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Bioanalysis of monomethyl fumarate in human plasma by a sensitive and rapid LC–MS/MS method and its pharmacokinetic application LC–MS/MS determination of monomethyl fumarate in human plasma

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ABSTRACT

Dimethyl fumarate (DMF) is the methyl ester of fumaric acid, after oral administration completely converts to its active metabolite monomethyl fumarate (MMF). A simple, rapid and sensitive LC–MS/MS method was developed and validated for the quantification of MMF in human plasma. Monomethyl fumarate d3 was used as an internal standard (IS). The analyte and the IS were extracted from plasma using a selective solid phase extraction technique. The clean samples were chromatographed on a C_{18} column using formic acid and acetonitrile (25:75, v/v) as mobile phase. An API-4000 LC–MS/MS system equipped with turbo ion spray (TIS) source and operated in multiple reactions monitoring (MRM) mode was used for the study. The method was validated for linearity in the range of 5.03-2006.92 ng/mL. Also, a number of stability tests were conducted to evaluate the stability of analyte, IS in plasma samples and in neat samples, the results comply with recent bioanalytical guidelines. A shortest run time helped us to analyze more than 300 samples in a day. The method was applied to a pharmacokinetic study in ten healthy male Indian subjects and the study data was authenticated by conducting incurred sample reanalysis (ISR).

1. Introduction

Multiple sclerosis (MS) is an autoimmune and neurodegenerative disease characterized by the inflammation of the brain and spinal cord in which focal lymphocytic infiltration leads to damage of myelin and axons [1]. Dimethyl fumarate (DMF) is a fumaric acid derivative approved by the US FDA and EMA for the treatment of relapsing-remitting forms of multiple sclerosis (MS) [2,3]. After oral administration, DMF is rapidly hydrolyzed in the intestinal mucosa to monomethyl fumarate (MMF) [4]. Peak concentrations of MMF are achieved within 5–6 h. MMF have been shown to activate the nuclear factor (erythroid-derived 2)-like 2 (Nrf2) pathway in vitro and in vivo in animals and humans [5–7]. DMF is not quantifiable in plasma following oral administration; hence, all pharmacokinetics related to DMF were performed with plasma MMF concentrations.

Very few analytical methods [8,9] were reported so far for the determination of DMF along with its metabolite MMF in rat blood and DMF along with other few disease-modifying agents of multiple sclerosis in human plasma. Recently, Junnotula et al., 2016 [8] was published LC–MS/MS method to quantify DMF and its metabolite MMF in rat blood. This method was complex with use of trapping reagent tiopronin to form DMF and MMF conjugates and capturing of free DMF and MMF fractions. A protein precipitation (PP) technique was employed to extract the conjugates. In the same year, another LC-UV and LC–MS method was reported by Suneetha *et al.*, 2016 [9] for the determination of DMF along with other drugs namely fampridine and teriflunomide, which are used in the treatment of MS. This method owing run time of 15 min is not a high throughput bioanalysis of DMF. Also, MMF was not quantified in plasma. For a bioavailability and bioequivalence and pharmacokinetic studies of DMF, it is necessary to quantify the MMF concentrations [10].

Till date, no LC–MS/MS method describes the complete method development and validation procedures for the determination of MMF in human plasma. For pharmacokinetic and bioequivalence studies of DMF, one should have a sensitive and selective analytical method to quantify MMF concentrations in plasma is needed. Tandem mass spectrometry (MS-MS) is a unique analytical tool for the quantification of drugs nanogram to picogram level from bio-

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