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A VALIDATED HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC SIMULTANEOUS METHOD FOR THE QUANTIFICATION OF IMATINIB MESYLATE AND ANASTRAZOLE BY PDA DETECTOR

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ABSTRACT

The aim of the project is to develop a fast, simple, precise, and cost-efficient RP-HPLC method for measuring the amounts of Imatinib mesylateand Anastrazole simultaneously in both pharmaceutical products and bulk samples. This technique has effectively achieved the separation of Imatinib mesylateand Anastrazole in large quantities. The separation was performed using a Phenomenex C18 150 x 4.6 mm, 5m analytical column at a wavelength of 280 nm. The mobile phase consisted of a mixture of methanol, water and acetonitrile in a ratio of 30:60:10. The pH of the buffer was adjusted to 5.5. The separation was carried out in isocratic elution mode with a flow rate of 1.0 ml/min. Imatinib mesylate had a retention time of 1.885 minutes, whereas Anastrazole showed a retention duration of 3.139 minutes.

ISSN:0975 -3583,0976-2833 VOL 15, ISSUE 01, 2024

Quantitative analysis of Imatinib mesylate and Anastrazole was achieved using PDA detection at 285 nm using a linear calibration curve. The concentration ranges of 10-50 µg/ml (with a correlation coefficient of 0.9995) and 1-5 µg/ml (with a correlation coefficient of 0.9995) were used for reliable quantification. The limit of detection (LOD) for Imatinib mesylate was 3.1245 µg/ml, whereas the LOD for Anastrazole was 0.3356 µg/ml. The proposed method is highly suitable for use in quality-control laboratories for the bulk and pharmaceutical quantitative analysis of pharmaceuticals, whether used individually or in combination. This technique is characterised by its simplicity and efficiency, while yet ensuring a high level of accuracy and precision.
Keywords: Imatinib mesylate, Anastrazole and RP-HPLC.

Introduction:

Anastrazole (ATZ) is a chemical compound with the molecular formula 2-[3-(2-cyanopropan-2-yl)-5-(1,2,4-triazol-1-ylmethyl) phenyl]-2-ethylpropanenitrile.This medication is a powerful nonsteroidal Aromatase inhibitor primarily used for the treatment of breast cancer in women who have gone through menopause. The substance is an off-white crystalline solid that lacks odor and has modest solubility in water. Anastrazole has high solubility in methanol, acetone, ethanol, and tetrahydrofuran, and it is particularly soluble in acetonitrile [3]. Anastrazole exhibits specific binding to and reversible inhibition of the enzyme aromatase [4]. A cytochrome P450 enzyme complex is present in several organs, including the ovaries, liver, and breasts of premenopausal individuals. Aromatase facilitates the conversion of androstenedione and testosterone into estrone and estradiol via a process known as aromatization [5]. Additionally, it may help reduce the likelihood of stroke, heart attack, chronic inflammation, prostatic enlargement, and prostate cancer [6]. Anastrazole has a prolonged plasma elimination half-life of 4050 hours.Based on the literature study, it has been discovered that very few various analytical techniques have been documented for the separation and quantification of anastrazole, including UV-spectrophotometric method and HPLC methods.

Imatinib mesylate (ITM) is a chemical compound with the chemical formula 4-[(4-Methyl-1-piperazinyl) methyl].The chemical compound is 4-methyl-3-[(4-(3-pyridinyl)-2-pyrimidinyl)amino]phenyl. The compound is called benzamid methane sulfonate. Imatinib mesylate is a derivative of 2-phenylaminopyrimidine.The compound is a tyrosine kinase inhibitor [8]. Imatinib is specifically formulated to hinder the activity of tyrosine kinase enzymes, such as Bcr-Abl. It is used for the therapeutic management of chronic myeloid leukemia (CML) and gastrointestinal stroma tumors (GISTs) [9]. The compound has high solubility in dimethyl sulfoxide, methanolacetonitrile, water, and ethanol, but it is insoluble in n-octanol and acetone.Chronic myelogenous leukemia is characterized by the presence of the Philadelphia chromosome, which results in the formation of a fusion protein called bcr-abl. This fusion protein is formed by the combination of abl and bcr (breakpoint cluster region) genes.

