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Letter to the Editor

Quantitative overestimation of free taurocyamine using short liquid cation-exchange chromatography columns?

Sir,

The quantitative determination of guanidino compounds in biological material of patients with hyperargininaemia or uraemia is important diagnostically, therapeutically, metabolically and pathophysiologically. These compounds, which include taurocyamine, are potent toxins [1, 2].

Using classic elution procedures for guanidino compounds, with cation-exchange chromatography, many reports [3, 4] suggest an increase in taurocyamine levels in serum and cerebrospinal fluid of uraemic patients. We would like to stress the fact that it is possible to overestimate the levels of taurocyamine using short cation-exchange columns. In the present work, we describe a method for preventing such overestimations.

We were unable to determine taurocyamine in biological fluids using our routine analysis method for guanidino compounds [5], because other unknown guanidino compounds have the same retention time as taurocyamine. This phenomenon was demonstrated by decreasing the flow-rate, pH and normality of the first buffer. Since the affinity of taurocyamine for strong-acidic cation-exchange resins is low or moderate, we decided to determine taurocyamine levels using a longer column system. The chromatographic equipment and conditions were the same as described earlier [5], except for the use of a jacketed glass column (1400 × 6.2 mm I.D.) filled with a resin (bed height 1300 mm) of Technicon Chromobeads® (type A). A sodium citrate buffer (0.2 *M* sodium ion, 0.05 *M* citrate ion) at pH 2.875 was pumped isocratically through the column at a flow-rate of 22.5 ml/h.

The sera from 57 patients with uraemia (33 dialysed and 24 non-dialysed) and 15 control sera were deproteinized by ultrafiltration using the Amicon Centriflo membrane (type CF 25) at 1000 *g* and 4°C. A 500-μl volume of filtrate, acidified with 10 μl of 2 *M* hydrochloric acid, was applied to the column. Fig. 1A shows the start of the elution pattern after applying a uraemic serum. Fig. 1B is identical to Fig. 1A, except for the addition of 5 nmol of standard taurocyamine. After elution of a first, unknown, guanidino com-

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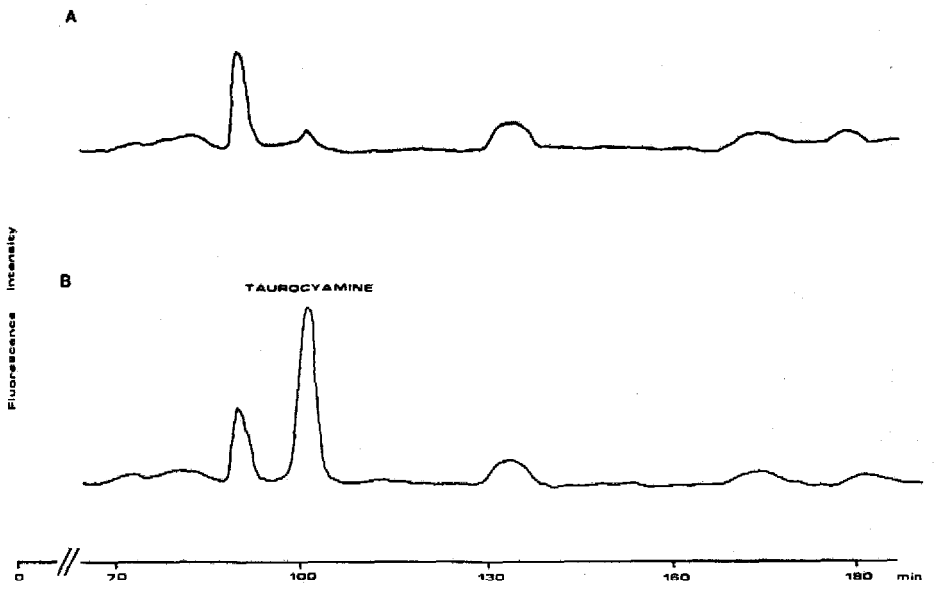

Fig. 1. (A) The start of the elution pattern of a uraemic serum, deproteinized by ultrafiltration. (B) The start of the elution pattern of the same uraemic serum, deproteinized by ultrafiltration plus 5 nmol of taurocyamine standard.

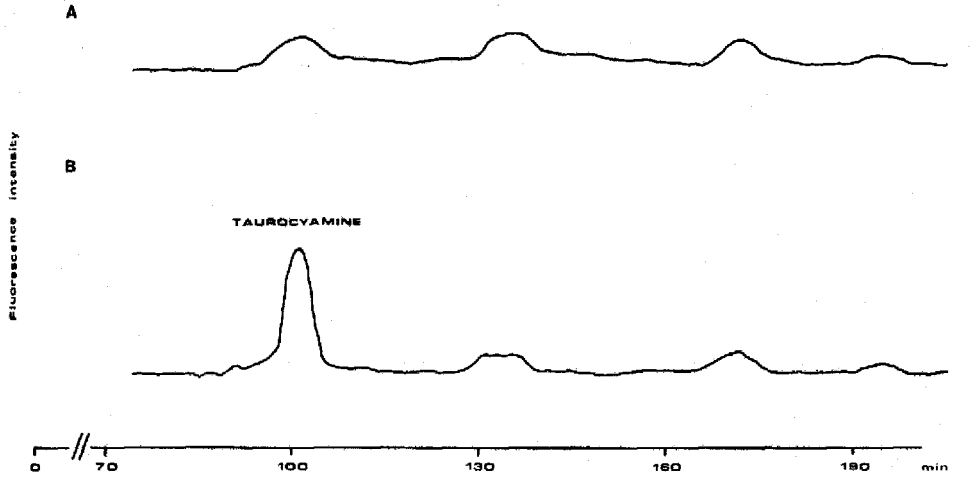

Fig. 2. (A) The start of the elution pattern of a uraemic serum, deproteinized with 20% trichloroacetic acid. (B) The start of the elution pattern of a uraemic serum deproteinized with 20% trichloroacetic acid plus 5 nmol of taurocyamine standard.

pound, a second is eluted with the same retention time as that for standard taurocyamine. With our detection system, the concentration values for the controls lie within the range < 200 to 300 nmol/l (200 nmol/l is our detection limit when applying 500 μl of serum). For the uraemic sera, we have obtained a range of < 200 to 1800 nmol/l. There was no significant difference between the taurocyamine values of dialysed and non-dialysed patients, and no correlation was found between taurocyamine values and sera urea values (sera urea values ranged from 1 to 4 g/l).

We found much lower taurocyamine values in uraemic and control sera compared to those obtained with short columns [3]. Yamamoto et al. [3] found ca. 4000 nmol/l taurocyamine in normal sera and about 26 000 nmol/l in uraemic sera. They also deproteinized their samples with the same ultrafiltration membrane system. This overestimation is probably due to co-elution of taurocyamine with other acidic guanidino compounds. Overestimation can also occur after deproteinization with trichloroacetic acid. Indeed, in this case, a broad peak (the base of the peak is at least twice as broad as that of standard taurocyamine only) is obtained with a similar retention time as that for taurocyamine (Fig. 2).

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