LC-MS/MS application for the simultaneous measurement of 12 biogenic monoamines

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Background

Correct measurement of biogenic monoamines is essential in the clinical diagnosis and follow-up of pheochromocytoma, paraganlioma and carcinoid tumours. Currently used fluorometric and electrochemical detection methods lack analytical specificity and sensitivity and are time consuming.

OBJECTIVE: To develop a fast, sensitive and specific LC-MS/MS assay for the simultaneous measurement of 12 biogenic monoamines.

Chromatographically Separation of 12 biogenic monoamines

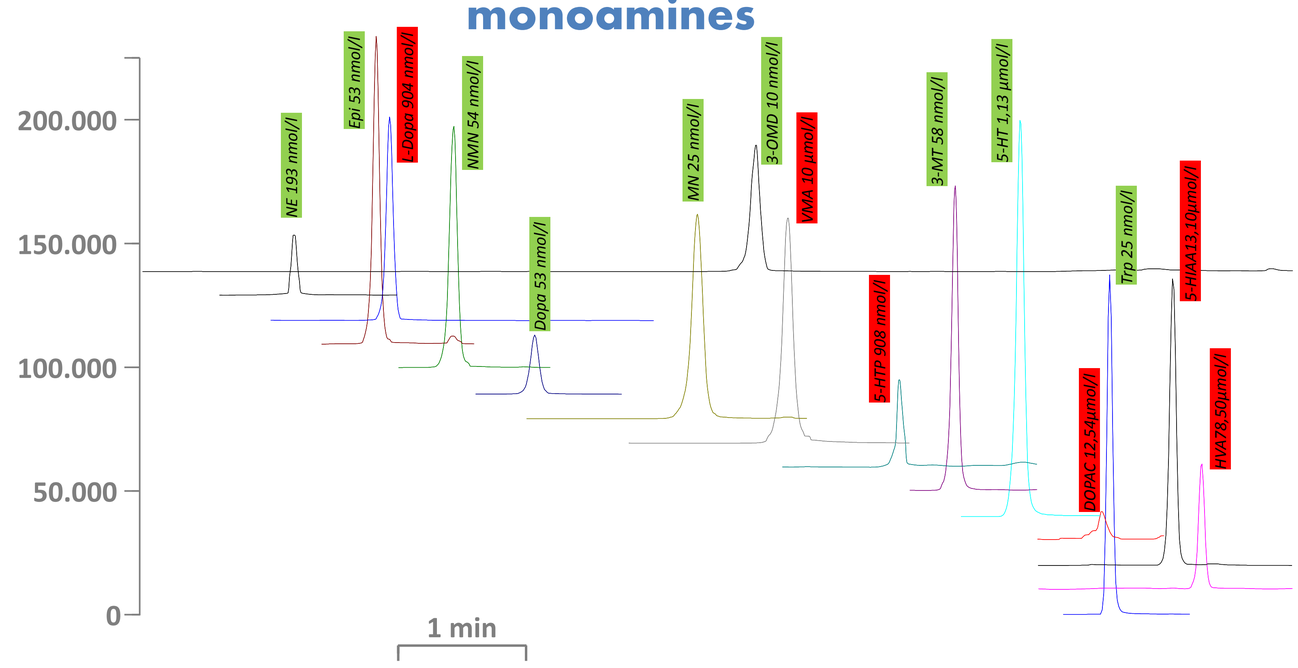

Figure 1: *Chromatographic separation of 12 biogenic monoamines. All 12 biogenic monoamines are chromatographically separated with a minimal resolution of 1.2. Acidic compounds are indicated in red, whereas basic compounds in green. Although medications like L-DOPA, 3-OMD and DOPAC were not fully chromatographically separated form the monoamines, they showed no interference.*

Results

Mass transitions and collision energies

Compound	Abbreviation	+/-	m/z	Qn	Ql	CE
Norepinephrine	NE	+	170.00	107.10	77.50	-22.0
Norepinephrine-d6	NE-d6	+	176.10	85.10	126.00	-20.0
Epinephrine	Epi	+	184.00	166.15	107.00	-12.0
Epinephrine-d6	Epi-d6	+	190.20	172.30	112.50	-12.0
L-Dopamine	L-Dopa	+	198.00	152.10	107.20	-14.0
Normetanephrine	NMN	+	166.20	79.30	77.30	-25.0
Normetanephrine-d3	NMN-d3	+	169.25	82.10	79.00	-28.0
Dopamine	Dopa	+	154.00	91.05	65.00	-24.0
Dopamine-d4	Dopa-d4	+	157.90	141.15	95.20	-14.0
Metanephrine	MN	+	198.20	148.30	165.30	-20.0
Metanephrine-d3	MN-d3	+	201.05	183.20	168.15	-11.0
3-O-Methyldopa	3-OMD	+	212.00	149.00	153.00	-16.0
Vanillylmandelic acid	VMA	-	197.10	137.00	108.00	+22.0
Vanillylmandelic acid -d3	VMA-d3	-	200.10	140.05	141.10	+21.0
5-hydroxytryptophan	5-HTP	+	221.10	204.15	162.10	-10.0
3-Methoxytyramine	3-MT	+	168.20	91.30	151.30	-25.0
d4-3-MT	3-MT-d4	+	171.90	155.25	95.20	-12.0
5-hydroxytryptamine	5-HT	+	176.90	160.20	115.10	-13.0
5-hydroxytryptamine-d4	5-HT-d4	+	181.05	164.35	99.10	-12.0
Tryptophan	Trp	+	205.15	188.05	146.10	-10.0
3,4-Dihydroxyphenylacetic acid	DOPAC	-	167.15	123.05	95.10	+12.0
5-hydroxyindoleacetic acid	5-HIAA	+	192.00	146.20	91.30	-15.0
5-hydroxyindoleacetic acid -d5	5-HIAA-d5	+	197.20	150.20	122.30	-15.0
homovanillic acid	HVA	-	180.95	122.20	105.20	+17.0
homovanillic acid-d5	HVA-d5	-	185.90	58.00	169.00	+21.0

