The Japanese Pharmacopoeia, 18th Edition Analysis of tranexamic acid

— 2.00 Change of conditions specified within General theory of chromatography —

Adjustable range of chromatography conditions has been specified in [2.00 General theory of chromatography] which has been stipulated based on the contents harmonized and agreed upon by Japanese/U.S./European Trilateral Pharmacopoeia at the first supplemental public comment of the Japanese Pharmacopoeia, 18th Edition, which was disclosed in September 2021.
Based on this, the speed-up of analysis can be expected by optimizing the column size and analytical conditions within the acceptable changeable range.
In this report, an example of analysis was reported, in which analysis time is shortened and solvents consumption is reduced by changing the conditions within the range specified in [2.00 General theory of chromatography] for JP18 Test for Quantitative Method of Tranexamic Acid.
* The change of analytical conditions is for the content to be applied from the first supplemental revision of J P18 and is not for the content announced in the past. Accordingly, this Technical Note is simply just for reference.

Changeable items in JP

In [2.00 General theory of chromatography], the change of LC column and instrumental conditions is allowed by satisfying the requirements of system suitability.

日本薬局方 〈令和3年9月バブコメ案〉 三薬局方国際調和		
カラム	官能基	変更不可
	粒子径	**全多孔性粒子：規定されたL/d_p比が-25%～+50%の範囲に変更可能** 表面多孔性粒子:理論段数が規定されたカラムの-25%～+50%の範囲であれば、 他のzと加 組み合わせも使用可能
	長さ	
	内径	粒子径やカラム長に変更がない場合に、カラム内径を調整する場合が而るかもしれない
装置	流量	カラムの内径と粒子径の両方の変更により、以下の計算式で変更可能 $F_2 = F_1 \times [(d_{c2}^2 \times d_{p1}) / (d_{c1}^2 \times d_{p2})]$ ※3 μmをまたぐ場合は細かい規定あり。カラムの粒子径と長さの変更による調整後、±50%の変更が許容される
	注入量	カラムの粒子径と長さを変更する場合、注入量の調整は以下の計算式が利用可能 $V_{inj2} = V_{inj1} \times (L_2 \times d_{c2}^2) / (L_1 \times d_{c1}^2)$
	検出波長	変更不可
	カラム温度	±10°C
	PH	±0.2
移動相	緩衝液濃度	変更可能なpH範囲内にて±10%
	混合比	50%以下の組成の移動相は±30% 0目対) ※ただし、全休に対して=10%以内

Flow rate

F_1 ： Flow rate of each medicinal product (mL/min)
F_2 ： Flow rate after change (mL/min)
d_{p1} ： Column particle diameter of each medicinal product (mm)
d_{p2} ： Particle diameter of column to use (mm)
d_{c1} ： Column inner diameter of each medicinal product (mm)
d_{c2} ： Column diameter to be used (mm)

Injection volume

V_{inj1} ： Injection volume of each medicinal product (μL)
V_{inj2} ： Adjusted injection volume (μL)
L_1 ： Column length of each medicinal product (cm)
L_2 ： New column length (cm)
* dc is synonymous with flow rate

Example: JP18 Tranexamic Acid　Quantitation Method

Referring to JP18 Tranexamic Acid Test as an analysis example, the column size was changed within the changeable range specified in [2.00 General theory of chromatography] and the analysis was implemented trying to find the speed-up conditions for analysis.

<Test conditions for JP18 Tranexamic Acid Quantitative Method>
Detector: UV absorption photometer (measuring wavelength: 220nm)
Column: Octadecylsilylated silica gel with 5 μm particle size for liquid chromatography is packed in a stainless steel tube with 6.0mm inner diameter and 25cm length.
Column temperature: Constant temperature at around 25°C
Mobile phase: 11.0g of anhydrous sodium dihydrogen phosphate is dissolved in 500ml water, and 5mL of triethylamine and 1.4g of sodium lauryl sulphate are added. After adjusting pH of the solution to pH 2.5 by using phosphoric acid or phosphoric acid solution (1→10), water is added to make the volume to be 600mL. Then, 400mL of methanol is added.
Flow rate: Flow rate is adjusted so that the retention time of tranexamic acid may be about 20 min.
* Using InertSustain AQ-C18, the result was compared with the application in which the retention time was adjusted by 1.4mL/min.
Injection volume: 20 μL
* For details, refer to our Technical Note LT092.
https://www.gls.co.jp/technique/app/detail.php?data_number=LT092

The column size written in JP is 25cm in column length (*L*) and 5μm in particle diameter (*dp*), and therefore, the value of *L/dp* becomes 50,000.
The changeable range of *L/dp* is within -25% - +50%, and so the changeable range of *L/dp* becomes as follows.

$$37{,}500 \leqq L/d_p \leqq 75{,}000$$

This time, the column of 3μm in particle diameter (*dp*) and 3.0mm in inner diameter (*dc*)　was selected to set up the speed-up conditions as in the table below.

