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1-(4-HYDROXYPHENYL)-, 1-(2,4-DIHYDROXYPHENYL)- AND 1-(2,5-DIHYDROXYPHENYL)-2-BROMOETHANONES: NEW LABELS FOR DETERMINATION OF CARBOXYLIC ACIDS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH ELECTROCHEMICAL AND ULTRAVIOLET DETECTION

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SUMMARY

A method is presented for the derivatization and determination of carboxylic acids by high-performance liquid chromatography with electrochemical and ultraviolet detection. The derivatizing reagents used in this study were synthesized, and their suitability was investigated for determination of drugs and metabolites with carboxylic acid groups. Quinoxaline-2-carboxylic, benzoic and salicylic acids each labeled with 1-(4-hydroxyphenyl)-, 1-(2,4-dihydroxyphenyl)- and 1-(2,5-dihydroxyphenyl)-2-bromoethanone were the principal esters studied; in addition, some antibiotics and their salts were also esterified. Conditions of derivatization are relatively mild at 60°C for 60 min or less, and the reaction is 76% complete. The detection limits are as low as 1 pmol for some acids. Clean-up steps are not required to remove excess derivatizing reagent.

INTRODUCTION

The analysis of foods for the presence of trace amounts of chemical contaminants is important for maintaining public confidence in the wholesomeness of food products. Many of these contaminants can be determined by high-performance liquid chromatography (HPLC) if an innate chromophoric, fluorescent or electrochemically active group is present. However, if these groups are absent, then chemical labeling of the contaminants is required for their determination. A chemical label should readily attach to the target compound, should be quantitatively attached, should allow measurement at low concentrations and should minimize the effects of interfering substances found in complex matrices. A variety of chemical labels have been developed in recent years. Although they were initially developed for determina-

tion of compounds by gas chromatography[1,2], chemical labels are now being produced for HPLC. The following compounds with labels for HPLC with ultraviolet (UV) detection have been reported in the literature: amine compounds using a phenylisothiocyanate label[3], dansyl chloride derivatives of phenols[4] and *p*-bromophenacyl bromide coupled with carboxylic acids[5]. Reagents sensitive to fluorescence detection include dansyl hydrazine, which reacts with aldehydes and ketones[6]; 9-anthryldiazomethane, which couples with carboxylic acids[7]; and 4-bromomethyl-6,7-dimethoxycoumarin, which also labels carboxylic acid groups[8]. In addition, a few reagents have been used for HPLC with electrochemical detection (ED): 2,4-dinitrofluorobenzene for amines[9], silver picrate for alkyl halides[10], *p*-nitrophenylhydrazine for aldehydes and ketones[11], *o*-phthalaldehyde for amino acids[12,13] and salicylaldehyde for hydrazines[14]. No derivatives of organic acids have been reported for HPLC with ED.

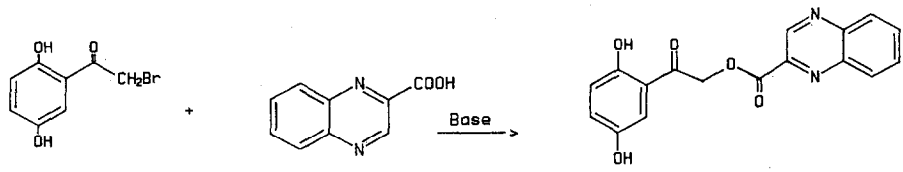
Many drug residues contain a carboxylic acid group. If these carboxylic acid groups are derivatized with an electrochemically active reagent, then the drug residues can be detected at low concentrations by using an electrochemical detector. Three electrochemically active reagents are 1-(4-hydroxyphenyl)-2-bromoethanone (4-HBE, CAS 2491-38-5), 1-(2,4-dihydroxyphenyl)-2-bromoethanone (2,4-DBE, CAS 2491-39-6) and 1-(2,5-dihydroxyphenyl)-2-bromoethanone (2,5-DBE, CAS 25015-91-2). The esterification reaction for an organic acid and 2,5-DBE is shown in Fig. 1. Both the syntheses and the results of a comparative study of the three compounds are presented, and potential applications are discussed.

