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DETERMINATION OF DISTEARYLCARBAMOYL CHLORIDE BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

A rapid and specific method for the determination of distearylcarbamoyl chloride in different extracts by high-performance liquid chromatography (HPLC) has been developed. In the method hexane extract containing this compound is evaporated and then treated with sodium thiophenolate to give the stabile thiocarbamate. This is determined by HPLC on a silica gel column with UV detection, using *n*-hexane–dichloromethane (7:3) as eluent. The sensitivity is about 0.1–0.5 μg.

INTRODUCTION

Carbamoyl chlorides are reactive compounds important as intermediates in the synthesis[1] of, *e.g.*, carbamates, thiocarbamates and substituted ureas. However, few methods for the analysis of these compounds have been published. Normally, large amounts of carbamoyl chlorides are analyzed[2] by titration of liberated chloride ions after reaction with an appropriate amine. Recently Rusch *et al.*[3] determined dimethylcarbamoyl chloride in air using a spectroscopic technique after reaction with 4-(*p*-nitrobenzyl)pyridine.

Distearylcarbamoyl chloride (DACC) has been found to be an excellent sizing agent for paper[4]. In the paper making process cellulose fibres are made hydrophobic by the reaction between cellulose hydroxyl groups and the carbamoyl chloride. Here we describe a high-performance liquid chromatographic (HPLC) method of determining distearylcarbamoyl chloride by derivatization with sodium thiophenolate (Fig. 1) to give the stable, UV-absorbing thiocarbamate. The method has been used routinely for small amounts of dialkylcarbamoyl chlorides in extracts from paper treated with a sizing agent (Kenotaf®) containing mainly distearylcarbamoyl chloride.

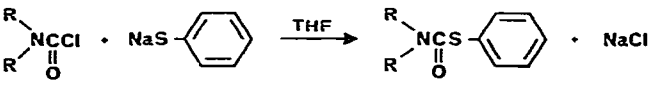

Fig. 1. Structure of distearylcarbamoyl chloride (DACC). On reaction with sodium thiophenolate a stable and UV-absorbing thiocarbamate is formed. R = mainly $C_{18}H_{37}$ containing about 10% $C_{16}H_{33}$ and small amounts of $C_{12}H_{25}$ and $C_{14}H_{29}$.

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MATERIALS AND METHODS

Reagents

n-Hexane, methylene chloride, chloroform and toluene were reagent grade from E. Merck (Darmstadt, G.F.R.). Tetrahydrofuran (THF) (E. Merck, reagent grade) was purified immediately before use by filtration through a column of Al_2O_3 (W 200, basic, activity super 1; ICN Pharm., Eschwege, G.F.R.). Sodium thiophenolate was prepared from thiophenol, pract. grade, (Fluka, Buchs, Switzerland) according to Jenden *et al.*[5].

Silica gel for column chromatography (Si 60, 0.063–0.2 mm and 0.2–0.5 mm) and silica gel thin-layer plates (HF_{254}) were purchased from E. Merck.

Chromatography system

An HPLC system based on a Waters 6000 pump (Waters Assoc., Milford, MA, U.S.A.) was used throughout this work in conjunction with an Altex 153 UV detector (Altex Scientific, Berkeley, CA, U.S.A.), fitted with filters for 254 nm, and a Waters U6K loop-injector. The column was a stainless-steel tube (30 cm × 3.9 mm I.D.) prepacked with 10-μm silica gel (Waters Assoc.). The system was kept at ambient temperature.

Distearylcarbamoyl chloride (DACC)

This compound, prepared from distearylamine and phosgene, is available from KemaNobel (Stockholm, Sweden) as Kenotaf®. Since the amine used as a starting material is synthesized from hydrogenated tallow fatty acids, DACC will comprise *ca.* 80% stearyl, 10% palmityl and 10% lauryl and myristyl carbon chains.

DACC technical grade (85% carbamoyl chloride), containing amine hydrochloride and alkylurea as impurities, was purified by passing through a silica gel column. About 100 g of DACC in 150 ml of carbon tetrachloride–chloroform (8:2) were rapidly passed through silica gel (200 g, 0.063–0.2 mm) in a glass filter (G3, diameter 10 cm). The gel was washed with 400 ml solvent. Evaporation of the combined washings yielded 75 g of DACC, free from amine hydrochlorides.

