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世界中医药学会联合会中药专业委员会

Specialty Committee of Chinese Materia Medica of WFCMS

SCM-C 0069-2025

阿胶

Asini Corii Colla

（发布稿，Specialty Committee Standard）

世界中联分支机构标准

International Standard of WFCMS

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前　言

请注意本文件的某些内容可能涉及专利。本文件的发布机构不承担识别专利的责任。

主要起草单位：东阿阿胶股份有限公司。

参与起草的单位：山东省食品药品检验研究院、南昌大学、四川大学、青岛海洋药物研究院、黑龙江中医药大学、江西师范大学、华润堂有限公司 CR Care Co., Ltd..、华辉济世（HYGIA HEALTH CARE）、德胜行有限公司、善康国际（澳门）药业有限公司、山东东阿黑毛驴牧业科技有限公司。

主要起草人：刘海滨、段小波、郭尚伟、张莹、田乐园、程杰、郑梁、葛楠、田潇、孙阳恩、王梦园、刘明浩、门翠翠、刘本玉、翟昌琪。

参与起草人及审阅专家（按姓氏拼音排序）：蔡英杰（中国澳门）、嵇传良、李剑扬（中国香港）、林永强、刘维岩、牛伟霞、庞慧慧、沙小梅、史兆松、涂宗财、唐文、王喜军、王辉、王亦芝（中国香港）、杨金波、于杰、周建飞、张守元。

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阿胶

1 范围

本文件规定了阿胶的术语和定义、质量要求及一般特征、试验方法、包装、运输、贮存、标志与标签。

本文件适用于阿胶药材的生产、运输和经营企业，检验检测机构等也可参照使用。

2 规范性引用文件

下列文件中的内容通过文中的规范性引用而构成本文件必不可少的条款。其中，注日期的引用文件，仅该日期对应的版本适用于本文件；不注日期的引用文件，其最新版本（包括所有的修改单）适用于本文件。

ISO 18664 Traditional Chinese Medicine — Determination of heavy metals in herbal medicines used in Traditional Chinese Medicine

ISO 21371 Traditional Chinese medicine — Labelling requirements of products intended for oral or topical use

ISO 23723 Traditional Chinese medicine — General requirements for herbal raw material and materia medica

ISO 22467 Traditional Chinese medicine — Determination of microorganisms in natural products

3 术语和定义

下列术语和定义适用于本文件。

3.1

阿胶

以驴的干燥皮或鲜皮，经浸泡去毛，切块洗净，水煎，浓缩，添加或不添加适量的黄酒、冰糖及豆油而制成的固体胶。

3.2

驴皮

为马科动物驴 Equus asinus L.的干燥皮或鲜皮。

4 质量要求

4.1 性状特征

本品呈长方形块、方形块或丁状。棕色至黑褐色，有光泽。质硬而脆，断面光亮，碎片对光照视呈棕色半透明状。气微，味微甘。

4.2 鉴别

以质荷比（m/z）539.8（双电荷）→612.4 和 m/z539.8（双电荷）→923.8 离子对提取的供试品离子流色谱中，应同时呈现与对照药材色谱保留时间一致的色谱峰。

4.3 特征图谱

以规定质荷比离子对提取的供试品离子流色谱中，应呈现与对照药材参照物色谱相对应的 7 个特征峰，其中 2 个峰应分别与相应对照品参照物峰的保留时间相对应，以峰 4（驴源多肽 A_1 参照物峰）为 S 峰，计算峰 1、峰 2、峰 3、峰 6、峰 7 与 S 峰的相对保留时间，应在规定值的±15%范围之内，规定值为：0.16（峰 1）、0.23（峰 2）、0.50（峰 3）、1.25（峰 6）、1.32（峰 7）。

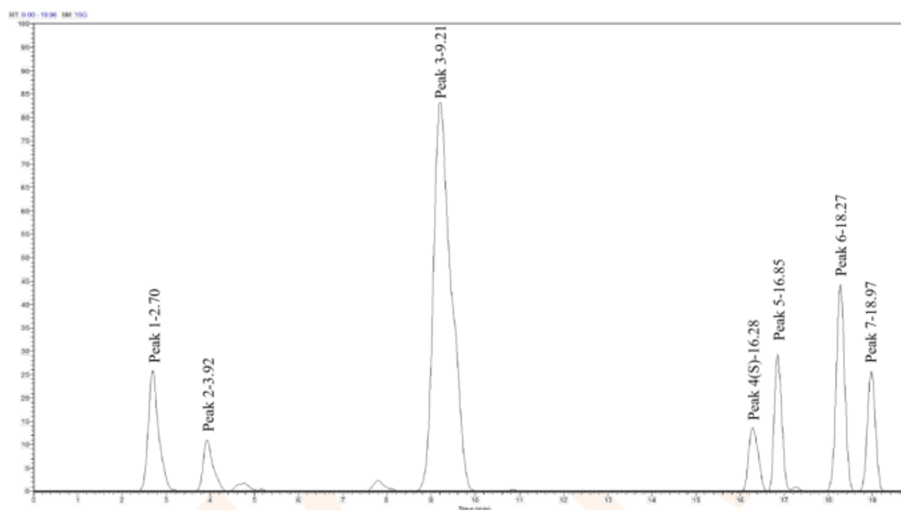

图 1 对照特征图谱

注：色谱柱：Syncronis C18，2.1mm×100mm，1.7μm

4.4 水分

水分含量不得过 15.0%。

4.5 水不溶物

水不溶物含量不得过 2.0%。

4.6 氨基酸

按干燥品计算，L-羟脯氨酸含量不得少于 8.0%、甘氨酸含量不得少于 18.0%、丙氨酸不得少于 7.0%、L-脯氨酸不得少于 10.0%。

4.7 特征多肽

按干燥品计算，含特征多肽以驴源多肽 A_1（$C_{41}H_{68}N_{12}O_{13}$）和驴源多肽 A_2（$C_{51}H_{82}N_{18}O_{18}$）的总量计应不得少于 0.15%。

