Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2016, 8(21): 94-105
(http://scholarsresearchlibrary.com/archive.html)

Scholars Research Library
ISSN 0975-5071
USA CODEN: DPLEB4

Development, Optimization and Validation of New Liquid Chromatographic Method for the Simultaneous Determination H1 Receptor Blockers in Bulk and their Pharmaceutical Formulations by Applying D-Optimal Mixture Design Methodology

Selvakumar Kanthiaha and Valliappan Kannappana

Department of Pharmacy, Annamalai University, Annamalai Nagar, Tamil Nadu, India

---

ABSTRACT

A simple, sensitive, and rapid HPLC method was developed for analyzing H1 receptor blockers viz, hydroxyzine, cetirizine, meclizine, loratadine, desloratadine, rupatadine and fexofenadine in bulk and drug products. Chromatographic separation was performed on Phenomenex cyano analytical column (150 mm x 4.6 mm i.d, 5 µm). D-optimal mixture design methodology, a chemometric tool was employed for chromatographic optimization. The mobile phase system comprising acetonitrile-methanol-ammonium acetate buffer (40 mM; pH 3.8 adjusted with acetic acid): 18/36/46% v/v/v respectively, was identified by using overlay plot of the design. Mobile phase delivered at 1.5 mL min-1 flow rate and peaks were detected at 222 nm. All analytes were baseline separated in less than 9.5 min. The proposed method was validated for linearity, accuracy, precision, limit of quantification, and robustness according to ICH Q2 (R1) guidelines. Calibration curves were linear over selected range (≥0.996) for all analytes. The proposed method was successfully applied for the quantitative analysis of seven commercially available tablet dosage forms. Good agreement was found between the assay results and the label claim of the marketed formulation by showing good %recovery and %CV. The study demonstrated that the proposed HPLC method can be employed for routine quality control purposes.

Keywords: HPLC, H1 receptor blockers, D-optimal mixture design methodology, Chemometric tool, ICH Q2 (R1) guidelines.

INTRODUCTION

Antihistamines or H_1 antagonist are class of medications that inhibit the action of histamine in the body by blocking the receptors of histamine [1]. Most of the second-generation H_1 antihistamines currently in use have been identified as structural modifications of pre-existing medications in this class [2]. For instance, cetirizine (CTZ), desloratadine (DES) and fexofenadine (FEX) are derived from its pre-existing antihistaminic medications *viz,* hydroxyzine (HYD), loratadine (LRT), and terfenadine (TER) respectively [3]. Similarly, DES is also derived from rupatadine (RUP) [4]. These antihistamines or H_1 antagonist are used to relief symptoms associated with seasonal allergic rhinitis, perennial allergic rhinitis and chronic idiopathic urticaria [5]. Other antihistaminic agent like meclizine (MEC) is a derivative of piperazine and is used for the prevention and treatment of motion sickness [6]. The chemical structure of HYD, LRT, FEX, DES, CTZ, MEC, and RUP are represented in Figure1.

The selected antihistaminic agents are widely used as single unit dosage forms and commercially available as tablets and syrups in Indian market. Antihistaminic drugs among the popularly known prescription and non-prescription (OTC) medications and the most commonly prescribed medicines for pediatric population. The use of any medication in this age group must adhere to the strictest safety criteria, and must offer the maximum guarantees of its therapeutic efficacy. Although, the functional moiety of these antihistamines is shared with each other, they differ significantly in terms of molecular properties, pharmacokinetics, membrane permeability, receptor affinity and toxicity potential [7-9]. Hence, it is necessary to develop an analytical method which could be applied to authenticate the standards of commercially available formulations.

