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CHROMATOGRAPHIC BEHAVIOUR OF ANTIBIOTICS ON THIN LAYERS OF AN INORGANIC ION-EXCHANGER

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SUMMARY

The chromatographic behaviour of amoxicillin, ampicillin, cephalexin, cloxacillin, doxycycline, tetracycline, erythromycin, gentamycin, streptomycin, and co-trimoxazole has been studied on thin layers of titanic silicate inorganic ion-exchanger with organic, aqueous, and mixed aqueous–organic mobile phases. Rapid separations of one antibiotic from numerous other antibiotics have been achieved, as have many binary and ternary separations. Salting-out TLC using aqueous ammonium sulphate solutions revealed the dependence of R_F values on the concentration of salt in the mobile phase, and the existence of a linear relationship between R_M and molarity of $(NH_4)_2SO_4$ for some antibiotics. The effect of varying the volume ratio of the binary mobile phase methanol–0.1 M formic acid on the R_F values of the antibiotics has also been studied.

INTRODUCTION

Analysis of antimicrobial drugs has been performed by many methods, e.g. spectrophotometry [1,2], fluorimetry [3,4] polarography [5,6], and high performance liquid chromatography (HPLC), which requires not only derivatization but also expensive equipment and extensive sample preparation [7,8]. When more than one antibiotic is present in a formulation, interaction between the drugs can occur [9] which necessitates their separation before determination. Thin-layer chromatography (TLC) is an ideal technique for screening drugs in toxicological analysis, because of its low cost, high speed, and easy maintenance [10,11]. In almost all TLC studies of drugs silica gel has been used as adsorbent [10–15]. During the last two decades synthetic inorganic ion-exchangers have been shown to be important adsorbents in TLC and their high selectivity toward some elements has

resulted in rapid and selective separations [16–23]. Literature survey shows that no attempt has been made to use these inorganic ion-exchangers in drug analysis. This paper reports the retention behaviour of ten common antibiotics on thin layers of the inorganic ion-exchanger titanic silicate [17]. Fast and selective methods have been developed for separation of one antibiotic from others in a single-step process; ternary and binary separations have also been achieved.

EXPERIMENTAL

Chemicals and Reagents

All chemicals and reagents were of analytical grade (Merck or BDH). The antibiotics studied were: amoxicillin (Farabi, Iran), ampicillin and cloxacillin (Kosar, Iran), cephalexin and cloxacillin (Pars Daru, Iran), doxycycline (Razak, Iran), tetracycline (Hakim Daru), erythromycin (Chaemie Daru), gentamycin (Alborz Daru, Iran), streptomycin (Jaber Bin Hayan, Iran), and co-trimoxazole (Tehran Daru, Iran).

Preparation of Ion-Exchange Plates

Titanium(IV) silicate was prepared by dropwise addition of sodium silicate solution (0.25 M, 640 mL) to titanic chloride solution (0.08 M, 2 L) in hydrochloric acid (0.2 M) with constant stirring. The pH of the mixture was adjusted to 6.5 by addition of sodium hydroxide solution (2 M). The white gel formed was left to settle overnight and then washed with distilled water until the supernatant was free from chloride, titanium, and silicate ions. The supernatant was removed completely and a slurry was prepared by mixing the gel (100 mL) with gypsum (15 g), as binder, in a 500-mL conical flask with Teflon stopper, and shaking the flask vigorously for 3 min. The slurry was then poured immediately into a Camag automatic TLC plate coater and used to coat eight 20 cm × 20 cm glass plates with a 300-μm layer. The plates were dried in an oven at 60°C for 2 h then stored at room temperature inside a desiccator.

Preparation of Test Solutions

Capsules (500 mg) of each of amoxicillin, ampicillin, cephalexin, cloxacillin, streptomycin, and tetracycline were dissolved in 10 mL ethanol and the solutions were filtered. Gentamycin injection solution (40 mg mL^{-1}) was used without treatment. A tablet of erythromycin (400 mg) was dis-

solved in 8 mL ethanol. A capsule of doxycycline (100 mg) was dissolved in 2 mL ethanol. A tablet of co-trimoxazole (80/400 mg) was dissolved in 10 mL ethanol and the solution was filtered.

