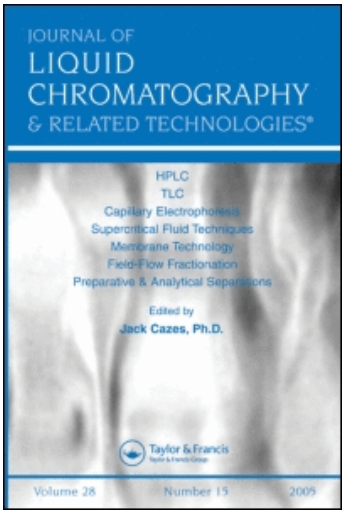

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Chemometrical Approach in Lansoprazole and Its Related Compounds Analysis by Rapid Resolution RP-HPLC Method

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Abstract: Lansoprazole has been determined in the presence of its five related compounds by the Rapid Resolution RP-HPLC method, using Zorbax Eclipse XDB C18 4.6 mm × 50 mm, 1.8 μm particle size column at 25°C and acetonitrile-1% TEA (41.0:59.0 v/v) at pH 6.6 as mobile phase. The total time for chromatographic separation was approximately 3 min. Experimental design was used for experimental screening (Full factorial 2^3 design) and optimization (Response surface methodology). The quality of separation is measured by resolution (Rs) and a Chromatographic Response Function. The method was validated for its selectivity, linearity, precision, and accuracy.

Keywords: Experimental design, Impurities, Lansoprazole, Rapid Resolution RP-HPLC

INTRODUCTION

Lansoprazole belongs to a class of antisecretory compounds that suppress gastric acid secretion by specific inhibition of the

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(H^+, K^+) – ATP ase enzyme system at the secretory surface of the gastric parietal cell. Because this enzyme system is regarded as the acid (proton) pump within the parietal cell, lansoprazole is characterized as a gastric acid pump inhibitor, as it blocks the final step of acid production. This effect is dose related and leads to inhibition of both basal and stimulated gastric acid secretion irrespective of the stimulus.[1]

Lansoprazole (**LS**), is a substituted benzimidazole with the chemical name 2-[[[3-methyl-4-(2,2,2-trifluoroethoxy)-2-pyridyl] methyl] sulfinyl] benzimidazole. For lansoprazole, five related compounds are specified as impurities: 2-[[[3-methyl-4-(2,2,2-trifluoroethoxy-2-pyridil]methyl]thio]-1H- benzimidazole (sulfideanalogue, **LN1**), 2-[[[3-methyl-4-(2,2,2-trifluoroethoxy-2-pyridil]methyl]sulfonyl]-1H-benzimidazole (sulfone analogue, **LN2**), 2-[[[3-methyl-4-(2,2,2-trifluoroethoxy-2-pyridil-1oxide]methyl]sulfonyl]-1Hbenzi-midazole (N-oxide analogue, **LN3**), 2-mercaptobenzimidazole (**MBI**), and 2- hydroxybenzimidazole (**HBI**). LN1, LN2, LN3, HBI, and MBI can be formed during the

