Evaluation of LC/MS/MS for the Determination of Amiodarone and its Metabolite Extracted From Dried Blood Spot Samples

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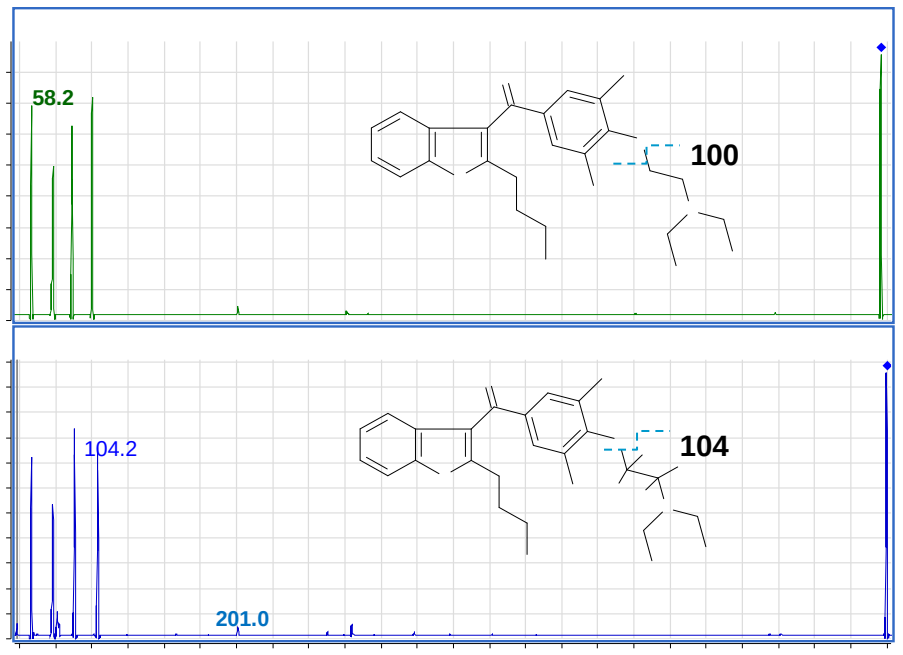
Introduction

A method for rapid detection and quantitation of amiodarone and des-ethylamiodarone in samples extracted from dried blood spots (DBS) was evaluated.
Amiodarone hydrochloride is an effective anti-arrhythmia medicine. However, as the disposition patterns of amiodarone in the human body show great differences between individuals, TDM (therapeutic drug monitoring) is usually necessary as part of the treatment.
In this study, using a small amount of sample extracted from dried blood spots, a rapid and easy-to-prepare LC/MS/MS method of the detection of amiodarone and amiodarone metabolites is evaluated.



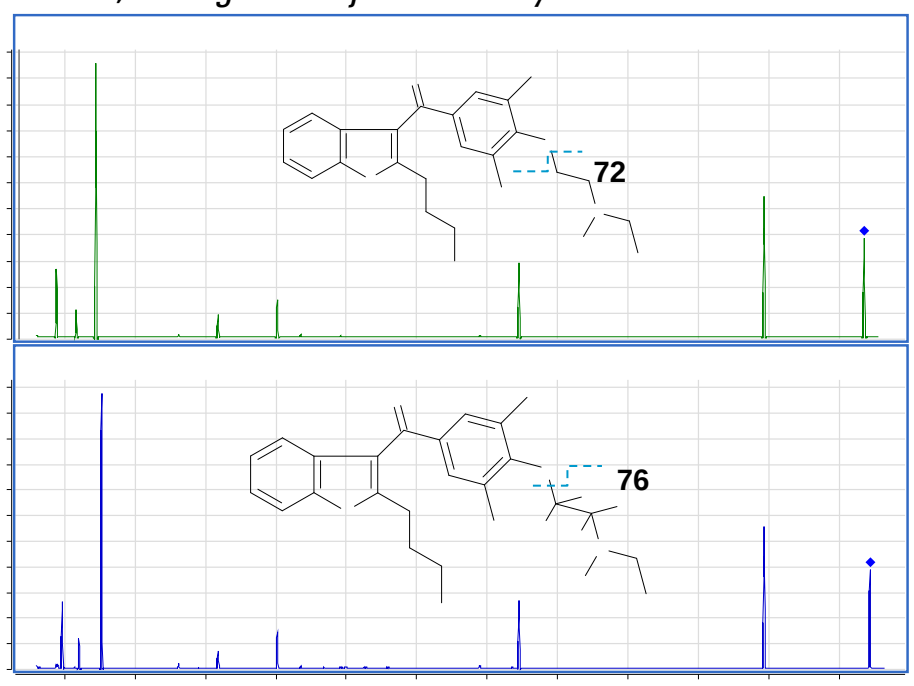
Product Ion Scan spectrum of Amiodarone Hydrochloride(upper)
and Amiodarone-d4 Hydrochloride (lower) , at collision energy=30eV

