EVALUATION OF THE RAPID DETECTION METHOD OF AMIODARONE BY LC/MS/MS

Yoshifumi Kogure, Hiroshi Sezaki
Agilent Technologies Inc., Takakura-cho 9-1, Hachioji-shi, Tokyo, Japan

Agilent Technologies

Introduction

The method for rapid detection and quantitation of amiodarone in the blood sample by LC/MS/MS was examined.

Amiodarone hydrochloride is an effective anti-arrhythmia treatment medicine classified in the Vaughan Williams class III . However, as amiodarone is a lipophilic compound, the disposition patterns of amiodarone in human bodies show the great differences between individuals and TDM (therapeutic drug monitoring) is applied.

In this study, following topics for the evaluation of LC/MS/MS method detectioning of amiodarone or amiodarone metabolite are examined;

I) Creating usual LC/MS/MS (MRM) detection method for amiodarone or desethylamiodarone
II) Usage of d4-amiodarone and d4-desethylamiodarone as the internal standards
III) Sample preparation by Dried Blood Spot (DBS) as the sample collection device
IV) Investigation of LC separation and condition suitable for rapid routine work cycles

Creating LC/MS/MS (MRM) detection method for amiodarone or desethylamiodarone

Using the standard reagent of Amiodarone Hydrochloride, LC/MS/MS conditions are examined.
Calbration curve was created by the diluted standard solution.

[Instruments]

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

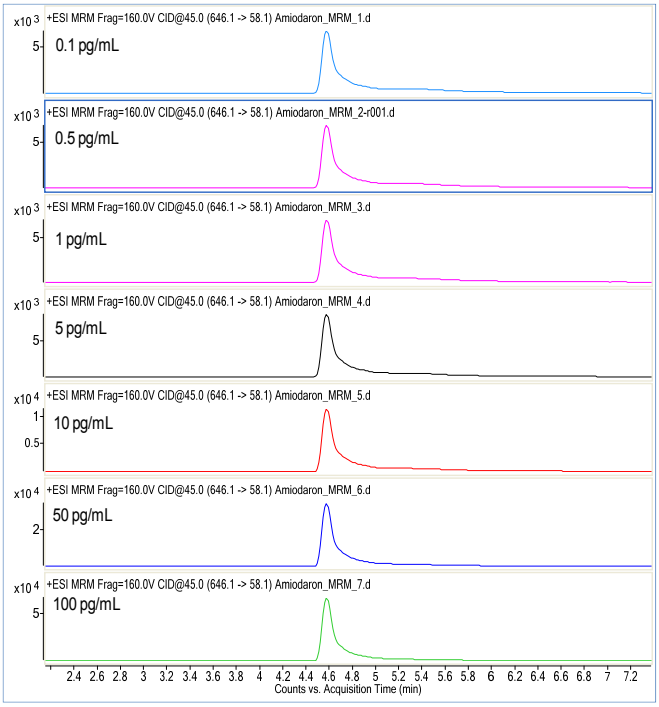
Optimization of instrument parameters for MRM mode acquisition was done by Agilent MassHunter Optimizer software.
In positive ESI ionization mode, *m/z* 646.1 were observed as [M+H]$^+$ precursor ion.

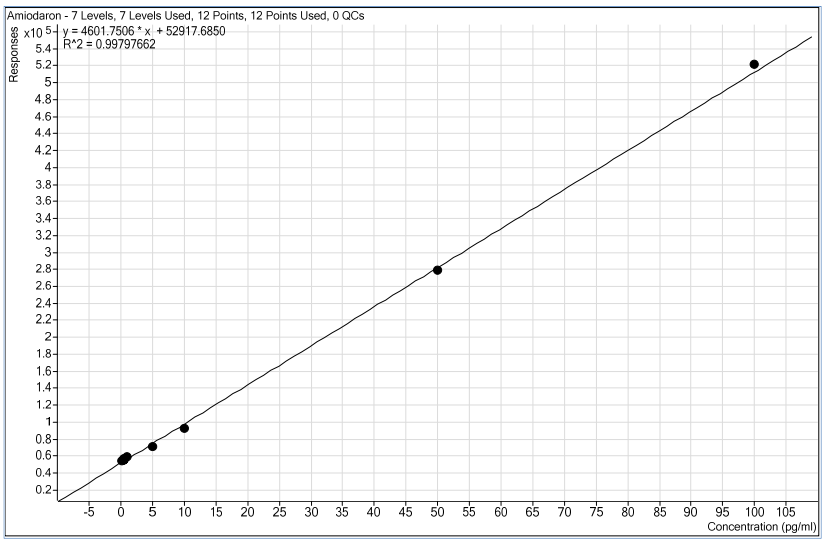
Precursor ion (*m/z*)	Product ion (*m/z*)	Fragmentor voltage (V)	Collision energy (eV)
646.1	58.1	160	45

