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中华人民共和国出入境检验检疫行业标准

SN/T 4258—2015

出口食品中水溶性维生素的测定方法

Determination of water-soluble vitamins in foods for export

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前 言

本标准按照 GB/T 1.1—2009 给出的规则起草。

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这些专利的责任。

本标准由国家认证认可监督管理委员会提出并归口。

本标准起草单位：中华人民共和国河北出入境检验检疫局、中华人民共和国上海出入境检验检疫局。

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出口食品中水溶性维生素的测定方法

1 范围

本标准规定了食品中维生素 B₁ (硫胺素)、B₂ (核黄素)、B₃ (烟酸、烟酰胺)、B₅ (泛酸)、B₆ (吡哆醇、吡哆醛、吡哆胺)、叶酸和 B₁₂ (钴胺素) 的液相色谱-质谱/质谱测定方法和维生素 Vc (L-抗坏血酸) 的高效液相色谱测定方法。

本标准适用于果汁、奶粉、含乳饮料、大米、饼干和果冻中维生素 B₁ (硫胺素)、B₂ (核黄素)、B₃ (烟酸、烟酰胺)、B₅ (泛酸)、B₆ (吡哆醇、吡哆醛、吡哆胺)、叶酸和 B₁₂ (钴胺素) 的测定和确证;适用于果汁、奶粉、含乳饮料、大米、果泥和果冻中维生素 Vc (L-抗坏血酸) 的测定。

2 规范性引用文件

下列文件对于本文件的应用是必不可少的。凡是注日期的引用文件,仅注日期的版本适用于本文件。凡是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。

GB/T 6682 分析实验室用水规格和试验方法

第一部分 食品中水溶性维生素 B₁ (硫胺素)、B₂ (核黄素)、B₃ (烟酸、烟酰胺)、B₅ (泛酸)、B₆ (吡哆醇、吡哆醛、吡哆胺) 和叶酸的测定

3 原理

试样中的维生素 B₁ (硫胺素)、维生素 B₂ (核黄素)、维生素 B₃ (烟酸、烟酰胺)、维生素 B₅ (泛酸)、维生素 B₆ (吡哆醇、吡哆醛、吡哆胺) 和叶酸用盐酸溶液、三氯乙酸溶液或温水(等电点法)提取,过膜后,用液相色谱-质谱/质谱仪检测,内标法定量。

4 试剂和材料

除非另有规定外,所有试剂均为分析纯,水为 GB/T 6682 规定的一级水。

- 4.1 甲醇:HPLC 级。
- 4.2 甲酸:HPLC 级。
- 4.3 乙酸铵:HPLC 级。
- 4.4 氨水。
- 4.5 盐酸。
- 4.6 三氯乙酸。
- 4.7 氢氧化钠。
- 4.8 BHT(二丁基羟基甲苯):纯度大于等于 99.3%, -18℃ 冷冻储藏。
- 4.9 0.1% 甲酸溶液(体积分数):量取 0.1 mL 甲酸(4.2),用水定容至 100 mL。
- 4.10 10 mmol/L 乙酸铵(pH=6.3)溶液:0.39 g 乙酸铵(4.3)加水溶解至 500 mL,混匀,用 0.1% 甲酸溶液(4.9)调节至 pH=6.3。

- 4.11 0.01 mol/L 盐酸溶液:量取 900 μ L 的盐酸(4.5),用水定容至 1 000 mL,混匀。
- 4.12 5 mol/L 盐酸溶液:量取 45 mL 的盐酸(4.5),用水定容至 100 mL,混匀。
- 4.13 5 mol/L 氢氧化钠溶液:称取 20 g 氢氧化钠(4.7),溶于 100 mL 水中,混匀。
- 4.14 40 g/L 三氯乙酸溶液:称取 40 g 三氯乙酸(4.6),溶于 1 000 mL 水中,混匀。
- 4.15 1%氨水溶液(体积分数):量取 1 mL 的氨水(4.4),用水定容至 100 mL,混匀。
- 4.16 1 mg/mL BHT 溶液:称取 10 mg BHT(4.8),溶于 10 mL 甲醇(4.1)中,混匀。
- 4.17 维生素标准物质:维生素 B₁(硫胺素)、维生素 B₂(核黄素)、维生素 B₃(烟酸、烟酰胺)、维生素 B₅(泛酸)、维生素 B₆(吡哆醇、吡哆醛、吡哆胺)、叶酸;内标物质:D4-烟酸、D4-烟酰胺、甲氨蝶呤和乙酰苯胺的纯度均为大于等于 95%,避光冷藏。各化合物基本信息参见附录 A 表 A.1。
- 4.18 标准储备液:精确称取适量标准品(4.17),配制成 1 mg/mL(维生素 B₂ 的浓度为 0.1 mg/mL)的标准储备液。叶酸用 1%氨水溶液(4.15)溶解;其他维生素用 0.01 mol/L 盐酸溶液(4.11)溶解。储备液 4 $^{\circ}$ C 避光保存,叶酸储备液保存期为 4 d,其他保持期为 6 d。
- 4.19 标准工作液:根据需要要用 0.01 mol/L 盐酸溶液(4.11)将标准储备液(4.18)逐级稀释,配制成一系列标准工作液,现用现配。
- 4.20 内标储备液:准确称取适量的 D4-烟酸、D4-烟酰胺、甲氨蝶呤和乙酰苯胺,分别用甲醇(4.1)配制成 500 μ g/mL 的标准储备液,4 $^{\circ}$ C 以下避光保存。
- 4.21 内标工作液:根据需要移取适量内标储备液(4.20),用甲醇(4.1)配制成 50 μ g/mL 的标准工作液,现用现配。
- 4.22 微孔滤膜:0.22 μ m,水系。

5 仪器和设备

- 5.1 液相色谱-质谱/质谱仪:配电喷雾离子源(ESI)。
- 5.2 分析天平:感量为 0.01 g 和 0.1 mg。
- 5.3 组织捣碎机。
- 5.4 离心机:转速不小于 4 000 r/min。
- 5.5 均质器:转速不小于 15 000 r/min。
- 5.6 涡旋混合器。
- 5.7 超声波清洗器。
- 5.8 聚丙烯具塞离心管:50 mL。
- 5.9 棕色容量瓶:10 mL,25 mL 和 100 mL。

6 试样制备与保存

6.1 制样要求

在制样的操作过程中,应防止样品受到污染或发生残留物含量的变化。

6.2 试样的制备

取同一批次 3 个完整独立包装样品(固体样品不少于 200 g,液体样品不少于 200 mL),固体或半固体样品粉碎混匀,液体样品混合均匀,分成两份,分别装入洁净容器内,一份作为试样供检测用,另一份作为留样保存并做好标识。

7 测定方法

7.1 样品的提取

7.1.1 果汁饮料

称取试样 2 g(精确至 0.01 g)于 10 mL 棕色容量瓶中,加入 40 μL 的内标工作液(4.21)和 100 μL 1 mg/mL BHT 溶液(4.16),用 10 mL 0.01 mol/L 盐酸溶液(4.11)定容至刻度,混匀,用 0.22 μm 微孔滤膜过滤后,供液相色谱-质谱/质谱仪测定。

7.1.2 大米、果冻、饼干

称取试样 2 g(精确至 0.01 g)于 50 mL 离心管中,加入 40 μL 的内标工作液(4.21)和 100 μL 1 mg/mL BHT 溶液(4.16),加入 10 mL 0.01 mol/L 盐酸溶液(4.11),涡旋混匀 1 min,超声 15 min,以 4 000 r/min 离心 10 min。取上清液用 0.22 μm 微孔滤膜过滤后,供液相色谱-质谱/质谱仪测定。

7.1.3 含乳饮料

称取试样 2 g(精确至 0.01 g),加入 40 μL 的内标工作液(4.21)和 100 μL 1 mg/mL BHT 溶液(4.16),准确加入 10 mL 40 g/L 三氯乙酸溶液(4.14),涡旋混匀 1 min,超声 15 min,以 4 000 r/min 离心 10 min。取上清液用 0.22 μm 微孔滤膜过滤后,供液相色谱-质谱/质谱仪测定。

7.1.4 奶粉

称取试样 2 g(精确至 0.01 g),加入 40 μL 的内标工作液(4.21)和 100 μL 1 mg/mL BHT 溶液(4.16),准确加入 20 mL 45℃~50℃水,涡旋混匀 1 min,超声 15 min。待溶液温度降至室温后,用 5 mol/L 盐酸溶液(4.12)调节 pH 至 1.90±0.5,放置 2 min,再用 5 mol/L 氢氧化钠溶液(4.13)调节 pH 至 4.70±0.5,以 4 000 r/min 离心 10 min。取上清液用 0.22 μm 微孔滤膜过滤后,供液相色谱-质谱/质谱仪测定。

上述提取步骤,均须避光进行。

7.2 测定

7.2.1 液相色谱参考条件

- 液相色谱参考条件如下:
- a) 色谱柱:UPLC HSS T₃ 50 mm×2.1 mm(内径),1.7 μm,或相当者;
 - b) 柱温:30℃;
 - c) 进样量:5 μL;
 - d) 流动相组成及梯度洗脱条件见表 1。

表 1 流动相洗脱程序

Time min	甲醇 %	10 mmol/L 乙酸铵(pH=6.3) %	流速 mL/min
0	1	99	0.3
1.0	1	99	0.3

表 1 (续)

Time min	甲醇 %	10 mmol/L 乙酸铵(pH=6.3) %	流速 mL/min
4.0	55	45	0.3
6.0	55	45	0.3
6.1	1	99	0.3
10.0	1	99	0.3

7.2.2 质谱参考条件

质谱参考条件如下：

- a) 离子源:电喷雾离子源；
- b) 扫描方式:正离子扫描；
- c) 检测方式:多反应监测(MRM)；
- d) 其他质谱参考条件参见附录 B 中表 B.1。

7.2.3 定性分析

在上述条件下测定样品和标准溶液,样品中待测物色谱峰保留时间与标准溶液对应的保留时间偏差在±2.5%之内;且样品中组分定性离子的相对丰度与浓度接近的标准工作溶液中对应的定性离子的相对丰度进行比较,最大允许偏差不得超过表 2 规定的范围,则可判定为样品中存在对应的待测物。9 种维生素的参考保留时间参见附录 B 中表 B.1。

