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INFLUENCE OF SURFACTANT TYPES ON CORRELATION OF RETENTION FACTOR AND HYDROPHOBICITY OF SELECTED TRIAZOLE FUNGICIDES IN MICELLAR ELECTROKINETIC CHROMATOGRAPHY

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Abstract

Application of micellar electrokinetic chromatography (MEKC) in quantitative structure-retention relationship (QSRR) has been studied for selected triazole fungicides. Effect of different surfactant types and concentrations of bile salts and sodium dodecyl sulfate on the correlation between logarithm of retention factor (log k) in MEKC and logarithm of octanol-water partition coefficient (log P_{ow}) was investigated. Five standard fungicides (cyproconazole, bromuconazole, epoxiconazole, bitertanol and difenoconazole) with known log P_{ow} values from 2.9 to 4.3 were used for constructing the calibration curve of log P_{ow} against the MEKC retention factor, log k. High correlations were observed between hydrophobicity (log P_{ow}) and log k in MEKC using two bile salt surfactants viz. sodium cholate and sodium deoxycholate and mixed bile salt systems, with squared correlation coefficient of linear regression greater than 0.98, due to the similar hydrogen bonding interaction patterns between bile salts MEKC systems and the octanol-water system.

Keywords: Surfactans, micellar electrokinetic chromatography, retention factor, hydrophobicity, triazole fungicides, quantitative structure-retention relationship.

Introduction

The hydrophobic character of organic molecules can be used to predict biomembrane transport, bioaccumulation in plants and animals, and soil adsorption. Solute hydrophobicity is usually expressed by the thermodynamic octanol-water partition coefficient (P_{ow}). The octanol-water partition coefficient is one of the most commonly reported physicochemical properties of drugs and industrial chemicals and the most widely employed descriptor for quantitaive structure-activity relationships (QSARs) for all kinds of biological, pharmaceutical and environmental property estimates (Poole et al., 2003). Determination of log P_{ow} of compounds was developed by direct and indirect methods. Traditionally, the shake-flask method combined with UV assay was used for direct measurement of log P_{ow} values (Danielsson and Zhang, 1996). This conventional method has some disadvantages: long analysis time (1 day/solute), interference from solute/solvent impurities and difficulty in temperature control. Reversed-phase high-performance liquid chromatography (RP-HPLC) (Donovan and Pescatore, 2002) and micellar electrokinetic chromatography (MEKC) for indirect log P_{ow} determination (Garcia *et al*., 1996) were used by using the linear relationship between log P_{ow} and log retention factor, log *k* (Equation 1). This equation is an example of quantitative structure-retention relationships (QSRR) (Jia *et al*., 2003; Woloszyn and Jurs, 1992).

$$\log P_{ow} = a \log k + b \qquad (1)$$

where *a* and *b* are constants that can be determined from the observed data. While retention factor, *k*, can be calculated from the experimental data. The advantages of these methods over the shake-flask method are rapid analysis, automation, temperature control, smaller sample requirements, and simultaneous separation of solutes.

Micellar electrokinetic chromatography (MEKC) is a mode of capillary electrophoresis technique which was first introduced in 1984 by Terabe and co-workers. In MEKC, an ionic surfactant (micelle) is usually used as a pseudostationary phase that corresponds to the stationary phase in conventional chromatography and the surrounding aqueous phase to the mobile phase. The separation principle of analytes is based on their differential partitioning between micellar phase and aqueous phase (Quirino and Terabe, 1999). The migration order for neutral analytes in MEKC generally relates to the hydrophobicity of the analyte and retention behavior of analytes depend strongly on the type of surfactant/micelle (Nishi and Terabe, 1996).

The correlation of MEKC retention data (log *k*) to octanol-water partition coefficient (log P_{ow}) was found high ($r^2 = 0.835$) for over 100 solutes with widely varying functionality (Herbert and Dorsey, 1995). This method reduces the laboratory-to-laboratory variability and the long analysis time due to the multiple mobile phase necessary in current HPLC methods for estimating log P_{ow}. It was also reported that bile salts micellar systems provide better correlation for log *k* vs log P_{ow} than SDS micellar systems (Yang *et al.*, 1996). In this paper, MEKC with different type of surfactants (bile salts and sodium dodecyl sulfate) and concentrations will be investigated on the correlation between retention factor (log *k*) in MEKC and hydrophobicity (log P_{ow}) of standard triazole fungicides (Figure 1). To the best of our knowledge, there is no MEKC study being reported on the log P_{ow} – log *k* correlation for the selected triazole fungicides.

