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Note

High-performance liquid chromatography of lipoic acid and analogues

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Lipoic (thioctic) acid is a naturally occurring cofactor which functions within transacylase subunits of α-keto acid dehydrogenase complexes[1]. Some analogues of lipoic acid have been prepared chemically[2] and others isolated as metabolites[3]; however, conventional chromatographic methods for purification and identification of such light-sensitive and polymerizable compounds commonly result in significant losses during the slower separations involved[4].

As demonstrated in the present paper, the closed nature of high-performance liquid chromatographic (HPLC) systems and the flexibility and speed of separation that can be achieved make this a good method for the study of lipoate derivatives.

MATERIALS AND METHODS

The separations were made with a Waters Assoc. (Milford, MA, U.S.A.) HPLC system consisting of: a 6000 A and M45 solvent delivery system, U6K injector, 450 variable-wavelength detector, data module and a stainless-steel (300 × 3.9 mm) μBondapak C_{18} column. Solvent programming was accomplished by using either a 660 solvent programmer or a 720 system controller.

Methanol was obtained from Waters Assoc., J. T. Baker (HPLC; Phillipsburg, NJ, U.S.A.) or Fisher Scientific (HPLC; Pittsburgh, PA, U.S.A.). Acetic acid was from Mallinckrodt (ACS; St. Louis, MO, U.S.A.). Water was deionized (Continental Water System, El Paso, TX, U.S.A.) and glass distilled. All solvent solutions were filtered through a 0.45-μm filter (Type HA Millipore) under vacuum before use. Mallinckrodt spectra dimethylformamide (DMF) was used as the sample solvent. Lipoic acid (LA) and dihydrolipoic acid (DIHLA) (Sigma, St. Louis, MO, U.S.A.), lipoamide (LAAM) and lipoyl glycinamide (LGYL) (Formochimica Cutolo-Calosi, Naples, Italy), 8-methyl-lipoic acid (MELA) and 6,9-dithiononanoic acid (DITNN) (Lederle Lab, Pearl River, NY, U.S.A.) were from commercial sources. Methyl tetranorlipoate S-oxide (TNS[O]ME), tetranorlipoic acid (TN), β-hydroxybisnorlipoic acid (HOBN), bisnorlipoic acid (BN) and methyl lipoate (LME) were obtained by syntheses[2] or isolation as metabolites[3].

Elution patterns were monitored at 330 nm (absorption maximum of the dithiolane ring) or at 240 nm (for detection of open-ring and oxidized analogues as well).

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RESULTS

The elution pattern of lipoic acid and analogues which is developed by linear gradient 1 (Table I) is illustrated in Fig. 1. The retention times and amounts of each component in the sample mixture are given in Table II. The peak heights measured at 330 nm are in reasonable agreement with the quantities of the dithiolanyl compounds,

TABLE I
CONDITIONS FOR LINEAR GRADIENTS
All gradients started with solvent A and ended with B; flow-rate was 2 ml/min.

Gradient	*Methanol (%)*		*Duration (min)*	*%/min*
	*Solvent A**	*Solvent B***		
1	40	70	15	2.0
2	37.5	75	20	1.9
3	30	75	25	1.8
4	25	75	30	1.7

* Contained 0.4 ml acetic acid per liter.
** Contained 0.2 ml acetic acid per liter.

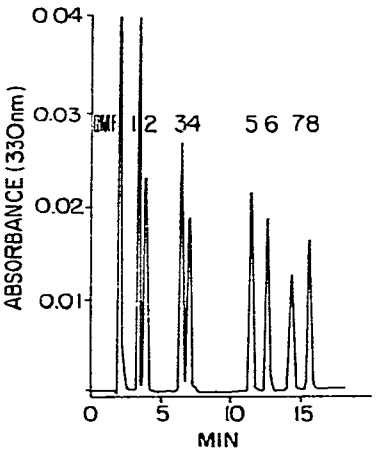

Fig. 1. Elution pattern for lipoic acid and analogues. 1 = TN; 2 = HOBN; 3 = BN; 4 = LAAM; 5 = LA; 6 = DITNN; 7 = MELA; 8 = LME. Conditions given in text.

but not for DITNN which has an absorbance maximum shifted to 285 nm as expected for this six-membered dithiane derivative. Separation of the more polar components can be increased by developing the column with gradients in which the methanol concentration of solvent A has been decreased. The series of gradients reported here (Table I) have essentially the same rate of increase in methanol and the separations of the less polar components are little changed. The elution behavior in gradient 1 of three other analogues, *viz.* methyl tetranorlipoate S-oxide, lipoyl glycinamide, and dihydrolipoic acid, are also included in results presented in Table II. The oxidized derivative (TNS[O]ME) has the shortest retention time of the series, eluting just after

TABLE II
RETENTION TIMES OF LIPOIC ACID AND ANALOGUES
Compositions of gradients are given in Table I; retention times are expressed in min.

Compounds	*Amounts (μmol)*	*Gradients*			
		1	*2*	*3*	*4*
TN	186	3.11	3.28	4.06	4.76
HOBN	82	3.56	3.88	5.33	6.78
BN	85	6.18	6.83	9.51	12.03
LAAM	77	6.76	7.60	10.78	13.73
LA	73	11.20	12.53	16.56	20.13
DITNN	221	12.36	13.83	17.96	21.63
MELA	74	14.06	15.73	20.03	23.86
LME	–	15.33	17.10	21.50	25.43
TNS[O]ME		2.15			
LGLY		6.60			
DIHLA		11.96			

the DMF peak. The presence of lipoic acid in samples of the slow-eluting dihydrolipoic acid, monitored at 240 nm, could be confirmed by the position of elution when monitored at 330 nm. Lipoyl glycinamide (dissolved in 25% methanol as it exhibited limited solubility in DMF) has a slightly shorter retention time than lipoamide in gradient 1.

While establishing the conditions given in Table I, sample mixtures were run with 25 to 75% methanol gradients in which acetic acid was absent from solvent A, B, or both. In these cases, variations in elution position, broadening, and generally less satisfactory separations were found, especially for the more polar acid derivatives. To confirm that this was associated with the ionization of the acidic group, one such shoulder/peak was collected from the lipoic acid position and rechromatographed in the acidified gradient system. A single symmetrical peak for lipoic acid was thereby obtained.

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