Will LC-MS/MS and VAMS improve live quality of patients suffering from cardiac arrhythmias?

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Introduction

Cardiac arrhythmia, observed as atrial fibrillation, atrial flutter, atrial tachycardia, ventricular tachycardia, and premature heartbeat, is a consequence of abnormal authority or conduction disturbance. Patients suffering from cardiac disorders are usually treated with pharmacotherapy, but therapeutic ranges for these pharmaceuticals are often narrow. Therapeutic Drug Monitoring (TDM), which is taking into account individual metabolism and condition of each patient, is becoming a common approach for adjusting appropriate dose of the drug, every year. However, collection of venous blood has many disadvantages e.g. requires the employment of trained medical personnel. The Volumetric Absorptive Microsampling (VAMS) is an interesting alternative. Sampling in this way is safe and comfortable for the patient. This approach does not require the involvement of medical personnel.

Arrhythmia in numbers:

Bradycardia < 60 bpm → 60-80 bpm (normal) → 100 bpm < Tachycardia

- Affects 2-3% of Europeans & Americans
- In Poland arrhythmia affects 1,8-2% of population (ca. 0,7-0,8 mln people)
- 80% of sudden cardic deaths are caused by ventricular arrhythmias.
- In Europe there are ca. 13,3 mln of people, which should be effectively and safely treated.
- Due to the aging of the populations: in 2030 the number of patients with arrhythmia will increase to 14-17 million, while in 2040 it may be doubled.

Methods

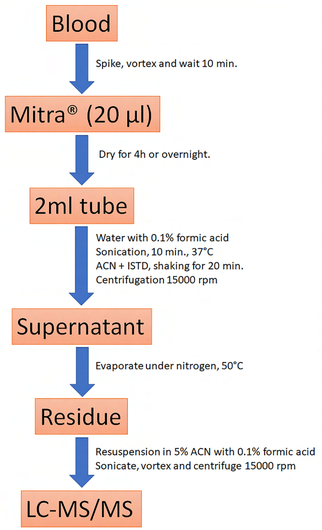


Fig. 1. Procedure workflow.

A sample preparation procedure and quantitative LC-MS/MS method for determination of selected drugs and its metabolites was developed, optimised and validated for the best coverage of the range of therapeutic concentrations.

Briefly - blood was collected with a MITRA® microsampling device (20 µl), dried at room temperature, sonicated with 0.1% formic acid in water and extracted using acetonitrile (ACN). Extracts were dried under nitrogen, resuspended in 5% ACN with 0.1% formic acid prior LC-MS/MS analysis (Fig. 1).

All the tested compounds were separated using reversed-phase chromatography and analyzed using QTRAP 5500+ LC-MS/MS system (SCIEX) operating in positive MRM mode. The MS/MS scanning was divided into 5 periods (Fig. 2) in which the ion source parameters were adjusted to increase the sensitivity for compounds that exhibit poor ionization. Data processing and quntitation analysis were performed using SCIEX OS software.

The validation included determination of limit of detection (LOD), lower limit of quantitation (LLOQ), linearity, recovery and intermediate precision of the method calculated on the basis of multiple repetitions ($p < 0.05$).