Experimental

Chemical and reagents

All triazole fungicides were obtained from Riedel-de Haen (Seelze, Germany). Sodium dodecyl sulfate (SDS) was obtained from Fisher Scientific (Loughborough, UK), sodium cholate from Anatrace (Ohio, USA), sodium deoxycholate from TCI Kasei (Tokyo, Japan), and borate buffer solution from Agilent (Germany). All other chemicals and solvents were common brands of analytical-reagent grade or better, and were used as received. Water was collected from a Millipore Water Purification System (Molsheim, France). The stock solutions of the individual triazole fungicides were prepared in methanol in the concentration range 2000 and 6000 ppm. Final dilutions (concentrations in the figures) were prepared by diluting the stock solution with methanol. The separation solutions were prepared by dissolving appropriate amounts of surfactants in borate buffer solution. All running buffers were filtered through a 0.45 µm nylon syringe filter from Whatman (Clifton, USA).

Apparatus and methods

The MEKC experiments were performed on an Agilent capillary electrophoresis system with a diode array UV-Vis detector. The uncoated fused-silica capillary (Polymicro Technologies, Phoenix, AZ, USA) of 64.5 cm total length and 56 to the detector (75 µm I.D. × 360 µm O.D.) was used and thermostated at 25ºC. Injections were made at 50 mbar for 1 s. A Voltage of +25 kV was applied for the CE separation. Prior to the first run, the capillary was flushed with 0.1 M NaOH 20 min, water for 10 min, and micellar system for 10 min. Between run, capillary was rinsed with 0.1 M NaOH, water, and micellar system for 2 min each.

The retention factor, *k*, was calculated from the MEKC migration time according to the equation 2 (Terabe *et al*, 1984):

$$k = [t_R - t_{EOF}]/\ t_{EOF}[1-(t_R/t_{mc})] \qquad (2)$$

where t_R , t_{EOF} , and t_{mc} are the migration times (min) of the solute, the EOF marker (methanol), and the micelle marker (phenantrene), respectively. The technique is performed by analyzing a set of standards of known log P_{ow} under the given MEKC conditions. Log *k* of each standard obtained is plotted against its log P_{ow} to form a linear calibration graph. When a standard gives two peaks (as isomer/enantiomer), log *k* is calculated from the last peak.

Table 1. Log P_{ow} data of selected triazole fungicide standards

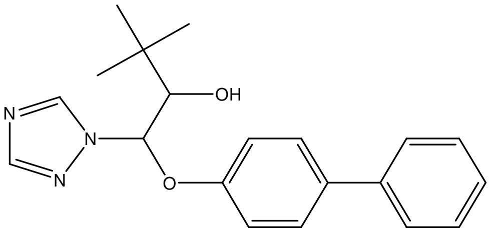
No	Standard compound	log P_{ow} (literature)[a]	log P_{ow} (prediction)[b]	Δ log P_{ow}
1	Cyproconazole	2.90	2.92	0.02
2	Bromuconazole	3.24	3.18	0.06
3	Epoxiconazole	3.44	3.43	0.01
4	Bitertanol	4.16	4.10	0.06
5	Difenoconazole	4.30	4.38	0.08

[a] Experimental log P_{ow} data from literature (sourch: http://www.syrres.com/esc)

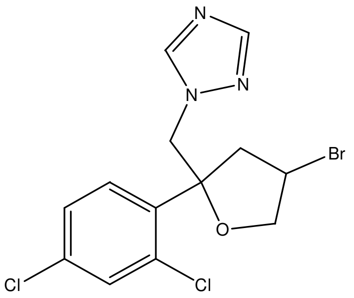
[b] Calculated log P_{ow} data from DRAGON software (Dragon Professional version 5.4-2006, TALETE srl)

