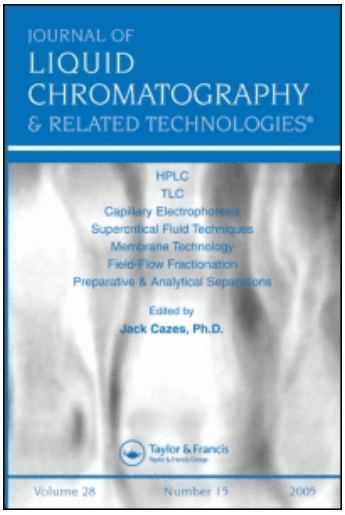


Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Determination of Six Pharmaceuticals and Their Degradation Products in Reversed-Phase High Performance Liquid Chromatography by Using Amine Additives

H. Yang[a]; F. C. Thyrion[a]
[a] Chemical Engineering Institute, Louvain University, Louvain-la-Neuve, Belgium

To cite this Article Yang, H. and Thyrion, F. C.(1998) 'Determination of Six Pharmaceuticals and Their Degradation Products in Reversed-Phase High Performance Liquid Chromatography by Using Amine Additives', Journal of Liquid Chromatography & Related Technologies, 21: 9, 1347 — 1357
To link to this Article: DOI: 10.1080/10826079808005882
URL: http://dx.doi.org/10.1080/10826079808005882

J. LIQ. CHROM. & REL. TECHNOL., 21(9), 1347–1357 (1998)

DETERMINATION OF SIX PHARMACEUTICALS AND THEIR DEGRADATION PRODUCTS IN REVERSED-PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY BY USING AMINE ADDITIVES

H. Yang, F. C. Thyrion

Chemical Engineering Institute
Louvain University
1 Voie Minckelers
B-1348 Louvain-la-Neuve, Belgium

ABSTRACT

Procaine, adiphenine, drofenine, nafronyl, tetracaine, and meclofenoxate and their degradation products were separated by reversed phase high performance liquid chromatography (HPLC) on Spherisorb ODS 2 columns (125 or 250 x 4 mm). The mobile phase was a mixture of acetonitrile or methanol and a buffer solution (pH=4.5) of sodium acetate (0.02 M) and acetic acid. Seven amines with different chemical structures were added respectively into the buffer solution in order to modify the peak tailing and retention behavior of the compounds analyzed. Significant improvement in peak shape and retention time of basic drugs was observed by adding some amines, while in the case of their degradated acids these properties were not modified. Triethylamine was chosen to analyze all the pharmaceuticals studied in this work except for drofenine where di-n-butylamine appeared the best amine.

INTRODUCTION

The difficulties connected with the use of reversed phase high performance chromatography in the analysis of drugs bearing amino group are well known. The silanophilic interaction of the amino group with the silanol function of the silica-bound hydrocarbonaceous stationary phase often causes a number of troubles, such as poor selectivity and unacceptable peak broadening and tailing. These problems can be overcome by the addition of appropriate mobile-phase modifier, such as triethylamine and morpholine which preferentially mask the silanol group on the packing materials.[1-6] We have succeeded in the separation of procaine, adiphenine, drofenine, nafronyl, tetracaine, and meclofenoxate and their degradation products on one type of column Spherisorb ODS 2, although the analysis of one or several of them were reported by other authors on different types of columns. [7-13] The choice of the amine additives and the influence of triethylamine concentration on the retention time and peak shape have also been discussed in this work.

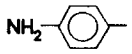
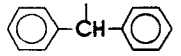
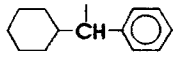
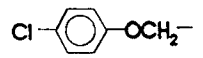
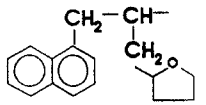
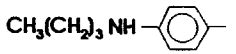
MATERIALS

A Hewlett-Packard 1050 liquid chromatography equipped with a variable wave-length detector (Hewlett Packard, 1050) was used in this study. A guard column (Hewlett Packard, LiChrospher100RP-18, 5μm, 4x4 mm) was fixed at the inlet of the stainless steel Spherisorb ODS 2 column (Hewlett Packard, 5μm, 125 or 250x4 mm).

