shaken prior to reading. This instability should be mentioned in the instruction sheet.

We thank the Hyland Division of Travenol Laboratories and the Behring Diagnostics Section of Hoechst (Australia) Limited for supplying the respective nephelometers and the reagents for this evaluation. We thank Mrs. Julie Lehman for performing the radial immunodiffusion assays.

References

Improved Determination of Serum Theophylline by Gas Chromatography with Use of a Nitrogen–Phosphorus Detector

B. Vinet and L. Zizian

We describe a method for the 7-min determination of theophylline in less than 100 μL of serum. The procedure requires no centrifugation or solvent evaporation after extraction. Butylation is done on the gas-chromatographic column by injecting the serum extract followed by a butylating mixture which contains 1-iodobutane as the alkylating agent. The method is precise, accurate, and free of interference. Results correlate well with those by ultraviolet spectrophotometry.

Additional Keyphrases: emergency procedure • pediatric chemistry

Gas chromatography has long been the most popular method for serum theophylline measurement. Several methods (1–3) are described in the literature, but generally they involve time-consuming extraction with an organic solvent and concentration of the extract before chromatography. In the method we propose, the serum extract is used without concentration and butylation is done on-column. Analysis time is essentially reduced to that required for the chromatographic separation. The method has been used in our laboratory for the analysis of many hundreds of patients' samples and found to be particularly useful in emergency and (or) pediatric cases.

Materials and Methods

All the drugs used in this study were in their pure form, obtained from our hospital's pharmacy. The various xanthine derivatives were from Sigma Chemical Co., Saint Louis, MO. 1-Iodobutane was from Eastman Kodak Co., Rochester, NY. The 25% methanolic solution of tetrabutylammonium hydroxide was from Matheson Coleman and Bell, Norwood, OH. 3-Isobutyl-1-methylxanthine was from Brickman and Co., Montreal, Que., Canada.

Internal standard: A 3 mol/L solution of NaH2PO4, pH 4.2, containing 50 μg of 3-isobutyl-1-methylxanthine per milliliter.

1. Zizian was supported by an MRC studentship. Received June 21, 1978; accepted Oct. 18, 1978.
This solution is stable at room temperature for at least a month.

**Organic-solvent mixture:** This is ether-dichloromethane-isopropanol (6/4/1 by vol), stored at 4 °C in a closed, amber-colored bottle for no longer than one month.

**Butylating mixture:** To 0.9 mL of 1-iodobutane, add 0.2 mL of methanol and 20 μL of the tetrabutylammonium hydroxide solution. This mixture must be prepared just before chromatography.

**Assay procedure:** To 100 μL of serum, add 50 μL of phosphate buffer (3 mol/L, pH 4.2) and 50 μL of methanol. Mix briefly, add 200 μL of the organic solvent mixture, and shake by hand, gently enough to avoid emulsion formation. After the mixture has stood for a few seconds the organic (upper) phase is ready to be injected. Rinse a 10-μL Hamilton gas-tight syringe with the butylating mixture and fill it to the 0.4-μL mark with the same mixture. Then aspirate 1.6 μL of the organic supernatant phase, which contains the theophylline. Inject the contents of the syringe during 10 s into the gas chromatograph (details in legend to Figure 1). The detector is operated in the nitrogen mode, with 3 mL of hydrogen and 300 mL of air per minute. The current bead setting is 600 and under such conditions the sensitivity is 0.7 C/g of nitrogen, as determined with methion.

For quantitation, measure the ratio of peak height of theophylline to that for the internal standard. Calibration sera are prepared by adding known amounts of theophylline to a pooled sample of human sera.

**Results**

On-column butylation requires less manipulation than pre-column derivatization. To avoid saturating the nitrogen-phosphorus detector, we butylate theophylline with the least-necessary amount of tetrabutylammonium hydroxide. Even then, the lowest concentration of this compound necessary for good sensitivity still gives an undesirably large interference at the beginning of the chromatogram.

In pre-column alkylation procedures (4), the quaternary ammonium salts are used as the bases for deprotonation, alkyl halides as the alkylating agents. The reaction is done in a solvent with a high dielectric constant. If theophylline is injected in 1-iodobutane alone, no butylation occurs. Addition of small amounts of tetrabutylammonium hydroxide and methanol to the iodobutane gives a mixture that promotes butylation of theophylline on injection at 300 °C. The alkylating agent is 1-iodobutane and the concentration of tetrabutylammonium hydroxide is low enough to minimize the peaks at the beginning of the chromatogram, but great enough for maximum alkylation yield. The methanol is necessary to keep the quaternary ammonium salt in solution and the butylating mixture must be prepared just before use, because it becomes cloudy and loses its effectiveness within a few hours.

The chromatographic sensitivity allows use of the organic solvent extract of serum without evaporation. When 1-iodobutane or n-butanol are used as the extracting solvents, recovery and sensitivity are poor and an emulsion forms. The organic solvent mixture we propose has a low tendency to form an emulsion and is less dense than serum. We have the same yield in butylation if theophylline is dissolved in the butylating mixture and if we use the two-zone injection as described.