Chromatography

All glassware used was acid washed and light-resistant. Antibiotic solutions were applied to the plates as circular spots by means of disposable fine glass capillaries. The spots were dried completely and the plates were developed in ascending mode (without conditioning) in a Camag twin-trough chamber. The development distance was always 12.5 cm from the origin. After development the plates were dried in an air oven and the antibiotics were detected with appropriate reagents (1% (*w*/*v*) ninhydrin in ethanol was used to locate amoxicillin, ampicillin, cephalexin, cloxacillin, gentamycin, and co-trimoxazole and 5% (*w*/*v*) potassium dichromate in concentrated H_2SO_4 was used to locate streptomycin, erythromycin, tetracycline and doxycycline).

RESULTS AND DISCUSSION

The results recorded in Tables I and II reveal that rapid and selective separations of the antibiotics can be achieved on titanic silicate ion-exchange plates. Erythromycin and tetracycline are readily and rapidly separated from many other antibiotics when 1:1:1 $CHCl_3$–MeOH–NH_4OH is used as mobile phase (Table I). Because of the high selectivity of titanic silicate, ternary and binary separations of antibiotics have also been achieved; these are recorded in Table II.

Salting-out thin layer chromatography is the separation of substances by using aqueous salt solutions as mobile phases [24]. Ammonium sulphate has an especially strong salting-out effect because of the ammonium [25] and sulphate ions [25,26]. A plot showing the dependence of the R_F values of the antibiotics on titanic silicate ion-exchanger on the concentration of aqueous ammonium sulphate in the mobile phase is shown in Fig. 1. A gradual increase in the concentration of ammonium sulphate results in a decrease in the R_F values of the antibiotics. The R_F values of substances separated by salting-out chromatography are known to decrease with increasing salt concentration in the mobile phase [27,28]. The R_F values of hydrophobic substances are lower than those of less hydrophobic compounds [24]. Fast binary separations of co-trimoxazole from ampicillin, amoxicillin, cephalexin, gentamycin, streptomycin, and cloxacillin were

Table I

Separation of one antibiotic from other antibiotics on thin layers of titanic silicate

Separation ($R_T - R_L$)[a]	Mobile phase	Interference	Time (min)
Amoxicillin (0.62–0.95) from five antibiotics	0.1 M Formic acid	Ampicillin, cephalexin, cloxacillin, erythromycin	9
Ampicillin (0.87–0.95) from five antibiotics	0.1 M Formic acid	Amoxicillin, cephalexin, cloxacillin, erythromycin	9
Cephalexin (0.79–0.95) from five antibiotics	0.1 M Formic acid	Ampicillin, amoxicillin, cloxacillin, erythromycin	9
Cloxacillin (0.97–1.00) from seven antibiotics	Acetone–methanol–acetic acid, 5:4.5:0.5	Co-trimoxazole, erythromycin	12
Co-trimoxazole (0.99–1.00) from nine antibiotics	Ethyl acetate	–	21
Doxycycline (0.00–0.00) from seven antibiotics	Acetone–methanol–aqueous ammonia, 5:4.5:0.5	Tetracycline, streptomycin	13
Erythromycin (0.23–0.29) from nine antibiotics	Chloroform–methanol–aqueous ammonia, 1:1:1	–	28
Gentamycin (0.00–0.03) from seven antibiotics	Acetone–0.05 M hydrochloric acid, 1:3	Tetracycline, doxycycline	22
Streptomycin (0.00–0.05) from seven antibiotics	Acetone–methanol–aqueous ammonia, 5:4.5:0.5	Tetracycline, doxycycline	13
Tetracycline (0.00–0.00) from seven antibiotics	Chloroform–methanol–aqueous ammonia, 1:1:1	Doxycycline, streptomycin	28

[a] $R_T = R_F$ of rear of spot, $R_L = R_F$ of front of spot

achieved by use of 1 M aqueous ammonium sulphate solution as mobile phase on titanic silicate ion-exchanger (Table II). In addition to ion-exchange the separation mechanism is based on non-specific hydrophobic interaction of the adsorbent with the substances being separated [29,30]. The theoretical basis of this mechanism was developed under the broader name ‘solvophobic interactions’ by Horvath and co-workers [31,32].

The dependence of R_M values on the molarity of the aqueous salt solution was first reported by Jakubec [26] for organic compounds in paper salting-out chromatography. A plot of R_M against molarity of ammonium sulphate for some of the antibiotics is recorded in Fig. 2, which shows that the dependence is linear.

