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# Simultaneous determination of pseudoephedrine and loratadine in syrups by HPLC using Cation exchange column and experimental design optimization

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**Abstract**
A simple and efficient liquid chromatographic method has been developed and validated for the simultaneous determination of pseudoephedrine (PS) and loratadine (LOR) in syrup dosage form. The separation was achieved within 7.0 min, employing a mixture of 53.5% v/v water-methanol containing 30.12 mM/L sodium dihydrogen orthophosphate adjusted to pH 5.22 as isocratic mobile phase, pumped at 1.0 ml/min through a strong cation exchange column (10µm particle size). The analytes were detected at 254 nm. Statistical experimental designs and graphic representations (response surface methodologies, Pareto charts) were used for optimizing the mobile phase composition. The linearity of the calibration ($r>0.99$, $n=15$) in the relevant ranges (up to 125% of the expected concentrations of the analytes in the formulation), method accuracy (RSD <2.0%), repeatability (RSD <2.0%) and intermediate precision, were verified. System suitability parameters were also determined. The validated method was successfully employed for the routine analysis of a syrup pharmaceutical preparation.

**Keywords:** Pseudoephedrine, loratadine, experimental design, HPLC, cation exchange column

## 1. Introduction
Loratadine (LOR) is ethyl 4-(8-chloro-5,6-dihydro-11-H-benzo-[5,6]cyclohepta[1,2-b] pyridin-11-ylidene)-1-piperidine-carboxylate (Figure 1). It is a long-acting, non-sedative second generation $H_1$ receptor blocker with no significant antimuscarinic activity. It is used for the symptomatic relief of allergic conditions including rhinitis and chronic urticaria [1].

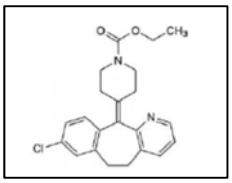

**Fig 1:** Chemical Structure of Loratadine

Pseudoephedrine (PS) is (1S, 2S)-2-(Methyl-amino)-1-phenylpropan-1-ol [1] (Figure 2). It is a direct and indirect sympathomimetic [1].

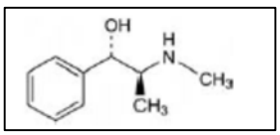

**Fig 2:** Chemical Structure of Pseudoephedrine

Pseudoephedrine and its salts are given orally for the symptomatic relief of nasal congestion. It is commonly combined with other ingredients in preparations intended for the relief of cough and cold symptoms.
Loratadine and pseudoephedrine sulfate are present together in dosage forms prescribed to relieve symptoms of allergic rhinitis [1]. Their commercial association exhibits important mass difference between the two analytes (PS: LOR up to 12:1, w/w),

moreover when presented as liquid formulations they require the addition of preservatives such as methylparaben (MP), prorylparaben (PP), or sodium benzoate (SB). All these aspects increase the analytical challenge.

The difference in terms of the intrinsic polarity between pseudoephedrine and loratadine is obvious, pseudoephedrine is characterized by a greater polarity (LogP octanol/water, =0.9) while loratadine is highly apolar (LogP octanol/water =5.20) [2]. This great difference in polarity between the two compounds offers an interesting analytical challenge.

The combination of LOR and PS in syrup formulation is not official in any pharmacoepia and it is not commonly used world-wide, accordingly there was no method developed so far for their determination in liquid formulations, the available reversed phase chromatographic methods were developed for the determination of the two analytes in tablets [3,4], however these methods indicated some difficulties to obtain optimum separation between the two analytes based on the fact that PS is hydrophilic while LOR is hydrophobic and hence under reversed phase conditions PSE tend elute with the solvent front indicating insignificant retention and possible interference with the formulation additives at the expense of preventing late elution of LOR. To resolve this problem and achieve optimum separation between the two analytes in tablets formulation an expensive approach which at the same time not suitable for routine application was developed where a $C_{18}$ and cyanopropyl column were used in series [5]. Other techniques including first derivative spectroscopy [3], multi-wavelength spectroscopy [4], chemometric treatment of spectrophotometric data [6], analysis of non-linear second-order spectrophotometric data generated by a pH-gradient flow injection technique and artificial neutral networks [7], and high performance thin layer chromatography [8] were also used.

The use of strong cation exchanger (SCX) materials, such as propylsulfonic acid, which are highly acidic ($pK_a$ <1) and effectively ionizes at all pH values, makes the development of separations for basic compounds relatively straightforward, besides its other advantage related to its specificity for the analysis of basic compounds when present in mixture with acidic/neutral compounds as the latter are either not retained or poorly retained and also improved peaks shape of the basic analytes [9-11].

