APPENDIX III

METHOD FOR DETERMINATION OF MOCA (TM) IN URINE BY GAS CHROMATOGRAPHY*

I. Scope and Application

This is a method for the determination of low levels of methylenebis-(ortho-chloroaniline) (MOCA) in ethyl ether extracts of urine samples.

The method can also be used to measure the approximate concentration of LD-813 present in urine. The LD-813 level can be estimated by measuring the MOCA content and dividing the result by 0.4.

The method was developed using a Hewlett-Packard 5750 gas chromatograph equipped with a dual flame ionization detector and a 1-mv recorder.

Component	Formula	Range of Method, μg	Expected composition, μg
Chloroform	CHCl3	-	Solvent
Triphenylamine	(C6H5)3N	-	Internal Standard
MOCA Derivative	C17H10O2N2Cl2F6	2-28	5

II. Sensitivity, Precision, and Accuracy

The precision of this method at the 95% confidence limits, for ten degrees of freedom, is ± 0.02 mg/liter at the 0.1 mg MOCA/liter urine level and ± 0.14 mg/liter at the 0.5 mg MOCA/liter urine level. This precision was determined by two technicians on two separate days.

The lower limit of detection is 0.04 mg MOCA/liter of urine. This sensitivity was determined by adding decreasing amounts of MOCA to urine, extracting with ether, and analyzing.

Samples were prepared at 0.1 and 0.5 mg/liter levels on five separate days and analyzed. Accuracy was within the limits of the precision.

III. Safety Precautions

1. Ethyl ether is extremely flammable. Handle with care in a well-ventilated hood away from sparks and flames. The TLV is 400 ppm.

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*This method is one employed by E.I. duPont de Nemours and Company, Inc., Elastomer Chemical Department, Wilmington, Delaware 19898, and is identified by their Code No. U860.5200S, 30 July 1975.

2. Trifluoroacetic anhydride is a poisonous lachrymator. Avoid inhalation of vapors and skin contact. If spilled on the skin, flush immediately with copious quantities of cold water.

3. Chloroform is poisonous. It should be handled with adequate ventilation. Avoid repeated or prolonged contact with the skin. The TLV is 25 ppm.

4. Ethyl alcohol is a flammable material. Handle in a well-ventilated hood away from sparks and flames. The TLV is 100 ppm.

5. Handle triphenylamine with care as it is an aromatic amine. Keep off skin; handle in well-ventilated area.

6. MOCA is a "cancer-suspect agent" and is regulated by the Department of Labor, Occupational Safety and Health Administration's "Carcinogens - Occupational Health and Safety Standards." As required by this Standard, the following are posted at the regulated area: definition of the regulated area, handling procedures, decontamination procedures, waste disposal, emergency procedures, regulated area entry log sheets, and inventory log sheets. Only those persons specifically designated to work with MOCA are allowed to enter the regulated area.

7. The volumetric flask used in the preparation of MOCA standard solution must be charged with MOCA inside the designated hood area. For weighing purposes the flask must be decontaminated with acetone prior to removal from the hood. Dispose of decontamination rinsings into containers approved for this purpose.

IV. Reagents

1. Chloroform, reagent grade

2. Ethyl ether, anhydrous ACS grade

3. Trifluoroacetic anhydride, Eastman white label No. 7386**

4. Triphenylamine, Eastman white label No. 1907

5. Trisodium phosphate, 1% aqueous solution

6. Nitric acid, 1 + 1.

7. Citric acid, 30% aqueous solution

8. Ethyl alcohol, absolute, U.S.P.

9. Triphenylamine Internal Standard Solutions

**The mentioning of product names does not constitute endorcement by the Department of Health, Education, and Welfare.

a. Internal Solution A

1) Weigh (to the nearest 0.0001 g) 0.045 - 0.055 g triphenylamine (TPA) into a 100-ml volumetric flask.

2) Dilute to volume with chloroform and mix.

3) Calculate TPA concentration as follows:

Conc TPA Solution A, μg/ml - wt TPA, g x 10,000

b. Internal Solution B

1) Add (pipet) 4.0 ml of TPA Standard Solution A to a 100-ml volumetric flask.

2) Dilute to volume with chloroform and mix.

3) Calculate TPA concentration as follows:

Conc TPA Solution B, μg/ml = Conc TPA Solution A x 0.04

10. MOCA Standard Solutions (See Safety Precautions, Section III)

a. MOCA Standard Solution A

1) Weigh (to the nearest 0.0001 g) 0.0045 - 0.0055 g of MOCA into a 100-ml volumetric flask.

2) Dilute to volume with ethyl alcohol and mix.

3) Calculate MOCA concentration as follows:

Conc MOCA Solution A, μg/ml = wt MOCA, g x 10,000

b. MOCA Standard Solution B

1) Add (pipet) 10 ml of MOCA Standard Solution A to a 100-ml volumetric flask.

2) Dilute to volume with ethyl alcohol and mix.

