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Review Article

Development of RP-HPLC Conditions for Separation of Oligostilbenes in 12 Dipterocarpaceae Crude Extracts

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ABSTRACT

Plants from the Dipterocarpaceae family have proven to be a rich source of oligostilbene. To date, about 100 out of more than 400 stilbene derivatives reported were isolated from Dipterocarpacae plants. A HPLC method was developed for separation of oligostilbenes in 12 extracts from 3 species of Dipterocarpaceae, *Shorea leprosula*, *Shorea parviflora* and *Shorea ovalis* from different plant parts and localities. For all samples, the wavelengths were set at 215, 254 and 283 nm, the column temperature was 35°C, the flow-rate was 1 ml/min and the injection volume was 5 µl. The chromatographic run was standardized for 20 minutes. The mobile phase were acetonitrile:water (ACN:H_2O) at different compositions. The suitable chromatographic condition for *S. leprosula* is a gradient elution of ACN: H_2O (15:85 to 55:45), *S. parviflora* (20:80 to 55:45) and *S. ovalis* (10:90 to 75:25).

Keywords: Chromatography, Oligostilbene, Dipterocarpaceae, *Shorea*.

INTRODUCTION

Dipterocarpaceae family is well-known in tropical rainforests, found predominantly in Southeast Asia. Comprising about 17 genera and approximately 600 species[1], they dominantly distributed in Malaysia with the greatest diversity in Borneo[2]. They are also found in other pantropical area including Seychelles, India, Northern South America, Africa, Indochina and Indonesia[3]. Besides providing valuable woods for timber industry, its phytochemicals especially oligostilbenes has various biological activities, including antibacterial[4-7], antifungal[4,8], antiinflammatory[9], cytotoxic[10,11] and HIV-inhibitory activities[12]. Plants from the Dipterocarpaceae family have proven to be a rich source of oligostilbenes[4,10,11,13-18]. In our previous study, we had successfully isolated oligostilbenes from *Neobalanocarpus heimii*[19,20] and *Dryobalanops* spp[21]. using HPLC. In this report, we have developed a method suitable for isolation of compounds in three species of Dipterocarpaceae, *Shorea leprosula*, *Shorea parviflora* and *Shorea ovalis*.

MATERIALS AND METHODS

Plant Materials

Dipterocarpaceous plant samples of *Shorea parvifolia*, *Shorea leprosula* and *Shorea ovalis* were provided by Atta-ur-Rahman Institute for Natural Products Discovery (AuRIns), Universiti Teknologi MARA, Malaysia. The plants were collected from two different forest reserves in Malaysia, in Jengka and Selai. Samples were obtained from the trunk wood (TW) and buttress bark (BB) of each plant. The samples are labelled as sample 1 to 12 as shown in Table 1.

Chemicals

Solvent for extraction was of analytical grade and chromatographic solvents are of HPLC grade. The ultra-pure water was obtained from PURELAB® Option water purification system (18.2 MΩ cm^{-1}, ±25°C).

Instrumentation

The HPLC system is a Dionex™ Ultimate® 3000 from Thermo Scientific™. The system is equipped with an ultra-pressure pump, a degasser, an auto sampler and a diode array detector (DAD). The chromatographic profiles and the integrated data were recorded using Chromeleon™ Chromatography software. The separations were achieved through a Phenomenex® Luna 5 µm C18 column (150 X 4.6 mm) equipped with a guard column of similar chemistry.

Sample preparation

10 g of each sample was extracted using acetone by sonication for 1 hour. The samples were individually filtered through a 0.45µm polytetrafluoroethylene (PTFE) membrane into their respective HPLC vials.

HPLC analysis

The HPLC analyses were carried out on a Phenomenex® LUNA C18 column (150 x 4.6 mm, 5µm). The mobile phase were acetonitrile:water (ACN:H_2O) at different compositions. The wavelengths were established at 215, 254 and 283 nm. The column temperature was maintained

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Table 1: Numbering of samples according to their species, localities and the plant parts.

