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EFFECT OF ACIDIC MOBILE PHASE ADDITIVES ON THE TLC BEHAVIOUR OF SOME ALKALOIDS ◈

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SUMMARY

The effect of two acids (phosphoric and acetic) and their derivatives (hydrogen di(2-ethylhexyl)orthophosphoric, chloroacetic, dichloroacetic, trichloroacetic, and trifluoroacetic acids) on the retention of selected alkaloids on silica gel plates has been investigated. The dependence of the chromatographic behaviour of the alkaloids on mobile phase diluent and on the concentration of the acids in mobile phase (0.001, 0.005, or 0.01 M) was also investigated. Higher concentrations of acid resulted in reduced adsorption and larger R_F values.

INTRODUCTION

Alkaloids are a large group of natural compounds found as secondary metabolites in plants (e.g. potatoes and tomatoes), animals (e.g. shellfish), and fungi. Most alkaloids are formed from amino acid molecules. Depending on their molecular structure alkaloids are divided into several groups (protoalkaloid, tropane, indole derivative, steroid derivative, terpenoid derivative, and isoquinoline and quinoline alkaloids).

Thin-layer chromatography, both non-instrumental and instrumental, used to be the method of choice for analysis of alkaloids [1–7]. Nowadays TLC is often used as a pilot method for HPLC [8,9] or to obtain alkaloids by preparative separation [10–15]. Silica gel is the adsorbent most often used in TLC. Its surface is covered with hydroxyl (silanol) groups which participate in the adsorption process by formation of hydrogen bonds.

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The effect of chromatographic conditions on the TLC behaviour of alkaloids has been investigated [16–18]. The objective of these investigations was to determine whether the interactions between some alkaloids and silica gel depend on the presence and concentration of a variety of acids in the mobile phase.

EXPERIMENTAL

TLC was performed on 5 cm × 10 cm silica gel $60F_{254}$ plates, with 0.25 mm layers, from Merck (Darmstadt, Germany). Methanol, ethyl acetate, acetic acid, and orthophosphoric acid were obtained from POCh (Gliwice, Poland), chloroacetic, dichloroacetic, trichloroacetic, and trifluoroacetic acid from Aldrich, and hydrogen di(2-ethylhexyl)orthophosphoric acid (HDEHP) from Merck. Atropine, colchicine, strychnine, vincamine, ephedrine, emetine, quinine, and quinidine were obtained from Aldrich, vinblastine and papaverine from Fluka, and veratrine, aconitine, and proto-pine from ICN Biochemicals and Reagents. The names and types of the sixteen alkaloids investigated are given in Table I and their structures are given in Table II.

Table I

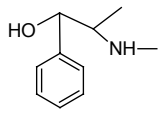
The alkaloids investigated

No.	Name	Type
1	Ephedrine	Pseudoalkaloids
2	Phenylethylamine	
3	Atropine	Tropane alkaloids
4	Homatropine	
5	Hyoscyamine	
6	Colchicine	
7	Strychnine	Indole alkaloids
8	Vincamine	
9	Vinblastine	
10	Veratrine	Steroid alkaloids
11	Aconitine	Terpenoid alkaloids
12	Protopine	Isoquinoline alkaloids
13	Papaverine	
14	Emetine	
15	Quinine	Quinoline alkaloids
16	Quinidine	

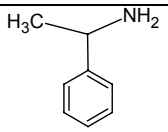
Table II

The chemical structures of the alkaloids investigated

Pseudoalkaloids

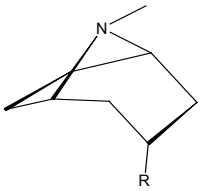


Ephedrine (1)

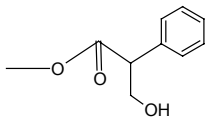


Phenylethylamine (2)

Tropane alkaloids

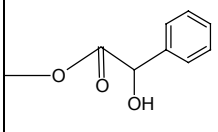


R =



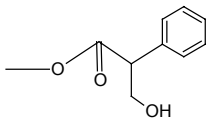
Atropine (3)

R =



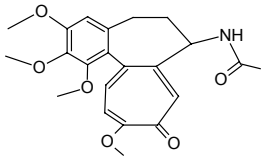
Homatropine (4)

R =



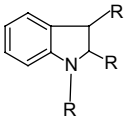
Hyoscyamine (5)