3) Calculate MOCA concentration as follows:

Conc MOCA Solution B, μg/ml = Conc MOCA Solution A x 0.1

Note: MOCA Solution B contains approximately 5 μg/ml

c. MOCA Standard Solution C

1) Add (pipet) 50 ml of MOCA Standard Solution A to a 100-ml volumetric flask.

2) Dilute to volume with ethyl alcohol and mix.

3) Calculate MOCA concentration as follows:

Conc MOCA Solution C, μg/ml = Conc MOCA Solution A x 0.5

11. Sodium bicarbonate, 10% aqueous

12. Brilliant Yellow indicator strips

13. MOCA recrystallized to a 109 C minimum melting point

V. Apparatus

1. Bottles, specimen, milk dilution plain, Corning No. 1365

2. Caps, gum rubber, Davol "Sani-Tab" No. 268, 1-1/2 in. od

3. Separatory funnel, Squibb, 125-ml with TEFLON(TM) stopcock

Clean all glass apparatus by soaking for a minimum of one hour, preferably 16 hours, in 1% aqueous trisodium phosphate. Rinse with distilled water, 1 + 1 nitric acid, and distilled water.

VI. Column Packing

Partitioning liquid	OV-1
Source	Supelco, Inc., Bellefonte, Pa.
Parts by weight liquid per 100 parts support	11.1%
Support	Supelcoport
Source	Supelco, Inc., Bellefonte, Pa.
Mesh size	80 - 100

This column packing is sold commercially by Supelco, Inc., Supelco Park, Bellefonte, Pa. 16823

VII. Operating Conditions (See Note 1)

Column size	5 ft x 0.085 in. (id)
Column packing	10% OV-1 on Supelcoport, 80 - 100 mesh
Column material	Stainless steel
Column temperature	280 C isothermal
Carrier gas	Argon
Flow rate	7 ml/min
Detector temperature	290 C
Injection port temperature	290 C
Sample size	3 µl

Component	Actual Retention Time Min	Sec	Relative Retention Time*	Sensitivity
Chloroform		40	0.2	128 x 10(4)
Triphenylamine	3	30	1.0	128 x 1
MOCA derivative	8	25	2.4	16 x 1

*Relative to TPA

VIII. Interferences

Any material having a chromatographic elution time identical to the MOCA derivative will yield high results. High MOCA analyses have been reported by individuals under medication at the time of sampling. Some medications interfere grossly with the determination. Each medication should be studied individually for its possible interference with this determination. The following medications have been evaluated.

Gross Interference	Slight Interference	No Interferences
Darvon (TM)	Talwin (TM)	Ananase (TM)
Aldoril (TM)	Norgesic (TM)	Tylenol (TM)
		Citrated Caffeine
		Nyquil (TM)
		Contac (TM)
		Anacin (TM)
		Anahist (TM)
		Aspirin
		APC

Effects of inhalation or ingestion of industrial chemicals upon this determination are not known. Some indication of possible interference can be obtained by adding the known chemical to human urine and analyzing the sample according to the method. This procedure, however, does not take into account any possible metabolite of the chemical under study. Materials tested under these conditions and found to produce chromatographic peaks in the MOCA derivative region and possibly interfere include HVA-2, POLYAC, and ZENITE. The following do not interfere: ADIPRENE, A-101 oil, Diak 3, Glycol E, HYLENE MP, HYLENE W, Micro-cel E, NA-22, PACM, VITON, HYLENE TRF, IML-1, CAYTUR 4, hexamethylenediamine, dimethylethanolamine, dimethoxyethyl phthalate and dimethylformamide.

IX. Procedure

A. Sample Preparation

1. Clean all glassware as directed in Section V, Apparatus.

2. To the clean specimen bottle, add (grad cyl) 2 ml of 30% citric acid stabilizer solution. Seal the bottle with the "snap-on" rubber cap.

3. After the urine specimen has been placed in the bottle, seal the bottle with the cap as quickly as possible. Mix the solutions by swirling.

   The urine solution can be stored for several hours in its stabilized form; however, the time should be kept as short as possible to prevent degradation of the MOCA. See Section XII, Calibration for degradation rate of MOCA in stabilized urine.

4. Add (grad cyl) 50 ml stabilized urine sample to the 125-ml separatory funnel.

   Start standard by adding 1 ml of MOCA Standard Solution B to 50 ml of uncontaminated urine; swirl to mix and let stand 5 min.

5. Add (pipet) 10 ml of ethyl alcohol and shake to mix. Let solution stand 5 min.

6. Add (grad cyl) 5 ml of 10% sodium bicarbonate solution and shake to mix, venting the CO2 through the stopcock.

7. Test the urine with Brilliant Yellow indicator paper. If the solution is not definitely alkaline, add additional 1-ml

increments of bicarbonate until a positive test is obtained.

Brilliant Yellow paper changes to a bright red color in an alkaline medium.

8. Add 50 ml ethyl ether and shake steadily for 2 min.

Do not use intermittent shaking as an emulsion will result.

