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Experimental design in reversed-phase high-performance liquid chromatographic analysis of imatinib mesylate and its impurity

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Abstract

For the determination of the optimal RP-HPLC chromatographic conditions for the separation of imatinib mesylate and its impurity STI 509-00 experimental design 2^4 was applied. All the factors that affect imatinib mesylate/STI 509-00 separation, as well as their mutial interations were investigated. Methanol and triethylamine content in the mobile phase, pH of the mobile phase and column temperature were independent variables or factors to be investigated in two levels: "low" and "high". Capacity factor was chosen as a dependent variable. From the experimentally determined capacity factor values, it was defined the factors that affect to chromatographic system the most. Applying response surface methodology the appropriate graphs were constructed from experimental points and optimal chromatographic conditions for the separation were defined. Optimal conditions for the separation of imatinib mesylate and STI 509-00 were obtained using X Terra 150 mm × 4.6 mm, particle size 5 μm column at 25 °C. Mobile phase consisted of 250 ml of methanol, 740 ml of water and 10 ml of triehylamine. pH of water phase was adjusted to 2.4 with 85% orthophosphoric acid and than methanol was added.

Keywords: Experimental design; Pharmaceutical analysis; Imatinib mesylate; STI 509-00

1. Introduction

Many experiments require a study of the effects of two or more factors. It can be shown that, in general, factorial experiments are the most efficient designs for this type analysis. By a factorial experiment we mean that in each complete trial or replication of the experiment all possible combinations of the levels of the factors are investigated. Factorial designs are widely used in experiments involving several factors where it is necessary to study the joint effect of these factor on a response [1].

A literature search showed many experimental design applications in analytical method development and validation, especially in the area of separation science. Experimental design has been used for separation optimization [2–4] and for validation in RP-HPLC method [5–7]. It is used for robustness testing in RP-HPLC method [8–10] and capillary electrophoresis [11]. Also, it is used for determination with artificial neural networks optimization [12].

In this paper, the full factorial design has been applied for defining optimal chromatographic conditions for the separation of imatinib mesylate and STI 509-00. Chemical structures of imatinib mesylate and STI 509-00 are shown in Fig. 1.

Imatinib mesylate is a protein-tyrosine Chinese (PTK) inhibitor and it is used for the treatment of chronic myeloid leukemia (CML) disease in adult patients. It is known as signal transudation inhibitor 571 (STI 571, formerly known as CGP 57148B). Chemical name of imatinib mesylate is benzamide, 4-[(-methyl-1-piperazinyl) methyl]-*N*-[4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]phenyl]-, monomethanesulfonate. Trade name is Glivec, *Novartis Pharma*, Switzerland. Each Glivec capsule contains 100 mg imatinib as mesylate (monomethansulphonate) salt and not more than 0.2% of impurity STI 509-00.

As a very novel and recently synthesized drug, for imatinib mesylate there are only a few references. It was approved by the US Food and Drug Administration (FDA) in 1999. and it is not official in any Pharmacopoeia.

Imatinib mesylate and its metabolite (GCP 74588, STI 509-00) were assayed in monkey plasma using a semi-automated solid-phase extraction procedure and liquid chromatography–mass spectrometry (LC–MS) [13]. The same technique was used for their quantification in human plasma [14].

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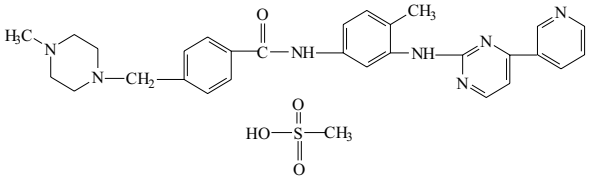
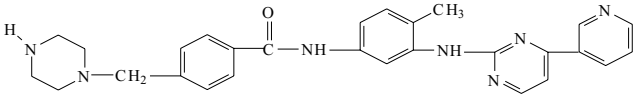


Imatinib mesylat



STI 509-00

Fig. 1. Structures of imatinib mesylate and STI 509-00.

Since, there is no reference, in the present literature, concerning analysis of the mentioned substances in pharmaceuticals, the proposed experimental design method for the investigation of separation of imatinib mesylate and STI 509-00 shortness the time of analysis and makes it more efficient.

2. Experimental

2.1. Methods

The primary objective of investigation was to establish the optimal conditions for the separation of structurally similar substances using factorial design and response surface methodology (RSM).