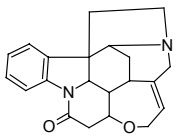
R =



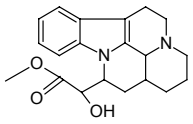
Colchicine (6)

Indole alkaloids

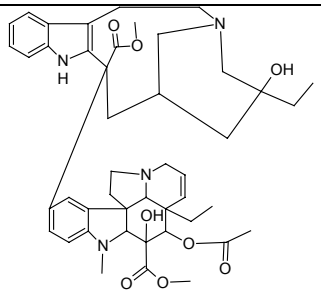




Strychnine (7)



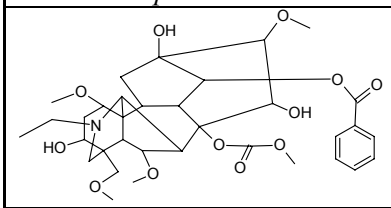
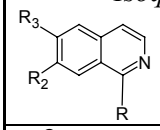
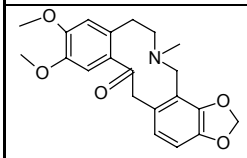
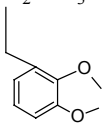
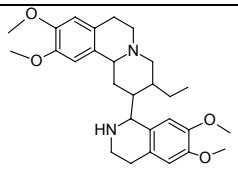
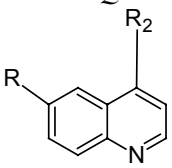
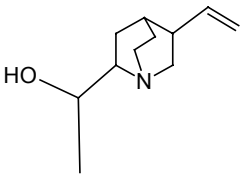
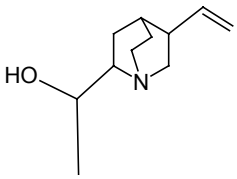
Vincamine (8)



Vinblastine (9)

Table II (continued)

The chemical structures of the alkaloids investigated

Steroid derivative		
Veratrine (mixture of several alkaloids) (10)		
Terpenoid derivative		
 Aconitine (11)		
Isoquinoline alkaloids 		
 Protopine (12)	$R_2 = R_3 = OCH_3$, R=  Papaverine (13)	 Emetine (14)
Quinoline alkaloids 		
$R = OCH_3$, R_2 =  Quinine (15)	$R = OCH_3$, R_2 =  Quinidine (16)	

Solutions (0.2%) of the alkaloids were prepared in chloroform. Plates were developed in horizontal sandwich DS chambers (Chromdes, Lublin, Poland); the mobile phases were either methanol–ethyl acetate, 40:60 (% *v*/*v*), mobile phase A, or methanol–ethyl methyl ketone 40:60 (% *v*/*v*), mobile phase B, or the same mobile phases containing a variety

of acids at different concentrations. Acetic acid, chloroacetic acid, dichloroacetic acid, trichloroacetic acid, trifluoroacetic acid, phosphoric acid, and hydrogen di(2-ethylhexyl)orthophosphoric acid (HDEHP) at concentrations of 0.001, 0.005, and 0.01 M were used in these investigations.

Before development the chromatographic chamber and plates spotted with the alkaloids were equilibrated for 15 min with mobile phase vapour. After chromatography the alkaloids were detected under UV light at 254 nm and by use of Dragendorff's reagent prepared by the Munier and Macheboeuf procedure. (Solution A was a solution of bismuth(III) nitrate and glacial acetic acid in water, and solution B was an aqueous solution of potassium iodide. Equal parts of both solutions were mixed. Before use one part of the mixture was added to two parts glacial acetic acid and ten parts water).

Two chromatograms were developed for each solute–solvent combination.

RESULTS AND DISCUSSION

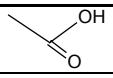
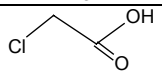
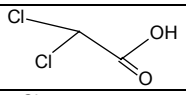
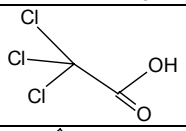
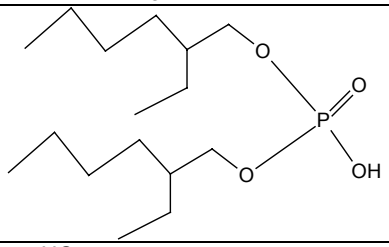
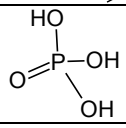
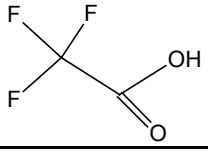
Because alkaloids are basic in character and the surface of silica gel is acidic, it may be expected that the interactions between an alkaloid molecule and the silanol groups of the silica gel will be strong or very strong. In consequence the alkaloids will have low R_F values. This is not true for all alkaloids, however. Some are strongly retained by the silica gel surface, others only weakly, depending both on the silica gel surface and on the mobile-phase modifier. The mobile-phase components could form complexes with the alkaloids and these complexes can have different bonding capacity with the stationary phase. The non-polar complexes could migrate with the mobile phase so their retention will be poor and their R_F values higher.