9. Let the solutions stand for 5 min to permit complete phase separation. Drain off the lower urine layer and discard.

10. Swirl the funnel to clear the interface and remove the last few ml of urine

11. Add (grad cyl) 5 ml of 10% sodium bicarbonate solution; shake for 10 sec (venting through the stopcock).

12. Let the solutions settle for 5 min and drain off the bottom layer.

B. Analysis for MOCA

1. Drain ether layer into a 30- x 80-mm vial. Evaporate the ether to dryness by blowing a stream of dry nitrogen into the vial.

Do not use air to evaporate the ether. A hot water bath may be used to hasten evaporation.

2. Add (medicine dropper) 1 ml trifluoroacetic anhydride. Allow the materials to react for a minimum of 10 min at room temperature.

3. Evaporate the contents of the vial to dryness using dry nitrogen.

4. Add (pipet) 1.0 ml TPA internal Standard Solution B and mix thoroughly.

5. Inject 3-μl samples into the chromatograph. (See Figure I)

Before analyzing any urine samples, condition the column by injecting several 6-μl samples of MOCA standard sample F. Preconditioning of the column is necessary to minimize adsorption and loss of MOCA.

X. Calculations

1. Determine the areas for MOCA and TPA using the formula:

Peak area, mm2 = peak height x width at 1/2 height x attenuation

2. Calculate the area ratio for MOCA

$$\text{Area ratio MOCA} = \frac{\text{area MOCA mm2}}{\text{area TPA mm2}}$$

3. Determine the weight ratio for MOCA from the calibration curve, Figure II. (See Section XII, A.)

4. Calculate μg MOCA as follows:

$$\text{MOCA, } \mu g = \text{wt ratio MOCA} \times \text{conc TPA Soln, } \mu g/ml$$

$$\text{MOCA extracted, mg/liter} = \frac{\mu g \text{ MOCA}}{\text{ml sample}}$$

5. Correct MOCA concentration for extraction efficiency as follows:

MOCA, mg/liter = MOCA extracted, mg/liter x Reciprocal Extraction Factor, see Figure III.

Report results to nearest 0.01 mg/liter.

XI. Column Standardization

Inject a sample which has been prepared according to the procedure (Section IX). Each peak must have the approximate retention time (± 5%), shape, and degree of resolution as those in a standard chromatogram. If these requirements are not met, check the operating conditions and/or replace the column.

XII. Calibration

A. Calibration Curve

1. Preparation of MOCA Stock Solution 1 (See Safety Precautions, Section III)

   a. Weigh (to the nearest 0.0001 g) 0.045 - 0.055 g MOCA into a 100-ml volumetric flask.

   b. Dilute to volume with ethyl ether.

   c. Calculate MOCA concentration as follows:

      Conc MOCA Soln 1, μg/ml = wt MOCA, g x 10,000

2. Preparation of MOCA Stock Solution 2

a. Pipet 10 ml MOCA Stock Solution 1 into a 1-liter volumetric flask.

b. Dilute to volume with ethyl ether.

c. Calculate MOCA concentration as follows:

Conc MOCA Soln 2, μg/ml = conc Soln 1, μg/ml x 0.01

3. Preparation of Standard Samples

a. Into five 30- x 80-mm vials, add (pipet) MOCA Stock Solution 2 as shown below:

Standard Sample	MOCA Stock Solution 2
A	0.4 ml (approximately 2 μg)
B	1.0 (approximately 5 μg)
C	2.0 (approximately 10 μg)
D	3.0 (approximately 15 μg)
E	4.0 (approximately 20 μg)
F	5.0 (approximately 25 μg)

b. Evaporate the standard samples to dryness under a nitrogen blanket. A water bath at 40 C may be used to aid evaporation.

c. Add (pipet) 1 ml trifluoroacetic anhydride and allow to react for 10 min.

d. Evaporate to dryness under a nitrogen blanket.

e. Add (pipet) 2 ml of triphenylamine Stock Solution B to each of the five standard samples.

f. Calculate weight ratio of MOCA/TPA in each standard sample, using the formula:

$$\text{Wt ratio MOCA/TPA} = \frac{\text{Conc MOCA Soln 2 x vol Soln 2 added, ml}}{\text{Conc TPA Soln x 1 ml}}$$

4. Preparation of Calibration Curve

a. Inject 3-μl sample of each standard sample into the chromatograph. Condition the column before injecting any samples (see Note 1).

b. Measure peak areas of MOCA derivative and TPA using formula:

Peak area mm2 = peak height x width at 1/2 height x attenuation.

c. Calculate area ratio of MOCA derivative and TPA as shown:

$$\text{Area ratio MOCA} = \frac{\text{area MOCA mm2}}{\text{area TPA mm2}}$$

d. Plot area ratio versus weight ratio for MOCA and draw a curve through the points. (See Figure II.)

