Journal of Chromatography, 218 (1981) 51–56
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 14,081

FLUORESCENCE DERIVATIZATION OF TERTIARY AMINES WITH 2-NAPHTHYL CHLOROFORMATE

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SUMMARY

2-Naphthyl chloroformate (NCF) was found to be a suitable fluorescence reagent for the derivatization of drugs containing a tertiary amino group. The tertiary amines undergo dealkylation when heated with NCF, forming fluorescent carbamates. The reaction has been applied to the pre-column derivatization of some antihistamines.

INTRODUCTION

A great problem in high-performance liquid chromatography (HPLC) is the detection of drugs with low UV absorbance. In such instances it is advantageous to form UV-absorbing or fluorescent derivatives for detection enhancement. Most drugs contain amino groups, and for primary and secondary amines a number of reagents have been described[1–4]. No satisfactory reactions, however, are known for the derivatization of tertiary amines. On the other hand, many drugs contain a tertiary amino group as the only functional group.

A reaction principle already used in gas chromatography[5–11] is the reaction of tertiary amines with chloroformates, which undergo dealkylation under suitable conditions, forming carbamates with the resulting secundary amine.

The aim of this work was the development of reagents on this basis for the HPLC analysis of tertiary amines with fluorescence detection.

EXPERIMENTAL

Apparatus

A Perkin-Elmer Series 2 liquid chromatograph and an LC 55 UV detector connected to a Perkin-Elmer MPF 44 fluorimeter were used with a 25-μl flow-through cell.

Reagents and chemicals

2-Naphthyl chloroformate (NCF) was synthesized according to the literature[12,13]. The reagents used were potassium carbonate (p.a. grade, anhydrous) and saturated methanolic potassium hydroxide. All solvents were of analytical-reagent grade.

0021-9673/81/0000–0000/$02.50

Derivatization procedure

About 10 pmol–5 nmol of the tertiary amine (as the free base) were heated with a 10-fold molar excess of NCF and 10 mg of potassium carbonate in 100 μl of dry benzene at 100°C for 1 h in a sealed conical vial. After cooling, the excess of the reagent was removed by shaking the reaction mixture with 300 μl of saturated methanolic potassium hydroxide for 1 min. After phase separation by adding 1 ml of water, an aliquot of the benzene layer was injected into the chromatograph.

Chromatography

The column contained Knauer RP-18 and the following mobile phases were used: I, methanol–water (80:20); II, methanol–water (70:30); and III, methanol–water–tetrahydrofuran (65:30:5).

RESULTS AND DISCUSSION

2-Naphthyl chloroformate can be synthesized easily by reaction of β-naphthol with phosgene[12,13]. The reagent is sensitive to moisture, but is stable for several months if stored under the cool and dry conditions.

In addition to primary and secondary amines, tertiary amines also form carba-

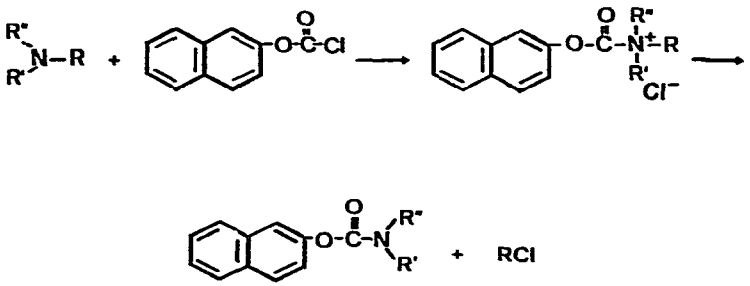


Fig. 1. Scheme of the reaction of NCF with tertiary amines.

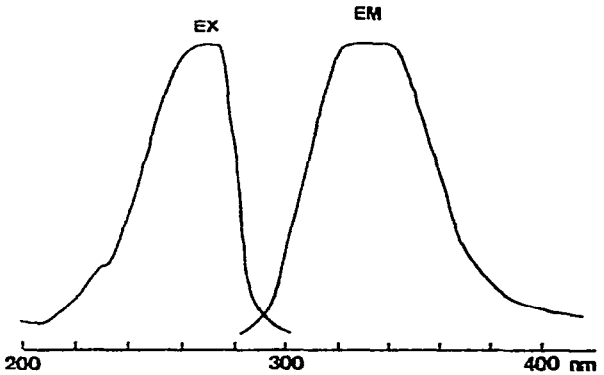


Fig. 2. Fluorecence spectrum of the diphenhydramine derivative in methanol–water (80:20). λ_{max}: excitation, 275 nm; emission, 335 nm.

