Scientific paper

Experimental Design in Chromatographic Analysis of Pramipexole and Its Impurities

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Abstract

Pramipexole is a dopamine D2-agonist/antiparkinsonian agent in which BI-II 546 CL, BI-II 751 xx and 2-amino benzothiazole are commonly found as impurities. Due to the lack of analytical data on pramipexole and its related substances in bulk drug and pharmaceuticals, we aim at the optimization and characterisation of the chromatographic behaviour of pramipexole and its related substances employing experimental design. The analysis was performed using a C18 column with mobile phases containing different ratios of acetonitrile and water phase (aqueous triethylamine/ortophosporic acid). The detection was performed at 262 nm for pramipexole, BI-II 751 xx and 2-aminobenzthiazole and at 326 nm for BI-II 546 CL. To define the influence of chromatographic parameters on separation, a central composite design was chosen. The content of acetonitrile, TEA and pH of the water phase were identified as the factors with important influences on retention. Using an appropriate mathematical model, we were able to predict retention under different conditions.

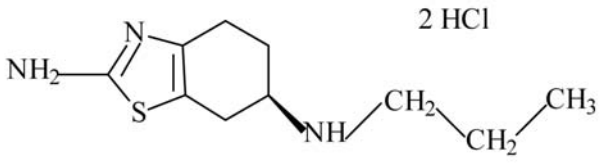
Keywords: pramipexole, impurity, central composite design, liquid chromatography

1. Introduction

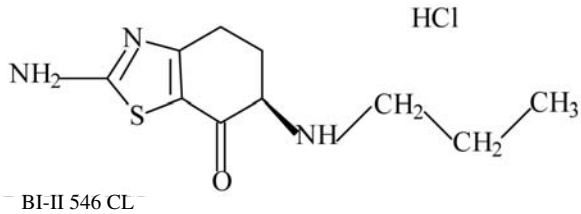
Pramipexole is a new drug used in therapy of Parkinson disease. Chemically it is (S)-2-amino-4,5,6,7-tetrahydro-6-(propylamino) benzothiazole. Chemical structures of active substances and its impurities are presented in Figure 1.

So far, only limited research was done concerning analysis of pramipexole. Analysis of pramipexole in biological samples was performed using HPLC with atmospheric pressure chemical ionization tandem mass spectrometry[1] and HPLC with electrochemical and UV detection.[2] Pramipexole and its enantiomer were separated using normal-phase HPLC.[3] There are no references concerning the analysis of pramipexole and its impurities in bulk substances and pharmaceuticals.

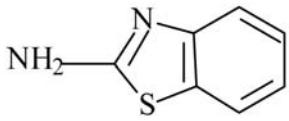
The aim of this study was the analysis of chromatographic separation of pramipexole, BI-II 546 CL, BI-II



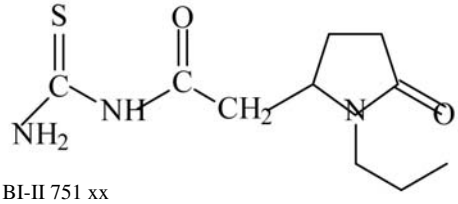
Pramipexole dihydrochloride



BI-II 546 CL



2-aminobenzothiazole



BI-II 751 xx

Figure 1. Structures of pramipexole and its impurities (2-aminobenzothiazole, BI-II 546 CL and BI-II 751 xx).

751 xx and 2-aminobenzothiazole using experimental design.

There are many experimental design applications in analytical method development and validation, especially in separation sciences. Experimental design has been used for optimisation of separation,[4–9] for validation in RP-HPLC[10–12] and for robustness testing in liquid chromatography[13–16] or in capillary electrophoresis.[17]

2. Experimental

2.1. Chemicals

All reagents used were of analytical grade. Acetonitrile (Lab Scan, Ireland), ortophosphoric acid (Carlo Erba Milan, Italy), triethylamine (TEA) (Acros Organics, Belgium) and water HPLC grade were used to prepare the mobile phase.