B. Extraction Factor

1. Preparation of MOCA/Urine Solutions

a. Add (grad cyl) 50 ml of stabilized, uncontaminated urine to 20 separatory funnels.

b. Add (pipet) 1 ml of MOCA Standard Solution B to each of 10 funnels and 1 ml (pipet) of MOCA Standard Solution C to the other 10.

c. Swirl to mix and let stand 5 min.

2. Preparation of Extraction Curve

a. Proceed starting from Section IX, A, step 5.

b. Determine mg/liter MOCA extracted from Section X, Calculation.

c. Calculate Reciprocal Extraction Factor as follows:

$$\text{Reciprocal Extraction Factor} = \frac{\mu\text{g/ml MOCA Standard Soln x 1 ml}}{\text{mg MOCA Extracted}}$$

d. Average Extraction Factors for each solution.

e. Plot mg/liter MOCA Extracted versus Extraction factors and draw a curve through the points. (See Figure III.)

C. Degradation Rate

1. Preparation of MOCA/Urine Solutions

a. Add (grad cyl) 50 ml of stabilized, uncontaminated urine to 14 specimen bottles.

b. Into seven bottles, add 1 ml of MOCA Standard Solution B and into the other seven add 1 ml of MOCA Standard Solution C.

c. Swirl to mix and store at room temperature

d. Analyze for MOCA, mg/liter on 1,2,3,4,5,9, and 13 days, starting with Section IX, A, step 5.

2. Preparation of Degradation Curve

a. Plot MOCA, mg/liter versus time and draw a curve through the points. (See Figures IV and V.)

XIII. Notes

1. Condition the column daily by injecting several 6-μl samples of MOCA derivative from standard sample F. The column must be conditioned before any urine samples are run for MOCA content.