4.8 重金属及有害元素

阿胶中铅（Pb）、镉（Cd）、汞（Hg）、砷（As）、铜（Cu）和铬（Cr）的限量应符合表 1 中的要求。

表 1 阿胶中重金属及有害元素限量

重金属及有害元素	指标要求/（mg/kg）
铅（以 Pb 计）	≤5
镉（以 Cd 计）	≤0.3
汞（以 Hg 计）	≤0.2
砷（以 As 计）	≤2
铜（以 Cu 计）	≤20
铬（以 Cr 计）	≤2

4.9 微生物指标

阿胶中需氧菌总数、霉菌和酵母菌总数、大肠埃希菌的限量应符合表 2 中的要求。

表 2 阿胶中微生物限量

微生物指标	指标要求
需氧菌总数（CFU/g）	$\leq 10^3$
霉菌和酵母菌总数（CFU/g）	$\leq 10^2$
大肠埃希菌	不得检出（1g）

5 抽样

抽样应按照 ISO 23723:2021 第 8 条所述方法进行。

6 试验方法

6.1 鉴别

按附录 A 的方法测定。

6.2 特征图谱

按附录 B 的方法测定。

6.3 水分测定

按照 ISO 23723:2021, 7.2.1 中规定的方法测定。

6.4 水不溶物测定

按附录 C 的方法测定。

6.5 氨基酸测定

按附录 D 的方法测定。

6.6 特征多肽测定

按附录 E 的方法测定。

6.7 重金属及有害元素测定

铅、砷、镉、汞、铜、铬含量测定按照 ISO 18664:2019 规定方法测定。

6.8 微生物测定

按照 ISO 22467:2021 规定方法测定。

7 包装、运输与贮存

7.1 包装

包装要牢固、防潮、整洁、美观、无异气味,便于装卸、仓储和运输。包装材料应洁净、干燥、无污染，符合药品安全包装材料要求，密封包装。

7.2 运输

运输应选择清洁、卫生、无污染、通风干燥、防潮的运输工具，运输过程应防止雨淋和曝晒。

7.3 贮存

置于干燥处，密闭保存。

8 标签及标志

包装储运图示标志及标签应符合 ISO 21317 的规定。

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附录 A

（规范性）

阿胶鉴别检测方法

A.1 供试品溶液的制备

取样品粉末 0.1 g，加 1 %碳酸氢铵溶液 50 mL，超声处理（功率 250 W，频率 40 kHz）30 分钟，用微孔滤膜滤过，取续滤液 100 μL，加入 10 μL 胰蛋白酶溶液（取序列分析级胰蛋白酶，加 1%碳酸铵溶液制成每 1mL 中含 1 mg 的溶液，临用前新制），混匀，37 ℃恒温酶解 12 小时，作为供试品溶液。

A.2 对照品溶液的制备

取阿胶对照药材 0.1g，同供试品溶液制备方法，制成对照药材溶液；

A.3 检测

A.3.1 液相条件

A.3.1.1 色谱柱

a）固定相：以十八烷基硅烷键合硅胶为填充剂；
b）色谱柱尺寸：内径为 2.1mm。

A.3.1.2 流动相

a）流动相 A：乙腈；
b）流动相 B：0.1%甲酸水溶液；
c）梯度洗脱程序见表 A.1；
d）流速：0.3mL/min；
e）进样量：5μl。

表 A.1 梯度洗脱条件

时间/min	流动相 A（%）	流动相 B（%）
0	3	97
12.0	5	95
20.0	15	85

A.3.2 质谱条件

采用三重四极杆质谱检测器，电喷雾离子化（ESI）正离子模式下多反应监测（MRM），驴皮源成分选择 m/z539.8（双电荷）→612.4、923.8 作为检测离子对。阿胶对照药材溶液中的各离子对的提取离子色谱峰的信噪比均应大于 3:1。

A.4 测定

吸取阿胶对照药材溶液、供试品溶液各 5 μl，注入高效液相色谱-质谱联用仪，测定。

A.5 结果判定

以 m/z 539.8→m/z 612.4、923.8 提取离子流图色谱中，应同时出现与对照药材溶液色谱保留时间相同的色谱峰，视为检出；否则，视为未检出。

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附录 B
（规范性）
阿胶特征图谱

B.1 供试品溶液的制备

取本品粉末 0.1g，精密称定，置 50mL 量瓶中，加 1%碳酸氢铵溶液 40ml，超声处理（功率 250 W，频率 40 kHz）30 分钟，加 1%碳酸氢铵溶液稀释至刻度，摇匀。精密量取 1 mL 至 5 mL 量瓶中，加胰蛋白酶溶液（取序列分析级胰蛋白酶，加 1%碳酸铵溶液制成每 1mL 中含 1 mg 的溶液，临用前新制）1mL，加 1%碳酸氢铵溶液稀释至刻度，摇匀，37℃恒温酶解 12 小时，滤过，取续滤液作为供试品溶液。

B.2 参照物溶液的制备

B.2.1 对照药材参照物溶液

取阿胶对照药材 0.lg，同供试品溶液的制备方法，制成对照药材参照物溶液。

B.2.2 对照品参照物溶液的制备

取驴源多肽 A_1 对照品、驴源多肽 A_2 对照品，精密称定，加 1%碳酸氢铵溶液制成每 1ml 各含 2.5μg 的混合溶液，作为对照品参照物溶液。

B.3 检测

B.3.1 液相条件

同 A 3.1。

B.3.2 质谱条件

采用三重四极杆质谱检测器，电喷雾离子化(ESI)正离子模式下多反应监测(MRM)，监测离子对见表 B.2，对照药材参照物溶液中的各离子对的提取离子色谱峰的信噪比均应大于 3:1。

表 B.2 监测离子对参数

测定成分	离子对①（m/z）	离子对②（m/z）
峰 1	421.71（双电荷）→656.40	421.71（双电荷）→585.39
峰 2	408.30（双电荷）→345.20	408.30（双电荷）→516.20
峰 3	393.22（双电荷）→556.40	393.22（双电荷）→499.40
峰 4	469.25（双电荷）→712.30	469.25（双电荷）→783.40
峰 5	618.35（双电荷）→779.40	618.35（双电荷）→850.40
峰 6	539.80（双电荷）→612.40	539.80（双电荷）→923.80
峰 7	691.30（双电荷）→598.30	691.30（双电荷）→780.40