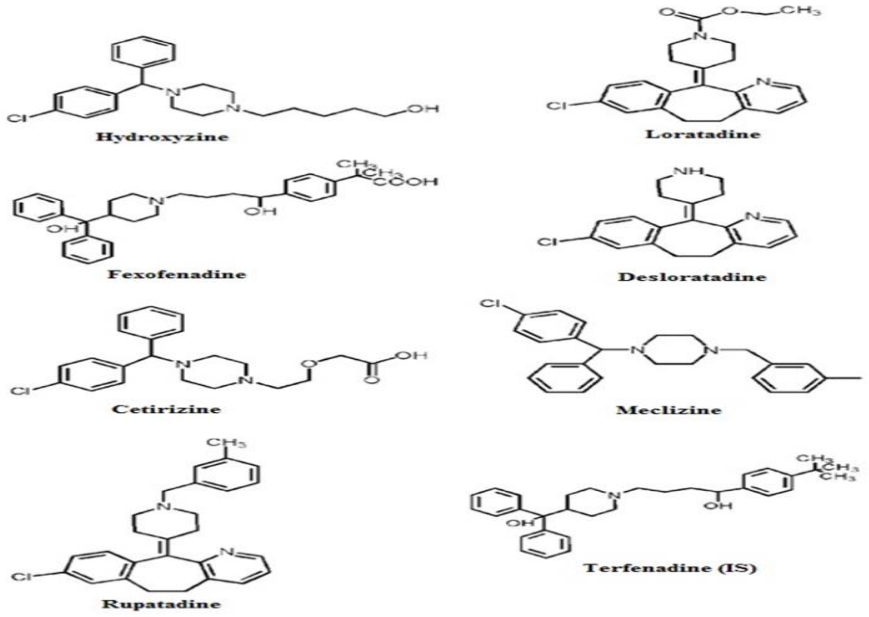

Figure-1: Chemical structure of analytes

In the literature, there have been many analytical methods were reported for determination of HYD [10-13], LRT [14-17], RUP [18], CTZ [19-22], DES [23-25], MEC [26,27] and FEX [28-34] in pharmaceutical formulations and biological matrices. Hammad et al, 2007 [35] proposed a HPLC method for quantitative estimation of HYD and CTZ in human serum. The method required an additional precolumn florescence labelling reaction for analyte detection. Srinubabu et al, 2007 [36] developed a high throughput liquid chromatography-tandem mass spectrometric method (HTLC-MS/MS) for simultaneous quantification of LRT and DES in human plasma. Wen et al, 2009 [37] proposed a LCMS/MS method for pharmacokinetics of RUP and DES in human plasma. Although the LC-MS technique is more sensitive compare with other analytical methods, MS is more expensive and requires sophisticated procedures than HPLC method. Emara et al, 2007 [38] developed a liquid chromatographic method for simultaneous determination of HYD, TER, CTZ and FEX respectively in human serum.

The method used two different wavelength ranges for peak identification, and envisages analyte separation as the only goal, not considering analyte retentivity and time of analysis as a major optimization criterion for analytical method development. Previously mentioned methods are not multi-purposeful since, it quantifies only two analytes under study. In addition, analyses of similar class drugs (antihistamines) involving different chromatographic methods consume organic solvents and reagents and demand more man power and time. To the best of authors' knowledge, no such method providing scope for simultaneous quantification of cited antihistaminic drugs in one chromatographic system and in such a shortest time has been described before.

As there is no reported method using experimental design technique, it was envisaged to develop, optimize and validate a new HPLC method to identify and quantify cited antihistaminic agents in pharmaceutical formulations and specified biological matrices by applying D-optimal mixture design methodology. D-optimal mixture design is

the most appropriate methodology where, the factors are the proportions of individual solvent in a mobile phase system [39]. This means that mixture factors are expressed as the fraction of total amount of their experimental ranges. Based on the initial screening studies, the levels of component of mobile phase were selected and subjected to mixture design experiments. Statistical study was performed to study the effect of mobile phase compositions on response of interest and finalizing the method condition. The proposed method was validated for linearity, accuracy, precision, limit of quantification, and robustness according to ICH Q2 (R1) guidelines [40]. The method successfully applied for the quantitative analysis of seven commercially available tablet dosage forms.

This is the first report where, D-optimal mixture design has been applied to optimize mobile phase composition to separate seven antihistamines simultaneously. The proposed method offers flexibility in customizing k value of the first peak, thus avoiding co-elution of the analyte along with the initial noises viz, solvent front and placebo excipients. This method show important advantages such as minimum experimental runs, high separation efficiency, short analysis time and fast method development.

MATERIAL AND METHODS

Instrumentation

The HPLC method development and validation was performed on Shimadzu HPLC (Shimadzu Corporation, Kyoto, Japan). The system consisted of two LC 20 AD solvent delivery modules, a SPD-M 20A PDA detector and a Rheodyne injector (model 7125, USA) valve fitted with a 20-µL loop. The system was controlled through a system controller (SCL-10A) and chromatographic data were collected and processed using LC Solutions® software (version 1.11SP1). Absorbance spectra were recorded using an UV-Visible spectrophotometer (Model UV-1601PC; Japan) using quartz cell of 1.00 cm path length. The mobile phase was degassed using Branson sonicator (Branson Ultrasonics, USA). Separation was performed on Phenomenex cyano analytical column (150 mm x 4.6 mm i.d, 5 µm) connected with a Phenomenex cyano guard cadridge (4 mm x 3 mm i.d, 5 µm) (Phenomenex®, USA).

Chromatographic condition

The chromatographic separation was carried out by using a mobile phase consisting of a mixture of ACN: MeOH: NH4OAc buffer. The pH was adjusted by using acetic acid. Prior to use, the mobile phase was degassed for 10 min in an ultrasonic bath and vacuum filtered through 0.45-µm membrane filter (Gelman Science, India). The mobile phase was prepared according to the plan defined by mixture design experiment. The analytes were detected at 222nm based on isobestic point. The HPLC system was used in an air-conditioned laboratory atmosphere (20± 2ºC).

Software tools

The D-optimal mixture design was performed by using Design expert®, 8.0 version (Stat-Ease, MN, USA). The rest of the calculations were computed using Microsoft Excel 2010 software (Microsoft, USA).

Chemicals and reagents

Working standards of HYD, LRT, RUP, DES, MEC, FEX, and TER (IS) was procured from Yarrow Chemical Ltd, Mumbai, India. CTZ was kindly donated by M/S. Sunglow Pharma, Puducherry, India. Acetonitrile (ACN) and methanol (MeOH) were of HPLC grade and purchased from M/S SD Fine chemicals, Mumbai, India. Ammonium acetate (NH4OAc) analytical grade (AR) was procured from Fischer Chemic Ltd, Chennai, India. Acetic acid (AA) was of analytical grade purchased from Spectrochem, Mumbai, India. High purity HPLC water was prepared by passing through a Millipore Milli-Q plus system (Millipore, Bangalore, India) and was used to prepare buffer solutions. The tablet dosage forms of Atarax (HYD-25mg), Lorfast Mel (LRT-10 mg), Allegra (FEX-120 mg), Deslor (DES-5mg), Okacet (CTZ 10 mg), Dilzan (MEC-25mg) and Rupanex (RUP-10mg) were procured from local pharmacy shop and evaluated for the amount present in the formulation.