The drugs studied in this work are listed in figure 1. Procaine hydrochloride 99% (Janssen, Belgium), hydrochloride salts of adiphenine, drofenine, tetracaine, and meclofenoxate (Sigma), nafronyl oxalate salt, 4-aminobenzoic acid 99%, diphenyl-acetic acid 99%, cyclohexylphenylacetic acid 99%, 4-chlorophenoxyacetic acid 98 % (Janssen), and 4-(butylamino)benzonic acid 98% (Aldrich) were used, as they were purchased, without further purification. Acetonitrile, methanol, and water were of HPLC grade (Labscan). Sodium acetate (UCB, Belgium), acetic acid (Aldrich), diethylamine, ethylethanolamine, N,N,N',N'-tetraethylethylenediamine, di-n-butyl-amine, N,N,N',N'-tetramethylethlenediamine, morpholine, triethylamine (Janssen, Belgium), used for the buffer solution were all of analytical grade.

METHODS

The drugs were dissolved in methanol solution for the HPLC injection except in the case of meclofenoxate where a mixture of chloroform and

Name	R1	R2
Procaine 4-Aminobenzoic acid		$-CH_2CH_3$
Adiphenine Diphenylacetic acid		$-CH_2CH_3$
Drofenine Cyclophenylacetic acid		$-CH_2CH_3$
Meclofenoxate 4-Chlorophenoxyacetic acid		$-CH_3$
Nafronyl [a]		$-CH_2CH_3$
Tetracaine 4-(Butylaminobenzoic) acid		$-CH_3$

a: Its degradation acid is not commercially available.

Figure 1. Structures of drugs R1COO-CH_2CH_2N(R2)2 and their degradation acids R1COOH.

isopropanol was used since meclofenoxate may react with methanol. All the separations were conducted at room temperature. Chromatographic retention data were expressed by the capacity factor $k' = (t_R-t_0)/t_0$ where t_R and t_0 are the retention times of the drug and the non-retained compound, respectively. The peak tailing of each drug was estimated by the asymmetry factor As = a/b, where a is the width of the leading half of the peak and b is the width of the tailing half of the peak, both measured at 10% of the peak height.

The analysis of the experimental data was realized by an integrator (Hewlett Packard 3396) and a PC software (Hewlett Packard, Peak 96). The asymmetry factors were calculated by a Matlab program.

Buffer solution was prepared by adding 0.02 M sodium acetate and a certain concentration of an amine to the aqueous solution, pH was then adjusted to 4.5 with acetic acid. The buffer solution was filtered though a nylon membrane (0.45μm, Gelman Science) before use.

Table 1

Capacity and Asymmetry Factors of Procaine and 4-Aminobenzoic Acid with Various Amines Added to the Buffer Solution

Amines	4-Aminobenzonic Acid		Procaine	
	k'	As	k'	As
Without amine	1.10	1.02	8.25	3.70
Di-n-butylamine	1.11	1.00	1.13	1.63
Diethylamine (11)	0.77	1.30	2.22	3.28
Diethylethanolamine (9.81)	0.77	1.18	2.22	3.55
Morpholine (8.36)	0.77	1.08	2.62	3.10
N,N,N',N'-Tetraethyl ethylenediamine	0.97	1.21	2.34	3.26
N,N,N',N-Tetramethyl ethylenediamine	1.02	1.23	1.93	4.35
Triethylamine (10.74)	1.06	1.09	2.08	2.48

On-line mixing of solvents was made by the quaternary pump system to insure the composition of the mobile phase. At each change of the eluent, the column was first washed with 50% methanol-water system, then the column was equilibrated with the new eluent for at least 30 min.

