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# AN OVERVIEW OF ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF SELECTED ANTI CANCER DRUGS STABILITY INDICATING HPLC ASSAY METHOD

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**Abstract**

The bio-analytical method is effective at determining the number of drugs and metabolites in a biological system. New methods, the validation of existing procedures, and the analysis of samples are one of the prominent tasks for bio-analysis. Above all, a compound can be measured using several methods and identified by different methods of analysis. Drugs may be tested by several extraction techniques, including liquid extraction, solid-phase extraction, and protein precipitation in complex plasma and biological samples. To determine how the environment, matrix, or procedures impact the matrix estimation to the time of the analysis, all steps in the process must be investigated. The more detailed study of drug products can be performed with higher-pressure analytical techniques, such as high- extraction(HPLC). At present, HPLC and GC usually perform biolysis. The parameters are linearity, repeatability, accuracy, selectivity, and continuity. We are proposing the development and validation of bio-analytical systems to assist in the quality assurance of drugs.

**Key words:** Anti cancer drugs, Bioanalytical, HPLC, Samples extraction techniques

## Introduction

To quantitatively determine different analytes in biological matrices, bio-analytical method validation is essential. Good preparation of samples and hyphenated instruments is crucial in modern bio-analysis. The development of complete bio-analytical methods in pharmaceutical research companies is critical during the drug discovery and development phase [1]. Bio-analysis covers identifying and quantifying biological sample analytes. Due to the sophistication

of the sample matrix, Bio-analysis is challenging [2]. There is a well-known need for intense sample preparation before being used in analytical instruments to use complex matrices such as blood, plasma, and urine. Modern bio-analysis requires high-performance sample preparation and hyphenated analytical methods.

**Dasatinib**

Dasatinib is a multi-tyrosine kinase inhibitor. Dasatinib has blocked the expression of BCR-AB1 by developing chronic leukemia myeloid and acute lymphoblastic leukemia lines. The IUPAC of Dasatinib N-(2-chloro- 6-methyl phenyl)-2-[[6-[4-(2-hydroxyethyl) piperazin-1- yl]-2-methylpyrimidin-4- yl] amino]-1, 3-thiazole-5- carboxamide with an empirical formula of $C_{22}H_{26}CLN_7O_2S$ with a molecular weight of 488.01 g.mol-1. Dasatinib is slightly soluble and very poorly soluble in ethanol, methanol, DMSO, acetone, acetonitrile. Dasatinib has a melting point of 280°-286°c and white or off-white powder (22. The recommended starting dose ofSprycel is100 mg given orally once daily for the chronic phase. Dasatinib has various strengths such as 20 mg, 50 mg, 70 mg, 80 mg, 100 mg, and 140 mg available on the market [3]. Dasatinib inhibits the kinase BCR-ABL, SRC (SRC,LCK,YES,FYN),C-KITA,EPHA2,andpdgfrßatNano molar concentrations. Based on modeling studies, Dasatinib is expected to be associated with several ABL kinase conformations.

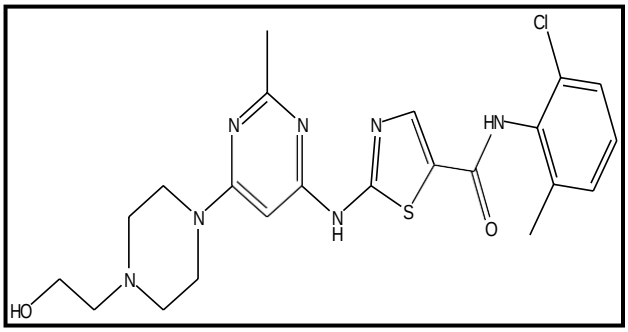


**Fig-1: Structure of Dasatinib**

**Gemcitabine hydrochloride**

Gemcitabine hydrochloride is a β-difluoro-nucleosides, purine anti-metabolite (4-amino-1-[(2R, 4R, 5R)-3, 3- difluoro-4-hydroxy-5-(hydroxymethyl) oxolan-2-yl] pyrimidin-2-one. A mixture of the diphosphate and the triphosphate nucleosides leading to inhibition in DNA synthesis Gemcitabine. It was approved by the FDA and has been shown for breast, ovary, non-small cell lung, and pancreatic cancer. Gemcitabine HCL is water-soluble, methanol-soluble, and ethanol and polar organic solvents are virtually insoluble. It is white and odorless crystalline and powder with a 168.64°c melting point. Gemcitabine's molecular formula is $C^9H_{11}F_2N_3O_4$, and 263.2 g/mol is molecular. It inhibits the growth of tumors by two mechanisms: first through the replacement of one nucleic acid buildingblock directly during DNA replication, leading to tumor cellapoptosis, and second, by irreversibly inactivating the ribonucleotide reductase enzyme, which prevents the development of deoxyribonucleotide and causes cell apoptosis. Gemcitabine is also used in pancreatic adeno-carcinoma. Patient supervision shows only a small improvement and its efficacy can be restrictedby thepoor drug administered, particularly for pancreatic adenocarcinoma tumors which are usually hypo-vascular and extensive desmoplastic stroma. Gemcitabine hasa half-life of 17 minutes [4].

