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Chromatographic method development for the simultaneous assay of pseudoephedrine hydrochloride and chlorphenamine maleate in oral dosage formulations

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ABSTRACT

Common cold medicines usually present as multi-component products and constitute one of the largest groups of 'over-the-counter' medicines marketed in Ghana. Due to their wide patronage and easy accessibility, their quality control is of utmost importance to medicine consumers. In this study, the Design of Experiment (DOE) concept is adopted to develop a chromatographic method for the simultaneous assay of pseudoephedrine hydrochloride and chlorphenamine maleate in commercially available products. The separation of the drugs was achieved on an Agilent Eclipse Plus C18 analytical column (4.6 × 150 mm; 5 µm) and detected at 252 nm, while maintaining column temperature at 30 °C. The method parameters, including acetonitrile concentration in the mobile phase, mobile phase pH and flow rate were optimized using a Central Composite Design study, while monitoring the method attributes, asymmetric factors, selectivity, retention times, and resolution. The outcome showed a good correlation between experimental and predictive values throughout the modeled Design Space, with a Desirability of 1.000. The conditions optimized included a mobile phase containing acetonitrile, 50 mM aqueous acetate buffer solution (pH = 3.19) and triethylamine (15.34:84.65:0.01 v/v/v), and a flow rate of 1.437 mL/min. The method was then validated following the International Council for Harmonization Q2(R) guidelines to establish linearity and range, accuracy, precision, robustness, and stability of the test solution. The developed method was successfully applied to estimate pseudoephedrine hydrochloride and chlorphenamine maleate contents in single and fixed-dose formulations commercially available (N = 38).

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Introduction

Common Cold is a common viral infection of the nasopharyngeal mucosa. It is contagious and spreads mainly by airborne droplets. Patients with the infection usually experience symptoms such as either runny or blocked nose, slight fever, cough with the cold, and sometimes muscle aches [1]. The medications indicated for the management of these symptoms usually present as fixed-dose combination products [2], varying greatly in their combinations of different active pharmaceutical ingredients (APIs) as well as in their relative proportions [3]. The combinations may include an analgesic, an antitussive, a nasal decongestant, and an antihistamine [4]. In Ghana, these medications form one of the largest groups commercially marketed, and a significant number of local pharmaceutical manufacturing industries are involved in their manufacture and distribution.

Many times, the problems associated with the use and application of poor-quality medicines, especially in LMIC, such as Ghana are attributed to variations in manufacturing-related issues. However, the role played by poor analytical methods, with respect to their robustness and reliability cannot be under-estimated. Particularly with common cold medicines, the presence of the different APIs with their unique physicochemical properties, coupled with the significant differences in the doses of the APIs in the drug products, poses analytical challenges for their quality control and assurance [4,5]. Thus, the use of improved and very robust analytical methods for their in-process controls and final batch analysis would significantly reduce the risk of poor-quality products reaching consumers or patients.

Over the years, different chromatographic methods have been developed to simultaneously assay the contents of some of these products [3–6]. The development of these methods, have usually been charted along the path of trial-and-error, where studies conducted have adopted a one-factor-at-a-time (OFAT) approach [7,8]. In this approach, a factor or factors that influence the analytical method are investigated within an appropriate range (or levels), while the other factors are kept constant. This approach, though widely adopted, has been shown to yield sub-optimal conditions, as a result of the narrow robust behavior of the method for instrumental variables used in the method development [8–10]. In an attempt to surmount these challenges, the Quality by Design (QbD)/Design of Experiment (DOE) methodology have been adopted by some authors to efficiently design and develop robust chromatographic methods for the quality assessments of medicines [11–13]. The International Council for Harmonization (ICH) defines QbD as a systematic approach to drug development (including analytical method development), which begins with predefined objectives, and uses science, and risk management approaches to gain product and process understanding, and ultimately process control [14]. Usually, the advantage with DOE as applied to analytical methods is that a design space is generated for each developed method [15–17], and this region provides the opportunity to improve upon the efficiency of the method in the near future [18]. Furthermore, analytical methods designed by pharmaceutical manufacturing companies and other certified quality control laboratories require the approval of national regulatory bodies for variations to the developed methods' post initial validations. However, with the use of the DOE approach, there would not be a need to apply for such regulatory approvals, when minor variations that do not significantly impact the outcomes of the method performance occur and such changes stay within the design space [8], during continuous improvements of the method.

The focus for the current study was to develop a simple and robust method for the quality assurance of formulations containing pseudoephedrine hydrochloride (PSD) and chlorphenamine maleate (CPM) in fixed-dose combination products used for the management of common cold symptoms in Ghana. An available method in the United States Pharmacopoeia recommends the separate assessments of PSD and CPM, due to their different doses in oral formulations, using a mobile phase of methanol and water (60:40) containing monobasic potassium phosphate, triethylamine hydrochloride, sodium lauryl sulfate and phosphoric acid [19]. This method recommends the separate sample preparations for independent analyses of the contents of CPM and PSD, accounting for increased cost of analysis. An earlier method developed by Yacobi *et al* for the simultaneous assay of the two compounds also recommended the use of acetonitrile, methanol, and sodium nitrate (35:40:25) as well as 1-heptanesulfonic acid as the mobile system [20]. Authors in the current study propose a relatively simple, sensitive, accurate and cost-effective reverse-phase chromatographic method for the simultaneous analysis of PSD and CPM with only one simple sample preparation. Considering the benefits of the DOE methodology mentioned above, the approach was adopted to optimize the chromatographic conditions for the separation and quantitation. We hereby present report of our investigations and recommend a design space applicable to the method for consideration in assuring the quality of PSD and CPM in common cold medications.