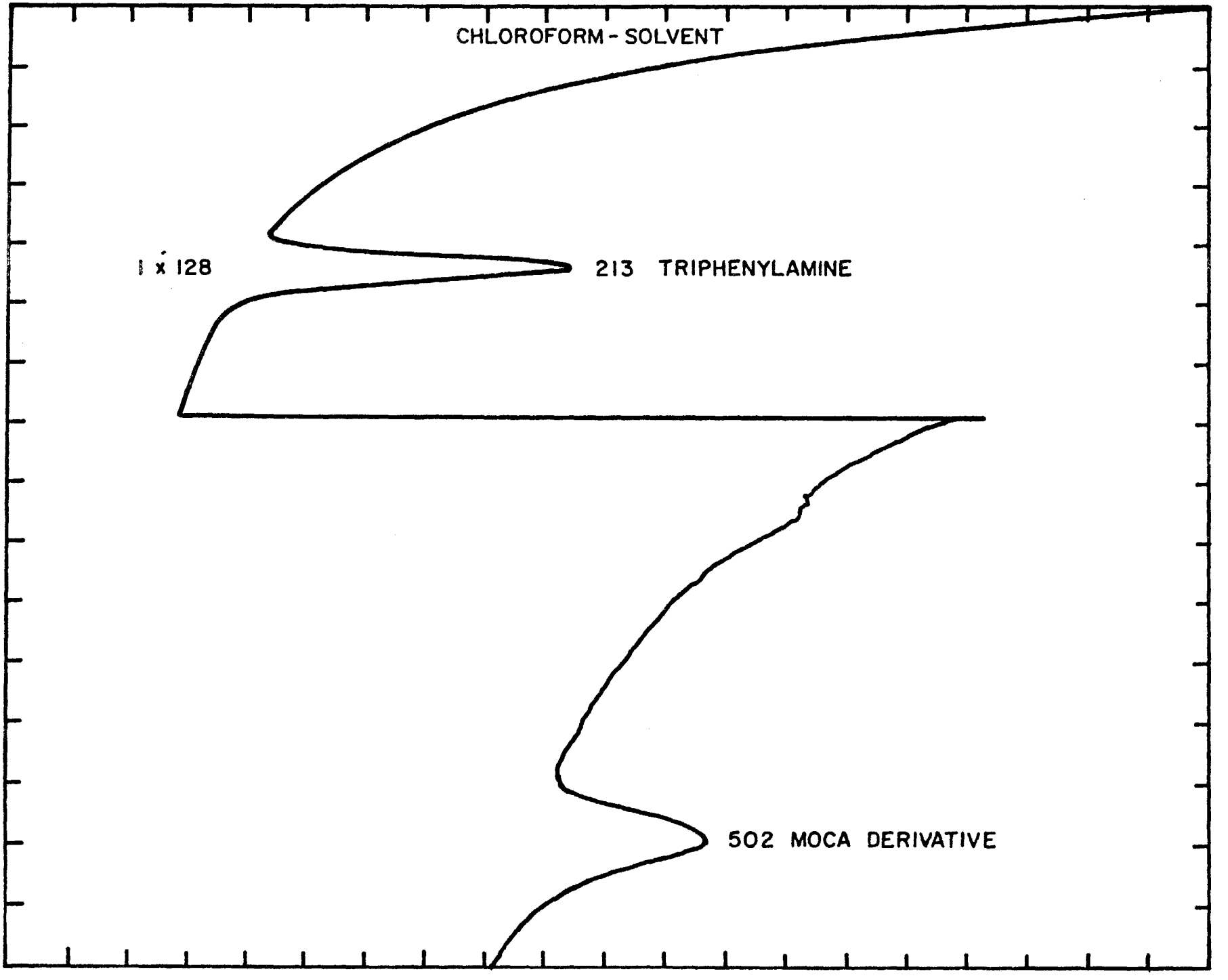

Figure 1 MOCA elution curve

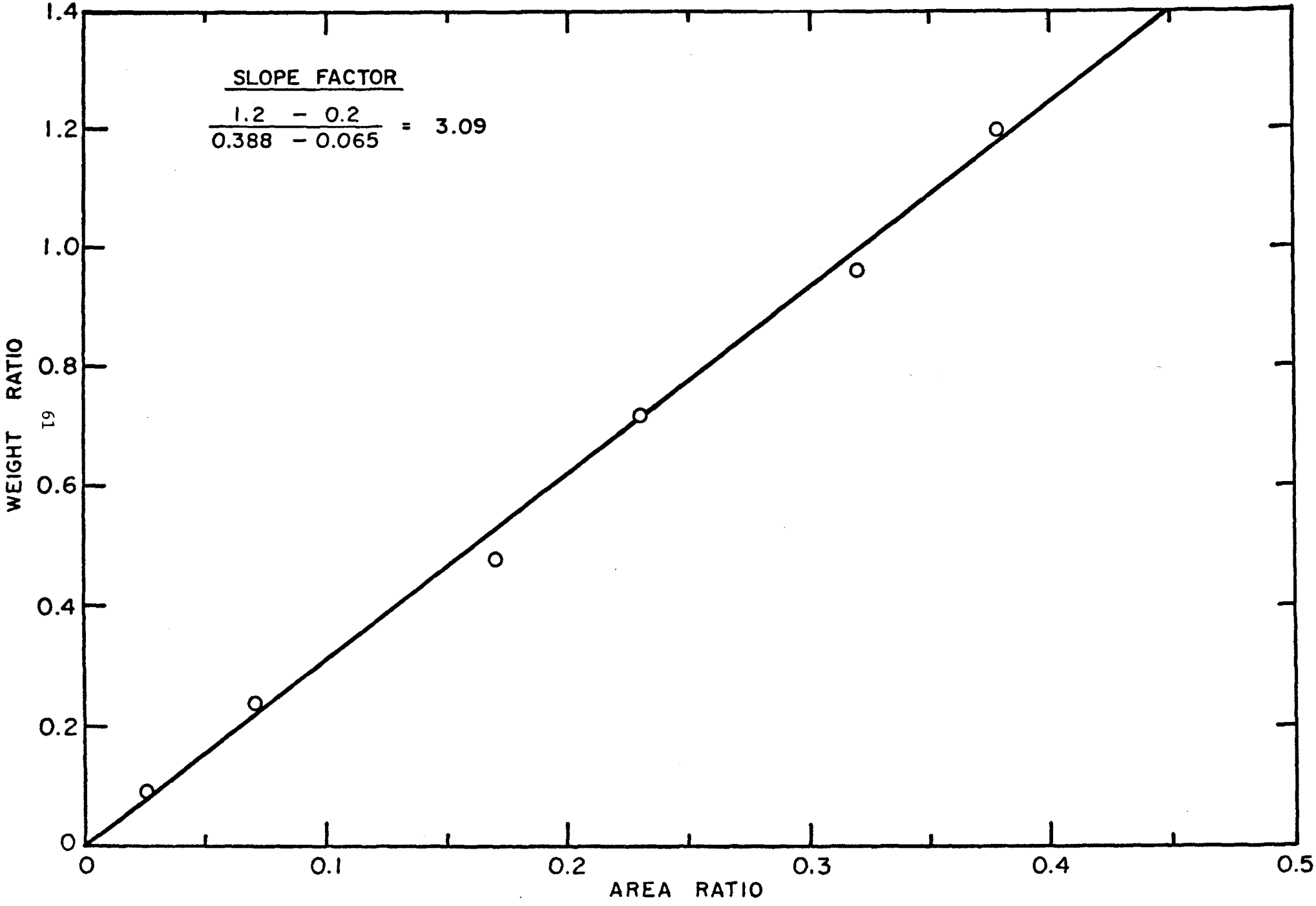

Figure 2. Calibration curve, moca in urine.

