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Fully Automated Online Sample Preparation AND Quantification of Amiodarone from Whole Blood using CLAM-LC-MS/MS

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Introduction

Amiodarone is an antiarrhythmic drug used to treat and prevent irregular heartbeats. Though being very effective and reliable, during the treatment with Amiodarone serious side effects can occur. Therefore this drug has to be monitored during the medication in order to minimize those side effects and their impact on the health of the treated patient.
The method of choice for therapeutic drug monitoring in clinical laboratories is quantification by LC-MS/MS – analysis. After a whole blood sample is taken from a patient, ideally a fully automatic analysis from whole blood is desired, starting with the sample preparation and transferring the prepared sample aliquot online to the LC-MS/MS-system for subsequent Amiodarone quantification.

Methods and Materials

Sample preparation

Whole blood patient samples were taken and centrifuged by 6000 rpm (Hettich Rotofix 32 A). The obtained blood serum then was directly put into the CLAM-unit, the barcode of the sample tubes were read automatically followed by automatic sample preparation and online LC-MS/MS-analysis.

Table 1: Analytical Conditions UPLC

Instrument	: Nexera X2
Eluents	: A/C: 0.2 % FA
	B/D: 70 % MeOH, 30 % ACN, 0,2% FA
Column1	: M&N Nucleodur C8, 100 A, 5 cm
Flow1	: 0,8 mL/min
Gradient1	: 0-1 min: 2% B, 1,2-2 min: 100% B, 2,2 min: 2 % B
Column2	: Kinetex F5, 2,6 µm, 100 A, 10 cm
Flow2	: 0,6 mL/min
Gradient2	: 0-0,2 min: 100 %B, 0,21-1,2 min: 70% B, 2,25 min: 85% B, 2,3: 100%B
Injection Volume	: 2 µL
Oven-Temp.	: 40°C

Table 2: Analytical Conditions MS

Instrument	: LCMS-8050
Ionization	: ESI; +4 kV
Nebulizing Gas Flow	: 3 L/min
Heating Gas Flow	: 10 L/min
Drying Gas Flow	: 10 L/min
Interface Temp.	: 300°C
DL Temp.	: 250°C
Heat Block Temp.	: 400°C
CID Gas pressure	: 270 kPa
Loop-Time	: 0,13 sec
Dwell Time	: 10 msec

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Sample measurement

The Shimadzu LCMS-8050 equipped with **CLAM** (**C**linical **L**aboratory **A**utomated sample preparation **M**odule) was used for sample measurement. MRMs and collision energies (CE) for Desethyl-Amiodarone and Amiodarone and their corresponding deuterated standards were automatically optimized by the LabSolutions software. All compounds were measured with one quantifier and at least two qualifiers. Patient samples were automatically prepared (Figure 1), online transferred to the autosampler and directly measured by LC-MS/MS. An automatic quantification was done subsequently.

Table 3: Automatic Sample Protocol

1	Reagent Dispense	Volume	15.0	Reagent	MeOH	Pre-Suck Reagent			
2	Sample Dispense								
3	Reagent Dispense	Volume	10.0	Reagent	ZnSO4	Pre-Suck Reagent			
4	Shaking	Time	30	Speed	1900				
5	Reagent Dispense	Volume	200.0	Reagent	IS Amio	Pre-Suck Reagent			
6	Shaking	Time	180	Speed	1900				
7	Filtration	Time	90						

Results

Figure 1 shows the full chromatogram of Desethyl-Amiodarone and Amiodarone in a patient sample in less than 2,5 minutes. Therefore, a high throughput in a clinical lab can be achieved. In addition, the CLAM provides measuring emergency samples prioritized, which is essential in the field of therapeutic drug monitoring.

