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A validated LC method for imatinib mesylate☆

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Abstract

An isocratic reversed-phase liquid chromatography method with UV detection has been developed for the purity evaluation of imatinib mesylate in bulk drug. The method is selective and is capable of detecting all process intermediates and other related compounds, which may be present at trace levels in the drug substance. The method was validated on a Symmetry Shield RP18 analytical column (150 × 4.6 mm, 5 μm), mobile phase consisting of 30 mM sodium octane sulphonic acid in 10 mM aqueous KH_2PO_4 (pH 2.5 with H_3PO_4): MeOH in the ratio of 42:58 v/v. The flow rate was set at 1.0 ml/min and the column was maintained at room temperature. The injection volume was set to 10 μl and the detector was set at a wavelength of 237 nm. The method was validated in terms of system precision, method precision, linearity, accuracy, limit of detection and limit of quantification.

Keywords: Imatinib mesylate; Purity; Isocratic reversed-phase and liquid chromatography

1. Introduction

Imatinib mesylate is a protein-tyrosine kinase inhibitor that inhibits the Bcr-Abl tyrosine kinase, inhibits proliferation and induces apoptosis in Bcr-Abl positive cell lines as well as fresh leukematic cells. Imatinib mesylate is indicated for the treatment of patients with chronic myeloid leukemia (CML) in blast crisis, accelerated phase or in chronic phase after failure of interferon—alpha therapy [1–3].

We have come across one publication regarding quantification of the anti-leukemia drug (Gleevec) and its metabolite in monkey plasma using semi-automated solid phase extraction procedure [4]. We did not however find any publication giving details for determining the chromatographic purity of imatinib mesylate in the open literature. In this manuscript, an isocratic reversed-phase method has been described to quantitate the impurities present in imatinib. It is likely that the unreacted intermediates and their precursors may remain as impurities in the final active pharmaceutical in-

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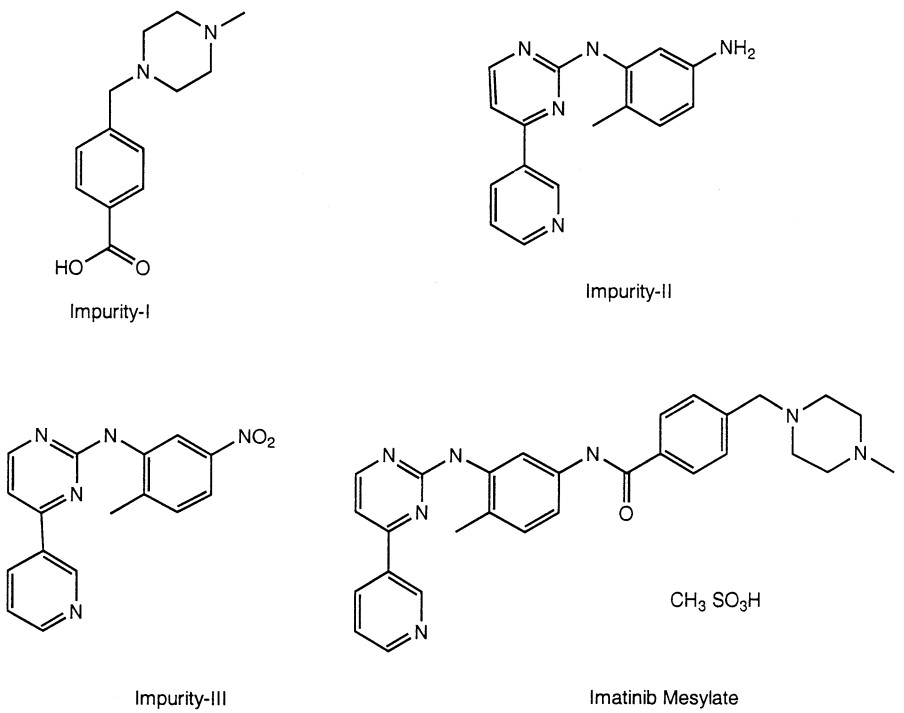

Fig. 1. Chemical structures of imatinib and its related impurities.

gredient (API) and affect its quality. In the present method, all the possible process impurities have eluted with adequate separation.