Preparation of the standard solution

A stock solution (1 mg mL-1) of HYD, LRT, FEX, DES, CTZ, MEC and RUP was prepared separately by dissolving 10 mg of each in a 10-mL volumetric flask using MeOH as diluent. From this stock solution, a mixture of working standard solution of 10 µgmL-1 strength was prepared by transferring 100µL of each stock solution in to a 10 mL volumetric flask using mobile phase as diluents. This mixture was considered as 100% solution. These stock solutions were stored at approximately 5ºC and were found to be stable for several weeks.

Preparation of the sample solution for the assay of tablets

The developed HPLC method was used for the determination of selected drugs in pharmaceutical formulations. Twenty tablets of each were weighed and powdered separately. An amount of powder equivalent to one tablet was accurately measured and transferred separately into each 10-mL volumetric flask containing 5 mL of the diluent (MeOH). Then the resulted solution of each was sonicated for 15 min to ensure complete solubility of the drugs. Finally, the volume was adjusted up to 10 mL with the same diluent. Further dilutions were made to obtain a assay solution containing HYD, LRT, FEX, DES, CTZ, MEC, RUP and TER (IS) as 25, 10, 12, 5, 10, 25, 10 and 5 µg mL-1 respectively. The resulted solution was centrifuged at 4000 rpm for 10 min; the clear supernatant was collected and filtered through a 0.2 µm membrane filter (Gelman Science, India) and 20µL of this solution was injected for HPLC analysis.

METHOD VALIDATION

The proposed liquid chromatographic method for estimation of selected antihistamines was validated by following ICH Q2(R1) guidelines [41]. Validation parameters like selectivity, specificity, linearity, limit of detection and quantification, accuracy, precision, stability, and robustness were addressed.

Selectivity

The quality control selectivity of the proposed method was assessed by comparing the chromatograms of placebo sample containing a mixture of the commonly used excipients with that of selected analytes [41].

Linearity

The linearity was established by analyzing five working solutions of LRT (2-10µg mL-1), FEX (5-25 µg mL-1), DES (2-10µg mL-1), LCT (2-10µg mL-1), PRZ (4-20 µg mL-1) and CIN (5-50µg mL-1) corresponding to 20–200 % of expected test conc.entration for quality control of HYD, LRT, FEX, DES, CTZ, MEC, RUP and TER (IS). Calibration curves were plotted by using recorded peak area ratios of analytes vs. Corresponding drug conc.entration with least squares linear regression analysis. The method was considered linear; if the coefficient of determination values were equal to or more than 0.99 [41].

Accuracy and recovery

The accuracy of the method was tested at three conc.entration levels of 80, 100 and 120 % of the expected assay value of the marketed formulation. QC samples were prepared as three replicates at each conc.entration level by spiking the standard drugs with the placebo excipients, which were left overnight to allow matrix-analyte interactions to occur, and then analyzed as described in "Preparation of Sample Solution" section. The % recovery of HYD, LRT, FEX, DES, CTZ, MEC, and RUP (n = 3) and mean % recovery (n = 9) were determined [41].

Precision

The precision was established by injecting three different conc.entrations of each analyte with IS, each in six replicates for intraday precision (repeatability) and on three consecutive days for the intermediate precision. Precision was expressed by %RSD of the analyte peak area [41].

Limit of detection and quantification

The LOD was determined based on signal to noise (S/N) ratio using analytical response of three times of the background noise. Calibration curves were plotted at five levels ranging from 0.05 to 1.0% of the nominal analyte conc.entration. The residual standard deviation of the response (σ) and slope (s) of the calibration curve was used to calculate the LOD as 3.3 σ/s and LOQ as 10 σ/s [41].

Robustness

The robustness of the proposed method was evaluated by D-optimal mixture design experiment and obtained data was used to establish robust domains with in experimental domain. In the present study, the effect of the percent mobile phase components (i.e, volume percentage of % ACN, MeOH and buffer, pH, and flow rate were selected. As response variables, viz. retention factor (k1), resolution between critical peaks (Rs1,2 and Rs5,6), and run time (tR8) were selected [41].

RESULTS AND DISCUSSION

Method development and optimization

Selection of column

Prior to the method development and optimization, a set of preliminary experiments were conducted to select the suitable stationary phase for the separation of HYD, LRT, FEX, DES, CTZ, MEC, RUP and TER (IS). The selectivity of the analytes was tested on Phenomenex monolithic C18 (100 mm x 4.6 mm id.), Phenomenex phenyl-hexyl (150 mm x 4.6 mm id, 5µm), and Phenomenex cyano (150 mm x 4.6 mm i.e. 5µm) analytical columns by using mixture of different ratios of water, MeOH and ACN as mobile phase. The experiment on C18 resulted was poor retention and lower selectivity of analytes under study. The phenyl moiety of the phenyl-hexyl phase may offer selectivity by interacting with phenyl ring of the analyte through π–π interactions, while the C6 chain present in phenyl-hexyl phase which offers classical reversed phase retention of analytes [42]. However, the experiment on phenyl-hexyl phase, baseline separated for HYD and LRT but failed to resolve TER, CTZ, FEX, DES and RUP peaks. The cyano column could separate HYD, LRT and CTZ but co-elution of peaks, poor peak shape and inadequate separations were observed for other selected analytes. The experiments on cyano column offered moderate analytes selectivity and that could separate HYD, LRT and CTZ peaks. Hence, cyano column was selected for further screening and optimization.