Experimental

Creating MS/MS (MRM) method for determination of amiodarone and desethylamiodarone
Reagents; Amiodarone Hydrochloride , Amiodarone-d4 Hydrochloride, Desethyl Amiodarone Hydrochloride , Desethyl Amiodarone-d4 Hydrochloride were purchaced from Toronto Research Chemicals Inc.

Mass Spectrometer ; Agilent 6460 Triple Quad LC/MS with Agilent Jet Stream Technology
Ion Source parameters ; ESI positive mode
Neblizer: 60 psi Sheath gas : 390°C, 12 L/min N_2 gas 300°C, 10 L/min
HPLC System; Agilent 1200 SL Binary Pump, Column Oven, Well Plate Autosampler

Optimization of MS/MS conditions; Optimization of MS/MS instrument parameters for MRM mode acquisition was performed using Agilent MassHunter Optimizer software, during flow injection analysis of standard solutions.



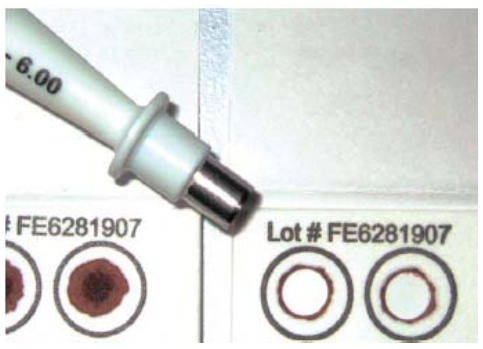
Product Ion Scan spectrum of Desethyl Amiodarone Hydrochloride(upper)
and Desethyl Amiodarone-d4 Hydrochloride (lower) , at collision evergy=15eV

Compound Information

Compound Name	Formula	Nominal Mass	Method	Precursor Ion	Fragmentor	Product Ion	Collision Energy	Abundance	Project Name
AM	C25H29I2NO3	645.02	D:\MassHunter\M	646	120	58.2	56	10927	Amiodarone2
						100.2	32	4622	Amiodarone2
						86.2	32	4245	Amiodarone2
						72.3	40	1078	Amiodarone2
DEA	C23H25I2NO3	616.99	D:\MassHunter\M	618	140	72.2	28	16613	Amiodarone2
						546.9	20	14741	Amiodarone2
						372.8	36	5413	Amiodarone2
						44.2	56	5417	Amiodarone2
d4AM	C25H33I2NO3	649.05	D:\MassHunter\M	650.1	180	58.2	56	7711	Amiodarone2
						88.2	36	2921	Amiodarone2
						104.2	32	3039	Amiodarone2
						73.2	36	2146	Amiodarone2
d4DEA	C23H29I2NO3	621.02	D:\MassHunter\M	622	100	76.3	28	34564	Amiodarone2
						546.9	20	27474	Amiodarone2
						372.8	36	10675	Amiodarone2
						48.3	60	9877	Amiodarone2

Result summary of MS/MS parameter optimization using MassHunter Optimizer software. Parameters with red circles were applied to further MRM analysis.

