POSTER NOTE

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Evaluation of the Analytical Parameters for Sensitive and Robust Quantitative Analysis of Catecholamines in Human Plasma with LC-MS for Clinical Research

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ABSTRACT

Purpose:

• To develop a sensitive and robust LC-MS method for analysis of catecholamines (epinephrine, norepinephrine and dopamine) in plasma.

•Evaluate calibration standards prepared in solvent (with and without SPE processing) and in plasma to support instances where analyte-free plasma is not available.

•Characterize method performance using RECIPE Quality Control (QC) samples and in-house prepared spiked donor plasma samples

Methods: Plasma samples were processed with SPE procedure and analytes were chromatographically separated with 9 minutes LC method. Analytes were detected with triple quadrupole mass spectrometer which collected two SRM transitions per each analyte to calculate ion ratio.

Results:

- The limit of quantitation was 5 ng/mL for dopamine and 25 ng/mL for epinephrine and norepinephrine.
- Method precision was better then 4.5% RSD.
- Method accuracy ranged from 86.6-119% for lowest QC sample.
- Matrix effects were observed only for epinephrine and were corrected by internal standard.

INTRODUCTION

Analysis of catecholamines in plasma requires senitive and robust analytical methods that meet clinical reasearch requirements. Mass spectrometry has become the prefered detection technique, successfully relaceing the traditionally used electrochemical detector.

MATERIALS AND METHODS

Sample Processing

- SPE columns: Biotage® EVOLUTE® Express WCX, 10 mg, 96-well plate
- Procedure
  - Mix 500 µL sample + 500 µL 10 mM NH_4•CH_3COO containing internal standards
  - Condition SPE plate
  - Load sample
  - Wash 1: 500 µL of water (2 times)
  - Wash 2: 500 µL of 2-propanol
  - Elute: 125 µL of 15% 2-propranol in 0.3% formic acid (2 times)

Liquid Chromatography

- Analytical Column: Restek® Ultra PFP Propyl, 3 µm ,150 x 2.1 mm
- Mobile phase:
  - A: 0.1% Formic acid in water
  - B: Methanol
- 9 min gradient
- Injection volume: 15 uL

Mass Spectrometry

- Detector: Thermo Scientific™ TSQ Quantiva™ triple quadrupole mass spectrometer
- HESI ionization source
- Polarity: positive
- 2 SRM's per each analyte to calculate ion ratio (Table 1)
- Q1 Resolution: 0.7 (FWHM)
- Q3 Resolution: 1.2 (FWHM)

Table 1. SRM Transitions for Catecholamines

Analyte	Ion	Precursor m/z	Product m/z
Norepinephrine	Quantifier	152.1	77.0
Norepinephrine	Qualifier	170.1	107.0
Norepinephrine-d6	Quantifier	158.1	81.0
Norepinephrine-d6	Qualifier	158.1	111.0
Epinephrine	Quantifier	166.1	77.0
Epinephrine	Qualifier	166.1	135.0
Ephedrine-d6	Quantifier	172.1	112.0
Ephedrine-d6	Quantifier	172.1	141.0
Dopamine	Quantifier	154.1	137.0
Dopamine	Quantifier	154.1	91.0
Dopamine-d4	Quantifier	158.1	141.0
Dopamine-d4	Quantifier	158.1	95.0

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Data Processing
Data were acquired and processed with Thermo Scientific™ TraceFinder™ software, version 4.1.

Calibration Standards
Calibration standards were prepared in house by spiking a) analyte-free plasma and b) 2-propanol:0.3% formic acid (15:85) (Table 2).

Note: Analyte-free plasma was prepared in-house by exposing plasma to UV light for 3 days.