MATERIALS AND METHODS

Apparatus

A Waters Assoc. Model 660 liquid chromatograph with a UK6 injector (Waters Assoc., Milford, MA, U.S.A.) was equipped for UV detection with a Model 163 Beckman (Berkeley, CA, U.S.A.) UV detector; for ED a Bioanalytical Systems (West Lafayette, IN, U.S.A.) Model LC4B amperometric electrochemical detector was used throughout. The cell consisted of a single glassy carbon indicator electrode, a 0.05-mm gasket and an Ag/AgCl reference electrode.

Separations of the esters were attained on a column (250 × 4 mm I.D.) of 7-μm RP-18 LiChrocart (Cat. No. 15539, E. Merck, Darmstadt, F.R.G.). The mobile phase consisted of 58 parts of methanol to which were added 42 parts of 0.1 *M* sodium acetate buffered at pH 6.5; the flow-rate was 1.0 ml/min. The UV determination was performed with a commercially prepared column (250 × 4.6 mm I.D.) of 5-μm RP-18



1-(2, 5-dihydroxyphenyl)-2-bromoethanone

Quinoxaline-2-carboxylic acid

2-(2, 5-dihydroxyphenyl)-2-oxo-2-ethyl quinoxaline-2-carboxylate

Fig. 1. Esterification reaction for an organic acid and 2,5-DBE.

Ultrasphere (Beckman) and a mobile phase of methanol–water (60:40) at 1.0 ml/min. An IBM (Danbury, CT, U.S.A.) Model NR80-B nuclear magnetic resonance spectrometer, a Hewlett-Packard (Palo Alto, CA, U.S.A.) Model 8452A diode array spectrophotometer and a Finnigan MAT (San Jose, CA, U.S.A.) 311A mass spectrometer were used.

Chemicals

The following chemicals were purchased: triethylamine (TEA), 18-crown-6 ether, dibenzo-18-crown-6 ether, 4-(4-nitrobenzyl)pyridine, aluminium bromide, bromoacetyl bromide, *p*-hydroxyacetophenone, cupric bromide, 1,4-dimethoxybenzene and 1,3-dihydroxybenzene (Aldrich, Milwaukee, WI, U.S.A.); acetonitrile, toluene, heptane, dimethylformamide, cyclohexane, hexane, ethyl acetate, chloroform, methanol, methylene chloride and benzene, all distilled-in-glass (Burdick & Jackson, Muskegon, MI, U.S.A.); quinoxaline-2-carboxylic acid (Q-2-C) (Pfizer, Groton, CT, U.S.A.); benzoic acid (BA) (Sigma, St. Louis, MO, U.S.A.); salicylic acid (SA) (Mallinckrodt,. St. Louis, MO, U.S.A.); [^{14}C]benzoic acid (Research Products International, Mount Prospect, IL, U.S.A.); hydrogen bromide (Matheson Gas Products, Rutherford, NJ, U.S.A.); acetone-d_6 (Norell, Landisville, NJ, U.S.A.). A single stock solution containing Q-2-C, SA and BA, each at $1.00 \cdot 10^{-6}$ mol/ml, was prepared in acetonitrile. Acetonitrile was the solvent for all other reagents used in the derivatizing step.

β-Lactam penicillins

Ampicillin, amoxicillin, cephapirin sodium and hetacillin potassium were USP (United States Pharmacopeial Convention, Rockville, MD, U.S.A.); penicillin G sodium was from Sigma; methicillin sodium, carbenicillin monosodium, oxacillin sodium, cloxacillin sodium and dicloxacillin sodium were obtained from Beecham-Massengill Pharmaceuticals (Bristol, TN, U.S.A.); and nafcillin sodium was from the Food and Drug Administration, National Center for Antibiotic and Insulin Analysis (Washington, DC, U.S.A.).

