

ANTIARRHYTHMICS IN PLASMA BY UV – FAST - Code Z78010

(Flecainide, Propafenone, Verapamil)

FLECAINIDE

Pharmacological effects: is a class IC antiarrhythmic drug according to the classification of Vaughan-Williams. It works by slowing the myocardial cell depolarization (phase 0), primarily on the conduction system of the His-Purkinje (conduction H-V) and, to a lesser extent, the nodal conduction and atrioventricular septal.

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

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

Metabolism: Flecainide is extensively metabolised to two primary metabolites, which are then conjugated and who are not considered pharmacologically active.

Elimination: excretion is essentially urinary, approximately 30% of the dose as unchanged Flecainide and the rest as metabolites: only 5% is excreted in the feces. In the case of the urine with $\text{pH} \geq 8$, such as, for example, in cases with renal tubular acidosis, or in patients at strict vegetarian diet, the elimination of Flecainide is very slow.

Notes: the plasma level monitoring is important in patients with severe hepatic or renal impairment, in which the elimination can be slowed. It is also recommended for patients with associated amiodarone and may also be useful in patients with congestive heart failure and renal impairment even if modest. The concentration of Flecainide should be measured after 3 or 4 days.

PROPAFENONE

Pharmacological effects: anti-arrhythmic drug blocking sodium channels (Class IC). Propafenone works by reducing the rate of rise of phase 0 of the action potential. Its predominant effect is thus made by depression of the conduction velocity at the level of all the cardiac structures.

The refractory period, which is not affected by the drug at normal heart rates, is instead extended (post-repolarization refractoriness) in the case of high frequency activation (tachycardias, atrial fibrillation or flutter). The level of sodium channel blockade is, characteristically, frequency-dependent. This explains why the effects of propafenone, both therapeutic and pro arrhythmic, are more pronounced at high frequencies activation.

Drug interactions: anticoagulant aswarfarin→ enhances action (bleeding risk); Digoxin: increases the plasma concentration of digoxin.

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

Plasma peak: following oral administration reaches the maximum of its action after 2-3 hours and the effect persists for more than 8 hours. Since the Propafenone is subject to first-pass hepatic metabolism and has a non-linear kinetics (exponential), with the extended release formulation, the amount of drug available in the blood appears to be, at equal dosage, the lower. (The formulation of this product really matter).

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

Elimination: Propafenone is extensively metabolized in the liver and excreted almost entirely as metabolites in the feces and urine.

VERAPAMIL

Pharmacological effects: Verapamil is an inhibitor of transmembrane flows of calcium ion, active on vascular smooth muscle and on myocyte in reducing myocardial contractility and tone; it is also equipped with antiarrhythmic properties. Verapamil exerts its antiarrhythmic action on the slow fibers "physiological" such as atrioventricular node which reduces conduction velocity and increases the refractory periods, and the slow fibers "pathological", ie of those normally fast, sodium-dependent (myocardium work, His-Purkinje system) which, in certain situations (such as ischemia), become just slow, calcium-dependents and may give rise to hyperkinetic arrhythmias focal or reentrant.

Drug Interactions: chronic treatment with Verapamil may increase the levels of digoxin in the serum of 50-75% during the first week of therapy and can lead to digitalis toxicity. Verapamil therapy may increase carbamazepine concentrations during combination therapy.

Elimination: Verapamil and its metabolites are excreted via the kidneys after an intense first-pass hepatic metabolism. The 3-4% of drug was found unchanged in the urine after 48 hours. Some of the major metabolites retain a significant clinical efficacy on smooth muscle tissue and / or infarction.

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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° 004	Antiarrhythmics in plasma by UV	11/2024
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TECHNICAL FEATURES

Principle of the Method:

This method is used to determine plasma concentrations of Flecainide, Propafenone, Verapamil after SPE purification; after the elution the sample is diluted with a suitable reagent (stabilizing solution) and injected into the HPLC chromatograph.

