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Evaluation of several solvent systems in the thin-layer chromatographic separation of cardiotonic aglycones and glycosides from cholesterol, phytosterols and their esters

Twenty-seven solvent mixtures were investigated and appear to give a good separation of fourteen cardiotonic steroids from cholesterol. The results are equally applicable to the separation of cardiac steroids from phytosterols and their esters. The R_F values of phytosterols are frequently close to the R_F value of cholesterol[1,2]. Moreover, with these solvent systems a better resolution is obtained for each cardenolide and aglycone.

Experimental

Thin-layer chromatography. Kieselgel F_{254} (Merck) 20 × 20 cm glass plates are heated at 110° for 60 min. After spotting 2 μl of sample, as described previously[3], the plates are developed in closed chambers (22 × 22 × 12 cm) with the solvent mixtures indicated in Table I. The plates, after development, are dried in the atmo-

TABLE I

SOLVENTS FOR SEPARATION OF AGLYCONES AND CARDENOLIDES BY TLC

Systems			*Migration (16-cm path) time (min) at 20°*	*Ref.*
Symbol	*Components*	*Proportion*		
I	Chloroform–acetone	9:10	90	2,17
II	Chloroform–methanol	97:3	90	3
III	Ethyl acetate–pyridine–water	5:1:4	120	1
IV	Ethyl acetate–chloroform–methanol	80:10:10	120	7
V	Chloroform–pyridine–water	50:10:40	130	7
VI	Dichloromethane–methanol–water	11:4:5	90	8
VII	Benzene–methanol–water	2:2:1	120	8
VIII	Benzene–isopropanol	4:1	115	9
IX	Chloroform–ethanol	90:10	105	10
X	Chloroform–methanol–water	90:10:0.25	100	11
XI	Hexane–diethyl ether–acetic acid (ac. ac.)	50:50:1	105	12
XII	Hexane–diethyl ether–ac. ac.	30:70:1	120	12
XIII	Hexane–diethyl ether–ac. ac.	10:90:1	90	12
XIV	Benzene–ethanol	40:10	90	10
XV	Benzene–ethanol	7:3	120	13
XVI	Ethyl methyl ketone–toluene–water–ac.ac.–methanol	40:5:3:1:2.5	120	14
XVII	Ethyl acetate–chloroform	9:1	120	14
XVIII	Methyl ethyl ketone–toluene	80:20	90	14
XIX	Chloroform–ethanol–*n*-butanol	2:1:1	150	18
XX	Toluene–*n*-butanol–ethyl acetate	2:0.3:1	90	18
XXI	Methyl ethyl ketone–ethyl acetate–ethanol–acetone	40:40:1:1	100	18
XXII	*n*-Butanol–toluene	3:2	180	18
XXIII	*n*-Butanol–ethanol	3:1	220	18
XXIV	*n*-Butanol–ethanol	1:3	220	18
XXV	Chloroform–cyclohexane–methanol–diethylamine	50:10:30:10	160	This paper
XXVI	Chloroform–ac.ac.–methanol	85:2:13	110	15
XXVII	Chloroform–cyclohexane–diethylamine	5:4:1	120	16

TABLE II

R_F, R_{Chol}[a] OF CARDIAC STEROIDS AND DETECTION

Kieselgel F_{254} (Merck) layer solvent, benzene–ethanol (7:3).

No.	*Aglycones*	*Cardenolides*	*-oses substituted in C-3*	*-R substituted in C-10*
1	Digitoxigenin[b]	—	—	$-CH_3$
2	Digitoxigenin	Digitoxin[b]	3-D-Digitoxose	$-CH_3$
3	Gitoxigenin[b]	—	—	$-CH_3$
4	Gitoxigenin	Gitoxin[b]	D-Digitoxose	$-CH_3$
5	Oleandrigenin	Oleandrin[b]	L-Oleandrose	$-CH_3$
6	Digoxigenin[b]	—	—	$-CH_3$
7	Digoxigenin	Digoxin[b]	D-Digitoxose	$-CH_3$
8	Digoxigenin	Lanatoside C[b]	β-D-Glucose D-Digitoxose D-Acetyldigitoxose	$-CH_3$
9	G-Strophanthidin	G-Strophantin[b]	L-Rhamnose	$-CH_2OH$
10	K-Strophanthidin[b]	—	—	$-CHO$
11	K-Strophanthidin	K-Strophanthin-α[b] (cymarin)	D-Cymarose	$-CHO$
12	K-Strophanthidin	Convallatoxin[b]	L-Rhamnose	$-CHO$
13	K-Strophanthidin	K-Strophanthin-β[b]	D-Cymarose β-D-Glucose	$-CHO$
14	K-Strophanthidin	K-Strophanthoside[b] (strophanthin)	D-Cymarose β-D-Glucose α-D-Glucose	$-CHO$

