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AIMS AND OBJECTIVES
Bioanalytical methods are used for the quantitation of drugs and their metabolites in biological matrices. The present discovery and drug development scenario calls for highly sensitive and selective methods to quantify drugs in matrices such as blood, plasma, serum or urine. Chromatographic methods (high-performance liquid chromatography [HPLC] or gas chromatography [GC] have been widely used for the analysis of small molecules, while liquid chromatography coupled to triple quadrupole mass spectrometry (LC-MS/MS) is the single most commonly used technology for bioanalysis. After developing a method with desired attributes, the method is validated to establish that it will continue to provide accurate, precise, and reproducible data during the complete duration of the study. Method validation is a process that demonstrates that the method will successfully meet or exceed the minimum standards recommended in the Food and Drug Administration (FDA) Guidance, for accuracy, precision, selectivity, sensitivity, reproducibility, and stability. Validation is performed using a control matrix spiked with the analyte/s to be quantified.

High-performance liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS) due to its high degree of sensitivity and selectivity represents the wide use for quantitative bioanalysis of small molecules. It can be routinely employed in a clinical environment and is attractive because of simplicity of sample processing and high throughput. Mass spectrometry can be regarded as a complementary technique having technical advantages over immunoassays and gas chromatography for the determination of steroids in biological matrices. The ability of mass spectrometry methods to detect low levels of steroids enhances their clinical use particularly at extremely low concentration levels.

Corticosteroids are used to treat various inflammatory and immunology mediated diseases. Prednisone, Prednisolone, Dexamethasone, Betamethasone, Fluticasone propionate, Triamcinolone acetonide and Budesonide are among the most frequently used corticosteroids and their determination in biological fluids is necessary. Corticosteroids and sex steroids can be estimated by immunoassay and Gas chromatography–mass spectrometry (GC–MS) methods. Immunoassays suffer from lack of selectivity and necessitates the use of different types of kits to cover the wide range of corticosteroids. Gas chromatography–mass spectrometry (GC-MS) methods suffer from drawbacks like long analysis time and time consuming derivatization. Prolonged or systemic use of corticosteroids leads to significant side effects. Low-dose corticosteroids may provide a favorable benefit/risk ratio for the
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therapeutic applications which requires quantification of drugs at very low concentrations in plasma. Developing the methods with high sensitivity and selectivity for corticosteroid pharmacokinetics (PK) is a challenging task; as a result LC–MS methods are now frequently applied for the determination of steroids.

The main problem associated with LC-MS/MS during the determination of analytes of interest in plasma is matrix effect. Molecules originating from the sample matrix that coelute with the compound(s) of interest can interfere with the ionization often resulting in false MS signals and responses.

The present work aims at the development of selective, sensitive and rapid LC-MS-MS method for determination of steroids in human plasma using selective reaction monitoring (SRM) mode. Efforts are made to eliminate co-eluting components of plasma, since their presence will reduce method sensitivity.

There are numerous investigations demonstrating that pharmacokinetics and drug effects can depend on the time of drug administration and on biological rhythms of physiological functions. Studies in animals and humans have provided evidence for regular and predictable circadian and circannual variations in pharmacodynamics and pharmacokinetics. Circadian (about 24 hr) rhythms exist at all levels of organization. There are circadian rhythms in sleep and activity patterns, in plasma cortisol levels (which reaches its maximal levels early in the morning in healthy humans) and in body temperature. These rhythms are driven by biological clocks and are endogenous in origin. A consequence of these rhythmic variations in biology is that both drug disposition and effects vary as a function of the time of drug administration. The temporal changes in drug effects include variations both in the desired (chronoeffectiveness) and undesired (chronotoxicity) effects. When considering circadian effect of the drugs, it is obvious that the fate of a drug may vary according to the time of its administration.

However, despite numerous experimental and clinical chronopharmcokinetic studies, the time of administration of a drug still remains a neglected factor in many kinetic studies; the reasons for which may be,

(a) Non availability of methodological aspects in order to correct the dose or therapy with circadian rhythm.
(b) Ignorance or denial of the relevance of chronobiological data. 

There is no doubt that the pharmacokinetics can be significantly influenced by time of administration. Time of day has to be regarded as an additional variable to influence kinetics of a drug. In present research work attempt is made to find circadian variation in the pharmacokinetic parameters of selected corticosteroids and sex steroids as application to developed methods.

**AIM OF THE WORK**

The present research work, aims at developing and validating the bioanalytical method for the selected synthetic corticosteroids and sex steroids and its application to chronopharmacokinetics.

**OBJECTIVES**

1. Bioanalytical method development and validation for simultaneous estimation of selected corticosteroids like Budesonide, Fluticasone, Prednisone, Prednisolone, Dexamethasone and Triamcinolone acetonide in human plasma as per the USFDA guidelines.

2. Bioanalytical method development and validation of Ethinyl estradiol in human plasma as per the USFDA guidelines.

3. Bioanalytical method development and validation of Levonorgestrel in human plasma as per the USFDA guidelines.

4. Bioanalytical method development and validation of Mifepristone in human plasma as per the USFDA guidelines.

5. Application of developed and validated bioanalytical methods to chronopharmacokinetic study of selected molecules like Prednisolone, Ethinyl estradiol, Levonorgestrel and interpretation of the data.