2. Experimental

2.1. Chemicals

Methanol (HPLC grade, Merck, USA), potassium dihydrogen ortho phosphate (A.R grade, Qualigens, India), sodium octane sulphonic acid (A.R grade, Spectrochem, India), ortho phosphoric acid (A.R grade, S.D. fine chemicals, India), HPLC grade water (Millipore Milli Q plus purification system). Samples of imatinib mesylate 4-(4-methyl-piperazin-1-ylmethyl)-*N*-[4-methyl-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-phenyl]-benzamide methanesulfonate and its impurities 4-(4-methyl–piperazin-1-ylmethyl)-benzoic acid (I), 4-methyl *N**3*-(4-pyridin-3-yl-pyrimidin-2-yl)-benzene-1, 3-diamine (II) and (2-methyl-5-nitro-phenyl)-(4-pyridin-3-yl-pyrimidin-2-yl)-amine (III) were received from Critical Care Division of Dr Reddy's Laboratories Limited, Hyderabad, India.

2.2. Equipment

A Waters LC system consisting of 510 HPLC pump, 717 plus Auto sampler, 486 tunable absorbance detector and 996 photodiode array detector was used for the development and validation studies. Chromatographic data were monitored, by using the Waters Millennium 32 Chromatography manager software. pH adjustment of aqueous phase was monitored using Elico pH meter Model No.L120.

2.3. Preparation of mobile phase

The mobile phase used under the chromatographic conditions was prepared by mixing aqueous buffer and methanol in the ratio of 42:58 (v/v). Aqueous buffer solution contains 30 mM sodium octane sulphonic acid in 10 mM aqueous potassium dihydrogen phosphate buffer and its