Effect of mobile phase pH

It is well known that the buffer type and pH are the key factors when multiple compounds to be analyzed. In the present study, effect of mobile phase pH range (3-5) was investigated by using ammonium acetate (NH4OAc) buffer and acetic acid (AA) as a pH modifier. NH4OAc an organic buffer was selected since, suitability with the selected pH range. The feasibility of ternary solvent mixtures containing ACN, MeOH and buffer with mobile phase pH range (3 to 5 adjusted with AA) was tested on selected cyano column. The separation of analytes was observed at pH 3.8. Hence, pH was fixed. However, critical peak pair Rs1,2 (HYD and LRT) and Rs5,6 (DES and CTZ) were observed. Hence, cyano column was further explored for optimization.

D-optimal mixture design optimization

The goal of mixture design in the preset study was to optimize the response of interest with respect to the proportions of the components, where optimization entails minimizing, maximizing, or targeting a value of the response of interest. In the present study, a D-optimal mixture design experiments was used to study the influence of changes in conc.entration of mobile phase compositions viz, ACN (A), MeOH (B) and buffer (C) on dependent variables and optimization of the response of interest. The mobile phase system containing ACN (A), MeOH (B) and buffer (C) that constituted to total 100% v/v/v was selected to establish an optimum condition in which all analytes separate from each other in a short analysis time.

In mixture experiments, the factors are the components of a mobile phase so their levels are not independent. This means that mixture factors are expressed as the fraction of total amount of their experimental ranges. Based on the preliminary studies, the levels of mobile phase components employed in the optimization where, volume percentage of ACN (16-24% v/v), MeOH (34-46% v/v) and NH4OAc buffer (34-46% v/v). In mixture design experiments, the sum of the mobile phase components viz, ACN, MeOH and buffer made equal to 100 % v/v/v. The pH of mobile phase was kept constant at 3.8 (adjusted with acetic acid) and mobile phase was delivered at 1.5 mL min-1. Terfenadine (TER) (Fig. 1) was used as an internal standard (IS) as it presented acceptable resolution and retention time with all these analytes. Analytes peaks were detected at 222nm. To judge the quality of the method under different experimental conditions, the following responses of interest were defined (i) retention factor of the first eluted peak HYD (k1), (ii) resolution between critical peak pair HYD, LRT (Rs1,2), and DES, CTZ (Rs5,6) and (iii) run time the method (tR8).

A total 14 runs (center, vertex, the edge centers, and axial check blends) obtained from the D-optimal mixture design were subjected to experiment to generate a response variables. The experiments were performed and results were summarized in Table 1.

Table-1: Experimental design matrix representing mobile phase composition and observed responses

Run	Type	ACN (A)	MeOH (B)	Buffer (C)	k_1	$Rs_{1,2}$	$Rs_{5,6}$	tR_8
1	Center	20	40	40	0.833	0.000	0.718	8.436
2	Cent Edge	16	42	42	0.929	1.102	0.431	9.201
3	Interior	21	37	42	0.877	0.618	0.000	9.038
4	Vertex	20	46	34	0.514	1.051	0.724	7.508
5	Vertex	20	34	46	1.019	2.044	1.579	10.191
6	Cent Edge	22	34	44	0.927	1.218	0.377	9.242
7	Vertex	20	46	34	0.517	1.049	0.722	7.506
8	Cent Edge	24	38	38	0.649	0.000	1.415	7.408
9	Vertex	16	46	38	0.773	0.000	1.323	8.483
10	Center	20	40	40	0.829	0.000	0.702	8.429
11	Vertex	24	42	34	0.481	1.267	1.081	7.551
12	AxialCB	20	37	43	0.942	0.985	0.000	9.343
13	Vertex	16	46	38	0.771	0.000	1.322	8.481
14	Cent Edge	18	36	46	1.089	2.029	1.851	10.451

The results for retention factor of first eluted peak of LRT (k1) were appeared to be significant improvements when decreasing the conc.entration of MeOH in the mobile phase. The improvement of resolution of critical peak pairs viz, (LRT and HYD) Rs1,2 and (DES and CTZ) Rs5,6 was observed in runs 5 and 14. This might be the effect of conc.entration of MeOH and buffer in the mobile phase. It was noted that conc.entration of organic polar solvent like MeOH and aqueous buffer have shown complementary effect on run time (retention time of last eluted peak= tR8) of the method.

Data interpretation

Statistical study was performed by using ANOVA. For all the reduced models, P value of <0.05 were obtained, implying these models are significant. For an experimental design a linear model equation generated by the design is portrayed below.

$$Y = \beta0 + \beta1X1 + \beta2X2 + \beta3X3 + \beta4X1\ X2 + \beta5X2\ X3 + \beta6X2\ X3 + \beta7X1\ X2\ X3$$

Where, Y is the response to be model, β is the regression coefficient and X1, X2 and X3 represents factors A (%ACN), B (%MeOH) and C (%buffer) respectively. The equation indicates coefficients (β0 to β7) of various model terms including the two factor and three factor interaction terms. It helps to analyze the effect of positive and/or negative interactions among the factors selected for the development of the HPLC method. A linear model equations generated by ANOVA are depicted below.

