INTERNATIONAL SORBENT TECHNOLOGY

SPE Application Note for Extraction of Catecholamines from Plasma

The following method has been developed for the extraction of norepinephrine, epinephrine and dopamine from plasma. The analytes, having formed a diphenyl boronate complex are retained on a non polar MFC18 column. Typical recoveries are > 98%.

EXTRACTION PROCEDURE

ISOLUTE® SPE Column: MFC18 50 mg / 10 ml Part # 240-0005-G

Pre-treatment: Draw venous blood in tubes containing 0.01M tripotassium ethylenediaminetetraacetate (50 ul) and immediately centrifuge at 2200g for 10 minutes at 4 C. Separate plasma and keep at -30 C.

To plasma (1 ml) add internal standard solution (0.1 ml), buffer containing the complexing reagent, pH 8.5 (1 ml) and 0.8% TBA buffer, pH 8.5 (2.1 ml). Sample should be in the range pH 8.3 - 8.5. Adjust if necessary with the addition of 2.0 M ammonium hydroxide.

Solvation: Condition the column with methanol (1 ml).

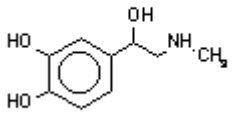
Equilibration: Rinse the column with 0.4% TBA buffer, pH 8.5 (1 ml).

Sample application: Apply the sample to the column at a flow rate of 1 ml / min.

Interference elution: Elute the interferences with 0.4% TBA buffer, pH 8.5 (1 ml) followed by 50/50 (v/v) 0.8% TBA buffer, pH 8.5 / methanol (0.5 ml).

Analyte elution: Elute the catecholamines with 10/90 (v/v) methanol / 0.1 M perchloric acid (0.4 ml) at a flow rate of 0.18 ml / min.

Structure Epinephrine is shown.



Structural considerations The analytes are relatively polar, but have two adjacent hydroxyl groups, which are used to form a less polar diphenyl boronate complex. This is extracted from the matrix using a non-polar retention mechanism.

Matrix considerations The matrix is aqueous, with high ionic strength.

Analytical method HPLC

Column: APEX II ODS, 3um x 5cm x 4.6 mm i.d.
Mobile phase: 15/8/77 (v/v/v) Methanol/Acetonitrile/50 mM Sodium Dihydrogen Orthophosphate, pH 2.8, containing 0.2 g/L sodium dodecyl sulphate.
Flow rate: 2 ml / min.
Detection: Coulometric Electrochemical Detector with conditioning cell, + 0.4V and Dual GCE. E1 = + 0.1V, E2 = -0.35V.

Reagents

General comments

1. Reagents
a) Internal Standard Solution. Dihydroxybenzylamine, 12.5 ng / ml in 0.01 M perchloric acid.
b) Buffer, pH 8.5 Stock Solution. Weigh ethylenediaminetetraacetic acid, disodium salt (5.0 g) and ammonium chloride (106.98 g) into a one litre volumetric flask. Dissolve in deionised water (950 ml), adjust to pH 8.5 (+/- 0.04) with 30% ammonium hydroxide and make up to the mark with deionised water.
c) Buffer Containing the Complexing Reagent, pH 8.5. To buffer, pH 8.5 stock solution (500 ml) add diphenylboronic acid, ethanolamine ester (1.0 g) and stir overnight. Adjust to pH 8.5 (+/- 0.04).
d) 0.8% TBA Buffer, pH 8.5. Weigh tetrabutylammonium bromide (4.0 g) into a 500 ml volumetric flask and dissolve in buffer, pH 8.5 stock solution (50 ml). Add deionised water (400 ml), adjust to pH 8.5 (+/- 0.04) and make up to the mark with deionised water.
e) 0.4% TBA Buffer, pH 8.5. Weigh tetrabutylammonium bromide (2.0 g) into a 500 ml volumetric flask and dissolve in buffer, pH 8.5 stock solution (50 ml). Add deionised water (400 ml), adjust to pH 8.5 (+/- 0.04) and make up to the mark with deionised water.
f) 50/50 (v/v) 0.8% TBA Buffer, pH 8.5 / Methanol. Add methanol (250 ml) and 0.8% TBA buffer, pH 8.5 (250 ml) to a reagent bottle and mix thoroughly. Adjust to pH 8.5 (+/- 0.04).
g) 10/90 (v/v) Methanol / 0.1M Perchloric Acid. Pipette 70% perchloric acid (7.8 ml) into deionised water (892.2 ml), add methanol (100 ml) and mix thoroughly.

2. The analytes being extracted are unstable at basic pH; keep the samples under these conditions for the shortest time.

3. Do not dry the column. This will avoid irreversible analyte adsorption.

4. The extracts are stable for one day at room temperature and for three days at 4 C.

5. All the reagents are stable at 4 C for six months.

6. Plasma samples are stable for two hours at room temperature and four hours at 4 C.

7. Reference. G. Grossi, M. L. Nemi, Poster presented at the 20th ISC, Bournemouth, UK, 19-24 June, 1994.

8. Previous # IST1001.

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ISOLUTE column part numbers represent the product configuration of choice for use with a vacuum sample processing station. For alternative configurations compatible with any SPE automation system, please consult your IST Distributor.

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