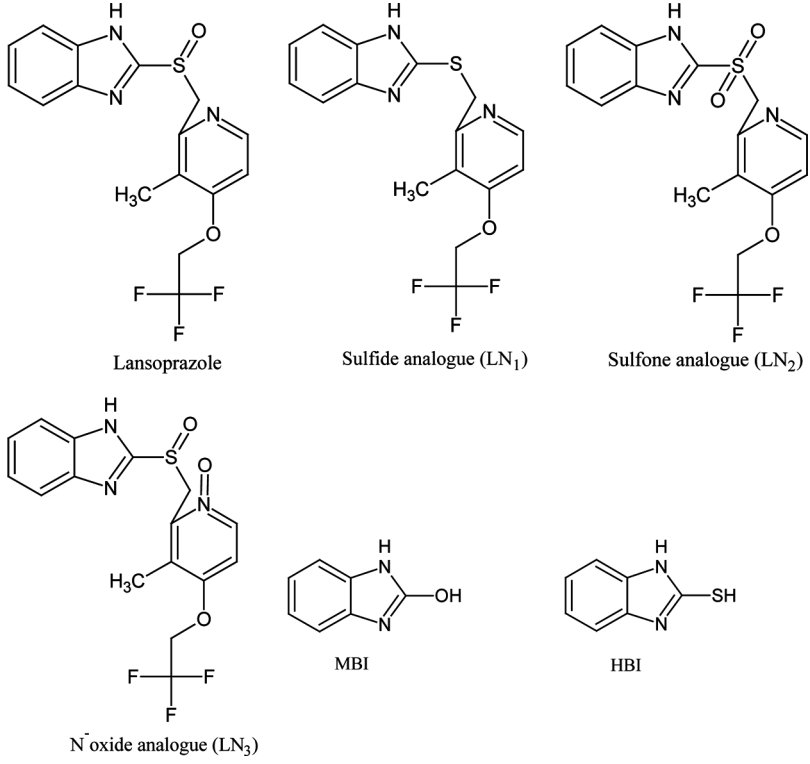

Figure 1. Chemical structure of lansoprazole and related substances.

manufacturing process, or by degradation of lansoprazole under acidic conditions or on exposure to light.[2] The chemical structure of lansoprazole and related compounds is shown in Figure 1. A literature search has shown same spectrophotometric methods for the determination of lansoprazole,[3,4] an anodic voltammetry assay for determination of lansoprasole,[5] a capillary electrophoresis method,[6] an RP HPLC method for determination of lansoprazole and its metabolites in plasma,[7,8] RP-HPLC methods for the determination of lansoprazole and related compounds sulfide analogue (LN1) and sulfone analogue (LN2) in pharmaceutical formulations.[9,11] USP 29 describes a method for determination of lansoprasole and sulfone analogue (LN2).[12] The aim of this investigation was development of a rapid, simple, robust rapid resolution RP-HPLC (RR RP-HPLC) method for simultaneous determination of lansoprazole and its impurities using design of experiments (DoE) approach. Since there are no references in the present literature concerning simultaneous determination of lansoprazole and the above mentioned related substances, the proposed method is a significant advance in pharmaceutical analyses.

EXPERIMENTAL

Chemicals and Reagents

All reagents used were of at least analytical grade. Acetonitrile, (ACN), gradient grade, (Merck, Darmstadt, Germany), water (HPLC grade), triethylamine (TEA) analytical grade, (Merck, Darmstadt, Germany), and 85% orthophosphoric acid (analytical grade, Merck, Darmstadt, Germany) were used to prepare the mobile phase. Lansoprazole, LN1, LN2, LN3, HBI, and MBI were kindly supplied by Krka d.d. Novo Mesto, Slovenia.

Standard Solutions

Standard stock solutions were prepared by dissolving the respective working standard substances in a mixture of ACN:1%TEA (40:60 v/v) to obtain the concentration of 0.1 mg mL^{-1} for lansoprazole and 10 μg mL^{-1} for LN1, LN2, LN3, HBI, and MBI. The standard stock solution was diluted with the same solvent to obtain working standard solutions.

Validation Procedure

In order to study linearity of the response, a series of working standard solutions of 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20 $\mu g\,mL^{-1}$ for lansoprazole and of 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, and 0.1 $\mu g\,mL^{-1}$ for each related compound were prepared. Five determinations were carried out for each solution. The linearity of peak area responses versus concentrations was studied. The correlation graph was constructed by plotting the peak areas obtained at the optimized conditions. Precision and accuracy were assessed using three different working standard solutions (5 $\mu g\,mL^{-1}$, 10 $\mu g\,mL^{-1}$, and 15 $\mu g\,mL^{-1}$ for lansoprazole; 0.02 $\mu g\,mL^{-1}$, 0.05 $\mu g\,mL^{-1}$ 0.08 $\mu g\,mL^{-1}$ of each related compound). Five determinations were carried out for each solution. For wavelength selection, standard solutions of 10 $\mu g\,mL^{-1}$ for lansoprazole and 0.05 $\mu g\,mL^{-1}$ of each related compound were prepared.

