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Determination of several sugars in serum by high-performance anion-exchange chromatography with pulsed amperometric detection

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Abstract

In this paper, a sensitive, simple and direct method for simultaneous determination of glucose, ribose, isomaltose and maltose in serum sample by high-performance anion-exchange chromatography coupled with integrated pulsed amperometric detection was developed. The four target analytes were easily and completely separated on an anion-exchange column at a flow-rate of 0.25 mL/min by binary step gradient elution in about 16 min and the two eluents were deionized water and 500 mM sodium hydroxide, respectively. The separated four analytes were detected directly by using a gold electrode and quadruple-potential waveform integrated pulsed amperometry without derivatization. Under the optimized conditions, when the injection volume was 25 μL, the detection limits (signal-to-noise ratio equal to 3) for glucose, ribose, isomaltose and maltose were 0.92, 7.50, 12.9 and 10.3 ng/mL, respectively. The calibration graphs of peak area for the four analytes were linear over two to three orders of magnitude with correlation coefficients greater than 0.998. R.S.D. of peak areas of the four analytes for five determinations were no more than 5.6%. The analytical method had been applied to the determination of glucose, ribose, isomaltose and maltose in real serum samples and good results with low relative standard deviation not more than 5.3% were obtained. The accuracy of the proposed method was tested by recovery measurements on spiked samples and good recovery results (98.1–107.9%) were obtained.

Keywords: Sugar; Serum; High-performance anion-exchange chromatography; Pulsed amperometric detection

1. Introduction

Sugars are among the most abundant organic compounds in the biosphere and they play very important roles in many life science systems. So the determination of them is very important in life science, food science, medical science, and agricultural science. The analysis of some sugars in serum has long been essential for the diagnosis of certain disease states and the effects of certain drugs. For example, monitoring blood glucose concentration levels is helpful to the control and treatment of diabetes, and the colorimetric assay of blood glucose has become a routine test in medical treatment. Ribose is a component of RNA, DNA, ATP, and it is required for the biosynthesis of these important biomolecules. In many human being and animal tissues and body fluids including blood, endogenous ribose is available from glucose via the hexose monophosphate shunt, so it is very necessary to develop simple, sensitive and reliable method for the determination of ribose in the life and medical science research. In recent years, it is showed that the maltose can act as an effective component in intravenous injections for the diabetes patients and it can replace glucose in some cases in medical treatment practice. After injection, the maltose undergoes a series of metabolic process passing through glucose intermediate in human body, therefore, it is also very important to monitor the concentration levels of maltose and related other compounds in blood for the medical research and medical treatment practice. Of the various determination methods available nowadays for the above-mentioned sugars, although simple colorimetric assay based on enzyme reactions and various enzyme sensors are the most often used

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methods, these methods cannot simultaneously determine multiple compounds [1–7]. In some cases, for some special reasons, it is very essential to get confirmatory information by using more specific and confirmatory analytical technique such as chromatographic methods. HPLC with refractive index detection has been widely used in sugar analysis field, but is limited by poor sensitivity and specificity [8,9]. The application of HPLC coupled with postcolumn derivatization and UV–vis detection in sugar analysis is impaired by the complicated and time-consuming experimental process and poor sensitivity [10,11]. Therefore, sensitive, selective and simple analytical methods that are suitable to direct and simultaneous determination of multiple sugars are in great demand. Recently, because of its advantages of high sensitivity, simplicity, organic solvent freedom and no need derivatization, high-performance anion-exchange chromatography at acidity higher than pH 13 in conjunction with integrated pulsed amperometric detection has been applied to analyze carbohydrates, amino acids, some life related compounds and some antibiotics in many different kinds of samples [12–17]. But this relatively new analytical method only has a very limited application in the analysis of sugars in blood samples. For example, Cox et al. [18] developed a method for the determination of mannitol and lactulose in serum for the small intestinal permeability study. In the Dionex Application Update 125 obtained from dionex website (http://wwwl.dionex.com/), a method for the determination of glucose and xylose in serum was recommended. However, up to now, there is no report of direct and simultaneous determination of glucose, ribose and maltose in serum sample by high-performance anion-exchange chromatography (HPAEC) separation and pulsed amperometric detection (PAD).

