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Note

Liquid chromatography and thin-layer chromatography of some substituted ureas

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A great deal of attention has been paid to the chromatographic behavior of urea-type compounds[1–4]. The main reasons for this interest are that these compounds constitute a widespread group of agricultural chemicals, they are of biochemical interest, and the group of compounds consists of a great number of structurally closely related compounds for which it is difficult to use classical analytical procedures for identification and quantitative assay. Traditional wet methods very often incur interferences; gas chromatography is amenable but subjects these compounds to possible decomposition. High-pressure liquid chromatography (HPLC) and thin-layer chromatography (TLC) techniques offer a rapid means of analysis for a large spectrum of substituted urea compounds and require a minimum of sample preparation.

EXPERIMENTAL

A Waters Model ALC 202/401 liquid chromatograph with an M6000 high-pressure pump, a U6K 2-ml loop injector, and a UV detector operating at a wavelength of 254 nm were used. A Hewlett-Packard Model 7130A recorder operated at 0.25 in/min and a Varian Model 620L 18K computer for integration were also employed. Chromatographic separation was effected using a stainless-steel column 1.3 m × 2 mm I.D. packed with octadecyltrichlorosilane (C_{18}) on Bondapak (37–50 μm grain size). Materials were obtained from Waters Assoc. (Framingham, Mass., U.S.A.). The mobile phase eluent was initially adjusted to 20% water and 80% acetonitrile, the polarity of which was adjusted by gradient elution for a 20-min period with a concave No. 7 curve to 90% water and 10% acetonitrile. A No. 7 curve is common to a Waters Model ALC 202/401 liquid chromatograph gradient elution curve selector. However, separation can be achieved with adequate success by eluting linearly for 20 min from 0–100% with water–acetonitrile (20:80). The column pressure was 2000 p.s.i. and the flow-rate 3 ml/min. The linear velocity was 2.8 cm/sec. A constant volume of 3 μl was injected by the stop-flow technique. The acetonitrile was Burdick and Jackson (Muskegon, Mich., U.S.A.) distilled-in-glass grade. Standards were obtained from CIBA-GEIGY (McIntosh, Ala., U.S.A.). Verification of purity was conducted by proton magnetic resonance and mass spectroscopy.

Thin-layer chromatography (TLC) was performed with silica gel G, neutral

(50% Merck silica gel–50% Bio-Rad Bio-Sil A) on 200 × 200 mm glass plates coated to a thickness of 200 μm and activated at 100° for 1 h. Solutions of chloroform–nitromethane (80:20) or benzene–chloroform–ethyl acetate (40:40:20) were used as a mobile phase. Solvents were Fisher reagent grade (Atlanta, Ga., U.S.A.). After elution the plates were air dried and then exposed to chlorine in a saturated chamber for 30 sec. The plates were then air dried for 2 min and sprayed with potassium iodide–starch solution.

RESULTS AND DISCUSSION

Fig. 1 shows the separation achieved with Bondapak/C_{18} on a typical sample. Resolution is similar to that from gas–liquid chromatograms. The linearity response

