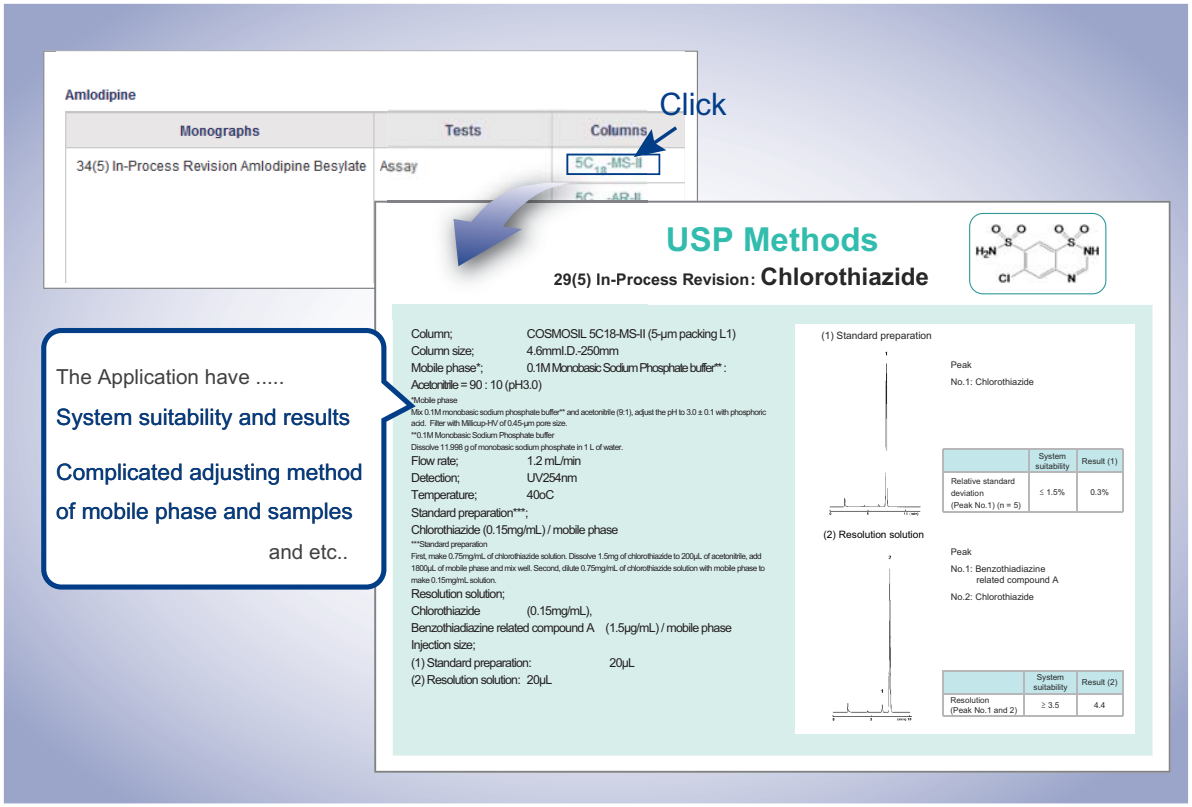
U.S. Pharmacopeia Methods for HPLC

Technical Note

Applications in accordance with U.S. Pharmacopeia Methods

Applications of USP Standars using COSMOSIL columns in accordance with the conditon specfied in USP-PF(Pharmacopoeial Forum) online is available on our website. For more information, please visit our web site at **http://www.nacalai.co.jp/global/cosmosil/**.

- ***Quickly establish QC processes for pharmaceuticals***
- ***Easily optimize analytical condition such as elution time etc.***
- ***Clearly identify your target peaks***



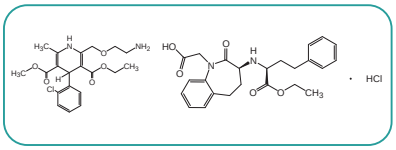
USP Standard List

1. Amlodipine
2. Amlodipine Besylate
3. Amoxicillin
4. Benazepril Hydrochloride
5. Chlorothiazide
6. Clavulanate Potassium
7. Chlorpheniramine Maleate
8. Esomeprazole Magnesium
9. Gabapentin
10. Glimepiride Glyburide
11. Hydrochlorothiazide
12. Losartan Potassium
13. Hydrochlorothiazide
14. Losartan Potassium
15. Metformin Hydrochloride
16. Omeprazole
17. Pantoprazole
18. Pantoprazole Sodium
19. Ramipril
20. Valsartan

*New Applications will be added accordingly.