Active halide test[15]

The α-bromo ketones were spotted and developed on silica gel by thin-layer chromatography (TLC) using ethyl acetate–hexane (1:1). The plate was dried, sprayed with a 1% solution of 4-(4-nitrobenzyl)pyridine in acetone and then sprayed with 10% sodium hydroxide solution. A purple spot appeared, indicating the presence of an active halide (*i.e.*, the presence of an α-halocarbonyl group).

Synthesis

(Method A) 4-HBE[16]. Cupric bromide (22.3 g, 0.100 mol) was added to a 250-ml flask equipped with a water condenser, and then 40 ml of ethyl acetate was added. The mixture was stirred, heated to boiling, and 40 ml of hot chloroform containing 8.17 g (0.060 mol) of *p*-hydroxyacetophenone was added rapidly. The mixture was heated to boiling for 1 h, filtered through Celite and then rotaryevaporated to dryness. The purple solid was dissolved in 100 ml of hot toluene, treated with 0.1 g of activated charcoal and 10 g of anhydrous sodium sulfate, vacuum-filtered through Celite and recrystallized. The purple crystals were dissolved in 150 ml of hot chloroform, treated

with 0.1 g of activated charcoal, vacuum-filtered through Celite, recrystallized and dried in a vacuum for 2 h at 80°C. White needles (2.0 g) gave a melting point of 126–127°C[16]. The needles gave a single TLC spot, a positive test for active halide, a molar absorptivity of 18 000 M^{-1} cm^{-1} at 288 nm in methanol and the following proton NMR data in acetone-d_6 (integration, chemical shift in ppm, peak type): 1H, 9.2, singlet; 2H, 8.0, doublet; 2H, 7.0, doublet; 2H, 4.6, singlet. The presence of about 5% of unreacted *p*-hydroxyacetophenone was indicated by a singlet at 2 ppm.

(Method B) 2,4-DBE[17]. A mixture of 16.5 g (0.150 mol) of 1,3-dihydroxybenzene, 32 g (0.16 mol) of bromoacetyl bromide and 150 ml of dry carbon disulfide was added to a 500-ml erlenmeyer flask. The mixture was cooled in an ice bath and stirred while 40 g of aluminum bromide (0.15 mol) was added over 15 min. The mixture was protected from moisture with a calcium chloride drying tube, stirred for 30 min more and allowed to set for 16 h at room temperature. The carbon disulfide was decanted and discarded, and the remaining brown solid was decomposed with 200 ml of ice water. The resulting slurry was shaken with two 100-ml portions of diethyl ether. The ether extract was dried with calcium chloride, treated with 0.5 g of activated charcoal, filtered through Celite and rotary-evaporated to dryness. The resulting oil was recrystallized three times from toluene–cyclohexane (1:1), once from chloroform and once from methanol–water (1:1) to yield 3.0 g of small white needles, which were dried in a vacuum at 80°C and had a melting point of 126–128°C[17]. The needles gave a single TLC spot, a positive test for active halide, molar absorptivities of 12 000 and 10 000 M^{-1} cm^{-1} at 290 and 318 nm, respectively, in methanol and the following NMR data in acetone-d_6 (integration, chemical shift in ppm, peak type); 1H, 10.8, singlet; 1H, 9.6, singlet; 1H, 8.1–7.8, multiplet; 2H, 6.5–6.3, multiplet; 2H, 4.6, singlet.

