Waters
Lab Highlights
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NOVA-PAK™ C_{18} RADIAL-PAK™ CARTRIDGE FOR DIRECT INJECTION ANALYSIS OF TIAPROFENIC ACID AND ITS METABOLITES IN PLASMA AND URINE

Tiaprofenic acid is a new non-steroidal, anti-inflammatory drug (NSAID) presently under clinical trials. Despite its relatively short half-life, tiaprofenic acid appears to be as effective as other NSAID's when given in 200 mg doses three times a day.

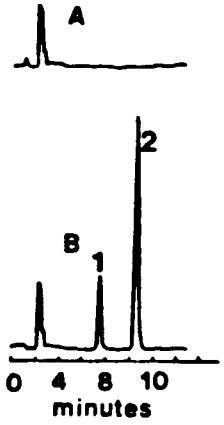
Jamali, et al. (1) have developed a rapid and sensitive method for the analysis of tiaprofenic acid and its metabolites in plasma and urine. Direct injections were made on a NOVA-PAK™ C_{18} Radial-PAK™ cartridge with quantifiable results to 0.5 µg/ml of tiaprofenic acid in plasma and urine.

The authors (1) used a relatively simple and rapid sample preparation. To 100-200 µl of plasma or urine, 50 µl of water or 50 µl 1M sodium hydroxide were added, as well as 50 µl of an internal standard (0.5 mg/ml naproxen in methanol) and 300 µl acetonitrile. The samples were vortexed, centrifuged at high speed for 2 minutes, filtered, and transferred to WISP™ limited volume inserts. Injections of 10-200 µl were made depending upon concentrations.

Experimental equipment consisted of an HPLC system from Waters Scientific, Mississauga, Canada. A Model 6000A pump, WISP™ Autosampler, Model 481 Variable Wavelength UV Detector, a Model 730 Data Module, and a 10 cm NOVA-PAK™ C_{18} Radial-PAK™ cartridge. A 5 cm guard column packed with 10 µm C_{18} preceded the analytical column. The mobile phase consisted of a 57:40:3 mixture of methanol:water:acetic acid at a flow rate of 1.5 ml/min. For quantitation, plasma samples were monitored at 315 nm and urine samples at 254 nm.

Blank plasma samples showed none of the metabolites to be present (Figure 1). Tiaprofenic acid and the internal standard eluted at 7.8 and 10.9 minutes, respectively.

FIGURE 1



Blank plasma (A) and plasma of patient (B) 8h after 200 mg dose of tiaprofenic acid Peak 1. Peak 2 is the internal standard.

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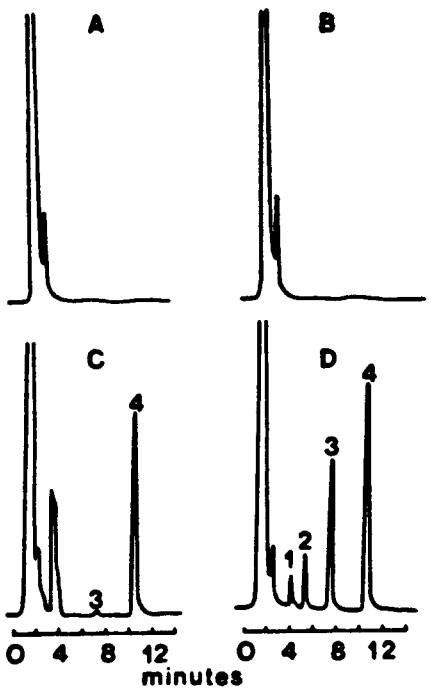
Urine samples presented a different picture. What appear to be ester conjugates of tiaprofenic acid and its metabolites may be eluting as a cluster of peaks at 3 to 4 minutes. Alkaline hydrolysis yielded the intact drug and metabolites (Figure 2).

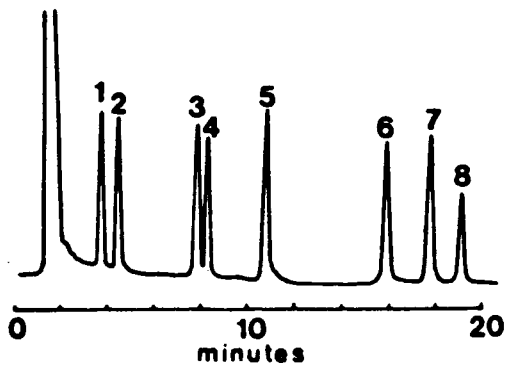
The authors also chromatographed other NSAID's on their system resulting in the ability to quantitate tiaprofenic acid and its metabolites in the presence of these other NSAID's (Figure 3).

FIGURE 2



Blank urine samples before (A) and after (B) alkaline hydrolysis. Patient urine samples 8h after a 400 mg dose before (C) and after (D) alkaline hydrolysis. Peaks 1, 2, 3 and 4 are reduced and oxidized metabolites, tiaprofenic acid, and internal standard, respectively.

FIGURE 3



Blank plasma with salicylic acid (1), piroxicam (2), tiaprofenic acid (3), ketoprofen (4), naproxen (5), fenprofen (6), flurbiprofen (7), and etodolac (8).

No changes in retention times throughout the experiment indicated to the authors a long, useful column lifetime.

The article is very comprehensive in its study and is recommended as a reference for this procedure.

FOR INVESTIGATIONAL USE ONLY.
THE PERFORMANCE CHARACTERISTIC FOR THIS
PROCEDURE HAS NOT BEEN ESTABLISHED.

1. Jamali, F., Russell, A., Berry, B. and Lehmann, C. J. Chromatogr. 310 (1984) 327-333.