Results and Discussion

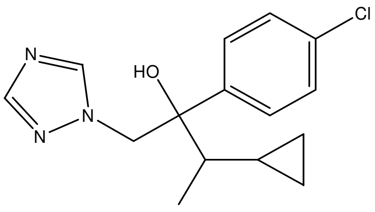
The five standard triazole fungicides (Fig. 1) have been selected to investigate the effect of surfactant types (sodium dodecyl sulfate and bile salts) and its concentrations on the correlation between log k in MEKC and log P_{ow}. The literature log P_{ow} value and the calculated log P_{ow} value from DRAGON software of five standard triazole fungicides are shown in Table 1. The difference between literature and calculated log P_{ow} value from DRAGON software was found ≤ 0.08.



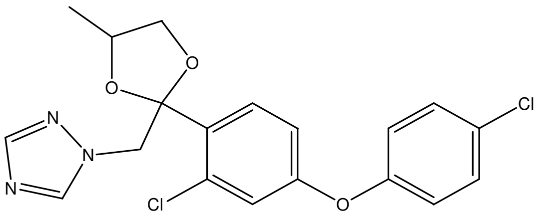
Bitertanol



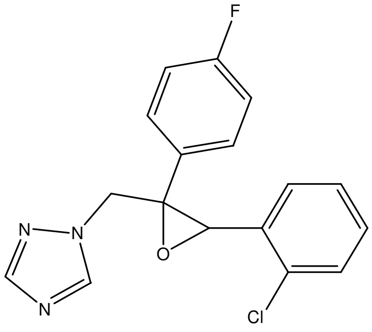
Bromuconazole



Cyproconazole



Difenoconazol



Epoxyconazole

Fig. 1: Structures of selected triazole fungicides

Sodium dodecyl sulfate (SDS) was first evaluated in the range of 30-50 mM in 4 mM borate buffer at pH 9.3. Separation of five standards of triazole fungicides was not successfully achieved by MEKC with SDS, as illustrated in Figure 2. It can be observed that only two fungicides can be separated (cyproconazole and bromuconazole). Three other fungicides (more hydrophobic fungicides) were co-eluted with the micelle marker. This is due to the more hydrophobic fungicides interact more strongly with the SDS micelle. SDS is a long-alkyl-chain surfactant. This surfactant is believed to form spherical micelle having ionic group on the surface and the hydrophobic core. When ionic interactions are strong in MEKC with SDS, analytes will migrate at around t_{mc}. Since the resolution was very poor, thus, effect of SDS as surfactant was not explored for further study on the correlation of log k and log P_{ow} of these fungicides.

Effect of bile salts (sodium cholate (SC), sodium deoxycholate (SDC), and mixed SC-SDC) as surfactants was then investigated. Bile salts are anionic surfactants found in biological sources. They have steroidal structures and form helical micelles having a reversed micelle conformation. Compared with SDS, bile salts have a relatively weak solubilization power. Figures 3-5 show the typical electropherograms of five triazole fungicides as a function of SC, SDC and mixed SC-SDC concentrations, respectively. Resolution was obtained for all five fungicides by MEKC with bile salt as surfactant in concentration range of 30-50 mM. All five fungicides are chiral compounds with two chiral centers and bile salts are chiral surfactants. For cyproconazole and bromuconazole, two isomer peaks were observed in all concentration of bile salt system, except at 30 mM SC. While for three other fungicides, only one peak was observed in all conditions. Log k is calculated from the last peak of cyproconazole and bromuconazole isomers. Correlation coefficient of linear regression between hydrophobicity and retention factor in MEKC is illustrated in Table 2. High correlations were observed with squared correlation coefficient of linear regression greater than 0.98 for all bile salt systems, due to the similar hydrogen bonding interaction patterns between bile salts MEKC systems and the octanol-water system (Yang *et al.*, 1996). The best correlation coefficient was obtained at 40 mM SDC system as surfactant ($r^2 = 0.9912$). The results obtained in this work on the log P_{ow} - log k correlation agree with those obtained by other authors who have found a good linear correlation log P_{ow} - log k in MEKC for different compounds and various MEKC micellar systems (Garcia et al., 1996; Herbert and Dorsey, 1995).

