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## Letter to the Editor

# Improvements to a high-performance liquid chromatographic assay for allopurinol and oxipurinol in plasma

Sir,

A recent publication from this laboratory [1] presented a simple and sensitive high-performance liquid chromatographic (HPLC) assay for allopurinol and oxipurinol in plasma. Additional experience with this assay obtained during a pharmacokinetic study [2] has led to two improvements which increase column life and sensitivity.

In the original method [1], 0.5 ml of plasma was mixed with 0.1 ml of internal standard and 0.2 ml 20% trichloroacetic acid (TCA), centrifuged, and 50 $\mu$l of the supernate injected onto a reversed-phase column eluted with a phosphate buffer (pH 6, 0.05 *M*). Due to the extreme acidity of TCA, the pH of the injected sample is very low and it was found that even though a very small volume was injected, column deterioration was occurring. To eliminate the problems, plasma samples are now precipitated using an excess of ammonium sulfate (AS) granules, with all other steps the same. This yields a supernate with a fairly neutral pH (ca. 6), well within the pH tolerance limits of the column. Sensitivity and recovery are the same as with the TCA method.

For some pharmacokinetic studies, increased sensitivity was required. Extraction of the supernate from the AS precipitation with two volumes (5 ml) of diethyl ether—propanol (6:1), combination of the organic phases, evaporation to dryness, reconstitution with 50 $\mu$l of the mobile phase and injection of 40—50 $\mu$l increased the sensitivity by a factor of 10 over that originally reported. Extraction with diethyl ether—propanol (6:1) had been suggested for fluorouracil [3]; although other ratios were tried, this combination yielded the highest and most consistent recoveries.

The two alterations to the procedure suggested above provide significant improvements to the assay. It is hoped that these will prove useful to others.

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