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QbD Approach in the Comparative Study of Azurin and Dabrafenib Anticancer Agents by UPLC Mass Septrometry

Elavarasi.E*, Dr. R.Srinivasan
Department of pharmaceutical analysis &chemistry
Faculty of pharmacy
Bharath institute of higher education and research
Selaiyur, Tambaram,Chennai-73

Corresponding Author
Elavarasi.E

Abstract:- In the present situation there is an number of death is cancer with 19.3 million deaths. Some usual therapies are immune therapy, chemotherapy, and radiotherapy for cancer. Through this therapy treatment strategies may yield even better outcomes. A redox protein is Azurin having 128 amino acids with a size of 14kDa and secreted by Pseudomonas aeruginosa as a periplasmic protein. And the family is cupredoxin. Azurin's anticancer properties have demonstrated that when it interacts with P53, more intracellular transcription factors are produced and P53 is stabilized. The idea of developing high-quality pharmaceutical goods with quality in mind (QbD) has replaced it. It is a significant component of the current trend in pharmaceutical quality; QbD is the best way to produce high-quality, comprehensive pharmaceutical products, but it also poses a significant challenge to the sector's historically established procedures. The C-terminal domain of azurin has four sections that make up the Alpha helix: CD, GH, FG, and EF. A compound can be measured using several methods and identified by different methods of analysis. High extraction is a higher-pressure analytical technique that can be used to conduct a more thorough investigation of medicinal products (HPLC), and liquid chromatography-mass spectrometry (LC-MS/MS). Several extraction methods can be used to evaluate drugs, including liquid-liquid extraction. Adding a combination drug is known as Dabrafenib. It is an anti-cancer medication used for the treatment of cancer. Its purity and dependability are ensured by using approaches like validation and analytical tests. Using MODDE software version 8.2, DOE(Design of experiment)was statistically designed (Umetrics Inc., NJ, USA). Responsive surface modeling (RSM) was used to calculate the non-linear multidimensional relationship between the independent components and CQAs.

Keywords:- *Dabrafenib, Azurin, LC-MS/MS, Pseudomonas aeruginosa.*

I. INTRODUCTION

Cancer is the most common cause of death. The International Agency for Research on Cancer estimated that there were 19.3 million new cases of cancer globally in 2020, mainly due to breast cancer, lung cancer, skin cancer, and colorectal cancer [1]. Despite major advances in targeted therapies such as immune therapy, chemotherapy, and radiotherapy [2].The main kinds of cancers include benign tumors and malignant cancers. Malignant and benign tumors are serious problems of Cancer. (3)Cancer is also categorized by the organ where it occurs, for example, Lung tumor, Breast tumor, and Colorectal tumor, etc.**(4)**

Dabrafenib chemically designated as N- {3- [5- (2-amino pyrimidin-4-yl) -2-tert- butyl-1, 3-thiazol -4-yl]-2-fluorophenyl} -2, 6-difluoro benzene-1-sulfonamide. This drug is related to the B-RAF enzyme, which will exhibit important activity in cellular growth regulation, and inhibits to produce anticancer action.

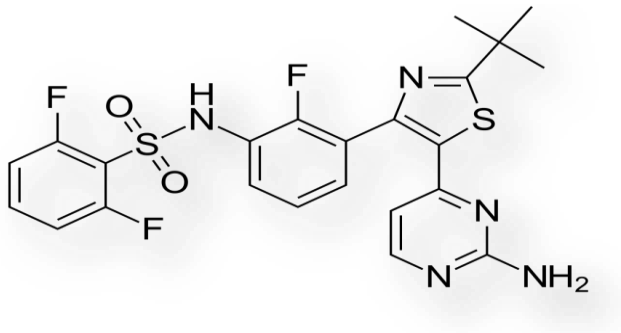

Fig 1 Structure of Dabrafenib

(5)Azurin which is secreted by Pseudomonas aeruginosa from the family of cupredoxin. It contains protein consisting of periplasmic copper in 128 AA of 14 KDa size. It is made up of eight antiparallel strands structure which is connected by 4 loops and linked by a disulfide bridge**.** Azurin has been used for the developmentof therapeutic proteins.which have anti-cancer agentsas well as antiviral and anti-bacterial effects. Azurin is a water-soluble and stable protein that is small in size and can be easily purified in a bacterial host. Azurin with electron transfer it is

known as ET. Anticancer activity of Azurin has shown the interaction between Azurin and P53 leads to the rise in intracellular transcription factors and stabilization of P53(6)Quality intentionally principles are wont to advance product and process quality in the industry, particularly the automotive industry, they need also been adopted by the U.S. Food and Drug Administration (FDA). (7)Quality intentionally (QbD) has become a replacement concept for the development of quality pharmaceutical products, it's an important part of the fashionable approach to pharmaceutical quality, QbD is the best solution to create a top quality altogether pharmaceutical products but it's also a serious challenge to the Pharmaceuticalindustry whose processes are fixed in time(8)

II. STRUCTURE OF AZURIN

Alpha helix consists of four regions it is found inthe C-terminal domain CD, GH, FG, and EF. An immunoglobulin fold and β-sandwich coreenable thepeptide to show anticancer activity by escaping the immune response (9)