A plot of R_F against the volume fraction of methanol in the binary mobile phase (methanol–0.1 M formic acid) is recorded in Fig. 3. As the volume fraction of methanol is gradually increased the R_F values of co-trimoxazole, cloxacillin, and erythromycin increase whereas those of streptomycin, tetracycline, gentamycin, and doxycycline gradually decrease.

Table II

Ternary and binary separations achieved on titanic silicate plates

Mobile phase	Separation ($R_T - R_L$)[a]	Time (min)
Chloroform–methanol–aqueous ammonia, 1:1:1	Tetra (0.00–0.00)–Erythro (0.17–0.30)–Ampi (0.87–0.95) Tetra (0.00–0.00)–Erythro (0.23–0.35)–Amoxi (0.85–0.93) Tetra (0.00–0.00)–Erythro (0.24–0.40)–Cepha (0.85–0.94) Tetra (0.00–0.00)–Erythro (0.17–0.36)–Cloxa (0.81–0.96) Tetra (0.00–0.00)–Erythro (0.23–0.43)–Genta (0.76–0.87) Tetra (0.00–0.00)–Erythro (0.22–0.63)–Co-Tri (0.85–0.93) Strepto (0.00–0.07)–Erythro (0.22–0.63)–Ampi (0.85–0.93) Strepto (0.00–0.08)–Erythro (0.29–0.63)–Amoxi (0.84–0.91) Strepto (0.00–0.07)–Erythro (0.26–0.63)–Cepha (0.86–0.93) Strepto (0.00–0.07)–Erythro (0.22–0.63)–Cloxa (0.80–0.95) Strepto (0.00–0.10)–Erythro (0.26–0.44)–Genta (0.78–0.88) Strepto (0.00–0.12)–Erythro (0.22–0.43)–Co-Tri (0.84–0.91) Doxy (0.00–0.00)–Erythro (0.10–0.38)–Ampi (0.85–0.96) Doxy (0.00–0.00)–Erythro (0.19–0.39)–Amoxi (0.86–0.95) Doxy (0.00–0.00)–Erythro (0.23–0.40)–Cepha (0.88–0.95) Doxy (0.00–0.00)–Erythro (0.29–0.39)–Cloxa (0.83–0.97) Doxy (0.00–0.00)–Erythro (0.26–0.33)–Genta (0.80–0.91) Doxy (0.00–0.00)–Erythro (0.25–0.33)–Co-Tri (0.80–0.91)	28
Ammonium sulphate (1 M)	Co-Tri (0.00–0.00)–Ampi (0.60–0.78) Co-Tri (0.00–0.00)–Amoxi (0.58–0.65) Co-Tri (0.00–0.00)–Cepha (0.35–0.76) Co-Tri (0.00–0.00)–Genta (0.65–0.76) Co-Tri (0.00–0.00)–Strepto (0.56–0.72) Co-Tri (0.00–0.00)–Cloxa (0.58–0.68)	20
0.1 M Formic acid	Tetra (0.00–0.45)–Ampi (0.61–0.82) Tetra (0.00–0.45)–Amoxi (0.51–0.80) Tetra (0.00–0.45)–Cepha (0.59–0.79) Tetra (0.00–0.45)–Cloxa (0.62–0.75) Genta (0.00–0.17)–Ampi (0.77–0.90) Genta (0.00–0.17)–Amoxi (0.78–0.91) Genta (0.00–0.17)–Cepha (0.61–0.80) Genta (0.00–0.17)–Cloxa (0.59–0.70) Genta (0.00–0.17)–Erythro (0.59–0.75)	9
20% Dipotassium hydrogen phosphate	Erythro (0.00–0.00)–Genta (0.45–0.71)–Amoxi (0.82–0.91) Co-Tri (0.00–0.00)–Tetra (0.63–0.71)–Amoxi (0.88–0.94) Co-Tri (0.00–0.00)–Genta (0.48–0.75)–Amoxi (0.88–0.93) Co-Tri (0.00–0.00)–Strepto (0.74–0.80)–Amoxi (0.89–0.94)	28