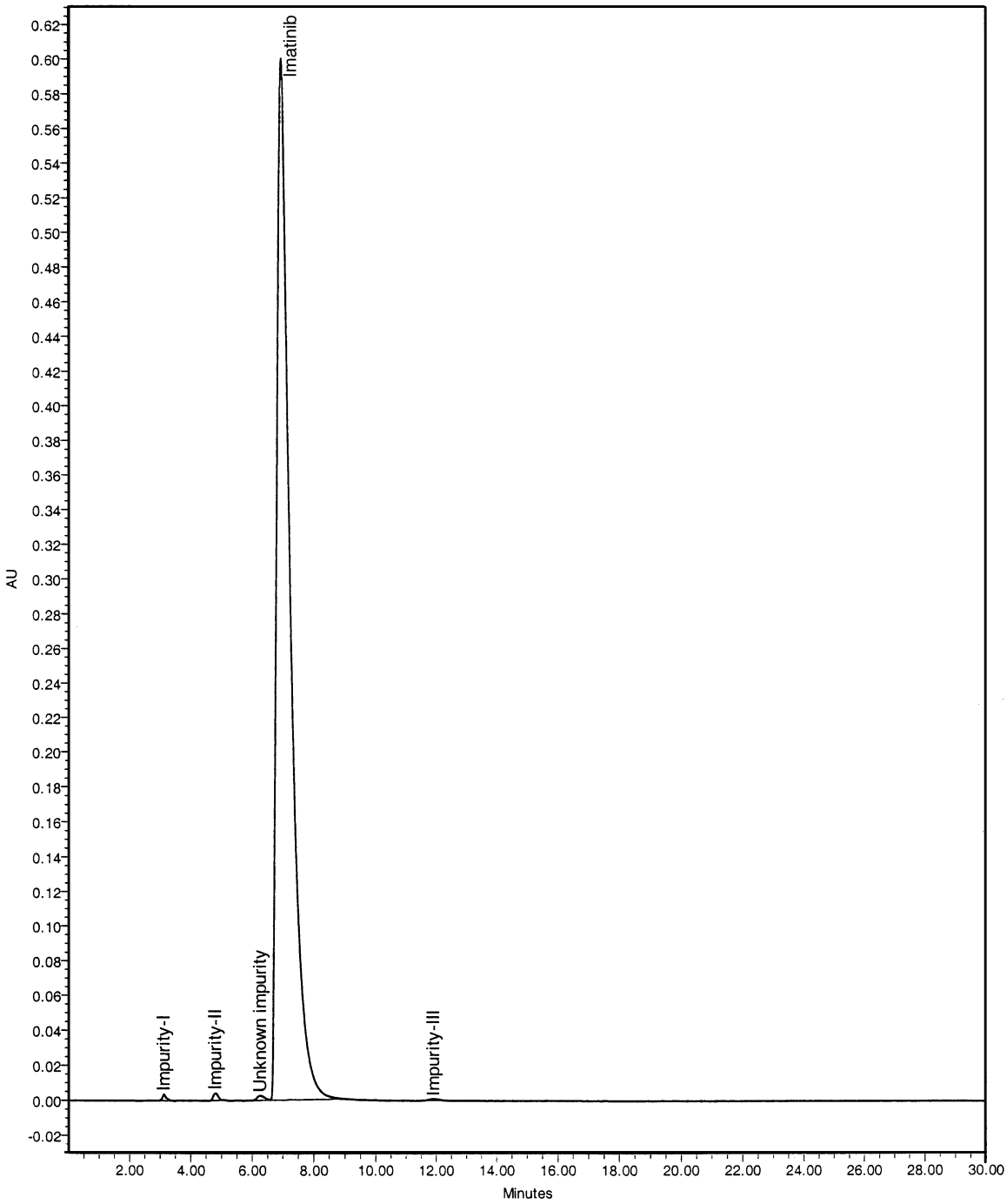

Fig. 2. Typical chromatogram of imatinib illustrating peak tailing in discovery RP amide C16 (250 × 4.6 mm, 5.0 μm) column, mobile phase consisting of 0.01 M KH_2PO_4:CH_3CN:MeOH (40:30:30) with a flow rate of 1.0 ml/min and UV detection at 237 nm.

Table 1

Compound name	Hichrom RPB (150 × 4.6 mm, 5.0 μm)			Novapak C18 (150 × 4.6 mm, 5.0 μm)			Symmetry C18 (150 × 4.6 mm, 5.0 μm)			Shimpak CLC ODS (150 × 4.6 mm, 5.0 μm)			Symmetry Shield RP18 (150 × 4.6 mm, 5.0 μm)		
	Rs	*T*	*k*	Rs	*T*	*k*	Rs	*T*	*k*	Rs	*T*	*k*	Rs	*T*	*k*
Impurity-I		1.4	2.8		1.2	2.2		1.4	2.9		1.1	3.4		0.9	2.1
Impurity-II	7.6	1.1	5.2	7.2	1.5	4.7	8.2	1.0	5.8	10.7	1.1	6.9	4.3	1.1	3.5
Impurity-III	13.0	1.1	11.1	12.5	1.5	12.8	15.2	1.0	13.5	16.8	1.1	16.4	3.8	1.1	13.5
Unknown impurity	3.1	1.9	13.2	2.3	1.5	15.4	4.7	1.0	16.9	6.1	1.0	21.3	12.7	1.3	9.4
Imatinib	3.3	1.3	16.3	2.7	2.4	19.4	5.6	1.2	22.4	5.6	1.4	27.4	2.0	1.2	11.0

Rs, resolution; *T*, tailing factor; *k*, retention factor.

pH was adjusted to 2.5 with ortho phosphoric acid. The resulting mixture was filtered through a 0.45 μm-nylon66 membrane using a Millipore vacuum filtration system.

2.4. Chromatographic conditions

A number of columns such as Hichrom RPB (150 × 4.6 mm, 5.0 μm), (Hichrom Ltd, UK) Novapak C18 (150 × 4.6 mm, 5.0 μm), (Waters, USA), Symmetry C18 (150 × 4.6 mm, 5.0 μm), (Waters, USA), Shimpak CLC ODS (M) (150 × 4.6 mm, 5.0 μm), (Shimadzu, Japan) and Symmetry Shield RP18 (150 × 4.6 mm, 5.0 μm), (Waters, USA) were used during method development. The method was optimized on a Symmetry Shield RP18 analytical column (150 × 4.6 mm, 5 μm). The flow rate was set at 1.0 ml/min and the column was maintained at room temperature. The injection volume was set to 10 μl and the detector was set at a wavelength of 237 nm.

2.5. Preparation of samples during method development

Stock solutions of imatinib and its impurities were prepared in acetonitrile in the concentration of 2.5 and 0.1 mg/ml, respectively. Two thousand micro litre of imatinib stock solution and 100 μl of each impurity stock solutions were transferred into a 10 ml of volumetric flask and made upto volume with mobile phase. This solution has imatinib mesylate at 0.5 mg/ml and various impurities are present at 0.2% level. This solution was used for the initial method development and method optimization.

2.6. Preparation of samples for validation

Stock solutions of imatinib and its impurities were prepared in methanol. These stock solutions were further diluted with mobile phase to get the required concentrations for validation studies.

