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Reversed-phase liquid chromatography analysis of imatinib mesylate and impurity product in Glivec® capsules

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Abstract

The reversed-phase high-performance liquid chromatographic (RP-HPLC) method has been developed and validated for the simultaneous determination of imatinib mesylate and of the impurity product in Glivec® capsules (Novartis, Switzerland). Separations were performed on a X Terra™ 150 mm × 4.6 mm, 5 μm particle size column at 25 °C. The mobile phase was a mixture of methanol–water–triethylamine (25:74:1, v/v/v) with flow rate of 1.0 ml min^{-1}. pH value of water–triethylamine (TEA) was adjusted to 2.4 with orthophosphoric acid before adding of methanol. UV detection was performed at 267 nm. Acetaminophen was used as an internal standard. The method was validated statistically for its selectivity, linearity, precision, accuracy and robustness. Due to its speed and accuracy, the method may be used for quality control analyses.

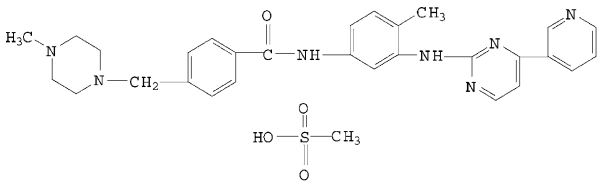
Keywords: Imatinib mesylate

1. Introduction

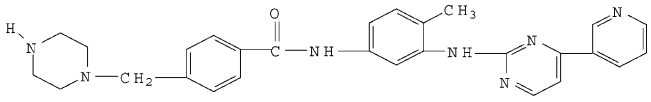
Imatinib mesylate is known as Signal Transduction Inhibitor 571 (STI 571, formerly known as CGP 57148B) and as an antineoplastic agent. It received fast track approval by the US Food and Drug Administration in 2001 [1]. It is a protein-tyrosine kinase (PTK) inhibitor. Its target kinase is a protein produced by DNA translocation (the "Philadelphia chromosome"). Imatinib mesylate is used for the treatment of chronic myeloid leukemia (CML) disease in adult patients.

Glivec® or imatinib mesylate, is designated chemically as benzamide 4-[(-methyl-1-piperazinyl)methyl]-*N*-[4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]phenyl]-, monomethanesulfonate (CAS Registry℠ Database) [2]. Each Glivec® capsule contains 100 mg of imatinib as mesylate (methansulphonate) salt and not more than 0.2% of impurity (in the following text STI 509-00).

This paper presents the reversed-phase high-performance liquid chromatographic (RP-HPLC) method applied for the simultaneous determination of imatinib mesylate and STI 509-00 as an impurity product. Chemical structures of imatinib mesylate and STI 509-00 are shown in the following sheme.



Imatinib mesylate



STI 509-00

Being a very novel and recently synthetized drug, there are only a few references for imatinib mesylate. It is not official in any Pharmacopoeia. Imatinib mesylate and its metabolite were assayed in monkey plasma using a semi-automated solid phase exstraction procedure and liquid chromatography–mass spectrometry (LC/MS) tandem. Separations were performed using a Waters Symmetry Shield™-RP8 50 mm × 4.6 mm column and methanol–water (72:28, v/v) containing 0.05% (w/v) ammonium acetate as a mobile phase [3]. Under the same experimental conditions

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a high-throughput quantification of imatinib mesylate and its main metabolite in human plasma, using LC/MS, were performed [4].

The aim of our investigation was to develope and validate the RP–HPLC method for the simultaneous determination of imatinib mesylate and STI 509-00 in a pharmaceutical dosage form. Since there is no reference, in the present literature, concerning analyses of the mentioned substances in pharmaceuticals, the proposed method is a significant advance in pharmaceutical analysis.

2. Experimental

2.1. Reagents and samples

All reagents used were of an analytical grade. Methanol-gradient grade (Lab Scan, Ireland), water-HPLC grade, TEA (Merck, Darmstadt, Germany) and 85% orthophosphoric acid (Carlo Erba, Milano, Italy) were used to prepare the mobile phase. Glivec® capsules (containing 100 mg of imatinib as mesylate salt) were manufactured by Novartis Pharma A. G., Switzerland. Working standards of imatinib mesylate and STI 509-00 were obtained from the same supplier.

