International Journal of Pharmaceutical Sciences and Drug Analysis

E-ISSN: 2788-9254
P-ISSN: 2788-9246
IJPSDA 2024; 4(1): 56-59
www.pharmacyjournal.info
Received: 06-10-2023
Accepted: 14-11-2023

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A study on therapeutic drug monitoring (TDM) of 5-fluorouracil

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Abstract
Finalizing an appropriate analytical method for identifying and quantifying pharmaceuticals and their metabolites in various bodily fluids is critical throughout method development. For the estimate of pharmaceuticals and their metabolites in biological fluid, the detection technology chosen should be very sensitive, precise, and accurate. The sample preparation method chosen should provide consistency in recovery and repeatability, as well as minimal or no interference with substances of interest during instrumental analysis. Different extraction methodologies such as protein precipitation extraction, liquid-liquid extraction, and solid phase extraction are evaluated based on the nature of the biological matrix and drug's physico-chemical properties such as molecular structure, polarity, partition coefficient, solubility, and dissociation constant, among others, to extract neat form of the compounds of interest free from endogenous substances such as proteins, phospholipids, sugars, and salts present in the biolo During mass spectrometric analysis, endogenous molecules such as phospholipids and proteins typically cause ion suppression, resulting in incorrect results. Furthermore, adjusting chromatographic settings is critical for maximum throughput, selectivity, and sensitivity when testing substances of interest. Flow rate, column, column temperature, mobile phase combination, and reconstitution solution are all variables that must be optimized. If any of the above parameters are not appropriately optimized, the analysis will suffer, resulting in poor sensitivity and uneven recoveries, and the method will need to be redeveloped. As a result, the technique development process is critical in bioanalysis.

Keywords: Study, therapeutic, drug, monitoring, 5-fluorouracil

Introduction
In India, cancer can have serious social and economic effects, typically resulting in family destitution and societal injustice. In a population of 12 billion people, slightly more than 1 million new instances of cancer are detected each year. In age-adjusted terms, this reflects around a fourth of the male and female incidence seen in Western Europe. Cancer, on the other hand, was responsible for an estimated 600000-700000 fatalities in India in 2012. This result is comparable to the death burden reported in high-income nations when adjusted for age. Low rates of early-stage identification and poor treatment results are partly to blame for these findings. Cancer is a huge cost on society in both economically developed and developing countries. Because of population expansion and age, as well as an increase in the frequency of proven risk factors such as smoking, obesity, physical inactivity, and changing reproductive patterns linked with urbanization and economic development, cancer is becoming more common. According to GLOBOCAN (the World Health Organization's international agency for cancer research, which provides current estimates of the incidence, mortality, and prevalence of major types of cancer for 184 countries around the world), about 14.1 million new cancer cases and 8.2 million deaths occurred worldwide in 2012. The burden of cancer has shifted over time, with less developed nations now accounting for around 57 percent of cases and 65 percent of cancer deaths worldwide.

Cancer research in India has risen in size and influence during the last 20 years. From low-tech, large-scale health outcomes to some of the most sophisticated fields of fundamental cancer science, Indian clinicians, scientists, and federal and state policymakers have championed cancer research. Cancer research in India is a complicated setting in which public policy must be balanced across numerous conflicting goals.

According to these predictions, several anticancer drug procedures using HPLC-UV or HPLC-flourimetry, GC and GC-MS. However, due to a variety of limiting factors such as low sensitivity, a high plasma requirement for establishing methods, cost, longer run times, thermal stability of molecules, and the difficulty in processing large samples using the

aforementioned analytical techniques, researchers have shifted their focus to developing more sensitive and high throughput methods using HPLC coupled with MS and MS/MS, which are used for clinical and pharmacokinetic applications.

The established bio-analytical methods must be sensitive enough to determine the drug and/or its metabolite (s) concentration in biological samples for at least five elimination half-lives after drug administration. A bio-analytical method is a collection of procedures for gathering, processing, storing, and analyzing analyte (s) in a biological matrix. The three phases of bio-analytical method analysis are: a) method development, b) method validation, and c) method implementation (sample analysis).

Selectivity, sensitivity, linearity, precision, accuracy, matrix effects, recovery, stability, and dilution integrity are all important factors for LC-MS/MS method validation. Validation should be done for each analyte in the biological matrix. Furthermore, the analyte's stability in spiked samples should be assessed.