USP methods

38(1) In-Process Revision: Amlodipine and Benazepril Hydrochloride Capsules



Impurities

Column; **COSMOSIL 5C$_{18}$-MS-II (5-μm packing L1)**

Column size; 4.6mmI.D.-250mm

Mobile phase; A: Acetonitrile : Buffer 1* = 20 : 80
B: Methanol : Buffer 2** = 80 : 20

*Buffer 1
0.7%(v/v) Triethylamine buffer (pH3.0) including tetrabutyl ammonium hydrogen sulfate. Dissolve 7.0mL of triethylamine in 800mL of water. Adjust the pH to 3.0 ± 0.1 with phosphoric acid. Add 1.2 g of tetrabutyl ammonium hydrogen sulfate, then dilute with water to 1 L, filter with Millicup-HV of 0.45-μm pore size.

**Buffer 2
0.7%(v/v) Triethylamine buffer (pH3.0). Dissolve 7.0mL of triethylamine in 800mL of water. Adjust the pH to 3.0 ± 0.1 with phosphoric acid, and dilute with water to 1 L, filter with Millicup-HV of 0.45-μm pore size.

Gradient;
B conc. 15→70% (0→100min), 70→15% (100→101min), 15% (101→110min)

Flow rate; 1.2 mL/min

Detection; UV237nm

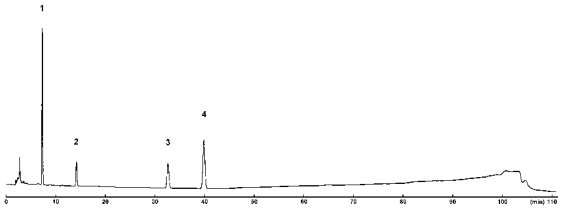
Temperature; 40ºC

Standard solution;
Amlodipine Besylate (1μg/mL),
Amlodipine related compound A (1μg/mL),
Benazepril·HCl (3μg/mL),
Benazepril related compound C (3μg/mL) / diluent***

***Diluent
Acetnitrile : Methanol : Buffer 2 = 20 : 30 : 50

Injection size; 40μL

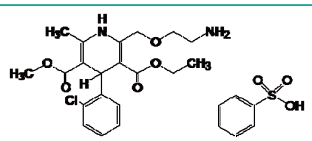
Peak
No.1: Benazepril related compound C
No.2: Amlodipine related compound A
No.3: Amlodipine
No.4: Benazepril



	System suitability	Result
Resolution (Peak No.3 and 4)	≥ 2.0	9.2
Tailing factor (Peak No.3)	≤ 2.0	1.0
Tailing factor (Peak No.4)	≤ 2.0	1.0

USP methods

34(5) In-Process Revision: Amlodipine Besylate



Assay

Column; **COSMOSIL 5C$_{18}$-MS-II (5-μm packing L1)**

Column size; 4.6mmI.D.-150mm

Mobile phase;
Methanol : Acetonitrile : 0.7%(v/v) Triethylamine buffer (pH3.0)* = 35 : 15 : 50

*0.7%(v/v) Triethylamine buffer (pH3.0)
Dissolve 7.0mL of triethylamine in 800mL of water. Adjust the pH to 3.0 ± 0.1 with phosphoric acid, and dilute with water to 1 L, filter with Millicup-HV of 0.45-μm pore size.