Preparation of reference sample

Purified DACC (5 g, 7.7 mmoles) and sodium thiophenolate (2.27 g, 17.2 mmoles) were refluxed in THF (30 ml) for 2 h under nitrogen. After evaporation the residue was distributed between 2 *M* sodium hydroxide (100 ml) and *n*-hexane (2 × 60 ml). The hexane layers were collected, dried (anydrous sodium sulphate) and evaporated. The residue was purified by silica gel column chromatography using toluene as eluent. The reaction and purification was followed by thin-layer chromatography (TLC) (silica gel, toluene). Three spots could usually be seen: DACC (R_F = 0.54, ninhydrin positive); unidentified by-product (R_F = 0.5, UV-absorbing); DACC-thiocarbamate (R_F = 0.39, UV-absorbing). The DACC-thiocarbamate was identified by NMR and mass spectrometry (see Discussion).

Extraction

The extraction studies were made on unbleached sulphate paper-board, base paper weight 300 g/m^2. The paper was coated on both sides with polyethylene, 60

g/m^2. However, both uncoated and coated paper were extracted. Either the paper was cut into pieces and extraced in a flask or the extraction cell test was used (ASTM F 34-68).

The extractions were performed with 10 g paper (or 6 dm^2 in the ASTM cell) using 200 ml *n*-heptane, 50% aqueous ethanol, 3% aqueous acetic acid and water. The aqueous solutions were extracted with *n*-hexane (2 × 50 ml). The combined hexane extracts were dried with anhydrous Na_2SO_4, filtered and the solvent was evaporated in a rotary evaporator at 45°C. The *n*-heptane extract was filtered directly and evaporated as above.

Derivatization

The hexane or heptane extracts were evaporated to near dryness in a rotary evaporator and transferred by means of a few millilitres of dichloromethane to a screw-cap vial (3 ml, No. 13222; Pierce, Rockford, IL, U.S.A.) and the solvent was evaporated using a nitrogen stream. Sodium thiophenolate (20 mg) and THF (1 ml) were added, the air was removed by flushing with nitrogen and the vial was closed (PTFE-lined caps) and heated at 60°C for 2 h.

The reaction mixture was transferred to a test-tube (the vial being thoroughly rinsed with small amounts of hexane and 2 *M* NaOH). A 15-ml volume of 2 *M* NaOH was added and the solution was extracted with *n*-hexane (2 × 5 ml). The combined hexane extracts were collected, dried with anhydrous Na_2SO_4, filtered (through a small glass-wool plug in a Pasteur pipette) and evaporated by means of a stream of nitrogen. The tubes and the Na_2SO_4 were rinsed several times with small amounts of *n*-hexane which were then filtered. The filtered solution was evaporated to dryness (nitrogen) and the residue dissolved in *n*-hexane–dichloromethane (7:3) (0.5 ml). A 5-μl volume was immediately injected into the chromatograph.

Liquid chromatography

An HPLC silica gel column containing 10-μm particles and with *n*-hexane–dichloromethane (7:3) at 1.5 ml/min as eluent was used. Before running the samples a standard solution of DACC-thiocarbamate (*e.g.*, 0.2 mg/ml solution in the eluent) was run as a control of the retention time and column efficiency. After a number of runs with samples from the paper extracts the column deteriorated and no adequate separation was obtained. The column was regenerated by successively washing with dichloromethane (150 ml), *n*-hexane (150 ml) and *n*-hexane–dichloromethane (7:3) (50 ml).

Sample clean-up by column chromatography

Some extracts especially from polyethylene-coated papers gave poor chromatograms due to impurities obscuring the DACC-thiocarbamate peak. In order to achieve better separation and longer HPLC column life, a clean-up was performed by chromatography on a small silica gel column (13 × 1 cm, 5 g, Merck silica gel 60, 0.2–0.5 mm particle size) in *n*-hexane–dichloromethane (8:2). After filtration and evaporation, the derivatized sample was dissolved in 1 ml *n*-hexane–dichloromethane (8:2) and placed on to the column. Most of the impurities comprising low-molecular-weight polyethylene and diphenyl disulphide were eluted with 60 ml *n*-hexane–dichloromethane (8:2) and discarded. The solvent was then changed to dichloromethane

and 70 ml were collected in a round-bottomed flask. After evaporation of the solvent the residue was transferred to a test-tube, again evaporated in a stream of nitrogen, dissolved in 0.5 ml *n*-hexane–dichloromethane (7:3) and submitted to HPLC as above.

