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Original Article

Development and Validation of a Liquid Chromatographic Method for the Determination of Selected Anti Cancer Drugs in Bulk and Pharmaceutical Formulations

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This research work discusses about developing and validating a RP-HPLC method for the determination of selected anti-cancer drugs in bulk and pharmaceutical formulations using Trial and error method. Analytes were separated on a Onyx monolithic- C18 (100×4.6mm) with mobile phase comprising Potassium dihydrogen orthophosphate (0.01M), Methanol and Acetonitrile in ratio of (30:30:40), with flow rate of 0.9 mL/min and pH: 4 adjusted with dilute orthophosphoric acid. Total chromatographic analysis time per samples was approximately 5 minutes with DST-Internal standard (IS), IMT, IBT and SFN eluting with retention times of 4.0, 4.78, 5.88 and 7.05 minutes respectively. Calibration curves were linear over selected range 0.997 for the all analytes. The method was sensitive with the LODs were 12.457, 13.07 and 29.169 ng/mL and LOQs were 43.68, 40.6 and 88.3ng/mL for IMT, IBT and SFN respectively. Inter and Intra-day precision data (in terms of %RSD) was fond to be less than ≥3 respectively, Recoveries ranged ≥102±2% for Imatinib- Gleevec, Ibrutinib-Imbruvica and Sorafenib- Nexavar. The obtained results corroborated the potential of the proposed method for determination of all the three anti-cancer dugs for routine analysis for products of similar type and composition.

Keywords: RP-HPLC, Dasatinib (IS), Imatinib, Ibrutinib and Sorafenib.

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1. INTRODUCTION

The deregulation of protein kinase has been identified to play a key role in the molecular pathogenesis of human cancers, such as chronic myelogenous leukemia and also in solid tumors [1]. In Chronic myeloid leukemia (CML) tyrosine kinase receptors are proteins playing an important role in the transduction of the signals involved in growth of cells [2]. The first generation drug used for the treatment of

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CML is imatinib. The failure of imatinib most likely arises from a combination of tumor and host related factors that contribute to pharmacokinetic variability and induction of resistance [3]. Dasatinib and Nilotinib have revolutionized the treatment of chronic myeloid leukemia and tumors. These drugs are second generation, approximately more potent than imatinib and it also inhibits a number of imatinib-resistant mutant proteins [4].

Dasatinib (DST), chemically N-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1-piperazinyl]-2-methyl-4-pyrimidinyl] amino]-5-thiazole carboxamide monohydrate as shown in (Figure 1a). Is a potent oral inhibitor of multiple oncogenic kinases. Used as internal standard.

The chemical name of imatinib mesylate is benzamide, 4-[(-methyl-1-1piperazinyl) methyl]-N-[[4-(3-pyridinyl)-2-pyrimidinyl] amino] phenyl]-, monomethanesulfonate as shown in (Figure 1b). Imatinib is an antineoplastic agent used to treat chronic myelogenous leukemia and it is protein tyrosine kinase inhibitor that inhibits the Bcr- Abl tyrosine kinase, the constitutive abnormal tyrosine kinase created by the Philadelphia chromosome abnormality in chronic myeloid leukemia.

The chemical name of ibrutinib is 1-{(3R)-[4-amino-3-(4-phenoxyphenyl)-1H-pyrazolo [3, 4-d] pyrimidin-1-yl] piperdin-1-yl} prop-2-en-1-one as shown in (Figure 1c). Ibrutinib is a small-molecule inhibitor of Burton's tyrosine kinase (BTK) That targets the ATP binding domain of BTK and forms a covalent bond with a cysteine residue (Cys-481) in the binding pocket that leads to sustained inhibition of BTK enzymatic activity. Ibrutinib is used in the treatment of mantle cell lymphoma (MCL), chronic lymphocytic leukemia, Waldenstroms macroglobulinemia [5].

