

NOTE NO. 48 - Dec 2 1994

SPS DETERMINATION OF ANTIPYRINE IN HUMAN SERUM

In this Note, the usual review (TECHNICAL) is followed by a novel exchange (QUESTIONS AND ANSWERS). Among the exchange subjects:

On grounds of cost, sensitivity, and analytical speed, is capillary electrophoresis (CE) a sure and obvious replacement for HPLC?

Aside from the CE comparison, was the RAM sensitivity adequate? At all limiting?

On grounds of cost, selectivity, reproducibility, and analytical speed, how does SPS stack up?

TECHNICAL

This technical section of this Note refers to a study by B. J. Gurley et al. (1, 2).

Analytes:

Antipyrine (AP), acetaminophen (APAP).

Also tested but negated as possible interferents:

antipyrine metabolites:

4-hydroxyantipyrine, norantipyrine (not detected in human serum, thus confirming that these are eliminated faster than formed [3,4]);

drugs:

adenosine, aminopyrine, atropine, butorphanol, gentamicin, indomethacin, penicillin, pentobarbital, phenacin.

Sample Matrix: Human serum

Sample Size: 25 μ L

SPS-C8 Columns: Guard cartridge: 5 micron, 1 cm x 3 mm ID

Regis Product Number: 785408

Analytical column: 5 micron, 25 cm x 4.6 mm ID

Regis Product Number: 785208

Mobile Phase: 99% 0.1M KH_2PO_4 and K_2HPO_4 (pH 7.4), 0.5% Acetonitrile, 0.5% Tetrahydrofuran

Flow Rate: 1.0 mL/min, 37° C

Detection: 244 nm

Retentions: AP, 8.5 min; APAP, 11.6 min.

Over the whole 0.25 to 20 μ g/mL serum concentration range, absolute AP recovery was $99.7 \pm 0.82\%$ and the analysis was linear. To avoid peak asymmetry and concurrent small increase in back pressure, guard columns were replaced at

200 injections. As illustrated in Figures 1 and 2, the method is fast and unique, requiring less than 13 minutes turnaround time and no sample preparation. [Reported only in this Note: An endogenous but AP-interfering peak from dog serum was eliminated by changing buffer strength from 0.1 M to 0.04 M.] The study demonstrates that the method can be conveniently used for pharmacokinetic monitoring of AP.

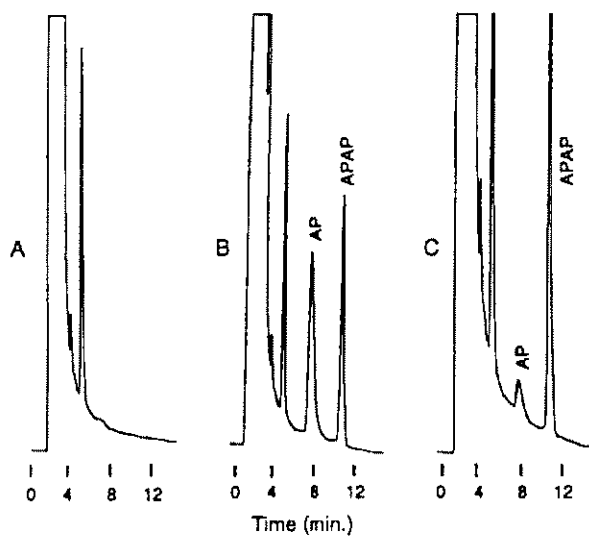


Figure 1. Typical study chromatograms. Detector sensitivity for chromatogram C was 4 times that for A and B (AUFS, A, B: 0.01; C: 0.04). Chromatograms A: Blank human serum; B: 5 µg AP and APAP, each/mL serum; C: 0.5 µg AP/mL serum, 5 µg APAP/mL serum. AP: antipyrine; APAP: acetaminophen.

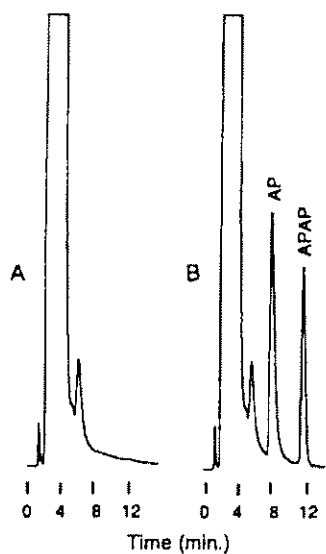


Figure 2. Chromatograms of serum samples from a 100-kg human at 0 hours (A) and 12 hours (B) after having received a 10 mg/kg dose of AP. AP: antipyrine; APAP: acetaminophen.

QUESTIONS AND ANSWERS

Question 1 (Dr. Perry to Dr. Gurley): (a) Could you expand more on your choice of RAM over capillary electrophoresis (HPCE)? (b) What nominal elution time is offered by HPCE? (c) What sensitivity?