Flow rate; 1.0 mL/min

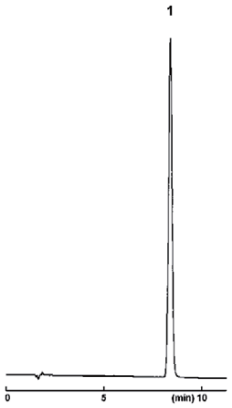
Detection; UV237nm

Temperature; 40ºC

Standard preparation;
Amlodipine Besylate (0.05mg/mL) / mobile phase

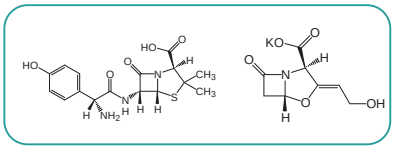
Injection size; 10μL

Peak
No.1: Amlodipine



	System suitability	Result
Relative standard deviation (n = 5)	≤ 2.0%	0.4%

USP methods



36(4) In-Process Revision: **Amoxicillin and Clavulanate Potassium for Oral Suspension**

Assay

Column; **COSMOSIL 5C$_{18}$-MS-II (5-μm packing L1)**

Column size; 4.6mmI.D.-250mm

Mobile phase; Methanol : pH 4.4 Sodium Phosphate buffer* = 5 : 95

* pH 4.4 Sodium Phosphate buffer
Dissolve 7.8 g of monobasic sodium phosphate in 900mL of water, adjust the pH to 4.4 ± 0.1 with phosphoric acid, dilute with water to make 1000 mL, and mix.

Flow rate; 2.0 mL/min

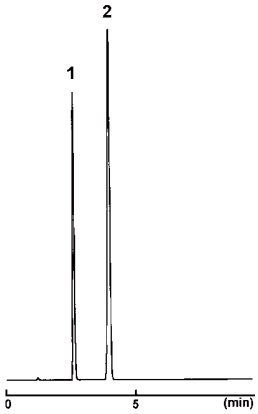
Detection; UV220nm

Temperature; 40ºC

Resolution solution;
Clavulanate Lithium (0.20mg/ml),
Amoxicillin (0.50mg/ml) / Water

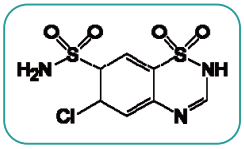
Injection size; 20 μL

Peak
No.1: Clavulanic Acid
No.2: Amoxicillin



	System suitability	Result (Peak No.1)	Result (Peak No.2)
Resolution (Peak No.1/No.2)	≥ 3.5	8.7	
Tailing factor	≤ 1.5	1.5	1.0
Relative standard deviation (n=6)	≤ 2.0%	0.3%	0.2%

USP methods



29(5) In-Process Revision: **Chlorothiazide**

Assay

Column; **COSMOSIL 5C$_{18}$-MS-II (5-μm packing L1)**

Column size; 4.6mmI.D.-250mm

Mobile phase*;
0.1M Monobasic Sodium Phosphate buffer** : Acetonitrile
= 90 : 10 (pH3.0)

*Mobile phase
Mix 0.1M monobasic sodium phosphate buffer** and acetonitrile (9:1), adjust the pH to 3.0 ± 0.1 with phosphoric acid. Filter with Millicup-HV of 0.45-μm pore size.

**0.1M Monobasic Sodium Phosphate buffer
Dissolve 11.998 g of monobasic sodium phosphate in 1 L of water.

Flow rate; 1.2 mL/min

Detection; UV254nm

Temperature; 40ºC

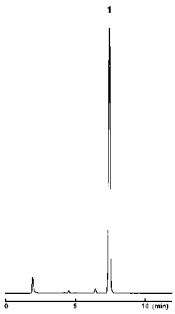
Standard preparation*;**
Chlorothiazide (0.15mg/mL) / mobile phase

***Standard preparation
First, make 0.75mg/mL of chlorothiazide solution. Dissolve 1.5mg of chlorothiazide to 200 μL of acetonitrile, add 1800 μL of mobile phase and mix well. Second, dilute 0.75mg/mL of chlorothiazide solution with mobile phase to make 0.15mg/mL solution.

