

ANTIARRHYTHMICS IN PLASMA BY LC/MS

Code LC99110





This product fulfills all the requirements of Directive 98/79/EC on in vitro diagnostic medical devices (IVD).

The declaration of conformity (CE) is available upon request.



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N° 001		10/2024
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INTRODUCTION

Amiodarone, Desethylamiodarone, Flecainide, Propafenone, Sotalol, Quinidine

Amiodarone Butil-2-(dihedral-3.5-N-diethylaminoethoxy-4-benzoyl)-3-benzofuran)PM=654.32 is an antiarrhythmic drug used to treat supraventricular and ventricular arrhythmias, where other antiarrhythmic molecules are ineffective.

Amiodarone has the ability to block adrenoreceptors.

There is a correlation between the oral dose taken and concentrations in target tissue and plasma, but plasma concentrations in different patients taking the same dose may vary significantly.

The efficiency of Amiodarone is expressed at concentrations close to 1.0 mg/litre, while at concentrations > 2.5 mg/litre it is toxic.

Amiodarone is bound to plasma proteins for about 96%.

Its major active metabolite is **N-desethyl-amiodarone**.

It is a highly unstable molecule; tests performed by placing 5 g/ml of Amiodarone in a saline solution at pH=7.4 in plastic tubes, have shown, after 12 minutes, at room temperature, a decrease in concentration of 20%; the same solution after 4 days at 5°C contained only 10% of the unmodified drug.

Amiodarone is instead stable in a 50% ethanol solution.

It is also stable for more than 6 weeks if stored in glass tubes at -20°C.

Flecainide is a class IC antiarrhythmic drug according to Vaughan-Williams' classification. It acts by slowing down the depolarization of the myocardiac cell (phase 0) mainly on the His-Purkinje conduction system (H-V conduction) and, to a lesser extent, on atrioventricular and interatrial nodal conduction.

Bioavailability: after oral administration, Flecainide is rapidly and almost completely absorbed by the gastrointestinal tract; bioavailability is almost complete (over 90% of the dose) and independent from food.

Steady-state: the established blood levels are reached 3-5 days after the start of therapy: there was no evidence of accumulation after prolonged treatment.

Metabolism: Flecainide is largely metabolized to two main metabolites, which are then conjugated and are not considered pharmacologically active.

Elimination: the excretion is essentially urinary, about 30% of the dose as Flecainide unchanged and the rest as metabolites: only 5% is eliminated with faeces. In the case of urine with pH 8, as, for example, in cases with tubular renal acidosis or in patients on a strictly vegetarian diet, the elimination of Flecainide is greatly slowed down.

Note: The monitoring of plasma levels is important in patients with severe hepatic or renal impairment, in which the elimination can be slowed down. It is also recommended in patients with associated Amiodarone therapy and may also be useful in patients with congestive heart failure and renal impairment, even if minor.

Flecainide concentrations should be measured after 3 or 4 days.

Propafenone is an antiarrhythmic drug blocking sodium channels (Class IC). Propafenone acts by reducing the rate of rise of phase 0 of the action potential. Its prevailing effect is therefore the depression of the conduction velocity at the level of all cardiac structures.

The refractory period, which is not modified by the drug at normal heart rates, is instead prolonged (post-ripolarization refractoriness) in case of high activation rates (tachycardias, fibrillation or atrial flutter).

The level of blockage of sodium channels is, characteristically, frequency-dependent. This explains why the effects of Propafenone, both therapeutic and pro-rhythmic, are more pronounced at high activation frequencies.

Drug interactions: dicumarolic anticoagulants (Warfarin) enhances action (risk of bleeding); Digoxin: increases the plasma concentration of Digoxin.

Bioavailability: it depends on the type of formulation (50 - 95 %) but increases when Steady-state is reached.

Plasma peak: after oral administration it reaches its maximum action after 2-3 hours and the effect persists for more than 8 hours. Since Propafenone is subject to first-pass liver metabolism and has non-linear (exponential) kinetics, with the extended-release formulation, the share of the drug available at the blood level is, at the same dosage, lower. (The formulation on this product is very important).

Hepatic metabolism, saturated type (cytochrome P450) with formation of pharmacologically active metabolites (5OH-propaphenone, N-depropionyl-propaphenone).

Elimination: Propaphenone is largely metabolized in the liver and excreted almost completely as metabolites in stool and urine.

Sotalol is an active ingredient of specific indication against cardiac arrhythmias. Among the non-selective beta-blockers, sotalol is one of the most water-soluble and in addition to beta-blocking activity, it is also able to block the potassium channel, acting as a class III antiarrhythmic. This allows the use of this drug for atrial fibrillation in conjunction with ventricular tachyarrhythmias.

Subtalol is a drug that has long been studied because of its unique pharmacological properties, showing both beta-blocker and potassium channel blocker activity.

The intake of sotalol is indicated in case of ventricular arrhythmias, both for the treatment of life-threatening ventricular tachyarrhythmias and for the symptomatic treatment of unsupported ventricular tachyarrhythmias. It is also used in supraventricular arrhythmias, particularly in the prophylaxis of paroxysmal atrial tachycardia, paroxysmal atrial fibrillation by reentry into the atrioventricular node through accessory pathways and in maintaining sinus rhythm after atrial fibrillation.

