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SEPARATION AND IDENTIFICATION OF PRIMARY AND SECONDARY ALIPHATIC AMINES AS *p*-(N,N-DIMETHYLAMINO)-BENZENE-*p'*-AZOBENZAMIDES BY PAPER AND THIN-LAYER CHROMATOGRAPHY

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SUMMARY

The benzoylating agent *p*-(N,N-dimethylamino)-benzene-*p'*-azobenzoyl chloride, which is used for the identification of alcohols, also reacts readily with primary and secondary amines. A series of *p*-(N,N-dimethylamino)-benzene-*p'*-azobenzamides of primary and secondary amines were prepared and optimum conditions for their synthesis were found. The chromatographic separation of amides is very good, amides of secondary amines, in particular, exhibiting extraordinarily good chromatographic properties, so that the above mentioned method of analysis is considered to be one of the best in use at present. Their derivatives are orange-red and scarlet substances and, therefore, no method of detection is necessary in their identification. This property could also be made use of in their photometric determination. The sensitivity of detection is very good, and spraying the chromatogram with 0.01 *N* sulphuric acid can prove the presence of less than 0.3 μg of amide in the spot.

INTRODUCTION

Primary and secondary amino groups contain reactive hydrogen, which can be substituted with various acid radicals. Amines, being basic substances, can combine with acids to form salts. The action of acylating agents on primary and secondary amines forms amides substituted on the nitrogen atom. The amides are mostly solids, and are therefore suitable for testing and identification by melting-point determinations and paper chromatography (PC) and thin-layer chromatography (TLC). The derivatives most frequently used for the identification of amines are substituted benzamides. Benzoylation is carried out with benzoyl chloride, but chloro-substituted benzoic acid is more frequently used in the Schotten–Baumann method[1]. The pyridine method was found to be convenient with slightly basic amines. Pyridine is useful as it not only forms a reaction medium that can absorb the hydrogen chloride evolved, but it also combines with benzoyl chloride to form benzoylpyridinium chloride, which is more reactive than benzoyl chloride itself. In addition to benzoyl chloride and *p*-nitrobenzoyl chloride[2], 3,5-dinitrobenzoyl chloride[3–5] is also used in the identification and preparative separation of amines. In the preparation of N-substituted amides from amines and diphenylketene, the

procedure consists of heating and subsequent decomposition of benzoyldiazomethane into diphenylketene, which reacts immediately with the primary or secondary amine present. The reaction gives rise to amides of diphenylacetic acid, which crystallize well and exhibit sharp melting-points[6].

The PC and TLC separation of free aliphatic amines cannot be carried out owing to the volatility of the lower members of the homologous series. Therefore, it is more suitable to use gas chromatography or to chromatograph the compounds as salts or appropriate derivatives.

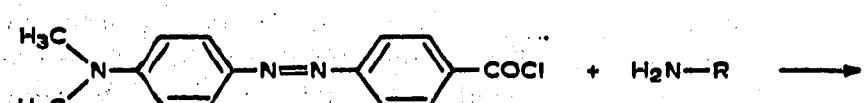
The PC of higher aliphatic monoamines and their salts requires a special condition to be fulfilled: the compounds must be chromatographed in systems preventing their hydrolysis[7–11].

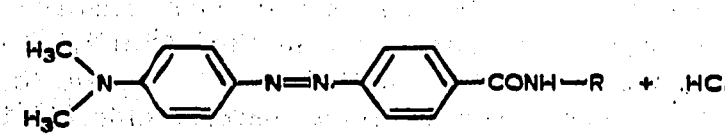
The separation of free amines of low volatility in a thin layer is based on the adsorptivities of the amine and the adsorbent. The increasing basicity of the amine is directly proportional to its adsorptivity. For the separation, alkaline aluminium oxide[12,13] is used, for example, or amines can be separated as salts[14–17]. Some other methods of identification of amines in a thin layer of sorbent involve the use of variously modified samples or layers[18–20]. Of the derivatives that have been separated by paper and TLC, 3,5-dinitrobenzamides[5,14,21] proved to be the most suitable. Amines were also chromatographed on thin layers as 4-dimethylamino-3,5-dinitrobenzamides[22]. In addition, for the separation of amines, other derivatives can also be used, *e.g.*, β-aminovinyl-*o*-hydroxyphenyl ketones[23] or derivatives that were obtained by the reaction of amines with 2,4-dinitrofluorobenzene[24]. The colour reaction of amines with 2,6-dichloroquinone-4-chlorimide[25] is an example of suitable and sensitive methods for the detection of free amines.

