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Validated RP-HPLC analytical method for simultaneous estimation of imatinib mesylate and anastrazole in pharmaceutical formulation

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Abstract
A simple, precise, accurate, efficient and reproducible, isocratic Reverse Phase- High Performance Liquid Chromatography (RP-HPLC) method was developed and validated for the simultaneous estimation of Imatinib mesylate and Anastrazole in pharmaceutical formulation. Imatinib mesylate and Anastrazole were separated using an Phenomenex Luna 3µ C8(2) 100A°, LC Column 150 x 45, Shimadzu of 2030 LC Prominence i-series with high detection capabilities of PDA detector and the mobile phase contained a mixture of 0.02M sodium dihydrogen phosphate (pH adjusted to 2 with 0.1% orthophosphoric acid), acetonitrile and water (30:55:15,v/v/v). The flow rate was set to 1ml/min with the response detected at 228nm. The retention time of Imatinib mesylate and Anastrazole was found to be 1.88min, 3.139 min. Linearity for imatinib mesylate, in the range of 100-500µg/ml, for anastrazole in the range of 1-5µg/ml with correlation coefficient of 0.9999. The percentage recovery of Imatinib mesylate and anastrazole was found to be 100.27, 99.75 respectively. Validation parameters such as specificity, linearity, precision, accuracy, robustness, and limit of detection (LOD), limit of quantification (LOQ) were evaluated for the method according to the International Conference on Harmonization (ICH) Q2 R1 guidelines.

Keywords: imatinib mesylate, anastrazole, RP-HPLC, ICH, LOD, LOQ

Introduction

Anastrazole

Anastrazole is chemically 2-[3-(2-cyanopropan-2-yl)-5-(1, 2, 4-triazol-1-ylmethyl) phenyl]-2-ethylpropanenitrile [1].It is a potent nonsteroidal Aromatase inhibitor mainly used in the treatment of breast cancer in postmenopausal women [2]. It is an off white crystalline solid, odourless and has moderate aqueous solubility. Anastrazole is freely soluble in methanol, acetone and ethanol, tetrahydrofuran and very soluble in acetonitrile [3]. Anastrazole selectively binds to and reversibly inhibits the aromatase [4]. A cytochrome P450 enzyme complex found in many tissues including those of premenopausal ovary, liver and breast. Aromatase catalyzes the aromatization of androstenedione and testosterone into estrone and estradiol [5]. It can also contribute to decrease the risk of stroke, heart attack, chronic inflammation, prostate enlargement and prostate cancer [6]. Anastrazole is eliminated slowly with a plasma elimination half-life of 40-50 hours .As per the literature survey it is revealed that very few analytical methods for the separation and estimation of anastrazole have been reported such as UV-spectrophotometric method and HPLC methods.

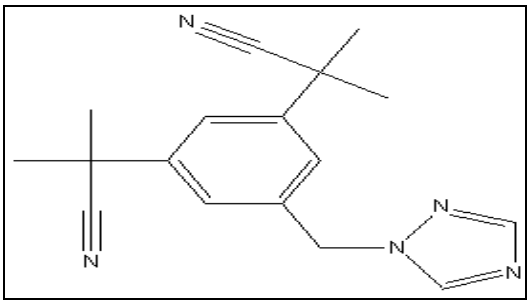

Fig 1: Structure of Anastrazole Imatinib mesylate:

Imatinib mesylate is chemically 4-[(4-Methyl-1-piperazinyl) methyl]-N-[4-methyl-3-[(4-(3-pyridinyl)-2 pyrimidinyl] amino] phenyl] benzamid methane sulfonate [7]. Imatinib mesylate is 2-phenylaminopyrimidine derivative.It is tyrosine kinase inhibitor [8]. Imatinib is designed to inhibit tyrosine kinase such as Bcr-Abl and is used in the treatment of chronic myeloid leukemia (CML), gastrointestinal stroma tumors (GISTs) [9]. It is freely soluble in dimethyl sulfoxide, methanolacetonitrile, water and ethanol and is insoluble in n-octanol, acetone.In chronic myelogenous leukemia, the Philadelphia chromosome leads to a fusion protein of *abl* with *bcr* (*breakpoint cluster region*), termed *bcr-abl*. Imatinib is used to decrease *bcr-abl* activity [10]. Imatinib mesylate is one of the newest anticancer drug on the market and was one of the first drugs to be pushed through the food and drug administration quick track approval designation [11]. The elimination halflife of imatinib mesylate and its active metabolite, N-desmethylimatinib (M1) were approximately 18 and 40hours respectively [12]. As per the literature survey it is revealed that very few analytical methods for the separation and estimation of anastrazole have been reported such as UVspectrophotometric method and HPLC methods.

