

AMIODARONE and DESETHYLAMIODARONE IN PLASMA BY UV FAST
CODE Z33610

INTRODUCTION

Amiodarone is an antiarrhythmic agent used for various types of tachyarrhythmias, both ventricular and supraventricular arrhythmias. Amiodarone is fat-soluble, and tends to concentrate in tissues including fat, muscle, liver, lungs and skin. This confers a high volume of distribution (5000 liters in a 70 kg adult) and a long half-life. Due to the long half-life of Amiodarone, oral loading typically takes days to weeks.

Amiodarone shows beta blocker-like and calcium channel blocker-like actions on the SA and AV nodes, increases the refractory period via sodium -and potassium – channel effects, and slows intra-cardiac conduction of the cardiac action potential, via sodium-channel effects.

Amiodarone is extensively metabolised in the liver, and can affect the metabolism of numerous other drugs. The major metabolite of Amiodarone is Desethylamiodarone (DEA), which also has antiarrhythmic properties. The metabolism of Amiodarone is inhibited by grapefruit juice, leading to elevated serum levels of Amiodarone.

Amiodarone potentiates the action of warfarin. Individuals taking both of these medications should have their warfarin dose halved and their anticoagulant status (measured as prothrombin time (PT) and international normalized ratio (INR)) measured more frequently. Amiodarone inhibits the action of the cytochrome P450 isozyme family. This reduces the clearance of many drugs, including cyclosporine, digoxin, flecainide, procainamide, quinidine, sildenafil, simvastatin, theophylline and warfarin.

Unlike most other drugs, which are excreted via the urine, excretion is primarily hepatic and biliary with almost no elimination via the renal route and it is not dialyzable. Elimination half-life average of 58 days for Amiodarone and 36 days for the active metabolite, Desethylamiodarone (DEA). There is 10-50 % transfer of Amiodarone and DEA in the placenta as well as presence in breast milk. Accumulation of Amiodarone and DEA occurs in adipose tissue and highly perfused organs, therefore, if an individual was taking Amiodarone on a chronic basis, if it is stopped it will remain in the system for weeks to months.

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CE

This product fulfills all the requirements of Directive 98/79/EC on in vitro diagnostic medical devices (IVD). The declaration of conformity is available upon request.

N° 007	Amiodarone and Desethylamiodarone in plasma by UV - FAST	09/2024
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TECHNICAL FEATURES

Principle of the Method:

Amiodarone and **Desethylamiodarone** are separated treating the serum/plasma with a suitable precipitant. 10 µl are directly injected into HPLC.

Recovery : 100 %

Sensitivity (LLOD) : 0,05 mg/l

Minimum Concentration analyzable (LLOQ) : 0,15 mg/l

Linearity of the Method : 0,15 – 30 mg/l

Accuracy intra serie (relative error %)

Amiodarone :

Ci	Cs
0,2 mg/l	2 mg/l
6,0%	4,0%

Accuracy inter serie (relative error %)

Amiodarone :

Ci	Cs
0,2 mg/l	2 mg/l
6,1%	4,0%

Reproducibility intra serie (coefficient of variation %)

Amiodarone :

C LLOQ	Cm	Cs
0,15 mg/l	1 mg/l	5 mg/l
4,5%	3,4%	2,4%

Reproducibility inter serie (coefficient of variation %)

Amiodarone :

C LLOQ	Cm	Cs
0,15 mg/l	20 mg/l	40 mg/l
6,0%	4,1%	2,9%

Accuracy intra serie (relative error %)

Desethylamiodarone :

Ci	Cs
0,2 mg/l	2 mg/l
6,1%	2,9%

Accuracy inter serie (relative error %)

Desethylamiodarone :

Ci	Cs
0,2 mg/l	2 mg/l
8,3%	4,4%

Reproducibility intra serie (coefficient of variation %)

Desethylamiodarone :

C LLOQ	Cm	Cs
0,15 mg/l	1 mg/l	5 mg/l
4,1%	2,4%	1,0%

Reproducibility inter serie (coefficient of variation %)

Desethylamiodarone :

C LLOQ	Cm	Cs
0,15 mg/l	20 mg/l	40 mg/l
8,7%	3,1%	1,6%

Coefficient of correlation R2 + Dev Std :

0,9937 ± 0,0036

Therapeutic Range in Plasma:**Amiodarone** 0,25 – 2,00 mg/l**Desethylamiodarone** 0,25 – 2,00 mg/l**Components of the kit :**

All the components are ready-to-use and stable 3 years at 2-8 °C. The Calibrator is stable 18 months at 2-8 °C.