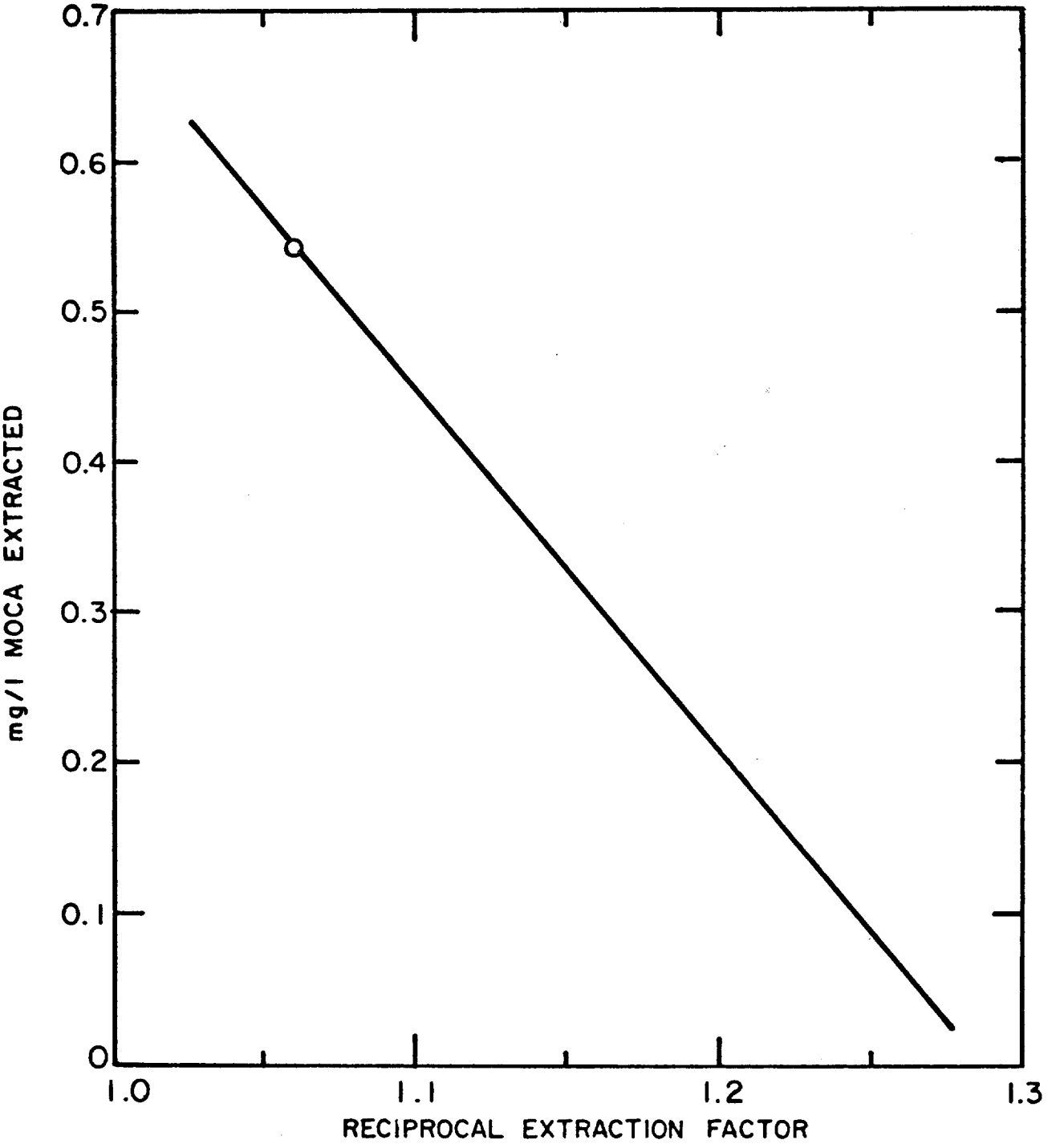

Figure 3. Extraction efficiency factor for MOCA in urine.