Quinidine is an antiarrhythmic drug, belonging to Vaughan Williams' class Ia. It is an alkaloid that was extracted from the bark of the Cinchona tree.

It is a diastereoisomer of quinine. Having very similar structures, they have similar, but opposite, stereochemical properties, such as to be defined quasi-enantiomers. It is used in the treatment of supraventricular arrhythmias, such as fibrillation and atrial flutter. It is also a P glycoprotein inhibitor (MDR1 or ABCB1) at the blood-brain barrier which is an ABC transporter involved in the elimination of xenobiotics.

TECHNICAL FEATURES

RECOVERY			Amiodarone	99,2%	
			Desethylamiodarone	92,9%	
			Flecainide	88,3%	
			Propafenone	82,7%	
			Sotalol	81,5%	
			Quinidine	115,9%	
SENSITIVITY (LLOD):			Amiodarone	0,003 mg/l	
			Desethylamiodarone	0,003 mg/l	
			Flecainide	0,003 mg/l	
			Propafenone	0,003 mg/l	
			Sotalol	0,01 mg/l	
			Quinidine	0,01 mg/l	
MINIMUM	CONCENTRATION	ANALYZABLE	Amiodarone	0,01 mg/l	
(LLOQ):			Desethylamiodarone	0,01 mg/l	
			Flecainide	0,01 mg/l	
			Propafenone	0,01 mg/l	
			Sotalol	0,05 mg/l	
			Quinidine	0,05 mg/l	
LINEARITY:			Amiodarone	0,01 – 25 mg/l	
			Desethylamiodarone	0,01 – 25 mg/l	
			Flecainide	0,01 – 25 mg/l	
			Propafenone	0,01 – 25 mg/l	
			Sotalol	0,05 – 50 mg/l	
			Quinidine	0,05 – 50 mg/l	
<u>Accuracy intra serie (relative error %)</u>			Ci	Cs	
<u>Amiodarone:</u>			0,1 mg/l	1 mg/l	
			5,45%	1,48%	
<u>Accuracy inter serie (relative error %)</u>			Ci	Cs	
<u>Amiodarone:</u>			0,1 mg/l	1 mg/l	
			7,62%	3,74%	
<u>Reproducibility intra serie (coefficient of variation %)</u>			C LLOQ	Cm	CUP
<u>Amiodarone:</u>			0,01 mg/l	1 mg/l	2,5 mg/l
			7,22%	2,41%	1,12%
<u>Reproducibility inter serie (coefficient of variation %)</u>			C LLOQ	Cm	CUP
<u>Amiodarone:</u>			0,01 mg/l	20 mg/l	40 mg/l
			8,22%	3,87%	2,90%
<u>Coefficient of Correlation R2 + Dev Std:</u>			0,9990 ± 0,0007 Amiodarone		
<u>Accuracy intra serie (relative error %)</u>			Ci	Cs	
<u>Desethylamiodarone:</u>			0,1 mg/l	1 mg/l	
			5,48%	1,45%	

<u>Accuracy inter serie (relative error %)</u> <u>Desethylamiodarone:</u>	<table><tr><td>Ci</td><td>Cs</td></tr><tr><td>0,1 mg/l</td><td>1 mg/l</td></tr><tr><td>7,55%</td><td>3,74%</td></tr></table>	Ci	Cs	0,1 mg/l	1 mg/l	7,55%	3,74%			
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0,01 mg/l	20 mg/l	40 mg/l								
12,19%	3,44%	2,62%								
<u>Coefficient of Correlation R2 + Dev Std:</u>	0,9987 ± 0,0003 Desethylamiodarone									
<u>Accuracy intra serie (relative error %)</u> <u>Flecainide:</u>	<table><tr><td>Ci</td><td>Cs</td></tr><tr><td>0,1 mg/l</td><td>1 mg/l</td></tr><tr><td>8,59%</td><td>4,83%</td></tr></table>	Ci	Cs	0,1 mg/l	1 mg/l	8,59%	4,83%			
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C LLOQ	Cm	CUP								
0,01 mg/l	20 mg/l	40 mg/l								
8,84%	6,37%	5,37%								
<u>Coefficient of Correlation R2 + Dev Std:</u>	0,9975 ± 0,0005 Flecainide									
<u>Accuracy intra serie (relative error %)</u> <u>Propafenone:</u>	<table><tr><td>Ci</td><td>Cs</td></tr><tr><td>0,1 mg/l</td><td>1 mg/l</td></tr><tr><td>2,86%</td><td>3,52%</td></tr></table>	Ci	Cs	0,1 mg/l	1 mg/l	2,86%	3,52%			
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C LLOQ	Cm	CUP								
0,01 mg/l	20 mg/l	40 mg/l								
8,40%	3,80%	3,17%								
<u>Coefficient of Correlation R2 + Dev Std:</u>	0,9987 + 0,0005 Propafenone									

