

## For setting separation conditions

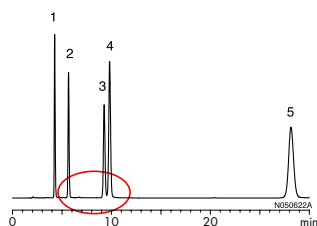
Establishment of simple separation conditions using Ph(Phenyl), TMS(C1) and CN(Cyano) columns.

S050719A

During the optimization of separation conditions, changing the composition of the mobile phase is an effective way to influence retention and resolution of compounds. Proper column selection can also change retention and resolution between peaks. Ph and CN can sometimes obtain excellent separation with more simple mobile phase conditions than that used with ODS columns.

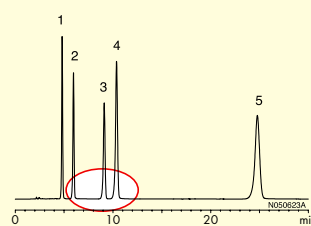
## Simple separation condition using Ph and CN column

**Pro C18**  
acetonitrile / water / TFA (12/88/0.1)



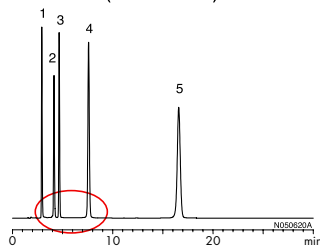
Change of column

**Ph**  
acetonitrile / water / TFA (12/88/0.1)

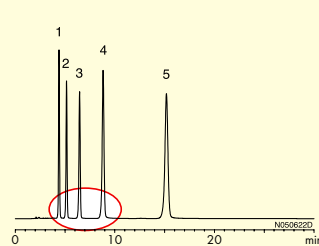


Optimization of mobile phase

**Pro C18**  
acetonitrile / ethyl acetate / water / TFA (9/3/88/0.1)



**CN**  
acetonitrile / water / TFA (12/88/0.1)



### Catechins

1. (-)-Epigallocatechin
2. (+)-Catechin
3. (-)-Epicatechin
4. (-)-Epigallocatechin gallate
5. (-)-Epicatechin gallate

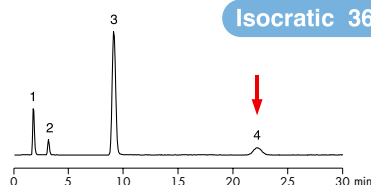
Column : 150 X 4.6 mm I.D.  
Flow rate : 1.0 mL/min  
Temperature : 37°C  
Detection : UV at 280 nm

Optimization of separation conditions of catechins on ODS results in complicated mobile phase containing ethyl acetate. In contrast, Ph or CN enables separations with a simple composition mobile phase.

## Simple separation condition using TMS and CN column

**Pro C18**

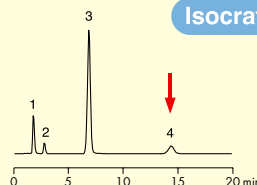
Isocratic 36%B



Change of column

**TMS**

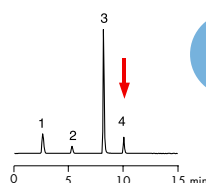
Isocratic 30%B



Gradient elution

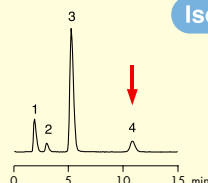
**Pro C18**

Gradient  
30-90%B(0-10min),  
90%B(10-15min)



**CN**

Isocratic 30%B



### Soy isoflavones

1. Daidzin
2. Genistin
3. Daidzein
4. Genistein

Column : 50 X 2.0 mm I.D.  
Eluent : A) water / formic acid (100/0.05)  
          B) acetonitrile / water / formic acid (50/50/0.05)  
Flow rate : 0.2 mL/min  
Temperature : 37°C  
Detection : ESI positive mode

TMS and CN are useful for quick and simple analysis of compounds that differ greatly in hydrophobicity, without the need to use gradient elution chromatography.