Research Article

Application of Factorial and Doehlert Designs for the Optimization of the Simultaneous Separation and Determination of Antimigraine Drugs in Pharmaceutical Formulations by RP-HPLC-UV

Sami Jebali,1,2 Chaouki Belgacem,2,3 Mohamed Radhouen Louhaichi,1 Senda Bahri,1 and Latifa Latrous El Atarche2,4

1Laboratoire National de Contrôle des Médicaments, 11 Bis Rue Jebel Lakhdar Bab Saadoun, 1006 Tunis, Tunisia
2Université de Tunis El Manar, Faculté des Sciences de Tunis, Laboratoire de Chimie Analytique et Electrochimie, Campus Universitaire El Manar II 2092, Tunis, Tunisia
3Institut National de Recherche et d’Analyse Physico-Chimique, Technopole, 2020 Sidi Thabet, Ariana, Tunis, Tunisia
4Université de Tunis El Manar, Institut Préparatoire Aux Etudes d’Ingénieurs d’El Manar, B.P.244 El Manar II 2092, Tunis, Tunisia

Correspondence should be addressed to Latifa Latrous El Atarche; latifa.latrous@ipeiem.rnu.tn

Received 27 February 2019; Revised 28 May 2019; Accepted 15 July 2019; Published 15 August 2019

Academic Editor: David Touboul

Copyright © 2019 Sami Jebali et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

A sensitive, precise, accurate, and specific isocratic reversed-phase high-performance liquid chromatographic (RP-HPLC) method for the simultaneous separation and determination of zolmitriptan, naratriptan, dihydroergotamine, ketotifen, and pizotifen in pharmaceutical formulations has been developed and validated. An experimental design was applied for the optimization of the chromatographic parameters. A two-level full factorial 2k was used for studying the interaction between the variables to be optimized: the percentage of acetonitrile in the mobile phase, mobile-phase pH, nature of the buffer, and column oven temperature. The most significant parameters are the percentage of acetonitrile and the mobile-phase pH. These significant parameters were optimized using the Doehlert matrix. The optimum separation was achieved by means of a Waters XBridge C18 column (250 mm × 4.6 mm, 5 μm) with a mobile phase consisting of acetonitrile and a 10 mM sodium perchlorate buffer (38:62, v/v) at a flow rate of 1.0 mL·min⁻¹ and UV detection at 220 nm. The selectivity, method linearity, accuracy, and precision were examined as part of the method validation. The described method shows excellent linearity over a range of 30 to 70 μg·mL⁻¹ for all compounds with correlation coefficients higher than 0.995. The standard deviations of the intraday and interday precision were between 0.75 and 1.94%. The validated method was successfully applied to perform routine analysis of these compounds in different pharmaceutical products such as syrups and tablets. In the presence of some preservatives, it was found that there were no peaks at the related peak locations.

1. Introduction

Migraine is the third most prevalent and sixth most disabling medical illness in the world. It consists of headache attacks lasting 4–72 h, of moderate to severe intensity, associated with nausea and photo- and phonophobia [1, 2]. This pathology affects 10% of the global adult population between the age of 25 and 55 with highest prevalence in women [3, 4]. Antimigraine therapy includes currently potent serotonin 5-HT13/1D receptor agonists, a collectively known triptan drug class [5, 6]. They work by stimulating serotonin receptors in the brain. This is the reason why the role of 5-hydroxytryptamine (5-HT) as the central mediator of migraine attack has received much attention [7–12].

Zolmitriptan (Zol), naratriptan (Nara), dihydroergotamine (DHE), and pizotifen malate (Pizo) are recommended as first-line drugs for acute migraine treatment. Clinical studies have shown Zol to be effective and well tolerated [13]. It stimulates
serotonin receptors in the brain causing blood vessels to narrow down, and consequently, it relieves the pain of migraines [14]. Likewise, Nara, a selective serotonin agonist, acts on 5-HT1 receptors to cause the vasoconstriction of cranial arteries. It is used also for the acute treatment of the headache phase of migraine attacks [15]. DHE, a semisynthetic ergopeptide, is widely used to prevent or treat vascular headaches such as migraine and cluster headaches [16, 17]. It acts by the stimulation of serotonergic receptors of neurons of the capacitance vessels [18, 19]. In addition, Pizo is an H1 receptor antagonist used for the prophylaxis of migraine [20, 21].

Various analytical procedures have been implemented for the determination of these compounds in pharmaceutical formulations and plasma samples. High-performance liquid chromatography (HPLC) with ultraviolet-visible detection [22–24], HPLC with fluorescence detection [16, 17, 25, 26], HPLC with coulometric detection [27, 28], and HPLC with mass spectroscopy [29–35] are the most used techniques. Additionally, a micellar electrokinetic capillary chromatography has been developed and validated to allow the analysis of Nara in pharmaceutical products [36]. The quantification of Pizo is also determined by various atomic absorption methods, colorimetric methods, and potentiometric methods [28, 37]. The electrochemical behavior of Nara by developing a differential pulse voltammetric assay was examined to study its content uniformity in the tablet form [38]. Recently, a new method for selective determination of triptans in rat plasma has been developed with an efficient zirconia-based reversed-phase chromatography [39]. Screen-printed silver electrodes are shown as cost-effective surface-enhanced Raman scattering substrates for the sensitive and quantitative detection of naratriptan [15].