Test Solutions

To prove the validity and applicability of the proposed RR RPHPLC method, and for the optimization procedure, a standard solution containing a mixture of lansoprazole and related compounds in a concentration ratio corresponding to the recommendations by the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH)[13] was used. The standard solution contained 10 $\mu g\,mL^{-1}$ lansoprazole, and 0.05 $\mu g\,mL^{-1}$ (corresponding to 0.5% of the concentration of lansoprazole) of each related compound was prepared using standard stock solutions. In all experiments, the test solution was used to acquire chromatograms and injections of individual samples (containing only one compound) were used for peak identification. Only chromatograms acquired with the test solution were used in the calculation of chromatographic responses.

Apparatus

The chromatographic system Agilent RR HPLC system, 1200 series (consisting of binary pump SL, DAD detector SL, and column compartment TCC SL) was used. The samples were introduced through a high performance auto sampler SL (HIP-ALS SL). A single UV absorbance was measured at 285 nm. The peak areas were integrated automatically with Windows NT based LC ChemStation Software. The Mat Lab software was used for generation and evaluation of the experimental design.

Chromatographic Conditions

Separations were performed using a Zorbax Eclipse XDB C18 rapid resolution high throughput (RR HT) 4.6 mm × 50 mm, 1,8 μm particle size column at 25°C. A flow rate of 1.0 mL min^{-1} was used. Wavelength was selected by scanning working standard solutions of all investigated compounds over 200 to 400 nm wavelengths. All measurements were made with 10 μL injection volume and UV detection at 285 nm as all components showed reasonably good response at this wavelength. Mobile phases were prepared using acetonitrile and water–triethylamine (TEA) mixtures. For the optimization procedure, the acetonitrile content in the mobile phase was varied between 30–50% (v/v). The water-TEA solutions with 0.5%, 1.0%, and 1.5% (v/v) were used. Solutions were prepared by mixing 5 mL, 10 mL, and 15 mL of TEA with 995 mL, 990 mL, and 985 mL water HPLC grade, respectively. The pH values of the TEA solutions were varied between 5.0 and 7.0. pH values were adjusted with 85% ortophosphoric acid before addition of ACN. The mobile phase was filtered through a 0.2 μm Millipore filter.

RESULTS AND DISCUSSION

Optimization

Chromatographic behavior of lansoprazole and its related substances was examined using mobile phases containing methanol-water and acetonitrile–water at various ratios, and considerable difference between the separation activity of methanol and acetonitrile was not observed. A clear separation could not be obtained because HBI, MBI, and LN3 coeluted and the LS peak was not resolved from the LN2 peak. Concerning basic characteristics of investigated substances, peaks showed tailing and bad symmetry. The addition of TEA in the water phase enabled a better separation and a better peak symmetry was obtained as well. pH adjustment of the mobile phase before adding an organic solvent was necessary. It was observed that pH variations between 6.0 and 9.0 did not significantly affect retention or quantification as expected, considering the pKa value of lansoprazole (pKa = 8.8) and structure similarity of related compounds. Acidic mobile phases were not suitable since lansoprazole is an acid labile substance.[9] As the objective of the method was to resolve lansoprazole and related impurities in a short analysis time with no compromise in resolution, sensitivity, and robustness, the Eclipse XDB C18 rapid resolution high throughput, 4.6 mm × 50 mm, 1.8 μm particle size column was preferred. The Eclipse XDB column is extra densely bonded and double end capped and it

can be used over a wide pH range. Columns with short (50 mm) length and 1.8 μm particle sizes provide very high resolution in a short analysis time.[14,15] On the basis of preliminary investigations, it was confirmed that acetonitril content (v/v %), triethylamine content (v/v %) in the mobile phase and pH value of mobile phase were the important factors affecting separation. In the case of a limited number of investigated factors (up to four) full factorial design is usually employed for screening experiments. Screening experiments are important to precisely define how the important factors affect the method, their useful ranges, and interactions between investigated factors. For a typical factorial design, it is necessary to determine how many factors are of interest and how many levels of each factor are studied. A k factor, l level design involves performing $N = l^k$ experiments.[16] Full factorial design at two levels is mainly used for screening so as to determine the influence of a number of effects on a response and to eliminate those that are not significant. The next stage undertaken is usually a more detailed study, e.g., optimization. For screening investigations of lansoprazole and related impurities a full factorial design at two levels ($l = 2$) was chosen. According to this a 2^3 full factorial design was applied and the levels of factors, upper ("+") and lower ("−") were defined (Table 1.)