In this paper, we present a sensitive, selective, simple and time-saving analytical method for direct and simultaneous determination of glucose, ribose, isomaltose and maltose in serum sample by HPAEC and PAD. (In view of the fact that maltose related reagents or products often contain isomaltose impurity, our research targets also include isomaltose.) We have developed the new gradient elution conditions for separation of the four target analytes. Under the optimized conditions, the four target analytes were easily and completely separated on an anion-exchange column at a flow-rate of 0.25 ml/min by binary step gradient elution in about 16 min, and then they were detected directly by using quadruple-potential waveform pulsed amperometry on a gold electrode without derivatization. The analytical method had been successfully used to the determination of glucose, ribose, isomaltose and maltose in real serum samples.

2. Experimental

2.1. Reagents

To prepare the standard solutions, sample solutions and the mobile phase, 18 MΩ purified water produced with a laboratory water purification system (Barnstead, IA, USA) was used throughout the experiments. D-Glucose, ribose, maltose, isomaltose and sodium azide were purchased from Sigma–Aldrich (St. Louis, MO, USA) and were used as received. The single component stock solutions of the four sugars with concentration of 1 mg/mL were prepared by dissolving suitable amount of sugar in 20 mg/L sodium azide diluent. The injected standard mixture solutions of the four sugars were made by diluting the four corresponding aliquots of single component stock solutions of sugars with an aqueous diluent containing 20 mg/L sodium azide. Guarantee grade reagent sodium hydroxide used to prepare mobile phase was purchased from Beijing Chemicals Corporation, Beijing, China. The 50% sodium hydroxide concentrated solution was prepared by dissolved suitable amount of solid sodium hydroxide into equal amounts of purified water. To precipitate as more amounts of sodium carbonate as possible before use, this solution was left undisturbed for at least 24 h. The separation of the four target analytes was obtained on an anion-exchange column by binary step gradient elution. Mobile phase I was 18 MΩ purified water vacuum filtered through a 0.2 μm nylon filter. Mobile phase II, a 500 mM sodium hydroxide solution, was prepared by diluting an aliquot of 50% sodium hydroxide concentrated solution. All of the mobile phases were kept under nitrogen to prevent contamination by atmospheric carbon dioxide.

2.2. Chromatographic conditions

The chromatography system used in our experiments was a DX-600 IC system (Dionex, Sunnyvale, CA, USA). This system consisted of a GS50 gradient pump with on-line degas, an AS50 thermal compartment with 25 μL injection loop, an AS50 autosampler, and an ED50 electrochemical detector equipped with a thin-layer type amperometric cell. The cell comprised a gold working electrode with about 1 mm diameter, a glass and Ag/AgCl combination reference electrode (Dionex) and a titanium counter electrode consisting of the cell body. The chromatographic separation of the four sugars was performed on an CarboPac PA10 analytical column (250 mm × 2 mm i.d. Dionex) and an CarboPac PA10 guard column (40 mm × 2 mm i.d. Dionex) at a flow-rate of 0.25 mL/min, both columns were packed with an identical microporous, polymeric anion-exchange material and they were installed in the thermal compartment at a controlled temperature of 30 °C. The sample injection volume was 25 μL. The gradient elution was performed with water and 500 mM sodium hydroxide mobile phases using a binary step gradient elution shown in Table 1. The four target sugars were detected directly by using quadruple-potential waveform pulsed amperometry without derivatization on a gold electrode and the detection conditions are given in Table 2. A personal computer equipped with a Chromeleon 6.5 chromatography software (Dionex) was used to acquire and process chromatographic data. Peak area was used as the analytical measurement.

Table 1
Gradient elution conditions for separation of glucose, ribose, isomaltose and maltose

Time (min)	Water (%)	500 mM NaOH (%)	Curve[a]
0.0	82.5	17.5	
10.0	82.5	17.5	
10.1	0.0	100.0	5
17.0	0.0	100.0	
17.1	82.5	17.5	5
30.0	82.5	17.5	

[a] Shapes of gradient curves are defined in the GS50 Gradient Pump Operator's Mannual, pp. 37–38 (Dionex Document No. 031612, Revision 2). Curve 5 is linear gradient.