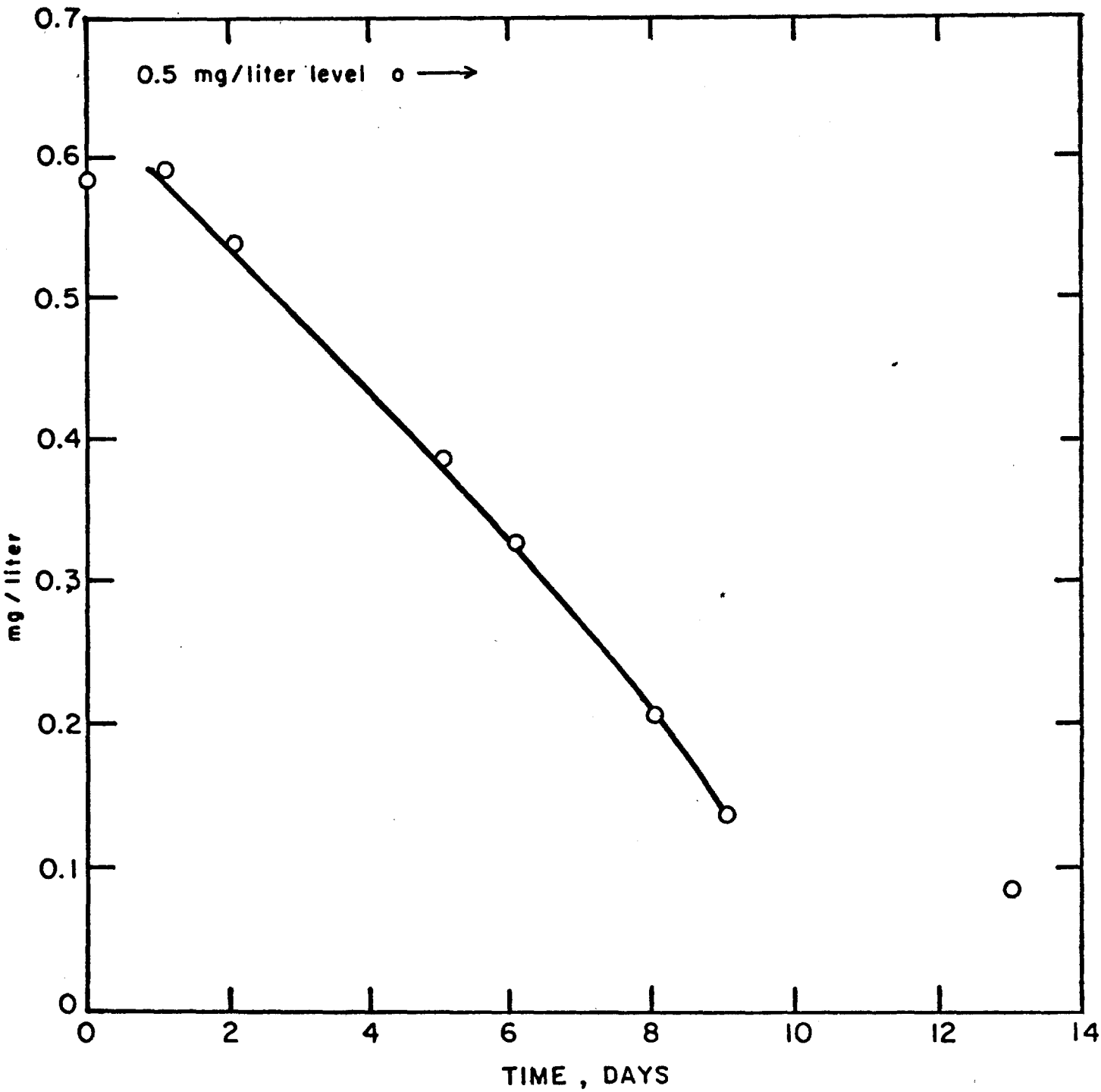

Figure 4. Moca in urine - degradation rate at room temperature.