Imatinib is used to reduce the activity of bcr-abl [10]. Imatinib mesylate is a recently developed anticancer medication that received expedited clearance from the Food and Drug Administration. The half-life of imatinib mesylate and its active metabolite, N-desmethylimatinib (M1), were about 18 and 40 hours, respectively [12]. According to the

ISSN:0975 -3583,0976-2833 VOL 15, ISSUE 01, 2024

literature study, only a small number of analytical techniques have been described for the separation and quantification of anastrazole, including the UV spectrophotometric method and HPLC approaches.

Materials and Methods:

Drug substance (Imatinib mesylate and Anastrazole),Mthanol (HPLC grade), Water (HPLC grade) and acetonitrile (HPLC grade), HPLC system (Shimadzu SPD-20A, Tokyo, Japan).

Instrumentation:

The HPLC investigations were carried out using a Shimadzu SPD-20A HPLC system manufactured by Tokyo, Japan. The system comprised of a separation module and a photodiode array detector. The experiments were conducted in isocratic mode, using an Autosampler. The data collection and processing were performed using laboratory solution software. The separation was conducted with a Phenomenex C18 150 x 4.6 mm, 5m analytical column. The supplementary apparatus used included a pH meter (Eutech), a weighing scale (Shimadzu), and an ultrasonicator (Unichrome, UCA701).

Preparation of mobile phase:

Mobile phase was prepared by mixing methanol, water and acetonitrile in a ratio of 30:60:10 and the pH of the buffer was adjusted to 5.5and was filtered through 0.45µ membrane.

Preparation of standard stock solution:

Using a digital microbalance, 10 mg of Imatinib mesylate and 1.0 mg of Anastrazole were measured and placed into a 100 millilitre volumetric flask. Following the addition of seven millilitres of diluent, the mixture was subjected to sonication in order to facilitate dissolution. Subsequently, the solution was diluted to its full capacity using the diluent, and ultimately, it was further diluted to achieve 30 µg/ml and 3 µg/ml of Imatinib mesylate and Anastrazolethe respectively.

Chromatographic conditions:

High Performance Liquid Chromatography equipped with PDA detector.

For Imatinib mesylate and Anastrazole (isocratic)

Column :	PhenomenexC18 150 x 4.6 mm, 5m analytical column
Wavelength :	2805nm
Injection Volume :	20µl
Column Temperature:	Ambient
Flow rate :	1.0 ml/min

The ITM peak was detected at 1.885 minutes and had an area of 958764, with a tailing factor of 1.12. Figure 1 and Table 1 demonstrate that the ATZ peak appeared at a retention time of 3.139 minutes, with a peak area of 58615, a tailing factor of 1.15, and a resolution of 2.56. The experiment was considered ideal since it yielded favorable results and had a shorter time of retention. The retention time of ITM is about 1.885 minutes, whereas the retention period of ATZ is 3.139 minutes.

ISSN:0975 -3583,0976-2833 VOL 15, ISSUE 01, 2024

Table 1: System suitability parameters

S.No.	Name of the Peak	Retention Time (Mins)	Peak Area	Tailing Factor	Resolution	Plate Count
01	Imatinib mesylate	1.885	958764	1.12	---	6578
02	Anastrazole	3.139	58615	1.15	2.56	5642

