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Sodium Deoxycholate Micelles Activated Separation of Coexisting Five-nucleobases by High-performance Thin-layer Chromatography

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Abstract

A novel high-performance thin-layer chromatographic method has been developed for the resolution of five-coexisting nucleobases (adenine, guanine, cytosine, thymine, and uracil). The nucleobases were separated on aluminum-backed cellulose 60 F_{254} plates with the aid of 5.0% aqueous sodium deoxycholate (NaDC)-acetonitrile (AcN), 1:3 (v/v) as mobile phase. All the nucleobases were viewed on HPTLC plates under 254nm UV light. The order of R_F value given in parentheses was guanine (0.12) < adenine (0.44) < cytosine (0.50) < uracil (0.72) < thymine (0.84). The effect of pH (acidity or basicity) of the mobile phase on the retention of individual nucleobases was examined. Furthermore, the effect of interference of mono- (Li^+, Na^+), and bivalent (Mg^{2+}, Ba^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Pb^{2+}) cations; mono- (Br^-, CH_3COO^-, NO_3^-, IO_4^-), and bivalent (CO_3^{2-}, SO_4^{2-} MoO_4^{2-}) anions, and complexing ligands (urea, and EDTA) on the retention behavior of nucleobases were also assessed. The chromatography of nucleobases was also performed on silica 60 F_{254}, RP-18 F_{254}, and kieselgel 60 F_{254} HPTLC plates. These TLC plates failed to separate the coexisting purines and pyrimidines. The detection limit of all nucleobases on cellulose 60 F_{254} layers was 5.4×10^{-2} µg $spot^{-1}$. The proposed method is rapid, easy, and reliable. It can be applied for routine analysis of DNA, and RNA nucleobases.

Keywords: HPTLC; Nucleobases; Purines; Pyrimidines; Bile salt; Sodium deoxycholate

Introduction

High-performance thin-layer chromatography (HPTLC) is well suited to the separation of nucleobases. Most of reports on this topic are limited to the use of PEI-cellulose, ODS (octadecyl silica), and silica gel as layer materials (Randerath and Struck, 1961; Bij and Lederer, 1983; Steinberg et al., 1996). Chiral plates have also been used for separation of nucleobases, and enantiomers (Hatzack and Rasmuseen, 1999). The first use of micellar mobile phase by Armstrong in 1979 for the analysis of nucleotides was 1.3 AOT in cyclohexane-water mixture. The suggested mobile phase was capable to resolve a mixture of nucleotides on silanized silica gel. Compared to normal micelle forming surfactants, bile salts are unique in forming helical aggregates in solution (Gillio et al., 1988; Campanelli et al., 1989; Williams et al., 1990). In nature, sodium deoxycholate (Figure 1) referred as "secondary bile acid" is produced in the intestine from the salts of glycocholic, and taurocholic acid by the action of bacterial enzymes. Applications of sodium deoxycholate range from cell lyses, liposome preparation, isolation of membrane proteins and lipids, a cell culture media as supplement, preventing non specific binding in affinity chromatography, micellar electrokinetic chromatography and other chromatographic techniques (Hofmann and Mysels, 1987; Williams et al., 1990; Thompson et al., 1995 Morgan et al., 2008). Versatility of bile salts led us to utilize its analytical potential as mobile phase for the separation of coexisting nucleobases from their mixtures. This is probably the first report to separate five nucleobases (two purines, and three pyrimidines) on cellulose 60 F_{254} HPTLC plates by utilizing sodium deoxycholate (aqueous 5.0%) plus acetonitrile (1:3, v/v) as mobile phase. Furthermore, we have successfully achieved an interesting separation of thymine from uracil. This separation is important because both pyrimidines are used to differentiate between the structures of DNA and RNA (Figure 2).

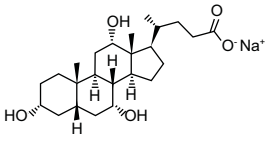

Figure 1: Structure of sodium deoxycholate.