The objective of the present work was to develop and validate a high-performance liquid chromatographic methodology for the simultaneous determination of LOR and PS in their combined syrup formulations, using cation-exchange stationary phase. Experimental design techniques were employed for development and validation of the proposed method, as a rational, cost-effective and convenient tool to speed up the process.

## 2. Materials and Methods

### 2.1 Apparatus and Software

Experiments were performed on a Shimadzu Prominence HPLC system consisted of: degasser (Model DGU-20A5), pump (Model LC-20AD), Rheodyne manual injector fitted with 20 µl loop, variable wavelength UV–Vis detector (Model SPD-20A).

Experimental design, data analysis and desirability function calculations were performed by using Design-Expert® trial version7.0.0. (Stat-Ease Inc., Minneapolis). Statistical analysis was carried using Microsoft Excel 2013 software (Microsoft, USA).

### 2.2 Reagents and Materials

The following reagents: sodium dihydrogen orthophosphate (BDH, Poole, England), orthophosphoric acid (Sdfine Chem. Ltd., India), methanol HPLC grade (Scharlau Chemie, Spain), Sodium hydroxide pellets (Sdfine Chem. Ltd., India) and double-distilled water were used for the preparation of the samples and for chromatographic analysis.

Loratadine (99.5%) was obtained from (Blue Nile Pharmaceuticals, Khartoum North, Sudan) and pseudoephedrine Sulphate (98.8%), was obtained from (SPIMACO, Al-Qassim, Kingdom of Saudi Arabia). Lorinase Syrup (SPIMACO, Kingdom of Saudi Arabia) Labeled to contain: Pseudoephedrine sulphate 60 mg and Loratadine 5 mg per each 5 ml, was purchased from pharmacies in Kingdom of Saudi Arabia.

### 2.3 Preparation of Solutions

#### 2.3.1 Optimization Standard

Five ml of a 0.5 mg/ml propyl p-hydroxybenzoate (PP) standard solution in methanol were added to a 20 ml volumetric flask containing 45 mg pseudoephedrine sulphate and 5 mg loratadine and the final volume was made to mark with 50% v/v aqueous methanol.

#### 2.3.2 Standard stock solution

Accurately weighed about 50 mg pseudoephedrine sulphate and 7 mg loratadine standards were transferred into a 10 mL volumetric flask, 5 mL of 50% v/v aqueous methanol diluent were added. The content of the flask was sonicated for 5 minutes, allowed to cool and the volume was adjusted to the mark with the same diluent.

#### 2.3.3 Preparation of calibration curve

Aliquot volumes (0.2- 2.5 ml) of the standard stock solution were transferred into a series of 20 ml volumetric flasks, and volumes were adjusted to the mark with the previous diluent.

#### 2.3.4 Sample preparation

The sample density was determined and then sample weight accurately equivalent to 5 mL was taken in 100 mL volumetric flask, 10 mL of the diluent were added. The sample was sonicated for 5 minutes, cooled and the volume was completed to mark with the same diluent. The sample was then filtered through 0.45 µm nylon filter and injected into the HPLC system.

### 2.4 Chromatographic Procedure

Chromatographic separations were carried out on a Partisil 10 SCX column (10 µm particle size, 250 mm×4.6 mm I.D.).The mobile phase consisted of methanol-water-sodium dihydrogen orthophosphate, adjusted to the required pH with 1% orthophosphoric acid or 1M NaOH. The analytes were monitored at 254 nm.

### 2.5 Validation

The optimized method was validated in agreement with the ICH guideline [12]. Accordingly, method linearity in the relevant working ranges, precision, and accuracy were evaluated. System suitability parameters were also determined.

## 3. Results and Discussion

### 3.1 Optimization Design and Analysis

Before starting an optimization procedure, the curvature term

was investigated using factorial design with center points investigates. ANOVA generated for $2^k$ factorial design showed that curvature is significant for all the responses (the resolution between each pair of adjacent analytes peaks) since p-value was less than 0.05. This implies that quadratic should be considered to model the separation process [13]. In order to obtain second order predictive model Box-Behnken design was employed [14], the optimum composition of the mobile phase was determined with series of mobile phases containing water: methanol mixtrues in the range of (45-50% v/v) containing ammonium acetate (35 - 45 mM/L) with pH varied between 5-6.