Calibration curve

A calibration curve was constructed using solutions containing known amounts of DACC and *n*-hexane extracts from non-DACC-treated papers (= blank extract) corresponding to *ca.* 10 g paper. A 1-ml chloroform solution of a blank extract was transferred to each of five screw-cap vials and evaporated by means of a stream of nitrogen. Sodium thiophenolate (20 mg) was added to each vial and then 1 ml THF containing 0.05, 0.1, 0.25 or 0.5 mg DACC. The derivatization, extractions and HPLC were performed as described above.

RESULTS AND DISCUSSION

Before considering the use of new chemicals in food or in contact with food, authorities in most countries not only demand toxicological studies but they also request specific studies of the amount of the chemicals which might contaminate the food. Since DACC is used for sizing of paper intended for food packaging a series of extractions have been performed. Under constant conditions, food simulating solvents, *e.g.*, *n*-heptane, 50 % aqueous ethanol, 3 % aqueous acetic acid and water, were used. DACC was to be determined in small amounts in these different types of extracts.

The complete extraction of DACC from water and the aqueous solutions described above with *n*-hexane was confirmed by the following experiment. DACC (0.5 mg) in hexane (50 ml) was extracted with 200 ml of the appropriate solvent. The DACC content in the hexane solutions was determined by HPLC and compared with the same amount of DACC in a hexane solution not partitioned with any solvent (see Table I). Thus, since DACC is a very lipophilic molecule and can be fully extracted with hexane the problem was reduced to determining DACC in dilute solutions of hexane (or heptane).

DACC is a reactive compound. It was not possible to use gas chromatography (GC) for quantitative analysis, due to the instability and the high molecular weight. Furthermore, GC separates DACC into four peaks due to the combinations of C_{14}.

TABLE I

EXTRACTION OF DACC FROM *n*-HEXANE WITH AQUEOUS SOLUTIONS

DACC content determined by HPLC.

Hexane phase	*DACC content in n-hexane phase (peak height in mm)*
Not extracted	153
Water extracted	157
3 % CH_3COOH extracted	163
50 % C_2H_5OH extracted	159

C_{16} and C_{18} carbon chains. Also underivatized DACC decomposed to the secondary amine when submitted to liquid chromatography on silica gel. However, by treating DACC with sodium thiophenolate (Fig. 1) a stable compound was formed which gave only one sharp symmetrical peak in HPLC. One further advantage of the derivatization was that UV detection could be used.

The structure of the thiophenyl carbamate of DACC was confirmed by nuclear magnetic resonance (NMR) and mass spectrometric studies. In ^{13}C NMR the signal from the carbonyl group is shifted from 149.0 ppm in DACC to 166.1 in the thiocarbamate, an expected low field shift due to the phenyl group. The thiocarbamate also exhibits signals in the region 128–136 ppm from aromatic carbons. It is interesting that CH_2–N in DACC gives two signals at 51.3 and 50.0 ppm, as for example in amides, but only one signal at 48.2 ppm is found for the thiocarbamate.

Mass spectral data for the thiophenyl carbamate of DACC are summarized in Table II. Using electron ionization it was impossible to differentiate between DACC and its thiophenyl carbamate derivative. The spectra of both compounds contained no M ion, but an ion with the same mass corresponding to M − Cl and M − thiophenyl respectively. By using chemical ionization the MH^+ ions were detected and a cleavage of MH^+ − thiophenyl confirmed the structure of the thiophenyl carbamate.

TABLE II

MASS SPECTRAL DATA OF THE THIOPHENYL CARBAMATE OF DACC (FIG. 1)

Collected on a Micromass 7070F instrument (V. G. Micromass, Winsford, Great Britain) using the direct inlet system. EI = Electron ionization; CI = chemical ionization with isobutane.

R groups in R_2N^-	*Theoretical m/e*		*Found m/e*		
	M	*M − thiophenyl* (S)	*with EI for M − thiophenyl*	*with CI*	
				MH^+	MH^+ − *thiophenyl*
C_{14}, C_{16}	573	464	464	574	464
C_{14}, C_{18}	601	492	492	602	492
C_{16}, C_{16}	601	492	492	602	492
C_{16}, C_{18}	629	520	520	630	520
C_{18}, C_{18}	657	548	548	658	548

The retention time of this synthetic thiophenyl carbamate of DACC was 4.5 min under the HPLC conditions stated above. A peak appeared at exactly the same retention time in chromatograms from derivatized extracts of DACC-treated papers. Typical chromatograms are shown in Figs. 2 and 3.

