BAS Plasma Catecholamine Kit

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Part MF-9078 **INSTRUCTION MANUAL**

For Research Purposes Only

Bioanalytical
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Section 1. Introduction

The BAS Plasma Catecholamine Kit is designed for in vitro determination of catecholamines, specifically epinephrine and norepinephrine, in human plasma. 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). Most patients who exhibit hypertension are screened for plasma epinephrine (E) and norepinephrine (NE), collectively referred to as "catecholamines," as a preliminary diagnostic indication of a pheochromocytoma or other catecholamine-producing malignancy. This is essentially a branch point for the clinician. Abnormally high levels of plasma catecholamines would indicate the potential presence of the above malignancies, while normal levels would tend to rule out this possibility. A positive result, however, would require the use of several additional recognized diagnostic techniques to confirm the presence of a pheochromocytoma, neuroblastoma, or other catecholamine-producing tumor.

One commonly accepted method of detecting a pheochromocytoma is the determination of catecholamines in a plasma sample. Liquid chromatography combined with electrochemical detection is considered the method of choice for the determination of free plasma catecholamines (3). This procedure has allowed researchers and clinicians to successfully separate and quantitate plasma 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 plasma matrix which can have a substantial influence on resolution due to the presence of interfering unidentified peaks.

The BAS Plasma Catecholamine Kit is optimized for the analysis of epinephrine (E) and norepinephrine (NE) and eliminates potential interferences from 28 of the most common precursors, metabolites, and drugs usually found in human plasma. (See Appendix I for a list of the compounds tested.) The resulting chromatogram is clean, with almost 100% resolved peaks for all analytes of interest. The kit can be adapted for use in small labs with less than 10 samples per week or be automated for large facilities analyzing 100 samples or more per day.

Principles of the Method

Aliquots (2.0 mL) of plasma spiked with 50 µL of a dihydroxybenzylamine (DHBA, 20 ng/mL) internal standard solution are initially treated with approximately 10 mg of Plasma Pretreatment Adsorbent. After shaking the sample tubes and spinning down the adsorbent, samples are transferred by disposable pipette to a conical-bottom test tube.

To the approximately 2.0 mL of sample are added approximately 50 mg of AAO and 1.5 mL of Reagent A. The tubes are then capped and shaken on a horizontal shaker for at least ten minutes. The tubes are placed upright and the AAO allowed to settle. The supernatants are aspirated from the tubes, leaving the AAO. The samples are then ready for washing.

The AAO is washed twice with Reagent B. The solution is dispensed with enough force to disrupt the AAO pellet. After the AAO settles, the wash solution is aspirated off. A final wash with Reagent C follows the same procedure. After Reagent C is aspirated the sample tubes are briefly centrifuged. Any Reagent C remaining on top of the AAO is aspirated, readying the samples for elution.

Elution of the catecholamines from the AAO is accomplished using 200 µL of the BAS Catecholamine Eluting Solution. After the eluting solution is added the tubes are vortexed, allowed to sit for approximately three (3) minutes, vortexed again, and centrifuged. To prevent injection of AAO particles into the LC system, only 150 µL of eluent is pipetted from the sample tubes to vials. The eluent is then ready for injection into an LC system.

An aliquot of the eluent is injected into the chromatographic system by overfilling a 100 µL loop with 120 - 150 µL of sample. Separation of the catecholamines and internal standard is achieved on the Catecholamine Cartridge column using the MP-2 mobile phase. The separation of the catecholamines occurs in under 20 minutes. There are at times small, late eluting peaks that are plasma dependent and must be taken into consideration. The retention time of these peaks depends upon the age of the column. Amperometric detection is attained using a glassy carbon electrode. An applied potential of +650 mV relative to a Ag/AgCl reference electrode is monitored at the working electrode.

Cautions

Hydrochloric acid, perchloric acid and sodium hydroxide may cause burns. Do not allow contact with eyes, skin or clothing. Avoid breathing vapors. Use adequate ventilation. Keep in tightly closed containers. Do not pipette by mouth. Wash hands thoroughly after use. In case of contact, immediately wash eyes or skin with large amounts of water for at least 15 minutes. Remove contaminated clothing.

