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# Determination of serum neopterin levels with two different HPLC methods

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This study aimed to compare serum neopterin levels in different HPLC methods

We used samples from serum pool which accepted to our routine biochemistry laboratory. We used mobil phase containing KH2PO4 and K2HPO4;and another one containing ammonium phosphate. For deproteinization TCA and acetonitrile were used. In first procedure, sample was prepared with 100 uL 2M TCA + 400 uL of serum, second one; 100uL serum + ascorbate + 10uL 10uL of TCA (50%) and the third one was; 100uL serum + acetonitrile (100%).

With these procedures, total and reduced neopterin levels were measured in each group except the procedure with ascorbate. Ascorbate was used to determine the reduced form, because in acidic conditions, reduced form (7-8 dihydroxyneopterin) is oxidized and ascorbate prevents this oxidation. Acidic iodit (5.4% KI in 1 M HCl I2/10.8%) was used to determine total neopterin, because acidic iodit oxidizes the reduce form, and whole neopterin turns to oxidize form.

Excitation and emission wavelengths were 275 and 345 nm. Column was C18, 5µ, 4.6x150 mm, RP. Injection volume was 10 µL. Flow rate was 0.8 mL/min and run time was 10 min. Results were calculated according to calibration curve obtained by external standards.

Retention times were 2.9th minute with ammonium phosphate buffer and 5th minute with KH2PO4/ K2HPO4 buffer. Peak areas were aproximately 30% bigger in ammonium phosphate than KH2PO4 and K2HPO4 buffer. Also baseline of chromatogram was a little bit noisy with KH2PO4 and K2HPO4 buffer. Limit of detection for neopterinin and sample preperations did not differ between two methods.

The chromatogram obtained by using mobil phase containing ammonium phosphate buffer with either TCA or acetonitrile procedures was good enough. Ammonium phosphate buffer provided short the run time, less noisy baseline and bigger peak areas. So, more sample could be assayed in same time using ammonium phosphate buffer as mobil phase and better chromatogram.

## Biography

Dr.Erbil has comleted his residency in clinical chemistry at the age of 30 years from Gülhane University school of Medicine. He is the chair of Laboratory Medicine in the same University.He has published more than 100 papers in reputed journals.He published two books.

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