The chemical name of sorafenib is 4-[4({[4Chloro3 (trifluorométhyl) phenyl] carbamoyl} amino) phénoxy] N-méthyl-2-pyridinecarboxamide as shown in (Figure 1d) Sorafenib an oral multi- kinase inhibitor is suppressing the activity of several cell surface receptor tyrosine kinases, like vascular endothelial growth factor receptor (VEGFR) and platelet-derived growth factor receptor and also intracellular members of the mitogen-activated protein kinase (MAPK) signal transduction pathway. It has been approved for the treatment of advanced renal-cell carcinoma (RCC) and hepato cellular carcinoma (HCC). [6, 7]

The Rational behind the selection of these anti leukemic drugs is that in cancer patients, the novel treatment regimen includes either any one of these drugs or in combinations with other TKIs. A typical combination is specific to individuals suffering from cancer this specificity of selecting combination of drugs (TKIs) mainly based on the genomics or gene coding that is specific to individuals. In recent years, there have been many reports on individual determination of selected (TKIs) in pharmaceuticals and biological matrices and additionally many liquid chromatographic methods are reported for the simultaneous determination of IMT, IBT and SFN tyrosine kinase inhibitors (TKI's) [8-17]. Furthermore most of these methods suffer from limitations such as poor retention, complicated procedure, expensive use of solvents and instrumentation to achieve better chromatographic separation and low detection capability for estimation in formulations. Till date, there are no methods reported in the literature with less retention, more resolution and in combination of these drugs.

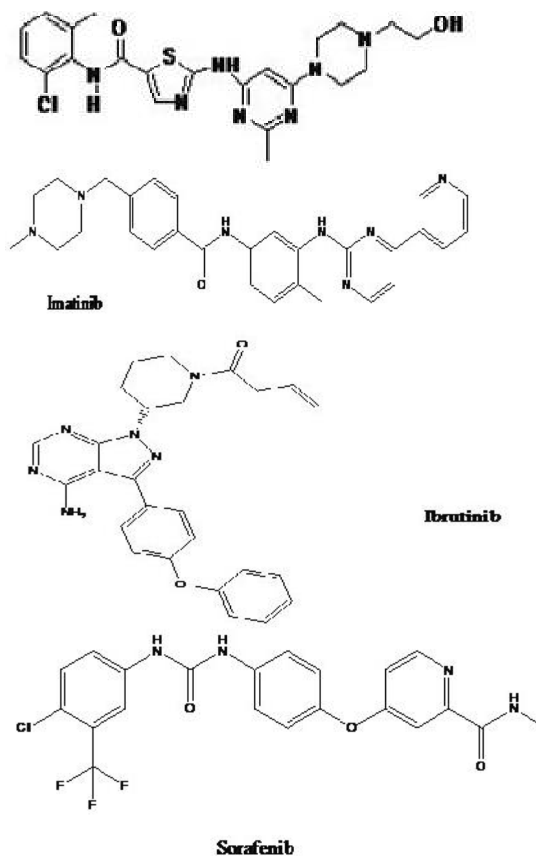

Fig 1: Chemical structures corresponding to four anti leukemic drugs 1a) Dasatinib (internal standard) 1 b) Imatinib 1 c) Ibrutinib 1 d) Sorafenib

Hence here in our research work we have developed a novel RP-HPLC method for the simultaneous determination of selected anti cancer drugs namely DST (IS), IMT, IBT and SFN (TKIs). This developed method can be utilized for the quantitative analysis of any one these drugs alone or in combination along with other TKIs drugs. This developed method could also be applied for TDM, Bio equivalence studies and drug-drug interaction studies.

2. MATERIALS AND METHODS

Instrumentation

The chromatographic method development and validation was performed on Shimadzu HPLC (Shimadzu Corporation, Kyoto, Japan). The system consisted of two LC-20AD solvent delivery modules: an SPD-M 20A PDA detector and a Rheodyne injector (model 7125, USA) valve fitted with a 20µL loop. Chromatographic data were collected and processed using LC solutions® software (Version 1.11SP1).

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The mobile phase was degassed using Branson sonicator (Branson Ultrasonic, USA).

Chromatographic Condition

The chromatographic separation was carried out using a mobile phase consisting of a mixture of potassium dihydrogen orthophosphate (aqueous content), MeOH and MeCN. The analytes were detected at 264 nm based on isobestic point. Prior to use, the mobile phase in different ratios was degassed for 10 min in ultrasonic bath and vacuum filtered through 0.45µm membrane filter (Gelman Science, India). The mobile phase was prepared by mixing appropriate proportions of aqueous content with MeOH and MeCN to the mixture as per (Table-1). The HPLC system was used at an ambient temperature (25±2°C)

Chemicals and Reagents

The working standards of Dasatinib (IS), Bms,India,Pvt. (DST) India, Imatinib-Gleevac, Novartis India [IMT], Ibrutinib-Imbruvica, Janssen Biotech [IBT], and Sorafenib-Nexavar, Bayer pharmaceuticals pvt. were donated by respected manufacturers. Methanol and Acetonitrile was HPLC grade, Orthophosphoric acid and KH_2PO_4 of analytical grade was purchased from SD fine Chemicals, Mumbai, India. High purity HPLC grade water was prepared by using Milli-Q Academic, Millipore (Bangalore, India).

