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Journal of Pharmaceutical and Biomedical Analysis 36 (2004) 649–651
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
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Short communication

HPLC determination of mycophenolic acid and mycophenolic acid glucuronide in human plasma with hybrid material

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Received 28 May 2004; received in revised form 16 July 2004; accepted 21 July 2004
Available online 8 September 2004

Abstract

Mycophenolic acid (MPA), the active metabolite of the prodrug mycophenolate mofetil is an immunosuppressive agent which inhibits inosine monophosphate dehydrogenase. MPA is metabolised to phenolic glucuronide (MPAG) that may be hydrolysed in vivo to form free MPA. Drug monitoring is required in patients with multi-organ failure. Here, we report a HPLC method with organic/inorganic hybrid material for the simultaneous analysis of MPA and MPAG in human plasma.

MPA and MPAG and carboxy butoxy ether mycophenolic acid (MPAC) used as internal standard were analysed on a bonded X-Terra column with a linear gradient elution mode using orthophosphoric acid and acetonitrile as eluents. Sample treatment procedure consists of deproteinisation with acetonitrile. Analytical recoveries were higher than 98 and 89% at concentrations ranging from 1 to 25 and 20 to 200 mg/L for MPA and MPAG, respectively. Calibration curves fitted by plotting the peak area ratio (compound of interest/internal standard) versus concentration were linear in the range 0.2–50 mg/L for MPA and in the range 1–500 mg/L for MPAG. The quantification limit was 0.2 mg/L for MPA and 1 mg/L for MPAG with a coefficient of variation less than 20% for a 500 μL sample volume. Intra- and inter-assay coefficient of variation was lower than 7% for all compounds. Detection was performed at 215 nm. Peak identity was confirmed through library matching by comparison with reference spectra. The X-Terra column provides good peak shape and may be used at low pH with a long life-time column.

This HPLC method using a simple sample treatment procedure appears suitable for therapeutic drug monitoring in organ-transplant patients. The method is sensitive enough for monitoring MPA and MPAG during pharmacokinetic studies.
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Keywords: HPLC; X-Terra; Mycophenolic acid; Mycophenolic acid glucuronide; Human plasma

1. Introduction

Mycophenolic acid (MPA), the active metabolite of the prodrug mycophenolate mofetil is an immunosuppressive agent which inhibits inosine monophosphate dehydrogenase. MPA is metabolised to phenolic glucuronide (MPAG) which is the major metabolite (Fig. 1).

MPAG may undergo an enterohepatic recirculation resulting in a second peak plasma of MPA [1]. MPA is also metabolised in minor metabolites, 7-*O*-glucoside and acyl glucuronide (AcMPAG) [2].

Drug monitoring of MPA and MPAG is required regarding: (1) the inter and intra individual variability in plasma concentrations and area under curve, (2) the potential drug–drug interactions with others immunosuppressant drugs [3] and (3) the influence of organ failure on free MPA [1].

Some HPLC methods for the analysis of MPA and MPAG in plasma have been reported [4–9]. The methods described

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doi:10.1016/j.jpba.2004.07.034

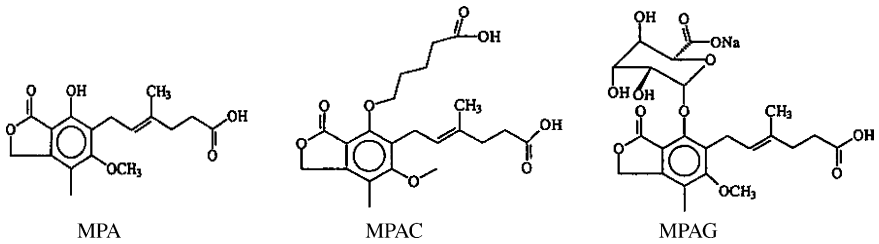

Fig. 1. Molecular structures of MPA, MPAG and MPAC.

used conventional stationary phases with [4,5] or without [7–9] gradient elution mode. Here, we report a HPLC method using an organic/inorganic material (X-terra phase) which is known to improve peak shape and to exhibit excellent stability for the simultaneous analysis of MPA and MPAG in human plasma. This hybrid material combines the best properties of silica and polymer. It contains methylsiloxane groups distributed homogeneously throughout the particle backbone.