Table 2
System suitability results obtained in Symmetry Shield RP18 (150 × 4.6 mm, 5.0 μm) column, mobile phase consisting of 30 mM sodium octane sulphonic acid in 0.01 M KH_2PO_4 (pH 2.5): MeOH (42:58) with a flow rate of 1.0 ml/min and UV detection at 237 nm

Compound name	USP resolution	Theoretical plates	Selectivity	USP tailing	Retention factor (k)
Impurity-I		1312		0.9	2.1
Impurity-II	4.3	3789	1.7	1.1	3.5
Unknown impurity	12.7	4409	2.7	1.3	9.4
Imatinib	2.0	4269	1.1	1.2	11.0
Impurity-III	3.8	6774	1.2	1.1	13.5

3. Results and discussions

3.1. Method development and column selection

Chemical structures of imatinib mesylate and its related substances are shown in Fig. 1. The impurities are labeled as impurity-I, impurity-II and impurity-III. Impurity-II is the precursor for imatinib. The laboratory batch sample of imatinib mesylate, which was selected for validation studies, contained an unknown impurity, which eluted at 10.5 min, (shown in Fig. 4). A resolution mixture containing imatinib and various impurities was used for method development.

Different mobile phases and stationary phases were employed to develop a suitable LC method for the quantitative determination of impurities present in imatinib mesylate. A number of columns containing various packing materials of ODS supplied by different manufacturers and different mobile phase compositions were tried to get good peak shapes and selectivity for the impurities present in imatinib. Peak tailing was observed when phosphate buffer with different compositions of acetonitrile and methanol was employed, a specimen chromatogram is shown in Fig. 2. USP tailing was found to be 1.9 for imatinib peak while it was found to be 1.3, 1.1, and 1.0 for impurity-I, impurity-II and impurity-III, respectively. In the next approach phosphate buffer and acetonitrile in a ratio of (75:25 v/v) was employed using a Symmetry C8 (150 × 4.6 mm, 5.0 μm) column. Under these conditions impurity-II eluted in close proximity to imatinib peak, moreover impurity-I showed an early retention at 1.2 min. Acetonitrile content was decreased to delay elution time of impurity-I, but decreasing the percentage of acetonitrile level did not increase the retention time significantly. In another attempt using Symmetry Shield RP18 (250 × 4.6 mm, 5.0 μm) and a mobile phase consisting of 0.05 M KH_2PO_4:CH_3CN:MeOH (70:20:10 v/v/v) imatinib peak eluted at 19 min with a good separation from impurity-II, however, impurity-I was not retained under these conditions and eluted at 2 min. Gradient elution was employed to ensure the retention time of impurity-I and the elution of imatinib peak and its other related impurities with good separation. In gradient elution the unknown impurity eluted very close to the imatinib peak. In another trial when different compositions of acetonitrile and phosphate buffer with pH adjusted to 3.0 were tried, early retention of imatinib was seen. In yet another trial Symmetry Shield RP18 (150 × 4.6 mm, 5.0 μm) column with a mobile phase consisting of 10 mM sodium octane sulphonic acid in 0.01 M KH_2PO_4 (pH 2.5 with H_3PO_4) and acetonitrile in a ratio of (70:30 v/v) was tried. In this solvent system the unknown impurity eluted very close to imatinib peak. Another solvent system consisting of 30 mM sodium octane sulphonic acid in 0.01 M KH_2PO_4 (pH 2.5 with H_3PO_4) and methanol in a ratio of (42:58 v/v) using a Symmetry Shield RP18 (150 × 4.6 mm, 5.0 μm) column was tried. Imatinib eluted around 11–13 min and the separation of imatinib from its impurities was found to be exceptionally good in this solvent system. Methane sulphonic acid showed an early elution at 1.7 min with poor response. Tailing factor, resolution and retention factor were generated on the columns from different manufacturers with the above mobile

