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A critical study on bio-analytical Lc-Ms/Ms method validation of therapeutic medication chemotherapeutic drug

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Abstract

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. During method development, carry-over should be handled and minimized. Carry-over should be tested during validation by injecting blank samples after a high concentration sample or calibration standard at the quantification's upper limit. Carry over in the blank sample after the high concentration standard should not exceed 20% of the lower limit of quantification, and 5% for the internal standard. If carry-over looks to be unavoidable, the study samples should not be randomized. Specific procedures should be considered, evaluated during validation, and implemented during the analysis of study samples to ensure that accuracy and precision are not compromised.

Keywords: Bio-analytical, validation, therapeutic, medication, chemotherapeutic, drug

Introduction

The measurement done in the laboratory of a parameter that, with appropriate interpretation, can directly alter prescription method," according to the International Association for Therapeutic Drug Monitoring and Clinical Toxicology. The measurement is typically a biological matrix of a prescription xenobiotic, but it might also be an endogenous chemical prescribed as a replacement therapy in a person who is physiologically or pathologically low in that compound." Watson and colleagues

Reasons for requesting TDM

- Suspected toxicity
- Lack of response of active drug
- Compliance with medication regime
- Change in clinical stage of patient
- Patient drug interaction
- Monitoring compliance
- Individualizing therapy -during early therapy –during dosage changes
- Diagnosing under treatment
- Monitoring and detecting drug interactions
- Guiding withdrawal of therapy

A clinical pharmacologist for patient diagnosis, a clinical pharmacist for drug measures in biological fluids, and an analytical scientist for patient care are all members of the ideal TDM team. A physician must individualize a patient's pharmacological therapy in order to achieve the best balance between therapeutic efficacy and the occurrence of undesirable effects. Because all patients have different pharmacokinetics (PK) and pharmacodynamics (PD), getting to the goal is always difficult. When both PK and PD variability are significant, the lack of control of drug concentration inhibits the patient's clinical response variability. New analytical techniques made it possible to measure the modest drug concentrations observed in biological fluids after drug treatment in the early 1960s. As a result, the TDM approach allowed for the reduction of pharmacokinetic variability in drug therapy by managing drug therapy using body concentrations rather than merely doses

Criteria of drugs to be a candidate for TDM

The following criteria should be used to select a suitable drug for TDM:

- The relationship between drug concentration and drug effect should be clarified. As a result, there is a link

between medication concentration in the matrix (plasma/serum) and efficacy and toxicity.

- Narrow therapeutic index range (little difference between therapeutic and potentially lethal doses); this means that the concentrations of separation between the drug's therapeutic benefit and those generating unfavorable effects should be modest.
- The difference in between-subject pharmacokinetic variability should be obvious, as should a poor or unexpected relationship between dose and drug concentration/response. For instance, a given dose may generate a desired pharmacological response in one patient but toxicity in another. These medicines are suitable for TDM because of significant variations in metabolism related to genetic makeup, age, sex, or disease.
- The dose-clinical result relationship is unpredictably complex. For instance, a given dose may generate a desired pharmacological response in one patient but toxicity in another. These medicines are suitable for TDM because of significant variations in metabolism related to genetic makeup, age, sex, or disease.
- TDM is frequently used with drugs that have nonlinear pharmacokinetic characteristics. A modest change in dosage for these medications might result in disproportionately high serum/plasma concentrations, which can lead to toxicity. Furthermore, drug buildup may result in toxicity as a result of a substantial interaction with another medicine.

Pharmacokinetic parameters and TDM

Pharmacokinetic studies on pharmaceutical drugs can be conducted in order to determine clinical efficacy, tolerance in the treated animal, and consumer safety. The main goals are to determine the parameters that influence absorption, distribution, and elimination (basic pharmacokinetic studies) and to assess the bioavailability of the active ingredient in two or more product formulations and/or delivery routes (bioequivalence studies). The determination of withdrawal periods and the measurement of residues delivered into the environment are two other essential parts of pharmacokinetic investigations.

Absorption

The rate and degree of systemic availability of the active ingredient or active moiety should be established. In most cases, only plasma/blood curve-time data may be used to evaluate the rate of absorption; urine data can only be used to determine the extent of absorption. The rate and degree of absorption of the active ingredient should be assessed regardless of the route of administration of the pharmaceutical product. Only intravenous (bolus or infusion) data allow for the evaluation of the degree of absorption, hence comparisons with an equivalent intravenous dose should be undertaken whenever possible (absolute systemic availability). If a systemic effect is expected (for example, a high intrinsic activity active component), the degree of systemic absorption should be measured.