p-(N,N-Dimethylamino)-benzene-*p'*-azobenzoyl chloride, used for the chromatographic identification of alcohols[26,27], also reacts readily with aliphatic primary and secondary amines. The preparation of the reagent is relatively simple[28] and its reactivity with amines is high. Its properties, particularly the intense coloration of its derivatives, can be made use of in the chromatographic separation and identification of the amines by both PC and TLC.

EXPERIMENTAL

The reagent *p*-(N,N-dimethylamino)-benzene-*p'*-azobenzoyl chloride reacts readily with primary and secondary amines to form the corresponding amides according to the equation:





In the chromatographic separation and identification of amines, it is not necessary to prepare amides on the preparative scale. The amides obtained need

not be purified by crystallization or by any other method, so that the overall synthesis of the amides is rapid and simple.

Micro-preparation

About 1 mg of the reagent is suspended in 1 ml of benzene. About 2 to 4 drops of amines are added to the suspension and the mixture is heated at the boiling-point of benzene; with lower amines, 1 or 2 min is sufficient, but with the middle and higher amines a period of 10–15 min is required. With aqueous solutions of amines, a dioxan–benzene mixture (2:1) is used as the reaction medium. The derivatives obtained need not be purified and the reaction mixture can be directly applied to the chromatogram.

Paper chromatography

Whatman No. 2 filter-paper was used, impregnated with dimethylformamide solutions of various concentrations.

The following systems were used for the separation of primary amines:

S_1: 30 % dimethylformamide/hexane–benzene (3:1) (at 18°)

S_2: 50 % dimethylformamide/cyclohexane–benzene (2:3) (at 18°).

The following system was used for the separation of secondary amines:

S_3: 60 % dimethylformamide/cyclohexane (at 5°).

Thin-layer chromatography

The procedure was carried out by using cast layers of Silica Gel G (according to STAHL; Lachema, Brno). Activitation was carried out at 110° for 1 h, and 2–5 μl of a 0.2 % benzene solution of the amide was applied and chromatographed at 21°.

The separation of primary amines was carried out in the following mobile phases:

S_4: cyclohexane–ethyl acetate (60:40)

S_5: cyclohexane–methyl ethyl ketone (70:30)

S_6: hexane–ethyl acetate (60:40)

S_7: hexane–methyl ethyl ketone (60:40)

S_8: benzene–ethyl acetate (70:30).

The separation of secondary amines was carried out in the following mobile phases:

S_5: cyclohexane–methyl ethyl ketone (70:30)

S_9: cyclohexane–ethyl acetate (70:30)

S_{10}: cyclohexane–ethyl acetate (80:20)

S_{11}: hexane–ethyl acetate (80:20)

S_{12}: cyclohexane–acetone (80:20).

Detection

p-(N,N-Dimethylamino)-benzene-*p'*-azobenzamides are coloured substances, so there is no need for a detection method to be used. Amides react towards pH by a colour change and, therefore, spraying the chromatogram with small amounts of 0.01 *N* H_2SO_4 is recommended. It was found that amounts of less than 0.3 μg of amide can be observed in the spot.

TABLE I

R_F AND R_M VALUES OBTAINED BY PAPER AND THIN-LAYER CHROMATOGRAPHY OF *p*-(N,N-DIMETHYLAMINO)-BENZENE-*p'*-AZOBENZAMIDES OF PRIMARY ALIPHATIC AMINES