<u>Accuracy intra serie (relative error %)</u> <u>Sotalol:</u>	Ci	Cs	
	0,5 mg/l	2,5 mg/l	
	10,43%	2,84%	
<u>Accuracy inter serie (relative error %)</u> <u>Sotalol:</u>	Ci	Cs	
	0,5 mg/l	2,5 mg/l	
	11,08%	2,41%	
<u>Reproducibility intra serie (coefficient of variation %)</u> <u>Sotalol:</u>	C LLOQ	Cm	CUP
	0,05 mg/l	1 mg/l	5 mg/l
	4,14%	3,88%	1,30%
<u>Reproducibility inter serie (coefficient of variation %)</u> <u>Sotalol:</u>	C LLOQ	Cm	CUP
	0,05 mg/l	20 mg/l	40 mg/l
	7,23%	4,37%	2,48%
<u>Coefficient of Correlation R2 + Dev Std:</u>	0,9986 ± 0,0006 Sotalol		
<u>Accuracy intra serie (relative error %)</u> <u>Quinidine:</u>	Ci	Cs	
	0,5 mg/l	2,5 mg/l	
	14,12%	8,16%	
<u>Accuracy inter serie (relative error %)</u> <u>Quinidine:</u>	Ci	Cs	
	0,5 mg/l	2,5 mg/l	
	14,99%	6,35%	
<u>Reproducibility intra serie (coefficient of variation %)</u> <u>Quinidine:</u>	C LLOQ	Cm	CUP
	0,05 mg/l	1 mg/l	5 mg/l
	5,93%	8,95%	6,06%
<u>Reproducibility inter serie (coefficient of variation %)</u> <u>Quinidine:</u>	C LLOQ	Cm	CUP
	0,05 mg/l	20 mg/l	40 mg/l
	12,69%	18,15%	12,46%
<u>Coefficient of Correlation R2 + Dev Std:</u>	0,9964 ± 0,0007 Quinidine		

COMPONENTS OF THE KIT (100 TESTS)

Reagent A – Deproteinization Solution	1 x 40 ml	
Reagent B – Deuterated Internal Standard Solution	1 x 5 ml	Store at -20 °C
Reagent C – Diluting Solution	1 x 30 ml	
Calibrator in plasma – Level 0	1 x 1 ml	Code LC99116 (Packed separately – see data sheets)
Calibrator in plasma – Level 1	1 x 1 ml	Code LC99116 (Packed separately – see data sheets)
Calibrator in plasma – Level 2	1 x 1 ml	Code LC99116 (Packed separately – see data sheets)
Calibrator in plasma – Level 3	1 x 1 ml	Code LC99116 (Packed separately – see data sheets)
Calibrator in plasma – Level 4	1 x 1 ml	Code LC99116 (Packed separately – see data sheets)
Calibrator in plasma – Level 5	1 x 1 ml	Code LC99116 (Packed separately – see data sheets)
Calibrator in plasma – Level 6	1 x 1 ml	Code LC99116 (Packed separately – see data sheets)
Reagente M1 – Mobile Phase M1	1 x 500 ml	
Reagente M2 – Mobile Phase M2	2 x 500 ml	

All reagents are ready to use and stable 3 years at room temperature, except Reagent B which should be stored at -20°C. The storage method of the Lyophilic Calibration Standard is described in the dedicated data sheet.

ACCESSORIES AND CONSUMABLES

CODE	DESCRIPTION	PACKAGING
LC99116	Calibrator in plasma for Antiarrhythmics	7 x 2 x 1 ml
LC99119	Control in plasma for Antiarrhythmics - Levels 1, 2 e 3	3 x 5 x 1 ml
SK99110	Starter kit for Antiarrhythmics in plasma	1 Pc
Z699675902	Poroshell 120 EC-C18 (50 x 2,1 mm, 1,9 µm) Analytical Column	1 Pc
S29057U	Standard Vial of 2 ml with screw cap	100 Pcs

MINIMUM INSTRUMENTAL EQUIPMENT REQUIRED

LC-MS/MS Triple Quadrupole
MRM mode, ESI positive

OPTIONAL EQUIPMENT

Autosampler
Operational Computer

MODE OF WHOLE BLOOD COLLECTION

Take 3 ml of venous blood in a test tube without gel for serum or with Heparin as anticoagulant for plasma.

Centrifuge at 4000 rpm for 5 minutes.

Separate the serum and store at - 20 °C. Stable 4 weeks.

ANALYTICAL PROCEDURE

STEP 1

IMPORTANT: Consider that, in STEP 2, 300 µl per sample of this diluted solution is required. Then prepare the necessary amount for the analytical session.

Dilute **Reagent B - Internal Standard Solution** with **Reagent A - Deproteinization Solution** 1:10.

Vortex for 10 seconds

The internal standard, once diluted, is stable one week from the day of preparation if stored at -20°C

STEP 2

Dispense in an Eppendorf tube:

- 100 µl of **Calibrator (C0-C6)/Controls/Samples**
- 300 µl di **Deproteinization Solution + Internal Standards** prepared in **STEP 1**

Vortex for 30 seconds

Centrifuge at 14.000 rpm for 10 minutes

STEP 3

Dispense in a vial:

- 200 µl of **supernatant**
- 300 µl of **Reagente C – Diluting Solution**

Vortex for 5 seconds

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

INJECTION

- Inject 1 µl of solution in HPLC (according to instrumental sensitivity)

