L-theanine Quality Standard and Operation Procedures for Test Standard

English Name: L-Theanine(N-ethylglutamine)
Molecular Formula:C7H14N2O3　Molecular Weight: 174.20
Character：White Crystal powder,odorless, a little sweet characteristic taste,threshold value:0.15%。Decomposition temperature:214~215℃。Soluble in water,insoluble in ethanol,aether

Quality Standard

Items	Index	Ref. edition
Character	White Crystal powder	
Assay	98.0%~102.0%	HPLC
Specific Rotation [α]20/D	＋7.7°~＋8.5°	ChP2010
Solution Appearance	Colourless,transparent	
Chloride	≤0.020%	ChP2010
Drying on loss	≤0.5%	ChP2010
Residual on ignition	≤0.2%	ChP2010
PH	5.0~6.0	ChP2010
Melting point	202～215℃	ChP2010
Heavy metal	≤10PPm	ChP2010
Arsenic	≤4PPm	ChP2010
Total Plate Count	≤1000cfu/g	ChP2010
Mold & Yeast	≤100cfu/g	ChP2010
E.Coli.,	Absent	ChP2010
Salmonella	Absent	ChP2010

Operation Procedures for Test Standard
1 Apparatuses
1.1 Oven
1.2 Resistance stove case
1.3 High efficiency liquid chromatograph
1.4 Rotation apparatus
2 .Operation Methods
2.1 Characters
2.1.1 Eyes:White Crystal powder,odorless, a little sweet characteristic taste.
Soluble in water,insoluble in ethanol,aether
2.1.2 Solution Appearance(1.0g/20mlH2O):colourless,transparent。
2.1.3 Melting point: 202～215℃ according to ChP2010（appendixⅦC）
2.2 Drying on loss
2.2.1 apparatus,equipment
2.2.2.1 weighing bottle with cover on grinding mouth-diameter:50mm height:30mm
2.2.2.2 Galvanothermy constant temperature drying case,able to keep temperature 105±2℃。
2.2.2 Analysis steps
Weigh 1g sample with precision to .0002g, put into weighing bottle dried to constant weight in105±2℃ in advance,slightly open the cover,and put into the

drying case near level position of thermometer's mercury ball,until constant weight in 105±2℃. Take final test data as test result.The loss weight is not over 0.5% based on ChP2010（appendixⅨG）

2.2.3 Analysis result description

Water(H2O)(X1),with mass percentage(%)as follows：

$$X1 = \frac{m1 - m2}{m} \times 100$$

m1 — mass for weighing bottle+sample before drying g；

m2 — mass for weighing bottle+sample after drying g；

m — sample mass g。

2.3 Residual on ignition:according to ChP2010（appendix ⅨJ),max.0.1%。

2.4 Chloride：weigh sample 0.5g，add thin nitric acid 10ml. After dissolving，add water to 50ml. Based on ChP2010（appendix ⅨC),compared with comparison solution made by standard sodium chloride 10.0ml ,not over (0.020%)。

2.5 PH value:weigh the sample 1g,put into 100ml volume flask, and add proper distilled water for dissolving and diluting to scale。With clean beaker taking qualified solution around 50ml ,and corrected acidity meter testing directly,PH should be 5.0~6.0 based on ChP2010（appendix ⅦG）

2.6 Specific Rotation:Take sample 2.5g dissolved by distilled water and diluted to 50ml,and adjust solution temperature to 20℃,put into1dm rotation tube.Test based on ChP2010 (appendix ⅦE),the specific rotation should be ＋7.7°~＋8.5°。

2.7 Arsenic content：Take sample1.0g，put into bottle A ,and according to preparation of standard arsenic spot,from "add potassium iodide reagent 5ml”on，operate ChP2010（appendix ⅨF）（No.1）,and make comparison between made arsenic spot and the counterpart made by standard arsenic solution 4.0ml,not more deeper(4ppm)

2.8 Heavy metal Take sample1.0g，after dissolving with distilled water 20ml，add acetate buffer (pH3.5)2ml and suitable water to 25ml，test based on ChP2010 (appendix ⅨE)（No 1）,the heavy metal cannot be over10PPm。

2.9 Assay

2.9.1 Apparatus:high-efficiency liquid chromatograph.

2.9.2 Reagents

2.9.2.1 Acetonitrile (chromatogram grade).

2.9.2.2 Phosphorus acid (analysis grade).

2.9.3 Standard sample

2.9.4 Chromatogram conditions

2.9.4.1 Chromatogram pole:filling L1（C18 pole）,4.6 mm×250mm

2.9.4.2 Flow phase: acetonitrile:1% phosphorus acid（5:95）

2.9.4.3 Detection wave length:199nm

2.9.4.4 Flow speed:1ml/min

2.9.4.5 Sample injection quantity:20μL。
2.9.5 Standard sample solution
Take L-theanine standard sample 0.5g,precisely weigh,and dissolved with flow phase and diluted to 50.0ml.Precisely take 2ml,put into 50ml volume flask,and diluted to scale with flow phase。
2.9.6 Intended sample solution
Take sample 0.5g,precisely weigh,and dissolved with flow phase and diluted to 50.0ml.Precisely take 2ml,and put into 50ml volume flask,diluted to scale with flow phase.
2.9.7 Test
Prepare apparatus according to operation procedures of high-efficiency liquid chromatograph,after balance,take standard solution and intended solution for injection,record chromatogram until 1.5 times of the peak reservation time.
2.9.8 Calculation formula

$$Assay(\%) = W_s X A_i X 100\% / As x Wi$$

A_i→ L-theanine peak area of intended sample solution
W_s →standard solution sample weight；
As→L-theanine peak area of standard solution；
Wi→Intended sample weight;