Experimental

All experiments were performed at 25 ± 2°C.

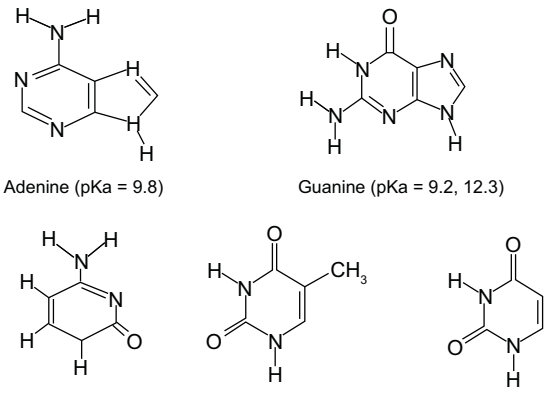

Figure 2: pKa value and chemical structure of nucleobases.

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Chemicals and reagents

Pre-coated cellulose 60 F_{254} HPTLC aluminum foils were of Merck, Darmstadt, Germany. Acetonitrile (Lichsolv, Merck, India), purines and pyrimidines (Himedia, Mumbai, India) and sodium deoxycholate (E-Merck, India) were used.

All chemicals and reagents were of analytical reagent (AR) grade. Double distilled water (DDW) was used throughout the experiment.

Working standard solutions

Solutions of purines (adenine, and guanine), and pyrimidines (cytosine, thymine, and uracil) were prepared in a mixture of methanol and water (4: 6) to give concentration of 1.0% w/v.

Detection

HPTLC plates containing fluorescent indicator were kept under short wave 254nm UV light to locate the position of analyte.

Stationary phases

Cellulose 60 F_{254}, silica gel 60 F_{254}, RP-18 F_{254}, and Kieselgel 60 F_{254} aluminium foils of size 6 x 6 cm were used as stationary phases.

Buffer solutions

The buffer solutions used to prepare the solution of NaDC (5.0%) at different pH levels are listed in Table 1.

Mobile phases

A variety of mobile phases listed in Table 2 were examined to identify the most suitable solvent system for separation of nucleobases.

HPTLC method

Precoated HPTLC plates were activated at 60 ± 2°C in an electrically controlled oven for 20 min and stored in closed chamber until used. Spotting of 1.0 µL of sample using micropipette (Tripette 0783178, Germany) is done at 1.0 cm from the base of HPTLC plates. Spots were air dried and developed in a closed presaturated Camag TLC twin-trough chamber with desired mobile phase by ascending technique up to the ascent of 5.0 cm from the point of application. After development, the HPTLC plates were withdrawn from glass chamber, air dried and then detected under UV light to locate the position of analyte as fluorescent spots under short wavelength.

The R_F values of visualized spots were calculated by using formula: $R_F = 0.5 (R_L + R_T)$ where $R_L = R_F$ of the leading edge, and $R_T = R_F$ of trailing edge.

In order to examine the effect of volume of acetonitrile added in aqueous NaDC, R_F of nucleobases, were chromatographed on cellulose layers. The affinity of the solvent systems with different concentrations of aqueous NaDC was assessed by calculating the capacity factor values.

The capacity factor (K) was determined as a function of the conventional mobility R_F

$$K = \left(\frac{1}{R_F} - 1 \right).$$

It is used to indicate the relative affinity of purines or pyrimidines

Composition	Volume ratio	pH
0.04M boric acid + 0.04 M phosphoric acid	50 : 50	2.3
0.04M boric acid + 0.04 M phosphoric acid + 0.24 M NaOH	50 : 50 : 8	3.4
0.04M boric acid + 0.04 M phosphoric acid + 0.24 M NaOH	50 : 50 : 10	5.7
0.04M boric acid + 0.04 M phosphoric acid + 0.24 M NaOH	50 : 50 : 14	7.2
0.04M boric acid + 0.04 M phosphoric acid + 0.24 M NaOH	50 : 50 : 60	11.9

Table 1: Buffer solutions used as solvents to prepare mobile phases containing 5.0 % NaDC.