ANTIARRHYTHMICS IN PLASMA by LC/MS - Warnings

COLUMN CONDITIONING

Install the new Poroshell EC-C18 analytical column (50 x 2.1 mm, 1.9 µm) thermostated at 25°C. Disconnect the detector and run a Mobile Phase M1 solution: Mobile Phase M2 (20 : 80 v/v) at a flow rate of 400 µl / minute for 10 minutes. Do not recycle washing liquids. Condition the column with a Mobile Phase M1 solution: Mobile Phase M2 (20 : 80 v/v) at 400 µl / minute flow for 10 minutes. Then flux a Mobile Phase M1 solution: Mobile Phase M2 (80 : 20 v/v) at a flow rate of 400 µl / minute, for 5 minutes. Then, flux Mobile Phase M1 : Mobile Phase M2 (65 : 35 v/v) at a flow rate of 400 µl / minute, for 10 minutes. Finally, perform 3 mobile phase injections before launching the session.

Phase recirculation analysis is NOT possible.

If the T Amb of the Laboratory is 25°C it is recommended to keep the Mobile Phase at 2-8°C between an analytical session.

COLUMN CLEANING

Disconnect the detector. Flushing a Mobile Phase M1 solution: Mobile Phase M2 (80 : 20 v/v) at a flow of 400 µl / minute for 5 minutes by discharging it, then flush Mobile Phase M1 : Mobile Phase M2 (10 : 90 v/v) at a flow rate of 400 µl / minute for 15 minutes. The column should be stored in a Mobile Phase M1 solution: Mobile Phase M2 (10 : 90 v/v).

INJECTION NEEDLE WASHING

Wash the syringe with Mobile Phase M2.

ESI+ PARAMETERS OPTIMIZED ON Waters TQ-S micro ESI+

Capillary	3,00 kV
Desolvation Temperature	200 °C
Desolvation Gas	600 L/hour
Dwell time	25 ms

GRADIENT SETTING

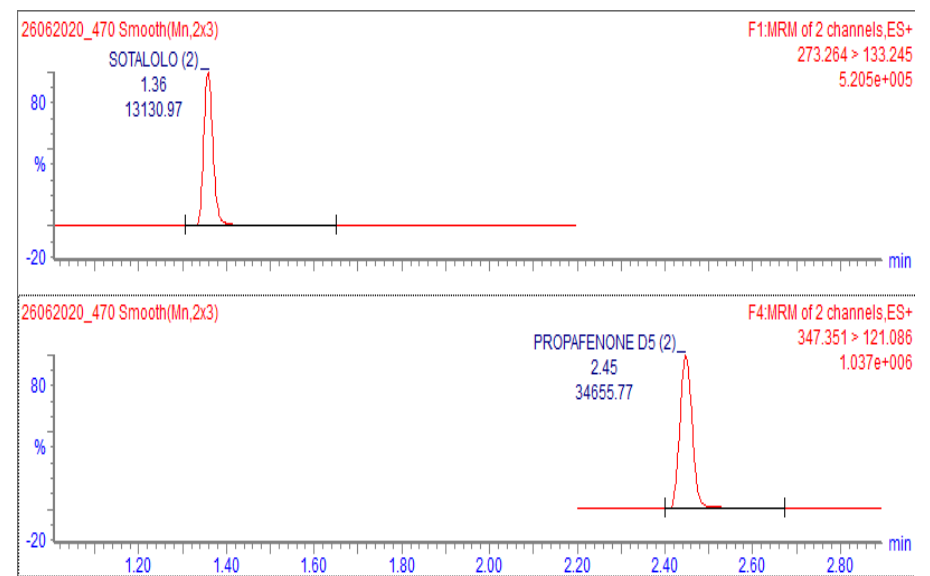
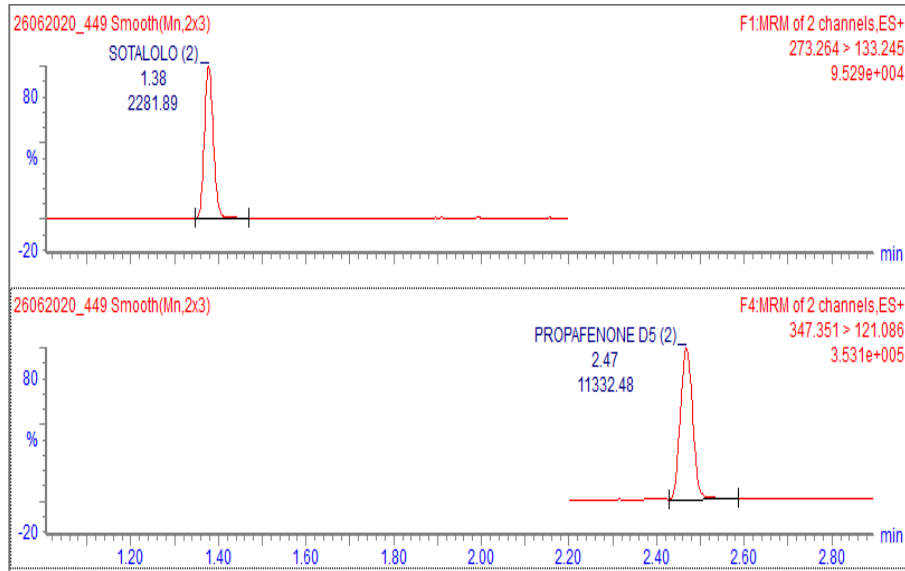
Time (min)	% M1 (PUMP A)	% M2 (PUMP B)	FLOW (µl/min)
0	100	0	500
1.0	75	25	500
3.5	10	90	500
4.01	10	90	500
4.10	100	0	500
5.0	100	0	500