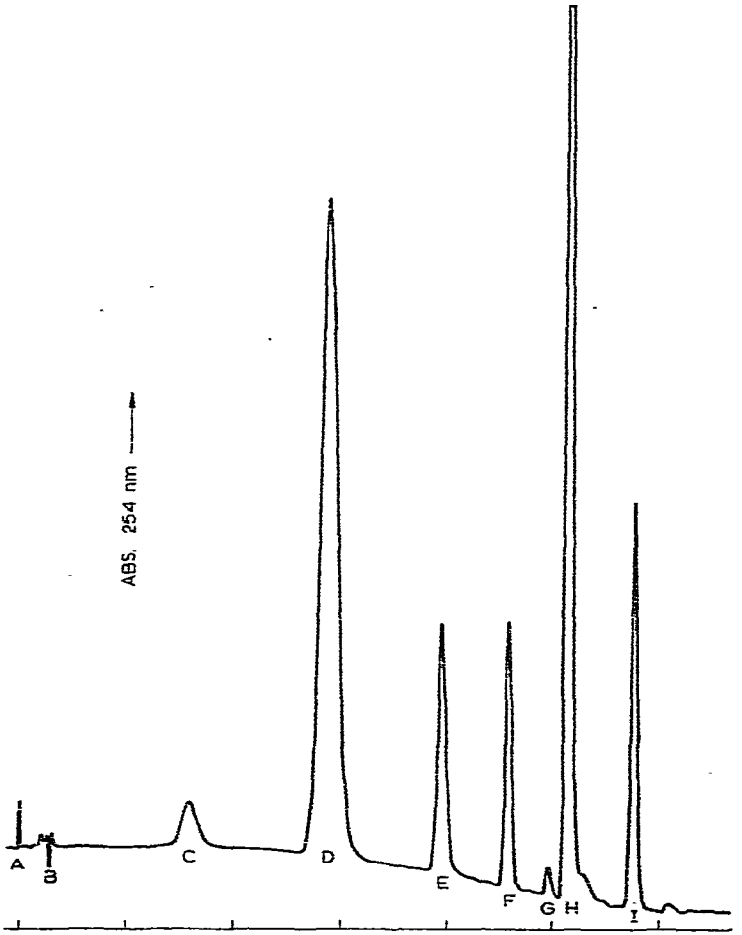

Fig. 1. Resolution of selected ureas. A = injection point; B = solvent front; C = 1,1-dimethyl-3-(1,1,1-trifluoro-*o*-tolyl)urea; D = 1,1-dimethyl-3-(1,1,1-trifluoro-*m*-tolyl)urea; E = 1,1-dimethyl-5-(1,1,1-trifluoro-*m*-tolyl)urea; F = 1,3-bis(1,1,1-trifluoro-*o*-tolyl)urea; G = impurity related to H; H = 1,3-bis(1,1,1-trifluoro-*m*-tolyl)urea; I = dimethylphthalate internal standard. Concentration adjusted to 8 mg/ml for sample components and 2 mg/ml internal standard. See text for experimental conditions. R_t/sec: C = 390; D = 690; E = 960; F = 1110; H = 1245; I = 1395.

of the UV detector to increasing concentrations is seen to be quite good. Substitution of the water–acetonitrile mobile phase by water–ethyl acetate–isopropanol (40:10:50) did not yield equivalent resolution. A water–methanol mobile phase did not allow separation of the bis compounds. Increasing the concentration of water tended to cause earlier elution of the compounds and loss of resolution. Increasing the acetonitrile concentration caused a corresponding increase in retention time for all compounds investigated.

Under the conditions specified, the number of theoretical plates was 2000. The detection sensitivity limits for compounds investigated were 50–300 ng by UV detection at 254 nm. Because the maximum absorbances of the individual ureas assayed are not always coincident with this wavelength, it is probable that an increase in sensitivity would occur by using a variable-wavelength spectrophotometer.

Dimethylphthalate was found to be useful as an internal standard. Areas determined by the Varian Model 620L 18K computer had a coefficient of variance of 0.51%. Response factor reproducibility was 0.82% relative standard deviation of the mean at 2 σ. Analyses of mixtures of varying concentrations produced an accuracy of 1.0% relative standard deviation of the mean at 2 σ. In Table I results are tabulated for various substituted ureas considered in this study.

TABLE I

LIQUID CHROMATOGRAPHY DATA COMPILATION

Results presented are for typical sample mixtures.

*Urea**	*Mixture 1*		*Mixture 2*		*Mixture 3*			*Retention time (sec)*	
	Present (%)	*Found (%)*	*Present (%)*	*Found (%)*	*Present (%)*	*Found (%)*		*Mixture 3(1)*	*Mixture 3(2)*
						1	*2***		
C	5.0	5.0	10.1	10.0	30.7	30.8	30.8	391	388
D	85.1	85.4	20.5	20.3	5.5	5.5	5.5	689	691
E	3.0	2.9	30.2	30.2	15.4	15.2	15.1	960	965
F	6.0	6.0	30.2	30.3	7.2	7.2	7.3	1110	1107
H	0.3	0.3	9.0	9.1	41.2	41.1	41.1	1246	1247

* For designations, see the legend to Fig. 1.