2.2. Chromatographic Conditions

The chromatographic system Waters Breeze was consisted of Waters 1525 Binary HPLC Pump, Waters 2487 UV/VIS dual absorbance detector and Breeze Software Windows XP for data collection. Separations were performed on the Zorbax Extend-C18 4.6 mm × 150 mm, 5 μm particle size column with UV detection at 262 nm for pramipexole, BI-II 751 xx and 2-aminobenzothiazole and at 326 nm for BI-II 546 CL. The flow rate was 1.0 mL min^{-1}. Temperature was 25 °C during all experiments. The samples were introduced through a Rheodyne injector valve with a 20 μL sample loop.

Mobile phases were prepared by mixing water phase (water, TEA and appropriate pH adjusted with orthophosphoric acid) with acetonitrile. pH was measured using PHM 210 standard pH meter, METERLAB®, Radiometer Analytical, Villeurbane Cedex, France. For the electrode calibration, standard buffer solutions were used (citrate buffer for pH = 4.0 ± 0.02 and phosphate buffer for pH = 7.00 ± 0.02 at 25 °C), by Radiometer Analytical, Villeurbane Cedex, France.

2.3. Standard Solutions

Laboratory mixture was prepared of 13 μg mL^{-1} of pramipexole, 6.5 μg mL^{-1} of BI-II 546 xx, 10 μg mL^{-1} of 2-aminobenzothiazole and 6 μg mL^{-1} of BI-II 751 xx in the appropriate medium (see Table 1).

Table 1: Factors and their low (–), high (+) and zero (0) values.

	Factor	Value		
		(–)	(+)	(0)
x_1	Acetonitrile (%)	5	15	10
x_2	pH of the water phase	2.5	4.0	7.0
x_3	Content of TEA (%)	0.6	1.0	0.8

3. Results and Discussion

To obtain an acceptable separation of basic compounds with good resolution and peak symmetry in a reasonable run time presents a problem because of strong interactions between the basic function and the free silanol groups of the non-polar stationary phase. Although many manufacturers produce columns with modified column packing suitable for the analysis of basic substances (stationary phases, which can be used at high pH values at which the basic compound exists in the non-ionized form), this is usually not enough to get expected results. At high pH values column lifetime is shortened. For this reason, water-phase additives are a better choice than high pH.

Pramipexole and its impurities are alkaline compounds, except BI-II 751 xx, so in their analysis TEA was chosen as the mobile phase additive. TEA blocks free silanol groups and provides better peak symmetry and better separation. Preliminary investigations included the analysis of chromatographic retention of the investigated substances on different non-polar stationary phases (C8 and C18) with various particle size and column length. Also, the influence of mobile phase polarity was investigated.

During the preliminary study, factors, which could have stronger influence, were extracted for further analysis (acetonitrile content, pH of the water phase and TEA content). The other factors, temperature (25 °C) and flow rate (1 mL min^{-1}) were kept constant during the study. For input factors, three value levels were defined, low, zero and high and are presented in Table 1.

The central composite design (CCD) was chosen. The CCD is build up of a full factorial 2^k designs to which

Table 2: Matrix of experiments for CCD.

		Factors		
	Exp. No	x_1	x_2	x_3
Full factorial design	1	+	+	+
	2	–	+	+
	3	+	–	+
	4	–	–	+
	5	+	+	–
	6	–	+	–
	7	+	–	–
	8	–	–	–
Star design	9	+	0	0
	10	–	0	0
	11	0	+	0
	12	0	–	0
	13	0	0	+
	14	0	0	–
Replications	15	0	0	0
	16	0	0	0
	17	0	0	0
	18	0	0	0

a star design is added. The CCD is completed by addition of a center point. The total number N of experiments with *k* factors is:

$$N = 2^k + 2k + c. \quad (1)$$

The first term is related to the full factorial design, the second to the star points and the third to the center point. From the repetition of the center point, the experimental variance at the center of the domain can be estimated. For three factors to be considered, at least 8 + 6 + 1 = 15 experiments are necessary.[18]

The matrix of experiment and results for retention factors obtained from 18 experiments (four experiments are replications) are presented in Tables 2 and 3, respectively, while in Figure 2 we present experiments for CCD in 3D. In Table 4 we present the predicted response values.

