



# Urinary Catecholamines Kit

October 2001

**MF-9056**

**INSTRUCTION MANUAL**

---

For Research Purposes Only

---

Bioanalytical  
Systems, Inc  
2701 Kent Avenue  
West Lafayette  
Indiana 47906  
[www.bioanalytical.com](http://www.bioanalytical.com)

Copyright June 2000, 2001

Bioanalytical Systems, Inc.  
2701 Kent Avenue  
West Lafayette, IN 47906 USA  
765-463-4527  
FAX 765-497-1102  
bas@bioanalytical.com

ALL RIGHTS RESERVED

---

**Table of Contents**

Section 1. Summary and Explanation .....	1
Section 2. Components.....	2
Section 3. Additional Materials Required.....	3
Section 4. Instrumentation .....	4
Section 5. Stock Reagents.....	4
Section 6. Specimen Collection Preservation and Storage.....	4
Section 7. Experimental Assay Procedure.....	5
7.1 Preparation of Assay Reagents .....	5
7.2 Preparation of Standards .....	5
7.3 Preparation of Calibration Standards.....	6
7.4 Calibration Schemes.....	7
7.5 Preparation of Pooled Quality Control Samples .....	8
Section 8. Assay Procedure .....	9
8.1 Chromatographic Conditions.....	10
Section 9. Liquid Chromatograph, Initial Setup.....	11
9.1 Idle Periods.....	11
9.2 Interfering Compounds .....	12
9.3 Quantitative Calculations .....	12
9.4 Linearity .....	12
9.5 Accuracy.....	12
9.6 Precision .....	12
9.7 Limit of Detection.....	12
9.8 Expected Values .....	13
9.9 Correlation Data .....	13

Section 10. Results and Discussion .....	13
Section 11. References.....	15
Section 12. Suggested Readings .....	16
Appendix I: Compounds Tested .....	17
Appendix II: Catecholamine Salts as a Percent of Free Base.....	18
Appendix III: Late-Eluting Peak .....	19
Appendix IV: Quantitative Calculations .....	20

**Intended Use**

The Urinary Catecholamines Kit is designed for the in-vitro determination of free catecholamines in human urine.

**Section 1. Summary and Explanation**

The clinical significance of catecholamines and their metabolites is well documented. Beer et al. postulated the relationship between catecholamine levels and pheochromocytoma as early as 1937 (1). Currently, most patients who exhibit hypertension are initially screened for catecholamine levels. This is essentially a branch point for the clinician. Abnormally high urinary or plasma catecholamine levels would indicate the potential presence of a catecholamine-producing malignancy, while normal catecholamine levels would tend to rule out this possibility. A positive result, however, requires the use of several additional recognized diagnostic techniques to confirm the presence of a pheochromocytoma, neuroblastoma, or other catecholamine-producing malignancies.

The most commonly accepted method for screening for pheochromocytoma is the determination of catecholamines in a 24-hour urine sample. This is preferred over the determination of plasma catecholamines due to the extremely low concentrations of these substances in plasma.

Liquid chromatography combined with electrochemical detection is considered the method of choice for the determination of free urinary catecholamines. This procedure has allowed researchers and clinicians to successfully separate and quantitate urinary catecholamines at both normal and abnormal levels. One of the inherent problems with this technique, however, is the potential interference from catecholamine metabolites, precursors and drugs, as well as the effects of the urinary matrix which can have a substantial influence on resolution due to the presence of interfering unidentified peaks.

The BAS Urinary Catecholamine Kit is optimized for the analysis of epinephrine, norepinephrine and dopamine, and has the added advantage of eliminating almost all interferences from the chromatogram. Using our proprietary chemistry, we have successfully eliminated any potential interference from 25 of the most common precursors, metabolites and drugs usually found in the urine. (See Appendix I for a list of compounds tested.) The resulting chromatogram is clean, with almost 100% resolved peaks for all analytes of interest (see Figures 1, 2 and 3, Results and Discussion section), if the enclosed instructions are faithfully followed. The entire procedure is essentially error-free and is guaranteed by Bioanalytical Systems. The kits can be adapted to the small lab with less than 10 samples per week, or be automated for the large facility where there is a requirement for 100 samples or more per day.

## Section 2. Components

### Urinary Catecholamines Kit, 100 Samples (MF-9020)

Quantity	Description
1 pkg.	Catecholamine Standards Kit
2 bottles	MP-2 Urinary Catecholamine Mobile Phase, one (1) liter/bottle
1 bottle	Reagent A, Urinary Catecholamine Pretreatment Solution, 125 mL/bottle
1 bottle	Reagent B, SPE Column Wash-1 Solution, 500 mL/bottle
1 bottle	Reagent C, SPE Column Wash-2 Solution, 250 mL/bottle
1 bottle	Reagent D, SPE Column Eluting Solution, 250 mL/bottle
1 ea.	Catecholamine Column
100 ea.	Solid Phase Extraction (SPE) Columns
1 ea.	Urinary Catecholamine Manual