Table 2
Detection waveform for glucose, ribose, isomaltose and maltose

Time (s)	Potential (V) vs. glass/Ag/AgCl combination electrode	Integration state
0.00	0.10	
0.20	0.10	Begin
0.40	0.10	End
0.41	−2.00	
0.42	−2.00	
0.43	0.60	
0.44	−0.10	
0.50	−0.10	

2.3. Preparation of sample solution

Serum sample was kindly provided by Shuanghuan Pharmaceutical Company. Before we got it, it had been undergone the following preparation procedure: after blood sample was collected from patients, to remove protein from the blood sample, 3 mL of acetonitrile was added in 1 mL of blood sample in a glass test tube. Following a mixing process on a vortex vibrator in about 2 min, this mixture was centrifuged at a speed of 6000 r/min for 8 min, and the supernatant serum was separated and filtered through a nylon membrane with pore size 0.25 μm, and then kept at 4 °C in refrigerator. Finally, the serum sample can be directly injected for analysis after appropriate dilution.

3. Results and discussion

3.1. Chromatographic conditions

Because of their weak acidity, carbohydrates including the four target sugars (glucose, ribose, isomaltose and maltose) can exist as anions under strong alkaline conditions, and therefore they can be retained and separated according to their pK_a and molecule weight by using anion-exchanger as stationary phase and using sodium hydroxide or sodium acetate solutions as mobile phase. In our preliminary test, we found that only by adopting 100 mM sodium hydroxide solution as isocratic elution mobile phase, the four sugars can be separated in about 30 min, the chromatogram is shown in Fig. 1. For simplicity, convenience and freedom of contamination from sodium acetate, we used water and sodium hydroxide solution as two eluents to conduct the separation of the four sugars by a binary gradient elution. In the four target sugars, glucose and ribose belong to monosaccharides, isomaltose and maltose belong to disaccharides. It is clear that the key problem to be solved in this separation system is that on the one hand we must guarantee the baseline separation of the four sugars especially glucose and ribose; on the other hand, we must make the entire separation time especially the retention time of maltose be as short as possible. To attain this goal, at first we used linear gradient elution, but we found that the experimental result was not good enough. First, if

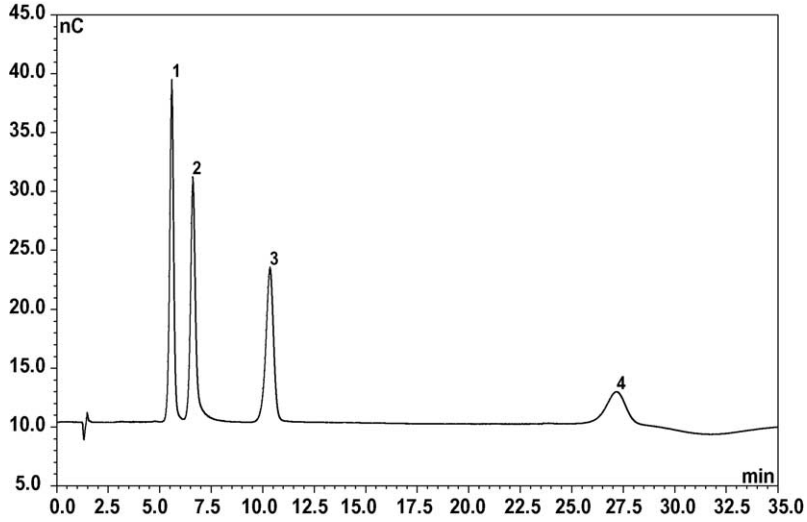

Fig. 1. Chromatogram of a standard mixture of four sugars obtained with isocratic elution. Chromatographic conditions: isocratic elution mobile phase is 100 mM sodium hydroxide solution; detection waveform is shown in Table 2; flow-rate of mobile phase is 0.25 mL/min; column temperature is 30 °C; injection volume is 10 μL and analyte concentration is 2.0 μg/mL. Peak: (1) glucose; (2) ribose; (3) isomaltose and (4) maltose.