表 2 定性确证时相对离子丰度的最大允许偏差

相对离子丰度/%	>50	>20~50	>10~20	≤10
允许相对偏差/%	±10	±15	±20	±50

7.2.4 定量测定

在上述条件下,对样液及标准工作溶液(参考线性浓度范围为:0.05~10.0 μg/mL;叶酸为 0.05~100 ng/mL)进样,样液中待分析物的响应值均应在仪器测定的线性范围内,内标法定量。标准物质的多反应监测(MRM)色谱图参见附录 C 图 C.1。

7.3 空白实验

除不称取试样外,均按上述步骤进行。

8 结果计算

按式(1)计算各种维生素的含量,计算结果需扣除空白值:

$$X_i = \frac{c_i \times V}{m} \times \frac{1\,000}{1\,000} \dots\dots\dots (1)$$

式中：

X_i ——试样中被测物含量,单位为毫克每千克(mg/kg)；

c_i ——从标准曲线上得到的样液中待测物的含量,单位为微克每毫升($\mu\text{g/mL}$)；

V ——样液最终定容体积,单位为毫升(mL)；

m ——试样溶液所代表试样的质量,单位为克(g)。

注：计算结果应扣除空白值。

9 测定低限、精密度、回收率

9.1 测定低限

本方法中各维生素在不同基质的测定低限见表 3。

表 3 不同基质中各维生素的测定低限 单位:mg/kg

标准物质	基质					
	饮料	大米	饼干	含乳饮料	奶粉	果冻
硫胺素	1	1	1	1	1	1
烟酸	1	5	5	1	1	1
吡哆醛	0.2	1	1	0.2	1	1
吡哆醇	0.2	1	1	0.2	1	1
烟酰胺	1	5	5	1	1	1
泛酸	1	4	1	1	1	1
核黄素	1	1	1	1	1	1
吡哆胺	0.2	1	1	0.2	1	1
叶酸	0.1	0.2	0.5	0.2	0.2	0.01

9.2 回收率

本方法分别在大米、果汁、含乳饮料、果冻、饼干和奶粉基质中不同添加水平的添加回收率范围参见附录 F 表 F.1。

第二部分 食品中水溶性维生素 B₁₂(钴胺素)的测定

10 原理

试样中的维生素 B₁₂(钴胺素)用盐酸溶液、三氯乙酸溶液或温水(等电点调节 pH)提取,用 HLB 固相萃取柱进行富集并去除部分杂质后,用液相色谱-质谱/质谱仪检测,外标法定量。

11 试剂和材料

除非另有规定外,所有试剂均为分析纯,水为 GB/T 6682 规定的一级水。

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11.1 乙腈:HPLC 级。

11.2 7%乙腈:量取 70 mL 乙腈(11.1),用水稀释定容至 1 000 mL。

11.3 25%乙腈:量取 250 mL 乙腈(11.1),用水稀释定容至 1 000 mL。

11.4 维生素 B₁₂(Vitamin B₁₂或 Cyanocobalamin)标准物质:纯度大于等于 99%,冷藏于冰箱中避光保存。参见附录 A 表 A.1。

11.5 标准储备液:精确称取适量标准品(11.4),用 0.01 mol/L 盐酸溶液(4.11)配制成 1 mg/mL 的标准储备液。4℃避光保存,有效期为 6 d。

11.6 标准工作液:根据需要用 0.01 mol/L 盐酸溶液(4.11)将标准储备液(11.5)逐级稀释,配制成一系列标准工作液,现用现配。

11.7 HLB 固相萃取柱:60 mg,3 mL 或相当者。使用前分别用 5 mL 甲醇和 5 mL 水预淋洗并保持柱体湿润。

11.8 其他同第 4 章。

12 仪器和设备

12.1 固相萃取装置。

12.2 其他同第 5 章。

13 试样制备与保存

同第 6 章。

14 测定方法

14.1 样品的提取

14.1.1 果汁饮料

称取试样 10 g(精确至 0.01 g)于 25 mL 棕色容量瓶中,用水定容至 25 mL,待净化。

14.1.2 大米、果冻、饼干

称取试样 2 g(精确至 0.01 g)于 50 mL 离心管中,加入 10 mL 0.01 mol/L 盐酸溶液(4.11),涡旋混匀 1 min,超声 15 min,以 4 000 r/min 离心 10 min。取上清液置于 25 mL 棕色容量瓶中,用水定容至刻度,待净化。

14.1.3 含乳饮料

称取试样 10 g(精确至 0.01 g)于 50 mL 离心管中,加入 5 mL 40 g/L 三氯乙酸盐酸溶液(4.14),涡旋混匀 1 min,超声 15 min,以 4 000 r/min 离心 10 min。取上清液置于 25 mL 棕色容量瓶中,用水定容至刻度,待净化。

14.1.4 奶粉

称取试样 2 g(精确至 0.01 g),加入 20 mL 45℃~50℃水,涡旋混匀 1 min,超声 15 min。待溶液

温度降至室温后,用 5 mol/L 盐酸溶液(4.12)调节 pH 至 1.90 ± 0.5 ,放置 2 min,再用 5 mol/L 氢氧化钠溶液(4.13)调节 pH 至 4.70 ± 0.5 ,以 4 000 r/min 离心 10 min,收集上清液,待净化。

14.2 净化

将 14.1 获得的待净化液通过固相萃取柱(11.7),用 5 mL 7%乙腈溶液(11.1)将干扰物质从固相萃取柱上淋洗下来,最后用 1.0 mL 25%乙腈溶液(11.2)将维生素 B₁₂洗脱,收集全部洗脱液,过 0.22 μm 微孔滤膜后,供液相色谱-质谱/质谱仪测定。

上述提取步骤,均须避光进行。

14.3 测定

14.3.1 仪器参考条件

同 7.2.1 和 7.2.2。

14.3.2 定性分析

在上述条件下测定样品和标准溶液,样品中待测物色谱峰保留时间与标准溶液对应的保留时间偏差在 $\pm 2.5\%$ 之内;且样品中组分定性离子的相对丰度与浓度接近的标准工作溶液中对应的定性离子的相对丰度进行比较,最大允许偏差不超过表 2 规定的范围,则可判定为样品中存在对应的待测物。在上述仪器条件下,维生素 B₁₂的参考保留时间约为 7.56 min。

14.3.3 定量测定

在上述条件下,对样液及标准工作溶液(参考线性浓度范围为:0.05~1 00 ng/mL)进样,样液中待分析物的响应值均应在仪器测定的线性范围内,外标法定量。标准物质的多反应监测(MRM)色谱图参见附录 D 图 D.1。

14.4 空白实验

除不称取试样外,均按上述步骤进行。

15 结果计算

试样中维生素 B₁₂的含量利用数据处理系统计算或按式(2)计算:

$$X_i = \frac{c_i \times V}{m} \times \frac{1\ 000}{1\ 000} \dots\dots\dots (2)$$

式中:

X_i ——试样中被测物含量,单位为微克每千克(μg/kg);

c_i ——从标准曲线上得到的样液中待测物的含量,单位为纳克每毫升(ng/mL);

V ——样液最终定容体积,单位为毫升(mL);

m ——试样溶液所代表试样的质量,单位为克(g)。

注:计算结果应扣除空白值。

16 测定低限、精密度、回收率

16.1 测定低限

本方法中维生素 B₁₂ 在果汁和含乳饮料的测定低限为 0.5 μg/kg, 饼干和果冻的测定低限为 1 μg/kg, 大米的测定低限为 2 μg/kg, 奶粉的测定低限为 5 μg/kg。

16.2 回收率

本方法分别在大米、果汁、含乳饮料、果冻、饼干和奶粉基质中不同添加水平的添加回收率范围参见附录 F 表 F.2。

第三部分 食品中水溶性维生素 Vc(L-抗坏血酸)的测定

17 原理

试样中的维生素 Vc(L-抗坏血酸)用偏磷酸溶液或温水(等电点调节 pH)提取后,用液相色谱紫外检测器检测,外标法定量。

18 试剂和材料

18.1 偏磷酸。

18.2 磷酸二氢钾。

18.3 磷酸。

18.4 3%偏磷酸溶液:称取 30 g 偏磷酸(18.1),用水溶解并稀释至 1 000 mL。

18.5 0.05 mol/L 磷酸二氢钾(pH=3):称取磷酸二氢钾(18.2)固体 6.80 g,用水溶解并稀释至 1 000 mL,用磷酸(18.3)调节 pH 为 3,用 0.22 μm 微孔滤膜过滤。

18.6 维生素 Vc(Ascorbic acid)标准物质纯度大于等于 99%,冷藏于冰箱中避光保存。详见附录 A 表 A.1。

18.7 标准储备液:精确称取适量标准品(18.6),用 3%偏磷酸溶液(18.4)配制成 1 mg/mL 的标准储备液。4℃避光保存,现用现配。

18.8 标准工作液:根据需要用 3%偏磷酸溶液(18.4)将标准储备液(18.7)逐级稀释,配制成一系列标准工作液,现用现配。

18.9 其他同第 4 章。

19 仪器和设备

19.1 高效液相色谱仪:配有紫外检测器或二极管阵列检测器。

19.2 其他同第 5 章。

20 试样制备与保存

同第6章。

21 测定方法

21.1 样品提取

21.1.1 果汁饮料

称取试样2 g(精确至0.01 g)于50 mL棕色容量瓶中,用3%偏磷酸溶液(18.4)定容至刻度,混匀,用0.22 μm 微孔滤膜过滤,所得滤液待进样测定。超出线性范围时用3%偏磷酸溶液(18.4)稀释到一定倍数后,用0.22 μm 微孔滤膜过滤,供液相色谱仪测定。

21.1.2 大米、果冻、果泥、含乳饮料

称取试样5 g(精确至0.01 g),用3%偏磷酸溶液(18.4)定容至100 mL,超声15 min且超声过程中需要加入冰块控制温度在10 $^{\circ}\text{C}$ ~20 $^{\circ}\text{C}$ 之间,4 000 r/min离心10 min。取上清液用0.22 μm 微孔滤膜过滤后,供液相色谱仪测定。