Table 2: Squared correlation coefficient (r^2) of linear regression between hydrophobicity (logP_{ow}) and retention factor (log k) in MEKC with bile salts as surfactant

Surfactant	30 mM	40 mM	50 mM
SC	0.9827	0.9865	0.9881
SDC	0.9909	**0.9912**	0.9898
SC+SDC (1:1)	0.9843	0.9879	0.9892

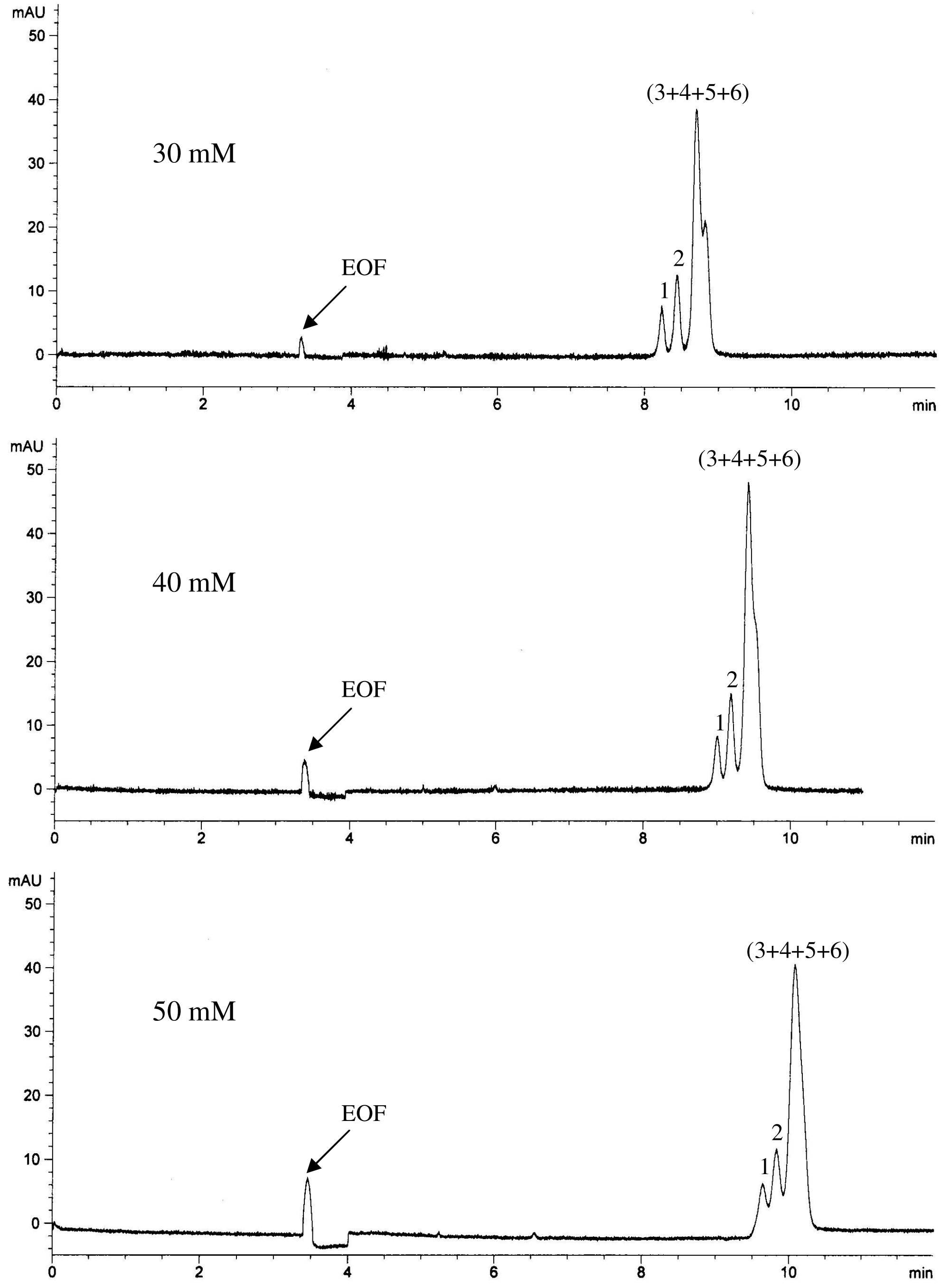


Fig. 2: Electropherogram of triazole fungicides with different SDS concentration. Sample, 100 ppm mixed standards (in methanol), injected hydrodynamically (HDI) for 1 s at 50 mbar (HDI); Separation solution: 30 - 50 mM SDS in 4 mM borate buffer (pH 9.3); capillary, 75 µm i.d. x 64.5 cm (effective length, 56 cm); applied voltage, +25 kV; detection wavelength, 200 nm; temperature, 20°C. Peaks identification: 1 (cyproconazole), 2 (bromuconazole), 3 (epoxiconazole), 4 (bitertanol), 5 (difenoconazole), and 6 (micelle marker).

