Application Note 66

Simple Solid Phase Extraction and HPLC Analysis of Catecholamines in Plasma

A sample cleanup procedure for catecholamines, using a disposable Supelclean LC-WCX SPE tube, takes only 20 minutes. Absolute recovery of an internal standard can be as high as 90%. Relative recoveries for pg amounts of plasma catecholamines can range from 96-106%. Subsequent analyses using ECD and SUPELCOSIL LC-18-DB columns are rapid and sensitive to as little as 20pg of catecholamine.

Key Words:
• catecholamines • plasma • epinephrine • norepinephrine

Several methods have been developed to measure blood levels of the catecholamines, epinephrine and norepinephrine. The preferred method, due to favorable sensitivity, analysis time, and cost, is HPLC with electrochemical detection. However, the many potentially interfering components of human plasma make even this analytical method difficult.

The most widely used sample cleanup procedure involves extracting catecholamines from plasma on alumina. This process takes 30 minutes to an hour or more, and absolute recovery rates are typically below 70% (1,2). Modifications of the procedure yield cleaner extracts, but do not improve recovery rates (3).

A sample cleanup procedure we have developed requires a single, disposable Supelclean™ LC-WCX solid phase extraction (SPE) tube and takes only 15 minutes. Absolute recovery of the internal standard is greater than 90%. Relative recoveries for picogram amounts of plasma catecholamines can be greater than 90%. The subsequent analysis using electrochemical detection and a SUPELCOSIL™ LC-18-DB HPLC column is rapid and sensitive to as little as 20pg of catecholamine. Because catecholamines normally are present in very small amounts, sharp, symmetrical peaks are especially important for quantifying these compounds. The catecholamines' basic nature, on the other hand, makes them difficult to elute as symmetrical peaks from conventional reversed phase columns. SUPELCOSIL LC-18-DB columns are deactivated specifically for analyses of basic compounds. They ensure sharp catecholamine peaks for the most reliable quantification.

Samples containing catecholamines were analyzed on a SUPELCOSIL LC-18-DB column and detected with an electrochemical detector. Plasma levels in some samples were enhanced by adding known amounts of epinephrine and norepinephrine, to simulate values within the normal range or elevated levels associated with certain neural and endocrine disorders (Figure A).

Figure A. Catecholamines in Human Plasma, Using SPE, HPLC, and Electrochemical Detection

Column: **SUPELCOSIL LC-18-DB, 15cm x 4.6mm, 5µm particle**
Cat. No.: **58348**
Mobile Phase: 0.025M citric acid, 0.025M Na_2HPO_4, 0.005mM Na_2EDTA, 34mg/L octanesulfonic acid (Na salt), pH to 3.4 with 85% H_3PO_4
Flow Rate: 1.5mL/min., 1205psig
Temp.: ambient
Det.: electrochemical (oxidative mode, applied potential: +650mV, range: 1.0nA, filter: 0.1Hz)
Inj.: 100µL 0.2M $HClO_4$ containing catecholamines extracted from human blood plasma, plus added catecholamines as listed (480pg dihydroxybenzylamine added to each sample as internal standard) or 100µL unextracted plasma.
Supelguard LC-18-DB guard column, 2cm x 4.6mm, 5µm particle

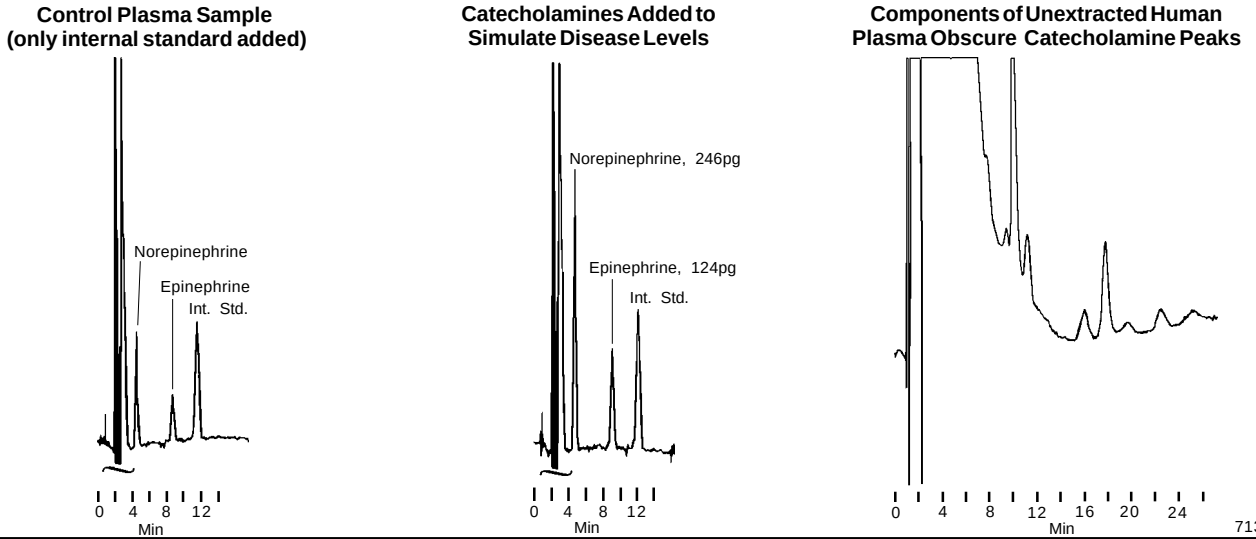

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Table 1. SPE Provides Consistent, High Recovery of Catecholamines Added to Human Plasma