Most of the optimized separation methods cited in the literature for the analysis of antimigraine drugs by RP-HPLC involve a variation of a large number of variables in the separation process. To our knowledge, no paper carried out changes of experimental conditions all along the chromatographic run, for the separation of antimigraine drugs. For this reason, it is more effective and time-saving to resort to the experimental design procedure.

Being a laboratory having as the main task the quality control of drugs intended for use in the local market, it was of big interest for our team to develop a reliable analytical technique for the detection and quantification of Zol, Nara, DHE, Keto, and Pizo in various pharmaceutical products. So far, to the best of our knowledge, no reversed-phase high-performance liquid chromatographic techniques with ultraviolet-visible detection (RP-HPLC-UV) were reported in the literature for the simultaneous separation and quantification of triptan pharmaceutical formulations. In the first step of this work, a two-level full factorial design was used to study the effects of factors which influence and interfere in the simultaneous separation and determination of Zol, Nara, DHE, and Pizo in both raw materials and pharmaceutical formulations. In the second step, the most influential factors are optimized using the response surface methodology with a Doehlert design. This method was fully validated according to the International Conference on Harmonization (ICH) validations rules [40]. The applicability of the developed method was fully and successfully verified by the analysis of several commercialized pharmaceutical products from the local market.

2. Materials and Methods

2.1. Reagents and Chemicals. The reference standards of Pizo, DHE, Nara, Keto, and Zol with a purity of 99.99% were obtained from Sigma-Aldrich (France). The chemical structures of all studied compounds are shown in Figure 1. HPLC-grade methanol (MeOH) and acetonitrile (ACN) were purchased from Prolabo (Paris, France). High-purity water was prepared by using the Diamond Reverse Osmosis System (United Kingdom). Sodium perchlorate, perchloric acid, sodium acetate, acetic acid, orthophosphoric acid, and sodium hydroxide were purchased from Prolabo (Paris, France). Sodium dihydrogenphosphate was purchased from Carlo Erba (France).

The commercialized pharmaceutical products used were Zomig tablets (2.5 mg Zol) produced by AstraZeneca United Kingdom; Naramig tablets (2.5 mg Nara) marketed by GlaxoSmithKline, France; Ikaran LP tablets (5 mg DHE) manufactured by Pierre Fabre Medicament Production in Tunisia; Pizofen syrup (0.25 mg/5 mL Pizo) manufactured by Simed, Tunisia; and Tefanyl tablets (1 mg Keto) and Pizofen tablets (0.5 mg Pizo) manufactured by Saiph, Tunisia.

2.2. Chromatographic Conditions. LC analyses were performed on an Agilent model 1200 consisting of a quaternary pump, an autosampler, a vacuum degasser, and a thermostat column compartment. Separation was achieved on a Waters XBridge® ODS column (250 mm × 4.6 mm ID, 5 μm particle size).

The mobile phase was composed of 38:62 (v/v) acetonitrile/10 mM sodium perchlorate buffer (pH 3.5). The system was equilibrated with the mobile phase before injection, and the flow rate was 1.0 mL·min⁻¹. The detector consisted of a diode-array detector (DAD) UV-visible model 1200.

2.3. Preparation of Solutions. Stock solutions of 500 μg·mL⁻¹ of each compound were prepared in MeOH. These solutions were used to prepare the working solutions by serial dilutions in ultrapure water in the range 30–70 μg·mL⁻¹.

Oral suspension samples and syrup were diluted with ultrapure water at a concentration of 50 μg·mL⁻¹. The obtained solutions were sonicated for 10 min and were filtered with a 0.45 μm filter prior to injection into the HPLC system.

Twenty units of tablet and capsule samples were accurately weighted and powdered separately in a mortar. An equivalent weight of 5 mg of the studied compound was dissolved in 100 mL ultrapure water. After 15 min of mechanical shaking, the solution was filtered through a 0.45 μm Millipore filter. All preparations were performed in three replicates.

3. Results and Discussion

The nature of the stationary phase was chosen on the basis of previous works [4, 32, 41]. The majority of the
published HPLC method uses octadecylsilica columns (C18) for the separation of antimigraine drugs. For the selection of the wavelength, as shown in the PDA spectra, the studied compounds have a good absorbance at 220 nm.

3.1. Study of the Influence of Factors on Resolution and Run Time. In order to quantify the influence of operating parameters on the simultaneous separation of antimigraine drugs, four main factors were chosen: % of ACN in the mobile phase \( (U_1) \), mobile-phase pH \( (U_2) \), nature of the buffer \( (U_3) \), and column oven temperature \( (U_4) \). These variables with their respective domain are chosen on the basis of the previous work [41] and preliminary studies, and they are presented in Table 1. Experiments were performed on a two-level full factorial design \( 2^k \) \cite{42, 43}. In these types of designs, variables \( (k) \) are set at two levels (minimum and maximum) normalized as \(-1\) and \(+1\). The experimental response \( (Y) \) associated with a \( 2^k \) factorial design (for four variables) is represented by a linear polynomial model with interaction as follows:

![Chemical structures of studied compounds.](image)
where $Y$ is the experimental response, $X_i$ the coded variable ($-1$ or $+1$), $b_i$ the estimation of the principal effect of the factor $i$ for the response $Y$, and $b_{ij}$ the estimation of the interaction effect between factors $i$ and $j$ for the response $Y$. The coefficients of the equation model were calculated in the experimental field, as listed in Table 1.