Resolution solution;
Chlorothiazide (0.15mg/mL),
Benzothiadiazine related compound A (1.5 μg/mL) / mobile phase

Injection size; (1) Standard preparation: 20 μL
(2) Resolution solution: 20 μL

(1) Standard preparation



Peak
No.1: Chlorothiazide

	System suitability	Result (1)
Relative standard deviation (Peak No.1) (n = 5)	≤ 1.5%	0.3%

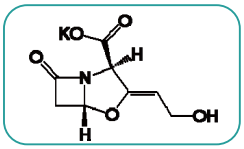
(2) Resolution solution

Peak
No.1: Benzothiadiazine related compound A
No.2: Chlorothiazide

	System suitability	Result (2)
Resolution (Peak No.1 and 2)	≥ 3.5	4.4

USP methods

34(6) In-Process Revision: Clavulanate Potassium



Chromatographic purity (1)

Column; **COSMOSIL 5C_{18}-MS-II (5-μm packing L1)**

Column size; 4.6mmI.D.-100mm

Mobile phase; A: 50mmol/l NaH_2PO_4 with H_3PO_4 (pH4.0)
B: Methanol : mobile phase A = 50 : 50

Gradient; B conc. 0%(0→4min), 0→50%(4→15min), 50%(15→18min)

Flow rate; 1.0 mL/min

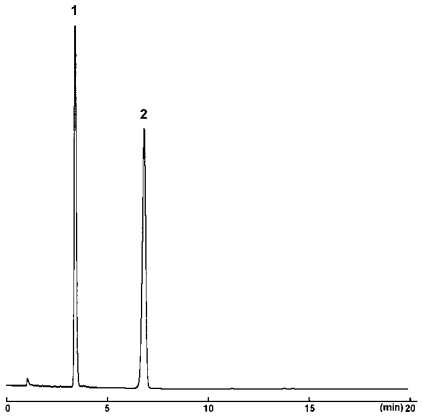
Detection; UV230nm

Temperature; 40ºC

Resolution solution;
Clavulanate Lithium (0.1mg/ml),
Amoxicillin (0.1mg/ml) / mobile phase A

Injection size; 20 μL

Peak
No.1: Clavulanic Acid
No.2: Amoxicillin

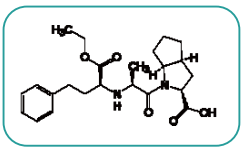


	System suitability	Result
Tailing factor (Peak No.1)	≤ 2.0	1.6
Theoretical plates (Peak No.1)	≥ 2,000	4,900
Resolution (Peak No.1/No.2)	≥ 13	13

USP-052

USP methods

31(3) In-Process Revision: Ramipril



Assay

Column; **COSMOSIL 5C_{18}-PAQ (5-μm packing L1)**

Column size; 4.6mmI.D.-150mm

Mobile phase*;
0.1% Sodium Dodecyl Sulfate Solution** : Acetonitrile = 55 : 45

*Mobile phase
Mix the 0.1% sodium dodecyl sulfate solution** and acetonitrile, adjust the pH to 2.75 ± 0.1 with phosphoric acid.

**0.1% Sodium Dodecyl Sulfate Solution
Prepare 0.1% solution of sodium dodecyl sulfate. Adjust the pH to 2.4 ± 0.1 with phosphoric acid, then filter with Millicup-HV of 0.45-μm pore size.

Flow rate; 1.8 mL/min

Detection; UV210nm

Temperature; 40ºC

System suitability preparation;
Ramipril (0.2mg/mL),
Ramipril related compound A (0.01mg/mL) / mobile phase

Injection size; System suitability preparation: 20 μL

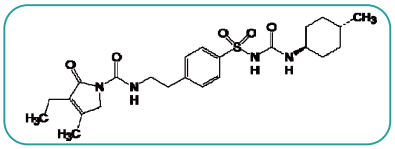
Peak
No.1: Ramipril related compound A
No.2: Ramipril

	System suitability	Result
Resolution (Peak No.1 and 2)	≥ 2.0	4.0
Theoretical plates (Peak No.2)	≥ 4,000	7,000
Relative standard deviation (n = 5) (Peak No.2)	≤ 1.0%	0.3%

USP-009

USP methods

33(3) In-Process Revision: Glimepiride Tablets



Assay

Column; **COSMOSIL 5C$_{18}$-MS-II (5-μm packing L1)**

Column size; 4.6mmI.D.-150mm

Mobile phase; Acetonitrile : Phosphate buffer* = 50 : 50

*Phosphate buffer
8.335mM monobasic sodium phosphate buffer (pH2.1 to 2.7). Dissolve 0.5 g of monobasic sodium phosphate in 500mL of water. Adjust the pH to approx. 2.6 with 10% phosphoric acid.