[a] R_{Chol} = R_F value relative to cholesterol.
[b] Compound tested.

sphere, sprayed with a detection reagent — 100 g of *p*-toluenesulphonic acid (PTSA) in 100 ml of water — and heated at 100° for 10 min (ref. 4). The spots are evaluated in visible light and in UV with a wavelength of 254 nm.

Preparation of the samples. Four aglycones, digitoxigenin, gitoxigenin, digoxigenin and K-strophanthidin, and ten cardenolides, digitoxin, gitoxin, oleandrin, digoxin, lanatoside C, G-strophanthin, K-strophanthin-α (cymarin), convallatoxin, K-strophanthin-β, and K-strophanthoside (strophanthin), were tested. The numerical arrangement of these cardiac steroids is indicated in Table II.

The aglycones are dissolved in 99.8 % methanol, the glycosides in several methanol–water mixtures or in methanol–chloroform (50:50), and cholesterol is dissolved in chloroform[5,6]. 20 % solutions of cardiac steroids are applied.

Discussion

The results discussed here are given in Tables III–V. The best separation of cardiac steroids from cholesterol, phytosterols and their esters can be obtained with system XV. System XXI separates substances 6, 2, and 10. Systems VIII, IX, X, and XIV provide a good separation of substances 11, 7 and 2. Systems XII, XIII, XVII, XXI develop substances 1 and 5. With systems VII and XI all cardiac steroids remain at the origin and the R_F value of cholesterol is 0.24 and 0.37 respectively. Systems II, VI, VIII, IX, X, XII, XIII, XIV, XVII, XVIII, XX, and XXVII give good resolution of cardiac steroids and separate them from cholesterol and their esters. System XXV separates substance 11. With systems VIII, IX, X, XV, XVI,

H substituted in	R_F	R_{Chol}	*Colour with PTSA reagent*			
			Visible light		*UV light*	
			After detection	*24 h after detection*	*After detection*	*24 h after detection*
14	0.66	0.83	yellow-green	yellow	green	bright-green
14	0.62	0.76	grey	blue-grey	dark yellow	dark yellow
14, 16	0.58	0.71	green	green	blue	bright blue
14, 16	0.52	0.65	grey-yellow	grey	white-blue	blue-grey
14, 16	0.64	0.80	grey-pink	grey-green	blue-green	blue
etyl						
12, 14	0.68	0.75	yellow-brown	grey	bright blue	bright blue
12, 14	0.63	0.70	grey	grey	blue-grey	blue-grey
12, 14	0.29	0.37	grey-violet	grey	blue-brown	blue-brown
5, 11, 14	0.06	0.08	yellow	yellow-pink	yellow-pink	yellow-pink
5, 14	0.69	0.77	yellow	yellow	pink	bright pink
5, 14	0.54	0.68	grey-brown	grey-blue	pink-yellow	pink-yellow
5, 14	0.33	0.49	yellow-green	yellow	pink-yellow	pink-yellow
5, 14	0.23	0.28	yellow-grey	brown	orange-pink	orange-pink
5, 14	0.05	0.07	grey-pink	grey-brown	brown-pink	brown-pink

and XXII an effective separation of substance 8 from 12 is achieved. Systems VIII, XIV, XV, XX, XXI, and XXVII clearly develop substances 5 and 7. Systems V, VIII, IX, X, XIV, XV, XVI, XVII, XXII, and XXIII achieve a good separation of substance 11 from 12.

