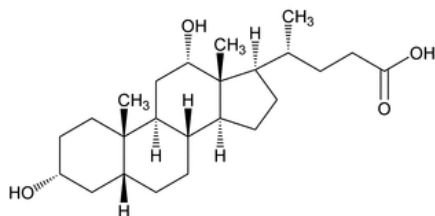


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Deoxycholic Acid



$C_{24}H_{40}O_4$ 392.58
Cholan-24-oic acid, 3,12-dihydroxy-, (3 α ,5 β ,12 α)-;
3 α ,12 α -Dihydroxy-5 β -cholan-24-oic acid CAS RN®: 83-44-3.

DEFINITION

Deoxycholic Acid contains NLT 97.0% and NMT 103.0% of deoxycholic acid ($C_{24}H_{40}O_4$), calculated on the anhydrous basis. Deoxycholic Acid can be of animal or synthetic origin. FDA has not approved the use of animal-derived Deoxycholic Acid as a drug substance.

IDENTIFICATION

- **A. SPECTROSCOPIC IDENTIFICATION TESTS (197), Infrared Spectroscopy:** 197A or 197K
- **B.** The retention time of the deoxycholic acid peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY

- **PROCEDURE**
Diluent: [Methanol](#) and water (80:20)
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 (%)
0.0	75.0	25.0
2.0	55.0	45.0
14.0	42.0	58.0
24.0	0.0	100.0
35.0	0.0	100.0

Standard solution: 0.01 mg/mL of [USP Deoxycholic Acid RS](#) in *Diluent*
Sample solution: 0.01 mg/mL of Deoxycholic Acid in *Diluent*
Chromatographic system
(See [Chromatography \(621\), System Suitability.](#))

Mode: LC**Detector:** Charged aerosol**Column:** 4.6-mm × 15-cm; 3-μm packing [L1](#)**Flow rate:** 1.0 mL/min**Injection volume:** 25 μL**Run time:** 35 min**System suitability****Sample:** *Standard solution*

[NOTE—The retention time of deoxycholic acid is about 13.0 min.]

Suitability requirements**Relative standard deviation:** NMT 3.0% for six injections**Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of deoxycholic acid ($C_{24}H_{40}O_4$) in the portion of Deoxycholic Acid taken:

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

 r_U = peak area of deoxycholic acid from the *Sample solution* r_S = peak area of deoxycholic acid from the *Standard solution* C_S = concentration of the [USP Deoxycholic Acid RS](#) in the *Standard solution* C_U = concentration of Deoxycholic Acid in the *Sample solution* P = labeled purity of [USP Deoxycholic Acid RS](#) (%)**Acceptance criteria:** 97.0%–103.0% on the anhydrous basis**IMPURITIES**• [RESIDUE ON IGNITION \(281\)](#)**Sample:** 1.0 g**Acceptance criteria:** NMT 0.2%• **LIMIT OF LEAD**

[NOTE—This test and *Acceptance criteria* are only applicable to Deoxycholic Acid of animal origin. Select reagents having as low a lead content as practicable, and store all solutions in high-density polyethylene containers. Rinse all plastic and glassware thoroughly with warm, 50% nitric acid followed by water.]

Standard solutions: [NOTE—Prepare these solutions on the day of use.] Transfer 10.0 and 50.0 mL of standard lead solution TS into two separate 100-mL volumetric flasks, add 10 mL of 3 N [hydrochloric acid](#) to each, and dilute with water to volume. The third standard, 10.0 μg/mL, is taken directly from standard lead solution TS.

Sample solution: Transfer 10.0 g of Deoxycholic Acid, weighed to the nearest 0.1 mg, into an evaporating dish. Add 5 mL of 25% sulfuric acid (made by adding 25 mL of [sulfuric acid](#) to 75 mL of water), and distribute the 25% sulfuric acid solution uniformly. Within a hood, place the dish on a steam bath to evaporate most of the water. Place the dish on a burner, and slowly pre-ash the remaining *Sample solution* by expelling most of the sulfuric acid. Place the dish in a muffle furnace that has been set at 525°, and ash the contents of the dish until the residue appears free from carbon. Cool, and cautiously wash down the inside of the evaporation dish with water. Add 5 mL of 1 N [hydrochloric acid](#). Place the dish on a steam bath, and evaporate to dryness. Add 1.0 mL of 3 N [hydrochloric acid](#) and approximately 5 mL of water, and heat briefly on a steam bath to dissolve any residue. Transfer to a 10-mL volumetric flask, dilute with water to volume, and mix.

Sample blank: Prepare by ashing 5 mL of 25% sulfuric acid solution. Cool, and cautiously wash down the inside of the evaporation dish with water. Add 5 mL of 1 N [hydrochloric acid](#). Place the dish on a steam bath, and evaporate to dryness. Add 1.0 mL of 3 N [hydrochloric acid](#) and approximately 5 mL of water, and heat briefly on a steam bath to dissolve any residue. Transfer to a 10-mL volumetric flask, dilute with water to volume, and mix.

Instrumental conditions(See [Atomic Absorption Spectroscopy \(852\)](#).)**Mode:** Atomic absorption**Analytical wavelength:** 283.3 nm**Lamp:** Lead electrodeless discharge