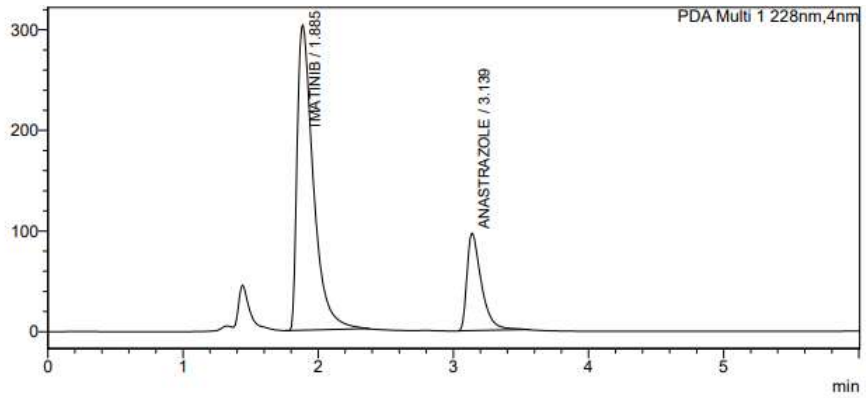

Fig.No. 01 : Typical Chromatogram of Imatinib mesylate and Anastrazole

Preparation of sample solution:

A total of 10 mg of each sample was measured and placed into a 10 ml volumetric flask, followed by the addition of 7 ml of diluent. The combination was thereafter subjected to sonication to facilitate the dissolution of the substance, and subsequently diluted to its final volume using a diluent. The solution was further diluted to a volume of 10 ml using the diluent and then passed through a 0.45µ Nylon syringe filter.

Procedure:

Five injections, each consisting of 20 µl, were administered for both the active ITM and ATZ reference solutions. Chromatograms were acquired and the peak responses were assessed. The system's appropriateness was determined by assessing its parameters. The measurement of ITM and ATZ in the sample was accomplished by the examination of the peak responses.

Method Validation:

The current investigation assessed several factors to validate the HPLC technique for measuring ITM and ATZ in line with the defined process, hence establishing its appropriateness for the intended use. The validation criteria were implemented in accordance with the standards established by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH).

Linearity and Range:

The concentrations of ITM and ATZ that exhibited a direct correlation with peak area were (10 - 50 µg/ml), (1 – 5 µg/ml). The results are shown in Figures 2 and 3, Tables 2 and 3, and the linearity of the calibration curve is verified by the strong correlation coefficient of the regression equation.

ISSN:0975 -3583,0976-2833 VOL 15, ISSUE 01, 2024

Table 2: Linearity data of ITM

S.No.	Concentration (µg/ml)	Peak Area
1	0	0
2	10	320588
3	20	642578
4	30	958764
5	40	1288352
6	50	1648598
Slope		**32750**
Intercept		**-8934.4**
Regression		**0.9995**

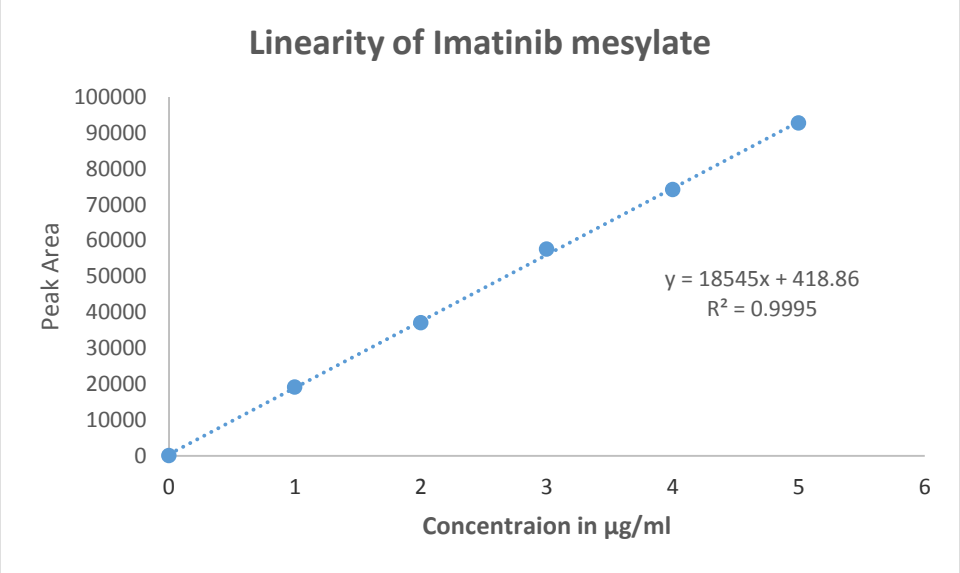

Fig.No. 02 : Linearity of Imatinib mesylate

Table 3: Linearity data of ATZ

S.No.	Concentration (µg/ml)	Peak Area
1	0	0
2	1	19139
3	2	37078
4	3	57615
5	4	74156
6	5	92695
Slope		**18545**
Intercept		**418.86**
Regression		**0.9995**

