



Urinary Metanephrine Kit

February, 1997

MF-9076

INSTRUCTION MANUAL

For In Vitro Diagnostic Use

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MANUFACTURER'S NOTE

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Section 1. SUMMARY AND EXPLANATION

The Urinary Metanephrine Kit is designed for the in vitro determination of metanephrines, specifically metanephrine, normetanephrine and 3-methoxytyramine in human urine.

The clinical significance of catecholamines and their metabolites is well documented. Beer *et al.* (1) postulated the relationship between catecholamine levels and pheochromocytoma as early as 1937 (1). Metanephrine and normetanephrine are metabolites of epinephrine and norepinephrine, respectively. Most patients who exhibit hypertension are screened for urinary metanephrine (M) and normetanephrine (NM), collectively referred to as "metanephrines" 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 urinary metanephrines 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 screening for a pheochromocytoma is the determination of metanephrines in a 24-hour urine sample. As the metanephrines are found in both free and conjugated states, the urine sample must be acid hydrolyzed prior to the determination of the total metanephrine concentration. Urinary metanephrines are better discriminators of essential hypertension and pheochromocytoma than urinary catecholamines, having a higher sensitivity and specificity than urinary catecholamines.

Liquid chromatography combined with electrochemical detection is considered the method of choice for the determination of total urinary metanephrines (3). This procedure has allowed researchers and clinicians to successfully separate and quantitate urinary metanephrines and 3-methoxytyramine, a metabolite of dopamine, 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 Metanephrine Kit is optimized for the analysis of metanephrine (M), normetanephrine (NM), and 3-methoxytyramine (3-MT). This kit has the added advantage of eliminating almost all interferences in the chromatogram. Using our proprietary chemistry, we have successfully eliminated any potential interferences from 27 of the most common precursors, metabolites, and drugs usually found in human urine. (See Appendix I for a list of the compounds tested.) The resulting chromatogram is clean with 100% resolved peaks for all analytes of interest (see Figures 1, 2, and 3: Results and Discussion section). The kit 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.

An additional advantage is that the same LC hardware can be converted to the analysis of urinary catecholamines in as little as one hour, using the BAS Urinary Catecholamine Kit, by simply equilibrating the system with the urinary catecholamine mobile phase.

Section 2. PRINCIPLES OF THE PROCEDURE

Aliquots (2.0 mL) of urine are combined with 2.0 mL of deionized water and 50 μ L of BAS Metanephrines Internal Standard. This mixture is adjusted to a pH of 1.25 - 1.05 with 1.0 M HCl and placed in a water bath at 90 to 95° C for 30 to 60 minutes. The sample is then removed from the water bath and 2 mL of Reagent A are added. The pH is adjusted to within the range of 6 - 7 using 1.0 M HCl or 1.0 M NaOH.

The assay utilizes Solid Phase Extraction (SPE) columns and anion exchange resin columns to extract the metanephrines. The SPE columns are prepared by wetting the columns with 2 mL of methanol followed by 2 mL of deionized water. Each hydrolyzed, pH adjusted sample is then transferred to an extraction column and drawn through by vacuum to waste. The column is washed with 5 mL of deionized water. The metanephrines are eluted with 4 mL of Reagent B.

The quick-snap columns containing the resin are shaken, and the resin is allowed to settle briefly. The tips are snapped off and the columns are allowed to drain. The eluent from each SPE column is then transferred to a resin column. After the sample has drained, the resin is washed with 5 mL of Reagent C and allowed to drain. Finally the metanephrines are eluted with 11 mL of Reagent D.

After mixing, an aliquot of the eluent is then injected into the chromatographic system by overfilling a 50 μ L loop. The metanephrines, 3-MT, and internal standard are separated on a BAS MF-6213-CL column. The separation of the metanephrines is achieved in under 15 minutes. Amperometric detection occurs at a glassy carbon electrode with an applied potential of +800 mV relative to a Ag/AgCl reference electrode.

