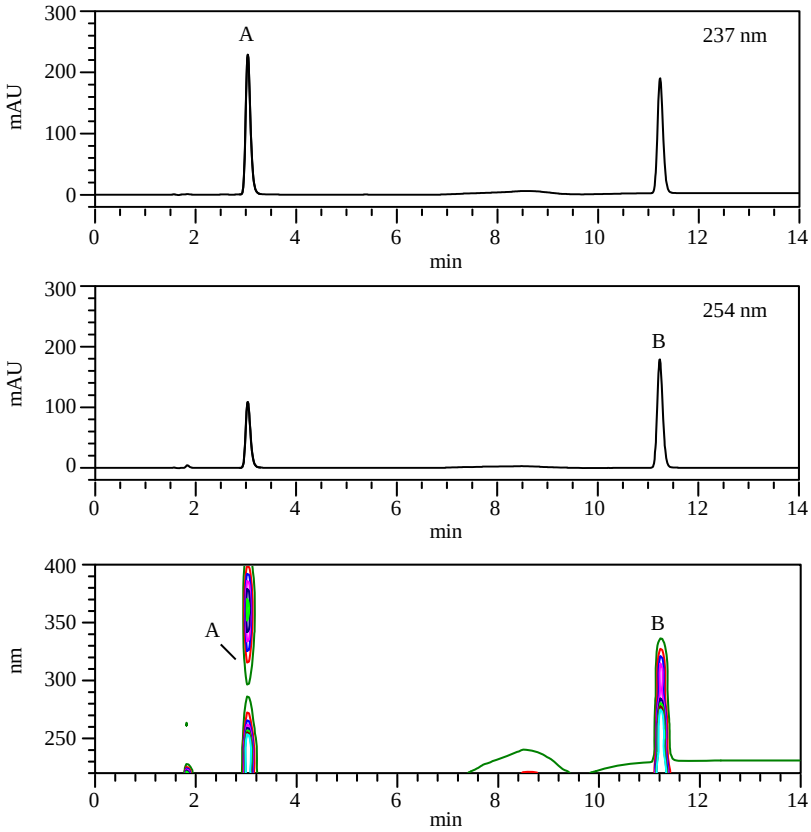
■ Analysis of Combination Drugs (Identification and Quantitative Analysis by DAD)

Prescription drugs generally contain a single type of active pharmaceutical ingredient (API). However, the number of combination drugs containing more than two types of APIs has been increasing in recent years. Benefits of combination drugs include the reduced number of medications and the prevention of missed doses. Presented here is the analysis of a combination drug containing two types of APIs by HPLC/DAD (diode array detector).
Diode array detection is capable of simultaneously providing chromatograms at specified wavelengths and absorption spectra of specified peaks. Thus, it is possible to perform a quantitative analysis based on the chromatogram and, at the same time, to confirm the maximum absorption of the absorption spectrum or to perform a qualitative analysis based on comparison with the spectrum of the standard sample.

■ Analysis Example of Standard Sample



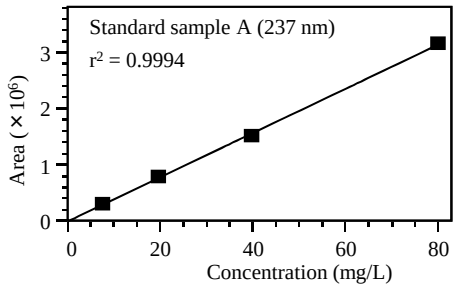
<Analytical Conditions>

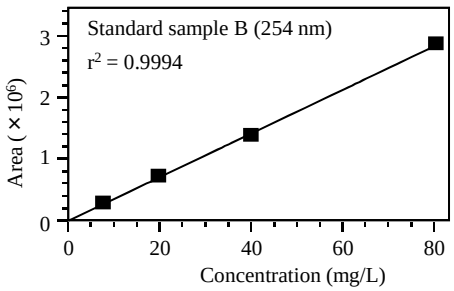
Column	:	HITACHI LaChrom C18 (5 μm) 4.6 mm I.D. × 150 mm
Eluent	:	Phosphoric acid buffer/Methanol (a) 30 / 70 (b) 10/ 90 (v/v) (a) (b) Gradient
Flow rate	:	1.0 mL/min
Column temperature	:	25°C
Detection wavelength:		DAD 220 - 400 nm (237 nm, 254 nm)*
Injection vol.	:	20 μL

*)The wavelength specified in the Pharmacopoeia was used for the detection of each component.

[Analysis Example for Standard Sample containing Components A and B (40 mg/L each)]

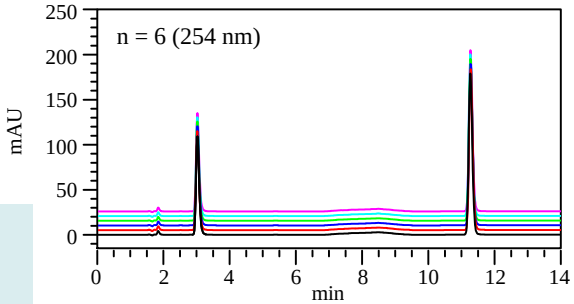
■ Linearity of Standard Sample (8 – 80 mg/L each)





■ Reproducibility of Standard Sample

	Retention time (% RSD)	Area value (% RSD)
A	0.06	0.27 (237 nm)
B	0.04	0.18 (254 nm)