Amine	*Solvent system*													
	S_1		S_2		S_4		S_5		S_6		S_7		S_8	
	R_F	R_M	R_F	R_M	R_F	R_M	R_F	R_M	R_F	R_M	R_F	R_M	R_F	R_M
Methyl	0.08	+1.061	0.45	+0.087	0.10	+0.954	0.17	+0.689	0.13	+0.825	0.33	+0.308	0.18	+0.659
Ethyl	0.15	+0.753	0.58	−0.140	0.21	+0.575	0.30	+0.368	0.25	+0.477	0.49	+0.017	0.32	+0.327
n-Propyl	0.25	+0.477	0.65	−0.269	0.32	+0.327	0.36	+0.250	0.36	+0.250	0.58	−0.140	0.44	+0.105
Isopropyl	0.24	+0.501	0.63	−0.231	0.36	+0.250	0.40	+0.176	0.39	+0.194	0.52	−0.035	0.46	+0.070
Allyl	0.20	+0.602	0.60	−0.176	0.32	+0.327	0.39	+0.194	0.38	+0.213	0.50	−0.000	0.44	+0.105
n-Butyl	0.33	+0.308	0.70	−0.368	0.39	+0.194	0.42	+0.140	0.43	+0.122	0.64	−0.250	0.53	−0.052
Isobutyl	0.31	+0.347	0.68	−0.327	0.43	+0.122	0.43	+0.122	0.44	+0.105	0.56	−1.050	0.51	−0.017
Isoamyl	0.42	+0.140	0.70	−0.368	0.47	+0.052	0.47	+0.052	0.49	+0.017	0.65	−0.269	0.59	−0.158
n-Hexyl	0.54	−0.070	0.78	−0.545	0.49	+0.017	0.47	+0.052	0.53	+0.052	0.71	−0.389	0.66	−0.288
Cyclohexyl	0.40	+0.176	0.70	−0.368	0.49	+0.017	0.51	−0.017	0.53	−0.052	0.68	−0.327	0.66	−0.288

TABLE II

R_F AND R_M VALUES OBTAINED BY PAPER AND THIN-LAYER CHROMATOGRAPHY OF *p*-(N,N-DIMETHYLAMINO)-BENZENE-*p'*-AZOBENZAMIDES OF SECONDARY ALIPHATIC AMINES

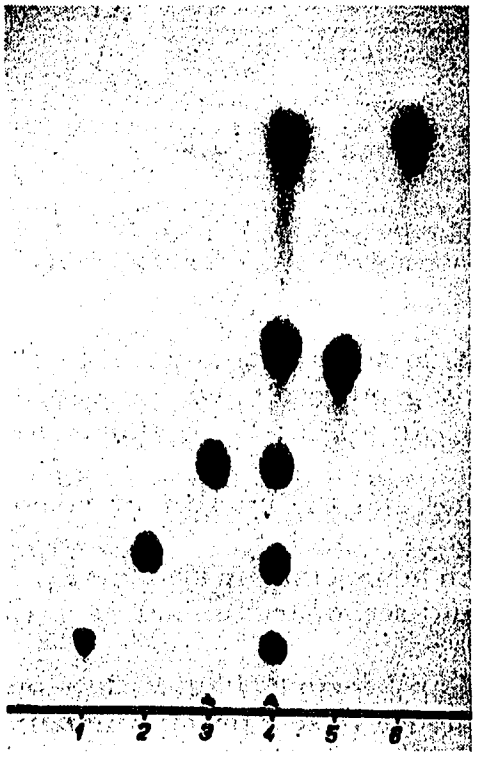
Amine	*Solvent system*											
	S_3		S_5		S_9		S_{10}		S_{11}		S_{12}	
	R_F	R_M	R_F	R_M	R_F	R_M	R_F	R_M	R_F	R_M	R_F	R_M
Dimethyl	0.03	+1.510	0.19	+0.630	0.12	+0.865	0.05	+1.279	0.03	+1.510	0.16	+0.720
Diethyl	0.12	+0.865	0.36	+0.250	0.27	+0.432	0.12	+0.865	0.08	+1.061	0.27	+0.432
Di-*n*-propyl	0.28	+0.410	0.44	+0.105	0.41	+0.158	0.20	+0.602	0.15	+0.753	0.34	+0.288
Diisopropyl	0.30	+0.368	0.47	+0.052	0.46	+0.070	0.24	+0.501	0.20	+0.602	0.34	+0.288
Di-*n*-butyl	0.49	+0.017	0.48	+0.035	0.49	+0.017	0.27	+0.432	0.22	+0.545	0.39	+0.194
Diisobutyl	0.48	+0.035	0.48	+0.035	0.51	−0.017	0.29	+0.389	0.25	+0.477	0.40	+0.176
Di-*n*-amyl	0.65	−0.269	0.51	−0.017	0.54	−0.070	0.32	+0.327	0.26	+0.454	0.42	+0.140
Di-*n*-hexyl	0.75	−0.477	0.57	−0.122	0.60	−0.176	0.37	+0.231	0.31	+0.347	0.48	+0.035
Dicyclohexyl	0.63	−0.231	0.56	−0.105	0.58	−0.140	0.38	+0.213	0.31	+0.347	0.47	+0.052
Di-*n*-octyl	0.89	−0.908	0.64	−0.250	0.66	−0.288	0.43	+0.122	0.36	+0.250	0.54	−0.070