21.1.3 奶粉

称取试样2 g(精确至0.01 g),加入20 mL 45 $^{\circ}\text{C}$ ~50 $^{\circ}\text{C}$ 水,涡旋混匀1 min,超声15 min且超声过程中需要加入冰块控制温度在10 $^{\circ}\text{C}$ ~20 $^{\circ}\text{C}$ 之间。后用5 mol/L盐酸溶液(4.12)调节pH至 1.90 ± 0.5 ,放置2 min,再用5 mol/L氢氧化钠溶液(4.13)调节pH至 4.70 ± 0.5 ,以4 000 r/min离心10 min,取上清液用0.22 μm 微孔滤膜过滤后,供液相色谱仪测定。

上述提取步骤,均应避光进行。

21.2 测定

21.2.1 液相色谱参考条件

液相色谱参考条件如下:

- a) 色谱柱:TechMate C₁₈-ST柱,250 mm \times 4.6 mm(内径),5 μm ;或相当者;
- b) 波长:266 nm;
- c) 进样量:10 μL ;
- d) 柱温:25 $^{\circ}\text{C}$;
- e) 流动相:0.05 mol/L磷酸二氢钾(pH=3)。

21.2.2 色谱测定

按照上述检测条件测定样液和标准工作溶液,以被测物峰面积为纵坐标,标准溶液浓度为横坐标绘制标准工作曲线,用标准工作曲线对样品进行定量,样品溶液中待测物的响应值均应在仪器测定的线性范围内。若其响应值超过线性范围,可调整定容体积使之满足定量测定线性范围的要求。在上述色谱条件下,待测物的参考保留时间约为3.42 min。标准溶液的液相色谱图参见附录E图E.1。

21.3 空白实验

除不称取试样外,均按上述步骤进行。

22 结果计算

同第 8 章。

23 方法的测定低限和回收率

23.1 测定低限

果汁、奶粉、含乳饮料、大米和果冻中维生素 C 的测定低限均为 100 mg/kg;果泥中维生素 C 的测定低限为 20 mg/kg。

23.2 回收率

本方法分别在大米、果汁、含乳饮料、果冻、果泥和奶粉基质中不同添加水平的添加回收率范围参见附录 F 表 F.3。

附 录 A
(资料性附录)
水溶性维生素标准品信息

表 A.1 水溶性维生素标准品信息

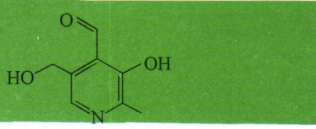
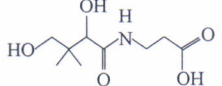
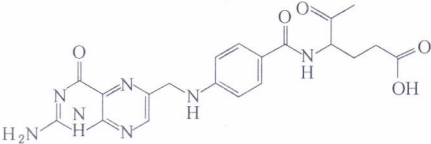
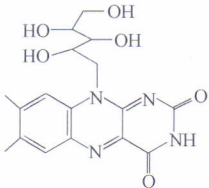
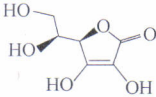
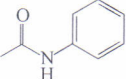
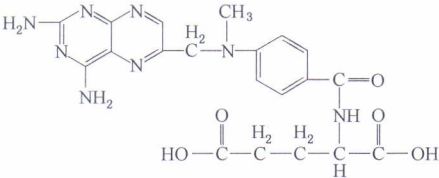
名称	英文名	结构式	CAS 号	分子式
硫胺素 (VB ₁)	Thiamine		67-03-8	C ₁₂ H ₁₇ ClN ₄ OS
烟酸 (VB ₃)	Nicotinic acid		59-67-6	C ₆ H ₅ NO ₂
吡哆醛 (VB ₆)	Pyridoxal		65-22-5	C ₈ H ₉ NO ₃
吡哆醇 (VB ₆)	Pyridoxine		8059-24-3	C ₈ H ₁₁ NO ₃
吡哆胺 (VB ₆)	Pyridoxamine		524-36-7	C ₈ H ₁₂ N ₂ O ₂
烟酰胺 (VB ₃)	Nicotinamide		98-92-0	C ₆ H ₆ N ₂ O
泛酸 (VB ₅)	Pantothenic acid		137-08-6	C ₉ H ₁₇ NO ₅
钴胺素 (VB ₁₂)	Cyanocobalamin		68-19-9	C ₆₃ H ₈₈ CoN ₁₄ O ₁₄ P

表 A.1 (续)

名称	英文名	结构式	CAS 号	分子式
叶酸 (VB ₉)	Folic acid		59-30-3	C ₁₉ H ₁₉ N ₇ O ₆
核黄素 (VB ₂)	Riboflavin		83-88-5	C ₁₇ H ₂₀ N ₄ O ₆
维生素 C	Ascorbic acid		50-81-7	C ₆ H ₈ O ₆
乙酰 苯胺	Acetanilide		103-84-4	C ₈ H ₉ NO
甲氨 蝶呤	Methotrexate		59-05-2	C ₂₀ H ₂₂ N ₈ O ₅

附 录 B
(资料性附录)

LC-MS/MS 系统电喷雾离子源参考条件¹⁾

表 B.1 被测物的参考保留时间、监测离子对、去簇电压和碰撞气能量

分析物	保留时间 min	离子对 m/z	去簇电压 (DP) V	碰撞气能 量(CE) eV	对应的内标化 合物名称
硫胺素	1.51	265.2/122.2*	70	6	乙酰苯胺
		265.2/144.2	70	6	
烟酸	2.21	124.1/78.1*	108	21	D4-烟酸
		124.1/80.1	108	17	
吡哆醛	2.65	168.2/94.0	80	25	乙酰苯胺
		168.2/150.0*	80	10	
吡哆醇	3.85	170.0/134.0*	88	20	乙酰苯胺
		170.0/152.0	88	37	
吡哆胺	1.30	169.0/134.0*	80	18	乙酰苯胺
		169.0/152.0	80	30	
烟酰胺	3.65	123.1/78.2*	108	22	D4-烟酰胺
		123.1/80.2	108	20	
泛酸	7.30	220.0/90.0*	90	5	甲氨蝶呤
		220.0/202.0	90	8	
钴胺素	7.56	678.5/147*	150	33	—
		678.5/358.8	150	21	
叶酸	7.47	442.4/176.9	135	29	甲氨蝶呤
		442.4/295.1*	135	7	
核黄素	7.91	377.4/171.8*	156	37	甲氨蝶呤
		377.4/243.1	156	21	
D4-烟酸	2.16	128.1/84.1	110	20	—
D4-烟酰胺	3.61	127.0/84.0	90	20	—
乙酰苯胺	8.76	136.0/94.0	80	15	—
甲氨蝶呤	7.62	455.0/308.0	135	20	—

注：* 为定量离子对，对于不同质谱仪器，仪器参数可能存在差异，测定前应将质谱参数优化到最佳。

1) 非商业性声明：附录 B 所列参考质谱条件是在安捷伦 6460 型液质联用仪上完成的，此外列出试验用仪器型号仅为提供参考，并不涉及商业目的，鼓励标准使用者尝试不同厂家或型号仪器。

附录 C (资料性附录)

水溶性维生素标准溶液选择性离子流图

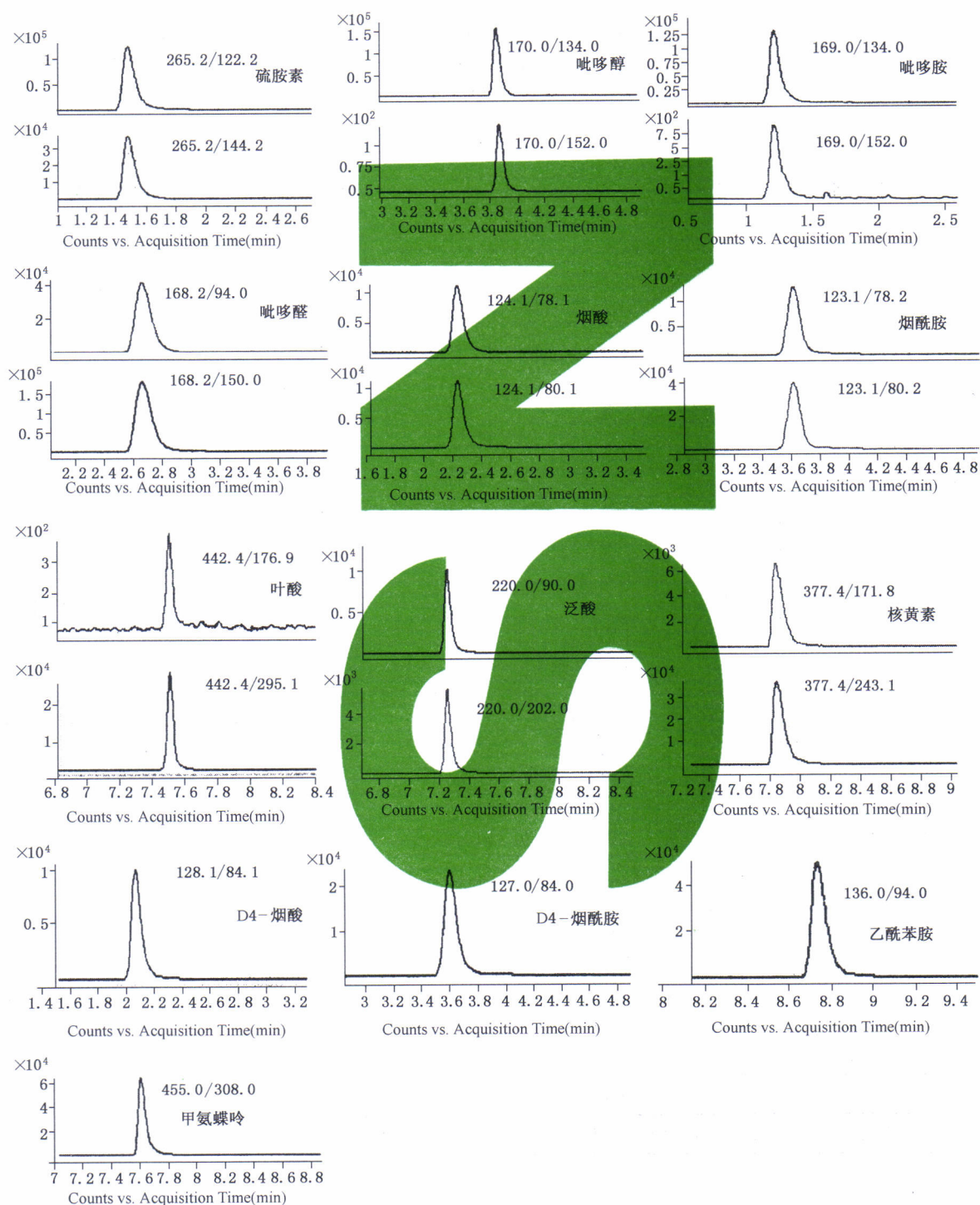


图 C.1 9 种水溶性维生素及其内标的选择性离子流图(浓度为 0.5 $\mu\text{g/mL}$, 叶酸为 20 ng/mL)