Code for mobile phases	Composition
M_1	DDW (double distilled water)
Aqueous micellar bile salt solution	
M_2	5.0% NaDC in DDW
Aqueous micellar solutions with organic additives	
M_3	M_2–AcN (9 : 1, v/v)
M_4	M_2–AcN (8 : 2, v/v)
M_5	M_2–AcN (7 : 3, v/v)
M_6	M_2–AcN (6 : 4, v/v)
M_7	M_2–AcN (5 : 5, v/v)
M_8	M_2–AcN (4 : 6, v/v)
M_9	M_2–AcN (3 : 7, v/v)
M_{10}	M_2–AcN (2.5 : 7.5, v/v)
M_{11}	M_2–AcN (2 : 8, v/v)
M_{12}	M_2–AcN (9 : 1, v/v)
M_{13}	M_2–Acetone (2.5 : 7.5, v/v)
M_{14}	M_2–MeOH (2.5 : 7.5, v/v)
M_{15}	M_2–HCOOH (2.5 : 7.5, v/v)
M_{16}	M_2–DMSO (2.5 : 7.5, v/v)
M_{17}	5.0% aqueous NaC–AcN (2.5 : 7.5, v/v)
M_{18}	5.0% aqueous NaTC–AcN (2.5 : 7.5, v/v)
Buffer micellar NaDC solutions with AcN (2.5 : 7.5, v/v)	
M_{19}	5.0% NaDC (pH 2.3)–AcN
M_{20}	5.0% NaDC (pH 3.4)–AcN
M_{21}	5.0% NaDC (pH 5.7)–AcN
M_{22}	5.0% NaDC (pH 7.2)–AcN
M_{23}	5.0% NaDC (pH 11.9)–AcN

AcN, acetonitrile; NaC, sodium cholate; NaTC, sodium taurocholate; MeOH, methanol; DMSO, dimethylsulfoxide.

Table 2: Solvent systems used as mobile phases in chromatographic studies.

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Mobile phase			Nucleobases		
	Purines			*Pyrimidines*	
	Adenine	Guanine	Cytosine	Thymine	Uracil
M_1	0.33T	0.57	0.78	0.80	0.80
M_2	0.50	0.54	0.60	0.70	0.70
M_3	0.53	0.50	0.75	0.75	0.74
M_4	0.55	0.48	0.75	0.80	0.80
M_5	0.56	0.50	0.75	0.90	0.86
M_6	0.60	0.57	0.80	0.89	0.86
M_7	0.58	0.48	0.77	0.85	0.82
M_8	0.55	0.40	0.71	0.85	0.77
M_9	0.49	0.24	0.58	0.85	0.75
M_{10}	0.44	0.12	0.50	0.84	0.72
M_{11}	0.44	0.10	0.48	0.84	0.73
M_{12}	0.12	0.00	0.14	0.53	0.41
M_{13}	0.32	0.10	0.46	0.67	0.38T
M_{14}	0.13	0.06	0.35	0.38	0.20
M_{15}	0.95	0.94	0.95	0.97	0.96
M_{16}	0.90	0.90	0.91	0.92	0.91
M_{17}	0.64	0.24	0.75	0.93	0.91
M_{18}	0.56T	0.18	0.70	0.83	0.80T

Each value is an average of four measurements; T = Tailed spot; nd = not detected.

Table 3: R_F values of five nucleobases on cellulose 60 F_{254} HPTLC plates developed with mobile phases (M_1-M_{18}).

between the solid substrate and the solvent. Strong affinity for the substrate is indicated by high value of capacity factor and vice versa.