RESULTS AND DISCUSSION

Choice of the Amine Additive

Procaine and its degradation product 4-aminobenzonic acid were used in the study of the choice of a suitable amine additive. Seven amines of 0.1% (v) concentration were each added to the buffer solution and the pH adjusted to 4.5 by acetic acid. The analysis were conducted in the 125x4 mm Shperisorb ODS 2 column at λ = 260 nm. The mobile phase consisted of 25% of methanol and 75% of buffer solution under a flow rate of 1.5 mL/min. Table 1 gives the capacity (k') and asymmetry factors (As).

Procaine retainted strongly on stationary phase when using eluent having no amine additives, it has a capacity factor (k') of 8.25 and an asymmetry factors (As) of 3.70. The capacity factor of procaine decreases dramatically as an amine is added to the mobile phase.

For 4-aminobenzonic acid, the addition of amine has almost no effect on the retention. This confirms the interaction of the amino group of procaine with the stationary phase. For the asymmetry factors, di-n-butylamine appears the best amine providing an acceptable As[4] (As<2). The masking effect of diethylethanolamine bearing a hydroxyl function appears to be much lower than that of triethylamine. This can be explained by the following reasons: 1) The binding of diethylethanolamine to the stationary phase is less effective due to the decrease of its hydrophobicity related to the presence of the hydroxyl function, 2) Its hydroxyl group may interact with its amino group. The As values (3.28 and 1.63 respectively) in the cases of diethylamine and di-n-butylamine demonstrate that an increase in chain length of the amine additives gives a significant improvement in peak shape. The silanol-amine complex might be stabilized by hydrophobic interactions between the hydrocarbonaeous ligates of the stationary phase and the bulky alkyl moiety of di-n-butylamine.

Table 1 shows that N,N,N',N'-tetraethylethylenediamine and N,N,N',N'-tetramethylethylenediamine have almost no positive influence on the asymmetry factors although the retention time of procaine decreases a lot in their presence. It can be assumed that the four methyl or ethyl groups hinder the interaction of the amino group with the silanol group of the packing phase. It seems that the base strength of the basic drugs has no effect on the retention parameters by the comparison of pKa.

Triethylamine was used for the study of the influence of the concentration of amine additive since 4-aminobenzonic acid and procaine can not be separated in the case of di-n-butylamine.

Influence of the Concentration of Triethylamine

Several concentrations of triethylamine were used in order to improve the asymmetry factors of procaine (PA) and 4-aminobenzoic acid (PABA). The evolution of capacity factors and asymmetry factors with the concentration of triethylamine are given in figures 2 and 3. The chromatograms in figure 4 show the improvement of the analysis between two conditions: A) without amine additive; B) the concentration of amine is 0.28% (v) of buffer solution.

The analysis conditions were similar with the ones used in the study of the choice of amine additive, however when the concentration of triethylamine reached 0.28%(v), the following gradient elution of the mobile phase was used in order to obtain a better separation of 4-aminobenzonic acid and procaine : 90