Materials and methods

Materials

Chemicals and reagents

Reagents and solvents used for preparing samples and carrying out analyses were of analytical grade and HPLC grade, respectively. The solvents and reagents used included acetonitrile (ACN) (HPLC gradient grade; Fisher Chemicals, UK; Exp: 09/2020: Lot No: 1738242), glacial acetic acid (AcOH) (BDH England, Exp Date: 03/20, Batch No.: 10C290524), sodium acetate trihydrate (NaOAc) (Daejung Chemicals and Metals Co. Ltd, Korea, Lot No.: S04640I1; Exp: 21/09/2021) and triethylamine (TEA) (Fisher Scientific, UK; 05/2020; Lot No: 1417770). Demineralized water was freshly produced in-house from an ion-

exchange system (Ahura Aqua Treat, Mumbai, India), and terminally sterilized with ultra-violet radiation and filtered through a 0.45 µm membrane filter before using to prepare all solutions and buffers.

Drug substances and products

Chlorphenamine maleate (CPM) working standard (Supriya Lifescience Ltd, India, Batch No: SLL/C/0115014, Mfg date: 01/15, Exp date: 12/19, Assay: 99.95%) and Pseudoephedrine HCl (PSD) working standard (Zhejiang Apeloa Kangyu Pharmaceutical Co. Ltd Batch No: KY-pH-M20180619, Mfg date: 06/18, Retest date; 06/23, Assay: 100.64%) were donated by Salom Pharmacy Limited (Asokore-Mampong, Kumasi). These materials were used as secondary reference standards after standardizing them with their respective primary reference standards. 38 commercially available oral solid and liquid dosage formulations containing either PSD or CPM or both, as part of their active pharmaceutical ingredients, were randomly purchased from retail pharmacies in the Kumasi Metropolis and used for the analysis.

Instrumentation

Chromatographic measurements were performed on an Agilent 1260 Infinity II LC System (Agilent Technologies, Germany), consisting of a Quaternary Pump 1260 VL (G1311C), UV-Visible detector 1260 Variable Wave Detector (VWD) VL (G1314B), Multi sampler 1260 ALS (G1329B), and Multicolumn Thermostat 1260 TCC (G1316A). The mobile phase was degassed using a 3 litre Dial Ultrasonic Cleaner Tank with a heated bath (Allendale Ultrasonics, England) and filtered using a sintered glass filter (5 μm) before use. A reverse-phase Agilent Eclipse Plus C18 analytical column (4.6 $\times$ 150 mm; 5 µm) (Madeia, USA) was used for chromatographic separation of the drug substances.

Software and data analyses

Chromatographic analysis and data integration were recorded on a windows computer system using Agilent OpenLab CDS ChemStation Edition for LC & LC-MS Systems (Agilent Technologies; 2016). The choice of experimental design runs, and design-related multivariate calculations were performed using the software package, Design-Expert 11.0.5.0 version for Windows 64 bit (Stat-Ease Inc., Minneapolis, 2018). GraphPad Prism for Windows (version 6.01, 2012) was used to perform validation related descriptive and inferential statistics, including standard deviations, relative standard deviations, standard errors of means and regression analysis. The stability data of the analytes in the test standard solution was evaluated using Minitab 18.1 (Minitab, Inc., USA, 2017).

Methods

Preparation of solutions

Preparation of diluent solution. The diluent used for the preparation of standard and sample solutions was prepared by combining volumes of 50 mM NaOAc solution (pH = 3.0) and ACN in a ratio of 90:10.

Preparation of standard solutions. Stock standard solutions of PSD (120 mg/mL) and CPM (8 mg/mL) were prepared and stored in light-resistant containers. The working standards of PSD (1.2 mg/mL) and CPM (0.08 mg/mL) representing 100% concentrations as well as the calibration standards (that is, 0.6, 0.72, 0.84, 0.96, 1.08, 1.20, 1.32, 1.44, 1.56, 1.68 and 1.80 mg/mL for PSD; 0.04, 0.048, 0.056, 0.064, 0.072, 0.08, 0.088, 0.096, 0.104, 0.112 and 0.12 mg/mL for CPM) were freshly prepared by diluting determined aliquots of the stock solutions with the diluent. Combined test standard solutions containing mixtures of the two analytes were prepared immediately before use by pipetting and mixing appropriate volumes of the corresponding stock standards and topping up to the required volumes with the diluent. All dilutions were performed in volumetric flasks.

Chromatographic conditions

Chromatographic separations were carried out on an Agilent Eclipse Plus C18 analytical column (4.6 $\times$ 150 mm; 5 µm) with the adopted conditions optimized based on DOE methodology as described under 2.2.3. A mobile phase consisting of a mixture of ACN, 50 mM acetate buffer (containing NaOAc and AcOH), and TEA was employed for the study. The pH of the buffer was adjusted with 1% AcOH (v/v). 0.01% TEA was employed as an ion-pairing agent in the mobile phase, to reduce the interaction of the compounds with free silanol groups in the stationary phase, thereby reducing the tailing effect, initially observed in the preliminary investigations. The injection volume of the sample was maintained at 20 µL throughout the study. The entire chromatographic system was also maintained in an air-conditioned laboratory atmosphere (16 – 20 ^{o}C). The column temperature was maintained at 30 ^{o}C and the detection of analytes was also achieved at a wavelength of 252 nm.