Derringer's desirability function was used to optimize the separation since the process involves optimization of three responses at a time [15]. The resolutions between the adjacent peak pairs were considered as the response for the optimization purpose. The use of response surface methodologies (Figure 3), indicated that optimum mobile phase composition was 53.5% v/v water-methanol mixture having ionic strength 30.12 mM/L and pH 5.22.

Figure 4 shows representative chromatograms of the optimization standard, standard mixture, and real sample under the optimized conditions, where baseline separation of the relevant analytes was achieved within a reasonable time.

Under the optimized chromatographic conditions it was observed that LOR had eluted first followed by PS, this observation can be explained with reference to the analytes acid dissociation constants ($pk_a$) and the mobile phase pH. The low acid dissociation constant of LOR ($pk_a$ = 5.25 - a much weaker base as compared to PS) which

Was almost matching the pH of the mobile phase under the optimized conditions; suggests that it was not significantly

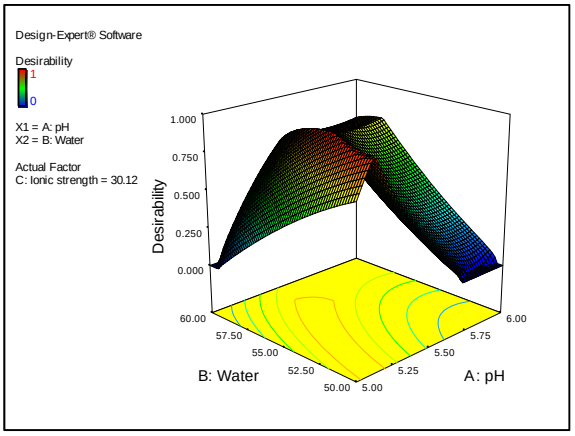


**Fig. 3:** Graphical representation of overall desirability function D (=1.0) were pH (A) of 5.22, percent water content (B) of 53.5, and ionic strength (C) of 30.12 mM

Ionized and retained in the column to a lesser extent followed by PS PSE ($pk_a$ = 9.8) which had undergone full ionization at this pH. This finding is consistent with the earlier observation [16], that if two bases are fully protonated then the stronger base is eluted before (has less affinity for the stationary phase) than the weaker.

Accordingly it is possible to predict the elution order of basic compounds in a mixture under cation exchange conditions through the knowdeldge of their $pk_a$ value, moreover this property can be used to adjust selectivity by proper selection of the mobile phase pH and manipulating its ionic strength and aqueous/organic ratio.

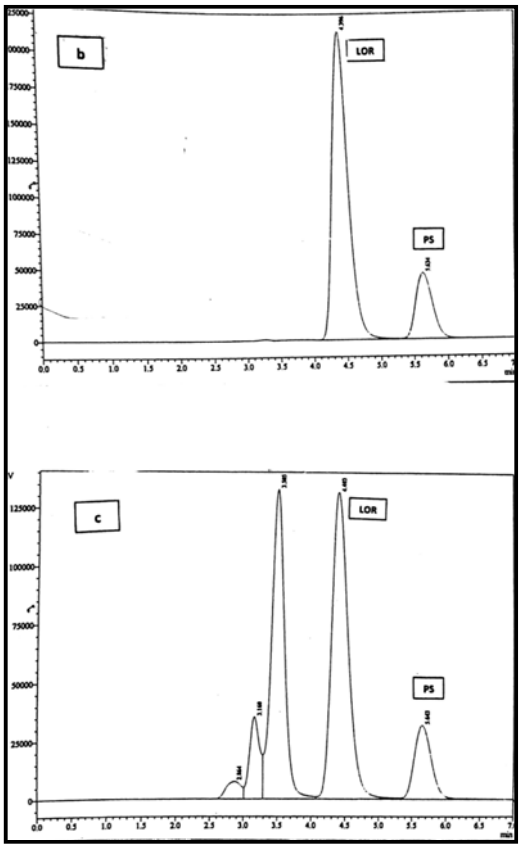


**Fig 4:** Chromatograms corresponding to b) standard Solution c) real sample of product B (Lorinase Syrup), under optimum condition (pH= 5.22, percent water content = 53.5 and ionic strength =30.12mM).

### 3.2 Method Validation

The optimized method was validated in agreement with the ICH guideline [12]. Accordingly, method linearity in the relevant working ranges, precision and accuracy were evaluated. System suitability was also determined.

