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Add the following:

^Cyanocobalamin Chewable Gels

DEFINITION
Cyanocobalamin Chewable Gels contain NLT 90.0% and NMT 155.0% of the labeled amount of cyanocobalamin ($C_{63}H_{88}CoN_{14}O_{14}P$).

IDENTIFICATION
• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the test for *Strength, Method 1* or *Method 2*.

STRENGTH
[NOTE—The requirements may be met by following any one of the methods specified in this section. The method used is stated in the labeling only if *Method 1* is not used.]

• **METHOD 1**
[NOTE—Protect all samples from light. Use cryogenic gloves when handling liquid nitrogen.]

Solution A: 0.1% [formic acid](#) in [water](#)
Solution B: 0.1% [formic acid](#) in [acetonitrile](#)
Mobile phase: See [Table 1](#).

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	98.0	2.0
7.0	80.0	20.0
8.0	65.0	35.0
9.0	65.0	35.0
10.0	98.0	2.0

System suitability solution: Dissolve 5 mg of [USP Cyanocobalamin \(Crystalline\) RS](#) in 10 mL of [water](#), warming if necessary. Allow to cool. Add 1 mL of a 1.0-mg/mL solution of [tosylchloramide sodium](#), 0.5 mL of 0.01 M [hydrochloric acid](#), and then dilute with [water](#) to 25 mL. Shake and allow to stand for 5 min. Dilute with *Solution A* to 1 µg/mL of cyanocobalamin, and inject immediately. [NOTE—This procedure generates 7β,8β-lactocyanocobalamin.]

Standard solution: 1 µg/mL of cyanocobalamin from [USP Cyanocobalamin \(Crystalline\) RS](#) in [water](#)

Sample solution: Immerse 25–30 Chewable Gels in liquid nitrogen in a cryogenic vessel for 10 min. Cool a blender jar by swirling liquid nitrogen for about 1 min and discard. Add frozen Chewable Gels to the cooled blender jar, and grind to a fine powder. Transfer a portion of the powder, nominally equivalent to 25 µg of cyanocobalamin, into a 50-mL centrifuge tube. [NOTE—Proceed to the next step immediately or keep the powdered Chewable Gels frozen until use.]

Add 200 mg of [pancreatin](#) and 25.0 mL of [water](#), accurately measured, and mix on a vortex mixer for 30 s. Shake on a water bath shaker at 40° until complete dissolution (about 10 min). Cool to room temperature, add 10 mL of [methylene chloride](#), and mix on a vortex mixer until well mixed. Shake on a horizontal shaker for 15 min. Centrifuge the sample at 2000 rpm for 5 min. Pass a portion of the top aqueous layer through a 0.2-µm nylon filter, and discard the first few milliliters of the filtrate.

Chromatographic system

(See [Chromatography \(621\), System Suitability.](#))

Mode: LC

Detector: UV 361 nm

Column: 2.1-mm × 5-cm; 1.8-μm packing [L96](#)

Column temperature: 25°

Flow rate: 0.5 mL/min

Injection volume: 10 μL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 5.0 between the cyanocobalamin and 7β,8β-lactocyanocobalamin peaks, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of cyanocobalamin ($C_{63}H_{88}CoN_{14}O_{14}P$) in the portion of Chewable Gels taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of cyanocobalamin from the *Sample solution*

r_S = peak area of cyanocobalamin from the *Standard solution*

C_S = concentration of cyanocobalamin in the *Standard solution* (μg/mL)

C_U = nominal concentration of cyanocobalamin in the *Sample solution* (μg/mL)

Acceptance criteria: 90.0%–155.0%

• METHOD 2

[NOTE—Protect all samples from light. Use cryogenic gloves when handling liquid nitrogen.]

Solution A, Solution B, System suitability solution, Standard solution, Sample solution, and Analysis: Proceed as directed in *Strength, Method 1*.

Mobile phase: See [Table 2](#).

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	98.0	2.0
13	80.0	20.0
14.5	65.0	35.0
16.0	65.0	35.0
17.0	98.0	2.0
20.0	98.0	2.0

Chromatographic system

(See [Chromatography \(621\), System Suitability.](#))

Mode: LC

Detector: UV 361 nm

Column: 2.1-mm × 10-cm; 1.8-μm packing [L1](#)

Column temperature: 30°

Flow rate: 0.5 mL/min

Injection volume: 10 μL