B.4 测定

分别精密吸取对照品参照物溶液 2μl，对照药材参照物溶液与供试品溶液各 5μl，注入高效液相色谱-质谱联用仪，测定，即得。

B.5 结果判定

以规定质荷比离子对提取的供试品离子流色谱中,应呈现与对照药材参照物色谱相对应的 7 个特征峰，其中 2 个峰应分别与相应对照品参照物峰的保留时间相对应，以峰 4（驴源多肽 A_1 参照物峰）为 S 峰，计算峰 1、峰 2、峰 3、峰 6、峰 7 与 S 峰的相对保留时间，应在规定值的±15%范围之内，规定值为：0.16（峰 1）、0.23（峰 2）、0.50（峰 3）、1.25（峰 6）、1.32（峰 7）。

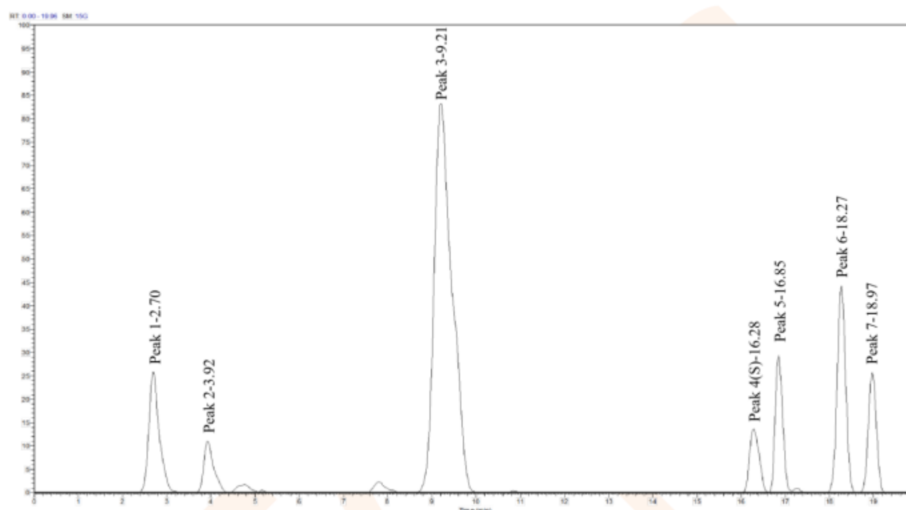

图 B.1 对照特征图谱

注：色谱柱：Syncronis C18，2.1mm×100mm，1.7μm

附录 C
（规范性）
阿胶中水不溶物含量的测定方法

C.1 方法概要

试样粉末经水溶解后，弃去溶液，采用烘干法去除水分，再通过烘干前后的称量数值计算出水不溶物的含量。

C.2 测定

取本品 1.0 g，精密称定，加水 5 mL，加热使溶解，转移至已恒重 10 mL 具塞离心管中，用温水 5 mL 分 3 次洗涤，洗液并入离心管中，摇匀。离心（转速为每分钟 2000 转）10 分钟，去除管壁浮油，倾去上清液，沿管壁加入温水至刻度，离心，依法清洗 3 次，倾去上清液，离心管在 105℃加热 2 小时，取出，置干燥器中冷却 30 分钟，精密称定，计算，即得。

C.3 结果计算

按公式（C.1）计算水分：

$$X_1 = \frac{m_1 - m_2}{m} \times 100 \quad \text{（C.1）}$$

式中：

X_1 —— 供试品的水不溶物含量，单位为克每百克（g/100g）；

m_1 —— 水不溶物和离心管的总质量，单位为克（g）；

m_2 —— 恒重后离心管的质量，单位为克（g）；

m —— 供试品的质量，单位为克（g）。

在重复性条件下获得的两次独立测定结果的绝对差值不应超过算术平均值的 10%。

附录 D
（规范性）
阿胶中氨基酸含量的测定方法

D.1 供试品溶液的制备

取本品粗粉约 0.25g，精密称定，置 25mL 量瓶中，加 0.1 mol/L 盐酸溶液 20mL，超声处理(功率 500 W，频率 40 kHz)30 分钟，放冷，加 0.1 mol/L 盐酸溶液至刻度，摇匀。精密量取 2mL，置 5 mL 安瓿中，加盐酸 2 mL，150℃水解 1 小时，放冷，移至蒸发皿中，用水 10 mL 分次洗涤，洗液并入蒸发皿中，蒸干，残渣加 0.1 mol/L 盐酸溶液溶解，转移至 25mL 量瓶中，加 0.1 mol/L 盐酸溶液至刻度，摇匀，即得。

D.2 对照品溶液的制备

取 L-羟脯氨酸对照品、甘氨酸对照品、丙氨酸对照品、L-脯氨酸对照品适量，精密称定，加 0.1 mol/L 盐酸溶液制成每 1 mL 分别含 L-羟脯氨酸 80 μg、甘氨酸 0.16 mg、丙氨酸 70 μg、L-脯氨酸 0.12 mg 的混合溶液，即得。

D.3 衍生化

精密量取上述对照品溶液和供试品溶液各 5 mL，分别置 25 mL 容量瓶中，各加 0.1 mol/L 异硫氰酸苯酯（PITC）的乙腈溶液 2.5 mL，1 mol/L 三乙胺的乙腈溶液 2.5 mL，摇匀，室温放置 1 h，加 50%乙腈至刻度，摇匀。取 10 mL 置于试管内，加正己烷 10 mL，振摇，静置 10 min。取下层溶液，滤过，备用。

D.4 色谱条件与系统适用性试验

a) 色谱柱：十八烷基硅烷键合硅胶为填充剂；
b) 柱温：43 ℃；
c) 检测波长：254 nm；
d) 流动相：以乙腈-0.1 mol/L 醋酸钠溶液（用醋酸调节 pH 至 6.5）（体积比 7:93）为流动性 A，以乙腈-水（体积比 4:1）为流动性 B，洗脱程序见表 D.1；理论板数按 L-羟脯氨酸峰计算不应低于 4000。

表 D.1 梯度洗脱程序

时间（分钟）	流动相 A（%）	流动相 B（%）
0～11	100→93	0→7
11～13.9	93→88	7→12
13.9～14	88→85	12→15
14～29	85→66	15→34
29～30	66→0	34→100