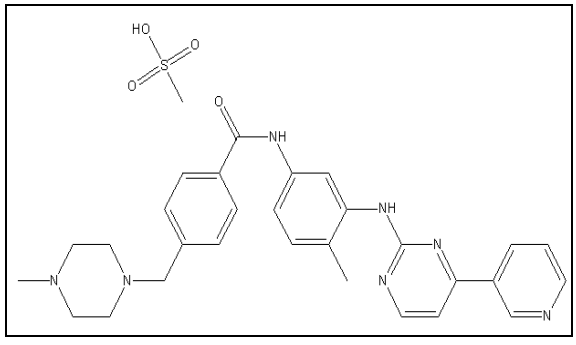

Fig 2: Structure of Imatinib mesylate

Materials and Methods

Ingredients

Anastrazole and Imatinib mesylate pharmaceutical formulation were kindly procured from National Scientific laboratories.commercial pharmaceutical formulations which are claimed to contain 1 mg of anastrazole and 100 mg of imatinib were used in analysis. HPLC grade water and acetonitrile were procured from merck manufactures, were used for analysis.

Instrumentation

Equipment used was manufactured by Shimadzu of 2030 LC Prominencei-series with high detection capabilities of PDA detector and Autosampler was used for sampling. Also used pH meter manufactured by LABINDIA, Analytical balance manufactured by ESSAE.

Preparation of Mobile Phase

0.02M of sodium dihydrogen phosphate buffer solution was prepared by taking 0.312gms of sodium dihydrogen phosphate is dissolved in 1000ml HPLC grade water in 1000ml volumetric flask and adjust to pH 2 with 0.1%OrthoPhosphoric acid solution .Then the resulting solution was filtered through 0.45µ membrane filter under vaccum filtration.

Diluent: Acetonitrile: water (50:50v/v).

Method development

Mobile phase consists of 0.02MSodim dihydrogen phosphate: acetonitrile: water in the ratio of 30:55:15v/v/v. The mobile phase was pumped from the solvent reservoir in the ratio of 30:55:15v/v/v. to the column at a flow rate of 1 ml/min whereas run time set was 6 mins .the separation was performed on Phenomenex 3µ mC8(2)100A^{O}, LC column 150×45mm.

The column was maintained at ambient temperature and the volume of each injection was 20µl.prior to injection of the solution column was equilibriated for atleast for 30 mins with mobile phase flowing through the system. The eluents were detected at 228nm.

Preparation of Standard Stock Solution

Weigh accurately about 100 mg of Anastrazole and 100 mg of Imatinib mesylate of working standards were transferred into 100 ml volumetric flasks separately .100 ml of diluent (acetonitrile : water (50:50v/v))was added, sonicated for few minutes, to dissolve and make up to volume with diluent to obtain a concentration of 1000µg/ml. Filter with 0.45µ membrane filter.

Preparation of Sample Solution

Twenty tablets (each tablet contains 100mg Imatinib mesylate and 1mg Anastrazole) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed .weight equivalent to 1tablet powder of Imatinib mesylate and Anastrazole and dissolved in sufficient diluents (Acetonitrile: water (50:50v/v)).After that filtered the solution using 0.45µ membrane filter and sonicated for 5 min and dilute to 100 ml with diluent.The optimized chromatographic conditions were shown in table: 1

Table 1: Optimised chromatographic conditions and system suitability parameters for proposed HPLC method of Imatinib mesylate and Anastrazole.

Parameters	Imatinib Mesylate	Anastrazole
Column	Phenomenex luna3umC8(2)100A^{O},LC column 150×45mm.	
Diluent	Acetonitrile: water (50:50v/v)	
Mobile phase	0.02Msodiumdihydrogen phosphate: acetonitrile: water(30:55:15v/v/v)	
Flow rate	1ml/min	
Column temperature	Ambient	
Sample temperature	NA	
Injection volume	20µl	
Wavelength	228nm	
Run time	6min	
Retention time	1.88,3.139	
The %RSD Peak areas of Imatinib Mesylate and Anastrazole	0.59	
Tailing factor	1.99,1.73	
Plate count	5362,5469	

Results and Discussion

Validation of Proposed Method

The developed method of analysis was validated as per the ICH guidelines for the parameters like system suitability, specificity, linearity, accuracy, precision, robustness, Ruggedness, limit of detection and limit of quantification