2.1 CAUTIONS

Acetic acid, hydrochloric 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 3. COMPONENTS**Urinary Metanephrine Kit, 100 Samples****MF-9022**

<u>Quantity</u>	<u>Description</u>
1 pkg.	Metanephrines Standards Kit. Store in sealed vials in a dark location.
2 bottles	MP-3 Urinary Metanephrine Mobile Phase, one (1) liter/bottle. Store at room temperature.
1 bottle	1.0 M Acetic Acid, 100 mL. Used to adjust pH of reagents. Store at room temperature.
1 bottle	1.0 M NaOH, 100 mL. Used to adjust pH of reagents. Store at room temperature.
1 bottle	Reagent A. 250 mL/bottle. Store at room temperature.
1 bottle	Reagent B. 500 mL/bottle. Store at room temperature.
1 bottle	Reagent C. Empty. To be prepared as described in assay procedure. Store at 2-8° C upon preparation.
1 bottle	Reagent D. Solid, (23.13± .01 g). To be prepared as described in assay procedure. Store at 2-8° C upon reconstitution.
1 bottle	BAS Metanephrine Internal Standard, 10 mL/bottle. Store at room temperature.
1 ea.	Metanephrine Cartridge Column. Store at room temperature. To be used only with MP-1, MP-2, or MP-3.
1 ea.	10 cm Cartridge Column Holder.
100 ea.	Solid Phase Extraction (SPE) Columns. Store at room temperature.
100 ea.	Resin Columns. Store at room temperature.
1 ea.	Urinary Metanephrine Manual.

Urinary Metanephrine Replacement Kit, 100 Samples MF-9023

<u>Quantity</u>	<u>Description</u>
2 bottles	MP-3 Urinary Metanephrine Mobile Phase, one (1) liter/bottle. Store at room temperature.
1 bottle	1.0 M Acetic Acid, 100 mL. Used to adjust pH of reagents. Store at room temperature.
1 bottle	1.0 M NaOH, 100 mL. Used to adjust pH of reagents. Store at room temperature.
1 bottle	Reagent A. 250 mL/bottle. Store at room temperature.
1 bottle	Reagent B. 500 mL/bottle. Store at room temperature.
1 bottle	Reagent C. Empty To be prepared as described in assay procedure. Store at 2-8° C upon preparation.
1 bottle	Reagent D. Solid, (23.13± .01 g). To be prepared as described in stock reagents. Store at 2-8° C upon reconstitution.
1 bottle	BAS Metanephrine Internal Standard, 10 mL/bottle. Store at room temperature.
100 ea.	Solid Phase Extraction (SPE) Columns. Store at room temperature.
100 ea.	Resin Columns. Store at room temperature.
1 ea.	Urinary Metanephrine Manual.

Section 4. ADDITIONAL MATERIALS REQUIRED

- Mechanical Pipettors
- Class A volumetric glassware
- 13 x 100 mm glass straight wall test tubes
- 16 x 125 mm glass test tubes with caps
- 13 x 100 mm glass screw cap test tubes
- Glass scintillation vials
- 1 L graduated cylinder
- 4 x 10 position plastic test tube rack
- 6 x 12 position plastic test tube rack
- Permanent markers
- Vacuum manifold
- Vacuum pump or aspirator with trap
- pH meter with a probe capable of fitting into a 13 x 100 mm test tube
- Mixer
- Analytical balance (0.1 mg)
- Top-loading balance (0.01 g)
- Water bath capable of maintaining 90-95° C

Section 5. STOCK REAGENTS

Deionized water (DI H ₂ O)	Type 1 reagent grade water (ASTM) or HPLC grade water.
1.0 M HCl	Reagent grade.
MP-3 Metanephrine Mobile Phase	See label for shelf life and storage.
Reagent A	See label for shelf life and storage.
Reagent B	See label for shelf life and storage.
Reagent C	Prepared from Reagent D. See label for shelf life and storage.
Reagent D	See label for shelf life and storage.
BAS Internal Standard	See label for shelf life and storage.
1.0 M Acetic Acid	See label for shelf life and storage.
1.0 M NaOH	See label for shelf life and storage.

Section 6. SPECIMEN COLLECTION, PRESERVATION AND STORAGE

Acidified Urine. Urine used in this assay is from a 24-hour collection. The 24-hour sample for metanephrines 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 STANDARDS

Metanephrine (M), Normetanephrine (NM), and 3-Methoxytyramine (3-MT) standard working solution.