The experimental design and results are presented in Table 2. The responses of interest are resolution $R_s$ between Zol and Nara ($Y_1$) and run time ($Y_2$).

According to the obtained results, the coefficients of the polynomial model were calculated using the NEMROD software [44]:

$$Y_1 = 3.54 - 1.33X_1 + 0.13X_2 + 1.31X_3 - 0.12X_4$$
$$+ 0.01X_1X_2 + 0.28X_1X_3 - 0.01X_1X_4 - 0.01X_2X_3 - 0.07X_2X_4$$
$$- 0.01X_3X_4 - 0.01X_1X_3X_4 + 0.01X_2X_3X_4$$
$$+ 0.01X_1X_2X_3X_4.$$  

(1)

$$Y_2 = 34.688 - 17.688X_1 + 11.313X_2 - 0.125X_3 - 0.6X_4$$
$$- 5.563X_1X_2 + 7.0X_1X_3 - 0.375X_1X_4 - 0.025X_2X_4$$
$$- 0.275X_2X_4 - 0.337X_3X_4 + 2.75X_3X_3X_4$$
$$- 0.15X_1X_3X_4 - 0.213X_1X_3X_4 - 0.287X_2X_3X_4$$
$$- 0.162X_1X_2X_3X_4.$$  

(2)

The effects and interactions of various investigated factors are presented in Figure 2(a). This figure shows that the most influential factors are % ACN, mobile-phase pH, and nature of the buffer. However, the temperature and the interaction variables have negligible effects. The % ACN has a negative effect on the studied response. As expected, the variation of solute retention with the mobile-phase composition follows the usual trend and shows a faster elution of all studied compounds upon increasing ACN percentage. The observed effect is due to a decrease in the dielectric constant of the mixture after progressively increasing the ratio of the organic modifier and, consequently, leads to a decrease in the retention factor. Mobile-phase pH is the second most significant factor of the separation of triptan drugs. Its effect is positive, especially on time analysis. The changes in the retention as a function of the pH result from the changes in the ionization form of these solutes, which is pKa dependent. The elution of Zol, Nara, and Keto was rapid compared to that of DHE and Pizo. Their retention times remained almost unchanged over the studied pH range because of their high pKa values (the pKa of Zol, Nara, and Keto was, respectively, 9.64, 9.7, and 8.43). In fact, at this pH range, Zol, Nara, and Keto were protonated, so they were not retained by the reversed phase. However, the retention time of DHE and Pizo increased with the pH because of their lower pKa values (6.75 and 6.95, respectively). Sodium dihydrogen phosphate and sodium perchlorate buffers (pH = 3.5) were tested to investigate their effect on both peak shape and retention time. Their proportions in the mobile phase were adjusted in order to obtain an acceptable separation in comparable analysis time. It appears that the retention order of the 5 compounds with the 2 buffers was similar. However, the retention time of the 5 compounds was affected by the nature of the solution buffer and especially by the perchlorate buffer. Pareto analysis [45] gives more significant information to interpret these results. In fact, this analysis calculates the percentage effect of each factor on the response, according to the following relation:

$$P_i = \left( \frac{b_i^2}{\sum b_i^2} \right) \times 100, \quad i \neq 0.$$  

(3)

Figure 2(b) presents the Pareto graphic analysis. The results show that the % of acetonitrile in the mobile phase and mobile-phase pH are the most determining factors of the (separationary) separation of the studied compounds. Therefore, 83.30% of the response is brought about by these two factors. The % of acetonitrile in the mobile phase represents 59.12% of the response.

3.2. Optimization of Chromatographic Conditions. In the second step, to determine optimum factors that ensure the chromatographic separation of the target antimigraine drugs in pharmaceutical formulations, predominant factors such as mobile-phase composition and mobile-phase pH were investigated.

For this study, the response surface methodology based on empirical mathematical modeling was used. A second-order polynomial model was used to study the possible nonlinear effects and curvature in the field of study:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3^2 + b_4X_4^2 + b_{12}X_1X_2.$$  

(4)

A Doehlert uniform shell design is performed [46–48]. The factors are given in the form of coded variables ($X_i$) without units in order to permit comparison of factors of different natures. The transformation of natural variables ($U_i$) into coded corresponding variables ($X_i$) is made on the basis of the following equation:

$$X_i = \left[ \frac{U_i - \bar{U}_i}{\Delta U_i} \right] \alpha,$$  

(5)

where $X_i$ is the value taken by the coded variable $i$; $U_i$ is the value taken by the factor $i$; $\bar{U}_i$ is the value taken by the factor $i$ in the centre of the experimental field; $\Delta U_i$ is the range of variation of the factor $i$; and $\alpha$ is the maximum coded value of $X_i$; $X_1 = 1, X_2 = 0.866$, and $X_3 = 0.816$. The levels of the independent variables (effective variables $U_i$) were calculated according to these following relations:
were determined within and between three different days.

Quantification. Recoveries were determined at five different concentrations, accuracy, recovery, limit of detection, and limit of quantification. Recoveries were determined at five different concentrations, and linearity, accuracy, and precision were determined within and between three different days.