2.2. Standard solutions

Stock solutions were prepared by dissolving the respective working standard substances in a mixture of methanol:water (25:75, v/v) to obtain the concentration of 2 mg ml^{-1} for imatinib mesylate and 10 μg ml^{-1} for STI 509-00. For the calibration curves, series of nine solutions were prepared in the concentration range from 0.05 to 1.0 mg ml^{-1} for imatinib mesylate and from 0.4 to 5 μg ml^{-1} for STI 509-00.

2.3. Laboratory mixtures

To prove the validity and applicability of the proposed RP–HPLC method, the laboratory mixture of imatinib mesylate and STI 509-00 was made in the ratio which corresponded to the Glivec® capsules. Stock solutions were prepared by dissolving the respective working standard substances in the 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. For the quantitative analysis of the mixture, three series (0.4, 0.5 and 0.6 mg ml^{-1} for imatinib mesylate; 0.8, 1.0 and 1.2 μg ml^{-1} for STI 509-00) were prepared, with ten solutions for each concentration. For the chromatographic determination acetaminophen was used as an internal standard in the concentration of 40 μg ml^{-1}.

2.4. Sample solutions

From Glivec® capsules, three series of solutions (0.4, 0.5 and 0.6 mg ml^{-1}, calculated to declared imatinib mesylate content) with ten solutions for each concentration were prepared in the mixture of methanol:water (25:75, v/v). Acetaminophen was added as an internal standard (40 μg ml^{-1}). Resulting solutions were injected on the column.

2.5. Chromatographic conditions

The chromatographic system Hewlett Packard 1100 consisted of a HP 1100 pump, HP 1100 UV-Vis detector and HP ChemStation integrator. Separations were performed on a X Terra™ 4.6 mm × 150 mm, 5 μm particle size column at 25 °C. The samples were introduced through a Rheodyne injector valve with the 20 μl sample loop.

The mobile phase was prepared by mixing of 740 ml of water with 10 ml of TEA, pH was adjusted to 2.4 with 85% orthophosphoric acid and than 250 ml of methanol were added and pH was adjusted to 2.6 with 85% orthophosphoric acid, if necessary. The mobile phase was filtered through a 0.2 μm Millipore filter and degassed in an ultrasonic bath. Acetaminophen was used as an internal standard. The flow rate of the mobile phase was 1.0 ml min^{-1}. UV detection was performed at 267 nm.

3. Results and discussion

Imatinib mesylate and STI 509-00 are structurally very similar. Imatinib mesylate possesses in addition a methyl group in piperazine part of molecule. Because of their like basic characteristics and similar structure, their separation is very difficult. A similar affinity to the stationary phase, bad symmetry of peaks and s long retention time characterize the RP-HPLC analysis of these substances.

Chromatographic behavior of imatinib mesylate and STI 509-00 was examined using a column X Terra™ with mobile phases of different polarities. The X Terra™ was a C_{18} column with a specific column packing. Free silanol groups were additionaly protected by methyl groups which enabled the application in a wide pH range.

Mobile phases with acetonitrile in content of 30% and more, produced a resolution factor for imatinib mesylate and STI 509-00 of <1. Acetonitrile as an organic solvent in the mobile phase gave a good separation with addition of a citrate buffer. Chromatographic behavior of both analytes in the range from 20 to 35% of acetonitrile with the citrate buffer (pH from 2.8 to 4.8) was tested. A satisfactory separation was obtained using the acetonitrile from 23 to 25% with the citrate buffer (pH from 4.0 to 4.4), column temerature at 30 °C and flow rate of 1.1 ml min^{-1}. Increase of the acetonitrile content, resulted in shorter retention times, but the selectivity factor was close to 1. Also, decrease of pH made the separation not satisfactory. The use of the mobile phase which consisted of 23.5% acetonitrile and citric buffer pH 4.0, with flow rate of 1.1 ml min^{-1} and at column temperature of 30 °C gave the retention times of 16.81 min and 15.42 for imatinib mesylate and STI 509-00, respectively.