2. Experimental

2.1. Materials

MPA and carboxy butoxy ether mycophenolic acid (MPAC, internal standard) were generously supplied by Hoffman Laroche, Basel, Switzerland. MPAG was obtained from Analytical Services, Catersham, Great Britain. Acetonitrile, orthophosphoric acid were purchased from Merck, France.

2.2. Equipment and HPLC conditions

The chromatographic system consisted of Hewlett Packard 1050 series using HPChem software (Agilent Technologies, France). Separation of MPA, MPAG and internal standard was achieved using X-Terra RP18, 5 μm, 150 mm × 3.9 mm (Waters, France). The mobile phase consisted of acetonitrile–orthophosphoric acid as eluents. The gradient conditions are the following: (A) 40 mM H_3PO_4, (B) 40 mM H_3PO_4/CH_3CN, 55/45 v/v, with the gradient: 0–4 min (55.5% B), 4–17 min (55.5–100% B), 17–20 min (100–55.5% B). The flow rate was maintained at 1.2 ml/min. The detection wavelength was 215 nm using a photodiode array detector. The system was used at ambient temperature (20 °C).

2.3. Sample treatment

The 500 μL of acetonitrile containing the internal standard were added to 500 μL of plasma. After mixing for 1 min,

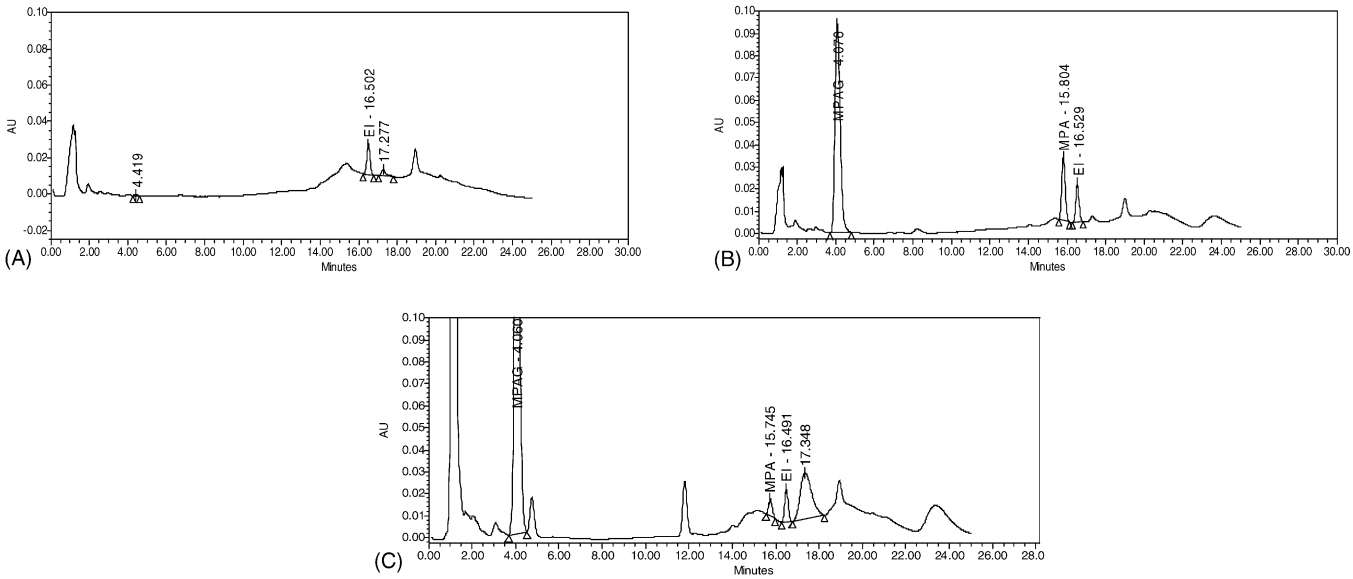

Fig. 2. Chromatograms of (A) blank plasma, (B) plasma spiked with 5 mg/L MPA, 50 mg/L MPAG and the internal standard and (C) patient sample: 2 mg/L MPA, 118 mg/L MPAG.