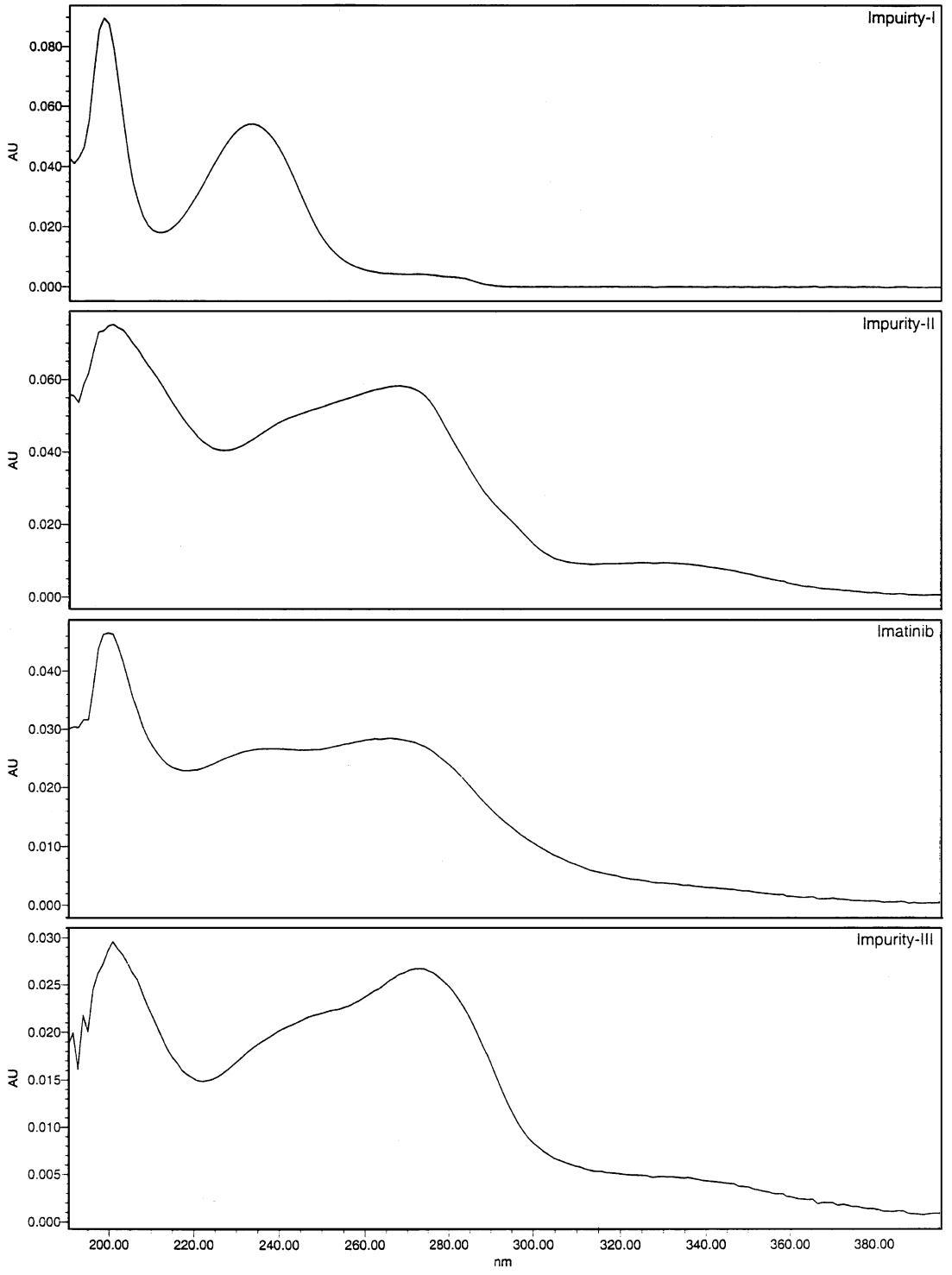

Fig. 3. UV spectra of imatinib and its impurities extracted from photodiode array detector in the final chromatographic conditions.