To study the effect of interference of mono- and bivalent cations or anions, urea, and EDTA on the R_F value of nucleobases, analyte sample (1.0 µL) was spotted onto the activated TLC plates followed by the spotting of 1.0 µL of the interfering species on the same spot. The plates were developed with M_{10} (5% aqueous NaDC-AcN, 1:3, v/v). After development, spots were visualized under short wave 254nm UV light. The R_F valued were determined and compared with those obtained in the absence of interfering species.

For the separation, equal volumes of all five nucleobases were mixed and 1.0 µL of the resultant mixture was applied on HPTLC plates. The plate was developed with M_{10}, the spots were visualized and R_F values of the separated spots of the nucleobases were calculated.

The limits of detection of nucleobases were determined by spotting a definite volume of different concentrations of nucleobases (0.01-1.0%) on the cellulose F_{254} HPTLC plates. The plates were then developed and the corresponding spots were detected. The method was repeated with successive lowering of the amounts of nucleobase (purine or pyrimidine) until no spot was detected. The minimum amount of nucleobase that could be visualized was taken as the limit of detection.

Results and Discussion

Selection of mobile phase

The aim of the present study was to select a useful solvent system to achieve the separation of coexisting five nucleobases (two purines, and three pyrimidines). For this, purpose chromatography of all nucleobases was performed on cellulose layers using different solvent systems. Both mono- component as well as binary mixed solvent systems was tested for the chromatography of these five nucleobases. When micellar solutions of cationic (cetyltrimethylammonium bromide, CTAB), non-ionic (Tween-20), and anionic (sodium dodecyl sulfate, SDS) surfactants were used as eluent, all nucleobases show smeared spots with high mobility. Mixed micellar solutions were also not successful for separating nucleobases; all show broadened spots. In view of these unfavorable results, linear anionic surfactant, SDS was replaced with helical sodium deoxycholate (bile salt). Aqueous 5.0% solution of sodium deoxycholate (NaDC) was selected for study because of its versatile nature of separating many isomeric compounds. It is well known that, the separation efficiency of pure micellar mobile phase system is improved by the addition of the organic additives (Sumina et al., 2003). Therefore, mixed mobile phases of 5.0% NaDC-acetonitrile in different ratio was tested, and the obtained results with these solvent systems are listed in Table 3. Addition of acetonitrile increases the hydrophobic nature of the mobile phase in order to retain the hydrophilic species. From the data of this Table, the following conclusions may be drawn:

- In mobile phase, M_1 (DDW) both purines show lower mobility compared to pyrimidines on cellulose 60 F_{254} layers.
- When 5.0% aqueous bile salt, NaDC (M_2) was used as the mobile phase, the R_F of all nucleobases slightly reduced as compared to their mobility in M_1.
- In both M_1, and M_2, the R_F of nucleobases was in the order: adenine < guanine < cytosine < thymine ≈ uracil.
- The R_F value of adenine, guanine, cytosine, thymine, and uracil was found to increase with the increase in volume ratio of acetonitrile in its mixture with 5.0%aqueous NaDC (M_3-M_6), followed by decline in R_F value from M_7 to M_{12}.

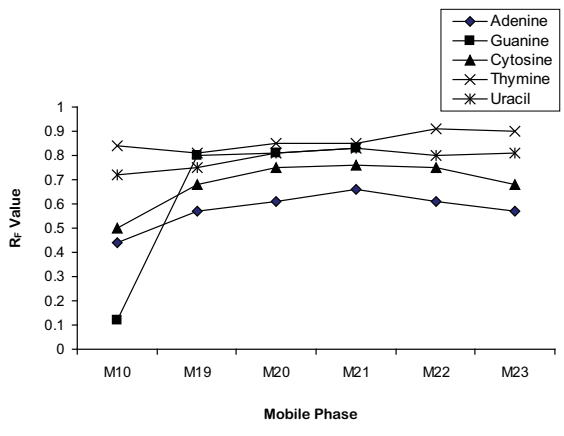

Figure 3: R_F value of five nucleobases on cellulose layers developed with micellar buffer mobile phases (M_{19}-M_{23}).