Thus, the experimental domain was defined (there is of course no guarantee that the result will be valid outside this region). A zero level (center) in which are all variables are fixed at their mean value, is included in order to minimize the risk of missing non-linear relationships. This experiment is not included in the calculation of coefficients.[17] Nine experiments were performed on the basis of matrix presented in Table 2.

For estimation of the system response the resolution (Rs) between peak pairs was chosen. Selecting Rs way of measuring response was done in order to avoid inaccurate conclusions, e.g., selecting wrong separation parameters in case of overlapping peaks. After performing the experiments, Rs was calculated for all the obtained peak pairs. The total number of detected peak pairs was five: (1) HBI/MBI; (2) MBI/LN3; (3) LN3/LS; (4) LS/LN2; (5) LN2/LN1. These results are presented in Table 3.

Table 1. Factors and their lower (−), upper (+) and zero (0) levels

		Factor levels		
	Factors	(−)	(+)	(0)
x_1	%(v/v) TEA in mobile phase	0.5	1.5	1.0
x_2	pH of mobile phase	5.0	9.0	7.0
x_3	% (v/v) ACN in mobile phase	30	50	40

Table 2. Matrix for experiments for a full factorial design 2^3. Combinations of low (−), high (+) and zero level of factors

	Factors		
Exp. no.	x_1	x_2	x_3
1	−	−	−
2	+	−	−
3	−	+	−
4	+	+	−
5	−	−	+
6	+	−	+
7	−	+	+
8	+	+	+
9	0	0	0

In experimental design, a linear mathematical model of the measured response is often applied for the evaluation of the influence of the investigated factors. An often used linear model is:

$$y = b_o + b_1x_1 + b_2x_2 + b_3x_3 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3 + b_{123}x_1x_2x_3 \quad (1)$$

where y presents the estimated response, b_o is the average experimental response, coefficients b_1, b_2 and b_3 are the estimated effects of the factors considered. The coefficients b_{12}, b_{13}, b_{23}, and b_{123} are called interaction terms. In this way, the factorial design provides information about the importance of interaction between factors.[18] The number of coefficients

Table 3. The *Rs* values of the eluted peak pairs

	Experimental values of Rs				
	Obtained peak pairs				
Exp. no	HBI/MBI	MBI/LN3	LN3/LS	LS/LN2	LN2/LN1
1	1.30	1.08	7.53	1.49	15.06
2	1.13	1.11	8.20	1.57	13.39
3	1.28	1.50	8.28	1.56	13.34
4	1.45	2.05	8.44	2.34	16.63
5	1.54	1.69	7.90	1.51	12.39
6	1.48	2.04	8.64	1.58	12.84
7	1.53	1.80	7.91	1.49	12.49
8	1.38	1.23	7.65	1.75	11.90
9	1.19	2.25	8.28	2.30	16.68

Table 4. Matrix for calculating coefficients

	Coefficients							
Exp. no.	b_1	b_2	b_3	b_{12}	b_{13}	b_{23}	b_{123}	b_0
1	−	−	−	+	+	+	−	+
2	+	−	−	−	−	+	+	+
3	−	+	−	−	+	−	+	+
4	+	+	−	+	−	−	−	+
5	−	−	+	+	−	−	+	+
6	+	−	+	−	+	−	−	+
7	−	+	+	−	−	+	−	+
8	+	+	+	+	+	+	+	+
divisor	4	4	4	4	4	4	4	8

is equal to the number of experiments (in our experiment 8). The zero level experiment was not included in the calculation of coefficients. Also, b_o is the intercept of the linear model, b_1, b_2, and b_3 are the main effects, b_{12}, b_{13}, and b_{23} are two–factor interactions, and b_{123} is a three factor interaction. For calculating the coefficients, the matrix given in Table 4 was applied. The values of obtained coefficients are listed in Table 5.

