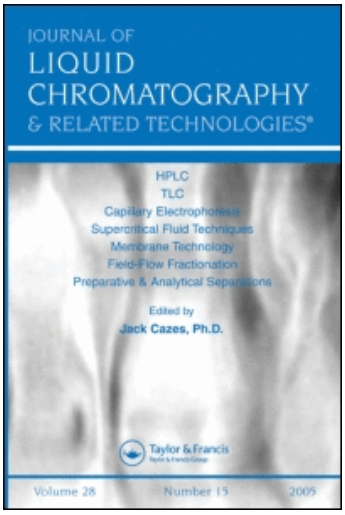

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A SIMPLIFIED METHOD FOR THE DETERMINATION OF METHYLGLYOXAL BIS(GUANYLHYDRAZONE), MGBG, IN BIOLOGICAL FLUIDS BY REVERSED-PHASE ION-PAIR HPLC

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ABSTRACT

Methylglyoxal bis(guanylhydrazone, MGBG, is described as an important agent in the fight against certain types of leukemia and other proliferative processes.

For this study, we developed a paired-ion HPLC method in order to measure MGBG concentrations in serum and urine samples. MGBG recovery in serum and urine being 95-98%, ultrafiltered samples were then injected onto a C_{18} column and eluted isocratically with a mobile phase consisting of a mixture of phosphate buffer 0.05 M, pH = 3.0, methanol (80:20) and 1.0 mM sodium heptane sulfonate, with UV (280 nm) detection. The lower limit of MGBG detection was 1.0 µg/ml in urine and 0.1 µg/ml in serum.

INTRODUCTION

S-adenosylmethionine decarboxylase (SAMDC) is one of the key enzymes in the biosynthesis of polyamines in eucaryotes. The use of inhibitors of this enzyme has in recent years been found to be one of the most rational phases in the therapeutical approach to proliferative processes as well as to certain types of infection. One of the main inhibitors of this type is 1,1'-[(methylethanediylidene)dinitrilo] diguanidine, MGBG (Figure 1), which has been found to be active against acute myelocyte leukemia (1), tripanosomes (2) (3) and coccidia (4).

The purpose of the present study is to establish an analytical HPLC method for the said compound in serum and urine. With a view to having sufficient data available we have determined quantitatively the influence of pH, organic solvent and the quantity, concentration and nature of the sodium alkyl sulfonates in the mobile phase (5) on the retention of MGBG by the stationary phase.

The present method, unlike previous ones (6) (7), by allowing for the preparation of blood and urine samples based on their ultrafiltration in diluted form through a 30,000-Dalton molecular weight cut-off filter, makes sample preparation much simpler, while very clear filtrates are obtained for chromatography, thus ensuring good results concerning sensitivity and recovery.

MATERIALS AND METHODS

Reagents and Materials

Standard MGBG was purchased from Sigma Chemicals (St. Louis,MO., USA). All water used was purified by the Milli-Q Water Purification System (Millipore Corporation Bedford, MA., USA). The sodium salts of butane, pentane, hexane and heptane sulfonic acids were purchased from Fluka Chemie (Buchs, Switzerland). HPLC grade methanol was supplied by Merck (Darmstadt, Germany). All other chemicals were of reagent grade.

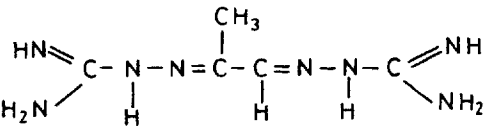

Figure 1. Structure of Methylglyoxal bis (guanylhydrazone) (MGBG)

The Ultrafree (MC, UFC3 TTK 00) microseparation system with a molecular weight cut-off of 30,000 Daltons was purchased from Millipore Corporation.

Instruments

For chromatography we used a component system comprising a Konik KNK-500-A pump (Konik Instruments, Barcelona, Spain), a Rheodyne 7210, 20 ul loop injector and a Waters 441 absorbance detector (Waters Assoc., Milford, MA., USA) operating at 280 nm. Peak areas were measured with a Waters 745-B, and the column used was a C_{18} Spherisorb ODS-2 (25 x 0,4 cm, 10 µ particle size) purchased from Teknokroma Coop., Barcelona, Spain.

Chromatographic procedure

The different mobile phases used were prepared by using phosphate buffer 0.05 M, while varying the pH, methanol percentage and the quantity and nature of the sodium alkyl sulfonate for each case (5). The mobile phase used for final analysis was a mixture of phosphate buffer 0.05 M, pH = 3.0, methanol (80:20) and 1.0 mM sodium heptane sulfonate, with the flow-rate set at 1.0 ml/min. All injection volumes for HPLC analysis were 50 µl (loop 20 µl), and all chromatographic operations were carried out under ambient conditions.