$$k_1 = + 0.31A + 0.56B + 1.25C + 0.13AB + 0.087AC + 0.16BC + 0.71ABC$$

$$Rs_{1,2} = - 1.30A – 0.51B + 4.09C + 9.04AB – 3.37AC – 3.18BC - 26.62ABC$$

$$Rs_{5,6} = + 13.21A + 4.54B + 6.38C – 31.39AB – 36.73AC -19.79BC + 58.71ABC$$

$$tR_8 = + 3.73A + 6.93B + 9.99C + 7.96AB +7.93AC + 3.58BC – 22.64ABC$$

From the above equations, factors with a positive sign indicated a positive effect on the selected responses. Positive interaction terms indicated the combined effect of factors on response variables. The individual components of the mixture viz, ACN (A), MeOH (B) and buffer (C), all interaction terms viz, AB, AC, and BC as well as the combined effect of all three component ABC have shown positive effect on retention factor of the first eluted peak (k1). It was

observed that the fraction of buffer (C) and interaction between ACN and MeOH (AB), ACN and buffer (AC) and MeOH and buffer (BC) have shown to have positive effect on response Rs1,2. As in the case of response Rs5,6, the individual components of the mixture viz, A, B and C as well as interaction between three factors A, B and C were exhibit positive effect. However, the combined effect of all three components of the mixture ABC was positive for response Rs5,6. The fraction of mixture components and interaction between two factors AB, AC, and BC were shown positive effect on run time of the last eluted peak (tR8). Trace plots (Fig. 2a-d) were presented to illustrate how response changes with variations in the proportion of each component of the mobile phase while keeping all the others constant at reference point. Fig.2a and b indicated that the increment in proportion of buffer conc.entration (v/v) significantly increased the retention factor of the first eluted peak k1 and resolution of Rs1,2. From Fig. 2c it was noticed that increasing the conc.entration of buffer, ACN, and MeOH in the mobile phase has shown synergistic effect on resolution Rs5,6. It was observed from Fig. 2d that increasing the proportions of organic solvents viz, MeOH and ACN in the mobile phase significantly reduces the run time of the method (tR8).

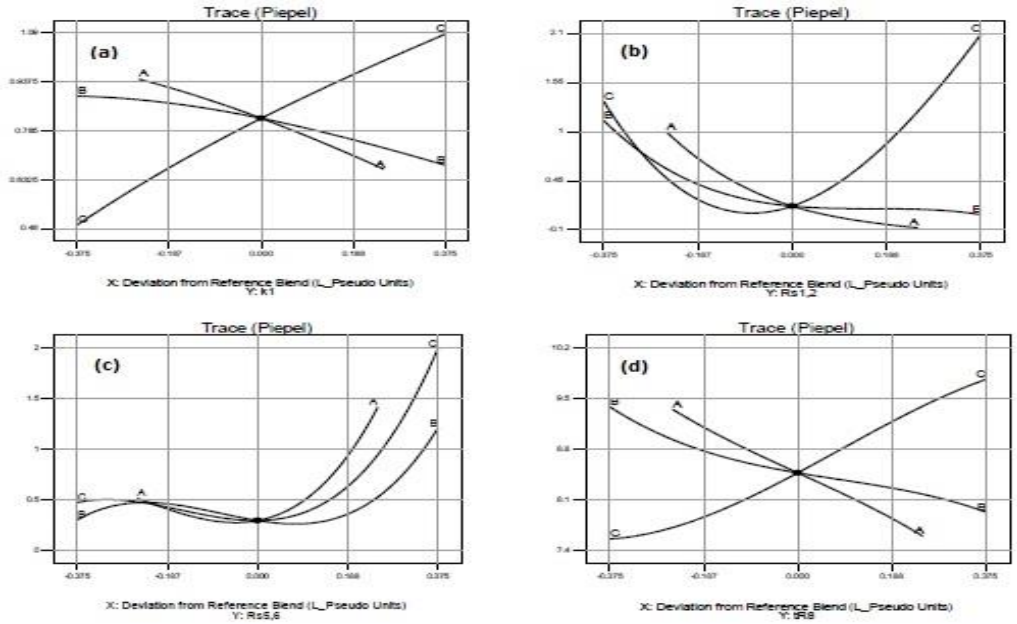

Figure-2: Trace plots of the response (a) retention factor k1, (b) and (c) resolution between Rs1,2 and Rs5,6 respectively and (d) retention time of tR8 with respect variations in the proportion of each component of the mobile phase viz, ACN, MeOH and buffer.

In the present study, a HPLC method developed intended for routine quality control of selected drugs in bulk and their pharmaceutical formulation. Therefore, it is essential to customize the retention factor of the first eluted peak (k1) thus avoiding co-elution of the analyte along with initial noises viz, solvent front and placebo excipients. Since, the selected responses were not affected in the same manner with the changes in experimental parameters, it was necessary to arrive a best possible combination of adequate retention of the first eluted peak of HYD (k1), adequate separation of critical peaks (Rs1,2 and Rs5,6) in less runtime tR8<10 min. Overlay plot of the design was explored to identify an optimal chromatographic condition of the method [43]. Overlay plots are projection of contour plots onto one figure. From this, a region with acceptable criterion values was identified and marked. From overlay plot (Fig. 3) the mobile phase composition containing ACN/MeOH/buffer at 18/36/46 v/v/v respectively was selected as an ideal condition for regular analysis. The experiments were carried out under optimal condition and respective chromatograms were shown in Fig. 4. The optimized condition gave an adequate resolution between critical peak pairs HYD, LRT (Rs1,2), and DES, CTZ (Rs5,6) within a less analysis time (9.5 min).