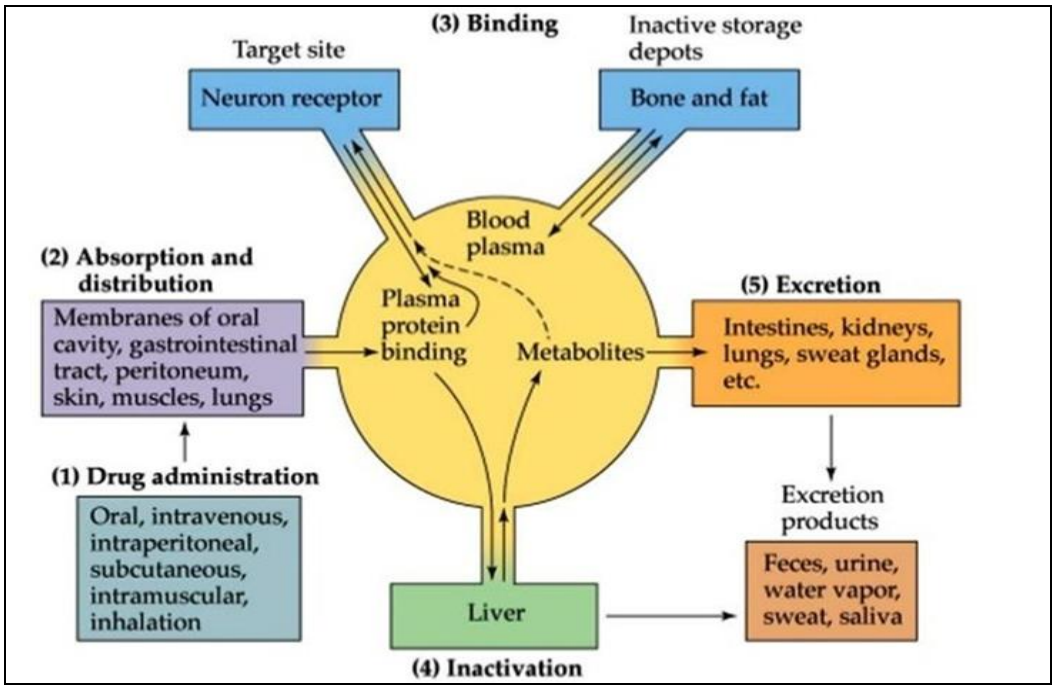

Fig 1: Pharmacokinetics Parameters For Drug

Measurement of the active ingredient's (or therapeutic moiety's or metabolite's) concentration in plasma as a function of time. Urinary excretion (active component, metabolites), as well as acute pharmacological effects, may be taken into account. Systemic availability is a measure of a product's availability in comparison to an intravenous (absolute) or extravascular (relative) reference product.

TDM in anticancer class of drugs

Anticancer medicines have a lot of pharmacokinetic diversity between people. Furthermore, toxicity is sometimes more closely related to matrix level than dosage. Furthermore, many anticancer medicines' pharmacokinetic characteristics are complicated by drug metabolism, and therapeutic ranges have yet to be established.

Several researches, however, have produced better findings when it comes to pharmacokinetics and drug responsiveness. Many anticancer medications, such as methotrexate, PTX, 5FU, Docetaxel, and Busulfan, are ideal candidates for routine monitoring. It is not acceptable to under-dose cancer patients with chemotherapy drugs. Certain serious adverse effects, such as myelo-suppression, can, on the other hand, be fatal. As a result, it is useful to create therapeutic limits for anticancer medications that produce matrix concentrations at the upper end of the nontoxic range. The use of TDM to personalize chemotherapy in order to improve anticancer medication efficacy while avoiding major drug toxicity would be extremely beneficial in patient care. TDM also has other advantages in cancer patients, including as improved compliance, reduced pharmacokinetic variability among patients, detection of drug-drug interactions, and dosage changes for patients with hepatic or renal failure.

A multidisciplinary strategy combining a nurse, phlebotomist, medical technologist, clinical chemist, and physician is required to successfully deploy TDM in cancer patients.

