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Short Communication

Utility of dansyl derivatization to the high-performance liquid chromatographic analysis of 2-phenylethylamine drugs

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ABSTRACT

This paper describes the utility of dansyl derivatization in the high-performance liquid chromatographic separation of a number of 2-phenylethylamines of pharmaceutical interest. The derivatives were formed in an alkaline acetone–aqueous sodium carbonate solution, and injected into the chromatograph without the need for their extraction from the reaction mixture. Separations were accomplished on an octadecylsilane column using methanol–water–acetic acid–triethylamine mobile phases and photometric detection at 249 or 335 nm. Comparison of the retention data for the non-dansylated and dansylated 2-phenylethylamines indicated the achievement of favorable alterations in the elution orders. The applicability of dansyl derivatization to the detection of an impurity was demonstrated with a sample of epinephrine containing norepinephrine at the 2% level.

INTRODUCTION

The 2-phenylethylamines are a group of drugs of pharmaceutical and pharmacological interest because of their widespread occurrence in commercial dosage forms and the diversity of their biological actions [1]. These compounds are primary or secondary amines of low molecular weight, often also containing one or more phenolic groups [2]. Owing to the close similarities of their structural features, the chromatographic resolutions of mixtures of 2-phenylethylamines is sometimes challenging, particularly when dealing with diastereomeric pairs such as ephedrine and pseudoephedrine [3,4].

Our laboratory is investigating the use of 5-dimethylaminonaphthalene-1-sulfonyl (dansyl) chloride as a derivatizing reagent for the high-performance liquid chromatographic (HPLC) analysis of drugs of pharmaceutical importance. In addition to

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imparting strong ultraviolet and fluorescent properties, the introduction of a dansyl moiety into the structure of an organic analyte will result in a derivative of higher molecular weight and lower polarity, effects which are all desirable in chromatographic separations [5,6]. Hence, it is not surprising to find that this reagent has been extensively used for the identification, detection and separation of a wide variety of drugs by thin-layer chromatography (TLC) [7–10] and HPLC [11–15]. However, HPLC methods for the analysis of those 2-phenylethylamines that are present in pharmaceutical dosage forms, and which make use of a dansylation step, appear to be limited to only a few compounds [16–19].

The purpose of this communication is to describe the general utility of dansyl derivatization for the HPLC separation of eighteen phenolic and non-phenolic 2-phenylethylamine drugs found in pharmaceutical samples and of which all but two, methamphetamine and norpseudoephedrine, are listed in the United States Pharmacopeia [20]. Furthermore, the applicability of this approach to the simultaneous detection of these compounds when they occur in mixtures in which one of the 2-phenylethylamine derivatives is present in much smaller concentrations than another one is also demonstrated.

EXPERIMENTAL

Apparatus and experimental conditions

The liquid chromatograph consisted of a Spectra-Physics (Santa Clara, CA, USA) Model 3500B solvent delivery system and a Model 770 variable-wavelength detector. Samples were injected through a Valco (Houston, TX, USA) injector valve fitted with a 10-μl sample loop. Elutions were monitored at 254 or 335 nm. Chromatograms were recorded with a Hewlett-Packard (Avondale, PA, USA) Model 3380A recording integrator. The chromatographic column was a Waters Assoc. (Milford, MA, USA) 10-μm μBondapak C_{18}, 300 × 3.9 mm I.D., protected by a Whatmann (Clifton, NJ, USA) 30–40-μm Co:Pell C_{18}, 70 × 2.1 mm I.D. guard column. The mobile phases were combinations of HPLC-grade methanol, reagent-grade acetic acid and triethylamine (TEA) (J. T. Baker, Phillipsburg, PA, USA) and water that had been doubly distilled in glass. All analyses were performed at ambient temperature, and with the mobile phases delivered at the flow-rate of 1.5 ml/min.

Chemicals

2-Phenylethylamines used were laboratory working standard samples of levonordefrin, hydroxyamphetamine hydrobromide, the sulfate salts of amphetamine and mephentermine, the bitartrate salts of epinephrine, metaraminol and norpinephrine, and the hydrochloride salts of dopamine, ephedrine, ethylnorepinephrine, methamphetamine, methoxamine, norephedrine (phenylpropanolamine), norpseudoephedrine, phenoxyphenamine, phentermine, phenylephrine and pseudoephedrine. Standard solutions of 2-phenylethylamines (1 mg/ml) were prepared by dissolving the individual compounds in methanol–water–acetic acid–TEA (50:38:1.5:0.5). Reagents for derivatization reactions were analytical-grade dansyl chloride (Aldrich, Milwaukee, WI, USA) and certified anhydrous sodium carbonate and spectroanalyzed acetone (Fisher Scientific, Fair Lawn, NJ, USA).

Reagents

The dansyl chloride solution was prepared in acetone to contain 5 mg/ml. After filtering, this solution was stored in an amber glass container. The basic solution was prepared by dissolving 0.367 g of sodium carbonate in 300 ml of water, adding 150 ml of acetone, and mixing.