As more compact spots, and differential R_F were realized in M_{10} (5.0% aqueous NaDC-acetonitrile, 1:3). This mobile phase was selected for further studies. To understand the effect of other organic additives on the R_F values of nucleobases, acetonitrile in M_{10} was replaced by other organic solvents (viz: acetone, methanol, formic acid, and DMSO), and the resultant mobile phase systems (M_{13}-M_{16}) were used for chromatography.

- In M_{13}, and M_{14}, all nucleobases showed lower mobility as compared to M_{10}. The order of R_F was: $M_{10} > M_{13} > M_{14}$.
- In M_{15} and M_{16} all nucleobases showed constant and high R_F value ($R_F > 0.90$). Compact spots were realized in M_{15}, whereas in M_{16} spots were diffused. For investigating the effect of other bile salts, NaDC in M_{10} was replaced by other biological bile salts (i.e. NaC or NaTC), and the resultant mobile phase systems (M_{17}, and M_{18}) were used for chromatography. It was found that there was no significant change in R_F values on substitution of NaDC by NaC or NaTC in M_{10}.

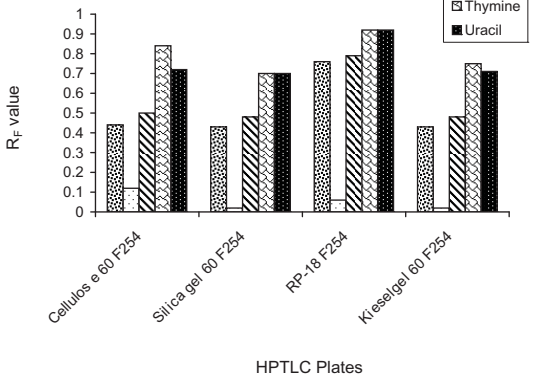

Figure 4: R_F values of nucleobases on various HPTLC plates developed with mobile phase, M_{10}.

The R_F data of nucleobases obtained with buffered 5.0% NaDC (pH 2.3, 3.4, 5.7, 7.2 or 11.9) plus acetonitrile (M_{19}-M_{23}) mobile phases are compared and presented in Figure 3. It is clear from this Figure that the R_F value of nucleobases is slightly influenced by pH of NaDC in all mobile phases (M_{19}-M_{23}), except guanine, which was not detected in M_{22}, and M_{23}. The R_F value of guanine was increased to 0.83 from its standard R_F value (0.12) in acidic media (M_{19}-M_{21}).

Effect of nature of sorbent layers

To establish the effectiveness of cellulose 60 F_{254} HPTLC layers, the retention behavior of nucleobases was also examined on different sorbent layers, and the obtained results have been plotted in Figure 4. Although more compact spots were realized on RP-18 F_{254} layers, but separation of coexisting five nucleobases is possible only on cellulose 60 F_{254} layers.

Effect of interference

Table 4 summarizes the effect of a variety of impurities on retention (R_F) of nucleobases. From the data listed in Table 4, it is evident that the R_F values of nucleobases were altered from its standard value in the presence of impurities in the sample. All nucleobases showed high R_F value in the presence of Li^+ as compared to other alkali or alkaline earth metal cations. Guanine, cytosine, thymine and uracil were not detected in the presence of Mg^{2+}. The R_F value (retention) of nucleobases was greatly influenced in the presence of transition metal cations, probably due to greater affinity of transition metal cations to bind with DNA or RNA nucleobases. In the presence of Ni^{2+} and Cu^{2+}, all nucleobases showed almost similar R_F values. Zn^{2+} converted the compact spot of nucleobases into a badly diffused spots.The detection of guanine cytosine, thymine, and uracil in the presence of Co^{2+} was difficult.