Flow rate; 1.0 mL/min

Detection; UV228nm

Temperature; 40°C

System suitability preparation;
Glimepiride (0.1mg/mL),
Glimepiride related compound B (0.02mg/mL),
Glimepiride related compound C (0.02mg/mL) / diluent**

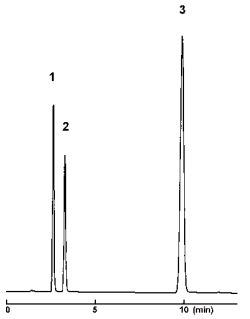
**Diluent
Acetonitrile : Water = 90 : 10

Standard preparation;
Glimepiride (0.1mg/mL) / diluent**

Injection size;
(1) System suitability preparation: 10μL
(2) Standard preparation: 10μL

(1) System suitability preparation

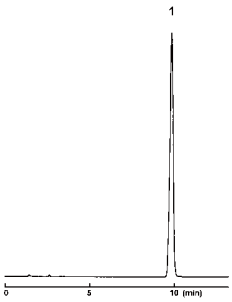
Peak
No.1: Glimepiride related compound B
No.2: Glimepiride related compound C
No.3: Glimepiride



	System suitability	Result (1)
Resolution (Peak No.1 and 2)	≥ 1.5	3.8
Tailing factor (Peak No.3)	≤ 2.0	1.2

(2) Standard preparation

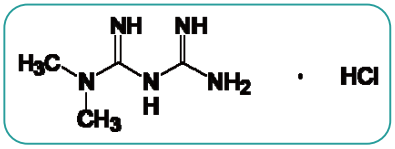
Peak
No.1: Glimepiride



	System suitability	Result (2)
Relative standard deviation (n = 5)(Peak No.1)	≤ 2.0%	0.2%

USP methods

35(6) In-Process Revision: Glyburide and Metformin Hydrochloride Tablets



Assay - Metformin Hydrochloride

Column; **COSMOSIL 5C$_{18}$-MS-II (5-μm packing L1)**

Column size; 4.6mmI.D.-250mm

Mobile phase; Acetonitrile : Buffer (pH3.85)* = 1 : 9

*Buffer (pH3.85)
0.5%(w/v) sodium heptanesulfonate / 0.5%(w/v) sodium chloride buffer. Dissolve 1.0 g of sodium heptanesulfonate and 1.0 g of sodium chloride in approx. 1.8 L of water, and mix. Adjust the pH to 3.85 with 0.06M phosphoric acid, dilute with water to 2 L, filter with Millicup-HV of 0.45-μm pore size.

Flow rate; 1.0 mL/min

Detection; UV218nm

Temperature; 30°C

Standard solution;
Metformin·HCl (0.25mg/mL) / Diluent**

**Diluent
Acetonitrile : water = 1 : 40

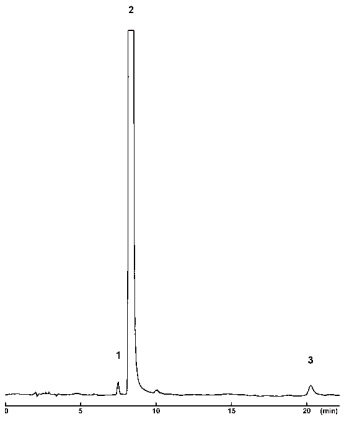
System suitability stock solution;
Metformin related compound B
Metformin related compound C
25μg/mL each / Diluent**

System suitability solution*;**
Metformin·HCl (0.25mg/ml),
Metformin related compound B (0.25μg/mL),
Metformin related compound C (0.25μg/mL) / Diluent**

***System suitability solution
Add standard solution to 0.5mL of system suitability stock solution to make 50mL

Injection size; 5μL

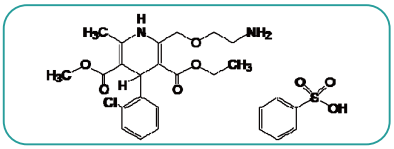
Peak
No.1: Metformin·related compound B
No.2: Metformin
No.3: Metformin related compound C



