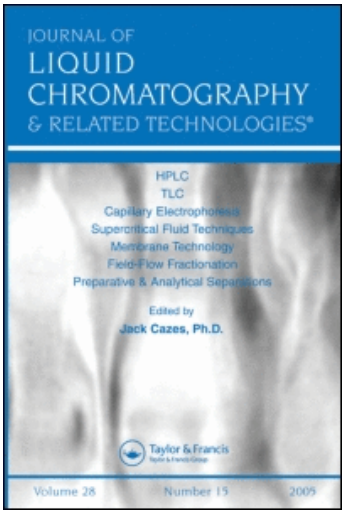

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Speciation of Thulium Porphyrin Complexes by Reversed-Phase HPLC

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ABSTRACT

Six new thulium porphyrin complexes have been separated by high performance liquid chromatography with triethanolamine (TEOLA) and acetic acid (HAc) as additives in the methanol–water mixture. The function of TEOLA and HAc has been described. The whole analysis was completed on a C_{18} column in 16 min at a flow rate of 0.5 mL min^{-1}, with the mobile phase of methanol–water (93 : 7,v : v) containing 75 mmol L^{-1} TEOLA and 200 mmol L^{-1} HAc. The detection limits (S/N = 3) of thulium porphyrins at 420 nm were 1.0, 1.0, 1.0, 1.0, 5.0, and 5.0 ng mL^{-1}, respectively. Compared with the methanol system, it can attain the equivalent separation with the mobile phase of ethanol–water (90 : 10,v : v) containing 25 mmol L^{-1} TEOLA and 200 mmol L^{-1} HAc pumped at 0.25 mL min^{-1}.

Key Words: Thulium; Porphyrin; HPLC.

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INTRODUCTION

In recent years, the analysis of metal elements is no longer simply concerned with their quantitation, while the study on the speciation of metal elements is more and more attraction to researchers because the biological and environmental activities of metal ions are still closely associated with their existence state, except for dependence on their physical properties.[1,2] Thus, in order to investigate the content and species of metal elements, in view of analysis, it is necessary to hyphenate the high performance separation technique with highly sensitive detection.[3]

Porphyrins are a class of naturally occurring macrocyclic compounds, such as, some metalloporphyrins having been found in living organisms or raw oil. Otherwise, with rare earth elements being extensively applied in agriculture, functional materials, etc., there have increasingly been possibilities for them to be exposed to the environment or to be taken in by biosome.[4] In natural plants and animals, rare earth binding proteins, DNA, and chlorophylls have been observed.[4–8] Therefore, the study of the separation of rare earth porphyrins has a potential purpose for the verification of the natural speciation of rare earth elements.

Being a significant approach of separation power, high performance liquid chromatography has played an important role in the separation of metal ions and its complexes. Particularly, the assay by HPLC with other specialized detection techniques has mainly been developed to study trace metal ions and its related species.[9] To date, there have been many reports on the analysis of metalloporphrins by HPLC, which principally center on the determination of metal ions with porphyrin as a type of highly sensitive chromogenic reagent.[10,11] However, only few papers have been presented dealing with the porphyrin complexes of rare earth elements. The separations of rare earth tetraphenylporphins[12] and rare earth octaethylporphyrins[13] on an octadecyl-bonded silica gel column, under basic conditions, have been reported by Saitoh et al. Parise et al. have developed a new HPLC method for the determination of motexafin gadolinium and motexafin lutetium in human plasma.[14] Owing to the time-consuming preparation of rare earth porphyrins, the study of complexes of porphyrins with certain rare earth ions by HPLC has not been found.

