INTERNATIONAL SORBENT TECHNOLOGY

SPE Method Development Recommendations for Extraction of VMA, DOPAC, 5-HIAA, and HVA from Urine

This represents recommendations for SPE method development. The proposed steps are based on experience with similar analytes and matrices, but have not been verified in IST laboratories. Please refer to section below for the analyte and matrix considerations that were made in developing this method.
As for all method development, this procedure should first be developed using pure solvent spiked with analyte. Only after the chemistry is established should spiked matrix samples be tested.
Non-aqueous samples: Spike a solvent similar to sample matrix.
Aqueous samples: Spike reagent water or 10 to 20 mM buffer. An appropriate buffer is usually the same as that used in the equilibration step.

The following method addresses the extraction of vanilmandelic acid (VMA), 3,4-dihydroxyphenylacetic acid (DOPAC), 5-hydroxyindoleacetic acid (5-HIAA) and homovanillic acid (HVA) from urine using an anion exchange retention mechanism.

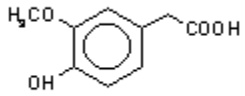
EXTRACTION PROCEDURE

ISOLUTE® SPE Column: SAX 100 mg / 1 ml (Part # 500-0010-A)

There may be more than one phase that could be effective in the extraction of this compound. The method development should include testing phases in parallel in order to optimize the procedure.

Pre-treatment:	To urine, add internal standard solution (50 ul) and EDTA buffer, pH 8.5, 1ml. Ensure the sample is in the range pH 7.0-8.5. Adjust if necessary with 0.5M sodium hydroxide.
Solvation:	Condition the column with methanol (0.6 ml).
Equilibration:	Rinse the column with EDTA buffer, pH 8.5 (250 ul) followed by 10:90 (v/v) methanol:water (0.5 ml).
Sample application:	Apply the diluted sample (200 ul) to the column at a flow rate of 0.5 ml / min.
Interference elution:	Elute interferences with 10:90 (v/v) methanol:water (1 ml).
Analyte elution:	Elute with 0.1M orthophosphoric acid (0.5 ml) at a flow rate of 1 ml / min.

Structure HVA is shown.



Structural considerations The analytes are capable of holding a negative charge, and can be extracted with the positively charged SAX sorbent.

Matrix considerations The matrix is aqueous and of relatively high ionic strength.

Analytical method HPLC

Column: APEX II ODS, 3 um x 5 cm x 4.6 mm i.d.
Mobile phase: 2:98 (v/v) acetonitrile:50 mM sodium dihydrogen orthophosphate, pH 2.8 containing 0.5 g / L 1-octanesulphonic acid, sodium salt.
Flow rate: 1 ml / min
Detection: Coulometric Electrochemical Detector with Dual GCE. E1 = 0.0V, E2 = 0.5V

Reagents

General comments

1. Reagents

a) Internal Standard Solution. Weigh isovanilmandelic acid (50 mg) into a one litre volumetric flask. Add 0.1M hydrochloric acid (900 ml), dissolve the internal standard and make up to the mark with 0.1M hydrochloric acid.

b) 0.1M Hydrochloric Acid. Add deionised water (200 ml) to a 250 ml volumetric flask. Pipette in concentrated hydrochloric acid (2.1 ml) and mix thoroughly. Make up to the mark with deionised water.

c). EDTA Buffer, pH 8.5. Weigh ethylenediaminetetraacetic acid, disodium salt (0.5 g) and ammonium chloride (10.7 g) into a reagent bottle and dissolve in deionised water (900 ml). Add methanol (100 ml) and mix thoroughly. Adjust to pH 8.5 (+/- 0.1) by the addition of 30% ammonium hydroxide.

d) 10/90 (v/v) Methanol/Water. Add methanol (10 ml) and deionised water (90 ml) to a regent bottle and mix thoroughly.

e) 0.1M Orthophosphoric Acid. Add deionised water (200 ml) to a one litre volumetric flask. Carefully pipette in orthophosphoric acid (6.8 ml), mix thoroughly and make up to the mark with deionised water.

2. The analytes being extracted are unstable under alkaline conditions. Samples should be kept under these conditions for as short a time as possible. In the case of DOPAC, it is advisable to perform the sample pre-treatment just prior to applying to the SPE column.

3. Do not dry the column. Irreversible analyte absorption may be observed.

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

5. Urine samples may be preserved with 0.1M hydrochloric acid.

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

7. Previous # IST1002.

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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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