Validation of an HPLC method for the simultaneous determination of eletriptan and UK 120.413

MIRA ZEČEVIĆ[*1], BILJANA JOCIĆ[1], SNEŽANA AGATONOVIĆ–KUŠTRIN[2] and LJILJANA ŽIVANOVIĆ[1]

[1]*Institute of Pharmaceutical Chemistry and Drug Analysis, Faculty of Pharmacy, Vojvode Stepe 450, P. O. Box 146, 11000 Belgrade, Serbia and* [2]*Pharmacy M315, School of Biomedical, Biomolecular and Chemical Sciences, The University of Western Australia, Crawley, W. A., 6009, Australia (e-mail: mzecevic@pharmacy.bg.ac.yu)*

Abstract: A rapid and sensitive RP HPLC method was developed for the routine control analysis of eletriptan hydrobromide and its organic impurity UK 120.413 in Relpax® tablets. The chromatography was performed at 20 °C using a C_{18} XTerra™ (5 μm, 150 × 4,6 mm) column at a flow rate 1.0 ml/min. The drug and its impurity were detected at 225 nm. The mobile phase consisted of TEA (1 %) – methanol (67.2:32.8 v/v), the pH of which was adjusted to 6.8 with 85 % orthophosphoric acid. Quantification was accomplished by the internal standard method. The developed RP HPLC method was validated by testing: accuracy, precision, repeatability, specificity, detection limit, quantification limit, linearity, robustness and sensitivity. High linearity of the analytical procedure was confirmed over the concentration range of 0.05 – 1.00 mg/ml for eletriptan hydrobromide and from 0.10 – 1.50 μg/ml for UK 120.413, with correlation coefficients greater than $r = 0.995$. The low value of the *RSD* expressed the good repeatability and precision of the method. Experimental design and a response surface method were used to test robustness of the analytical procedure and to evaluate the effect of variation of the method parameters, namely the mobile phase composition, pH and temperature. They showed small deviations from the method setting. The good recovery and low *RSD* confirm the suitability of the proposed RP HPLC method for the routine determination of eletriptan hydrobromide and its impurity UK 120.413 in Relpax® tables.

Keywords: validation, RP HPLC, eletriptan hydrobromide, UK 120.413.

INTRODUCTION

Eletriptan, 3-{[(*R*)-1-methyl-2-pyrrolidinyl]methyl}-5-[2-(phenylsulfonyl)eth-yl]indole hydrobromide (Fig. 1a), is a new orally active 5-HT$_{1B/1D}$ agonist, recently approved by the Food and Drug Administration for the acute treatment of migraine headache.[1–4] The related impurity in bulk drug samples is (R)-5-ethyl-3-(1-met-
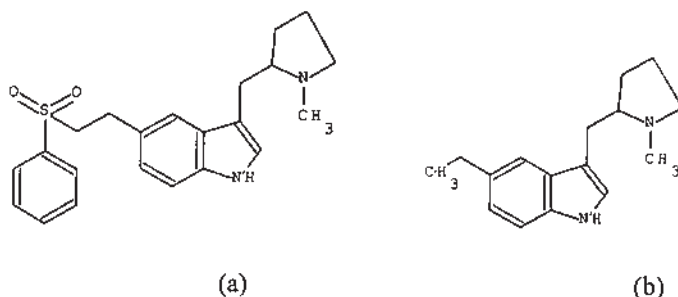

Fig. 1. Structures of eletriptan (a) and UK 120.413 (b)