System Suitability Tests

System suitability tests parameters were checked by repetitively injecting the drug solution at the concentration level of 10ug/ml for anastrazole and imatinib mesylate to check the reproducibility of the system. System suitability parameters like number of theoretical plates (N),tailing factor ,resolution ,and relative standard deviation of peak height or peak area or repetitive injections were studied .The %RSD values are below 2%,theoretical plate count is above 2000 and tailing factor is less than 2,indicating that the method is suitable the results were shown in table: 2

Table 2: system suitability tests for Imatinib mesylate and Anastrazole

Drug	Theoretical plates	Tailing factor	Retention time	Resolution
Imatinib Mesylate	5362	1.454	1.885	NA
Anastrazole	5469	1.234	3.139	5.808

Linearity

Linearity for the developed method was checked by the standard solutions containing Imatinib mesylate in the concentration range of 100-500ug/ml, Anastrazole in the concentration range of 1-5ug/ml were prepared and injected into the chromatographic system and chromatograms were recorded. A linear relationships between the peak areas versus concentration was observed in the range of study. The slope, y-intercept and correlation coefficient of Imatinib mesylate was found to be 4242, 1847 and 0.9999, the slope, y-intercept and correlation coefficient of anastrazole was found to be 89565,476.1 and 0.9999 respectively.

