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TLC Separation of Cephalosporins on Stannic Arsenate Layers

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Abstract*: The chromatographic behaviour of some cephalosporins has been studied on synthetic stannic arsenate layers using citrate and borate buffers as mobile phases. Several ternary and quaternary separations have been achieved. The utility of these separations has been demonstrated for estimation of cephalosporins in blood serum from patients.This method used is simple, rapid ,reproducible and can also be applied in the separation and determination of cephalosporins in other biological samples. The limit of detection was found to be 0.20 µg/l.*
KeyWords: *TLC, Cephalosporins, Stanic arsenate*

I. Introduction

The use of inorganic ion exchangers as adsorbents in thin layer chromatography has afforded promising results in the separation of metal ions, anions [1–4], organic compounds [5,6], phenols [7] and organic acids [8]. The widespread application of ion-exchange resins for separating and purifying amino acids from protein hydrolysis has received considerable attention [9,10]. Stannic arsenate has been used for TLC because it has been found to be quite stable in acids, bases, and common organic solvents; it has been used for separation of metal ions [11], anions [12],and amino acids [13].Addition of inorganic salts to conventional adsorbents and use of organic and inorganic solvents as mobile phases has been reported to result in improved separations of some pharmaceutical products compared with the untreated adsorbent [14]. Double hydroxide adsorbent layers have been used for TLC separation of cephalosporins [15]. The cephalosporin anti-biotics are a large family of therapeutically useful compounds

Sporadic publications on the identification of cephalosporin anti-biotics by densitometry on hydrocarbon-impregnated silica gel HPTLC plates have appeared in the literature [16-17] and TLC of cephalosporins has also been performed on silanized silica gel [18-19]. In this work we have reported the utility of stannic arsenate and simple mobile phases for separation of cephalosporins and their subsequent quantitative determination in the blood serum of patients.

II. Experimental

Chemicals

Sodium arsenate(Loba Chemic,India),Stenic Chloride(Loba Chemic,India),Methanol (AR grade), citric acid, sodium hydroxide, potassium di-hydrogen phosphate, ammonia solution, borax, sodium bicarbonate, ferric ammonium sulphate, and hydroxylamine hydrochloride were from Merck (Mumbai, India) . Boric acid was from Ranbaxy (S.A.S. Nagar, India). Ni-ckel(II) chloride hexahydrate was from Qualigens Fine Chemical (Mum-bai, India).

Samples

Cefaclor was from Aristo pharmaceuticals (Nani Daman, Mumbai, India), ceftriaxone from Otomotive Products (Navi Mumbai, India), cefta-zidime from Biochem Pharmaceuticals (Mumbai, India), cefoperazone from

Unimed Technologies (Halol, Gujarat, India), cefotaxime from Starry Health Care (Vikhroli (W) Mumbai, India), cefadroxil from Comed Chemicals (Baroda, Gujarat, India), and cephalexin from Glaxo India (Mumbai, In-dia). The structures of the compounds are shown in Fig. 1.

III. Preparation of Ion- exchange material and development of TLC plates

Stenic arsenate ion-exchanger was prepared by mixing 0.5 molar sodium arsenate with 0.5 molar stenic chlotide in 1:1 ratio at pH 10.0 and subsequent addition of 0.1M HCl . The resulting precipitate was digested at room temp. for 24 hours,filtered by suction and then dried in an oven at 40±5o C.The material obtained was cracked in dimineralized water,mixed with 1 M Nitric ad. and kept for over night so that it is converted in to protonated form.It was washed with demineralized water to remove exciss ad. and finally dried in an oven at 40^0C

The TLC plates were prepared in the usual manner from demineralized water . The slurry was spread over TLC plates with 0.2 mm thickness and dried in air overnight, and activated at 60°C for 1 h before spot application.

Solvent System used

At least 20 different solvents were used as mobile phases but sepa-ration of cephalosporins on stannic arsenate was achieved solely by use of citrate and borate buffers of different pH. The mobile phases used are listed in Table I.

Qualitative Separations in synthetic mixtures

For qualitative studies solutions (1.0 mg $mL^{-1)}$ of each cephalospo-rin in DMW were applied at one end of the plate. The glass plates (18cm x 20 cm) were developed, at room temperature (25 ± 3°C), in rectangular chambers (20 cm × 22 cm × 9 cm) previously equilibrated by conditioning with mobile phase for at least 1 h. The time required for chromatographic development of the plates varied with the mobile phase used. After development plates were dried with a stream of cold air and the spots were visualized.

by placing the plates in a chamber of iodine vapour. Brown spots on the plate revealed the location of the compounds. hR_F values were calculated by means of the formula:

$$hR_F = \frac{\text{distance travelled by the geometrical centre of the solute spot}}{\text{distance travelled by solvent front from the point of application}} \times 100$$

The hR_F values obtained are listed in Table II.