In this paper, the chromatographic behavior of six thulium porphyrin complexes was investigated. It was found that triethanolamine (TEOLA) additive in the mobile phase played a dominant role in the elution of thulium porphyrins from an ODS column. The two mobile phase systems, which consisted of methanol–water (93 : 7, v : v) containing 75 mmol L^{-1} TEOLA and 200 mmol L^{-1} acetic acid (HAc), as well as ethanol–water (90 : 10,v : v) containing 25 mmol L^{-1} TEOLA and 200 mmol L^{-1} HAc, respectively, were

suggested for rapid isolation of these six complexes. At 420 nm, the detection limits for these six compounds were 1.0, 1.0, 1.0, 1.0, 5.0, and 5.0 ng mL^{-1}, respectively.

EXPERIMENTAL

Materials

The free acid form of the porphyrins (L_{1-6}) and their complexes with Tm(III) which are presented as [Tm(L_{1-6})(H_2O)$_3$Cl] were synthesized according to the procedure described in the literature.[15,16] When examined by UV–Vis spectrophotometry, all of these thulium porphyrins gave two sharp absorption peaks, corresponding to the so-called α-band and β-band peaks in the 500–600 nm region with respect to the metalloporphyrins. In the mass spectrum, each of the six complexes gave a characteristic peak at m/z 845, 901, 781, 837, 919, and 949, corresponding to the six molecular ions [Tm-L_{1-6}]$^+$, respectively. The structural formula of these complexes is illustrated in Fig. 1.

Methanol, ethanol, HAc, TEOLA, acetone, and dichloromethane were of analytical-reagent grade. Doubly distilled water was used for all solution preparation.

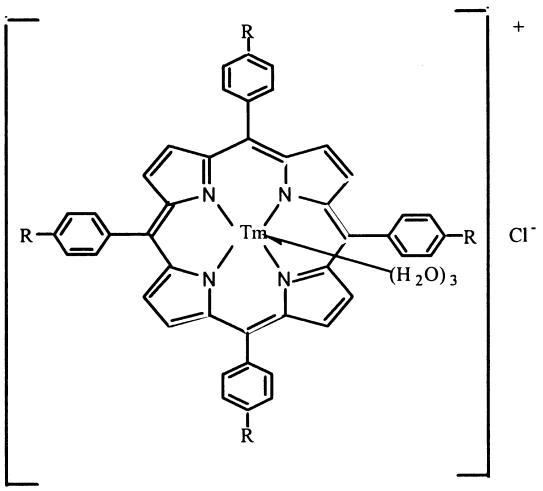

Figure 1. Structures of thulium porphyrin complexes. (1) —OH; (2) —OCH_3; (3) —H; (4) —CH_3; (5) —Cl; (6) $CH(CH_3)_2$.

High Performance Liquid Chromatography System

The HPLC system consisted of an LC-10A model pump, SPD-10AV UV–Vis detector, Shimpak SBC-ODS analytical column (5 μm, 2.6 × 150 mm), C-R7A recorder and a 5 μL injection loop (Shimadzu, Japan). The isocratic mobile phase was pumped at 0.5 mL min^{-1} for the methanol system and 0.25 mL min^{-1} for the ethanol system. The detection wavelength was set at 420 nm.

The mobile phase was principally prepared with methanol or ethanol and water by the addition of TEOLA together with HAc. The stock solutions of each thulium porphyrin complex and its mixture were prepared at a concentration of 0.1 mg mL^{-1} with the mixture of dichloromethane–methanol (1 : 4, v : v) and diluted to different concentrations with methanol when used. All the experiments were performed at room temperature.