Fragmentations (optimized on XEVO TQ-S micro)

Analita	Q1	Q3	CONO (V)	ENERGIA DI COLLISIONE
Sotalol (1)	273	33	28	32
Sotalol (2)	273	213	28	24
Quinidine (1)	325	184	58	28
Quinidine (2)	325	172	58	46
Propafenone (1)	342	116	46	28
Propafenone (2)	342	98	46	28
Propafenone-D5 (1)	347	121	16	24
Propafenone-D5 (2)	347	103	16	26
Flecainide (1)	415	301	48	42
Flecainide (1)	415	232	48	54
Flecainide-D3 (1)	418	304	2	40
Flecainide-D3 (1)	418	98	2	34
Desethylamiodarone (1)	618	72	74	48
Desethylamiodarone (2)	618	58	74	60
Desethylamiodarone-D4 (1)	622	373	60	50
Desethylamiodarone-D4 (1)	622	201	60	44
Amiodarone (1)	646	58	58	76
Amiodarone (2)	646	100	58	44
Amiodarone-D4 (1)	650	88	10	50
Amiodarone-D4 (2)	650	104	10	50

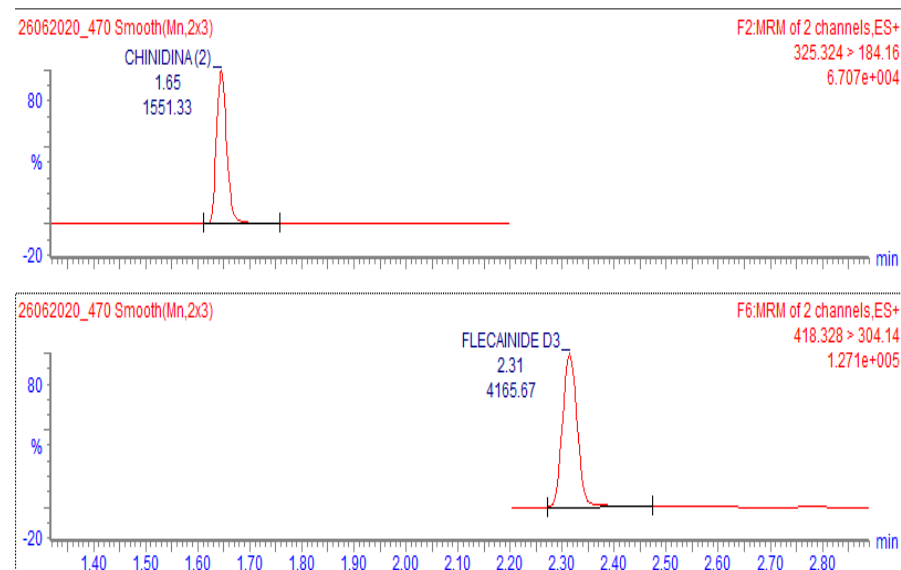
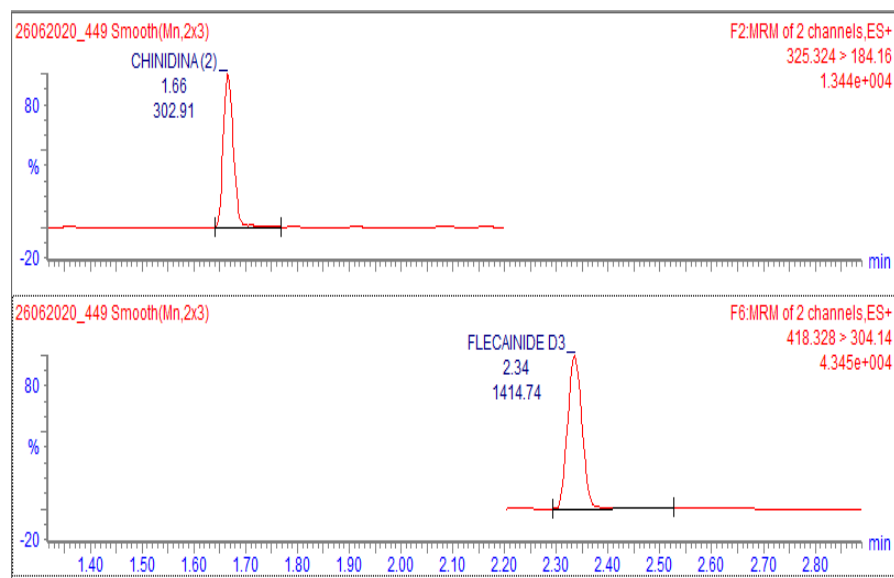
ANTIARRHYTHMICS IN PLASMA BY LC/MS

(Reference Chromatograms)



