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Chromatographic Separation Studies of Cephalosporins on CTAB Modified Silica Layers with Different Buffer Solvent Systems

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Abstract: Various surfactant modified silica layers were used for the chromatography of five different Cephalosporins. The 4% methanolic CTAB impregnated silica layer was useful for the chromatography of Cephalosporins. The acidic buffer solvent systems were used with the 4% methanolic CTAB impregnated silica layers for the separation of different mixtures [A (Cefaclor, Ceftriaxone and Cefadroxil), B (Ceftriaxone, Cefoprazone and Cephalexin) and C (Cefaclor, Ceftriaxone and Cephalexin)] of Cephalosporins. The interference due to the presence of sodium and potassium salts, glucose and urea on the identification and mobility of all the five Cephalosporins were also examined.
Keywords: Cephalosporins,Thin Layer Chromatography,Surfactants,Buffer solvent systems.

Introduction

Cephalosporins (fig:1) structurally differ from penicillin's by the heterocyclic ring system. Cephalosporins are pencillinase resistance antibiotics with significant activity against both gram-positive and gram-negative bacteria. They are among the safest and the most effective broad spectrum bactericidal antimicrobial agents, therefore they are the most prescribed of all the available antibiotics [1-2]. Because of the high therapeutic importance of cephalosporins several analytical techniques have been used for their analysis such as, titrimetric[3-4], High performance liquid chromatography [5], Voltametric [6], Spectrophotometry [7-8] Mass-spectrophotomtery [9]. The most of these analytical methods required expensive and sophisticated instruments. This report is an attempt in the direction of developing a simple and reliable method for on plate identification and separation of cephalosporins in pharmaceutical formulations. Amongst all chromatographic techniques, thin layer chromatography (TLC) has been the most popular for routine analysis due to its simplicity of use, simultaneous analysis of large number of samples, use of specific and colorful reactions, the possibility of two-dimensional separation and easier manipulation of stationary and mobile phases. The aim of this study was to develop a new chromatographic system by impregnating silica gel with solutions of surfactants.

In this report we have utilized the versatile amphiphotheric nature of surfactants in the impregnation of silica layers. It is reported that the preliminary impregnation of the adsorbent with surfactant solutions leads to the change in elution order and their behavior was observed in the impregnation of both normal and reversed stationary phase. The separation of primary aliphatic and aromatic mono- and polyamines [9-11], amino acids [12] peptides and dipeptides [13-14]. The introduction of surfactant into the stationary phase leads to dynamic modifications in the stationary phase. According to the literature survey, previously Cephalosporins were analyzed by various TLC methods on silica gel layers [15-16] but the use of methanolic surfactant modified stationary phase is lacking.

Experimental:

Instrumentation and reagents

A TLC applicator (Toshiwal India) and pH meter Elico India Ltd was used. Chemical required like silica Gel 'H', Sodium dodecyl sulfate (SDS), N-cetyl-N, N, N-trimethylammonium bromide (CTAB), Methanol, were purchased from Merck India, Iodine crystals, Glacial acetic acid, Boric acid, Phosphoric acid and Sodium hydroxide were obtained from CDH India,

Samples
Cefaclor, Ceftriaxone, Cefaperozone, Cefadroxil and Cephalexin (pure & formulated) were from Lupin labs, Mandideep, M.P., India as a gift samples.

General procedure

Preparation of Test Solution
The drug powder (250 mg) was transferred into a 50 mL standard volumetric flask and then 25 mL methanol was added and the resulting mixture was sonicated for 40 mins. The solution was filtered through Whatman filter paper (No.41) and the residue obtained was washed again with methanol. The total filtrate was transferred in a 50 mL standard volumetric flask and made-up to the mark with methanol. This solution contains 5 mg/mL (w/v) of drug.

Detector Iodine Vapors were used as detector

.**Stationary Phase:** The following (**Table 1**) stationary phases were used for the chromatography

Mobile phase: The solvent systems were used (**Table 2**) as mobile phases for chromatography of all the drugs.

Chromatography

(a) Preparation of TLC plates

The TLC plates were prepared by mixing silica gel G with double distilled water in 1:3 ratios by weight with constant shaking to obtain homogeneous slurry. The resultant slurry was applied on the glass plates with the help of a manual applicator to give a 0.25 mm-thick layer. The plates were dried at room temperature and then activated at 100 ± 2 ^{0}C by heating in an electrically controlled oven for one hr. The activated plates were stored in a close chamber at room temperature until used.