D.5 测定

分别精密吸取衍生化后的对照品溶液与供试品溶液各 5 μL，注入液相色谱仪，测定，备用。

D.6 结果计算

按公式（D.1）计算氨基酸含量：

$$X_2 = \frac{c \times v \times 12.5}{m \times 10^3} \times 100 \quad \text{（D.1）}$$

式中：

X_2 —— 供试品的氨基酸含量，单位为克每百克（g/100g）；

c —— 待测液中氨基酸的测定值，单位为毫克每毫升（mg/mL）；

v —— 供试品的溶解体积，单位为毫升（mL）；

12.5 —— 稀释倍数；

m —— 供试品（折干后）的质量，单位为克（g）。

平行样的相对偏差不应过 2.0%。

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附录 E
（规范性）
阿胶中驴源多肽含量的测定方法

E.1 供试品溶液的制备

同 B.1。

E.2 对照品溶液的制备

同 B.2.2。

E.3 色谱、质谱条件与系统适用性

E.3.1 液相条件

液相色谱参考条件同 A.3.1。

E.3.2 质谱条件

采用三重四极杆质谱检测器，电喷雾离子化（ESI）正离子模式下多反应监测（MRM），监测离子对见表 E.1，理论塔板数按驴源多肽 A_1 峰计算应不低于 4000。

表 E.1 监测离子对参数

测定成分	定量离子对（m/z）	定性离子对（m/z）
驴源多肽 A_1	469.25（双电荷）→712.30	469.25（双电荷）→783.40
驴源多肽 A_2	618.35（双电荷）→779.40	618.35（双电荷）→850.40

E.4 测定

精密量取对照品溶液 1mL、2mL、5mL、10mL、20mL 和 25mL，分别置 50mL 量瓶中，加 1%碳酸氢铵溶液稀释至刻度，制成标准曲线溶液。分别精密吸取不同浓度的标准曲线溶液与供试品溶液各 5μL，注入高效液相色谱-质谱联用仪，以对照品峰面积为纵坐标，对照品浓度为横坐标制备标准曲线。从标准曲线读出供试品溶液中相当于驴源多肽 A1 和驴源多肽 A2 的量，计算即得。

E.5 结果计算

试样中驴皮源成分含量按公式（E.1）计算：

$$X_3 = \frac{(C_1 + C_2) \times 5 \times V}{m \times 10^6} \times 100 \quad \text{(E.1)}$$

式中：

X_3 —— 试样中驴源多肽含量，单位为克每百克（g/100g）；

C_1 —— 从标准曲线中读出的待测液中驴源多肽A_1的浓度，单位为微克每毫升（μg/mL）；

C_2 —— 从标准曲线中读出的待测液中驴源多肽A_2的浓度，单位为微克每毫升（μg/mL）；

V —— 试样的定容体积，50 mL;

5 —— 稀释倍数;

m —— 称取试样（折干后）质量，单位为克（g）。

以两个平行试样测定结果的算术平均值报告结果，保留两位小数。

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参考文献

[1] 国家药典委员会，《中华人民共和国药典》2025 年版一部，人民卫生出版社，2025

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Foreword

Attention is drawn to the possibility that some of the contents of this document may be subject to patent rights. The issuing organization of this document shall not be held responsible for identifying patent rights.

Main Drafting Organization: Dong-E E-Jiao Co., Ltd.

Participating Drafting Organizations: Shandong Provincial Institute for Food and Drug Control, Nanchang University, Sichuan University, Qingdao Institute of Marine Drugs, Heilongjiang University of Chinese Medicine, Jiangxi Normal University, CR Care Co., Ltd. , HYGIA HEALTH CARE , Deshenghang Co., Ltd. , Sun Kong International (Macao) Pharmaceutical Co., Ltd. , Shandong Dong-E Black Donkey Animal Husbandry Technology Co., Ltd.

Main Drafters: Liu Haibin, Duan Xiaobo, Guo Shangwei, Zhang Ying, Tian Leyuan, Cheng Jie, Zheng Liang, Ge Nan, Tian Xiao, Sun Yang'en, Wang Mengyuan, Liu Minghao, Men Cuicui, Liu Benyu, Zhai Changqi.

Participating Drafters and Reviewing Experts (sorted alphabetically by last name): Cai Yingjie (Macao, China), Ji Chuanliang, Li Jianyang (Hong Kong, China), Lin Yongqiang, Liu Weiyan, Niu Weixia, Pang Huihui, Sha Xiaomei, Shi Zhaosong, Tu Zongcai, Tang Wen, Wang Xijun, Wang Hui, Wang Yizhi (Hong Kong, China), Yang Jinbo, Yu Jie, Zhou Jianfei, Zhang Shouyuan.

The drafting procedure for this document complies with SCM1.1-2021 "*Guidelines for Standardization Work - Part 1: Development, Revision, and Publication of Standards*" issued by the World Federation of Chinese Medicine Societies.

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Asini Corii Colla

1 Scope

This document specifies the terms and definitions, quality requirements and general characteristics, test methods, packaging, transportation, storage, marking, and labeling for Asini Corii Colla.

This document is applicable to production, transportation, and trading enterprises of Asini Corii Colla as a medicinal material. Inspection and testing institutions may also refer to it for use.

2 Normative References

The following referenced documents are indispensable for the application of this document, For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 18664 Traditional Chinese Medicine — Determination of heavy metals in herbal medicines used in Traditional Chinese Medicine
ISO 21371 Traditional Chinese medicine — Labelling requirements of products intended for oral or topical use
ISO 23723 Traditional Chinese medicine — General requirements for herbal raw material and materia medica
ISO 22467 Traditional Chinese medicine — Determination of microorganisms in natural products

3 Terms and Definitions

For the purpose of this document, the following terms and definitions apply.

3.1

Asini Corii Colla

a solid gelatin prepared from the dried or fresh skin of *Equus asinus* L.(donkey), through processes including soaking to remove hair, cutting into pieces, washing, decocting in water, concentrating, and optionally adding appropriate amounts of yellow rice wine, crystal sugar, and soybean oil.

3.2

Donkey skin

dried or fresh skin of the equine animal Equus asinus L.

4 Requirements

4.1 Morphological characteristics

This product appears as rectangular pieces, cubical pieces, or small cubes. It is brown to dark brown in color, with a glossy surface. The texture is hard and brittle. The fractured surface is smooth and glossy, and the fragments are brownish and semi-translucent when viewed against light. It has a slight odor and a slightly sweet taste.