Accurately weigh 29.62 ± 0.10 mg metanephrine-HCl, 44.96 ± 0.10 mg normetanephrine-HCl, and 30.45 ± 0.10 mg 3-methoxytyramine-HCl. Transfer quantitatively into a single 25.0 mL volumetric flask and dilute to 25.0 mL with 0.5 M HCl. Mix well. Sonicate if necessary. Transfer 6.00 mL of the resulting 1 mg/mL M, 1.5 mg/mL NM, and 1 mg/mL 3-MT solution to a 100.0 mL volumetric flask. Dilute to 100.0 mL with 0.5 M HCl and mix well to assure homogeneity. This standard working solution is 60.0 $\mu\text{g/mL}$ M, 90.0 $\mu\text{g/mL}$ NM, and 60.0 $\mu\text{g/mL}$ 3-MT. This solution can be stored in 0.5 mL aliquots at -80°C for up to six months.

7.2 PREPARATION OF CALIBRATION STANDARDS

When extracted samples are used for the purpose of calibration, acid-stabilized urine is spiked and serially diluted on the day of assay and used directly as shown below.

Preparation of Multi-Point Calibration Standards Acidified Urine

Spiked Conc. of Metanephrines in $\mu\text{g/mL}$	Volume of Unspiked Urine	Spiking Solution and/or Volume of Spiked Urine Added
2400, M and 3-MT 3600, NM	9.6 mL	400 μL of the M, NM 3-MT standard working solution
1200, M and 3-MT 1800, NM	5.0 mL	5 mL of the 2400 ng/mL M urine pool
600, M and 3-MT 900, NM	5.0 mL	5 mL of the 1200 ng/mL M urine pool
300, M and 3-MT 450, NM	5.0 mL	5 mL of the 600 ng/mL M urine pool
150, M and 3-MT 225, NM	5.0 mL	5 mL of the 300 ng/mL M urine pool
75, M and 3-MT 112.5, NM	5.0 mL	5 mL of the 150 ng/mL M urine pool

7.3 CALIBRATION SCHEMES

Different calibration schemes can be used depending on the preference of the user and the availability of a matrix to be used for the calibration. A multi-point calibration line can be used as defined in the calibration 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 II.

A single point calibration scheme can be used if a urine standard of known metanephrine concentration is available. There are lyophilized urines of known metanephrine concentration on the market. The user can pool urine samples from several individuals to create a single point calibration matrix and determine the 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 a y-intercept. The absolute value of the x-intercept can be calculated using the equation:

$$x = \left| -b/m \right|$$

where: b = y intercept
m = slope

The value for x is the concentration of the unspiked single point pool. Extraction of the unspiked pool whose metanephrine concentrations are calculated using Method 1 in Appendix II should confirm this.

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

7.4 PREPARATION OF POOLED QUALITY CONTROL SAMPLES

Separately prepared M, NM, 3-MT stock and working solutions are used for the pooled quality control (QC) samples as follows: Accurately weigh 29.62 ± 0.10 mg metanephrine-HCl, 44.96 ± 0.10 mg normetanephrine-HCl, and 51.77 ± 0.10 mg 3-methoxytyramine-HCl. Transfer quantitatively into a 25.0 mL volumetric flask and dilute to volume using 0.5 M HCl. Mix well. Transfer 3 mL of this 1 mg/mL M, 1.5 mg/mL NM, 1.7 mg/mL 3-MT stock solution to a 100 mL volumetric flask and dilute to volume with 0.5 M HCl. The final concentration of this working solution is 30 $\mu\text{g/mL}$ M, 45 $\mu\text{g/mL}$ NM, and 51 $\mu\text{g/mL}$ 3-MT.

Low QC Pool

Urine collections can be combined into one pool and used as the source matrix for all the QC pools. This pool should be properly acidified as indicated in Section 6. This combined pool can be used directly as the low QC pool. It contains only endogenous levels of metanephrines; i.e. no spike is added. 500 mL can be reserved for the low QC pool.

Middle QC Pool

Pipette 1.5 mL (glass, class A) of the working solution into a 500 mL volumetric flask and dilute to the mark with the urine pool. Mix well. This middle QC pool represents an added metanephrine concentration of 90 ng/mL M, 135 ng/mL NM, and 153 ng/mL 3-MT.

