

# Urinary riboflavin determination by C18 reversed-phase open-column chromatography.

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## Abstract

Reverse phase open column chromatography was used to determine urinary riboflavin. An aliquot of a urine specimen was added directly onto the reverse phase open column. The riboflavin was collected and measured as the compound was eluted. The mobile phase (MeOH/H<sub>2</sub>O/HOAc 37/63/0.1 v/v/v) and stationary phase (C18, 50 microns) were used. The method shows good reproducibility. Recovery of added Vit.B2 was 98.89-100%. The coefficient variation of reproducibility of within day was 1.53%. The coefficients of variation of reproducibility of day-to-day were 0.39% and 1.74% for low and high concentration samples, respectively. The reverse phase open column chromatography was compared to the traditional fluorometric method and HPLC method for riboflavin. The coefficient of linear regression of these comparisons are  $Y = 0.331 + 1.010X$  (n = 37, r = 0.984) and  $Y' = -0.004 + 1.036X'$  (n = 15, r = 0.999) for RP open column vs. traditional method and RP open vs. HPLC, respectively, where X or X' is the reverse phase open column chromatography method and Y or Y' is the traditional fluorometric method or HPLC, respectively. Concentrations of riboflavin as low as 0.05 micrograms/ml were measured with a linear relation of response to concentration to at least 10 micrograms/ml.