PTSA, used for visualisation, permits a great sensitivity and prevent interferences of other lipidic substances[4].

TABLE III

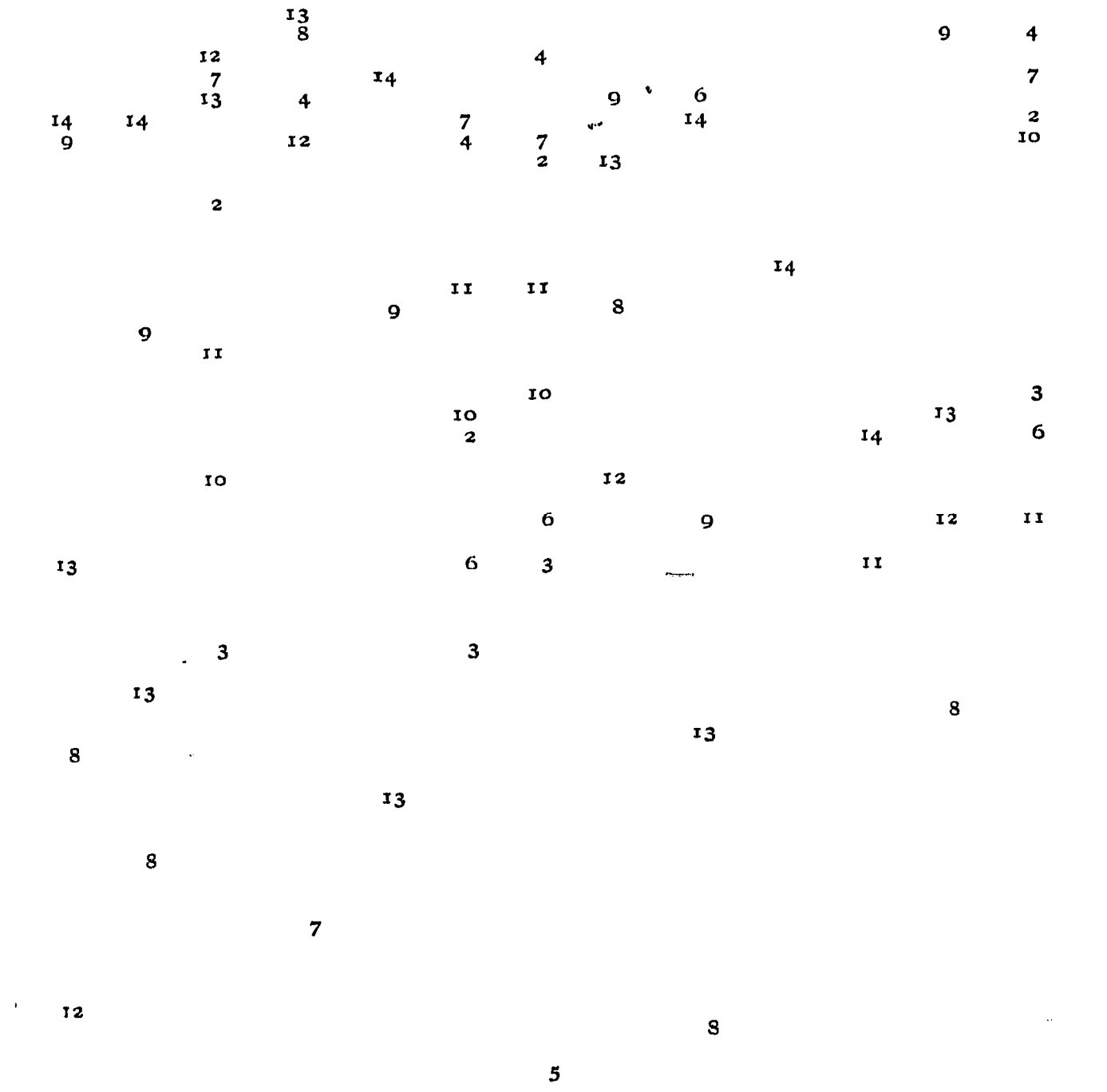
R_F VALUES OF THE REFERENCE COMPOUND CHOLESTEROL IN SOLVENT SYSTEMS I–XXVII (*cf.* TABLE I)