Preparation of Standard Solutions

Stock solutions of MGBG for assay in serum and urine were prepared by dissolving it in 2N hydrochloric acid to obtain a solution of 500 µg/ml, which

was then diluted in water, giving solutions in the concentration range 0.01-300 µg/ml.

Preparation of Serum and Urine Samples

0.2 ml aqueous MGBG stock solution and the same quantity of a mixture (8) of ethanol, acetonitrile and water (40:10:50) were added to 0.2 ml of serum or urine to obtain standard calibration solutions in the concentration range 0.01-100 µg/ml. These were vortexed for 20 seconds, added to the Ultrafree-MC ultrafiltration system (m.w. cut-off filter, 30,000 Daltons), and centrifuged for 15 minutes at 3000 g, the clear filtrate being used directly for chromatographic analysis.

RESULTS AND DISCUSSION

Influence of Mobile Phase pH

This was studied by using mobile phases comprising 0.05 M phosphate buffer of different pH = 2.5 , 3.5 , 5.0 , 6.0 , 7.0 and 8.0, methanol (85:15) and 2.5 mM sodium alkyl sulfonate (butane, pentane, hexane or heptane). Figure 2 shows representation of k' values contrasted with pH.

The values of k' are seen to diminish slightly as pH = 5, to increase markedly later for the sodium alkyl sulfonates with the shortest chains (C_4 - C_6).

It should be observed that, in the case of sodium heptane sulfonate, k' values are very high and remain constant when the pH of the mobile phase is altered.

Influence of Methanol Contents

A mobile phase comprising 0.05 M phosphate buffer pH = 5.0, methanol in proportions of 10 ; 20 ; 30 ; 40 ; 50 and 60 % , and 2.5 mM of sodium alkyl sulfonate (butane, pentane, hexane or heptane) was used. The representation of k' values contrasted with the percentage of methanol for (C_4 - C_7) sodium

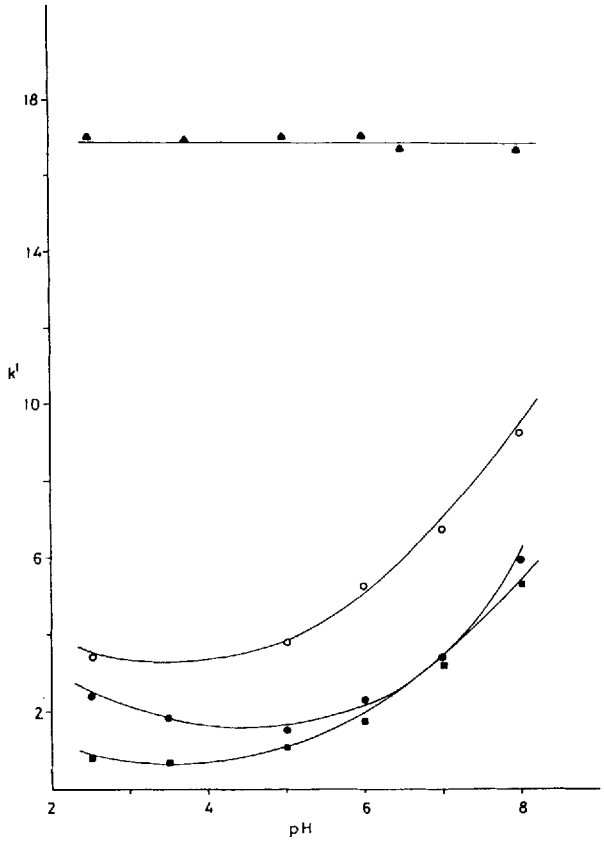

Figure 2. Effect of variation of the capacity factor of the MGBG in a mobile phase containing 0.05 M phosphate buffer with different pH = 2.5 ; 3.5 ; 5.0 ; 6.0 ; 7.0 and 8.0, methanol (85:15) and 2.5 mM sodium alkyl sulfonates. (■) Butane; (●) Pentane; (○) Hexane; (▲) Heptane.