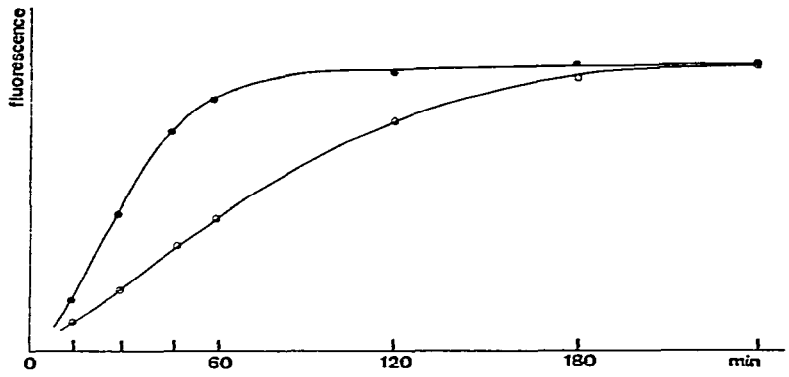


Fig. 3. Kinetics of the reaction of NCF with diphenhydramine. ○, Without catalyst; ●, with K_2CO_3 catalyst.

mates under dealkylation when heated with chloroformates. The scheme of the reaction of NCF with tertiary amines is shown in Fig. 1.

Highly fluorescent carbamates were formed, which showed fluorescence stability for more than 24 h. The fluorescence spectrum showed an excitation maximum at 275 nm and an emission maximum at 335 nm (Fig. 2).

Optimization of the reaction conditions was carried out with the antihistamine diphenhydramine as a model compound. As can be seen from Fig. 3, the use of potassium carbonate as a catalyst permits a higher reaction rate. The excess of the chloroformate can be destroyed by shaking the reaction mixture with alcoholic alkali[9] followed by the addition of water to obtain a better phase separation.

In addition to methyl groups, ethyl and benzyl groups can also be split off. The reaction is also applicable to N-methyl groups in heterocyclics such as N-methylpiperidine. As far as more complicated compounds are concerned, however, the reaction does not take place homogeneously. For example, by-products were formed during the reaction of mebhydroline, clemizol and antazoline. In comparison with the main

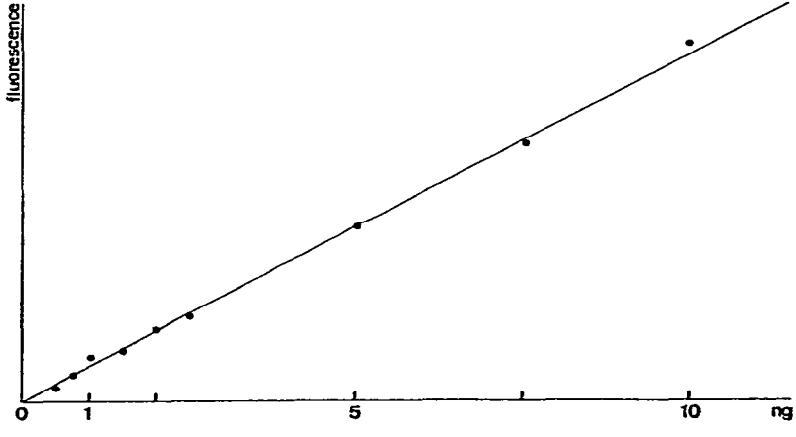


Fig. 4. Calibration graph for diphenhydramine.