High QC Pool

Pipette 12.5 mL (glass, class A) of the working solution into a 500 mL volumetric flask and dilute to the mark with urine pool. Mix well. This high QC pool represents an added metanephrine concentration of 750 ng/mL M, 1125 ng/mL NM, and 1275 ng/mL 3-MT.

These QC pools can be dispensed in convenient aliquots, sealed, frozen, and used for at least one month.

7.5 PREPARATION OF REAGENTS D, C AND HCl SOLUTIONS

Preparation of Reagent D

Dissolve Reagent D in 900 mL deionized water. Adjust to pH 6.5 using 1.0 M acetic acid or NaOH. Dilute to 1500 mL with DI H₂O. After preparation of Reagent C, store 1150 mL of Reagent D in the Reagent D bottle. Discard excess Reagent D. Store at 4° C.

Preparation of Reagent C

Add 10 mL of Reagent D to 900 mL of deionized water. Adjust to pH 6.5 using 1.0 M acetic acid or NaOH. Dilute to one (1) liter with DI H₂O. Store at 4° C.

Preparation of 1.0 M Hydrochloric Acid (HCl)

Add 10 mL of concentrated HCl to 106 mL of water. Store at room temperature.

Section 8. ASSAY PROCEDURE

1. All samples to be assayed should be brought to room temperature. If used, reconstitute lyophilized standards and controls according to the manufacturer's instructions.
2. Label one 13 x 100 mm screw cap test tube, one 13 x 100 mm straight wall test tube, and one 16 x 125 mm test tube per sample.
3. Label and prepare one resin column for each sample by shaking until the resin is completely suspended. Place the columns in a rack and allow the resin to settle.
4. Vortex each sample to ensure homogeneity and transfer 2.0 mL aliquots of each sample to a labeled 13 x 100 mm screw cap test tube.
5. With a mechanical pipette, transfer 50 μ L of internal standard working solution to each test tube.
6. Pipette 2.0 mL of deionized water to each test tube and vortex.
7. With a calibrated pH meter, adjust the pH of each sample to 1.05-1.25 using 1.0 M HCl. Rinse the pH electrode between each sample. Cap each test tube after the sample pH has been adjusted.
8. Hydrolyze each sample in a water bath set at 90 to 95° C for 30 to 60 minutes.
9. Remove the samples from the bath and allow them to cool.
10. Uncap the test tubes. Add 2.0 mL of Reagent A to each sample and vortex.
11. With a calibrated pH meter, adjust the pH of each sample to 6.0 - 7.0 using 1.0 M HCl or 1.0 M NaOH if necessary. Rinse the pH electrode between each sample.
12. Affix the SPE columns to a vacuum manifold.
13. Pipette 2 mL of methanol into the SPE columns and apply the vacuum to draw the solvent through to waste. **(Note: It is imperative over the course of the extraction that the column beds are not dried out by drawing air through them.)**
14. Pipette 2 mL of deionized water into the SPE columns and apply the vacuum to draw it through to waste.
15. Transfer the pH-adjusted urine samples into the SPE columns and apply a vacuum to draw the urines through to waste. Flow rates should not exceed 2 mL/min.

16. Wash the SPE columns with 5 mL of deionized water. Draw the wash through to waste.
17. Place the labeled 13 x 100 straight wall test tubes in the vacuum manifold. Pipette 4 mL of Reagent B into the SPE columns, apply the vacuum, and collect the eluent.
18. Remove the test tubes from the vacuum manifold and vortex briefly.
19. Remove the caps from the resin columns (from step 3), then snap off their tips. Allow them to drain completely.
20. Transfer the eluent from the SPE column to the resin column. Allow to drain completely.
21. Wash the resin column with 5.0 mL of Reagent C, allowing it to drain completely.
22. Place each resin column into a labeled 16 x 125 mm glass test tube.
23. Elute the metanephrines into the glass test tubes from the resin column with 11 mL of Reagent D. Allow to drain completely.
24. Cap the test tubes containing the eluent from the resin columns, and mix well.

Optional Procedure Use with Autosamplers

25. Transfer portions of the eluent from step 24 to autosampler vials and inject. It is recommended that the vials be capped.