(b) Chromatographic procedure

Test solutions (10µL) were applied on (15 × 3 cm) silica gel thin layer plates with the help of micropipette at about 2 cm above the lower edge of the plates. The solvent ascent was fixed to 10 cm in all cases for the determination of R_F values of all individual drugs. Linear ascending development was carried out in a vapor equilibrated TLC twin trough chamber. The optimized chamber saturation time for the mobile phase was 15 min at room temperature (25 ± 1 ^{0}C). Subsequent to the development, TLC plates were dried at room temperature. The plates were then detected by using iodine vapors and all the drugs are visualized as colored spots. The R_F values of drug were determined by the following relation –

$R_F = 0.5 (R_L + R_T)$

where $R_L = R_F$ of leading front.

$R_T = R_F$ of trailing front.

Separation
For the separation, equal amount of drugs to be separated were mixed [A (Cefaclor, Ceftriaxone and Cefadroxil), B (Ceftriaxone, Cefoprazone and Cephalexin) and C (Cefaclor, Ceftriaxone and Cephalexin)] and 10µL of the analyte from the resultant mixtures were loaded on CTAB impregnated TLC plates (S_{10}). The plates were developed with different solvent systems (M_1, M_2 and M_3 for A, B and C mixtures respectively) and the spots were detected and the R_F values of the separated drugs were determined.

Interference
For investigating the interference of various organic and inorganic compounds like sodium and potassium salts, urea and glucose on the identification and mobility of all the five cephalosporins studied, an aliquot (10µL) of all drugs were spotted on CTAB impregnated TLC plates (S_{10}) followed by spotting of 10µL of the interfering species (1mg/mL) on the same spot. The chromatography was performed with solvent system M_1 on CTAB impregnated silica layers (S_{10}). The spots were detected and the R_F values of drugs were calculated and compared.

Results and discussion:

The chromatography of cephalosporin was performed on various stationary phases impregnated with cationic and anionic surfactants at different concentrations. The various buffer systems were also used as solvent systems. The buffer system having pH 3.15 (M_1) was first used with all the stationary phases in order to select the most useful chromatographic system for the identification and separation of Cephalosporin. The results of the present study are presented in Table 3. The following conclusions are drawn from the results.

1. On silica surface (unimpregnated) the Cefaclor and Cefaprozone are not detected while Ceftriaxone and Cefadroxil show tailed spots and Cephalexin remains at the point of application.

2. In case of SDS impregnated silica layers (S_2 – S_6), the mobility of Cefaclor, Cefadroxil and Cephalexin increases with the increase in concentration of SDS as an impregnant, while the mobility of Cefatriaxone decreases and also Cefoperazone remains at the point of application.

3. On CTAB impregnated silica layers (S_7–S_{11}), the mobility of all the Cephalosporins increases with the increase in the concentration of CTAB as an impregnant.

It is clear from the results that the lower R_F values are observed for all the Cephalosporins on SDS impregnated silica layers as compared to CTAB impregnated layers except Ceftriaxone where the reverse trend was observed. This may be due to the electrostatic interactions between the SDS and the Cephalosporins. This situation is caused because SDS is impregnated on negatively charged silica via the hydrophobic group and its negatively charged hydrophilic group is oriented away from the surface. As compared to SDS, CTAB is impregnated on negatively charged silica via positively charged head group and its hydrophobic group is oriented away from the surface. On the other hand, the acidic buffer solvent systems show significant interactions with

all the drugs on CTAB impregnated layers as compared to SDS impregnated layers. The significant influence of solvent system M_1 on the stationary phase S_{10} (S_1 Impregnated with 4 % MeOH solution of CTAB) results in the beneficial mobilitys of all the cephalosporins and high compactness and high visibility of spots. The mutual separation possibility of Cephalosporins also increases on the stationary phase S_{10}. The stationary phase S_{10} (S_1 Impregnated with 4 % MeOH solution of CTAB) was used for the further study. Different buffer systems were used as eluents for the chromatography of Cephalosporins on the stationary phase S_{10}. The results are presented in Table 4. From the point of chromatographic conditions all the Cephalosporins provide efficient results in the acidic buffer solvent systems as compared to the alkaline buffer solvent systems. This might be due to the presence of Na^+ ion in Cephalosporins which facilitates the release of an electron, leading to the formation of a polar compound. In acidic medium it was possible to resolve three combinations of Cephalosporins (Table 5) such as: (a) Cefaclor, Ceftriaxone and Cefadroxil, (b) Cefaclor, Ceftriaxone and Cephalexin (c) Ceftriaxone, Cefaprozone and Cephalexin in the solvent systems M_1, M_2 and M_3 respectively. These separations are not possible in the alkaline buffer solvent systems. The results of the influence of interfacing species on the chromatography of Cephalosporins on stationary phase S_{10} developed with solvent system M_1 are presented in Table 6. It is clear from the results that in NaCl as KCl as interfering species, ceftriaxone and cefadroxil show tailed spots while all other three drugs are not greatly influenced by their presence. In urea, all the cephalosporins are not detected. In case of glucose as an interfering species cefaclore and cefadroxil show tailed spots while all other three drugs are not detected. Therefore, it is concluded that the presence of these interfering species greatly influence the chromatography of Cephalosporins.