#### 3.2.1 Range and Linearity

Method range and linearity were evaluated with six mixtures of standards at the following concentrations: 44.5 -555.7µg/ml and 7.4-92.5 µg/ml for PS and LOR respectively, covering up to 125% of the expected analytes concentration in the formulation. Triplicate injections were made and calibration curves were constructed from the standard drug concentrations versus peak areas of the individual drugs. The calibration curves were defined by the equations shown in Table 1, the residuals were spread uniformly and at random around the regression lines, passing the normality distribution test ($p<0.05$). In addition, correlation coefficient values were greater than 0.99 and the confidence intervals of the intercepts contained the zero, confirming method linearity.

#### 3.2.2 Precision

Method precision was verified in its repeatability and intermediate precision aspects according to the ICH guideline. Six replicate determinations of the samples containing 100% of their corresponding expected concentrations in the pharmaceutical product were injected and the RSD (%) of their recoveries were determined. Satisfactory RSD levels below 2% were obtained (Table 1). For verification of the intermediate precision the process was repeated on another day using fresh reagents and samples, the samples were injected at random during two different days. This evidenced that the outcome of the determination was statistically similar regardless the day of the assay and the reagents preparation in the determinations. In addition, in all cases almost quantitative and consistent (RSD<2%) drug recoveries were recorded [17]. These results

confirmed that the method is precise.

**Table 1:** Results of Method validation

| Parameter | PS | LOR |
|---|---|---|
| Slope (b) | 1192.73 | 39674.56 |
| Intercept (a) | 6683.68 | 38554.68 |
| Correlation coefficient ($r^2$) | 0.9999 | 0.9999 |
| Std dev. of slope ($S_b$) | 6.80 | 6.04 |
| Std dev. of intercept ($S_b$) | 185.57 | 1504.54 |
| LOD (µg/ml) | 3.67 | 0.70 |
| LOQ (µg/ml) | 11.11 | 2.12 |
| Repeatability (% ±SD) | 98.34 (0.90) | 105.76 (0.95) |
| Int. precision (% ±SD) | 99.10 (1.10) | 105.22 (1.86) |
| Recovery (% ±SD) | 101.31 (1.00) | 102.45 (1.51) |

### 3.2.3 Accuracy
The method accuracy was demonstrated using standard addition method by evaluating analytes recoveries from a pre-assayed pharmaceutical formulation sample, containing 60% of the declared amounts of the drugs, which was fortified with known amounts of the two analytes, to reach concentration levels of 60% -120% of the expected drug concentrations in the pharmaceutical dosage form. The observed RSD levels (< 2%), were considered satisfactory and confirmed the suitability of the method for the accurate determination of the two analytes.

### 3.2.4 Limits of Detection (LOD) and Quantification (LOQ)
In order to assess that the validated concentration ranges of the analytes were above their LOQ values, the LOD and LOQ were determined employing the ICH method based on the calibration curve [35]. The LOD values were 0.70 µg/mL for LOR, 3.67 µg/mL for PS; the corresponding LOQ values, determined using the same method, were 2.12 µg/mL and 11.11 µg/mL, respectively. These values are well below the lowest expected analyte concentrations in the samples; hence the suitability of the method is supported.

### 3.2.5 System Suitability Parameters
System suitability parameters must be checked to ensure that the system is working correctly during the analysis. The test was carried out by injecting five replicates of the optimization standard. The method performance data including column efficiencies (N), resolutions between adjacent peeks ($R_s$), and asymmetry factor ($A_s$) are listed in Table 2. All parameters were in good agreements with the theoretically required ones [18].

**Table 2:** Optimization System Suitability Parameters

| Analyte | $R_s$ | $A_s$ | N |
|---|---|---|---|
| LOR | 2.96* | 1.33 | 2417.8 |
| PS | 2.69** | 1.26 | 4489.2 |

*Resolution between PP and LOR ** resolution between LOR and PS

## 4. Conclusion
A reliable and rapid liquid chromatography method for the simultaneous determination of LOR and PSE in syrup has been developed and validated. Experimental designs were employed for rational method optimization and demonstration of its suitability for the intended purpose. The use of strong cation exchange column combined with a very simple, relatively cheap and easy to prepare mobile phase enabled separating the active principles within 7.0 min, despite their widely different physicochemical properties. It was also possible to achieve their quantification in spite of the important abundance differences between the analytes (PSE: LOR up to 12:1, w/w). In addition, the results indicated that the method is sensitive, linear, precise, and accurate, with regard to the analytes under investigation. Therefore it can be safely applied to the quality control of syrup formulations containing LOR and PSE without interference from the formulation additives.

### 4.1 Conflict of interest
Authors do not have any conflict of interest with the commercial identities mentioned in this article.

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