4-Amino-7,8-dihydro-L-biopterin

Product number 11.281

Step 1: Reduction of 4-amino-L-biopterin
1.5 g of 4-amino-L-biopterin and 6.0 g of Na2S2O4 are placed in a 250 ml round bottom flask. 60 ml of water are added and a glass stopcock is mounted. The round bottom flask is evacuated and swirled for a short time. Attention, the suspension can foam.
The round bottom flask is warmed in a water bath at 60°C for 40 minutes. The solution becomes light yellow.
HPLC analysis shows that this solution still contains about 6% of 4-amino-L-biopterin. More Na2S2O4 or longer reaction time did not reduce the amount of 4-amino-L-biopterin.
The round bottom flask is immersed in cold water for 20 minutes. If the purification cannot be started immediately, the round bottom flask has to be stored at 2°C overnight.
In contrast to dihydrobiopterin, 4-amino-7,8-dihydro-L-biopterin is readily soluble in water and must therefore be purified by column chromatography.

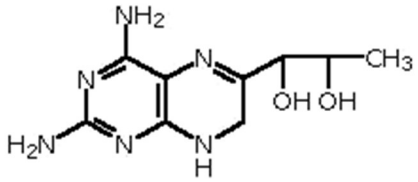
Step 2: Column chromatography (Whatman CC 31)
Preparation of the column:
Column diameter: 8 cm, height of the stationary phase: 20 cm
A slurry of cellulose CC31 from Whatman and NH4AcOH solution 5% is poured through a funnel into the column. The top of the cellulose is protected by filter papers.
The column is equilibrated with H2O.
The solution with the crude 4-amino-7,8-dihydro-L-biopterin (60 ml) is loaded onto the column.
The column is run with H2O.
The fractions containing Na2S2O4 are recognized by the smell.
The fractions are examined by TLC.
TLC foil: cellulose 400, developing solvent: water
The spots can be visualized by using ultraviolet light.
Solutions of 4-amino-7,8-dihydro-L-biopterin are very sensitive to oxygen.
They must therefore be protected by argon or stored in a freezer. All work must be done as quickly as possible.
When a container for the next fraction is changed, a small sample (3 drops) should be placed in a small vial and this vial and the container with the fraction should be placed immediately in a freezer.
The TLC is performed with the small samples in the vials.
The best fractions are collected and the water is evaporated to about
30 ml.

Step 3: Column chromatography (Sephadex G-10)
Column diameter: 5 cm, height of the stationary phase: 20 cm
The column is washed with 0.2% NaOH and then with a lot of water.
The eluent (water or water containing 0.5% of AcOH) is degassed and argon is bubbled through the eluent from above.

The solution of step 2 (30 ml) is loaded onto the column.
In order to protect the 4-amino-7,8-dihydro-L-biopterin from oxygen, argon is bubbled though the fractions.
The fractions are collected as described in step 2 and tested by HPLC.
Column: Spherisorb S5 ODS1
Eluant: 10 mM Na2HPO4 pH 6.0 plus 15% methanol
Detection: 254 nm
The best fractions are collected, the water is evaporated and the residue dried in a vacuum desiccator over NaOH.
The columns have to be washed immediately, so that the remaining 4-amino-7,8-dihydro-L-biopterin and the impurities do not oxidize in the columns.

Purity: 97.5% (HPLC)

Description: light yellow



4-Amino-7,8-dihydro-L-biopterin

$C_9H_{14}N_6O_2$
238.24
238.117823
C 45.37% H 5.92% N 35.27% O 13.43%

Product no. 11.281

HPLC		
	Sample	2 mg/ml
	Column	Spherisorb S5 ODS 1
	Eluent	10 mM PB pH 6.0 plus 15% MeOH
	Flow	1 ml/min
	Detection	254 nm