IV. Quantitative Separations

For quantitative work, stock solutions of cephalosporins were pre-pared in demineralized water. Solutions of different cephalosporins were mixed, spotted by means of a microsyringe, and developed with a selected mobile phase. A pilot plate was run simultaneously to visualise exact position of the spot on the TLC plate. The regions containing the cephalosporins were scraped from the plates, mixed in demineralized water and then filtered. The clear solution containing the cephalosporin content of each spot was then analysed by a spectrophotometric method [20-24]. Results are shown in Table III.

Quantitative Estimation of Cephalosporins in Blood Serum Samples

Anhydrous sodium sulphate (40 g) and ethanol (95%, 2 mL) were mixed with 10 mL of oxalate blood. The mixture was centrifuged and after 2.5 hours the supernatant liquid was decanted. Because of the presence of sodium sulphate and alcohol, water was eliminated. Finally the mixture was mixed with diethyl ether for 45 min. The ether layer was separated and the mixture was concentrated to approximately 0.10 mL under vacuum at 50°C. Blood serum samples from different patients were collected and analysed for specific antibiotics by a spectrophotometric method. Known amount of the concentrated solution of cefalosporine was then applied to the TLC plates and the plates were developed with an appropriate mobile phase. The region containing the spot was scraped from the plate, mixed with demineralised water, and same procedure was applied as in quantitative separation.

V. Results And Disicussion

Antibiotics are chemically defined reproducible chemical substan-ces produced in and isolated from living cells, or are chemical or biological derivatives of these [25]. Non-specific methods for analysis of anti-biotics, for example microbiological and spectrophotometric methods, do not differentiate between structurally similar byproducts from the synthesis or degradation of the antibiotics of interest. More specific methods such as TLC, GC, and HPLC that differentiate among different structures are preferable for analysis of antibiotics [26]. Because of the unstable nature of antibiotics, decomposition of the drugs or polymerization can occur during chromatography, although this occurs less in TLC than in paper chromatography [27].

Stannic arsenate is regarded as quite stable, amorphous, and hydrated to a variable extent. TLC plates are, therefore, activated at 60°C to desorb physically bonded water. The spots were detected by placing the TLC plates in an iodine vapour chamber. The iodine vapour dissolves in or forms weak charge transfer complexes with organic compounds and the cephalosporins show up as brown spots on a pale yellow background within few minutes. After marking the zones for further reference exposure of the plates to air causes the iodine to sublime and the spots fade. The hR_F values of the different cephalosporins after chromatography with mobile phases S1 to S9 are shown in Table II. It is clear from the hR_F values that cefaclor, which contains a Cl group, has higher hR_F values for most of the mobile phases than cephalexin (OCH_3 group) or cefadroxil (CH_3 group). This might be because of the negative inductive effect – Cl^- has a stronger negative inductive effect than OCH_3 (i.e. attracts electrons more strongly).

It was found that the behaviour of cefoperazone was peculiar in almost all of the mobile phases except ammonia and potassium dihydrogen phosphate. The positive inductive effects of the methyl and ethyl groups in

cefoperazone reduce chemical interaction with the mobile phase and hence migration is suppressed. On the other hand, the presence of the Na^+ ion in cefotaxime, ceftazidime, and cefoperazone facilitates release of an electron, leading to the formation of polar compounds that are highly soluble in water and, therefore, of low R_F in acidic media and high R_F value in basic media, as expected.

It is apparent from Table II that separation of most of the cephalo-sporins is poor with the ammonia mobile phase, probably because of solvation of the alkali metals by the ammonia molecule. The exceptional behaviour of ceftriaxone chromatographed with ammonia and potassium dihydrogen phosphate enables selective separation of this antibiotic from the other cephalosporins. On the basis of the different R_F values of the cephalosporins when chromatographed with different mobile phases it is possible to achieve some important binary and ternary quantitative sepa-rations on stannic arsenate layers in a few minutes only. It is worth noting one advantage of using stannic arsenate layers – the possibility of selective separation of some components of a synthetic mixture of cephalosporins. The results are reported in Table III.

This TLC method is simple, rapid, selective, reproducible, and applicable to identification and separation of cephalosporins. The practical utility of this method was demonstrated by quantitative identification of common cephalosporins in serum samples from patients .The results are summarized in Table IV. The percentage recovery, accuracy, and reproducibility of the method were checked statistically.