RESULTS AND DISCUSSION

It was found that the *p*-(N,N-dimethylamino)-benzene-*p'*-azobenzamides of primary and secondary aliphatic amines exhibit good chromatographic properties. They are fairly soluble in polar solvents and can be successfully chromatographed on both paper and thin layers. The best results were obtained in the following solvent systems.

For the PC of the amides prepared from primary amines, system S_1 (30% dimethylformamide/hexane–benzene, 3:1) proved to be the best. The results obtained are illustrated in Fig. 1. For secondary amines, system S_3 (60% dimethylformamide/cyclohexane) proved to be the best system, as shown in Fig. 2. The choice of the solvent system for the PC of amides that were derived from secondary amines is no problem. The homologous series of secondary amines is readily separated in a considerable number of mixed solvents over a wide range of concentrations, with dimethylformamide serving as the stationary stage.

In the TLC of primary amines, very good results were obtained in systems S_4 (cyclohexane–ethyl acetate, 60:40) and S_8 (benzene–ethyl acetate, 70:30), as



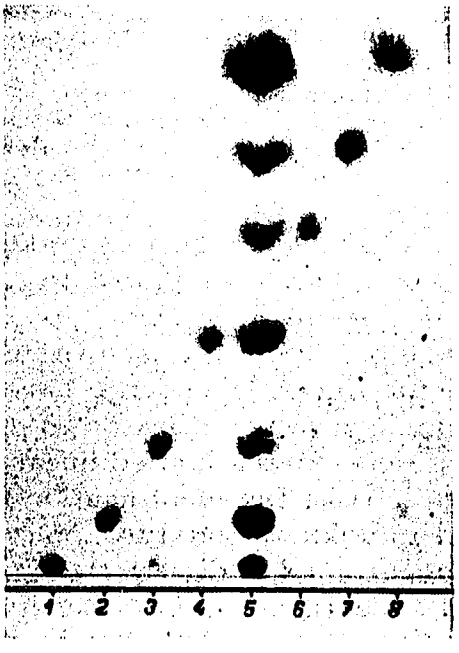


Fig. 1. Paper chromatogram obtained with system S_1(30% dimethylformamide/hexane–benzene, 3:1) on Whatman No. 2 filter-paper at 18° for *p*-(N,N-dimethylamino)-benzene-*p'*-azobenzamides of primary amines: 1 = methyl; 2 = ethyl; 3 = *n*-propyl; 4 = mixture of C_1–C_6 primary amines; 5 = *n*-butyl; 6 = *n*-hexyl.

Fig. 2. Paper chromatogram obtained with System S_3 (60% dimethylformamide/cyclohexane) on Whatman No. 2 filter-paper at 5° for *p*-(N,N-dimethylamino)-benzene-*p'*-azobenzamides of secondary amines: 1 = dimethyl; 2 = diethyl; 3 = di-*n*-propyl; 4 = di-*n*-butyl; 5 = mixture of C_1–C_6 and C_8 secondary amines; 6 = di-*n*-amyl; 7 = di-*n*-hexyl; 8 = di-*n*-octyl.

