A Sensitive Assay for the Determination of DOPA and Dopamine in Human Cerebrospinal Fluid (CSF)

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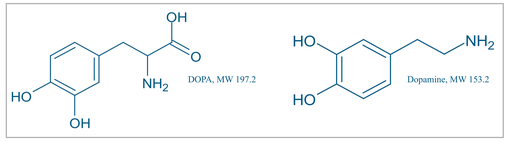
Introduction:

Dopamine is a catecholamine neurotransmitter, responsible for conducting signals in the central nervous system as well as in the sympathetic nervous system. Dopamine is responsible for many physiological functions affecting behavior, mood, and involuntary action. Dopamine is produced through the decarboxylation of dihydroxyphenylalanine (DOPA), which can occur in the brain as well as in the periphery. In human CSF, DOPA and dopamine can be indicators of uptake across the blood-brain barrier (DOPA), neurological conversion/enzyme activity, and reuptake of released neurotransmitters (dopamine).



In general, catechols are extremely sensitive to oxidation, requiring acid pretreatment of samples in order to improve stability. In addition, the catecholamine neurotransmitters have low molecular weights and are very polar compounds, making them very difficult to measure by traditional bioanalytical means (HPLC, LC-MS). Presented here is a very sensitive method for the determination of DOPA and dopamine in acid stabilized human CSF.

Methodology:

Calibration Ranges	50 to 2500 pg/mL DOPA 25 to 1250 pg/mL Dopamine

Sample Extraction

Sample Volume	0.200 mL
Internal standard solution added	10 µL of Dopamine-D4 (20 ng/mL) / DOPA-D3 (40 ng/mL)
Reagent added	0.400 mL 0.1N HCl solution
SPE	96-Well Strata SCX (Phenomenex, 50 mg) Preconditioned with acetonitrile, HCl solution
Wash	0.4 mL HCl solution 2 × 0.4 mL acetonitrile
Derivatize/Elute	0.2 mL propionic anhydride 2 × 0.4 mL elution solution (acetonitrile/ propionic anhydride/pyridine) Heat @ 60° C for 30 min.
Evaporate	Under nitrogen, 65° C for 60 min.
Reconstitute	0.2 mL water/acetonitrile (9:1)

LC Analysis

Column (temp.)	Phenomenex Kinetex C18 1.7µ, 100 X 2.1 mm (60°C)
Mobile phase A	Water/formic acid/ammonium hydroxide (1000:2.0:0.4)
Mobile phase B	Acetonitrile/methanol/formic acid/ammonium hydroxide (500:500:2.0:0.4)
Gradient conditions	25% to 45% solvent B over 6.5 minutes, linear gradient, 0.5 mL/min
Injection volume	10 µL

MS-MS

Instrument	Sciex 4000
Ion transitions	366→208 DOPA 322→137 Dopamine

Synthetic (Mock) CSF Used:

In CSF, endogenous DOPA and dopamine are present in control matrix obtained commercially. A synthetic solution was used as blank control matrix for calibration samples in order to avoid errors related to background subtraction. The composition of the synthetic CSF solution was based on concentration values found in literature. (Geigy Scientific Tables, 8th edition, CIBA-GEIGY Limited 1981.)

Calcium chloride 1 mg/mL
Urea 3 mg/mL
Sodium chloride 5 mg/mL
Albumin (bovine) 2 mg/mL

Results:

Examples of chromatography for each analyte are presented in figures 1 through 10. The ion transitions for DOPA and dopamine in free base form provided limited sensitivity by LC-MS (LLOQ in the low ng/mL range). Using the mass transitions of the propionyl derivatives on a Sciex 5000 instrument yielded approximately a 10 to 20-fold improvement in sensitivity. Preliminary screening of human CSF samples suggested baseline endogenous levels of dopamine would be low, in the range of 10 to 50 pg/mL. In addition, availability of sample volume in CSF is very restricted, so increasing the sample aliquot volume for extraction to improve sensitivity is of limited value. Changing the ion transition setting by utilizing a larger derivative proved beneficial to sensitivity to where background noise was reduced and the detection limit improved.