Experimental design model in method development

A review was conducted to identify method parameters and method attributes (for example, resolution, selectivity, asymmetric factor, etc.) that affect the performance of chromatographic methods. A number of these factors were then considered and investigated empirically in the initial stages of the method development. Based on the outcome of such preliminary investigations, three method parameters were selected: concentration of ACN in mobile phase [A], mobile phase pH [B], and flow rate [C]. These parameters were explored further using a Central Composite Design (CCD) model. The method attributes

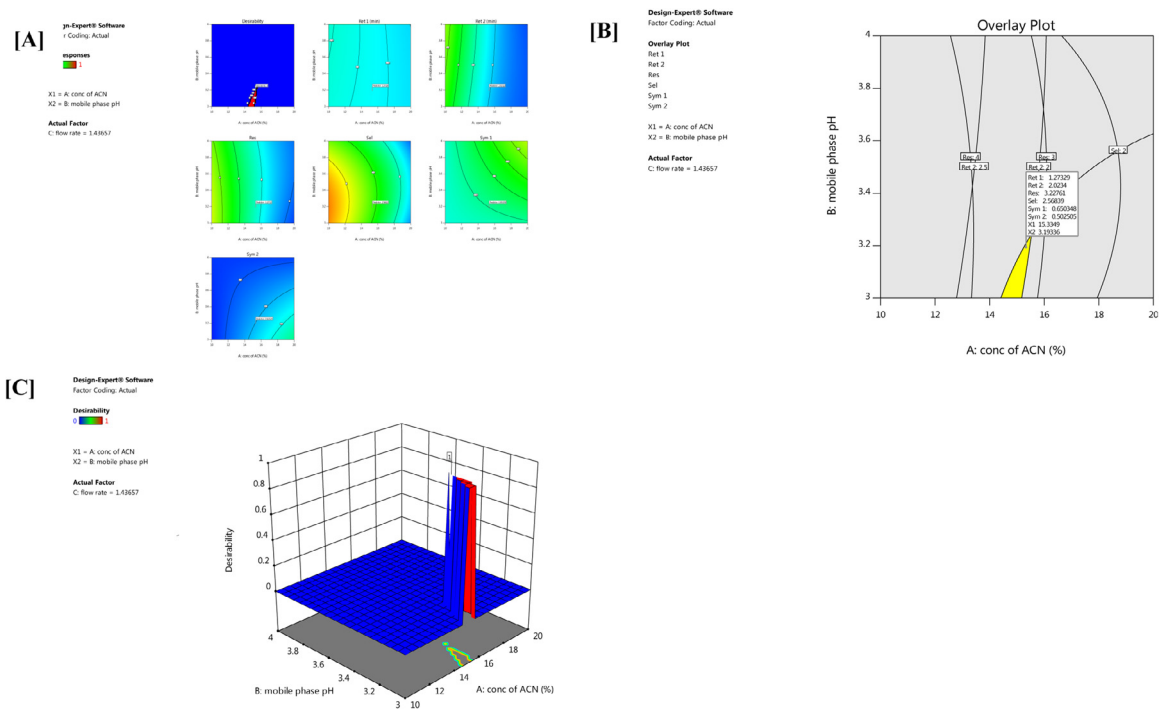


Fig. 1. Results from optimization studies conducted.
[A] – Contour plots illustrating the interaction between the Critical Method Parameters and Method Attributes in the Optimization Design studies. [B] – 2D Overlay Plot showing Method Operable Design Region for developed method. [C] – 3D Surface plot showing Method Operable Design Region for developed method.

monitored included the retention times of CPM (Rt_1) and PSD (Rt_2), selectivity factor (Sel), the resolution between the two drug substances (Res), and the asymmetric factors for both compounds (Sym_1 & Sym_2).

The experimental results from the CCD were fitted to a second-order polynomial model, which included interaction and quadratic terms. The interaction terms were effects resulting from interactions between any two of the studied parameters (for e.g., interaction between A & B, A & C and B & C) whiles the quadratic terms were the squared effects from each of the parameters (for e.g. A^2, B^2 and C^2). The qualities of the models were examined by estimating the coefficients of correlation (R^2), for the responses studied, using multi-linear regression analysis (MLR).

The general MLR model was represented by the following equation:

$$R = \beta_o + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{22} X_2^2 + \beta_{33} X_3^3 \quad (1)$$

Where, R represented the measured response (including Rt_1, Rt_2, Sym_1, Sym_2, Sel and Res); X_1, X_2, and X_3, represented the selected method parameters (which are [A], [B] and [C]); β_o was the intercept; β_1 and β_2 were first-order parameters; β_{12}, β_{13} were interaction parameters; and β_{11}, β_{22} and β_{33} were second order (quadratic) parameters, respectively. The optimized Method Operable Design Region [16] or design space (Fig. 1b & 1c) was then predicted using the Derringer's desirability function.

System suitability test

The system suitability test was carried out by analyzing the quality attributes of the method from replicate experimental runs (N = 10) using a freshly prepared combined standard solution (100% concentration of each analyte) in the mobile phase. The quality attributes monitored included injection precision, the resolution between the analytes, relative retention time, selectivity factor, and asymmetric factors of the analyte peaks.

Method validation. The developed method was validated in accordance with the ICH Guideline Q2(R1) [21]; following recommendations from literature [22–24].

Specificity and selectivity. Specificity and selectivity were assessed by comparing the chromatogram from a matrix without expected analytes with that of a matrix containing the expected analytes (that is, 1.2 mg/mL of PSD and 0.08 mg/mL of CPM) [24]. The selectivity was confirmed by comparing the Mean ± SD of the retention times for the analytes using Student *t*-test analysis [22].

