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Greener bioanalytical approach for LC/MS–MS assay of enalapril and enalaprilat in human plasma with total replacement of acetonitrile throughout all analytical stages☆

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ABSTRACT

Green bioanalytical approaches are oriented toward minimization or elimination of hazardous chemicals associated to bioanalytical applications. LC/MS–MS assay of enalapril and enalaprilat in human plasma was achieved by elimination of acetonitrile from both sample preparation and chromatographic separation stages. Protein precipitation (PP) by acetonitrile addition was replaced by liquid–liquid extraction (LLE) in 1-octanol followed by direct large volume injection of the organic layer in the chromatographic column operated under reversed phase (RP) separation mechanism. At the mean time, acetonitrile used as organic modifier in the mobile phase was successfully replaced by a mixture of propylene carbonate/ethanol (7/3, v/v). Three analytical alternatives ((I) acetonitrile PP + acetonitrile based chromatographic elution; (II) 1-octanol LLE + acetonitrile based chromatographic elution; (III) 1-octanol LLE + propylene carbonate/ethanol based chromatographic elution) were validated and the quality characteristics were compared. Comparison between these alternative analytical approaches was also based on results obtained on incurred samples taken during a bioequivalence study, through application of the Bland–Altman procedure.

1. Introduction

The introduction of the concept of green analytical chemistry dates from the late nineties [1,2]. The definition of this concept was comprehensively formulated by Keith [3,4]: "the use of analytical chemistry techniques and methodologies that reduce or eliminate solvents, reagents, preservatives and other chemicals that are hazardous to human health or the environment and that may also enable faster and more energy-efficient analyses without compromising performance criteria". More often the most difficult task is to translate from existing methods to greener ones keeping unaltered the performance criteria. Topics on greening of analytical chemistry were extensively discussed and reviewed [5–9]. Aspects focusing specifically on chromatographic methods are also available [10–12]. A discussion about green bioanalytical principles was recently published [13]. Implementation of the green chemistry principles in sample preparation conducted to the following development directions [14]: miniaturization of liquid–liquid extraction [15,16], elimination or minimization of solvent use [17], increasing the role of solid phase extraction in sample preparation [18,19]; increased use of membrane supported liquid phase (micro)extraction [20,21]; use of ionic liquids as extraction media [22] or ionization media from the solid state [23,24]. In liquid chromatography, two main directions for greener alternatives are currently explored. The first one deals with reduction of solvents and additives through reducing columns internal diameters (from analytical to narrow or micro bore ranges) and dimensions of packings (from 5 to 3 and sub-2 μm particle sizes) [25,26]. The second one refers to replacement of acetonitrile and/or methanol in the mobile phases with less harmful and environmental friendly alternatives such as water [27], ethanol (EtOH) or iso propanol [28], propylene carbonate (PC) [29] and carbon dioxide (either in sub-critical or supercritical state) [30–32].

We actually tried to turn a somewhat "classical" bioanalytical method in a greener one. The first step consisted in the replacement of acetonitrile (ACN) during the sample preparation stage (protein precipitation). Liquid–liquid extraction in 1-octanol was considered as a possible alternative. Direct large volume injection from the organic layer is thus possible. The fundamentals referring to large volume injection of diluents non-miscible with the mobile

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can observe that slopes of the probability plots are closely approximating the unit, and linear correlations with the inverse normal cumulative distribution of a data set having a null mean and a standard deviation of 1 are also significant. Analysis of data resulting after comparison between the experimental approaches clearly indicates their equivalence.

4. Conclusions

Elimination of acetonitrile from the sample preparation stage and the chromatographic separation of a bioanalytical approach used to assay enalapril and enalaprilat in plasma samples was successfully achieved. Protein precipitation by means of acetonitrile addition was replaced by liquid–liquid extraction in 1-octanol. Direct large volume injection from the organic layer in the chromatographic column avoids solvent removal by evaporation and compensate for reduced recoveries of the target compounds on LLE. Chromatographic elution based on acetonitrile was replaced by a mobile phase composed from propylene carbonate, ethanol and water. Similar retention data were produced without major modifications of the gradient profile used during the chromatographic separation. "Greening" the bioanalytical approach through acetonitrile elimination was possible without any compromise of the performance criteria. The "classic" approach was compared in terms of performance characteristics with the two alternatives which progressively eliminate ACN from use. Validation and different comparison models based on data produced through analysis of incurred plasma samples lead to similar conclusions: no compromise of global quality characteristics are produced though the progressive elimination of acetonitrile from the analytical procedures. Both features highlighted in the work (large volume injection of diluents non-miscible with the mobile phase and acetonitrile replacement by propylene carbonate/ethanol mixtures in the mobile phase composition) may be successfully applied in bioanalytical applications without affecting performance.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jchromb.2012.11.023.

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