The small volume of the organic phase is responsible for the low extraction (about 10%), which is not improved by more vigorous extraction followed by centrifugation; the small volume is necessary for good sensitivity.

The within-run precision of the method was 5.2% and the day-to-day precision, measured during one month, 7.3%; both values are for a theophylline concentration of 20 mg/L.

Figure 1B represents the analysis of a patient's sample. From Figure 1A one can see that no normal serum constituent interferes and that the background value is <1 mg/L. Theophylline concentrations as low as 5 mg/L are easily measured.

The method is free of interference from other xanthises (including theobromine); the most commonly used barbiturates give peaks that are well resolved from both the theophylline peak and the internal standard peak.

It has been proposed to use pentylation instead of butylation with an OV-17 column to separate the phenobarbital and the 3-isobutyl-1-methylxanthine peaks (5). Under our conditions they give discrete peaks (5.3 and 5.6 min, respectively), and phenobarbital in a concentration of 30 mg/L gave no interference.

The method is unaffected by hemolytic and icteric sera. Highly lipemic sera produce in some cases an emulsion with the organic solvent mixture; it is then necessary to increase the organic phase volume to 0.8 mL, to evaporate the extract in siliconized cups, and redissolve the residue in 10 μL of methanol. Even under such conditions, the method is still relatively rapid, because the organic solvent mixture is highly volatile.

Results by the method correlate well \( (r = 0.959 \text{ for } n = 25) \) with those by the ultraviolet procedure of Jatlow (6), giving the following regression line: \( y = 0.98x + 0.15 \) as calculated by the least squares method, \( y \) representing the values by our method and \( x \) those by the ultraviolet procedure.

The participation of P. Lemieux and A. Belmonte was greatly appreciated.

**References**

Quality of Sweat Test Performance in the Diagnosis of Cystic Fibrosis

Harry Shwachman and Abbas Mohmoodian

The sweat test, correctly performed, appears to be reliable, but results were found to be unreliable about half the time in 84 small community hospitals assessed. The reasons are inexperienced and untrained technicians, the infrequency of test performance, the use of unstandardized equipment, and the lack of appropriate standards. It behooves all clinical laboratory directors to re-examine and standardize their procedures and use the recommendations of the Cystic Fibrosis Foundation. Physicians should be made aware of the current situation and send their patients only to laboratories that perform the test reliably.

Additional Keyphrases: quantitative pilocarpine iontophoresis • conductivity • chloride selective electrode • chloride plate test • interlaboratory performance • pediatric chemistry • analysis of nail clippings • age-related effects

The purpose of this report is to document our experience during two years, comparing sweat-test results for patients referred to us because of the diagnosis of possible cystic fibrosis (CF). Some patients with known cystic fibrosis, also referred because of uncommon complications or for therapeutic suggestions, had been diagnosed and treated in other Cystic Fibrosis Centers. Most of the patients coming to us were referred by pediatricians who had considered the diagnosis of cystic fibrosis and had sweat tests performed in their local institutions. During 1976 and 1977 a total of 143 patients were referred to us who had already had sweat tests.

Procedure

The sweat test performed in our laboratory since 1960 is the standard quantitative pilocarpine iontophoresis test (1). The detailed procedure has been described elsewhere (2). First, sweat glands on the forearm are stimulated with pilocarpine and a 2.5-mA current. This step, which requires 5 min, has not caused a burn or a serious local reaction in more than 24,000 tests. Next, sweat is collected for 25 min onto weighed gauze pads, previously tested for contamination. Evaporation of sweat is prevented. Any condensate on the plastic seal is included in the weighed gauze pad. At least 50 mg of sweat is required for the analysis, but much larger amounts usually are produced. The error in the chemical assay is inversely related to the volume of sweat. When a small sample volume is anticipated, sweat collected from both the left and right forearms is combined, although this theoretically is undesirable because a low sweat rate may yield lower electrolyte values. Sodium and potassium are determined by flame photometry, chloride by the Schales and Schales method (3).

These analyses were done in duplicate in about half the patients in this study when the patients were first seen, in addition to a chloride plate test (4). In many cases the clinical history, including family history, was obtained and a physical examination made. Additional tests, done only if deemed to be useful, included chest roentgenograms, sweat testing of siblings, stool-trypsin tests, sputum or throat cultures, serum protein analysis, allergy studies, and follow-up visits. Several patients were seen on subsequent visits and had repeat sweat tests. To determine the reproducibility of the sweat test, we repeated the test on 107 patients who served as controls and on 75 patients with cystic fibrosis. The patients' age range was three months to 40 years, the interval between tests was less than two months.

Results

A total of 143 patients were referred to our center for diagnostic or therapeutic reasons who had had sweat tests before our examination. We repeated the sweat tests as outlined above. Fifteen patients had been diagnosed and treated for cystic fibrosis at nine different Cystic Fibrosis Centers recognized by the Cystic Fibrosis Foundation. Our results in testing these 15 patients is given in Table 1. The mean values for sodium, potassium, and chloride are consistent with the