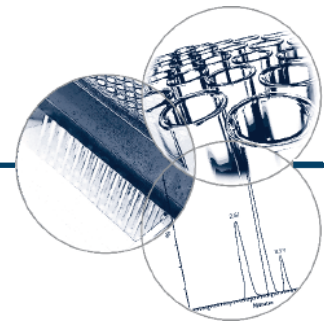
RESEARCH ARTICLE

SPECIAL FOCUS: BIOANALYTICAL SAMPLE PREPARATION



Large-volume injection of sample diluents not miscible with the mobile phase as an alternative approach in sample preparation for bioanalysis: an application for fenspiride bioequivalence

Background: Liquid–liquid extraction of target compounds from biological matrices followed by the injection of a large volume from the organic layer into the chromatographic column operated under reversed-phase (RP) conditions would successfully combine the selectivity and the straightforward character of the procedure in order to enhance sensitivity, compared with the usual approach of involving solvent evaporation and residue re-dissolution. Large-volume injection of samples in diluents that are not miscible with the mobile phase was recently introduced in chromatographic practice. The risk of random errors produced during the manipulation of samples is also substantially reduced. **Results:** A bioanalytical method designed for the bioequivalence of fenspiride containing pharmaceutical formulations was based on a sample preparation procedure involving extraction of the target analyte and the internal standard (trimetazidine) from alkalinized plasma samples in 1-octanol. A volume of 75 µl from the octanol layer was directly injected on a Zorbax SB C18 Rapid Resolution, 50 mm length × 4.6 mm internal diameter × 1.8 µm particle size column, with the RP separation being carried out under gradient elution conditions. Detection was made through positive ESI and MS/MS. Aspects related to method development and validation are discussed. **Conclusions:** The bioanalytical method was successfully applied to assess bioequivalence of a modified release pharmaceutical formulation containing 80 mg fenspiride hydrochloride during two different studies carried out as single-dose administration under fasting and fed conditions (four arms), and multiple doses administration, respectively. The quality attributes assigned to the bioanalytical method, as resulting from its application to the bioequivalence studies, are highlighted and fully demonstrate that sample preparation based on large-volume injection of immiscible diluents has an increased potential for application in bioanalysis.

The simplest and most straightforward way for enhancing the sensitivity of an analytical chromatographic method is through the increase of the amount of target compounds loaded onto the column. As samples are characterized by proper concentrations of the target analytes, this is equivalent to the increase of the injected sample volume. If the inherent sensitivity of the detection device is less than the threshold imposed to the analytical method, dedicated procedures for concentration of the analytes are required. If the target compounds are placed in complicated matrices (and this is specifically the case of bio-samples), sample preparation techniques are used prior to chromatographic separation for the isolation of analytes and/or to control for/avoid interferences. Liquid–liquid extraction (LLE) is one of the oldest techniques used for isolation of analytes from cumbersome matrices. Such an approach is efficient, easy to develop, does not require complicated and expensive laboratory equipment and automatically involves a concentration step through removal of the extraction solvent(s) by evaporation and redissolution of the dried extract in a convenient combination of other solvents, compatible with the mobile phase used in the consequent chromatographic separation technique. The extensive sample manipulation represents one of the main disadvantages of the LLE technique, directly affecting the final quantitative response variability through incorporation of random errors induced during the sample preparation procedure. Some of the variability induced by the sample preparation procedure and/or detection (i.e., ionization

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