Journal of Chromatography, 178 (1979) 271–276

CHROM. 12,066

SENSITIVE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR THE DETERMINATION OF WATER IN VARIOUS SAMPLES

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(Received May 29th, 1979)

SUMMARY

Water and phenyl isocyanate react to yield N,N'-diphenylurea, a very stable and highly UV-absorbing compound. The reaction is quantitative and is completed in half an hour.

N,N'-diphenylurea can easily be chromatographed on a reversed-phase high-performance liquid chromatographic system. The smallest detectable amount, theoretically, is *ca.* 1 ng corresponding to <100 pg of water.

Applications to both solid and liquid samples are given as well as examples of crystal water determinations. The results are compared to results obtained by the Karl Fischer method.

INTRODUCTION

The Karl Fischer titration is the most usual method for the determination of water. It is not very sensitive and an acidic medium is formed during the titration procedure. This can lead to sample degradation and liberation of additional water from organic samples. The results are also dependent on the solvent used, *e.g.* the water content of fructose is doubled when changing from methanol to formamide.

Proton nuclear magnetic resonance (NMR) and near-infrared methods are also used but they are neither very specific nor sensitive.

During the development of the phenyl isocyanate method for the liquid chromatographic determination of aliphatic alcohols[1], an unknown peak appeared in almost every chromatogram. It proved to be N,N'-diphenylurea (DPU), a very stable and highly UV-absorbing derivative of water. The reaction of phenyl isocyanate and water is shown in Fig. 1.

The reaction is quantitative and usually needs no catalysts when an excess of the derivatizing agent is used. DPU can be easily separated and determined on a reversed-phase column with water–acetonitrile as mobile phase. UV detection of DPU allows determinations down to the nanogram level, which corresponds to water amounts below one nanogram.

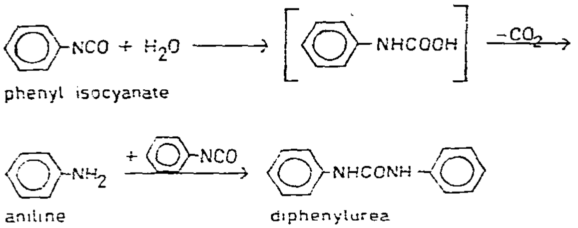


Fig. 1. Reaction of phenyl isocyanate and water.

EXPERIMENTAL

Apparatus

A Hupe & Busch 1010 A liquid chromatograph equipped with a Hewlett-Packard 1030 B variable-wavelength UV-detector (both from Hewlett-Packard, Karlsruhe, G.F.R.), a Goerz Servogor 541 recorder (Goerz Electro, Vienna, Austria) and a balanced-density self-packed LiChrosorb RP-18, 10 μm (E. Merck, Darmstadt, G.F.R.) reversed-phase column (250 mm × 4 mm I.D.) were used for the chromatographic separation and detection of DPU. Injections were made by a pressure-tight 10-μl SGE syringe (Scientific Glass, North Melbourne, Australia). A Perkin-Elmer (Beaconsfield, Great Britain) 402 scanning spectrophotometer, a Jeol (Tokyo, Japan) FX-60 Fourier transform ^{1}H and ^{13}C NMR spectrometer and a Büchi (Flavil, Switzerland) melting point apparatus together with the liquid chromatograph were used to check the purity of DPU.

The derivatization procedure was carried out in 10-ml measuring flasks placed in a desiccator equipped with a magnetic stirrer.

Caution: It is best to handle phenyl isocyanate (PHI) in a fume cupboard because it is an eye irritant.

Micropipettes (0.5–1.0 ml) were used to transfer samples and reagents.

Reagents

The following reagents were used: water, twice distilled; acetonitrile (ACN), redistilled (Rathburn Chemicals, HPLC grade); phenyl isocyanate (PHI) (Merck) stored in desiccator; N,N-dimethylformamide (DMF), distilled under vacuum, dried over powdered molecular sieve 4A, stored in desiccator; N,N-dimethylformamide (Merck); O-ethyl-N-phenylurethane (EPU), recrystallized from light petroleum until no DPU was detectable[1]; *n*-heptanol, dried over powdered molecular sieve 4A; triethylene diamine.

