and sulfamethoxazole) reportedly encounter a high incidence of side effects, mainly leukopenia, thrombocytopenia, and deterioration of kidney function (1, 2). A possible mechanism for this impaired kidney function is the occurrence of crystalluria within the tubular lumen (3). Pharmacokinetic monitoring during treatment may decrease drug-associated adverse effects (4, 5). Methods published for sulfamethoxazole (SMZ) applied various solvents and extraction techniques, have chromatographic retention times of about 20 min (6, 7).

We have developed a simple and rapid liquid-chromatographic method for determining serum SMZ, in which only a protein separation step is required; the retention time for SMZ is about 1 min. The HPLC system (Series 4; PerkinElmer Corp., Norwalk, CT 06682) was equipped with a Rheodyne sample injector, a Model LC-85B detector, and a Model Sigma-16 integrator. The chromatographic analysis was performed with a 0.46 x 3 cm C18 column (PerkinElmer). The mobile phase was methanol/phosphoric acid (10 mmol/L), 30/70 by vol, at a flow rate of 1.5 mL/min. The column effluent was monitored at 250 nm.

SMZ stock standard (10 mg/mL) was prepared in 0.1 mol/L phosphoric acid containing, per liter, 0.3 mL of 10 mol/L sodium hydroxide. Working standard solutions were made by adding appropriate amounts of stock standard solution to drug-free pooled serum to give the following concentrations: 500, 250, 125, and 62.5 mg/L.

The deproteinization was carried out by adding 50 μL of perchloric acid (70%) solution to 500 μL of serum samples. The sample was vortex-mixed and centrifuged at 10 500 x g for 4 min, then a 20-mL aliquot of clear supernate was applied to the column. The sample was assayed in the same manner. The peak height for the sample was compared with those of the standard curve.

Figure 1 shows a chromatogram of SMZ in deproteinized serum.

The recovery of SMZ was calculated by dividing the peak-height ratio for samples by that obtained from injection of nondeproteinized standards in mobile phase. The mean recovery was 81.99 (SD 5.85%) (n = 12).

The concentration–response relationship was linear between 3 and 500 mg/L (y = 0.754x + 6.38; r = 0.995).

The between-day coefficient of variation was 2.61% (n = 22) for a 200 mg/L control.

References
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Fig. 1. Chromatogram of serum containing sulfamethoxazol, 62.5 mg/L (retention time 1.09 min) and sulfamethoxazole with blood levels in AIDS patients treated for Pneumocystis carinii (Abstract 1226). ICACC Congress, 1988.


Several drugs, including ampicillin, amoxycillin, and penicilamine (I), react with ninhydrin and therefore interfere with qualitative or quantitative methods for amino acids. The appearance of such extraneous bands or peaks may lead to unnecessary further investigation of patients if they are not recognized as artifacts (false positives) or, more seriously, they could obscure genuine abnormalities (false negatives).

Vigabatrin (Merrell Dow Pharmaceuticals Ltd., Staines, England) is an anticonvulsant that inhibits 4-aminobutyrate aminotransferase (EC 2.6.1.19), leading to increased

Fig. 1. High-voltage electrophoresis (pH 2.0) of urine, after staining with ninhydrin
N, normal urine; P1, P2, urines from patients receiving vigabatrin; V, vigabatrin; arrows, major abnormal bands
concentrations of 4-aminobutyric acid in the central nervous system. It has not been released for general prescription but is available on a named patient basis for treatment of intractable convulsions. It reacts with ninhydrin and can be assayed by automated amino acid analysis (2).

High-voltage electrophoresis of urine samples from two patients receiving vigabatrin yielded bands that stained strongly with ninhydrin and did not correspond to known amino acids (Figure 1). A less-intense band was also visible on thin-layer chromatography of a sample from one of the patients, but this was not a consistent finding; however, the formulation of the ninhydrin staining solution was different for electrophoresis and for thin-layer chromatography. Amino acid analysis of these samples (Biotronik LC 5001; Wissenschaftliche Gerate GmbH, München, F.R.G.) revealed several unidentified peaks. We have also observed an increase in the excretion of pyroglutamic acid in these patients, presumably owing to enzyme inhibition by vigabatrin affecting the γ-glutamyl cycle (3).