Sample extraction and preparation using Dried Blood Spots (DBS)
Standard solutions with each concentration shown in the table were prepared by dilution with 2 mM ammonium formate containing 0.1% formic acid . 2 µL of internal standard solution (10 µg/mL of d4-amiodarone and d4-desethylamiodarone, respectively) and standard solution (12 concentration steps of amiodarone and desethylamiodarone) were spiked in 96 µL of rat blood.

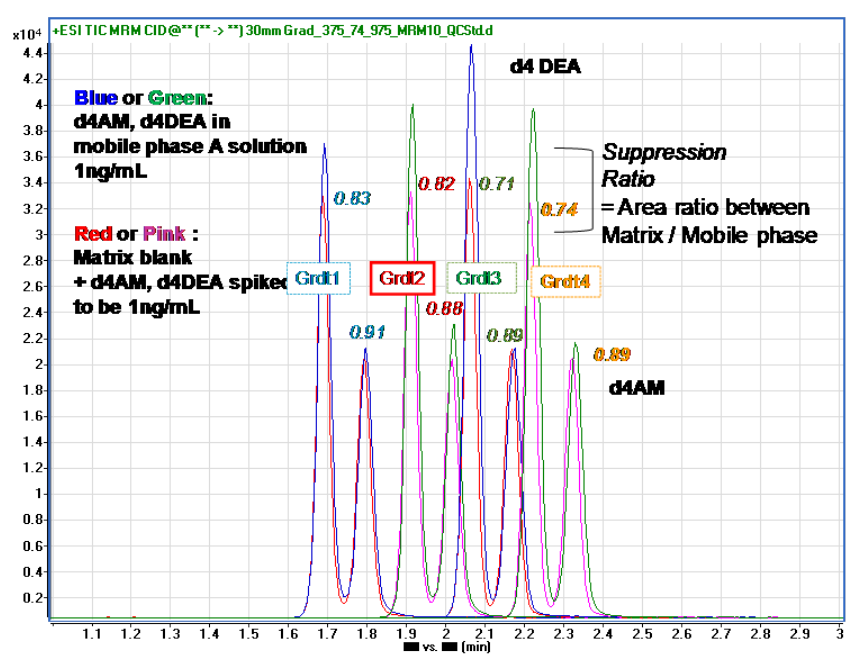


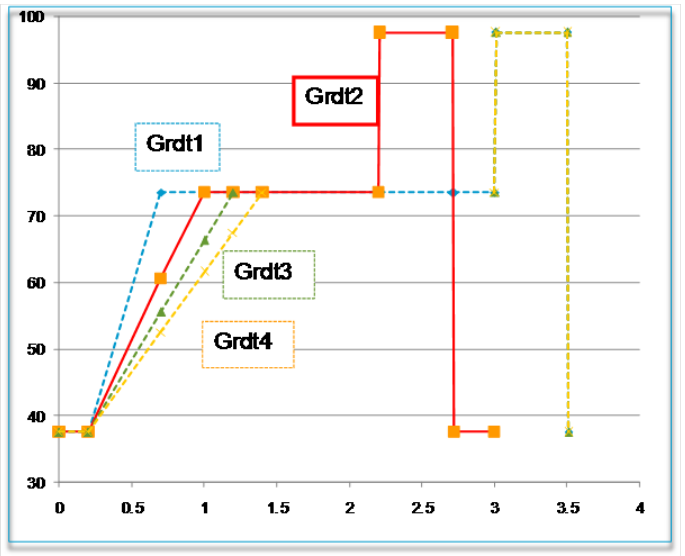
Picture of FTA-cards and the 6 mm ID puncher.
Left: A 15 µL blood spot. Right: Spots in which a 6 mm ID disk was punched.
(Reference ; Agilent Technologies Application Note 5990-4705)

Evaluation of LC/MS/MS for the Determination of Amiodarone and its Metabolite Extracted From Dried Blood Spot Samples