To show that quantitative determinations were made on the correct compound, several extracts from DACC-treated paper were combined and submitted to preparative HPLC in which peak A (Fig. 2) was collected. The ^{13}C NMR spectrum of the collected substance was identical with that obtained from a reference thiophenyl carbamate of DACC.

The HPLC method was calibrated by adding known amounts of DACC (0.05–

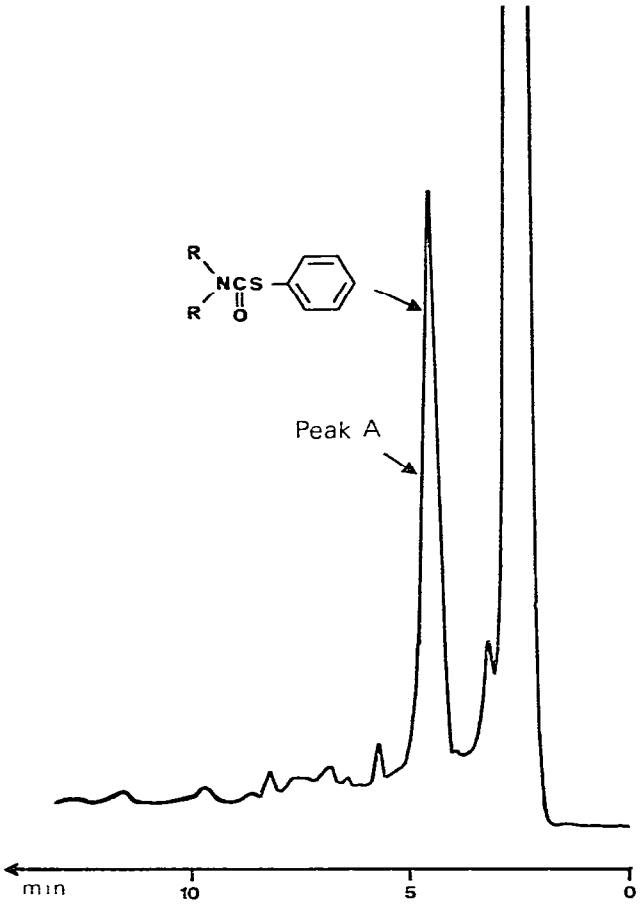

Fig. 2. Chromatogram of 5 μl of derivatized heptane extract from a paper containing 0.35 mg DACC. Conditions: 250 × 3.9 mm I.D. column containing silica gel (10 μm) with *n*-hexane–dichloromethane (7:3) as mobile phase, flow-rate 1.5 ml/min; UV detection at 254 nm with 0.08 a.u.f.s.

0.5 mg) to extracts from paper not treated with DACC and taking these standards through the assay procedure. The amount of DACC in paper extracts was then determined from a calibration curve (Fig. 4) constructed by plotting the height of the thiophenyl carbamate peak *versus* the amount DACC added to the extract.

It was found important to investigate the specificity of the derivatization reaction since both tetraalkylurea and the ethyl carbamate (Fig. 5) could be formed from DACC during extractions of DACC-treated papers in water and alcohol solutions respectively. However, no peak with the same retention time as the thiophenyl carbamate of DACC was detected in HPLC when 0.5 mg of the tetraalkylurea or the ethyl carbamate were submitted to the derivatization procedure.

The reproducibility of the method was investigated by taking four replicate samples of 0.05–0.5 mg through the whole procedure, including extraction from a water solution. The results are shown in Table III. Each solution was injected four times into the chromatograph.