Stock and working Standard Solutions

Stock standard solutions of Dasatinib(Internal standard), Imatinib, Ibrutinib and Sorafenib, at 1000µg/mL, were prepared individually using mixture of MeOH and water in 80:20 v/v and stored at 4°C protected from light. The solutions of DST(IS), IMT, IBT and SFN further diluted with the mobile phase to give a series of standard mixtures having a final concentration range 2-25µg/mL The solution prepared for the optimization procedure comprised of DST, IMT, IBT and SFN, at10µg/mL

Preparation of the sample solution

Ten tablets of and Ten capsules of were Imatinib- Gleevec, Ibrutinib- Imbruvica and Sorafenib- Nexavar. weighed and analyzed separately. An amount of powder equivalent to 10mg was weighed and transferred in a 10mL volumetric flask, and 5mL of mobile phase was added. This mixture was subjected to sonication for approximately 15 min to ensure complete solubility of drugs, and the solution was made up to the mark with mobile phase and further dilutions were made to obtain concentration of IMT 8.0µg/mL, IBT 10µg/mL and SFN 20µg/mL The resulted solutions were centrifuged at 4000 rpm for 10 min, and clear supernatant was collected and filtered through a 0.2µm membrane filter (Gelman Sciences, India). A 20µl of the final solution was injected in triplicate and chromatographed.

Validation

Validation studies were conducted using the optimized assay conditions based on the principles of validation described in the ICH guidelines "Text on validation of Analytical Procedures [19] "and "Q2B, Validation of Analytical Procedure: Methodology [20]". Key analytical parameters, including, accuracy, precision, linearity, detection limit, quantitation limit was evaluated

3. RESULTS AND DISCUSSION

A RP-HPLC method was developed, optimized and validated for determining the various TKI's namely DST, IMT, IBT and SFN. Trial and error method as shown in (Table 1).was utilized for screening and optimizing the chromatographic separation problems due to interferences between drugs, excipients and solvent components.

Table 1: Trial and error method for DST, IMT, IBT and SFN.

Trail No.	Buffer concentration (mM)	Acetonitrile %v/v	Methanol %v/v	Flow rate ml/min	pH	K^1	$Rs_{(1,2)}$	$Rs_{(2,3)}$	tR_4
1	0.005	35	35	0.6	3.6	0.47	1.78	0.890	5.9
2	0.01	35	35	0.9	3.2	0.386	1.297	2.3	3.676
3	0.01	30	35	0.75	3.6	0.3	1.39	2.7	4.4
4	0.005	30	30	0.6	3.6	0.2	1.8	2.9	5.4
5	0.005	30	30	1	3.2	0.5	2.0	4.3	9.88
6	0.005	35	45	0.8	3.6	0.6	1.9	4.2	8.4
7	0.01	45	25	0.6	3.2	0.3	1.8	3.8	4.0
8	0.005	35	25	0.9	3.2	0.4	2.1	0	3.2
9	0.005	35	25	0.6	3.6	0.4	2.5	0	5.1
10	0.01	35	35	0.6	3.6	0.3	1.5	3.3	6.5
11	0.01	30	30	0.9	4.0	0.4	1.6	2.9	7.12
12	0.007	35	30	0.6	3.6	0.2	1.85	3.4	6.5

Assay method validation

The last step of the study was to check method validation for specificity, linearity, intra/inter-day precision, and robustness. The optimized HPLC method was specific in relation to the placebo used in the study. All placebo chromatograms showed no interference peaks (figure 4). An excellent linearity was established at five level in the range of 2-25 µg/ml for IMT, IBT and SFN and 7µg/ml of DST(IS) with R^2 of more than 0.99 for all the analytes. The slope and intercept of the calibration curve were 0.521 and + 0.131 for IMT and 0.510 and +0.211 for IBT and 0.100 and +0.279 for SFN respectively as shown in (Figure 2a, 2b, 2c,).