hyl-2-pyrrolidinylmethyl)-1*H*-indole, (UK 120.413), (Fig. 1b). Control of pharmaceutical impurities is currently a critical issue in the pharmaceutical industry. The International Conference on Harmonization (ICH) has formulated a workable guideline regarding the control of impurities.[5] Organic impurities associated with the active pharmaceutical are the unwanted chemicals which are developed during drug synthesis or formulation. The presence of these unwanted chemicals, even in small amounts, may influence the efficacy and safety of the pharmaceutical products. Impurity profiling (identification and quantification) is now receiving increased attention from regulatory authorities. A number of recent articles[6,7] described a designed approach and guidance for the isolation and identification of process-related impurities and degradation products. In general, according to ICH guidelines on impurities in new drug products,[8] identification of impurities below the 0.1 % level is not considered to be necessary unless the potential impurities are expected to be unusually potent or toxic. In all cases, impurities should be quantified. If data are not available to quantity the proposed specification level of an impurity, studies to obtain such data may be required. UK 120.413 is an unwanted organic impurity developed during the manufacture of the bulk drug. Its presence in dosage form is limited to 0.2 % due to side effects. Different side effects, such as cardiac events, asthenia, nausea, dizziness and somnolence, can develop as a consequence of a loss of the selectivity for the $5HT_{1B/1D}$ receptor subtypes and selectivity for other receptors, such as dopamine and different subtypes of $5HT_1$ receptors.[9]

A highly sensitive method for the determination of eletriptan in biological fluids (saliva and plasma) is based on HPLC analysis with gradient elution.[10] However, to the best of our knowledge, no analytical method for the quantitative analysis of the chemical purity of eletriptan in dosage forms is yet reported in the literature. This the aim of this study was to develop a new appropriate chromatographic method for impurity profiling of eletriptan bulk drug and pharmaceutical formulations.

EXPERIMENTAL

Chemicals and solutions

The standard substances, eletriptan and impurity UK 120.413, and the examined preparation, Relpax® tablets containing 40 mg of eletriptan in a form of the hydrobromide, were obtained from Pfizer, H. C. P. Corporation. Water was deionised using a System Simplicity 185 (*Millipore*, U.S.A.).

All reagents were of analytical grade. Triethylamine (TEA) (Merck, Germany), methanol-gradient grade (Lab Scan, Ireland) and 85 % orthophosphoric acid (Carlo Erba, Italy) were also used.

Solutions

The stock solutions were prepared by dissolving the standard substances in methanol to obtain concentration of 2.00 mg/ml for eletriptan hydrobromide and 5.00 µg/ml for UK 120.413. A 1.00 mg/ml solution of phenobarbital as the internal standard in the mobile phase was also prepared.

Sample preparation

An accurately weighed quantity of ten finely powdered Relpax® tablets, equivalent to 100.00 mg of eletriptan hydrobromide, was transferred with 25 ml of methanol into a 50-ml volumetric flask. The film had previously been removed from the tablets. After sonicating and shaking the mixture for 25–30 min, it was made up to volume with the same solvent, mixed and passed through a Whatman 42 filter. An aliquot (1.50 ml) of this solution was transferred into a 10-ml volumetric flask, 0.50 ml of internal standard solution was added and made up to volume with the mobile phase solutin. The concentration of eletriptan hydrobromide was 0.30 mg/ml.

Chromatographic conditions

A Hewlett-Packard HP 1100 (Palo Alto, CA, USA) chromatographic system equipped with an HP 1100 binary pump and an HP 1100 UV-VIS detector. The sample was injected *via* a Rheodyne injector valve with a 20 µl sample loop and the detection was performed at 225 nm. A Waters XTerra^TM (5 µm, 150 mm × 4.6 mm) column was used. The mobile phase flow rate was 1.0 ml/min and the column temperature was 20 °C. The mobile phase consisted of an aqueous solution of TEA (pH 6.8, 1 %) – methanol (67.2:32.8 v/v). The mobile phase was filtered and degassed before use.

RESULTS AND DISCUSSION

The developed method was tested for accuracy, precision, repeatability, specificity, detection limit (LOD), quantification limit (LOQ), linearity, robustness and sensitivity.

The specificity of the method was investigated by observing potential interference between eletriptan hydrobromide and its impurity with tablet excipient (Fig. 2). No interfering peaks were present in the chromatograms.

The linearity of the relationship between the peak area and concentration was determined by analysing nine standard solutions over the concentration range $0.05 - 1.00$ mg/ml for eletriptan hydrobromide and $0.10 - 1.50$ µg/ml for UK 120.413. There samples (20 µL) of each of these solutions were injected into the chromatographic system. For all analytes, the relationship between the peak area ratio of drug to internal standard and concentration was highly linear over the entire examined concentration range (Table I). The correlation coefficients of the calibration curves being greater than $r = 0.995$. The relative standard deviations (*RSD*) and the standard errors of the slope, as well as of the intercept for both analytes confirm the excellent linearity.