3.3. Validation of the Method. The optimized method was validated in relation to the following properties: specificity, accuracy, recovery, limit of detection, and limit of quantification. Recoveries were determined at five different concentration levels, and linearity, accuracy, and precision were determined within and between three different days.

Table 2: Factorial design, experimental conditions, and experimental results.

| Experiment no. | Experimental design | Experimental plan | Results |
|---------------|---------------------|------------------|---------|
|               | $X_1$ | $X_2$ | $X_3$ | $X_4$ | $U_1$ | $U_2$ | $U_3$ | $U_4$ | $Y_1$ | $Y_2$ (min) |
| 1             | 1     | 1     | −1   | −1   | 25    | 2     | Phosphate 30 | 3.7 | 40  |
| 2             | −1    | −1    | −1   | −1   | 40    | 2     | Phosphate 30 | 0.6 | 6.8 |
| 3             | −1    | 1     | −1   | −1   | 25    | 7     | Phosphate 30 | 4.1 | 80  |
| 4             | 1     | 1     | −1   | −1   | 40    | 7     | Phosphate 30 | 0.8 | 13.5|
| 5             | −1    | −1    | −1   | −1   | 25    | 2     | Perchlorate 30 | 6.0 | 32  |
| 6             | 1     | −1    | 1    | −1   | 40    | 2     | Perchlorate 30 | 3.8 | 16  |
| 7             | −1    | 1     | 1    | −1   | 25    | 7     | Perchlorate 30 | 6.1 | 60  |
| 8             | 1     | 1     | 1    | −1   | 40    | 7     | Perchlorate 30 | 4.2 | 34  |
| 9             | −1    | −1    | −1   | 1    | 25    | 2     | Phosphate 40 | 3.6 | 39  |
| 10            | 1     | −1    | −1   | 1    | 40    | 2     | Phosphate 40 | 0.5 | 6.7 |
| 11            | −1    | 1     | −1   | 1    | 25    | 7     | Phosphate 40 | 4.0 | 79  |
| 12            | 1     | 1     | −1   | 1    | 40    | 7     | Phosphate 40 | 0.6 | 13.5|
| 13            | −1    | −1    | −1   | 1    | 25    | 2     | Perchlorate 40 | 5.7 | 31  |
| 14            | 1     | −1    | −1   | 1    | 40    | 2     | Perchlorate 40 | 3.4 | 15.5|
| 15            | −1    | 1     | 1    | 1    | 25    | 7     | Perchlorate 40 | 5.8 | 58  |
| 16            | 1     | 1     | 1    | 1    | 40    | 7     | Perchlorate 40 | 3.8 | 30  |

$Y_1$: resolution $R_s$ (Zol-Nara); $Y_2$: run time.

3.3.1. Specificity. According to the International Conference on Harmonization (ICH) validations rules (Validation of Analytical Procedures: Text and Methodology Q2 (R1)), specificity is the ability to assess unequivocally the target compound in the presence of components, which may be expected to be present [40]. The resolution factors between all peaks in standard solutions (Figure 4) and in real samples (Figure 5) are satisfactory, reflecting the specificity of the method. As shown in Figure 5, the presence of preservative peaks did not interfere with the studied compounds. Peaks of the placebo components were adequately separated from those of active compounds which indicates the selectivity of the method.

On the contrary, as shown in Table 4, the purity factors of all peaks are more than 950, and the peak purity of each active compound and PDA spectra shows that no interferences with any component (related impurities, degradation products, or excipients) are observed. Therefore, the proposed method is applicable to the selective determination of these compounds.

3.3.2. Linearity. The linearity of the method was evaluated by injecting solutions of each antimigraine drug over a wide concentration range (30 to 70 $\mu$g·mL$^{-1}$). For each analyte, calibration curves were constructed. Calibration data could fit a linear model for all analytes with typical correlation coefficients exceeding 0.995, as shown in Table 5.

$F$ values (Fisher’s test for the existence of a significant slope) that are greater than the Fisher $F$ critical value deduced from the table ($\alpha$, 1, and $N - 2$) for $\alpha = 0.05$ and $N = 15$ are 4.67 for all the analytes, since we can conclude that the regression is acceptable at the risk of error of 5%.

3.3.3. Precision. Method precision was determined by replicate analyses of 6 independent preparations at 100% of the test concentration according to ICH validation rules [40]. The

$$U_1 = 7.5X_1 + 32.5,$$

$$U_2 = 2.9X_2 + 4.5.$$  

$\mu$: slope that are greater than the Fisher's test for the existence of a significant slope.
intermediate precision was obtained by repeating the intra-assay experiment for three days.

The repeatability and intermediate precision values were calculated as relative standard deviation (RSD) of the found values. The mean RSD values for intra- and interday precision varied from 0.78 to 1.84% and from 0.75 to 1.94%, respectively (Table 6).