The values of coefficients b_2 for the second (MBI/LN3) and the last (LS/LN1) peak pair, and especially values of coefficients b_3 for all peak pairs, demonstrates that separation of the investigated substances, as measured by the Rs values, is most affected by the acetonitrile content (factor x_3) and the pH of the mobile phase (factor x_2). The value of the coefficients for the two-factor interaction, b_{23} for the first (HBI/MBI) and second (MBI/LN3) peak pairs confirmed the main factor effects. Content of TEA in the mobile phase had the lowest influence on investigated system responses, so in further experiments it was kept constant at 1.0% TEA (v/v). In order to investigate the chromatographic behavior of the investigated substances for the given experimental range, and to define the optimum separation conditions further, optimization

Table 5. The values of obtained coefficients

	The values of obtained coefficients							
The peak pairs	b_o	b_1	b_2	b_3	b_{12}	b_{13}	b_{23}	b_{123}
HBI/MBI	1.661	0.048	0.076	−1.124	−0.128	−0.086	−0.839	−0.092
MBI/LN3	2.762	−0.227	0.792	−0.982	−0.262	0.047	0.612	0.505
LN3/LS	2.068	0.001	0.126	−0.793	−0.003	0.038	0.003	0.021
LS/LN2	1.213	−0.036	0.637	−1.085	0.028	−0.038	0.001	−0.001
LN2/LN1	3.225	0.010	0.060	−0.808	0.052	−0.045	−0.082	−0.072

of the method was performed using RSM. This is a collection of mathematical and statistical techniques useful for analyzing problems where several independent variables influence a dependent variable or response and the goal is to optimize this response.[19,20] A response surface can be defined as a graph in one dimension (one factor of interest) or a surface in two dimensions (two factors of interest) when a response variable is plotted as a function of one or more (quantitative) factors. A chromatographic response function (CRF) was used for optimizing the separation quality of lansoprazole and its impurities in such a way, that maximum resolution with the minimum assay time was obtained. The CRF is a coefficient which characterizes the quality of the separation in quantitative manner; preferably, a flexible function that allows desirable time and resolution criteria to be specified.[21,22] The corresponding terms in the chromatogram are then compared to these criteria. One such CRF can be represented by the following equation:

$$CRF = \Sigma R_i + L^a - b/T_m - T_l/ - c(T_1 - T_o) \tag{7}$$

where R_i is resolution between adjacent pairs of peaks. L is the total number of peak detected, T_m is an acceptable analysis time, T_l is the retention time of the last eluted peak, T_o is a specified minimum retention time and a, b, c are the arbitrary weighting factors. In this work a very simple, but very useful CRF is used:

$$CRF = \prod_{i=1}^{L-1} R_s(i, i+1) \tag{7b}$$

where $R_s(i, i+1)$ is the resolution between peak No. i and peak No. $i+1$.

A minimum obtained value of individual *Rs*-values of 2.5 as a selection criterion was used. The total number of detected peaks (L) was six.

As the screening experiments have shown that the two most important factors are acetonitrile content and pH value of mobile phase, these are simultaneously varied. Resolution (Rs) of obtained peak pairs were estimated for a combination of five pH values (5.0, 6.0, 7.0, 8.0, and 9.0) and six combinations of acetonitrile-1%TEA (v/v) ratio in mobile phase (33:67, 35:65; 38:62, 40:60, 43:57, and 45:55). CRF values were calculated for these 30 experiments (Table 6). Contour diagrams of the CRF (Figure 2a) and analysis time (Figure 2b) were constructed.

The results obtained using this methodology proposed clearly shows that it was possible to simultaneously, systematically optimize the influence of organic modifier content and pH of the mobile phase on the separation of the investigated substances. Evaluation of the influence of simultaneous variations of the factors on the considered

Table 6. The values of chromatographic response function

	Values of CRF				
	pH values				
% (v/v) ACN	5.0	6.0	7.0	8.0	9.0
33	13.76	29.67	34.45	24.34	25.89
35	17.52	37.81	40.91	25.56	25.63
38	16.30	45.15	48.08	20.87	23.09
40	15.53	47.96	52.11	21.48	21.11
43	13.98	40.38	54.54	24.65	25.24
45	14.05	36.43	53.60	17.85	22.15

response, at the same time, was a measure of the robustness of the method. When pH of the mobile phase is less than 6.0 and more than 9.0 the resolution between peaks are not adequate. Organic modifier content had the largest influence on resolution factor, yielding sufficient resolution and short analysis time in the range 40% and 45% ACN. Decreasing ACN content resulted in longer analysis times. Increasing ACN content leads to decreased analysis time, but also decreases the resolution between peaks. According to the experimental results, it was obvious that relatively small changes of ACN content and mainly mobile phase pH have a great influence on the separation. The best result, which corresponds to high values of the CRF was obtained using a