Section 2. Components and Required Materials

The following components are included in BAS Plasma Catecholamine Kits (MF-9016) and Plasma Catecholamine Replacement Kits (MF-9017):

Plasma Catecholamine Kit

1 pkg.	Catecholamine Standards Kit (0.5 g each of epinephrine, norepinephrine, dopamine, L-DOPA and DHBA internal standard)*
2 bottles	MP-2 Catecholamine Mobile Phase, one (1) liter/bottle
1 bottle	Reagent A, 250 mL/bottle (Buffer, pH 8.8)
1 bottle	Reagent B, One (1) liter/bottle (Aqueous Wash Solution, pH 7.0, contains no irritants)
1 bottle	Reagent C, 500 mL/bottle (Aqueous Wash Solution, pH 7.0, contains no irritants)
1 bottle	Catecholamine Eluting Solution, 50 mL/bottle (**Caution:** contains less than 60 mM Phosphoric and Monochloroacetic acid)
1 vial	AAO Acid Washed Alumina, 10 g/vial
1 scoop	Used to dispense AAO
1 vial	Catecholamine Pretreatment Adsorbant, 2g/vial
1 ea.	Catecholamine Cartridge Column
1 ea.	10 cm Cartridge Column Holder
1 ea.	Plasma Catecholamine Manual

Plasma Catecholamine Replacement Kit

2 bottles	MP-2 Catecholamine Mobile Phase, one (1) liter/bottle
1 bottle	Reagent A, 250 mL/bottle (Buffer, pH 8.8)
1 bottle	Reagent B, one (1) liter/bottle (Aqueous Wash Solution, pH 7.0, contains no irritants)
1 bottle	Reagent C, 500 mL/bottle (Aqueous Wash Solution, pH 7.0, contains no irritants)
1 bottle	Catecholamine Eluting Solution, 50 mL/bottle (**Caution:** contains less than 60 mM Phosphoric and Monochloroacetic acid)
1 vial	AAO Acid Washed Alumina, 10 g/vial
1 scoop	Used to dispense AAO
1 vial	Catecholamine Pretreatment Adsorbant, 2g/vial
1 ea.	Plasma Catecholamine Manual

* Store sealed vials refrigerated at 2-8° C when not in use. For stability of epinephrine and norepinephrine working solution and DHBA internal standard stock solution see Preparation of Standards, Section 4.

The Catecholamine Standards Kit is used to prepare epinephrine and norepinephrine standard working solutions and DHBA internal standard stock solution. The Plasma Catecholamine Kits are sufficient for the analysis of 100 samples. MP-2, Catecholamine

Eluting Solution, and Reagents A, B and C have finite shelf lives and should be purchased only in the amounts that will be used during the shelf life period. The shelf life and storage for each reagent is listed on the bottle. Opening reagents does not affect shelf life.

Expired reagents, chemicals, etc. should not be used.

Required Materials

In addition to the materials provided in the Plasma Catecholamine Kits, the following materials are required:

Mechanical Pipettors
Class A volumetric pipette
Class A volumetric flasks
Borosilicate glass scintillation vials
Test tube rack
Beakers
Small spatula
Vials and caps for autosampler, if used
Gloves, disposable
Permanent markers
Side arm vacuum flask
Tubing and stopper for aspirator trap
Blunt-tip pipetting needle
Vacuum pump or aspirator
Horizontal shaker
Centrifuge
Vortexer
Analytical Balance
Top-loading balance
-80° C ultra-low-temperature freezer
Conical test tubes and caps
Liquid Chromatograph
Electrochemical Detector

NOTE: Polypropylene conical-bottom test tubes (12 x 75 mm) and polyethylene caps may be obtained from the following:

Vendor	Part No. (Tubes)	Part No. (Caps)
Sarstedt	57.512 PP	65.809 (push-type)
VWR/Baker	60818-099	60819-003 (plug-type)
Fisher	TC-5002	14-959-17 (push-type)

Section 3. Specimen Collection, Preservation and Storage

To obtain truly basal levels, the patient must minimize the influence of stress and other factors. Epinephrine and epinephrine-like drugs, as well as Aldomet and Inderal, interfere with the test procedure. Inderal, for example, interferes due to its physiological effect. Such drugs should be discontinued one week prior to obtaining a sample. The patient must not use tobacco, drink coffee or teas, or eat anything for at least four hours before blood is drawn for the test. Preparation should also include the placement of an indwelling, heparinized intravenous catheter, accompanied by complete instructions and reassurance regarding the procedure. The patient should then rest for 30 minutes in the supine position in a quiet room before the specimen is obtained.