附 录 D

(资料性附录)

维生素 B₁₂ 标准品的选择性离子流图

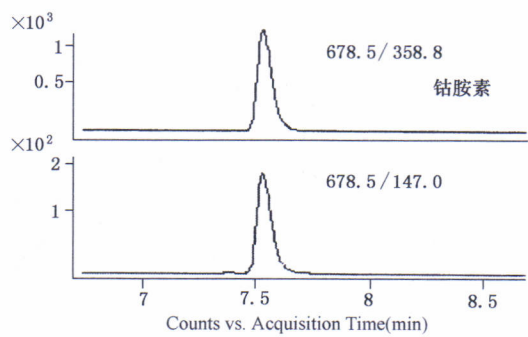


图 D.1 维生素 B₁₂ 的选择性离子流图 (浓度为 5 ng/mL)

附录 E
(资料性附录)
维生素 C 的液相色谱图

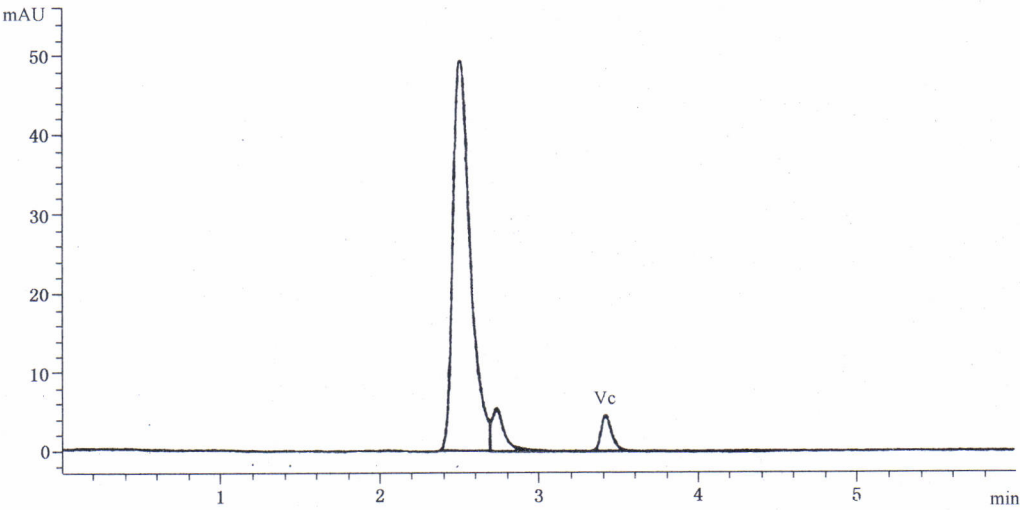


图 E.1 维生素 C 标准品的液相色谱图(2.0 µg/mL)

附 录 F
(资料性附录)

水溶性维生素的在不同基质中的回收率范围

表 F.1 九种维生素在不同基质中的回收率范围

组分名称	果汁		果冻		大米		饼干		含乳饮料		奶粉	
	添加水平 mg/kg	回收率 %	添加水平 mg/kg	回收率 %	添加水平 mg/kg	回收率 %	添加水平 mg/kg	回收率 %	添加水平 mg/kg	回收率 %	添加水平 mg/kg	回收率 %
硫胺素	1	80.6~109	1	86.4~110	1	80.7~97.5	1	80.2~103	1	80.0~103	1	80.7~99.2
	5	81.6~102	5	83.4~110	5	86.6~108	5	81.6~104	5	80.8~104	5	82.7~97.8
	10	80.7~99.6	10	80.8~110	10	80.9~105	10	82.4~110	10	80.2~98.6	25	80.1~102
烟酸	1	81.0~95.8	1	80.0~109	5	80.2~108	5	81.1~96.1	1	80.0~106	1	80.6~105
	5	81.4~105	5	80.4~104	20	81.1~101	20	81.3~99.1	5	80.4~101	10	84.0~94.1
	25	82.6~106	10	80.1~99.7	100	90.3~105	100	90.0~105	25	85.2~94.3	150	93.7~102
吡哆醛	0.2	80.9~106	1	81.0~109	1	81.6~105	1	80.8~110	0.2	81.8~103	1	81.1~97.9
	1	90.1~110	5	81.6~104	5	87.4~103	5	85.4~97.0	1	97.5~108	5	82.3~92.0
	5	86.3~106	10	80.5~100	10	85.3~104	10	81.1~100	5	81.7~105	25	83.4~98.5
吡哆醇	0.2	81.0~106	1	81.0~110	1	80.6~109	1	80.5~108	0.2	82.9~97.8	1	87.1~101
	1	80.1~102	5	81.6~101	5	81.7~102	5	83.5~102	1	83.0~97.0	5	89.6~104
	5	82.4~101	10	80.7~101	10	84.6~104	10	80.0~101	5	84.2~106	25	83.8~103
烟酰胺	1	93.2~105	1	80.0~92.0	5	87.8~103	5	84.0~102	1	81.3~108	1	81.0~108
	5	94.3~109	5	80.2~102	20	80.2~109	20	82.9~96.7	5	80.2~101	10	80.6~98.1
	25	89.7~104	10	80.1~101	100	91.6~98.3	100	90.4~103	25	80.9~101	150	90.7~103
泛酸	1	80.6~96.1	1	83.1~103	4	86.3~103	1	80.9~100	1	80.7~101	1	80.1~107
	5	82.7~106	5	80.8~101	8	87.8~106	5	80.2~105	5	81.1~110	10	80.6~104
	10	81.7~102	10	80.0~105	20	84.5~105	10	81.2~100	10	80.3~106	100	90.6~104
吡哆胺	0.2	80.4~108	1	80.1~104	1	90.7~102	1	82.8~110	0.2	80.5~102	1	88.3~101
	1	80.3~110	5	81.0~102	5	88.1~108	5	81.7~108	1	80.1~105	5	81.9~106
	5	80.9~107	10	80.1~90.7	10	84.4~95.7	10	81.7~101	5	81.0~94.5	25	80.7~104
叶酸	0.1	80.6~108	0.01	75.9~108	0.5	81.6~94.6	0.1	84.6~109	0.2	81.0~99.9	0.2	81.2~98.2
	0.5	81.4~105	0.02	72.0~101	1	80.7~99.4	0.5	80.3~106	0.5	82.0~105	0.5	87.8~99.9
	1	83.7~104	0.10	78.6~100	5	90.3~101	1	80.2~102	5	82.9~98.1	10	83.3~106
核黄素	1	80.7~101	1	84.0~110	1	86.4~102	1	89.6~104	1	80.8~104	1	82.0~100
	5	89.8~108	5	82.2~103	5	90.7~107	5	80.4~96.3	5	81.6~97.0	5	82.4~99.7
	10	90.2~106	10	80.5~110	10	89.4~101	10	80.8~105	10	85.0~102	25	80.9~103

表 F.2 维生素 B₁₂ 在不同基质中的回收率范围

基质	添加水平 μg/kg	回收率范围 %	基质	添加水平 μg/kg	回收率范围 %
果汁	0.5	71.6~92.0	饼干	1	75.0~105
	2	70.1~97.7		5	72.2~98.2
	5	76.5~101		20	83.6~103
果冻	1	71.4~100	含乳饮料	0.5	73.6~95.8
	2	74.9~98.9		2	73.8~91.4
	10	70.4~103		5	73.1~96.2
大米	2	70.0~106	奶粉	5	72.0~99.0
	5	80.0~102		20	72.9~94.9
	10	74.7~99.1		100	80.9~98.1

表 F.3 维生素 C 在不同基质中的回收率范围

基质	添加水平 mg/kg	回收率范围 %	基质	添加水平 mg/kg	回收率范围 %
果汁	100	90.8~105	果泥	20	83.5~106
	200	92.3~107		50	82.2~107
	500	91.2~106		250	91.3~105
果冻	100	94.5~107	含乳饮料	100	90.4~105
	150	93.4~104		200	97.9~106
	300	98.1~103		500	93.1~101
大米	100	90.8~106	奶粉	100	90.5~107
	200	90.0~105		500	91.1~102
	1 000	96.9~107		2 000	96.0~105

Foreword

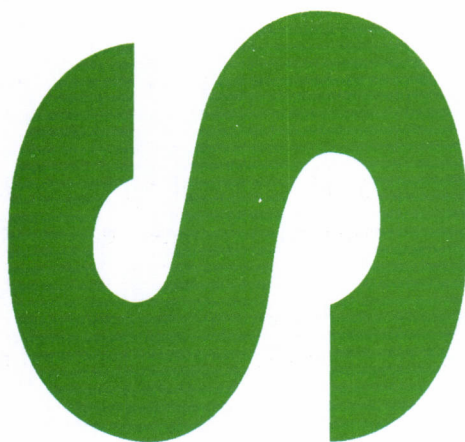
This standard was drafted according to GB/T 1.1—2009.

Attention is required to the certain contents of this text which might be related to some patents. This file is not responsible to identify these.

This standard was proposed by and is under the charge of National Regulatory Commission for Certification and Accreditation.

This standard was drafted by Hebei Entry-Exit Inspection and Quarantine Bureau of the People's Republic of China and Shanghai Entry-Exit Inspection and Quarantine Bureau of the People's Republic of China.

Main drafters of this standard are: Ai Lianfeng, Duan Yongsheng, Wang Jing, Guo Chunhai, Ma Yusong, Song Ge, Qu Li.



Determination of water-soluble vitamins in foods for export

1 Scope

This standard specifies the determination method for residues of vitamin B₁ (thiamine), vitamin B₂ (riboflavin), vitamin B₃ (nicotinic acid, nicotinamide), vitamin B₅ (pantothenic acid), vitamin B₆ (pyridoxine, pyridoxal, pyridoxamine), folic acid and vitamin B₁₂ (cyanocobalamin) in foods for export by HPLC-MS/MS, and vitamin C (L-ascorbic acid) in foods for export by HPLC.