(Method C) 2,5-DBE[18]. 1,4-Dimethoxybenzene (27.6 g, 0.200 mol) and 28 ml (0.32 mol) of bromoacetyl bromide were added to a 500-ml erlenmeyer flask. The mixture was cooled in an ice bath and stirred while 53.4 g (0.200 mol) of aluminum bromide was added over 10 min. Within 10 min after the addition was completed, an exothermic reaction took place that filled the flask with a red solid. After the contents of the flask had set for 12 h at room temperature, 100 ml of 48% hydrogen bromide solution and 100 g of ice were added. The mixture was stirred for 1 h and was shaken with two 200-ml portions of diethyl ether. The ether extract was shaken with three 200-ml portions of water, dried with 40 g of magnesium sulfate, filtered and rotary-evaporated to dryness. The resulting dark solid (49 g) was recrystallized three times from absolute ethanol to yield 11 g of the monobromoacetate of 2,5-DBE (m.p. 105–107°C; NMR results indicated that a small amount of the 5-methoxy derivative was present). The 11 g was dissolved in 200 ml of warm, dry methanol which was saturated with hydrogen bromide. After the mixture had been stirred for 18 h, 200 ml of water was added. The mixture was cooled to −10°C in a refrigerator. The yellow solid was collected by vacuum filtration and dried under vacuum at 50°C for 48 h. One recrystallization from toluene–heptane (1:1) followed by one recrystallization from toluene yielded 5.0 g of small yellow needles, which had a melting point of 117–119°C. The needles gave a single TLC spot, a positive test for active halide, a molar absorptivity of 4000 M^{-1} cm^{-1} at 372 nm in methanol and the following NMR data in acetone-d_6 (integration, chemical shift in ppm, peak type): 1H, 11.1, singlet; 1H, 8.3, singlet; 3H, 7.3–6.7, multiplet; 2H, 4.7, singlet.

(Method D) 2-(2,5-dihydroxyphenyl)-2-oxoethyl ester of BA. A mixture of 92.0 mg (0.398 mmol) of 2,5-DBE, 28.0 μl of TEA and 12.2 mg of BA was dissolved in 3.0 ml of acetonitrile. The mixture was sealed in a vial and heated at 45°C for 4 h. The mixture was transferred to a separatory funnel with 100 ml of water and was shaken with three portions of methylene chloride. The methylene chloride extract was concentrated to a few milliliters for mass spectrometric analysis.

General derivatization procedure

Each reaction was carried out in a 1-ml vial with a PTFE-lined cap. The vials were heated in an aluminum block heater. In most cases, a 4:2:1 molar ratio of derivatizing reagent, TEA and the organic acid was used. Dimethyl formamide was used to dissolve the antibiotics. Temperature and reaction time for 2,4-DBE and 2,5-DBE were 2 h and 45°C, whereas for 4-HBE they were 1 h and 80°C.

RESULTS AND DISCUSSION

Electron-ionization mass spectrometry was used to verify the structure of the ester formed from the reaction of 2,5-DBE and BA [2-(2,5-dihydroxyphenyl)-2-oxoethyl benzoate]. Method D was used to form the ester, and the mass spectrum was obtained. The parent peak at *m/z* 272 and both the P + 1 and P + 2 isotope peaks were observed. The intensities of the P + 1 and P + 2 peaks were 17.1 and 2.26% of that of the parent peak, respectively, which agree well with the theoretical values of 16.9 and 2.27% calculated for $C_{15}H_{12}O_5$. The ion fragments at *m/z* 65, 69, 77, 109, 137, 150, 164 and 254, as well as the base peak at *m/z* 105, can be attributed to this ester.

The determination of carboxylic acids by HPLC with UV detection has been applied to derivatives of either phenacyl bromide or *p*-bromophenacyl bromide[5]. 4-HBE was synthesized for use as a derivatizing reagent so that carboxylic acids could be determined electrochemically after a similar esterification process, which is described above in the general derivatization procedure. Although ED is sensitive at low concentrations of acid, the time required for the detector to equilibrate at the minimum operating potential of 1.2 V made the use of the 4-HBE ester impractical. We found, however, that the 4-HBE ester was very useful for HPLC determinations with UV detection. Of the three derivatizing reagents evaluated, 4-HBE showed the strongest absorption with a molar absorptivity of 17 000 M^{-1} cm^{-1} at 289 nm in methanol–water (1:1). The wavelength of maximum absorption and the molar absorptivity of each derivatizing reagent were similar to those of the corresponding ester. The usefulness of 4-HBE was further tested by reacting it with several aliphatic and aromatic carboxylic acids. The detection limit is 8 pmol (1 ng) for the 4-HBE derivative of BA, which gave a peak at 8.1 min that was well separated from the peak for unreacted 4-HBE. The minimum excess of 4-HBE compared to BA needed to obtain complete derivatization was 4 mol. A plot of the absorbance of the ester *versus* the amount of BA (Table I) was linear between 30 and 1400 pmol of BA.