4.2 Identification

In the extracted ion chromatograms of the test solution using the ion pairs with mass-to-charge ratio (m/z) 539.8 (doubly charged) → 612.4 and m/z 539.8 (doubly charged) → 923.8, chromatographic peaks consistent with the retention times of those in the reference medicinal material chromatogram should appear simultaneously.

4.3 Characteristic chromatogram

In the extracted ion chromatograms obtained using the specified ion pairs, the test solution shall exhibit seven characteristic peaks corresponding to those in the reference standard chromatogram. Among these, two peaks should match the retention times of the respective reference standard peaks. Using Peak 4 (donkey-derived peptide A_1 reference peak) as the reference peak (S), calculate the relative retention times of Peak 1, Peak 2, Peak 3, Peak 6, and Peak 7 against the S peak. The values must fall within ±15% of the specified reference values: 0.16 (Peak 1), 0.23 (Peak 2), 0.50 (Peak 3), 1.25 (Peak 6), and 1.32 (Peak 7).

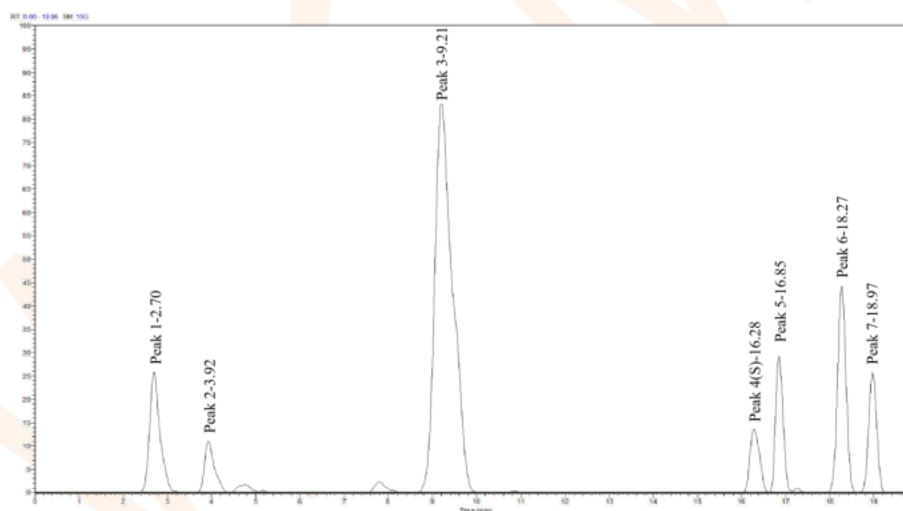

Figure 1 — Reference Chromatographic Profile

Note: Chromatographic column:Syncronis C18, 2.1mm×100mm, 1.7μm

4.4 Moisture

The Moisture content should not be more than 15.0%.

4.5 Water-insoluble matter

The mass fraction of water-insoluble matter should not be more than 2.0%.

4.6 Amino acids

The contents, calculated on the dried basis, shall not be less than 8.0% for

L-Hydroxyproline; 18.0% for Glycine; 7.0% for Alanine; and 10.0% for L-Proline.

4.7 Characteristic peptides

Calculated on the dried basis, the total content of the characteristic peptides, calculated as Donkey-derived Peptide A1 ($C_{41}H_{68}N_{12}O_{13}$) and Donkey-derived Peptide A2 ($C_{51}H_{82}N_{18}O_{18}$), shall not be less than 0.15%.

4.8 Heavy metals and harmful elements

The limits for lead, cadmium, mercury, arsenic, copper, and chromium in Asini Corii Colla shall comply with the requirements specified in Table 1.

Table 1 — Limits for Heavy Metals and Harmful Elements in Asini Corii Colla

Heavy Metals and Harmful Elements	Specifications/（mg/kg）
Lead	≤5
Cadmium	≤0.3
Mercury	≤0.2
Arsenic	≤2
Copper	≤20
Chromium	≤2

4.9 Microbiological limits

The limits for Total Aerobic Microbial Count, Total Combined Molds and Yeasts Count, and Escherichia coli in Asini Corii Colla shall comply with the requirements specified in Table 2.

Microbiological Limits	Specifications
Total Aerobic Microbial Count（CFU/g）	$\leq 10^3$
Total Combined Molds and Yeasts Count（CFU/g）	$\leq 10^2$
Escherichia coli	Not detected per 1g

Table 2 — Microbiological Limits for Asini Corii Colla

5 Sampling

Sampling of Asini Corii Colla shall be carried out in accordance with the method described in ISO 23723:2021, Clause 8.

6 Test methods

6.1 Identification

Method of measurement shall comply with the method specified in Annex A.

6.2 Characteristic chromatogram

Method of measurement shall comply with the method specified in Annex B.

6.3 Determination of moisture

The testing method specified in ISO 23723:2021, 7.2.1 shall apply.

6.4 Determination of water-insoluble

Method of measurement shall comply with the method specified in Annex C.

6.5 Determination of amino acids

Method of measurement shall comply with the method specified in Annex D.

6.6 Determination of characteristic peptides

Method of measurement shall comply with the method specified in Annex E.

6.7 Determination of heavy metals and harmful elements

The testing method specified in ISO 18664:2019 shall apply.

6.8 Determination of microorganisms

The testing method specified in ISO 22467:2021 shall apply.

7 Packaging, transportation, and storage

7.1 Packaging

The packaging shall be secure, moisture-proof, clean, neat, and aesthetically pleasing, free from any abnormal odor, and facilitate loading, unloading, storage, and transportation. The packaging materials shall be clean, dry, uncontaminated, comply with the safety requirements for pharmaceutical packaging materials, and be hermetically sealed.

7.2 Transportation

Vehicles used for transportation shall be clean, sanitary, free from contamination, well-ventilated, dry, and moisture-proof. During transportation, the products must be protected from rain and direct sunlight exposure.

7.3 Storage

Preserve in a dry place, tightly closed.

8 Marking and labeling

The pictorial markings for packaging, handling, and storage, as well as the labels, shall comply with ISO 21317.