Solvent system	R_F *value*	*Solvent system*	R_F *value*	*Solvent system*	R_F *value*
I	0.598	X	0.762	XIX	0.945
II	0.76	XI	0.37	XX	0.736
III	0.875	XII	0.6105	XXI	0.67
IV	0.80	XIII	0.85	XXII	0.93
V	0.978	XIV	0.80	XXIII	0.93
VI	0.78	XV	0.835	XXIV	0.909
VII	0.24	XVI	0.945	XXV	0.916
VIII	0.75	XVII	0,855	XXVI	0.93
IX	0.845	XVIII	0.89	XXVII	0.81

TABLE IV

R_{Chol} VALUE FOR CARDIAC STEROIDS 1–14 (*cf.* TABLE II) IN SOLVENT SYSTEMS I–XXVII (*cf.* TABLE I)

R_{Chol}	*Solvent system*												
	I	*II*	*III*	*IV*	*V*	*VI*	*VII*	*VIII*	*IX*	*X*	*XI*	*XII*	*XIII*
0.00									13				
		2			8							3	
		7											10
	3							13	8				11
						13							6
		10				4							
								8					3
0.10		13.6				2			12	13		5	
		3											
						6				8			
		11				10							
												1	
						3							
								12					
0.20										12			
	1				6								
0.30													5
									10				1
									6				
								11					
						11			4				
0.40									7				
										7			
					4								
					7								
								7					
									3				
						1							
0.50													
					10								
						5		4					



(Continued on p. 284)

TABLE IV (*continued*)

R_{Chol}	Solvent system												
	I	*II*	*III*	*IV*	*V*	*VI*	*VII*	*VIII*	*IX*	*X*	*XI*	*XII*	*XI*
										6			
									2				
								3					
0.60								10					
		5			2								
										10			
					3				11	4			
								2					
0.70								6					
								1					
					11								
										3			
				4									
										2			
				2									
0.80									1	1			
				3									
			4										
			6										
			11							11			
								5					
0.90			3										
				1									
					1								
			2						5				
					12								
				5									
					5								
1.00													
										5			
			5										
			7										
1.10													

IV	XV	XVI	XVII	XVIII	XIX	XX	XXI	XXII	XXIII	XXIV	XXV	XXVI	XXVII
								11	12	9			1
					12								
				2									
		12											
					8								
							1						
										13			
	4												
								4					
	11							7					
								6					
	7												
	3					1		10					
			1										
						5				12			
			5										
	6			11								6	
	2									8		10	
	10							2				4	
	5			10					11				
				3								7	5
												3	
	1												
									4				
		7							7				
									10				
					11			3					
					10								
		11			7				2	11			
								5		10	9	1	
		4								4	13		
					4			1	5	7	12	11	
		2			2				3	2, 3	6	2	
		1			6					6	8, 10		
		10								5	3, 4	5	
		3			3					1	1, 2		
					5				1		7		
					1						5		
		5											
				1									
				5									

TABLE V

AGLYCONES AND CARDENOLIDES 1–14 (*cf.* TABLE II) REMAINING AT THE ORIGIN IN SOLVENT SYSTEMS (I–XXVII *cf.* TABLE I)

Solvent system	*Substance*
I	2, 4, 5, 7, 8, 9, 10, 11, 12, 13, 14
II	4, 8, 9, 12, 14
III	9
IV	—
V	9, 13, 14
VI	7, 8, 9, 12, 14
VII	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
VIII	9, 14
IX	9, 14
X	9, 14
XI	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
XII	2, 4, 6, 8, 10, 11, 12, 13, 14
XIII	2, 4, 7, 8, 9, 12, 13
XIV	9, 14
XV	—
XVI	—
XVII	4, 8, 9, 14
XVIII	9, 14
XIX	—
XX	8, 9, 12, 13, 14
XXI	8, 9, 12, 13, 14
XXII	14
XXIII	—
XXIV	—
XXV	—
XXVI	14
XXVII	8, 9, 12, 13, 14

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