Dansylation procedure

To the 2-phenylethylamine compound (*ca.* 5 mg), contained in a 50-ml glass-stoppered erlenmeyer flask, 10 ml of dansyl chloride solution and 15 ml of basic solution were added in succession. After dissolving the sample with swirling, the flask was stoppered and allowed to stand in the dark and at room temperature for at least 2 h. The solution was then injected into the chromatograph.

RESULTS AND DISCUSSION

The dansylation procedure described in the present study is based on the experimental conditions used by Fishman [21] for the dansylation of the phenolic groups of estrogen compounds. Such experimental conditions are especially advantageous for the derivatization of the *o*-diphenolic functionalities of 2-phenylethylamine compounds like the catecholamines because, once dansyl moieties become attached to these positions, the possibility of their fast base-catalyzed oxidative degradation to non-reactive quinone artifacts is precluded [22]. However, since the dansylation of the amino group of 2-phenylethylamines by this procedure has not been previously described, we conducted first a reaction-rate study using model compounds. Based on the temporal changes in detector responses at 335 nm, the non-phenolic 2-phenylethylamines, phenylpropanolamine and pseudoephedrine, were dansylated more slowly than phenolic estrogens. Moreover, the reaction rates for both compounds became maximal in about 2 h and remained fairly constant thereafter. Similar results were obtained with those 2-phenylethylamines capable of forming di- and tridansylated derivatives.

Table I lists the capacity ratio, k', values of the non-dansylated and dansylated forms of the 2-phenylethylamines studied. As expected, the data show that the dansyl derivatives are more strongly retained than the corresponding non-dansylated parent compounds, a situation that made necessary the use of a higher concentration (65 *vs.* 5%) of methanol in the mobile phase to bring about their elution. Furthermore, dansylation altered the order of elution of several groups of 2-phenylethylamines. For instance, whereas the non-dansylated compounds were retained in the increasing order non-phenolic > monophenolic > diphenolic, dansylation caused the order of retention to change to that of diphenolic > monophenolic > non-phenolic. The same effect was noted with the diastereomeric pair phenylpropanolamine–norpseudoephedrine. In addition, the dansyl derivative of methoxamine was eluted ahead of the dansyl derivatives of non-phenolic 2-phenylethylamines even though they were similarly retained when in the non-dansylated state. This change in elution behavior is probably related to structural differences among these compounds since methoxamine in the only one possessing an alkoxy group.

Fig. 1. shows the HPLC separation of a mixture of several non-phenolic 2-phenylethylamines known to undergo dansylation only at a primary or secondary

TABLE I

COMPARISON OF k' VALUES OF 2-PHENYLETHYLAMINES AND THEIR CORRESPONDING DANSYL DERIVATIVES

$k' = (t_R/t_0) - 1$, where t_R = retention time and dead time t_0 = 2.0 min. Mobile phases: methanol–water–acetic acid–TEA (non-dansylated = 5:93:1.5:0.5; dansylated = 65:33:1.5:0.5).

Compounds	k'	
	Non-dansylated (254 nm)	Dansylated (335 nm)
Diphenolic		
Norepinephrine	0.2	23.0
Levonordefrin	0.2	27.4
Ethylnorepinephrine	0.2	31.0
Epinephrine	0.3	39.1
Dopamine	0.3	45.7
Monophenolic		
Metaraminol	0.6	8.6
Phenylephrine	0.6	10.7
Hydroxyamphetamine	1.1	15.1
Non-phenolic		
Phenylpropanolamine	1.6	1.8
Norpseudoephedrine	2.0	1.7
Ephedrine	2.3	2.5
Pseudoephedrine	2.9	3.0
Methoxamine	3.3	1.6
Amphetamine	3.7	3.7
Methamphetamine	5.0	5.5
Phentermine	7.6	6.1
Methoxyphenamine	8.7	6.6
Mephentermine	9.8	7.6

amino group. Relative to their non-dansylated forms, dansylation generally led to improved peak symmetry and, as in the case of the diastereomeric pair ephedrine and pseudoephedrine, also to improved resolution (R_s = 2.67 *vs.* 2.00) in spite of the closeness of their relative retention values (α = 1.25 for the dansylated form; α = 1.23 for the non-dansylated form). However, in the case of the related compounds amphetamine and methamphetamine it was surprising to find that both resolution (R_s = 7.03 *vs.* 3.70) and relative retention (α = 1.70 *vs.* 1.38) were improved since these compounds differ from each other by only one N-methyl group. A similar trend was noted with the pair phentermine–mephentermine, for which the difference is just one methyl group.