The chromatograms were developed and shown in figure 3 and figure: 4

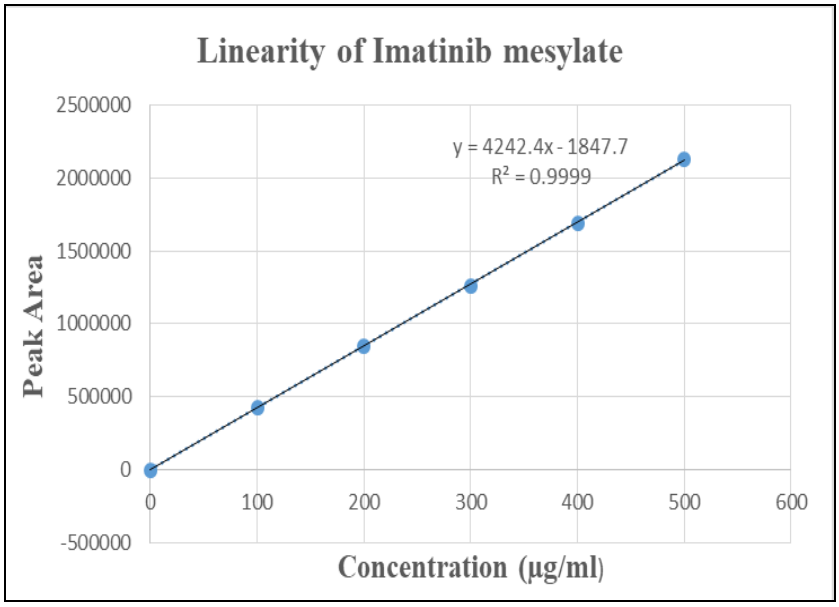

Fig 3: Linearity graph of Imatinib mesylate

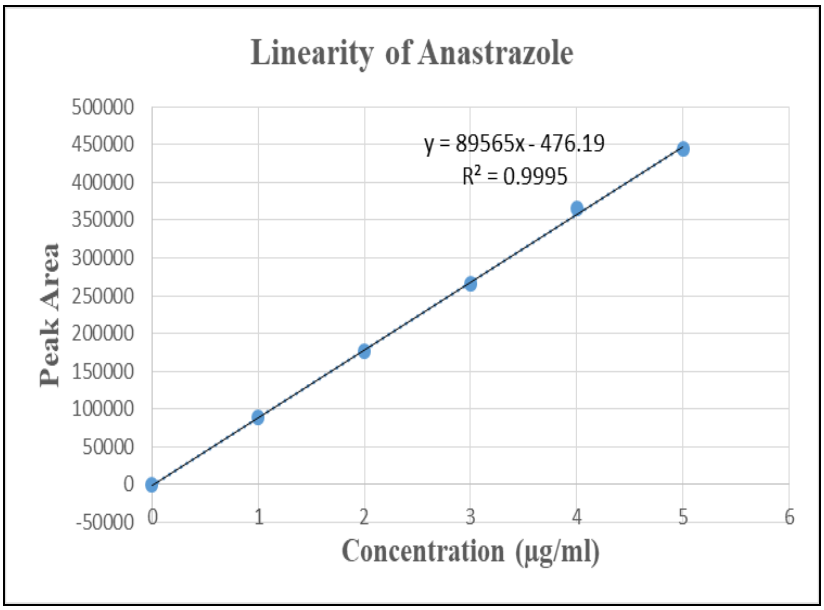

Fig 4: Linearity graph of Anastrazole

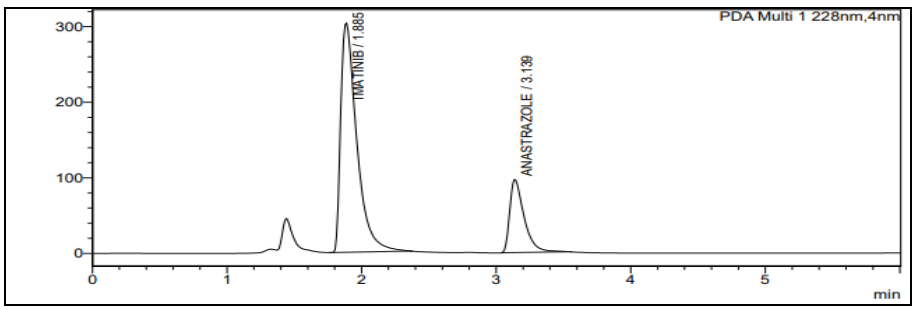

Fig 5: Standard chromatogram of Imatinib mesylate and Anastrazole

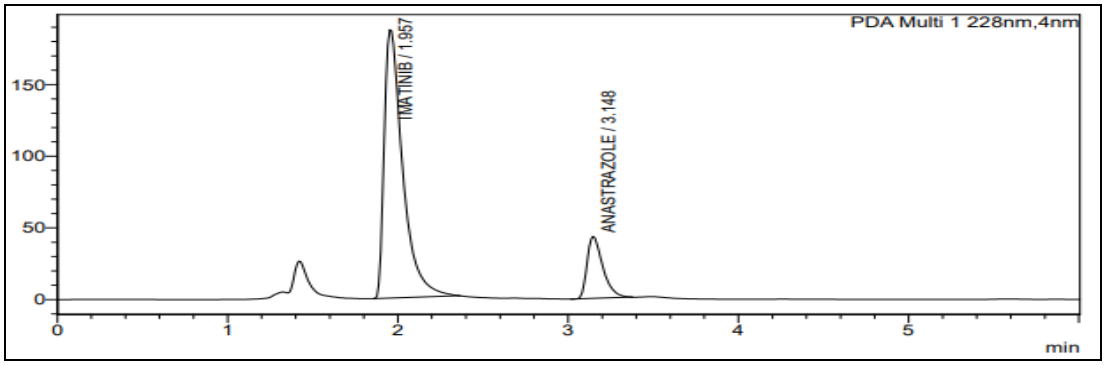

Fig 6: Sample chromatogram of Imatinib mesylate and Anastrazole

Precision

Method Precision

Precision is the measure of closeness of the data values to each other for a number of measurements under the same analytical conditions.

Precision of the test method was determined by six replicates (n=6) solutions were prepared and each solution was injected in duplicate under the same conditions and mean value of peak area response for each solution were considered.

The results were given in Table: 5

Intermediate Precision

The intermediate precision of the method was evaluated by performing precision on different lab by different analyst and different days.

The standard preparation concentrations of 100mg of imatinib mesylate and 1mg of anastrazole was injected six times in to the HPLC and the %RSD for the area of 6 replicate injections was calculated. The results were given in Table: 5

Table 3: Precision data of Imatinib mesylate and Anastrazole

S.no	Imatinib Mesylate			Anastrazole		
	Retention time	Peak area		Retention time	Peak area	
		I.P	M.P		I.P	M.P
1.	2.001	1264306	1265506	3.128	263378	264668
2.	1.976	1264570	1266670	3.132	264888	266458
3.	1.968	1262941	1267741	3.132	263314	263414
4.	1.962	1273391	1272391	3.132	268917	265517
5.	1.961	1265701	1283301	3.135	262258	262158
6.	1.959	1283007	1272207	3.135	263979	264379
Avg	1.9711	1268986	1271303	3.1323	264455.7	264432.3
SDV	0.0158	7806.781	6537.747	0.002	2349.984	1519.724
% Rsd	0.804	0.615	0.514	0.082	0.889	0.575

Acceptance Criteria

The %RSD for the peak area of six standard injections should not be more than 2.0%.

Accuracy

The accuracy of the method was determined by calculating recovery of Imatinib and Anastrazole at 50%, 100%,150% was added to a pre quantified sample solution and injected in to the HPLC system. The mean percentage recovery of imatinib and anastrazole at each level was calculated and given in Table: 6, 7.