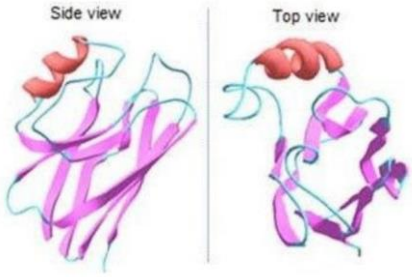

Fig 2 Structure of Azurin

III. METHODS AND MATERIALS

➢ *Chemical reagents*

The dabrafenib (purity: 99.68%)
standard and sorafenib (purity: 99.87%)
Internal standards (IS) were obtained from theNovitum Labs,Chennai, India.
Acetonitrile (ACN) and methyl alcohol of LC purity were acquired from Merck, Mumbai, India.
Water has been processed from the Milli-Q waters system

➢ *Analytical methods HPLC:*

The specific form of column chromatography is high-performance liquid chromatography (high-pressure liquid chromatography). which is used in analysis and biochemistry to separate, identify and quantify active compounds. Pumps in HPLC, are used to passthe major clinical treatment which is **8** Cancer can be treated by using several chemopreventive agents that cause toxicity that restricts the usage.

➢ *Extraction Methods Liquid-Liquid Extraction*

Liquid-liquid extractionprovides differential solubility and will spread the two immiscible fluids. The two phases are inseparable. It is general, there are two stages, of the solutionone is aqueous and the other contained in the living organisms. the extraction step can be removed from thematrix.

➢ *LC-MS/MS:*

liquid chromatography (or HPLC) with mass spectrometry MS is liquid chromatography-mass spectrometry (LC-MS/MS). The qualitative and quantitative analysis of the drug substances, drug products, and biological samples used in LC-MS/MS. It plays a vital role it forms the evaluation and interpretation of bioavailability and bioequivalence in pharmacokinetic data.**11**

- **Drug name:** Dabrafenib
- **Analytical techniques:**UPLC-MS/MS
- **Description of techniques:**
- **System:**Acquity H-class UPLC system, Coupled to a Xevo TQ-S Micro Tandem Mass Spectrometer
- **Column:** CORTECS UPLC C18 Column (2.1× 50 nm, 1.6 µm Particle size, Waters)
- **Mobile Phase:**Methanol and water: CAN
- **Linearity:** 10-2500 µg/ml
- **Flow Rate:** 0.7 ml/min
- **LLOQ:**0.9µg/ml
- **HLOQ:** 2599.2µg/ml
- **Analytical techniques:**LC/MS/MS
- **Description of techniques**:
- **Mass spectrometric detection:** Mode-Multiple reaction mode(MRM) andAPI5500 (TQMS)Triple-quadrupole mass spectrometry positive ion mode, software version 1.5.2 (Sciex)
- **Mobile Phase:**Acetonitrile in water: methanol
- **Column:**i C18 Column (5.0 µm 50×2.0 mm)
- **Flow Rate:** 0.150 ml/min
- **Linearity:** 2.0-210ng/ml
- **Injection Volume:**1.5 µl
- **LLOQ:** 5; 2ng/ml

➢ *Design of experiment(DOE).*

DOE was statistically designed using MODDE software version 8.2 (Umetrics Inc., NJ, USA). A D-optimal design was selected to account for the asymmetry in the settings of critical process parameters, namely process speed, and airflow. The asymmetric nature of the selected D-optimal design suggests that sometimes reproducibility (i.e. variation of response under the same conditions, pure error) could be tested at levels other than middle values [10]. Hence reproducibility runs were set at the high-level values by the software. The chance for error during the model design was minimized by ensuring that both G-efficiency (a measure of model efficiency) and condition number (a measure of model sphericity) were at their optimal levels of 85.15% (recommended > 60–70%) and 10.55 respectively [22]. The model was quadratic polynomial fitted using the partial least squares (PLS) method.

➢ *DOE model analysis*

After model fitting, model verification was carried out to ensure its validity and reproducibility through sequential elimination of the insignificant runs using distance to model plots while evaluating the effect of the elimination on the

model using lack of fit plots. Furthermore, the model terms were reviewed individually and those with no statistical significance were eliminated because their influence on the values of CQAs was negligibly small [10]. Two eliminations were carried out; the insignificant runs and the insignificant model terms. ANOVA analyses for total variations of the responses at two levels were carried out (variances attributed to the regression model and variances obtained from residuals and replicate errors).

IV. CONCLUSION

A therapeutic effect with limited anticancer agents of drugs. Azurin is the biomolecules against the protein causes apoptosis by stabilizing P53 protein LC-MS technique was produced for the quantitation of a US-FDA-approved dabrafenib anticancer drug. The development and validation of analytical methods in QbD play a vital role in the essential step for developing any pharmaceutical products. This review represents anti-cancer drugs; based on the literature review, it can be concluded that Drugs are performed in theHPLC, LC-MS it is for identification, purification, and quantification. The main activity for the analytical Low detection limits, inthe ability to produce azurin as the protein which is binding with the drug Dabrafenib and, are The application of QbDis a multifactorial design that is used in the experimental analysis targeted functionalities. In conclusion, the systematic application of QbD principles served two broad aims in this work. Firstly, to identify the influence of variations and interactions of the chosen factor by using the quality drug design the drug is bound with the protein and reacts as the dabrafenib**.**

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