Laboratories screening for amino acid abnormalities need to be aware of drug-related and other artifacts that might interfere with the detection of genuine abnormalities. This is particularly important for drugs that are used to treat children who are likely to be undergoing investigation for metabolic disease.

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References

Evaluation of an Alveolar Carbon Monoxide Monoxida

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We report here our experience with an alternative to direct measurement of carboxyhemoglobin (COHb) in blood: analysis for alveolar carbon monoxide (CO). The sampling procedure is based on that of Jones et al. (1). Subjects, standing upright, emptied their lungs, took a deep breath, and held it for 20 s. They then exhaled the first 900 mL (anatomic dead space) into a polyvinyl bag (900-mL urine-collection bags; Méridon, Montreal, Canada), and, without taking another breath, completely inflated two other 900-mL bags connected with plastic tubing (1.8 L of alveolar air in equilibrium with blood).

Within 12 s we analyzed the alveolar air samples with a CO analyzer (Model 8501 BA infrared spectrometer; Bendix, Lewisburg, WV). To obtain precise digital readings, we transformed the analog output of our CO analyzer by using a digital multimeter (Model 310B; Beckman Industrial, Brea, CA), averaging the values of two multimeter readings taken 15 s apart, 2 min after the beginning of the sample aspiration.

To study the analytical recovery, we filled 15 of the 1.8-L bags with commercial CO (Médigaz, Québec, Canada), 57.8 μL/L, analyzing five immediately, five after 12 h, and five after 24 h. At 12 and 24 h values were respectively 98.1% and 95.2% of the first measured value (CV = 0.58% for each set of measurements).

To establish the correlation with COHb, we measured concurrently in 24 volunteers (13 smokers, 11 nonsmokers) a duplicate alveolar CO sample and a venipuncture blood sample for determination of COHb. The blood samples were analyzed by standard methods with an OSM-2 Hemoxymeter (Radiometer, Copenhagen, Denmark).

The correlation between COHb and alveolar CO is expressed by the following linear least-squares regression equation: CO, alveolar (μL/L) = 539.3 COHb – 3.3 (n = 24), standard error of estimate = 1.904, standard error of the slope = 13.12, r2 = 0.9335). Values ranged from 0.6 to 52.6 μL/L (mean 13.64 μL/L). From analyses of the duplicate samples we estimated the total CV for the method (within-run analytical variation plus variation introduced by the sampling method) to be 4.16% (2). For smokers and nonsmokers the results were respectively as follows: CV = 3.14% and 6.76%, mean = 24.8 and 1.4 μL/L.

Repeated analyses of controls gave a within-run analytical CV ≤0.4% (n = 10), a cumulative CV (total analytical imprecision) ≤0.87% (n = 35). No significant drift was observed during the four-month study.

The alveolar CO analysis we used is a good method for assessing CO intoxication: it is noninvasive, inexpensive, and can be easily performed by untrained subjects. Its reproducibility was excellent, showing very low variability. This procedure can be used to document most environmental and occupational exposures to CO.

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References

Improvement in the Head-Space Gas-Chromatographic

Method for Determination of Three Ketone Bodies in Plasma, Masako Kimura,1 Naomi Ogashara,1 Kunio Kobayashi,1 Akira Hitoi,1 Akira Matsuoka,2 and Yukio Kinura2 (1 Dept. of Clin. Pathol. and Clin. Lab., Hyogo College of Med., 1-1, Mukogawa-cho, Nishinomiya 663, Japan; and 2 Faculty of Pharm. Sci., Mukogawa Women’s Univ., Koshien Kyuban-cho, 11-68, Nishinomiya 663, Japan)

We made two improvements on the procedure of the headspace gas-chromatographic method reported previously for determining the three ketone bodies in plasma (1). First, the