This standard is applicable to the determination and the confirmation of vitamin B₁ (thiamine), vitamin B₂ (riboflavin), vitamin B₃ (nicotinic acid, nicotinamide), vitamin B₅ (pantothenic acid), vitamin B₆ (pyridoxine, pyridoxal, pyridoxamine), folic acid and vitamin B₁₂ (cyanocobalamin) in juice, milk power, milk drinks, rice, biscuit and jelly; and is applicable to the determination of vitamin C (L-ascorbic acid) in juice, milk power, milk drinks, rice, puree and jelly.

2 Normative references

The following documents is necessary for this standard. For dated references, only dated editions shall apply to this standard. For undated references, the latest edition of the normative document referred to applies.

GB/T 6682 Water for analytical laboratory use-Specification and test methods

Part 1: Determination of vitamin B₁ (thiamine), vitamin B₂ (riboflavin), vitamin B₃ (nicotinic acid, nicotinamide), vitamin B₅ (pantothenic acid), vitamin B₆ (pyridoxine, pyridoxal, pyridoxamine) and folic acid in foods

3 Principle

The vitamins in the test sample are extracted with hydrochloric acid solution, trichloroacetic acid solution or warm water (isoelectric point method). After filtered through a membrane, the extract is detected by liquid chromatography-tandem mass spectrometry and quantified by internal standard method.

4 Reagents and materials

Unless specifically noted, all reagents used should be of analytically grade; water is the first grade water prescribed by GB/T 6682.

4.1 Methanol: HPLC grade.

4.2 Formic acid: HPLC grade.

4.3 Ammonium acetate: HPLC grade.

4.4 Ammonium hydroxide.

4.5 Hydrochloric acid.

4.6 Trichloroacetic acid.

4.7 Sodium hydroxide.

4.8 BHT (butylated hydroxytoluene), purity $\geq 99.3\%$, stored at $-18\text{ }^{\circ}\text{C}$.

4.9 0.1% formic acid solution: Accurately measure 0.1 mL formic acid (4.2) into a 100 mL volumetric flask, dilute with water to 100 mL, mix adequately.

4.10 10 mmol/L ammonium acetate solution ($\text{pH} = 6.3$): Dissolve 0.39 g ammonium acetate (4.3) into 500 mL water, mix adequately, and then the pH of the solution was adjusted pH with 0.1% formic acid solution (4.9) to 6.3.

4.11 0.01 mol/L hydrochloric acid solution: Transfer 900 μL hydrochloric acid (4.5) into a 1 000 mL volumetric flask, dilute with water to 1 000 mL, mix adequately.

4.12 5 mol/L hydrochloric acid solution: Transfer 45 mL hydrochloric acid (4.5) into a 100 mL volumetric flask, dilute with water to 100 mL, mix adequately.

4.13 5 mol/L sodium hydroxide solution: Dissolve 20 g sodium hydroxide (4.7) into 100 mL water, mix adequately.

4.14 40 g/L trichloroacetic acid solution: Dissolve 40 g trichloroacetic acid (4.6) into 1 000 mL water, mix adequately.

4.15 1% ammonium hydroxide solution: Dissolve 1 mL ammonium hydroxide (4.4) to final volume of 100 mL with water, mix adequately.

4.16 1 mg/mL BHT solution: Dissolve 10 mg BHT (4.8) into 10 mL methanol (4.1), mix adequately.

4.17 Vitamin standards: the purity of vitamin B₁ (thiamine), vitamin B₂ (riboflavin), vitamin B₃ (nicotinic acid, nicotinamide), vitamin B₅ (pantothenic acid), vitamin B₆ (pyridoxine, pyridoxal, pyridoxamine), folic acid; and internal standards: D4-nicotinic acid, D4-nicotinamide, methotrexate and acetanilide are no less than 95%, respectively, and relative information, see Table A.1 in Annex A.

4.18 Stock standard solution: Accurately weigh an adequate amount of standard (4.17), dissolve in 0.01 mol/L hydrochloric acid solution and prepare of 1 mg/mL (0.1 mg/mL for vitamin B₂), store in a refrigerator at 4 °C avoiding light, assign a shelf life of 6 days; the folic acid was dissolved in 1% ammonium hydroxide solution (4.15) with a shelf life of 4 days.

4.19 Standard working solution: Prepare a standard working solution by diluting the above stock solution (4.18) with 0.01 mol/L hydrochloric acid solution (4.11) just before use.

4.20 Stock internal standard solution: Accurately weigh a certain amount of each standard, such as D4-nicotinic acid, D4-nicotinamide, methotrexate and acetanilide and dissolve it in methanol (4.1) to make the standard stock solution of 500 µg/mL, store in a refrigerator at 4 °C avoiding light.

4.21 Internal standard working solution: Accurately measure an appropriate volume of stock internal standard solution (4.20) and dilute to 50 µg/mL with methanol (4.1) just before use.

4.22 Water-phase filter membrane: 0.22 µm.

5 Apparatus and equipment

5.1 Liquid chromatography-mass spectrograph, equipped with electrospray ion source.

5.2 Balance: accuracy to 0.01 g and 0.1 mg.

5.3 Organ blender.

5.4 Centrifuge, speed of no less than 4 000 r/min.

5.5 Homogenizer, speed of no less than 15 000 r/min.

5.6 Homogenizer.

5.7 Ultrasonic cleanser.

5.8 Polypropylene centrifuge tube with cap: 50 mL.

5.9 Brown glass volumetric flask: 10 mL, 25 mL and 100 mL.

6 Preparation and storage of test sample

6.1 Requirement

In the course of sample preparation, precaution must be taken avoid the contamination or any factors which may cause the change of residue content.

6.2 Preparation of test sample

Take three independent packing samples from the same batch (not less than 200 g for the solid sample and not less than 200 mL for liquid sample). Grind the solid and semisolid samples with a muller, mix thoroughly the liquid sample, then divide into two equal portions and then place in clean containers. One uses as a test sample and the other as a preserved sample.

7 Procedure

7.1 Extraction

7.1.1 Juice

Weigh 2 g (accurate to 0.01 g) into a 10 mL brown glass volumetric flask. Add 40 μ L internal standard solution (4.21) and 100 μ L 1 mg/mL BHT solution (4.16), and dilute with 0.01 mol/L hydrochloric acid solution (4.11) to 10 mL, mix adequately. Then the solution is passed through 0.22 μ m filter and ready for HPLC-MS/MS determination.

7.1.2 Rice, jelly and biscuit

Weigh 2 g (accurate to 0.01 g) into a 50 mL polypropylene centrifuge tube with cap. Add 40 μ L internal standard solution (4.21), 100 μ L 1 mg/mL BHT solution (4.16) and 10 mL 0.01 mol/L hydrochloric acid solution (4.11), homogenize for 1 min, ultrasonicate for 15 min. After centrifugation at 4 000 r/min for 10 min, the supernatant is passed through 0.22 μ m filter and ready for HPLC-MS/MS determination.

7.1.3 Milk drinks

Weigh 2 g (accurate to 0.01 g) into a 50 mL polypropylene centrifuge tube with cap. Add 40 μ L internal standard solution (4.21), 100 μ L 1 mg/mL BHT solution (4.16) and 10 mL 40 g/L trichloroacetic acid solution (4.14), homogenize for 1 min, ultrasonicate for 15 min. After centrifugation at 4 000 r/min for 10 min, the supernatant is passed through 0.22 μ m filter and ready for HPLC-MS/MS determination.

tion.

7.1.4 Milk power

Weigh 2 g (accurate to 0.01 g) into a 50 mL polypropylene centrifuge tube with cap. Add 40 μ L internal standard solution (4.21), 100 μ L 1 mg/mL BHT solution (4.16) and 20 mL 45 $^{\circ}$ C ~50 $^{\circ}$ C water, homogenize for 1 min, ultrasonicate for 15 min. After cooling, the pH of the solution was adjusted with 5 mol/L hydrochloric acid solution (4.12) to 1.90 ± 0.5 , and then adjusted with 5 mol/L sodium hydroxide solution (4.13) to 4.70 ± 0.5 for protein precipitation. Subsequently the mixture was centrifuged at 4 000 r/min for 5 min, the supernatant is passed through 0.22 μ m filter and ready for HPLC-MS/MS determination.

During the extraction process, samples are always protected from direct exposition to light.

7.2 Determination

7.2.1 HPLC operating conditions

Reference HPLC operating conditions is as following:

- a) Column: UPLC HSS T₃ 50 mm \times 2.1 mm (i.d.), 1.7 μ m particle size or equivalent;
- b) Column temperature: 30 $^{\circ}$ C ;
- c) Injection volume: 5 μ L;
- d) Mobile phases and gradient elution conditions are listed in Table 1.

Table 1—Mobile phase and gradient elution condition

Time min	Methanol %	10 mmol/L ammonium acetate solution(pH=6.3) %	flow rate mL/min
0	1	99	0.3
1.0	1	99	0.3
4.0	55	45	0.3
6.0	55	45	0.3
6.1	1	99	0.3
10.0	1	99	0.3

7.2.2 MS conditions

Reference MS conditions is as following:

- a) Ionization mode: ESI;
- b) scan mode: positive mode;
- c) Monitor mode: multiple reaction monitoring (MRM);
- d) Other reference mass operating conditions are listed in Annex B Table B.1.

7.2.3 Qualitative analysis

Under the above determination condition, the variation range of the retention time for the peak of analyte in unknown sample and in the standard working solution can not be out of range of $\pm 2.5\%$. For the same analysis batch and the same compound, the variation range of the ion ration between the two daughter ions for the unknown sample and the standard working solution at the similar concentration can not be out of range of table 2, and then the corresponding analyte must be present in the sample. Under the above operating condition, retention time of each vitamin is shown as Table B. 1 in Annex B.

Table 2—Maximum permitted tolerances for relative ion intensities while confirmation

Relative intensity/%	>50	>20~50	>10~20	≤ 10
Permitted tolerance/%	± 10	± 15	± 20	± 50

7.2.4 Quantitative analysis

Under the above conditions of the apparatus, inject series of working standard solutions (the reference concentration range for folic acid is 0.05~100 ng/mL, and 0.05~10.0 $\mu\text{g/mL}$ for the other vitamins) and sample solutions separately. Use the curve to quantify the each analyte in unknown sample by internal standard method. The responses of the analyte in the standard working solution and the sample solution should be within the linear range of the instrument detection. Under the above operating condition, the chromatogram of the standard can be found by Figure C.1 in Annex C.