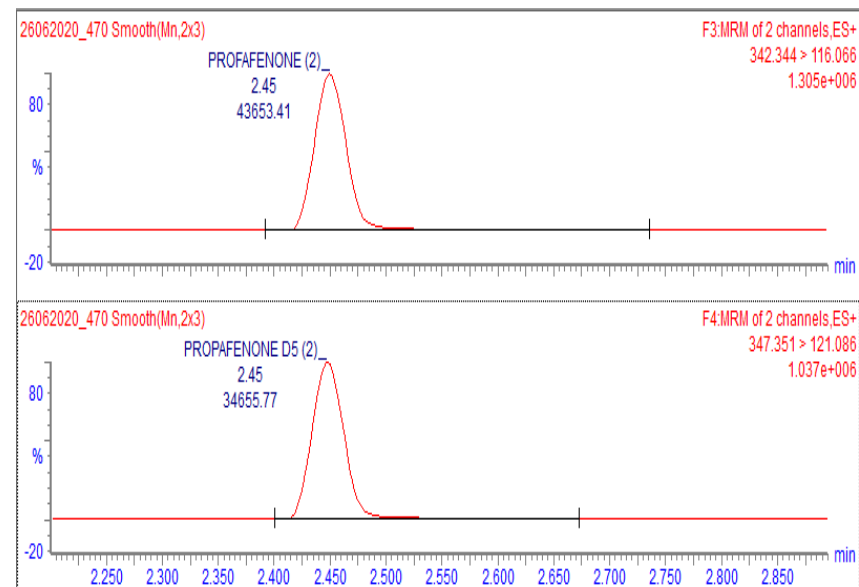
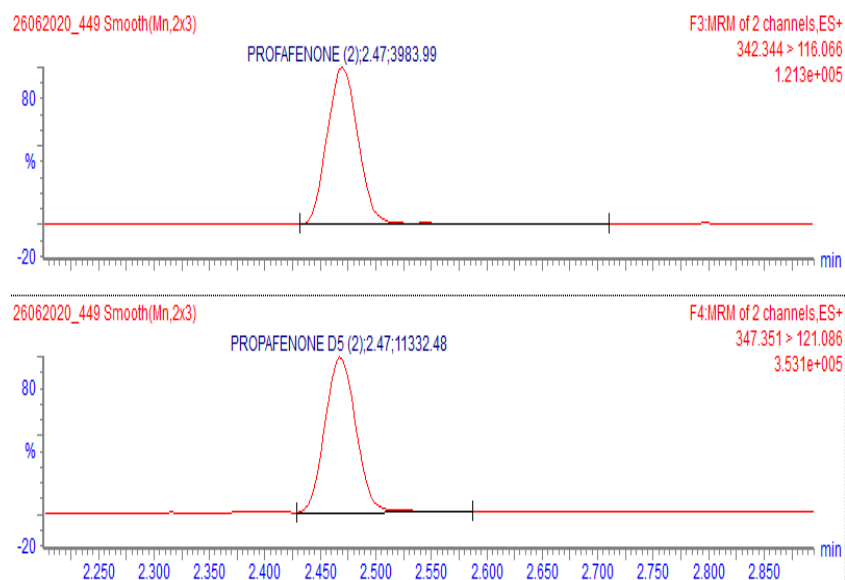
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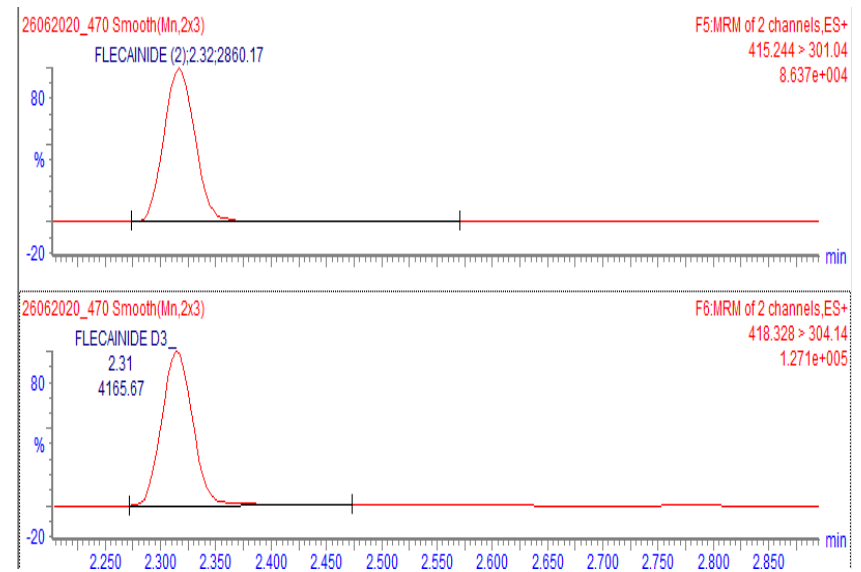
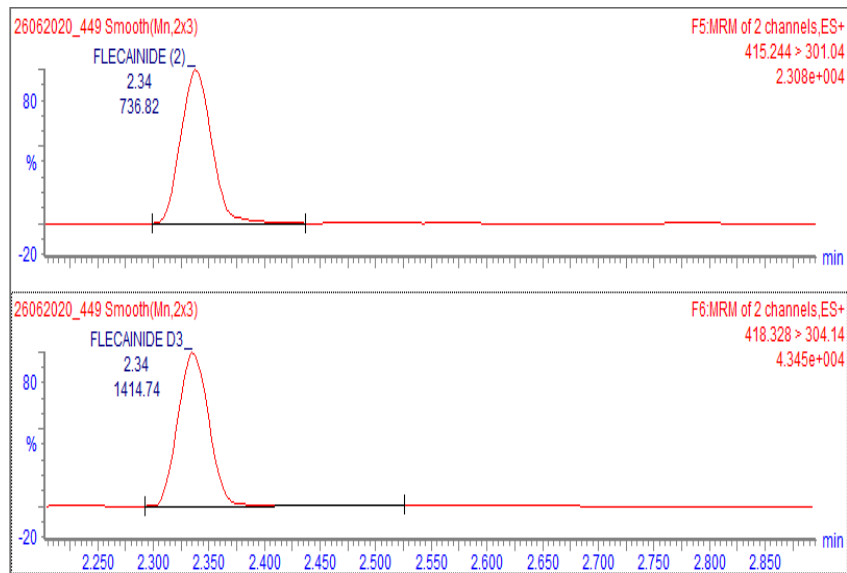