Results

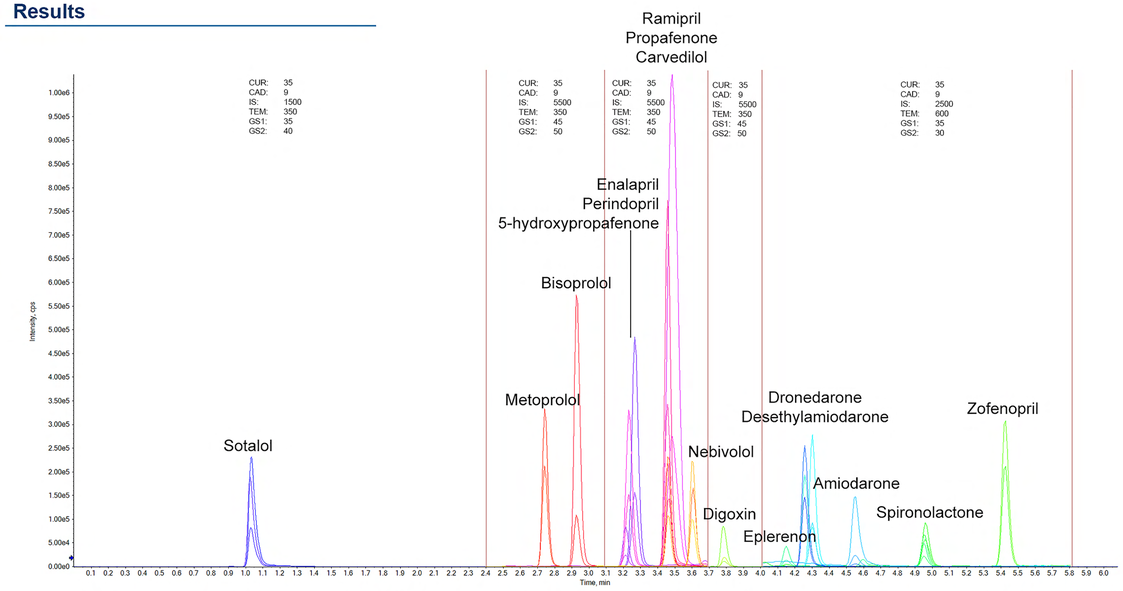
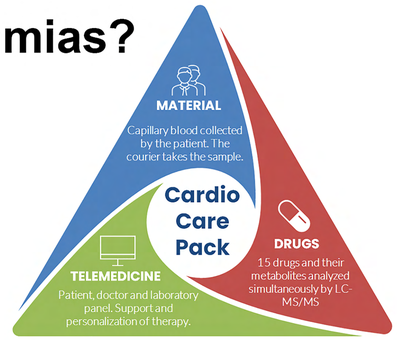


Fig. 2. XIC chromatogram of the selected compounds with periods and ions source settings applied.



Tab. 2. Recovery - 80-120% ($p \leq 0.05$). *Recovery [%]*

Analyte	QC Low (0.5/5/50 µg/L)	QC Medium (2.5/25/250 µg/L)	QC High (10/100/1000 µg/L)
Sotalol	102.17	116.45	98.82
Metoprolol	105.07	109.34	92.20
Bisoprolol	105.24	107.32	85.18
5-hydroxypropafenone	96.39	113.65	96.25
Perindopril	102.83	114.50	95.65
Enalapril	98.02	109.50	88.35
Propafenone	98.42	89.39	80.54
Ramipril	101.96	88.75	95.66
Carvedilol	94.07	111.10	95.55
Digoxin	81.48	113.05	90.62
Nebivolol	93.71	110.78	92.06
Eplerenone	115.75	111.96	112.26
Spironolactone	100.26	108.36	92.76
Dronedarone	96.14	107.40	93.96
Desethylamiodarone	86.56	94.12	84.51
Zofenopril	111.93	104.88	99.05
Amiodarone	98.08	97.96	86.50

Tab. 1. Therapeutic index, LOD, LLOQ (S/N ≥ 6) and linearity (R ≥ 0.995) of the method.

Analyte	*Therapeutic index [µg/L]*	*Regression coefficient (R)*	*Linearity [µg/L]*	*LOD [µg/L]*	*LLOQ [µg/L]*	*S/N*
Sotalol	1400 - 1700	r = 0.99806	10 - 2500	< 10	< 10	3406
Metoprolol	3 - 270	r = 0.99974	1 - 250	< 1	< 1	261
Bisoprolol	3 - 50	r = 0.99917	2.5 - 250	< 1	< 1	438
5-hydroxyropafenone	153 - 337	r = 0.99875	50 -2500	< 10	< 10	2677
Perindopril	80 - 150	r = 0.99697	10 -250	< 1	< 1	811
Enalapril	10 - 100	r = 0.99504	2.5 - 250	< 1	< 1	561
Propafenone	100 - 1000	r = 0.99704	1 - 250	< 1	< 1	1136
Ramipril	10 - 10 000	r = 0.99869	2.5 - 250	< 1	< 1	435
Carvedilol	6.93 - 77	r = 0.99628	2.5 - 250	< 1	< 1	298
Digoxin	0.5 - 2	r = 0.99754	0.25 - 25	< 0.25	0.25	7
Nebivolol	0.5 - 1.5	r = 0.99749	0.25 - 25	< 0.1	0.1	6
Eplerenone	200 - 1700	r = 0.99808	10 - 2500	< 1	< 1	95
Spironolactone	10 - 300	r = 0.99851	2.5 - 250	< 1	< 1	33
Dronedarone	80 - 170	r = 0.99805	2.5 -250	< 1	< 1	127
Desethylamiodarone	200 - 1000	r = 0.99688	10 -2500	< 10	< 10	456
Zofenopril	50 - 170	r = 0.99744	2.5 - 250	< 1	< 1	57
Amiodarone	1000 - 2500	r = 0.99726	10 -2500	< 10	< 10	438

Tab. 3. Intermediate precision - 90-110% ($p \leq 0.05$).