N,N′-diphenylurea (DPU) was prepared by mixing water and PHI in a molar ratio of 1:2.2. The mixture was allowed to react overnight in an open vessel, and the white precipitate was collected by suction filtration and washed with *n*-hexane until the melting point corresponded to the value in the literature, 515 K. Purity was also checked by ^{13}C-NMR and UV spectroscopy and liquid chromatography.

Sample derivatization

Dried DMF was used as solvent for almost all samples because it seems to catalyze the PHI reaction and is the best solvent for urethanes[1]. Because of their poor solubility in DMF it is advisable to add about one fifth (of the DMF amount) of dioxane to pure hydrocarbon samples. The samples were weighed into acetone washed and dried (383° K) 10-ml measuring flasks, equipped with a magnetic rod (5 mm) and a plastic stopper.

Liquid samples. A sample amount (max. 6 ml) corresponding to at most 10 mg of water was weighed into the measuring flask. 1.0 ml of dried DMF and 0.5 ml of PHI were added and the mixture allowed to react in the desiccator–magnetic stirrer system for half an hour. 2.0 ml of dried *n*-heptanol was added to destroy the excess of PHI. After 15 min the measuring flask was filled to the mark with DMF, which need not be dried at this stage.

Solid samples. If the solid sample is totally DMF-soluble it can be weighed directly into the measuring flask and treated like liquid samples.

Samples not totally DMF-soluble must first be powdered and then either extracted with dried DMF and treated like liquid samples or weighed directly into the measuring flask. The error in total volume must be taken into account in quantitation.

Blanks. A blank value must be determined for each batch of dried DMF and *n*-heptanol, because they always contain some water. The blank value must be subtracted from the total water amount obtained.

Liquid chromatographic separation and quantitation

A 0.5-ml volume of the clear DMF solution (if it is not clear it must be centrifuged) and 0.5 ml of the EPU solution (*e.g.* 1 mg/ml in DMF) used as internal standard (I.S.) were pipetted into a glass-stoppered test tube. 1–10 μl of this solution was injected with a syringe into the chromatographic system using water–acetonitrile (60:40) at a flow-rate of 2 ml/min as eluent. The LiChrosorb RP-18 column was in a thermostated oven at 313° K. The peaks were monitored at 230 nm. Under these conditions DPU eluted at *ca.* 5.5 min and EPU at *ca.* 4.2 min (see Fig. 2). Heptylphenylurethane was eluted from the column by a short gradient program to 95% ACN.

Peak height measurements were used for quantitation using the following equations:

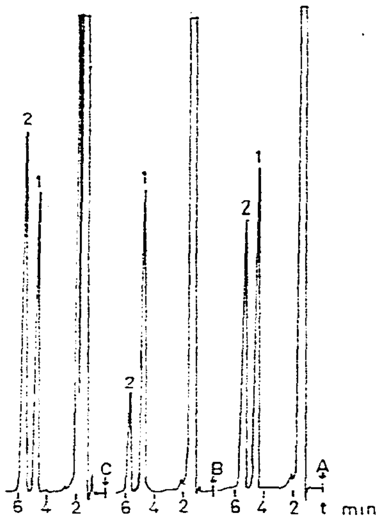
$$k = \frac{\dfrac{C_{\mathrm{I.S.}}}{h_{\mathrm{I.S.}}}}{\dfrac{C_{\mathrm{ST}}}{h_{\mathrm{ST}}}} \qquad (1)$$

where k is a constant, $C_{\mathrm{I.S.}}$ is the I.S. concentration (mg/ml) in the calibration run, C_{ST} is the DPU concentration (mg/ml) in the calibration run, and $h_{\mathrm{I.S.}}$ and h_{ST} are the corresponding peak heights; and

$$C_s = \frac{C_{\mathrm{I.S.}} \times h_{\mathrm{S}}}{k \times h_{\mathrm{I.S.}}} \qquad (2)$$

where C_{S} is the DPU concentration (mg/l) in the sample. The blank value must be subtracted from the value obtained from eqn. 2.