At the end of the 30-minute period 3.0 mL of blood should be withdrawn to flush the heparin from the catheter before obtaining the specimen. This 3.0 mL of blood is discarded. Blood should be collected in heparin tubes and immediately immersed in an ice bath. The tubes should be centrifuged at 2-8° C to separate the plasma within 30 minutes of collection. At least 2.5 mL of plasma is needed for each sample to be assayed.

For samples that are not extracted within five hours after collection it will be necessary to add an antioxidant. Make the antioxidant solution by dissolving 5.0 g of sodium metabisulfite and 3.5 g of disodium EDTA in 100.0 mL of water. The collected plasma should be placed in a polypropylene test tube and spiked with the antioxidant solution (40 µL of the antioxidant solution per mL of plasma). The tubes should then be frozen at -70° C. Samples prepared and stored in this manner are stable for up to eight months.

Section 4. Assay Procedure

Preparation of 0.1 M Perchloric Acid

Commercial perchloric acid can be obtained as 60% or 70% by weight, representing 9.2 and 11.7 moles/liter, respectively. To prepare a 0.1 M solution dilute the acid in the following proportions: 1.0 mL of the 60% perchloric acid with 91 mL of distilled water, or 1.0 mL of the 70% perchloric acid with 117 mL of distilled water.

Caution: Never add water to an acid. Always add the acid to the water.

Preparation of Standards

E, NE standard working solution. Accurately weigh 45.48 ± 0.10 mg epinephrine bitartrate and 74.75 ± 0.10 mg norepinephrine bitartrate · 1.0 H_2O. Transfer quantitatively into a 250.0 mL volumetric flask and dilute to 250.0 mL with 0.1 M perchloric acid (PCA). Mix well. Sonicate if necessary. Label this solution as standard solution A. The catecholamine concentrations for standard solution A are 100 µg/mL E and 150 µg/mL NE.

Transfer 100.0 µL of standard solution A to a 100.0 mL volumetric flask. Dilute to 100.0 mL with 0.1 M PCA and mix well to assure homogeneity. Label this solution as the standard working solution. The catecholamine concentrations for the standard working solution are 100 ng/mL E and 150 ng/mL NE. This solution can be aliquoted and stored at -80° C for up to one year. Refrigerate aliquots in use at 2-8° C for up to one week.

Internal standard stock solution. Place 16.00 ± 0.10 mg of DHBA · HBr into a 100.0 mL volumetric flask and dissolve with 0.1 M PCA. Dilute to the mark with 0.1 M PCA. Mix well. Sonicate if necessary. Label this solution as internal standard solution A. Solution A is 100.0 µg/mL DHBA.

Dilute 1.0 mL of internal standard solution A to 100.0 mL with 0.1 M PCA in a 100.0 mL volumetric flask. Mix well. Label this solution as internal standard solution B. Solution B is 1.0 ug/mL in DHBA.

Dilute 2.0 mL of internal standard solution B to 100.0 mL with 0.1 M PCA in a 100.0 mL volumetric flask. Mix well. Label this solution as internal standard working solution. The internal standard working solution is 20.0 ng/mL DHBA. Store aliquots at -80° C for up to one year and refrigerate at 2-8° C while in use for up to three months.

Preparation of Pooled Quality Control Samples

Low QC Pool. 200 µL of the working standard solution is pipetted into a 200.0 mL volumetric flask and diluted to the mark with stabilized plasma. The QCs are mixed to assure homogeneity. Low QCs can be aliquoted into labeled test tubes. This low QC pool represents an added catecholamine concentration of 100 pg/mL E and 150 pg/mL NE.

Note: Commercially available lyophilyzed controls can be used.

Middle QC Pool. 600 µL of the working standard solution is pipetted into a 200.0 mL volumetric flask and diluted to the mark with stabilized plasma. The QCs are mixed to assure homogeneity. Middle QCs can be aliquoted into labeled test tubes. This middle QC pool represents an added catecholamine concentration of 300 pg/mL E and 450 pg/mL NE.

High QC Pool. 1500 µL of the working standard solution is pipetted into a 200.0 mL volumetric flask and diluted to the mark with stabilized plasma. The QCs are mixed to assure homogeneity. High QCs can be aliquoted into labeled test tubes. This high QC pool represents an added catecholamine concentration of 750 pg/mL E and 1125 pg/mL NE.