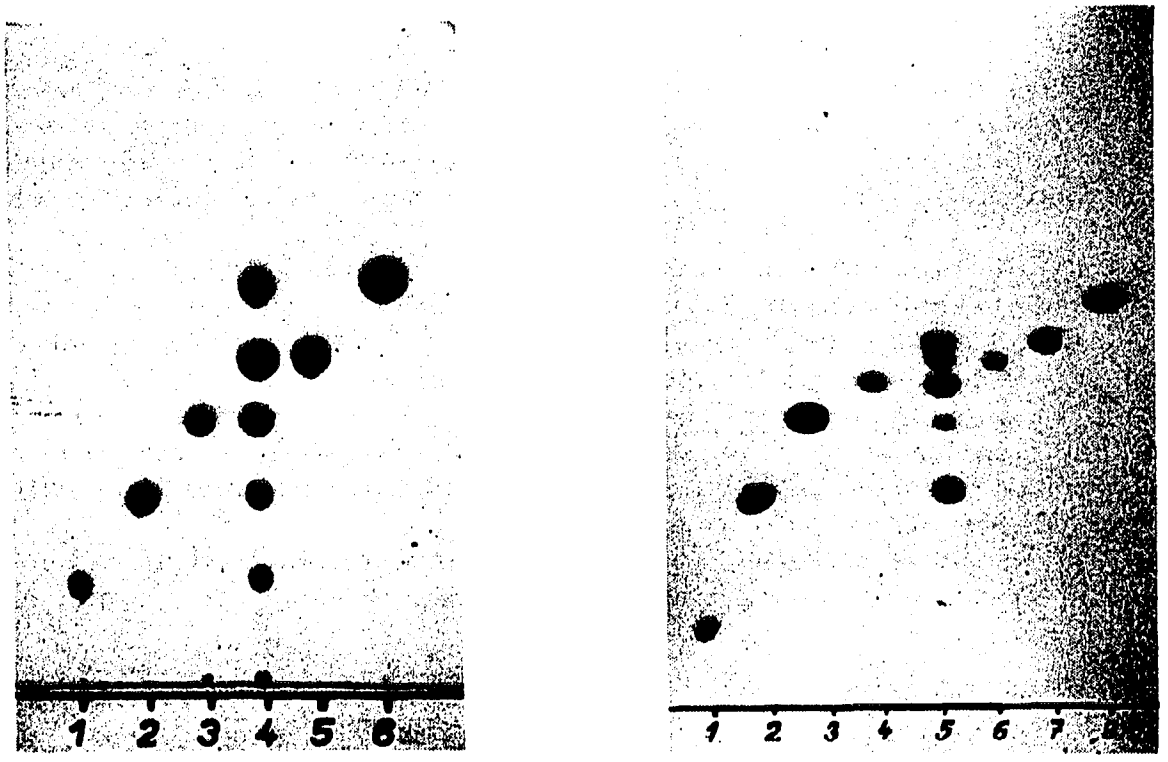


Fig. 3. Thin-layer chromatogram obtained with system S_8 (benzene–ethyl acetate, 70:30) at 21 ° for *p*-(N,N-dimethylamino)-benzene-*p'*-azobenzamides of primary amines: 1 = methyl; 2 = ethyl; 3 = *n*-propyl; 4 = mixture of C_1–C_4 primary amines; 5 = *n*-butyl; 6 = *n*-hexyl.

Fig. 4. Thin-layer chromatogram obtained with system S_5 (cyclohexane–methyl ethyl ketone, 70:30) at 21 ° for *p*-(N,N-dimethylamino)-benzene-*p'*-azobenzamides of primary amines: 1 = dimethyl; 2 = diethyl; 3 = di-*n*-propyl; 4 = di-*n*-butyl; 5 = mixture of C_2–C_6 secondary amines; 6 = di-*n*-amyl; 7 = di-*n*-hexyl; 8 = di-*n*-octyl.

shown in Fig. 3. The separation of amides, derived from secondary amines, in the thin layer, can be achieved in almost any system. Fairly good results were obtained, for example, in systems S_9 (cyclohexane–ethyl acetate, 70:30) and S_5 (cyclohexane–methyl ethyl ketone, 70:30), as shown in Fig. 4.

There is no linear dependence between R_m values and the number of carbon atoms in an amine molecule in any one of the given systems (as shown in Figs. 5 and 6) except in PC; there exists a limited linear dependence in homologues in systems S_1, S_2 and S_3. On this basis, the identification of primary and secondary amines can still be carried out in unknown mixtures.