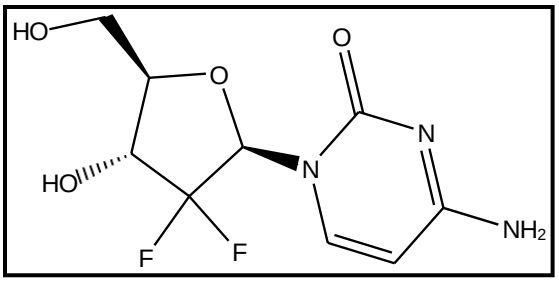


**Fig-1: Structure of Gemcitabine hydrochloride**

**Ruxolitinib**

Ruxolitinibis an active selective inhibitor indicatedfor the treatment of moderate-and high-risk myelo-fibrosis, including primary ice lophibrosis, post-polycythemia, and post-essential thrombocy the miamyelofibrosis. The food and drug a dministration(FDA) approved Ruxolitinib. Ruxolitinib with a (3R)-3-cyclopentyl-3-[4-(7H- pyrrolo [2,3-d]pyrimidin-4-yl)pyrazol-1-yl]propane nitrile molecular formulation, known chemically as(r)-3- 3-(4-(7h-pyrrolo[2,3-d].WhenJAK2mutationswere detected in myelofibrosis, the emphasis was moved towards selective JAK inhibitors to control diseases. Ruxolitinibis a potent, first-class, and selective FDA-approved inhibitor for myelofibrosis therapy. It is soluble with the inert gas in organic solvents such as ethanol, DMSO, and DMF [5].

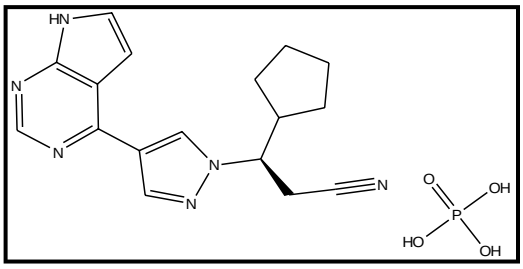


**Fig-3: Structure of Ruxolitinib**

**Table-1: Extraction Values of Gemcitabine**

| DRUG | MAT RIX | HPLC-COLUMN | SAMPLE PREPARATION | LLOQ | REFERENCE |
|---|---|---|---|---|---|
| Gemcitabine | Humanplasma | AcquityuplchssT3column (100A, 2.1 × 150 mm,1.8m) | Solid-phaseextraction | 50,000Pg/ml | 6 |
| | Tumortissue | Hypercarbcolumn(100x 2.15µm)Thermo Fisher scientific) | Liquid-liquidextraction | 0.2 ng/ml | 7 |
| | Humanplasma | Altimac18column(2.1× 100 Mm, 5µm) | Proteinprecipitation | 0.9 ng/ml | 8 |
| | Driedbloodsample | BDSHypersilc18,(100x 4.6 mm, 5µ) | Proteinprecipitation | 50 ng/ml | 9 |
| | Humanperipheral Blood mononuclear Cells | ABiobasic5µm,50x2.1 Mm column | Proteinprecipitation | 25 ng/ml | 10 |