The method has been successfully developed and validated and can be used in pharmacokinetic and bioequivalence research, as well as therapeutic medication monitoring. Anticancer medications must be determined in biological metrics alone as well as in their combination dose forms in the contemporary pharmaceutical environment, with LCMS/MS technologies being used to analyze anticancer agents that are more sensitive. As a result of the current significance, the current suggested effort is to develop the most selective, sensitive, and rapid bio analytical methods for analyzing anticancer drugs, with the goal of reducing the numerous challenges that researchers encounter. For their usefulness, these designed and validated methodologies could be employed in clinical pharmacokinetic investigations and therapeutic medication monitoring.

Therapeutic drug monitoring (TDM) and chromatographic techniques in TDM

TDM is essential to optimize the treatment of critical dose medications with a narrow therapeutic range (the difference between a therapeutic and possibly deadly dose is tiny) and a high risk of overdosing or underdosing. The drug concentration can be used to guide drug dose in order to maximize therapeutic effectiveness and pharmacological responses while reducing negative effects. Furthermore, it is used to track a patient's adherence to a drug regimen and to spot potential drug-drug, drug-herb, or food-drug interactions.

In the 1970s, TDM was utilized in clinical practice to personalize drug therapy. TDM has been carried out using immunoassay for many years, although it is now recognized that immunoassay methods can be hampered by non-specific interference from similar substances, metabolite interference, or matrix effects.

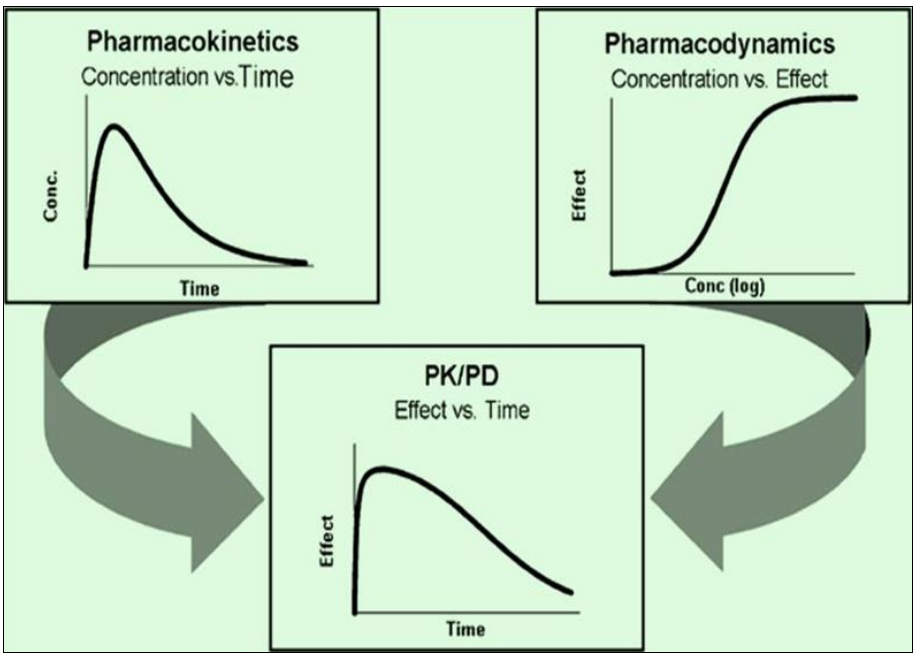

Fig 1: Correlations between pharmacokinetic and pharmacodynamic profile of drug which is the prime base for therapeutic drug monitoring

TDM is a clinical laboratory measurement of a chemical parameter that, when combined with competent medical interpretation, can have a direct impact on drug prescribing practices. TDM, on the other hand, refers to the individualization of drug dosage by keeping drug concentrations in the plasma or blood within a therapeutic range or window. TDM allows for the evaluation of a medication's efficacy and safety in a range of clinical contexts by combining knowledge of pharmaceutics, pharmacokinetics, and pharmacodynamics. This procedure aims to personalize therapy regimens for maximum patient benefit. TDM is traditionally defined as the measurement of drug concentrations in various biological fluids and the interpretation of these concentrations in terms of clinically relevant factors. Pharmacokinetic concepts are used by clinical pharmacists and pharmacologists to evaluate these interpretations. With the publishing of the first pharmacokinetic studies relating mathematical theories to patient outcomes in the 1970s, the science of TDM brought a new facet of clinical practice. As a result, the bio-analytical technique is ultimately responsible for correlating pharmacokinetic characteristics with pharmacodynamic variability among patients' drug responses

Research methodology: Therapeutic Drug Monitoring (TDM) of 5-Fluorouracil in plasma was performed in 12 patients with various kinds of cancer using the proposed LCMS/MS bio-analytical approach.