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Representative Chromatograms:

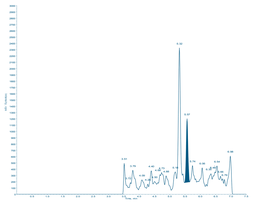

Figure 1. DOPA LLOQ (50 pg/mL) Sample

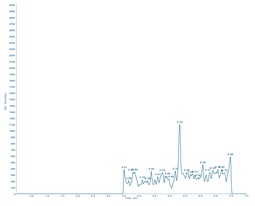

Figure 2. DOPA Synthetic CSF Control Blank Sample

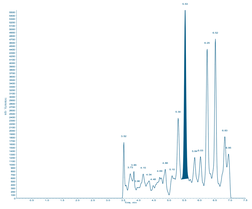

Figure 3. DOPA Authentic Sample at 84.2 pg/mL

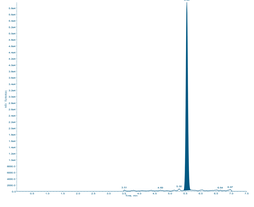

Figure 4. DOPA ULOQ (2500 pg/mL) Sample

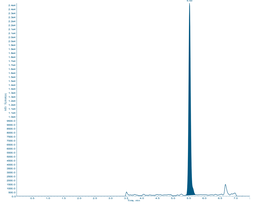

Figure 5. DOPA Internal Standard (DOPA-D3) Chromatogram

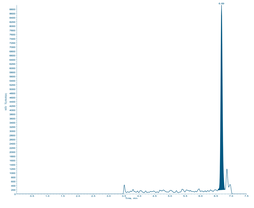

Figure 6. Dopamine LLOQ (25 pg/mL) Sample

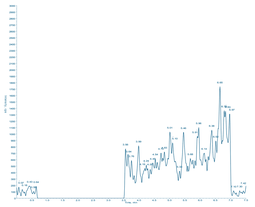

Figure 7. Dopamine Synthetic CSF Control Blank Sample

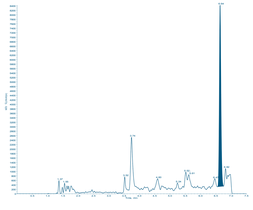

Figure 8. Dopamine Authentic Sample at 26.2 pg/mL

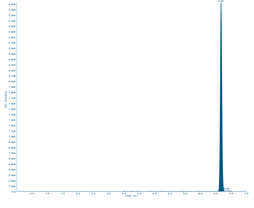

Figure 9. Dopamine ULOQ (1250 pg/mL) Sample

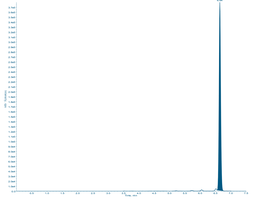

Figure 10. Dopamine Internal Standard (Dopamine-D4) Chromatogram

Validation Data:

Extraction recovery of DOPA was very good, ranging from 83% to 95%. Recovery for dopamine was similar at 83% to 91%. The matrix factor (relative response in the presence of CSF extract) was measured to be 0.9 to 1.0 for both analytes.

LLOQ (50.0 pg/mL)	Low (150 pg/mL)	Mid (610 pg/mL)	High (890 ng/mL)
Overall %CV			
8.5	3.5	2.7	2.8
Overall %Bias			
3.7	-3.4	-2.5	-1.5

DOPA Precision and Accuracy

	LLOQ (50.0 pg/mL)	Low (150 pg/mL)	Mid (610 pg/mL)	High (890 ng/mL)		LLOQ (50.0 pg/mL)	Low (150 pg/mL)	Mid (610 pg/mL)	High (890 ng/mL)		LLOQ (50.0 pg/mL)	Low (150 pg/mL)	Mid (610 pg/mL)	High (890 ng/mL)
Run 1	51.9	153	590	844	Run 2	45.5	150	623	854	Run 3	46.0	149	598	876
	50.0	140	583	881		47.9	144	585	868		55.7	146	593	901
	55.5	147	605	901		49.1	150	577	855		51.4	141	595	876
	50.1	146	591	881		51.9	153	578	856		47.1	140	634	889
	54.4	143	584	915		53.5	138	579	905		61.4	148	614	910
	53.9	136	588	827		48.8	144	584	876		59.6	141	600	858

Precision and accuracy data for both analytes are given above and below.