The investigation of the mobile phases with methanol resulted in a good separation of both analytes with significantly shortened retention times (approximately 12 min run time). The influence of methanol content from 21 to 31% was investigated. pH of the mobile phase was adjusted in the range from 2.4 to 3.1 with 85% orthophosphoric acid. An optimal separation was achieved with the mobile phase containing 25% of methanol and pH adjusted to 2.6. Concerning basic characteristics of imatinib mesylate and STI 509-00 paeks showed tailing and bad symmetry. The addition of TEA or other short-chain bases to the mobile phase reduced peak tailing of basic compounds. pH adjustment of the mobile phase before adding an organic solvent was necessery [5,6]. Consequent addition of TEA in the water phase, varying its content from 0.1 to 1.4%, enabled a better separation of the investigated substances and a better peak symmetry was obtained as well. TEA improved the peak shape by interacting strongly with the acidic silanol groups on the silica surface. Overloading the surface with TEA reduced secondary retention of other amines and thus improved tailing. The content of TEA of 1% gave the best results.

Peak tailing and bad peak symmetry can be prevented by addition of modificators to the mobile phase, but more acceptable approach is to adjust the pH value of the mobile phase.

With the mobile phase, given in Section 2, separation was performed in 7 min and retention times of STI 509-00 and imatinib mesylate were 5.096 and 6.858 min, respectively. Increasing the pH of the water phase to 3.1 lead to an increase of the separation time to 26 min and the retention times were 21.032 min for STI 509-00 and 25.798 min for imatinib mesylate. According to the experimental results it is obvious that small changes of methanol content and mainly mobile phase pH have a great influence on the separation.

After establishing the optimal conditions for the separation, the selectivity, linearity, precision, robustness, limit of quantitation and limit of detection were determined.

The representative chromatograms of the laboratory mixture and sample are presented in Fig. 1.

The assay was selective, no significant interfering peaks were observed at the retention time of imatinib mesylat, STI 509-00 and internal standard. All excipients were eluated at a different times and did not interfere with the analysed compounds.

Linear relationships of the peak area over the concentration range from 0.05 to 1.0 mg ml^{-1} for imatinib mesylate and from 0.4 to 5 μg ml^{-1} for STI 509-00 were obtained. The important calibration curve parameters: slope (a), intercept (b), correlation coefficient (r) and standard deviation of the intercept (S_b) are present in the Table 1.