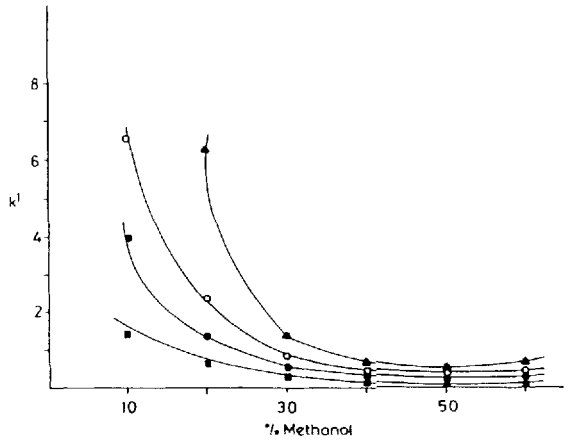

Figure 3. Effect of variation of the percentage of methanol content in the mobile phase on he capacity factor of MGBG. Mobile phase containing 0.05 M phosphate buffer pH=5.0, methanol in proportions : 10 ; 20 ; 30 ; 40 ; 50 and 60 % and 2.5 mM of sodium alkyl sulfonate. (■) Butane; (●) Pentane; (○) Hexane; (▲) Heptane.

alkyl sulfonates is given in figure 3. A noticeably sharp increase occurs in k' values in all cases when methanol decreases to below 30 %. From the shape of these curves regions can be selected where great increases in methanol concentration have very noticeable effects on the capacity factor, and therefore on chromatographic separations.

Influence of Sodium Alkyl Sulfonate Concentration

This was determined by working whith mobile phases constituted by 0.05 M phosphate buffer pH = 3.5, methanol (85:15) and sodium alkyl sulfonate (butane, pentane, hexane or heptane) in concentrations of 0.0 ; 1.0 ; 2.0 ; 2.5 ; 3.0 ; 4.0 and 5.0 mM, the variation affect the values of the capacity factor (Fig. 4). It was observed that the presence of the salts produced a very slight

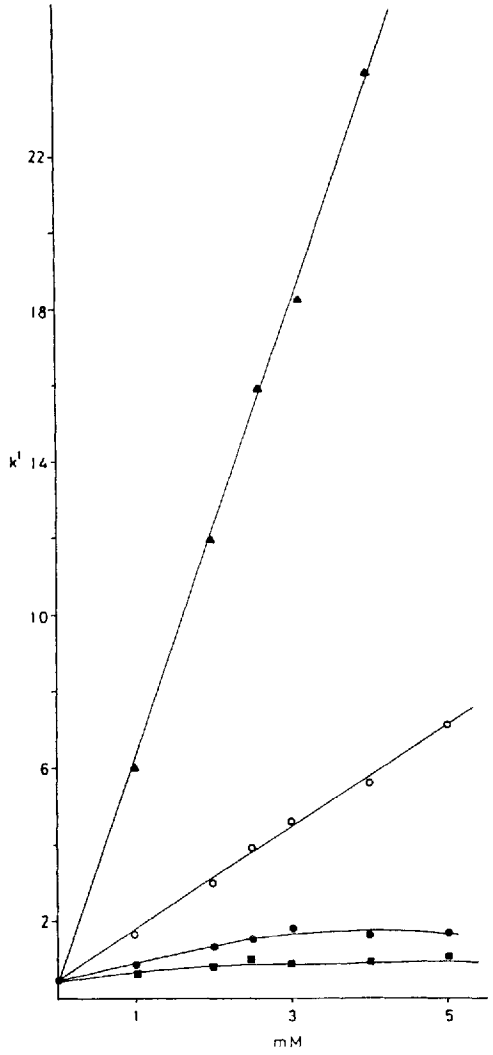

Figure 4. Effect of sodium alkyl sulfonate concentration in mobile phases on the capacity factor of MGBG. Mobile phase containing 0.05 M phosphate buffer pH = 3.5, methanol (85:15) concentration of sodium alkyl sulfonate: 0.0 ; 1.0 ; 2.0 ; 2.5 ; 3.0 ; 4.0 and 5.0 mM. (■) Butane, (●) Pentane, (○) Hexane, (▲) Heptane.

