Quantitative Analysis of Triclocarban in Blood

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Abstract □ A method is presented for quantitatively determining triclocarban in blood. Triclocarban is extracted from blood with ether, isolated by TLC, and measured through its UV absorption at 265 nm in methanol. This method is sensitive to 250 ng (50 ppb in 5 ml of blood) of free triclocarban with a relative standard deviation of 5.2%, correlated with a radiotracer analysis of ^{14}C-labeled triclocarban. It has been applied successfully to the analysis of triclocarban in human and rabbit blood.

Keyphrases □ Triclocarban—quantitative analysis in human and rabbit blood □ TLC—quantitative analysis, triclocarban in human and rabbit blood □ UV spectroscopy—quantitative analysis, triclocarban in human and rabbit blood

Recently, hexachlorophene was found to be absorbed into the blood after topical application (1–3). Other bacteriostatics used in skin products must also be examined for skin absorption. This requires detection methods sensitive to parts per billion (ppb) in blood. Triclocarban[1], a bacteriostatic currently used in personal care products, is effective against common Gram-positive bacteria (*e.g., Staphylococcus* type) and fungi (4, 5).

An attempt by the authors to measure triclocarban by GC[2] with electron capture was unsuccessful due to its low volatility. The authors were also unable to derivatize the possible enol (6) to increase volatility. Results are now presented concerning a method for quantitatively determining triclocarban in blood. This procedure employs the extraction of triclocarban from blood with ether, isolation by TLC, and measurement through its UV absorption in methanol.

EXPERIMENTAL

Reagents—The following were used: triclocarban[3] (99% pure as received), anhydrous ether (reagent grade), acetic acid (reagent grade), absolute methanol (reagent grade), and silica gel G-325[4] (325 mesh and above).

Procedure—A 5.0-ml blood sample (0.5% sodium citrate was added as an anticoagulant) was frozen in an ethylene glycol ethyl ether[5]–dry ice bath (−20°) for 10 min and then warmed to room temperature. The first extraction was made with 15 ml anhydrous ether on a mixer[6] for 5 min. Ten milliliters of the supernate was removed and 10 ml of fresh ether was added for the second extraction; this step was repeated for the third extraction. All ether extracts were collected, evaporated to 3.0 ml on a water bath[7] at 60°, mixed well with approximately 100 mg of silica gel G, and centrifuged. Then 2.5 ml of the supernate was transferred to a 2.5-ml centrifuge tube and evaporated to dryness.

The residue was taken up in 0.2 ml of methanol and spotted on a diethylaminoethylcellulose[8]-coated glass plate. On each plate, at least three standards of triclocarban were spotted along with unknown samples by adding known amounts of triclocarban stock solution (1 μg/μl of methanol) to each 5.0-ml blood sample and treating in the same way as other samples. The plate was developed with carbon tetrachloride to remove the interfering background from the original triclocarban spot. Finally, the plate was developed with 4.0% acetic acid in absolute methanol to approximately three-fourths of the length of the plate, and the triclocarban spot was located under shortwave UV light (254 nm). The spot was scraped off, collected in a 2.5-ml centrifuge tube, eluted with 0.3 ml of methanol for 10 min in a water bath at 60°, and centrifuged. The UV absorption spectrum of the eluate was recorded[9] using 50-μl capacity microcells[10]. Peak heights (absorbances) of the spectrum at 265 nm were used to determine the triclocarban.

Table I—Triclocarban Blood Levels by Different Analytical Methods (Rabbit Blood)

Standard Triclocarban, ppb	Radioisotope Technique: Counting Whole Blood, ppb	Radioisotope Technique: Counting Ether Extracts, ppb	TLC–UV Method, ppb
0	0	0	0
200	199	209	205
400	398	413	393
1000	969	1054	899
2000	2127	2100	1800
2400	2620	2382	2800
3000	3246	3225	2900
4000	4187	3881	4100
4000	4199	4278	4400
5000	5074	5336	5800

RESULTS AND DISCUSSION

The recovery of bacteriostatic from whole blood[11] is 89.8 ± 14.4% (average ± *SD*). This value was established by extracting blood samples with known amounts of triclocarban, from 100 to 50,000 ppb, and comparing with ether extracts to which the same amount of triclocarban was added after the extraction of blank whole blood. Although percent recovery was not compared below 100 ppb in this manner, it was possible to detect 50 ppb from blood samples inoculated with triclocarban, showing a good correlation with samples of higher concentrations. Recoveries were identical with both human and rabbit blood.

It was also established that the three repetitive extractions gave maximum recovery.

Recoveries decreased to 68% when samples were stored in a refrigerator for 6 weeks. However, samples stored for 1 month at 0° gave identical recoveries as fresh samples.

The linearity of the method was confirmed with blood samples through the standardization curves. Correlation coefficients (*r*) for linearity were calculated (7) for seven different sets of experiments, with three to five points for each set. They were found to be 0.999, 0.989, 0.995, 0.998, 1.000, 0.995, and 0.999, and all were very close to perfect linearity ($r = 1.000$).