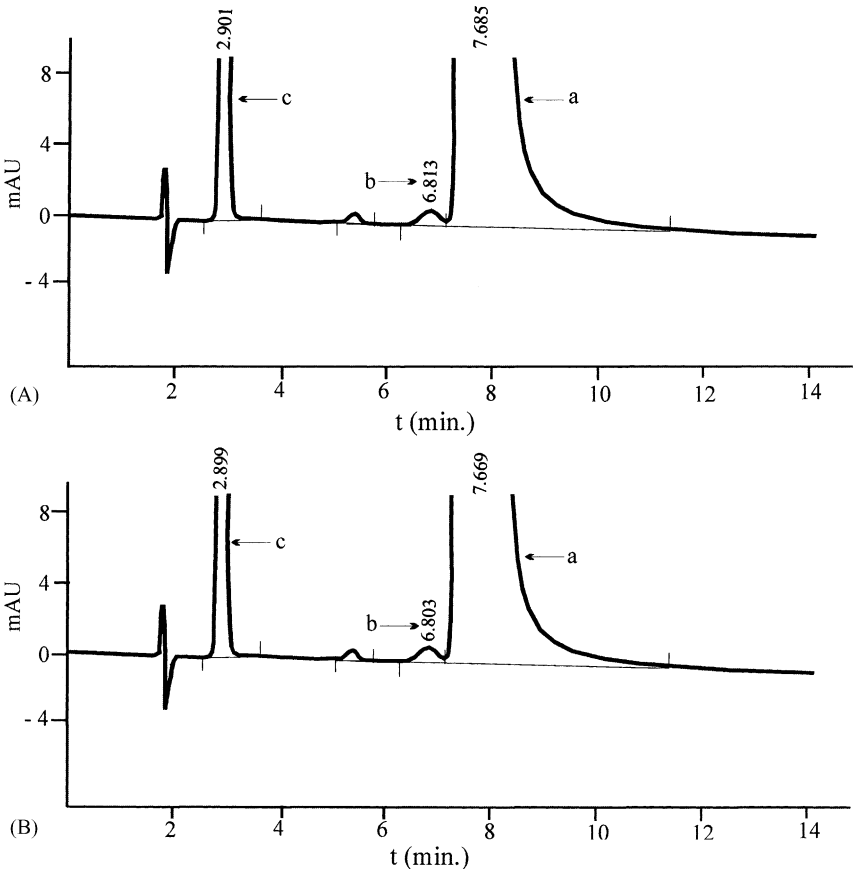

Fig. 1. The chromatogram of Imatinib mesylate (a), STI 509-00 (b) and internal standard (c): A, laboratory mixture; B, Glivec® capsules; (mobile phase: methanol−water (25:75, v/v); flow rate 1.0 ml min^{-1}; UV detection 267 nm).

Table 1
The important parameters for the calibration curves

Parameter	Imatinib mesylate	STI 509-00
Concentration range	0.05–1.0 mg ml^{-1}	0.4–5 μg ml^{-1}
$y = ax + b$	66 493x + 209.83	50.54x − 0.84
r	0.9997	0.9994
S_b	232.5	2.4

r the correlation coefficient; S_b the standard deviation of the intercept.

Table 2
Precision of the RP-HPLC method for Glivec® capsules assay

Compound (concentration)	Injected	Found	CV (%)	R (%)
Imatinib mesylate (mg/ml)	0.4	0.399 ± 0.007[a]	1.7	99.6
	0.5	0.507 ± 0.004	0.7	101.3
	0.6	0.598 ± 0.007	1.1	99.7
STI 509-00 (μg/ml)	0.8	0.826 ± 0.018	2.2	103.2
	1.0	1.025 ± 0.023	2.3	102.5
	1.2	1.243 ± 0.025	2.0	103.6

[a] S (n = 10).

The results for precision of the proposed RP-HPLC method are given in Table 2. 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 0.7 to 1.7% for imatinib mesylat and from 2.0 to 2.3% for STI 509-00; recovery values from 99 to 103%).

The results of the content determination of imatinib mesylate and STI 509-00 in Glivec® capsules are given in Table 3. The content of the imatinib mesylat was in the range from 97.3 to 97.6% and the content of the impurity was lower than 0.2%.

As defined by the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), the robustness of an analytical procedure refers to its capability to remain unaffected by small and deliberate variations in method parameters [7]. Typical variations in the case of liquid chromatography are: influence of variations of pH in a mobile phase, influence of variations in mobile phase composition, different columns, temperature and flow rate [8]. The robustness test starts with a selection of the factors that might influence the performance of the method [9]. In this case, the analysis influenced by four factors, temperature, % TEA, % methanol and pH of the mobile phase for chromatographic separation of imatinib mesylate and STI 509-00. The experimental domain of the selected factors is reported in Table 4.

Table 4
Investigated range of robustness testing

Variable	Range investigated
Temperature (°C)	20–55
Triethylamine content (%)	0.1–1.4
Methanol content (%)	21–31
pH of the mobile phase	2.4–3.1

The study was done on related influence of: (1) temperature and % TEA (2) % TEA and pH of the mobile phase (3) % TEA and % methanol and (4) % methanol and pH of the mobile phase. For each of them 64 experiments were performed.

For robustness testing, the response surface methodology (RSM) was applied. The RSM is a collection of mathematical and statistical techniques useful for analysing problems where several independent variables influence a dependent variable or response, and the goal is to optimize this response [10].