TABLE I. Linear regressions analysis of eletriptan hydrobromide and UK 120.413

	Eletriptan hydrobromide	UK 120.413
Concentration range	$0.05 - 1.00$ mg/ml	$0.10 - 1.50$ µg/ml
$Y = ax + b$	$79.8566\,x - 0.0928$	$0.1346\,x + 0.0014$
r	0.9998	0.9972

TABLE I. Continued

	Eletriptan hydrobromide	UK 120.413
Concentration range	0.05 – 1.00 mg/ml	0.10 – 1.50 μg/ml
S_b	0.3499	0.0033
S_a	0.6022	0.0050
t_a	0.2651	0.4129
RSD	1.40%	2.65%

r – Correlation coefficient; S_a,S_b – standard deviations of the intercept and slope, respectively; t_a – calculated deviation value for the intercept; $t_{0.05}$ – 2.821; *RSD* – relative standard deviation
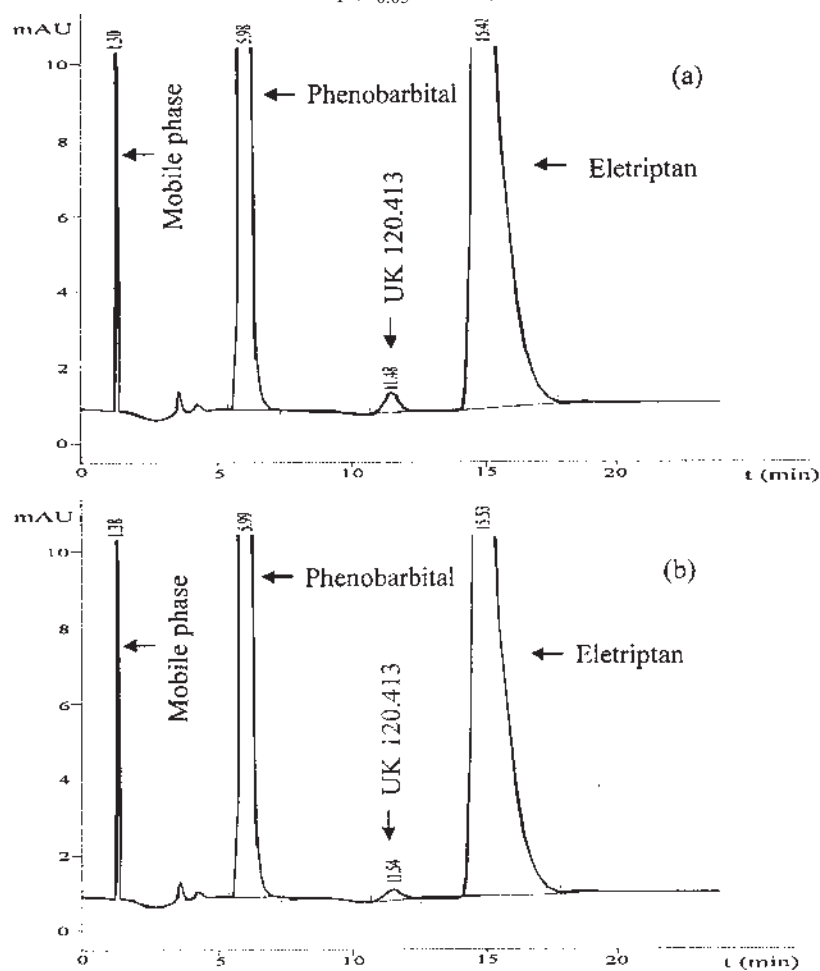

Fig 2. HPLC Chromatogram of the working solution of eletriptan, UK 120.413 standard substance and phenobarbital as the internal standard (a) and HPLC chromatogram of the solution of eletriptan, UK 120.413 from the sample of Relpax® tablets with phenobarbital as the internal standard (b); Mobile phase: TEA (pH 6.8, 1 %) – methanol (67.2:32.8 v/v), column temperature 20 °C and flow rate 1.0 ml/min.