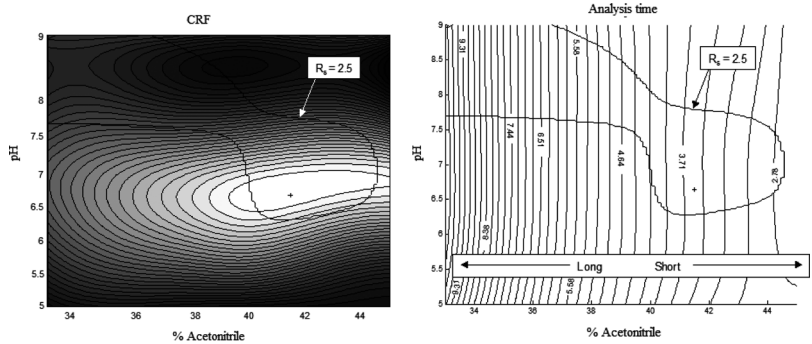

Figure 2. Contour diagrams of the CRF (a) and analysis time (b). The contour corresponding to all R_s-values ≥ 2.5 is marked. The point corresponding to the optimal chromatographic parameters is marked with "+" (41.0 % acetonitril, $pH = 6.6$).

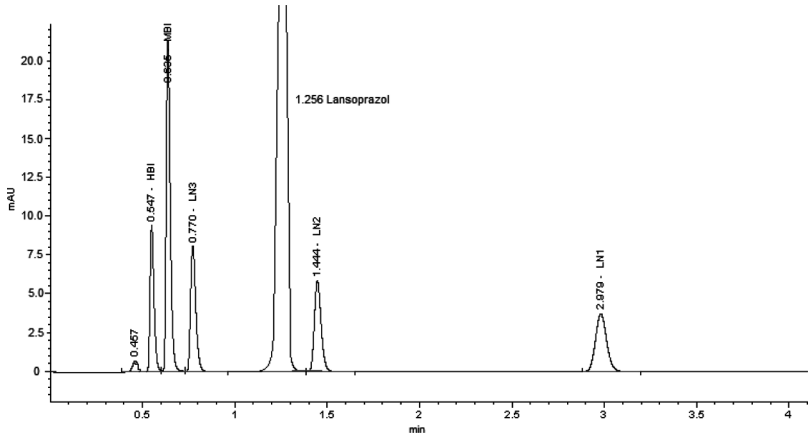

Figure 3. The chromatogram of the test solution, mobile phase ACN-1%TEA (41:59 v/v), pH = 6.6.

mobile phase composition of ACN-1%TEA (41:59 v/v) at a pH of 6.6. A representative chromatogram of the test solution is presented in Figure 3.

Resolution between peaks of HBI/MBI, MBI/LN3, and LS/LN2 were 2.52, 2.99, and 3.00, respectively. The peaks tailing were not observed. All respective compounds were clearly separated and their corresponding peaks were sharply developed. Separation was obtained in 3 min. The chromatographic separation parameters of a representative chromatogram are presented in Table 7.

Table 7. The chromatographic parameters for the representative chromatogram

	Eluted peaks					
	HBI	MBI	LN3	LS	LN2	LN1
t_r(min)	0.55	0.63	0.77	1.26	1.44	2.98
k	1.14	1.94	1.69	3.88	2.77	4.16
	Eluted peak pairs					
	HBI/MBI	MBI/LN3	LN3/LS	LS/LN2	LN2/LN1	
Rs	2.52	2.99	8.79	3.00	17.41	
α	1.40	2.54	3.67	2.68	5.54	

t_r-retention time, k-capacity factor, α-selectivity, Rs-resolution

Method Validation

After establishing the optimal conditions for the separation, the selectivity, linearity, limit of detection, limit of quantization, precision, and accuracy were determined for all the investigated substances.

Selectivity

The assay is selective as no significant interfering peaks are observed at the retention time of the investigated substances.