Segregate tubes according to concentration and store at -80° C.

Preparation of Multi-Point Calibration Standards

Normally, when an extracted calibration line is used for the purpose of calibration, plasma is spiked and serially diluted on the day of assay and used directly as shown below. The use of the lowest calibrator shown in the scheme below is optional.

Spiked Concentration of Catecholamines in pg/mL	Volume of Unspiked Plasma	Spiking Solution and/or Vol. of Spiked Plasma Added
2,000 E 3,000 NE	9.80 mL	200 µL of the E and NE standard working solution
1,000 E 1,500 NE	5.0 mL	5 mL of the 2,000 pg/mL E plasma pool
500 E 750 NE	5.0 mL	5 mL of the 1,000 pg/mL E plasma pool
250 E 375 NE	5.0 mL	5 mL of the 500 pg/mL E plasma pool
125 E 187.5 NE	5.0 mL	5 mL of the 250 pg/mL E plasma pool
62.5 E 93.75 NE	5.0 mL	5 mL of the 125 pg/mL E plasma pool
31.25 E 93.75 NE	5.0 mL	5 mL of the 62.5 pg/mL E plasma pool
15.6 E 23.9 NE	5.0 mL	5 mL of the 31.25 pg/mL E plasma pool

Note: A lyophilyzed plasma of known concentration can be used as a single point calibrator.

Calculations

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

$$C = y/m$$

where:
- C = concentration of catecholamine in the 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 is ignored or, effectively, forced to zero to correct for the presence of endogenous catecholamines in the pooled plasma used for the calibration line.

Calibration Method 2: When a single point calibration scheme is used, the catecholamine concentrations are calculated as follows. A spiked plasma of known catecholamine concentration is extracted with the other samples. Peak height ratios of the endogenous catecholamines/internal standard are calculated. All concentrations are calculated as:

$$C = (S \times U)/P$$

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

Extraction Procedure

1. Label two 12 x 75 mm, disposable, conical-bottom, polypropylene tubes and one sample vial for each sample to be extracted.

2. If used, reconstitute lyophilized standards and controls according to the manufacturer's instructions. If needed, thaw pooled plasma and catecholamine working standard solution for spiking calibrators. Prepare calibration standards, if used.

3. Keep plasma samples (subject and QC) at room temperature until thawed.

4. Vortex each sample after it is thawed or reconstituted, centrifuge if necessary, and quantitatively transfer 2.0 mL aliquots of each sample to a labeled test tube.

5. Dispense 50 µL of the internal standard working solution into each test tube.

6. Using a small spatula, place approximately 10 mg of the Plasma Pretreatment Adsorbent into each of the sample tubes. This is about a spatula tip full of the sorbent (precision is not necessary).

Note: Shake the samples by hand if a mechanical shaker is not available. Do not use rockers. They do not provide adequate mixing.

7. Cap each tube. Shake the samples by hand to assure that the sorbent is well dispersed and shake the tubes vigorously in a horizontal position on a horizontal shaker for at least ten minutes.

8. Centrifuge for at least five minutes at maximum rpm appropriate for the test tube.

9. Transfer as much sample as possible to the other labeled test tube with a disposable pipette. Take care not to disturb or transfer any of the adsorbent pellet at the bottom of the tube.

Note: Make sure that the AAO is being well dispersed in the solution and not simply collecting on the bottom of the tube. Shake the samples by hand if a mechanical shaker is not available. Do not use rockers.

10. Using the scoop provided, dispense approximately 50 mg of AAO into each test tube.

11. Dispense 1.5 mL of Reagent A into each test tube.

12. Cap each tube and vortex or shake by hand briefly to disperse the AAO in the solution.

13. Shake the tubes vigorously in a horizontal position on a horizontal shaker for at least fifteen minutes.

14. Centrifuge for at least five minutes at the maximum rpm appropriate for the test tube.

15. Uncap the tubes and aspirate all supernatants to waste. A system that works well if many samples are being assayed consists of a vacuum source with a liquid trap connected by plastic tubing to a blunt end pipetting needle.

16. Disrupt the AAO pellet by washing with 3.0 mL of Reagent B. A repeating pipettor can provide sufficient force when the wash solution is dispensed to disrupt the pellet and swirl the AAO. An alternative would be to recap and vortex the tube.