By analysing seven solutions of three different concentrations for each analyte, the precision (repeatability) of the chromatographic procedure was assessed. The low value of the *RSD* (Table II) indicates satisfactory repeatability of the method.

TABLE II. Precision of the RP HPLC method

Compound	Injected	Found	RSD/%	R/%
Eletriptan hydrobromide/(mg/ml)	0.200	0.204 ± 0.004	1.90	101.98
	0.300	0.303 ± 0.003	0.95	101.07
	0.400	0.391 ± 0.009	2.25	97.68
UK 120.413/(µg/ml)	0.600	0.551 ± 0.008	1.42	91.86
	0.900	0.901 ± 0.022	2.42	100.10
	1.200	1.140 ± 0.037	3.28	94.98

$S(n = 7)$

The limit detection (LOD) was measured as the lowest amount of analyte that may be detected to produce a response which is significantly different from that of a blank. The limit of detection was derived from the lowest concentration which could be detected with reasonable certainty. It was calculated as the ratio of the response (σ) and the slope (S) of the calibration curve at the levels approaching the limits according to equation LOD = 3.3 (σ/S).[11] The LOD for eletriptan hydrobromide was 5 ng/ml and 10 ng/ml for UK 120.413. The limits of quantification (LOQ), calculated as the lowest amount of analyte which can be reproducibly quantified above the baseline noise with a *RSD* ≤ 3 % for replicate injections, were 10 ± 1.4 ng/ml and 30 ± 2.65 ng/ml for eletriptan and UK 120.413, respectively.

According to ICH, the robusstness of an analytical method refers to its capability to remain unaffected by small and deliberate variations in the method parameters. Experimental design was used to evaluate the robustness of the method in order to study the simultaneous variation of the factors on the considered response.[12–17] Recognizing that HPLC separation can be influenced by many factors, such as the composition of the mobile phase, the pH of the mobile phase, temperature, flow rate, stationary phase properties, *etc,* it is important to precisely define the factors having the greatest effect and their domains. It was experimentally confirmed that the following chromatographic parameters should be closer examined as variables: the pH of the mobile phase (factor X_1), the composition of the mobile phase (the concentration of methanol) (factor X_2) and the column temperature (factor X_3). To screen the relative influence of each of the factors and their possible interactions in the experimental domain, central composite design was applied.

The central composite design is built up of a full factorial 2^k design to which a star design is added. The central composite design is completed by the addition of a center point. The total number N of experiments with k factors is: $N = 2^k + 2\,k + c$. The first term is related to the full factorial design, the second to the star points and

the third to the center point. When three factors are to be considered, at least $8 + 6 + 1 = 15$ experiments are necessary. Replicates of the center point allow an accurate evaluation of the experimental error, hence enabling the significance of the effects to be estimated. Considering $k = 3$ factors, the experimental domain has the compositon of a cube, the eight corner points of which represent the locations of the experiments. The lengty of the arms of the star plays a major role in the appearance of the central composite design. If the length his in the middle of the range of the investigated factors, the star points lie on the faces of the cube and the experimental domain is the same as defined by a 2^k full factorial design. This kind of design is called a face-centred cube design.

The ranges investigated during the robustness testing of the proposed RP HPLC method were: column temperature; 20 °C, 25 °C and 30 °C, methanol in the mobile phase 32 %, 33% and 34 % and 6.2, 6.5 and 6.8 pH of the mobile phase.

The relationship between the inputs (for the three factors) and the output in the central composite design can be presented as a second order polynom of the following from: $y = b_0 + b_1 x_1 + b_2 x_2 + b_3 x_3 + b_{12} x_1 x_2 + b_{13} x_1 x_3 + b_{23} x_2 x_3 + b_{11} x_1^2 + b_{22} x_2^2 + b_{33} x_3^2 + b_{123} x_1 x_2 x_3$.

In order to obtain a better idea of the significance of the influence of a factor, it is useful to put each variable on a comparable scale, *i.e.,* it is common to code the experimental data. The highest coded value of each variable equals $+1$ and the lowest -1. The central point of each factor is 0 and the design is symmetric around this value. Then, the corresponding regression coefficients are approximately on the same scale. Provided that the data are correctly coded, the larger the coefficient, the greater is its significance.[18] The model matrix for a face-centred cube design for three factors and the capacity factors for the investigated substances as the observed responses are presented in Table III.