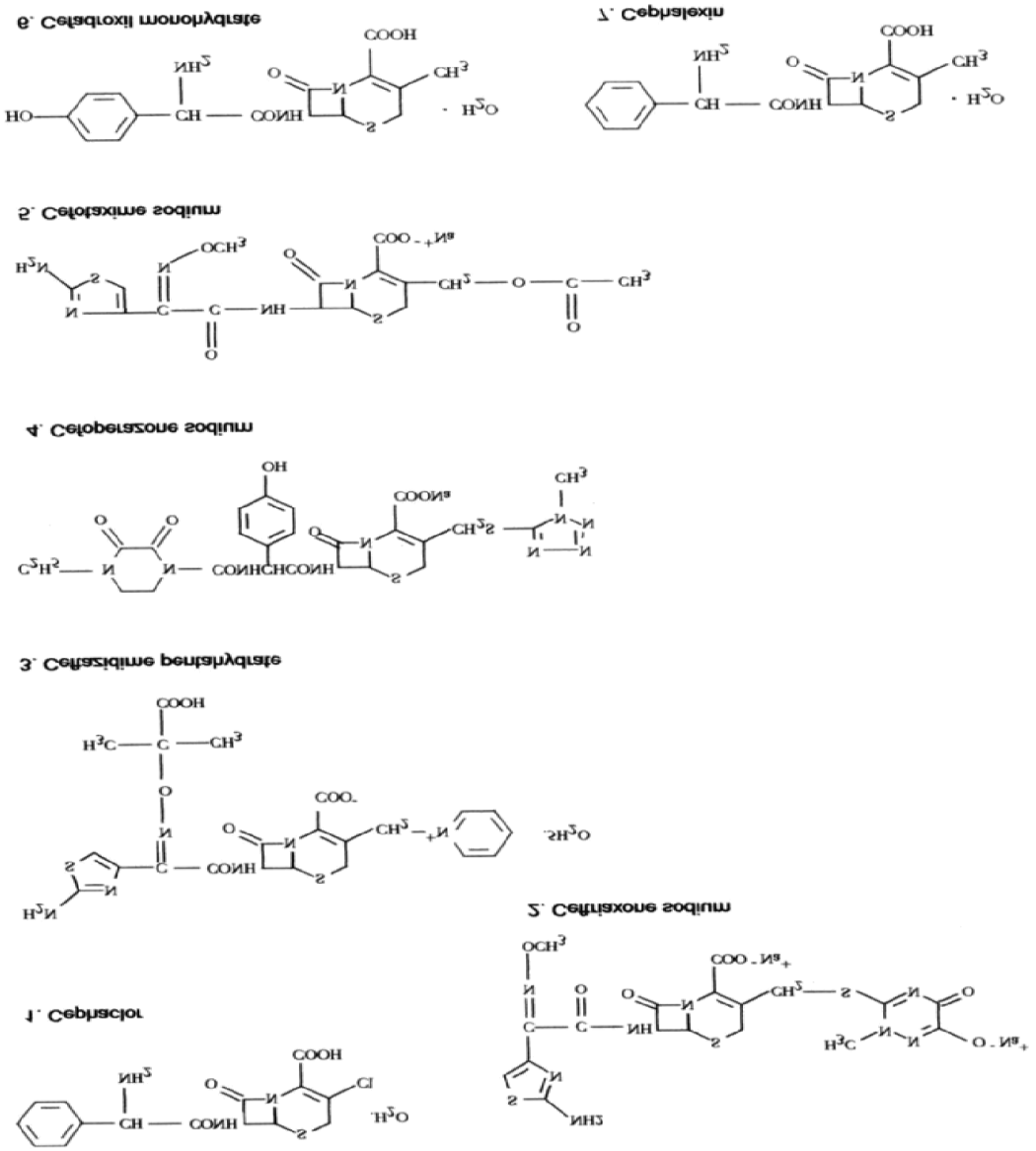

Fig. 1. The molecular structures of the cephalosporins studied

Table I
Solvent System used

Components	pH	Abbreviation
0.2 M Boric acid	1.20	S1
Citrate buffer (0.2 M citric acid + 0.2 M sodium hydroxide)	3.10	S2
Citrate buffer (0.2 M citric acid + 0.2 M sodium hydroxide)	4.15	S3
Citrate buffer (0.2 M citric acid + 0.2 M sodium hydroxide)	5.55	S4
Borate buffer (0.2 M boric acid + 0.2 M sodium hydroxide)	9.20	S5
Borax	9.22	S6
Borate buffer (0.2 M boric acid + 0.2 M sodium hydroxide)	10.23	S7
Aqueous ammonia (30%)	13.35	S8
0.1 M Potassium dihydrogen phosphate	5.35	S9