RESULTS AND DISCUSSION

Choice of Composition of the Mobile Phase

All of the six thulium porphyrins are easily dissolved in non-polar organic solvent; thus, in the separation mode of reversed-phase HPLC, it is appropriate to select the organic solvent as a principal composition of the mobile phase. However, the experimental results indicated that compounds 4–6 could not elute from the ODS column within 60 min, and the elution peak areas of compounds 2–3 were very small when the binary solvents such as methanol–water (95 : 5,v : v), methanol–acetone (80 : 20,v : v), methanol–dichloromethane (80 : 20,v : v), or pure methanol were used as the mobile phase. All six free porphyrin ligands could be eluted under the same conditions. The solubility of thulium porphyrin was slightly greater than its corresponding free porphyrin ligand. Therefore, it could be concluded that compounds 2–6 were strongly adsorbed onto the alkyl-bonded silica gel stationary phase during the process of chromatographing them, which is analogous to the retention behaviors of the trivalent transition metal porphyrin complexes in reversed-phase HPLC reported by Suzuki et al.[17] These thulium porphyrin complexes could be effectively eluted by the addition of TEOLA, together with HAc, to the mobile phase.

Effect of Methanol Content in the Mobile Phase

When the concentration of TEOLA and HAc in the mobile phase were kept constant, the ratio of methanol and water markedly influenced the retention behaviors of these thulium porphyrin complexes, with no change of their elution sequence (Fig. 2).

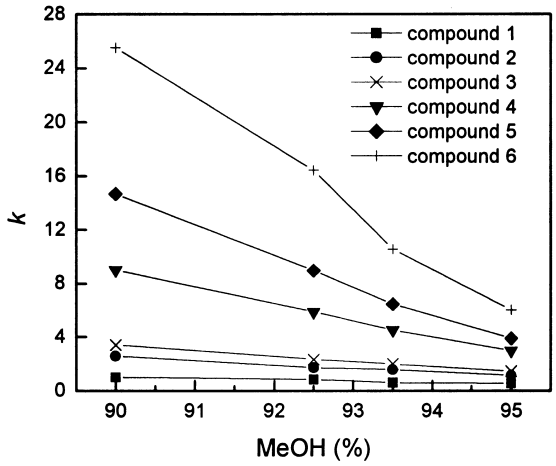

Figure 2. Effect of methanol content on the retention of thulium porphyrin complexes. Mobile phase: methanol–water containing 50 mmol L^{-1} TEOLA and 200 mmol L^{-1} HAc.

From Fig. 2, when the content of methanol was below 90%, the retention time of compound 6 was too long, and the peak shape was poor. If the methanol content was above 95%, the peaks of compounds 2 and 3 interfered with each other. Therefore, a satisfactory separation could only be obtained within a narrow range of methanol content.

Effect of Triethanolamine Concentration in the Mobile Phase

Compounds 2–6 could barely be eluted from the ODS column, except for compound 1, in the mobile phase of methanol–water (93 : 7,v : v) containing 200 mmol L^{-1} acetic acid only. When adding TEOLA to the mobile phase, all of the thulium porphyrin complexes could be properly eluted. Moreover, with increasing concentration of TEOLA, the elution rates of most of these complexes were accelerated, while that of compound 1 was almost unchanged, as shown in Fig. 3.

It is well known that the octadecyl-bonded stationary phase material is usually prepared by the silanization modification of silanol groups on the silica gel surface. In general, the surface of packing material retains incompletely-reacted silanols as a consequence of the influence of the steric hindrance of the alkyl moiety and the experimental conditions. Otherwise, in the polar mobile phase, it is possible for these thulium porphyrins to possess one net positive charge by dissociating, and there is a probability for them to be

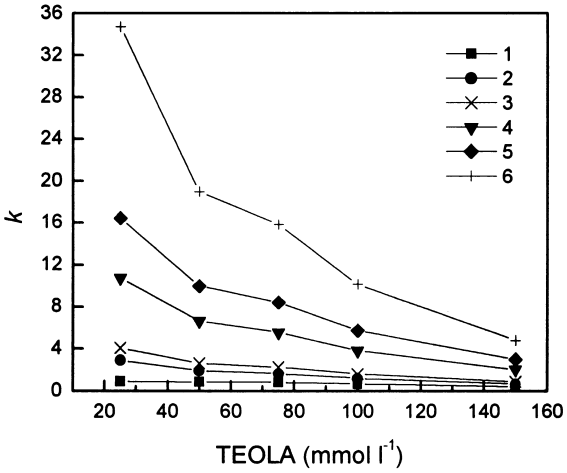

Figure 3. Effect of TEOLA concentration on the retention of thulium porphyrin complexes. Mobile phase: methanol : water = 93 : 7 (v : v) containing X mmol L^{-1} TEOLA and 200 mmol L^{-1} HAc.