The following parameters may be required for successful TDM implementation

- Approval by the Human Ethics Committee
- Authorized hospital permission for authorized methodology
- Patient compliance
- A bio-analytical method for a specific drug has been developed and validated.
- Proper specimen collection, handling, processing, and storage
- Mathematical pharmacokinetic calculation expertise

Liquid chromatography mass spectrometry (LC-MS)

Peptide mapping, glycoprotein mapping, natural product de-replication, Bio affinity screening, in vivo drug screening, metabolic stability screening, metabolite identification, impurity identification, degradation identification, quantitative bio-analysis, and quality control is all common uses for LC-MS in drug development.

The technique of mass spectrometry is very useful for identifying substances. MS has the distinct benefit of having a high level of molecular specificity and detection sensitivity. The molecular weight and structure-specific fragmentation ion information are provided by MS in the form of a fingerprint mass spectrum. The main disadvantage of MS is its inability to handle compound mixes. Tandem mass spectrometry (MS/MS) can only help to a certain amount with this problem. The high resolution separation capabilities of MS are thus mutually beneficial when HPLC and MS are interfaced. MS is on the verge of becoming a universal LC detector.

LC/MS/MS is a hybrid technology that combines the separating capacity of HPLC with the detecting power of mass spectrometry. A mass spectrometer is a comparatively expensive detector in comparison to these. To put it another way, chromatography is the separation of the components of a mixture in order to identify and/or quantify some or all of them. The chromatographic retention characteristic is used to begin the identification process. Because the likelihood of more than one analyte having essentially identical retentions makes this insufficient for definitive identification, LCMS/MS is employed for compound identification with the following advantages.

Advantages

- It can give absolute identification, not only by providing structural information from the molecule under inquiry,
- But also by providing the analyte's molecular weight
- Sensitivity – from picogram (pg) levels of analyte, a full-scan spectrum and perhaps identification can be acquired. It can be utilized to produce quantitative data with high accuracy and precision, usually at low levels.

Research methodology

Analytical variables and TDM

The quality of the sample tested determines the quality of the specimen results. The preanalytical phase includes everything from patient preparation to specimen analysis. In vivo or in vitro, preanalytical mistakes can occur. Many of these artifacts might skew test findings by causing changes that aren't representative of the patient's physiological state. The laboratory has a harder time controlling in vivo factors, but some can be addressed by enforcing specimen collection and handling criteria. TDM is influenced by primarily two sorts of elements (Green, 2008).

1. Pre-analytical variables *in vivo* (Patient associated pre analytical factors)
2. Pre-analytical variables *in vitro*

***In vivo* pre analytical factors (Goodman, 2009)**

- Patient compliance
- Patient's physiological condition (age, sex, diet, body weight, exercise)
- Hemolysis or lipemia
- Drug interactions
- Degree of protein binding
- Time of dose vs time of collection

***In vitro* pre analytical factors**

- Primary tube selection for matrix collection
- Site of matrix collection
- Fill volume
- Sample preparation precedure
- Time of storage
- Temperature of storage

Sampling for TDM

For pharmacokinetic studies, suitable biological fluids (blood, plasma, serum, urine, etc.) and tissues should be chosen. Plasma is often thought to be the most effective biological fluid for such research because it has a faster turnaround time because blood coagulation is not required.

Blood sampling

The place of blood collection, the sample process, the material utilized for sampling, the blood collecting tubes, the anticoagulant, the delay, and the conditions of centrifugation to obtain plasma should all be considered. The substance's stability should be checked while collection and while being stored until analysis. The quantity of blood

samples taken and when they are taken should be sufficient to determine absorption, distribution, and excretion. In the post-absorption phase, blood samples should be collected for as long as is required for the inquiry (often up to four "half-lives")

Other biological fluids and tissues

Other biological fluids may be of general interest (e.g., if analytical constraints limit the usefulness of blood samples, urine samples may be used to determine the terminal disposition slope) or of particular interest (e.g., if analytical constraints limit the usefulness of blood samples, urine samples may be used to determine the terminal disposition slope) (e.g. local distribution to support a claim). The collection of some of these fluids necessitates extra care (e.g. immediate pH measurement of urine, conditions of storage, etc.). Repeated biopsies under local anesthetic for tissues could be approved for scientific purposes, as this saves animals and enables for individual-based research. When employing a biopsy procedure, however, particular care should be taken to ensure that there is no pain or suffering.

Chromatographic techniques in TDM

Different chromatographic techniques are used in TDM depend on drug and availability. These all techniques are describe with their justification.