Table II
hR_f values of the cephalosporins on stannic arsenate layers developed with different mobile phases

No.	Cephalosporin	S1	S2	S3	S4	S5	S6	S7	S8	S9
1	Cephalexin	87.55	72.42	11.24	77.27	94.50	84.61	87.50	43.32	44.40
2	Cefadroxil	42.90	72.32	0.00	77.27	43.82	15.30	38.40	61.35	55.50
3	Cefaclor	99.95	87.27	76.73	85.50	99.40	99.50	97.85	71.52	52.50
4	Cefotaxime	85.54	0.00	76.47	0.00	87.50	92.50	74.80	73.83	65.42
5	Ceftriaxone	0.00	0.00	0.00	0.00	0.00	0.05	0.10	62.55	17.35
6	Ceftazidime	0.00	0.00	0.00	0.00	45.00	98.45	3050	74.55	71.50
7	Cefoperazone	58.15	0.00	0.00	31.35	43.75	37.40	44.55	75.80	70.40

Table III
Quantitative separations of cephalosporins from synthetic mixtures on stannic oxide layers

No.	Separations achieved	Amount taken (µg)	Amount found (µg)[a]	Recovery (%)	Error (%)	*SD*[a]	Mobile phase[b]
1	Cefaclor	50.00	49.50	99.00	-1.00	0.018	S2
	Ceftriaxone	50.00	50.00	100.00	0.00	0.070	
	Cefadroxil	50.00	49.85	99.70	-0.30	0.007	
2	Ceftriaxone	50.00	50.00	100.00	0.00	0.079	S4
	Cefoperazone	50.00	50.04	100.08	0.08	0.048	
	Cephalexin	50.00	50.22	100.44	0.44	0.062	
3	Ceftazidime	50.00	49.72	99.44	-0.56	0.090	S1
	Cefotaxime	50.00	49.73	99.46	-0.54	0.065	
	Cefadroxil	50.00	49.61	99.22	-0.78	0.097	
4	Cefotaxime	50.00	49.83	99.66	-0.34	0.014	S9
	Cefadroxil	50.00	49.72	99.44	-0.56	0.013	
	Ceftriaxone	50.00	50.01	100.02	0.02	0.008	
5	Cefaclor	50.00	49.50	99.00	-1.00	0.017	S3
	Ceftriaxone	50.00	50.0	100.00	0.00	0.083	
	Cephalexin	50.00	50.18	100.36	0.36	0.021	
6	Cefaclor	50.00	49.50	99.00	-1.00	0.017	S6
	Ceftriaxone	50.00	50.00	100.00	0.00	0.070	
	Cefoperazone	50.00	49.92	99.84	-0.16	0.168	
	Cefadroxil	50.00	49.65	99.30	-0.70	0.097	
7	Ceftriaxone	50.00	50.00	100.00	0.00	0.079	S5
	Ceftazidime	50.00	49.73	99.46	-0.54	0.183	
	Cefotaxime	50.00	49.84	99.68	-0.32	0.014	
	Cephalexin	50.00	49.50	99.00	-1.00	0.013	

[a]Average from five replicate determinations
[b]S1, Boric acid (pH 1.25); S2, citrate buffer (pH 3.15); S3, citrate buffer (pH 4.05); S4, citrate buffer (pH 5.05); S5, borate buffer (pH 9.10); S6, borax buffer (pH 9.18); S9, po-tassium dihydrogen phosphate (pH 5.30)

Table IV
Quantitative separations of cephalosporins from samples of patients' serum

Serum sample no.	Cephalosporin	Amount taken (µg)	Amount found (µg)[a]	Recovery (%)	a *SD*	Mobile phase[b]
1	Cefaclor	50	49.90	99.80	0.05	S 1
2	Cefotaxime	100	49.95	99.90	0.125	S 6
3	Ceftriaxone	100	49.20	9840	0.038	S 8
4	Ceftazidime	50	49.45	98.90	0.022	S 6

[a]Average from four replicate determinations
[b]S1, boric acid (pH 1.25); S6, borax buffer (pH 9.18); S8, aqueous ammonia (pH 13.55)

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5	Ceftriaxone	0.00	0.00	0.00	0.00	0.00	0.05	0.10	62.55	17.35
6	Ceftazidime	0.00	0.00	0.00	0.00	45.00	98.45	3050	74.55	71.50
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