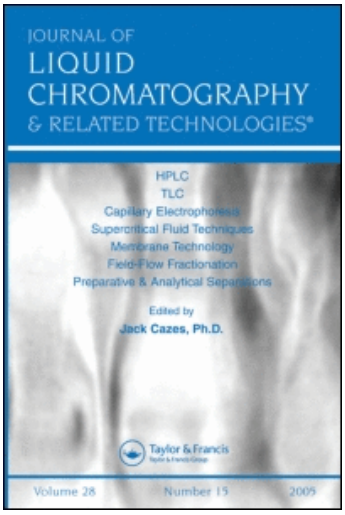

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HIGH-PRESSURE LIQUID CHROMATOGRAPHY ASSAY FOR DANE SALT POTASSIUM (-)-N-(1-METHOXYCARBONYLPROPENE-2YL)-p-HYDROXYPHENYLGLYCINE

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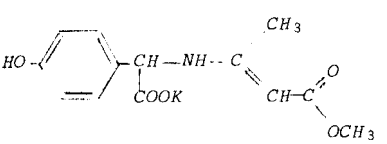
ABSTRACT

A rapid, ion-pair, high-pressure liquid chromatographic method for analysis of Dane Salt Potassium (-)-N-(1-Methoxycarbonylpropene-2-yl)-p-Hydroxyphenylglycine was developed. Tetrabutylammonium hydroxide was used as a counter-ion in the mobile phase. A fixed wavelength detector (λ = 280 nm) and a μ-Bondapak C-18 column were employed. The percent relative range of the method (precision) was 0.6% (n = 3).

INTRODUCTION

Enamine protected amino acids are commonly used during the manufacture of penicillins and cephalosporins (1–9).

These compounds are referred to as Dane Salts. Potassium (-)-N-(1-Methoxycarbonylpropene-2-yl)-p-Hydroxyphenylglycine (I) is such a compound.



I

A method to determine the purity of this compound has not been reported in the literature. Quality of this compound has a bearing on the overall yield and quality of the penicillin or cephalosporin being manufactured by it. Therefore, it was decided to develop a specific HPLC assay for determination of purity of Dane Salt Potassium (-)-N-(1-Methoxycarbonylpropene-2-yl)-p-Hydroxphenylglycine.

EXPERIMENTAL

Reagents:

Methanol, Burdick & Jackson, Muskegon, MI 49442.

Tetrabutylammonium Hydroxide, Eastman Chemical Company, Rochester, New York 14650.

Potassium Hydroxide Solution (45%, w/v), Fisher Scientific Company, Fair Lawn, New Jersey 07410.

Dane Salt Potassium (-)-N-(1-Methoxycarbonylpropene-2-yl)-p-Hydroxyphenylglycine (Reference Standard)
Bristol Laboratories, Syracuse, New York 13201.

Phosphoric Acid (85%, w/v), Fisher Scientific Company, Fair Lawn, New Jersey 07410.

HPLC Conditions:

Column:	μ-Bondapak C-18, 30 cm x 3.9 mm I.D., from Waters Associates, Milford, Mass., Catalog No. 27324.
Pump:	Milton Roy mini-pump, 5000 p.s.i. or equivalent.
Mobile Phase:	MeOH/Water (50/50) with 0.1N potassium hydroxide, 0.00693M tetrabutylammonium hydroxide adjusted to pH 7.0 with concentrated phosphoric acid.
Flow Rate:	1.5 ml/minute.
Injector:	Valco Loop Injector, 7000 p.s.i., 40-μl loop, Valco Instrument Co., Inc., Houston, Texas.
Detector:	Waters Model 440 or equivalent, 280 nm.
Chart Speed:	0.2 inch/minute.

