TABLE I

Sample number	*Component*	*Concentration (%)*	*Average range found from all Laboratories* ($\times 10^{-3}$)	*Range predicted by equation* ($\times 10^{-3}$)
1	Benzene	98.04	6.67	3.28
	Toluene	0.73	2.11	2.17
	Ethylbenzene	0.80	2.88	2.23
	Cyclohexane	0.43	1.66	1.88
2	Toluene	90.64	25.00	10.89
	Benzene	1.82	4.00	4.44
	Ethylbenzene	2.69	8.62	5.24
	p-Xylene	3.72	14.00	6.17
	n-Nonane	1.13	2.78	3.79

due to the change in number of components and the variation in the concentration of these components.

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Received February 5th, 1964

Paper chromatography of short chain aliphatic amides

During studies on the use of aliphatic amides by the bacterium *Pseudomonas aeruginosa* as sole source of carbon and nitrogen for growth[1–3], it became necessary to check the purity of the amides and identify very small amounts of amide remaining in the culture media after growth. The use of direct paper chromatography of amides was investigated since, although it is possible to convert amides to their hydroxamates[4] and there exist a number of suitable solvents for chromatography of hydroxamates[5,6], the procedure does not allow distinction to be made between amides such as acetamide, N-methylacetamide and N-acetylacetamide, as these amides give the same hydroxamate. A few methods for direct chromatography of amides have been published but these are for long chain amides[7] or involve treatment of the paper, *e.g.* with 5 % polycaprolactam–formic acid[8]. In the search for a suitable solvent, it was found that many of the usual chromatography solvents were unsatisfactory because the amides travelled with the solvent front, did not move at all, or in the case of *n*-butanol–acetic acid–water did not give adequate separation of the amides. The solvent finally selected was toluene–ethanol (75:25 v/v); amides were detected by conversion to hydroxamates and the hydroxamates visualised with ferric chloride[9,10].

Experimental procedure

1.0 *M* aqueous solutions of amides were used with the exception of glutamine, asparagine and malonamide; saturated solutions of these three amides were used (approx. 0.3, 0.2 and 0.8 *M*, respectively). Approximately 0.002 ml of each amide was applied to Whatman No. 1 chromatography paper, 18 × 22 in., and after the spots had dried the paper was equilibrated for 30 min in a Shandon all glass chromatography tank arranged for descending chromatography and containing a beaker of toluene–ethanol (75:25). At the end of the equilibration period, the solvent was introduced to the edge of the paper and the chromatogram was developed for $2^1/_2$ h in the machine direction of the paper; it was then removed from the tank, the solvent front marked, and dried in an oven at 100° for 3 min. The chromatogram was then sprayed with reagent of the following composition: 80 % methanol, 20 % water (v/v) containing 3 g of sodium hydroxide/100 ml and saturated with hydroxylamine hydrochloride. Although alkaline hydroxylamine is unstable, satisfactory results were obtained with reagent that had been stored for 1 week at 5°. The sprayed chromatogram was placed in an oven at 100° for 10 min and then lightly sprayed with 1 % ferric chloride hexahydrate in 90 % methanol–10 % conc. HCl (v/v); the hydroxamates of each amide appeared as pink spots against a yellow background. Although these pink spots faded slowly, they were still clearly visible after 72 h and since the yellow background due to ferric chloride faded more rapidly, examination of the chromatograms for very faint spots was carried out after 3–6 h when the background was practically white. Using the above procedure, it was possible to detect 5 μg of acetamide; the amount of the other amides detectable varied with their rate of reaction with alkaline hydroxylamine[9].

Some amides could be distinguished on the basis of their hydroxamate colour; thus the hydroxamate of iodoacetamide gave a brown colour with ferric chloride; the pink coloured hydroxamate of cyanoacetamide darkened to become almost black after 24 h and that of thioacetamide became blue around its edge. In addition, thioacetamide can be detected directly by spraying with 5 volumes hydrogen peroxide containing 1 % ferric chloride; the reagent gives an ephemeral red colour with μg amounts of thioacetamide but it destroys the ability of thioacetamide to form a hydroxamate with alkaline hydroxylamine. None of the other amides tested gave any colour with ferric chloride–hydrogen peroxide.

Results

The rates of migration of a number of amides relative to acetamide (R_A) were determined using toluene–ethanol solvent; these are listed in Table I together with their actual R_F's.

Discussion

The above procedure is a rapid way of separating short chain aliphatic amides and locating their position on the chromatogram. The time chosen for heating amides with alkaline hydroxylamine to convert them to hydroxamates is a compromise since there is considerable variation in the times necessary for complete conversion of amide to hydroxamate; *e.g.* formamide requires 60 min at 26° whereas N-methylacetamide requires 420 min at 60°. Therefore if substituted amides are known to be present, it might be necessary to prolong the heating period with alkaline hydroxyl-

TABLE I

R_F AND R_A VALUES (RATE OF MIGRATION RELATIVE TO ACETAMIDE) FOR SOME SHORT CHAIN ALIPHATIC AMIDES

Amide	R_F	R_A
Formamide	0.08	0.6
N-Methylformamide	0.18	1.4
Acetamide	0.13	1.0
Glycolamide	0.07	0.54
N-Methylacetamide	0.32	2.5
N-Acetylacetamide	0.53	4.1
Thioacetamide	0.32	2.5
Cyanoacetamide	0.06	0.45
Fluoroacetamide	0.19	1.5
Iodoacetamide	0.27	2.1
Propionamide	0.26	2.0
Acrylamide	0.21	1.6
Lactamide	0.10	0.8
β-Hydroxypropionamide	0.07	0.54
Butyramide	0.4	3.2
Isobutyramide	0.51	3.9
Malonamide	0.01	0.1
Glutamine	0.0	0.0
Asparagine	0.0	0.0
Glycine amide	0.0	0.0

amine in order to obtain sufficient conversion of the amide to hydroxamate for a detectable colour with ferric chloride to be observed. The procedure can be made quantitative by running duplicate spots and after locating each amide by the above method on one strip, eluting it from the corresponding portion of the second strip and applying the quantitative method involving the correct temperature and time for that particular amide[9,10].

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Received February 3rd, 1964

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