SIMULTANEOUS DETERMINATION OF ANTIHYPERTENSIVE DRUGS IN SERUM BY LC-MS/MS

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Introduction

Antihypertensives (AHT) is a class of drugs that are used to treat hypertension. The compliance to antihypertensive therapy is critical to achieve adequate blood pressure and avoid complications such as stroke or myocardial infarction. Therapeutic drug monitoring (TDM) is a reliable approach to assess AHT compliance, moreover TDM helps to distinguish non-compliance from patients with true resistant hypertension.

The aim of this work, based on the physicians' requests, was to develop, validate and implement a method for quantification of AHT in serum samples suitable for use in routine laboratory practice.

Antihypertensive Drugs

Method I: ACEBUTOLOL, ATENOLOL, BETAXOLOL, BISOPROLOL, CARVEDILOL, METOPROLOL, NEBIVOLOL

Method II: IRBESARTAN, LOSARTAN, TELMISARTAN, VALSARTAN, AMLODIPINE, NITRENDIPINE, INDAPAMIDE

LC-MS/MS Methods

Instrumentation:

- Agilent 6460 Triple Quadrupole LC/MS System, ESI+
- Agilent Poroshell 120 (EC-C18, 2.1×100 mm, 2.7 µm) column (Agilent, USA)

Sample preparation:

- 50 µl of sample (calibrator, control, serum)
- Protein precipitation with 150 µl MeOH (I)/ACN (II) (containing IS)

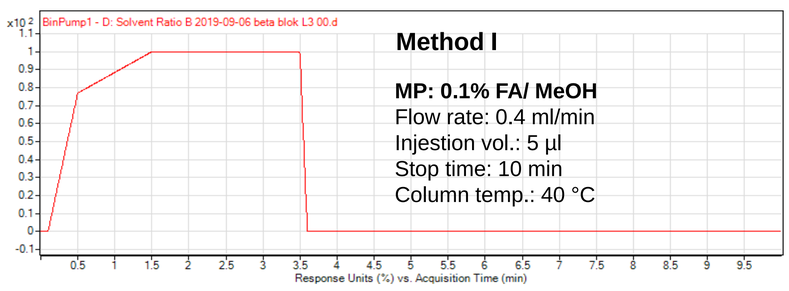
Materials used for determination of methods parameters:

- Calibrators, controls inhouse spiked (TRC, Canada; HPC Standards GmbH, Germany)
- Deuterated internal standards (TRC, Canada)
- Formic Acid (Honeywell Fluka™)
- Solvents: Inhouse purified H_2O; ACN, MeOH LC-MS grade (Honeywell)
- Patient samples: Serum samples

Mass Spectrometry Conditions

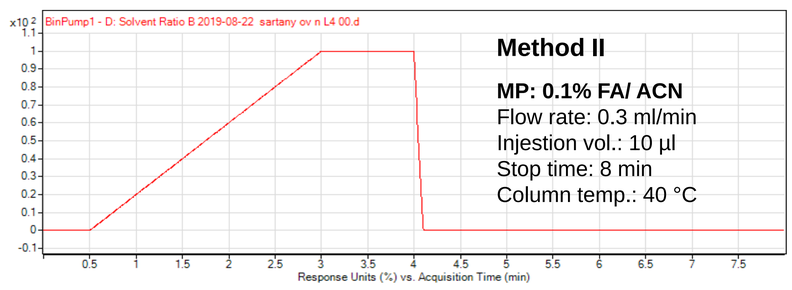
	Method I	Method II	
Ionization	positive	positive	
Gas temperature	300	180	ºC
Gas flow	6	8	l/min
Nebulizer	25	25	psi
SGT	350	350	ºC
SGF	10	11	l/min
Capillary	3500	3500	V
Nozzle voltage	0	0	V

LC Conditions



Method I

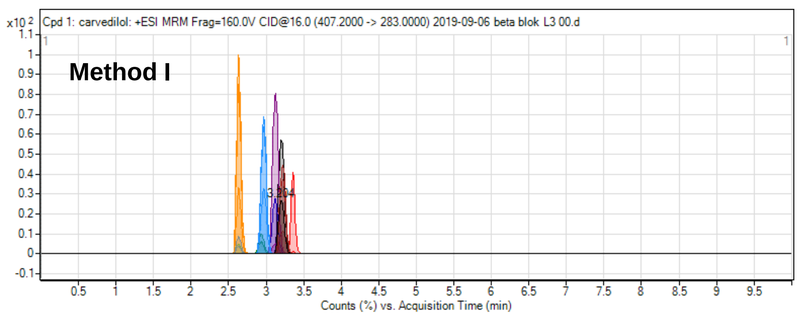
MP: 0.1% FA/ MeOH
Flow rate: 0.4 ml/min
Injestion vol.: 5 µl
Stop time: 10 min
Column temp.: 40 °C