** Reanalysis of mixture 3 with different sample weights.

Normal principles of adsorption chromatography were applied to effect a TLC separation procedure using activated silica gel. The time for elution varied from 30–40 min at ambient temperatures. Fig. 2 shows typical TLC separations with the corresponding eluents used. Concentrations were less than 5%. Samples should be double-spotted in order to give the necessary concentration to evaluate sample quantification at the normal standard range of: 1.0% (w:v), 2.5% (w:v), and 5% (w:v). The standard deviation of the analysis representing the relative standard deviation of the mean at 2 σ was found to be approximately 25%. Spots were visually estimated by comparing to known standards. Good sensitivities were obtained for the substituted ureas considered in this study. Spots appeared as purple spots on a light gray background. It was found necessary to prepare fresh solutions prior to analysis and to allow the solutions to equilibrate for 30 min.

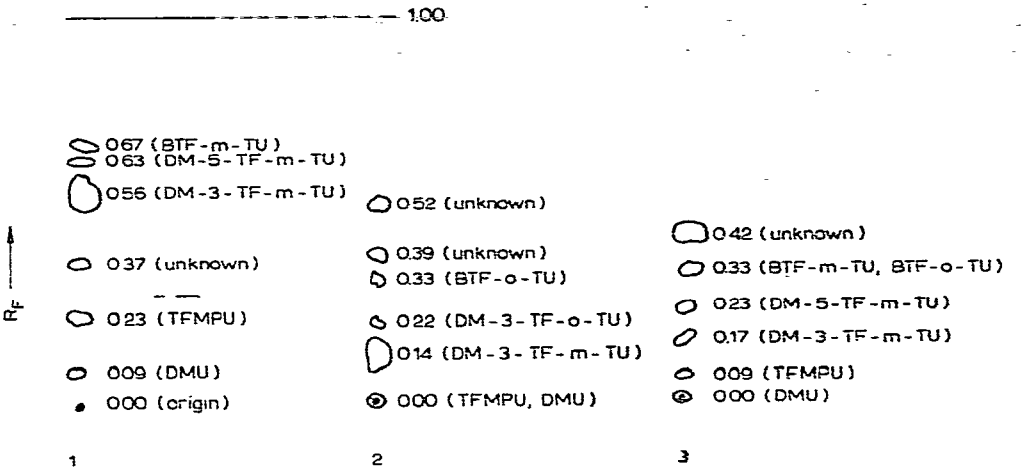

Fig. 2. TLC separation of selected ureas. 1 = chloroform–nitromethane–methanol (80:20:5); 2 = benzene–chloroform–ethyl acetate (40:40:20); 3 = chloroform–nitromethane (80:20). DMU = Dimethyl urea; TFMPU = trifluoromethylphenylurea; DM-3-TF-*m*-TU = dimethyl-3-trifluoro-*m*-tolyl urea, etc.; BTF-*m*-TU = bis-trifluoro-*m*-tolyl urea, etc.

It should be noted that no one TLC system described will separate all of the compounds studied. Combinations of two systems were generally used to assay mixtures. Slight variations in eluent component ratios may be necessary for certain applications, depending on the original sample matrix.

CONCLUSIONS

The present paper offers a TLC and HPLC method for the possible quantification of a very wide range of substituted ureas. With the modern pumping systems, flow gradients, and mixture gradients available many similar substituted urea compounds can be separated. Separation of the complex isomeric system investigated in this work suggests the above statement. Combination of the liquid chromatography method with the TLC procedure provides a most powerful analytical methodology for investigating highly substituted isomeric urea compounds.

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