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Large volume injection of 1-octanol as sample diluent in reversed phase liquid chromatography: Application in bioanalysis for assaying of indapamide in whole blood

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ABSTRACT

Large volume injection of samples in strong diluents immiscible with the mobile phases used in reversed phase liquid chromatography (RPLC) has been recently introduced in practice. In the present work, the potential of the technique has been evaluated for bioanalytical applications. The process consists of the liquid–liquid extraction of indapamide from whole blood into 1-octanol, followed by the direct injection from the organic layer into the LC. Detection was made through negative electrospray ionization (ESI) and tandem mass spectrometry (MS^2). The method was developed, validated, and successfully applied to a large number of samples in two bioequivalence studies designed for indapamide 1.5 mg sustained release and 2.5 mg immediate release pharmaceutical formulations. The performance of the analytical method is discussed based on data resulting from the validation procedure and the completion of the bioequivalence studies.

1. Introduction

The main purpose of bioanalysis is the determination of selected compounds in biological matrices [1,2]. Two challenging problems relate to the sample preparation step in bioanalytical processes: elimination of the biological matrix to sustain method's selectivity and enrichment of the target compounds to achieve low quantitation limits. Protein precipitation methods and extraction processes are more often used to support the above mentioned goals.

Liquid–liquid extraction readily isolates analytes in water immiscible solvents. The sample transfer to the chromatographic system is usually preceded by the removal of the extraction solvent (under gas stream, eventually thermally assisted) and redissolution of the dry residue in a solvent compatible with the mobile phase, to further support a large injected volume. Evaporation step may add random errors to the experimental results and seriously lengthens the duration of the analytical process. It would be highly preferable to inject large volumes of the organic phase directly to the chromatographic column. However, it is generally accepted that if the injection solvent is stronger than the mobile phase, the chromatographic peaks will be broadened and/or distorted [3–8]. Some practical solutions to accommodate stronger diluents to large volume injection – reversed phase liquid chromatography (RPLC) through application of pulsed elution gradients have been proposed [9]. Recent studies demonstrated that band broadening/peak distortion does not occur if the dilution solvent has an increased retention compared to target compounds [10]. This also applies for dilution solvents, which are not miscible with the mobile phase and exhibit enhanced affinity for the stationary phase compared to target analytes [11,12]. The reduction of the retention factors characterizing the target compounds should be considered, because the highly retained dilution solvent "saturates" a proportional amount of the stationary phase [11]. The use of water-immiscible solvents as diluents in RPLC has been recently highlighted for the assay of related impurities in active ingredients [13], antioxidants in pharmaceutical formulations [14] and ginkgolic acid in standardized extracts [15]. Additional phenomena related to similarity/dissimilarity of the viscosities characterizing injection solvent and the chromatographic eluent, namely the viscous fingering problem, should also be taken in consideration under these particular conditions [16]. Accordingly, if the injection solvent is less viscous than the eluent, chromatographic peaks tend to have fronting. By the opposite, a more viscous injection solvent should be fingered by the backward eluent, leading to peak tailing [17,18].

The basic phenomena relating to large volume injection of diluents non-miscible with the mobile phase, more precisely

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