adsorbed onto the active sites of the silanols. Thus, most of these thulium porpohyrins could not be eluted in the mobile phase without TEOLA. As for compound 1, because the four hydroxy groups on its structure electrostatically repel the silanols, it could be easily eluted from the ODS column in all cases. The function of TEOLA could be considered in view of three favorable aspects. First, in the weakly acidic medium, the protonized TEOLA could be preferable to be adsorbed by the active sites of silanols, which accordingly reduced the opportunity of the adsorption of thulium porphyrins. Secondly, compared with water and methanol, TEOLA is a strong electron donor; therefore, it is possible for TEOLA to coordinate with thulium ion by replacement of water in these thulium porphyrin complexes. This is helpful for the improvement of the separation selectivity of thulium porphyrins. Thirdly, the protonized TEOLA could increase the solubility of thulium porphyrins in the polar mobile phase, which is similar to a "salt effect". Consequently, adding TEOLA to the mobile phase could hasten the elution of these thulium complexes.

Effect of the Content of Acetic Acid in the Mobile Phase

The primary intent of adding HAc to the mobile phase was to control the acidity of the mobile phase and protect the reversed phase packing material.

However, the experimental results indicated that an increase in the concentration of HAc could also accelerate the elution rates of thulium porphyrins, as shown in Fig. 4. Compared with Fig. 3, the effect of HAc on the retention time of thulium porphyrins was weaker than that of TEOLA. Moreover, when the mobile phase consisted of methanol–water (93 : 7,v : v) containing 200 mmol L^{-1} HAc only, except for compound 1, the other thulium porphyrins could not be effectively eluted from the ODS column. Thus, it could be concluded that the function of the HAc was mainly to modify the species of TEOLA in the mobile phase, as well as to partly suppress the dissociation of silanols.

Effect of Ethanol and Triethanolamine in the Mobile Phase

From Figs. 2–4, when methanol was used as the main additive in the mobile phase, there were longer intervals between compounds 3 and 4, 5, and 6, while compounds 1, 2, and 3 had shorter intervals. This led to the narrow range of methanol content in the mobile phase. As a rule, for the mode of reversed-phase HPLC, if the solvent polarity parameter of the mobile phase is reduced, the retention factor of solute is decreased, that is, the solute of long retention time was remarkably influenced, more than the solute of short retention time. Therefore, the retention behaviors of thulium porphyrins were also investigated with ethanol as the principal composition of the mobile phase. The results are shown in Figs. 5 and 6. The effect of HAc was not tested

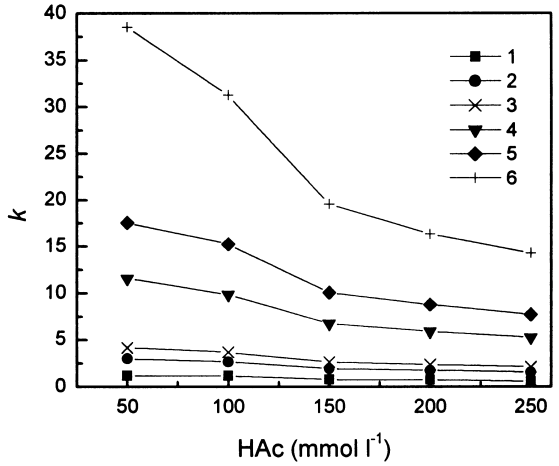

Figure 4. Effect of HAc concentration on the retention of thulium porphyrin complexes. Mobile phase: methanol : water = 93 : 7 (v : v) containing 50 mmol L^{-1} TEOLA and X mmol L^{-1} HAc.

