	1000	3545.96±40.48	1.14
1000	1887.56±20.54	1.09	2000	3532.46±41.90	1.19	2000	6432.08±64.88	1.01
1500	2834.40±27.85	0.98	3000	5148.72±59.19	1.15	3000	8745.88±76.27	0.87
2000	3927.92±43.91	1.12	4000	6589.84±64.46	0.98	4000	10877.76±88.40	0.81
2500	4774.68±50.64	1.06	5000	7848.12±78.59	1.00	5000	13124.80±99.71	0.76

Table 9: Summary of validation parameters

Parameter	CPM	DEX	GUA
Linearity Range (ng/band) (*n*=5)	500–2500	1000–5000	1000–5000
Correlation Coefficient (R^2)	0.9984	0.9973	0.9964
Regression line equation	y=1.9081x+27.74	y=1.4914x+533.6	y=2.3603x+1464.3
Precision			
Repeatability of Sample application (*n*=7)	0.76	0.86	0.65
Intraday precision (*n*=3)	0.97–1.35	0.97–1.24	0.74–0.85
Interday precision (*n*=3)	1.23–1.41	1.21–1.44	0.95–1.04
%Recovery (*n*=3)	99.53–100.05	98.71–99.61	98.66–99.08
Limit of Detection (LOD) (ng/band)	36.40	127.98	82.22
Limit of Quantification (LOQ)(ng/band)	110.30	387.82	249.14

Accuracy

The developed method shows %recovery of 99.53–100.05, 98.71–99.61, and 98.66–99.08 for CPM, DEX, and GUA, respectively, which indicates accuracy of the method in quantification of all three drugs.

LOD and LOQ

Sensitivity of the method was established by finding out LOD and LOQ where, LOD was found 36.40 ng/band, 127.98 ng/band, and 82.22 ng/band while LOQ was found 110.30 ng/band, 387.82 ng/band, and 249.14 ng/band for CPM, DEX, and GUA, respectively.

Table 9 shows summary of validation parameters.

Assay of Marketed Formulation

Chromatogram of marketed formulation shows no interferences of excipients or additives in estimation as no additional peaks were observed. The drug content was found to be 98.0 ± 0.82, 98.40 ± 0.43, and 99.10 ± 0.62 for CPM, DEX, and GUA, respectively, in their combined dosage form [Table 10].

Table 10: Analysis of marketed formulation

Drug	Amount of drug in syrup (mg)	Amount of drug found in syrup (mg)	Assay (%) (*n*=3) Mean±SD
CPM	2.5	2.45	98.0±0.82
DEX	5	4.92	98.40±0.43
GUA	50	49.55	99.10±0.62

CONCLUSION

HPTLC method was developed for simultaneous estimation of CPM, DEX, and GUA in its combined dosage form utilizing systematic analytical quality by design approach. Seven potential method parameters were identified from preliminary trials and scientific knowledge. Two critical method parameters-volume of water and migration distance were screened by Taguchi OA screening design that significantly affects resolution-1 and resolution-2. 3^2 full factorial response surface methodology was applied to

screened critical method parameters to identify its relationship with resolutions. Design space was obtained from the response surface model from which control strategy was prepared and optimized chromatographic condition was selected for quantification of all three drugs. Developed method was validated according to ICH guideline. Lower values of LOD and LOQ indicate good sensitivity of method. The method was also found specific, precise, accurate and repeatable. The developed method was applied for assay of syrup formulation and results were found to be in great agreement with the label claim. The proposed method can be applied for routine analysis of CPM, DEX, and GUA in its combined syrupdosage form.

ACKNOWLEDGMENTS

The authors are thankful to Maliba Pharmacy College, UkaTarsadia University, for providing research facility.

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