Reagent A – Deproteinization Sol. with Internal Standard, **1 x 30 ml****Reagent B** – Test Solution, **1 x 2 ml****Reagent C** – Stabilization Solution, **1 x 5 ml****Plasma Calibrator lyophil.**, **1 x 2 ml****Code Z33016 (packed separately - see data sheet)****Reagent M** – Mobile Phase, **3 x 500 ml****Minimum Instrumental equipment required:**

Isocratic HPLC System with loop of 10 µl.
Spectrophotometric Detector UV/VIS $\lambda=242$ nm
Chromatograms Recorder

Optional Equipment:

Autosampler
Operational Computer

Blood Collection Procedure:

Collect 3 ml of blood in a suitable test tube without gel and without anticoagulant. Centrifuge at 4000 rpm for 5 min. Plasma samples should be stored at -20 °C for no longer than 4 weeks.

PREANALYTICAL PROCEDURE

Test Solution Preparation

Pipette in a tube :

- 900 µl of H₂O HPLC grade
- 100 µl of **Reagent B – Test Solution**

INJECTION :

- Inject 10 µl of this solution in the chromatographic system.

Verify that the Test Solution has retention time similar to fig. below. If the Test is all right you can start with the analytical procedure; if not, check the functionality of the analytical system.

Important : Don't use this solution to calibrate

ANALYTICAL PROCEDURE

STEP 1 : Samples Preparation

Pipette in a tube with cap:

	Calibrator	Samples
Calibrator	200 µl	
Samples		200 µl
Reagent A – Deproteinization Sol.	300 µl	300 µl

Warning: deproteinize directly on Vortex for at least 10 seconds

STEP 2 : Centrifuge at 14.000 rpm for 10 minutes

STEP 3 : Take 300 µl of clear supernatant and add 50 µl of **Reagent C – Stabilization Solution**

Vortex for 10 seconds

N.B.: at this step, the sample is stable 3 days at 2-8 °C

INJECTION :

- Inject 10 µl of this solution in the chromatographic system.

AMIODARONE / DESETHYLAMIODARONE FAST - Warnings

REAGENT B : TEST SOLUTION

Amiodarone	About 16,9 mg/l
Desethylamiodarone	About 7,6 mg/l

SPECTROPHOTOMETRIC DETECTOR PARAMETERS

λ	242 nm
GAIN	0,001 AUFS
INTEGRATION TIME	10 sec.

HPLC COLUMN PROTECTION

To save the analytical column Reverse Phase POROSHELL 120 EC C-18 4,6 x 50 mm, 2,7 μ m, the use of Javelin Colfilters (1 x 10 pcs.) cod. S90199511 is obligatory.

HPLC COLUMN CONDITIONING

Install a new analytical C₁₈ Reversed-Phase column POROSHELL 120 EC-C18 (50 x 4,6 mm, 2,7 μ m).
Disconnect the detector and filter 30 ml of **H₂O : Acetonitrile (20 : 80 v / v)** set flow of 1,0 ml/minute. **Don't recycle the washing solutions.** Flux 30 ml of a solution of **H₂O : Acetonitrile (80 : 20 v / v)** at flow of 1,0 ml/minute. **Don't recycle the washing solutions.** Condition the column with the mobile phase at a flow of 1,0 ml/minute and discharge the first 20 ml. Finally inject the Chemical Standard and verify the quality of the HPLC run.
And it is possible to make analysis at recycling phase, as long as you have the foresight to filter the same mobile phase with 0.22 μ filter before each analytical run. The signal that the mobile phase must be changed is given by the significant increase of the background noise of the detector Fluorimetric. If room temperature is > 20 °C store the Mobile Phase at 2-8 °C between an analytical session and another.

COLUMN CLEANING

Disconnect the detector. Flux 15 ml of **H₂O : Acetonitrile or Methanol (80 : 20 v / v)** set flow of 1,0 ml/minute and discharge. Flux a solution of **H₂O : Acetonitrile or Methanol (30 : 70 v / v)** for 30 minutes discharge it. **It is recommended to perform the washing of Analytical Column at the end of each analytical run.** Store the column in a solution of **H₂O : Acetonitrile or Methanol (30 : 70 v / v).**

INJECTION NEEDLE WASHING

Wash with a solution of Water / Ethanol or Methanol or Acetonitrile (80 : 20 v/v).

HPLC PARAMETERS

LOOP	10 μ l
FLOW	1,5 ml/min.
PRESSURE	About 110 bar

ACCESSORIES AND CONSUMABLES

CODE	DESCRIPTION	PACKAGING
Z33016	Calibrator in plasma for Amiodarone/Desethylamiodarone	4 x 2 ml
Z33019	Control in plasma for Amiodarone/Desethylamiodarone, Levels 1 and 2	2 x 5 x 2 ml
Z699975902	Poroshell 120 EC C18 (50 x 4,6mm –2,7 u) Analytical Column	1 Pc
S90199511	Javelin Colfilters	1 x 10 Pcs
S51843550	Clear glass vials with reduced volume from 1,5 ml to 15 ul	1 x 100 Pcs
S51820717	Caps for Clear glass vials with reduced volume from 1,5 ml to 15 ul	1 x 100 Pcs

