INTRODUCTION

India’s pharmaceutical industry has evolved from almost nonexistent to a one of world leader in the production of high quality generic drugs. India has garnered a worldwide reputation for producing high quality, low cost generic drugs. The industry currently meets India’s demand for bulk drugs and nearly all its demand for formulations, with the remainder supplied by foreign multinational corporations. The world has witnessed a sharp increase in the demand for cost-effective generic drugs in a decade. In such a scenario, India’s rise as a generic destination is the blessing for people in under developed countries or patients looking for cheap medicines. Factors like patent expiries, investment in research and Development participation will boost the sector.

A generic drug must contain the same active ingredients as the original formulation. According to the U.S. Food and Drug Administration (FDA), generic drugs are identical or within an acceptable bioequivalent range to the brand-name counterpart with respect to pharmacokinetic and pharmacodynamic properties. By extension, therefore, generics are considered identical in dose, strength, route of administration, safety, efficacy, and intended use. Thus, because of the importance of generic drugs it must be demonstrated that the safety and efficacy of generic are comparable to the safety and efficacy of the Innovator drugs. Ascertainment between the generic and innovator product is carried out by a study of bioequivalence.\(^{(1)}\)

Recently medication is not by a single drug, but with multi-drug therapy, which could be the use of combinations of different drugs in the treatment of patients suffering from multiple diseases. One method, which is reported for the quantitation of a drug, need not necessarily indicate for the multiple analytes assay. Hence the research for a more accurate and precise method, using multiple analytes and higher throughput of samples with better sensitivity can be developed.

Bioanalysis of study samples is a important phase of Bioequivalence assessment, The analytical method used in an in vivo bioavailability or bioequivalence study to measure the concentration of the active drug ingredient in body fluids. It shall be demonstrated to be accurate and of sufficient sensitivity to measure, with appropriate precision, the actual concentration of the active drug ingredient achieved in the body.\(^{(2)}\)

Thus, bioanalytical method employed for the quantitative determination of drugs and their metabolites in biological fluids, plays a significant role in the evaluation and interpretation of bioequivalence, pharmacokinetic studies. The quality of these studies, which are often used to support regulatory filings, is directly related to the quality of the underlying bioanalytical data. It
is therefore important that guiding principles for the validation of these analytical methods to established and spread to the pharmaceutical community. (3)

Bioanalytical methods are used for the quantitation of drugs and their metabolites in biological matrices. In today’s drug development environment, highly sensitive and selective methods are required to quantify drugs in matrices such as blood, plasma, serum, or urine. Chromatographic methods high-performance liquid chromatography [HPLC] have been widely used for the bioanalysis of small molecules, with liquid chromatography coupled to triple quadrupole mass spectrometry detector (LC/MS/MS) being the single most commonly used technology. After developing a method with desired attributes, the method requires validation to establish that it will continue to provide accurate, precise, and reproducible data during study-sample analysis. (4)

High-performance liquid chromatography (sometimes referred to as high-pressure liquid chromatography), HPLC, is a chromatographic technique that can separate a mixture of compounds and is used in analytical chemistry to identify, quantify and purify the individual components of the mixture. HPLC typically utilizes different types of stationary phases, a pump that moves the mobile phase(s) and analyte through the column and a detector to provide a characteristic retention time for the analyte. The MS detector may also provide selectivity and sensitivity related to the analyte. Impressive progress has been made in the technology and application of combined liquid chromatography-mass spectrometry (LC–MS) in the past years. LC–MS has become the method-of-choice of analytical support in many stages of drug development in pharmaceutical industries. (5)

Liquid chromatography/electrospray ionization tandem mass spectrometry (LC/ESI-MS/MS) is one of the most prominent analytical techniques owing to its inherent selectivity and sensitivity. In LC/ESI-MS/MS is often used to enhance the detection sensitivity. UPLC improves the chromatographic separation, and enhances the mass spectrometric ionization efficiency and MS/MS detectability. (6)

Conventional liquid–liquid extraction (LLE), protein precipitation (PP) are now been considered as methods of the past. The last decade has witnessed a rapid development of novel sample preparation techniques in bioanalysis. Developments in SPE techniques such as selective sorbents and in the overall approach to SPE, such as hybrid SPE and polymer SPE, have been addressed. Considerable literature has been published in the area of solid-phase extraction and its different versions and their application in the development of selective and sensitive bioanalytical methods. Techniques such as dispersive solid-phase extraction, disposable pipette extraction and
micro-extraction by packed sorbent offer a variety of extraction phases and provide some unique advantages to bioanalytical methods.\textsuperscript{(7)}

Bioanalytical method validation as per current guidelines is an essential tool to establish that the method continues to provide accurate, precise, and reproducible data during study-sample analysis. It includes all of the procedures that demonstrate that a particular method used for quantitative measurement of analytes in a given biological matrix, such as blood, plasma, serum, or urine, is reliable and reproducible for the intended use.

The fundamental parameters for this validation include (1) accuracy, (2) precision, (3) selectivity, (4) sensitivity, (5) reproducibility, and (6) stability. Full validation is required for a new drug entity and when implementing a developed method for the first time in the bioanalytical laboratory. Partial validation is required if modifications are made to an already validated method, the number of validation exercises depends on the extent of modification and the impact on the integrity of the validation data.\textsuperscript{(8)}

In this proposal, it is planned to develop and validate chromatographic methods for sample preparation of drugs from varying therapeutic categories, in single or in combination, such as combination of Lornoiroxicam and paracetamol belonging to the anti-inflammatory class of compounds.

Milnacipran hydrochloride is a selective norepinephrine and serotonin reuptake inhibitor; it inhibits norepinephrine uptake with greater potency than serotonin. It is a racemic mixture with the chemical name: (±)-[1R(S),2S(R)]-2-(aminomethyl)-N,N-diethylphenylcyclopropane carboxamide hydrochloride.

Fingolimod a derivative of ISP-1 (myriocin), a fungal metabolite of the Chinese herb Iscaria sinclarii as well as a structural analogue of Sphingosine. It is a novel immune modulator is a sphingosine 1-phosphate receptor modulator. Chemically, fingolimod is 2-amino-2-[2-(4 octylphenyl)ethyl] propan-1,3-diol hydrochloride.

Similarly, drugs from different therapeutic categories can be selected and methods for their estimation individually or simultaneously can be developed if the literature reveals that none are available for the same