HIGH PERFORMANCE LIQUID CHROMATOGRAPHY OF SAFRAMYCINS, HETEROCYCLIC QUINONE ANTITUMOR ANTIBIOTICS

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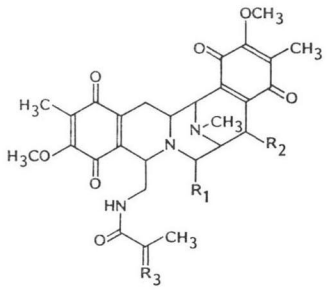
Saframycins are members of a new family of antitumor antibiotics isolated from the streptothricin-producing strain of *Streptomyces lavendulae* No. 314. Ten components[1~5], A (**1**), B (**2**), C (**3**), G (**4**), H (**5**), Y3 (**6**), D (**7**), F (**8**), R (**9**) and S (**10**), have been isolated from the culture broth and their structures have been established as shown in Fig. 1. All saframycins bear the dimeric quinone skeleton as a common structure unit. Thin-layer chromatographic method is available for separation of saframycins[6,7]. However, this method does not suffice for identification and precise quantification. In this paper, we report an HPLC method for separation and quantification of saframycins. All chromatograms were obtained using BIP-I pump, UVIDEC-100-IV variable-wavelength UV detector with absorbance expressed in absorbance units full scale (Aufs), RC-250 chart recorder, all from Jasco, Tokyo, Japan. Saframycin S (**10**) was excluded from this study because of its labile property.

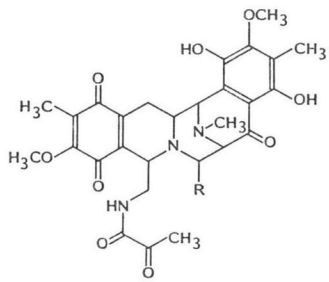
Fig. 2 indicates an isocratic reversed-phase HPLC elution profile of a mixture of nine components. This chromatogram was obtained by the following systems; packing: Cosmosil $5C_{18}$ (4.6×150 mm, Nakarai Chemicals, Kyoto, Japan); mobile phase: acetonitrile - H_2O (1: 1.8), containing TFA (0.05%); flow rate: 1.0 ml/minute; sensitivity: 270 nm, 0.04 Aufs; chart speed: 5 mm/minute. These chromatographic conditions afforded a good separation of the seven components, **9**, **7**, **2**, **6**, **3**, **5** and **4**, although the elution of the last two components, **5** and **4**, was too prolonged. On the other hand, they were unsuitable for two components, **1** and **8**, because of a retention time being more than 1 hour and a lack of sufficient separation.

In Fig. 3, a mixture of the nine components was subjected to separation by normal phase HPLC. Cosmosil 5SL column (4.6×150 mm, Nakarai Chemicals) was used as stationary phase. Among a variety of solvents tested, the mixed solvent, $CHCl_3$ - 2-PrOH (99: 1), was selected as the most suitable mobile phase. All other conditions were the same as in the case of Fig. 2. Five components, **8**, **1**, **4**, **3** and **2**, were well separated from other components. The com-

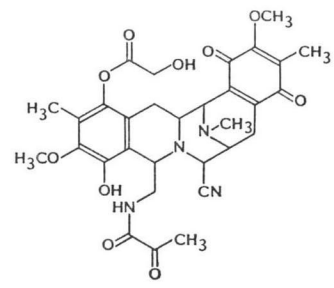
Fig. 1. Structures of saframycins.



	R_1	R_2	R_3
Saframycin A (**1**)	CN	H	O
Saframycin B (**2**)	H	H	O
Saframycin C (**3**)	H	OCH_3	O
Saframycin G (**4**)	CN	OH	O
Saframycin H (**5**)	CN	H	OH, CH_2COCH_3
Saframycin Y3 (**6**)	H	H	H, NH_2
Saframycin S (**10**)	OH	H	O



	R
Saframycin D (**7**)	H
Saframycin F (**8**)	CN



Saframycin R (**9**)

Fig. 2. Reversed-phase HPLC chromatogram of saframycins.