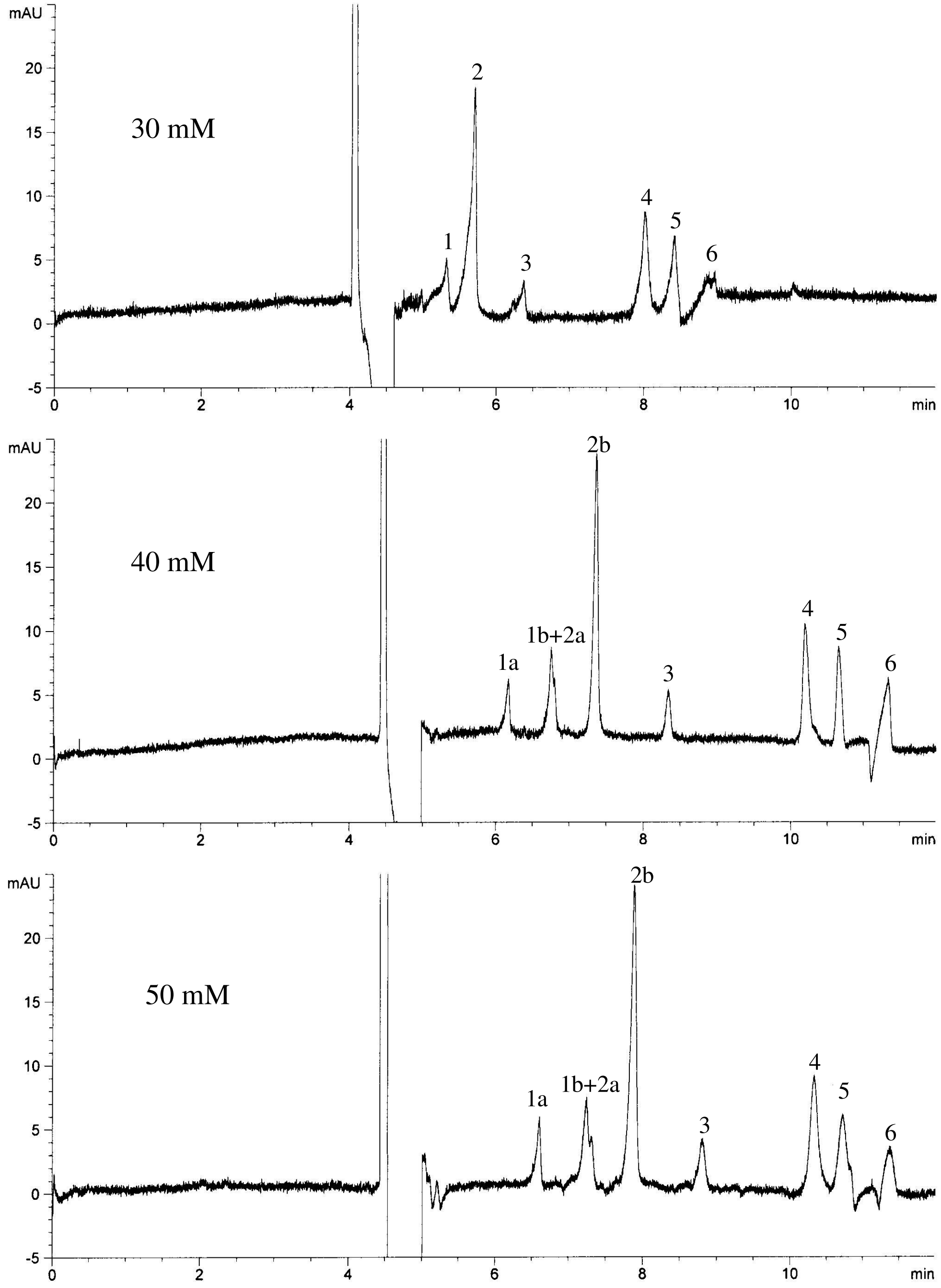


Fig. 3: Electropherogram of triazole fungicides with different SC concentration. Analysis conditions as in Figure 2; Peaks identification: 1a, 1b (cyproconazole isomers); 2a, 2b (bromuconazole isomers); 3 (epoxiconazole); 4 (bitertanol); 5 (difenoconazole); and 6 (micelle marker).