In mobile phases A and B the diluents were ethyl acetate and ethyl methyl ketone, respectively. The organic modifier was the same – methanol. It can be assumed that interaction between the alkaloid molecules, the silica gel, and the mobile phases would be the same, because of the presence of methanol. Different R_F values are obtained by use of the two mobile phases, however. Somewhat higher values were observed for ethyl methyl ketone as diluent than for ethyl acetate. This is opposite to their solvent strengths (ε^o is 0.58 and 0.51 for ethyl acetate and ethyl methyl ketone, respectively). It is possible that ketone molecules displace alkaloid molecules from the stationary phase more strongly than does ethyl acetate.

In the second part of our research the character of the mobile phase was changed by addition of different acids (Table III). This sometimes led

Table III

Chemical structures and pK_A of the acids

Chemical name	Abbre-viation	Chemical structure	pK_A
Acetic acid	AA		4.8
Chloroacetic acid	CAA		2.8
Dichloroacetic acid	DCAA		1.5
Trichloroacetic acid	TCAA		1.7
bis(2-Ethyl-hexyl)orthophospho-ric acid	HDEHP		3.95
Phosphoric acid	PhA		2.12
Trifluoroacetic acid	TFAA		0.67

to alteration of the interactions between the alkaloid molecules and the silica gel surface. Interactions between alkaloid and acid molecules occurred in the mobile phase and changed retention of the alkaloid molecules by the silica gel surface. The R_F values obtained for the alkaloids are listed in Tables IV and V.

For all the mobile phases R_F values were lowest for some of the tropane alkaloids. R_F values were highest for papaverine. All systems led

to poor separation of atropine, homatropine, and hyoscyamine. The fourth compound of this group (colchicine) has a very bulky molecule which could interact with both the stationary phase and mobile phases (R_F values in the range 0.6–0.8). Interaction of this alkaloid with the mobile phase is slightly stronger.

Table IV

R_F values of alkaloids when chromatographed with mobile phase A, methanol–ethyl acetate, 40:60 (*v*/*v*), containing a variety of acids at different concentrations

Alkaloid	Mobile phase A	+ 0.001 M PhA	+ 0.001 M HDEHP	+ 0.001 M AA	+ 0.001 M CAA	+ 0.001 M DCAA	+ 0.001 M TCAA	+ 0.001 M TFAA
1	0.65	0.2	0.8	0.16	0.23	0.9	0.21	0.2
2	0.57	0.6	0.58	0.56	0.62	0.63	0.58	0.52
3	0.05	0.05	0.04	0.05	0.05	0.06	0.06	0.05
4	0.04	0.08	0.06	0.08	0.04	0.05	0.09	0.12
5	0.04	0.1	0.04	0.11	0.18	0.06	0.29	0.4
6	0.76	0.6	0.68	0.64	0.61	0.67	0.64	0.64
7	0.09	0.09	0.07	0.1	0.11	0.1	0.11	0.1
8	0.64	0.6	0.6	0.6	0.63	0.6	0.6	0.57
9	0.44	0.5	0.38	0.4	0.4	0.42	0.4	0.58
10	0.34	0.52	0.26	0.36	0.37	0.38	0.5	0.47
11	0.26	0.2	0.25	0.22	0.23	0.26	0.25	0.31
12	0.29	0.23	0.23	0.24	0.23	0.35	0.24	0.28
13	0.81	0.77	0.8	0.86	0.88	0.9	0.7	0.72
14	0.24	0.22	0.21	0.5	0.41	0.26	0.26	0.26
15	0.29	0.3	0.34	0.63	0.65	0.3	0.35	0.33
16	0.25	0.32	0.28	0.3	0.31	0.27	0.33	0.35
Alkaloid	Mobile phase A	+ 0.005 M PhA	+ 0.005 M HDEHP	+ 0.005 M AA	+ 0.005 M CAA	+ 0.005 M DCAA	+ 0.005 M TCAA	+ 0.005 M TFAA
1	0.65	0.62	0.68	0.58	0.61	0.68	0.57	0.21
2	0.57	0.61	0.59	0.64	0.62	0.65	0.6	0.63
3	0.05	0.05	0.07	0.06	0.095	0.08	0.08	0.06
4	0.04	0.06	0.08	0.094	0.12	0.08	0.1	0.14
5	0.04	0.05	0.06	0.05	0.07	0.1	0.3	0.43
6	0.76	0.73	0.7	0.79	0.77	0.73	0.73	0.68
7	0.09	0.1	0.1	0.11	0.14	0.11	0.12	0.1
8	0.64	0.64	0.65	0.62	0.64	0.64	0.61	0.59
9	0.44	0.46	0.42	0.42	0.45	0.46	0.44	0.6
10	0.34	0.54	0.39	0.37	0.39	0.47	0.5	0.5
11	0.26	0.21	0.27	0.23	0.26	0.31	0.33	0.32
12	0.29	0.22	0.32	0.23	0.28	0.36	0.24	0.32
13	0.81	0.83	0.86	0.87	0.91	0.9	0.88	0.75
14	0.24	0.22	0.25	0.51	0.45	0.28	0.58	0.27
15	0.29	0.33	0.3	0.64	0.69	0.32	0.63	0.4
16	0.25	0.3	0.3	0.34	0.39	0.32	0.37	0.39