Fig. 2 shows the separation of the dansyl derivatives of representative 2-phenylethylamines capable of adding from one to three dansyl groups to their structures. As expected, the order of elution was found to be directly related to the number of dansyl groups added, with the monodansylated derivatives eluting the earliest and the tridansylated derivatives eluting the latest. As with the mixture of dansyl derivatives

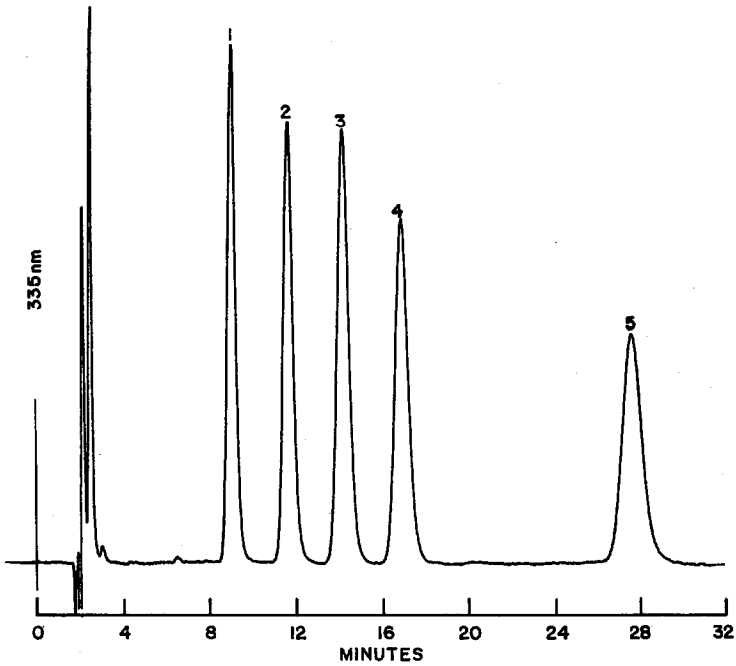

Fig. 1. Chromatographic separation of the monodansyl derivatives of: 1 = phenylpropanolamine; 2 = ephedrine; 3 = pseudoephedrine; 4 = amphetamine; 5 = methamphetamine. Mobile phase: methanol–water–acetic acid–TEA (60:38:1.5:0.5).

shown in Fig. 1, the separation was only achievable when the concentration of methanol in the mobile phase was raised even further, namely to 70%. An interesting finding was the order of elution of the pair materaminol–phenylephrine, two monophenolic compounds differing only in the position of the methyl group in the alkyl side chain, and whose mixture was not resolvable when in the underivatized form. After dansylation, metaraminol, a primary amine, eluted ahead of phenylephrine, an N-methylated secondary amine.

To test the applicability of the dansylation reaction to the simultaneous analysis of a mixture of two or more 2-phenylethylamines, and in which one of the components is present in much smaller concentrations relative to another, a sample of epinephrine found to contain less than 4% of norepinephrine by the compendial TLC procedure [20], was analyzed by the HPLC method with dansylation. By comparison with the results obtained with a solution of pure epinephrine that had been spiked with different concentrations of norepinephrine, and on the basis of the ratio of the norepinephrine peak area to the sum of the norepinephrine plus epinephrine peak areas, the suspected epinephrine sample was found to contain about 2% norepinephrine (duplicate analyses). Hence, the proposed dansylation procedure may also prove valuable in the HPLC detection and quantitation of very low levels of 2-phenylethylamine compounds that may be present as contaminants of other 2-phenylethylamines.

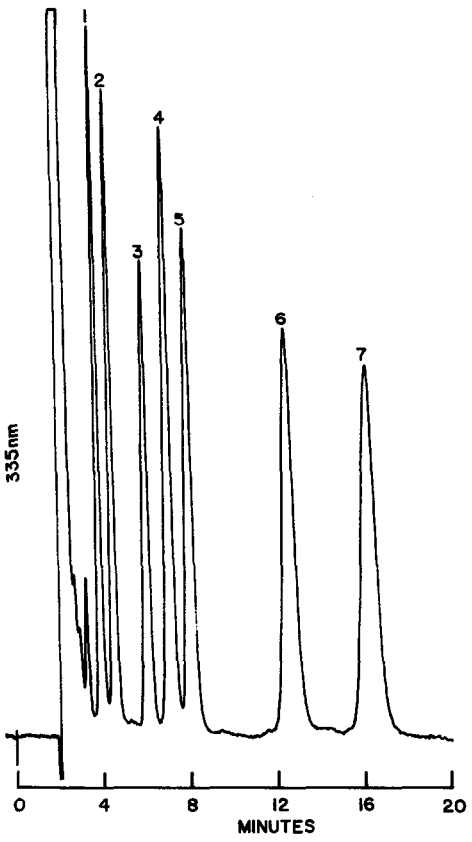

Fig. 2. Chromatographic separation of the mono-, di- and tridansylderivatives of: 1 = phenylpropanolamine; 2 = ephedrine; 3 = metaraminol; 4 = phenylephrine; 5 = hydroxyamphetamine; 6 = norepinephrine; 7 = epinephrine. Mobile phase: methanol–water–acetic acid–TEA (70:28:1.5:0.5).

In summary, dansylation is a simple and effective means of improving the detectability and resolution of 2-phenylethylamines of pharmaceutical interest during their HPLC analysis. In addition, comparison of the retention times of the dansylated and non-dansylated forms of a given 2-phenylethylamine may provide the analyst with additional confirmatory evidence on the identity of these compounds.

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