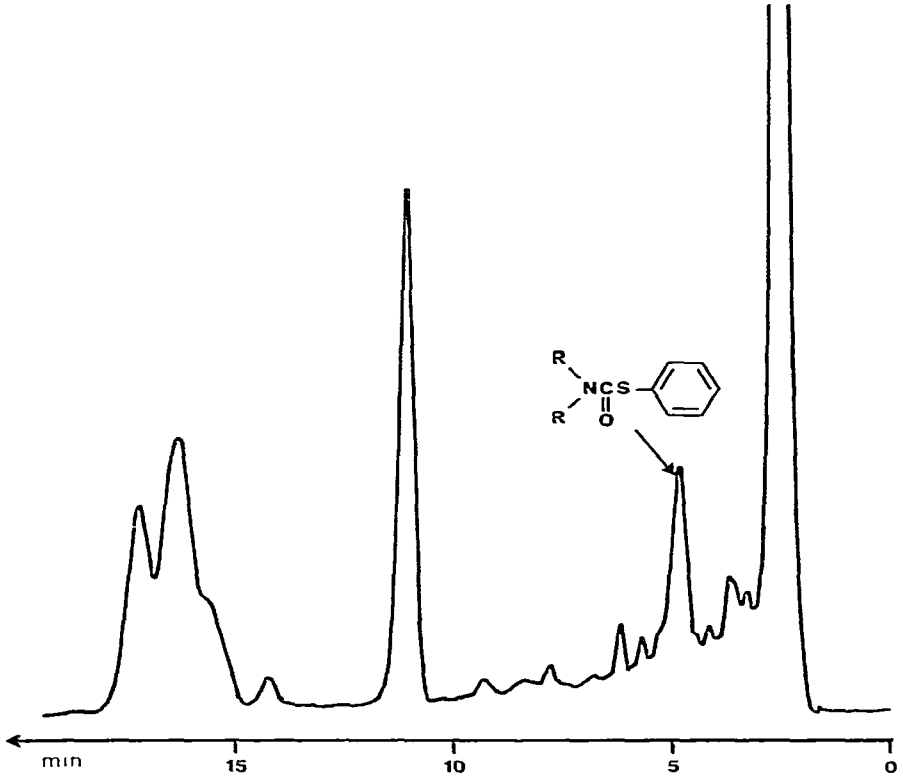

Fig. 3. Chromatogram of 0.05 mg of DACC in *ca.* 9 mg of an extract from bleached untreated paper (140 g/m^2). Conditions as in Fig. 2.

The recovery was evaluated by adding 0.25 mg DACC to a water–hexane mixture and then analyzing the hexane phase by the described method. The recovery was determined to be 82 % by comparing the peak height with that obtained by direct injection of the same amount of pure thiophenyl carbamate of DACC.

The minimum amount of DACC detectable depended on a number of factors, *e.g.*, paper qualities, presence of coating and purification of the extract by silica gel chromatography. In extracts from 10 g unbleached paper (300 g/m^2), 0.05 mg added, corresponding to an injected amount of 0.5 μg, gave a peak height of 40 mm at a detector attenuation of 0.08. It was possible under these conditions to detect 0.1 μg, but no efforts have been made to reach a better sensitivity.

Some results obtained are shown in Table IV. The paper-board was produced with 0.2 % DACC in a full scale trial, and assuming 50 % retention the DACC content in the paper should be 3 mg/dm^2. DACC reacts with the fibres during the drying procedure and the storage. The curing was followed by measuring the decreasing amount of absorbed lactic acid. Thus a more hydrophobic paper should show a smaller amount of DACC extractable by heptane. Table IV shows the extractable amounts of DACC and the absorption of lactic acid *versus* storage time.

From toxicological studies a No Toxic Effect Level (NTEL) for a substance can be deduced. In this case it was concluded that 10 mg DACC per kg bodyweight

