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Ultrapressure liquid chromatography–tandem mass spectrometry assay using atmospheric pressure photoionization (UPLC-APPI-MS/MS) for quantification of 4-methoxydiphenylmethane in pharmacokinetic evaluation

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ABSTRACT

4-Methoxydiphenylmethane (4-MDM), a selective augmenter of Leukotriene A_4 Hydrolase (LTA_4H), is a new anti-inflammatory compound for potential treatment of chronic obstructive pulmonary disease (COPD). Currently, there is no liquid chromatography tandem mass spectrometric (LC–MS/MS) method for the quantification of 4-MDM. A major barrier for developing the LC–MS/MS method is the inability of electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) to ionize 4-MDM due to its hydrophobicity and lack of any functional group for ionization. With the advent of atmospheric pressure photoionization (APPI) technique, many hydrophobic compounds have been demonstrated to ionize by charge transfer reactions. In this study, a highly sensitive ultrapressure liquid chromatography tandem mass spectrometry assay using atmospheric pressure photoionization (UPLC–APPI-MS/MS) for the quantifications of 4-MDM in rat plasma has been developed and validated. 4-MDM was extracted from the plasma by solid phase extraction (SPE) and separated chromatographically using a reverse phase C_8 column. The photoionization (PI) was achieved by introducing anisole as a dopant to promote the reaction of charge transfer. The assay with a linear range of 5 (LLOQ)–400 ng mL^{-1} met the regulatory requirements for accuracy, precision and stability. The validated assay was employed to quantify the plasma concentrations of 4-MDM after an oral dosing in Sprague Dawley (SD) rats.

1. Introduction

4-Methoxydiphenylmethane, (4-MDM, Fig. 1A) is a selective augmenter of Leukotriene A_4 Hydrolase (LTA_4H) aminopeptidase enzyme activity and a new anti-inflammatory compound for chronic obstructive pulmonary disease (COPD) [1]. LTA_4H has been targeted in discovery of anti-inflammatory drugs for COPD as it plays a major role in the generation of leukotriene B_4 (LTB_4), a potent modulator associated with the chemotaxis of leukocytes in the lung such as neutrophils and monocytes [2–4]. LTA_4H is a bifunctional enzyme having an epoxy hydrolase site and an aminopeptidase site [5,6]. Recent work by Snelgrove et al. demonstrated that proline-glycine-proline (PGP), a potent chemoattractant for neutrophils, is a physiological substrate of the aminopeptidase site of LTA_4H [7]. The authors concluded that aminopeptidase activity of LTA_4H plays an anti-inflammatory role during influenza virus infection, whereas the epoxy hydrolase activity plays a proinflammatory role. Binding of 4-MDM to LTA_4H enhances the degradation of PGP resulting in the decrease of neutrophil recruitment in the lung, and subsequently the reduction of inflammation with no effect on the epoxy hydrolase activity of LTA_4H. Recent studies have demonstrated that oral administration of 4-MDM is effective against emphysema in mice induced by elastase and cigarette smoke. [1,8].

Abbreviations: 4-MDM, 4-methoxydiphenylmethane; ACN, acetonitrile; APPI, atmospheric pressure photoionization; BCS, biopharmaceutical classification system; CAD, collision activated dissociation; CE, collision energy; COPD, chronic obstructive pulmonary disease; CEP, collision cell entrance potential; CXP, collision cell exit potential; DP, decluttering potential; EP, entrance potential; IE, ionization energy; LLOQ, lower limit of quantification; LTA_4H, leukotriene A_4 hydrolase; LTB_4, leukotriene B_4; PA, proton affinity; PI, photoionization; PGP, proline-glycine-proline; PK, pharmacokinetics; RCF, relative centrifugal force; SD, Sprague Dawley.

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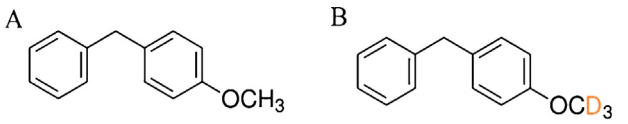

Fig. 1. Chemical structures of (A) 4-MDM (MW 198.2), and (B) Labelled internal standard containing 3 deuterium (MW 201.2).

To establish the future clinical therapeutic dose and dosing regimen of 4-MDM, preclinical pharmacokinetic (PK) study is essential to characterize its PK in rodent and nonrodent animals. A sensitive liquid chromatography tandem mass spectrometry (LC–MS/MS) assay for 4-MDM in plasma samples is a prerequisite for establishing the PK profiles. However, the development of an LC–MS/MS for 4-MDM is challenging due to its high hydrophobicity and lack of any functional group that can be ionized by electrospray Ionization (ESI) or atmospheric pressure chemical ionization (APCI). Atmospheric pressure photoionization (APPI), introduced in 2000, uses photons to ionize both polar and non-polar compounds [9,10]. Previous works have demonstrated successful applications of APPI ever since, in analysis of pharmaceuticals [11,12], polyaromatic hydrocarbons [13,14], hydrophobic peptides [15,16], apolar lipids [17] and steroids [18].

This manuscript reports, for the first time, the development, validation and application of a sensitive assay for 4-MDM in rat plasma using ultra pressure liquid chromatography (UPLC)–APPI-MS/MS. The assay was validated in linearity, accuracy, precision, matrix effect, recovery and stability. The validated assay was subsequently used to establish the plasma profile of 4-MDM in Sprague Dawley rats after an oral dosing to demonstrate its application.