Table 2. Concentrations of In-House Prepared Calibrators

Analyte	Cal1	Cal2	Cal3	Cal4	Cal5	Cal6	Cal7	Cal8
	Concentration (pg/mL)							
Epinephrine	25	50	100	250	500	1000	2500	5000
Norepinephrine	25	50	100	250	500	1000	2500	5000
Dopamine	5	10	20	50	100	200	500	1000

Quality Control Samples: See Table 3

Table 3. Recipe® ClinCheck® plasma controls

Analyte	QC Level I	QC Level II
	Mean concentration/concentration range (pg/mL)	
Epinephrine	106 / 84.8-127	569 / 455-683
Norepinephrine	373 / 298-448	2173 / 173-2608
Dopamine	101 / 70.7-131	508 / 406-610

Method performance evaluation

Precision
Intra- and inter-assay precision of calibrators in plasma: 5 replicates in 3 batches
Intra and inter-assay precision of QC samples: 4 replicates in 3 batches

Accuracy
Analysis of QC samples: 4 replicates in 3 batches

Spike recovery
Spike plasma from 6 donors to 500 pg/mL of epinephrine and norepinephrine and 100 pg/mL of dopamine. Spike plasma from a single donor to 250 pg/mL of epinephrine and norepinephrine and 50 pg/mL of dopamine. Analyze donor plasma before and after spiking with analytes. Calculate analyte concentrations and % recovery.

Matrix Effects

Spike analyte and internal standard into SPE elution solvent and SPE-processed blank plasma. Calculate %recovery as ratio between analyte peak area in spiked SPE eluted blank plasma and analyte peak area in SPE elution solvent.

Calibration Curve Evaluation

The following calibration curves were evaluated by performing quantitative analysis of QC samples and calculating %recovery against vendor-provided mean concentration:

- Calibrators prepared in injection solution and not processed with SPE method
- Calibrators prepared in injection solution and processed with SPE method
- Calibrators prepared in analyte free plasma and processed with SPE method

RESULTS

Limits of quantitation and calibration ranges met clinical research requirements and are summarized in Table 4. Representative plasma calibration curves are presented at Figure 1.

Method precision obtained for calibrators in plasma and QC samples was better then 13.1% RSD and 4.6% RSD respectively. Method accuracy for all calibrators and QC samples were within +/- 20%. The method precision data for calibration standards and QCs are presented in Table 5 and Table 6, respectively.

Chromatograms of Level I and Level II QC samples are presented in Figure 2.

Method accuracy evaluated in spike recovery experiment ranged from 91.2-112%. Results are presented in Table 7.

Matrix effects (Table 8) were negligible for dopamine and norepinephrine. Significant matrix effects were observed for epinephrine, but those were corrected by internal standard as demonstrated by method accuracy.

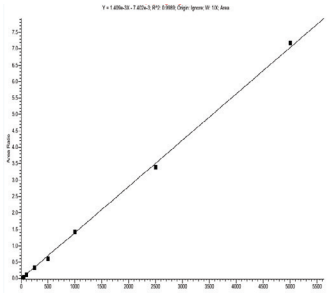
Quantitative analysis of QC samples using calibration curves prepared in solvent and prepared in plasma showed no difference in data accuracy. Table 9 compares accuracy of data collected for QC samples using calibration curve prepared in solvent without SPE processing and plasma with SPE processing.

Table 4. Limits of quantitation and calibration ranges.

Analyte	LOQ (pg/ml)	Calibration range (pg/mL)
Epinephrine	25	25-5000
Norepinephrine	25	25-5000
Dopamine	5	5-1000

FIGURE 1. Representative plasma calibration curves and chromatograms of lowest calibration standard.

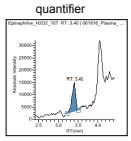
a) Epinephrine



pg/mL	%Diff
25	9.99
50	2.33
100	-3.38
250	0.07
500	-9.33
1000	1.20
2500	-3.13
5000	2.25

25 pg/mL

quantifier



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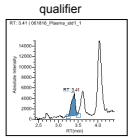
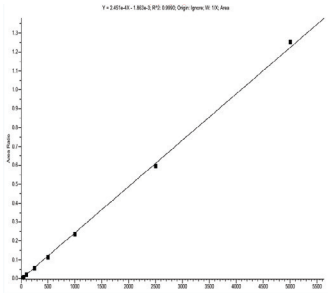