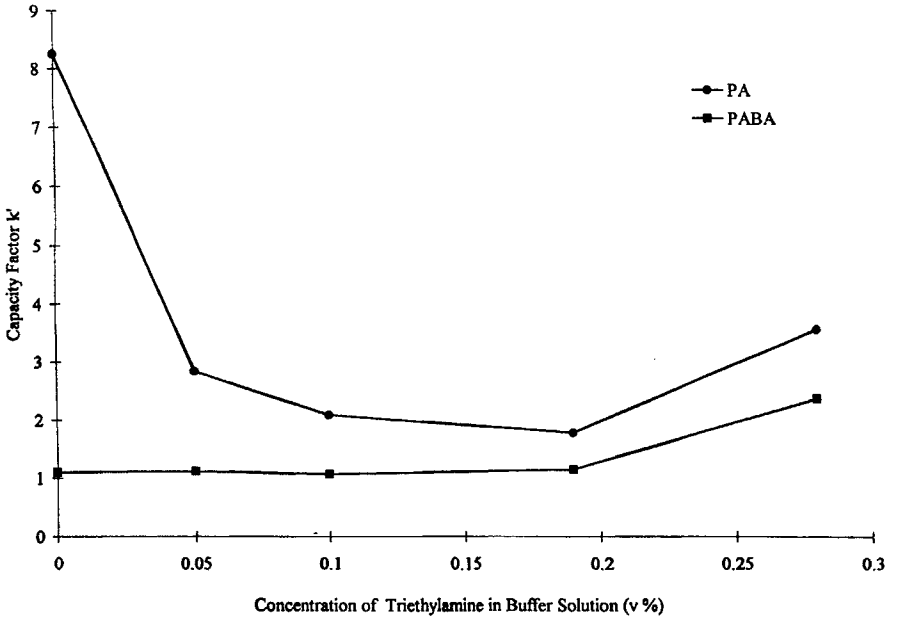


Figure 2. Evolution of the capacity factors of procaine (PA) and 4-aminobenzonic acid (PABA) with the concentration of triethylamine (Conditions: Spherisorb ODS 2 125x4 mm; λ= 260 nm; 25% of methanol and 75% of 0.02 M sodium acetate buffer. flow rate: 1.5 mL/min; when triethylamine reaches 0.28% (v), mobile phase changed to : 90 to 70% (v) of buffer solution from 0 to 5 min, then 70 to 90% (v) of buffer solution from 5.01 to 7 min).

to 70% (v) of buffer solution from 0 to 5 minute, then 70 to 90% (v) of buffer solution from 5.01 to 7 minute. Significant improvement in peak shape and retention time of procaine was observed by increasing the triethylamine concentration, while in the case of 4-aminobenzoic acid these properties were not influenced.

Separation of the Other Pharmaceuticals

Having chosen the amine additives and determined the influence of triethylamine concentration through the analysis of procaine and 4-aminobenzonic acid, the other pharmaceuticals listed in figure 1 were also separated.

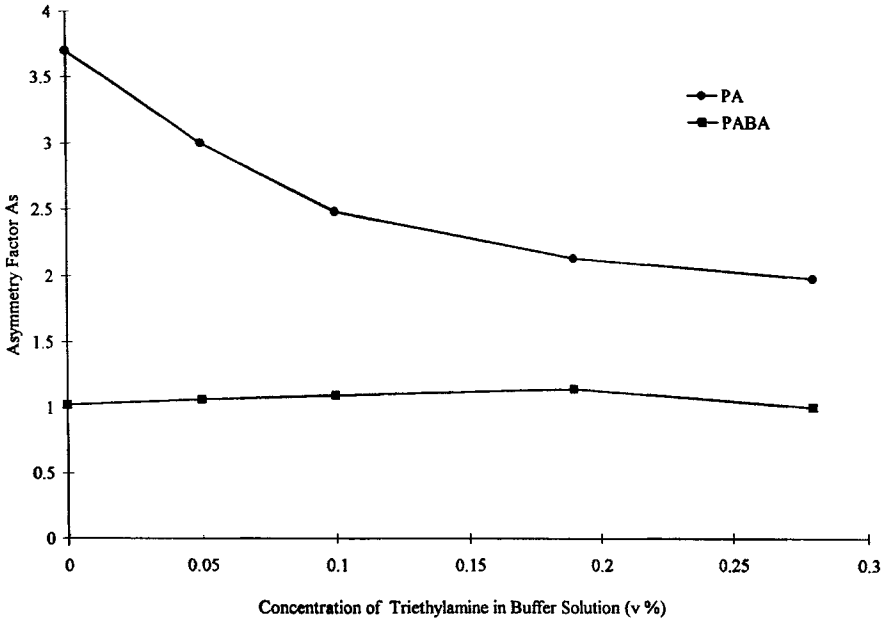


Figure 3. Evolution of the asymmetry factors of procaine (PA) and 4-aminobenzonic acid (PABA) with the concentration of triethylamine (Same analytical conditions as these of figure 2).