However, a significant increase in R_F values of adenine, guanine, and cytosine was noted in the presence of anions, urea, and EDTA. Both purines (adenine, and guanine) produced double spots in the presence of MoO_4^{2-} showing effective complexing tendency of molybdate ion.

Interfering ions			Nucleobases		
	Purines			*Pyrimidines*	
	Adenine	Guanine	Cytosine	Thymine	Uracil
Without impurities	0.44	0.12	0.50	0.80	0.80
Cations (mono- and bivalent)					
Li^+	0.60	0.82	0.74	0.70	0.70
Na^+	0.39	0.32	0.50	0.75	0.74
Mg^{2+}	0.36	nd	nd	0.80	0.80
Ba^{2+}	0.55	0.43	0.66	0.90	0.86
Co^{2+}	0.00	0.nd	0.81	0.89	0.86
Ni^{2+}	0.77	0.77	0.70	0.85	0.77
Cu^{2+}	0.83	0.79	0.85	0.85	0.82
Zn^{2+}	0.54	0.47	0.60	0.85	0.75
Cd^{2+}	0.58	0.45	0.69	0.84	0.72
Pb^{2+}	0.59	nd	0	0.84	0.73
Anions (mono- and bivalent)					
Br^-	0.56	0.38	0.14	0.53	0.41
CH_3COO^-	0.54	0.39	0.46	0.67	0.38T
NO_3^-	0.50	0.39	0.35	0.38	0.20
IO_4^-	0.54	0.41	0.91	0.92	0.91
CO_3^{2-}	0.49	0.40	0.95	0.97	0.96
SO_4^{2-}	0.50	0.41	0.75	0.83	0.86
MoO_4^{2-}	0.45	0.43	0.73	0.80	0.76
Complexing ligands					
Urea	0.55	0.40	0.70	0.82	0.77
EDTA	0.51	0.38	0.66	0.81	0.74

Table 4: R_F values of nucleobases on cellulose 60 F_{254} HPTLC plates developed with mobile phase, M_{10} in the presence of organic and inorganic impurities in the working standard sample.

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JBABM/Vol.2 Issue 2

Compared to adenine, the R_F value of guanine was greatly modified in the presence of impurities (cations, anions, urea,or EDTA) in the sample. This might be due to the presence of two attractive centres in guanine to interact with impurities, whereas in the case of adenine there is only one attractive centre (Barda et al., 1996).

Separation

The position of spots appeared on cellulose fluorescent plate is depicted in Figure 5. From Figure 5 it is clear that, with mobile phase M_{10} (5% aqueous NaDC + AcN, 1: 3, v/v) purines and pyrimidines could be separated easily from their mixtures.

Limit of detection

The lowest possible detectable microgram amounts of all five nucleobases obtained on cellulose F_{254} HPTLC plates developed with M_{10} was ≈ 0.054 µg $spot^{-1}$. It shows that the developed method is reasonably suitable for identifying these nucleobases at trace level.

Conclusion

The proposed thin layer chromatographic system comprising of cellulose 60 F_{254} as stationary phase , and 5.0% aqueous sodium deoxycholate (NaDC)-acetonitrile (AcN), 1:3 (v/v) as mobile phase is most favorable for the separation of coexisting five-nucleobases (adenine, guanine, cytosine, thymine, and uracil). IT is highly selective, reliable, and rapid requiring 5.0-7.0 min for resolution of DNA and RNA nucleobases.

