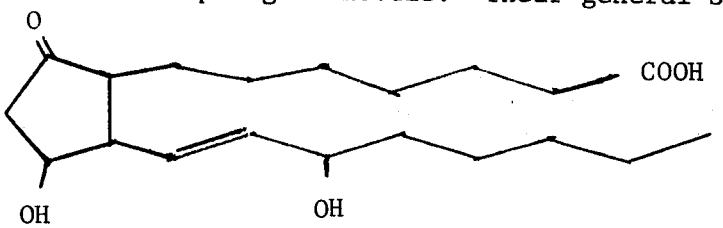
Waters
Lab Highlights
An Internal Communication of Applications and Techniques
LAH 0102 2/83

LITERATURE CORNER

μBONDAPAK™ COLUMNS FOR THE ANALYSIS OF PROSTAGLANDINS AT PHYSIOLOGICAL LEVELS

Prostaglandins are extremely potent and important biological hormones found in blood and tissue at picogram levels. Their general structure is



Currently, prostaglandins are quantitated using GC/MS, radioimmunoassay, or bioassay. These procedures have major drawbacks - selectivity, sample size, recovery, and expense. None of these methods are adequate for multiple prostaglandin profiles where the type and amount are important in the understanding of smooth muscle stimulation (induction of labor, abortion, menstruation; dilation of blood vessels and effects on blood pressure; and inhibition of blood clotting).

Recently, Watkins and Petersen from Mass. General Hospital developed a quantitative LC method for several prostaglandins (1). Their method involves esterification of the carboxylic acid group with panacyl bromide (2) to form a highly UV absorbing and highly fluorescent derivative.

The derivatized sample is prepared using a silica SEP-PAK® cartridge with prostaglandin recovery greater than 90%. The derivatized prostaglandins are separated on a μBONDAPAK™ C_{18} or μBONDAPAK™ Fatty Acid column using a mobile phase of acetonitrile and 0.1% acetic acid in an isocratic or gradient mode. Figure 1 shows a representative chromatogram of 6 prostaglandins.

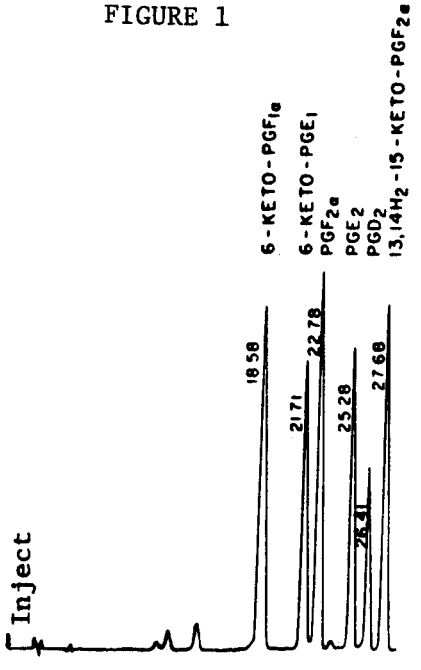
The derivatized prostaglandins are detected by UV at 254 nm with a detection limit of ≃ 140 picograms at 0.005 AUFS. By using a Schoeffel FS-970 Fluorometer, a detection limit of ≃ 60 picograms can be attained easily with 249 nm excitation/413 nm mission at a sensitivity of 0.1 μA. Figure 2 shows a comparison of the detectors and their linearity.

Table 1 shows they found no significant difference between radioimmunoassay and the new LC method. They also demonstrated the feasibility of the derivatization, sample prep, and chromatography for analysis of prostaglandins in cell tissue cultures.

Jim Krol

1. W. D. Watkins and M. B. Petersen, Anal. Biochem., 125, (1982) 30-40.
2. Source for panacyl bromide soon to be available from Polysciences, Inc. 215/343-6484

FIGURE 1



HPLC of 6 prostaglandin standards on μBONDAPAK™ Fatty Acid Column.

Conditions are:

Mobile Phase: A = 0.1% Acetic Acid
B = Acetonitrile

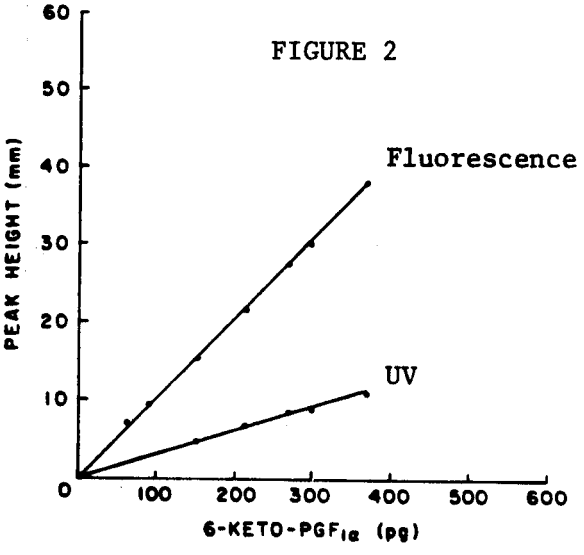
56% B to 65% B; Curve 6 (linear) for 15 mins; then held at final conditions for 15 mins.

Flow Rate: 1.2 ml/min

Detection: 254 nm

UV vs. Fluorescence Detection. UV at 54 nm at 0.005 AUFS, Fluorescence at 249 nm excitation/ 413 nm emission at sensitivity of 0.1 μA.
Linear correlation coefficient > .99 for both methods of detection.

FIGURE 2



Sample	HPLC (μg 6-keto-$PGF_{1\alpha}$)	RIA (μg 6-keto-$PGF_{1\alpha}$)
1	2.50	2.24
2	3.17	2.98
3	2.50	2.95
4	2.50	2.46
	2.67 ± 0.34	2.66 ± 0.37

TABLE 1: Each value given as mean ± 1 standard deviation for 2 determinations. Approximately 500-1500 pg of 6-keto-$PGF_{1\alpha}$ was assayed by each method and the derived value corrected to total initial volume.