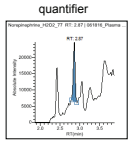
b) Norepinephrine



pg/mL	%Diff
25	11.39
50	7.65
100	-5.51
250	-4.33
500	-6.40
1000	-3.22
2500	-2.10
5000	2.53

25 pg/mL

quantifier



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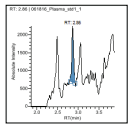
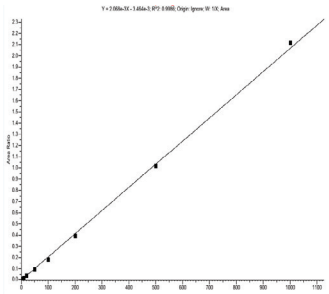


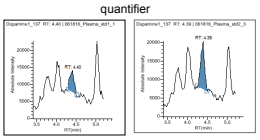
c) Dopamine



pg/mL	%Diff
5	9.62
10	6.47
20	4.45
50	-8.26
100	-9.61
200	-3.99
500	-1.27
1000	2.61

5 pg/mL 10 pg/mL

quantifier



qualifier

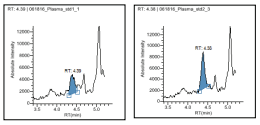


TABLE 5. Method precision and accuracy obtained for calibration standards in plasma.

a) Intra-assay

Analyte		Std1	Std2	Std3	Std4	Std5	Std6	Std7	Std8
Dopamine	pg/mL	5	10	20	50	100	200	500	100
	%RSD	4.65-7.05	3.17-7.13	4.35-6.34	1.47-1.8	1.31-1.51	1.07-3.75	0.67-3.17	0.18-0.56
	%Rec	106-117	108-110	103-116	94.5-115	86.6-95.2	94.5-101	97.0-103	105-107
Epinephrine	pg/mL	25	50	100	250	500	1000	2500	5000
	%RSD	4.28-4.78	1.32-7.81	3.74-7.40	2.70-8.65	2.48-7.74	3.15-10.6	1.05-10.6	0.59-8.56
	%Rec	89.7-99.7	88.3-93.8	83.8-86.6	86.3-89.9	82.3-93.0	89.9-99.2	87.0-96.0	91.2-94.3
Norepinephrine	pg/mL	25	50	100	250	500	1000	2500	5000
	%RSD	0.97-7.05	2.07-6.94	1.04-4.50	0.57-2.42	0.82-1.72	1.04-1.62	0.43-1.98	1.11-1.78
	%Rec	94.7-97.1	96.2-99.8	92.8-99.8	95.4-98.1	93.3-94.7	98.7-99.6	98.4-100	102-106

Table 5 (cont.)
b) Inter-assay

Analyte		Std1	Std2	Std3	Std4	Std5	Std6	Std7	Std8
Dopamine	pg/mL	5	10	20	50	100	200	500	100
	%RSD	13.1	5.0	5.79	10.0	4.1%	2.51	2.02	1.27
	%Rec	97.3	94.1	99.4	99.6	88.1	94.5	97.7	103
Epinephrine	pg/mL	25	50	100	250	500	1000	2500	5000
	%RSD	5.16	5.43	6.69	6.06	5.49	6.48	7.52	6.83
	%Rec	111	103	94.5	96.3	96.3	104	100	101
Norepinephrine	pg/mL	25	50	100	250	500	1000	2500	5000
	%RSD	5.52	4.73	3.55	1.62	1.19	1.19	1.37	1.87
	%Rec	106	103	97.9	96.7	93.2	97.8	98.0	102

TABLE 6. Method precision (a) and accuracy (b) obtained for QC samples

a)

Analyte	Precision (%RSD)			
	Intra-assay		Inter-assay	
	QC Level I	QC Level II	QC Level I	QC Level II
Dopamine	<2.4	<1.5	2.0	2.7
Epinephrine	<1.3	<1.3	3.2	4.6
Norepinephrine	<2.8	<2.1	2.7	1.5

b)

Analyte	Method Accuracy (Average% Recovery)			
	Intra-assay		Inter-assay	
	QC Level I	QC Level II	QC Level I	QC Level II
Dopamine	115-119	101-108	117	104
Epinephrine	110-117	101-111	115	107
Norepinephrine	86.6-90.6	88.0-89.3	88.8	88.6

FIGURE 2. Chromatograms of Level I and Level II QC samples showing both quantifying and qualifying ions.