7.3 Blank test

The operation of the blank test is the same as the described in the method of determination, but with the omission of sample addition.

8 Calculation an expression of result

Calculation the content of vitamins in test sample by data processor or according to formula (1)

$$X_i = \frac{c_i \times V}{m} \times \frac{1\,000}{1\,000} \dots\dots\dots (1)$$

where:

X_i —the content of vitamin in test sample,mg/kg;

c_i —the concentration of vitamin in the test sample calculated by calibration curve,µg/mL;

V —the final volume of the sample solution,mL;

m —the corresponding mass of test sample in the final solution,g.

note: the blank value shall be subtracted from the result of calculation.

9 Limit of determination

9.1 The limits of quantification of the method for vitamins in different matrixes are listed as Table 3.

Table 3—Limits of quantification for vitamins in different matrixes (mg/kg)

standards	matrix					
	juice	rice	biscuit	milk drinks	milk power	jelly
Thiamine	1	1	1	1	1	1
Nicotinic acid	1	5	5	1	1	1
Pyridoxal	0.2	1	1	0.2	1	1
Pyridoxine	0.2	1	1	0.2	1	1
Nicotinamide	1	5	5	1	1	1
Pantothenic acid	1	4	1	1	1	1
Riboflavin	1	1	1	1	1	1
Pyridoxamine	0.2	1	1	0.2	1	1
Folic acid	0.1	0.2	0.5	0.2	0.2	0.01

9.2 Recovery

The ranges of recovery in rice, juice, milk drinks, jelly, biscuit and milk power are listed as Table F.1 in Annex F.

Part 2: Determination of vitamin B₁₂ (Cyanocobalamin) in foods

10 Principle

The vitamin B₁₂ (Cyanocobalamin) in the test sample is extracted with hydrochloric acid, trichloroacetic acid and or warm water (isoelectric point method). After extracted, the solution is cleaned up with SPE cartridge of HLB, the elute is detected by liquid chromatography-tandem mass spectrometry and quantified by external standard method.

11 Reagents and materials

Unless specifically noted, all reagents used should be of analytically grade; water is the first grade water prescribed by GB/T 6682.

11.1 Acetonitrile, HPLC grade.

11.2 7% acetonitrile aqueous solution: Accurately measure 70 mL acetonitrile (11.1) into a 1 000 mL volumetric flask, dilute with water to 1 000 mL, mix adequately.

11.3 25% acetonitrile aqueous solution: Accurately measure 250 mL acetonitrile (11.1) into a 1 000 mL volumetric flask, dilute with water to 1 000 mL, mix adequately.

11.4 Vitamin standards: the purity of vitamin B₁₂ (cyanocobalamin) is no less than 99%, which should be stored at 4 °C in dark and relative information listed in Table A.1 in Annex A.

11.5 Stock standard solution: Accurately weigh an adequate amount of standard (11.4), dissolve in 0.01 mol/L hydrochloric acid solution and prepare of 1 mg/mL, store in a refrigerator at 4 °C, assign a shelf life of 6 days.

11.6 Middle working solution: Prepare a standard working solution by diluting the above stock solution (11.5) with 0.01 mol/L hydrochloric acid solution just before use.

11.7 Oasis HLB cartridge: 60 mg, 3 mL or equivalent. The column is activated with 5 mL methanol and 5 mL water before use. The column should be kept wet always.

11.8 The other apparatuses are the same as section 4.

12 Apparatus and equipment

12.1 Solid phase extraction.

12.2 The other apparatuses are the same as section 5.

13 Preparation and storage of test sample

The apparatuses are the same as section 6.

14 Procedure

14.1 Extraction

14.1.1 Juice

Weigh 10 g (accurate to 0.01 g) into a 25 mL brown volumetric flask, and dilute to the volume with water for cleaning up.

14.1.2 Rice, jelly and biscuit

Weigh 2 g (accurate to 0.01 g) into a 50 mL polypropylene centrifuge tube with cap. Add 10 mL 0.01 mol/L hydrochloric acid solution (4.11), homogenize for 1 min, ultrasonicate for 15 min. After centrifugation at 4 000 r/min for 10 min, the supernatant is placed in a 25 mL brown volumetric flask and the volume is filled with water for SPE cleanup.

14.1.3 Milk drinks

Weigh 10 g (accurate to 0.01 g) into a 50 mL polypropylene centrifuge tube with cap. Add 5 mL 40 g/L trichloroacetic acid solution (4.14), homogenize for 1 min, ultrasonicate for 15 min. After centrifugation at 4 000 r/min for 10 min, the supernatant is placed in a 25 mL brown volumetric flask and the volume is filled with water for SPE cleanup.

14.1.4 Milk power

Weigh 2 g (accurate to 0.01 g) into a 50 mL polypropylene centrifuge tube with cap. Add 20 mL

45 °C ~50 °C water, homogenize for 1 min, ultrasonicate for 15 min. After cooling, the pH of the solution was adjusted with 5 mol/L hydrochloric acid solution (4.12) to 1.90 ± 0.5 , and then adjusted with 5 mol/L sodium hydroxide solution (4.13) to 4.70 ± 0.5 for protein precipitation. Subsequently the mixture was centrifuged at 4 000 r/min for 10 min, the supernatant is waited for cleanup.

14.2 Cleaning-up

Transfer the extract solution (14.1) into the HLB column (11.7). Rinse the column with 5 mL 7% acetonitrile aqueous solution (11.2) and then elute with 1 mL 25% acetonitrile aqueous solution (11.3) at a flow rate of 1 mL/min. The eluted solution is collected in a 5 mL volumetric flask and filtered through a 0.22 μm membrane before HPLC-MS/MS analysis.

During the extraction process, samples are always protected from direct exposition to light.

14.3 Determination

14.3.1 Operating conditions

The apparatuses are the same as section 7.2.1 and 7.2.2.

14.3.2 Qualitative analysis

Under the above determination condition, the variation range of the retention time for the peak of analyte in unknown sample and in the standard working solution can not be out of range of $\pm 2.5\%$. For the same analysis batch and the same compound, the variation range of the ion ration between the two daughter ions for the unknown sample and the standard working solution at the similar concentration can not be out of range of table 2, and then the corresponding analyte can be present in the sample. Under the above operating condition, retention time of vitamin B₁₂ is 7.56 min.

14.3.3 Quantitative analysis

Under the above conditions of the apparatus, inject series of working standard solutions (the reference concentration range is 0.05 ~ 100 ng/mL) and sample solutions separately. Use the curve to quantify the each analyte in unknown sample by internal standard method. The responses of the analyte in the standard working solution and the sample solution shall be within the linear range of the instrument detection. Under the above operating condition, the chromatogram of the standard can be found by figure D.1 in annex D.

14.4 Blank test

The operation of the blank test is the same as the described in the method of determination, but with the omission of sample addition.

15 Calculation an expression of result

Calculation the content of vitamin B₁₂ in test sample by data processor or according to formula (2)

$$X_i = \frac{c_i \times V}{m} \times \frac{1\ 000}{1\ 000} \dots\dots\dots (2)$$

where:

X_i —the content of vitamin in test sample, µg/kg;

c_i —the concentration of vitamin in the test sample calculated by calibration curve, ng/mL;

V —the final volume of the sample solution, mL;

m —the corresponding mass of test sample in the final solution, g.

note: the blank value shall be subtracted from the result of calculation.

16 Limit of determination, recovery

16.1 Limit of determination

The limits of quantification are 0.5 µg/kg for juice and milk drinks; 1 µg/kg for biscuit and jelly; 2 µg/kg for rice and 5 µg/kg for milk power, respectively.

16.2 Recovery

The ranges of recovery in rice, juice, milk drinks, jelly, biscuit and milk power ate listed as table F.2 in Annex F.

Part 3: Determination of vitamin C (L-ascorbic acid) in foods

17 Principle

The vitamin C (L-ascorbic acid) in the test sample is extracted with metaphosphoric acid or warm water(isoelectric point method). After filtered through a membrane, the extract is detected by liquid

chromatography with a UV detector and quantified by external standard method.

18 Reagents and materials

18.1 Metaphosphoric acid.

18.2 Potassium dihydrogen phosphate.

18.3 Phosphate acid.

18.4 3% metaphosphoric acid solution: Accurately measure 30 g metaphosphoric acid (18.1) into a 1 000 mL volumetric flask, dilute with water to 1 000 mL, mix adequately.

18.5 0.05 mol/L potassium dihydrogen phosphate solution (pH=3): Accurately measure 6.80 g potassium dihydrogen phosphate (18.2) into a 1 000 mL water, mix adequately, then the pH of the solution was adjusted with phosphoric acid (18.3) to 3. Filter the solution with 0.22 μ m membrane.

18.6 Vitamin standard: the purity of vitamin C (L-ascorbic acid) is no less than 99%, which should be stored at 4 $^{\circ}$ C in dark and relative information in Tabel A.1 in Annex A.

18.7 Stock standard solution: Accurately weigh an adequate amount of standard (11.4), dissolve in 3% metaphosphoric acid solution (18.4) and prepare of 1 mg/mL. The standard stock solution can be stored in a refrigerator at 4 $^{\circ}$ C by avoiding light just before use.

18.8 Middle working solution: Prepare a standard working solution by diluting the above stock solution (18.7) with 3% metaphosphoric acid solution (18.4) just before use.

18.9 The other apparatuses are the same as section 4.

19 Apparatus and equipment

19.1 Liquid chromatography, equipped with a UV detector or a diode array detector.

19.2 The other apparatuses are the same as section 5.

20 Preparation and storage of test sample

The apparatuses are the same as section 6.

21 Procedure

21.1 Extraction

21.1.1 Juice

Weigh 2 g (accurate to 0.01 g) into a 5 mL brown glass volumetric flask and dilute with 3% metaphosphoric acid solution (18.4) to 10 mL, mix adequately. Then the solution is passed through 0.22 μm filter and ready for analysis. Dilute with 3% metaphosphoric acid solution (18.4) for high concentration samples, then pass through 0.22 μm filter and wait for HPLC determination.