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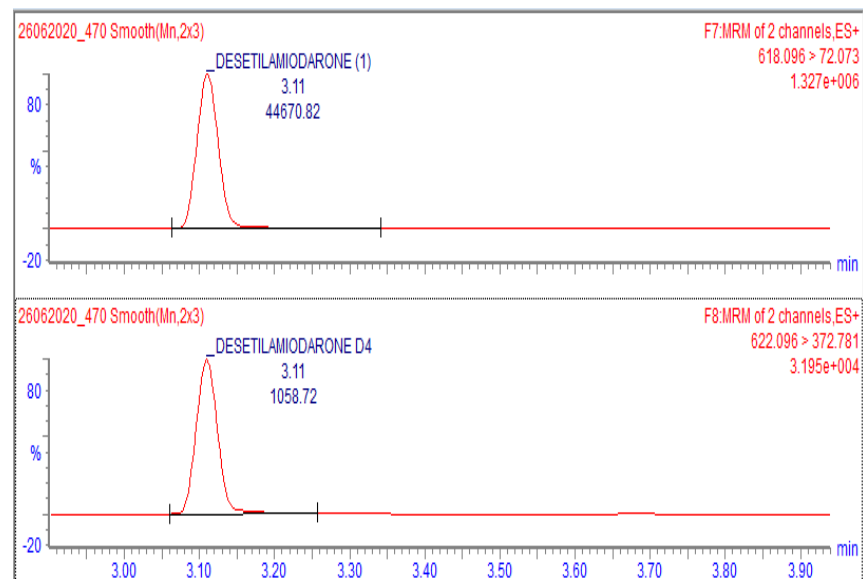
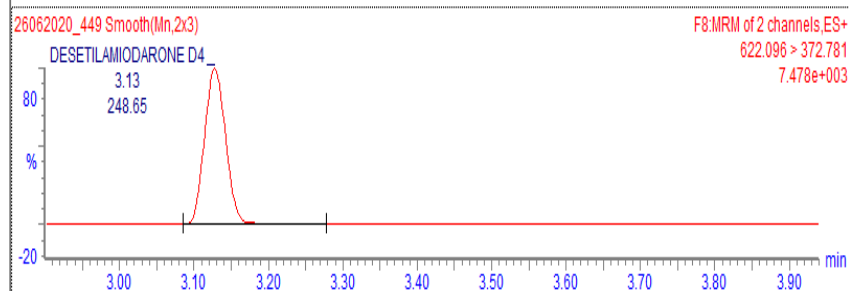
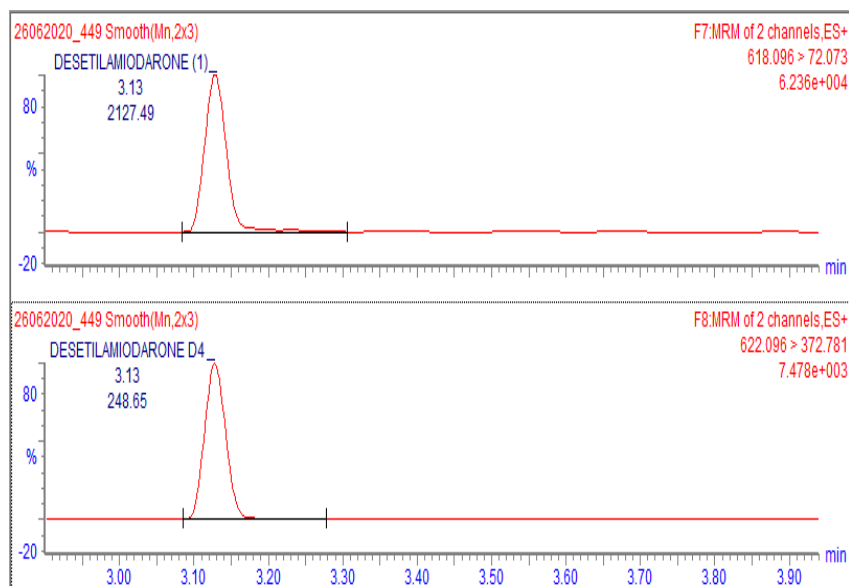
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(Reference Chromatograms)