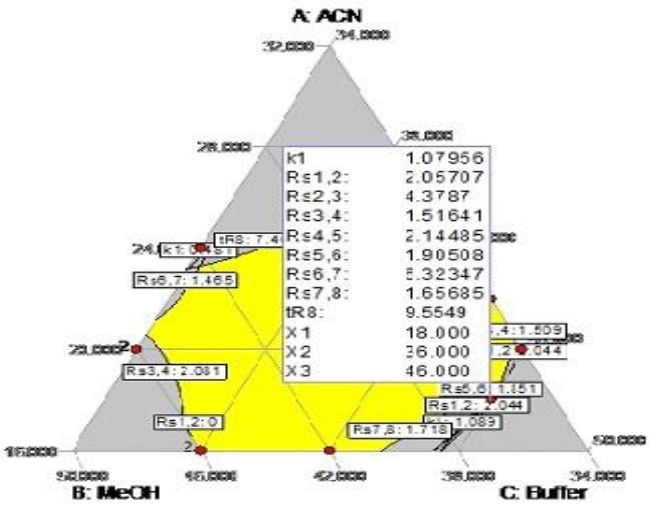

Figure-3: Overlay plot of the experimental design showing the region of optimum mobile phase composition.

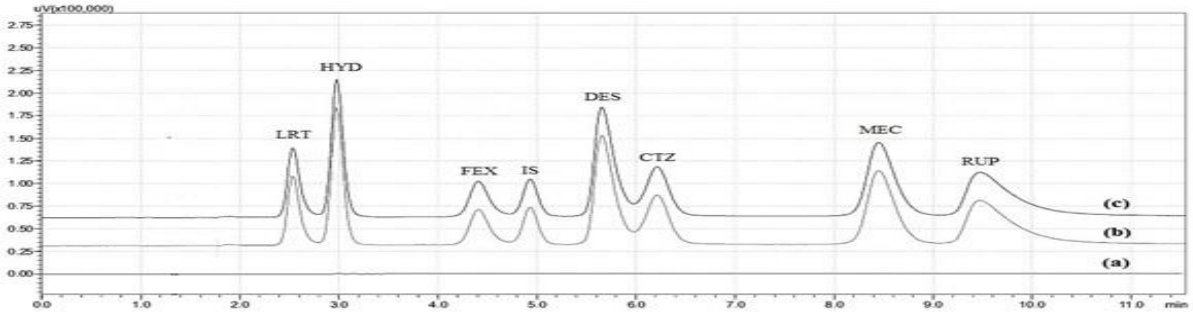

Figure-4: Representative overlaid chromatograms corresponding to (a) placebo solution (b) laboratory made mixtures and (c) marketed tablet dosage forms as per elution order: loratadine (LRT), hydroxyzine (HYD), fexofenadine (FEX), terfenadine (IS), desloratadine (DES), cetirizine (CTZ), meclizine (MEC) and rupatadine (RUP) under optimum condition.

Validation of formulation assay method

The selectivity of the method was assessed by placebo sample containing a mixture of the commonly used excipients (starch, lactose monohydrate, hydroxypropyl methyl cellulose, titanium dioxide and magnesium stearate) with that of selected analytes; no interfering peaks were noticed in the chromatogram (Fig. 4a). Calibration curves were plotted using peak area ratios of all the analytes. The obtained regression equations are summarized in Table 2. The obtained correlation coefficients were found to be > 0.9 that indicated high linearity over the entire conc.entration range. The % recovery (n = 3) and mean % recovery (n = 9) were determined, and data are presented in Table 2. The recoveries of analytes at each level were found to lie within the acceptable criteria of the bias 2 %. The method precision was evaluated by injecting six replicates at three conc.entration levels for intra- and inter-day precision and the results were expressed as % RSD. The results are summarized in Table 2. Limit of detection (LOD) and limit of quantification (LOQ) values for HYD, LRT, FEX, DES, CTZ, MEC and RUP were estimated by plotting calibration curves at five levels ranging from 0.05 to 1.0 % of the nominal conc.entration, and the values are presented in Table 2. The stock and the sample solutions were stable throughout the period of study (30 days). No significant degradation was found within the period of evaluation, indicating that solutions are stable. Peak areas of all the analytes were almost identical to that obtained during initially prepared solutions and additional peaks were not observed. The D-optimal mixture design experimental results were used to verify the robustness of the method. The variations in percent mobile phase components i.e, %ACN (18%±1.5), MeOH (36%±1.5) and buffer (46%±1.5), flow rate (1.45-1.55 mL min-1) and pH (3.8±0.02) were did not alter the retention factor, resolution, and analysis time values more than 2% demonstrating the robustness of the method.

Application to formulation

To assess the applicability of the developed method for intended purpose, an attempt was made to determine content of HYD, LRT, FEX, DES, CTZ, MEC and RUP in commercially available tablets. For assay, the sample solution was prepared as described under section "Preparation of sample solution". The assay results are summarized in Table 2. From Table 2, the recoveries of cited antihistamines from tablets was high (% recovery), consistent (low %RSD) and agreed with the label claim of corresponding marketed formulations. The obtained results demonstrate high accuracy and reproducibility of the proposed method. A typical chromatogram of tablet analysis is shown in fig. 4(c).