21.1.2 Rice, jelly, puree and milk drinks

Weigh 5 g (accurate to 0.01 g) into a 100 mL polypropylene centrifuge tube with cap. Add 3% metaphosphoric acid solution (18.4) to the volume, ultrasonicate for 15 min, and add ice to maintain the system temperature at 10 $^{\circ}\text{C}$ ~ 20 $^{\circ}\text{C}$ during ultrasonic process. After centrifugation at 4 000 r/min for 10 min, the supernatant is passed through 0.22 μm filter and wait for HPLC determination.

21.1.3 Milk powder

Weigh 2 g (accurate to 0.01 g) into a 50 mL polypropylene centrifuge tube with cap. Add 20 mL 45 $^{\circ}\text{C}$ ~ 50 $^{\circ}\text{C}$ water, homogenize for 1 min, ultrasonicate for 15 min, and add ice to maintain the system temperature at 10 $^{\circ}\text{C}$ ~ 20 $^{\circ}\text{C}$ during ultrasonic process. The pH of the solution was adjusted with 5 mol/L hydrochloric acid solution (4.12) to 1.90 ± 0.5 , and then adjusted with 5 mol/L sodium hydroxide solution (4.13) to 4.70 ± 0.5 for protein precipitation. Subsequently the mixture was centrifuged at 4 000 r/min for 5 min, the supernatant is passed through 0.22 μm filter and wait for HPLC determination.

During the extraction process, samples are always protected from direct exposition to light.

21.2 Determination

21.2.1 HPLC operating conditions

Reference HPLC operating conditions is as following:

- a) Column: TechMate C₁₈-ST, 250 mm \times 4.6 mm (i.d), 5 μm particle size or equivalent;
- b) Wavelength: 266 nm;
- c) Injection volume: 10 μL ;
- d) Column temperature: 25 $^{\circ}\text{C}$;
- e) Mobile phases: 0.05 mol/L potassium dihydrogen phosphate solution (pH=3).

21.2.2 HPLC analysis

Under the above operating condition, the standard working solution should be randomly injected in-

between the injections of the sample solution of equal volume. The respond of the analyte is Y-axis, concentration of standard working solution is X-axis,protract standard working curve. Quantity with standard working curve,the responses of the sample solution should be within the linear range of the instrument detection. Under the above operating conditions,the reference retention time of vitamin C is 3.42 min. The chromatogram of the standard can be found in Figure E.1 of Annex E.

21.3 Blank test

The operation of the blank test is the same as the described in the method of determination,but with the omission of sample addition.

22 Calculation an expression of result

The apparatuses are the same as section 8.

23 Limit of quantification and recovery

23.1 Limit of quantification

The limits of quantification are 100 mg/kg for rice,juice,milk drinks,and milk power,and 20 mg/kg for puree.

23.2 Recovery

The ranges of recovery in rice,juice,milk drinks,jelly,puree and milk power are listed as Table F.3 in Annex F.

Annex A
(Informative Annex)
Standard information of water-soluble vitamins

Table A.1—Standard information of water-soluble vitamins

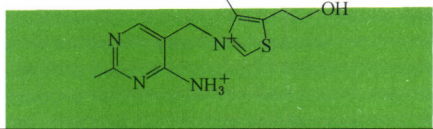
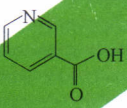
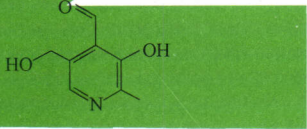
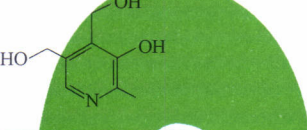
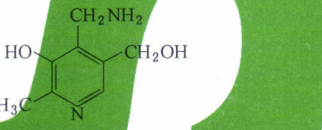
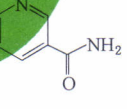
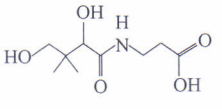
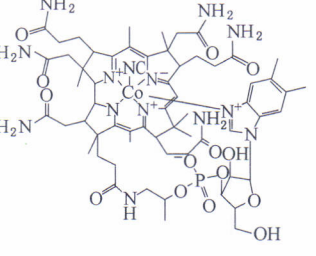
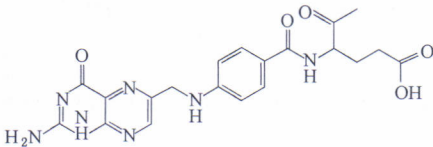
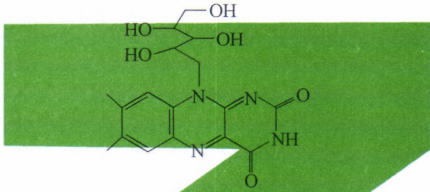
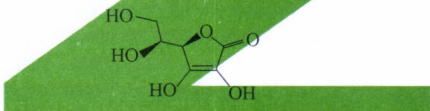
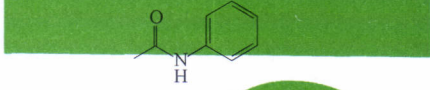
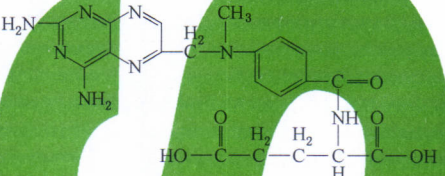
Compound	Structure formula	CAS No.	Molecular formula
thiamine		67-03-8	$C_{12}H_{17}ClN_4OS$
nicotinic acid		59-67-6	$C_6H_5NO_2$
pyridoxal		65-22-5	$C_8H_9NO_3$
pyridoxine		8059-24-3	$C_8H_{11}NO_3$
pyridoxamine		524-36-7	$C_8H_{12}N_2O_2$
nicotinamide		98-92-0	$C_6H_6N_2O$
pantothenic acid		137-08-6	$C_9H_{17}NO_5$
cyanocobalamin		68-19-9	$C_{63}H_{88}CoN_{14}O_{14}P$

Table A.1 (continue)

Compound	Structure formula	CAS No.	Molecular formula
folic acid		59-30-3	$C_{19}H_{19}N_7O_6$
riboflavin		83-88-5	$C_{17}H_{20}N_4O_6$
ascorbic acid		50-81-7	$C_6H_8O_6$
acetanilide		103-84-4	C_8H_9NO
methotrexate		59-05-2	$C_{20}H_{22}N_8O_5$

Annex B
(Informative Annex)
Mass spectral acquisition of Agilent 6460 LC-MS/MS¹⁾

Table B.1—Retention times, transitions, declustering potential and collision energy

Compound	Retention times min	Transitions m/z	Declustering potential V	collision energy eV	Corresponding internal compound
thiamine	1.51	265.2/122.2 *	70	6	Acetanilide
		265.2/144.2	70	6	
nicotinic acid	2.21	124.1/78.1 *	108	21	D4-Nicotinic acid
		124.1/80.1	108	17	
pyridoxal	2.65	168.2/94.0	80	25	Acetanilide
		168.2/150.0 *	80	10	
pyridoxine	3.85	170.0/134.0 *	88	20	Acetanilide
		170.0/152.0	88	37	
pyridoxamine	1.30	169.0/134.0 *	80	18	Acetanilide
		169.0/152.0	80	30	
nicotinamide	3.65	123.1/78.2 *	108	22	D4-Nicotinamide
		123.1/80.2	108	20	
pantothenic acid	7.30	220.0/90.0 *	90	5	Methotrexate
		220.0/202.0	90	8	
cyanocobalamin	7.56	678.5/147 *	150	33	—
		678.5/358.8	150	21	
folic acid	7.47	442.4/176.9	135	29	Methotrexate
		442.4/295.1 *	135	7	
riboflavin	7.91	377.4/171.8 *	156	37	Methotrexate
		377.4/243.1	156	21	
D4-Nicotinic acid	2.16	128.1/84.1	110	20	—
D4-Nicotinamide	3.61	127.0/84.0	90	20	—
Acetanilide	8.76	136.0/94.0	80	15	—
Methotrexate	7.62	455.0/308.0	135	20	—

Note: * mark is the quantification ion pair. for the different MS equipment, the parameters may be different, and the MS parameters should be optimized to the best before analysis.

1) Non-commercial statement; the reference mass parameters in Annex B are accomplished by Agilent 6460 LC-MS/MS, the equipment and its type involved in the standard method is only for reference and not related to and commercial aim, and the analysts are encouraged to use equipment of different corporation of different type.

Annex C (Informative Annex)