- 5 people with breast cancer.
- 2 people with pancreatic cancer.
- 2 people with stomach cancer.
- 1 patient with head and neck cancer.
- 1 patient with BM (Buccal Mucosa) cancer.
- 1 patient with Tongue Cancer.
- To get the requisite pharmacokinetic parameters, blood samples from patients were obtained at 5 points and the concentration of 5-FU was evaluated in each sample at each of these locations for therapeutic drug monitoring (TDM).
- 0.5 hr.
- 1 hr.
- 2.5 hr.
- 3.5 hr.
- 5.5 hr.
- Quantitative analysis of the proposed bio-analytical approach was used in conjunction with LAB-SOLUTION software in an LCMS/MS system to determine 5-FU. Each patient's 5-FU dose (750 mg) and elimination points (LZ =3) were set. During chemotherapy, the 5-FU medication was given as a 1 or 2 hour infusion with saline (NS Glass) or D5W (5 percent Dextrose in water).
- Pharmacokinetic software PK-SOLVER II was used to assess the pharmacokinetic parameters and pharmacokinetic profile of each patient for Cmax of 5-FU treatment in TDM.
- The following are the chemotherapy protocols for all 12 patients, together with primary intent data, quantitative data from LCMS/MS-LABSOLUTION, and pharmacokinetic data from PKSOLVER II, from case I to case XII.

Results and Discussion

Case-I: Chemotherapy: 5-Fluorouracil (750 mg) (2 hrs IV Infusion).

Table 1: Diagnosis and lab investigation of Case-I

Diagnosis: Cancer of Breast		
Age: 41 yrs	Sex: Female	
Height: 154 cms	Weight: 57 kgs	
BSA:1.5	Other: 5FU + Doxorubicin + Cyclophosphamide	
Lab Investigation		
Hb: 13.3%	TC: 9020	Plate: 346000

Table 2: Detection by LCMS-MS for Case-I

Time	0	0.5	2.5	3.5	5.5
Conc. (ng) by LCMS/MS method	0	0.499	0.289	0.016	0.05

Case-II: Chemotherapy: 5-Fluorouracil (750 mg) (2 hrs Iv Infusion).

Table 3: Diagnosis and lab investigation of Case-II

Diagnosis: Cancer of Breast		
Age: 50 yrs	Sex: Female	
Height: 164 cms	Weight: 65 kgs	
BSA:1.6	Other: 5FU + Epixtoa + Cyclophosphamide	
Lab Investigation		
Hb: 12.2%	TC: 7043	Plate: 333080

Table 4: Detection by LCMS-MS for Case-II

Time	0	0.5	2.5	3.5	5.5
Conc. (ng) by LCMS/MS method	0	1.490	1.689	0.343	0.048

Case-III: Chemotherapy: 5-Fluorouracil (750mg) (2hrs IV Infusion).

Table 5: Diagnosis and lab investigation of case-III

Diagnosis: Cancer of Breast		
Age: 45yrs	Sex: Female	
Height: 152 cms	Weight: 50 kgs	
BSA:1.4	Other: 5FU+Cyclophosphamide+Epixtra	
Lab Investigation		
Hb: 12.3%	TC: 7750	Plate: 2.5L

Table 6: Detection by LCMS-MS for case-III

Time	0	0.5	2.5	3.5	5.5
Conc. (ng) by LCMS/MS method	0	0.946	0.369	0.105	0.008

Case-IV: Chemotherapy: 5-Fluorouracil (750mg) (2hrs IV Infusion).

Table 7: Diagnosis and lab investigation of case-IV

Diagnosis: Cancer of Breast		
Age: 49 yrs	Sex: Female	
Height: 156 cms	Weight: 56 kgs	
BSA:1.6	Other: 5FU+Cyclophosphamide+Epixtra	
Lab Investigation		
Hb: 11.8%	TC: 7260	Plate: 2.53L

Table 8: Detection by LCMS-MS for case-IV

Time	0	0.5	2.5	3.5	5.5
Conc. (ng) by LCMS/MS method	0	1.842	1.709	0.396	0.099

Case-V: Chemotherapy: 5-Fluorouracil (750mg) (1.5 hrs IV Infusion).