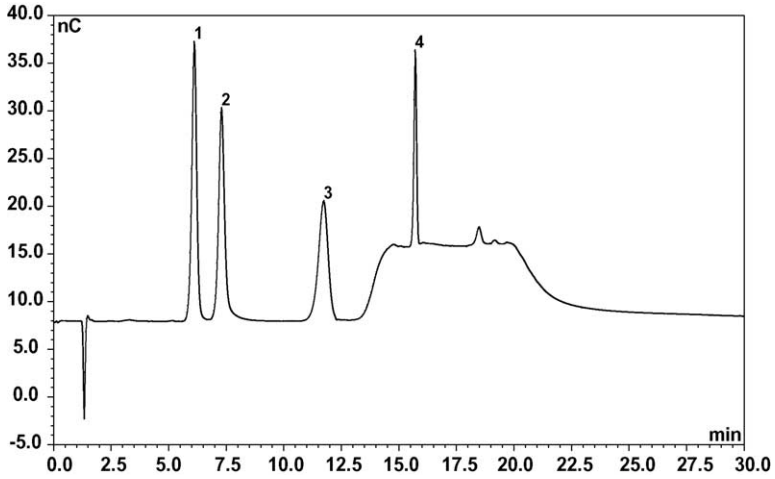

Fig. 2. Chromatogram of a standard mixture of four sugars obtained with binary step gradient elution. Chromatographic conditions: gradient elution conditions is shown in Table 1; injection volume is 25 μL; analyte concentration is 0.80 μg/mL and other chromatographic conditions and peak identities are the same as those in Fig. 1.

we began linear gradient elution too early or we adopt a too steep linear gradient elution, the resolution between glucose and ribose was not ideal. If we began linear gradient elution too late or we adopt a too gent linear gradient elution, the long retention time did not be reduced greatly. What is more, the peak of maltose was both low and wide, and therefore the detection limit of maltose was very poor. Second, if we adopted a linear gradient elution, always there was some peaks situated at the slope, in this case, it is very hard to get a low detection limits for these compounds. Finally, in the retention time window between the peak of isomaltose and maltose, there is no other impurity peak observed. In the light of above mentioned fact, finally, a binary step gradient elution using water and 500 mM sodium hydroxide as two eluents was selected in our experiments. In the first ten minute, we conducted an isocratic elution by using a binary mixture of 82.5% of water and 17.5% of 500 mM sodium hydroxide, then we increased the percentage of sodium hydroxide eluent to 100% in 0.1 min, and after that, we maintained 100% of 500 mM sodium hydroxide elution 7 min. Finally, we restored the gradient eluent to its initial eluent, a binary mixture solution of 82.5% of water and 17.5% 500 mM sodium hydroxide. Following the above gradient elution condition, we got a very flat baseline and complete separation for the first three analytes (glucose, ribose and isomaltose) at the lower platform of the chromatogram in the range of 0–12.5 min, after that, we also got the peak of maltose at the higher platform of the chromatogram in the range of 12.8–20.0 min. In addition, in this step gradient elution procedure, the 7 min lasting elution of the column by a relatively concentrated 500 mM sodium hydroxide was very helpful to elute some strong retention impurities before the next chromatographic run. In the chromatogram obtained by this gradient condition, all peaks of four analytes were high, narrow, symmetrical and situated at platform of the chromatogram, which was very favorable to get a high analytical sensitivity for us, especially for the analysis of maltose. Fig. 2 is the chromatogram obtained with 0.80 μg/mL mix standard solution of the four sugars using this gradient condition.

As a generally accepted rule, column temperature also is an important factor influencing the chromatography separation. We examined the effect of column temperature on the separation of the four target sugars under the gradient conditions selected above. From the experimental results, it was found that the retention time of the four sugars decreased slightly with increasing the column temperature in the range of 25–40 °C, which indicated that the retention process is a exothermic process. Although column temperature had some extent influence on the separation, compared

Table 3
Detection limit, linearity range and reproducibility

Analyte	Detection limit[a] (ng/mL)	Linearity range (ng/mL)	r[b]	R.S.D. for peak area[c] (%)
Glucose	0.92	0.0050–25	0.9983	2.7
Ribose	7.50	0.010–25	0.9988	4.6
Isomaltose	12.9	0.050–25	0.9985	5.6
Maltose	10.3	0.050–25	0.9984	3.6

[a] LOD at a signal-to-noise radio of 3.
[b] r = correlation coefficient.
[c] Relative standard deviation for five replicate determinations.

to the gradient conditions, column temperature only was a minor factor affecting the separation of the four sugars. In our experiments, 30 °C was chosen as column temperature.