	System suitability	Result
Resolution (Peak No.1 and 2)	≥ 1.5	2.7
Tailing factor (Peak No.2)	0.8~2.0	2.0
Relative standard deviation (n = 6) (Peak No.1)	≤ 10%	2%
Relative standard deviation (n = 6) (Peak No.2)	≤ 1.5%	0.5%
Relative standard deviation (n = 6) (Peak No.3)	≤ 10%	6%

USP methods

36(2) In-Process Revision: Amlodipine Besylate Tablets



Assay

Column; **COSMOSIL 5C_{18}-MS-II (5-μm packing L1)**

Column size; 4.6mmI.D.-150mm

Mobile phase;
Methanol : Acetonitrile : 0.7%(v/v) Triethylamine buffer (pH3.0)*
= 35 : 15 : 50

*0.7%(v/v) Triethylamine buffer (pH3.0)
Dissolve 7.0mL of triethylamine in 800mL of water. Adjust the pH to 3.0 ± 0.1 with phosphoric acid, and dilute with water to 1 L, filter with Millicup-HV of 0.45-μm pore size.

Flow rate; 1.0 mL/min

Detection; UV237nm

Temperature; 40°C

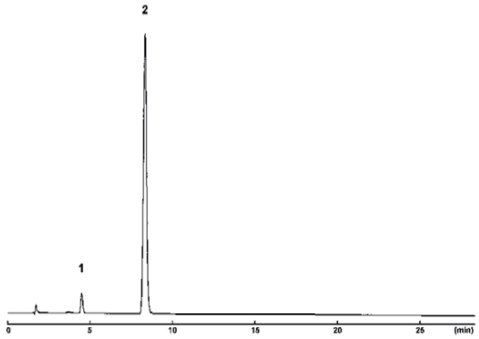
System suitability solution;
Amlodipine Besylate (20 μg/mL),
Amlodipine related compound A (2 μg/mL) / mobile phase

Injection size; 50 μL

Peak

No.1: Amlodipine related compound A

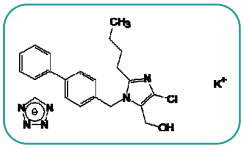
No.2: Amlodipine



	System suitability	Result
Resolution (Peak No.1 and 2)	≥ 8.5	10.8
Tailing Factor (Peak No.1)	≤ 2.0	1.2
Tailing Factor (Peak No.2)	≤ 2.0	1.3
Relative standard deviation (n = 6) (Peak No.1)	≤ 5.0%	3.5%
Relative standard deviation (n = 6) (Peak No.2)	≤ 1.0%	1.0%

USP methods

34(3) In-Process Revision: Losartan Potassium



Assay

Column; **COSMOSIL 5C_{18}-MS-II (5-μm packing L1)**

Column size; 4.6mmI.D.-250mm

Mobile phase;
0.1% (v/v) Phosphoric Acid* : Acetonitrile = 60 : 40

*0.1% (v/v) phosphoric acid
Dissolve 1176.5 μL of phosphoric acid (85%) to water, add water to 1 L.

Flow rate; 1.0 mL/min

Detection; UV254nm

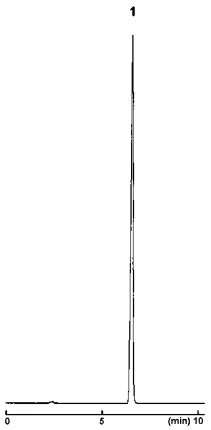
Temperature; 35°C

Standard preparation;
Losartan Potassium (0.25mg/mL) / Methanol

Injection size; 10 μL

Peak

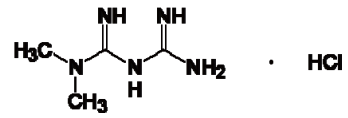
No.1: Losartan



	System suitability	Result
Theoretical plate	≥ 5,600	15,200
Tailing factor	≤ 1.4	1.0
Relative standard deviation (n = 5)	≤ 0.5%	0.4%

USP methods

33(2) Second Interim Revision: Metformin Hydrochloride Tablets



Dissolution–Test 3

Column; **COSMOSIL 5C$_{18}$-MS-II (5-μm packing L1)**

Column size; 4.6mmI.D.-250mm

Mobile phase; Acetonitrile : Buffer (pH3.0)* = 1 : 19

*Buffer (pH3.0)
10mM Sodium 1-pentanesulfonate / 0.05M monobasic sodium phosphate buffer. Dissolve 1.38 g of monobasic sodium phosphate in approx. 1.8 L of water. Add 3.484 g of 1-pentansulfonic acid sodium salt, and mix. Adjust the pH to 3.00 ± 0.05 with diluted phosphoric acid. Add water to make 2 L, filter with Millicup-HV of 0.45-μm pore size.

