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Editorial

Determination of Pharmaceutical Active Substances or their Metabolites in Biological Fluids

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Biopharmaceutical analysis deals with the qualitative identification/confirmation and quantitative measurement of drugs or drug metabolites in biological fluids. The biological fluids include whole blood, plasma, serum, saliva, cerebrospinal fluid, urine etc. The qualitative identification/confirmation determines the organoleptic and physicochemical characteristics whereas quantitative analysis could be for bioequivalence, pharmacodynamics, pharmacokinetics, toxicokinetics purposes. Biopharmaceutical analysis also applies to drugs used for illicit purposes, forensic investigations, anti-doping testing in sports. This analytical technique unlike chemical analytical technique could face challenges arising from components of the sample matrix interfering with the analyte(s) of interest. Because these drugs/metabolites are in these complex and varied matrices, detection limits vary depending on the drug/metabolite. As the drugs/metabolites very often occur at very low concentrations (trace or ultra trace levels), sensitive and selective methods that are easily automated and within minimum pretreatment are preferable.

A number of sample preparation techniques have been developed for this purpose. It could involve filtration, extraction (liquid-liquid, liquid-solid adsorption or solid phase extraction using cartridge), desalting and protein precipitation with the primary objective of isolating the analyte(s) of interest from the interfering compounds in the sample matrix and dissolving them in a suitable solvent followed by pre-concentration prior to quantification. Therefore, the goals of any sample preparation are to have the analyte(s) of interest in solution (leading to quantitative recovery), free from interfering matrix components and at a concentration appropriate for detection and measurement.

Protein precipitation mainly in plasma or serum samples can be accomplished by use of polar organic solvents (methanol, acetonitrile, acetone etc. or their mixture) or treating the biological fluid with acids or inorganic salts, such as formic acid, perchloric acid, trichloroacetic acid, ammonium sulfate, sodium sulfate, or zinc sulfate. In some cases, acid hydrolysis (for basic compounds) or base hydrolysis (for acidic compounds) is used to ensure that the analyte(s) of interest are freely solvated in the urine sample. Also enzymatic hydrolysis that frees bound analyte(s) also may be used in urine analysis.

A number of analytical methods exit for the identification/confirmation and determination of drugs/metabolites in biological fluids and they include: immunoassay methods (radioimmunoassay, enzyme immunoassay, fluorescence polarization immunoassay etc.), chromatographic method (thin-layer chromatography, high performance liquid chromatography, gas chromatography, ultra performance liquid chromatography and supercritical fluid chromatography etc.), hyphenated method (liquid chromatography–mass spectrometry, gas chromatography–mass spectrometry, liquid chromatography–diode array detection and capillary electrophoresis–mass spectrometry etc.), spectroscopic method (fluorescence, ultraviolet/visible etc.). The choice of any of these analytical methods depends on the physicochemical properties of the analytes, number and structures of the analytes, the range over which analytes has to be measured, the matrices of interest and the stability of the analytes in the biological matrices.

In conclusion, the process adopted for the isolation of analyte(s) of interest such as sample preparation procedure and isolation steps have to ensure the stability of the analyte(s) until the completion of the analytical estimation. Sample preparation is also very necessary in order to achieve sufficient sensitivity and selectivity. Finally, preservation techniques can be used to minimize changes such as adsorption, diffusion, microbiological degradation, oxidation and volatilization between collection and analysis.

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