Results and Discussion

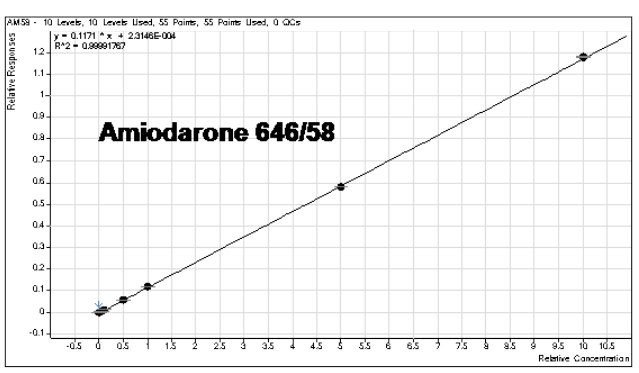
Investigation of LC separation suitable for high throughput LC/MS analysis
HPLC conditions for the separation of amiodarone and desethylamiodarone are examined. A gradient profile was developed which considered both peak separation and the peak intensities.
Column ; ZORBAX Eclipse plus C18 (2.1 x 30 mm, 1.8 µm) Column temp. ; 50°C
Mobile Phase ; A: 2 mM ammonium formate with 0.1% formic acid B: 2 mM ammonium formate with 0.1% formic acid in MeOH
Flow rate ; 0.8 mL/min Injection Volume ; 5 µL
Suppression effects for blood extracted samples in matrix varied according to the gradient profile. Peak area differences between standard solution and the standard spiked in blank matrix at the same concentration were compared.
Considering both intensity and suppression ratio results, gradient profile 2 (Gradient 2) appears to be most suitable for further quantitation work.

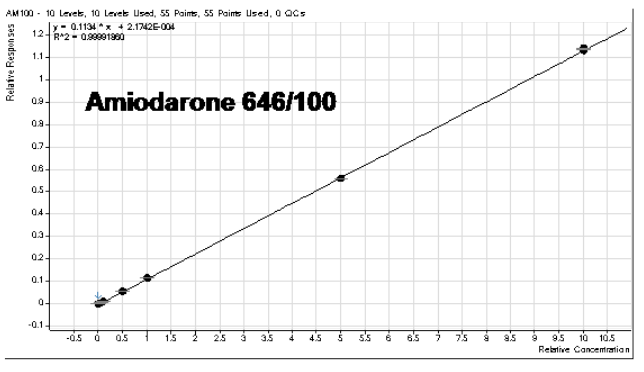


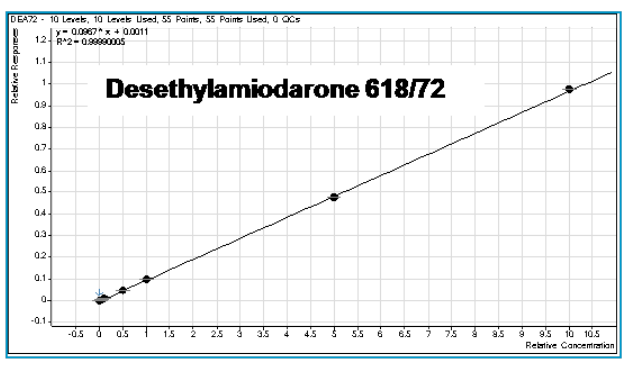


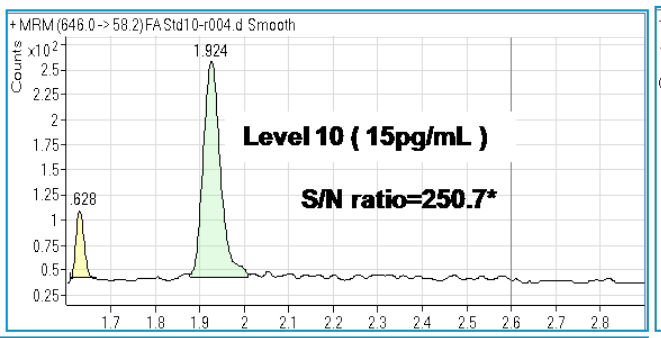
Time (min)	0	0.2	1.0	2.2	2.21	2.71	2.72	3.0
%B	37.5	37.5	73.5	73.5	97.5	97.5	37.5	37.5