Linearity and range. Linearity and range of the method were evaluated using the external calibration approach by testing serially prepared calibration standards (corresponding to 50% - 150%) from the stock solution as described in section 2.2.1.2. The concentrations prepared for PSD included 0.6, 0.72, 0.84, 0.96, 1.08, 1.20, 1.32, 1.44, 1.56, 1.68 and 1.80 mg/mL whiles that of CPM included 0.04, 0.048, 0.056, 0.064, 0.072, 0.08, 0.088, 0.096, 0.104, 0.112 and 0.12 mg/mL. Replicate determinations (n = 10) were carried out for each test concentration in a randomized manner and the peak areas, reported as Mean ± SD were plotted against test concentrations and statistical analysis was performed by the method of least squares [22]. Linearity was predicted by estimating the regression coefficient (R^2), and the linear regression y-intercept of the response versus concentration plot. The regression model was also tested for fitness by determining the level of significance of the *F* value of the model in an ANOVA analysis at a 5% risk level [22,24]. Additionally, the residual plots for the sets of test data were generated.

Limits of detection and quantitation. The limit of detection (LOD) and limit of quantitation (LOQ) were calculated from the y-intercepts of regression lines of the linearity curve using the expression below:

$$\mathrm{LOD} = 3.3\sigma/s \tag{2}$$

$$\mathrm{LOQ} = 10\sigma/s \tag{3}$$

Where σ is the standard deviation of response; s is the slope of the calibration curve.

Precision. Precision was demonstrated by determining repeatability and intermediate precision. Repeatability was initially proven on six replicate injections of 100% test concentrations of analytes. A second determination on repeatability considered triplicate determinations of 80%, 100%, and 120% working concentrations of the analytes, to investigate the effect of concentration change on the precision of the method. Intermediate precision, on the other hand, was demonstrated by 3 independent analysts on three consecutive days using 100% test concentrations of working standards. In each of the determinations, the relative standard deviations (RSD) of the peak areas were determined and compared to literature [21].

Accuracy. The method accuracy was performed by determining the percentage recoveries of PSD and CPM from the addition of their respective working standards to matrices of excipients. The samples, prepared in triplicates and corresponding to 80%, 100%, and 120% of working concentrations of the drug substances, were used for the determination. The respective peak areas were recorded and the Mean ± SD of recoveries for each concentration term used was calculated [5,21,24]. Accuracy was further evaluated by analysing the working standards with the developed method and comparing the outcomes with that from the use of the USP compendial method [19].

Robustness. The robustness of the method was performed as described in the literature [22]. Briefly, this was carried out by monitoring the effects of deliberate changes in flow rate (± 10%), injection volume (± 5 µl), column temperature (± 5%), acetonitrile content of the mobile phase (± 2%), and wavelength of detection (± 5%) on method quality attributes like retention times, selectivity, resolution, and peak symmetry. The results from the modification were compared to the established specification or acceptance criteria for the attribute in the developed method.

Stability of solutions. The stability of PSD and CPM in freshly prepared combined working standard solutions were tested over 48 hours at room temperature. Triplicate determinations of percentage assays of each analyte in the test standard solution (containing PSD – 1.2 mg/mL; CPM – 0.08 mg/mL) were carried out at predefined intervals and analyzed using regression analysis, to estimate the shelf lives of the analytes in the test standard solution [22].

Analysis of dosage formulation

The validated method was applied to assay the content of PSD and CPM in selected commercially available oral dosage formulations (N = 38). The samples were handled differently depending on their dosage forms and these are described below:

Tablets. For tablets, an equivalent amount of the formulation containing the equivalent weight of 60 mg of PSD or 4 mg of CPM or both as per label claim (depending on the product composition) and uniformity of weight determinations, was accurately weighed after powdering 20 of such tablets. The weighed amount was quantitatively transferred into a 50 mL volumetric flask and diluted with 20 mL of the diluent. The solution was sonicated for 10 min, to completely extract the active ingredients, and then topped up to the required volume with the diluent to obtain expected concentrations of PSD as 1.2 mg/mL and CPM as 0.08 mg/mL. The resultant solution was then filtered through 0.45 µm filters and analyzed. Replicate determinations (N = 5) were carried out using the optimized conditions for the developed method. Qualitatively, the presence of the analytes in the products was confirmed by the consistency of the retention times for the analytes with that of the working standards. Their respective concentrations were then evaluated from the peak areas recorded using the linear regression models with respect to their calibration standards.

Table 1
ANOVA and Regression analysis for selected factorial models for each method attribute studied in the CCD experiments.

Response	Name	Observations	Transform	Fit Model	Adjusted R^2	Predicted R^2	p-value	F-value	PRESS[§]
R1	Ret_1	19	None	Quadratic	0.9622	0.8564	< 0.0001	51.87	0.6011
R2	Ret_2	19	None	Quadratic	0.9936	0.9751	< 0.0001	311.46	0.7299
R3	Res	19	None	Quadratic	0.9593	0.8413	< 0.0001	48.20	7.45
R3	Sel	19	None	Quadratic	0.9493	0.8110	< 0.0001	38.45	1.66
R6	Sym_1	19	None	2FI	0.4182	-0.9877	0.0427	3.16	0.4255
R7	Sym_2	19	None	2FI	0.5752	-0.5178	0.0083	5.06	1.40

[§] PRESS stands for Predicted Residual Error Sum of Squares

Syrups. For syrups, an equivalent volume of each product containing the equivalent weight of 60 mg of PSD or 4 mg of CPM or both as per label claim (depending on the product composition) and uniformity of content determinations, was accurately pipetted into a 50 mL volumetric flask, and subsequently prepared using a similar procedure as tablets above. The resultant solution was then filtered through 0.45 µm filters and analyzed. Also, the concentrations of the analytes were evaluated using a similar approach as that for the tablets.