Table 1
Recoveries

Compounds	Concentration added (mg/L)	Recovery (%, mean ± S.D.)
MPA	0.5	105.1 ± 8.7
	5.0	111.9 ± 4.0
	20.0	102.1 ± 4.6
MPAG	5.0	103.6 ± 7.1
	50.0	97.6 ± 3.4
	200.0	93.4 ± 5.3

50 μL of 40 mM H_3PO_4/acetonitrile, 55/45 v/v was added. The mixture was mixed for 30 s and then centrifuged at 2000 × *g* for 10 min. The supernatant was diluted five times in 40 mM H_3PO_4/acetonitrile, 75/25 v/v, and 100 μL were injected into the column.

Calibrators for MPA and MPAG were prepared from stock solutions of 100 mg/L MPA and 1000 mg/L MPAG in acetonitrile/water (80/20 v/v), diluted in drug free plasma to final concentrations of 0.5, 1.0, 5.0, 20.0 mg/L and 5, 10, 50, 200 mg/L, respectively. In-house control samples were prepared by spiking drug free plasma with MPA and MPAG stock solutions to obtain final concentration of 10 and 100 mg/L, respectively. Plasma from heart/lung transplant patients receiving oral mycophenolate mofetil (500 mg twice a day) were analysed.

3. Results and discussion

Chromatograms of a blank plasma, a plasma sample spiked with the compounds of interest and a patient sample are shown in Fig. 2. Mean analytical recovery for each compound was determined by comparison of peak area obtained from plasma supplemented at known concentrations and those obtained by direct injections of calibrators. Results are shown Table 1.

Calibration curves fitted by plotting peak area ratio (compound of interest/internal standard) versus concentration were linear in the range 0.2–50.0 mg/L for MPA and 1–500 mg/L for MPAG. The correlation coefficient was higher than 0.999 for the two compounds. The typical regression equation were $y = 0.331x + 0.032$ or MPA and $y = 0.166x - 0.044$ for MPAG. The intra-day and inter-day precision ($n = 5$) were assessed by replicate analysis of plasma samples spiked with the compounds of interest at two different concentrations. Intra-day coefficient of variation ranged from 1.9 to 3.9% for MPA and 1.9 to 5.0% for MPAG at concentration of 5 and 20 mg/L for MPA and 50 and 200 mg/L for MPAG. Inter-day coefficient of variation were below 6% for all compounds.

The quantification limit was 0.2 mg/L for MPA and 1 mg/L for MPAG with a CV inferior to 20% for 500 μL sample volume. Compared to values reported using UV detection [4,6,8,9], the limits of quantification obtained are lower, particularly for MPAG.

X-Terra RP18 stationary phase has demonstrated a long lifetime with about 700 biological samples injected without any deterioration. The stationary phase used provides higher efficiency and improves peak shape of the compounds especially MPAG compared with conventional reversed phase column. Tailing factor of MPAG was 1.07 and those of MPA was 0.96. The use of acidic solutions in the mobile phase does not affect the stationary phase which exhibits excellent stability at low pH (pH 1).

The HPLC method described is simple and rapid considering the sample treatment procedure. The method appears suitable for drug monitoring in organ-transplant patients and sensitive enough for monitoring MPA and MPAG during pharmacokinetic studies.

References

[1] L.M. Shaw, R. Mick, I. Nowak, M. Korecka, K.L. Brayman, J. Clin. Pharmacol. 38 (1998) 268–275.
[2] R. Bullingham, A.J. Nicholls, B.R. Kamm, Clin. Pharmacokinet. 34 (1998) 429–455.
[3] K. Zucker, A. Tsaroucha, L. Olson, V. Esquenazi, A. Tzakis, J. Millller, Ther. Drug Monit. 21 (1999) 35–43.
[4] M. Shipkova, P.D. Niedmann, V.W. Armstrong, E. Schutz, E. Wieland, L.M. Shaw, M. Oellerich, Clin. Chem. 44 (1998) 1481–1488.
[5] D.G. Watson, F.G. Araya, P.J. Galloway, T.J. Beattie, J. Chromatogr. 35 (2004) 87–92.
[6] D. Teshima, K. Otsubo, N. Ktagawa, S. Yoshimur, Y. Itoh, R. Oishi, J. Clin. Pharmacol. Ther. 28 (2003) 17–22.
[7] C.E. Jones, P.J. Taylor, A.G. Johnson, J. Chromatogr. 708 (1998) 293–300.
[8] G. Khoschsorur, W. Erwa, J. Chromatogr. 799 (2004) 355–360.
[9] J.O. Svensson, C. Brasyystrom, J. Save, Ther. Drug Monit. 21 (1999) 322–324.