Suggested Attenuations & Retention Times:	*RT (min.)*	*Attenuations*
Dane Salt Potassium (-)-N-(1-Methoxy-carbonylpropene-2-yl)-p-Hydroxphenylglycine	*4.0 ± 0.5*	*0.05*
Benzocaine (Internal Standard)	*5.5 ± 0.5*	*0.05*

Mobile Phase Preparation:

Transfer 500 ml of methanol and 450 ml of distilled water to a 1-liter volumetric flask and mix the contents. Pipet 18.0 ml of tetrabutylammonium hydroxide titrant, and 8.5 ml of potassium hydroxide reagent to the flask and mix the contents. Adjust to pH 7.0 with phosphoric acid (approximately 6 ml). Dilute to volume with water. Filter the resulting solution through Whatman No. 5 filter paper (5.5 cm) placed in a Millipore filter holder.

Diluent Preparation:

Transfer 1.0 ml of Potassium Hydroxide Solution (2.25%, w/v) and 40.0 ml of methanol to a 100-ml volumetric flask. Dilute to volume with distilled water and mix well.

Internal Standard (Benzocaine Solution) Preparation:

Transfer 25 ± 1 mg of Benzocaine Solution to a 100-ml volumetric flask. Dissolve and dilute to volume with methanol and mix well. Transfer 10 ml of the resulting solution to a 100-ml volumetric flask, dilute to volume with methanol and mix well.

Master Standard or Sample Solution Preparation:

Transfer 30 ± 2 mg of Dane Salt Potassium (-)-N-(1-Methoxy-carbonylpropene-2-yl)-p-Hydroxyphenylglycine Reference Standard or sample, accurately weighed, into a 200-ml volumetric flask. Add 100 ml of methanol and swirl to dissolve (1) the contents. Dilute to volume with methanol and mix well.

Working Standard or Sample Solution Preparation:

Transfer 5.00 ml of Dane Salt Potassium (1)-N-(1-Methoxycarbonylpropene-2-yl)-p-Hydroxyphenylglycine Reference Standard or sample solution and 10.00 ml of Benzocaine Solution into a 50-ml volumetric flask. Dilute to volume with methanol and mix well. Transfer 4.0 ml of the resulting solution to a 10-ml volumetric flask, dilute to volume with the diluent (2) and mix well.

NOTES

(1) If the standard or sample is not dissolved in methanol after swirling, sonicate for five minutes.

(2) Diluent should be added to the working solution immediately before injecting the final solution into the HPLC system.

Calculations:

1. *Peak Height Ratio (R)* $= \frac{\text{Peak Height of Analyte}}{\text{Peak height of Internal Standard}}$

   $R_{Std.}$ = *Peak height ratio of standard solution.*

   R_{Sple} = *Peak height ratio of sample solution.*

   Analyte = Dane Salt Potassium (-)-N-(1-Methoxycarbonylpropene-2-yl)-p-Hydroxyphenylglycine.

2. *Standard Factor (F)* $= \frac{\text{Std. wt. (mg) x Std. Purity (\%)}}{R_{Std.} \times 100}$

3. *Assay Results:*
   Percent Dane Salt Potassium (-)-N-(1-Methoxycarbonylpropene-2-yl)-p-Hydroxyphenylglycine

$$= \frac{R_{Sple} \times F \times 100}{\text{Sample wt. (mg)}}$$

RESULTS & DISCUSSION

The purpose of this work was to develop a specific assay procedure for Dane Salt Potassium (-)-N-(1-Methoxycarbonylpropene-2-yl)-p-Hydroxy-

TABLE I

Spike Recoveries

Amount Added		Amount Found	
% Target	mg	mg	% Recovery
60	19.08	19.13	100.3
100	31.80	32.38	101.8

phenylglycine with a suitable level of precision and accuracy. Standard linearity was checked by assaying standards ranging from 25 to 200% of the target value with Benzocaine as internal standard. Relative standard deviation (2s%) for chromatographic variability was determined by five injections of the standard solution and was found to be 0.6%. Accuracy of the method was determined by spiking authentic Dane Salt Potassium (-)-N-(1-Methoxycarbonylpropene-2-yl)-p-Hydroxyphenylglycine at the 60 and 100% levels, respectively, to the actual sample solution (containing 27.75 mg Dane Salt Potassium (-)-N-(1-Methoxy-carbonyl-propene-2-yl)-p-Hydroxyphenylglycine. The percent recoveres are shown in Table I.