Catecholamine Level	Relative Recovery (%) Trial 1	Trial 2	Trial 3	Mean ± SD
Normal Plasma Level				
Norepinephrine (410pg/mL)	92.8	102	95.9	96.9 ± 4.7
Epinephrine (206pg/mL)	95.8	89.5	108	97.8 ± 9.4
Simulated Disease Level				
Norepinephrine (1230pg/mL)	110	100	109	106 ± 5.5
Epinephrine (618pg/mL)	98.1	90.7	102	96.9 ± 5.7

SPE Tube: Supelclean LC-WCX (1mL tube)

Tube Conditioning Solution: 500µL 0.5N hydrochloric acid in water (remove excess acid with 1mL water)

Sample Addition: Mix 500µL plasma with 500µL water, pass sample through tube at 250µL/min.

Tube Wash Procedure: two 1mL aliquots of water, then add internal standard (144pg in 30µL water) and 250µL water at a slow, dropwise rate

Elution Solution: 250µL 0.2M perchloric acid in water (elute at a slow dropwise rate, collect eluate in a silanized glass vial)

Under the conditions we used, the detection limit for a 2:1 signal:noise ratio was 20pg on column for both epinephrine and norepinephrine. Figure A also shows that the extraction process effectively separates plasma components from the catecholamines.

Catecholamine recovery rates, determined by the method of standard addition at low and high levels of introduction, are summarized in Table 1. Recovery from the proprietary SPE packing was significantly better than with alumina.

Several other factors must be considered for successful analysis of catecholamines. First, all glassware must be silanized.◆ Catecholamines bind irreversibly to untreated borosilicate glass.

Second, fresh, chilled heparinized plasma must be used. A very late eluting peak (about 90 minutes) appears if the plasma is stored for as little as one day. This peak could interfere with subsequent analyses.

Third, freshly purified water must be used in preparing the standards and mobile phase, and in the extraction process. After only a few days, bacteria will begin to grow in water stored in polyethylene containers. Bottled HPLC grade water can also contain bacteria. This water should be distilled and filtered before being used in catecholamines analyses.

Fourth, best results are obtained when a dual electrode electrochemical detector is used. These detectors provide better sensitivity than single electrode detectors. An applied potential of +650mV was optimum for detection in this analysis. At higher potentials, noise was increased relative to the response for catecholamines. Mobile phase components were oxidized, causing evolution of gas bubbles in the detector cell. These bubbles produced large noise peaks on the chromatograms.

Fifth, the citric acid/disodium phosphate mobile phase (4), of all mobile phases tested, gave the cleanest chromatograms in the shortest analysis time. The absence of organic solvent makes this mobile phase compatible with both glassy carbon and carbon paste electrodes in electrochemical detectors.

And last, the analytical column will last longer if it is protected from any traces of serum components in the extracted samples. Supelguard™ LC-18-DB guard columns contain the same packing as the analytical column, thus increasing the efficiency of the analysis while prolonging the lifespan of the analytical column. Our Supelguard column kits contain one of these disposable 2cm cartridge-type columns, a column holder, and the fittings needed to connect the column holder to 1/16" tubing.

For accurate, rapid, and consistent analyses of catecholamines in human blood plasma, we recommend sample cleanup on Supelclean LC-WCX SPE tubes, coupled with analysis on a SUPELCOSIL LC-18-DB column and electrochemical detection.

A Supelco vacuum manifold proved highly useful in the sample extraction process by enabling us to simultaneously extract as many as 24 samples. For information on the Visiprep™ SPE Vacuum Manifold, request publication 494059.

Ordering Information:

Description	Cat. No.
Supelclean LC-WCX SPE Tubes, 1mL, pk. of 100	**57060-U**
SUPELCOSIL LC-18-DB Column, 15cm x 4.6mm	**58348**
Supelguard LC-18-DB Guard Column Kit	**59555**
Supelguard LC-18-DB Columns, pk. of 2	**59565**
Sylon™ CT, pint	**33065-U**

References

1. Kremer, R., *et al.*, J. Chromatogr. (Biomed. Appins.), *344*, 313 (1985).
2. Gerlo, E. and R. Malfait, J. Chromatogr. (Biomed. Appins.), *343*, 9 (1985).
3. Frayn, K.N. and P.F. Maycock, Clin. Chem., *29*, 1426 (1983).
4. Williams, C.H., *et al.*, J. Chromatogr. (Biomed. Appins.) *344*, 71 (1985).

◆Silanization Procedure:

1. Bring silanizing reagent (Sylon CT) in contact with glass surface for 30 sec.
2. Repeat step 1 with fresh reagent.
3. Rinse surface 2X with toluene.
4. Rinse surface 3X with methanol.
5. Dry glassware with nitrogen, using the Drying Attachment for the Supelco Solid Phase Extraction Vacuum Manifold.

Contact our Technical Service Department (phone 800-359-3041 or 814-359-3041, FAX 800-359-3044 or 814-359-5468) for expert answers to your questions.