Table 2. Summary validation report for formulation assay condition

Parameters	**HYD**	**LRT**	**FEX**	**DES**	**CTZ**	**MEC**	**RUP**
Linearity	2-10µg mL^{-1}	2-10µg mL^{-1}	5-25 µg mL^{-1}	2-10µg mL^{-1}	2-10µg mL^{-1}	4-20 µg mL^{-1}	5-50µg mL^{-1}
Results	$y = 0.029x + 0.003$	$y = 0.088x + 0.032$	$y = 0.053x + 0.041$	$y = 0.102x + 0.022$	$y = 0.054x + 0.019$	$y = 0.051x + 0.021$	$y = 0.029x + 0.003$
r^2	0.999	0.992	0.995	0.994	0.995	0.998	0.999
LOD	2.57 $ngmL^{-1}$	1.51 $ngmL^{-1}$	5.32 $ngmL^{-1}$	8.51 $ngmL^{-1}$	1.77 $ngmL^{-1}$	4.04 $ngmL^{-1}$	5.57 $ngmL^{-1}$
LOQ	8.28 $ngmL^{-1}$	4.60 $ngmL^{-1}$	16.05 $ngmL^{-1}$	25.81 $ngmL^{-1}$	5.37 $ngmL^{-1}$	12.24 $ngmL^{-1}$	16.90 $ngmL^{-1}$
Specificity	The method is specific with respect to tablets ingredients						
Accuracy	**Mean Recovery± SD (%), (n = 3)**						
at 80%	102.35±0.04	101.02±0.16	100.37±0.58	101.41±0.28	100.60±0.09	102.29±0.07	102.35±0.04
at 100%	99.83±0.03	99.85±0.97	101.00±0.01	100.30±0.19	101.47±0.13	99.82±0.02	99.83±0.03
at 120%	99.02±0.04	99.67±0.55	98.61±0.39	98.99±0.29	98.68±0.05	99.02±0.04	99.02±0.04
Mean recovery ± SD (%), (n = 9)							
	100.40±1.50	100.18±0.85	99.99±1.12	100.23±1.07	100.25±1.23	100.37±1.47	100.40±1.50
Precision	**n=6**						

a. Intraday	Conc.	%RSD	Conc.	%RSD	Conc.	%RSD	Conc.	%RSD	Conc.	%RSD	Conc.	%RSD	Conc.	%RSD
	2µg	0.95	2µg	2.44	5µg	2.65	2µg	2.80	2µg	1.11	4µg	2.31	5µg	0.95
	5µg	1.02	6µg	1.28	15µg	2.49	6µg	1.30	6µg	1.04	12µg	1.18	25µg	1.02
	10µg	0.82	10 µg	1.78	25µg	2.14	10µg	1.30	10 µg	1.23	20µg	2.10	50µg	0.82
b. Inter day	Conc.	%RSD	Conc..	%RSD	Conc.	%RSD	Conc.	%RSD	Conc.	%RSD	Conc.	%RSD	Conc.	%RSD
	2µg	0.84	2µg	2.48	5µg	2.44	2µg	2.78	2µg	1.10	4µg	2.24	5µg	0.84
	5µg	1.32	6µg	1.32	15µg	2.32	6µg	1.31	6µg	1.02	12µg	1.21	25µg	1.32
	10µg	0.96	10 µg	1.82	25µg	2.12	10µg	1.34	10 µg	1.24	20µg	2.08	50µg	0.96

Assay data for tablet dosage forms							
Brand	Atarax	Lorfast Meltabs	Allegra	Deslor	Okacet	Dilzan	Rupanex
Label claim	25 mg	5 mg	120 mg	5 mg	10 mg	25 mg	10 mg
Found	24.55±0.06	4.99±0.02	119.4±0.01	4.97±0.02	9.96±0.02	24.98±0.02	9.92±0.04
%RSD	1.25	1.48	1.14	1.30	1.06	1.18	1.03

Advantages of the proposed method

This proposed chromatographic system is a new approach to simultaneous determination of basic hydrophobic HYD and zwitterionic CTZ in bulk and their pharmaceutical formulations. The analysis of hydrophobic basic analytes viz, LRT and DES is usually challenging because of the tendency of peak tailing and poor resolution of these analytes. The separation of these studied analytes is achieved in the same chromatographic system. In the present study, a HPLC method developed intended for routine quality control where the analysis time need to be optimized without losing resolution. Furthermore, it is essential to customize the retention factor of the first eluted peak (k1) thus avoiding initial noises viz, solvent front and placebo excipients. The D-optimal mixture design methodology in this study aided in establishing the optimum mobile phase composition for successful quality separation of analytes. The proposed method can be extended for screening parent antihistaminic molecules along with their respective active metabolite without changing chromatographic conditions. For instance, HYD and CTZ, LRT and DES, and RUP and DES were analyzed in a run time of 6.5, 5.8, and 9.5 min respectively in a single chromatographic system was hopefully increases the working efficiency of analyst. The proposed method reduces overall 30 different methods into one single method. In addition, the organic buffer system may favors for LC-MS studies.