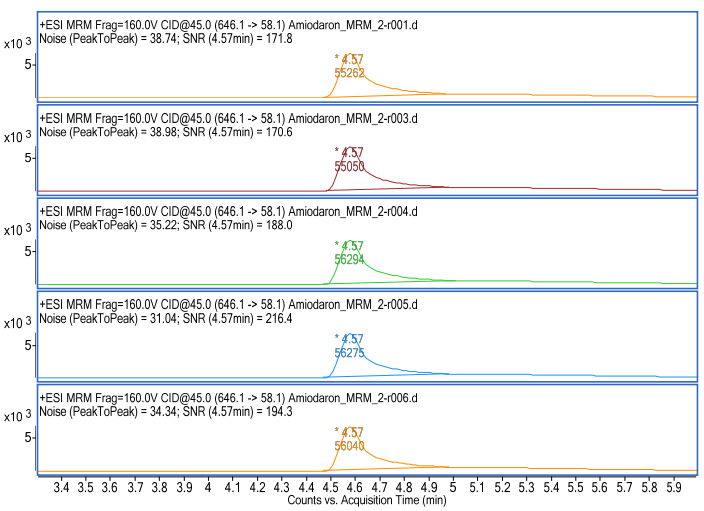
[HPLC]

Column ; ZORBAX Eclipse plus C18 (2.1 x 50 mm, 1.8 µm) Column temp. ; 50° C
Mobile Phase ; A: 10 mM ammonium formate with 0.02% formic acid B: Acetonitrile Flow rate ; 1 mL/min
Gradient ; Time (min) / %B 0min (10%) – 20min (30%) – 30min (60%) – 40min (90%) – 45min(90%)
Injection Volume ; 1 µL

Standard solution of amiodarone was diluted by mobile phase A to create the calbration sample with 0.1pg/mL to 100pg/mL concentration. There is fine linearity through the range 0.1pg/mL to 100pg/mL.







Five replicated injection of 0.5pg/mL sample solution : RSD (%) = 1.05

Reference : For the sample obtained from a patient who was administrated amiodarone, above LC/MRM method could detect amiodarone at pg/mL order as a concentration of final sample solution. Also, LC/MS/MS (product ion scan) for desethylamiodarone could be performed to check the fragment ions. Then, desethylamiodarone could be detected with same pg/mL order by LC/MRM method. (data not shown.)