The mobile phase consisted of a mixture of 0.02 M sodium acetate buffer solution containing 0.28 %(v) triethylamine and methanol or acetonitrile. Table 2 summarizes the capacity and the asymmetry factors for all the substances analyzed.

The optimum analytical conditions are given below: 1) Adiphenine and diphenylacetic acid were separated by using the 250x4 mm Shperisorb ODS 2 column at λ = 260 nm. The mobile phase consisted of 40% of buffer solution and 60% of acetonitrile (Flow rate, F = 1.5 mL/min.). 2) Nafronyl was analyzed under the same condition as adiphenine but at λ = 280 nm. 3) Meclofenoxate and 4-chloro-phenoxyacetic acid were analyzed in the 125x4 mm Spherisorb ODS 2 column at λ = 280 nm, with a gradient elution of the mobile phase from 15 to 30 % of acetonitrile in 0 to 5 min. (F = 1.5 mL/min.). 4) Tetracaine and 4-butylaminobenzonic acid were separated in the 125x4 mm Spherisorb ODS 2 column (λ = 280 nm) with a mobile phase constitued of 65% of buffer solution and 35% of acetonitrile (F=1.5 mL/min.). 5) Since drofenine retained in the column when triethylamine was used as buffer additive, di-n-

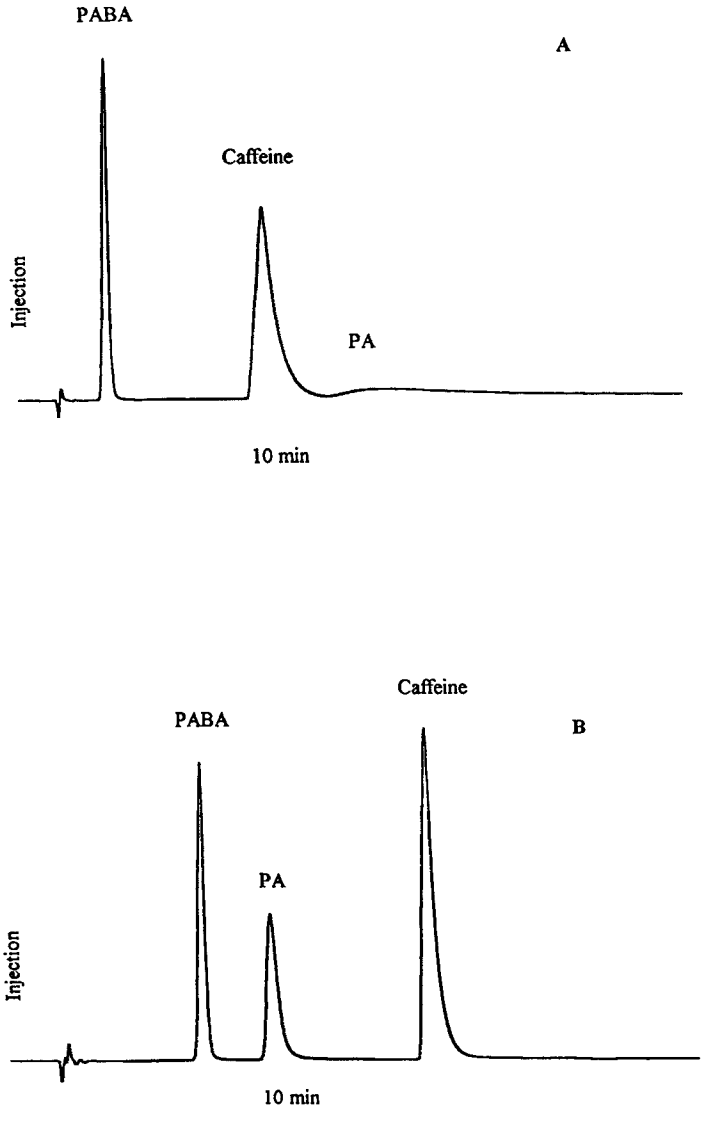


Figure 4. Chromatograms of procaine (PA) and 4-aminobenzoic acid (PABA) in the absence of triethylamine (A) and 0.28% (v) of triethylamine (B) (Same analytical conditions as these of figure 2, caffeine used as internal standard).

