



Determination of Epinephrine Concentrations in Human Plasma by High Performance Liquid Chromatography with Tandem Mass Spectrometry

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Overview

A sensitive liquid chromatography tandem mass spectrometry (LC/MS/MS) method has been developed and validated for the quantitative analysis of epinephrine in human plasma containing K₂-EDTA and sodium metabisulfite. The positive ion electrospray method has a quantitation range of 50.0 pg/mL to 2000 pg/mL. Reversed phase chromatography was achieved using a stationary phase that has the ability to withstand 100% aqueous conditions for extended periods. A stable, isotopically labeled internal standard (epinephrine-d₃) facilitated method precision and accuracy. Human plasma was prepared for analysis using phenylboronic acid (PBA) solid phase extraction (SPE). The selectivity using this phase was critical in removing an endogenous interference observed with cation exchange phases. This method has proven to be linear, accurate, precise, selective, and rugged.

Introduction

The objective of this work was to develop a bioanalytical LC/MS/MS method for the quantitation of epinephrine that met FDA guidances set forth in the Guidance for Industry document dated May, 2001. The following challenges were addressed:

- Blank plasma: Plasma used for method blanks, method controls, calibration standards, and quality control samples was screened prior to use to ensure that widely varying endogenous epinephrine levels did not affect method performance.
- Interferences: Interferences were removed during extraction by utilizing covalent interactions between epinephrine and the solid phase (phenylboronic acid). In addition, a secondary epinephrine confirmation ion was monitored to aid in confirmation and troubleshooting.
- Stability: Sodium metabisulfite was added to all plasma samples to reduce epinephrine oxidation.
- Sensitivity: A lower limit of quantitation (LLOQ) of 50.0 pg/mL was achieved.

Methods

Sample Preparation:

- Add internal standard (epinephrine-d₃) to 1.00 mL human plasma containing K₂-EDTA and sodium metabisulfite
- Add methanol, mix, and centrifuge
- Transfer the supernatant to a clean tube and add ammonium hydroxide
- Condition Varian Bond Elut LRC-PBA SPE sorbent
- Load the sample and wash with basic aqueous and organic
- Elute with formic acid in methanol
- Dry the eluent and reconstitute

Sample Analysis:

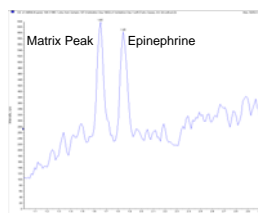
- Column: Phenomenex Synergi Hydro-RP
- Elution: 100% 50 mM ammonium formate in water with 0.1% formic acid
- Detector: Applied Biosystems/MDS Sciex API 4000 MS/MS

Data Analysis:

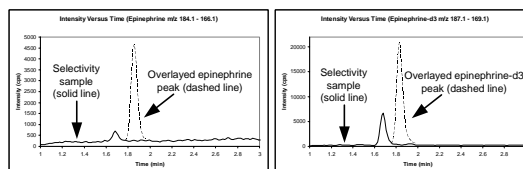
- Weighing: 1/(concentration²)
- Chromatographic Peak Integrations: Applied Biosystems/MDS Sciex Analyst 1.4
- Regression and Analysis: Thermo Watson LIMS 7.1.0.01

Results

Sensitivity at the LLOQ (50.0 pg/mL): Signal to noise at the LLOQ was approximately 9:1. The epinephrine signal baseline was elevated due to low molecular weight noise (i.e. mobile phase contribution).

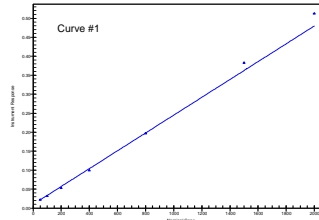


Selectivity: All potential interferences were chromatographically separated from epinephrine (epinephrine R_t = 1.85 minutes). The SPE phase used (PBA) exhibited greater selectivity compared to polymeric and cation exchange phases.



Linearity: The average coefficient of determination (R²) for epinephrine was 0.9967.