Plant	Place	Parts*	Sample No.
Shorea leprosula	Jengka	BB	1
		TW	2
	Selai	BB	3
		TW	4
Shorea parvifolia	Jengka	BB	5
		TW	6
	Selai	BB	7
		TW	8
Shorea ovalis	Jengka	BB	9
		TW	10
	Selai	BB	11
		TW	12

*BB=buttress bark, TW=trunk wood

at 35°C, and the flow-rate was set at 1 ml/min. and the injection volume was 5 □l. The chromatographic run was 20 minutes, followed by a column flushing at 95% ACN for 5 minutes and a post-run column condition for another 5 minutes. A good chromatographic condition for each sample was achieved by adjusting the slope of the gradient elution. The slopes were systematically adjusted by gradually changing the solvent composition at the end of a chromatographic run while maintaining the initial solvent composition, and vice versa.

RESULTS AND DISCUSSION

In a preliminary analysis, the initial solvent composition was maintained at ACN:H_2O (5:95), while at the end of the chromatographic run, the solvents were adjusted as ACN:H_2O (95:5, 85:15, 75: 25, 65:35 and 55:45). The chromatographic profiles for all samples were analyzed individually by examining their peak resolutions. Based on this result, further analyses were carried out by maintaining the selected solvent composition at the end of the chromatographic run, while adjusting the initial composition at ACN:H_2O (10:90, 15:85, 20: 80, 25:75 and 35:65). Figure 1 shows the gradual improvements of the retention time and peak resolutions with the change of the solvent composition. All 12 samples underwent this analysis. Based on these 10 chromatographic profiles, the best chromatographic condition was individually selected for each sample. The selection was based on the peak resolution and the chromatographic run-time. Based on