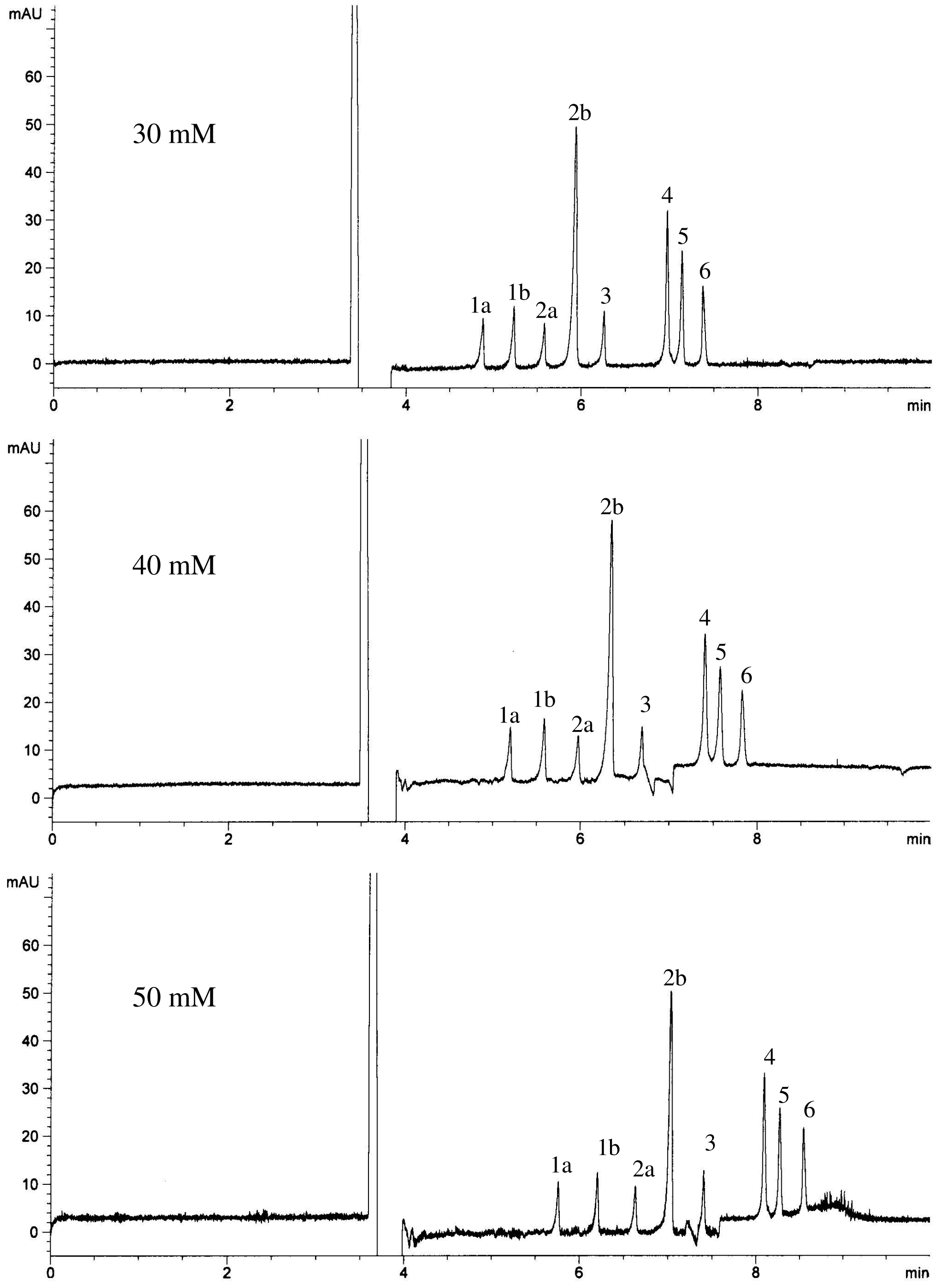


Fig. 4: Electropherogram of triazole fungicides with different SDC concentration. Analysis conditions and peaks identification as in Fig. 3.