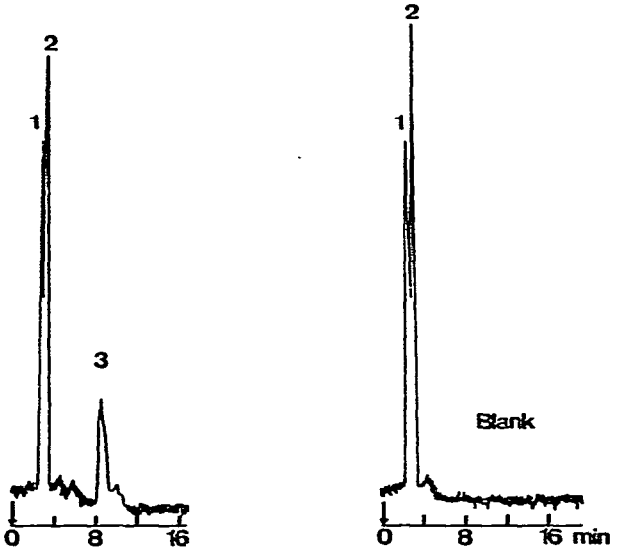


Fig. 5. HPLC of 500 pg diphenhydramine after derivatization with NCF. Column: RP-18, 25 × 0.4 cm I.D. Mobile phase: methanol–water (80:20). Flow-rate: 2 ml/min. Detection: fluorescence, λ_{ex} = 275 nm, λ_{em} = 335 nm. 1, 2 = Decomposition products of the reagent; 3 = 500 pg of diphenhydramine.

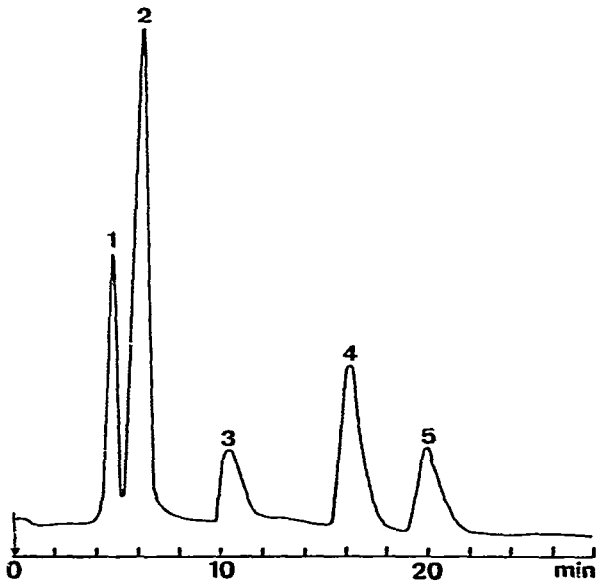
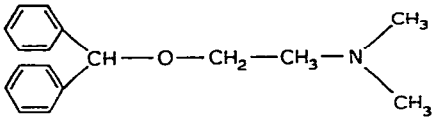
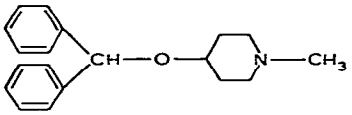
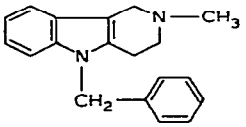
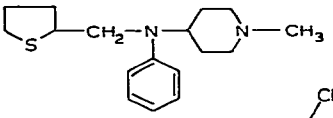
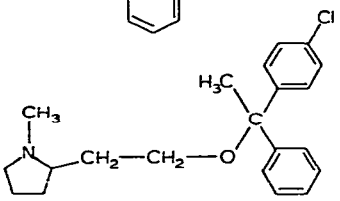
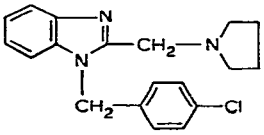
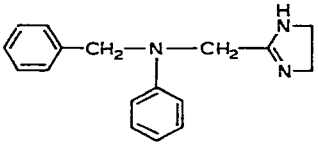


Fig. 6. HPLC separation of antihistamine derivatives. Conditions as in Fig. 5, except mobile phase is methanol–water–tetrahydrofuran (65:30:5). 1, 2 = Decomposition products of the reagent; 3 = diphenylpyraline; 4 = diphenhydramine; 5 = thenalidine.