### Urinary Catecholamines Replacement Kit, 100 Samples (MF-9021)

Quantity	Description
2 bottles	MP-2 Urinary Catecholamine Mobile Phase, one (1) liter/bottle
1 bottle	Reagent A, Urinary Catecholamine Pretreatment Solution, 125 mL/bottle
1 bottle	Reagent B, SPE Column Wash-1 Solution, 500 mL/bottle
1 bottle	Reagent C, SPE Column Wash-2 Solution, 250 mL/bottle
1 bottle	Reagent D, SPE Column Eluting Solution, 250 mL/bottle
100 ea.	Solid Phase Extraction (SPE) Columns
1 ea.	Urinary Catecholamine Manual

The Urinary Catecholamine Kits are sufficient for the analysis of approximately 100 samples. MP-2 and Reagents A, B, C and D have finite shelf lives and should be purchased only in the amounts that will be used during the shelf life period. The shelf life for each individual reagent is listed on the bottle. See page 5. (*Warning: Do not use expired reagents, chemicals etc.*)

### **Section 3. Additional Materials Required**

Mechanical pipettors  
Class A volumetric pipettes  
Class A volumetric flasks  
2.0 mL polypropylene microtube (Sarstedt 72.609.001) or equivalent; screw cap with integral safe seal "O" ring (Sarstedt 65.716.00X) or equivalent  
Borosilicate glass disposable straight wall test tubes, 13 × 100 mm  
Borosilicate glass scintillation vials or equivalent  
1 L graduated cylinder, glass  
Vials for autosampler (if required)  
Crimp rings and seals for autosampler vials (if required)  
Crimper for autosampler vials (if required)  
3 mL plastic transfer pipettes or equivalent  
6 × 12 position plastic test tube rack  
Gloves, disposable  
Solid phase extraction vacuum manifold  
Vacuum pump or aspirator with trap  
pH meter  
Vortex mixer  
Analytical balance (0.1 mg)  
Electronic top loading balance or equivalent (0.01 g)  
–80 °C ultra low temperature freezer or equivalent

### **Section 4. Instrumentation**

Liquid chromatograph

Single channel electrochemical detector with dual glassy carbon electrode

Suitable data reduction software or integrator

Strip chart recorder

### **Section 5. Stock Reagents**

Deionized water (DI H <sub>2</sub> O)	Type I reagent grade water (ASTM) or HPLC-grade distilled water
MP-2 Catecholamine Mobile Phase	CF-1102 (See label for shelf life)
Reagent A	CF-1103 (See label for shelf life)
Reagent B	CF-1104 (See label for shelf life)
Reagent C	CF-1105 (See label for shelf life)
Reagent D	CF-1106 (See label for shelf life)
Methanol	HPLC-grade
Acetic acid (Glacial)	
Disodium EDTA	
L-Cysteine	

### **Section 6. Specimen Collection Preservation and Storage**

Acidified Urine	Urine used in this assay is typically from a 24-hour collection. A 24-hour urine sample for catecholamines is collected in an appropriate receptacle which contains 10 mL of 6 N HCl. The acidified urine should be refrigerated at 2–8 °C when not in use.
-----------------	---

## Section 7. Experimental Assay Procedure

### 7.1 Preparation of Assay Reagents

0.5 M Acetic Acid: Dilute 28.5 mL of glacial acetic acid to 1.0 L with DI H<sub>2</sub>O. Shelf life is one year at room temperature.  
(Warning: Do not use expired reagents, chemicals, etc.)

### 7.2 Preparation of Standards

#### Epinephrine (E), Norepinephrine (NE) and Dopamine (DA) Standard Working Solutions:

Accurately weigh  $45.50 \pm 0.10$  mg Epinephrine Bitartrate and  $76.80 \pm 0.10$  mg Norepinephrine Bitartrate. (See Appendix II for calculations.) Transfer quantitatively to a 25.0 mL volumetric flask. Dissolve and dilute to volume with 0.5 M acetic acid (sonicate if necessary). Transfer 1.00 mL of this 1 mg/mL E, 1.5 mg/mL NE stock solution to a 100 mL glass volumetric flask. Accurately weigh  $12.38 \pm 0.10$  mg DA HCl and transfer quantitatively to the same 100 mL glass volumetric flask. Mix until dissolved. Dilute to 100.0 mL with 0.5 M acetic acid and mix well to insure homogeneity. This standard working solution contains 10.0 µg/mL E, 15.0 µg/mL NE and 100 µg/mL DA. This solution can be dispensed into convenient aliquots, sealed, and stored at  $-80$  °C for up to one year. Refrigerate aliquots in use at 2–8 °C for up to one week. Henceforth, this will be termed the "Standard Working Solution." (Warning: Do not use expired reagents, chemicals etc.)

#### Internal Standard Stock Solution:

Place  $15.82 \pm 0.10$  mg of DHBA HBr into a 100 mL glass volumetric flask. Dissolve and dilute to volume with 0.5 M acetic acid. This solution contains 100 µg/mL DHBA. Store at  $-80$  °C and refrigerate at 2–8 °C while in use. (Warning: Do not use expired reagents, chemicals, etc.)

