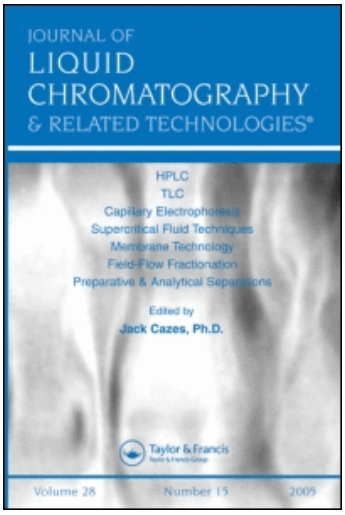


Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

TLC and HPLC of Mixture of Anthracyclinones

Jiří Matějů[a]; Marian Beran[a]; Josef Jizba[a]; Miloslav Podojil[a]
[a] Department of Biogenesis of Natural Substances, Institute of Microbiology Czechoslovak Academy of Sciences, Prague 4, Czechoslovakia

To cite this Article Matějů, Jiří , Beran, Marian , Jizba, Josef and Podojil, Miloslav(1981) 'TLC and HPLC of Mixture of Anthracyclinones', Journal of Liquid Chromatography & Related Technologies, 4: 6, 977 — 983
To link to this Article: DOI: 10.1080/01483918108059599
URL: http://dx.doi.org/10.1080/01483918108059599

JOURNAL OF LIQUID CHROMATOGRAPHY, 4(6), 977-983 (1981)

TLC AND HPLC OF MIXTURE OF ANTHRACYCLINONES

Jiří Matějů, Marian Beran
Josef Jizba and Miloslav Podojil
Department of Biogenesis of Natural Substances
Institute of Microbiology
Czechoslovak Academy of Sciences
CS-142 20 Prague 4, Czechoslovakia

ABSTRACT

A combination of TLC and HPLC was used to separate a natural mixture of anthracyclinones /daunomycinone, 7-deoxydaunomycinone, 7-deoxy-13-dihydrodaunomycinone, 13-dihydrodaunomycinone, carminomycinone, 13-dihydrocarminomycinone, "bisanhydrocarminomycinone" and ε-rhodomycinone/. The method is suitable for routine analysis of anthracyclinones in the course of their biosynthesis in production cultures and during in vitro biotransformations.

INTRODUCTION

Analysis of anthracyclines and anthracyclinones by the TLC method was first used to isolate dihydrodaunomycinone /1/ and to separate adriamycin and dihydrdaunomycin /2/. A number of studies concerned the formation of this group of substances during fermentation /3-8/ and during in vitro and in vivo conversions /9, 10/.were published. The analysis was made more effective with the aid of the HPLC technique, especially in the study of

metabolism and distribution of anthracyclines and anthracyclinones in organisms /11-14/ and recently in the studies of their biosynthesis and bioconversions /15, 16/. Our work deals with the application of a combination of TLC and HPLC to the qualitative analysis of a group of anthracyclinones /Figure 1/ formed in our biosynthetic and biotransformation experiments.

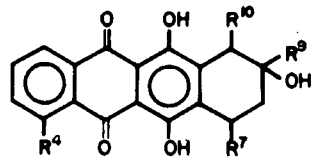
MATERIALS AND METHODS

Chemicals

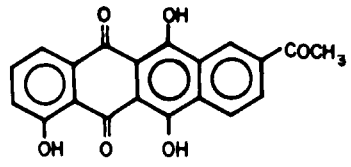
Daunomycinone /I, ref. 6/, 7-deoxydaunomycinone /II, ref. 17/, 7-deoxy-13-dihydrodaunomycinone /III, ref. 18/, 13-dihydrodaunomycinone /IV, ref. 19/, carminomycinone /V, supplied by prof. Gauze, Inst. New. Antibiot., Acad. Med. Sci., Moscow/, 13-dihydrocarminomycinone /VI, ref. 20/, ε-rhodomycinone /VII, ref. 21/, "bisanhydrocarminomycinone" /VIII, isolated and identified in Dept. Biogenesis Nat. Substances, Inst. Microbiol. ČSAV, Prague/. All solvents were distilled before use.