Usage of d4-amiodarone and d4-desethylamiodarone as the internal standards

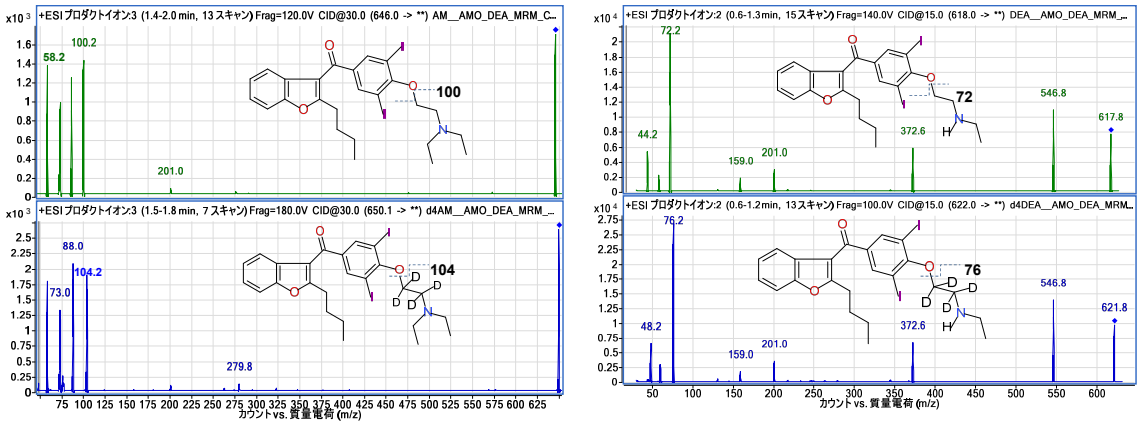
Application of deuterium isotopic standards of amiodarone and desethylamiodarone are examined.

< Reagents> Amiodarone Hydrochloride , Amiodarone-d4 Hydrochloride , Desethyl Amiodarone Hydrochloride , Desethyl Amiodarone-d4 Hydrochloride were purchaced from Toronto Research Chemicals Inc,

<Optimization of MS/MS conditions> Optimization of MS/MS instrument parameters for MRM mode acquisition was done by Agilent MassHunter Optimizer software.

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	10527	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



Sample preparation by Dried Blood Spot (DBS) as the sample collection device

Extraction procedure from slight amount of blood sample is also examined.

Rat blood was provided of 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.

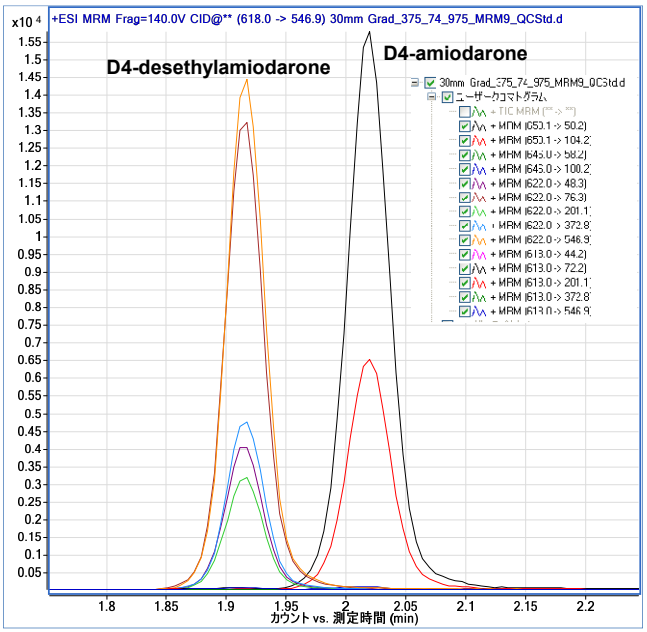
< Dried Blood Spot (DBS) extraction>

Standard solutions with each concentration shown below were prepared by dilution with HPLC mobile phase A. 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) are spiked in 96 µL of rat blood. 15 µL of spiked blood was spotted on FTA-cards. FTA-cards were dried at room temperature for about 5hours.

Disks of 6 mm ID were punched from the dried blood spot using a puncher. Disks were placed into a 2 mL Eppendorf tube in which 100 µL of ACN/water 60:40 v/v containing 2% folic acid was added. Extraction was achieved by vortex-mixing for a few seconds before ultra sonication for 15 min. After centrifugation at 12,000 rpm, the blood-extracts were transferred into clean vials. In order to simplify sample preparation, the 60% ACN-extracts were injected directly onto the LC/MS system without reconstitution or dilutions.

CAL level	0	1	2	3	4	5	6	7	8	9	10	11	12
Standard solution (µg/mL)	500	100	50	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)	1500	300	150	30	15	3	1.5	0.3	0.15	0.03	0.015	0.003	0.0015

Investigation of LC separation suitable for rapid cycles



HPLC conditions for the separation of amiodarone and desethylamiodarone are examined.