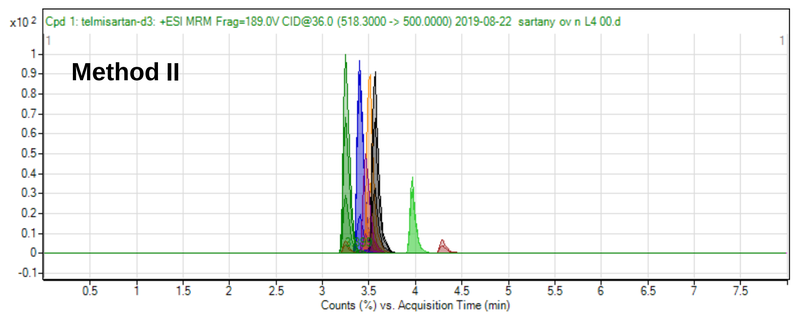


Method II

MP: 0.1% FA/ ACN
Flow rate: 0.3 ml/min
Injestion vol.: 10 µl
Stop time: 8 min
Column temp.: 40 °C

LC-MS/MS Chromatograms





MRM Transitions

Analyte	Parent	Product (quantifier/ qualifier)
Method I		
ACETOBUTOLOL	337.2	260/180
ATENOLOL	267.2	145/190
ATENOLOL-d7	274.2	190/-
BETAXOLOL	308.2	72/116
BISOPROLOL	326.2	116/74
BISOPROLOL-d5	331.2	121/-
CARVEDIOL	407.2	224/283
METOPROLOL	268.2	116/159
NEBIVOLOL	406.2	151/388
Method II		
IRBESARTAN	429.2	195/211.1
IRBESARTAN-d4	433.3	195.1/211.1
LOSARTAN	423.2	207/405
TELMISARTAN	515.2	276/497
TELMISARTAN-d3	518.3	279/500
VALSARTAN	436.2	235/207
VALSARTAN-d3	439.2	235/207
AMLODIPINE	409.2	238/294
AMLODIPINE-d4	413.2	238/298
NITRENDIPINE	361.1	315/329
INDAPAMIDE	366.1	132/117
INDAPAMIDE-d3	369.1	117/132

Analytical Parameters

Calibration Range: 5-1000 µg/l

Analyte Method I	LLQ (n=10)		Intra Assay (n=10)		Recovery 1 (n=5)	Recovery 2 (n=5)	R^2
	[µg/l] Mean ± SD	CV [%]	[µg/l] Mean ± SD	CV [%]	[%]	[%]	
ACEBUTOLOL	4.94±0.40	8.11	611.7±14.4	2.4	110.96	111.23	0.999
ATENOLOL	4.55±0.36	7.84	600.91±11.81	1.96	103.60	99.23	0.999
BETAXOLOL	5.06±0.30	5.86	521.52±15.35	2.94	102.75	98.66	0.999
BISOPROLOL	4.64±0.26	5.70	605.39±9.51	1.57	110.11	100.14	0.999
CARVEDILOL	5.41±0.48	8.78	618.76±17.37	2.81	103.71	104.90	0.999
METOPROLOL	4.74±0.30	6.26	605.12±18.38	3.04	114.08	114.81	0.999
NEBIVOLOL	5.03±0.38	7.59	593.37±18.80	3.17	93.37	92.40	0.999

Analyte Method II	LLQ (n=10)		Intra Assay (n=10)		Recovery 1 (n=5)	Recovery 2 (n=5)	R^2
	[µg/l] Mean ± SD	CV [%]	[µg/l] Mean ± SD	CV [%]	[%]	[%]	
IRBESARTAN	4.90±0.27	5.48	88.44±1.71	1.93	100.23	97.46	0.999
LOSARTAN	5.64±0.51	9.07	120.09±2.84	2.36	93.53	90.68	0.999
TELMISARTAN	5.48±0.17	3.08	85.89±3.13	3.65	99.44	105.25	0.999
VALSARTAN	5.28±0.47	8.89	91.69±5.66	6.18	107.31	109.26	0.999
AMLODIPINE	4.99±0.50	9.94	85.95±5.29	6.16	97.36	98.05	0.999
NITRENDIPINE	5.15±0.50	9.78	92.44±6.46	6.99	107.25	107.17	0.999
INDAPAMIDE	5.18±0.31	5.94	96.32±7.46	7.75	96.10	96.31	0.999

Conclusions

Simple and fast approach

- Methods are simple, fast and ensure grater throughput of samples
- Easy and fast sample preparation

Satisfactory analytical parameters

- LLQs, Recoveries
- Methods are reliable in terms of precision
- The calibration curves provide linearity in the relevant range of drug concentrations

LC-MS/MS system configuration

- 14 analytes in 2 methods with only change in MPB
- Provides TDM with relatively quick response

Methods are suitable for routine practice in diagnostic lab.