Factorial designs are an enormously popular class of experimental designs that are often used to investigate multifactor response surfaces. Important descriptions of factorial designs are the number of factors involved in the design and the number of levels of each factors. In general, if k is the number of factors being investigated, and m is the number of levels for each factor, then m^k factors combinations are generated by a full factorial design. Full factorial designs have been especially useful for describing the effects of qualitative and quantitative factors [15]. These are most useful where the number of factors is relatively limited. These designs are organized as follows. First, it is necessary to establish a region over which each factor is to be studied. The second step is the choose design. The third step is to choose a response and the fourth, a mathematical model can then be produced relating the response to the factors. Often a linear model of the form:

$$y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + \cdots + b_{N-1}x_{N-1} + b_Nx_N + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3 + \cdots + b_{(N-1)N}x_{N-1}x_N$$

where y presents the estimate response, b_0, is the average experimental response, the coefficients b_1 to b_N are the estimated effects of the factors considered and the extend to which these terms affect the performance of the method is called main effect. The coefficients b_{12} to $b_{(N-1)N}$ are called the interaction terms. We can see that the factorial design provides information about the importance of interaction between the factors [16]. Factors which have the greatest influence have to be closely investigated during the optimization applying RSM. That 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 [1].

2.2. Reagents and standards

All reagents used were of an analytical grade. Methanol (gradient grade; *Lab Scan*, Ireland), water (HPLC grade), triethylamine (TEA, *Merck*, Darmstadt, Germany) and 85% orthophosphoric acid (*Carlo Erba*, Milan, Italy) were used to prepare the mobile phase. Working standards of imatinib mesylate and STI 509-00 were obtained from *Novartis Pharma*, Switzerland.

2.3. Apparatus

The chromatographic system Hewlett Packard 1100 consisted of a HP 1100 pump, a HP 1100 UVVIS detector and a HP ChemStation integrator. Separations were performed on a X Terra 150 mm × 4.6 mm, 5 μm particle size column. UV detection was performed at 267 nm. The flow rate was 1.0 ml/min. The samples were introduced through a Rheodyne injector valve with a 20 μl sample loop. For the statistical analysis program STATISTICA version 5.0 was used.

2.4. Laboratory mixtures

Stock solutions were prepared by dissolving the respective working standard substances in mixture of methanol–water (25:75 (v/v)) to obtain the concentration of 2 mg ml^{-1} for imatinib mesylate and 4 μg ml^{-1} for STI 509-00. Working solution was prepared in concentrations of 0.5 mg ml^{-1} for imatinib mesylate and 1.0 μg ml^{-1} for STI 509-00.

3. Results and discussion

Considering structural similarity and basic characteristics of imatinib mesylate and STI 509-00, and also, great difference of investigated concentrations of these compounds, applied experimental design enabled rapid determination of optimal chromatographic conditions for their separation.

During the preliminary investigations the chromatographic behavior of investigated substances was investigated using different C_{18} columns (Beckman ODS 150 mm × 4.6 mm, Alltech C_{18} 250 mm × 4.6 mm, Bio-Rad C_{18} 250 mm × 4.6 mm and X Terra C_{18} 150 mm × 4.6 mm), varying content of organic modifier, TEA, pH of the mobile phase and temperature. Because of the basic characteristics and similar structure of imatinib mesylate and STI 509-00,

Table 1
Factors and their levels

Factors	Levels	
	(−)	(+)
(A) Temperature (°C)	20	50
(B) TEA (%)	0.1	1.4
(C) pH of the mobile phase	2.4	3.1
(D) Methanol (%)	21	31

(−) and (+) are "low" and "high" levels.

they exhibit similar affinity for a column, bed symmetry of peaks and long time of separation on classical C_{18} stationary phases. X Terra is C_{18} column with a specific column packing. Free silanol groups are additionally protected with methyl groups which resulted in excellent peak shape and significantly shortened separation.

On the basis of the preliminary experiments, the big influence on the separation of compounds had temperature (factor A), % TEA (factor B), content of organic modifier (factor C) and pH of the mobile phase (factor D). Factors and their "low" (−) and "high" (+) levels are presented in the Table 1.

For the analysis of imatinib mesylate and its impurity 2^k the full factorial design was chosen. To screen the relative influence of these factors and their possible interactions in the experimental domain, factorial design 2^4 was chosen, which will study the effects of the selected four factors in sixteen runs.

As the parameter to define chromatographic behavior of investigated substance and its impurity, capacity factor (retention parameter which represents affinity of investigated substance towards stationary phase) was chosen. Scheme of the experiment is given in Table 2.

The results for capacity factors of imatinib mesylate (k'_1) and STI 509-00 (k'_2) are presented in Table 3.

