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Application News

High Performance Liquid Chromatograph iSeries LC-2050C

HPLC Analysis for Rebamipide in Accordance with Japanese Pharmacopoeia

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User Benefits

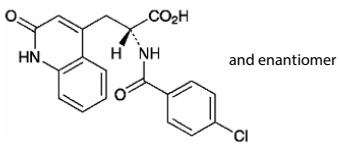
- Purity test for Rebamipide (related substances) can be performed in accordance with Japanese Pharmacopoeia.
- Shim-pack™ GIST C18 provides good separation for rebamipide and related substances.

■ Introduction

Rebamipide has been used as a therapeutic drug for gastric ulcers for a long time. It promotes production of prostaglandins to mucosa that protects the stomach wall, resulting in suppression of inflammation.

This article presents an analysis conducted in accordance with Japanese Pharmacopoeia using HPLC LC-2050 3D, an integrated HPLC.

■ Analysis Conditions (in accordance with Japanese Pharmacopoeia)



and enantiomer

Fig. 1 Structural formula of rebamipide

In accordance with purity test of rebamipide (related substances) described in 18th Revised Japanese Pharmacopoeia, it is required to confirm three items as system suitability: confirmation of detection, system performance, and system repeatability.

These items were confirmed as follows,

Purity (4) Related substances:

In “test for required detectability”, the standard solution and the detectability confirming solution were analyzed to be compared with peak areas. In “system performance”, the system performance confirming solution was measured to confirm the peak resolution. In “system repeatability”, the standard solution was analyzed six times repeatedly to confirm the relative standard deviation of peak areas.

The analytical conditions for the purity test of rebamipide (related substances) are shown in Table 1.

Shim-pack GIST C18 was used in this study since the stationary phase of the analytical column is specified as octadecylsilylated silica gel (ODS).

Table 1 Analytical conditions employed for LC-2050C 3D

System	:	LC- 2050C 3D
Column	:	Shim-pack GIST C18 *1 (250 mm × 4.6 mm I.D., 5 µm)
Column Temp.	:	40 ℃
Mobile Phase	:	Sodium 1-decanesulfonate aqueous solution*2/ methanol/phosphoric acid =100:100:1(v/v/v)
Flow Rate	:	1.0 mL/min*3
Injection Volume	:	10 µL
Detection	:	UV at 232 nm
Vial	:	Shimadzu LabTotal for LC 1.5mL, Glass *4

*1 P/N: 227-30017-08

*2 Mixture of 1000 mL of 2.44 g/L sodium 1-decanesulfonate aqueous solution, 1000 mL of methanol, and 10 mL of phosphoric acid (85%)

*3 Adjusted to make the retention time of rebamipide as around 12 min

*4 P/N: 227-34001-01

■ Sample Preparation

The preparation methods for the standard and sample solutions and the solutions used for the system suitability test are listed in Table 2.

The solutions for this purity test (related substances) were the standard and sample solutions for the purity test (rebamipide *m*-chloro isomer).

Table 2 Preparation methods for solutions

Sample solution	:	Dissolve 40 mg of rebamipide in 100 mL of sample preparation solvent*5.
Standard solution	:	Pipet 2 mL of sample solution, and add sample preparation solvent to make exactly 20 mL. Pipet 2 mL of this solution, and add sample preparation solvent to make exactly 20mL.
<System suitability>		
Test for required detectability	:	Pipet 5 mL of standard solution, and add sample preparation solvent to make exactly 50 mL
System performance	:	Dissolve 20 mg of 4-chlorobenzoate in methanol to make 50 mL. To 5 mL of this solution add 5 mL of sample solution and sample preparation solvent to make 50 mL.
System repeatability	:	Standard solution

*5 Sample preparation solvent：Mixture of water/0.05 mol/L phosphate buffer (pH=6.0)*6 /methanol=7:7:6

*6 0.05 mol/L phosphate buffer (pH=6.0) : To 50 mL of 0.2 mol/L test solution for buffer*7, add 5.7 mL of 0.2 mol/L sodium hydroxide test solution*8 and water to make 200 mL.

*7 Dissolve 27.218 g of potassium dihydrogen phosphate for pH measurement to water to make 1000 mL.

*8 Dissolve 8.0 g of sodium hydroxide to freshly boiled and cooled water to make 1000 mL (To be prepared on demand) .

■ System Suitability

Chromatograms obtained in "test for required detectability" and "system performance" are shown in Fig. 2 and Fig. 3, respectively.