Results and discussion

Method development

Attempts have been made in existing literature to simplify the chromatographic conditions needed to analyze CPM and PSD containing products. Earlier works showed that with either isocratic or gradient elution systems involving phosphate, ammonium acetate or formate buffers (pH = 3–7), in combination with acetonitrile or methanol, and ion-pairing agents like 1-heptanesulphonate and triethylamine, good separation and quantitation of PSD, CPM, and their combinations with other analytes such as paracetamol, phenylephrine, bromhexine, dextromethorphan, etc. were observed at wavelengths between 215 nm – 310 nm [5,6,25–27]. However, there are concerns with the use of inorganic buffers as they are not volatile and could precipitate on the column after use, and this requires extra column care, with sometimes extra cost. For this reason, the use of organic buffers such as acetate buffer was considered in the current study. Also, most of the above-mentioned methods developed were through empiric approaches and this has been showed to sometimes lead to narrowly robust conditions; calling for frequent revalidations [8]. In the quest to address such challenges, a DOE approach involving Plackett-Burman and central composite designs was used to screen and select key chromatographic conditions, and optimize them for the analysis of bromhexine, chlorpheniramine, paracetamol, and pseudoephedrine in oral tablet and syrup preparations by Vignaduzzo and Kaufman [27]. Notwithstanding this feat, the optimized conditions reported are 'not friendly' to resource-limited settings, where the luxury of possessing more than one column is limited or non-existent and almost all analyses are performed with the commonly available C18 columns. For routine analytical purposes therefore, both the compendial method and the experimentally robust methods developed have limited uses in Ghana and other parts of the developing world.

For this reason, authors in the current study, aimed to develop a chromatographic method, whose sensitivity is not affected by the significant dosage difference for PSD and CPM being 15:1 (usually observed with the fixed-dose combinations), that can be easily adopted in the developing world for the simultaneous analysis of PSD and CPM. This approach maximises its robustness with the use of the DOE approach to optimize the chromatographic conditions. Initially, six method parameters, including column temperature, ACN and TEA concentrations in the mobile phase, mobile phase pH, flow rate and the wavelength of detection, were considered for the method development. Preliminary investigations showed that the change in flow rate and the ACN concentration in the mobile phase, significantly altered the retention times, selectivity factor and resolution of PSD and CPM. Similarly, a change in the TEA concentration also had some effects on the peak asymmetric factors. In the absence of TEA, some degree of tailing was observed. However, when TEA was added to the mobile system, the tailing reduced significantly and beyond 0.01% concentration, there were no significant differences in the peak asymmetric factors for the analytes. Thus, 0.01% was maintained for the method optimization. A change in the mobile phase pH also demonstrated significant effects on the tailing of CPM. The other two parameters, column temperature and wavelength of detection (**Figure SM1**) were thought to offer minimal effects on the performance of the method, and so, were also maintained at constant parameters during the CCD experimental study. The results from the CCD experimental studies are shown in Table 1 and Fig. 1a.

The regression models developed to establish the quantitative effects of the three parameters, with their interaction effects are shown below. In the following models, A, B, and C represented the main effects of ACN concentration in the mobile phase, mobile phase pH, and flow rate, respectively. AB, AC, and BC represented the effects of two-factor interactions (between). A^2, B^2, and C^2 represented quadratic effects.

$$Ret_1 = 1.21312 - 0.0647124A + 0.0029823B - 0.511298C - 0.012875AB + 0.023875AC - 0.006125BC - 0.00152942A^2 - 0.00347396B^2 + 0.181965C^2 \quad (4)$$

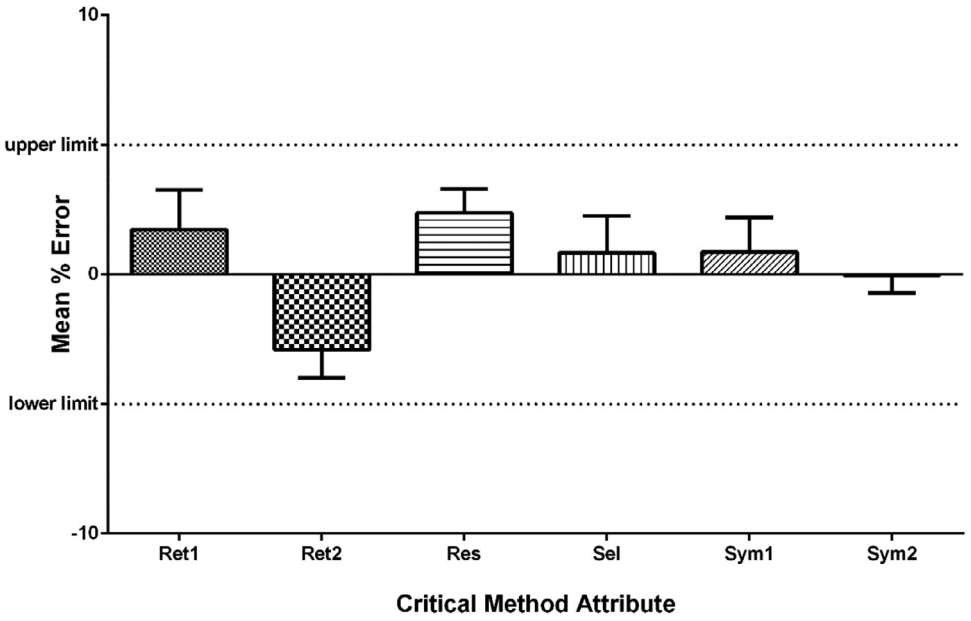


Fig. 2. Verification of Method Operable Design Region for the developed method.