Annex A
(Normative)
Test Method for Identification of Asini Corii Colla

A.1 Preparation of test solution

Weigh accurately 0.1 g of the powdered Asini Corii Colla sample being examined and add to 50 mL of 1% ammonium bicarbonate solution. Sonicate the mixture (power:250 W, frequency:40 kHz) for 30 minutes, then filter through a microporous membrane. Take 100 μL of the filtrate, add 10 μL of trypsin solution (prepare using sequencing grade trypsin, dissolve in 1% ammonium bicarbonate solution to obtain a solution containing 1 mg per 1 mL, prepare freshly before use), mix thoroughly, and enzymatically digest at a constant temperature of 37°C for 12 hours as the test solution.

A.2 Preparation of reference solution

Weigh accurately 0.1 g of the Asini Corii Colla reference medicinal material, prepare similarly to the test solution method to obtain the reference medicinal material solution.

A.3 Detection

A.3.1 Liquid chromatographic conditions

A.3.1.1 Column

a) Stationary phase: Packing with octadecylsilane chemically bonded silica;
b) Column dimensions: Inner diameter 2.1 mm.

A.3.1.2 Mobile phase

a) Mobile phase A: Acetonitrile;
b) Mobile phase B: 0.1% formic acid aqueous solution;
c) Gradient elution program as per Table A.1;
d) Flow rate: 0.3 mL/min;
e) Injection volume: 5 μL.

Table A.1 — Gradient Elution Conditions

Time (min)	Mobile Phase A (%)	Mobile Phase B (%)
0	3	97
12.0	5	95
20.0	15	85

A.3.2 Mass spectrometric conditions

A triple quadrupole mass spectrometer detector is used, operating in electrospray

ionization positive ion mode with multiple reaction monitoring. For the detection of Asini Corii Colla-specific components, the ion transitions m/z 539.8 (doubly charged) → 612.4 and m/z 539.8 → 923.8 are selected as the monitoring pairs. For the Asini Corii Colla reference standard solution, the signal-to-noise ratio (S/N) of the extracted ion chromatogram peaks for each ion pair must exceed 3:1.

A.4 Determination

Aliquots of 5 μL each from the Asini Corii Colla reference standard solution and the test solution are injected into a high-performance liquid chromatography-mass spectrometry (HPLC-MS) system for analysis.

A.5 Result judgment

In the extracted ion chromatograms for the transitions m/z 539.8→612.4 and m/z 539.8→923.8, peaks must exhibit retention times identical to those observed in the chromatogram of the Asini Corii Colla reference standard solution to confirm detection; otherwise, the result is interpreted as not detected.

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Annex B
(Normative)
Test Method for Characteristic Chromatogram of Asini Corii Colla

B.1 Preparation of test solution

Weigh accurately 0.1 g of the powdered Asini Corii Colla sample being examined and transfer to a 50 mL volumetric flask. Add 40 mL of 1% ammonium bicarbonate solution, and sonicate (power: 250 W, frequency:40 kHz) for 30 minutes. Dilute to volume with 1% ammonium bicarbonate solution and mix thoroughly. Precisely measure 1 mL into a 5 mL volumetric flask, add 1 mL of trypsin solution (prepare using sequencing grade trypsin, dissolve in 1% ammonium bicarbonate solution to obtain a solution containing 1 mg per 1 mL, prepare freshly before use), then dilute to volume with 1% ammonium bicarbonate solution and mix thoroughly. Incubate at 37°C for 12 hours for enzymatic digestion. Filter the solution, and use the subsequent filtrate as the test solution.

B.2 Preparation of reference solutions

B.2.1 Reference medicinal material solution

Weigh accurately 0.1 g of Asini Corii Colla reference medicinal material following the same procedure as for the test solution.

B.2.2 Reference substance solution

Weigh accurately donkey-derived peptide A_1 reference substance and donkey-derived peptide A_2 reference substance, and dissolve in 1% ammonium bicarbonate solution to prepare a mixed solution containing 2.5 μg/mL of each peptide as the reference standard solution.

B.3 Detection

B.3.1 Liquid chromatographic conditions

Same as A.3.1.

B.3.2 Mass spectrometric conditions

Use a triple quadrupole mass spectrometer detector, in electrospray ionization positive ion mode with multiple reaction monitoring. The monitored ion pairs are listed in Table B.2. The signal-to-noise ratio of the extracted ion chromatographic peaks for each ion pair in the reference medicinal material solution should be greater than 3:1.

Table B.2 — Monitored ion pair parameters

Component	Ion Pair ①（m/z）	Ion Pair ②（m/z）
Peak 1	421.71 (doubly charged) →656.40	421.71 (doubly charged) →585.39

Peak 2	408.30 (doubly charged) →345.20	408.30 (doubly charged) →516.20
Peak 3	393.22 (doubly charged) →556.40	393.22(doubly charged) →499.40
Peak 4	469.25 (doubly charged) →712.30	469.25(doubly charged) →783.40
Peak 5	618.35 (doubly charged)→779.40	618.35(doubly charged) →850.40
Peak 6	539.80 (doubly charged)→612.40	539.80(doubly charged) →923.80
Peak 7	691.30 (doubly charged)→598.30	691.30(doubly charged) →780.40

B.4 Determination

Precisely inject 2 μL of the reference substance solution, and 5 μL each of the reference medicinal material solution and the test solution into the HPLC-MS system for analysis.

B.5 Result Assessment

The extracted ion chromatograms of the test solution at the specified m/z transitions must exhibit seven characteristic peaks corresponding to those in the reference medicinal material solution. Among these, two peaks should match the retention times of the respective reference standard peaks. Using Peak 4 (donkey-derived peptide A_1 reference peak) as the reference peak (S), calculate the relative retention times of Peak 1, Peak 2, Peak 3, Peak 6, and Peak 7 against the S peak. The values must fall within ±15% of the specified reference values: 0.16 (Peak 1), 0.23 (Peak 2), 0.50 (Peak 3), 1.25 (Peak 6), and 1.32 (Peak 7).