Table 2

Capacity and Asymmetry Factors for Five Basic Drugs and Their Degradation Acids

Drugs	As	k'
Adiphenine	3.72	2.30
Diphenylacetic acid	1.05	0.62
Drofenine	4.6	3.72
Cyclohexanephenylacetic acid	2.42	0.29
Meclofenoxate	1.57	4.48
4-Chlorophenoxy acetic acid	1.46	171
Nafronyl	3.46	5.25
Tetracaine	1.28	2.85
4-Butylaminobenzonic acid	2.13	5.70

butylamine was used as additive. Thus drofenine and cyclohexane-phenylacetic acid can be separated in the 250x4 mm column (λ = 260 nm). The mobile phase contained 65% of acetonitrile and 35% of sodium acetate buffer solution with 0.59%(v) of di-n-butylamine (F = 1.5 mL/min.). Among the nine drugs studied, most of them gave acceptable asymmetry factors (As < 2).

However, in the case of adiphenine, drofenine, and nafronyl, poor peak shapes were still observed, which can be improved by using more hydrophobic amine additives such as long chain aliphatic amines. This will be the objective of further study.

CONCLUSION

The present study has shown a successful separation of procaine, adiphenine, drofenine, nafronyl, tetracaine, and meclofenoxate and their degradation products on reverse-phase HPLC by using one type of column, Spherisorb ODS 2.

The use of amine additives has proved to be an effective method for the improvement of peak shape and retention time of basic drugs. The retention parameters (As, k') provided in this paper can be useful when it becomes expensive to buy one column for each group of compound to be separated.

Finally, it is worth noting that the columns used in this work have been used intensively for six months; a better result would be obtained with a new column.

ACKNOWLEDGMENTS

The authors wish to thank Christieans-Nycomed for kindly purchasing the drugs used in this work. One of the authors,Dr. H. Yang, is now working at the Separation Laboratory, Department of Chemical Engineering, The National University of Singapore.

REFERENCES

1. A. Sokolowski, K. G. Wahlund, J. Chromatogr., **189**, 299-316 (1980).

2. L. R. Snyder, J. L. Glajch, J. J. Kirkland, **Practical HPLC Method Development**, John Wiley, New York, 1988.

3. A. Nahum, C. Horvath, J. Chromatogr., **203**, 53-63 (1981).

4. K. E. Bij, C. Horvath, W. R. Melander, V. Nahum, J. Chromatogr., **203**, 65-84 (1981).

5. R. Gill, S. P. Alexander, A. C. Moffat, J. Chromatogr., **247**, 39-45, (1982).

6. R. M. Smith, J. P. Westlake, R. Gill, M. D. Osselton, J. Chromatogr., **514**, 97-109 (1990).

7. T. Daldrup, P. Michalke, Chromatogr. Newsl., **10(1)**, 1-7 (1982).

8. M. Bauer, C. Degude, L. Mailhe, J. Chromatogr., **315**, 457-464 (1984).

9. H. G. Eigendorf, G. Moschwitzer, R. Budde, Pharazie., **44**, 645-647 (1989).

10. G. Facchini, F. Zaccheo, M. Nannetti, Boll. Chim. Farm., **122**, 405-411 (1983).

11. L. Gagliardi, A. Amato, A. Basili, G. Cavazzutti, J. Chromatogr., **362**, 450-454 (1986).

12. R. Gill, M. D. Osselton, J. Chromatogr., **386**, 65-77 (1987).

13. F. Li, Yao Wu Fenxi Za Zhi, **9(3)**, 169-171 (1989).

Received June 1, 1997
Accepted August 4, 1997
Manuscript 4511