The constant k was determined using standard DPU and EPU solutions and it was checked daily. The factor for transforming DPU into water is 1/11.78 (0.0849).



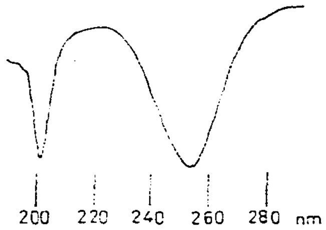


Fig. 2. Typical chromatograms of ethylphenylurethane (1) used as internal standard and diphenylurea (2). A, standard; B, blank; C, sample. Conditions: column LiChrosorb RP-18, 10 μm, 25 cm × 4 mm I.D.; eluent, acetonitrile–water (50:50); flow-rate, 2 ml/min; detection, UV at 230 nm, 1.0 a.u.f.s.; injection volume, 10 μl of *ca.* 1 mg/ml.

Fig. 3. UV scan of diphenylurea.

An integrator system was also used, but the peak height measurement for quantitation was found to be more reliable.

RESULTS AND DISCUSSION

Drying of reagents

Although DMF is the best solvent for the derivatizing reaction it has the disadvantage of being very hydrophilic. To keep the blank value as low as possible and thus increase sensitivity DMF must be dried. Distillation under reduced pressure followed by 4A powdered molecular sieve treatment was found to be quite effective (water content <0.01 %). *n*-Heptanol was treated the same way. Both these reagents were centrifuged with the molecular sieve and stored in a desiccator.

Phenyl isocyanate was also stored in a desiccator. *(DPU is formed in pipettes once used for PHI and exposed to open air).*

All glassware must be dry.

Effect of the amount of phenyl isocyanate and time on the derivatization reaction. Destruction of the excess of phenyl isocyanate

It is advisable to use at least a 10-fold molar excess of PHI, because all active hydrogen-containing functional groups also react with the derivatizing agent[1]. Insufficient PHI leads to formation of aniline (see Fig. 1), which elutes much faster than DPU. The first step in Fig. 1 is slow. Aniline reacts very fast. A total derivatization time of half an hour was found satisfactory.

The excess of PHI must be destroyed before injection, because water is a constituent of the eluent. *Iso*-butyl alcohol would be ideal but *n*-heptanol was chosen because it is easier to dry. The alcohol was allowed to react for 15 min.

Stability of the derivatives

Both standards and derivatized samples were stored in DMF for several days with no noticeable changes in the chromatograms compared with those of fresh samples.

Detection and linearity

DPU has two absorption maxima in the UV region, one at *ca.* 200 nm and the other at 256 nm (in ethanol, Fig. 3). At the latter, which is more useful, DPU has a molar extinction coefficient of 36 100 l $mole^{-1}cm^{-1}$. This allows detection down to the 1–10 ng level. In order to avoid multiple sample dilutions it is sometimes advisable to detect DPU at a lower wavelength (*e.g.* 230–254 nm).

To check quantitation and linearity of detection a series of different known water concentrations (in DMF) was derivatized. The quantitation calibration was made using standard DPU. The linearity curve is shown in Fig. 4. The reaction was quantitative. The relative error was greater in the low concentration region because of relatively larger blank values (Fig. 2).