$$\text{Ret}_2 = 2.02659 - 0.958503A + 0.089838B - 0.947899C - 0.111625AB + 0.318625AC - 0.041125BC + 0.392649A^2 - 0.0268422B^2 + 0.331484C^2 \quad (5)$$

$$\text{Res} = 3.3391 - 1.75229A - 0.0501926B - 0.182758C + 0.22875AB + 0.09125AC + 0.12875BC + 0.397088A^2 - 0.140314B^2 + 0.16551C^2 \quad (6)$$

$$\text{Sel} = 2.6112 - 0.726803A - 0.164119B - 0.0043934C + 0.1225AB + 0.0075AC - 0.0025BC - 0.109699A^2 - 0.236978B^2 - 0.0372203C^2 \quad (7)$$

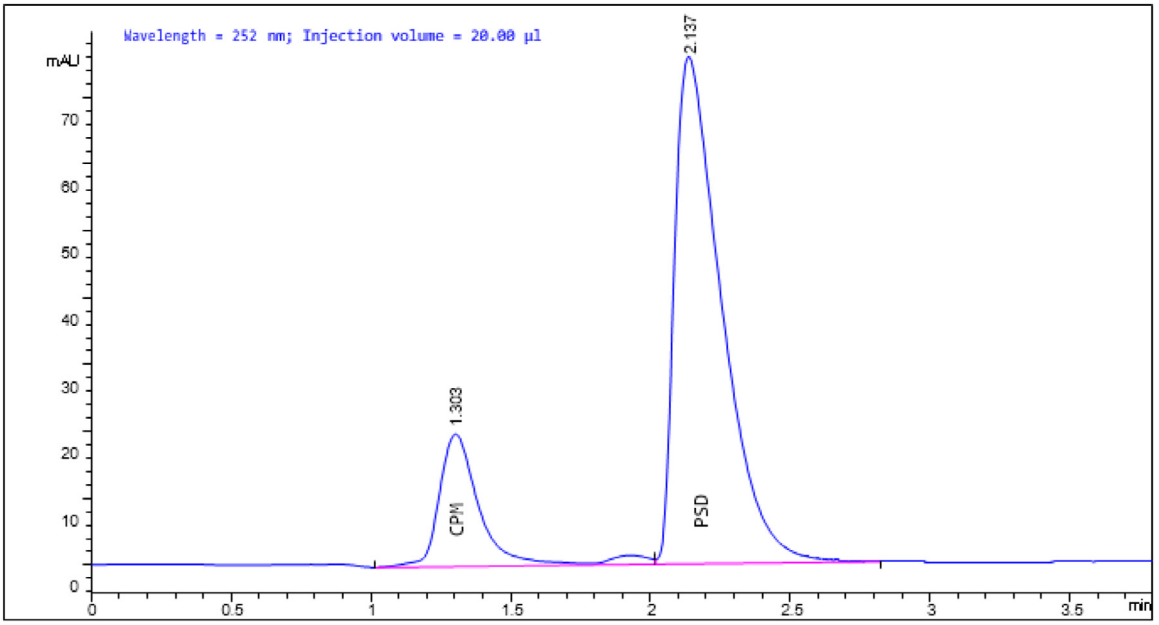
$$\text{Sym}_1 = 0.680526 + 0.0572135A + 0.0468629B + 0.00199692C + 0.0475AB - 0.0525AC - 0.045BC \quad (8)$$

$$\text{Sym}_2 = 0.459474 + 0.106906A - 0.0891327B + 0.0925276C - 0.08625AB + 0.11125AC - 0.12375BC \quad (9)$$

Having estimated the effects of the method parameters on each of the method attributes, the design space was modeled using conditions showed in [SM1]. The results for the modeled design space are shown in Fig. 1b & 1c. The accuracy of the predicted conditions within the design space **[SM2]** was verified by randomly investigating 5 of the predicted set of conditions generated from the optimization algorithm, and comparing the experimental outcomes with ones from the predicted space [28]. It was observed that the average percentage errors associated with the predicted set of conditions were less than ± 5.00% (Fig. 2; **[SM3]**). This proved the robustness of the design space and showed that the space was desirable and well suited for adoption. Thus, with a Desirability of 1.000, the following conditions were employed for use as optimized conditions for the developed method: The mobile phase contained ACN, 50 mM sodium acetate buffer (pH = 3.19) and TEA, in the ratio of 15.34: 84.65:0.01 (v/v/v). The chromatographic separation was achieved at a flow rate of 1.437 mL/min. The column temperature was maintained at 30 ºC and the detection of analytes was achieved at a wavelength of 252 nm. A system suitability test was carried out with the optimized conditions and the results showed that the method was suitable for the intended analytical purpose **[SM4]**. The chromatogram for the separated compounds using the optimized conditions is illustrated in Fig. 3.