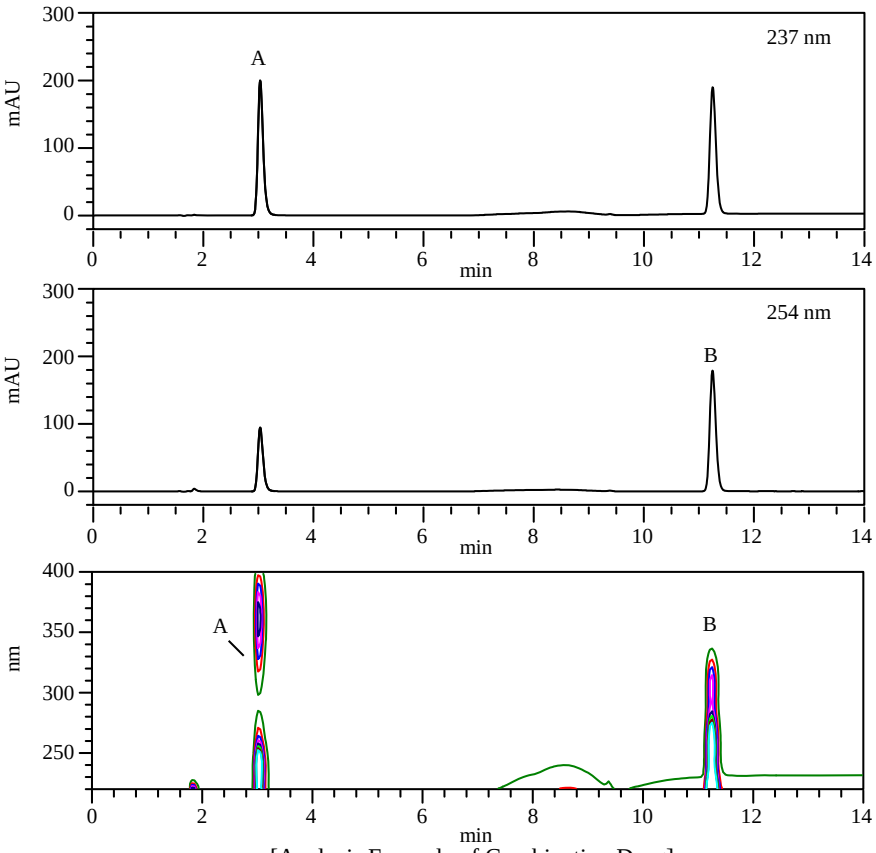


[Overlaid Chromatograms]

A good linearity was obtained for both components A and B over the concentration range of 8 – 80 mg/L.
A good result for the reproducibility with the RSD = 0.04% – 0.06% (retention time) and RSD=0.18%– 0.27% (area value) was also obtained.

■ Analysis Example of Combination Drug (Identification and Quantitative Analysis by DAD)

■ Analysis Example of Combination Drug



[Analysis Example of Combination Drug]

<Preparation Method for Combination Drugs>

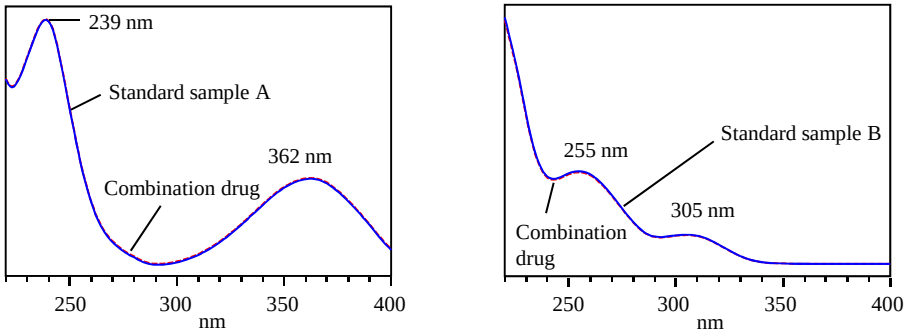
Sample (1 tablet)
| ← Dissolve in about 100 mL of eluent (a)
Let it stand for 20 min and then, sonicate
|
Make up to 200 mL with eluent (a)
|
Centrifuge (3000 rpm, 10 min)
|
Filter twice through a 0.2 µm filter
|
Analytical sample (20 µL)

■ Quantitative Analysis based on Chromatogram

[Quantitative Value for Combination Drug]

	Result of Quantitative Analysis
A	34.11 mg/L
B	39.24 mg/L

■ Identification based on Spectrum



[Spectra of Standard Sample and Detected Peak]

DAD allows for confirmation of the maximum absorption of the absorption spectrum for each component.

In the section of Liquid Chromatography under the General Tests of the Japanese Pharmacopoeia Sixteenth Edition, it is stated "If a detector which is able to obtain chemical structural information of the component at the same time is used, highly specific identification can be achieved by confirming identity of the chemical structure of the component and that of an authentic specimen, in addition to the identity of their retention times." This statement suggests the use of the LC/DAD for identification.
The preparation of the draft for the Japanese Pharmacopoeia Seventeenth Edition is currently in progress and the rationalization which allows for performing the identification by using the highly specific chromatography for quantitative analysis method is also being considered as a mean for the "rationalization for identification." [1)2)]

1) **Guideline for Drafting the Japanese Pharmacopoeia, Seventeenth Edition (Draft)**
2) **Opinions, etc. received for "Opinion Survey Concerning Guideline for Drafting the Japanese Pharmacopoeia, Seventeenth Edition Draft (Draft)" (April, 2012)**

* The Japanese Pharmacopoeia does not specify the test methods for combination drugs.
* This analytical sample was provided by Division of Physical Pharmaceutical Chemistry, Faculty of Pharmacy, Keio University.

System Configuration: Chromaster 5110 pump, 5210 Auto Sampler, 5310 Column Oven, 5430 DAD

NOTE : These data are an example of measurement; the individual values cannot be guaranteed.