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Note

Determination of methylglucamine in urine using high-performance liquid chromatography

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Methylglucamine (N-methyl-D-glucamine), introduced in 1949 as a salt-forming base[1], is now widely used in pharmaceutical preparations. The methylglucamine salts of diatrizoic acid (3,5-diacetamido-2,4,6-triiodobenzoic acid) and iothalamic acid (5-acetamido-2,4,6-triiodo-N-methylisophthalamic acid) are used as radiographic contrast media. The renal pharmacodynamics of methylglucamine are at present being investigated and a simple assay was needed. The concentration of methylglucamine in urine samples of interest ranges from 0.2 to 10% (w/w), for which the use of high-performance liquid chromatography with a refractive index detector is suitable.

EXPERIMENTAL

Materials

Methylglucamine iothalamate in 60% solution (Conray 60; Mallinckrodt, St. Louis, Mo., U.S.A.), of nominal concentration 600 mg/ml was used as the source of methylglucamine. The concentration found by iodine assay[2] was 583 mg/ml. Other chemicals were of reagent grade.

Chromatographic system and procedure

A modular liquid chromatograph was used (Varian Model 4100, Varian Aerograph, Palo Alto, Calif., U.S.A.). The system consisted of a syringe-type pump, a vertical water-jacketed column, a Fresnel reflection-type refractive index (RI) detector and a recorder equipped with a disc integrator. The sensitivity setting was $1 \cdot 10^{-5}$ RI unit for full-scale deflection. A Varian A-25 recorder equipped with a disc integrator was used for quantitation of chromatographic peaks. The chromatographic column and RI detector were thermostatically controlled at $45 \pm 0.5°$ by a circulating water-bath. The column was equipped with a stop-flow septumless injector. The vertical water-jacketed stainless-steel column (1 m × 2 mm) with a fritted-disc outlet was packed by the tap–fill procedure. The packing used was a pellicular strong cation exchanger of the sulfonic acid type. Solutions of methylglucamine in dog urine were applied in 3-μl aliquots. Conditions of eluate concentration, pH and flow-rate were varied.

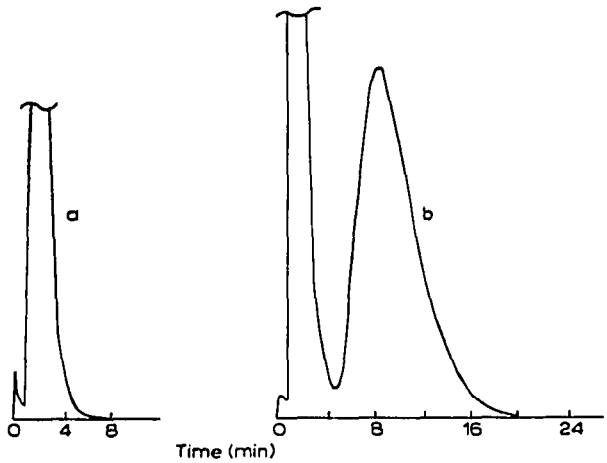

Fig. 1. Chromatogram of dog urine containing (a) 0 mg/ml and (b) 36 mg/ml of methylglucamine separated on strong pellicular cation-exchanger column (1 m × 2 mm). Eluent: 0.005 M KH_2PO_4, pH 7.2. Flow-rate: 40 ml/h. Temperature: 45°.

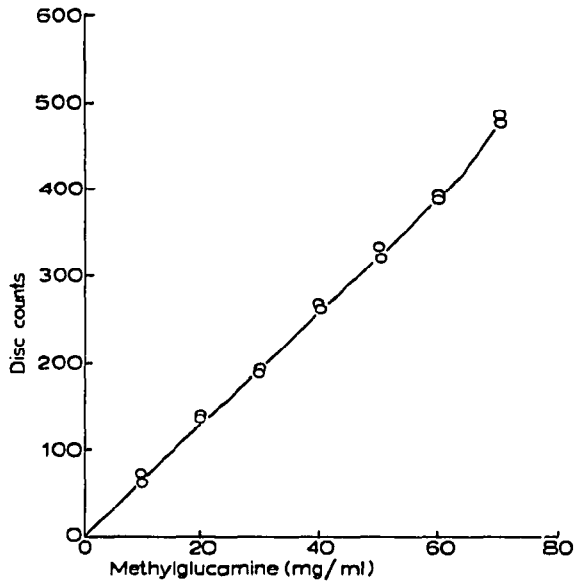

Fig. 2. Standardization plot for methylglucamine in urine

RESULTS AND DISCUSSION

Methylglucamine (pK 9.62)[3] can be separated from neutral and anionic substances by cation exchange at sufficiently low pH. It will not necessarily be separated from other cations near neutral pH but the normal cationic urine constituent of greatest weight concentration (ammonium) is usually present at a concentration below the detection limit of the refractive index detector, at the sensitivity employed.

At pH 3.2 (0.01 *M* orthophosphoric acid), a cation peak was present (possibly a combination of ammonia, amino acids and creatinine) which interfered with the methylglucamine peak. At higher pH, this interference disappeared. At pH 7.2, the optimum concentration of potassium dihydrogen orthophosphate was about 0.005 *M*: as the concentration increased, the separation from the void volume peak progressively decreased and the peak became progressively less sharp. At flow-rates much above 40 ml/h the resolution deteriorated. On the basis of these findings, the conditions chosen for the elution were 0.005 *M* potassium dihydrogen orthophosphate, pH 7.2, flow-rate 40 ml/h at 45°. The void volume peak appeared at 2.0 min and methylglucamine appeared at 8.3 min (Fig. 1). Known solutions of methylglucamine iothalamate in canine urine were used for calibration. A standard curve is shown in Fig. 2.

ACKNOWLEDGEMENTS

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