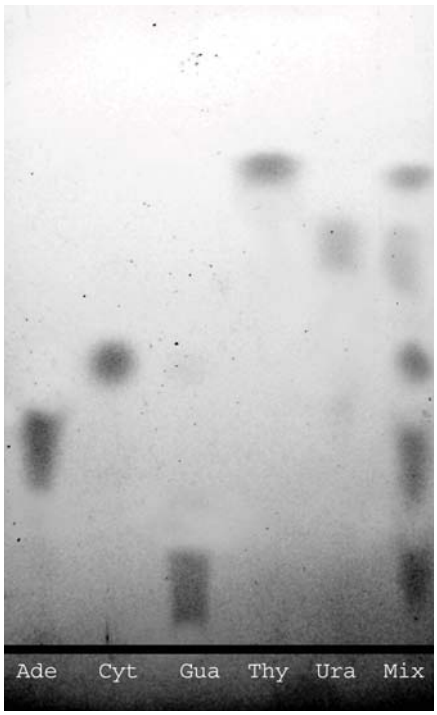

Figure 5: Separation of five coexisting nucleobases (adenine, cytosine, guanine, thymine, and uracil on cellulose 60 F_{254} layer developed with mobile phase M_{10}.

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References

1. Armstrong D, Terril RQ (1979) Thin layer chromatographic separation of pesticides, decachlorobiphenyl, and nucleosides with micellar solutions. Anal Chem 51: 2160-2163. » CrossRef » PubMed » Google Scholar
2. Barda JV, Sponer J, Hobza P (1996) Ab initio study of the interaction of guanine, and adenine with various mono-, and bivalent metal cations (Li^+, Na^+, K^+, Rb^+, Cs^+; Cu^+, Ag^+, Au^+; Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}; Zn^{2+}, Cd^{2+}, and Hg^{2+}). J Phys Chem 100: 7250-7255. » CrossRef » PubMed » Google Scholar
3. Bij KE, Lederer M (1983) Thin-layer chromatography of some nucleobases and nucleosides on cellulose layers. J Chromatogr 268: 311-314. » CrossRef » PubMed » Google Scholar
4. Campanelli AR, Sanctis SCDe, Giglio E, Pavel NV, Quagliata C (1989) From crystal to micelle: A new approach to the micellar structure. J Inclusion Phenomena 6: 391-400. » CrossRef » PubMed » Google Scholar
5. Giglio E, Loreti S, Pavael NV (1988) EXAFS: A new approach to the structure of micellar aggregates. J Phys Chem 92: 2858-2862. » CrossRef » PubMed » Google Scholar
6. Hatzack H, Rasmussen SK (1999) High-performance thin-layer chromatography method for inositol phosphate analysis. J Chromatogr B Biomed Sci Appl 736: 221-229. » CrossRef » PubMed » Google Scholar
7. Hofmann AF, Mysels KJ (1987) Bile salts as biological surfactants. Collo Surf 30: 145-173. » CrossRef » PubMed » Google Scholar
8. Morgan WA, Thanh NK, Ding Y (2008) The use of high performance thin layer chromatography to determine the role of membrane lipid composition in bile salt- induced kidney cell damage. J Pharmacol Toxicol Methods 57: 70-73. » CrossRef » PubMed » Google Scholar
9. Randerath K, Struck H (1961) Thin layer chromatography: separation of nucleic acid derivatives in cellulose layers. J Chromatogr 6: 365-367. » CrossRef » PubMed » Google Scholar
10. Steinberg JJ, Cajigas A, Oliver G (1996) Nucleic acid and their derivatives, Hand Book of Thin-Layer Chromatography, 3rd edn : Chromatographic Science Series, New York, Wiley Press 89: 733-747. » CrossRef » PubMed » Google Scholar
11. Sumina EG, Shtykov SN, Tyurina NV (2003) Surfactants in thin-layer chromatography. J Anal Chem 58: 808-818. » CrossRef » PubMed » Google Scholar
12. Thompson CO, Trenerry VC, Kemmery B (1995) Micellar electrokinetic capillary chromatographic determination of artificial sweeteners in low-Joule soft drinks and other foods. J Chromatogr A 694: 507-514. » CrossRef » PubMed » Google Scholar
13. Williams RW, Fu Z, Hinze WL (1990) Micellar bile salt mobile phases for the liquid chromatographic separation of routine compounds and optical, geometrical, and structural isomers. J Chromatogr Sci 28: 292-302. » CrossRef » PubMed » Google Scholar