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

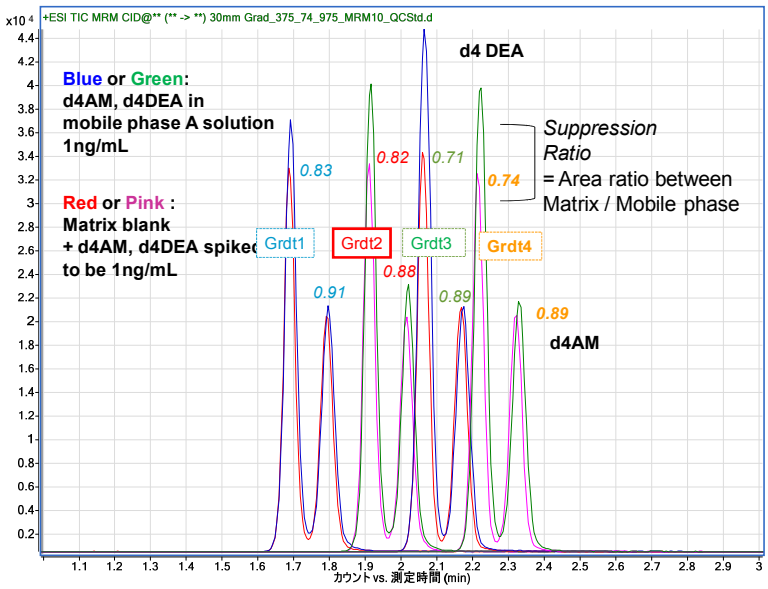
Gradient profile is investigated under considerations with the separation and the peak intensities.

One example of gradient profile shown in the left chromatogram ;

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

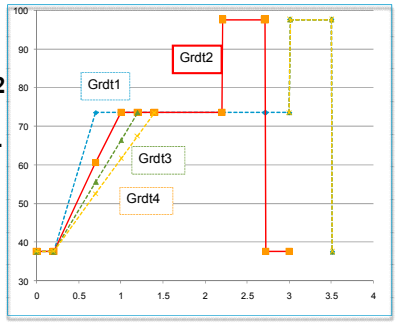
Suppression influence is considered with the gradient profiles ; defferences of intensity between standard solution and matrix-added sample was observed (next page).

Investigaion of LC separation suitable for rapid Cycles (cont.)



Suppression effect by blood extracted matrix supposed to be changed according to the gradient profile. Peak area differences between standard solution and the standard which is spiked in blank matrix to be the same concentration is compared.

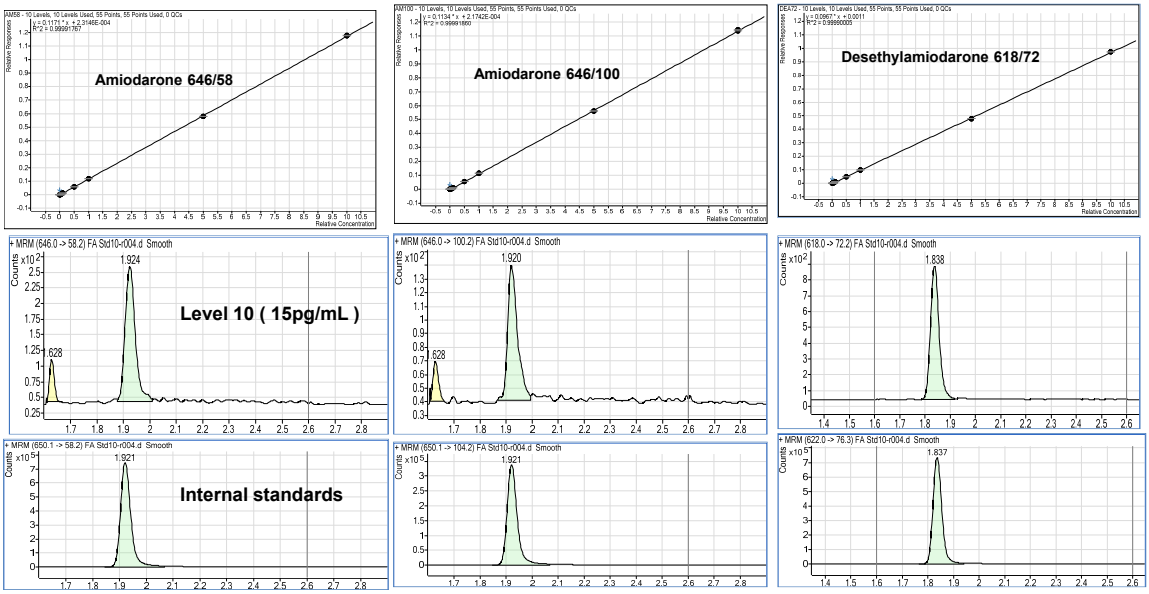
Considering both intensity and suppression ratio results, gradient profile 2 Seemed to be most suitable For further quantitation work.