TABLE III. Model matrix for a face-centred cube central composite design for three factors and the capacity factors k' for the investigated substances

Exp. No.	Factors			$k'_{\text{Eletriptan}}$	$k'_{\text{UK 120.413}}$
1	+1	+1	+1	6.84	0.00
2	+1	+1	−1	8.80	6.36
3	+1	−1	+1	6.42	0.00
4	+1	−1	−1	8.04	5.68
5	−1	+1	+1	8.52	6.43
6	−1	+1	−1	10.95	7.72
7	−1	−1	+1	7.50	0.00
8	−1	−1	−1	10.48	6.85
9	+1	0	0	7.72	0.00
10	−1	0	0	9.12	6.58
11	0	+1	0	10.24	7.43

TABLE III. Continued

Exp. No.	Factors			$k'_{\text{Eletriptan}}$	$k'_{\text{UK 120.413}}$
12	0	−1	0	7.70	5.52
13	0	0	+1	7.22	0.00
14	0	0	−1	9.32	6.48
15	0	0	0	8.24	6.06
16	0	0	0	8.23	6.07
17	0	0	0	8.20	6.07
18	0	0	0	8.24	6.04

Exp. No.: 1–8, 9–14 and 15–18 are Full factorial design, Star points and center point with replicates, respectively

For calculating coefficients, MATLAB 6.5 was used and the results are presented as the following polynoms:

$$k'_{\text{Eletriptan}} = 8.373 - 0.875\, x_1 + 0.521\, x_2 - 1.109\, x_3 - 0.039\, x_1 x_2 + 0.229\, x_1 x_3 + 0.026\, x_2 x_3 - 0.098\, x_1^2 + 0.453\, x_2^2 - 0.248\, x_3^2 - 0.111\, x_1 x_2 x_3$$

$$k'_{\text{UK 120.413}} = 5.442 - 1.554\, x_1 + 0.989\, x_2 - 2.666\, x_3 - 0.828\, x_1 x_2 - 0.488\, x_1 x_3 + 0.681\, x_2 x_3 - 1.534\, x_1^2 + 1.651\, x_2^2 - 1.584\, x_3^2 - 0.780\, x_1 x_2 x_3$$

$k'_{\text{Eletriptan}}$ and $k'_{\text{UK 120.413}}$ are capacity factors for the investigated substances.

The obtained values for the coefficients indicate that the column temperature (factor X_3) and the content of methanol in the mobile phase (factor X_1) have the greatest impact on the chromatographic behaviour of the system. On the other hand, the separation and capacity factors were less sensitive to variations in the pH of the mobile phase (factor X_2). In order to maximise the chromatographic performance and to obtain a better understanding of the separation process, it is always recommended to attempt to observe the system graphically. Hence, and additional ten experiments were performed and ten new solutions were examined at 20, 25 and 30 °C column temperature, with 32, 33 and 34 % of methanol in the mobile phase, with a replicate at the mid-point (25 °C temperature and 33 % of methanol). Being the factor having the least influence, the pH was held constant in these experiments. Based on the results from these experiments, 3D-graphs were constructed, Fig. 3, and the data was completed with theoretical equations which correlate the capacity factors of eletriptan and UK 120.413 with the most important chromatographic conditions: $k'_{\text{Eletriptan}} = -1577.303 + 97.215(X_1) - 0.050(X_2) - 1.485(X_1^2) - 0.001(X_2)^2 - 0.002(X_1)(X_2)$; $k'_{\text{UK 120.413}} = -3339.786 + 200.835(X_1) + 6.092(X_2) - 2.984(X_1^2) + 0.040(X_2)^2 - 0.254(X_1)(X_2)$. X_1 is content of methanol in the mobile phase (%); X_2 is column temperature (°C).