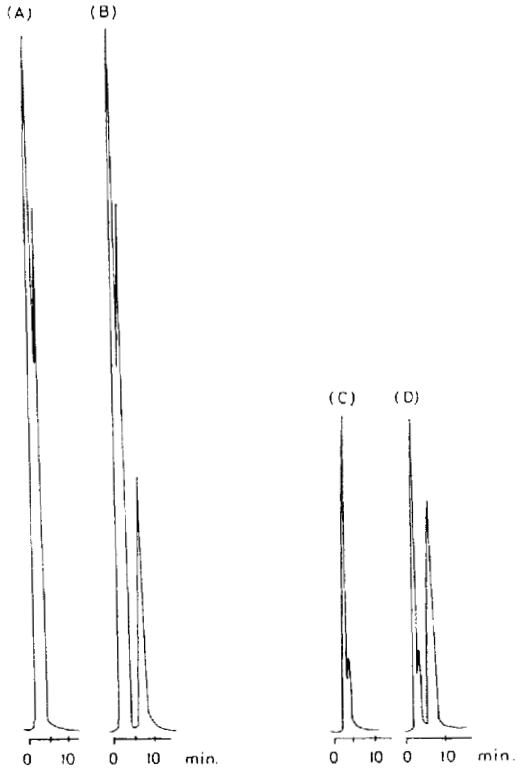

Figure 5. Chromatograms: (A) Blank urine. (B) Urine containing 10.0 μg/ml MGBG. (C) Blank serum. (D) Serum containing 1.0 μg/ml MGBG. Separation was carried out on octadecylsilane bonded-phase column. Mobile phase containing 0.05 M phosphate buffer pH = 3.0, methanol (80:20) and 1.0 mM sodium heptane sulfonate. Flow-rate : 1.0 ml/min.

increase in capacity factor values for butane and pentane sulfonates, while in the cases of hexane and heptane sulfonates the increase in k' is marked. It is also remarkable that in these two cases the increase in k' was totally linear as the concentration of sodium alkyl sulfonate increased.

Chromatographic Separation

The results obtained from the chromatographic study allow us to select the best chromatographic conditions for the determination by HPLC of MGBG in serum and urine. We have established that the most suitable mobile phase is that formed by mixing 0.05 M phosphate buffer pH = 3.0, methanol (80:20) and 1.0 mM sodium heptane sulfonate. The chromatograms obtained for serum and urine samples are shown in figure 5.

Extraction of MGBG

The extraction of MGBG from serum and urine samples was carried out using Ultrafree-MC ultrafiltration units with low-binding 30,000 Dalton polysulphone membranes, a solution made up of ethanol, acetonitrile, water (40:10:50) being used to avoid the binding of MGBG to serum and urine proteins (8). Analysis by HPLC of the filtrate after application of the serum or urine samples to the Ultrafree-MC units revealed no binding of MGBG to proteins, and that nothing was retained in the ultrafiltration membrane.

Sensitivity, Recovery and Linearity

The limits of detection for MGBG (determined by a signal-to-noise ratio greater than 3) were 1.0 ug/ml in urine and 0.1 ug/ml in serum, as determined by direct analysis of MGBG-supplemented urine and serum samples. (n = 5, standard deviation: serum $\pm$ 5.0 ; urine $\pm$ 7.0 %).

Detector responses of serum and urine samples spiked with MGBG were compared with those of directly injected aqueous solutions with identical concentrations of the compound in question. Recovery monitored in serum and urine ranged from to 94 to 99 % with a coefficient of variance never exceeding $\pm$ 6 %.

Linearity was checked by measuring 10 different concentrations in the range 0-100 µg/ml for samples of MGBG in water, serum and urine. The resulting lines of representations of peak areas of MGBG contrasted with concentrations of MGBG is represented by the equations: $y = 0.52 + 2.5\,x$; CC = 0.997 for serum and $y = 0.60 + 1.53\,x$; CC = 0.996 for urine samples.

CONCLUSION

An isocratic, ion-paired HPLC method was established for monitoring methylglyoxal bis(guanylhydrazone) (MGBG) in serum and urine. The drug was retained as an ion-pair, with sodium heptane sulfonate and determined by UV absorbance detection at 280 nm. A novel feature of the method is that it involves a simple ultrafiltration sample, Ultrafree-MC, system, a molecular weight cut-off of 30,000 Daltons which simplifies the method of analysis without impairing its accuracy, as has been established that the binding of MGBG to protein is negligible.

REFERENCES

1. Corti, A., Dave, C., Williams-Ashman, H.G., Mihich, E. and Schenone, A., Biochem.J. 139, 351 (1974).
2. Chang, K.P., Steiger, R.F., Dave, C. and Cheng, Y.C., J. Protozool. 25, 145 (1978).
3. Litchfield, J.T. and Wilcoxon, F.A., J. Pharmacol. 196, 99 (1948).
4. San Martin, B., Alunda, J., Balaña-Fouce, R. and Ordóñez, D., Comp. Biochem & Physiol. 87B, 863 (1987).
5. Salto, F., Negro, A. and Martin, J., J. Liq. Chromatogr. 9, 2443 (1986).
6. Marsh, K.C., Repta, A.J. and Sternson, L.A., J. Chromatogr. 18, 101 (1980).
7. Rosenblum, M.G. and Loo, T.L., J. Chromatogr. 183, 363 (1980).
8. Tyczkowska, K., Aucoin, D.P., Richardson, D.C. and Aronson A.L., J. Liq. Chromatogr. 10 (12) 2613 (1987).