**8.1 INSTRUMENTATION
AND CHROMATOGRAPHIC
CONDITIONS**

Column: BAS Metanephrine column, Part # MF-6213-CL

Mobile Phase: BAS MP-3 Metanephrine Mobile Phase

Detector: BAS LC-4C Amperometric Detector with dual glassy carbon electrodes in series or equivalent. The applied potential is set at +800 mV for the upstream electrode relative to a Ag/AgCl reference electrode. Recommended gain is set at +50 nA full scale.

Chromatograph: BAS 200B, BAS 480 or equivalent

Flow rate: 1.1 mL/min.

Autosampler: BAS Sample Sentinel or equivalent

Loop volume: 50 μ L

Run time: 15 minutes

Column temperature: 40° C

Recorder: BAS RYT-DP or equivalent

Data Reduction: BAS ChromGraph or equivalent

Approximate Retention times: M, 4-5 minutes
NM, 4-6 minutes
3-MT, 9-11 minutes
BAS-IS, 11-13 minutes

Section 9. LIQUID CHROMATOGRAPH, INITIAL SETUP

1. Connect the Urinary Metanephrine Column (MF-6213-CL) to the system.
2. Set LC pump to a flow rate of 1.1 mL/min.
3. Pump approximately 50 mL of MP-3 mobile phase through the column to remove any trapped air bubbles from the column. **(Warning: Never Wash the Metanephrine Column with Solvent. Use Only MP-3)**
4. Connect the column to the 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 the electrochemical detector to STANDBY. Turn on power. Set potential and gain. Turn the mode switch to CELL.
7. Allow system to equilibrate for approximately one hour.
8. The pressure should be 3200 to 4000 psi. The system pressure should be stable. **(Warning: 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 either troubleshoot the pressure problem or replace the column or both.)**
9. The background current should not exceed 10 nA. **(Note: You can obtain useful data at a background higher than 10 nA, but if the system exceeds 10 nA, you need to 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-3 mobile phase reservoir and recirculate the mobile phase at 0.1 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 manual for shutdown procedures.

9.2 LINEARITY

Calibration curve data and linear regression parameters from three (3) 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 from 75 to 2400 ng/mL for M and 3-MT, and 112.5 to 3600 ng/mL for NM. Correlation coefficient (r) were all greater than 0.999.

9.3 ACCURACY

The accuracy of the method was assessed by subtracting the low QC mean concentration from the respective QC mean concentration (either medium or high) and then dividing by the spike value of that QC. This calculated value is the % recovery determined in these experiments. These % recoveries were then averaged for the medium and high QCs and reported as the interday % recoveries. These were 96.9% for metanephrine, 97.5% for normetanephrine, and 95.2% for 3-methoxytyramine.

9.4 PRECISION

The within-day precision of the method was determined from the relative standard deviations (RSD) of 3-6 replicate analyses of the three pooled quality control levels. This parameter was determined for each of the three different analytical runs. The interday precision of the method was determined from the RSDs of the mean of the three control pools over the three analytical runs.

Within-Day Precision			
	Low QC	Medium QC	High QC
Metanephrine	3.7-6.3% 68.9 ng/m	2.9-5.1% 151 ng/mL	1.4-1.9% 802 ng/mL
Normetanephrine	2.3-4.5% 167 ng/mL	2.9-5.5% 301 ng/mL	1.0-1.9% 1255 ng/mL
3-Methoxytyramine	0.5-4.5% 92.7 ng/mL	2.2-3.7% 239 ng/mL	0.7-2.0% 1326 ng/mL

Interday Precision 7 days			
	Low QC	Medium QC	High QC
Metanephrine	5.3% 68.9 ng/m	4.9% 151 ng/mL	2.7% 802 ng/mL
Normetanephrine	3.4% 167 ng/mL	4.9% 301 ng/mL	2.5% 1255 ng/mL
3-Methoxytyramine	6.7% 92.7 ng/mL	5.7% 239 ng/mL	3.1% 1326 ng/mL

9.5 LIMIT OF DETECTION

A standard solution of the metanephrines was injected on the LC system at a gain of 50 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 assayed sample concentration of 10 ng/mL for metanephrine, normetanephrine, and 3-methoxytyramine. Thus, assay limit of detection (ALOD) is a concentration of 10 ng/mL, since we are recommending a gain setting of 50 nA full scale for the assay. Obviously, at more sensitive gain setting the (ALOD) would increase substantially.

