SPE Method Development Recommendations for 2,5-Hexanedione 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 Biotage 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.

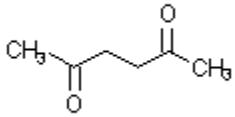
The following method addresses the extraction of total and free 2, 5-hexanedione from urine using a non-polar retention mechanism. Analysis is by HPLC, following derivatization with dansylhydrazine.

EXTRACTION PROCEDURE

ISOLUTE® SPE Column: C18(EC), 500mg/3mL (Part # 221-0050-B)

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:	Centrifuge urine sample (6.5 mL) for 10 minutes at 3000 rpm. Add 1.3g / L 1, 3 diacetylbenzene, internal standard (50 uL) and mix thoroughly. Divide the sample into two parts. 4 mL of sample is applied directly to the SPE column. The remaining 2 mL is hydrolysed thus: Add 12 N hydrochloric acid (200 uL) to the urine sample, mix thoroughly and incubate for 45 minutes at 100 C. Cool to room temperature and centrifuge at 3000 rpm for 10 minutes. The sample is then extracted in the same way as the non-hydrolysed sample.
Solvation:	Condition the column with methanol (3 mL).
Equilibration:	Rinse the column with 0.1M hydrochloric acid (5 mL).
Sample application:	Apply the appropriate sample to the column at a flow rate of 1-2 mL/min.
Interference elution:	Elute interferences with deionized water (5 mL).
Analyte elution:	Elute the analytes with 50/50 (v/v) acetonitrile/water containing 2% (v/v) orthophosphoric acid (2.5 mL). Add derivatizing solution (15 uL) to eluted sample (10 uL) and mix thoroughly. After exactly 16 minutes analyse reaction mixture (10 uL) by HPLC.

Structure	As shown. 
Structural considerations	The analyte is a small non-polar molecule.
Matrix considerations	The matrix is aqueous, and of high ionic strength.
Analytical method	HPLC Column: Apex II ODS, 3um x 15cm x 4.6 mm I.D. Mobile phase A: 25mM Phosphate Buffer, pH 6.4 Mobile phase B: Acetonitrile. Gradient Profile: 0 min. - 41% A 12 min. - 41% A 12-15 min. linear to 25% A 15-20 min. - 25% A 20-23 min. linear to 41% A 23-30 min. - 41% A Temperature: Ambient Flow rate: 1mL/min Detection conditions (fluorometry) I EXC=340nm; I EM=525nm
Reagents	
General comments	1. Reagents. a) Internal Standard Solution. Weigh 1,3-diacetylbenzene (130 mg) into a 100 mL volumetric flask. Add methanol (75 mL), mix thoroughly and make up to the mark with methanol. b) 50/50 acetonitrile / water containing 2% (v/v) orthophosphoric acid. Add acetonitrile (50 mL) and deionized water to a reagent bottle and mix thoroughly. Carefully add concentrated orthophosphoric acid (2 mL) and mix thoroughly. c) derivatizing solution. Dissolve dansylhydrazine (30 mg) in dimethylformamide (100 uL), add acetonitrile (250 uL) and mix thoroughly. Filter solution through a hydrophobic membrane, 0.45 um porosity. NB Solution may be stored at 2-8 C under darkness for one week. Stable at -20 C for one month. d) 25 mM phosphate buffer, pH 6.4. Weigh dipotassium hydrogen orthophosphate (1.15 g) and potassium dihydrogen orthophosphate (2.49 g) into a one litre volumetric flask. Dissolve in deionized water (900 mL). Adjust to pH 6.4 and make up to the mark with deionized water. 2. Ensure the sorbent bed is not allowed to dry out between the conditioning and sample application steps. This will give reproducible recoveries from extraction to extraction.

3. It is important to check for possible loss of analyte during the sorbent drying stage. Recoveries could be adversely effected if this step is too harsh.

4. The derivatization time is critical and should adhered to as the derivatized 2, 5-hexanedione is unstable at room temperature. It is recommended that an autosampler is used for automatic mixing and injection.

5. Previous # IST1003

Reference: Maestri,-L et al. Determination of 2,5-Hexandione by high-performance liquid chromtography after derivatisation with dansylhydrazine
J. Chromatography B, 657 (1994) 111-117

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.

United States and Canada
T: + 1 434 9792319
Toll-Free: +1 800 446 4752
ordermailbox@biotage.com

Sweden
Biotage
T: + 46 18 56 59 00
order@eu.biotage.com

United Kingdon, EIRE
Biotage
T: + 44 1443 811811
eurosales@eu.biotage.com

Japan
Biotage
T: + 81 422 281233
order@biotage.co.jp