Waters
Lab Highlights

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SPEED, RESOLUTION, AND SENSITIVITY MAKE RESOLVE™ C_{18} RADIAL COMPRESSION CARTRIDGES IDEAL FOR NUCLEOSIDE QUANTITATION IN SERUM AND URINE

"Because of improved resolution, speed, and sensitivity, the method using [a]Radial-Pak™ cartridge is the method of choice for determining modified nucleosides from urine and sera and is routinely used in our laboratory (1)."

This is one of the comments recently reported by several researchers who adapted a μBondapak™ C_{18} steel column method to radial compression technology using a 5μ Radial-Pak™ cartridge with Resolve™ packing material.

Patients with cancer excrete elevated urine levels of modified nucleosides, most of which stem from the breakdown of transfer RNA. A possible mechanism of the origin of these elevated nucleoside levels in urine has been observed by finding high rates of turnover of transfer RNA in tumor tissues. A number of investigators are exploring the usefulness of the determination of nucleoside markers in the urine as a non-invasive method for determining pre-cancerous conditions.

Prior work in this area has been characterized by more complicated, ternary gradients and analysis time as long as 2-5 hours. In an attempt to improve this situation, several columns and buffer systems were evaluated. Sample preparation and isolation consisted of extraction under controlled pH conditions, centrifugation, affinity chromatography on a phenylboronate column, and lyophilization. A μBondapak™ C_{18} stainless steel column was found to be superior to Supelco C_{18} DB (5μm), and Nova-Pak™ C_{18} (5μm) columns or cartridges for these applications. Several nucleosides were clearly resolved on μBondapak™ C_{18} columns in 100 minutes by applying a linear gradient of 1-15% methanol in 0.01M phosphate (pH 5.1). However, a second isocratic run was required to resolve two additional nucleosides of interest.

A more rapid and complete separation of all of the nucleosides of interest was accomplished in a single 30 minute run using the Radial-Pak™ C_{18} cartridge, as shown in Figure 1. The reliability of the Radial-Pak™ cartridge method was compared with the separation accomplished on the μBondapak™ C_{18} column by determining the nucleoside content of twelve specimens of normal adult male urine by both methods. An excellent correlation ($r > 0.995$) was obtained in less than 1/3 the time. Detection limits as low as 5 picomoles were reported.

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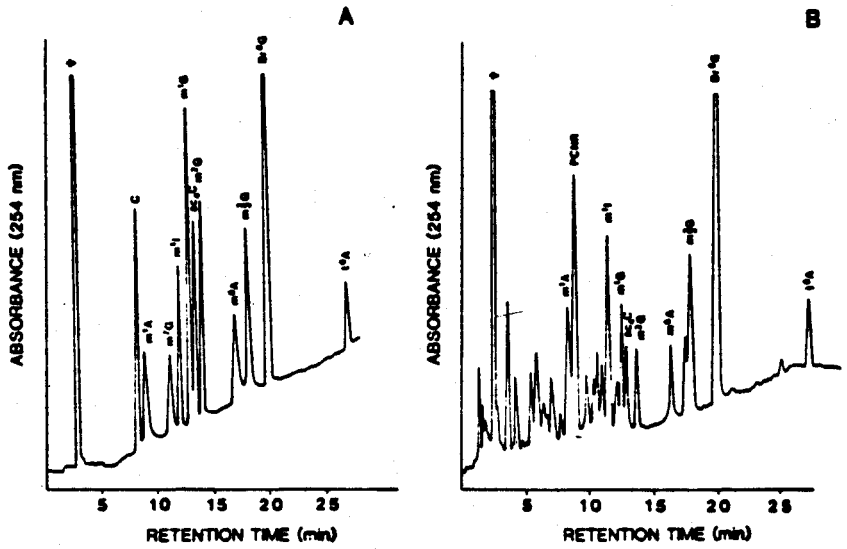

Figure 1: Reverse-phase HPLC separation of modified nucleosides on a Radial-Pak™ cartridge using Resolve™ C_{18} packing material. (A) Standard nucleoside mixture consisting of 100 pmole of each nucleoside; (B) 4µl urine sample from a cancer patient. Peak identification and quantitation as outlined in reference (1). A 30-minute linear gradient from 100% Buffer A (0.01 M phosphate, pH 3.0, 2 x 10^{-5} M dibutylamine, and 5% methanol) to 100% Buffer B (Buffer A containing 30% methanol) at 2ml/min was used.

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THE PERFORMANCE CHARACTERISTIC FOR THIS PROCEDURE HAS NOT BEEN ESTABLISHED.

In addition to the urine samples, sera samples were also analyzed. The authors concluded that the frequency of elevation of modified nucleosides was lower in serum than in the urine of the same cancer patient, indicating that determinations in urine is a more reliable indicator of malignancy.

Increased sensitivity, and resolution of all compounds of interest in one third the previously reported analysis time should indeed make this the method of choice in this area.

1. Apell, G., Buschman, F. L., and Sharma, O. K., *J. Chromatogr.*, 374 , 149 (1986).