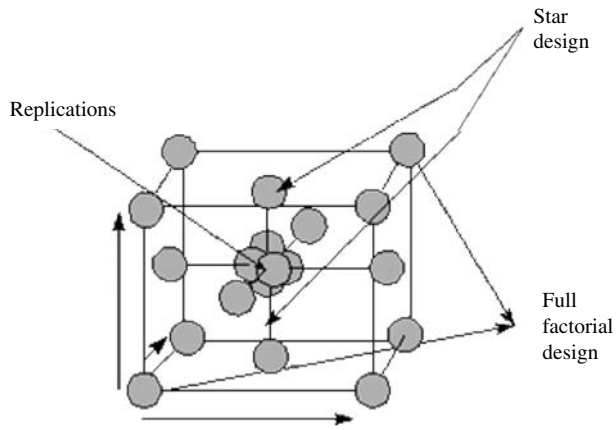


Figure 2. Presentation of the CCD experiments in 3D.

Table 3: Response factor values.

Exp. No.	Responses k_1	k_2	k_3	k_4
1	0.3186	1.684	2.705	9.526
2	1.260	10.944	17.309	35.570
3	–0.042	0.116	2.737	0.787
4	–0.210	0.514	20.434	2.332
5	0.1052	1.3818	2.050	8.154
6	1.616	14.517	17.892	34.681
7	–0.095	0.067	2.794	0.671
8	–0.201	0.6065	22.398	2.402
9	–0.251	–0.151	1.878	4.153
10	–0.224	0.4321	12.904	17.334
11	0.239	0.730	6.023	16.795
12	–0.2147	0.0035	6.213	1.312
13	–0.171	–0.005	5.231	12.080
14	0.0347	0.0369	5.093	10.074
15	–0.260	–0.020	5.720	7.285
16	–0.219	–0.013	5.759	7.589
17	–0.227	–0.020	5.759	7.628
18	–0.227	–0.020	5.767	7.667

Legend:
k_1 – retention factor of pramipexole,
k_2 – retention factor of BI-II 546 CL,
k_3 – retention factor of BI-II 751 xx,
k_4 – retention factor of 2-aminobenzothiazole.

Table 4: Predicted response values.

Exp. No.	Responses k_1	k_2	k_3	k_4
1	0.2933	1.5288	3.3654	9.4435
2	1.1312	9.7388	16.7873	35.3313
3	0.0343	1.1767	3.0151	1.6948
4	–0.2372	0.5247	19.5299	3.0836
5	0.1161	1.0688	2.5854	7.7856
6	1.5234	13.1545	17.2452	34.1565
7	0.0175	0.9704	2.9470	1.2929
8	–0.1920	0.4590	21.3689	2.8678
9	–0.4255	–1.6474	0.2511	3.0742
10	0.0156	3.1357	16.0057	16.8798
11	0.4749	3.7654	5.9957	18.0092
12	–0.3854	–1.8247	7.7151	–1.4352
13	–0.0660	0.2841	5.7183	10.7418
14	–0.0052	0.9550	6.0805	9.8792
15	–0.2658	–0.6218	5.0138	8.3088
16	–0.2658	–0.6218	5.0138	8.3088
17	–0.2658	–0.6218	5.0138	8.3088
18	0.2933	–0.6218	5.0138	8.3088

Legend:
k_1 – retention factor of pramipexole,
k_2 – retention factor of BI-II 546 CL,
k_3 – retention factor of BI-II 751 xx,
k_4 – retention factor of 2-aminobenzothiazole.