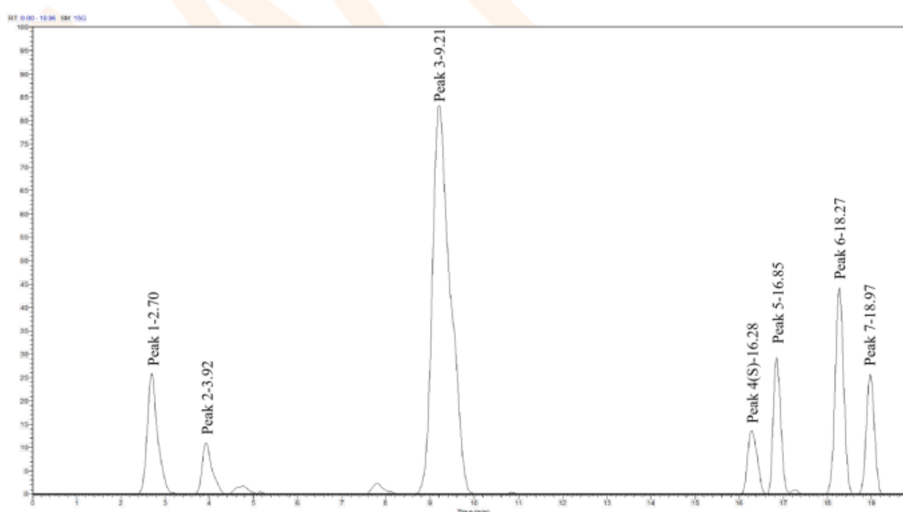


Figure B.1 — Reference Chromatographic Profile

Note: Chromatographic column: Syncronis C18，2.1mm×100mm，1.7μm

Annex C
(Normative)
Determination of Water-Insoluble Matter in Asini Corii Colla

C.1 Summary of method

After the sample powder is dissolved in water, the solution is discarded. The moisture is removed using the oven-drying method, and the content of water-insoluble matter is calculated based on the weight measurements before and after drying.

C.2 Determination

Weigh accurately 1.0 g of the sample, add 5 mL of water, and heat to dissolve. Transfer to a previously tared 10 mL centrifuge tube with stopper, rinse with 5 mL of warm water in three equal portions, combine the rinsings into the centrifuge tube, and mix well. Centrifuge (at 2000 rpm) for 10 minutes, remove floating oil from the tube wall, pour off the supernatant, add warm water along the tube wall to the mark, centrifuge, repeat the washing three times in the same manner, pour off the supernatant, heat the centrifuge tube at 105°C for 2 hours, remove, cool in a desiccator for 30 minutes, weigh accurately, and calculate.

C.3 Calculation of results

Calculate the water-insoluble matter content using formula (C.1):

$$X_1 = \frac{m_1 - m_2}{m} \times 100 \quad \text{(C.1)}$$

Where:

X_1 ——the water-insoluble matter content of the test sample, in grams per hundred grams (g/100g);

m_1 —— Mass (g) of the water-insoluble residue and centrifuge tube;

m_2 —— Constant mass (g) of the centrifuge tube;

m —— Mass (g) of the test sample.

Annex D
(Normative)
Determination of Amino Acid Content in Asini Corii Colla

D.1 Preparation of test solution

Weigh accurately approximately 0.25 g of the coarsely powdered sample and transfer to a 25 mL volumetric flask. Add 20 mL of 0.1 mol/L hydrochloric acid solution, sonicate (power: 500 W, frequency: 40 kHz) for 30 minutes, and allow to cool. Dilute to volume with 0.1 mol/L hydrochloric acid solution and mix well. Precisely pipette 2 mL of this solution into a 5 mL ampoule, add 2 mL of hydrochloric acid, and hydrolyze at 150°C for 1 hour. After cooling, transfer the solution to an evaporating dish, rinse the ampoule with 10 mL of water in portions, combine the washings in the dish, and evaporate to dryness. Dissolve the residue in 0.1 mol/L hydrochloric acid solution, transfer to a 25 mL volumetric flask, dilute to volume **with the same solvent, and mix thoroughly to obtain the test solution.**

D.2 Preparation of reference solution

Weigh accurately appropriate amounts of L-hydroxyproline, glycine, alanine, and L-proline reference standards. Dissolve in 0.1 mol/L hydrochloric acid solution to prepare a mixed solution containing 80 μg/mL of L-hydroxyproline, 0.16 mg/mL of glycine, 70 μg/mL of alanine, and 0.12 mg/mL of L-proline.

D.3 Derivatization

Precisely measure 5 mL each of the above reference solution and test solution, place into separate 25 mL volumetric flasks. Add 2.5 mL of 0.1 mol/L phenyl isothiocyanate (PITC) in acetonitrile and 2.5 mL of 1 mol/L triethylamine in acetonitrile to each flask. Mix well, let stand at room temperature for 1 hour, then dilute to volume with 50% acetonitrile and mix thoroughly. Transfer 10 mL of the solution to a test tube, add 10 mL of n-hexane, shake vigorously, and let stand for 10 minutes. Collect the lower layer solution, filter, and reserve for analysis.

D.4 Chromatographic conditions and system suitability test

a) Column: Packing with octadecylsilane chemically bonded silica;
b) Column temperature: 43°C;
c) Detection wavelength: 254 nm;
d) Mobile phase:
Mobile phase A: Acetonitrile-0.1 mol/L sodium acetate solution (adjusted to pH 6.5 with acetic acid) (7:93, v/v);
Mobile phase B: Acetonitrile-water (4:1, v/v);
Elution program as per Table D.1. The theoretical plate number for the L-hydroxyproline

peak should be not less than 4,000.

Time (min)	Mobile Phase A (%)	Mobile Phase B (%)
0～11	100→93	0→7
11～13.9	93→88	7→12
13.9～14	88→85	12→15
14～29	85→66	15→34
29～30	66→0	34→100

Table D.1 — Gradient Elution Program

D.5 Determination

Inject 5 μL each of the derivatized reference standard solution and test solution into the liquid chromatograph for analysis.

D.6 Calculation of results

Calculate the amino acid content using formula (D.1):

$$X_2 = \frac{c \times v \times 12.5}{m \times 10^3} \times 100 \quad \text{(D.1)}$$

Where:

X_2 —— Amino acid content of the test sample (g/100g);

c —— Measured concentration of the amino acid in the test solution (mg/mL);

v ——Volume of the test solution (mL);

12.5 —— Dilution factor;

m —— Mass of the test sample (dried basis) (g).