#### Internal Standard Working Solution (10 µg/mL):

Transfer 10.00 mL of the 100.0 µg/mL internal standard stock solution to a 100 mL volumetric flask. Dilute to the mark with 0.5 M acetic acid. Refrigerate at 2–8 °C while in use for up to three months. This solution can be stored at  $-80$  °C for up to one year. Henceforth, this will be referred to as the "Internal Standard Working Solution." Do not confuse this with the "Standard Working Solution" described previously. (Warning: Do not use expired reagents, chemicals etc.)

#### Aqueous Standard Solution:

An aqueous standard containing the three catecholamine analytes and internal standard can be made and injected to assure the proper function of the chromatographic system. The system check solution can be made as follows:

Pipette 50 µL of the working standard solution into a 10-mL volumetric flask. Pipette 100 µL of internal standard working solution into the same flask. Dilute to 10 mL with DI H<sub>2</sub>O.

The resulting solution has a concentration of 50 ng/mL of E, 75 ng/mL of NE, 500 ng/mL of DA, and 100 ng/mL of DHBA.\*

\* This solution is only useful as a chromatographic system check, and should not be used as a means of calibration for the samples. The accuracy and precision of this assay depend upon calibration standards extracted from the urine matrix.

### 7.3 Preparation of Calibration Standards

BAS has used two different approaches to calibrating the catecholamine separation. At one extreme, we have used a 6-point calibration curve that included all the concentrations in the table below. At the other extreme, we have calibrated with a single concentration from the table below. The accuracy of the assay was the same in each case, because the results were linear using the higher level of the concentration range. The analyst may pick any of the first three concentrations listed in the table below as a single-point calibration, after the concentration of the catecholamines has been determined, without any degradation in performance of the assay. The calibrating solution should be freshly diluted with acidified urine, and freshly spiked with standards, on the day of the assay.

#### Preparation of Multi-Point Calibration Standards

##### Acidified Urine Pool

Spiked urine calibrator number	Final desired added concentration of catecholamines in ng/mL	Volume of unspiked urine	Spiking solution and/or volume of spiked urine added
1	* 150 ng/mL E 225 ng/mL NE 1500 ng/mL DA	9.85 mL (acidified urine)	150 $\mu$ L Standard Working Solution
2	* 75 ng/mL E 112.2 ng/mL NE 750 ng/mL DA	5 mL (75 ng/mL E urine pool)	5 mL Urine Calibrator 1
3	* 37.5 ng/mL E 56.3 ng/mL NE 375 ng/mL DA	5 mL (37.5 ng/mL E urine pool)	5 mL Urine Calibrator 2
4	18.8 ng/mL E 28.1 ng/mL NE 187.5 ng/mL DA	5 mL (18.8 ng/mL E urine pool)	5 mL Urine Calibrator 3
5	9.4 ng/mL E 14.1 ng/mL NE 93.8 ng/mL DA	5 mL (9.4 ng/mL E urine pool)	5 mL Urine Calibrator 4
6	4.7 ng/mL E 7.0 ng/mL NE 46.9 ng/mL DA	5 mL (4.7 ng/mL E urine pool)	5 mL Urine Calibrator 5

\* can be used as a single point calibration standard  
E = epinephrine, NE = norepinephrine, DA = dopamine

## 7.4 Calibration Schemes

Different calibration schemes can be used depending on the preference of the user and the availability of matrix to be used for the calibration. A multi-point calibration line can be used as defined in the preparation table. In this case a least-squares linear regression is used to determine the slope and the concentrations of the unknowns as calculated using Method 1 in Appendix IV.

A single point calibration scheme can be used if a urine standard of known catecholamine concentration is available. There are lyophilized urines of known catecholamine concentration on the market. The user can pool urine samples from several individuals to create a single-point calibration matrix and determine its concentration using the standard addition method. Spiked calibration standards are prepared as seen in the preparation table. These are extracted and analyzed by LCEC. A least-squares linear regression performed on the spiked calibrators will yield a slope and y-intercept. The absolute value of the x-intercept can be calculated using the equation:

$$x = | -b/m |$$

where:    b = y-intercept  
          m = slope

The value for x is the concentration of the unspiked single point pool. An extraction of the unspiked pool whose catecholamine concentrations are calculated using Method 1 in Appendix IV should confirm this.

A pool whose catecholamine concentrations are determined in this manner can be dispensed into convenient aliquots, sealed, frozen, and used for at least one month. Extended stability of such a pool should be determined by the user.

### 7.5 Preparation of Pooled Quality Control Samples

Pooled quality control samples should be used to verify the assay on a daily basis. This will insure that the unknown values are valid under the above experimental conditions. The QCs can be prepared in the following manner.