LLOQ (25.0 pg/mL)	Low (75.0 pg/mL)	Mid (240 pg/mL)	High (920 ng/mL)
Overall %CV			
10.7	5.5	4.4	3.3
Overall %Bias			
3.6	-0.9	1.9	0.2

Dopamine Precision and Accuracy

	LLOQ (25.0 pg/mL)	Low (75.0 pg/mL)	Mid (240 pg/mL)	High (920 ng/mL)		LLOQ (25.0 pg/mL)	Low (75.0 pg/mL)	Mid (240 pg/mL)	High (920 ng/mL)		LLOQ (25.0 pg/mL)	Low (75.0 pg/mL)	Mid (240 pg/mL)	High (920 ng/mL)
Run 1	23	70.6	257	917	Run 2	22.7	73.8	241	922	Run 3	31.3	72.4	239	926
	25.4	71.5	249	962		24.9	74.6	257	865		28.9	73.9	243	939
	26.0	73.9	253	931		24.6	77.1	241	922		30.0	79.0	226	909
	23.8	68.0	251	961		25.5	79.6	267	866		28.1	78.0	246	918
	23.7	71.2	250	964		22.3	74.7	235	902		27.8	70.8	227	913
	24.1	84.5	247	962		24.2	73.8	242	935		29.9	69.8	232	917

Stability testing was done in unaltered, authentic CSF samples. These samples were acidified and then analyzed to assess an anchor value for comparison after stressing. The high stability pool had a mean value of 230 pg/mL for DOPA and 200 pg/mL for dopamine. The low stability pool had a mean value of 75.0 pg/mL for DOPA and dopamine.

DOPA Stability

	High Stability (230 pg/mL)	%Bias	Low Stability (75.0 pg/mL)	%Bias		High Stability (230 pg/mL)	%Bias	Low Stability (75.0 pg/mL)	%Bias		High Stability (230 pg/mL)	%Bias	Low Stability (75.0 pg/mL)	%Bias
24 hrs @ RT	235	2.2	84.5	12.7	4 cycle freeze/thaw	225	-2.2	77.0	2.7	70 hrs, extract stab.	245	6.5	77.0	2.7
	231	0.4	77.9	3.9		250	8.7	80.4	7.2		245	6.5	82.2	9.6
	241	4.8	80.2	6.9		235	2.2	67.5	-10		259	12.6	79.7	6.3

Dopamine Stability

	High Stability (200 pg/mL)	%Bias	Low Stability (75.0 pg/mL)	%Bias		High Stability (200 pg/mL)	%Bias	Low Stability (75.0 pg/mL)	%Bias		High Stability (200 pg/mL)	%Bias	Low Stability (75.0 pg/mL)	%Bias
24 hrs @ RT	229	14.5	67.6	-9.9	4 cycle freeze/thaw	207	3.5	74.5	-0.7	70 hrs, extract stab.	218	9.0	69.8	-6.9
	210	5.0	65.7	-12.4		207	3.5	67.0	-10.7		215	7.5	72.7	-3.1
	214	7.0	68.8	-8.3		215	7.5	68.7	-8.4		203	1.5	70.3	-6.3

Matrix effects in CSF were tested by measuring endogenous levels in 10 different lots of unaltered matrix. These same lots were fortified with additional DOPA and dopamine by spiking an aliquot of each with a standard solution. A target value was calculated for each matrix lot and compared to the found value. These results are presented below.

Matrix Effect for DOPA in Unaltered Human CSF

CSF Lot #	Endogenous Amount Found (pg/mL)	Amount Added (pg/mL)	Total Amount Expected (pg/mL)	Total Amount Found (pg/mL)	% Bias
1	84.2	400	484	474	-2.0
2	186	400	586	555	-5.4
3	269	400	669	812	21
4	148	400	548	508	-7.2
5	307	400	707	668	-5.6
6	147	400	547	513	-6.2
7	284	400	684	650	-4.9
8	223	400	623	607	-2.6
9	587	400	987	957	-3.0
10	219	400	619	606	-2.1

Matrix Effect for Dopamine in Unaltered Human CSF

CSF Lot #	Endogenous Amount Found (pg/mL)	Amount Added (pg/mL)	Total Amount Expected (pg/mL)	Total Amount Found (pg/mL)	% Bias
1	<25.0	200	200	206	3.0
2	<25.0	200	200	205	2.5
3	<25.0	200	200	202	1.0
4	26.2	200	226	226	0
5	<25.0	200	200	198	-1.0
6	<25.0	200	200	193	-3.5
7	<25.0	200	200	201	0.5
8	37.9	200	238	231	-2.9
9	24.4	200	224	230	2.7
10	<25.0	200	200	192	-4.0

Conclusion:

Dopamine is an important endogenous compound for neurological/physiological health. The relative abundance of DOPA and dopamine can be a useful biomarker for certain therapeutic applications. The low molecular weight, endogenous presence, and relatively low target concentrations of DOPA and dopamine make a quantitative method challenging in biological fluids. The approach taken with this method has demonstrated sensitivity, accuracy, and robustness in human CSF.