Toxicologic Studies on Hydrocarbons. VI. A Colorimetric Method for the Determination of Kerosine in Blood

Horace W. Gerarde and Paul Skiba

During the course of a study on the toxicity of kerosine, there arose need for a method for the quantitative analysis of kerosine in blood. The ultraviolet spectrophotometric method developed for the analysis of alkylbenzenes in blood (1) was found to have limited application to the determination of kerosine blood levels, primarily because of its lack of sensitivity as well as the erratic readings that resulted from unusual background absorptions.

The purpose of this paper is to describe a simpler, more reliable, and more sensitive method for the determination of kerosine in blood. This procedure can be used in clinical hospital laboratories which do not have recording ultraviolet spectrophotometers. The method is based on the production of a color by the reaction of the aromatic hydrocarbons in kerosine with formaldehyde-sulfuric acid reagent (2). The kerosine is extracted from a hemolyzed blood sample with the use of carbon tetrachloride.

A photoelectric colorimeter is used to measure the color produced in the reaction of the aromatic hydrocarbons in kerosine with the formaldehyde–sulfuric acid reagent. The intensity of this characteristic color is recorded, and the concentration of kerosine is determined by reference to a previously prepared calibration curve. The method described has been used for the determination of kerosine in a 5-ml blood sample.

From the Medical Research Division, Esso Research and Engineering Co., Linden, N. J., and The Bureau of Biological Research, Rutgers State University, New Brunswick, N. J.

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MATERIALS AND METHODS

APPARATUS

1. Photoelectric colorimeter, Klett-Summerson Model 800-3, with filter no. KS-50. (Any photoelectric colorimeter of suitable sensitivity may be used. The intensity of the color is measured at a characteristic wave length of 490 mp.)
2. Test tubes, Klett No. 802, calibrated, graduated at 5 ml. and 10 ml.
3. Clinical centrifuge, International, with 4-place head for 15-ml. tubes and attached trunnion rings, and speeds variable up to 3000 rpm.
4. No. 3 hollow polyethylene stoppers.

REAGENTS

1. 0.1 N hydrochloric acid
2. C.P. carbon tetrachloride
3. 37% U.S.P. formaldehyde
4. C.P. concentrated sulfuric acid

CALIBRATION

1. Prepare primary standards of the kerosine dissolved in carbon tetrachloride. Suitable concentrations, by volume, are 5, 10, 15, 20, 25, 50, and 100 ppm.
2. Prepare the formaldehyde–sulfuric acid reagent by mixing 1.0 ml. of 37% U.S.P. formaldehyde with 100 ml. of C.P. concentrated sulfuric acid.
3. Pipet 5.0 ml. of the standard kerosine sample into the test tubes used in the colorimeter; to this add 5.0 ml. of the formaldehyde–sulfuric acid reagent. Cover the test tube with a polyethylene stopper and shake vigorously for 2 minutes. Note: Ordinary rubber stoppers or corks are not satisfactory; on contact with the acid reagent, they form colored solutions.
4. Centrifuge the tube for 5 minutes at 2000 rpm.
5. Measure the absorbency of each of the standard solutions including a 5.0-ml. sample of the formaldehyde–sulfuric acid reagent (to give 0.00 absorbency).
6. Prepare a curve by plotting absorbency versus concentration of aromatic hydrocarbons (ppm).
PROCEDURE

1. Place 35 ml. of approximately 0.1 N HCl in a 2-oz. wide-mouth bottle.
2. Deliver 5.0 ml. of blood from a syringe into the wide-mouth bottle. Cap the bottle tightly and invert it several times to allow mixing of the blood and acid.
3. Add 5.0 ml. of carbon tetrachloride to the hemolyzed blood by means of a pipet. Cap tightly and shake the bottle vigorously for 3–5 minutes.
4. Remove the upper (aqueous) layer from the bottle by using a blunt hypodermic needle and a vacuum. Exercise care not to remove any of the carbon tetrachloride layer when the last few milliliters of aqueous layer are being removed.
5. Centrifuge the carbon tetrachloride extract and carefully remove the supernatant aqueous layer.
6. Transfer the carbon tetrachloride extract to a Klett 802 test tube.
7. Pipet 5.0 ml. of the formaldehyde–sulfuric acid reagent into the tube containing the carbon tetrachloride extract of the blood.
8. Cap the test tube with a polyethylene stopper and shake it vigorously for 2 minutes.
9. Centrifuge the tube for 5 minutes at 2000 rpm.
10. Measure the absorbency (using a no. 50 filter).
11. Determine the concentration of kerosine by reference to the previously prepared calibration curve.