Methods

TLC samples were chromatographed on precoated silica gel sheets Silufol^{R} /Kavalier, Czechoslovakia/ with dimensions of 150 x 150 mm. For analytical purposes about 5 μg substance dissolved in 10 μl chloroform-methanol mixture was applied in a 10 mm wide strip. R_F values were determined with a front migration distance of 100 mm. The solvent systems used for chromatography are given in Table 1. The zones were detected in a visible and UV-light /CAMAG-lamp, λ = 254 and 366 nm/, sometimes after a treatment with ammonia fumes, and identified by comparing them with standards.



		R^4	R^7	R^9	R^{10}
I	daunomycinone	OCH_3	OH	$COCH_3$	H
II	7-deoxydaunomycinone	OCH_3	H	$COCH_3$	H
III	7-deoxy-13-dihydrodaunomycinone	OCH_3	H	$CHOHCH_3$	H
IV	13-dihydrodaunomycinone	OCH_3	OH	$CHOHCH_3$	H
V	carminomycinone	OH	OH	$COCH_3$	H
VI	13-dihydrocarminomycinone	OH	OH	$CHOHCH_3$	H
VII	ε-rhodomycinone	OH	OH	CH_2CH_3	CO_2CH_3



VIII " bisanhydrocarminomycinone "

FIGURE 1

Structures of compounds studied

High performance liquid chromatograph SP-8000/ Spectra-Physics Corp., Santa Clara, CA/ with variable wavelength detector SP-770 at 470 nm was used. Chromatography was performed on 250 mm x 4.6 mm i.d column, packed with 10 micron LiChrosorb RP-8. Samples were dissolved in methanol and injected /in an amount of 0.10--0.16 μg/ by a 10 μl sample loop. The chromatograph operated at a temperature of 20 °C, flow rate 1.5 ml min^{-1} and pressure 8.9-10.3 MPa.

TABLE 1

R_F Values of Anthracyclinones

Compound	R_F* /x 100/									
	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
I	73	41	63	31	31	70	16	28	68	59
II	79	61	78	55	33	80	31	34	63	72
III	42	20	28	18	19	37	7	22	45	35
IV	30	11	16	8	9	29	3	12	28	26
V	86	55	71	61	37	78	38	45	66	77
VI	36	16	22	19	12	32	8	25	37	37
VII	89	67	82	74	49	87	56	52	83	85
VIII	98	83	92	90	75	99	87	65	99	94

*S1, benzene-chloroform-ethyl acetate-methanol /7:7:2:2/; S2, chloroform-acetone /6:1/; S3, chloroform-acetone--methanol /90:15:1/; S4, benzene-acetone /4:1/; S5, heptane-chloroform-methanol /5:5:1/; S6, chloroform--methanol-water /190:10:1/; S7, benzene-ethyl acetate--methanol /40:10:1/; S8, heptane-ethyl acetate-ethanol /6:4:1/; S9, chloroform-ethanol-acetic acid /90:10:0.25/; S10, benzene-chloroform-ethyl acetate-methanol /7:5:5:2/.

RESULTS AND DISCUSSION

Table 1 shows R_F values obtained by chromatography of mixtures containing substances I-VIII. Acetone-containing solvent systems /S2, S4/ yielded less sharply defined zones, sharply delineated zones being achieved with S2 by adding methanol /S3/. Increased content of ethyl acetate caused a better separation of substances V and VII.

The capacity ratios of the HPLC technique are summarized in Table 2. During separation, substance III

TABLE 2

Capacity ratios obtained from HPLC data*

Compound		IV	I	VI	III	VII	II
k'	A	1.51	2.03	2.74	3.38	3.38	4.29
	B	1.09	1.50	2.00	2.00	4.01	2.75

*k'-capacity ratio; dead time was determined by injection of methanol; A, methanol-water /70:30/; B; methanol--water-acetonitrile /35:35:30/. All compounds were separated in less than 12 min.

interfered with substance VII in mobile phase A and with substance VI in mobile phase B. In the mobile phases used, substances V and VIII exhibited excessively long retention times and were not therefore included in the set of substances studied by HPLC. Although the limit of detection by HPLC is about 100 times lower, TLC has the advantage of being suitable for the separation of all substances under study.

The data indicate that the full chromatographic characterization of an anthracyclinone in a mixture requires the use of several chromatographic systems. Because of its simplicity and experimental feasibility the TLC technique is suitable for studying anthracyclinones during their biosynthesis.

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