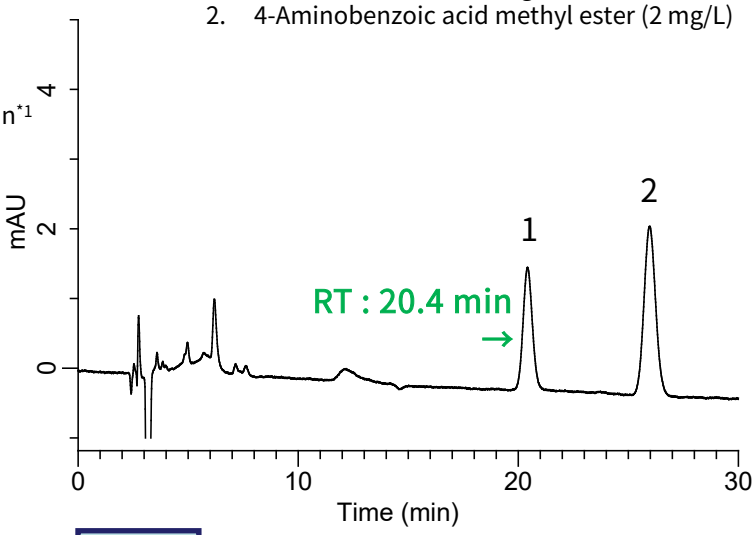
	JP条件	変更可能範囲	高速化条件
カラム長さ（L, mm）	250 mm	113~225 mm	150 mm
カラム粒子径(d_p, μm)	5 μm	-	3 μm
L/d_p	50,000	37,500 ~75,000	50,000
内径（d_c,mm）	6.0 mm	-	3.0 mm
流量(*F,* mL /min)	1.4 mL /min	0.35 ~1.06 mL/min	0.35 mL/ min
注入量（V_{inj},μL）	20μL	3 μL	3 μL

Conditions written in JP <before change>

HPLC conditions

Column	: InertSustain AQ-C18 (5 µm, 250 x 6.0 mmI.D.)
Eluent	A) Phosphoric acid buffer solution*1 B) CH_3OH A/B = 60/40, v/v
Temperature	: 25 °C
Detector	: UV 220 nm
Injection volume	: 20 µL
Flow rate	: 1.4 mL/min

1. Tranexamic acid (200 mg/L)
2. 4-Aminobenzoic acid methyl ester (2 mg/L)



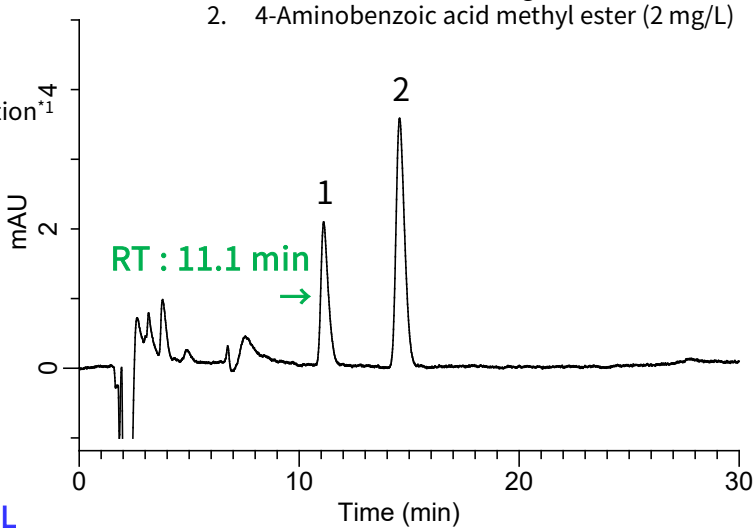
Analysis time : About 30 min.
Volume of solvent used: About 42mL

Speed-up conditions <after change>

HPLC conditions

Column	: InertSustain AQ-C18 HP : (3 µm, 150 x 3.0 mmI.D.)
Eluent	A) Phosphoric acid buffer solution*1 B) CH_3OH : A/B = 60/40, v/v
Temperature	: 25 °C
Detector	: UV 220 nm
Injection volume	: 3 µL
Flow rate	: 0.35 mL/min

1. Tranexamic acid (200 mg/L)
2. 4-Aminobenzoic acid methyl ester (2 mg/L)



Analysis time : About 15 min.
Volume of solvent used: About 5.25mL

[Analysis time, shortened to approx. 1/2] and [Volume of solvent used, saved to approx. 1/8] were achieved.

By downsizing the column, it is expected that equilibration time of mobile phase can be shortened.

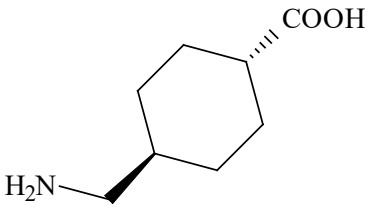
*1 Phosphoric acid buffer solution
Refer to the test conditions in the previous page.

<Result of system suitability test>

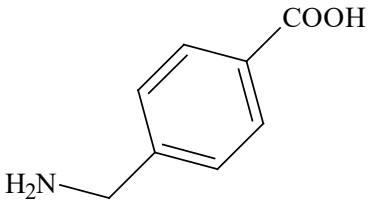
基準		JP記載条件		高速化条件	
分離度（1,2）	5以上	6.4	PASS	5.3	PASS
RSD%（1）（n=6）	0.6%以下	0.02%	PASS	0.55%	PASS

The good results for the conditions written in JP and the speed-up conditions were obtained as shown in the table above, by satisfying the requirements specified in the system suitability for both conditions.

Structure



Tranexamic acid



4-aminomethylbenzoic acid

Structures are created using Chemistry 4-D Draw which is provided by ChemInnovation Software, Inc.

*This data is for reference only when selecting the columns for the customer considering the analysis based on pharmacopoeia and is not to guarantee the system suitability of the customer’ s instruments.

Products used

●Analytical column
InertSustain AQ-C18 HP
3 µm, 150 x 3.0 mm I.D.
Cat.No. 5020-89930