Waters
Lab Highlights
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OXALIC ACID IN URINE

Oxalic acid is an end product of mammalian carbohydrate and protein metabolism. It is produced in the liver and is transferred to the kidneys, where, under some conditions, it can form kidney stones (which are mainly composed of calcium oxalate).

Oxalic is mildly toxic to humans and is excreted by normal humans in small amounts. It occurs naturally in a number of plants such as spinach, rhubarb and tomatoes.

Occasionally people suffer from a disease called Hyperoxaluria which is believed to be caused by a lack of specific enzymes. This leads to excessive amounts of oxalic acid being produced and deposited in the kidneys. The victims excrete excessive quantities of oxalic, glyceric, and glycolic acid in their urine.

In two recent papers, Hughes et al. (1) and Murray et al. (2) have published a sensitive and specific method for measuring oxalic acid in urine using HPLC.

In this method, oxalic acid in the urine is reacted with o-phenylene diamine to form the derivative 2,3-dehydroxyquinoxaline (DHQX). This derivative has strong UV absorbtivity and strong fluorescence. Although fluorescence detection is more sensitive, the signals obtained by UV detection (using a 440 detector at 313 nm) give more than adequate sensitivity.

Murray et al. studied 19 potentially interfering urinary constituents and concluded that the other compounds tested contributed only 5% or less to the oxalate assay. Urine samples were spiked with oxalate at three levels. They then carried out 13 tests which gave a mean recovery of 106% with a coefficient of variation of 13.9%. Hughes et al. reported recoveries of $100.2 \pm 6.65\%$ (mean and S.D.).

Recently, Oscar Lazo of the Catholic University, Santiago, Chile, visited Waters headquarters to check out this method. Initial feasibility had been shown by work done by Sara Abelaira at a Waters representative in Argentina, Lab Rodriguez Corswant.

Oscar Lazo found that the recommended reaction temperature for the derivatization (130°C) could be modified to a more practical temperature (90°C).The results of his reaction time study are shown in Figure 1. Oscar also adapted the chromatography to suit a μBondapak™ C_{18} column (Fig. 2) and demonstrated, by spiking samples, that excellent recoveries and linearities were obtained.

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FIGURE 1

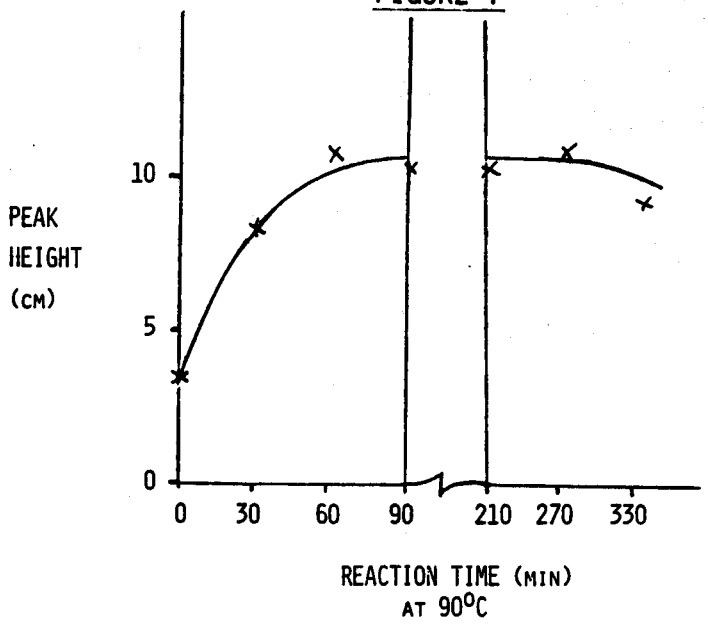
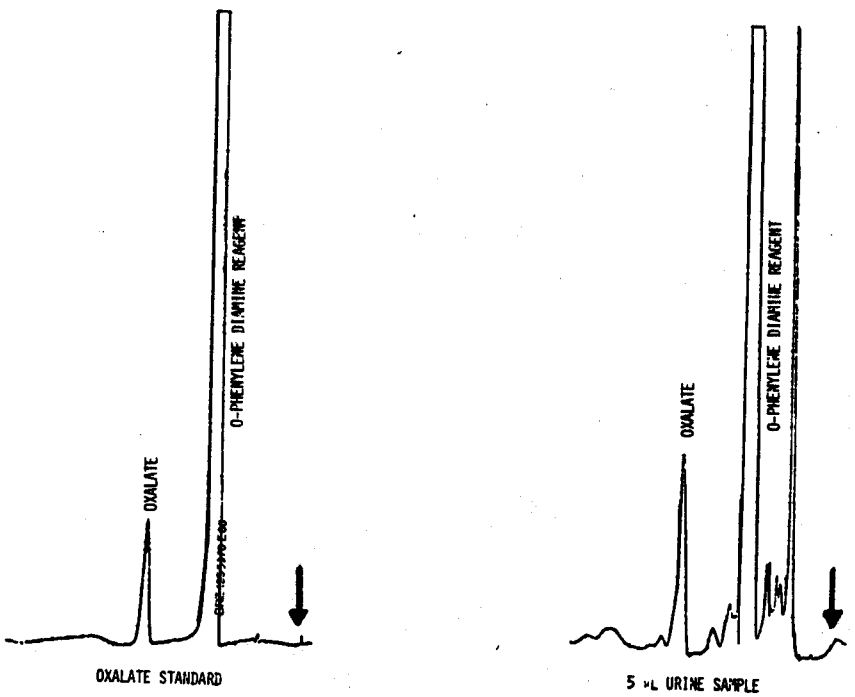


FIGURE 2

CONDITIONS

Column:	μBONDAPAK™ C_{18} s/s 3.9 x 300 mm
Mobile Phase:	20:Methanol
	80: 0.1 M ammonium Acetate
Flow Rate:	1 ml/min.
Detector:	M440 at 313 nm 0.05 AUFS



1. Hughes, H., Hagen, L. and Sutton, R.A.L. Anal. Biochem. 119, 1-3 (1982).
2. Murray, J.F., Nolen, H.W., Gordon, G.R. and Peters, J.H. Anal. Biochem. 121, 301-309 (1982).