RESULTS AND DISCUSSION

The accuracy and precision of the method have been determined only for the analysis of samples containing known kerosines. The method determines the aromatic hydrocarbon fraction of the sample rather than the total kerosine. It can be applied to any pure aromatic hydrocarbon or hydrocarbon mixture which gives a color with the sulfuric acid–formaldehyde reagent.

Difficulty has been experienced in preparing standard, known concentrations of kerosine in blood because of its limited solubility. It has been found that mixtures containing more than 30 ppm kerosine in blood were not reproducible.

To study the recovery of kerosine from blood, known amounts of kerosine in a 0.1% concentration in acetone were added to oxalated rat blood. The recovery and typical results are presented in Table 1.
The kerosine used contained 13.8 per cent aromatics (Fluorescent Indicator Adsorption analysis—ASTM Method D-2, D1319)(3). A concentration of 10 ppm is equivalent to measuring 1.4 ppm of aromatic hydrocarbons. The blood samples containing lesser amounts give values that are similar to a blank sample free of kerosine. It appears that the lower limit of detection in blood is about 10 ppm.

The reproducibility of the analyses of the color measurement for a period of several weeks for standard carbon tetrachloride solutions of kerosine are shown in Table 2. Separate samples were treated with fresh reagent on each date.

Table 2. Precision of Analysis Using Standard CCl₄ Solutions of Kerosine

<table>
<thead>
<tr>
<th>Concentration prepared (ppm)</th>
<th>Concentration found (ppm)</th>
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<th>Mar. 25</th>
<th>Apr. 8</th>
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APPLICATION OF METHOD

Rats were deeply anesthetized with ether and forced to aspirate 0.1 ml of kerosine by retracting the tongue and placing the kerosine in the mouth while holding the animal's head in a vertical position. Blood was collected with a syringe at the bifurcation of the abdominal aorta at various intervals after dosing. It was immediately hemolyzed, and the hydrocarbons were extracted with carbon tetrachloride as described. The concentrations of kerosine found in the blood of these animals are shown in Fig. 1. The assumption is made...
that all of the hydrocarbons in the kerosine are absorbed into the blood stream from the alveolar capillaries at the same rate.

The kerosine is rapidly removed from the blood stream, as shown in Fig. 1. The level changes from 125 ppm 5 minutes after dosing to 52 ppm in 25 minutes. The concentration in the next 30 minutes (1 hour after dosing) changes from 52 ppm to 38 ppm. It is probable that the kerosine hydrocarbons are removed from the blood principally by storage in the fat deposits of the body. Another possible route of elimination is by urinary excretion.

The animals dosed with 0.1 ml. of kerosine by aspiration had severe and extensive pulmonary edema and hemorrhage. The LD₅₀ of kerosine by this route of administration is approximately 0.2 ml./kg. of body weight. The oral LD₅₀ of kerosine for the rat is approximately 28 ml. (4).

**SUMMARY**

A photoelectric colorimetric method is described for the quantitative determination of kerosine in blood. The procedure involves hemolysis of 5 ml. of the sample followed by extraction of the kerosine with carbon tetrachloride. The extract is reacted with a formaldehyde–sulfuric acid reagent to produce a characteristic color. The intensity of this color is measured photometrically, and the concentration of kerosine is determined by reference to a previously prepared calibration curve. Concentrations as low as 10 ppm can be conveniently determined.

**REFERENCES**