Trichloroacetic Acid (TCA)

Application	Determination in urine
Analytical principle	Photometry
Completed in	1976
Revised in	May 1983

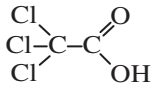
Summary

Trichloroacetic acid is excreted with the urine as the metabolic product of several chlorinated hydrocarbons. Hence, the method described here is diagnostically unspecific. However, due to the ease with which it can be carried out and its good analytical reliability, this method is still quite useful for screening purposes.

The TCA is reacted with pyridine in an alkaline medium at 65 °C to give a red reaction product whose extinction is measured photometrically at 530 nm and in a cuvette with a path length of 10 mm. The calibration curve is linear for concentrations between 3.0 and 55.0 mg/L.

Sensitivity:	Reciprocal calibration factor k' = 83.4 mg/L for the given experimental conditions	
Within-series imprecision:	Standard deviation (rel.)	s = 1.0%
	Prognostic range	u = 2.0%
	At a concentration of 30.4 mg/L TCA in urine and where n = 25 determinations	
Between-day imprecision:	Standard deviation (rel.)	s = 4.1%
	Prognostic range	u = 8.6%
	At a concentration of 25.0 mg/L TCA in urine and where n = 20 days	
Inaccuracy:	Recovery rate	r = 92–96%
Detection limit:	3.0 mg/L TCA in urine	

Trichloroacetic acid (TCA)



At room temperature trichloroacetic acid is a white, very hygroscopic and very caustic crystalline substance (mp 57.5 °C).

In humans TCA is formed as a metabolic product of chlorinated hydrocarbons such as 1,1,2-trichloroethylene (TRI); 1,1,1-trichloroethane; 1,1,1,2-tetrachloroethane; pentachloroethane; and tetrachloroethylene.

Since most of the TCA in the body is bound to plasma proteins [13,14], its excretion via the kidneys is very slow, having a half-life of about 100 h [15]. TCA thus tends to accumulate in the body [16–18].

Author: *A. Eben*
Examiners: *D. Henschler, W. Pilz*

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Contents

1 General principles

The trichloroacetic acid excreted in the urine is reacted with pyridine in an alkaline medium, according to the method of *Tanaka* and *Ikeda* [3]. The extinction of the red reaction product is determined photometrically at 530 nm.

Aqueous solutions with known concentrations of trichloroacetic acid are used to set up a calibration curve for quantitative analysis of the urine samples.

2 Equipment, chemicals and solutions

2.1 Equipment

UV-vis photometer with filter Hg 546 nm or spectrophotometer
Waterbath
10 mm Cuvettes (no plastic material!)
20 mL Reagent flasks (graduated) with ground-glass rim (14.5) and fitted glass tube (inner diameter, 3 mm; length, 100 mm)
0.5, 1, 2, 4 and 5 mL Volumetric pipettes
50 and 100 mL Volumetric flasks
10 mL Graduated test tubes with ground-glass stoppers
Desiccator
Vacuum pump

2.2 Chemicals

Trichloroacetic acid (TCA) p.a.
Phosphorus pentoxide for drying (e.g., Sikapent from Merck)
Pyridine p.a.
Toluene p.a.
Potassium hydroxide p. a.
The commercial trichloroacetic acid is dried under vacuum (10^{-2} mbar) over phosphorus pentoxide until its weight is constant.
Double-distilled water

2.3 Solutions

7.8 M Potassium hydroxide solution:
43.76 g Potassium hydroxide is made up to 100 mL with double-distilled water.

2.4 Calibration standards

Stock solution:
About 50 mg trichloroacetic acid (very hygroscopic!) is weighed out as quickly and accurately as possible and made up to 100 mL with double-distilled water (0.5 g/L). Calibration standards, ranging in TCA concentration from 5–50 mg/L, are made by diluting the stock solution with double-distilled water according to the following pipetting schedule:

Stock solution Volume mL	Calibration standards Final volume mL	Concentration mg/L
0.5	50	5
1.0	50	10
2.0	50	20
4.0	50	40
5.0	50	50

3 Specimen collection and sample treatment

A 1 mL urine sample is pipetted into a 20 mL reagent flask to which 2.5 mL 7.8 M potassium hydroxide solution, 5.0 mL pyridine and 0.5 mL toluene are added consecutively. After the contents of the flask have been thoroughly mixed, the glass tube is inserted into the flask and the flask is placed in a waterbath for 50 min at 65 °C. The sample solution is then cooled to room temperature and 3.0 mL of the pyridine layer is pipetted into 10 mL test tubes and mixed well with 0.6 mL water. A reagent blank is prepared in the same way, using double-distilled water instead of urine.

4 Analytical determination

Within 20 min after the pyridine layer and water have been mixed thoroughly, the extinction of the sample is measured against the reagent blank at 530 nm and in a cuvette with a path length of 10 mm.