Table 1: Stationary Phase.

Code	Composition
S_1	Silica gel G
S_2	S_1 Impregnated with 0.1% MeOH solution of SDS
S_3	S_1 Impregnated with 1% MeOH solution of SDS
S_4	S_1 Impregnated with 2 % MeOH solution of SDS
S_5	S_1 Impregnated with 4 % MeOH solution of SDS
S_6	S_1 Impregnated with 7 % MeOH solution of SDS
S_7	S_1 Impregnated with 0.1% MeOH solution of CTAB
S_8	S_1 Impregnated with 1 % MeOH solution of CTAB
S_9	S_1 Impregnated with 2 % MeOH solution of CTAB
S_{10}	S_1 Impregnated with 4 % MeOH solution of CTAB
S_{11}	S_1 Impregnated with 7 % MeOH solution of CTAB

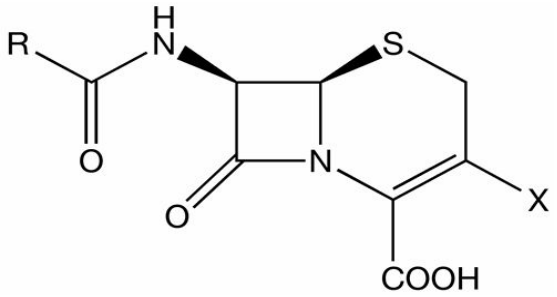

Fig: 1 Structure of Cephalosporin

Table 2: Mobile Phases.

Code	Composition
M_1	Citrate buffer (0.2M citric acid + 0.2 M NaOH) pH 3.15
M_2	Citrate buffer (0.2M citric acid + 0.2 M NaOH) pH 4.05
M_3	Citrate buffer (0.2M citric acid + 0.2 M NaOH) pH 5.05
M_4	Borate buffer (0.2M boric acid + 0.2 M NaOH) pH 9.10
M_5	Borate buffer (0.2M boric acid + 0.2 M NaOH) pH 10.03

Table 3: R_F values of Cephalosporins developed on unimpregnated and impregnated silica layers with solvent system M_1.

Drugs	Stationary Phases										
	S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8	S_9	S_{10}	S_{11}
Cefaclor	ND	0.54	0.57	0.57	0.59	0.61	0.81	0.81	0.82	0.86	0.88
Ceftriaxone	0.91 (T)	0.87 (T)	0.83 (T)	0.82 (T)	0.80 (T)	0.80 (T)	0.00	0.07	0.10	0.14	0.13
Cefadroxil	0.98 (T)	0.47 (T)	0.48 (T)	0.48	0.51	0.57	0.61	0.61	0.68	0.71	0.71
Cefaprozone	ND	ND	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.04
Cephalexin	0.00	0.00	0.28	0.31	0.31	0.38	0.57	0.58	0.61	0.67	0.68

Table 4: R_F value of Cephalosporin's developed with CTAB impregnated silica layers (S_{10}) using different buffer solvent systems.

Drugs	Solvent Systems				
	M_1	M_2	M_3	M_4	M_5
Cefaclor	0.86	0.76	0.81	0.92	0.97
Ceftriaxone	0.14	0.00	0.00	0.14	0.36
Cefadroxil	0.71	0.00	0.76	0.41	0.36
Cefaprozone	0.04	0.00	0.31	0.43	0.43
Cephalexin	0.67	0.11	0.70	0.91	0.43

Table 5: Separations (R_F values) of different Cephalosporins achieved from their mixtures on CTAB impregnated silica layers (S_{10}) using with different solvent systems.

Mobile Phase	Drug Mixture			
M_1	A	Cefaclor (0.91)	Ceftriaxone (0.09)	Cefadroxil (0.66)
M_3	B	Ceftriaxone(0.00)	Cefaprozone (0.37)	Cephalexin (0.81)
M_2	C	Cefaclor (0.79)	Ceftriaxone(0.00)	Cephalexin (0.17)

Table 6: Effect of Various Organic and Inorganic Impurities on the IdentificationMobility (R_F) of different Cephalosporins on Silica gel layers (S_{10}) developed with Solvent System M_1

Impurity	Drugs				
	Cefaclor	Ceftriaxone	Cefadroxil	Cefaprozone	Cephalexin
NaCl	0.84	0.11 T	0.72 T	0.00	0.66
KCl	0.81	0.09 T	0.74 T	0.14	0.67
Urea	ND	ND	ND	ND	ND
Glucose	T	ND	T	ND	ND

ND = Not detected ,T = Trailing

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