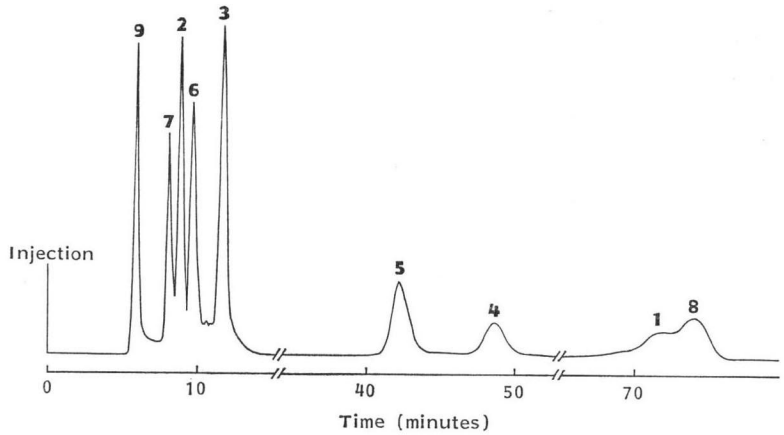
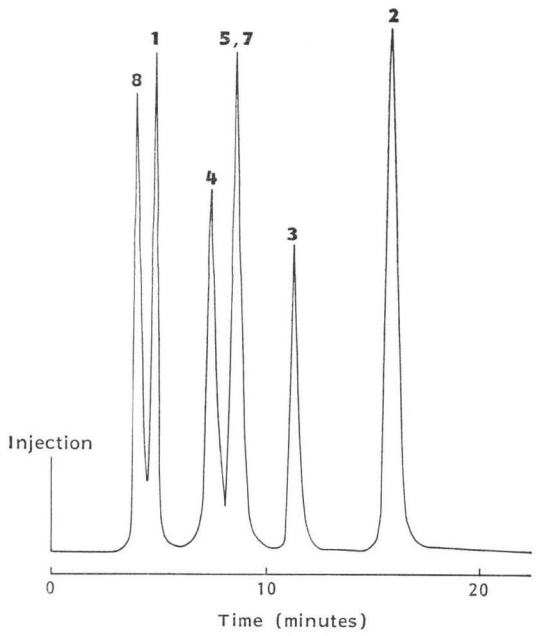


Fig. 3. Normal phase HPLC chromatogram of saframycins.



ponents, **7**, was overlapped with **5**, and the two components, **6** and **9**, were not eluted within 1 hour. The elution order of the components, other than **7**, obtained in Fig. 2, went into reverse in Fig. 3. Through this study, the analytical condition necessary for the assay method by which all saframycins can be determined on a single chromatographic run, was not found. However, it was confirmed that each component can be analyzed accurately by using either the reverse or the normal phase mode described above.

The calibration for saframycin A (**1**) was accomplished in a series of experiments, with varying amounts of ranging from 4.4 to 44 ng/ml. The chromatographic system applied was the same as in Fig. 3 with the exception of sensitivity of 0.01 Aufs. The linear relationship of the peak height to the quantity injected was obtained. A signal to noise ratio (S/N) at 4.4 ng of **1** was 8: 1. The detection limit of **1**, based on a S/N (4 : 1), was 2.2 ng, which was about 1/230 that of the TLC-a dual wavelength chromatogram scanner method[6,7].

References

1) Arai, T. & A. Kubo: Isoquinoline quinones from actinomycetes and sponges. *In* The Alkaloid. XXI. *Ed.*, A. Brossi, pp. 56~100, Academic Press, New York, 1983
2) Arai, T.; K. Takahashi & A. Kubo: New antibiotics, saframycins A, B, C, D and E. J. Antibiotics 30: 1015~1018, 1977
3) Lown, J. W.; C. C. Hanstock, A. V. Joshua, T. Arai & K. Takahashi: Structure and conformation of saframycin R determined by high field ^{1}H and ^{13}C NMR and its interactions with DNA in solution. J. Antibiotics 36: 1184~1194, 1983
4) Mikami, Y.; K. Yokoyama, H. Tabeta, K. Nakagaki & T. Arai: Saframycin S, a new saframycin group antibiotic. J. Pharm. Dyn. 4: 282~286, 1981
5) Kishi, K.; K. Yazawa, K. Takahashi, Y. Mikami & T. Arai: Structure-activity relationships of saframycins. J. Antibiotics 37:

847~852, 1984

6) ARAI, T.; K. TAKAHASHI, K. ISHIGURO & K. YAZAWA: Increased production of saframycin A and isolation of saframycin S. J. Antibiotics 33: 951~960, 1980

7) ARAI, T.; K. YAZAWA, K. TAKAHASHI, A. MAEDA & Y. MIKAMI: Directed biosynthesis of new saframycin derivatives with resting cells of *Streptomyces lavendulae*. Antimicrob. Agents Chemother. 28: 5~11, 1985