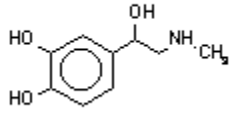
SPE Application Note for Catecholamines from Urine

This method was proposed for the extraction of norepinephrine, epinephrine and dopamine from urine. 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:	To urine (250 ul) add internal standard solution (50 ul), buffer containing the complexing agent, pH 8.5 (500 ul) and 0.8% TBA buffer, pH 8.5 (750 ul). Sample pH should be in the range 8.3-8.5. Adjust if necessary with the addition of 2.0M 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 (1 mL) to the column at a flow rate of 1 mL/min
Interference elution:	Elute 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 0.08M acetic acid (1 mL) at a flow rate of 0.5 mL/min
Structure	Epinephrine is shown. 
Structural considerations	The analytes are relatively polar, but have two adjacent hydroxy 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 4.6mm i.d. Mobile phase: 15:8:77 (v/v) Methanol/Acetonitrile/50mM Sodium Dihydrogen Orthophosphate, pH 2.8, containing 0.2g/L sodium

dodecyl sulphate.
Flow rate: 2mL/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, 1.5mg/L in 0.01M perchloric acid.
b) Buffer, pH 8.5 Stock Solution. Weigh ethylenediaminetetraacetic acid, disodium salt (5.0g) and ammonium chloride (106.98g) into a one litre volumetric flask. Dissolve in deionized water (950mL), adjust to pH 8.5 (+/- 0.04) with 30% ammonium hydroxide and make up to the mark with deionized water.
c) Buffer Containing the Complexing Reagent, pH 8.5. To buffer, pH 8.5 stock solution (500mL) add diphenylboronic acid, ethanolamine ester (1.0g) and stir overnight. Adjust to pH 8.5 (+/- 0.04).
d) 0.8% TBA Buffer, pH 8.5. Weigh tetrabutylammonium bromide (4.0g) into a 500 mL volumetric flask and dissolve in buffer, pH 8.5 stock solution (50mL). Add deionized water (400mL), adjust to pH 8.5 (+/- 0.04) and make up to the mark with deionized water.
e) 0.4% TBA Buffer, pH 8.5. Weigh tetrabutylammonium bromide (2.0g) into a 500 mL) 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) 0.08M Acetic Acid. Pipette glacial acetic acid (2.3 mL) into a 500 mL volumetric flask. Add deionized water (450 mL), mix thoroughly and make up to the mark with deionized water.

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. Collect 24 hour urine samples into plastic bottles releasing reduced quantities of plastic residues. Preserve by the addition of concentrated hydrochloric acid to a concentration of 8 mL/L. Record the total volume.

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

8. Previous # IST1000

ISOLUTE column part numbers represent the product configuration of choice for use with a vacuum sample processing station. For 96-well and alternative column configurations compatible with any SPE automation system, please contact Biotage.

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