3.2.S.3 Characterization

3.2.S.3.1 Description of structure and other characteristics

L-Ornithine L-Aspartate is a complex of basic amino acids (ornithine) and acidic (aspartic acid) amino acids. Ornithine has α amino and α carboxyl groups, with an amino group at the other end, and aspartate has α amino and α carboxyl groups, with a carboxyl group at the other end. The molecular ions of ornithine and aspartic acid have positive and negative charges respectively. Ornithine aspartic acid can be obtained by the reaction of ornithine and aspartic acid in the aqueous phase. The crystallization of L-Ornithine L-aspartate is soluble in water, and the amino and carboxyl groups are dissociated in the divalent ionic solution.

Confirm that the L-Ornithine L-Aspartate manufactured by our company meets the specification. (Refer to 3.2.S.5 Reference standard)

1) The nuclear magnetism

1.1) Instrument: Germany Brooker

1.2）Specifications：AV II- 500 BKUKER

1.3) Equipment name：nuclear magnetic resonance instrument, made in Switzerland.

1.4) Solvent: D_2O

1.5) Method: take the appropriate amount of L-Ornithine L-Aspartate sample (Batch No. C552202010) and reference standard (Batch No. 07125-SLBW4848) at room temperature dissolve it in D2O and analyzed by NMR at 298K.

1.6) Result

1.6.1) Hydrogen (H) spectrum analysis results of L-Ornithine L-Aspartate sample and reference standard

Chemical shift(δ)		Proton number	Multiplicity	Hydrogen identification
Sample (C552202010))	Standard product (07125-SLBW4848)			

1.716-1.743	1.694-1.709	2	m	H-2
1.787-1.805	1.769-1.783	2	m	H-3
2.622-2.809	2.615-2.783	2	m	H-8
3.011-3.041	2.989-3.019	2	t	H-1
3.745-3.769	3.727-3.751	1	t	H-4
3.858-3.883	3.838-3.862	1	dd	H-7

1.6.2) Carbon (C) spectrum analysis results of L-Ornithine L-Aspartate sample and reference standard

Chemical shift(δ)		Carbon type	Carbon identification
Sample (C552202010))	Standard product (07125-SLBW4848)		
22.12	22.13	Secondary carbon	C-2
26.79	26.81	Secondary carbon	C-3
35.93	35.93	Secondary carbon	C-1
38.24	38.27	Secondary carbon	C-8
51.56	51.62	Tertiary carbon	C-7
53.48	53.51	Tertiary carbon	C-4
173.53	173.51	Quaternary carbon	C-5
173.53	173.64	Quaternary carbon	C-6
176.92	176.96	Quaternary carbon	C-9

1.7) Hydrogen spectrum analysis 1H-NMR

In the hydrogen nuclear magnetic resonance spectrum (^{1}H-NMR), the hydrogen proton patterns of the sample and the standard are consistent. Both integral values show 10 hydrogen protons instead of 19, which is due to the rapid exchange of 9 active hydrogens (3 -OH and 3 -NH_2) with deuterium in the molecular formula. Therefore, these nine hydrogen proton peaks are not shown.

1.8) Carbon spectrum analysis ^{13}C-NMR

In the hydrogen nuclear magnetic resonance spectrum (^{13}C-NMR), the carbon spectra of the sample and the standard are consistent. The total number of peaks in each group is 9. It is shown

that there are 9 carbons in the molecular structure, which is consistent with the molecular carbon number of L-ornithine-L-aspartate.

1.9) Conclusion

It can be seen from the H NMR spectrum that the carbon spectrum and H spectrum of the L-ornithine-L-aspartate sample are consistent with the standard substance, the carbon and hydrogen protons are consistent, and the two structures are completely consistent.

1.10) Nuclear magnetic spectrum

Appendix 8: Hydrogen spectrum analysis ^{1}H-NMR

Appendix 9: Sample hydrogen spectrum ^{1}H-NMR

Appendix 10: Standard carbon spectrum ^{13}C-NMR

Appendix 11: Sample carbon spectrum ^{13}C-NMR

2) Mass spectrum

2.1) Instrumentation: Thermofisher LTQ

2.2) Test data

Batch number	Mass-to-charge ratio(m/z)	Relative abundance	Remarks
Sample	264.90	100	---
Standard product	264.18	100	---

2.3) Analysis

The comparison between the sample and the standard sample was basically consistent.

2.4) Mass spectrogram

Appendix 12: Mass spectrum

3) Elemental analysis

3.1) Instrument: Model Vario EL III CHNSO, Germany

3.2) Results

Element		Actual test value (%)	Theoretical value (%)	Atomic weight
Standard	C	39.49%	40.71%	12.0107
	H	7.50%	7.22%	1.00794
	N	15.21%	15.84%	15.9999
sample	C	39.96%	40.71%	12.0107
	H	7.49%	7.22%	1.00794
	N	15.63%	15.84%	15.9999

3.3) Conclusion

The results of elemental analysis showed that the proportion of C, H and N in L-Ornithine L-aspartate sample was consistent with that of reference standard.

Appendix 13 Elemental Analysis Spectrum

4) X-ray diffraction

4.1) Instruments: Bruker D8 Advance, Germany

4.2) Sample preparation: Sample (Batch No. C552202010), Reference Standard (Batch No. 07125-SLBW4848) are directly compressed to tablet and tested on the machine.

4.3) Conclusion: the main diffraction peak location of the sample is the same with that of the reference standard, and the crystal from is comparable.

4.4) X-ray Diffraction Pattern

Appendix 14: X-ray diffraction patterns of Sample

Appendix 15: Standard X-ray diffraction patterns

5) Infrared

5.1) Instrument: FTMIC-IR-RAM Type: VERTEX 70 (Fourier transform microinfrared Raman HYPERION-VERTEX70-RAMII)，BRUCKNER

5.2) Test method: KBr tabletting

5.3) Test results

The infrared absorption spectrum of the L-ornithine-L-aspartate sample ((Batch No. C552202010)is consistent with the infrared absorption spectrum of the reference substance（(Batch No. 07125-SLBW4848）

5.4) Infrared spectrum

Appendix 16: Sample infrared spectrum

Appendix 17:Standard infrared spectrum