2. Materials and methods

2.1. Chemicals

Acetonitrile, methanol and water of LC–MS grade, toluene and carbon disulfide of LC grade, and heparin sodium salt were purchased from EMD Millipore Corp (Billerica, MA, USA). LC grade acetone, and anisole (analytical standard, purity 99.9%) were obtained from Sigma Aldrich (St. Louis, MO, USA). Heparinized pooled blank rat plasma was purchased from Equitech-Bio Inc. (Kerrville, TX, USA). For solid phase extraction, Waters Oasis HLB columns (Milford, MA, USA) were used. For cosolvent formulation, polyethylene glycol 400 (PEG 400), propylene glycol and glycerin were purchased from Avantor Performance Materials Inc. (Center Valley, PA, USA). Deionized ultrapure water was purified by Milli-Q water purification system (Bedford, MA, USA).

2.2. Synthesis of deuterated-4-MDM-d_3

To a solution of 0.60 g (3.0 mmols) of 4-benzylphenol in 3 mL of DMF, 0.20 g (1.0 mmols) of KOH dissolved in 10 mL DMF was added, followed by 0.20 mL (3.6 mmols) of deuterated iodomethane (CD_3I). The reaction was stirred at room temperature overnight and the reaction progress was monitored by TLC. The retention factor (R_f) for the starting material and the product on a normal phase TLC plate developing with a 1:3 ethyl acetate-hexane solvent system were R_f = 0.48 and R_f = 0.74, respectively. Both the starting material and the product were visualized under a UV lamp (254 nm). When the reaction was complete as determined by TLC, the solution was taken up in 25 mL of ethyl acetate, washed with saturated aqueous LiCl solution (2 × 10 mL), and then concentrated under reduced pressure to afford crude product as a yellow oil. Purification by Kugelrohr distillation (210–215 °C at approximately 1 Torr pressure) afforded 0.40 g (2.0 mmols) of deuterated-4MDM in 65% yield, confirmed by ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.39–7.36 (m, 2H), 7.31–7.26 (m, 3H), 7.22 (d, J = 8.4 Hz, 2H), 6.92 (d, J = 8.4 Hz, 2H), 4.00 (s, 2H).

Table 1
Transitions and compound dependent parameters for 4-MDM.

Transition	*DP*	*EP*	*CE*	*CEP*	*CXP*
m/z 198 → 167	30.4	2.7	21.1	13.5	1.4
m/z 198 → 121	21	7.1	29.7	12.5	3.4
m/z 198 → 183	26	7.3	31.3	15.1	2.6

Table 2
Transitions and compound dependent parameters for the IS.

Transition	*DP*	*EP*	*CE*	*CEP*	*CXP*
m/z 201 → 124	22.1	3.5	30.4	15.4	1.6
m/z 201 → 167	20	7	20.7	19.6	2.4

2.3. LC–MS/MS

4-MDM and 4-MDM-d_3 (I.S) were analyzed by Waters Aquity UPLC system (Waters Corp., Milford, Ma, USA) coupled with a Photospray™ ion source attached to an API Qtrap 3200 triple quadruple mass spectrometer (Applied Biosystem/MDS SCIEX, Foster City, CA, USA). A dopant (toluene, anisole, acetone or carbon disulfide) was added to the Photospray source by a separate LC pump (Shimadzu Corp. Kyoto, Kyoto Prefecture, Japan) to ionize the analytes.

2.3.1. Chromatographic conditions

20 μL of the sample was injected into the UPLC and separated in a Kinetex silica bonded reverse phase C_8 column (50 mm × 2.1 mm, 1.7 μm particle size, Phenomenex Inc. Torrance, California, USA) at 45 °C. Water and Acetonitrile were used as mobile phases A and B, respectively, at a flow rate of 150 μL min^{-1}. The gradient was 0–10% B (0–0.5 min), 10–30% B (0.5–1 min), 30–70% B (1–4 min), 70% B (4–6.5 min), 90% B (6.51–7.2 min), and 10% B (7.21 min). The eluent from the column was diverted to the mass spectrometer from 3 to 6.5 min. To eliminate any carryover, the needle was washed with 2 mL of acetonitrile (strong wash) and 4 mL of 15% methanol (weak wash) after each injection.

2.3.2. Mass spectrometer conditions

The dopant (toluene, acetone, anisole or carbon disulfide) was pumped directly into the Photospray source at a flow rate of 7 μL min^{-1}. Pure nitrogen, generated by Genius 3030 gas generator (PEAK Scientific, Scotland, UK), was used as nebulizer and UV lamp gases. The instrument parameters for the assay were as follows: ion source temperature was set at 100 °C; curtain gas, gas 1 (nebulizer gas), and gas 2 (lamp gas) were set at 15 psi, 20 psi and 15 psi, respectively. The collision activated dissociation (CAD) gas was kept at "medium settings" to optimally break down the parent ion to generate MS2 ions and to achieve maximum sensitivity. The ionization of the analyte was carried out in positive mode with ion transfer voltage of 700 V. At least two different transitions were monitored: one as quantitative and another as qualitative transition. The compound dependent parameters, including declustering potential (DP), entrance potential (EP), collision energy (CE), collision cell entrance potential (CEP) and collision cell exit potential (CXP), were optimized separately by manual scanning for each of the transitions (Tables 1 and 2). Analyst® 1.5 (Applied Biosystem/MDS SCIEX, Foster City, CA, USA) was used for quantification of the analyte.