A separately prepared E, NE and DA stock solution is used for the pooled quality control (QC) samples as follows: Accurately weigh  $18.20 \pm 0.10$  mg of Epinephrine Bitartrate and  $40.90 \pm 0.10$  mg Norepinephrine Bitartrate. Transfer quantitatively to a 250.0 mL volumetric flask. Dissolve and dilute to volume using an aqueous solution containing 100 mg/L each L-Cysteine and Na<sub>2</sub>EDTA. Mix well. The final concentration of this "Stock A, QC solution" is 40 µg/mL E and 80 µg/mL NE. (See Appendix II for calculations.)

Accurately weigh out  $14.85 \pm 0.10$  mg DA HCl and transfer quantitatively to a clean 250 mL volumetric flask. Using a Class A pipette transfer 25.0 mL of the "Stock A, QC solution" to this volumetric flask and dilute to volume using the L-Cysteine/Na<sub>2</sub>EDTA solution. Mix well. The concentrations of the catecholamines in this "Stock B, QC solution" are 4.0 µg/mL E, 8.0 µg/mL NE and 48 µg/mL DA.

#### Low QC Pool:

Pipette (glass, class A) 1.0 mL of "Stock B, QC solution" into a 200.0 mL volumetric flask and dilute to the mark with acidified urine. Mix by inversion or magnetic stirring. Aliquot 1.25 mL each into labeled Sarstedt microtubes. This low QC pool represents an added catecholamine concentration of 20 ng/mL E, 40 ng/mL NE, and 240 ng/mL DA.

#### Middle QC Pool:

Pipette (glass, class A) 3.0 mL of "Stock B, QC solution" into a 200.0 mL volumetric flask and dilute to the mark with acidified urine. Mix by inversion or magnetic stirring. Aliquot 1.25 mL each into labeled Sarstedt microtubes. This medium QC pool represents an added catecholamine concentration of 60 ng/mL E, 120 ng/mL NE, and 720 ng/mL DA.

#### High QC Pool:

Pipette (glass, class A) 5.0 mL of "Stock B, QC solution" into a 200.0 mL volumetric flask and dilute to the mark with acidified urine. Mix by inversion or magnetic stirring. Aliquot 1.25 mL each into labeled Sarstedt microtubes. This high QC pool represents an added catecholamine concentration of 100 ng/mL E, 200 ng/mL NE, and 1200 ng/mL DA.

Separate the microtubes according to concentration and store at  $-80$  °C in properly labeled freezer boxes. These QC samples are stable for one year if stored at  $-80$  °C. (*Warning: Do not use expired reagents, chemicals, etc.*)

---

## Section 8. Assay Procedure

1. Keep all experimental and QC samples frozen until ready to use.
2. Thaw urine samples (subject and QC) at room temperature. If lyophilized standards and controls are used, reconstitute according to the manufacturer's instructions.
3. For each sample to be assayed, label two sets of 13 × 100 mm disposable glass test tubes. Set-1 is for sample preparation, and Set-2 is for extract collection.
4. Vortex each sample after it has thawed or been reconstituted. With a pipettor, transfer 1.0 mL aliquots of each sample to a labeled glass test tube of Set-1.
5. With a pipettor, transfer 20 µL of internal standard working solution to each test tube of Set-1.
6. With a pipettor, transfer 1.0 mL of Reagent A to each test tube in Set-1. Vortex for at least one (1) second.
7. Affix the urinary SPE columns equal to the number of samples to be processed into the vacuum manifold, and connect to a vacuum source.
8. Pipette 2 mL of methanol into each SPE column and apply a vacuum to draw it through to waste.

**Warning:** *It is imperative over the course of the extraction that the column beds not be dried out by drawing air through them, with the exception of the final elution step. Adjust the vacuum so that the rate of draw through the SPE columns does not exceed 1 mL/min.*

9. Pipette 2 mL of Reagent B into the SPE columns and apply a vacuum to draw it through to waste.
10. Transfer the prepared urine samples from step 5 above into the SPE columns and apply a vacuum to draw the urines through to waste.
11. Wash each SPE column with 2 mL of Reagent B followed by 2 mL of Reagent C. Draw each wash through to waste.
12. Place the Set-2 labeled test tubes in the Vac Elute and reorient the lid to the collect position. Pipette 2 mL Reagent D into each SPE column, apply the vacuum and collect all the effluent into the labeled Set-2 test tubes. In this case it is acceptable to draw air through the SPE columns.
13. Remove the test tubes from the vacuum manifold and vortex them briefly.

14. Load 100  $\mu\text{L}$  or more of the effluent into a 50  $\mu\text{L}$  loop (50  $\mu\text{L}$  sample injection) and inject these samples in the liquid chromatograph for quantitation.
15. The excess effluent can be stored up to one week in capped test tubes at room temperature. (*Warning: Do not use expired reagents, chemicals, etc.*)

Optional. For Automated Use with Autosampler

16. Pipette 200  $\mu\text{L}$  of each effluent into a labeled autosampler vial. Crimp seals onto the autosampler vials. Place vials in autosampler and initiate analysis.