Flow rate; 1.0 mL/min

Detection; UV230nm

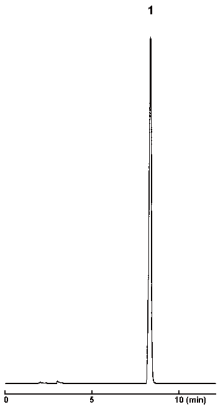
Temperature; 40°C

Standard solution;
Metformin·HCl (0.1mg/mL) / 0.2M Monobasic Potassium Phosphate buffer (pH6.8)

Injection size; 40 μL

Peak

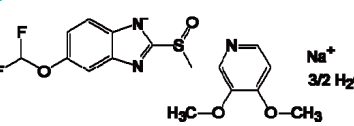
No.1: Metformin·HCl



	System suitability	Result
Tailing factor	≤ 2.0	1.1
Theoretical plate	≥ 1,500	17,800
Relative standard deviation (n = 5)	≤ 2.0%	0.2%

USP methods

34(3) Third Interim Revision Announcement: Pantoprazole Sodium



Assay

Column; **COSMOSIL 5C$_{18}$-MS-II (5-μm packing L1)**

Column size; 4.6mmI.D.-150mm

Mobile phase; A; Acetonitrile - Methanol Mixture* : Buffer** = 15 : 85
B; Acetonitrile - Methanol Mixture*

*Acetonitrile - Methanol Mixture
Acetonitrile : Methanol = 70 : 30

**Buffer
10mM Ammonium phosphate buffer. Dissolve 1.32 g of dibasic ammonium phosphate in 1 L of water. Adjust the pH to 7.5 with phosphoric acid. Filter with Millicup-HV of 0.45-μm pore size.

Gradient; B conc. 14% (0→10min), 14→58% (10→35min), 58→14% (35→36min), 14% (36→46min)

Flow rate; 1.0 mL/min

Detection; UV285nm

Temperature; 30°C

System suitability preparation*;**
Pantoprazole·Na
Pantoprazole related compound A
Pantoprazole related compound B
5 μg/mL each in Acetonitrile : Water = 1 : 1, Diluent†

***System suitability preparation
Dissolve pantoprazole·Na, pantoprazole related compound A and pantoprazole related compound B in the mixture of acetonitrile and water (1:1) to obtain 0.5mg of each component per mL. Dilute this solution with diluent† to obtain 5 μg of each component per mL.

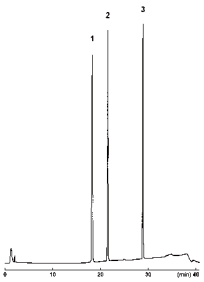
†Diluent
0.28% Ammonia water. Transfer 2.5mL of ammonium hydroxide to 50-mL volumetric flask, and dilute with water to volume.

Standard preparation††;
Pantoprazole·Na (0.06mg/mL) / Acetonitrile : Water = 1:1, Diluent†

††Standard preparation
Transfer 4.0mg of pantoprazole·Na to a 10-mL volumetric flask, dissolve in 1.4mL of a mixture of acetonitrile and water (1:1), and dilute with diluent† to volume. Further dilute with diluent† to obtain a solution contains 0.06mg of pantoprazole·Na per mL.