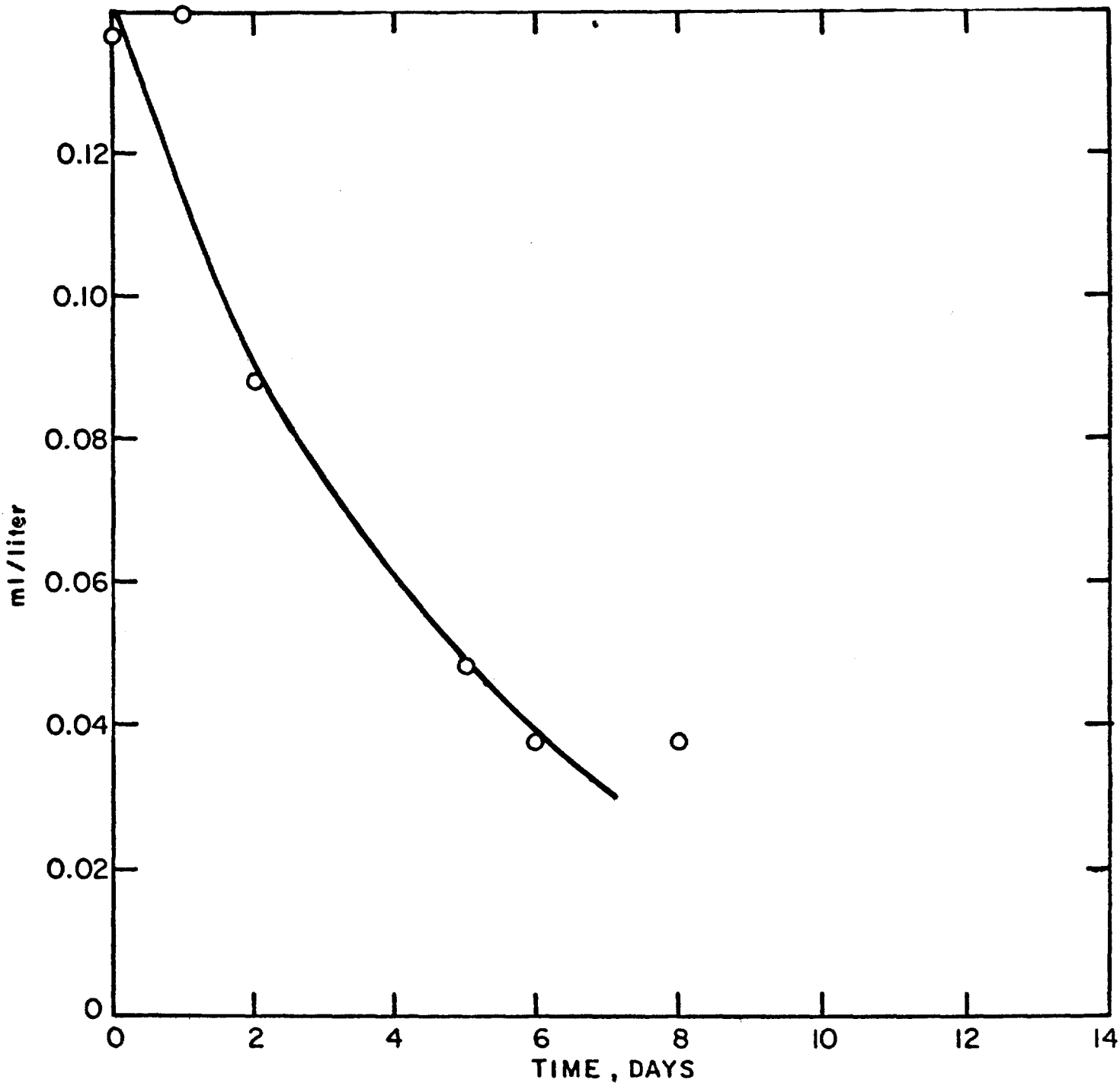

Figure 5. Moca in urine degradation rate at room temperature 0.1 mg/liter level.

APPENDIX IV.

METHOD FOR DETECTION OF MOCA (TM) CURING AGENT ON ENVIRONMENTAL SURFACES BY COLORIMETRIC SPOT TEST*

I. Scope and Application

This colorimetric method is applicable to the detection of MOCA on environmental surfaces and on protective clothing in the absence of other primary aromatic amines. The method is simple and can be readily used in the field.

II. Principle

The method is based upon the formation of a color complex by the reaction of the amine group of MOCA with nitrous acid to form the diazonium salt and subsequent coupling with itself. The nitrous acid is prepared "in situ" by reacting an aqueous solution of sodium nitrite with acetic acid.

III. Interferences

This method is a general colorimetric spot test for primary aromatic amines and not specific for MOCA.

Environmental surfaces and protective clothing may be contaminated with material which is colored originally. A positive test is one in which color is formed or increased after addition of the last reagent.

IV. Sensitivity, Precision, and Accuracy

The lower limit for the detection of MOCA using the colorimetric spot test was determined by swabbing an 0.05 sq. meter stainless steel area. Solutions of decreasing concentrations of MOCA were evaporated off the tray. The tray was then swabbed. The lower limit of one milligram per sq. meter gave readily seen color.

V. Apparatus

1. Cotton swabs
2. Glass vials

VI. Reagents (Reagent Grade)

1. Dimethylformamide (DMF)
2. Acetic acid

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*This method is one employed by E.I. duPont de Nemours and Company, Inc., Elastomer Chemical Department, Wilmington, Delaware 19898, and is identified by their Code No. E350.5200S, 15 March 1976.

3. "Nitrite" solution

Dissolve 20 g sodium nitrite in 100 ml distilled water.

VII. Safety Precautions

1. DMF is rapidly absorbed through the skin and can carry other chemicals into the skin. DMF can pave the way for dermatitis. DMF is classified as a teratogen and females of childbearing age should not work with this chemical. If DMF comes in contact with the skin, wash well with water immediately.

2. Reagent grade acetic acid can cause serious burns and blisters to the skin. Wash well with water immediately if acetic acid is spilled on the skin.

3. MOCA may cause cancer based on tests with laboratory animals. Avoid skin contact and inhalation of vapor and dust. For details on safe handling practices, read bulletin AP 710.1.

VIII. Procedure

1. Place 10 ml of DMF in a glass vial.

2. Place a cotton swab into the glass vial containing DMF.

3. Wait 30 sec.

4. Wipe an area 8 in. x 10 in. with the cotton swab. Go over the area three times.

5. Place the cotton swab back into the DMF.

6. Wait 30 sec.

7. Add approximately 1 ml of acetic acid to the vial.

8. Add approximately 1 ml of "nitrite" solution to the vial.

9. Observe solution for development of an orange-brown color. The color which forms within 1 minute indicates that MOCA may be present (see Note 1).

IX. Notes

1. If the solution has color after swabbing, it is advisable to obtain two swabs of the same area (adjacent locations). One vial acts as a blank and compensates for any color pickup due to swabbing. The blank is treated exactly the same as the sample vial except that nitrite solution is not added. The two vials can be viewed side by side; any color increase in the nitrite treated sample can be easily noted.

A quantitative method for detection of certain carcinogens on metal, painted and concrete surfaces has been developed by Weeks, R.W. Jr., Dean, B.J. and Yasuda, S.K. and has been published in Analytical Chemistry Vol 48: 2227-2233, 1976.

☆ U.S. GOVERNMENT PRINTING OFFICE: 1978— 757-141/1839

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