Table 2
Scheme of the experiment

Number of experiment	% Methanol	pH of the mobile phase	% TEA	T (°C)	Treatment combination
1	−	−	−	−	(1)
2	−	−	−	+	a
3	−	−	+	−	b
4	−	−	+	+	ab
5	−	+	−	−	c
6	−	+	−	+	ac
7	−	+	+	−	bc
8	−	+	+	+	abc
9	+	−	−	−	d
10	+	−	−	+	ad
11	+	−	+	−	bd
12	+	−	+	+	abd
13	+	+	−	−	cd
14	+	+	−	+	acd
15	+	+	+	−	bcd
16	+	+	+	+	abcd

(−) and (+) are "low" and "high" levels.

Table 3
Capacity factors of imatinib mesylate and STI 509-00

Treatment combination	Capacity factor	
	Imatinib mesylate	STI 509-00
1	10.61	8.83
a	6.8	5.46
b	6.04	5.61
ab	3.59	3.11
c	56.84	45.74
ac	30.26	26.96
bc	21.99	17.72
abc	15.43	12.44
d	3.96	3.56
ad	2.77	2.38
bd	1.87	1.87
abd	1.28	1.28
cd	11.65	9.76
acd	7.77	7.03
bcd	5.89	4.96
abcd	4.39	3.7

Traditional tabular presentation of data for calculating the classical factor effects in a 2^4 factorial design is shown in Table 4.

On the basis of the obtained results values for factors effects were calculated and shown in Table 5.

Obtained values showed the greatest influence of factors C, D and B, respectively, on the chromatographic behavior of mentioned substances. Also, mutual interactions of these factors were of great importance. According to the results presented in Table 5 factor A (temperature) has no influence on separation of investigated compounds and in the following experiments it was kept constant at 25 °C.

This way, further work was directed in the course of investigation of influence of factors C, D and B and their mutual interactions. Optimization of the method was carried out applying RSM for defining the optimal chromatographic conditions.

In this case, the analysis of three factors, % TEA, pH of the mobile phase and % methanol, for chromatographic behavior of imatinib mesylate and STI 509-00 was investigated. The study was done on related influence of: (1) % TEA and pH of the mobile phase; (2) % TEA and % methanol and (3) % methanol and pH of the mobile phase. For each of them 64 experiments were performed.Based on the performed experiments, coefficients were calculated characterizing the polynomes of second order and three-dimensional graphs were constructed as well.

For the % TEA/pH of the mobile phase system the equation for k'_1 was obtained:

$$z = 100.839 + 15.433x - 90.335y + 2.162x^2 - 7.897xy + 20.874y^2$$

where x is the % TEA, y the pH of the mobile phase and z the capacity factor for imatinib mesylate.

Table 4
Data for calculating the classical factor effects in a 2^4 factorial design

	A	B	AB	C	AC	BC	ABC	D	AD	BD	ABD	CD	ACD	BCD	ABCD
(1)	−	−	+	−	+	+	−	−	+	+	−	+	−	−	+
a	+	−	−	−	−	+	+	−	−	+	+	+	+	−	−
b	−	+	−	−	+	−	+	−	+	−	+	+	−	+	−
ab	+	+	+	−	−	−	−	−	−	−	−	+	+	+	+
c	−	−	+	+	−	−	+	−	+	+	+	−	+	+	−
ac	+	−	−	+	+	−	−	−	−	+	−	−	−	+	+
bc	−	+	−	+	−	+	−	−	+	−	−	−	+	−	+
abc	+	+	+	+	+	+	+	−	−	−	+	−	−	−	−
d	−	−	+	−	+	+	−	+	−	−	−	−	+	+	−
ad	+	−	−	−	−	+	+	+	+	−	+	−	−	+	+
bd	−	+	−	−	+	−	+	+	−	+	+	−	+	−	+
abd	+	+	+	−	−	−	−	+	+	+	−	−	−	−	−
cd	−	−	+	+	−	−	+	+	−	−	+	+	−	−	+
acd	+	−	−	+	+	−	−	+	+	−	−	+	+	−	−
bcd	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−
abcd	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
divizor	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8

A three-dimensional graph is presented in Fig. 2.

For the % TEA/pH of the mobile phase system the equation for k'_2 was obtained:

$$z = 70.971 + 9.163x - 64.668y + 2.254x^2 - 5.479xy + 15.274y^2$$

where x is the % TEA, y the pH of the mobile phase and z the capacity factor for STI 509-00.