3.3.4. Accuracy and Recovery. The accuracy of the method was evaluated in triplicate by recovery tests at five concentration levels (30, 40, 50, 60, and 70 μg·mL⁻¹). Therefore, recovery tests were performed by spiking different samples at five levels, and the results obtained as well as the respective relative standard deviation (RSD) are presented in Table 7. The higher values of recoveries and the lower values of the

Table 3: Doehlert’s experimental design, experimental conditions, and responses.

| Experiment no. | Design of experiments | Operating conditions | Experimental conditions |
|----------------|-----------------------|----------------------|-------------------------|
|                | X₁                   | X₂                   | ACN (%) | pH | Y₁ (min) | Y₂ (min) |
| 1              | 1.0000               | 0.0000               | 40.0     | 4.5 | 4.01     | 23.00     |
| 2              | −1.0000              | 0.0000               | 25.0     | 4.5 | 6.05     | 44.00     |
| 3              | 0.5000               | 0.8660               | 36.3     | 7.0 | 4.20     | 37.00     |
| 4              | −0.5000              | −0.8660              | 28.8     | 2.0 | 5.70     | 29.00     |
| 5              | 0.5000               | −0.8660              | 36.3     | 2.0 | 3.80     | 16.00     |
| 6              | −0.5000              | 0.8660               | 28.8     | 7.0 | 5.80     | 55.00     |
| 7              | 0.0000               | 0.0000               | 32.5     | 4.5 | 5.04     | 35.50     |
| 8              | 0.0000               | 0.0000               | 32.5     | 4.5 | 5.06     | 35.40     |

Y₁: resolution Rₖ (Zol-Nara); Y₂: run time.

Figure 2: (a) Graphic analysis of effects. (b) Pareto graphic analysis.

The higher values of recoveries and the lower values of the...
RSD of the assay indicate that the method is precise and accurate. The results depict that the present method is useful for bulk drug analysis as well as commercial pharmaceuticals in different forms and different types of formulations.

3.3.5. Limit of Detection (LOD) and Limit of Quantification (LOQ). The LOD and LOQ were estimated for all compounds based on signal-to-noise ratio (S/N). The baseline noise was measured in a blank experiment in the region of retention time of the compound using chromatographic software. The limits of detection (S/N = 3) and of quantification (S/N = 10) of Pizo, Keto, DHE, Nara, and Zol ranged from 0.075 to 0.10 μg·mL⁻¹ and 0.25 to 0.33 μg·mL⁻¹, respectively.

3.4. Application of the Method. The potential of the method for the analysis of pharmaceutical formulations from the local market such as tablets and syrup has been demonstrated. The outcome of this study has shown a good agreement between the experimental and label claims (Table 8). The recovery percentage, with respect to the label claims, ranged between 97.45 and 102.60%, indicating a good accuracy of the proposed method. In order to further assess the accuracy of the proposed method and taking into consideration the matrix effect, a new set of recovery experiments were carried out by spiking the drug.
### Table 5: Linearity details of selected compounds.

| Compound | Equation | Correlation coefficient | $F$ (Fisher) |
|----------|----------|--------------------------|--------------|
| Zol      | $y = 64153.333x + 16393.667$ | 0.998 | 2535.39 |
| Nara     | $y = 51618.667x - 7266.333$ | 0.997 | 2057.85 |
| Keto     | $y = 22653.000x - 6153.333$ | 0.997 | 2154.05 |
| DHE      | $y = 33653.000x - 961.667$ | 0.997 | 2121.47 |
| Pizo     | $y = 31270.333x - 37732.333$ | 0.996 | 2325.35 |

### Table 6: Data on intra- and interday method precision.

| Compound | Interday mean RSD (%) | Intraday mean RSD (%) |
|----------|-----------------------|-----------------------|
| Zol      | 0.78                  | 0.75                  |
| Nara     | 0.85                  | 0.86                  |
| Keto     | 1.03                  | 1.03                  |
| DHE      | 1.41                  | 1.41                  |
| Pizo     | 1.63                  | 1.63                  |

### Table 7: Method precision and accuracy for the recovery of studied compounds.

| Compound | Amount spiked ($\mu$g·mL$^{-1}$) | Amount recovered ($\mu$g·mL$^{-1}$) | Mean recovery (%) | RSD (%) |
|----------|----------------------------------|------------------------------------|-------------------|---------|
| **At 60% level** | | | | |
| Zol      | 30                               | 29.96                              | 97.92             | 0.43    |
| Nara     | 30                               | 30.19                              | 100.56            | 0.93    |
| Keto     | 30                               | 29.40                              | 97.99             | 0.11    |
| DHE      | 30                               | 30.22                              | 100.73            | 0.69    |
| Pizo     | 30                               | 30.58                              | 100.94            | 0.16    |
| **At 80% level** | | | | |
| Zol      | 40                               | 41.07                              | 101.17            | 0.85    |
| Nara     | 40                               | 39.84                              | 99.60             | 1.16    |
| Keto     | 40                               | 40.55                              | 101.38            | 0.94    |
| DHE      | 40                               | 40.12                              | 100.31            | 0.89    |
| Pizo     | 40                               | 40.28                              | 99.95             | 1.54    |
| **At 100% level** | | | | |
| Zol      | 50                               | 51.39                              | 101.57            | 0.83    |
| Nara     | 50                               | 50.59                              | 101.18            | 0.96    |
| Keto     | 50                               | 50.89                              | 101.78            | 1.55    |
| DHE      | 50                               | 49.88                              | 99.77             | 2.36    |
| Pizo     | 50                               | 50.55                              | 100.49            | 1.90    |
| **At 120% level** | | | | |
| Zol      | 60                               | 60.11                              | 99.20             | 0.69    |
| Nara     | 60                               | 58.63                              | 97.72             | 2.02    |
| Keto     | 60                               | 58.98                              | 98.30             | 1.00    |
| DHE      | 60                               | 58.99                              | 98.31             | 1.47    |
| Pizo     | 60                               | 58.74                              | 97.41             | 0.77    |
| **At 140% level** | | | | |
| Zol      | 70                               | 70.44                              | 99.78             | 0.24    |
| Nara     | 70                               | 70.17                              | 101.11            | 1.42    |
| Keto     | 70                               | 70.18                              | 100.26            | 0.35    |
| DHE      | 70                               | 70.79                              | 101.12            | 0.50    |
| Pizo     | 70                               | 71.35                              | 101.50            | 0.57    |