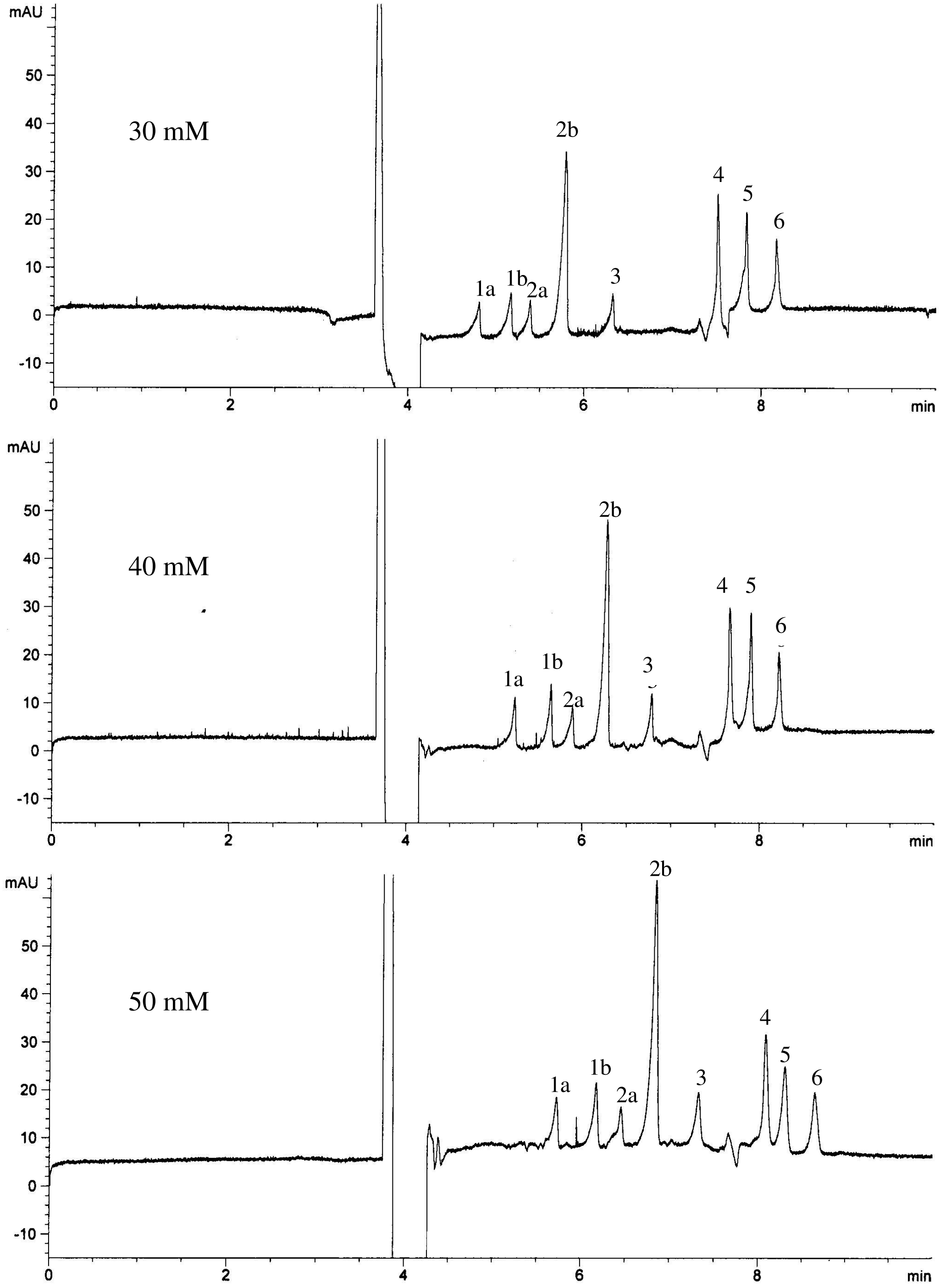


Fig. 5: Electropherogram of triazole fungicides with different mixed SDC-SC concentration. Analysis conditions and peaks identification as in Fig. 3.

Conclusion

High correlations were observed between hydrophobicity (log P_{ow}) and retention factor (log k) in MEKC using two bile salt surfactants *viz.* sodium cholate and sodium deoxycholate and mixed bile salt systems. The best correlation coefficient of linear regression ($r^2 = 0.9912$) was obtained by MEKC with 40 mM SDC in 4 mM borate buffer (pH 9.30). Log P_{ow} estimation of test triazole fungicides by the best MEKC system will be evaluated in the further study.

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References

1. Danielsson, L.G., Zhang, Y.H., 1996, Methods for Determining *n*-Octanol-Water Partition Constants; Trends Anal. *Chem.; 15*, 188-196.
2. Donovan, S.F., Pescatore, M.C., 2002, Method for Measuring the Logaritm of Octanol-Water Partition Coefficient by Using Short Octadecyl-Poly(Vinyl Alcohol) High-Performance Liquid Chromatography Columns; *J. Chromatogr. A.; 952*, 47-61.
3. Garcia, M.A., Diez-Masa, J.C., Marina, M.L., 1996; Correlation Between the Logarithm of Capacity Factors for Aromatic Compounds in Micellar Electrokinetic Chromatography and Their Octanol-Water Partition Coefficients; *J. Chromatogr. A.;742,* 251-256.
4. Herbert, B.J., Dorsey, J.G., 1995; *n*-Octanol-Water Partition Coefficient Estimation by Micellar Electrokinetic Capillary Chromatography; Anal. *Chem.; 67*, 744-749.
