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 JP18 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.

Japanese Pharmacopoeia <Public comment proposal in September 2021> International harmonization of Trilateral pharmacopoeia		
Column	Functional group	Non-changeable
	Particle diameter	Totally porous particle: Specified ratio of L/dp is changeable within the range of - 25% - +50% Surface porous particle: Combination of other L and dp is allowed if theoretical plate number is within the range of - 25% - +50% of specified column.
	Length	
	Inner diameter	If there is no change in particle diameter or column length, an adjustment to the column inner diameter may be needed.
Instrument	Flow rate	By changing both column inner diameter and particle diameter, changeable by the equation below. $F_2 = F_1 \times [(d_{c2}^2 \times d_{p1}) / (d_{c1}^2 \times d_{p2})]$ * There are detailed provisions if the diameter crosses 3 um and after adjustment by changing the column particle diameter and the column length, the change is allowed within the range of +/-50%.
	Injection volume	When changing the column particle diameter and the length, injection volume can be adjusted by the equation below. $V_{inj2} = V_{inj1} \times (L_2 \times d_{c2}^2) / (L_1 \times d_{c1}^2)$
	Detection wavelength	Non-changeable
	Column temperature	+/-10℃
Mobile phase	pH	+/-0.2
	Buffer solution temperature	+/-10% within changeable pH range
	Mixing ratio	+/-30%(relative) for mobile phase with the composition of less than 50% * Within +/-10% against total

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)
* d_c 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℃
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.

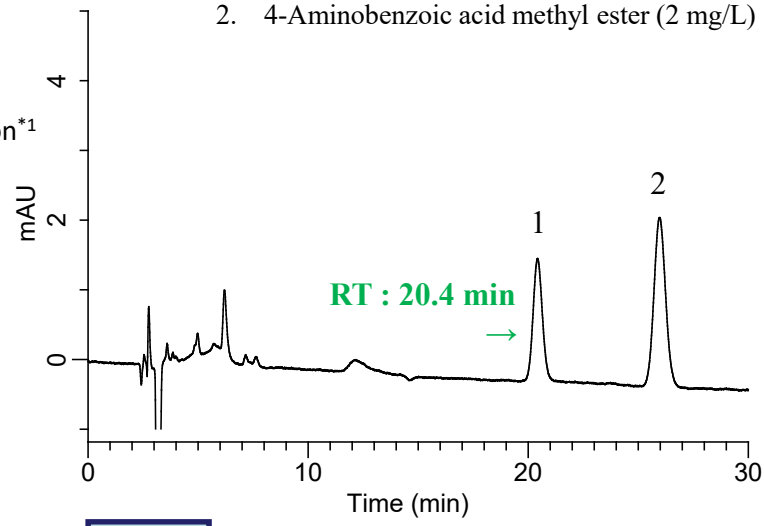
	JP conditions	Changeable range	Speed-up conditions
Column length (*L*, mm)	250 ㎜	113 – 225mm	150 mm
Column particle diameter (d_p, µm)	5 µm	-	3 µm
L/d_p	50,000	37,500 – 75,000	50,000
Inner diameter (d_c, mm)	6.0 mm	-	3.0 mm
Flow rate (*F*, mL/min)	1.4 mL/min	0.35 – 1.06mL/min	0.35mL/min
Injection volume (V_{inj}, µL)	20 µL	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 ℃
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)



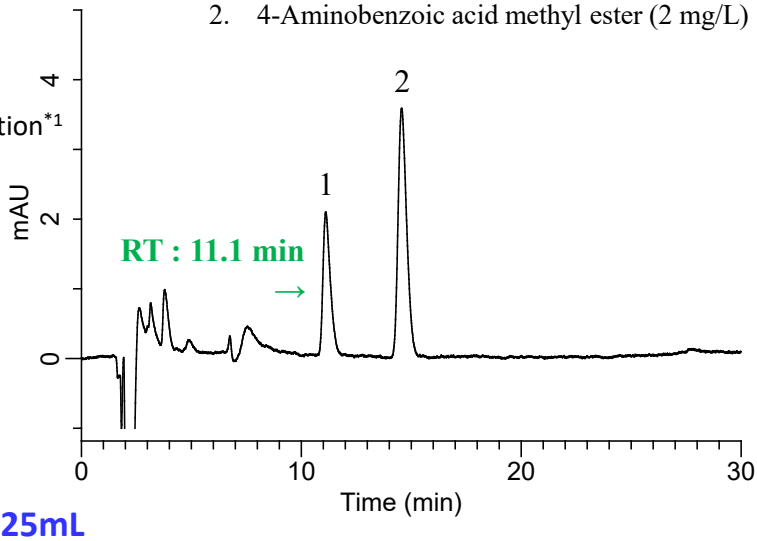
Analysis time : About 30 min.
Volume of solvent used : About 42mL

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 ℃
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.

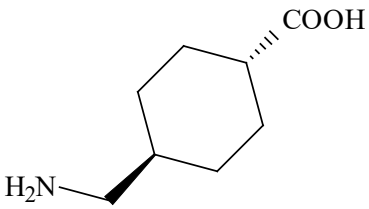
*1 Phosphoric acid buffer solution
Refer to the test conditions in the previous page.

<Result of system suitability test>

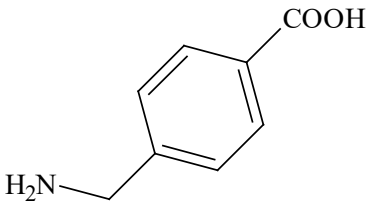
Criteria		Conditions written in JP		Speed-up conditions	
Resolution (1,2)	More than 5	6.4	PASS	5.3	PASS
RSD%(1) (n=6)	Less than 0.6%	0.02%	PASS	0.55%	PASS

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

GL Sciences Inc. Japan
22-1 Nishishinjuku 6-chome
Shinjuku-ku, Tokyo
163-1130, Japan

Phone: +81-3-5323-6620
Fax: +81-3-5323-6621
Email: world@gls.co.jp
Web: www.glsciences.com

GL Sciences Inc. USA
4733 Torrance Blvd. Suite 255
Torrance, CA 90503
USA

Phone: +1-310-265-4424
Fax: +1-310-265-4425
Email: info@glsciencesinc.com
Web: www.glsciencesinc.com

GL Sciences B.V.
Dillenburgstraat 7C
5652AM, Eindhoven
The Netherlands

Phone: +31-40-254-9531
Email: info@glsciences.eu
Web: www.glsciences.eu

GL Sciences (Shanghai) Limited
Tower B, Room 2003
Far East International Plaza
No.317 Xianxia Road, Changning District
Shanghai, China 200051

Phone: +86-21-62782272
Email: contact@glsciences.com.cn
Web: www.glsciences.com.cn

GL Sciences
International Distributors
Visit our Website at www.glsciences.com/distributors