### Table 8: Content of industrial pharmaceutical forms with respect to the label amount claimed.

| Sample       | Compounds | Label content | Norms (%) | Found                        | Recovery (%) |
|--------------|-----------|---------------|-----------|------------------------------|--------------|
| Zomig tablets| Zol       | 2.5 mg/tablet | 100.0±5.0 | 2.44 mg/tablet               | 97.45        |
| Naramig tablets| Nara    | 2.5 mg/tablet | 100.0±5.0 | 2.49 mg/tablet               | 99.87        |
| Ikaran LP tablets | DHE    | 5 mg/tablet  | 100.0±5.0 | 5.13 mg/tablet               | 102.60       |
| Pizofen tablets          | Pizo    | 0.5 mg/tablet | 100.0±5.0 | 0.49 mg/tablet               | 98.04        |
| Pizofen syrup             | Pizo    | 0.25 mg/5 mL | 100.0±5.0 | 0.24 mg/5 mL                 | 98.24        |
| Tefanyl tablets           | Keto    | 1.0 mg/tablet | 100.0±5.0 | 0.99 mg/tablet               | 98.61        |
solutions with known amounts of standard target compound solutions.

The typical chromatograms related to the separation of the five end products are illustrated in Figure 5. The peaks were identified by comparison of the retention time of the separated compounds and standards. No interference between peaks was observed in the chromatograms of the commercial formulations under the described conditions. Therefore, the excipients present in the commercial preparations have no interference with the analysis of Zol, Nara, Keto, DHE, and Pizo.

4. Conclusion

Applying fractional factorial design (FFD) with the purpose of screening of variables and Doehlert design for optimization of the screened variables, the simultaneous determination of Zol, Nara, DHE, and Pizo in pharmaceutical preparations was conducted by reversed-phase high-performance liquid chromatography (RP-HPLC).

The factorial designs have demonstrated that the percentage of acetonitrile in the mobile phase and mobile-phase pH are the most influential parameters of optimization of chromatographic separation. In addition, the method was validated according to ICH guidelines; the results indicate that the method is sensitive, precise, accurate, and applicable to various commercial pharmaceutical preparations: syrups and tablets containing Zol, Nara, DHE, and Pizo. Therefore, the method can be used for routine quality control analysis in the pharmaceutical environment. The results of this study demonstrate the benefit of applying the experimental design methodology in selecting optimum conditions for the determination of drugs in pharmaceutical formulations.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Disclosure

This research was performed as part of employment of the authors Sami Jebali and Senda Bahri.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Supplementary Materials

Figure S1: purity spectra of studied compounds. Figure S2: PDA spectra of antimigraine drugs. Figure S3: linearity graphs of selected compounds. Figure S4: placebo chromatograms of studied commercialized pharmaceutical products. Table S1: placebo compositions of studied commercialized pharmaceutical products. (Supplementary Materials)