3.2. Analytical performance

Under the selected chromatographic conditions mentioned above, the analytical performance of the proposed method was examined and the results are shown in Table 3. From this table, we can found that there is a linear correlation between peak area and concentration over two or three orders of magnitude for four sugars with a correlation coefficients better than 0.998 and the detection limits measured as three times the peak height signal-to-noise ratio for the four sugars were in the range of 0.92–12.9 ng/mL. R.S.D. of peak areas of the four analytes for five determinations were no more than 5.6%. The data in Table 3 indicated that the method we present here is fairly sensitive and reproducible.

Table 4
Results for the determination of four sugars in serum sample

Analyte	Concentration[a] (μg/mL)	R.S.D. (%)	Spiked concentration (μg/mL)	Recovery (%)
Glucose	305.6	1.9	400.0	98.1
Ribose	3.90	4.5	4.0	104.6
Isomaltose	0.00	nd	4.0	107.9
Maltose	9.42	5.4	4.0	104.1

[a] Mean for five determinations; nd: not detected.

3.3. Sample analysis

To examine the accuracy of the proposed method and its applicability to the analysis of real samples, the proposed method had been applied to simultaneously analyze glucose, ribose, isomaltose and maltose in real serum sample. Because of the great difference existing in the concentration levels of glucose and the other three analytes, it is necessary for us to

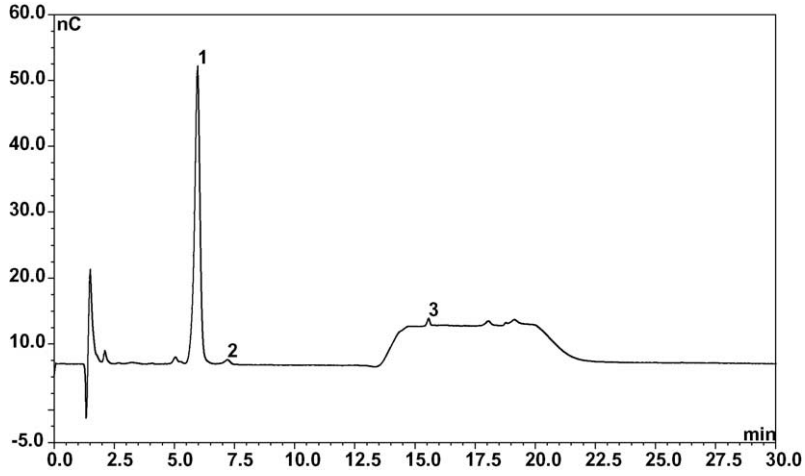

Fig. 3. Chromatogram of serum sample after it was diluted 200 times for the analysis of glucose. Chromatographic conditions: gradient elution conditions is shown in Table 1; injection volume is 25 μL and other chromatographic conditions are the same as those in Fig. 1. Peak: (1) glucose; (2) ribose and (3) maltose.

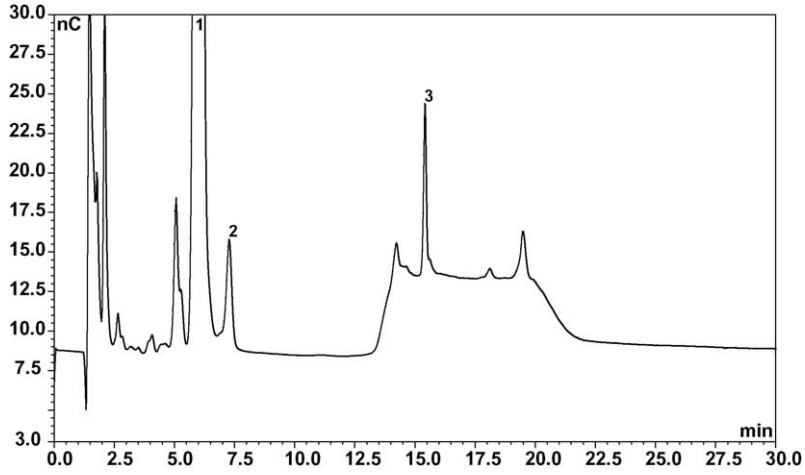

Fig. 4. Chromatogram of serum sample after it was diluted 10 times for the analysis of ribose and maltose. All chromatographic conditions and peak identities are the same as those in Fig. 3.

