Quantitative Analysis of Tribromsalan in Blood and Urine

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Abstract □ Tribromsalan can be quantitatively measured in whole blood and urine by a technique involving extraction with ethyl acetate, treatment with silica gel, separation by TLC, and quantitative measurement by fluorescent spectrophotometry. This method has a sensitivity down to 125 ng (25 ppb in 5.0 ml of sample) of free tribromsalan and shows an average 90% recovery of tribromsalan in blood and urine with standard deviations of 9.7 and 7.4%, respectively.

Keyphrases □ Tribromsalan—analysis, blood and urine, TLC, fluorometry □ TLC—analysis, tribromsalan, blood and urine □ Fluorometry—analysis, tribromsalan, blood and urine □ Bacteriostatic agents—tribromsalan, analysis, blood and urine, TLC, fluorometry

Tribromsalan[1] is a bacteriostat which was widely used in soap and detergent products. It is effective against common Gram-positive bacteria (*e.g., Staphylococcus* type) (1–3).

Since recent studies demonstrated the absorption of hexachlorophene through human skin into the blood (4–6), other bacteriostats used in skin products must also be examined for skin absorption. Such study requires detection methods sensitive to parts per billion in blood. Tribromsalan was analyzed in toilet soaps by rapid liquid chromatography (7) with a detection limit of 100 ng. This method was not applicable to tribromsalan in blood or urine because of extracted interference.

A method is now presented for quantitatively determining tribromsalan in blood. It involves extraction of tribromsalan with ethyl acetate, isolation by TLC, and measurement through fluorescence spectrophotometry.

EXPERIMENTAL

Reagents—Tribromsalan was 99% pure as analyzed by the manufacturer. The solvents used were ethyl acetate, acetic acid, and absolute methanol, all reagent grade. Silica gel G-325[2] was used.

Procedure—The standards (25–1000 ppb) were prepared by adding known amounts of tribromsalan stock solution (1 μg/10 μl of ethanol) to each 5.0-ml blood sample; they were treated the same way as other samples to be described.

A 5.0-ml blood sample (5 mg of sodium citrate or 0.2 mg of heparin/ml of blood was added as anticoagulant) was frozen in an ethylene glycol–ether–dry ice bath (−20°) for 10 min for complete hemolysis and then thawed. Extraction was made with 15 ml of ethyl acetate on a mixer[3] for 2 min followed by centrifuging at 3500 rpm for 5 min.

Ten milliliters of the supernate was transferred to a 15-ml graduated centrifuge tube and evaporated to 3.0 ml on a water bath[4] at 60°. Approximately 100 mg of silica gel G was added, mixed well, and centrifuged. The supernate, 2.5 ml, was then transferred to a 2.5-ml centrifuge tube and evaporated to exactly 0.2 ml. If the final volume became less than 0.2 ml, it was adjusted with additional ethyl acetate.

Sixty microliters of this aliquot was spotted on a diethylaminoethylcellulose-coated glass plate[5]. At least three standards of tribromsalan were spotted on each plate along with unknown samples. The plate was developed to the top three times with absolute methanol to remove the interfering background from the original tribromsalan spot. Finally, the plate was developed with 7.5% acetic acid in methanol to approximately one-third of the plate (7–8 cm, R_f value for tribromsalan = 0.43). The plate was air dried thoroughly (see *Results and Discussion*).

The tribromsalan spot was located visually under UV light (350 nm). The plate was run on a fluorescent spectrophotometer[6] with a TLC scanner for quantitative measurements. A No. 390-nm cutoff filter was used before the emission monochromator with a 0.5 × 0.5-cm scanning slit. Spectra for tribromsalan were obtained at an excitation wavelength of 362 nm and an emission wavelength of 422 nm.

Spectra were photocopied, cut out, and weighed for quantitative measurement. Since the instrument setting for the TLC scanner can be varied from one reading to another, peak height measurement alone was not satisfactory. For area measurement, a photocopying and weighing method was convenient and as accurate as measuring height times width at half-height.

For the analysis of urine samples, the procedure was followed as described for blood samples with these exceptions:

1. The pH of urine samples was adjusted to 6.8 with monobasic or dibasic potassium phosphate before extraction with ethyl acetate, since the natural pH of urine samples varies a great deal.
2. The samples were not frozen in dry ice, since they did not need hemolysis.
3. The silica gel treatment was omitted, and the 10-ml aliquot of the ethyl acetate extract was evaporated to a final volume of 0.3 ml before spotting.

RESULTS AND DISCUSSION

The average recovery of tribromsalan from human blood was 90.6 ± 9.7% (14 samples). This value was established by extracting blood samples containing known amounts of tribromsalan (100–2500 ppb) and comparing with ethyl acetate solutions of known amounts of tribromsalan.

The linearity of the method was confirmed with human blood, rabbit blood, and human urine samples through standard curves. Correlation coefficients (r) for linearity were calculated (8) for eight different sets of experiments with three to five points for each set. They were found to be 0.976, 0.983, 1.000, 0.996, 0.992, and 1.000 for blood samples (25–1000 ppb) and 0.982 and 0.960 for urine samples (100–1000 ppb). Greater background interferences were noticed for urine samples than for blood samples. With urine, the lower limit of sensitivity was 100 ppb.