17. Allow the AAO to settle and aspirate the liquid to waste.

18. Repeat the 3.0 mL Reagent B wash and aspirate the liquid to waste.

19. Wash the AAO pellet with 3.0 mL of Reagent C and aspirate the liquid to waste. For this aspiration step draw off as much Reagent C from the pellet as possible without removing the AAO. Also, remove any droplets clinging to the side of the tube. As an option, the tubes can be spun at this point to create an AAO pellet. Any excess Reagent C will remain on top of the pellet and can be easily aspirated.

20. Add 200 µL of Catecholamine Eluting Solution to each test tube.

21. Vortex for at least fifteen seconds and then allow the tubes to stand for at least three minutes. Briefly vortex the tubes again. Be sure that the AAO pellet is completely dispersed in the eluting solution while vortexing and that it does not remain at the bottom of the tube.

22. Centrifuge for at least five minutes at the maximum rpm appropriate for the test tube.

23. Transfer 150 µL of the supernatant into a labeled sample vial. Be careful not to disrupt or aspirate any of the AAO pellet. Do not allow the extracted samples to sit on the AAO pellet for more than 30 minutes. Readsorption of the catecholamines to the AAO will occur and reduce recovery.

24. If using an autosampler, transfer portions of the eluent from step 23 to autosampler vials and inject. It is recommended that the vials be capped.

Section 5. Instrumentation and Chromatographic Conditions

Chromatographic Conditions

Column: BAS Catecholamine column (MF-6213-CL)

Mobile Phase: BAS MP-2 Catecholamine Mobile Phase (CF-1102)

Detector: Amperometric with dual glassy carbon electrodes in series. The applied potential is set at +650 mV for the upstream electrode relative to a Ag/AgCl reference electrode. Gain is set at +5 nA for the electrode. Filter setting is 0.1 Hz.

Chromatograph: BAS 200B, BAS 480 or equivalent

Flow rate: 1.0 mL/min

Autosampler: BAS Sample Sentinel or equivalent

Loop volume: 100 µL

Sampled volume: 100-150 µL

Run time: 22 minutes (may vary depending on how the late eluting peak is dealt with)

Column temperature: 40° C

Recorder: BAS RYT-DP or equivalent

Approximate retention times: E, 7-8 minutes; NE, 8-9 minutes; DHBA, 13-16 minutes. Times are dependent on column age.

Data Reduction: BAS ChromGraph or equivalent

Liquid Chromatograph Initial Setup

Warning: Never wash the catecholamine column with solvent. Use only MP-2 or MP-3.

1. Connect the Catecholamine Column (MF-6213-CL) to the system.
2. Set LC pump to a flow rate of 1.0 mL/min.
3. Pump approximately 50 mL of MP-2 mobile phase through the column to remove any trapped air bubbles from the column.
4. Connect the column to the electrochemical cell.
5. Pump approximately 20 mL through the system with the effluent going to waste.

6. Turn the mode switch on the electrochemical detector to STANDBY. Turn on power. Set potential and gain. Apply potential to the cell.

7. Allow system to equilibrate for approximately one hour and ensure that the column temperature is 40° C.

8. The pressure should be 3200 to 4000 psi. The system pressure should be stable. While you can obtain useful data at the high end of this pressure range, you should not allow the system to exceed 4000 psi. Should the pressure exceed 4000 psi, shut the system down and troubleshoot the pressure problem, replace the column, or both.

9. The background current should not exceed 10 nA. While you can obtain useful data at a background higher than 10 nA, for best results you should clean the system and replace the mobile phase at the first available opportunity if the system exceeds 10 nA offset.

10. When the baseline is stable, inject samples.

11. Allow the effluent to flow to waste during the analysis.

LC Injection Procedure

1. Ensure that the LC system is equilibrated under the proper LC parameters and a stable baseline is achieved.

2. An aqueous standard diluted in the Catecholamine Eluting Solution can be injected as an LC system check. This solution is made by diluting 500 µL of the working standard solution and 2.0 mL of the working internal standard solution to 10.0 mL using the Catecholamine Eluting Solution. It is recommended that this system check be injected prior to injections of extracted samples to prove system suitability. An injection of this solution in the middle and at the end of sample injections is also recommended.