Method validation

The results of the validation are summarized in Table 2. It was observed that the retention times for CPM and PSD were significantly different from each other ($t = 5.979$; $df = 4$; $p = 0.0039$). This meant that the developed method was able to



Peak #	RetTime [min]	Width [min]	Area [mAU*s]	Height [mAU]	Asymmetric factor	Resolution	Selectivity
1	1.303	0.1562	205.94980	19.81868	0.67	-	-
2	2.137	0.1756	886.27698	75.86712	0.45	3.27	2.59
Totals:			1092.22678	95.68580			

Fig. 3. Chromatogram produced from the optimized method for the analysis of CPM and PSD with associated quality attributes.

detect both analytes independently of each other without any interference from the test solution matrix. Also, the wavelength for detection ensured that only the analytes of interest were detected; the excipients were not detectable at the levels necessary for quantitative purposes. Specificity was therefore proven for the method [**SM5**]. In establishing method accuracy [**SM6**], the percentage recoveries obtained complied with the acceptance criteria. Method accuracy was demonstrated over a concentration range (80% - 120% of test concentration) for both analytes (Table 2). Method accuracy was further confirmed when the outcomes from the use the presently developed method was compared with that from the use of the USP compendial method. The results were observed to be comparable; indicating that the method produced comparable results as the existing one. Precision [**SM7**] was also demonstrated accordingly for the developed method. Similarly, the responses for both CPM and PSD were showed to be linear within the ranges 0.040 mg/mL – 0.120 mg/mL and 0.60 mg/mL – 1.80 mg/mL [**SM8**]. *F*-value from the linear regression model was shown to be significant, further demonstrating a strong correlation between the concentration of the analytes and peak area response [22]. The LOD and LOQ [**SM9**] were also estimated. Due to the design approach adopted for the study, the robustness of the method within the modeled design space was demonstrated with modifications in injection volume, column temperature, ACN content in the mobile phase, and wavelength of detection [**SM10**]. It was observed that since most of the modifications were within the range of the design space, the quality attributes monitored were mostly consistent with the acceptance criteria. However, in the case of the flow rate, it was observed that when the modifications fell outside the range of the design space, the retention times for CPM and PSD were not consistent with the applicable specification. This further proved that the robustness of the method could be better demonstrated with the modeled design space. Change of method parameters within the design space, would not constitute a change in the method; however, a change outside the space would require a revalidation. The test solution prepared for the assay was showed to be stable beyond 48 hours [**SM11**]. Thus, for large batch analyses, several injections could be carried out since solutions could last more than 48 hours, and this is critical.

Analysis of commercial formulations

The validated HPLC method was then used to simultaneously estimate the contents of CPM and PSD in commercially available products. Both solid dosage (n = 23, 60.53%) and liquid dosage formulations (n = 15, 39.47%) were considered. While 19 (50%) of the products contained CPM, 10 (26.3%) contained PSD and 9 (23.7%) of the products contained both CPM

Table 2
Summary of validation results of CPM and PSD.

Validation parameter		CPM	PSD	Remarks
Specificity/selectivity [S5]	Retention time (Mean ± SD)	1.303 ± 0.0023 mins (N = 3)	2.307 ± 0.2908 mins (N = 3)	Retention times are significantly different from each other (t = 5.979; df = 4; p = 0.0039). Method selectively and specifically identifies both analytes.
Accuracy [S6]	% Recovery (Mean ± SD)			Developed method accurately estimates the content of the analytes. Acceptance criteria – [98.00% - 102.00%]
		0.064 mg/mL = 99.72% ± 1.433 (N = 3)	0.960 mg/mL = 99.97% ± 0.3717 (N = 3)	
		0.080 mg/mL = 99.11% ± 1.325 (N = 3)	1.200 mg/mL = 99.40% ± 0.7504 (N = 3)	
		1.200 mg/mL = 100.2% ± 0.1721 (N = 3)	1.440 mg/mL = 101.2% ± 0.6940 (N = 3)	
	new method vs compendial method (%)	101.14 ± 0.535 vs 100.65 ± 0.757	99.78 ± 0.992 vs 101.08 ± 0.178	Assays from the two methods were comparable (p > 0.05)
Linearity and Range [S8]	Regression equation	y = 1628x + 42.62	y = 608.6x + 5.018	Acceptance Criteria – [R^2 > 0.99] Linearity of responses established for the specified analytes' concentration ranges.
	R^2	0.9935	0.9979	
	Sy.x	3.362	10.57	
	F-value, p-value	16,502, p < 0.0001 (N = 10)	52,547, p < 0.0001 (N = 10)	
	Range	0.040 mg/mL – 0.120 mg/mL	0.60 mg/mL – 1.80 mg/mL	
LOD [S9]		0.002155 mg/mL	0.018158 mg/mL	
LOQ [S9]		0.006529 mg/mL	0.0549 mg/mL	
Precision [S7]	Repeatability (same conc)	RSD = 1.19%, N = 6	RSD = 0.93%, N = 6	Acceptance Criteria – [RSD < 2.0%] Developed method responses were precise.
	Repeatability (3 conc terms)	RSD = 0.4867% ± 0.4611, N = 3	RSD = 0.5167% ± 0.3453, N = 3	
	Intermediate precision (3 analysts for 3 days)	RSD = 0.10% - 1.72%, N = 3	RSD = 0.07% - 0.85%, N = 3	
Robustness [S10]		Robust within design space	Robust within design space.	Altering method parameters within design space did not affect method attributes. On the other hand, altering method parameters outside design affected compliance of retention times.
Stability of test solution (N = 3) [S11]	Predicted Shelf life	54.5576 hours	69.3775 hours	Analytes were very stable in the test solution for more than 48 hours.
	Regression Equation	Y = 100.357 – 0.0840x	Y = 100.529 – 0.0698x	
	F-value, p-value	80.90, < 0.0001	130.13, < 0.0001	
	R^2	0.7639	0.8388	

and PSD. For the analysis of the content of CPM, 18 (64.29%) of the products were found to be compliant with the release specification of 90 – 110% of the label claim as adopted from the United States Pharmacopoeia [19], whiles in the case of the PSD, 16 (84.21%) were compliant with reference to a similar acceptance criteria from the same compendial standard. There were 11 products which failed either in the content of CPM (n = 8, 72.7%) or PSD (n = 1, 9.1%) or both (n = 2, 18.2%). The outcome of the analysis showed that the method was suitable for the intended purpose of the quality control of CPM and PSD contents in oral formulations, and rightly distinguishing between compliant and non-compliant products. The outcome of the analysis is showed in Table 3.