The relationship between inputs (for the three factors) and output can be presented as second order polynoms with the following equation:

$$y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3 + b_{11}x_1^2 + b_{22}x_2^2 + b_{33}x_3^2 + b_{123}x_1x_2x_3 \quad (2)$$

To assess the investigated system realistically, retention factor was chosen as output. MATLAB 6.5 was used for calculating the coefficients. In order to have a better idea of the significance of factor influences, it is useful to observe the variable on comparable scales, e.g. it is common to code experimental data. The obtained coefficients are approximately on the same scale and the significance could be determined by examining the magnitude of coefficients.[19] Provided that the data are coded correctly, a bigger value means greater significance. The results for the coefficients are presented in Eqs.(3–6) for pramipexole, BI-II 546 CL, BI-II 751 xx and 2-aminobenzothiazole, respectively:

$$y = -0.2658 - 0.2205x_1 + 0.4302x_2 - 0.0304x_3 + 0.0609x_1x_2 + 0.3105x_1x_3 + 0.2302x_2x_3 - 0.3408x_1x_2x_3 + 0.0789x_1^2 - 0.0233x_2^2 + 0.0634x_3^2, \quad (3)$$

$$y = -0.628 - 2.3915x_1 + 2.7950x_2 - 0.3355x_3 + 1.366x_1x_2 + 1.5922x_1x_3 + 1.2414x_2x_3 - 2.6824x_1x_2x_3 + 0.502x_1^2 - 0.4035x_2^{2+} + 0.4669x_3^2, \quad (4)$$

$$y = 5.0138 - 7.8773x_1 - 0.8597x_2 - 0.1811x_3 + 3.1146x_1x_2 + 1.8416x_1x_3 + 0.8856x_2x_3 + 0.8569x_1x_2x_3 + 0.3931x_1^2 + 0.2616x_2^2 - 0.0836x_3^2, \quad (5)$$

$$y = 8.3088 - 6.9028x_1 + 9.7222x_2 + 0.4313x_3 + 1.6682x_1x_2 - 0.0217x_1x_3 + 2.0018x_2x_3 - 6.1619x_1x_2x_3 + 0.0836x_1^2 + 0.2769x_2^2 + 0.0371x_3^2. \quad (6)$$