dilute the serum sample with different times for the determination of glucose and the other three analytes, respectively. In our case, the concentration level of glucose was relative high, when it was analyzed, the serum sample was diluted 200 times; while the concentration level of the other three analytes was relative low, when they were analyzed, the serum sample was diluted 10 times. After dilution, the serum sample can be directly injected for analysis. Table 4 shows the analytical results for the four sugars in serum sample. It is observed that good results with relative standard deviations no more than 5.4% were obtained. To test the reliability of the proposed method, recovery tests were carried out with standard spiked serum sample and the results are also presented in Table 4. From the data in this table, we found that satisfactory recovery results (98.1–107.9%) were achieved for the four sugars. Figs. 3 and 4 are the chromatograms we got for the analysis of glucose and the other three analytes, respectively.

4. Conclusion

In this paper, a simple, rapid, sensitive and reliable chromatography method was established for direct and simultaneous determination of glucose, ribose, isomaltose and maltose in real serum sample by binary step gradient elution high-performance anion-exchange chromatographic separation followed by quadruple-potential waveform pulsed amperometric detection. By this method, one single chromatography run can be completed in about 16 min, the detection limits (S/N = 3) for glucose, ribose, isomaltose and maltose are 0.92, 7.50, 12.9 and 10.3 ng/mL, respectively, and there is a linear correlation between peak area and concentration of four sugars over two to three orders of magnitude. The analytical method can be applied to the determination of glucose, ribose, isomaltose and maltose in real serum samples.

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References

[1] K. Schugeri, L. Brandes, X. Wu, J. Bode, J.L. Ree, J. Brandt, B. Hitzmann, Anal. Chim. Acta 279 (1993) 3.
[2] M. Rank, J. Gram, B. Danielsson, Anal. Chim. Acta 281 (1993) 521.
[3] H. Qu, X. Zhang, S. Zhang, Food Chem. 52 (1995) 187.
[4] M. Varadit, N. Adanyi, G. Nagy, J. Rezessy-Szabo, Biosens. Bioelectron. 8 (1993) 339.
[5] I. Lager, M. Fehr, W.B. Frommer, FEBS Lett. 553 (2003) 85.
[6] J.R. Moody, W.C. Purdy, Anal. Chim. Acta 53 (1971) 239.
[7] M. Kai, T. Kamada, Y. Baba, M. Shitomoto, S. Setoyama, S. Otsuji, Clin. Chim. Acta 108 (1980) 259.
[8] T. Buttler, L. Gordon, G. Marko-Varga, Anal. Chim. Acta 279 (1993) 27.
[9] N.C. Van de Merbel, H. Lingeman, U.A.Th. Brinkman, A. Kolhorn, L.C. de Rijke, Anal. Chim. Acta 279 (1993) 39.
[10] N.C. Van de Merbel, I.M. Kool, H. Lingeman, U.A.Th. Brinkman, A. Kolhorn, L.C. de Rijke, Chromatographia 33 (1992) 525.
[11] G. Marko-Varga, E. Dominguez, B. Hahn-Hagerdal, L. Gorton, H. Irth, G.J. De Jong, U.A.Th. Brinkman, J. Chromatogr. 523 (1990) 173.
[12] R.D. Rocklin, A.P. Clarke, M. Weitzhandler, Anal. Chem. 70 (1998) 1496.
[13] P. Jandik, A.P. Clarke, N. Avdalovic, D.C. Andersen, J. Cacia, J. Chromatogr. B 732 (1999) 193.
[14] H. Yu, Y. Ding, S. Mou, P. Jandik, J. Cheng, J. Chromatogr. A 966 (2002) 89.
[15] J. Cheng, P. Jandik, N. Avdalovic, Anal. Chem. 75 (2003) 572.
[16] A.P. Clarke, P. Jandik, R.D. Rocklin, Y. Liu, N. Avdalovic, Anal. Chem. 71 (1999) 2774.
[17] M.B. Jensen, D.C. Johnson, Anal. Chem. 69 (1997) 1776.
[18] M.A. Cox, T.H. Iqbal, B.T. Cooper, K.O. Lewis, Clin. Chim. Acta 263 (1997) 197.