[a] $R_T = R_F$ of rear of spot, $R_L = R_F$ of front of spot

Amoxi = amoxicillin, Cloxa = cloxacillin, Erythro = erythromycin, Co-Tri = co-trimoxazole, Ampi = ampicillin, Doxy = doxycycline, Genta = gentamycin, Cepha = cephalexin, Tetra = tetracycline, Strepto = streptomycin

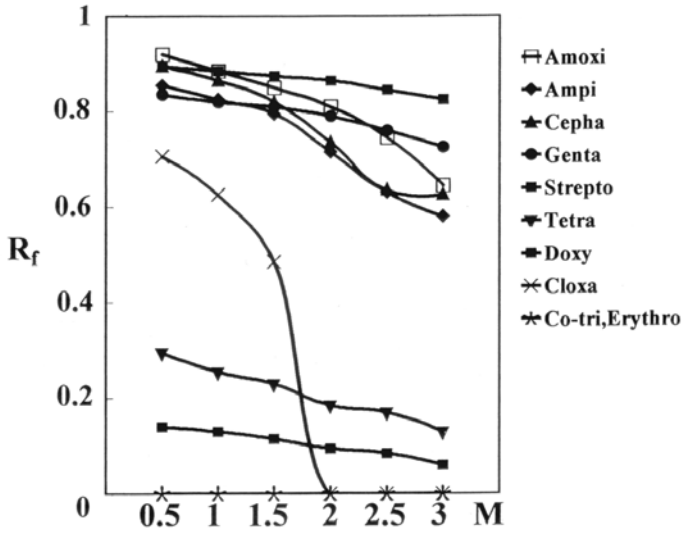

Fig. 1

Plot of R_F against concentration of aqueous ammonium sulphate

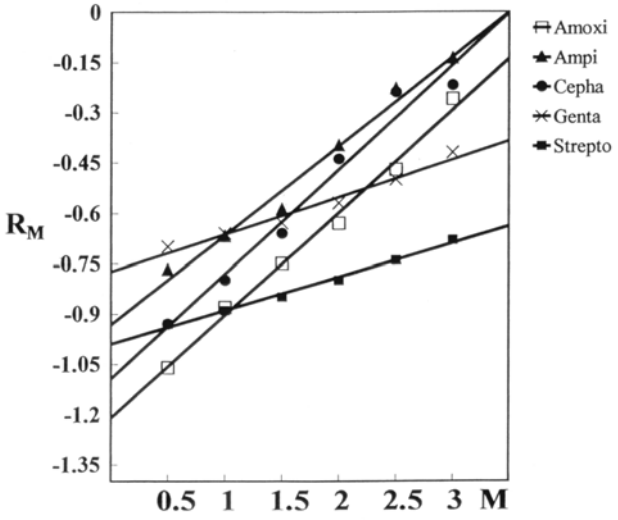

Fig. 2

Dependence of the R_M values of some of the antibiotics on the molarity of ammonium sulphate in the mobile phase

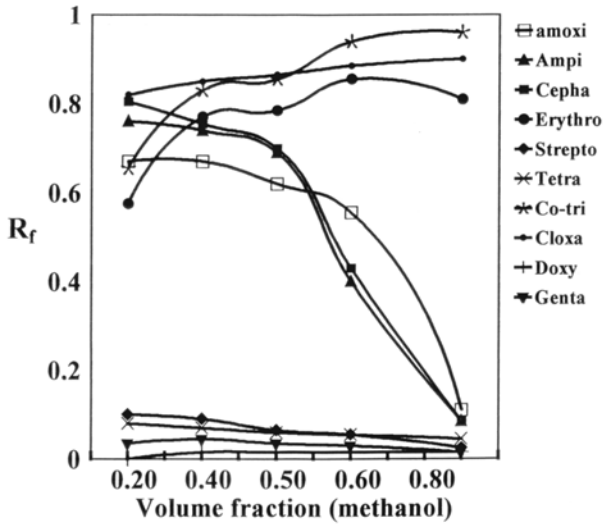

Fig. 3

Plot of R_F against volume fraction of methanol in the binary mobile phase methanol–0.1 M formic acid

The R_F values of cephalexin, ampicillin, and amoxicillin, however, drop sharply when the volume fraction of methanol is >0.5. These variations in the chromatographic behaviour of the antibiotics in this binary mobile phase offer new possibilities of speedy separations.

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