The precision was determined by several replicate analyses of a

[1] 3,4,4′-Trichlorocarbanilide, TCC.

[2] Hewlett Packard model 7610 A, column OV-17, 2% on Chromosorb WHP with 5% methane in argon as flow and purge gas.

[3] 3,4,4′-Trichlorocarbanilide including radioactive compound (ring labeled as ^{14}C), Monsanto Co., St. Louis, Mo.

[4] Research Specialties Co., Richmond, Calif.

[5] Dowanol, Dow Chemical, Midland, Mich.

[6] Vortex Genie mixer, Scientific Industries, Mass.

[7] N-EVAP-A water bath with fitted test tube rack that has air flow device on the top, Organomation Associates Inc., Shrewsbury, MA 01545

[8] S&S material, 100–125 μm, 20 × 20 cm.

[9] Shimadzu MFP-50L, American Instrument Co., Silver Spring, Md., or Cary model 14, Cary Instrument Co., Division of Varian Co., Palo Alto, Calif.

[10] Beckman.

[11] Fresh human blood samples were donated by volunteers, and outdated human blood samples (3–6 weeks old) were obtained from Middlesex General Hospital, N.J. All samples were kept refrigerated until use.

human blood sample containing 500–1000 ppb of triclocarban. These blood samples were prepared by adding known amounts of triclocarban solution in absolute methanol (1.0 μg/μl). Four replicate samples for 500 ppb were spotted on one TLC plate and five replicate samples for 1000 ppb were spotted on another. The average relative standard deviation was 5.2%.

Samples for the radiotracer comparison were prepared by inoculating rabbit blood with a 0.1% methanol solution of ^{14}C-triclocarban. Ten samples ranged from 0 to 5000 ppb. Aliquots of each sample were analyzed for triclocarban using the present method (TLC–UV) and a radioisotope technique with both whole blood and their ether extracts. For isotope analysis, 100 μl of each blood sample was placed directly in 15 ml of 2,5-bis-2-(5,5-butylbenzoxazolyl)thiophene cocktail solution and counted in a liquid scintillation counter[12]. Ether extracts were evaporated to dryness, taken up in 1.0 ml of methanol, and counted in the same manner. The parts per billion values recovered for the unknown samples with three methods are compared in Table I. An analysis of variance (ANOVA test) showed no significant differences in recoveries among the three methods.

This method shows an average 90% recovery from blood with a good correlation with the amounts of triclocarban inoculated in blood. Results of *in vivo* studies with this method with animals following oral intubation and topical application of triclocarban will be published subsequently.

[12] Tricarb, Hewlett Packard.

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Clindamycin Phosphate: Neuromuscular and Blood Pressure Effects in Cats

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Abstract □ Three doses (10, 20, and 40 mg/kg) of clindamycin phosphate were tested in each of three feline neuromuscular preparations in a Latin-square sequence, and the muscular responses and blood pressures were compared. No statistically significant dose-related neuromuscular or blood pressure effects were observed.

Keyphrases □ Clindamycin phosphate—neuromuscular and blood pressure effects, cats □ Neuromuscular and blood pressure effects—clindamycin phosphate, cats □ Blood pressure and neuromuscular effects—clindamycin phosphate, cats

Clindamycin phosphate, a semisynthetic antibiotic produced by chlorination of lincomycin, is very similar in structure and antibacterial activity to its parent compound.

Neuromuscular depressive effects of lincomycin were demonstrated in experimental animals using doses comparable to those used clinically (1, 2). Lincomycin has been noted to interact with tubocurarine in a potentially clinically significant fashion to augment neuromuscular depression (3, 4).

Rapid administration of lincomycin has been associated with syncope, hypotension, and cardiac arrest (2, 5, 6).

Because of similarities between these drugs, this pilot experiment, employing the feline neuromuscular preparation, was undertaken to study the potential neuromuscular and blood pressure effects of clindamycin phosphate to determine if further studies were indicated.

Table I—Neuromuscular Effects

Dose, mg/kg	Depression of Time-Tension Integral Response, %
10	24 ± 15
20	15 ± 10
40	40 ± 30

EXPERIMENTAL

Three mongrel cats were anesthetized with halothane, nitrous oxide, and oxygen. A sciatic nerve–gastrocnemius muscle preparation was established as previously described (4, 7). Blood pressure was monitored *via* an arterial cannula and recorded electronically. Each animal received three doses (10, 20, and 40 mg/kg iv) of clindamycin phosphate according to a Latin-square design. Contractions were allowed to return to the control level prior to each administration. The data were examined by analysis of variance and the Student *t* test. The effect of calcium chloride (50 μg/kg) on recovery also was evaluated.

RESULTS

Neuromuscular depression in the cat was produced by all doses of clindamycin phosphate administered. The mean percent depression of the time–tension integral response with 1 *SD* for each dose is shown in Table I. Wide variation between the effects of the