Table 4: Accuracy data of Imatinib mesylate

Recovery level	Accuracy of Imatinib mesylate				
	Peak area		%Recovery	% of mean recovery	Average %recovery
	Sample	Standard			
50	635614	127219	99.87	100.13	100.27
	638452	127219	100.39		
	637582	127219	100.13		
100	1283037	127219	100.92	100.71	
	1279112	127219	100.56		
	1280574	127219	100.66		
150	1897099	127219	99.56	99.98	
	1912045	127219	100.27		
	1911112	127219	100.13		

Table 5: Accuracy data of Anastrazole

Recovery level	Accuracy of Anastrazole				
	Peak area		%Recovery	% of mean recovery	Average %recovery
	Sample	Standard			
50	132864	266039	99.82	99.92	99.75
	133158	266039	100.12		
	132956	266039	99.84		
100	265960	266039	100.03	99.99	
	265898	266039	99.96		
	266014	266039	99.98		
150	395425	266039	99.23	99.36	
	396457	266039	99.41		
	396921	266039	99.44		

Acceptance Criteria

The % Mean recovery should be within 99.00 -102.00%

Limit of Detection and Limit of Quantitation

The LOD of an individual analytical procedure is the lowest amount of components in a sample which can be detected but not necessarily quantitated as an exact value .The LOQ is a parameter of quantitative assay for low levels of compounds in a sample and is used particularly for the determination of impurities and degradation products.

The LOD for this method was found to be 7.58µg/ml, 018µg/ml and the LOQ value was found to be 22.97µg/ml, 0.53µg/ml.

Robustness

In the study of Robustness, chromatograms were recorded for flow rate and mobile phase composition variation, and chromatographic parameters were evaluated. It was found that there was no considerable variation in retention time and wavelength for these variations .In the present investigation robustness of the proposed method was demonstrated between different flow rates and different mobile phase compositions.

Theoritical plates of Imatinib mesylate and Anastrazole was found to be respectively. The results were shown in table: 8

Table 6: Robustness data of Imatinib mesylate

S.No	Parameter	Imatinib mesylate			
		RT(min)	Average	Tailing	%RSD
1	Change in flow rate-0.8 ml/min	2.428	1642638	1.983	0.02
	Change in flow rate-1.2ml/min	1.665	1091341	1.921	0.01
2	Change in mobile phase composition (35:50:15)	2.000	1332475	2.00	0.005
	Change in mobile phase composition (40:45:15)	2.155	1285006	1.933	0.08

Table 7: Robustness data of Anastrazole

S.No	Parameter	Anastrazole			
		RT(min)	Average	Tailing factor	%RSD
1	Change in flow rate- 0.8 ml/min	3.892	336442	1.452	0.04
	Change in flow rate- 1.2 ml/min	2.647	218669	1.389	0.08
2	Change in mobile phase composition (35:50:15)	3.607	283886	1.561	0.28
	Change in mobile phase composition (40:45:15)	4.569	265918	1.528	0.56

Acceptance Criteria

The %RSD for the peak area by changing flow rate and mobile phase proportion should not be more than 2.0%.

Assay

20 tablets each tablet contains the 100mg of Imatinib mesylate and 1 mg of Anastrazole were weighed and taken into a mortar and crushed into a fine powder and uniformly mixed .weight equivalent to 1tablet powder is dissolved in sufficient mobile phase and filtered with 0.45µ membrane filter and sonicated for 5min and diluted with mobile phase. Peak area of both standard and test was determined. The percent of assay was calculated from the peak area of standard and sample.

The percent assay was calculated by using the following formula the results were shown in table: 9

$$\text{Assay} = \frac{\text{Response of test}}{\text{Response of standard}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of test}}{\text{Weight of test}} \times \frac{\text{Potency of API}}{100}$$

$$\times \frac{\text{Average weight of formulation}}{\text{Labelled claim}} \times 100$$

Table 8: Assay of Anastrazole and Imatinib mesylate

Drugs	Sample peak area	Standard peak area	Labelled amount (mg/tab)	%Assay
Imatinib	1293037	1272119	100mg	101.68
Anastrazole	265814	266039	1mg	99.95

Acceptance Criteria

The %Assay should be within 99.00-102.00%.

Conclusion

The present developed isocratic RP-HPLC method was found to be specific, simple, accurate and rapid for the determination of Imatinib mesylate and Anastrazole in Pharmaceutical formulation.

Acknowledgement

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