The compounds 2,4-DBE and 2,5-DBE were synthesized to lower the working potential to a more useful voltage. The ED responses to Q-2-C, a metabolite of Carbadox, coupled to 2,4-DBE and to 4-HBE as well as to 2,5-DBE were measured over a range of potentials (Fig. 2). Of the three derivatives of Q-2-C, the 2,5-DBE ester oxidizes at the lowest voltage, thereby providing stability since most potentially

TABLE I

LINEAR EQUATIONS AND CORRELATION COEFFICIENTS (r) FOR PLOTS MADE WITH VARIOUS DERIVATIVES

$y = mx + b$.

*Plot No.**	m ($pmol^{-1}$)	b	r
1	$2.78 \cdot 10^{-5}$	$-4.11 \cdot 10^{-4}$	0.9998
2	0.0170	4.60	0.9913
3	0.0681	2.00	0.9977
4	0.109	2.82	0.9939

* (1) Plot of absorbance at 289 nm *vs.* picomol of BA derivatized with 4-HBE; (2) plot of response (nA) at 1.2 V *vs.* picomol of Q-2-C derivatized with 4-HBE; (3) plot of response (nA) at 0.6 V *vs.* picomol of Q-2-C derivatized with 2,5-DBE; (4) plot of response (nA) at 1.1 V *vs.* picomol of Q-2-C derivatized with 2,4-DBE.

interfering substances are not oxidized at this lower potential of 0.4 V. At 1.1 V, the baseline was stable for the 2,4-DBE ester of Q-2-C, but the response was lower than those for the other labeled esters. All esters were well separated from the unreacted reagents, which elute early.

Variables such as reagent ratio, temperature, reaction time and reaction solvent were investigated. The optimum molar ratio of derivatizing reagent–base–sample is 4:2:1 for all three derivatizing reagents. No improvement in peak height was obtained when the relative proportion of derivatizing reagent was greater than 4. Since the unreacted reagents elute near the "solvent front" and their peaks show little tailing, a large molar excess of reagent would cause interference for only those derivatives with short retention times. When the ratio of derivatizing reagent to base was 1:1, the

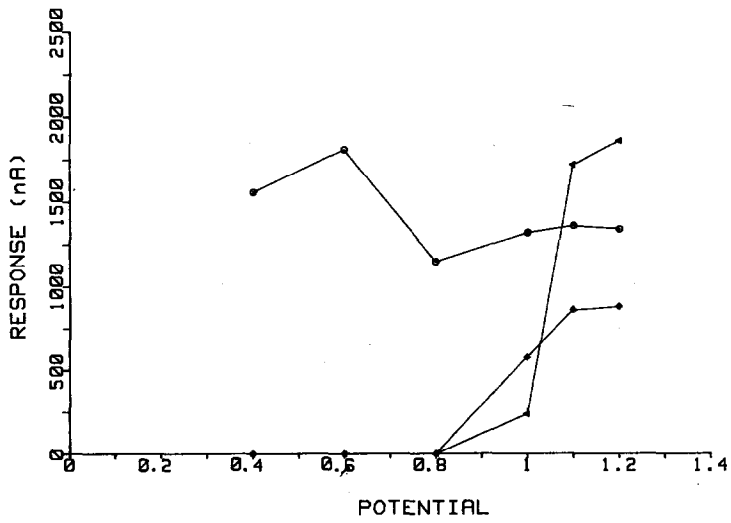


Fig. 2. Plot showing working potential (V) of three derivatives of Q-2-C. Key to symbols: ○ = 2,5-DBE–Q-2-C ester; ◇, 2,4-DBE–Q-2-C ester; △, 4-HBE–Q-2-C ester.

chromatogram showed that excess derivatizing reagent was destroyed with a somewhat lower peak response for the esters. To ensure an excess of derivatizing reagent, the molar proportion of base was decreased to 0.5. The ratios of reagents to the acid salts were not investigated extensively, and the ratios were generally kept at 4:2:1 when a crown ether was used instead of base.