Based on the performed experiments, coefficients were calculated characterizing the polynomes of second order and three-dimensional graphs were constructed as well.

For the T/% TEA system the equation was obtained:

$$z = 1.021 + 0.031x + 0.009y - 0.005x^2 - 0.003xy - 6.861e^{-5}y^2$$

where x is % TEA, y the temperature (°C) and z the selectivity factor. Three-dimensional graph is presented in Fig. 2.

The changes of the examined parameters in the given range did not influence the separation significantly, i.e. to the selectivity factor of those components. For the % TEA/pH system of the mobile phase, respectively, the equation was

Table 3
Glivec® capsules determination

Compound	Taken (mg/ml)	Found (mg/ml)	Found (mg/cps)	CV (%)	R (%)
Imatinib mesylate	0.4	0.390 ± 0.014[a]	97.4	3.5	97.4
	0.5	0.486 ± 0.050	97.3	0.9	97.3
	0.6	0.586 ± 0.050	97.6	0.9	97.6
Compound	MAC[b] (μg/ml)	Found (μg/ml)	Found (%)	CV (%)	
STI 509-00	0.8	0.232 ± 0.004	0.06	1.9	
	1.0	0.268 ± 0.009	0.06	3.3	
	1.2	0.301 ± 0.009	0.05	3.1	

[a] S (n = 10).
[b] MAC: maximum allowed content.

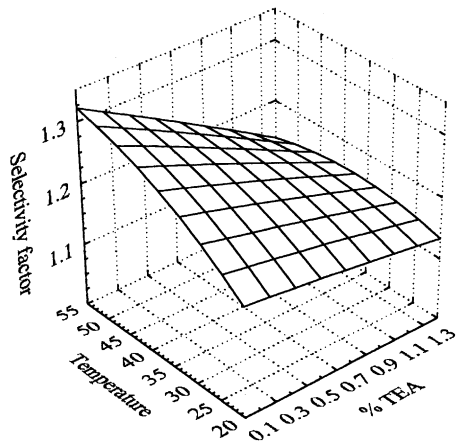

Fig. 2. Three-dimensional graph $\alpha = f$ (% TEA, t °C).

attained:

$$z = 5.156 + 1.31x - 3.12y - 0.044x^2 - 0.438xy + 0.607y^2$$

where x is % TEA, y the pH of the mobile phase and z the selectivity factor.

Three-dimensional graph is presented in Fig. 3. Within the range of pH from 2.7 to 3.1 and % TEA from 0.8 to 1.4% it may be said that the method is robust, while in the remaining part small changes of the examined factors esentially influenced the selectivity factor.

For the % TEA/% of methanol the obtained polynom of second order equation was

$$z = 2.055 - 0.001x - 0.05y + 0.002x^2 - 0.001xy + 0.001y^2$$

where x is % methanol, y the % TEA and z the selectivity factor.

Three-dimensional graph is presented in Fig. 4. Chromatographic separation of imatinib-mesilate and STI 509-00 was more successful with small changes of the methanol content.

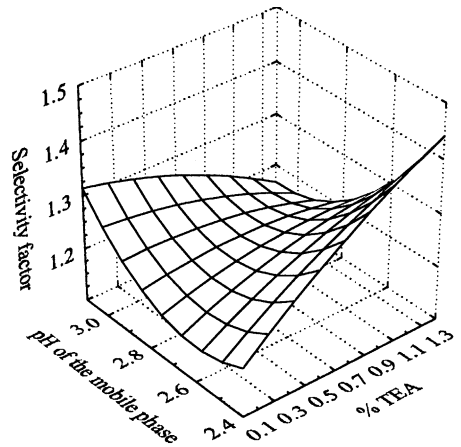

Fig. 3. Three-dimensional graph $\alpha = f$ (% TEA, pH).