Curve #	Slope	Intercept	R-Squared	LLOQ (pg/mL)	ULOQ (pg/mL)
1	0.000234804610	0.0100232396	0.9946	50.0	2000
2	0.000251382782	0.00457080229	0.9959	50.0	2000
3	0.000270103403	0.00011175439	0.9991	50.0	2000
4	0.000220118537	0.00154310384	0.9970	100	2000
Mean	0.000244102333	0.00406210279	0.9967		



Calibration Standard Performance: Two calibration standards were outside of method acceptance criteria and were excluded from the regression. The greatest percent bias was -3.5% and the greatest %CV was 7.4%.

Run #	Std 1 50.0 pg/mL	Std 2 100 pg/mL	Std 3 200 pg/mL	Std 4 400 pg/mL	Std 5 800 pg/mL	Std 6 1500 pg/mL	Std 7 2000 pg/mL
1	52.3	95.3	183	363	736	1550	2140
2	50.6	103	178	369	835	1560	2020
3	48.4	124	209	406	805	1460	1950
4	72.0	102	200	366	806	1510	2000
Mean	50.8	100	193	387	812	1530	2050
S.D.	1.86	4.19	14.2	15.7	16.2	57.2	84.6
%CV	2.9	4.2	7.4	4.1	2.0	3.7	4.1
%Bias	1.6	0.0	-3.5	-3.3	1.5	2.0	2.5
n	3	3	4	4	4	4	4

* This standard was outside of acceptance criteria and was not included in the regression.

Quality Control Sample Performance: Six out of seventy-nine QC samples failed to meet acceptance criteria. Four were at the LLOQ. Method performance was the worst at the LLOQ, exhibiting inter-run bias of -5.8% and %CV of 17.6%.

Parameter	LLOQ QC 50.0 pg/mL	LOW QC 100 pg/mL	MD QC 200 pg/mL	10x QC 2000 pg/mL	10x QC Mean 1600 pg/mL
Mean Concentration Found for Runs 1-4 (pg/mL)	47.1	149	736	1630	1610
Internal SD	8.28	14.7	33.5	105	69.0
Inter-run %CV	17.6	6.9	3.8	6.4	4.3
Inter-run %Bias	-5.8	-0.7	-0.5	1.0	0.6
n	14	20	20	5	20

Recovery: Analyte and internal standard recoveries were precise and were approximately 20% using this method. Greater recoveries were observed during method development using other SPE phases. Unfortunately, these phases were not selective enough for epinephrine.

Epinephrine Stability Determinations: With the addition of sodium metabisulfite, epinephrine was stable in solution, extracts, and in matrix for the stability timeframes and temperatures tested.

Stability Timeframe	%Difference from Fresh
-70°C Solution Stability (5 week, 5 day)	8.2%
-70°C Solution Stability (12 week, 4 day)	6.2%

Stability Experiment	Low-Level QC Mean (pg/mL)	High-Level QC Mean (pg/mL)
3 Cycles Freeze-Thaw Plasma	144 ng/mL (-4.0%)	1580 ng/mL (-1.3%)
24 Hour Room Temperature Plasma	146 ng/mL (-3.3%)	1520 ng/mL (-5.0%)
6 Day Extracts	147 ng/mL (-2.0%)	1520 ng/mL (-5.0%)
12 Week, 4 Day -70°C Plasma	160 ng/mL (6.7%)	1660 ng/mL (6.0%)
30 Week, 4 Day -70°C Plasma	160 ng/mL (6.7%)	1660 ng/mL (6.0%)

Conclusions

This method was successfully validated for the quantitation of epinephrine from 50.0 pg/mL to 2000 pg/mL. It was linear, accurate, precise, selective, and rugged. While method precision at the lower limit of quantitation was not ideal, it met FDA suggested precision requirements for bioanalytical LC/MS/MS methods (%CV ≤ 20%). Following method validation, this method was successfully used to analyze human plasma study samples.