**Table-2: Extraction Values of Dasatinib**

| DRUG | MATRIX | HPLC–COLUMN | SAMPLE PREPARATION | LLOQ | REFERENCE |
|---|---|---|---|---|---|
| Dasatinib | Human plasma | Lunacolumn(50mm×2.0Mm 3- micron particle size | Solid-phase extraction | 0.92ng/ml | 11 |
| | Ratplasma | AcquityUPLC$C_{18}$analyticalcolumn (Waters, Dublin, Ireland), with dimensions 100 × 1.0 mm, i.d., 1.7μmparticle size. | Solid-phase extraction Liquid-liquid extraction | 1.0ng/ml | 12 |
| | Ratplasma | Reversed-phaseC18column(504.6 mmi.d.,3mm)YMC-PackODS- AM | Protein precipitation and liquid-liquid extraction | 5.41ngm-1 | 13 |
| | Rat plasma | Reversed-phaseC18column(504.6 mm i.d., 3 mm)YMC-Pack ODS-AM | Liquid-liquidextraction | 1.0ng/ml | 14 |
| | Ratplasma | Reverse phaseC18column (50 mm × 3 mm i.d., 4.6 μ) (YMC- PACK, Japan) | Proteinprecipitation | 10ng ml−1. | 15 |
| | Human Plasma | Reversed-phaseC18 column(50 4.6mmi.d.,3mm)YMC-PackODS- AM | Solid-phase extraction | 1 ng/ml | 16 |
| | | | Liquid-liquid extraction | 62.5 ng/ml | |
| | | | Liquid-liquid extraction | 1 ng/ml | 17 |
| | | | Solid-phase extraction | 0.1 ng/ml | 18 |

**Table-3: Extraction Values of Ruxolitinib**

| DRUG | MATRIX | COLUMN | SAMPLE PREPARATION | LLOQ | REFERENCE |
|---|---|---|---|---|---|
| Ruxolitinib | Humanplasma | Phenomenex,synergipolarRP, 50X 2 mm, 4Mm | Liquid-liquidextraction | 4.89 ng/ml | 19 |
| | Ratplasma | Ymc packodsam (150mm ×4.6mm, 5 m) | Proteinprecipitation | 0.86ng/ml | 20 |

The appliance of the analytical approach is one of the drug developments. To quantitatively

determine different analytes, analytical method validation is necessary. Good preparation of samples and hyphenated instruments is vital in Pharmaceutical Analysis. The development of whole analytical methods in pharmaceutical research companies is significant during the drug discovery and development phase. Pharmaceutical Analysis covers identifying and quantifying sample analytes. Pharmaceutical Analysis is a fundamental science in many fields of study. Due to the complexity of the sample matrix, Pharmaceutical Analysis is challenging [21]. There is a well-known need for intense sample preparation before being used in analytical instruments to use complex matrices such as blood, plasma, and urine. Modern Pharmaceutical Analysis requires high-performance sample preparation and hyphenated analytical methods.

**Sample preparation**

In terms of time and the complexity of extracting the desired analyst from the matrix, the analysis's sample preparation phase is often the most important and complicated part. Moreover, there are unique obstacles to each matrix. Urine has high levels of salt; for example, plasma has plenty of phospholipids. Whole blood comprises red, lysed blood cells, and so on. Each analyte and matrix often have different properties, which dictate the type of extraction method to use.

**Goals of Sample preparation** [22]

- Reduce the impact of matrix
- Remove sample for variability sample
- Reduce the variability of assays
- Enhanced sensitivity
- Samples should be cleaner

**Challenges in method development**

- The matrix
- The number of specimens
- Fast procedure
- The quantification number of analyses
- Analyte pharmaceutical profile .

**Drug Development**

It takes approximately 10 years and takes about $1 billion to find and produce a new drug. The average annual approval of large molecules is nine and twentythree small molecules per year. Drug failures at various stages of production and patent expiry periods, primarily for existing organic products, contributed to a pharmaceutical R&D recession, as biologics are expected to reach a market value of $54 billion off- patent in the next five years. Pharmaceutical companies are working hard to reduce their internal capacities and fixed costs, and analytical research the need to make analytical sound tests help as a key tool for drug discovery and production must be well known, adding to the list of significant pharmaceutical expenditure. Pharmaceutical Analysis and the different stages of drug development are generally recognized as central to the pharmacokinetic/Pharmacodynamic characterization of the novel chemical entity since it was discovered. Thus it led to its market authorization. There are a few general ideas in this field that are the foundation of an overarching structure for approaching Pharmaceutical Analysis from the outset and through different phases of drug production [23].

Analytical method validation Need for validation of the analytical process[24] Accurate, reliable findings need to be satisfactorily interpreted by applying well-characterized and validated analytical methods. Analytical methods and techniques are continuously being changed and improved. It is also important to stress that each bio-analytical technique's specific characteristics differ from analysis to analysis. For each study, particular requirements for validation can be

required. When testing samples for a given study at more than one site, the analytical methods must be validated at each site and validated in the various areas for interlaboratory reliability must be adequately provided.[25-27].

**Linearity**

Linearity evaluates the system's ability to produce test results that are directly proportional to the sample analysis. Regardless of the process of drug production, the linear range of the procedure must be calculated. During the accuracy analysis, the starting and ending levels must be based on the five concentration levels. [28]. The following concentration levels are recommended for evaluation during method validation by ICH guidelines:

**Assay:** 80% –120% of concentrations of samples [finished product or pharmaceutical substances]. However, this range needs to be based on precise analysis. The linearity should be increased to a minimum of 75-125 percent of the nominal value, and accuracy should be prepared at 80, 100, and 120% of the nominal value.