In can be seen that the effect of the temperature depends on the concentration of methanol for the chromatographic behavior of UK 120.413 while this interaction is less significant in the case of eletriptan. Therefore, fine tuning of the temperature and the mobile phase composition can improve the separation between these two analytes.
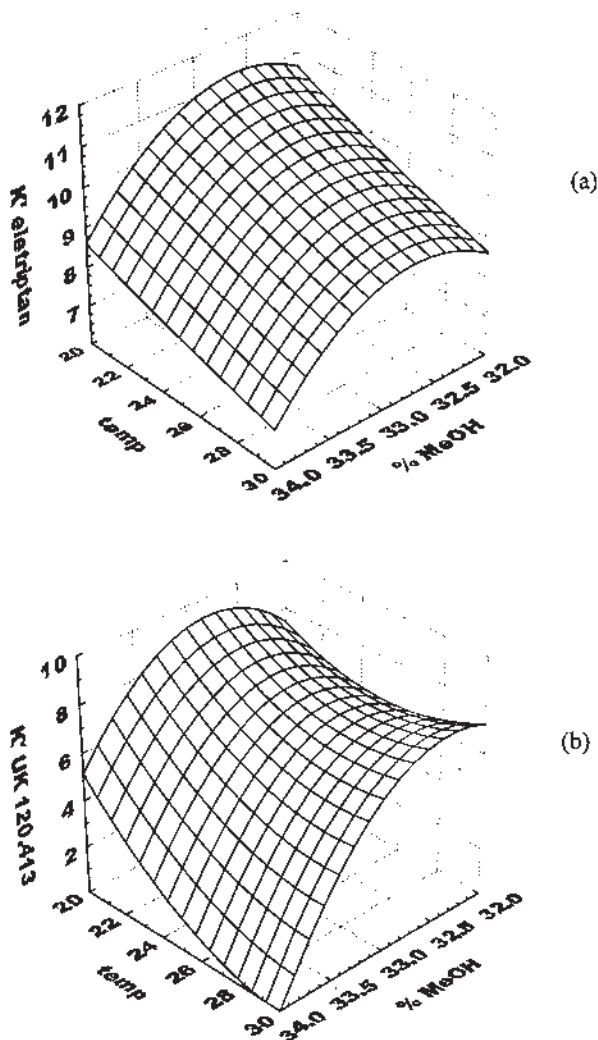
Fig 3. Three-D plots of the response surface for the capacity factors k' for eletriptan (a) and the impurity UK 120.413 (b): variation of the response k' as a function of % methanol and temperature; fixed factor pH 6.8.

This was confirmed by plotting the capacity factors as a function of the methanol content and the column temperature. A good chromatographic behaviour requires capacity factors to be neither too low (tendency of the molecules to be in the mobile phase rather than in the stationary phase), nor too high (long analysis time). It seems that, due to the similar lipophilic/hydrophilic characteristics of both substances, increasing the content of methanol is followed by a reduction in the retention times and capacity factors. However, the combination of higher methanol contents and higher column temperature results in an un satisfactory separation. Thus, good separation and optimum run time could only be assessed with lower levels of the investigated factors.

For the analysis of the variance, the ANOVA method was used to analyse the results in order to obtain an adequate elution model.[5] Since the chosen factor had a

significant effect on the responses, that is on the capacity factors, for eletriptan hydrobromide and UK 120.413, the variance in the data set asigned to the factors was larger than that of the residuals. It was confirmed by the Fisher variance ratios for significance of the regression, *i.e.,* significance of the factor effect. According to the data set for eletriptan hydrobromide and UK 120.413, F = 33.52 and F = 7.17, respectively. (F_{crit} = 6.26) and it was significant at the 95 % level of confidence. The test for the lack of fit was used to compare the variance due to the lack of fit with the variance due to purely experimental uncertainty. The data showed F_{lof} = 174.35 for eletriptan hydrobromide and F_{lof} = 207.52 for UK 120.413 (F_{crit} = 215.70) and it was not significant. Therefore, it can be concluded that there was no significant amount of variation in the measured responses and the measured responses could be explained by the model. As was to be expected, the residuals were very small. The values of R^2 = 0.977 for eletriptan hydrobromide and R^2 = 0.900 for UK 120.413 indicate that the factors explained the data very well. Taking the degrees of freedom into account, they were adjusted to R^2 = 0.948 for eletriptan hydrobromide and R^2 = 0.774 for UK 120.413.