Significance of the developed method

The development of this method using the DOE approach demonstrates the applicability of the use of experimental designs to correctly predict the optimized set of chromatographic conditions required for analytical purposes. Through the predictive modeling algorithm, it is possible and relatively easy to select the optimum conditions to attain the analyst's expected outcomes. In this case, it has been possible to achieve a successful resolution of the analytes, with all other required quality attributes, whiles maximizing the total time of analysis. Considering the intended use of the method in routine analyses, this outcome was deemed desirable. It is also possible that, as part of the lifecycle management of the method [7], periodic continuous improvements could be initiated within the design space to improve on the outcomes of the analytical method with ease, and such improvements would not require prior regulatory approval, granted that they do not significantly impact on the performance of the method. This could help inform policy in analytical method development and validation.

Table 3
Summary of results from assay of commercial products.

Sr No	Type of formulation	Batch number*	Product details Man. Date:	Exp. Date:	Active ingredient Assay ± SD (%, N = 5) CPM	PSD
1	Solid	X001	03/19	03/22	101.87 ± 0.219	101.80 ± 1.452
2	Solid	X002	03/19	03/22	100.92 ± 0.541	101.59 ± 0.635
3	Solid	X003	03/19	03/22	100.75 ± 1.045	100.88 ± 0.820
4	Liquid	X004	06/18	06/21	97.98 ± 0.234	-
5	Solid	X005	04/18	04/21	95.22 ± 0.582	-
6	Liquid	X006	01/19	01/22	104.93 ± 1.277	-
7	Liquid	X007	01/19	01/22	77.43 ± 0.981	99.12 ± 1.142
8	Liquid	X008	09/19	09/22	-	103.26 ± 0.114
9	Solid	X009	09/19	09/22	-	115.47 ± 1.872
10	Solid	X010	02/20	02/23	120.02 ± 1.324	110.09 ± 0.279
11	Solid	X011	08/20	08/23	65.38 ± 2.143	76.44 ± 2.497
12	Liquid	X012	10/19	10/22	105.22 ± 0.089	-
13	Liquid	X013	06/18	06/21	102.93 ± 0.247	-
14	Solid	X014	07/18	07/21	101.28 ± 0.554	-
15	Solid	X015	02/19	02/22	-	98.71 ± 2.001
16	Solid	X016	01/20	01/23	-	101.90 ± 0.140
17	Solid	X017	01/20	01/23	-	109.17 ± 0.150
18	Solid	X018	01/18	01/21	89.43 ± 0.014	-
19	Solid	X019	03/18	03/21	153.04 ± 0.252	-
20	Liquid	X020	12/18	12/21	100.11 ± 0.165	104.15 ± 0.007
21	Solid	X021	07/18	07/21	97.26 ± 0.026	-
22	Liquid	X022	01/19	01/22	108.48 ± 0.042	-
23	Liquid	X023	04/18	04/21	167.18 ± 0.283	-
24	Liquid	X024	12/17	12/20	113.08 ± 0.987	-
25	Liquid	X025	12/17	12/20	96.24 ± 0.924	100.29 ± 0.421
26	Solid	X026	05/19	05/22	-	101.49 ± 0.235
27	Solid	X027	05/19	05/22	-	105.43 ± 0.156
28	Liquid	X028	12/17	12/20	98.67 ± 0.273	-
29	Liquid	X029	09/18	09/21	102.99 ± 0.026	-
30	Solid	X030	08/18	08/21	95.55 ± 1.294	-
31	Solid	X031	09/18	09/21	86.73 ± 0.453	-
32	Liquid	X032	06/19	06/22	-	101.61 ± 0.552
33	Liquid	X033	07/19	07/22	-	104.53 ± 0.921
34	Solid	X034	07/19	07/22	-	103.11 ± 0.601
35	Solid	X035	08/18	08/21	52.49 ±1.432	-
36	Solid	X036	02/20	02/23	99.27 ± 0.127	98.98 ± 1.54
37	Solid	X037	03/19	03/22	101.24 ± 0.006	-
38	Solid	X038	11/19	11/22	88.19 ± 1.099	-
		Acceptance criteria			90.0% – 110.0%	90.0% – 110.0%

* Original product information withheld for confidentiality purposes. [-] means the API was not present in the sample.

Conclusion

A simple, robust, rapid, accurate, sensitive, and reproducible HPLC method for the simultaneous identification and assay of chlorphenamine maleate and pseudoephedrine hydrochloride in oral dosage formulation has been developed and validated using the Design of Experiment approach. This involved the use of the central composite design model to optimize the composition of acetonitrile in the mobile phase, mobile phase pH and flow rate whiles maintaining the wavelength of detection and column temperature at constant parameters. The method presents with a design space within which permissible alteration of chromatographic conditions were investigated and verified. The method was successfully validated and applied to quantify simultaneously the studied active ingredients in different batches of commercially available solid and liquid dosage products. 64.29% of products containing CPM and 85.21% of products containing PSD were found to be compliant with the release specification 90 – 110% of the label claim as adopted from the United States Pharmacopoeia. Effectively, the developed method demonstrates that in its use for routine analysis, can distinguish between good quality and substandard products containing CPM and PSD.

Data availability

All processed data applicable to the conduct of this study are included in the article and in the Supplementary Data [SM1 – SM11].

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Declaration of Competing Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.sciaf.2022.e01109.

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