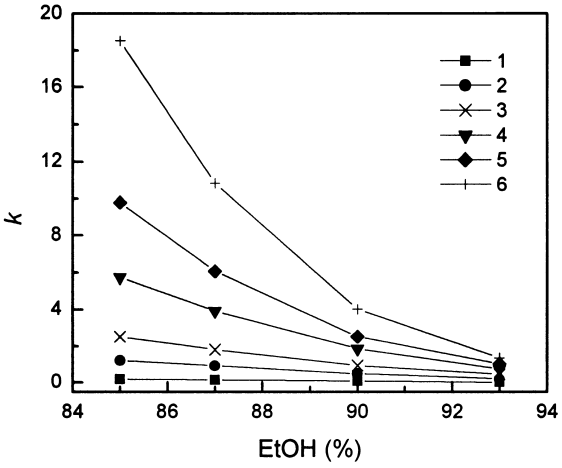

Figure 5. Effect of ethanol content on the retention of thulium porphyrin complexes. Mobile phase: ethanol–water containing 25 mmol L^{-1} TEOLA and 200 mmol L^{-1} HAc.

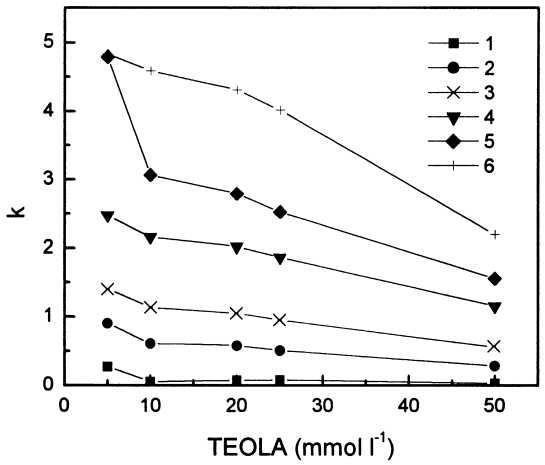

Figure 6. Effect of TEOLA concentration on the retention of thulium porphyrin complexes. Mobile phase: ethanol : water = 90 : 10 (v : v) containing X mmol L^{-1} TEOLA and 200 mmol L^{-1} HAc.

in this ethanol system, as it played a subordinate role as described previously. From Figs. 5 and 6, with the lower content of ethanol and TEOLA and the lower rate of elution, the equivalent elution can be attained for these thulium porphyrins with ethanol as the main constituent of the mobile phase, compared with that of the methanol system. In addition, from Fig. 6, when the concentration of TEOLA in the ethanol system was less than 5 mmol L^{-1}, the peaks of compounds 5 and 6 overlapped significantly. This further illustrated that TEOLA acted as the main role in the elution of thulium porphyrins.

Separation, Linear Range, and Detection Limit for the Thulium Porphyrins

Under optimum conditions, separation of these thulium porphyrin complexes was achieved with the mobile phase of methanol–water (93 : 7, v : v), containing 75 mmol L^{-1} TEOLA and 200 mmol L^{-1}HAc (Fig. 7) and the mobile phase of ethanol–water (90 : 10,v : v) containing 25 mmol L^{-1} TEOLA

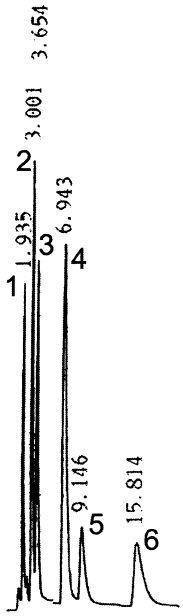

Figure 7. HPLC separation of thulium porphyrin complexes in methanol system. Column, Shimpak SBC-ODS (5 μm, 2.6 × 150 mm); Mobile phase: methanol : water = 93 : 7 (v : v) containing 75 mmol L^{-1} TEOLA and 200 mmol L^{-1} HAc; flow rate, 0.5 mL min^{-1}.