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(Reference Chromatograms)

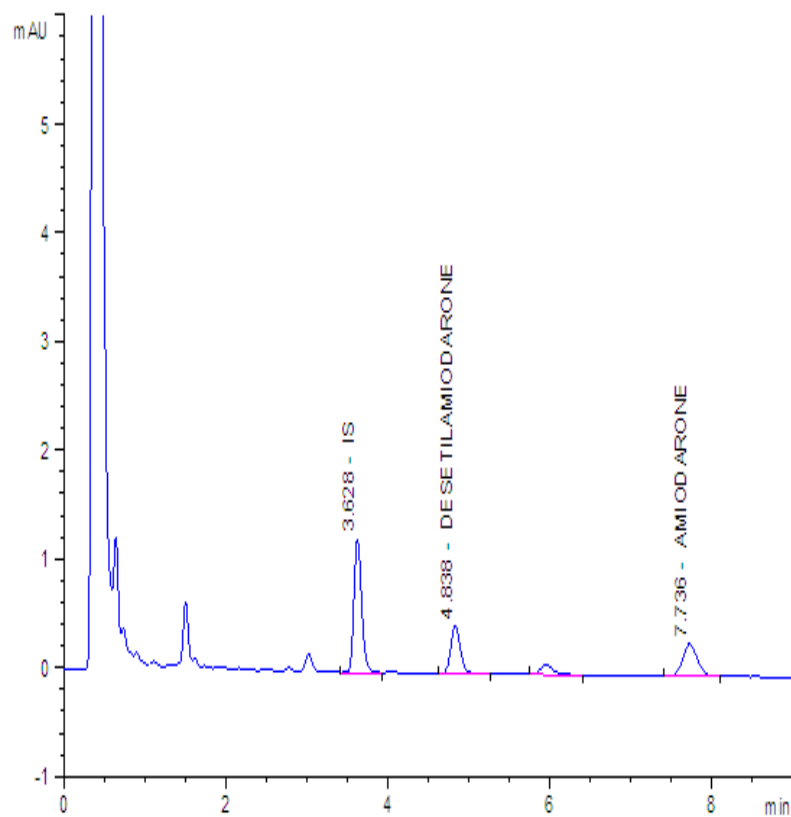


Fig. 1	Plasma Control Level 1
	R.T. 3.62 Internal Std
	R.T. 4.83 Desethylamiodarone 0,9 mg/l
	R.T. 7.73 Amiodarone 0,94 mg/l

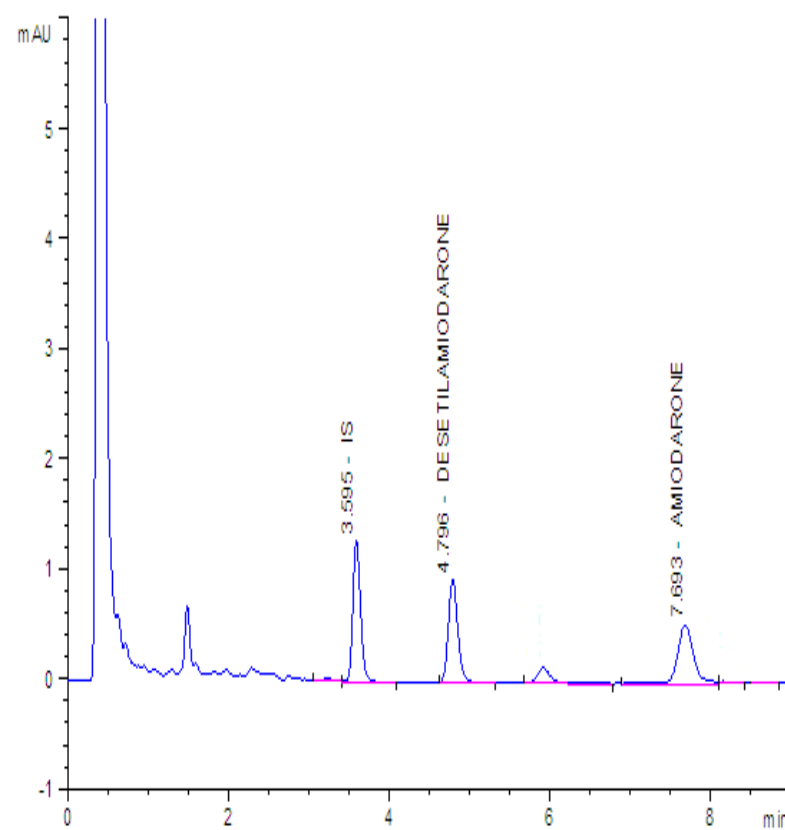


Fig. 2	Plasma Calibrator
	R.T. 3.59 Internal Std
	R.T. 4.79 Desethylamiodarone 2,0 mg/l
	R.T. 7.69 Amiodarone 2,1 mg/l