Table IV (continued)

R_F values of alkaloids when chromatographed with mobile phase A, methanol–ethyl acetate, 40:60 (*v*/*v*), containing a variety of acids at different concentrations

Alkaloid	Mobile phase A	+ 0.01 M PhA	+ 0.01 M HDEHP	+ 0.01 M AA	+ 0.01 M CAA	+ 0.01 M DCAA	+ 0.01 M TCAA	+ 0.01 M TFAA
1	0.65	0.23	0.88	0.08	0.29	0.60	0.27	0.22
2	0.57	0,63	0.6	0.65	0.62	0.66	0.63	0.76
3	0.05	0.07	0.08	0.058	0.11	0.15	0.12	0.08
4	0.04	0.15	0.1	0.15	0.15	0.17	0.15	0.15
5	0.04	0.14	0.08	0.14	0.21	0.13	0.33	0.47
6	0.76	0.68	0.72	0.68	0.66	0.65	0.67	0.71
7	0.09	0.11	0.12	0.095	0.16	0.16	0.17	0.11
8	0.64	0.64	0.68	0.65	0.59	0.68	0.62	0.62
9	0.44	0.45	0.49	0.42	0.39	0.68	0.46	0.61
10	0.34	0.6	0.41	0.25	0.42	0.54	0.52	0.59
11	0.26	0.24	0.3	0.21	0.34	0.36	0.33	0.36
12	0.29	0.24	0.36	0.19	0.3	0.38	0.28	0.41
13	0.81	0.78	0.9	0.89	0.9	0.38	0.77	0.77
14	0.24	0.25	0.3	0.53	0.51	0.38	0.51	0.3
15	0.29	0.32	0.36	0.67	0.62	0.38	0.38	0.47
16	0.25	0.3	0.4	0.37	0.39	0.38	0.43	0.45

The abbreviations used for the acids are explained in Table III and the identities of the alkaloids are given in Table I

Table V

R_F values of alkaloids when chromatographed with mobile phase B, methanol–ethyl methyl ketone, 40:60 (*v*/*v*) containing a variety of acids at different concentrations

Alkaloid	Mobile phase B	+ 0.001 M PhA	+ 0.001 M HDEHP	+ 0.001 M AA	+ 0.001 M CAA	+ 0.001 M DCAA	+ 0.001 M TCAA	+ 0.001 M TFAA
1	0.67	0.24	0.9	0.27	0.3	0.25	0.27	0.24
2	0.62	0.6	0.62	0.6	0.55	0.62	0.58	0.47
3	0.1	0.11	0.08	0.08	0.1	0.12	0.12	0.12
4	0.09	0.16	0.11	0.16	0.17	0.09	0.15	0.12
5	0.08	0.15	0.11	0.12	0.18	0.09	0.28	0.58
6	0.74	0.68	0.71	0.72	0.7	0.65	0.71	0.8
7	0.14	0.17	0.16	0.14	0.15	0.15	0.18	0.15
8	0.47	0.48	0.5	0.5	0.46	0.51	0.5	0.51
9	0.38	0.46	0.52	0.53	0.42	0.42	0.47	0.84
10	0.42	0.49	0.45	0. 47	0.47	0.4	0.45	0.44
11	0.39	0.34	0.38	0.36	0.4	0.3	0.41	0.55
12	0.22	0.23	0.26	0.26	0.25	0.2	0.25	0.28
13	0.9	0.8	0.87	0.81	0.8	0.9	0.8	0.75
14	0.06	0.21	0.08	0.26	0.24	0.08	0.27	0.25
15	0.45	0.45	0.5	0.7	0.7	0.5	0.5	0.47
16	0.44	0.45	0.48	0.45	0.46	0.42	0.47	0.48