**Contents uniformity:** 70 to 130% of the sample concentration is based on the type of dosage unless a more comprehensive, more adequate range is justified; [e.g., metered dose inhalers].

**Dissolution method:** 20% of the specified range is needed. If dissolution profiles are expected, the linearity range should begin at less than 120 percent of the total drug content recovered during the initial stage.

**Impurity detection method:** the degree of reporting is 120 percent. Impurity and testing are combined in this form: a standard of 100 percent is used to quantify and reveal impurity at 120 percent of the test specification.

**Accuracy**

The accuracy of an analysis method is known as the degree of agreement between the value taken as a typical true or agreed on reference value and the value found. [29-34]

**Bias**

Bias may be expressed as a percentage deviation from an agreed-upon reference value. The term "trueness" refers to the mean value of a generally accepted reference value for a variety of measurements. It can be described in terms of partiality. Due to the high workload associated with evaluating such a complete sequence, truthfulness is usually not determined during process validation but rather from the results of numerous QCS during routine application [35].

**Precision**

Precision is a term that refers to the degree to which a set of measurements are compiled under specific conditions from several samples of the same homogeneous sample. Precision is further subdivided into interday, intraday, and different analysts. Precision or repeatability tests have been carried out to assess accuracy in time. The use of different observers, instruments, reagents, and laboratories may have included these controls.[36].

**Intermediate precision**

In laboratories, the term "intermediate precision" applies to variations: different days, observers, and equipment, for example. The ISO description referred to the expression "intermediate precision m-factor varies" when the m-factor denotes the number of variables that change between determinations [operator, equipment, or time]. Occasionally, intermediate precision is referred to as intermediate precision [37] between, between, or between days.

**Limit of Detection** [LOD] The LOD is fully dependent on the boundary test. The smallest amount of analysis in a sample can be detected but not necessarily quantified under the given experimental conditions. The term "detection" is often used in conjunction with percentages,

components per million, or parts per billion.

**Limit OF Quantification [LLOQ]**

LLOQ is a small sample quantity of study which can be calculated with appropriate precise and accurate measurements. LLOQ based on accuracy can be chosen as the most practical approach. The LLOQ is the lowest sample concentration but can be reliably and accurately quantified. For example,chromatographical methods are used LLOQ only when basic noise is used according to signal and noise ratios. [38]

**Robustness**

According to the ICH guidelines, a robust analytical procedure is measuring its capacity to maintain its reliability during regular use without being influenced by minor but deliberate differences in process parameters. The ability to replicate the [analytical] technique in various lab sites or other conditions, and the robustness test, which is an experimental set-up to determine the robustness of a system, without the incidence of uneven variations between the results achieved, can be identified. [39-41]

**Ruggedness**

This covers various analysts, labs, columns, instruments, reagent sources, chemicals, and solvents. The research process's ruggedness is the degree of reproductiveness of test results obtained under many standard test conditions by analyzing the same samples.

**Recovery study**

Although the recovery study cannot be perfect, the scope of the analytes recovered and the internal norm should be consistent, reliable, and reproducible. For the extracted samples' analytics at three concentrations, recovery tests should be performed using unextracted parameters that indicate 100 percent of reconstruction [low, medium, and high]. [42-44]

**Matrix effect**

The matrix effect is defined as the effect of the coeluting residual matrix of a biological sample on the ionization of the target. The matrix effect may be caused by organic and inorganic substances such as amines, urea, and carbohydrates. The variance in the coefficients calculated from a six-lot matrix is less than 15% of the normalized matrix factor [mf]. This can be accomplished at both low and high concentrations [up to three times the lower LLOQ] and near to the upper quantification limit [ULOQ]. The concentration is calculated to have an average coefficient of variation [CV] of less than 15%. [45-47]

**Stability**

The stability of the analyte is a necessary precondition for accurate quantification in the analysis process. The complete validation of a system must also involve stability tests at the various research steps, including pre-analysis storage. [48].

## Conclusion

The bio-analytical method used to quantify drugs and their metabolites in biological fluids is important in the evaluation and interpretation of bio equivalence, pharmacokinetic [PK], and toxic kinetic studies. Low detection limits, the ability to produce structural information, the need for minimal sample preparation, and the ability to cover a wide range of analytes with varying polarities are all advantages of HPLC.

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