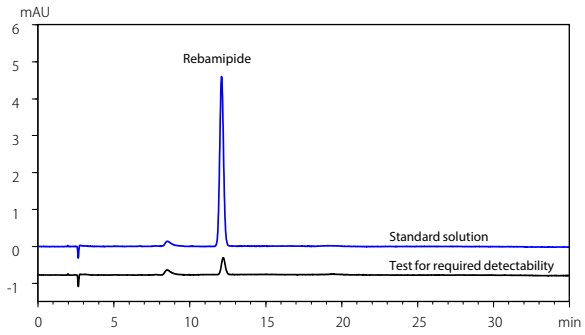


Fig. 2 Chromatogram- test for required detectability

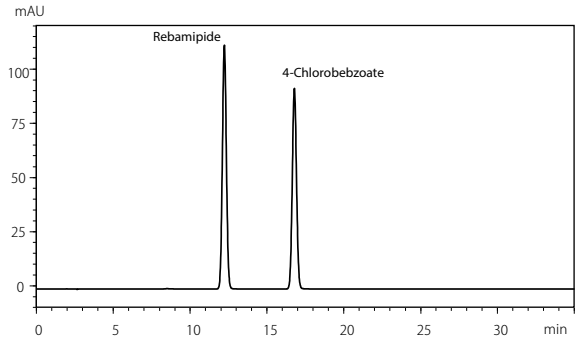


Fig. 3 Chromatogram- system performance

In "test for required detectability", 10-fold diluted standard solution was analyzed and the peak area ratio was 9.9%, which met the criterion.

In "system performance", 4-chlorobenzoic acid and rebamipide eluted in that order, with a resolution of 9.3. "System repeatability" showed that the relative standard deviation of peak areas of rebamipide in the standard solution (6 replicates) was 0.12%. A summary of criteria and results for the system suitability test is shown in Table 3.

■ Analysis of Sample Solution

Fig. 4 shows the chromatogram of the sample solution and its enlarged chromatogram around 5 to 20 min.

For the sample solution, two compounds are specified to be confirmed: rebamipide *o*-chloro isomer with a relative retention time of 0.5 and rebamipide debenzoyl form with a relative retention time of 0.7. The relative retention times of these two compounds were 0.53 and 0.68 respectively, which were confirmed to be close to the mentioned values.

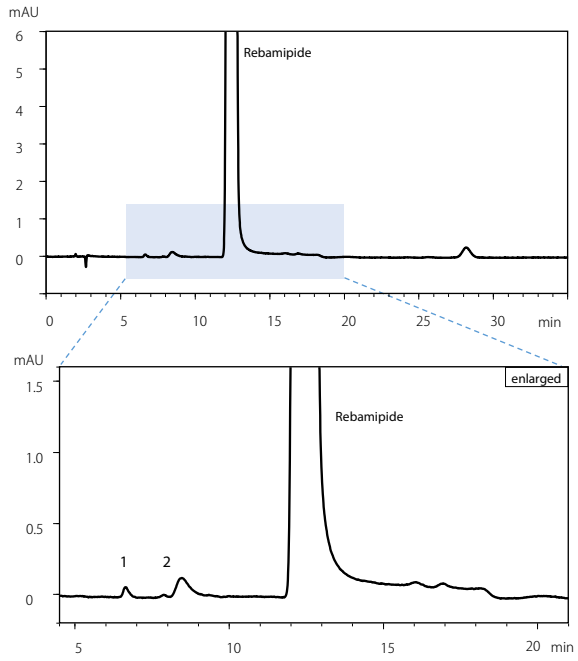


Fig. 4 Chromatogram- sample solution

1: Rebamipide *o*-chloro isomer Relative retention time 0.5
2: Rebamipide debenzoyl form Relative retention time 0.7

■ Conclusion

In this article, HPLC analyses were conducted using integrated HPLC LC-2050 3D in accordance with the system suitability test for the purity test of rebamipide (related substance) listed in Japanese Pharmacopoeia, 18th Revised Edition. As a result, it was confirmed that test for required detectability, system performance, and system reproducibility all met the criteria of Japanese Pharmacopoeia.

Table 3 Results of system suitability test

System Suitability items	Test item	Criterion	Result	Judgement
Retention selectivity	Area ratio	7 – 13 %	9.9 %	passed
System performance	Resolution	≥ 8	9.3	passed
System repeatability (standard solution)	Relative standard deviation	≤ 2.0 %	0.12 %	passed

<References>

"Rebamipide", Japanese Pharmacopoeia, 18th Revised Edition

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