Linearity

Linear relationships of the peak area over the concentration range from 2 to 20 $\mu g\,ml^{-1}$ for lansoprazole and from 0.01 to 0.1 $\mu g\,mL^{-1}$ for each related compound were obtained. The linear regression analysis indicated that the response of the HPLC system was linear for all investigated substances. Limit of detection values were determined at a signal to noise ratio of 3 ($S/N = 3$) and limit of quantification values at *a* $S/N = 10$. Important calibration curve parameters: slope (a), intercept (b), correlation coefficient (R^2), limit of detection (LOD), and limit of quantification (LOQ) are present in Table 8.

Precision and Accuracy

The important statistical values, such as standard deviation (S) and coefficient of variation (CV), as well as good recoveries indicate that the assay was precise (standard deviations are very small; CV range from 1.30 to 1.57% for lansoprazole and from 1.19 to 2.58%

Table 8. Calibration parameters

Parameter	LS	LN1	LN2	LN3	HBI	MBI
a	2.36	0.07	0.03	0.09	0.29	0.03
b	16.28	79.74	77.75	82.13	166.54	70.554
R^2	0.9993	0.9990	0.9996	0.9989	0.9997	0.9994
LOD $\mu g\,ml^{-1}$	0.83	5.03×10^{-3}	9.05×10^{-3}	8.83×10^{-3}	6.85×10^{-3}	5.95×10^{-3}
LOQ $\mu g\,ml^{-1}$	2.50	$15,24 \times 10^{-3}$	27.59×10^{-3}	26.78×10^{-3}	19.56×10^{-3}	17.86×10^{-3}

a-intercept, *b*-slope, R^2-coefficient of correlation, LOD-limit of detection, LOQ-limit of quantification.

Table 9. Precision of the proposed RR RP-HPLC method

	Injected ($\mu g\,ml^{-1}$)	Determined ($\mu g\,ml^{-1}$)	CV (%)	R (%)
LS	5	4.97 ± 0.08*	1.59	99.40
	10	9.97 ± 0.13	1.32	99.70
	15	15.12 ± 0.19	1.30	100.8
	Injected ($ng\,ml^{-1}$)	Determined ($ng\,ml^{-1}$)	CV (%)	R (%)
LN1	20	19.81 ± 0.51	2.58	99.05
	50	51.32 ± 0.74	1.43	102.64
	80	81.15 ± 1.45	1.78	101.43
LN2	20	20.23 ± 0.45	2.23	101.15
	50	49.78 ± 0.78	1.56	99.56
	80	80.64 ± 1.95	2.41	100.8
LN3	20	19.93 ± 0.42	2.06	99.65
	50	50.65 ± 0.97	1.91	101.30
	80	80.39 ± 1.87	2.32	100.48
HBI	20	20.45 ± 0.34	1.67	102.25
	50	50.83 ± 0.75	1.47	101.66
	80	79.42 ± 2.05	2.58	99.27
MBI	20	19.85 ± 0.55	2.75	99.25
	50	49.67 ± 0.59	1.19	99.34
	80	79.97 ± 1.31	1.64	99.96

*S-standard deviation ($n = 5$).

for its related compounds and accurate (recovery values from 99.7 to 100.8% for lansoprazole and from 99.7 to 102.6 for its related compounds). Accuracy has been calculated as a percentage of the nominal concentration. The results for precision and accuracy of the proposed RR RP-HPLC method are given in Table 9.

CONCLUSION

The methodology proposed represents an efficient and easily accomplishable approach in resolving the problem of searching for optimum RR RP-HPLC conditions. The linear models obtained demonstrate a large influence of acetonitril content and pH of mobile phase to the resolution between investigated substances. Valuable information about retention modeling was obtained by RSM, using resolution as an important component of CRF. The developed isocratic RR RP-HPLC method permits simultaneous determination of haloperidol and its related compounds specified as impurities due

to good separation and resolution of the chromatographic peaks and robustness towards reasonable changes in chromatographic parameters. The method is a simple, rapid, and robust assay for impurity determination and can provide acceptable linearity, accuracy, precision, and selectivity. The method allows determination of haloperidol, purity and level of impurities in drug substance. Thus, the amount and purity of the active substance, the percent level of impurities, and a total chromatographic purity can be determined in a single analysis.

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