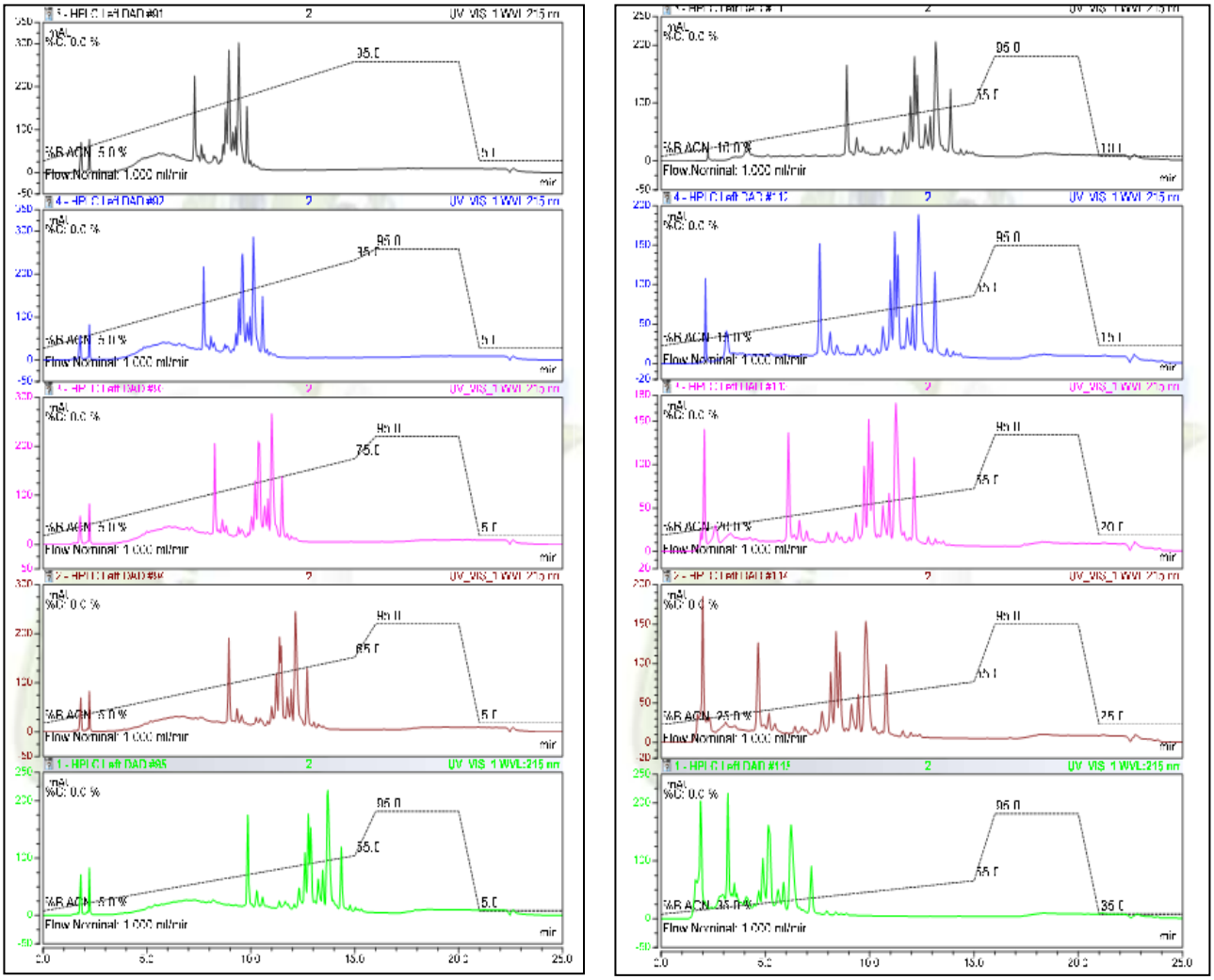

Figure 1: The chromatographic profiles of a plant sample when the solvent composition was changed at the end of the chromatographic run (left) and when the initial solvent composition is changed (right).