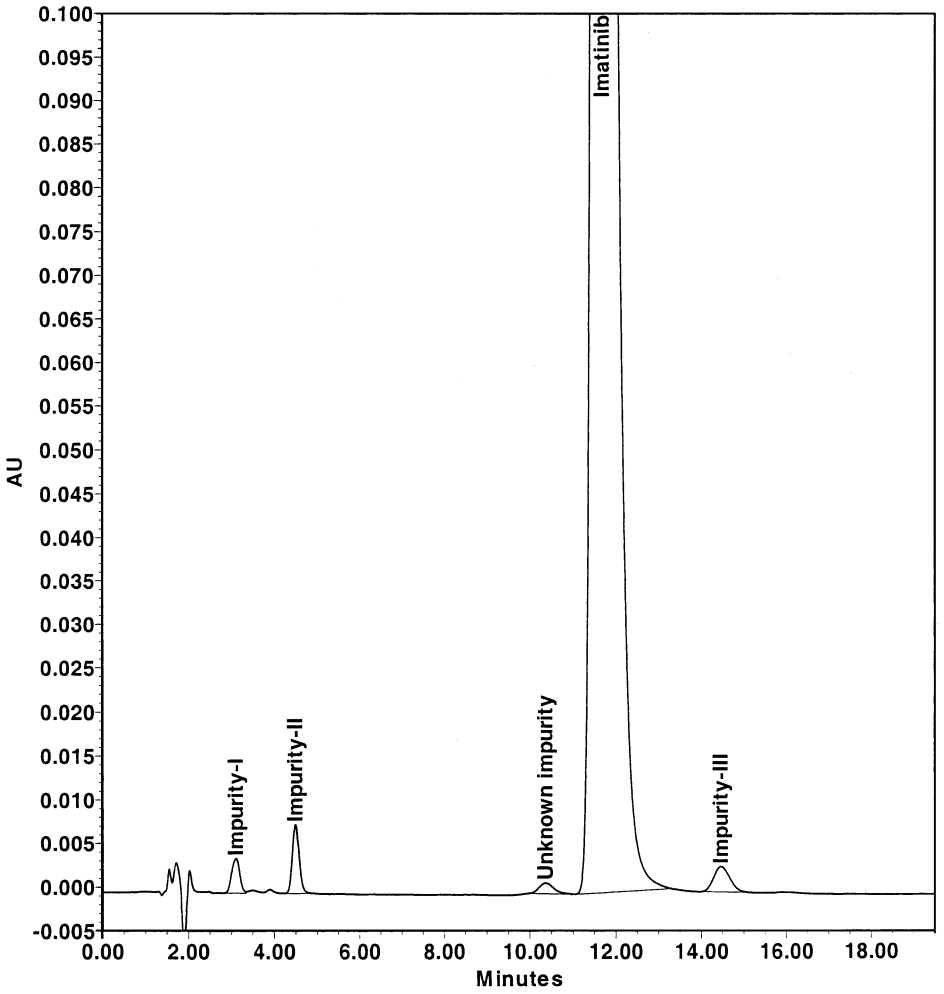

Fig. 4. Resolution chromatogram between imatinib and its impurities in Symmetry Shield RP18 (150 × 4.6 mm, 5.0 μm) column, mobile phase consisting of 30 mM sodium octane sulphonic acid in 0.01 M KH_2PO_4 (pH 2.5):MeOH (42:58) with a flow rate of 1.0 ml/min and UV detection at 237 nm.

phase. These results are shown in Table 1. The solvent system consisting of 30 mM sodium octane sulphonic acid in 0.01 M KH_2PO_4 (pH 2.5 with H_3PO_4) and methanol in a ratio of (42:58 v/v) using a Symmetry Shield RP18 (150 × 4.6 mm, 5.0 μm) column was selected for the validation studies. The system suitability results obtained using the final chromatographic conditions is shown in Table 2. Detection wavelength was selected as 237 nm to detect all the impurities. UV spectra of imatinib and its impurities are shown in Fig. 3. These spectras were extracted from photo diode array detector in the final chromatographic conditions.

3.2. Method validation

3.2.1. Specificity

Specificity of the method is its ability to detect and separate all the impurities present in the drug. Specificity of the method is demonstrated in terms of spectral as well as peak purity data of the drug and the impurities present in the drug.

Table 3
Accuracy results for impurity-I

Added (μg) ($n = 3$)	Recovered (μg)	% Recovery	%RSD
1.144	1.122	98.1	3.0
	1.190	104.0	
	1.148	100.4	
1.430	1.407	100.5	2.4
	1.412	98.8	
	1.371	95.9	
1.716	1.599	93.2	1.7
	1.643	95.8	
	1.650	96.2	

n, number of determinations.

Table 4
Accuracy results for impurity-II

Added (μg) ($n = 3$)	Recovered (μg)	% Recovery	%RSD
0.616	0.624	101.3	1.2
	0.629	102.1	
	0.614	99.7	
0.770	0.770	100.0	1.5
	0.750	97.5	
	0.770	100.0	
0.924	0.895	96.9	0.9
	0.897	97.1	
	0.882	95.5	

n, number of determinations.

Table 5
Accuracy results for impurity-III

Added (μg) ($n = 3$)	Recovered (μg)	% Recovery	%RSD
0.6168	0.533	86.5	1.7
	0.516	83.7	
	0.526	85.4	
0.7710	0.654	84.9	7.6
	0.761	98.8	
	0.727	94.3	
0.9252	0.823	88.9	3.2
	0.775	83.8	
	0.816	88.3	

n, number of determinations.

Impurities I, II and III were injected separately to confirm the retention times. Resolution mixture was then injected and specificity verified by performing peak purity test for each peak. All peaks passed the peak purity test. Resolution between imatinib and its impurities is shown in Fig. 4. Some of the small peaks observed in the chromatogram are due to impurities present in the batch sample, which were less than 0.02%.