Table 1. Regression equations, linear range, and detection limits of thulium porphyrin complexes.

Compound number	Regression equation[a]	r	Linear range ($\mu g\,mL^{-1}$)	Detection limit ($\mu g\,mL^{-1}$; S/N = 3)
1	$y_1 = 8.886 \times 10^3 + 3.164 \times 10^5 x_1$	0.9996	0.05–2.5	0.001
	$y_2 = 1.098 \times 10^3 + 5.505 \times 10^5 x_2$	0.9988	0.05–2.5	0.001
2	$y_1 = 1.931 \times 10^4 + 4.759 \times 10^5 x_1$	0.9975	0.05–10	0.001
	$y_2 = 2.261 \times 10^4 + 9.790 \times 10^5 x_2$	0.9996	0.05–10	0.001
3	$y_1 = 1.608 \times 10^4 + 4.811 \times 10^5 x_1$	0.9983	0.05–10	0.001
	$y_2 = 4.474 \times 10^3 + 9.619 \times 10^5 x_2$	0.9992	0.05–10	0.001
4	$y_1 = 1.641 \times 10^4 + 8.611 \times 10^5 x_1$	0.9979	0.05–10	0.001
	$y_2 = 4.823 \times 10^4 + 1.748 \times 10^6 x_2$	0.9993	0.05–10	0.001
5	$y_1 = 1.444 \times 10^4 + 2.847 \times 10^5 x_1$	0.9975	0.1–10	0.005
	$y_2 = 5.690 \times 10^3 + 5.904 \times 10^5 x_2$	0.9997	0.1–10	0.005
6	$y_1 = 2.722 \times 10^4 + 3.182 \times 10^5 x_1$	0.9976	0.1–5	0.005
	$y_2 = -1.347 \times 10^4 + 7.193 \times 10^5 x_2$	0.9979	0.1–5	0.005

[a]x_1, x_2, concentration of thulium porphyrin complexes ($\mu g\,mL^{-1}$); y_1, y_2, peak area of the corresponding complex (arbitrary unit). Subscripts (1, 2) 1, methanol system; 2, ethanol system.

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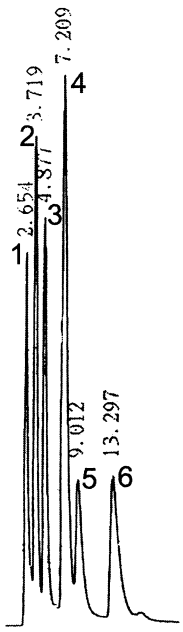

Figure 8. HPLC separation of thulium porphyrin complexes in ethanol system. Column, Shimpak SBC-ODS (5 μm, 2.6×150 mm); Mobile phase: ethanol : water = 90 : 10 (v : v) containing 25 mmol L^{-1} TEOLA and 200 mmol L^{-1} HAc; flow rate, 0.25 mL min^{-1}.

or 200 mmol L^{-1}HAc (Fig. 8). The entire analysis was completed in 16 min and 14 min, correspondingly. The regression equations, linear ranges, and detection limits of these thulium porphyrins are listed in Table 1.

CONCLUSIONS

Six water-insoluble thulium porphyrin complexes can be eluted from an ODS column with the addition of TEOLA, together with HAc, to the mobile phase of methanol (or ethanol)–water mixture. Increases of the contents of TEOLA together with HAc can accelerate the elution of these thulium porphyrins. Compared with the methanol system, the ethanol system can achieve an equivalent effect for the separation of these thulium porphyrins at lower flow rates. This indicates that the ethanol system is favorable to hyphenate other detect approaches in the analysis of water-insoluble rare earth porphyrin complexes.

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