3. Inject sample extracts in random order.

4. Injections of the calibrators and QCs should be interspersed throughout the unknown samples. If a single point calibration scheme is used it is a good idea to bracket the unknowns with the calibrators, then calculate the unknown concentrations based on the mean response of the calibrators.

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 at 1.0 mL/min until you are ready to process new samples. This will ensure minimum equilibration time 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 chromatograph manufacturer's manual for shutdown procedures.

Linearity

Calibration curve data and linear regression parameters from four (4) batches of plasma 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 from 31.3 to 2000 pg/mL for E and 49.6 to 3000 pg/mL for NE. Correlation coefficients (r) were all greater than 0.99736 for E and 0.99713 for NE.

Recovery

The recovery of the method was assessed by subtracting the mean concentration of the blank plasma samples from the respective QC mean concentration (either low, medium or high) and then dividing by the spike concentration of that QC. This calculated value is the percentage recovery determined in these experiments. The percentage recoveries were then averaged for the low, medium, and high QCs and reported as the overall percentage recovery (95.3% for epinephrine and 101.2% for norepinephrine).

QC Pools	Epinephrine	Norepinephrine
Low	82.3 - 100.5	99.4 - 107.5
Medium	91.6 - 100.6	95.3 - 102.1
High	98.6 - 106.8	98.0 - 106.4

Precision

The within-day precision of the method was determined from the relative standard deviations (RSD) of 5-10 replicate analyses of the three pooled quality control samples. This parameter was determined for each of the four different analytical runs. The inter-day precision of the method was determined from the RSDs of the mean of the three control pools over the four analytical runs.

Within-Day Precision

Analyte	Low QC	Medium QC	High QC
Epinephrine	4.3 - 8.6% 150 pg/mL	2.5 - 8.4% 344 pg/mL	3.3 - 9.5% 762 pg/mL
Norepinephrine	2.1 - 7.4% 504 pg/mL	3.6 - 7.4% 784 pg/mL	3.6 - 8.2% 1413 pg/mL

Inter-Day Precision (7 days)

Analyte	Low QC	Medium QC	High QC
Epinephrine	7.6% 150 pg/mL	7.3% 344 pg/mL	7.9% 762 pg/mL
Norepinephrine	7.3% 504 pg/mL	6.8% 784 pg/mL	9.1% 1413 pg/mL

Limit of Detection

A standard solution of the catecholamines was injected on the LC system. 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 assayed sample concentration of 5.0 pg/mL for epinephrine and 7.5 pg/mL for norepinephrine.

Expected Values

Normal plasma catecholamine levels expected for a patient who has been in a supine position for 30 minutes are < 88 pg/mL for epinephrine and 104 - 548 pg/mL for norepinephrine.[3]

Correlation Data*

Mean Concentration for All Samples Assayed in pg/mL				
	Epinephrine		Norepinephrine	
	BAS Procedure	Commercially Available Device	BAS Procedure	Commercially Available Device
Total # of Samples	70	70	70	70
Range (pg/mL): High Low	2434 15.8	2369 12.2	3488 130	3285 163
# of Spiked Samples	10	10	10	10
Range (pg/mL): High Low	1174 954	1005 801	1730 1503	1886 1355
Mean	441	393	827	760
Standard Deviation	558	514	720	653
Correlation Coeff. (R Value)*	0.994412		0.993926	
Y Intercept	13.35		-9.17	
Slope	1.075183		1.091721	
* Correlation coefficient is based on the correlation of the assayed concentrations for the two kits.				

Limitations

Chromatographic interference may occur from precursors, metabolites and drugs found in human plasma. Appendix I contains a list of 28 compounds tested that do not interfere with the assay.

Despite best efforts and precautions, some patients' plasma will still exhibit interferences that may obscure the catecholamines. Some of these interferences could be severe and there may be no recourse but to obtain another specimen from the patient to determine if the interferences were transient.

