DETERMINATION OF SIX NON-STEROIDAL ANTI-INFLAMMATORY AGENTS IN PLASMA AND URINE BY HPLC

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The determination of anti-inflammatory agents in plasma and urine assists in the establishment of dosage levels for new treatments and can also provide checks on patient compliance. From the range of anti-inflammatory agents currently in use at the Royal National Hospital for Rheumatic Diseases, Bath, U.K., we report on the determination of five established agents and one at present on clinical trial, as listed in the Table. In plasma, non-steroidal anti-inflammatory agents occur both free and bound to plasma protein, from which they may be released by precipitation of the protein using hydrochloric acid followed by centrifugation. In urine, non-steroidal anti-inflammatory agents occur free but mainly as their glucuronides, from which they may be hydrolysed either by refluxing in hydrochloric acid for 30 minutes or by incubation with β-glucuronidase in acetate buffer, pH 7.5 at 37°C for at least 12 hours.

A rapid and selective method for the determination of free and conjugated drug levels is provided by reverse phase hplc, using short (50 x 5 mm) columns packed with Spherisorb-5 ODS and a mobile phase of aqueous methanol at pH3 to suppress solute ionization. Injection of diluted urine, protein-free plasma, or ether extracts of plasma means minimal preparation of samples is required. Analysis times are less than 5 minutes.

Table. hplc CONDITIONS

	%methanol	UV λ_{nm}	internal standard	detection limit, ng.
Fenoprofen	40	272	indomethacin	25
Indomethacin	40	260	phenylbutazone	5.0
Ketoprofen	35	260	oxyphenbutazone	2.5
Naproxen	35	245	oxyphenbutazone	2.5
Phenylbutazone	40	254	indomethacin	5.0
Benoxaprofen	45	300	indomethacin	2.5

Detector response is linear over the concentration range 2.0 - 80μg ml^{-1}, determined by peak height measurement in the presence of an internal standard. Correlation coefficients of calibration graphs are greater than 0.995 with limits of error of less than ± 0.08 at 95% Confidence level. The percentage of added drug recovered from plasma or urine is greater than 95% in each case. The method is simple, rapid and more sensitive than previous methods published for fenoprofen (Nash, Bopp and Rubin, 1971), indomethacin (Skellern and Salole 1975), ketoprofen (Ballerini, Cambi and Del Soldato, 1977), naproxen (Thompson and Colins, 1973), phenylbutazone (Pound and Sears, 1975) and benoxaprofen (Chatfield and Woodage, in press).

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