### 8.1 Chromatographic Conditions

Column:	BAS catecholamine column, MF-6213-CL
Mobile Phase:	BAS MP-2 urinary catecholamine mobile phase, CF-1102
Detector:	Amperometric with dual glassy carbon electrodes in series. The applied potential is set at +600 mV for the upstream electrode relative to Ag/AgCl reference electrode. Gain is set for 20 nA for the working electrode.
Chromatograph:	BAS 200B, BAS 480 or equivalent
Flow Rate:	1.0 mL/min
Autosampler:	CMA 200, BAS Sample Sentinel or equivalent
Loop Volume:	50 $\mu\text{L}$
Sample Volume:	100 $\mu\text{L}$
Run Time:	21.5 minutes (may vary depending on how the late eluting peak is dealt with; see Appendix III)
Column Temperature:	40 °C
Recorder:	BAS dual-pen strip chart recorder
Approximate Retention Times:	E, 6–7 minutes NE, 7–8 minutes DHBA, 12–14 minutes DA, 16–18 minutes

---

## Section 9. Liquid Chromatograph, Initial Setup

1. Connect the Urinary Catecholamine Column (MF-6213-CL) to the system.
2. Set LC pump to a flow rate of one (1) mL/min.
3. Pump approximately 50 mL of MP-2 (CF-1102) mobile phase through the column to remove any trapped air bubbles from the column. *(Warning: Never wash the catecholamine column with solvent. Use only MP-2 Mobile Phase.)*
4. Connect column to electrochemical cell.
5. Pump approximately 20 mL through the system with the effluent going to waste. Ensure that there are no air bubbles in the electrochemical cell.
6. Turn mode switch on electrochemical detector to standby. Turn on power. Set potential and gain. Turn mode switch to CELL.
7. Allow the system to equilibrate for approximately one (1) hour.
8. The pressure should be 3000 psi  $\pm$  400 psi. The system pressure should be stable. *(Warning: While you can obtain useful data at pressures above 3400 psi, you should not allow the system to exceed 4000 psi. Should the pressure exceed 4000 psi, shut the system down and either troubleshoot the pressure problem or replace the column, or both.)*
9. The background or offset current should not exceed 10 nA. *(Note: While you can obtain useful data with a background higher than 10 nA, for best results, should the system exceed 10 nA offset, clean the system and replace the mobile phase at the first available opportunity.)*
10. When the baseline is stable, inject samples.
11. Allow the effluent to flow to waste during the analysis.

### 9.1 Idle Periods

Should the system not be used in a continuous mode, place the waste line directly into the MP-2 mobile phase reservoir and recirculate the mobile phase until you are ready to process new samples. This will insure minimum equilibration when you are ready to use the system again. The system may be left in the idle mode for an indefinite period. To remove the system from operation consult the manual for shutdown procedures.

**9.2 Interfering Compounds** None observed. See Appendix I for compounds tested. See Appendix III for precautions against a late-eluting peak.

**9.3 Quantitative Calculations** See Appendix IV.

**9.4 Linearity** Calibration curve data and linear regression parameters from five batches of urine extracts were determined using the BAS procedure. Calibration curves were weighted using a weighting factor of 1/concentration. The calibration curves were linear in the concentration range 3.7 to 900 ng/mL for E and NE, and 12.3 to 3000 ng/mL for DA. Correlation coefficients (r) were all greater than 0.9992.

**9.5 Accuracy** The accuracy of the BAS method was assessed by subtracting the blank urine sample mean concentration (either low, medium, or high), and then dividing by the spike value of that QC. This calculated value is the % recovery determined in these experiments. The % recoveries were then averaged for the low, medium, and high QCs and reported as the interday % recovery, which is 99.4% for E, 101.0% for NE, and 100.6% for DA.

**9.6 Precision** The within-day precision of the method was determined from the relative standard deviations (RSD) of 5 to 10 replicate analyses of the three pooled quality control samples and a blank pool of samples. The interday precision of the method was determined from the RSDs of the mean of the four control pools.

WITHIN-DAY PRECISION				
	Blank Control	Low QC	Medium QC	High QC
E	5.3–9.6%	0.8–3.3	1.3–2.2%	0.6–3.6%
NE	1.0–4.0%	0.7–2.9%	0.8–1.9%	0.3–3.0%
DA	1.3–4.9%	0.8–3.1%	1.3–2.1%	0.3–4.2%
INTERDAY PRECISION OVER 11 DAYS				
	Blank Control	Low QC	Medium QC	High QC
E	10.4%	6.1%	5.6%	5.5%
NE	6.1%	5.6%	5.0%	4.9%
DA	7.0%	6.1%	5.7%	5.6%

**9.7 Limit of Detection** A standard solution of the catecholamines was injected on the HPLC system at a gain of 20 nA full scale. At this gain, the noise level was very low and the width of the baseline was measured as the noise. A signal-to-noise ratio of 3:1 corresponded to an injected concentration of 0.5 ng/mL for the catecholamines. Thus, the limit of detection (LOD) is an injected concentration of 0.5 ng/mL, since we are recommending a gain setting of 20 nA full scale for the assay. When the sensitivity was increased to 2.0 nA full scale, the LOD was measured as 0.2 ng/mL for the catecholamines.