3.2.2. Linearity

Linearity of the method was checked by preparing solutions at nine concentration levels of 0.08, 0.12, 0.16, 0.20, 0.30, 0.40, 0.50, 0.60 and 1.0% for impurity-I, impurity-II, impurity-III and imatinib. Each sample solution was injected three times. The mean responses recorded for each impurity were plotted against concentration. The correlation coefficient for impurity-I, impurity-II and impurity-III was found to be 0.9993, 0.9989 and 0.9994, respectively, which indicated good linearity. The calibration equations for impurity-I, impurity-II and impurity-III was found to be $y = 21278.63x - 2225.083$, $y = 37411.37x - 2212.65$ and $y = 40087.90x - 2138.615$, respectively.

3.2.3. Response factor

The response factors were calculated by taking the ratios of slopes of impurity-I, impurity-II and impurity-III with the slope of imatinib obtained from the linearity curves. The response factor for impurity-I was found to be 0.7 while it was 1.3 for impurity-II and impurity-III. These response factors were used to quantitate the amount of various impurities present in different batch samples of imatinib in weight percentage.

3.2.4. Accuracy

Imatinib solution was spiked with each impurity solution at different concentrations at 0.16, 0.20 and 0.24% of analyte concentration 0.5 mg/ml. Each spiked solution was prepared in triplicate and injected. The recovery percentage and %RSD were calculated for each impurity. Recovery of impurity-I, II and III ranged from 93.0–104.0, 95.0–102.0, and 83.0–98.0%, respectively. The results are shown in Tables 3–5, respectively. The acceptance criteria for recovery of an impurity at a concentration level of 0.2% is between 80 and 120% as per our in-house validation protocol.

Table 6
LOD and LOQ results for impurities

Compound name	LOD (ng/ml)	%RSD ($n = 6$)	LOQ (ng/ml)	%RSD
Impurity-I	57.14	6.7	214.29	2.8
Impurity-II	23.08	6.8	76.92	1.6
Impurity-III	38.46	11.9	192.31	4.5

n, number of determinations.

Table 7
Results obtained from three different batches of imatinib mesylate

Compound code	Impurity-I % area	Impurity-II % area	Unknown impurity % area	Imatinib % area	Impurity-III % area
B.No.IM/221	0.23	0.19	0.04	99.24	0.12
B.No.IM/245	0.20	0.16	0.04	99.40	0.13
B.No.IM/317	0.20	0.18	0.04	99.39	0.12

3.2.5. Limit of detection

The sensitivity for detection can be demonstrated by determining the limit of detection (LOD). A signal-to-noise (S/N) ratio between 2 and 3 is generally considered to be acceptable for estimating detection limit. S/N ratios of individual peaks were determined at different concentrations to estimate LOD and the respective %RSD was calculated for replicate injections ($n = 6$). The LOD was found to be 0.011% (57.14 ng/ml) for impurity-I, 0.005% (23.08 ng/ml) for impurity-II and 0.008% (38.46 ng/ml) for impurity-III. The results are shown in Table 6.

3.2.6. Limit of quantification

The quantitation limit is the lowest concentration of a substance that can be quantified with acceptable precision and accuracy. A typical S/N ratio of 9–10 is generally considered to be acceptable for estimating the limit of quantification. S/N ratios of individual peaks were determined at different concentrations to estimate limit of quantitation (LOQ) and the respective %RSD was calculated for replicate injections ($n = 6$). The LOQ was determined to be 0.043% (214.29 ng/ml) for impurity-I, 0.015% (76.92 ng/ml) for impurity-II and 0.038% (192.31 ng/ml) for impurity-III. The results are shown in Table 6.

3.2.7. System and method precision

System precision for various impurities in imatinib was checked for repeatability. The sample was prepared by spiking imatinib with the impurities at a concentration of 0.2% of target analyte concentration and injected six times. The %RSD was found to be less than 8.0% for system precision.

To determine the method precision six independent solutions were prepared by spiking imatinib with the impurities at a concentration of 0.2% with respect to target analyte concentration. Each solution was injected once. The variations in the results for the various impurities were expressed in terms of %RSD. The values calculated were found to be below 7.0% RSD for the impurities, indicating satisfactory method precision.

3.2.8. Stability in analytical solution

A solution of imatinib containing impurities was prepared and kept at room temperature. This solution was injected at intervals of 0, 6, 12, 18 and 24 h. Areas of all impurities were nearly identical to that obtained at 0 h and additional peaks were not observed which indicates solution stability.