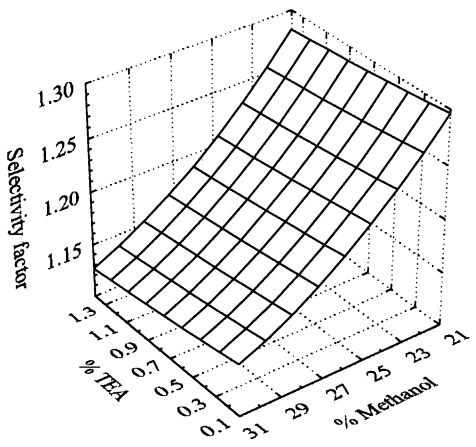

Fig. 4. Three-dimensional graph $\alpha = f$ (% TEA, % methanol).

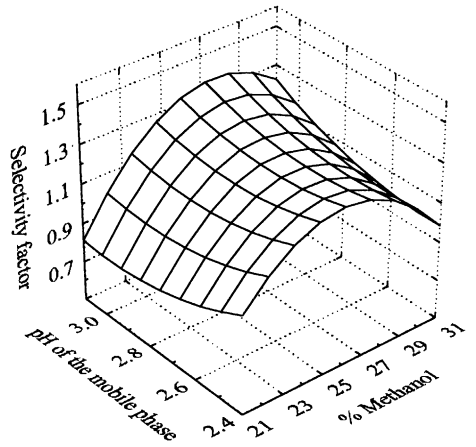

Fig. 5. Three-dimensional graph $\alpha = f$ (% methanol, pH).

For the % methanol/pH of the mobile phase, respectively, equation was obtained

$$z = 1.125 + 0.493x - 4.796y - 0.012x^2 + 0.062xy + 0.586y^2$$

where x is % methanol, y the pH and z the selectivity factor.

Three-dimensional graph is presented in Fig. 5. Small changes of methanol content and pH of the mobile phase essentially influence the separation.

Limits of detection (LOD) and limits of quantitation (LOQ) were experimentally determined and they are presented in Table 5.

Table 5
Limit of detection (LOD) and limit of quantification (LOQ)

Compound	LOQ[a] (μg/ml)	LOD[a] (μg/ml)
Imatinib mesylate	0.1	0.01
STI 509-00	0.2	0.01

[a] Experimentally determined values.

The best resolution was obtained using acetaminophen as an internal standard. The concentrations of imatinib mesylate and STI 509-00 were calculated using the internal standard method.

4. Conclusions

The proposed RP-HPLC method permits simultaneous determination of imatinib mesylate and STI 509-00 impurity product due to good separation and resolution of the chromatographic peaks. The method is applicable for a qualitative and quantitative analysis of Glivec® capsules. The results obtained are in a good agreement with the declared contents. The results are accurate and precise and confirmed by statistical parameters. There was no interference of the excipient (microcrystalline cellulose, crospovidon, colloidal silicon dioxide and magnesium stearate) in capsules. The proposed method is rapid, precise and estimates imatinib mesylate indipendently of STI 509-00.

References

[1] M. Habeck, The Lancet Oncol. 3 (2002) 6.
[2] CAS Registry^{SM} Database (2003) http://www.cas.org/motw/imatinib.html.
[3] R. Bakhtiar, L. Khemani, M. Hayes, T. Bedman, F. Tse, J. Pharm. Biomed. Anal. 28 (2002) 1183.
[4] R. Bakhtiar, J. Lohne, L. Ramos, L. Khemani, M. Hayes, F. Tse, J. Chromatogr. B 768 (2002) 325.
[5] J. W. Dolan, LC GC International, March (1999) 156.
[6] J. W. Dolan, LC GC International, July (1996) 400.
[7] International Conference on Harmonisation, Topic Q2B, Validation of Analytical Methods: Methodology, in : Proceedings of the Third International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human USE (ICH), Yokohama, Japan, 1996.
[8] EUROPEAN PHARMACOPEIA Technical Guide for the Elaboration of Monographs, third edition, Council of Europe, Strasbourg, Cedex, France, 1999.
[9] E. Hund, Y. Vander Heyden, M. Haustein, D.L. Massart, J. Smeyers-Verbeke, J. Chromatogr. A 874 (2000) 167.
[10] C. M. Douglas, Design and Analysis of Experiments, Wiley, USA, 1976, p. 340.