## 9.8 Expected Values

Expected values in normal healthy children and adults are listed below. For clinical significance of catecholamine levels, refer to Fundamentals of Clinical Chemistry (Ed. Norbert Tietz) (2). Representative disease state values can be found in reference 3.

Expected Catecholamine Levels, $\mu\text{g}/24$ h Urine Collection*, Healthy Individuals				
Age Range, yr	3-6 (4)	6-10 (4)	10-16 (4)	18-60 (4)
Epinephrine	1.5-3.3	1.9-6.3	3.2-6.4	7.3-18.3
Norepinephrine	8.0-17.4	14.8-26.2	25.7-39.5	16.9-101
Dopamine	107-219	126-274	236-348	153-306

\* Some laboratories may prefer to report concentrations of each catecholamine/g creatinine. In this case, the operator should determine the total creatinine concentration in the 24-hour urine sample and normalize each catecholamine concentration to one gram of creatinine.

## 9.9 Correlation Data

The BAS Urinary Catecholamines procedure was compared to a predicate device. The results are listed in the following table.

Mean Concentration for All Samples Assayed in Batches 2EQI-7EQI, in ng/mL						
	Epinephrine		Norepinephrine		Dopamine	
	BAS	CAD	BAS	CAD	BAS	CAD
# of Samples	177	175	177	175	177	175
Mean	36.2	40.2	104	109	416	417
Standard Deviation	61.8	63.0	104	109	543	529
Correlation Coefficient	0.9902		0.9969		0.9988	

BAS = BAS method; CAD = commercially available device method

## Section 10. Results and Discussion

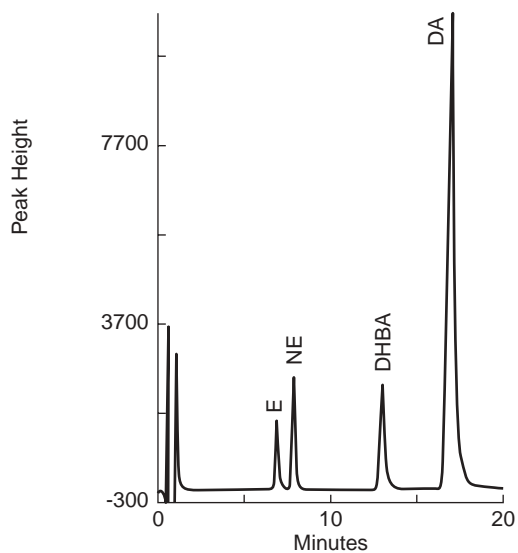
A chromatogram of an aqueous standard can be seen in Figure 1. A chromatogram of an unknown normal catecholamine level urine sample can be seen in Figure 2, while a chromatogram of an unknown abnormally high catecholamine level urine sample can be seen in Figure 3. These figures represent the typical chromatography one would expect using the HPLC conditions described.

The results of testing the most commonly encountered interfering compounds can be seen in Appendix I. The only compound among those tested that presents the possibility of interfering under these conditions is metanephrine. Its retention time is close to that of NE. However, experimentation demonstrated that the response of metanephrine under these conditions is 280 times less than the response for an equal quantity of NE. Additionally, the extraction procedure is less selective for the O-methylated metabolites; thus the recovery of

the free metanephrine would be diminished. These factors, plus the fact that metanephrine is for the most part in a conjugated form in urine, would indicate that interference from metanephrine in quantitating NE would be negligible. Over the course of these experiments, no metanephrine-induced interference with NE was observed using the BAS catecholamine kit.

---

Figure 1. BAS System Check (batch 3EQI run 101)



---

Figure 2. Normal Sample Using BAS Extraction Method (batch 3EQI run 40)

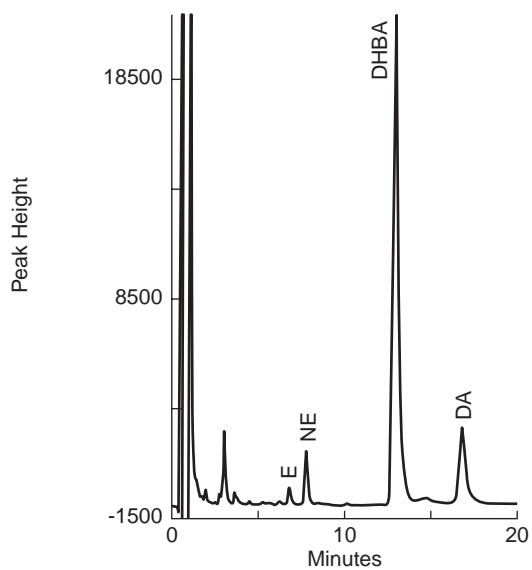
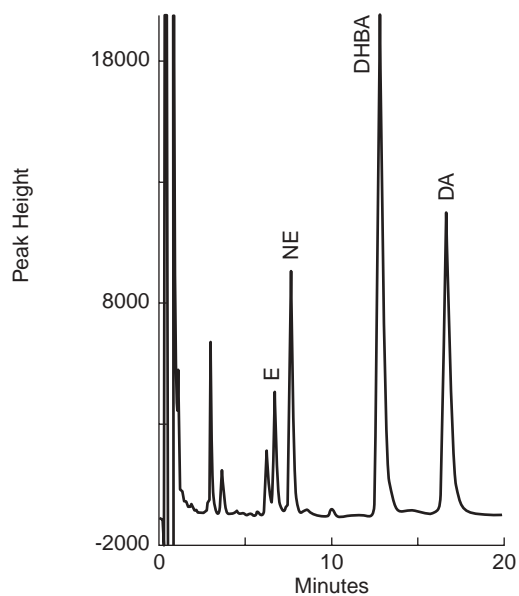


