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**ADVANCED ANALYTICAL METHODS FOR DETECTING MULTIPLE
PHARMACEUTICAL RESIDUES IN COMPLEX SAMPLES**

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The importance of science in enhancing the standard of human life is closely connected to the necessity of consuming clean and nutritious food and living in a safe environment. The use of pharmaceutical drugs in medicine and in veterinary medicine is permanently growing thus leading to environmental contamination.

Pharmaceutical contaminants include compounds from various drug classes such as analgesics, antidepressants, hormones and other endocrine-disrupting compounds, antitumor medications, antibiotics. These substances are repeatedly discharged into the environment either unchanged or in the form of active metabolites.

Pharmaceuticals are regularly introduced into the environment through various pathways such as wastewater treatment plants, runoff from large-scale livestock farming, unrestricted application of manure amended with veterinary pharmaceuticals. It results in the presence of pharmaceutical contaminants in food and natural settings.

The presence of pharmaceutical contaminants in food can potentially pose several risks to human health such as carcinogenicity, mutagenicity, or teratogenicity. Long-term intake of contaminated food can also disrupt the normal gut microbiome, trigger allergic reactions and lead to antimicrobial resistance.

The precise and accurate analytical methodologies are crucial for protecting public health and minimizing human exposure to harmful pharmaceutical contaminants. Techniques like ultra-high performance liquid chromatography paired with mass spectrometry detection and time-of-flight (UHPLC-ToF-MS) represent premier solutions for obtaining the desired accuracy levels. Using UHPLC-ToF-MS for analysis of pharmaceutical residues in complex samples has several advantages: excellent fast chromatographic separation of analytes prior to mass analysis; exact mass measurements for accurate identification of compounds; high resolution and sensitivity to detect and identify a wide range of pharmaceutical residues; a wide analytical coverage of a diverse range of compounds: small molecules, large biomolecules, and complex mixtures; non-targeted screening without prior knowledge of the analytes present.

The typical workflow of UHPLC-ToF-MS for determination of pharmaceuticals in complex samples involves the following key steps:

1. Sample collection includes acquiring samples of the appropriate matrices and performing pre-processing such as taking size measurements, weighing, cleaning, freezing, lyophilisation and storage.

2. Analyte extraction through homogenization and extraction of the targeted compounds using methods like protein lyophilisation, liquid-liquid extraction (LLE) and the Quick, Easy, Cheap, Effective, Rugged, and Safe extraction (QuEChERS).

3. The “Clean-up and preconcentration” step removes unwanted components and concentrates the target compounds via methods of solid phase extraction (SPE), filtering, centrifugation, lipid precipitation and evaporation.

4. UHPLC-ToF-MS analysis step encompasses instrument setup, sample injection, chromatographic separation, and mass spectrometry analysis.

5. Data interpretation involves quantification, peak detection and data validation including the limit of detection (LoD), the limit of quantification (LoQ), precision, accuracy and recovery.

Thus, determining pharmaceutical contaminants in complex samples is vital for safeguarding human health and well-being. The UHPLC-ToF-MS is the cutting-edge technique for achieving the high accuracy and precision results.