Other factors that influence the degree of derivatization are temperature, reaction time and reaction solvent. The derivatization reaction that was most affected by temperature was the formation of the 4-HBE esters; a reaction temperature of 80°C produced products that gave the best peak response. On the contrary, little increase in peak response was noted from esters of 2,4-DBE and 2,5-DBE produced at reaction temperatures above 40°C; these esters of salicylic acid did, however, have a higher yield at 60°C, suggesting that each acid should be tested for temperature effect. Subsequent measurements to determine the optimum reaction time were carried out at 45°C for reactions involving 2,4-DBE and 2,5-DBE and 80°C for those involving 4-HBE. Generally there was an increase in peak response over time for esters of all three derivatizing reagents, but when this relationship was plotted, the slopes of the lines were greatest for the 4-HBE esters of all acids tested. Esters of 2,4-DBE had the lowest slopes.

The last variable investigated for completeness of reaction was solvent effect. The solvents which gave the best yield were acetonitrile and benzene; dimethylformamide, acetone and ethyl acetate gave lower yields. With the use of [^{14}C]benzoic acid, the yield of the 2,5-DBE ester was estimated. The derivative, prepared as described in the general derivatization procedure, was injected into the liquid chromatograph and 1.0-ml fractions were collected. The radioactivity of each fraction was measured and, after subtracting the baseline counts, the counts for the ester fraction were calculated to be 76% of the total (Fig. 3).

The linearity of the standard curves for the esters of the three derivatizing reagents and Q-2-C was verified as shown in Table I. The slope for the 4-HBE ester is greater than the slopes for the esters of the other labels. The point scattering for the

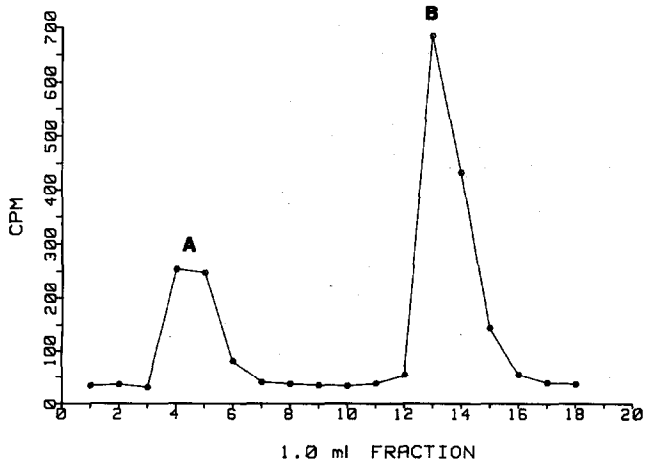


Fig. 3. Radioactivity of fractions collected during HPLC separation of (A) unreacted [^{14}C]benzoic acid from (B) 2,5-DBE ester of [^{14}C]benzoic acid.

4-HBE and 2,4-DBE derivatives is due mainly to the instability of the detector at the working voltage (1.2 and 1.1 V, respectively), whereas the low potential (0.60 V) used for the 2,5-DBE ester resulted in points that fall along the curve.

To determine the detection limits, a series of standard solutions containing each of the three organic acids was prepared, and the general derivatization procedure was carried out with 2,5-DBE. The injected solutions contained the equivalent of 0.5–10 pmol of each acid per 10-μl injection. The lowest concentration detected that was greater than two times the background noise was 1 pmol each for Q-2-C and SA, determined as the 2,5-DBE esters. Fig. 4 shows these chromatograms and that of the 2,5-DBE ester of benzoic acid, which has a detection limit of 2 pmol. The noise level at 0.2 nA was acceptable, although it was difficult to maintain with repeated injections.