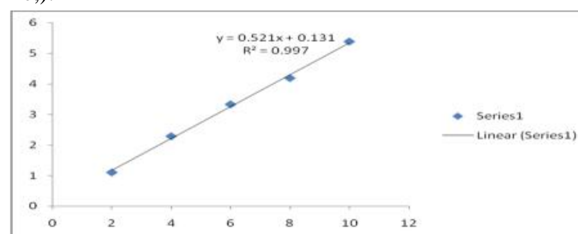

Fig 2a: Calibration Curve of Imatinib

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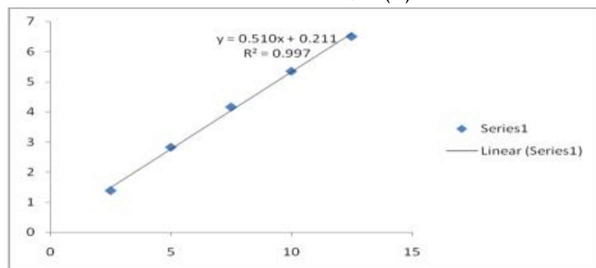

Fig 2b: Calibration Curve of Ibrutinib

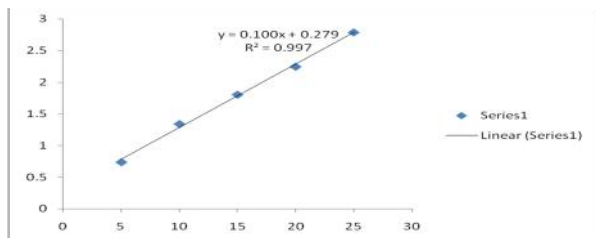

Fig 2c: Calibration Curve of Sorafinib

Since the correlation coefficients are not good indicators of linearity performance of an analytical procedure a one way ANOVA was performed. For all the analytes, the calculated F- Value (F calc) was found to be less than the theoretical F-value (F *crit*) at 5% significance level, indicating that there was no significance difference between replicate determinations for each concentration level. The method was sensitive with the LODs were 12.457, 13.07 and 29.169 ng/mL and LOQs were 43.68, 40.6 and 88.3ng/mL for IMT, IBT and SFN respectively. Inter and Intra-day precision data (in terms of %RSD) was fond to be less than 3 respectively, Recoveries ranged 102±2% for Imatinib-Gleevec, Ibrutinib- Imbruvica and Sorafenib- Nexavar. The obtained optimal conditions and their respective chromatograms were shown in (Figure 3) results corroborated the potential of the proposed method for determination of all the three anti-cancer drugs for routine analysis for products of similar type and composition.

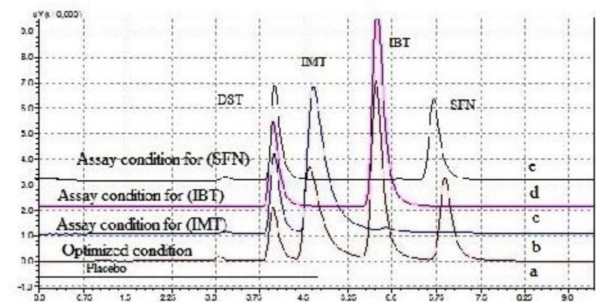

Fig 3: Representative overlaid Chromatograms corresponding to (a) Placebo solution; (b) optimized condition (c) Assay condition for IMT 8µg/ml (d) Assay condition for IBT 10µg/ml (e) Assay condition for SFN 20µg/ml with IS under optimal conditions

Accuracy (n=9), assessed by spike recovery, were found to be 102.47, 99.8 and 101.7 for IMT,IBT& SFN respectively, with were within acceptable ranges of 100± 2% [34]. The intra and inter-assay precision (n=6) was confirmed since, the %CV were well within the target criterion of 2 and 3, respectively. Robustness study reveals that small changes did not alter the retention times, retention factor, and resolution and therefore it would be concluded that the method conditions are robust.

4. CONCLUSIONS

It is concluded that the Mobile phase comprising of Potassium dihydrogen orthophosphate (0.01M), Methanol and Acetonitrile in ratio of (30:30:40), with flow rate of 0.9 mL/min and pH: 4 adjusted with dilute orthophosphoric acid gave good results .Total chromatographic analysis time per samples was approximately 7 minutes with DST(IS),IMT,IBT and SFN eluting with retention times of 4.0, 4.78, 5.85 and 7.05 minutes respectively. The obtained results corroborated the potential of the proposed method for determination of all the three anti-cancer drugs for routine analysis for products of similar type and composition.

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