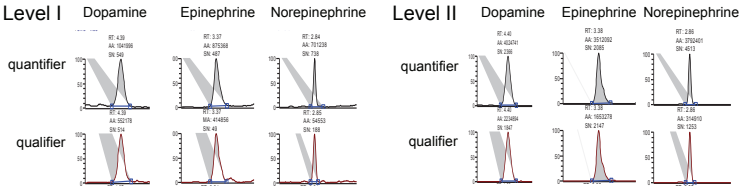


Table 7. Spike recovery experiment results. Concentrations are in pg/mL.

Analyte	Plasma →	#1	#2	#3	#4	#5	#6	#7	#8
Dopamine	Blank Donor Plasma	<5.00	<5.00	<5.00	<5.00	<5.00	<5.00	<5.00	<5.00
	Expected After Spike	100	100	100	100	100	100	50	500
	Detected After Spike	98.7	96.8	112	109	107	101	51.9	525
	%Recovery	**98.7**	**96.8**	**112**	**109**	**107**	**101**	**104**	**105**
Epinephrine	Blank Donor Plasma	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0
	Expected After Spike	500	500	500	500	500	500	250	2500
	Detected After Spike	506	481	530	527	518	538	273	2394
	%Recovery	**101**	**96.2**	**106**	**105**	**104**	**108**	**109**	**95.8**
Nor-epinephrine	Blank Donor Plasma	<25.0	<25.0	<25.0	45.7	83.73	41.88	<25.0	<25.0
	Expected After Spike	500	500	500	546	584	541.9	250	2500
	Detected After Spike	483	464	489	537	559	494	275	2533
	%Recovery	**96.5**	**92.8**	**97.8**	**98.5**	**95.8**	**91.2**	**110**	**101**

Table 8. Matrix Effects: Peak area recoveries in plasma versus solvent.

Peak Area	Dopamine	Dopamine-d4	Epinephrine	Epinephrine-d4	Nor-epinephrine	Nor-epinephrine-d4
Solvent	2043705	9048724	7745833	9091941	2446738	19217448
	2049921	8962708	7709421	9152761	2509643	20337832
	2113823	9309636	7991230	9451668	2654854	21443888
	2129558	9496382	8153433	9601191	2695811	21847306
Average	2084252	9204363	7899979	9324390	2576762	20711619
% RSD	2.10%	2.65%	2.66%	2.60%	4.57%	5.71%
Plasma	1958665	8319667	1950279	2436252	2150196	16667574
	1920204	8163811	1943066	2443034	2199082	16885748
	1942370	8200247	1925746	2441893	2204519	17061057
	1922009	8151569	1951801	2435355	2185281	16941968
Average	1935812	8208824	1942723	2439134	2184770	16889087
% RSD	0.94%	0.93%	0.61%	0.16%	1.12%	0.98%
Recovery	**92.9%**	**89.2%**	**24.6%**	**26.2%**	**84.8%**	**81.5%**

Table 9. QC concentration recoveries calculated against calibrators prepared in water or plasma.

Analyte	QC Level I		QC Level II	
	% Recovery			
	Solvent curve	Plasma curve	Solvent curve	Plasma curve
Dopamine	119-121	115-119	105-108	101-108
Epinephrine	101-107	110-117	94.6-103	101-111
Norepinephrine	87.5-91.7	86.6-90.6	89.3-90.9	88.0-89.3

CONCLUSIONS

- We demonstrated LC-MS analytical method for quantitation of catecholamines in plasma for clinical research.
- The method is sensitive and accurate.
- Method allows for use of calibration curve prepared in solvent if analyte-free plasma is not available.
- Sample preparation procedure does not require evaporation step to pre-concentrate samples which simplifies and reduce analysis cost.
- The throughput of the 9-minute LC method can be doubled to 12 samples/hour by implementing the method on a 2-channel LC system (Thermo Scientific Transcend™ II).

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