CONCLUSION

A simple, isocratic, rapid, accurate, and precise HPLC method has been developed and optimized utilizing D-optimal mixture design methodology for the simultaneous separation of seven antihistaminic drugs. The D-optimal mixture design methodology was used effectively for the optimization of mobile phase composition for the separation of analytes under study. This method show important advantages such as minimum experimental runs, high separation efficiency, short analysis time (9.5 min) and fast method development. The validation study supported the selection of the assay conditions by confirming that the assay was accurate, linear, precise, and robust. Higher sensitivity, adequate analyte retention, better resolution and shorter analysis time of the present method demonstrated that it can be employed for routine quality control purposes.

ACKNOWLEDGEMENTS

The author is grateful to UGC-SAP DRS Phase-II sponsored Department of Pharmacy, Annamalai University, Tamil Nadu, India, UGC Major research project [MRP-MAJOR-BIOT-2013-39967] for providing the facilities to carry this research work and for the financial assistance through UGC-BSR fellowship 55.

REFERENCES

1. Baroody FM, Naclerio RM, *Allergy*, **2000**, 55, 17-27.
2. Simon FER, Simons KJ, *World Allergy Organ J*, **2008**, 1, 145–155.
3. Obach RS, *Pharmacol Rev*, **2013**, 65, 578-640.
4. Rao MS, et al, *Indian J Otolaryngol Head Neck Surg*, **2009**, 61, 320-332.
5. Camelo-Nunes IC, *J Pediatr (Rio J)*, **2006**, 82, S173-180.
6. Al-Abdullah Al-Kafri N, AL-Mardini MA, *Int. J. Pharm. Sci. Rev. Res*, **2013**, 21, 138-142.
7. Bizikova P, Papich MG, Olivry T, *Vet Dermatol*, **2008**, 19, 348-357.
8. Agrawal DK, *Expert Opin Investig Drugs*, **2001**, 10, 547-560.
9. Peña J, Carbo ML, Solans A, Nadal T, Izquierdo I, Merlos M, *Eur J Drug Metab Pharmacokinet*, **2008**, 33, 107-116.
10. Péhourcq F, *J Pharmacol Toxicol Methods*, **2004**, 50, 41-44.
11. Briône W, Brekelmans M, *J Pharm Biomed Anal*, **2015**, 115, 69-73.
12. Kintz P, Godelar B, Mangin P, *Forensic Sci Int*, **1990**, 48, 139-143.

13. Javanbakht M, Fard SE, Mohammadi A, Abdouss M, *Anal Chim Acta*, **2008**, 612, 65-74.
14. Chen J, Zha YZ, Gao KP, *Pharmazie*, **2004**, 59, 600-603.
15. Kunicki PK, *J Chromatogr B Biomed Sci Appl*, **2001**, 755, 331-335.
16. Xu XJ, *Yao Xue Xue Bao*, **2004**, 39, 123-126.
17. Salem II, *J Pharm Biomed Anal*, **2004**, 34, 141-151.
18. Tian Y, et al, *J Pharm Biomed Anal*, **2008**, 47, 899-906.
19. Ren XL, et al. *Arzneimittelforschung*, **2011**, 61,287- 295.
20. Rosseel MT, Lefebvre RA, *Chromatogr*, **1991**, 565, 504-510.
21. Kim CK, *Pharm Biomed Anal*, **2005**, 37, 603-609.
22. Zaater MF, *J Pharm Biomed Anal*, **2000**, 22, 739-744.
23. Lu XY, *Zhejiang Da Xue Xue Bao Yi Xue Ban*, **2005**, 372-374.
24. Liu L, et al. *J Pharm Biomed Anal*, **2004**, 34,1013-1019.
25. El-Enany N, *Chem Pharm Bull (Tokyo)*, **2007**, 55, 1662-1670.
26. Wang Z, et al. *J Chromatogr B Analyt Technol Biomed Life Sci*, **2011**, 879, 95-99.
27. Peraman R, et al. *J Chromatogr Sci*, **2015**, 53, 793-799.
28. Hofmann U, Fromm, *Chromatogr B Analyt Technol Biomed Life Sci*, **2002**, 766, 227-233.
29. Fu I, et al. Matuszewski, *J Pharm Biomed Anal*, **2004**, 35, 837-846.
30. Tanaka Y, Yoshikawa Y, *Biol Pharm Bull*, **2012**, 35, 698-704.
31. Uno T, *J Pharm Biomed Anal*, **2004**, 35, 937-942.
32. Nirogi RV, *Biomed Chromatogr*, **2007**, 21, 209-216.
33. Arayne MS, *Med Chem Res*, **2011**, 20, 55-61.
34. Helmy SA, El Bedaiwy HM, *Biomed Chromatogr*, **2016**, 30, 1059-1064.
35. Hammad SF, et al. *Biomed Chromatogr*, **2007**, 21, 1030-1035.
36. Srinubabu G, et al. *J Chromatogr B Analyt Technol Biomed Life Sci*, **2007**, 860, 202-208.
37. Wen J, et al. *J Pharm Biomed Anal*, **2009**, 49, 347-353..
38. Emara S, et al. *J AOAC Int*, **2007**, 90, 384-390.
39. Hibbert DB, *J. Chromatogr. B Analyt*, **2012**, 910, 2-13.
40. Jason A, Advanced Topics in Experimental Design, Statistics.
41. ICH, Validation of analytical procedures, proceedings of the International Conference on Harmonisation, Geneva, 2005.
42. Kayillo S, *J. Chromatogr. A*, **2006**, 1126, 283-297.
43. Chiguru V, *Anal. Methods*, **2014**, 6, 4610-4616.