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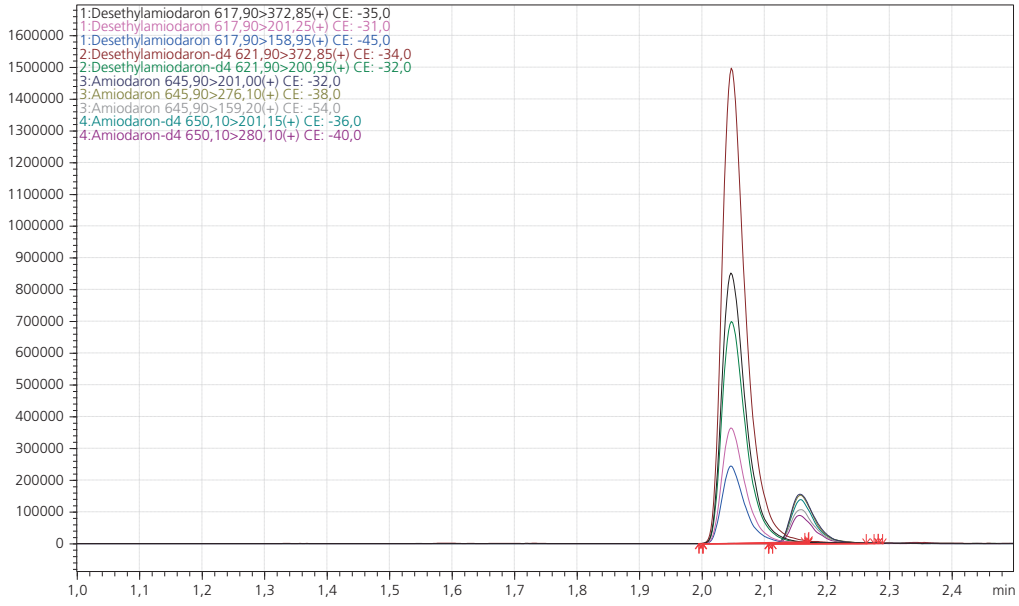
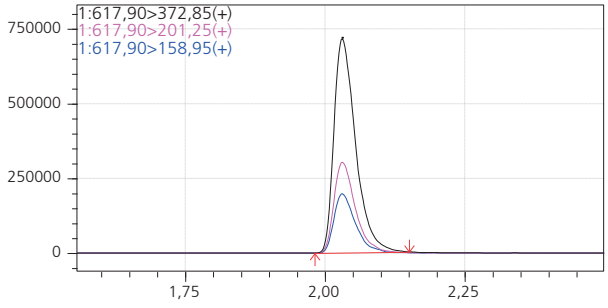
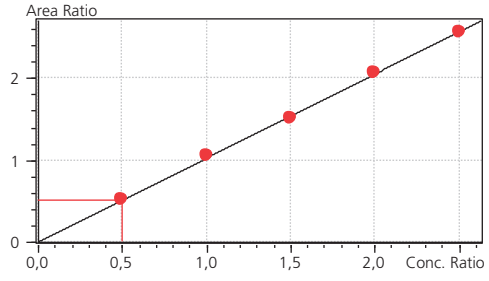


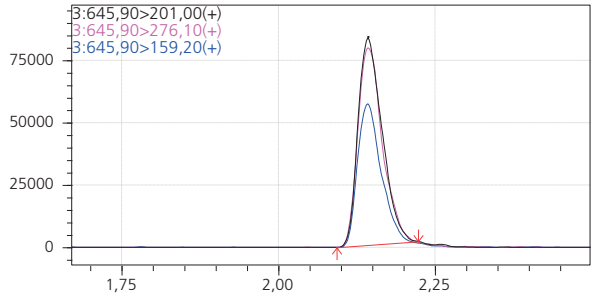
Figure 1: MS-Chromatogram of patient sample



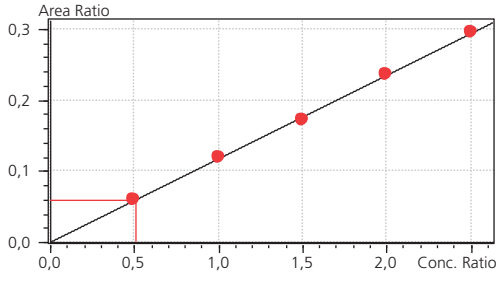
0.5 ng/ nL of Desethyl-Amiodarone



r^2 = 0,9994



0.5 ng/ nL of Amiodarone



r^2 = 0,9994

Figure 2: Peak profile at 0.5 ng/µL and calibration curves (0.5-3 ng/µL)