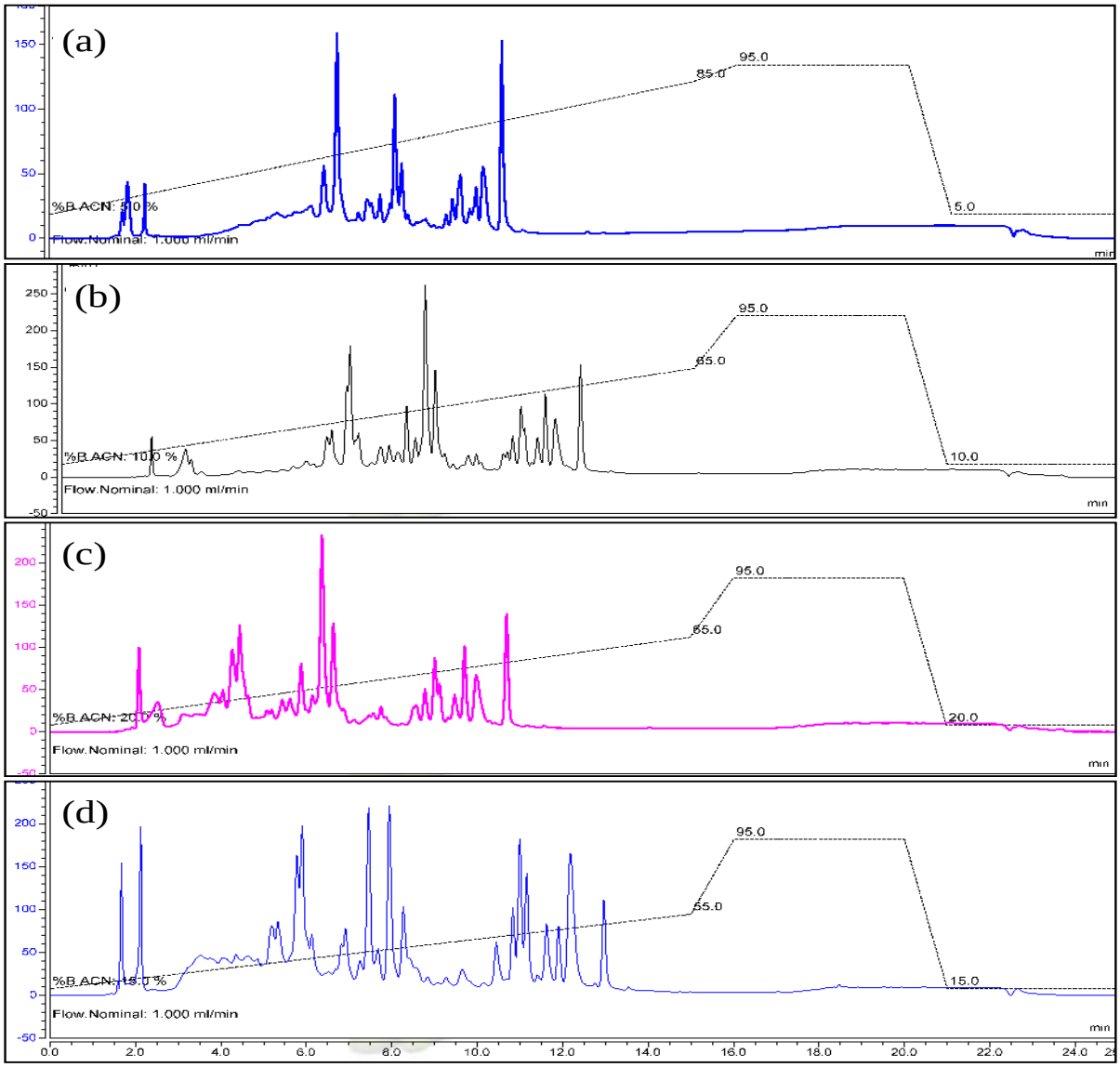

Figure 2: The chromatograms of *Shorea leprosula* in 20 minutes chromatographic run; (a) ACN: H_2O (5:95 to 85:15), (b) ACN:H_2O (10:90 to 65:25), (c) ACN:H_2O (20:80 to 65:25) and (d) ACN:H_2O (15:85 to 55:45).

their profiles, the solvent composition then further adjusted to improve the separation.

Determination of the practically suitable chromatographic condition

Apart from some minor differences, analysis of all chromatograms showed that samples from the same species having similar chromatographic profiles regardless their plant parts and localities. Due to these similarities, the results are discussed as a group of the same plant species, instead of individual samples.

Shorea leprosula

At the gradient of ACN:H_2O (5:95 to 85:15) the chromatogram shows rather a good separation of compounds. The compounds eluted between minute-6 to minute-10.5 with some co-elution. The peak shapes are acceptably narrow, indicating an efficient separation. The resolution however is poor based on the narrow space between peaks and some even overlapped (figure 2a). Reducing the gradient slope (ACN:H_2O; 10:90 to 65:25) had significantly improved the resolution. The peaks spread in a wider range of retention time, from minute-6 to minute-12. Some overlapping peaks were resolved and some are not (figure 2b). Increasing the solvent strength at the initial of a chromatographic run (ACN:H_2O; 20:80) reduced the retention time by 2 minutes while maintaining the peak resolution (figure 2c). This is very useful in order to reduce the solvent consumption and analysis time. Further improvement by a slight decrease of the gradient slope produced a well resolve chromatogram with minimum overlapping peaks. Furthermore, the compounds were eluted in an acceptable chromatographic range (figure 2d). Based on this analysis, the suitable chromatographic condition for *Shorea leprosula* is a gradient elution of ACN:H_2O (15:85 to 55:45 in 20 minutes).

Shorea parvifolia

The initial chromatographic condition, which was ACN:H_2O (5:95 to 95:5 in 20 minutes) resulted a not very promising profile. Only 3 apparent peaks were observed and the rest were heavily overlapping to the extent that