5. Jia, Z., Mei, L., Lin, F., Huang, S., Killion, R.B., 2003; Screening of Octanol-Water Partition Coefficients for Pharmaceuticals by Pressure-Assisted Microemulsion Electrokinetic Chromatography; *J. Chromatogr. A.; 1007*, 203-208.
6. Nishi, H., Terabe, S., 1996; Micellar Electrokinetic Chromatography Perspectives in Drug Analysis; *J. Chromatogr. A.*; 735, 3-27.
7. Poole, S.K., Patel, S., Dehring, K., Workman H., Dong, J., 2003; Estimation of Octanol-Water Partition Coefficient for Neutral and Weakly Acidic Compounds by Microemulsion Electrokinetic Chromatography using Dynamically Coated Capillary Columns; *J. Chromatogr. B.; 793*, 265-274.
8. Quirino, J.P., Terabe, S., 1999; Electrokinetic Chromatography; *J. Chromatogr. A.*; 856, 465-482.
9. Terabe, S., Otsuka, K., Ichikawa, K., Tsuchiya, A., Ando, T., 1984; Electrokinetic Separations with Micellar Solutions and Open-Tubular Capillaries; Anal. *Chem.; 56*, 111-113.
10. Woloszyn, T.F., Jurs, P.C., 1992; Quantitative Structure-Retention Relationship Studies of Sulfur Vesicants; Anal. *Chem.; 64*, 3059-3063.
11. Yang, S., Bumgarner, J.G., Kruk, L.F.R., Khaledi, M.G., 1996; Quantitative Structure-Activity Relationships Studies with Micellar Electrokinetic Chromatography Influence of Surfactant Type and Mixed Micelles on Estimation of Hydrophobicity and Bioavailability*; J. Chromatogr. A.; 721*, 323-335.