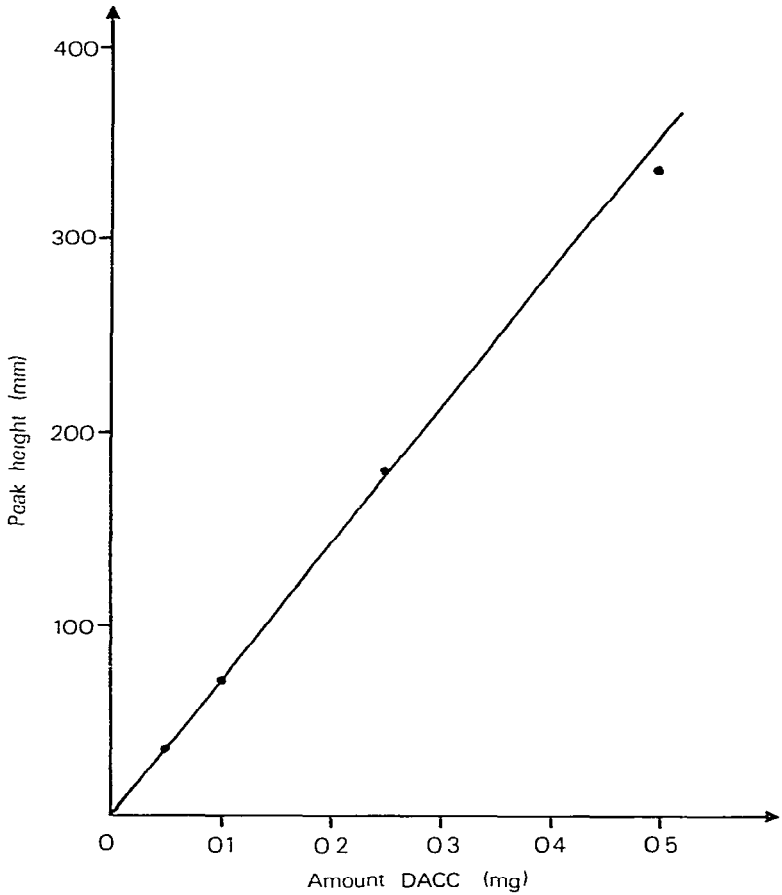

Fig. 4. Calibration curve from paper extracts containing 0.05–0.5 mg DACC. Conditions as in Fig. 2.

per day was a very conservative estimate of the NTEL value, *i.e.*, 600 mg/day (100 mg/dm^2) for a human being. The extracted amounts of DACC from the paper were far below 600 mg, even with a safety factor of 100 (see Table V).

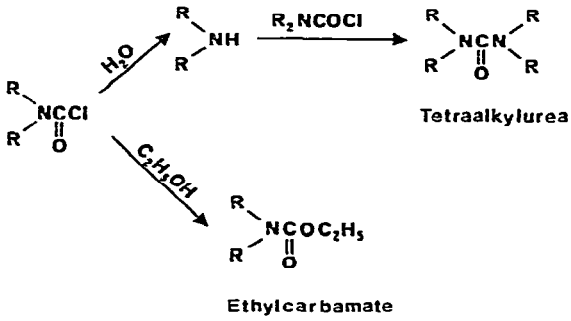

Fig. 5. Reaction products formed when DACC is exposed to water and ethanol.

TABLE III

REPRODUCIBILITY OF THE DETERMINATION OF DACC

DACC was added to a hexane extract from blank paper not treated with DACC. The solution was then extracted with water and taken through the whole derivatization procedure.

Amount of DACC added (mg)	*Mean peak height of 4 samples (mm)*	*S.D. of peak height of 4 samples (mm)*	*Coefficient of variation (%)*
0.5	335.3	15.7	3.14
0.25	179.2	7.3	3.71
0.1	69.2	2.6	4.05
0.05	35.6	1.1	4.67

TABLE IV

INFLUENCE OF CURING TIME ON EXTRACTION OF DACC BY HEPTANE AND PAPER HYDROPHOBICITY MEASURED BY LACTIC ACID ABSORPTION

Age of paper (days)	*Extracted amount of DACC (mg/dm^2)*	*Lactic acid absorption (g/m edge)*
7	0.395	0.91
29	0.28	0.46
80	0.27	0.38
125	0.145	0.38
Fully cured	0.0155	0.36

TABLE V

EXTRACTION OF DACC FROM FULLY CURED PAPER BY DIFFERENT SOLVENTS

Solvent	*Extraction time*	*Temperature (°C)*	*Extracted amount of DACC (mg/dm^2)*
Heptane	48 h	50	0.0155
50% C_2H_5OH	5 days	50	< 0.01
Water	5 days	50	< 0.01
3% CH_3COOH	5 days	50	< 0.01

REFERENCES

1 *Ullmans Encyklopädie der technischen Chemie*, Vol. 9, Verlag Chemie, Weinheim/Bergstr., 4th ed., p. 115*ff*.
2 T. Lesiak and L. Szczepkowski, *Chem. Anal. (Warsaw)*, 15 (1970) 165.
3 G. M. Rusch, S. L. Mendola, G.V. Katz and S. Laskin, *Anal. Chem.*, 48 (1976) 2259.
4 U. Helmer and A. Reuterhäll, *U.S. Pat.*, 3,887,427.
5 D. J. Jenden, I. Hanin and S. I. Lamb, *Anal. Chem.*, 40 (1968) 125.
6 *Code of Federal Regulations*, Title 21, U.S. Government Printing Office, Washington DC, 1971.