The relative deviation between parallel samples should not exceed 2.0%.

Annex E
(Normative)
Determination of Donkey-Derived Peptide Content in Asini Corii Colla

E.1 Preparation of test solution

Same as B.1.

E.2 Preparation of reference solution

Same as B.2.2.

E.3 Chromatographic, mass spectrometric conditions and system suitability

E.3.1 Liquid chromatographic conditions

Liquid chromatographic reference conditions same as A.3.1.

E.3.2 Mass spectrometric conditions

A triple quadrupole mass spectrometer detector is used with electrospray ionization (ESI) in positive ion mode and multiple reaction monitoring (MRM). The monitored ion transitions are listed in Table E.1. The theoretical plate number calculated for the donkey-derived peptide A1 peak should be not less than 4,000.

Table E.1 — Monitored Ion Pair Parameters

Component	Quantitative Ion Transition (m/z)	Qualitative Ion Transition (m/z)
Donkey-derived peptide A_1	469.25(doubly charged)→712.30	469.25(doubly charged)→783.40
Donkey-derived peptide A_2	618.35(doubly charged)→779.40	618.35 (doubly charged) →850.40

E.4 Determination

Precisely measure 1 mL, 2 mL, 5 mL, 10 mL, 20 mL, and 25 mL of the reference substance solution, place into separate 50 mL volumetric flasks, dilute to volume with 1% ammonium bicarbonate solution to prepare standard curve solutions. Precisely inject 5 μL each of the different concentration standard curve solutions and the test solution into the HPLC-MS system. Prepare the standard curve using the reference substance peak area as the ordinate and the reference substance concentration as the abscissa. Read the amounts equivalent to donkey-derived peptide A_1 and donkey-derived peptide A_2 in the test solution from the standard curve, and calculate.

E.5 Calculation of results

Calculate the donkey-derived peptide content in the sample using formula (E.1):

$$X_3 = \frac{(C_1 + C_2) \times 5 \times V}{m \times 10^6} \times 100 \quad \text{(E.1)}$$

Where:

X_3 —— Content of donkey-derived peptides in the sample (g/100g);

C_1 ——Concentration of donkey-derived peptide A1 in the test solution obtained from the standard curve (μg/mL);

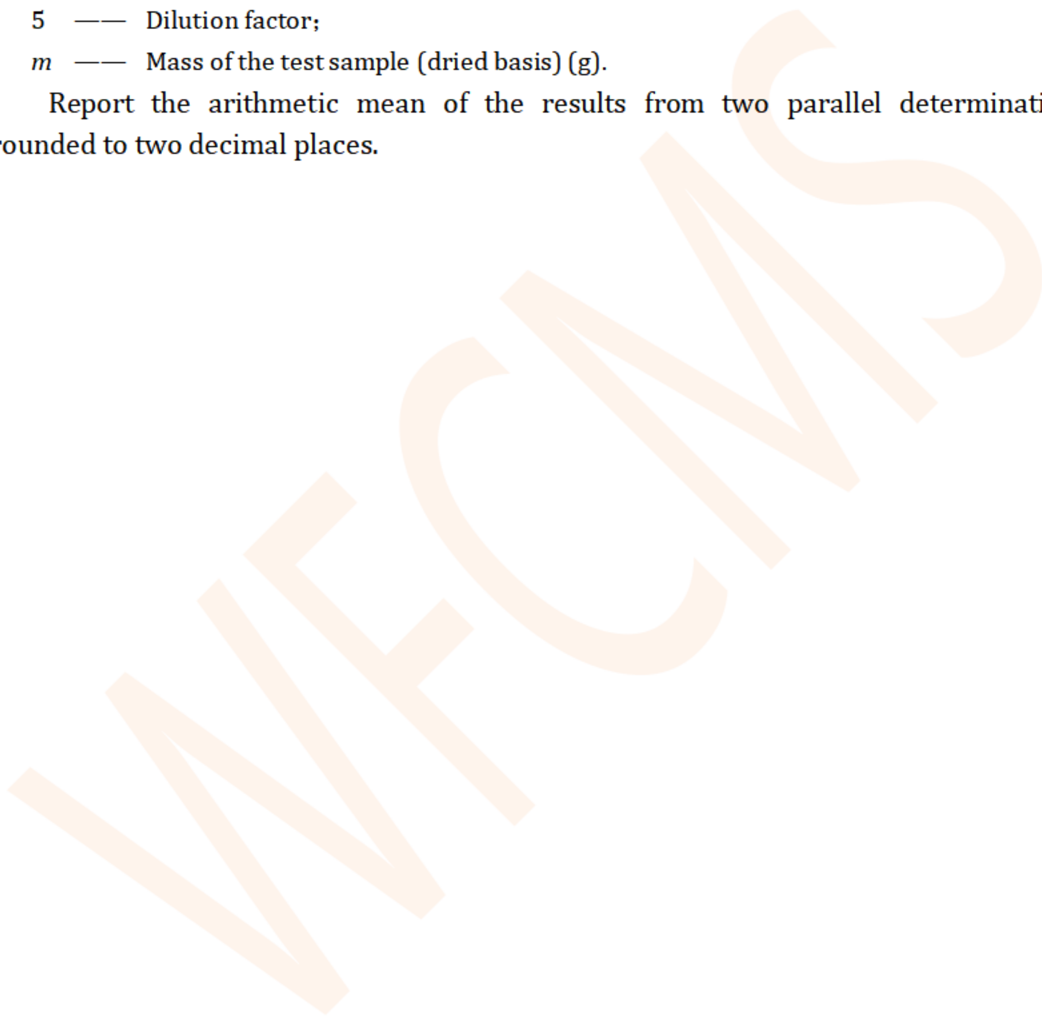
C_2 —— Concentration of donkey-derived peptide A2 in the test solution obtained from the standard curve (μg/mL);

V —— Final volume of the test solution (50 mL);

5 —— Dilution factor;

m —— Mass of the test sample (dried basis) (g).

Report the arithmetic mean of the results from two parallel determinations, rounded to two decimal places.



Bibliography

[1] Chinese Pharmacopoeia Commission, Pharmacopoeia of the People's Republic of China 2025 Edition, Volume I, People's Medical Publishing House, 2025

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