A three-dimensional graph is presented in Fig. 3.

For the % TEA/% methanol system the equation for k'_1 was obtained:

$$z = 87.392 - 8.282x - 5.394y + 1.139x^2 + 0.194xy + 0.087y^2$$

where x is the % TEA, y the % methanol and z the capacity factor for imatinib mesylate.

A three-dimensional graph is presented in Fig. 4.

Table 5
Factor effects values for imatinib mesylate and STI 509-00

Factors effects	Imatinib mesylate	STI 509-00
A	−5.820	−4.461
B	−8.772	−7.379
AB	3.045	2.054
C	14.662	12.026
AC	−3.810	−2.551
BC	−5.932	−5.289
ABC	2.555	1.689
D	−13.997	−11.416
AD	4.030	3.021
BD	5.592	4.649
ABD	2.555	1.689
CD	−9.707	−7.936
ACD	2.910	1.996
BCD	4.542	3.954
ABCD	−2.110	−1.469

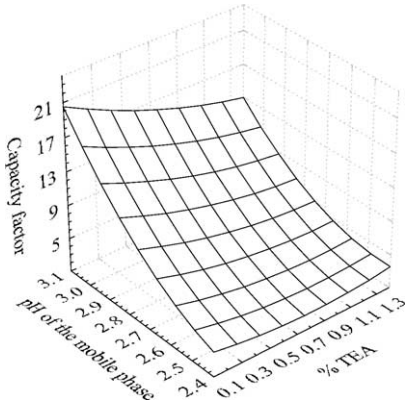


Fig. 2. Three-dimensional graph of $k'_1 = f$ (% TEA, pH) for imatinib mesylate.

For the % TEA/% methanol system the equation for k'_2 was obtained:

$$z = 70.283 - 5.097x - 4.378y + 0.762x^2 + 0.119xy + 0.071y^2$$

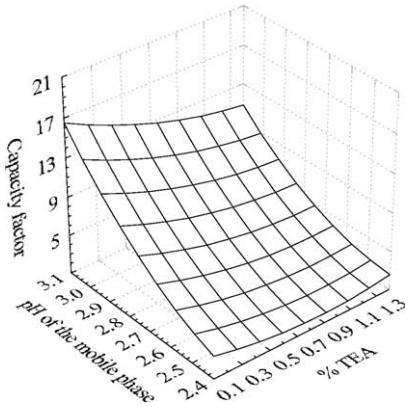


Fig. 3. Three-dimensional graph of $k'_2 = f$ (% TEA, pH) for impurity product.

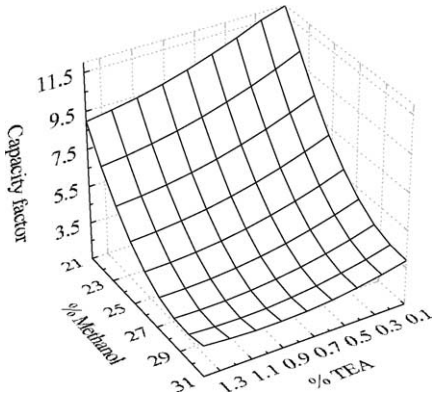


Fig. 4. Three-dimensional graph of $k'_1 = f$ (% TEA, % methanol) for imatinib mesylate.

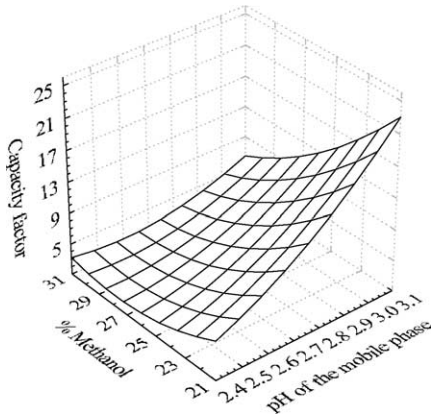


Fig. 6. Three-dimensional graph of $k'_1 = f$ (% methanol, pH) for imatinib mesylate.

where x is the % TEA, y the % methanol and z the capacity factor for STI 509-00.A three-dimensional graph is presented in Fig. 5.

For the % methanol/pH of the mobile phase system the equation for k'_1 was obtained:

$$z = 21.417 - 19.033x + 0.258y + 15.408x^2 - 1.972xy + 0.081y^2$$

where x is the % methanol, y the pH of the mobile phase and z the capacity factor for imatinib mesylate.

A three-dimensional graph is presented in Fig. 6.