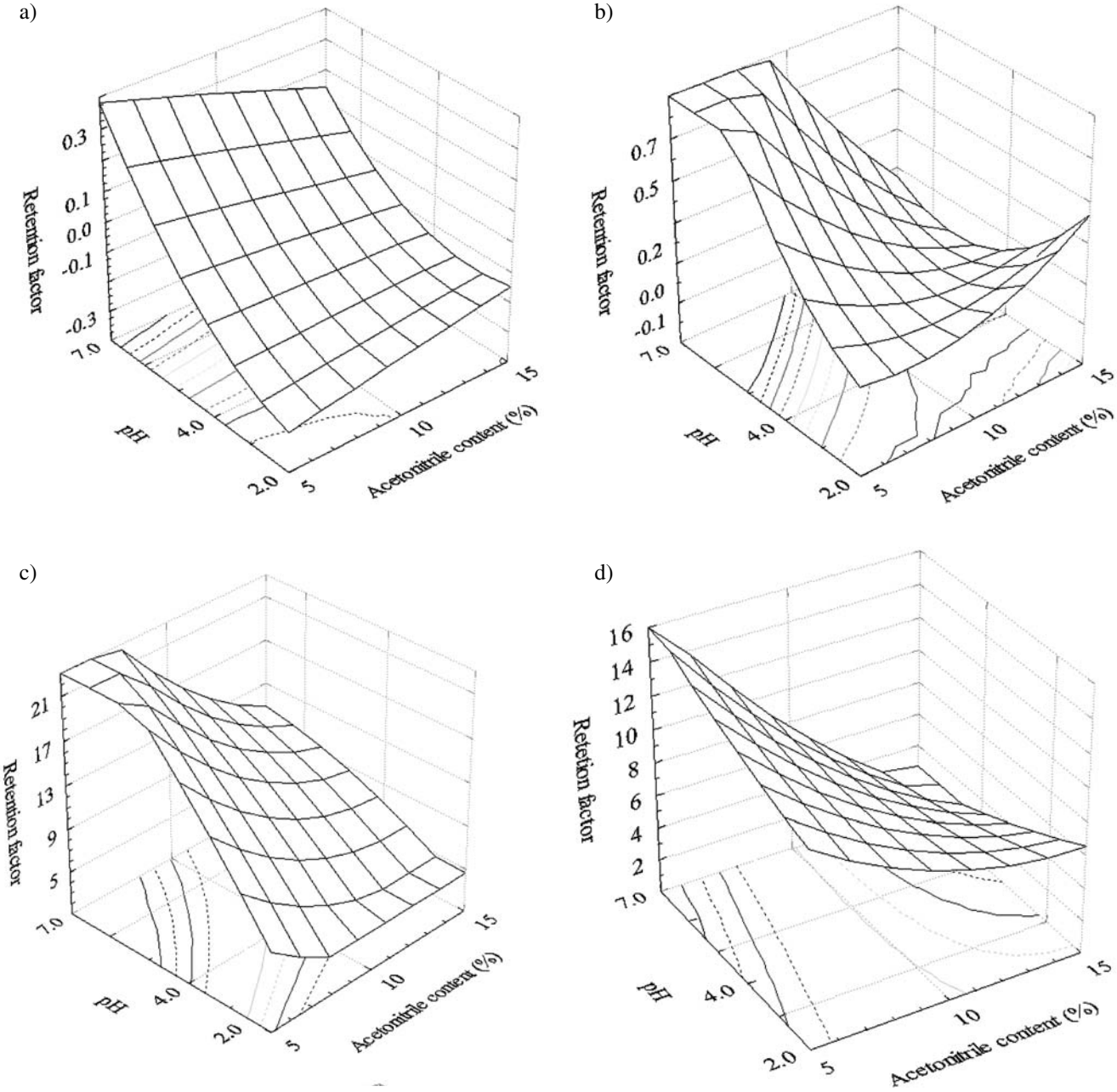


Figure 3. 3D graphs: a) *k*(pramipexole) = *f*(ACN, pH); b) *k*(BI-II 546 CL) = *f*(ACN, pH); c) *k*(BI-II 751 xx) = *f*(ACN, pH) and d) *k*(2-aminobenzothiazole) = *f* (ACN, pH).

The obtained results showed that the chromatographic retention of pramipexole, BI-II 546 CL and 2-aminobenzothiazole are affected mostly by pH of the water phase. On the other hand, the retention of BI-II 751 xx was under the greatest influence of acetonitrile content. The presence of two or more basic centres in pramipexole, BI-II 546 CL and 2-aminobenzothiazole structure is the reason for the strong basic character of these substances. Thus, a change of the mobile phase pH modifies the form of the substances from ionised to unionised leading to changes in retention. The BI-II 751 xx impurity has an acidic character because of the presence of thioamide and cyclic amide groups in its structure; so acidic and neutral pH had no influence on its retention. The second important factor, content of acetonitrile as organic modifier in mobile phase, had an expected influence on retention. A decrease in the content of acetonitrile leads to an increase of retention.

The Eqs.(3–6) describe a hyper surface and the presentation as 3D surface is possible, if one of the factors is kept constant at a medium value. The content of TEA was chosen as factor, which had the lowest influence on retention, and other factors (acetonitrile content and pH) were analyzed. The obtained graphs are presented in Figure 3.