Table 9: Diagnosis and lab investigation of case-V

Diagnosis: Cancer of Stomach		
Age: 50 yrs		Sex: Male
Height: 154 cms		Weight: 60 kgs
BSA:1.5		Other: Epirubicin + Cisplatin + 5FU
Lab Investigation		
Hb: 12.7%	TC: 8740	Plate: 366000

Conclusion

The most effective and promising chemotherapy treatment now available for different types of cancer is PTX and 5-FU, given intravenously every three weeks. A more thorough comprehension of the pharmacokinetic and pharmacodynamic characteristics of PTX and 5-FU by TDM may enhance the effectiveness of the customised dosage regimen. Therefore, paclitaxel (PTX) and 5-fluorouracil (5-FU) were chosen for the current TDM study based on the literature review.

Using internal standards (CBZ for PTX and 5-BU for 5-FU) and the liquid-liquid extraction (LLE) method, the bio-analytical LCMS/MS method was optimised for its appropriateness in human biological matrix plasma in terms of chromatographic and mass spectrometric characteristics.

Both of the currently available optimal methods were created and thoroughly validated in accordance with USFDA and EMEA standards.

The binary mode of mobile phase is the foundation of the bioanalytical technique, which is then followed by a measurement using multiple reaction monitoring during tandem mass spectrometry (MRM).

The developed and validated LCMS/MS techniques were linear for PTX over the range of 5- 3000 ng/mL (R^2 = 0.997) and for 5-FU over the range of 10-10000 ng/mL (R2 = 0.996).

It is crucial for the LCMS/MS analysis to reduce the matrix effect, ensure consistent recovery, and increase sensitivity (LOD=5 ng/mL for PTX and LOD=10 ng/mL for 5-FU) without interference from endogenous compounds. Our methods have the advantage of a smaller auto sampler injection volume (10 L for both drugs).

The fact that the existing bio-analytical procedures only take a short amount of time to complete (4 minutes for PTX and 3 minutes for 5-FU) shows that both of the approaches were quick and accurate for quantifying a specific drug.

Both assay methods were determined to be accurate and precise (inter batch 99.72-102.27% for PTX; 98.64-102.61 for 5-FU and intra batch 98.29-105.56% for PTX; 97.07-103.83% for 5-FU) (inter batch 1.67-13.07% CV for PTX; 2.69-7.24% CV for 5-FU and intra batch 0.79-14.22% CV for PTX; 1.34-8.26% CV for 5-FU).

By measuring and quantifying plasma concentration and determining pharmacokinetic parameters (by pksolver) in a number of cancer patients receiving 260 mg dose of PTX and 750 mg dose of 5-FU by intravenous route during chemotherapy, the clinical applicability of both bio-analytical methods to TDM was demonstrated (3 weeks of cycle).

We may draw the conclusion that the created and approved bio-analytical LCMS/MS assay method for PTX and 5-FU will allow for the thorough processing of numerous plasma samples from cancer patients for pharmacokinetic studies and bio-availability/bioequivalence (BA/BE) research.

The routine TDM of PTX and 5-FU will benefit from the simple, quick, selective, accurate, and precise bio-analytical assay methodologies, broadening the potential for clinical research to successfully treat cancer.

Major findings

According to USFDA and EMEA regulations, a highly accurate and straightforward approach was created and validated in human matrix-plasma for the measurement of Paclitaxel and 5-Fluorouracil. It was effectively used to carry out sample analysis for pharmacokinetic and bioavailability investigations of Paclitaxel and 5-Fluorouracil during chemotherapy in cancer patients. As evidenced by the data, TDM of Paclitaxel was successfully performed in 11 cancer patients and TDM of 5-Fluorouracil in 12 patients. Compared to previously published approaches, this one has a number of benefits, including reduced sample amount, high throughput, speed, increased sensitivity and simplicity, and appropriate retention without matrix interferences.

The current LC-MS/MS approach for the quantification of Paclitaxel and 5-Fluorouracil in human plasma offers a straightforward, reliable, fast, and sensitive analytical instrument. It can also be successfully used in clinical studies of pharmacokinetic profiles and TDM.

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