TABLE I

k' VALUES AND DETECTION LIMITS OF SOME ANTIHISTAMINE DERIVATIVES

Column: RP-18, 25 × 0.4 cm I.D. Mobile phase: methanol–water (70:30). Flow-rate: 2 ml/min. Detection: fluorescence, λ_{ex} = 275 nm, λ_{em} = 335 nm.

Compound	*Formula*	*Detection limit (ng per 20 µl)*	k'
Diphenhydramine		0.5	17.9
Diphenylpyraline		1	16.6
Mebhydroline		5	18.5
Thenalidine		1	20.1
Clemastine		5	19.9
Clemizol		10	19.0
Antazoline		5	17.9

product, however, the by-products amounted to only 1–10%. Quantitative determination is not possible in these instances. It has been found that the reaction also fails with pyridyl-substituted compounds[14].

The absolute yield of the carbamate from diphenhydramine is 81%, estimated

by comparison with a known amount of a synthetic authentic sample. The reaction, however, although not absolutely quantitative, shows good reproducibility. The relative standard deviation determined for 200 ng of diphenhydramine is 1.6% and for 20 ng it is 3% ($n = 9$).

The linearity of the reaction was investigated by preparing calibration graphs for various antihistamines. Linearity was observed over a range of more than one order of magnitude. The correlation coefficients lay between 0.997 and 0.999. A calibration graph for diphenhydramine in the lower nanogram range is shown in Fig. 4.

The capacity ratios (k') and detection limits for some antihistamines are given in Table I. A high sensitivity was observed for diphenhydramine. Fig. 5 shows a chromatogram for 500 pg diphenhydramine after derivatization. The use of a UV detector is also possible, although of course it is much less sensitive.

Fig. 6 shows the separation of a mixture of three antihistamine derivatives.

In addition to the application of the method to antihistamines, it was also applied to examples of other groups of drugs containing tertiary amino groups, such as analgesics, local anaesthetics and psychotropic drugs. The adaption of this method to the determination of drugs in plasma samples is now being examined. Further promising reagents, such as anthrylmethyl chloroformate and fluorenylmethyl chloroformate[16,17], are under investigation.

This derivatization procedure has also been applied with good results to thin-layer chromatography with fluorodensitometric evaluation[15].

REFERENCES

1 N. Seiler and J. Wiechmann, *Experientia*, 20 (1966) 559.
2 M. Roth, *Anal. Chem.*, 43 (1971) 880.
3 P. B. Gosh and M. W. Whitehouse, *Biochem. J.*, 108 (1968) 155.
4 S. Udenfriend, S. Stein, P. Bohler and W. Dairman, *Science*, 178 (1972) 871.
5 P. Hartvig and J. Vessman, *Acta Pharm. Suecica*, 11 (1974) 115.
6 P. Hartvig and J. Vessman, *Anal. Lett.*, 7 (1974) 223.
7 P. Hartvig, N. O. Ahnfelt and K. E. Karlsson, *Acta Pharm. Suecica*, 13 (1976) 181.
8 P. Hartvig, K. E. Karlsson, L. Johansson and C. Lindberg, *J. Chromatogr.*, 121 (1976) 235.
9 P. Hartvig and W. Handl, *Anal. Chem.*, 48 (1976) 390.
10 P. Hartvig, K. E. Karlsson, C. Lindberg and L. O. Boreus, *Eur. J. Clin. Pharmacol.*, 11 (1977) 65.
11 K. E. Karlsson and P. Hartvig, *Acta Pharm. Suecica*, 17 (1980) 249.
12 *Ger. Pat.*, 251,805; *Friedländer*, 11 (1912) 82.
13 L. C. Raiford and G. O. Inman, *J. Amer. Chem. Soc.*, 56 (1934) 1586.
14 L. A. Sternson and A. D. Cooper, *J. Chromatogr.*, 150 (1978) 257.
15 G. Gübitz, R. Wintersteiger and A. Hartinger, *Sci. Pharm.*, in preparation.
16 L. A. Carpino and G. Y. Han, *J. Org. Chem.*, 37 (1972) 3404.
17 H. A. Moye and A. J. Boning, *Anal. Lett.*, *B*, 12, No. 1 (1979) 25.