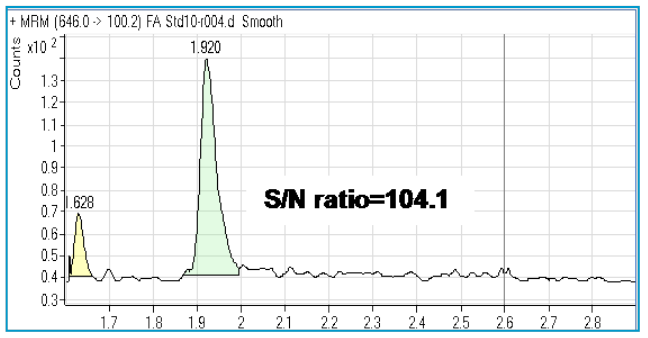
With the standard solution made up with the mobile phase A solution, there was excellent linearity for Level 10 (15 pg/mL) to Level 1 (300 ng/mL) for both amiodarone and desethylamiodarone.

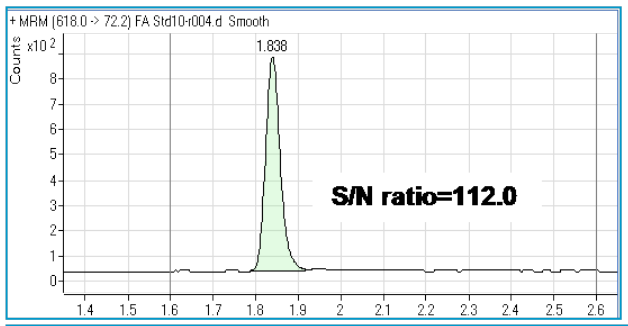


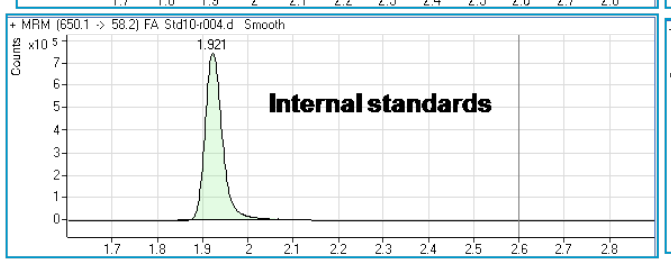


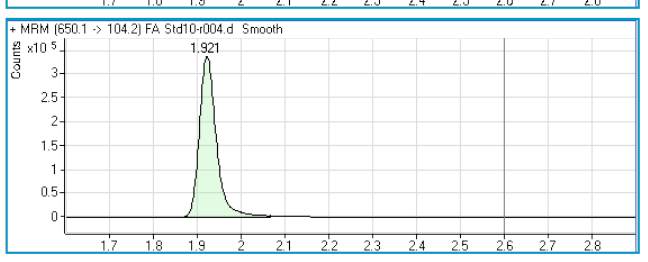


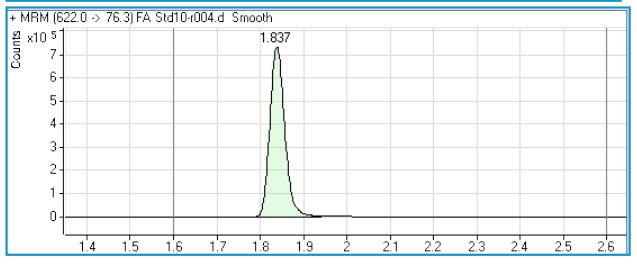












*** S/N ratio calibration is based on RMSx5 for all chromatograms.**

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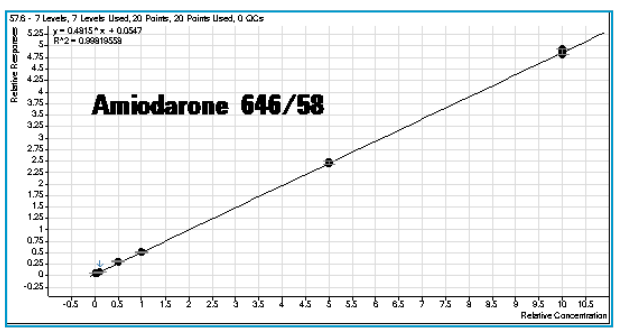
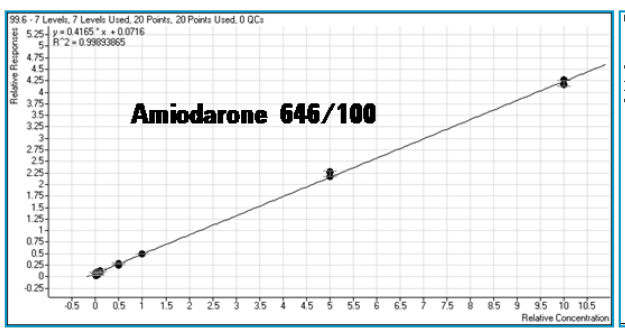
Results and Discussion