Figure 3. Abnormal Sample Using BAS Extraction Method (batch 3EQI run 17)



## Section 11. References

1. G. Beer, F.H. King, and M. Prinzmetal, *Ann. Surg.* 106 (1937) 85.
2. *Fundamentals of Clinical Chemistry* (N.W. Tietz, Ed.), W.B. Saunders Co., 595–603 (1987).
3. N. Racadot-Leroy and P. Fontaine, Assay of Urinary Free Catecholamines by High Performance Liquid Chromatography with Electrochemical Detection, *Rev. Fr. Endocrinol. Clin.* 26 (1985) 49–52.
4. A. Premel-Cabic, A. Turcant, and P. Allain, Normal Reference Intervals for Free Catecholamines and Their Acid Metabolites in 24-h Urines from Children, as Determined by Liquid Chromatography with Amperometric Detection, *Clin. Chem.* 32 (1986) 1585–1587.
5. Y. Ohkura and H. Nohta, Fluorogenic Reagents for the Derivatization of Catecholamines and Related Compounds for Liquid Chromatographic Analysis of Biological Samples, *Trends Anal. Chem.* 11 (1992) 74–79.

## Section 12. Suggested Readings

1. R.E. Shoup, Liquid Chromatography/Electrochemistry, *High Performance Liquid Chromatography, Advances and Perspectives*, Vol. 4 (C.S. Horvath, Ed.), Academic Press, 91–94 (1986).
2. A.A. Nanj and D. Blank, Catecholamines in Urine, *Clin. Chem.* 29 (1983) 595.
3. D.L. Palazzolo and S.K. Quadri, Reduced Variation in Retention Times of Biogenic Amines by Temperature Control in Liquid Chromatography with Electrochemical Detection, *J. Chromatogr.* 479 (1989) 216–219.
4. R.M. Riggins and P.T. Kissinger, Determination of Catecholamines in Urine by Reverse-Phase Chromatography and Electrochemical Detection, *Anal. Chem.* 49 (1977) 2109–2111.
5. R. Causon and M.J. Brown, Catecholamine Measurements in Pheochromocytoma: A Review, *Ann. Clin. Biochem.* 19 (1982) 396–404.
6. R. Kremer, J.C. Crawhall, and R. Kolanitch, Rapid and Reliable Estimation of Urinary Free Catecholamines in Patients with Pheochromocytoma: Comparison with Plasma Catecholamines and Vanillylmandelic Acid Excretion, *J. Chromatogr.* 344 (1985) 313–318.
7. G.A. Smythe, G. Edwards, P. Graham, and L. Lazarus, Biochemical Diagnosis of Pheochromocytoma by Simultaneous Measurement of Urinary Excretion of Epinephrine and Norepinephrine, *Clin. Chem.* 38 (1992) 486–492.
8. E.A. Gerlo and C. Stevens, Urinary and Plasma Catecholamines and Urinary Catecholamine Metabolites in Pheochromocytoma: Diagnostic Value in 19 Cases, *Clin. Chem.* 40 (1994) 250–256.
9. G. Stenstrom, B. Sjogren, and J. Waldenstrom, Excretion of Adrenaline, Noradrenaline, Vanillylmandelic Acid and Metanephrines in 64 Patients with Pheochromocytoma, *Acta. Med. Scand.* 214 (1983) 145–152.

**Appendix I: Compounds Tested****Retention Times of Catecholamines, Metabolites and Other Possible Interfering Compounds**

Compound	CAS Registry No.	Retention Time (min)*
DOPEG	[28822-73-3]	0.14
DL-DOPA	[63-84-3]	0.54
Acetaminophen	[103-90-2]	0.58
DOMA	[14883-87-5]	0.58
MHPG	[534-82-7]	0.58
VMA	[2394-20-9]	0.58
DOPAC	[120-32-9]	0.76
HVA	[306-08-1]	0.90
$\alpha$ -MethylDOPA	[555-29-3]	1.69
<b>E</b>	<b>[51-43-4]</b>	<b>7.21</b>
<b>NE</b>	<b>[138-65-8]</b>	<b>8.46</b>
Metanephrine	[881-95-8]	8.96
Normetanephrine	[1011-74-1]	10.85
<b>DHBA</b>	<b>[16290-26-9]</b>	<b>14.62</b>
Isoproterenol	[51-31-0]	16.40
Salsolinol	[70681-20-8]	18.10
<b>DA</b>	<b>[62-31-7]</b>	<b>19.37</b>
3-O-Methyldopamine		24.88
N-Methyldopamine	[62-32-8]	35.68
Isoetharine	[7279-75-6]	36.06
4-O-Methyldopamine	[645-33-0]	36.85
Chloramphenicol	[56-75-7]	NPD
Sodium Salicylate	[54-21-7]	NPD
Diphenylhydantoin	[57-41-0]	NPD
Theophylline	[58-55-9]	NPD
Caffeine	[58-08-2]	NPD
Diazepam	[439-14-5]	NPD
Labetalol	[32780-64-6]	NPD
5-HIAA	[54-16-0]	NPD
Mandelamine	[587-23-5]	NPD