Response 1 (Dr Gurley): (a) Our reason for choosing RAM HPLC technology over capillary electrophoresis was primarily one of cost. Commercially available capillary electrophoresis instrumentation is substantially more expensive than a typical isocratic HPLC configuration. Our laboratory was not equipped with a capillary electrophoresis instrument, however, we did have several HPLCs; consequently, it was economically more feasible to procure and adapt an SPS-C8 HPLC column than to purchase a capillary electrophoresis apparatus. I'm sure that many other

laboratories, especially those in university settings, are in a similar predicament. From a budgetary standpoint, it is much easier to adapt a \$600.00 RAM column to existing HPLC instrumentation than it is to purchase a \$40,000.00 capillary electrophoresis instrument. (b) The electrophoresis migration time for antipyrine as reported by Brunner et al (J. Chromatog. **1993**, 622, 98-102) was 7.2 minutes. This value was only 0.5 minutes earlier than the elution time achieved by our RAM method. (c) The sensitivity reported by Brunner et al was 0.5 μ g/mL. Their absolute recovery for AP at 0.5 μ g/mL was 89%. Our sensitivity was 0.25 μ g/mL and the absolute recovery at this concentration approached 100%.

Perry, Question 2: (a) For the AP determination, what sensitivity is desirable? Is the RAM HPLC approach at all limiting in this regard, or is the quoted sensitivity more than adequate?

Gurley, Response 2: (a) A sensitivity of 0.25 μ g/mL is more than desirable for both human and animal studies. Typical pharmacokinetic studies of AP in humans using a 10 mg/kg dose require sampling over 24 hours in order to establish the terminal linear phase of the concentration-time profile. At the end of 24 hours AP concentrations are usually well above 1.0 μ g/mL. In dogs, AP is eliminated more rapidly and concentrations are typically less than 0.5 μ g/mL after 8 hours; however, this is not a major concern because AP pharmacokinetics are best described by a one compartment model with first order elimination. As such, AP concentrations obtained beyond 8 hours in dogs provide no additional information to the pharmacokinetic profile. (b) The sensitivity of our RAM method is not a limitation. If anything it is an advantage, because not only is our RAM method more sensitive than most previously reported AP HPLC methods, it requires no sample pretreatment.

Perry, Question 3: (a) Why was 244 nm chosen for detection? (b) In setting up the method, was the AP/serum response measured as a function of wavelength?

Gurley, Response 3: (a & b) 244 was chosen as the wavelength for detection because AP exhibits a U.V. absorption maximum at this wavelength.

Perry, Question 4: Was there any interest felt or effort expended in making the analysis faster?

Gurley, Response 4: An effort was expended in making the analysis faster. The end result was the chromatographic conditions described in the method. Organic modifier (acetonitrile and THF) concentrations greater than 1.0% reduced the column's capacity for AP such that AP eluted with the serum front. Given the relative weakness of this mobile phase we decreased analysis time by heating the column. Optimal analysis time and peak symmetry were achieved by pumping a mobile phase of potassium phosphate buffer (0.1M, pH 7.4), acetonitrile and tetrahydrofuran (99.0:0.5:0.5, v/v/v) through a 25 cm SPS-C8 column heated to 37° C. We also found that the SPS-C8 column provided better efficiency and capacity for AP than either an SPS-C8 or GFF-II ISRP column.

Perry, Question 5: Was the main 25 cm RAM column ever replaced?

Gurley, Response 5: All validation studies were performed on the same column. The column was cleaned after 400 injections per instructions provided in the care and use pamphlet. After 400 injections AP and APAP peaks became less symmetrical. Cleaning the column restored peak symmetry without affecting retention. Since submitting the manuscript, we have installed a new SPS-C8 column. Under the same chromatographic conditions, this replacement column produced chromatograms identical to those depicted in the manuscript.

References: (1) **DETERMINATION OF ANTIPYRINE IN HUMAN SERUM BY DIRECT INJECTION RESTRICTED ACCESS MEDIA LIQUID CHROMATOGRAPHY.** Gurley, B. J.; Zermatten, S.; Skelton, D. J. Pharm. Biomed. Anal., in press.

(2) **RAPID AND SENSITIVE DETERMINATION OF ANTIPYRINE IN HUMAN SERUM VIA RESTRICTED ACCESS MEDIA HPLC.** Presented at the Annual Meeting of the American Association of Pharmaceutical Scientists, San Diego, CA, Nov. 1994. Gurley, B.; Zermatten, S. For abstract, see J. Pharm. Res. **1994** (Oct. Suppl.), 11, S-24: APQ 1055.

(3) Brodie, B. B.; Axelrod, J. *Pharmacol.* **1950**, 98, 97-104.

(4) St. Peter, J. V.; Abul-Hajj, Y.; Awni, W. M. *Pharm. Res.* **1991**, 8, 1470-1476.

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