Section 6. Chromatographic Results

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

Figure 1. Aqueous Standard Solution for LC System Check

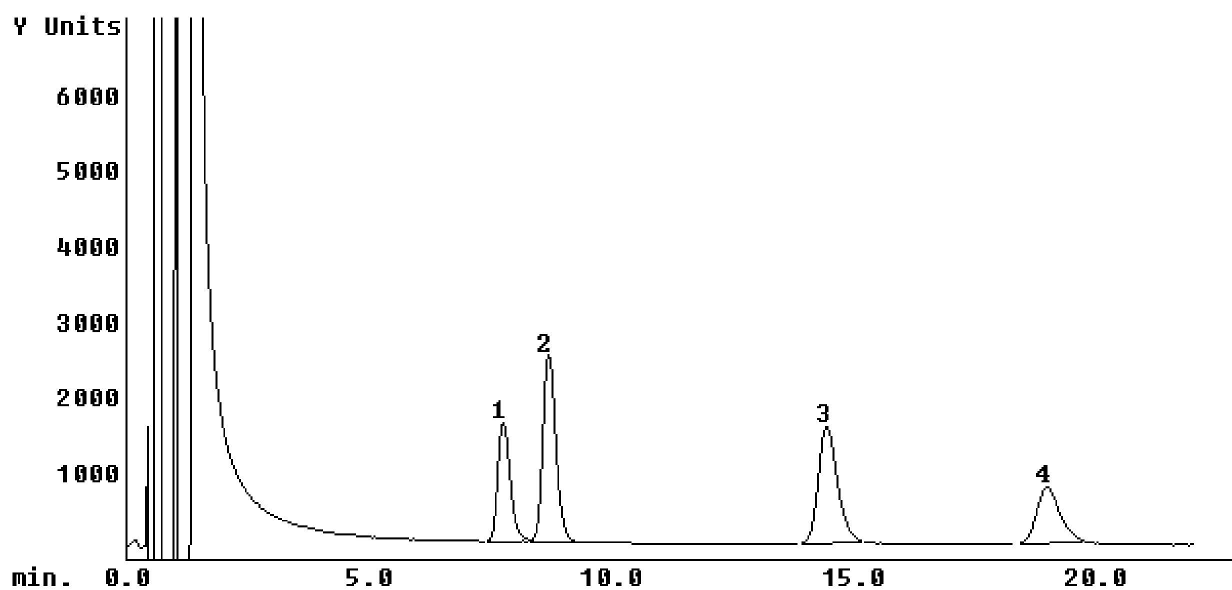


Figure 2. Extracted Plasma Sample within Normal Limits

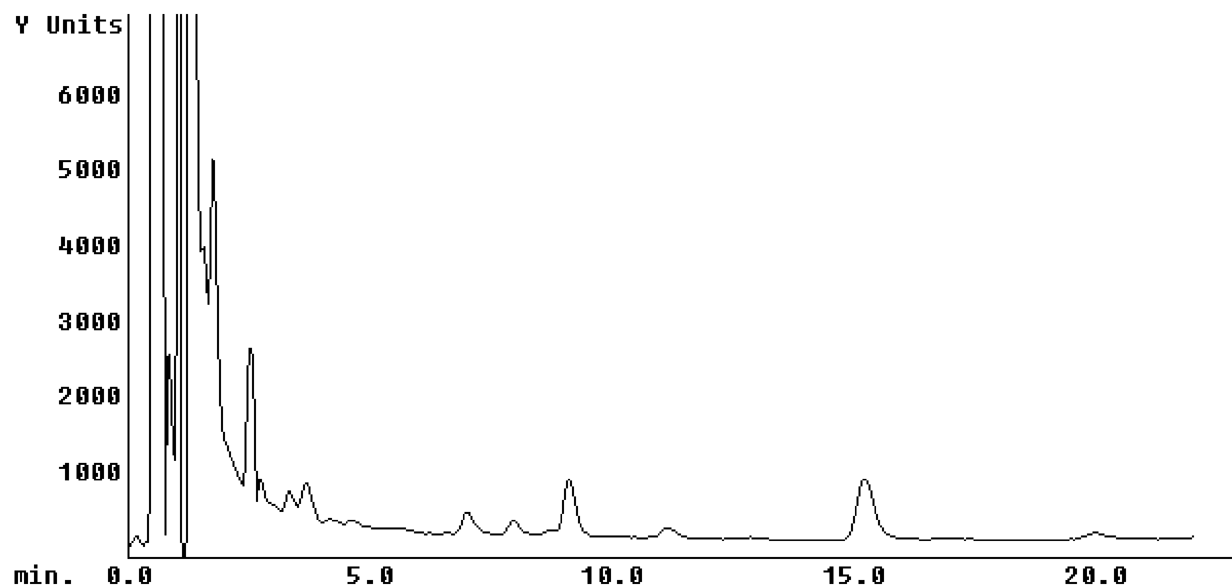
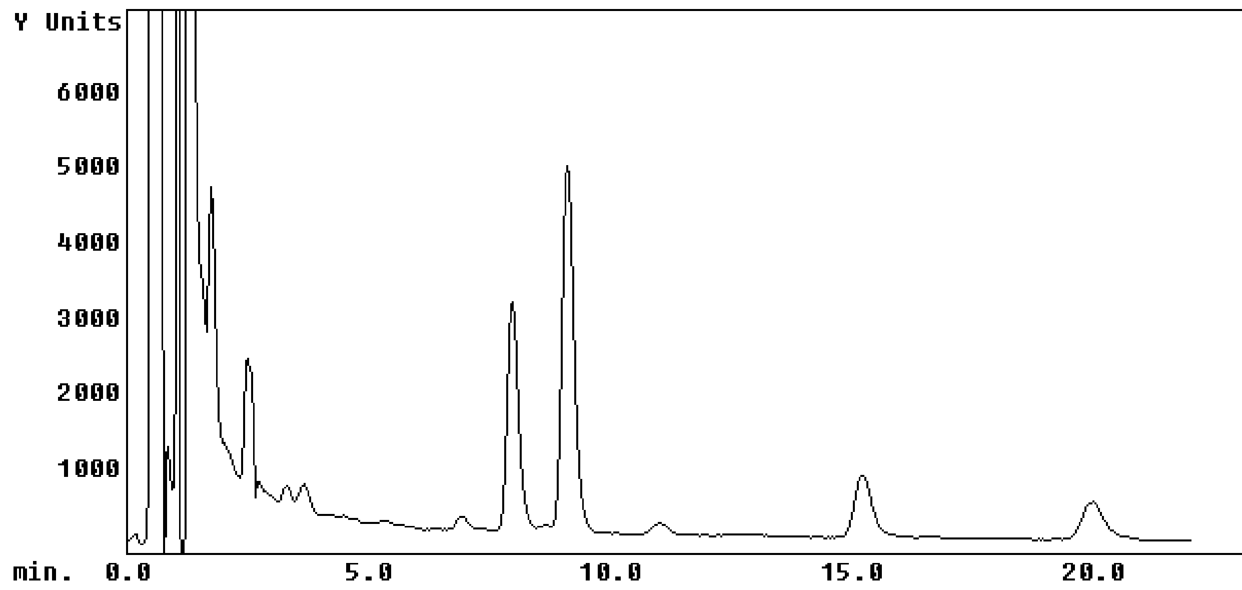


Figure 3. Spiked “Abnormal” Sample



Section 7. Resources

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4. *1991 Test Catalog*, MAYO Medical Laboratories, 264.

Suggested Readings

1. N.W. Tietz, ed., *Fundamentals of Clinical Chemistry*, W.B. Saunders Co. (1987).

Appendix I. Retention Times: Catecholamines & Possible Interfering Compounds

Compound	CAS Registry Number	Retention Time (corrected)
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
α–MethylDOPA	555-29-3	1.69
Epinephrine	**51-43-4**	**7.21**
Norepinephrine	**138-65-8**	**8.46**
DHBA	**16290-26-9**	**14.62**
Isoproterenol	51-31-0	16.40
Salsolinol	70681-20-8	18.10
Dopamine	62-31-7	19.37
N-Methyldopamine	62-32-8	35.68
Isoetharine	7279-75-6	36.06
4-O-Methyldopamine	645-33-0	36.85
Metanephrine	881-95-8	No Peak Detected
Normetanephrine	1011-74-1	No Peak Detected
3-Methoxytyramine	1477-68-5	No Peak Detected
Chloramphenicol	56-75-7	No Peak Detected
Sodium Salicylate	54-21-7	No Peak Detected
5,5-Diphenolhydantoin	57-41-0	No Peak Detected
Theophylline	58-55-9	No Peak Detected
Caffeine	58-08-2	No Peak Detected
Diazepam	439-14-5	No Peak Detected
Labetalol	32780-64-6	No Peak Detected
5-HIAA	54-16-0	No Peak Detected
Metoclopramide	7232-21-5	No Peak Detected
Mandelamine	587-23-5	No Peak Detected