Table 1: *M/z values of the precursor, quantifier and qualifier and collision energy of all compounds and internal standards*

Analytical sensitivities and recoveries

Compound	Recovery WCX (%)	Recovery WAX (%)	LoQ (nmol/l)	Normal range plasma (nmol/l)	Normal range urine (nmol/24 uur)
NE	48	<<	0.10	0.8-4.3 γ	<580 γ
Epi	48	<<	0.44	0.04-0.80 γ	<180 γ
L-Dopa	<5	<<	25.0	- γ	- γ
NMN	72	<<	0.01	0.23-1.07 γ	<4400 γ
Dopa	34	<<	0.35	0.03-0.18 γ	<2800 γ
MN	79	<<	0.01	0.07-0.33 γ	<2000γ
3-OMD	<5	<<	2.00	- γ	- γ
VMA	<<	78	1.08	<4.0 γ	<125000γ
5-HTP	<<	7	63.0	<8.5 γ	<227000 γ
3-MT	86	<<	0.01	<0.04 γ	<2390 γ
5-HT	82	<<	0.38	2.8-5.4 γ	<1190 γ
Trp	<<	41	33.0	40-70γ	<84000 γ
DOPAC	<<	17	45.0	- γ	- γ
5-HIAA	<<	27	1.70	18-108 γ	<56000 γ
HVA	<<	98	90.0	<5.5 γ	<125000γ

Table 2: *Recoveries, LoQ and normal ranges of 12 biogenic monoamines. Basic biogenic monoamines recover best with WCX and acid biogenic monoamines recover best with WAX sample preparation columns. γ indicates sufficient and γ indicates insufficient low LoQ value for measuring within or below reference range in plasma or urine samples.*

Conclusions

- All 12 monoamines are chromatographically separated
- Medication like 3-OMD, DOPAC en L-DOPA do not interfere
- Separate solid phase extraction methods are required for acidic (WAX) and basic (WCX) monoamines
- Analytical sensitivity is sufficient low for measuring all biogenic monoamines in urine
- Analytical sensitivity is sufficient low for most biogenic monoamines in plasma, except for HVA and 5-HTP

Material & Method

Chromatography	UHPLC Nexera
Column	ACE 3 C18-PFP 2 µm (150 x 3.0 mm)
Column temperature	40° C
Mobile phase	A: H_2O + ammonium formate (2mM) + 0.05% formic acid B: Acetonitrile (ACN) + ammonium formate (2mM) + 0.05% formic acid
Flow	0.4 mL/min
Gradient	0 – 3.0 min 0% => 1.5% phase B 3.0 – 4.5 min 1,5% => 11.5% phase B 4.5 - 9.0 min 11.5% => 27.5% phase B 9.0 - 11.5 min 85% phase B 11.5 - 14 min 0% phase B

Mass spectrometry	8060 (Shimadzu)
Drying gas	3mL/min
Heating gas	17 ml/min
Nebulizing gas	2 ml/min
DL temperature	300°C
Interface temperature	400°C
Heating block	500°C
Interface voltage	2 kV

Step	Strata-X-AW Phenomenex	WCX Waters
Dilute sample	0.1 % acetic acid (1:1)	water (1:1)
Condition step 1	0.3 mL MeOH	1 mL MeOH
Condition step 2	0.3 mL H_2O	1 mL AmAC 50 mM
Load sample	0.5 mL	0.5 mL
Wash step 1	0.3 mL 2% formic acid in H_2O	10% MeOH 1 ml
Wash step 2	----------	1 mL IPA
Eluate	0.3 mL 5% ammonia in MeOH	2 x 500µL 2% formic acid in ACN
Dry	N_2	N_2
Reconstitution	100 µL of mobile phase A	100 µL of mobile phase A

Weak cation exchange (WCX, Waters Oasis) and weak anion exchange (WAX, Phenomenex Strata™ X-AW) columns were used to extract respectively basic and acidic biogenic amines. Extracted samples were N_2-dried and reconstituted in 100 µl mobile phase A before injection (25 µl).

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