No reaction was observed between the mobile phase and the three derivatizing reagents, which are stable in acetonitrile for at least four weeks. The esters of most organic acids appear to be stable in acetonitrile for two days or more.

The retention times of derivatives of some β-lactam penicillins are given in Table II. The ester formation for antibiotics in the acid form must be carried out under mild conditions to prevent the hydrolysis of the β-lactam ring by TEA. When the reaction

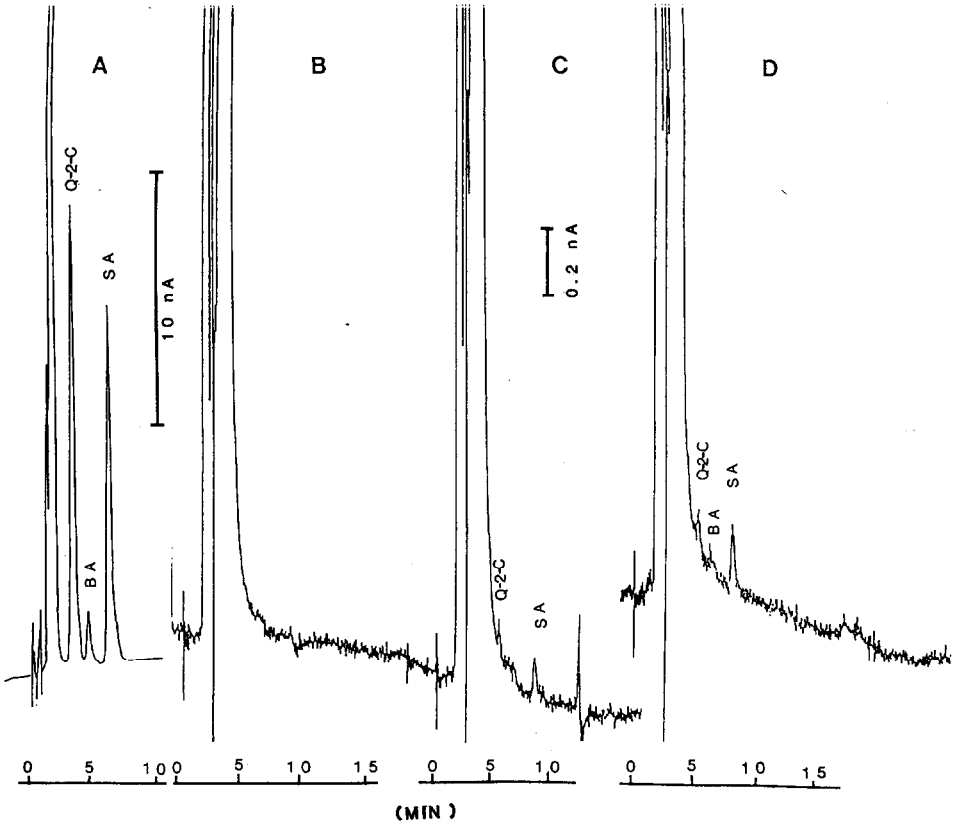


Fig. 4. Chromatograms of the 2,5-DBE esters of Q-2-C, BA and SA, obtained at a potential 0.6 V: (A) 1 nmol of each acid; (B) blank; (C) 1 pmol of each acid; (D) 2 pmol of each acid.

TABLE II

RETENTION TIMES (t_R) AT 0.8 V FOR SOME β-LACTAM PENICILLINS DERIVATIZED WITH 2,5-DBE

*Antibiotic derivatized**	t_R *(min)*	*Relative* t_R**
Ampicillin***	10.3	–
Amoxicillin***	16.7	–
Cephapirin sodium***	19.3	–
Cephapirin sodium§	6.3	0.30
Hetacillin potassium	7.8	0.38
Methicillin sodium	10.7	0.48
Penicillin G sodium	12.8	0.60
Carbenicillin monosodium	13.7	0.61
Oxacillin sodium	19.0	0.89
Cloxacillin sodium	21.0	0.98
Dicloxacillin sodium	28.8	1.32
Nafcillin sodium	30.7	1.46

* 18-Crown-6 ether was used for potassium salts; dibenzo-18-crown-6 ether was used for sodium salts.