Table 1: Chromatographic techniques used for tdm

Sr. no.	Chromatographic technique	Justification
1.	Thin layer chromatography (TLC)/ Paper chromatography	▪ Lack of specificity for compound identification, ▪ Time consuming, ▪ Sample preparation and sample pretreatment requirement ▪ Lack of effectiveness and sensitivity
2.	Gas chromatography	▪ Selective and specific method ▪ Internal standard can be used ▪ Used Only for thermally stable and volatized drugs ▪ Not suitable for polar drugs ▪ Derivatization is required
3.	High performance liquid chromatography	▪ Highly sensitive and specific method ▪ Used for thermally stable and thermo labile drugs ▪ Suitable for polar as wel as non polar drugs ▪ No requirement of derivatization for hydroxyl group identification

Results and Discussion

bio-analytical lc-Ms/Ms method validation for paclitaxel

According to USFDA and EMEA criteria, the proposed approach was validated.

System suitability: The percent CV of system appropriateness was 0.38 for PTX retention time, 0.56 percent for ISTD retention time, and 1.35 for PTX area ratio, all of which were below the acceptance criterion of 4.00 percent.

Table 2: Results of system suitability parameters for paclitaxel

Sample name	Area ratio	RT of PTX	RT of ISTD
MQC-1	0.520	1.456	1.6
MQC-2	0.430	1.450	1.395
MQC-3	0.432	1.454	1.389
MQC-4	0.506	1.465	1.6
MQC-5	0.436	1.457	1.41
MQC-6	0.439	1.451	1.39
Mean	0.442	1.455	1.395
SD	0.008	0.007	0.009
% CV	1.39	0.40	0.58

Carry over check

During the experiment, there was no substantial carry over, i.e. the area of the peak at the drug retention time in standard blank samples was not more than 20.00 percent of the area in LLOQ. As per acceptance requirements, the area of the peak during the retention time of ISTD in STD BL samples was not more than 5.00 percent of its area in LLOQ.

Table 3: Result of auto sampler carryover

Sample ID	Peak area	
	PTX	ISTD
STD BL	0	0
ULOQ	246475	217486
STD BL	0	0
LLOQ	1030	232007
% Carry over	0	0

Linearity

A weighted least square regression analysis of standard plots associated with an eight-point standard calibration curve was used to determine the method's linearity. A peak area ratio versus concentration calibration curve with the best match was shown. Quality control standards were also displayed as chromatograms. The PTX calibration curve was linear from 5.00ng/mL to 3000.00ng/mL, with a r2 0.9970 correlation coefficient.

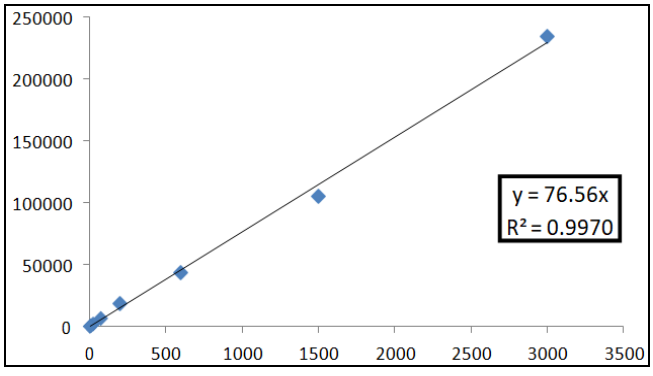

Fig 2: Concentration ratio (ng/mL)

Table 4: Summary of calibration parameters for PTX

P and A	Slope	Intercept	r2
P and A I	115.8	0	0.9950
P and A II	60.28	0	0.9960
P and A III	76.56	0	0.9970
Mean	84.21	0	0.9960

Conclusion

Only after a satisfactory chromatogram has been acquired can optimization begin. A acceptable chromatogram is one in which all of the chemicals are detected by more or less symmetrical peaks on the chromatogram. The shifting of the peaks can be predicted by a modest change in the mobile phase composition. Within the range of examined modifications, the position of the peaks can be predicted from a few experimental observations. A chromatogram that is optimized is one in which all of the peaks are symmetrical and well separated in a short amount of time. Using a more efficient column with a higher theoretical plate number can improve peak resolution.

The mass spectrometer is usually programmed to scan a specified mass range. This mass scan can be broad, such as in complete scan analysis, or very narrow, such as in selective ion monitoring. Depending on the type of scan, a single mass scan can take anywhere from 10 milliseconds to 5 seconds. During an LC-MS analysis, many scans are obtained. The ion current in individual mass scans is added up, and the 'totaled' ion current is plotted as an intensity point against time in LC-MS data. The resulting figure closely resembles that of an HPLC UV trace.

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