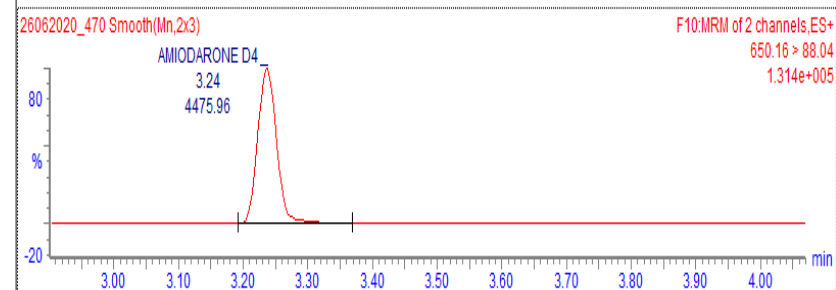
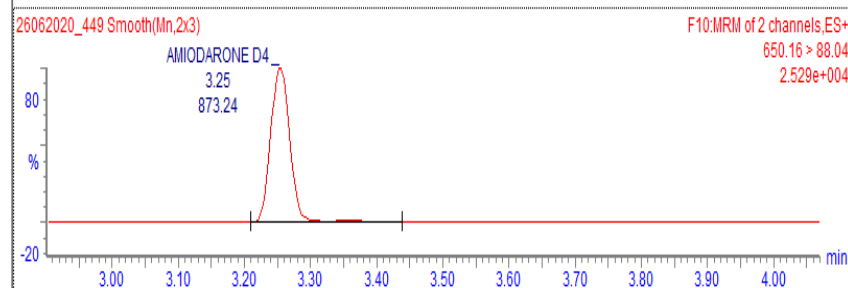
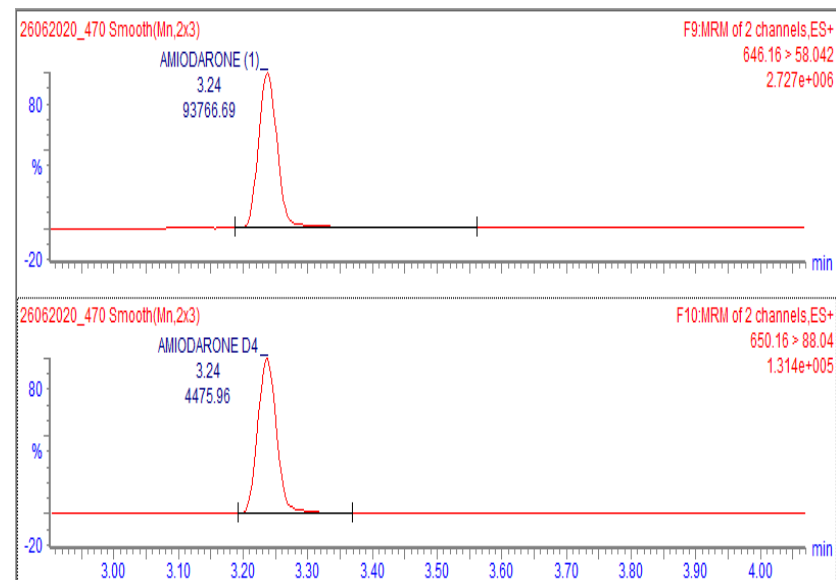
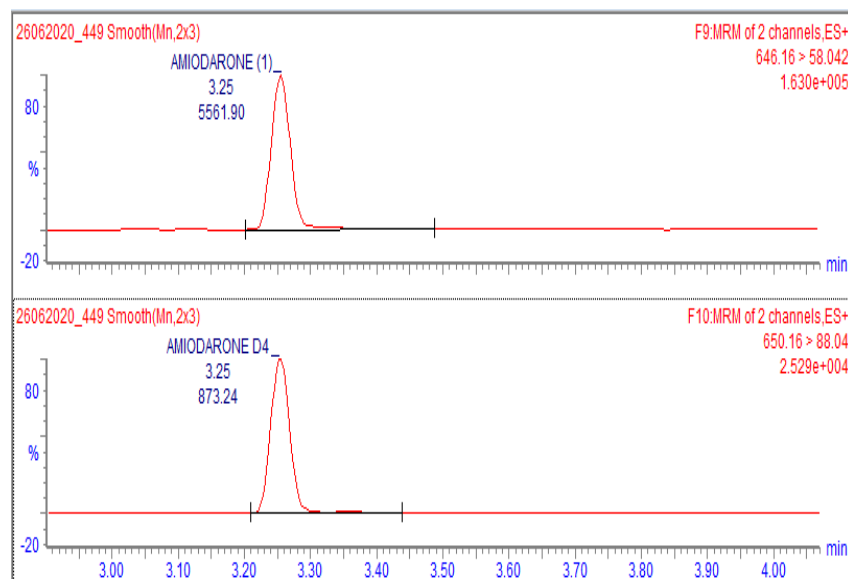
ANTIARRHYTHMICS IN PLASMA BY LC/MS

(Reference Chromatograms)



ANTIARRHYTHMICS IN PLASMA BY LC/MS

(Reference Chromatograms)





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