TABLE IV. Determination of the substances under study in Relpax[®] tublets

Compound	Taken/mg mL^{-1}	Found/mg mL^{-1}	Found/(mg/tbl)	*RSD*/%	*R*/%
Eletriptan hydrobromide	0.300	0.303 ± 0.003	48.62 ± 0.99	2.04	100.33
Compound	MAC/μg mL^{-1}	Found/μg mL^{-1}	Found/%	*RSD*/%	
UK 120.413	0.900	0.061	0.02	5.51	

S(*n* = 10); MAC – Maximum allowed content; *RSD* – Relative standard deviation; *R* – Recovery value

The validated method was then applied to assay eletriptan hydrobromide and UK 120.413 in Relpax[®] tablets. A summary of the results is presented in Table IV. The content of eletriptan hydrobromide was 100.33 % and the content of the impurity was lower than 0.2 %. The good recovery and low *RSD* confirm the suitability of the proposed RP HPLC method.

CONCLUSIONS

An isocratic method for the RP HPLC separation of eletriptan hydrobromide and UK 120.413 was developed. The metod is simple, selective, repeatable, linear and sensitive. The method robustness was demonstrated using experimental design techniques, taking into consideration the selectivity of the RP HPLC method. The central composite design method was first employed to evaluate the important chromatographic variables and further investigation of the robustness was carried out by application of a response surface design. The described method can be used for the simultaneous determination of eletriptan hydrobromide and its impurity product in bulk drug and in pharmaceutical products in routine control analysis.

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ВАЛИДАЦИЈА HPLC МЕТОДЕ ЗА ИСТОВРЕМЕНО ОДРЕЂИВАЊЕ ЕЛЕТРИПТАНА И UK 120.413

МИРА ЗЕЧЕВИЋ[*1], БИЉАНА ЈОЦИЋ[1], СНЕЖАНА АГАТОНОВИЋ-КУШТРИН[2] и ЉИЉАНА ЖИВАНОВИЋ

[1]*Инсūиūуū за фармаколошку хемију и анализу лекова, Фармацеуūски факулūеū, Војводе Сūеūе 450, ū. ūр. 146, 11000 Беоīрад и* [2]*Pharmacy M315, School of Biomedical, Biomolecular and Chemical Sciences, The University of Western Australia, Crawley, W. A., 6009, Australia*

У овом раду је представљена брза и осетљива RP HPLC метода намењена за рутинско испитивање и контролу елетриптан хидробромида и његове органске нечистоће UK 120.413 у Relpax® таблетама. Хроматографски поступак је изведен уз коришћење колоне $C_{18 \text{ XTerra}}$™ (5 μm, 150 × 4.6 mm) при протоку мобилне фазе од 1.0 ml/min и на 20 °C, а детекција лековите супстанце и њене нечистоће је вршена на 225 nm. Мобилна фаза се састојала из смеше TEA (1 %) – метанол (67,2:32,8 v/v), pH водене фазе је подешен на 6.8 са 85 % ортофосфорном киселином. Квантитативна анализа је вршена методом интерног стандарда. Предложена RP HPLC метода је валидирана, а испитивани су тачност, прецизност, поновљивост, специфичност, лимит детекције, лимит квантификације, линеарност, робустност и осетљивост методе. Висока линеарност аналитичког поступка је потврђена у опсегу концентрација 0,05–1,00 mg/ml за елетриптан хидробромид и 0,10–1,50 μg/ml за UK 120.413, са коефицијентима корелације који су већи од $r = 0,995$. Ниска вредност релативне стандардне девијације потврђује добру поновљивост и прецизност методе. Експериментални дизајн и метода површине одговора система су коришћени у току испитивања робустности да би се проценио утицај варирања вредности хроматографских параметара методе. Тест робусности је обухватао састав мобилне фазе, pH и температуру у малим варирањима вредности око номиналне. Добре "recovery" вредности и ниска релативна стандардна девијација потврђују да је предложена RP HPLC погодна за рутинско одређивање елетриптан хидробромида и његове нечистоће UK 120.412 у Relpax® таблетама.

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