For the % methanol/pH of the mobile phase system the equation for k'_2 was obtained:

$$z = 8.148 - 5.336x - 0.112y + 10.02x^2 - 1.488xy + 0.066y^2$$

where x is the % methanol, y the pH of the mobile phase and z the capacity factor for STI 509-00.A three-dimensional graph is presented in Fig. 7.

Analyzing the obtained three-dimensional graphs, similar chromatographic behavior of imatinib mesylate and STI 509-00 was observed. It was caused by the small structural differences (methyl group on the piperazine part of the molecule), as well as similar polarity of investigated substances. Imatinib mesylate as a tertiary amine is less polar so it was retained in the column. Within investigated content of TEA and methanol, as well as pH of the mobile phase, good separation was obtained, because STI 509-00 as a weaker base than imatinib mesylate, with a polar mobile phase, will be eluted first. Optimal chromatographic conditions were defined according to duration of the separation and peak symmetry.

Optimal conditions for the separation of imatinib mesylate and STI 509-00 were obtained using X Terra 150 mm × 4.6 mm, particle size 5 μm column at 25 °C. Mobile phase consisted of 250 ml of methanol, 740 ml of water and 10 ml of triehylamine. pH of water phase (mixture of water and triehylamine) was adjusted to 2.4 with 85% orthophosphoric acid and than methanol was added. pH of mobile phase adjusted to 2.6 with 85% orthophosphoric acid if necessary. Flow rate was 1 ml min^{-1}.

A representative chromatogram of laboratory mixture given in Fig. 8.

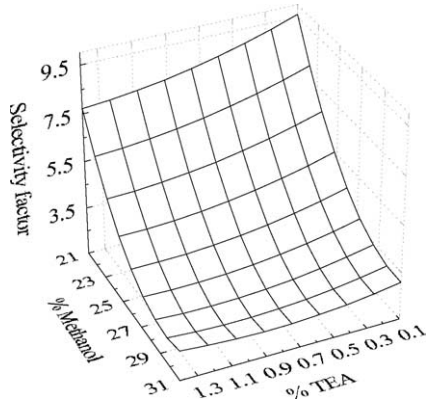


Fig. 5. Three-dimensional graph of $k'_2 = f$ (% TEA, % methanol) for impurity product.

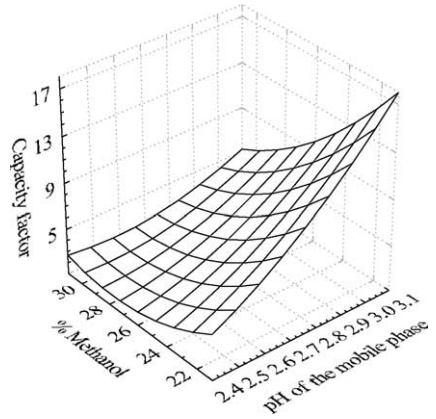


Fig. 7. Three-dimensional graph of $k'_2 = f$ (% methanol, pH) for impurity product.

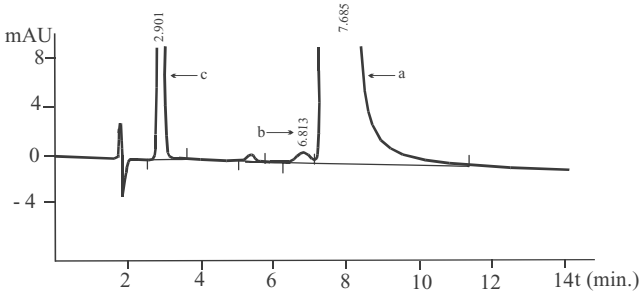


Fig. 8. The chromatogram of the laboratory mixture of imatinib mesylate (a), STI 509-00 impurity product (b) and internal standard paracetamol (c) (mobile phase: methanol–water (25:75 (v/v)); flow rate 1.0 ml min^{-1}; UV detection 267 nm).

4. Conclusions

The method for the RP-HPLC separation and analysis of the imatinib mesylate and its impurity product STI 509-00 has been developed using an experimental design. We demonstrated that a combination of considerations, such as column selectivity and the need to obtain analytical sensitivity, could be coupled with experimental design techniques to optimize the separation conditions for both speed and selectivity of the RP-HPLC method. A four factors two levels screening experiment was first used to identify the important chromatographic variables in 16 experiments. The similar chemical structure of the investigated substances caused a further optimization of the separation, for both speed and resolution, with a response surface design in 64 more experiments. The optimized method can be used for separation, identification and simultaneous determination of imatinib mesylate and its impurity product in bulk drug and pharmaceutical dosage forms.

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