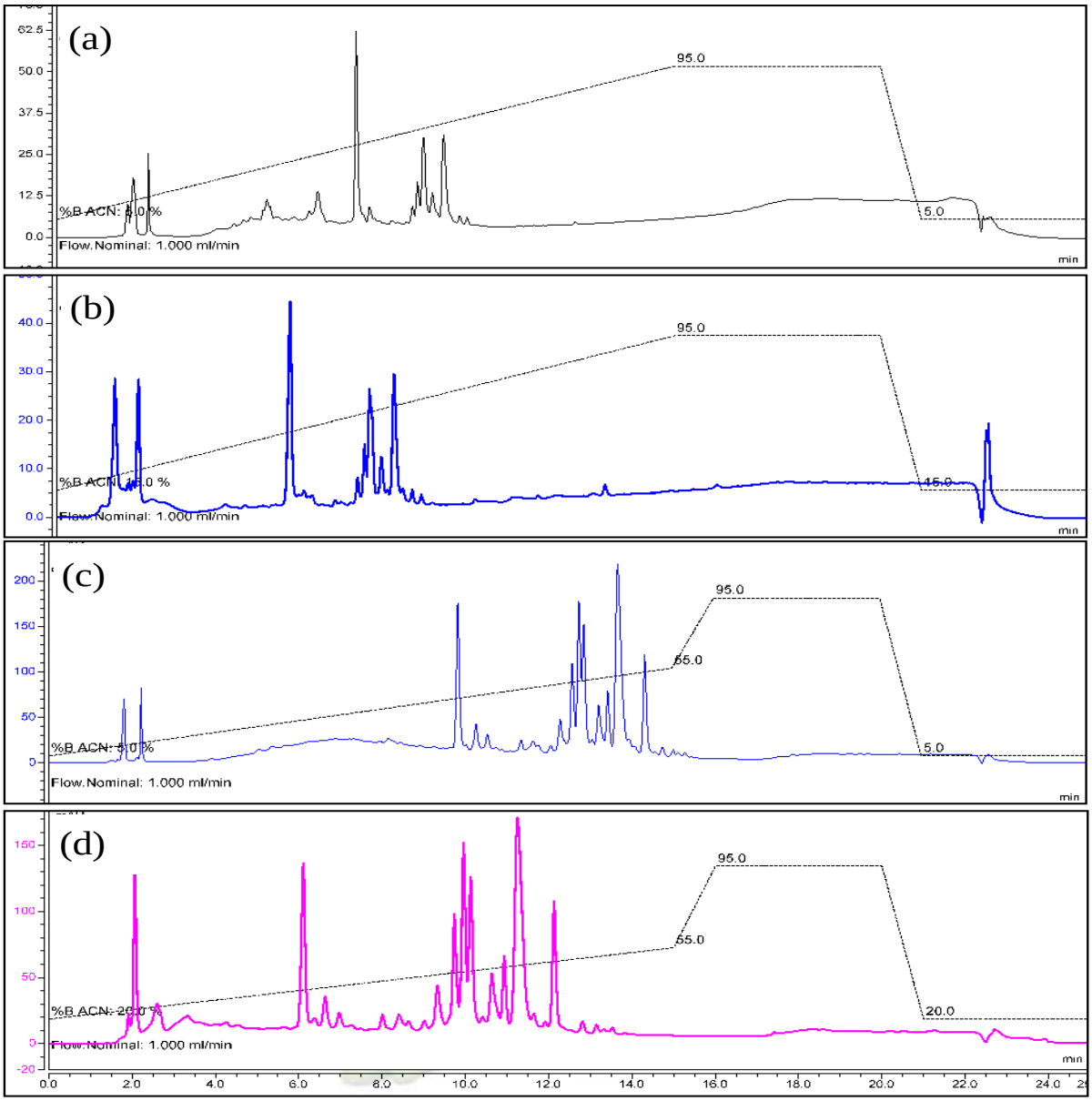

Figure 3: The chromatograms of *Shorea parviflora* in 20 minutes chromatographic run; (a) ACN:H_2O (5:95 to 95:5), (b) ACN:H_2O (15:85 to 95:5), (c) ACN:H_2O (5:95 to 55:45) and (d) ACN:H_2O (20:80 to 55:45).

they form a hump above the baseline (figure 3a). Increasing the initial solvent strength to ACN:H_2O (15:85) reduced the retention time but the crowded peaks remained overlapped. The first two peaks disappeared in the injection peak. A group of compounds that eluted between minute-8 and 10 however showed some improvements (figure 3b). With the hope to recapture the missing peaks, the initial solvent composition was switch to ACN:H_2O (5:95), while the end-composition was drastically changed to ACN:H_2O (55:45). The strategy was to obtain better separation by reducing the gradient slope. The separation was improved, however the elution of the first two compounds were dragged for 6 minute, forming a combined hump between minute-4 to 10 (figure 3c). The retention time was also increased significantly. Increasing the initial solvent strength (ACN:H_2O; 20:80) and keeping the end-solvent composition at ACN:H_2O (55:45) reduced the retention time while maintaining the resolution (figure 3d). Therefore, the chromatographic condition suitable for *Shorea parviflora* is a gradient elution of ACN:H_2O (20:80 to 55:45 in 20 minutes).

Shorea ovalis

The chromatogram in figure 4a was obtained when a gradient elution was at ACN:H_2O (25:75 to 75:25). The compounds eluted at early retention time due to the strength of the solvent at the initial chromatographic run. Since the column retention was low, the compounds were not well separated. Reducing the initial solvent strength composition to ACN:H_2O (20:80) while maintaining the end composition improved the resolution as the peaks spread in wider range of retention time (figure 4b). Further reduction of the initial solvent strength (ACN:H_2O; 5:75) did not improve the resolution, but increased the retention time (figure 4c). Compromising the initial solvent strength at the composition of ACN:H_2O (10:90) resulted a better resolution, but do not improve the retention time (figure