The precision of the method was determined by 10 replicate analyses of a human blood sample containing 500 ppb of tribromsalan. These blood samples were prepared by adding 50 μl of an ethanolic tribromsalan solution (1 μg of tribromsalan/μl of alcohol) to 100 ml of human blood. After thorough mixing, 10 5-ml samples of this blood were analyzed on the same TLC plate. The relative standard deviation was 7.4%.

Lowering the pH of blood to 5.9 using 10% acetic acid did not affect the recoveries. Since the minor variations (pH 7.0–7.4) that

[1] 3,4′,5-Tribromosalicylanilide, TBS, Fine Organics, Inc.
[2] Research Specialties Co.
[3] Vortex Genie, Scientific Industries.
[4] N-EVAP-A water bath with fitted test tube rack which has an air flow device on the top, Organomation, Inc.
[5] S&S material, 100–125 μm thickness, 20 × 20 cm.
[6] Hitachi model MPF-2A with TLC scanner (an accessory for model MPF-2A), Perkin-Elmer Corp.

occur with human or animal blood would not be expected to have any effect, no buffering was used. Urine samples, however, vary significantly in pH and need pH adjustment to 6.8.

The character of the fluorescent spectrum changed as the plate dried. The peak became larger and more defined at the given excitation and emission wavelengths (362 and 422 nm, respectively). There was also a significant shift of both the excitation and emission maximum wavelengths to 330 and 480 nm, respectively, when tribromsalan was in 7.5% acetic acid in methanol. Spectra obtained with wet plates resembled those in 7.5% acetic acid in methanol. The change of fluorescence in the presence of acid was also observed by other investigators with aspirin (9). Drying the plate for more than 24 hr in the air provided peaks with consistent intensities without a shift of maximum wavelengths on the fluorescent spectrophotometer.

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pH-Dependent Secretion of Procainamide into Saliva

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Abstract □ The relationship between serum and stimulated, mixed saliva concentrations of procainamide was determined in 12 chronically medicated patients. Samples were obtained at times chosen to approximate the maximum and minimum serum concentrations of the drug during a dosing interval. Marked intersubject variability was found in the ratio of saliva to serum concentration of the drug (0.27–8.93). There was no correlation between the dose (milligrams per kilogram per day) and the minimum serum or saliva concentration of procainamide. Saliva pH ranged from 6.3 to 8.0 in eight subjects. The ratio of saliva to serum concentration of procainamide increased with decreasing pH. This result can be largely explained by the pH-dependent ionization and distribution of procainamide, a weak base.

Keyphrases □ Procainamide—pH-dependent secretion into saliva, ratio of saliva to serum concentration, humans □ Saliva—concentration of secreted procainamide, pH dependent, compared to serum concentration, humans

Procainamide, an antiarrhythmic compound, demonstrates marked intersubject variability in absorption and elimination (1). The range of therapeutic serum concentrations, however, has been defined (1). Mean plasma concentrations ranging from 4 to 8 μg/ml are generally effective in controlling arrhythmias, while toxicity is likely to occur at concentrations above 12 μg/ml. Thus, serum concentration measurements are useful in monitoring therapy in individual patients. However, procainamide has a short half-life, approximately 3–6 hr, which necessitates multiple venipunctures to characterize the peak and minimum serum concentrations during a dosing interval and during continued therapy. These requirements may be excessively traumatic for routine clinical application. The purpose of this study was to assess whether saliva concentrations of procainamide can be used to monitor drug concentrations in the plasma of patients receiving the drug.

The salivary secretion of other drugs has been the subject of several investigations. Good correlation between either total or unbound serum and mixed saliva concentrations was demonstrated in humans for theophylline (2), lithium (3), digoxin (4), acetaminophen (5), salicylate (at low concentrations) (6), phenytoin (7), and several sulfonamides (8).

EXPERIMENTAL

Twelve patients in an acute coronary care unit[1], who had been receiving a fixed dose of procainamide hydrochloride[2] for at least 2 days, were studied. Daily doses, dosage intervals, and times of sample collection in relation to dose are listed in Table I for the individual patients. The clinical records indicated that renal and liver functions were essentially normal in all subjects.

Blood and mixed, stimulated saliva were collected simultaneously before and after a regularly scheduled dose of procainamide. Saliva flow was stimulated by having the patients chew on waxed film[3]. For Patients K and L, only one sample set was obtained. A third sample set was obtained at approximately 1 week after the initial samples for Patients A and E. All samples were obtained between 10 and 11 am and between 1 and 3 pm to avoid mealtimes and to minimize the possible effects of diurnal variation in saliva composition (9).

Saliva pH was determined immediately after sample collection for Patients E–L. The serum and saliva samples were refrigerated at 4° and analyzed within 2 days. Serum and saliva concentrations of procainamide were assayed by a double-extraction, spectrophotofluorometric method (1, 10).

[1] At the Millard Fillmore Hospital.

[2] Pronestyl.

[3] Parafilm.