Gas-Chromatographic Determination of Dyphylline in Serum and Saliva

Z. K. Shihabi and R. P. Dave

We describe a simple determination of dyphylline in serum and saliva by gas chromatography after solvent extraction. Dyphylline concentrations were found to be about 36% higher in saliva than in the corresponding serum.

Dyphylline [7-(2,3-dihydroxypropyl)-theophylline], a bronchodilator with low toxicity, is often used in asthmatic patients who cannot tolerate theophylline. Concentrations in serum and saliva and pharmacokinetics of this drug have not yet been adequately studied (1). Spectrophotometric methods for such study (2) are subject to many interferences. A gas-chromatographic method based on extraction on a neutral resin column has been described recently (3).

Here, we describe a gas-chromatographic assay for dyphylline in serum and saliva after extraction with isopropanol/chloroform. The method is simple and rapid enough to be suitable for routine work.

Materials and Methods

Reagents

Stock dyphylline standard: This was an aqueous 400 mg/liter standard of 7-(2,3-dihydroxypropyl)-theophylline (Aldrich Chemical Co., Milwaukee, Wis.; 53233).

Working dyphylline standard: Five milliliters of the stock solution was diluted to 100 ml with water to prepare a 20 mg/liter working solution.

Stock internal standard: A 150 mg/liter of 7-(β-hydroxypropyl)-theophylline (Aldrich Chemical Co.) in chloroform.

Working internal standard: Add 10 ml of the stock internal standard to 100 ml of isopropanol and dilute to 1000 ml with chloroform.

Specimens

Unstimulated saliva (1 ml) and a tube of blood were collected at the same time from patients who had been receiving dyphylline medication. The patients were instructed to drink a cup of water before saliva collections.

Procedure

Serum or saliva, standard, or control, 0.5 ml, is added to 100 µl of 1 mol/liter HCl in a test tube, followed by 5 ml of working internal standard. The contents of the tube are mixed on a vortex-type mixer for 30 s. The organic layer is transferred to another test tube, centrifuged to remove any droplets of serum, transferred again to vials, and evaporated at 55 °C under a gentle stream of air. The residue is dissolved in 25 µl of bis-(trimethylsilyl)trifluoroacetamide containing 10 µl of trimethylchlorosilane (Sigma Chemical Co., Saint Louis, Mo. 63178) per liter and left at 80 °C for 30 min.

Gas-Chromatographic Conditions

A 3-µl aliquot of the vial contents was chromatographed on a 90 cm x 2 mm (i.d.) column packed with 100/120 Supelcoport coated with 10% SP 2250 (Supelco Inc., Bellefonte, Pa. 16823) in a gas chromatograph, Model 2600 (Bendix Process Instruments, Ronceverte, W. Va. 24970), equipped with a flame ionization detector. The temperature was maintained at 245 °C for the column and 260 °C for the detector and injector. Nitrogen flow was at 35 ml/min. Standards were injected at the beginning and the end of each run.

Results and Discussion

Dyphylline contains nitrogen, so it can be expected to undergo silylation with the silylating agent at elevated temperatures (4) as well as methylation on the column with trimethylphenylammonium hydroxide. Methylation of dyphylline is easier and quicker, but silylation results in a better baseline and better reproducibility. Figure 1 shows the separation of the silylated dyphylline from the common methylxanthenes. Retention times for dyphylline and the internal standard were 200 and 130 s, respectively.

Assays performed on sera from 30 patients with different pathological disorders who had not received dyphylline therapy revealed no peaks with the retention time of dyphylline (Figure 2), indicating that the present extraction procedure is satisfactory.
The average analytical recovery of 10, 20, and 40 mg of dyphylline per liter added to pooled drug-free sera was 105%, as compared to aqueous standards. Although recovery of dyphylline was not affected by changes in extraction pH, peaks eluting close to dyphylline were smaller if an acidic pH was used. For this reason, hydrochloric acid was incorporated in the extraction procedure. Analytical recovery of dyphylline was improved by incorporating isopropanol or 1-propanol in the extracting solvent.

This method is sensitive in the therapeutic range (5–15 mg/liter) and peak height and concentration are linearly related to a concentration of 40 mg/liter. Concentrations of dyphylline in the sera of 60 patients on different dosages ranged from 0 to 24 mg/liter (mean, 11 mg/liter), while concentrations in the saliva of 16 patients ranged from 0 to 46 mg/liter (mean, 15 mg/liter), 36% greater than for the corresponding sera, which could be attributed to the short half-life of this drug in serum. The correlation coefficient between data for saliva and serum was 0.8. Saliva has been shown by many workers to be a suitable fluid to use for frequent drug monitoring. Unlike plasma, it can be collected without discomfort to the patient and in general represents the therapeutically active form of the drug.

Among the column packings we have tried are 3% OV 1, 3% OV 17, 3% OV 225, 5% SP2250, and 10% SP2250. The last two packings gave the best separation on the 90-cm column, and were also found to be satisfactory for determining theophylline in the free form.

We thank Dr. John Hartz for reviewing this paper.

References