	QC Low (0.5/5/50 [µg/L])				*QC Medium (2.5/25/250 [µg/L])*				*QC High (10/100/1000 [µg/L])*			
Analyte	Mean	SD	CV [%]	Intermediate precision [%]	Mean	SD	CV [%]	Intermediate precision [%]	Mean	SD	CV [%]	Intermediate precision [%]
Sotalol	50.16	2.82	5.62	100.34	249.44	24.81	9.95	99.78	1044.68	66.36	6.35	104.47
Metoprolol	4.92	0.41	8.28	98.42	27.08	1.91	7.08	108.33	98.19	8.39	8.55	98.20
Bisoprolol	5.25	0.52	9.99	105.03	24.58	2.01	8.21	98.34	93.75	6.17	6.58	93.75
5-hydroxypropafenone	47.29	2.09	4.42	94.58	249.50	21.45	8.60	99.80	1033.68	86.88	8.40	103.37
Perindopril	4.93	0.45	9.09	98.78	25.51	1.59	6.23	102.04	101.52	6.37	6.28	101.52
Enalapril	4.99	0.43	8.65	99.86	24.59	1.48	6.04	98.37	95.85	5.60	5.85	95.86
Propafenone	4.78	0.37	7.68	95.62	25.22	2.34	9.30	100.89	97.14	4.71	4.86	97.14
Ramipril	4.95	0.29	5.95	98.99	26.11	1.90	7.30	104.45	102.40	6.21	6.06	102.40
Carvedilol	4.84	0.36	7.47	96.77	25.64	1.99	7.78	102.57	100.72	4.96	4.92	100.73
Digoxin	0.49	0.04	9.05	98.48	2.52	0.10	4.21	100.87	9.65	0.42	4.44	96.56
Nebivolol	0.49	0.04	9.16	98.01	2.49	0.17	7.08	99.51	9.53	0.63	6.61	95.37
Eplerenone	51.12	2.73	5.35	102.25	257.61	13.66	5.31	103.05	1004.70	87.76	8.74	100.47
Spironolactone	4.67	0.36	7.83	93.51	25.27	1.10	4.39	101.09	104.07	4.31	4.14	104.07
Dronedarone	4.90	0.41	8.33	97.98	25.05	1.92	7.68	100.19	99.99	7.74	7.74	99.99
Desethylamiodarone	46.14	4.13	8.96	92.28	253.49	22.05	8.70	101.40	1045.36	58.49	5.60	104.54
Zofenopril	4.91	0.55	11.33	98.17	26.30	2.40	9.13	105.23	98.16	8.66	8.83	98.16
Amiodarone	47.74	4.46	9.35	95.47	258.76	17.06	6.60	103.51	994.18	74.51	7.49	99.42

Summary

The method development was focused on covering therapeutic ranges of all tested compounds in one run. Because of both high intensity and broad therapeutic range samples from patients treated with propafenone and ramipril may have to be diluted to fit in the working range of the LC-MS/MS method. Assay was validated with satisfactory results obtained for each tested parameter and demonstrated for all 17 compounds (Fig. 2): good linearity (regression coefficient in the range: 0.995 – 0.999) (Tab.1), %CV – 0.9-6.6% (Tab. 3), recovery from 80% to 116% (Tab. 2) and intermediate precision in the range of 92-108% (Tab. 3). Obtained data showed that VAMS coupled with optimized targeted LC-MS/MS analysis can be successfully applied for TDM in patients suffering from cardiac disorders. In order to check the correlation between the concentration of a given antiarrhythmic drug in the serum and in the capillary blood collected on Mitra device, a clinical trial is currently underway. The final product will include software platform that will connect patient, doctor and laboratory to help the doctor with the treatment of patient that can collect the sample on his/her own.

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CardioCarePack - a package of modern drug-monitored therapy solutions in patients with cardiac arrhythmias in development of personalized medicine