ISSN:0975 -3583,0976-2833 VOL 15, ISSUE 01, 2024

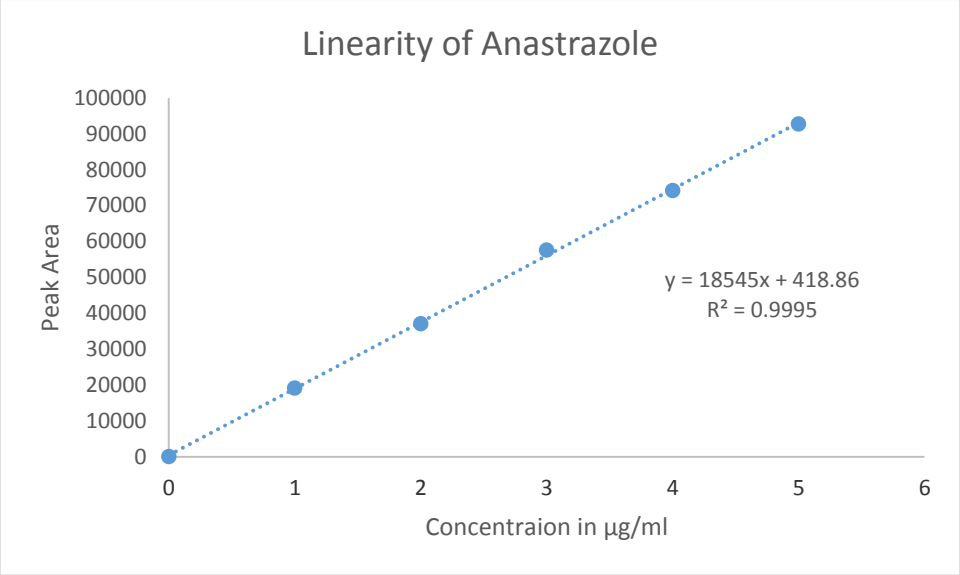

Fig.No. 03 : Linearity of Anastrazole

Accuracy and Precision:

The accuracy of recovery was assessed by adding additional Standard medication at three distinct concentration levels to a previously tested test solution. Our observations indicate that the recommended approach is very accurate for simultaneously estimating both ITM and ATZ, with a relative standard deviation (RSD) of less than 2%. The recovery rates for ITM and ATZ were found to be 99.70% and 9.92% respectively. The Method's reliability is shown by its great repeatability and low RSD readings. The tables numbered 4 and 5.

Table 4: Precision data of ITM and ATZ

Injection Number	ITM				ATZ			
	Retention Time	Peak Area	Plate Count	Peak Symmetry	Retention Time	Peak Area	Plate Count	Peak Symmetry
1	1.885	958764	6578	1.11	3.039	57615	5642	1.15
2	1.882	955674	6645	1.12	3.012	57256	5642	1.12
3	1.886	958624	6689	1.11	3.045	57895	5687	1.24
4	1.885	953265	6642	1	3.048	57426	5689	1.15
5	1.88	954268	6654	1.14	3.012	57143	5602	1.23
6	1.881	951235	6635	1.15	3.056	57246	5628	1.33
Average	1.883	955305			3.035	57430		
Standard Deviation	0.0025	2997			0.0189	282		
% RSD	0.1319	0.3138			0.6222	0.4906		

ISSN:0975 -3583,0976-2833 VOL 15, ISSUE 01, 2024

Table 5: Accuracy data of ITM and ATZ

Sample Preparation No.	ITM Assay (%)	ATZ Assay (%)
1	99.56	100.21
2	100.12	100.45
3	100.23	99.5
4	99.95	99.56
5	99.23	99.23
6	99.12	100.56
Mean	**99.70**	**99.92**
SD	**0.47**	**0.56**
RSD (%)	**0.4697**	**0.5584**

Robustness:

The results of the robustness analysis are shown in Table no.6. Both components exhibited comparable tailing factors, elution orders, resolutions, relative standard deviations, and recoveries. The analysis revealed that the relative standard deviation (RSD) of the peak sites was much below 2.0%.