9.6 EXPECTED VALUES

Normal Metanephrine Ranges $\mu\text{mol/mol}$ creatinine*, mean S.D. (4)			
Age Range, yr.	2-6 years	6-13 years	20-50 years
Metanephrine	204 32	109 29	73.2 19.2
Normetanephrine	204 41	93.5 8.5	85.2 38.9
3-Methoxytyramine	183 30	88.5 18.5	59.6 15.5

*Some laboratories prefer to report concentrations for each metanephrine/gm or mole creatinine. In this case, the operator should determine the total creatinine concentration in the 24-hour sample and normalize each metanephrine concentration to creatinine.

9.7 CORRELATION DATA

Mean Concentration for All Samples Assayed in ng/mL							
		Metanephrine		Normetanephrine		3-Methoxytyramine	
		BAS	CAD	BAS	CAD	BAS	CAD
# of Samples		137	137	136	136	135	135
Range	High	2132	2362	3275	3614	1488	1342
	Low	16	21	65	49	33	38
Mean		274	316	513	457	308	303
Std. Deviation		410	498	631	583	389	
Cor. Coef. (R value)		0.9867		0.9264		0.9813*	
Y Intercept		0.009641		0.02773		0.2351	
Slope		1.0159		0.9619		0.9546	

*Correlation Coefficient is based on the sample for sample regression of the $\ln[\text{concentration}]$. BAS = BAS procedure. CAD = Commercially available device.

9.8 LIMITATIONS

Chromatographic interference may occur from precursors, metabolites, and drugs found in human urine. Appendix I contains a list of 27 compounds tested that do not interfere with the assay.

Section 10. RESULTS AND DISCUSSION

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

The results of testing the most commonly encountered interfering compounds can be seen in Appendix I. Of the 27 different compounds tested, none interfered with the assay.