Injection size; (1) System suitability preparation: 20 μL
(2) Standard preparation: 20 μL

(1) System suitability preparation



Peak

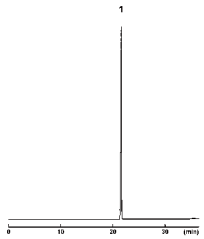
No.1: Pantoprazole related compound A

No.2: Pantoprazole

No.3: Pantoprazole related compound B

	System suitability	Result (1)
Resolution (Peak No.1 and 2)	≥ 10.0	12.5

(2) Standard preparation



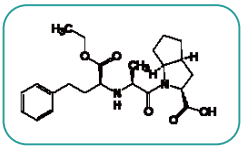
Peak

No.1: Pantoprazole

	System suitability	Result (2)
Relative standard deviation (n = 5) (Peak No.1)	≤ 2.0%	0.6%

USP methods

31(3) In-Process Revision: Ramipril



Assay

Column; **COSMOSIL 5C_{18}-MS-II (5-μm packing L1)**

Column size; 4.6mmI.D.-150mm

Mobile phase*;
0.1% Sodium Dodecyl Sulfate Solution** : Acetonitrile = 55 : 45

*Mobile phase
Mix the 0.1% sodium dodecyl sulfate solution** and acetonitrile, adjust the pH to 2.75 ± 0.1 with phosphoric acid.

**0.1% Sodium Dodecyl Sulfate Solution
Prepare 0.1% solution of sodium dodecyl sulfate. Adjust the pH to 2.4 ± 0.1 with phosphoric acid, then filter with Millicup-HV of 0.45-μm pore size.

Flow rate; 1.8 mL/min

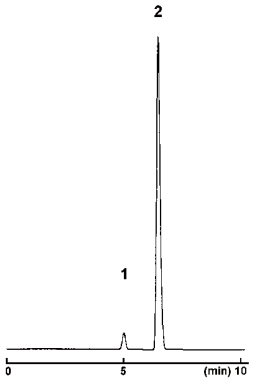
Detection; UV210nm

Temperature; 40°C

System suitability preparation;
Ramipril (0.2mg/mL),
Ramipril related compound A (0.01mg/mL) / mobile phase

Injection size; System suitability preparation: 20 μL

Peak
No.1: Ramipril related compound A
No.2: Ramipril



	System suitability	Result
Resolution (Peak No.1 and 2)	≥ 2.0	5.1
Theoretical plates (Peak No.2)	≥ 4,000	8,200
Relative standard deviation (n = 5) (Peak No.2)	≤ 1.0%	0.7%

USP-007

COSMOSIL USP List

USP No.	Phase	USP Description	Product Name
L01	C_{18}	Octadecyl silane <ODS or C_{18}> chemically bonded to porous silica or ceramic particles, 1.5 to 10 µm in diameter, or a monolithic rod.	COSMOSIL 3C_{18}-EB COSMOSIL 5C_{18}-MS-II COSMOSIL 5C_{18}-AR-II COSMOSIL 5C_{18}-PAQ COSMOSIL 5C_{18}-AR-300 COSMOSIL 2.5C_{18}-MS-II
L03	SIL	Porous silica particles, 5 to 10 µm in diameter, or a monolithic rod.	COSMOSIL 5SL-II
L07	C_8	Octylsilane <C_8> chemically bonded to porous silica particles, 1.5 to 10 µm in diameter, or a monolithic rod.	COSMOSIL 5C_8-MS COSMOSIL 5C_8-AR-300
L10	CN	Nitrile groups <CN> chemically bonded to porous silica particles, 3 to 10 µm in diameter.	COSMOSIL 5CN-MS
L11	Ph	Phenyl groups chemically bonded to porous silica particles, 1.5 to 10 µm in diameter.	COSMOSIL 5PE-MS COSMOSIL 5Ph-AR-300
L13	C_1	Trimethylsilane <C_1> chemically bonded to porous silica particles, 3 to 10 µm in diameter.	COSMOSIL 5TMS-MS
L20	Diol	Dihydroxypropane groups chemically bonded to porous silica particles, 5 to 10 µm in diameter.	COSMOSIL Diol-120-II COSMOSIL Diol-300-II
L26	C_4	Butyl silane <C_4> chemically bonded to porous silica particles, 3 to 10 µm in diameter.	COSMOSIL 5C_4-MS COSMOSIL 5C_4-AR-300
Lxx (Coming soon)	Pyrene	Pyrenyl groups chemically bonded to porous silica particles, 1.5 to 10 µm in diameter, or a monolithic rod	COSMOSIL PYE

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