** Relative to hexesterol.

*** Mobile phase: methanol–0.1 *M* sodium acetate, pH 6.5 (45:55).

§ Mobile phase: methanol–0.1 *M* sodium acetate, pH 6.5 (58:42).

involves the salt of an antibiotic, the ester is formed rapidly since it is catalyzed by the presence of dibenzo-18-crown-6 ether for sodium salts or 18-crown-6 ether for potassium salts. Hexesterol was used as an internal standard in order to determine the relative retention times of the esters. The working potential was set at 0.80 V to obtain these data because hexesterol is oxidized at this voltage.

CONCLUSION

The requirements of a good label mentioned at the outset have been met for the most part, that is, the derivatization reactions occur under mild conditions, and although not quantitative, they are fairly complete. The detection limits are very low for both UV and ED determinations of the esters of 4-HBE and 2,5-DBE, respectively. The absorption maximum for 4-HBE derivatives occurs at a wavelength that avoids most of the interferences found at lower wavelengths. Similarly, the oxidation of the derivatives of 2,5-DBE at the lower potentials eliminates those substances that are oxidized above 0.4–0.6 V, making the use of these chemical labels a practical alternative in the HPLC determination of carboxylic acids.

REFERENCES

1 R. K. Munns and J. E. Roybal, *J. Assoc. Off. Anal. Chem.*, 65 (1982) 1048.
2 *Pierce 1986–87 Handbook & General Catalog*, Pierce Chemical Company, Rockford, IL, p. 184.
3 R. L. Heinrikson and S. C. Meredith, *Anal. Biochem.*, 136 (1984) 65.
4 P. Tijssen and E. Kurstak, *Anal. Biochem.*, 99 (1979) 97.
5 H. Menard, A. Gaboury, D. Belanger and C. Roy, *J. Anal. Appl. Pyrolysis*, 6 (1984) 45.
6 R. K. Munns, W. Shimoda, J. E. Roybal and C. Vieira, *J. Assoc. Off. Anal. Chem.*, 68 (1985) 968.

7 E. E. Martinez and W. Shimoda, *J. Assoc. Off. Anal. Chem.*, 68 (1985) 1149.
8 R. Farinotti, P. Siard, J. Bourson, S. Kirkiacharian, B. Valeur and G. Mahuzier, *J. Chromatogr.*, 269 (1983) 81.
9 W. A. Jacobs and P. T. Kissinger, *J. Liq. Chromatogr.*, 5 (1982) 881.
10 I. S. Krull, C. M. Selavka, C. Duda and W. Jacobs, *J. Liq. Chromatogr.*, 8 (1985) 2845.
11 K. Shimada, M. Tanaka and T. Nambara, *Anal. Lett.*, 13 (1980) 1129.
12 L. A. Allison, G. S. Mayer and R. E. Shoup, *Anal. Chem.*, 56 (1984) 1089.
13 P. Leroy, A. Nicolas and A. Moreau, *J. Chromatogr.*, 282 (1983) 561.
14 P. E. Kester and N. D. Danielson, *Current Separations*, 5 (1984) 68.
15 B. R. Baker and J. A. Hurlbut, *J. Med. Chem.*, 11 (1968) 233.
16 L. C. King and G. K. Ostrum, *J. Org. Chem.*, 29 (1964) 3459.
17 G. N. Naik and T. H. Crawford, *Indian J. Chem.*, 4 (1966) 273.
18 M. C. Kloetzel, R. P. Dayton and B. Y. Abadir, *J. Org. Chem.*, 20 (1955) 38.