Figure 1. BAS System Check

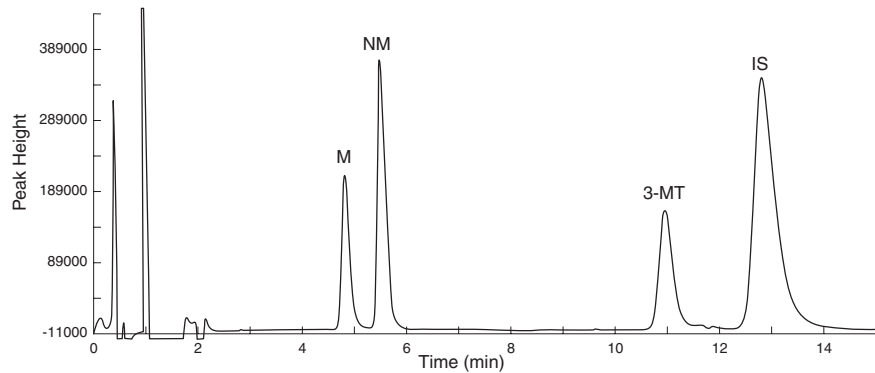


Figure 2. Normal Sample Using BAS Procedure in Full Scale

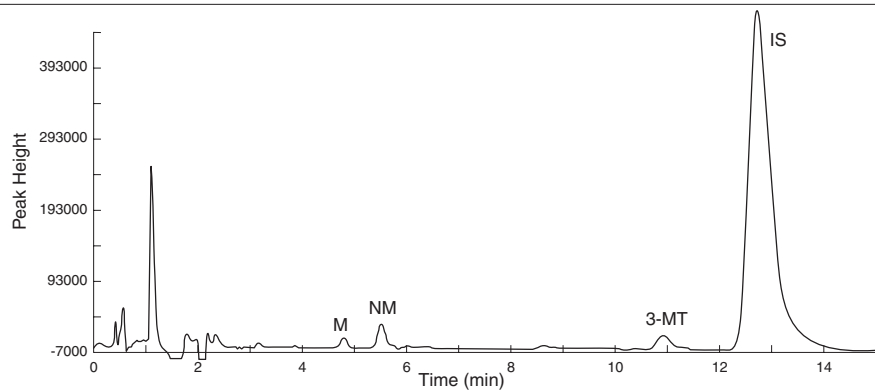
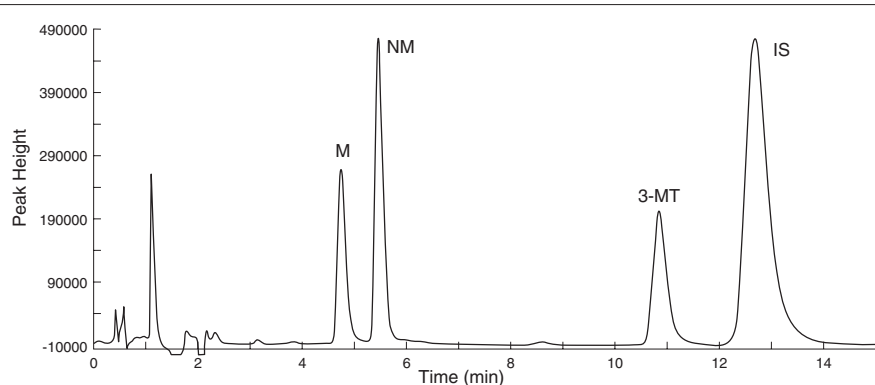


Figure 3. Abnormal Sample Using BAS Procedure



Section 11. REFERENCES AND SUGGESTED READING

REFERENCES

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2. T. G. Rosano, T. A. Swift and L. W. Hayes, *Clin. Chem.* 10B, (1991) 1859.
3. R. E. Shoup and P. T. Kissinger, *Clin. Chem.* 23, (1977) 1268.
4. J. Jouve, *et. al.* *J. Chrom.*, 274, (1983) 53.

SUGGESTED READING

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

Appendix I: COMPOUNDS TESTED**Table 1. Retention times of metanephrines and possible interfering compounds.**

Compound	CAS Registry Number	Retention Time
MHPG	[67423-45-4]	0.60
DOMA	[14883-87-5]	0.62, 0.81, 12.67**
VMA	[2394-20-9]	0.63
Acetaminophen	[103-90-2]	0.84
L-DOPA	[59-92-7]	0.95, 6.39
DOPAC	[120-32-9]	1.20, 1.45
HVA	[306-08-1]	1.28, 1.62
5-HIAA	[66866-39-5]	1.38, 1.62
Epinephrine	[51-43-4]	3.83
Norepinephrine	[138-65-8]	4.29**
-MethylDOPA	[555-29-3]	4.45 12.43**
Metanephrine	[881-95-8]	4.57
Normetanephrine	[1011-74-1]	5.18
N-Methyldopamine	[62-32-8]	6.74
Isoproterenol	[51-31-0]	6.89
Dopamine	[62-31-7]	8.11
Salsolinol	[70681-20-8]	8.25
3-Methoxytyramine	[1477-68-5]	10.29
BAS internal standard	NA	12.60
Isoetharine	[7279-75-6]	13.81
Serotonin	[61-47-2]	21.49
Cimetidine	[5148-61-9]	*
Chloramphenicol	[56-75-7]	*
Sodium Salicylate	[54-21-7]	*
5,5-Diphenolhydantoin	[57-41-0]	*
Theophylline	[58-55-9]	*
Caffeine	[58-08-2]	*
Diazepam	[439-14-5]	*
Labetalol	[32780-64-6]	*
Metoclopramide	[7232-21-5]	*
Mandelamine	[587-23-5]	*

* No Peak Detected

** No Peak Detected After Extraction

Appendix II: QUANTITATIVE CALCULATIONS

CALIBRATION METHOD 1: When serially diluted, spiked extracts were used for calibration purposes. Peak height ratios of the metanephrines/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 metanephrine in sample
y = peak height ratio of metanephrine 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 metanephrines in the urine used for the calibration line.

CALIBRATION METHOD 2: When a single calibration scheme was used, the metanephrine concentrations were calculated in the following manner: a spiked urine of known metanephrine concentration was extracted with the other samples. Peak height ratios of the endogenous metanephrine/internal standard were calculated. All concentrations were calculated as:

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

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