Metoclopramide	[7232-21-5]	NPD
Cimetidine	[51481-61-9]	NPD

\* Corrected retention time ( $t_R - t_0$ )

NPD = No Peak Detected

## Appendix II: Catecholamine Salts as a Percent of Free Base

The catecholamines contained in the BAS catecholamine standard kit, PN CF-1032, are only provided as catecholamine salts. Any standard solution made from these compounds must be corrected for the amount of free base per unit volume. The most common catecholamine salts are the Hydrochloride (HCl), Hydrobromide (HBr) and Bitartrate. A simple equation can be used to determine either the amount of free base in a given amount of salt, or, the amount of salt required to obtain a specific amount of free base. The simple, algebraic manipulation of the terms in the following equation make both calculations possible:

$$\frac{\text{Weight of Free Base}}{\text{Molecular Weight of Free Base}} = \frac{\text{Weight of the Salt}}{\text{Molecular Weight of the Salt}}$$

### Example:

You have weighed out a 5.0 mg sample of norepinephrine HCl. To find out how much free base is present in this sample:

$$\frac{\text{Weight of Free Base}}{169.2 \text{ g}} = \frac{5.0 \text{ mg}}{205.6 \text{ g}}$$

Therefore, the amount of free base = 4.1 mg.

### Example:

You need to find out how much Dopamine HCl is equivalent to 2.0 mg of free base. To determine how much Dopamine HCl to weigh out:

$$\frac{2.0}{153.2} = \frac{\text{Weight of Salt}}{189.6}$$

Therefore, you would need to weigh out 2.5 mg of Dopamine HCl.

The catecholamines and their common salts are listed below with respective molecular weights. Please verify the weight on the label of each compound.

Compound	Molecular Weight
Dopamine (Free Base)	153.2
Dopamine HCl	189.6
Dopamine HBr	234.1
Epinephrine (Free Base)	183.2
Epinephrine HCl	219.7
Epinephrine HBr	264.1
Epinephrine Bitartrate	333.3
Norepinephrine (Free Base)	169.2
Norepinephrine HCl	205.6
Norepinephrine HBr	250.1
Norepinephrine Bitartrate 1.5 H <sub>2</sub> O	346.3
Norepinephrine Bitartrate 1.0 H <sub>2</sub> O	337.3
3,4-Dihydroxybenzylamine (DHBA)	139.2
DHBA HCl	175.6
DHBA HBr	220.1

### ***Appendix III: Late-Eluting Peak***

There is a late-eluting compound that is extracted from the urine samples. Depending on the age of the column, this late eluter has a retention time of around 40 minutes. The retention time gradually decreases as more injections are made on the column. Users of the assay should be aware of this late eluter so that it can be dealt with properly. Since the retention time of this compound does not change much over the course of 50–70 injections, the user can deal with it by timing the injections so that the late eluting peak occurs at an innocuous time in the subsequent chromatogram. For example, using the BAS Urinary Catecholamine Kit, and making an injection every 21.5 minutes, this peak from the previous injection will consistently elute between DHBA and DA of the current injection without inter-

fering with either peak of interest. With a new column, the user should check this procedure with standards to insure that the late eluter is in the area described.

## **Appendix IV: Quantitative Calculations**

### **Calibration Method 1:**

When serially diluted, spiked extracts were used for calibration purposes, peak height ratios of the catecholamines/internal standard were calculated. Calibration curves were obtained using 1/concentration weighting in a least squares linear regression. All concentrations were calculated as:

$$C = y/m$$

where: C = concentration of catecholamine in sample  
y = peak height ratio of catecholamine to internal standard  
m = slope from linear regression

In calculating the concentration using this equation the y-intercept was ignored, or effectively forced to zero, to correct for the presence of endogenous catecholamines in the urine used for the calibration line.

### **Calibration Method 2:**

When a single point calibration scheme was used, the catecholamine concentrations were calculated in the following manner: a spiked urine of known catecholamine concentration was extracted with the other samples. Peak height ratios of the endogenous catecholamines/internal standard were calculated. All concentrations were calculated as:

$$C = \frac{(S \times U)}{P}$$

where: C = concentration of the catecholamine in the unknown sample  
S = known catecholamine concentration of the standard  
U = peak height ratio of catecholamine to internal standard in the unknown  
P = peak height ratio of catecholamine to internal standard in the known