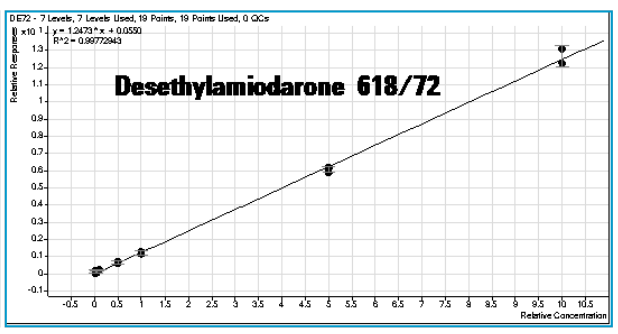
Sample solution preparation – Standard solution and Blood spot spiked samples

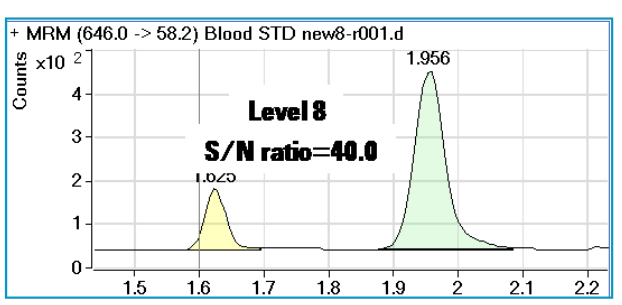
Calibration level		0	1	2	3	4	5	6	7	8	9	10	11	12
Standard solution	(µg/mL)	500	100	500	10	5	1	0.5	0.1	0.05	0.01	0.005	0.001	0.0005
Conc. In Blood spot	(ng/mL)	10,000	2,000	1,000	200	100	20	10	2	1	0.2	0.1	0.02	0.01
Final conc. (ng/mL)		1,500	300	150	30	10	3	1.5	0.3	0.15	0.03	0.015	0.003	0.0015

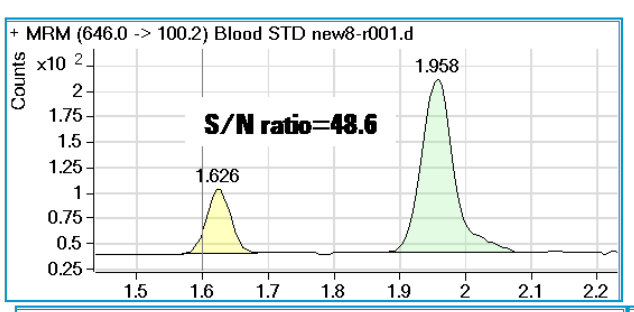
For DBS extracted standard solution, there was excellent linearity for Level 8 (150 pg/mL=1ng/mL blood) to Level 1 (300 ng/mL=2 µg/mL blood) for both amiodarone and desethylamiodarone.