Selected ion chromatograms of water-soluble vitamins

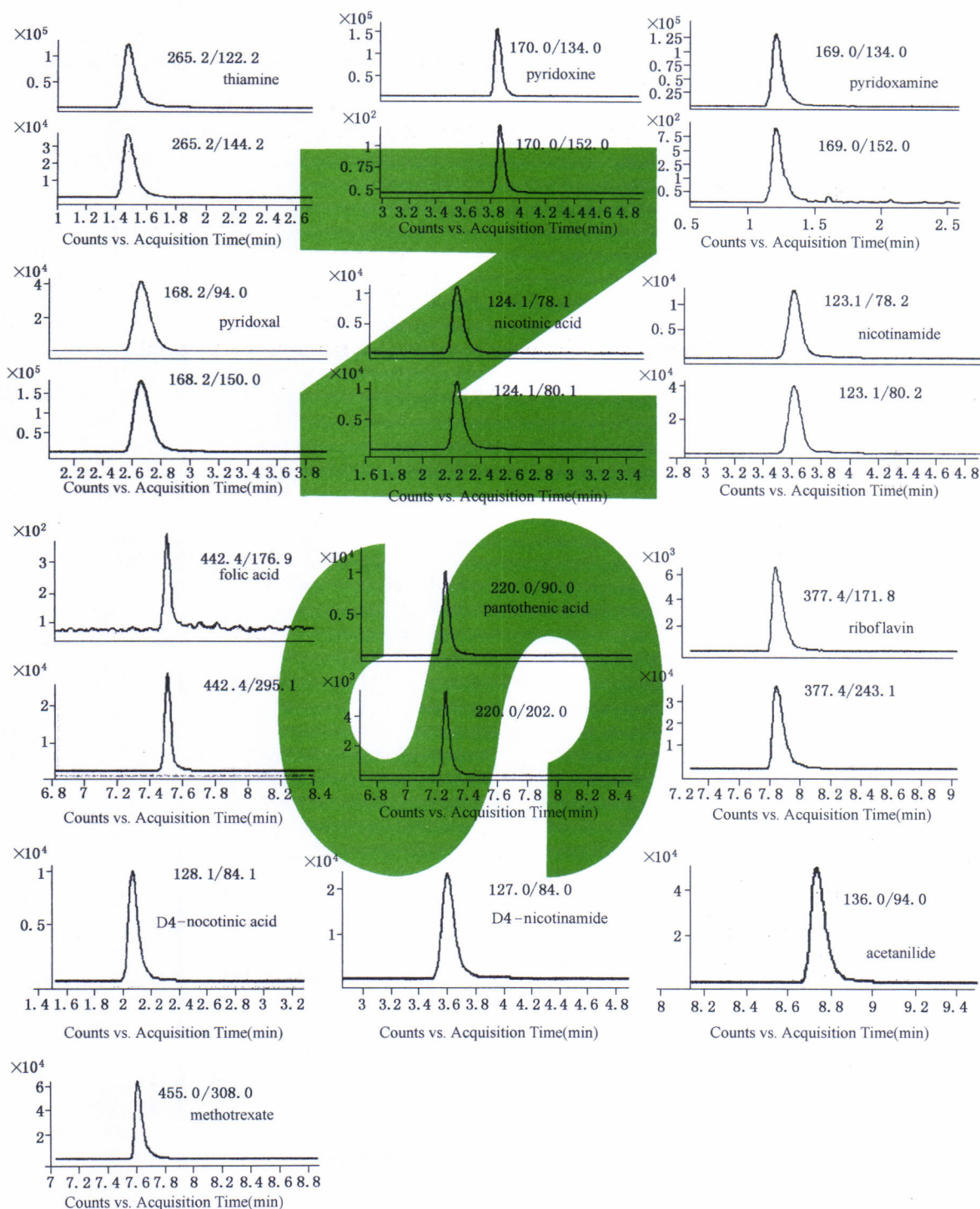


Figure C.1—Selected ion chromatograms of 9 kinds of water-soluble vitamins and their internal standards(0.5 $\mu\text{g/mL}$ and 20 ng/mL for folic acid)

Annex D
(Informative Annex)
Selected ion chromatograms of vitamin B₁₂

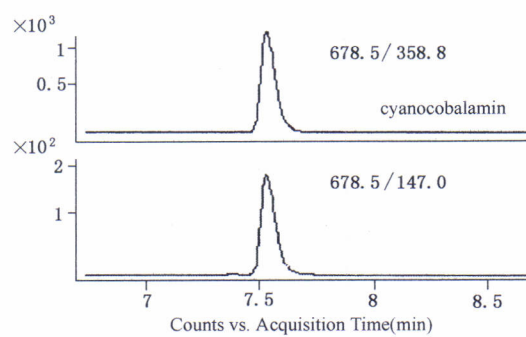


Figure D.1—Selected ion chromatograms of vitamin B₁₂ at 5 ng/mL

Annex E
(Informative Annex)
HPLC chromatogram of vitamin C

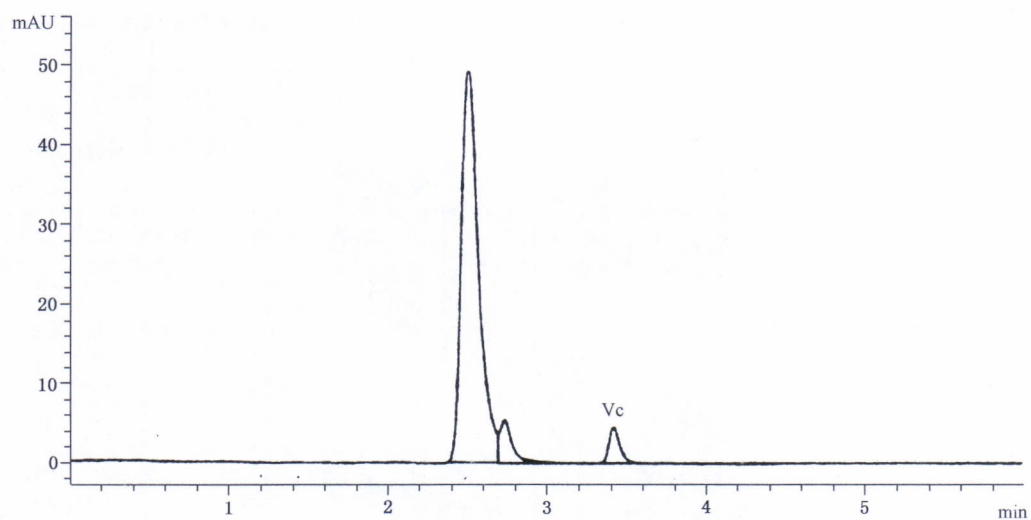


Figure E.1—HPLC chromatogram of standard at 2.0 µg/mL

Annex F

(Informative Annex)

Recovery of water-soluble vitamins in different matrix at different spike levels

Table F.1—Recovery of 9 water vitamins in different matrix at different spike levels

Compound	Juice		Jelly		Rice		Biscuit		Milk drinks		Milk power	
	Spike mg/kg	Recovery %	Spike mg/kg	Recovery %	Spike mg/kg	Recovery %	Spike mg/kg	Recovery %	Spike mg/kg	Recovery %	Spike mg/kg	Recovery %
thiamine	1	80.6~109	1	86.4~110	1	80.7~97.5	1	80.2~103	1	80.0~103	1	80.7~99.2
	5	81.6~102	5	83.4~110	5	86.6~108	5	81.6~104	5	80.8~104	5	82.7~97.8
	10	80.7~99.6	10	80.8~110	10	80.9~105	10	82.4~110	10	80.2~98.6	25	80.1~102
nicotinic acid	1	81.0~95.8	1	80.0~109	5	80.2~108	5	81.1~96.1	1	80.0~106	1	80.6~105
	5	81.4~105	5	80.4~104	20	81.1~101	20	81.3~99.1	5	80.4~101	10	84.0~94.1
	25	82.6~106	10	80.1~99.7	100	90.3~105	100	90.0~105	25	85.2~94.3	150	93.7~102
pyridoxal	0.2	80.9~106	1	81.0~109	1	81.6~105	1	80.8~110	0.2	81.8~103	1	81.1~97.9
	1	90.1~110	5	81.6~104	5	87.4~103	5	85.4~97.0	1	97.5~108	5	82.3~92.0
	5	86.3~106	10	80.5~100	10	85.3~104	10	81.1~100	5	81.7~105	25	83.4~98.5
pyridoxine	0.2	81.0~106	1	81.0~110	1	80.6~109	1	80.5~108	0.2	82.9~97.8	1	87.1~101
	1	80.1~102	5	81.6~101	5	81.7~102	5	83.5~102	1	83.0~97.0	5	89.6~104
	5	82.4~101	10	80.7~101	10	84.6~104	10	80.0~101	5	84.2~106	25	83.8~103
nicotinamide	1	93.2~105	1	80.0~92.0	5	87.8~103	5	84.0~102	1	81.3~108	1	81.0~108
	5	94.3~109	5	80.2~102	20	80.2~109	20	82.9~96.7	5	80.2~101	10	80.6~98.1
	25	89.7~104	10	80.1~101	100	91.6~98.3	100	90.4~103	25	80.9~101	150	90.7~103
pantothenic acid	1	80.6~96.1	1	83.1~103	4	86.3~103	1	80.9~100	1	80.7~101	1	80.1~107
	5	82.7~106	5	80.8~101	8	87.8~106	5	80.2~105	5	81.1~110	10	80.6~104
	10	81.7~102	10	80.0~105	20	84.5~105	10	81.2~100	10	80.3~106	100	90.6~104
pyridoxamine	0.2	80.4~108	1	80.1~104	1	90.7~102	1	82.8~110	0.2	80.5~102	1	88.3~101
	1	80.3~110	5	81.0~102	5	88.1~108	5	81.7~108	1	80.1~105	5	81.9~106
	5	80.9~107	10	80.1~90.7	10	84.4~95.7	10	81.7~101	5	81.0~94.5	25	80.7~104
folic acid	0.1	80.6~108	0.01	75.9~108	0.5	81.6~94.6	0.1	84.6~109	0.2	81.0~99.9	0.2	81.2~98.2
	0.5	81.4~105	0.02	72.0~101	1	80.7~99.4	0.5	80.3~106	0.5	82.0~105	0.5	87.8~99.9
	1	83.7~104	0.10	78.6~100	5	90.3~101	1	80.2~102	5	82.9~98.1	10	83.3~106
riboflavin	1	80.7~101	1	84.0~110	1	86.4~102	1	89.6~104	1	80.8~104	1	82.0~100
	5	89.8~108	5	82.2~103	5	90.7~107	5	80.4~96.3	5	81.6~97.0	5	82.4~99.7
	10	90.2~106	10	80.5~110	10	89.4~101	10	80.8~105	10	85.0~102	25	80.9~103

Table F.2—Recovery of vitamin B₁₂ in different matrix at different spike levels

Matrix	Spike μg/kg	Recovery %	Matrix	Spike μg/kg	Recovery %
Juice	0.5	71.6~92.0	Biscuit	1	75.0~105
	2	70.1~97.7		5	72.2~98.2
	5	76.5~101		20	83.6~103
Jelly	1	71.4~100	Milk drinks	0.5	73.6~95.8
	2	74.9~98.9		2	73.8~91.4
	10	70.4~103		5	73.1~96.2
Rice	2	70.0~106	Milk power	5	72.0~99.0
	5	80.0~102		20	72.9~94.9
	10	74.7~99.1		100	80.9~98.1

Table F.3—Recovery of vitamin C in different matrix at different spike levels

Matrix	Spike mg/kg	Recovery %	Matrix	Spike mg/kg	Recovery %
Juice	100	90.8~105	Puree	20	83.5~106
	200	92.3~107		50	82.2~107
	500	91.2~106		250	91.3~105
Jelly	100	94.5~107	Milk drinks	100	90.4~105
	150	93.4~104		200	97.9~106
	300	98.1~103		500	93.1~101
Rice	100	90.8~106	Milk power	100	90.5~107
	200	90.0~105		500	91.1~102
	1 000	96.9~107		2 000	96.0~105