5 Calibration

The calibration standards (compare Sect. 2.4) are subjected to the same treatment as the urine samples and determined photometrically (compare Sects. 3 and 4) to set up a calibration curve. The measured extinction values are plotted as a function of TCA concentration (Fig. 2). In the range of 3–55 mg/L the calibration curve is linear. The reciprocal calibration factor k' for a cuvette with a path length of 10 mm is:

$$k' = \frac{\rho}{\Delta E_{530\,\text{nm}/10\,\text{mm}}} = 83.4\ \text{mg/L}$$

where
ρ = mass concentration of TCA in the urine in mg/L
$E_{530\,nm/10\,mm}$ = extinction measured at 530 nm and in a cuvette with a path length of 10 mm

6 Calculation of the analytical result

The TCA concentration in the urine in mg/L that corresponds to the measured extinction value of the urine sample is read off the calibration curve.

7 Reliability of the method

7.1 Precision

To determine the imprecision within a series, 25 1 mL samples of urine collected over 24 h from a person who had been exposed to trichloroethene were analyzed. The relative standard deviation *(s)* was 1% and the prognostic range *(u)* was 2% for a TCA concentration of 30.4 mg/L.

Between-day imprecision was determined using commercially available synthetic urine with an average TCA concentration of 25.0 mg/L. Analyses over 20 days had a relative standard deviation *(s)* of 4.1% and a prognostic range *(u)* of 8.6%.

7.2 Accuracy

To determine the recovery rate, various concentrations of pure TCA were added to urine. As shown in Table 1, 92–96% of the TCA was recovered.

7.3 Detection limit

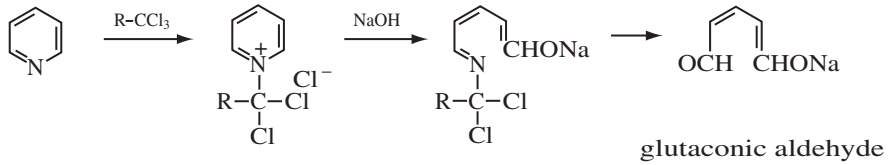
Under the given analytical conditions the detection limit was 3.0 mg/L TCA in urine.

7.4 Sources of error

There was no interference from trichloroethanol under the given conditions. Normally human urine contains no TCA or only very low concentrations (< 1 mg/L urine) [1, 2].

8 Discussion of the method

The method of *Tanaka* and co-workers [3] is based on the *Fujiwara* reaction [11], which was first used for the determination of chloroform. The mechanism of this reaction is still not completely understood. According to *Moss* and *Rylance* [4] glutaconic aldehyde is one of the end products:



Further details on the determination of TCA according to the pyridine-alkali method [5–7] can be found in the literature, as well as descriptions of a few gas chromatographic methods [8–10, 12].

Figure 1 shows the absorption spectrum between 430 and 610 nm of the reaction product of TCA and pyridine in alkaline solution under the conditions described here. The maximum is at 530 nm. The calibration curve for TCA at 530 nm and in a cuvette with a path length of 10 mm is linear for the concentration range 3.0–55.0 mg/L (Fig. 2). Thus, the conditions for the Lambert-Beer law are fulfilled in this concentration range and the analytical result can be determined directly from the measured extinction values using the reciprocal calibration factor *k'*.

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Table 1. Inaccuracy of the determination of TCA in urine.

Calculated concn. mg/L	Measured concn. mg/L	Recovery rate %
3.0	2.8	93
5.4	5.2	96
14.2	13.0	92
19.4	17.8	92
27.0	25.8	96
30.4	28.5	94
48.4	45.0	93
58.1	54.0	93

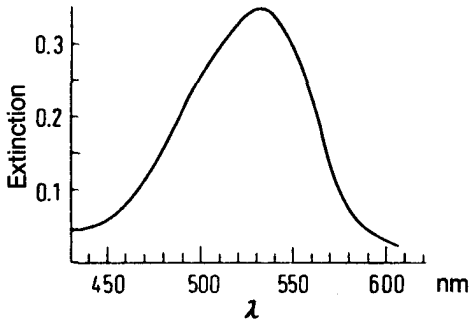

Fig. 1. Absorption spectrum of the reaction product of TCA and pyridine in alkaline solution (30.4 mg/L, 10 mm cuvette).

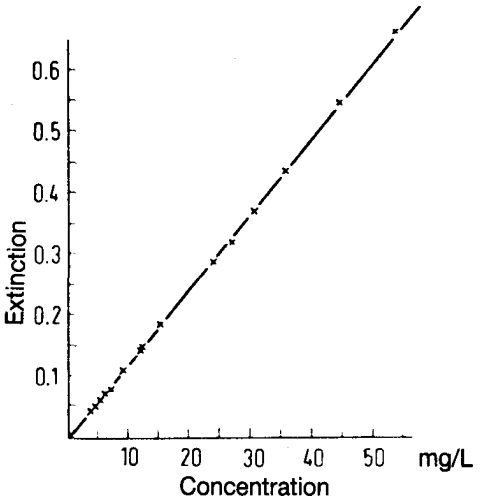

Fig. 2. Calibration curve for the determination of TCA in urine at 530 nm and a 10 mm cuvette.