Table V (continued)

R_F values of alkaloids when chromatographed with mobile phase B, methanol–ethyl methyl ketone, 40:60 (*v*/*v*) containing a variety of acids at different concentrations

Alkaloid	Mobile phase B	+ 0.005 M PhA	+ 0.005 M HDEHP	+ 0.005 M AA	+ 0.005 M CAA	+ 0.005 M DCAA	+ 0.005 M TCAA	+ 0.005 M TFAA
1	0.67	0.64	0.7	0.6	0.65	0.58	0.6	0.26
2	0.62	0.67	0.63	0.64	0.62	0.64	0.65	0.53
3	0.1	0.12	0.14	0.14	0.15	0.13	0.14	0.13
4	0.09	0.11	0.14	0.17	0.15	0.12	0.2	0.13
5	0.08	0.1	0.13	0.11	0.14	0.17	0.31	0.62
6	0.74	0.7	0.76	0.74	0.71	0.7	0.72	0.82
7	0.14	0.2	0.17	0.19	0.2	0.16	0.22	0.16
8	0.47	0.46	0.5	0.51	0.47	0.53	0.5	0.53
9	0.38	0.38	0.41	0.39	0.38	0.45	0.44	0.88
10	0.42	0.51	0.46	0.48	0.47	0.45	0.42	0.47
11	0.39	0.4	0.42	0.4	0.39	0.38	0.44	0.6
12	0.22	0.27	0.27	0.3	0.3	0.26	0.33	0.29
13	0.9	0.8	0.88	0.87	0.82	0.92	0.86	0.86
14	0.06	0.24	0.1	0.23	0.23	0.1	0.18	0.27
15	0.45	0.45	0.52	0.72	0.72	0.66	0.53	0.5
16	0.44	0.44	0.5	0.49	0.52	0.66	0.51	0.48
Alkaloid	Mobile phase B	+ 0.01 M PhA	+ 0.01 M HDEHP	+ 0.01 M AA	+ 0.01 M CAA	+ 0.01 M DCAA	+ 0.01 M TCAA	+ 0.01 M TFAA
1	0.67	0.64	0.7	0.65	0.62	0.7	0.68	0.29
2	0.62	0.68	0.65	0.65	0.62	0.67	0.65	0.73
3	0.1	0.14	0.18	0.12	0.17	0.17	0.18	0.14
4	0.09	0.13	0.16	0.2	0.19	0.14	0.24	0.15
5	0.08	0.11	0.15	0.12	0.17	0.19	0.36	0.66
6	0.74	0.72	0.73	0.71	0.73	0.75	0.72	0.84
7	0.14	0.22	0.2	0.16	0.24	0.19	0.25	0.19
8	0.47	0.47	0.52	0.55	0.51	0.56	0.51	0.54
9	0.38	0.43	0.48	0.37	0.43	0.47	0.49	0.88
10	0.42	0.52	0.48	0.48	0.53	0.5	0.47	0.51
11	0.39	0.44	0.45	0.37	0.42	0.42	0.49	0.64
12	0.22	0.3	0.29	0.3	0.35	0.3	0.35	0.31
13	0.9	0.81	0.92	0.83	0.85	0.92	0.86	0.88
14	0.06	0.24	0.14	0.21	0.26	0.12	0.29	0.31
15	0.45	0.46	0.55	0.69	0.7	0.7	0.64	0.53
16	0.44	0.45	0.52	0.45	0.55	0.49	0.55	0.54

The abbreviations used for the acids are explained in Table III and the identities of the alkaloids are given in Table I

The molecules of the indole alkaloids are more complicated than those of the tropane alkaloids. Their bulkiness resulted in their interactions with the stationary phase being the weakest (R_F values higher than for the tropane alkaloids). The exception was vinblastine, the molecules of which contain several groups which can form hydrogen bonds with the

stationary phase; this resulted in lower R_F values (strongest retention) than for other alkaloids in this group.