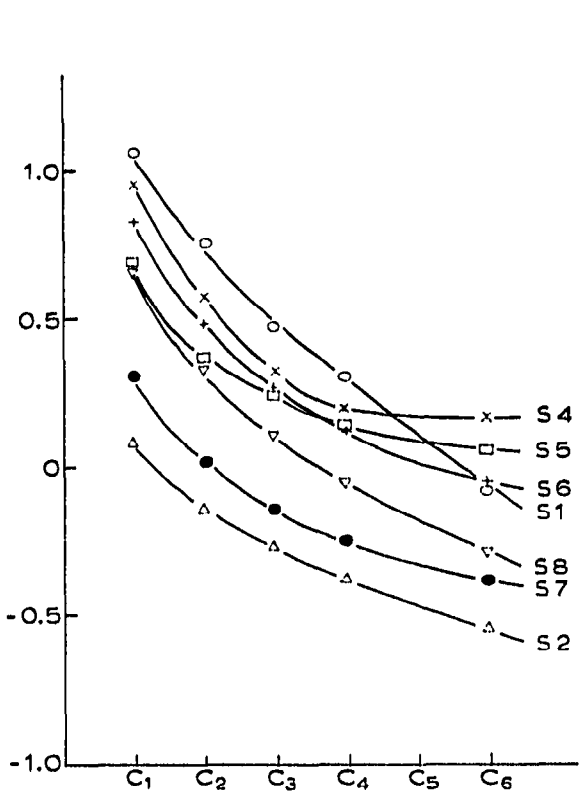
The reagent *p*-(N,N-dimethylamino)-benzene-*p'*-azobenzoyl chloride does not interfere in the identification in either its original or hydrolyzed form, and remains on the start of the chromatogram.

The advantages of the method described for the chromatographic identification of primary and secondary aliphatic amines are as follows:

(a) the reagent used, *p*-(N,N-dimethylamino)-benzene-*p'*-azobenzoyl chloride, is highly reactive and is not hydrolyzed on exposure to air; it is also stable, unlike other agents.

(b) the preparation of derivatives is simple and rapid;

(c) the derivatives are coloured, so that no detection is needed;



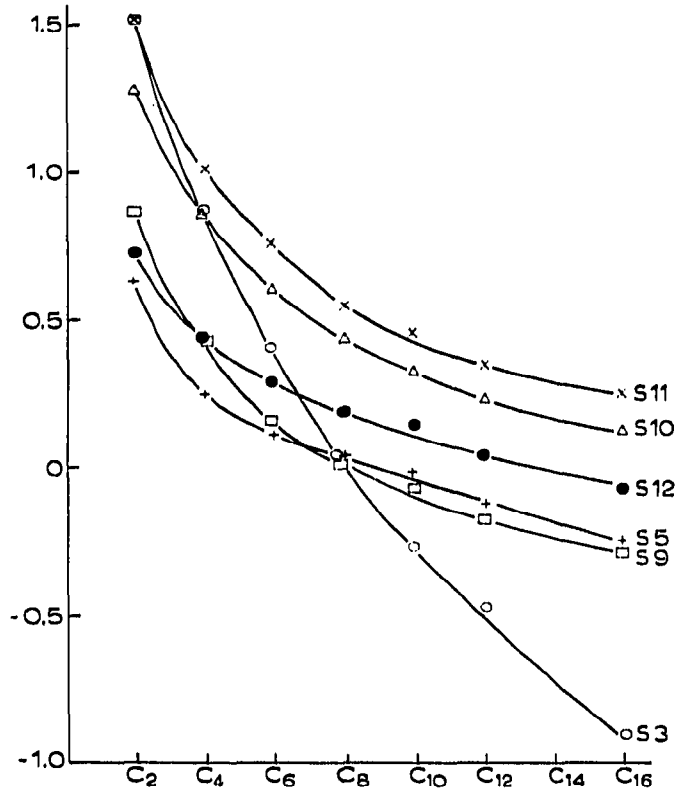


Fig. 5. Dependence of R_M values on the number of carbon atoms in an amide molecule derived from primary amines, in both paper and thin-layer chromatography.

Fig. 6. Dependence of R_M values on the number of carbon atoms in an amide molecule derived from secondary amines, in both paper and thin-layer chromatography.

(d) the derivatives can easily be chromatographed in currently used systems and, particularly in the chromatography of derivatives of secondary amines, there is no difficulty in the choice of the solvent system for either PC or TLC;

(e) the sensitivity of the method is so high that as little as 0.3 μg of amide can be observed in the spot, which corresponds to *ca.* 0.05 μg of amine;

(f) coloured derivatives can also be made use of in the quantitative chromatographic analysis of amines, as the Beer–Lambert law is obeyed at low concentrations.

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