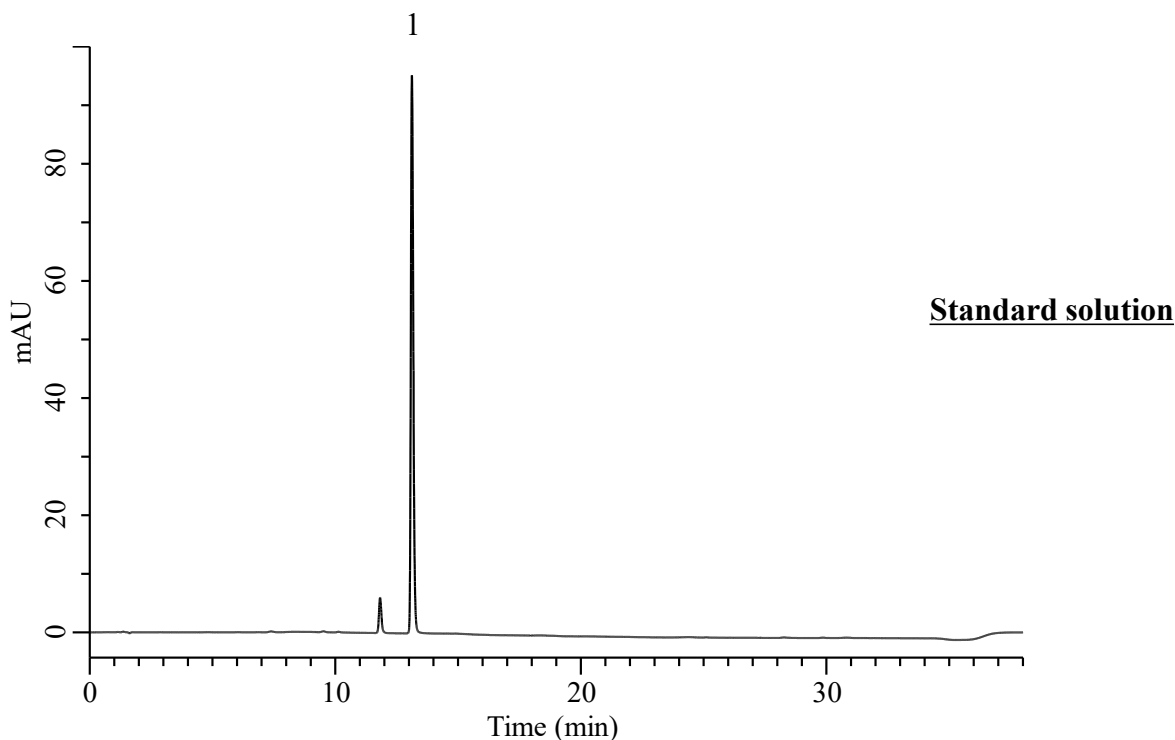


Analysis of Methylene blue

(Under the Condition of draft for USP PF52(3), Methylene Blue, Assay)



Conditions

System : Chromaster PLUS (HITACHI)
Column : InertSustain Phenylhexyl (GL Sciences Inc.)
 (3.5 μ m, 100 x 4.6 mm I.D.)
Column Cat. No. : 5020-93005
Eluent : A) CH₃CN
 B) 0.1% TFA in H₂O

Analyte:

1. Methylene blue 0.05 mg/mL

Tailing factor : (0.8 $\leq$) 1.32 ($\leq$ 1.8)

RSD of the
peak area (%) (n=6) : 0.232 ($\leq$ 1.10)

Time (min)	A (vol %)	B (vol %)
0.0	20	80
5.0	20	80
25.0	70	30
32.0	70	30
33.0	20	80
38.0	20	80

Flow Rate : 1.0 mL/min
Col. Temp. : 30 $^{\circ}$ C
Detection : UV 246 nm (Chromaster 5420 UV-VIS)
Injection Vol. : 5 μ L
Sample : Standard in Eluent A/Eluent B=30/70, v/v