HPLC ANALYSIS OF SULPHASALAZINE AND ITS METABOLITES

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Sulphasalazine is one of the drugs of first choice in the treatment of Crohn's disease and ulcerative colitis. A small fraction (10-30%) of orally ingested sulphasalazine is absorbed but the remainder reaches the colon intact where it is metabolised by bacterial azo-reduction to sulphapyridine and 5-aminosalicylic acid. Sulphapyridine and to a lesser extent 5-aminosalicylic acid are absorbed from the colon and undergo further metabolism: predominantly acetylation. The acetylation of sulphapyridine is under genetic control, the human population being approximately evenly distributed between fast and slow acetylators. In general, slow acetylators of suphapyridine face a greater risk of toxicity from sulphapyridine due to higher blood concentrations of free sulphapyridine.

Classically sulphasalazine is assayed spectrophotometrically, sulphapyridine and its metabolites are assayed using the Bratton-Marshall reaction, whilst 5-aminosalicylic acid and its metabolites are assayed spectrofluorimetrically. HPLC assays for sulphapyridine and its metabolites (Fischer & Klotz 1978) and 5-aminosalicylic acid and its metabolites (Fischer & Klotz 1979) have recently been described. The purpose of the present work was to develop a rapid, sensitive HPLC assay that would measure simultaneously all of sulphasalazine's major metabolites.

The assay developed was in the reverse phase mode using a short alkyl chain bonded silica packing (Hypersil-5µm SAS; 10 cm x 5 mm column). The mobile phase consisted of a mixture of methanol and 0.05M pH 7.4 phosphate buffer (15 : 85). At pH 7.4, 5-aminosalicylic acid and its N-acetylated metabolite are ionized and so it was necessary to use a pairing ion, tetrabutyl ammonium (0.15%), in order to retain the salicylates on the column. The pairing ion had a negligible effect on sulphapyridine and its acetylated metabolite. Due to the low concentrations of 5-aminosalicylic acid observed in plasma, fluorimetric detection was required and as sulphapyridine does not fluoresce at pH 7.4 it was necessary to couple a spectrophotometric detector (wavelength 260 nm) and a fluorimetric detector (excitation 310 nm; emission 410 nm) in series. Sulphasalazine is far more lipophilic than any of its metabolites and consequently it was not possible to chromatograph it isocratically using the conditions described above. Initial experiments using a much higher organic content proved more promising.

Using a flow rate of 1 ml. min^{-1} the observed retention times were: 5-aminosalicylic acid - 3.5 min; sulphapyridine - 5 min; N-acetyl-5-aminosalicylic acid - 8 min; N-acetylsulphapyridine - 11.5 min. The absolute sensitivities for these compounds based on a 10 µl injection, UV detection and using salicylic acid as an internal standard were: sulphapyridine - 0.05 $mg.l^{-1}$; N-acetylsulphapyridine - 0.4 $mg.l^{-1}$; 5-aminosalicylic acid - 0.3 $mg.l^{-1}$; N-acetyl-5-aminosalicylic acid - 0.3 $mg.l^{-1}$. With fluorimetric detection the sensitivities for 5-aminosalicylic acid and its acetylated metabolite were lowered to 0.05 $mg.l^{-1}$ and 0.06 $mg.l^{-1}$ respectively. Based on these sensitivities, plasma, after protein precipitation with acetonitrile, could be injected directly onto the column leading to a rapid and sensitive assay capable of monitoring the plasma concentrations of sulphasalazine metabolites seen in the normal therapeutic range.

Fischer, C., Klotz, U. (1978) J. Chromatog. 146: 157-162
Fischer, C., Klotz, U. (1979) Ibid. 162: 237-243