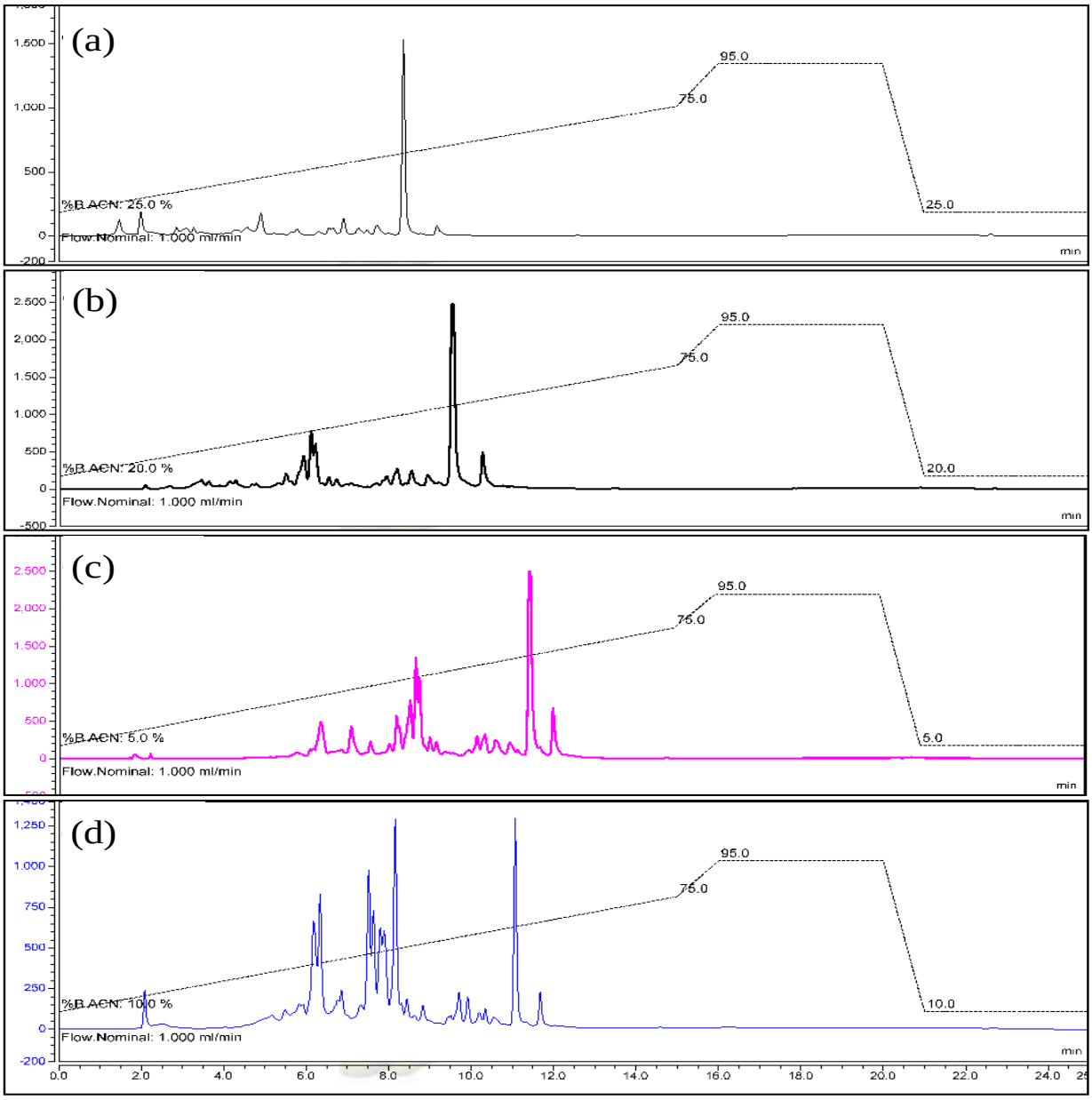

Figure 4: The chromatograms of *Shorea ovalis* in 20 minutes chromatographic run; (a) ACN:H_2O (25:75 to 75:25), (b) ACN:H_2O (20:80 to 75:25), (c) ACN:H_2O (5:95 to 75:25) and (d) ACN:H_2O (10:90 to 75:25).

4d). The chromatographic condition for *Shorea ovalis* was decided at a gradient elution of ACN:H_2O (10:90 to 75:25 in 20 minutes).

CONCLUSION

In the development of chromatographic method, solvent compositions at the initial and the end of a chromatographic run are significant factors in the resolution of compounds in a mixture. Increasing/reducing the solvent strength at the initial chromatographic run changes the retention factor of compounds in a column. Adjusting the gradient slope changes the separation behavior of the compounds. In general, the less steep the gradient, the better separation of the compounds.

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