

QUANTIFICATION OF UNBOUND MYCOPHENOLIC ACID BY HPLC-ATMOSPHERIC PRESSURE CHEMICAL IONISATION-TANDEM MASS SPECTROMETRY

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Mycophenolic acid (320.4 Da) is an immunosuppressant drug that is highly bound to human serum albumin. To facilitate the investigation of free (i.e. unbound) mycophenolic acid concentrations, we developed a HPLC tandem mass spectrometry method using indomethacin as an internal standard.

Free drug was isolated from plasma samples (500 μ l) using the ultrafiltration conditions reported by Shaw and Nowak [1]. The resultant ultrafiltrate (200 μ l), containing internal standard, was extracted using C18 solid phase cartridges (100 mg, Waters). Chromatography was performed on a Novapak C18 column (150 x 2.1 mm I.D., 4 μ m, Waters) operated at 35°C. The mobile phase consisted of 55% methanol:45% ammonium formate buffer (2 mM, pH 3.8) pumped at a flow rate of 0.5 ml/min. Detection was by selected reactant monitoring of mycophenolic acid (m/z 318.9_190.9) and the internal standard (m/z 356.0_297.1). The atmospheric pressure chemical ionisation interface was operated at 500°C in negative ionisation mode. The deprotonated species, [M-H]⁻ were the predominant precursor ions of the analytes produced under these conditions. The energy for collision induced fragmentation was -21.3 V. The discharge current and orifice potential were set a -2.5 μ A and -40 V, respectively.

The method was found to be linear over the range investigated, 2.5 to 200 μ g/l ($r > 0.990$, $n = 6$). The relative recovery of the method for the control samples studied (7.5, 40.0 and 150 μ g/l) ranged from 95% to 104%. The imprecision of the method, expressed in terms of intra- and inter-day coefficients of variation, was < 8% and < 9%, respectively. Further, analysis of pooled patient plasma produced an intra-day imprecision of 6.6%. The signal to noise ratio at the limit of quantification (2.5 μ g/l) was approximately 5:1. The mean absolute recovery ($n = 6$) of mycophenolic acid and the internal standard were $76.0 \pm 13.5\%$ and $86.0 \pm 9.1\%$, respectively.

The method reported provides an accurate and precise quantification of free mycophenolic acid over a wide analytical range and thus can be used for routine monitoring and pharmacokinetic studies. Further, this method shows the application of HPLC-mass spectrometry in the investigation of free drug pharmacokinetics.

(1) I. Nowak and L.M. Shaw, Clin. Chem. 41 (1995) 1011.