ISSN:0975 -3583,0976-2833 VOL 15, ISSUE 01, 2024

Table 6: Robustness data of ITM and ATZ

Condition	Imatinib mesylate			Anastrazole		
	% RSD	Tailing Factor	% Recovery	% RSD	Tailing Factor	% Recovery
1) Change in Flow rate						
Normal Condition (1.0 ml per minute)	0.12	1.12	99.23	0.20	1.18	99.56
Flow rate(0.8ml per minute)	0.11	1.13	99.56	0.25	1.15	99.66
Flow rate(1.2 ml per minute)	0.25	1.21	99.89	0.28	1.10	99.86
2) Change in minor component in the mobile phase						
Normal Condition (Methanol, water, and acetonitrile mixture with a ratio of 30:60:10)	0.44	1.11	99.75	0.51	1.18	99.89
(Methanol, water, and acetonitrile mixture with a ratio of 40:50:10)	0.41	1.12	99.23	0.52	1.15	99.41
(Methanol, water, and acetonitrile mixture with a ratio of 20:70:10)	0.42	1.10	99.98	0.61	1.18	99.99
3) Change in Wave Length						
Normal:Wave Length 285 nm	0.21	1.11	99.56	0.12	1.21	99.56
Wave Length 280 nm	0.22	1.12	99.85	0.21	1.25	99.65
Wave Length 290 nm	0.19	1.21	99.87	0.31	1.29	99.85
4) Change in pH						
Normal:pH 5.5	0.21	1.01	99.89	0.85	1.21	99.90
pH 5.0	0.23	1.08	99.45	0.45	1.22	98.57
pH 6.0	0.28	1.09	99.57	0.65	1.24	99.45

Ruggudness:

Imatinib mesylate and Anastrazole had respective mean peak areas of 955205 and 57570 with an RSD of 0.21 and 0.25%, respectively.

SUMMARY:

A novel and validated RP-HPLC method has been developed to determine ITM (Impurity to Main component ratio) and ATZ (Active to Total Z component ratio) in large quantities and in the pharmaceutical industry. Based on the results of the literature review, which showed a

ISSN:0975 -3583,0976-2833 VOL 15, ISSUE 01, 2024

lack of methodologies for accurately measuring ITM and ATZ in large amounts, there is an immediate need for a direct, cost-effective, and accurate solution to tackle this problem.

The concentrations of ITM and ATZ were quantified by injecting a methanol, water, and acetonitrile mixture with a ratio of 30:60:10 and a pH of 5.5 onto a Phenomenex C18 column with dimensions of 150 x 4.6 mm and a particle size of 5m. The flow rate was adjusted to 1.0 milliliters per minute, while the injection volume was 20 microliters. The ITM peak had a retention time of 1.885 minutes, whereas the ATZ peak showed a retention time of 3.139 minutes.

Following its improvement, the method was verified in accordance with ICH guidelines to assess its compatibility with the system, linearity, sensitivity parameters, precision, accuracy, and robustness. All validation parameters yielded results that were within acceptable ranges. The experiments had RSD values below 2. The range of recoveries varied between 98% and 102%.

CONCLUSION:

The suggested RP-HPLC technology provides a time-efficient and easy method that is straightforward, fast, accurate, specific, durable, and cost-effective. Hence, it is a preferred technique for the concurrent quantification of Imatinib mesylate and Anastrazole. The adopted approach underwent comprehensive verification in compliance with ICH principles, covering all aspects.

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