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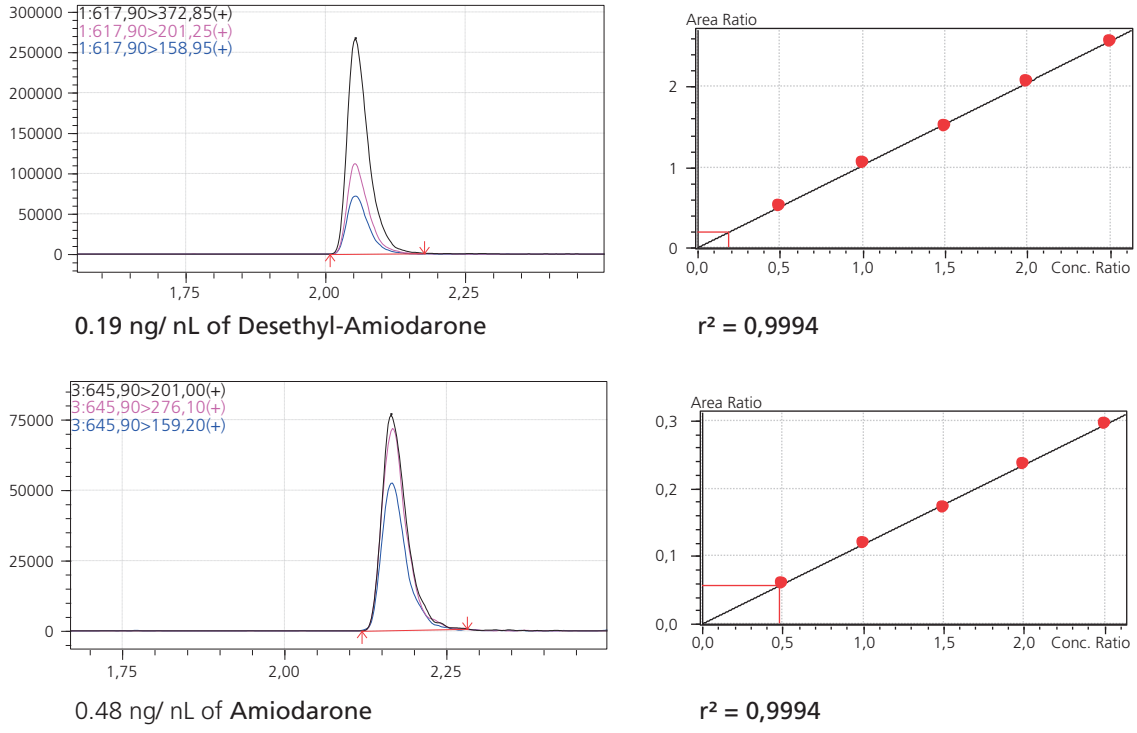

Figure 3: Peak profiles from patient sample

Peak profiles shown in figure 2 were obtained measuring five calibration levels of Desethyl-Amiodarone and Amiodarone from 0,5 to 3 ng/µl. The linear correlation factor was 0.9994 for both components indicating very good linearity. Both components were detectable at the lowest concentration of 0.5 ng/µL with high peak intensity of 750.000 for Desethyl-Amiodarone and 400.000 for Amiodarone, repectively.

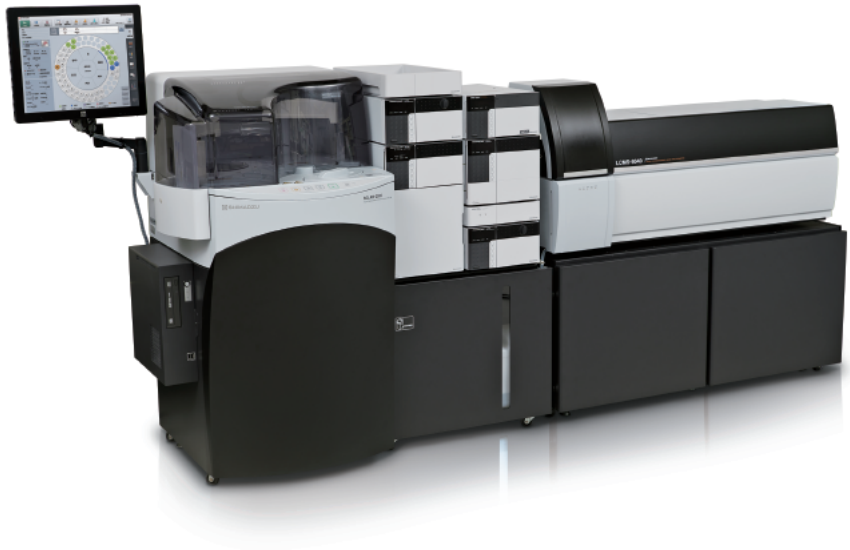

Figure 4: Shimadzu CLAM-LC-MS/MS

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Figure 3 shows peak profiles and the calculated concentration derived from the calibration curve. Even very low amounts could be detected with high peak intensities. Though measuring in a very harsh matrix, there is nearly no background noise thanks to a well established CLAM-preparation protocol and the use of a pre-column.

Conclusions

This study shows the successful **online** coupling of an automatic sample preparation module (**CLAM**) with a LC-MS/MS-system for accurate and reliable quantification for clinical drugs in whole blood . Herein it was possible, to establish a short and fast duty cycle below 2,5 min, in order to gain a high throughput of clinical samples, which is essential in the field of therapeutic drug monitoring. An excellent calibration curve linearity and -even measuring in harsh matrices like blood- good peak shapes with low background could be shown. In addition, this fully automatic system can handle clinical emergency samples, that can be measured prioritized.

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