Percent relative range for precision of the method was 0.6% and was determined by a single injection of triplicate sample preparations for each of two samples.

Specificity of the assay was shown by injecting two known degradation products, methylacetoacetate and p-hydroxyphenylglycine. No interferences were noted. A search was conducted in order to find a diluent in which Dane Salt Potassium (-)-N-(1-Methoxycarbonyl-propene-2-yl)-p-Hydroxyphenylglycine could be dissolved without significant degradation before injection. Dane Salt Potassium (-)-N-(1-Methoxycarbonylpropene-2-yl)-p-Hydroxyphenylglycine was more stable

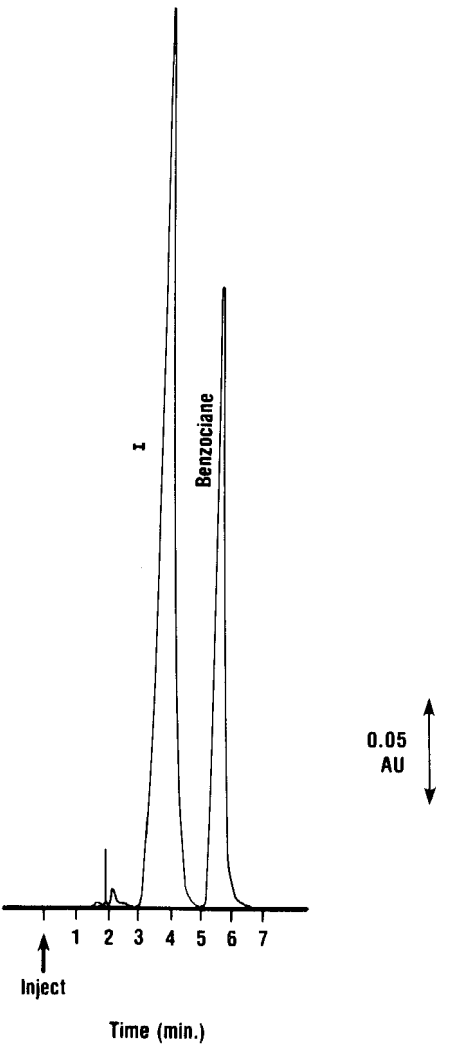


FIGURE 1 Sample Chromatogram

in methanol than in water or mobile phase alone over time. When methanol was used as a diluent, distorted peak shapes were produced during chromatography. When 40% methanol in H_2O, containing 0.023% potassium hydroxide solution was used in the final dilution step, Gaussian peak shapes and less than 1% loss after 10 minutes of Dane Salt Potassium (-)-N-(1-Methoxycarbonylpropene-2-yl)-p-Hydroxphenylglycine was observed. A typical sample chromatogram is shown in Figure 1.

In summary, an ion-pair HPLC assay method has been validated. The precision, accuracy and specificity of the method have been shown to be good.

REFERENCES

(1) Ishisuro, Susuru; Ishibashi, Kenichi., Japan Patent 7959296 (1977).

(2) Diaso Meseuer, Jose; Estere Bianchini, Asuncion; Sans Pitarch, Esteve., Spain Patent 474440 (1978).

(3) Wright, Ian G., U.S. Patent 4148817 (1976).

(4) VanderDrift, Johannes K.; Henninser, Peter W.; VanVeen, Gerard J., U.S. Patent 4128547 (1977).

(5) Estere Bianchini, Asuncion; Oalomo Coll, Antonio Luis, German Patent 2822876 (1977).

(6) Cuixart Grande, Jesus, Spain Patent 454986 (1977).

(7) Crast, Leonard B., Jr., Canada Patent 1024507 (1973).

(8) Crast, Leonard B., Jr., Gottstein, William J., U.S. Patent 516047 (1972).

(9) Lons, Anthony A. W.; Nayler, John, H. C., German Patent 1942693 (1968).