A strong influence of pH is evident. Higher pH values resulted in longer retention for all compounds. This influence is especially important for pramipexole and BI-II 546 CL because they were eluted earlier then solvent when pH of the mobile phase was lower than 4.0. Smaller influence on BI-II 546 CL retention than on pramipexole retention could be explained by the keto functional group in BI-II 546 CL, which increases its lipophilicity and decreases the basic character of the secondary amine group. As it was observed in CCD, acetonitrile influence on the BI-II 751 xx retention is stronger than pH influence. The ionization of BI-II 751 xx at pH < 7.0 is suppressed and pH could not have a strong influence. 2-Aminobenzothiazole is the most basic compound in mixture, so its retention strongly depends on pH. It is important to understand that at different pH of the mobile phase the elution order of BI-II 751 xx and 2-aminobenzothiazole can change, i.e. when pH is very low (2.0) 2-aminobenzothiazole is eluted before BI-II 751 xx, but higher pH causes BI-II 751 xx to elute first.

In general, pH of the mobile phase must be strictly controlled in order to achieve a good reproducibility of retention times. In our case, at pH > 4.0 we obtained acceptable results. The optimal content of acetonitrile is between 10% and 15% resulting in satisfactory resolution and run time. An example of good separation and retention of the investigated mixture is presented in Figure 4.

4. Conclusion

We studied chromatographic behaviour of pramipexole and its impurities BI-II 546 CL, BI-II 751 xx and 2-aminobenzothiazole. Experimental design was applied and gave the necessary information from only 18 experiments. The analyzed factors were related to structural and chemical characteristics of substances.

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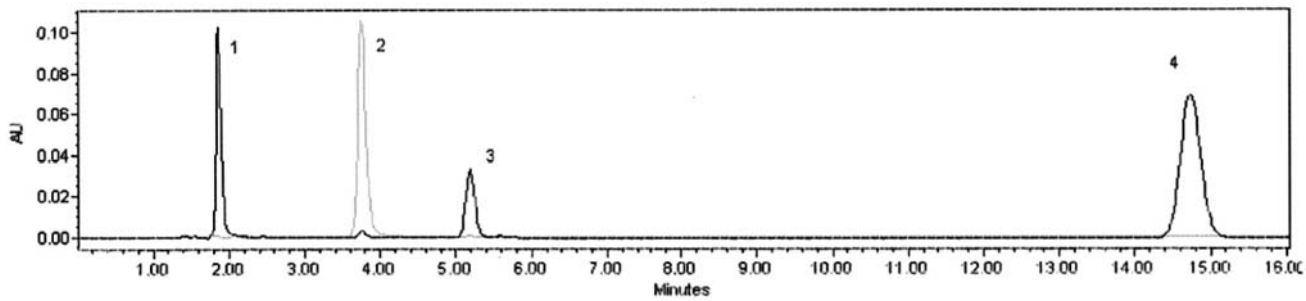

Figure 4. Representative chromatograme of pramipexole (1); BI-II 546 CL (2); BI-II 751 xx (3) and 2-aminibenzothiazole (4). Mobile phase: acetonitrile/water phase 15:85 V/V (water phase: 1% TEA, pH adjusted to 7.0 with ortophosphoric acid).

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Povzetek

Pramipexol je dopamine D2-agonist za zdravljenje Parkinsonove bolezni, v katerem BI-II 546 CL, BI-II 751 xx in 2-amino benzothiazole običajno najdemo kot nečistoče. Optimizirali in preučili smo kromatografsko ločitev teh spojin z uporabo eksperimentalnega načrta. Uporabili smo C18 kromatografsko kolono, eluent je vseboval acetonitril in vodno raztopino trietilamina in fosforne kisline. Detekcijo smo izvajali pri 262 nm za pramipexol, BI-II 751 xx in 2-aminobenzthiazole in pri 326 nm za BI-II 546 CL. Da bi definirali vpliv kromatografskih parametrov na separacijo, smo uporabili centralni sestavljeni eksperimentalni načrt.