References

[1] E. M. Abdou, S. M. Kandil, and H. M. F. ElMinnawy, “Brain targeting efficiency of antimigrain drug loaded mucoadhesive intranasal nanoemulsion,” International Journal of Pharmaceutics, vol. 520, no. 1-2, pp. 667–677, 2017.
[2] D. Hans-Christoph and W. D. David, “Headache research in 2015: progress in migraine treatment,” The Lancet Neurology, vol. 15, no. 1, pp. 4–5, 2016.
[3] Headache Classification Committee of the International Headache Society (IHS), “The international classification of headache disorders, 3rd edition (beta version),” Cephalalgia, vol. 33, no. 9, pp. 629–808, 2013.
[4] D. Vandelli, F. Palazzoli, P. Verri et al., “Development and validation of a liquid chromatography-tandem mass spectrometric assay for quantitative analyses of triptans in hair,” Journal of Chromatography B, vol. 1017-1018, pp. 136–144, 2016.
[5] P. E. Rolan, “Understanding the pharmacology of headache,” Current Opinion in Pharmacology, vol. 14, pp. 30–33, 2014.
[6] M. Mizerai, A. Krause, P. Zalewski, R. Skibiński, and J. Cielecka-Piontek, “Quantitative structure-retention relationship model for the determination of naratriptan hydrochloride and its impurities based on artificial neural networks coupled with genetic algorithm,” Talanta, vol. 164, pp. 164–174, 2017.
[7] S. Evers, J. Afra, A. Frese et al., “EFNS guideline on the drug treatment of migraine—revised report of an EFNS task force,” European Journal of Neurology, vol. 16, no. 9, pp. 968–981, 2009.
[8] P. P. A. Humphrey, W. Feniuk, M. J. Perren, I. J. M. Beresford, M. Skingle, and E. T. Whalley, “Serotonin and migraine,” Annals of the New York Academy of Sciences, vol. 600, no. 1, pp. 587–598, 1990.
[9] M. A. Moskowitz, “Basic mechanisms in vascular headache,” Neurologic Clinics, vol. 8, no. 4, pp. 801–815, 1990.
[10] T. S. Olsen, “Migraine with and without aura: the same disease due to cerebral vasospasm of different intensity. A hypothesis based on CBF studies during migraine,” Headache: The Journal of Head and Face Pain, vol. 30, no. 5, pp. 269–272, 1990.
[11] P. P. A. Humphrey, “5-Hydroxytryptamine and the pathophysiology of migraine,” Journal of Neurology, vol. 238, no. S1, pp. S38–S44, 1991.
[12] E. Loder, “Triptan therapy in migraine,” New England Journal of Medicine, vol. 363, no. 1, pp. 63–70, 2010.
[13] R. M. A. Abd-Elal, R. N. Shamma, H. M. Rashed, and E. R. Bendah, “Trans-nasal zolmitriptan novasomes: in-vitro preparation, optimization and in-vivo evaluation of brain targeting efficiency,” Drug Delivery, vol. 23, no. 9, pp. 3374–3386, 2016.
[14] P. Girotra, S. Kumar Singh, and G. Kumar, “Development of zolmitriptan loaded PLGA/poloxamer nanoparticles for migraine using quality by design approach,” International Journal of Biological Macromolecules, vol. 85, pp. 92–101, 2016.
[15] C. N. Hernández, M. B. Martín-Yerga, M. B. González-García, D. Hernández-Santos, and P. Fanjul-Bolado, “Evaluation of electrochemical, UV/VIS and Raman spectroelectrochemical detection of naratriptan with screen-printed electrodes,” Talanta, vol. 178, pp. 85–88, 2018.
[16] S. G. Romeijn, E. Marttin, J. C. Verhoef, and F. W. H. M. Merkus, “Simplified solid-phase extraction method for determination of dihydroergotamine in rabbit and human serum using high-performance liquid chromatography with fluorescence detection,”
International Journal of Analytical Chemistry