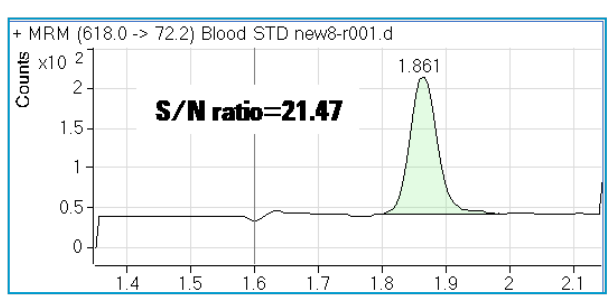


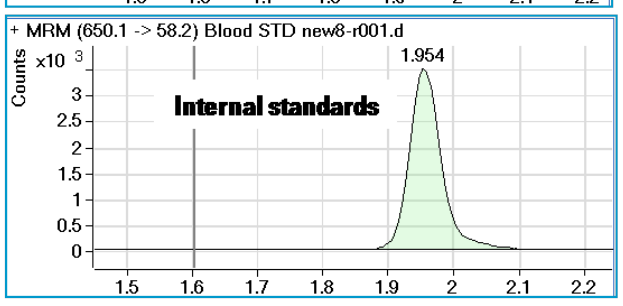


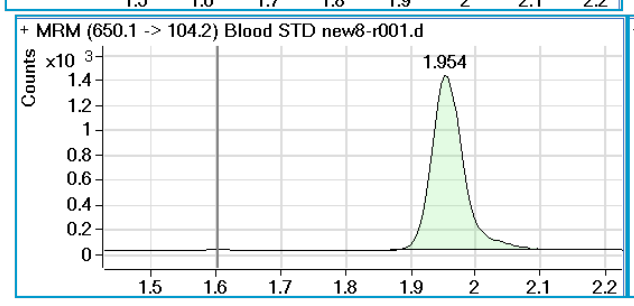


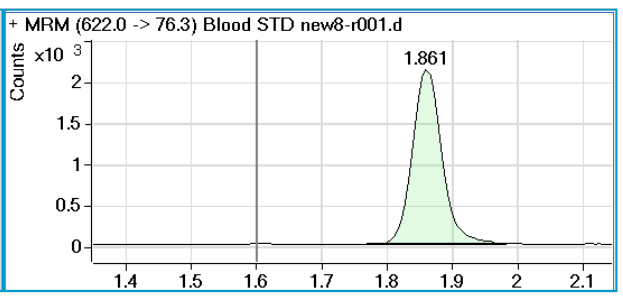












Each sample injection were replicated 3 times. At all the calibration level, accuracy values are between 85 to 115%. Calibration curves were weighed 1/x.

LC/MS/MS analysis For the detection of amiodarone, two MRM transitions (*m/z* of Q1/Q3 = 646/58 or 646/100) were monitored.

Detection Limit For both amiodarone and desethylamiodarone, the S/N ratio for chromatograms with Level 9 (0.2 ng/mL blood) were at least 5 times higher than that of Level 0 sample (Chromatograms are not shown here) . Detection limit of the method shown here is estimated at 0.2 ng/mL blood.

Application Typically for TDM, the concentrations of amiodarone in blood are usually monitored between the range of 600 ng/mL blood to 2000 ng/mL blood. The method developed in this work can be used to determine drug levels.

Further discussion Further studies will evaluate method precision and robustness.

Conclusions

Reference 1) *J. Kuhn et al. J. Pharm. Biomed. Analysis 51(2010) 210-216*
2) *Agilent Technologies Inc. Application Note 5990-4705*

Rat blood was provided by the kindly courtesy of Department of Biophysical Chemistry, Kyoto Pharmaceutical Univ.
FTA Elute MicroCards (FTA-cards) were provided by the kindly courtesy of GE Healthcare Japan Corporation.