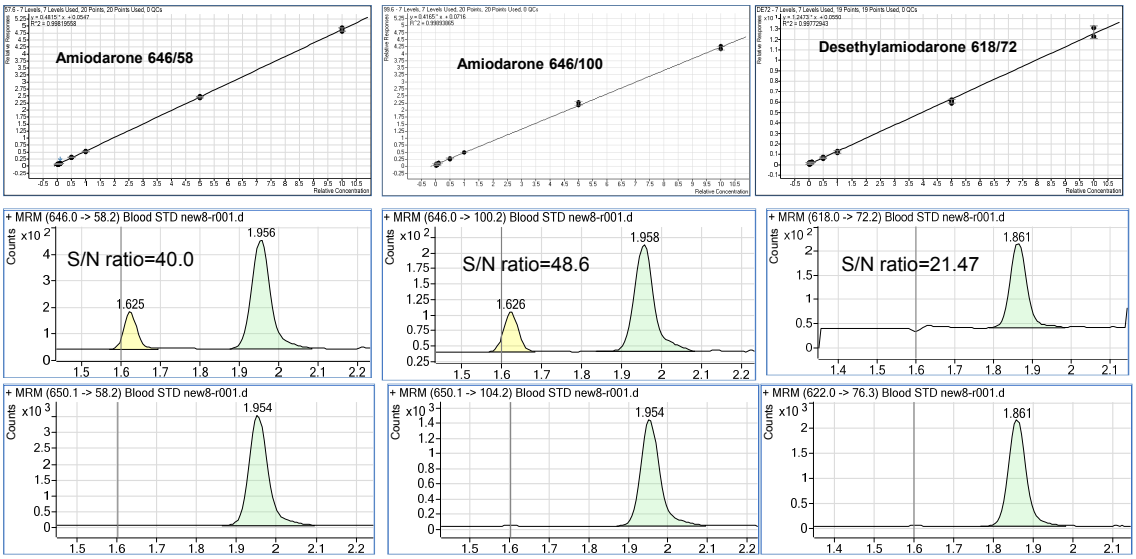
Suppression ratio is calculated based On the average peak area of three Replicate injections for each sample.

Calibration with standard solution or DBS sample

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



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



S/N ratio calicration is based to RMSx5.

Conclusion and further discussions

@ Quantitative LC/MS/MS detection for amiodarone and desethylamiodarone was examined. With Agilent 6460 Triplequad LC/MS system, the detection limit of standard amiodarone solution was 0.1pg/mL, and the limit of quantitation was 0.5pg/mL at final concentration. Reproductivity at the quantitation limit was as good as Area%RSD=1.05 (n=5).

@ Usage of isotopic labeled reagents as the internal standards combined with DBS extraction could be adapted for quantitation analysis.

@ With the blood- spiked standard, extracted through DBS method, calbration curve showed fine linearty between 1ng/mL blood to 2ug/mL.

@Further studies for prescision, reproducity , will be expected.

@Reference

J. Kuhn et al. J. Pharm. Biomed. Analysis 51(2010) 210-216