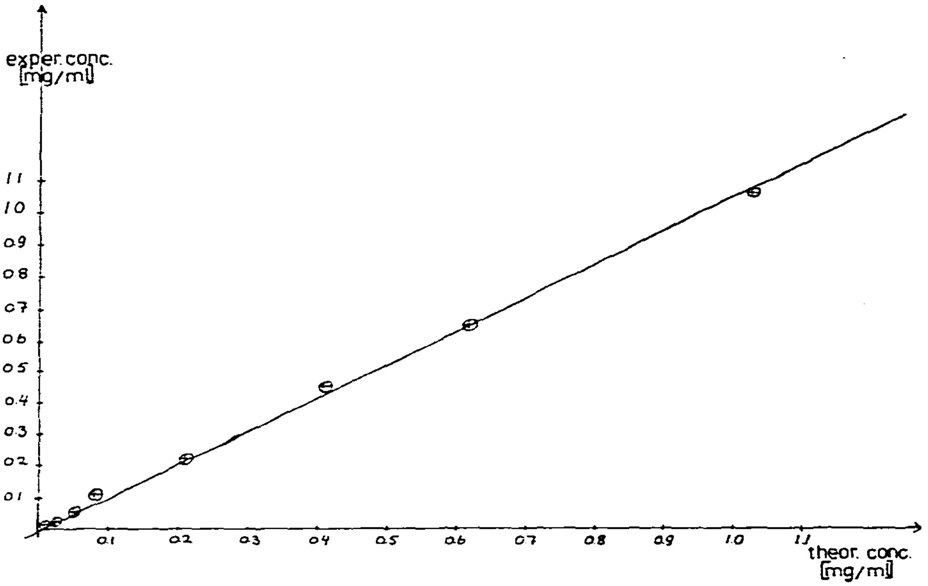


Fig. 4. Linearity curve for diphenylurea.

TABLE I

DETERMINATION OF WATER IN A SYNTHETIC OIL WITH VARIOUS SAMPLE AMOUNTS

Sample size (g)	*Water content (ppm, w/w)*
1	53
2	63
3	51
4	51

TABLE II

COMPARISON OF WATER DETERMINATION WITH THE DIPHENYLUREA AND KARL FISCHER METHODS IN VARIOUS SAMPLES

Sample	*The DPU method*	*The KF method*
A fodder based on molasses	0.86%	0.79%
A NPK fertilizer	1.68%	1.40%
White spirit	0.001%	—
Salpetre I	0.12%	0.17%
II	0.32%	0.35%
III	1.11%	0.24%
Paper	9.1%	
Wood	6.1%	

Applications

The water content in both organic and inorganic samples, liquids and solids, has been determined by the method described here. The Karl Fischer method was used as reference and the results were in good agreement with each other except for some inorganic salts.

A synthetic condensator oil was used to test the sensitivity of the method. Different sample sizes gave the results listed in Table I. The Karl Fischer method gave an average of *ca.* 75 ppm. Table II lists the results from different samples.

Inorganic salts containing the water of crystallization caused some problems. The results were improved using triethylenediamine as catalyst (1% in DMF) and changing the solvent from DMF to a mixture of DMF–ACN (50:50) (Table III).

The reason for the incomplete derivatization of aluminium sulfate and trisodium citrate could not be found.

TABLE III

EFFECT OF CATALYST AND SOLVENT ON DERIVATIZATION OF WATER OF CRYSTALLIZATION

Sample	*Solvent*	*Catalyst*	*Result*	*Theoretical*	*KF*
$CuSO_4 \cdot 5H_2O$	DMF	—	28.9%	36.1%	29.4%
	DMF	+	37.8%	36.1%	
$ZnSO_4 \cdot 7H_2O$	DMF	—	35.2%	43.8%	43.1%
	DMF–ACN	+	44.8%	43.8%	
$MgSO_4 \cdot 7H_2O$	DMF	—	37.5%	51.1%	49.7%
	DMF	+	36.6%	51.1%	
	DMF–ACN	+	49.3%	51.1%	
$Al_2(SO_4)_3 \cdot 18H_2O$	DMF	—	14.5%	48.6%	41.6%
	DMF	+	14.5%	48.6%	
	DMF–ACN	+	14.0%	48.6%	
$(COOH)_2 \cdot 2H_2O$	DMF	+	36.2%	31.6%	
Trisodium citrate $\cdot$ $2H_2O$	DMF–ACN	+	0.36%	12.2%	

ACKNOWLEDGEMENTS

We thank Lahja Leppänen, Arja Teerikoski and Tiia Kaila for technical assistance.

REFERENCE

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