Journal of Chromatography B: Biomedical Sciences and Application, vol. 692, no. 1, pp. 227–232, 1997.
[17] M. Murday, A. M. McLean, E. Slaughter, and R. A. Couch, “Determination of dihydroergotamine in human plasma by high-performance liquid chromatography with fluorescence detection,” Journal of Chromatography B: Biomedical Sciences and Applications, vol. 735, no. 2, pp. 151–157, 1999.
[18] B. G. McCarthy and S. J. Peroutka, “Comparative neuropharmacology of dihydroergotamine and sumatriptan (GR 43175),” Headache: The Journal of Head and Face Pain, vol. 29, no. 7, pp. 420–422, 1989.
[19] N. H. Raskin, “Pharmacology of migraine,” Annual Review of Pharmacology and Toxicology, vol. 21, no. 1, pp. 463–478, 1981.
[20] M. A. McShane and S. J. Hughes, “Managing headache in children,” Paediatrics and Child Health, vol. 21, no. 11, pp. 518–520, 2011.
[21] G. D. Solomon, “The pharmacology of medications used in treating headache,” Seminars in Pediatric Neurology, vol. 2, no. 2, pp. 165–177, 1995.
[22] M. A. Abounassif, H. A. El-Obeid, and E. A. Gadkariem, “Stability studies on some benzocycloheptane antihistaminic agents,” Journal of Pharmaceutical and Biomedical Analysis, vol. 36, no. 5, pp. 1011–1018, 2005.
[23] M. K. Srinivasa, B. M. Rao, G. Sridhar, P. R. Kumar, K. B. Chandrasekhar, and A. Islam, “A validated chiral LC method for the determination of zolmitriptan and its potential impurities,” Journal of Pharmaceutical and Biomedical Analysis, vol. 37, no. 3, pp. 453–460, 2005.
[24] M. K. Srinivasa, B. M. Rao, G. Sridhar, K. B. Chandrasekhar, and P. R. Kumar, “A validated chiral LC method for the enantiomeric separation of zolmitriptan key intermediate, ZTR-5,” Journal of Pharmaceutical and Biomedical Analysis, vol. 39, no. 3–4, pp. 796–800, 2005.
[25] J. Chen, X.-G. Jiang, W.-M. Jiang, N. Mei, X.-L. Gao, and Q.-Z. Zhang, “High-performance liquid chromatographic analysis of zolmitriptan in human plasma using fluorescence detection,” Journal of Pharmaceutical and Biomedical Analysis, vol. 35, no. 3, pp. 639–645, 2004.
[26] H. Humbert, J. Denouel, J. P. Chervet, D. Lavene, and J. R. Kiechel, “Determination of sub-nanogram amounts of dihydroergotamine in plasma and urine using liquid chromatography and fluorimetric detection with off-line and on-line solid-phase drug enrichment,” Journal of Chromatography B: Biomedical Sciences and Applications, vol. 417, pp. 319–329, 1987.
[27] E. Clement and M. Franklin, “Simultaneous measurement of zolmitriptan and its major metabolites N-desmethylzolmitriptan and zolmitriptan N-oxide in human plasma by high-performance liquid chromatography with coulometric detection,” Journal of Chromatography B, vol. 766, no. 2, pp. 339–343, 2002.
[28] N. El-Kousy and L. I. Behawy, “Determination of some antihistaminic drugs by atomic absorption spectrometry and colorimetric methods,” Journal of Pharmaceutical and Biomedical Analysis, vol. 20, no. 4, pp. 671–679, 1999.
[29] X. Chen, D. Zhong, H. Xu, B. Schug, and H. Blume, “Sensitive and specific liquid chromatographic-tandem mass spectrometric assay for dihydroergotamine and its major metabolite in human plasma,” Journal of Chromatography B, vol. 768, no. 2, pp. 267–275, 2002.
[30] F. Gerhard, A. Kurt, R. Thorsten, M. Wangemann, M. Althaus, and K. Rissler, “Determination of dihydroergocryptine in human plasma and urine samples using on-line sample extraction–column-switching reversed-phase liquid chromatography–mass spectrometry,” Journal of Chromatography B, vol. 808, no. 2, pp. 131–139, 2004.
[31] T. Yunbiao, Z. Limei, W. Yingwu, J. P. Fawcett, and G. Jingkai, “Rapid and sensitive liquid chromatography–tandem mass spectrometry method for the quantitation of levodropropizine in human plasma,” Journal of Chromatography B, vol. 819, no. 1, pp. 185–189, 2005.
[32] A. Dalpiaz, N. Marchetti, A. Cavazzini et al., “Quantitative determination of zolmitriptan in rat blood and cerebrospinal fluid by reversed phase HPLC–ESI-MS/MS analysis: application to in vivo preclinical pharmacokinetic study,” Journal of Chromatography B, vol. 901, pp. 72–78, 2012.
[33] K. Vishwanathan, M. G. Bartlett, and J. T. Stewart, “Determination of antimigraine compounds rizatRIPTan, zolmitriptan, naratriptan and sumatriptan in human serum by liquid chromatography/electrospray tandem mass spectrometry,” Rapid Communications in Mass Spectrometry, vol. 14, no. 3, pp. 168–172, 2000.
[34] F. Q. Alali, B. M. Tashtoush, and N. M. Najib, “Determination of ketotifen in human plasma by LC-MS,” Journal of Pharmaceutical and Biomedical Analysis, vol. 34, no. 1, pp. 87–94, 2004.
[35] M. Gergov, I. Ojanperä, and E. Vuori, “Simultaneous screening for 238 drugs in blood by liquid chromatography–ionspray tandem mass spectrometry with multiple-reaction monitoring,” Journal of Chromatography B, vol. 795, no. 1, pp. 41–53, 2003.
[36] K. D. Altria and R. McLean, “Development and optimisation of a generic micellar electrokinetic capillary chromatography method to support analysis of a wide range of pharmaceuticals and excipients,” Journal of Pharmaceutical and Biomedical Analysis, vol. 18, no. 4–5, pp. 807–813, 1998.
[37] The Stationery Office, British Pharmacopoeia, Vol. II, Her Majesty’s Stationary Office, London, UK, 8th edition, 2016.
[38] C. Velasco-Aguirre and A. Álvarez-Lueje, “Voltammetric behavior of naratriptan and its determination in tablets,” Talanta, vol. 82, no. 2, pp. 796–802, 2010.
[39] S. Ahmed and N. N. Atia, “Fast and efficient zirconia-based reversed phase chromatography for selective determination of triptans in rat plasma,” Journal of Pharmaceutical and Biomedical Analysis, vol. 145, pp. 241–251, 2017.
[40] International Conference on Harmonization (ICH) of Technical Requirements 366 for the Registration of Pharmaceutical for Human Use, Validation of Analytical Procedures: Text and Methodology Q2 (R1), ICH, Geneva, Switzerland, 2005.
[41] M. R. Louhaichi, S. Jebali, M. Kallel, N. Adhoum, N. Benhamida, and L. Monser, “Development and validation of a stability-indicating HPLC method for the determination of pizotifen, methylparaben, and propylparaben in syrup,” Journal of Liquid Chromatography & Related Technologies, vol. 37, no. 7, pp. 988–1003, 2014.
[42] J. Goupuy, “Les plans d’expériences,” in Techniques de l’ingénieur, vol. 4, pp. PE 230/1–PE 230/26, Editions of T.I (ETI), Paris, France, 1997.
[43] J. Goupuy, La Méthode des Plans d’expériences, Dunod, Paris, France, 1996.
[44] D. Mathieu and R. P. T. Luu, Software NEMROD, Université d’Aix-Marseille III, Marseille, France, 1980.
[45] D. P. Haaland, Experimental Design in Biotechnology, Marcel Dekker Inc., New York, NY, USA, 1989.
[46] D. H. Dochertl, “Uniform shell designs,” Applied Statistics, vol. 19, no. 3, pp. 231–239, 1970.
[47] L. Latrous El Atrache, R. Ben Sghaier, B. Bejaoui Kefi, V. Haldys, M. Dachraoui, and J. Tortajada, “Factorial design optimization of experimental variables in preconcentration of carbamates pesticides in water samples using solid phase extraction and liquid chromatography-electrospray-mass spectrometry determination,” *Talanta*, vol. 117, pp. 392–398, 2013.

[48] R. Ouertani, L. Latrous El Atrache, and N. B. Hamida, “Chemometrically assisted optimization and validation of reversed phase liquid chromatography method for the analysis of carbamates pesticides,” *Chemometrics and Intelligent Laboratory Systems*, vol. 154, pp. 38–44, 2016.