Of the pseudoalkaloids, phenylethylamine had R_F coefficients in the range 0.6–0.8 for all the mobile phases and, with some mobile phases, retention of ephedrine was strongest (R_F values 0.16 to 0.23). It is possible the hydroxyl groups in the ephedrine molecules formed hydrogen bonds with the stationary phase. Higher concentrations of acids in the mobile phase also reduced retention. The same effects were observed when TFAA was present in the mobile phase.

Of the isoquinoline alkaloids the papaverine molecule interacts very weakly with the stationary phase and its retention was very small (R_F values approx. 0.9). With mobile phases containing TFAA or TCAA, however, retention of this compound was strongest. Papaverine–TFAA and papaverine–TCAA complexes interacted strongly with the stationary phase (R_F values were lowest).

Emetine and protopine interacted similarly with stationary phase (R_F coefficient in the range 0.20–0.30) except for emetine with mobile phase B containing HDEHP or DCAA (when retention was strongest) and with mobile phase A containing AA or TCAA (highest R_F values).

It could be expected that because of the increasing acid strength (pK_A) the effect on retention of the alkaloids would be:

AA < HDEHP < CAA < PhA < TCAA < DCAA < TFAA

Our results were not in agreement with this, however, and the sequences of R_F values of the alkaloids were different for both mobile phases. For mobile phase A (Table IV) variation of the R_F values of the alkaloids was greater than for mobile phase B (Table V). The effect of addition of 0.001 M acid to the mobile phase resulted in R_F values in the order:

HDEHP < PhA < AA < TCAA < CAA < TFAA < DCAA

Use of a higher concentration of the acids (0.005 M) changed the sequence to:

AA < PhA < HDEHP < DCAA < TCAA < TFAA < CAA

and at a still higher concentration (0.01 M) the sequence of acids was:

CAA < PhA < AA < TCAA < HDEHP < DCAA < TFAA

Quinine and quinidine were well separated by almost all mobile phase A systems except for those containing DCAA and TFAA. Lower concentrations of acid (0.001 and 0.005 M) resulted in better separation of

quinine and quinidine ($\Delta R_F = 0.34–0.30$; for AA and CAA). Of all the mobile phases investigated these were best for the separation of quinine and quinidine.

It was apparent that acid strength (pK_A) had no effect on retention. Increasing the concentration of acid in the mobile phase resulted in increased R_F values (reduced retention) except for phosphoric acid (PhA). This acid had little or no effect on retention of the alkaloids.

For mobile phase B the lowest R_F values were usually obtained when no acid was added (Table V). Addition of acid resulted in weaker interactions with the silica surface and increased R_F values. On addition of a small amount of acid (0.001 M) the retention of alkaloids was in the sequence:

PhA < AA < DCAA < CAA < HDEHP < TCAA < TFAA

Addition of more acid (0.005 M) affected retention in a similar sequence (for most of the acids) and R_F values increased in the sequence:

PhA < HDEHP < AA < CAA < TCAA < DCAA < TFAA

Addition of the highest concentration of acid (0.01 M) also affected retention. The sequence of R_F values became:

PhA < AA < HDEHP < CAA < DCAA < TCAA < TFAA

It was noticed that with mobile phase B the strength of the acid (pK_A) affected retention. The highest values of R_F were observed for the strongest acid (TFAA, pK_A 0.67). Quinine and quinidine were separated when this mobile phase containing acetic or chloroacetic acid (0.001 or 0.005 M) was used.

Higher concentrations of the acids (0.005 or 0.01 M) usually led to reduced adsorption and increased R_F values.

CONCLUSIONS

Addition of acids to the mobile phase slightly reduced binding of the alkaloid molecules by the silica gel surface and slightly increased the R_F values of the alkaloids. Higher R_F values were obtained with the methanol– ethyl methyl ketone mobile phases. Better resolution of quinine and quinidine was achieved by use of methanol–ethyl acetate mobile phases. Higher concentrations of the acids in the mobile phase led to greater R_F values. The eluent strength of mobile phases containing acid depended

not only on the type of acid but also on the other components of the mobile phase.

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