Journal of Chromatography, 431 (1988) 471–473
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 4329

Letter to the Editor

Simultaneous determination of creatine and creatinine in serum by high-performance liquid chromatography

Sir,

The amount of creatine in serum is increased in primary myopathy, myositis, muskelatrophie and hyperthyroidism. The amount of creatinine is used as an index of renal diseases.

These two compounds have been conventionally determined by the colorimetric Jaffé method [1,2]. However, the sensitivity and reproducibility of this method are not satisfactory. There are reports [3,4] of the simultaneous determination of these two compounds by high-performance liquid chromatography (HPLC), by gradient elution with fluorimetric detection in combination with post-column derivatization. This method may be too complicated to determine only two compounds. There are also reports [5,6] of the isocratic HPLC determination of creatinine in serum with spectrophotometric detection.

This letter discusses an isocratic HPLC assay for creatine and creatinine in serum with spectrophotometric detection at 210 nm.

EXPERIMENTAL

Apparatus

A Shimadzu LC-5A high-performance liquid chromatograph equipped with an SPD-2A spectrophotometric detector, an SIL-1A sample injector and a CTO-2A column oven was used.

Chemicals

Acetonitrile and water were HPLC grade. Sodium lauryl sulphate was biochemical grade. Inorganic reagents were analytical grade. All these reagents and chemicals (creatine and creatinine) were purchased from Wako (Tokyo, Japan).

Chromatographic conditions

A Shim-pack CLC-ODS reversed-phase column (150 mm×6 mm I.D., 5 μm) obtained from Shimadzu (Kyoto, Japan) was used. The mobile phase was 100 m*M* sodium phosphate solution containing 30 m*M* sodium lauryl sulphate–ace-

0378-4347/88/$03.50

tonitrile (3:1, v/v) and the pH was adjusted to 2.1 with phosphoric acid. The column temperature was 55 °C and the flow-rate was 2.0 ml/min. The absorbance was monitored at 210 nm.

Sample pretreatment

Bond Elut C_{18} columns obtained from Analytichem International (Harbor City, CA, U.S.A.) were used for sample pretreatment. Serum was pretreated as follows: 100 μl of 1 *M* perchloric acid were added to 200 μl of serum, and the sample was shaken and centrifuged at 16 000 *g* for 2 min. A 100-μl sample of the supernatant was placed on a Bond Elut C_{18} column, and creatine and creatinine were eluted with 400 μl of 400 m*M* sodium phosphate solution (pH 2.0). A 50-μl sample was analysed by HPLC.

RESULTS AND DISCUSSION

The best result was obtained by reversed-phase ion-pair chromatography with a Shim-Pack CLC-ODS column. Fig. 1A shows a chromatogram of the standard sample. Fig. 1B shows a chromatogram of a serum sample, which was deprotein-

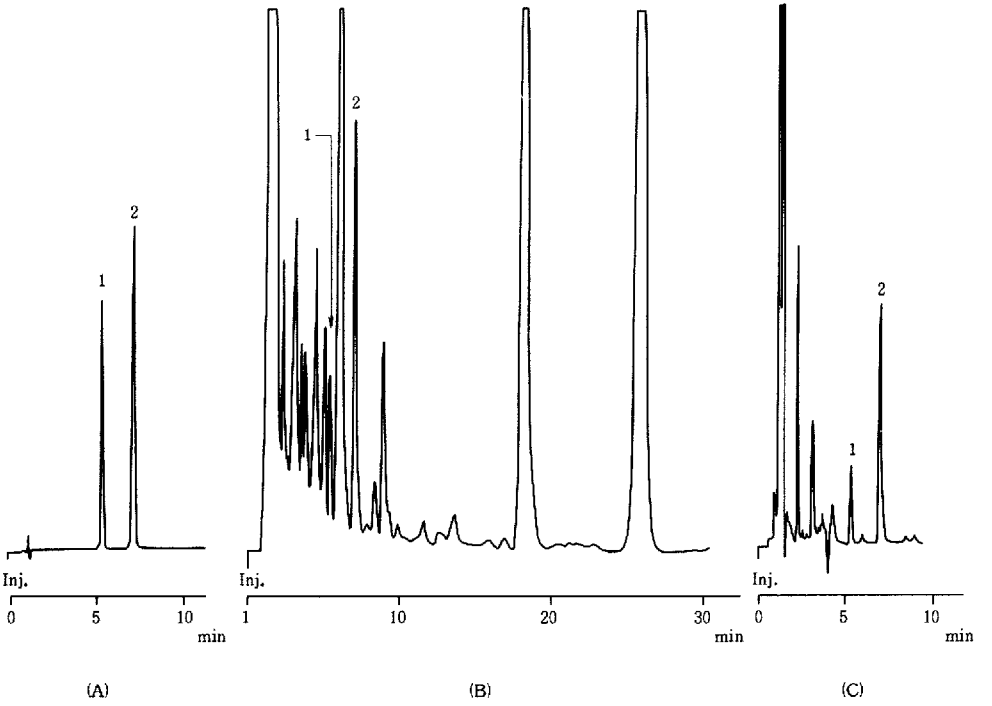

Fig. 1. (A) Chromatogram of a standard mixture: concentration, 1 mg/ml for each compound; injection volume, 50 μl; see text for conditions. (B) Chromatogram of a serum sample deproteinized with 1 *M* perchloric acid solution before injection: injection volume, 50 μl; analytical conditions are the same as in (A). (C) Chromatogram of a serum sample pretreated by the method described in *Sample pretreatment*: injection volume, 50 μl; analytical conditions are the same as in (A). Peaks: 1 = creatine; 2 = creatinine.

ized with perchloric acid. Though creatine and creatinine were separated from the other endogenous compounds, several peaks were observed after the creatinine peak.

In order to reduce the analysis time, it is necessary to remove the components eluting after the creatinine peak. A Bond Elut C_{18} column was tried for this purpose. Fig. 1C shows a chromatogram of a normal serum sample extracted by solid-phase extraction. No peaks were eluted after creatinine, and the analysis was completed within 10 min.

The mean recoveries at 10 mg/l for creatine and creatinine were 100.4% (coefficient of variation = 2.6%) and 100.5% (coefficient of variation = 2.7%), respectively ($n=5$). The detector response was linear up to 100 mg/l in serum. The linear regression equations for the calibration curve were: $y=5658x-1347$ ($r=0.9998$) and $y=8995x+1659$ ($r=0.9998$) for creatine and creatinine, respectively. The detection limits at a signal-to-noise ratio of 2 were 0.42 and 0.3 mg/l for creatine and creatinine, respectively.

The average concentrations for creatine and creatinine in normal serum were 5.4 mg/l and 11.5 mg/l, respectively.

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(First received February 22nd, 1988; revised manuscript received May 29th, 1988)