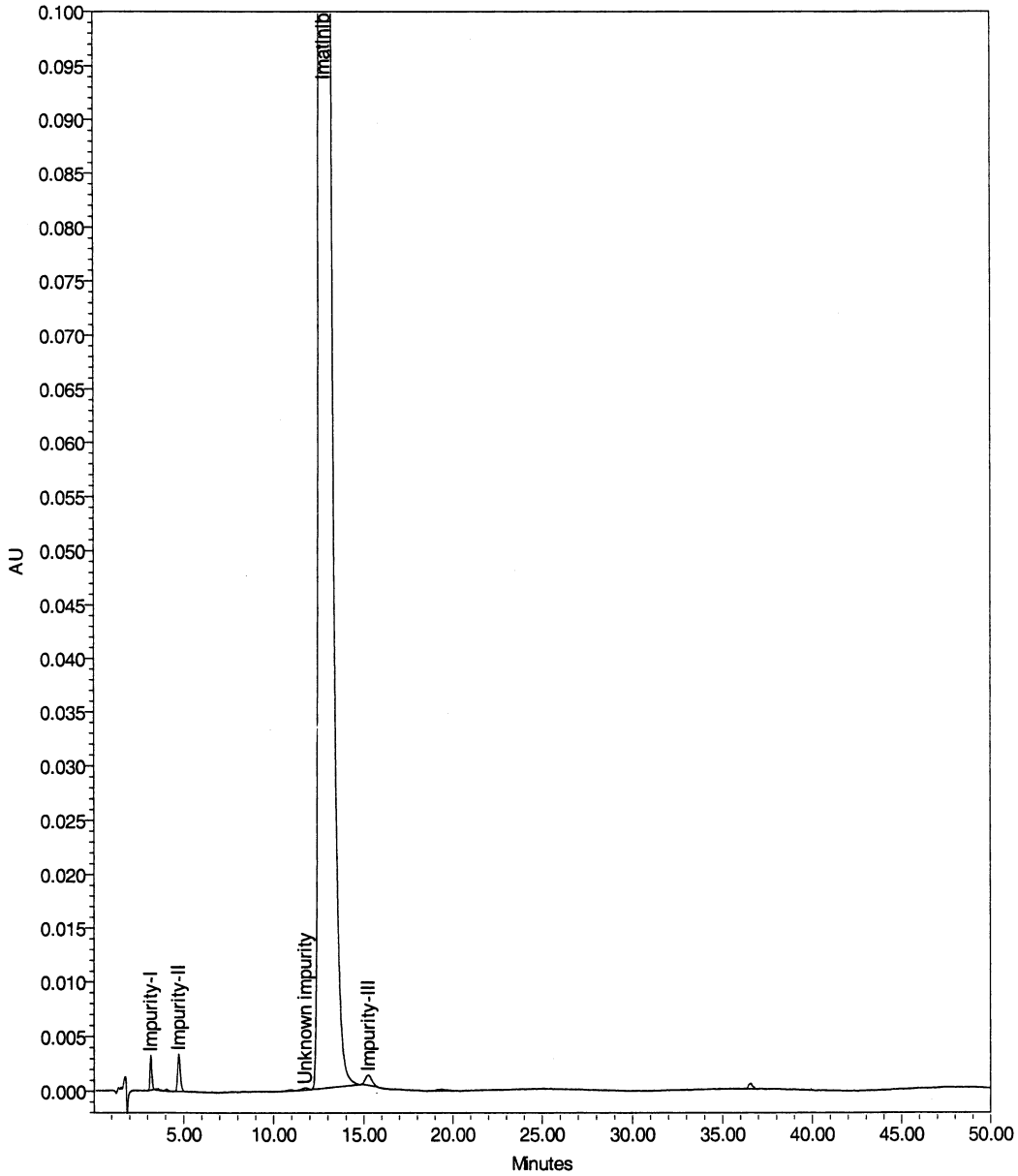

Fig. 5. The chromatogram for a typical batch of imatinib obtained using the method with which the validation was conducted.

4. Preparation of imatinib sample solution in routine analysis

Weighed 5.0 mg of imatinib mesylate sample in 10 ml of volumetric flask, dissolved in minimum amount of methanol and made up to the volume with mobile phase. Injected this solution into HPLC to determine the amount of impurities present in the sample. Three different batches of imatinib mesylate was analyzed under developed conditions and presented the results in Table 7. The chromatogram obtained after analysis was

shown in Fig. 5. Some of the small peaks observed in the chromatogram are due to impurities present in the batch sample, which were less than 0.1%.

5. Conclusions

The proposed LC method is selective for the quantification of impurities present in imatinib. The method is capable of detecting all process intermediates and other related compounds. Hence this method is useful for the purity determination of imatinib.

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