Recovery :

Flecainide	91,8 %
Propafenone	108,5 %
Verapamil	93,5 %

Sensitivity (LLOD) :

Flecainide	0,01 µg/ml
Propafenone	0,01 µg/ml
Verapamil	0,006 µg/ml

Minimum concentration analyzable (LLOQ) :

Flecainide	0,03 µg/ml
Propafenone	0,03 µg/ml
Verapamil	0,02 µg/ml

Linearity :

Flecainide	0,03 - 5 µg/ml
Propafenone	0,03 - 5 µg/ml
Verapamil	0,02 – 2,5 µg/ml

Normal Range :

Flecainide	0,4 – 0,8 µg/ml; tox> 2 µg/ml
Propafenone	0,3 – 2 µg/ml; tox> 2 µg/ml
Verapamil	0,05 – 0,25 µg/ml; tox> 1 µg/ml

Accuracy intra serie (relative error %) Flecainide :

Ci	Cs
0,1 µg/ml	1 µg/ml
13,8%	6,4%

Accuracy inter serie (relative error %) Flecainide :

Ci	Cs
0,1 µg/ml	1 µg/ml
9,2%	3,9%

Reproducibility intra serie (coefficient of variation % Flecainide :

C LLOQ	Cm	Ch
0,03 µg/ml	0,5 µg/ml	2 µg/ml
6,1%	4,0%	3,6%

Reproducibility inter serie (coefficient of variation % Flecainide :

C LLOQ	Cm	Ch
0,03 µg/ml	20 µg/ml	40 µg/ml
13,1%	4,5%	3,4%

Coefficient of correlation R² + Dev Std

0,9997 ± 0,0002 Flecainide

Accuracy intra serie (relative error %) Propafenone :

Ci	Cs
0,1 µg/ml	1 µg/ml
8,8%	2,2%

Accuracy inter serie (relative error %)**Propafenone :**

Ci	Cs
0,1 µg/ml	1 µg/ml
9,4%	2,2%

Reproducibility intra serie (coefficient of variation %)
Propafenone :

C LLOQ	Cm	Ch
0,03 µg/ml	0,5 µg/ml	2 µg/ml
3,5%	3,5%	3,0%

Reproducibility inter serie (coefficient of variation %)
Propafenone :

C LLOQ	Cm	Ch
0,03 µg/ml	0,5 µg/ml	2 µg/ml
8,3%	3,8%	2,6%

Coefficient of correlation R² + Dev Std

0,9998 ± 0,0001 Propafenone

Accuracy intra serie (relative error %) Verapamil :

Ci	Cs
0,05 µg/ml	0,5 µg/ml
5,2%	2,6%

Accuracy inter serie (relative error %) Verapamil :

Ci	Cs
0,05 µg/ml	0,5 µg/ml
7,6%	2,0%

Reproducibility intra serie (coefficient of variation %)
Verapamil :

C LLOQ	Cm	Ch
0,02 µg/ml	0,25 µg/ml	1 µg/ml
6,2%	4,0%	3,9%

Reproducibility inter serie (coefficient of variation %)
Verapamil :

C LLOQ	Cm	Ch
0,02 µg/ml	0,25 µg/ml	1 µg/ml
9,8%	3,2%	3,1%

Coefficient of correlation R² + Dev Std

0,9998 ± 0,0001 Verapamil

Components of the kit :

All the reagents are ready to use and stable 3 years at Room Temperature.

Reagent A – Diluting Solution, 1 x 150 ml**Reagent B – Internal Standard Sol., 1 x 5 ml****Store at 2 - 8 °C****Reagent C – Conditioning Solution 1, 1 x 100 ml****Reagent D – Conditioning Solution 2, 1 x 100 ml****Reagent E – Wash Solution, 1 x 100 ml****Reagent F – Eluting Solution, 1 x 30 ml****Reagent G – Stabilizing Solution, 1 x 30 ml****Reagent H – Test Solution, 1 x 1 ml****Calibrator in plasma lyophil., 2 x 2 ml****Code Z78016 (packed separately – see data sheets)**

Clean-up Columns, 100 Pcs

Reagent M – Mobile Phase, 2 x 500 ml

Minimum Instrumental equipment required:

Isocratic HPLC System (flow 1 ml/min)

Loop of 20 μ l

Detector UV λ = **207 nm**

Chromatograms Recorder

Autosampler

Operational Computer

Optional Equipment:

Whole Blood Collection :

Collect 3 ml of blood in a suitable test tube with EDTA or Heparin as anticoagulant. Centrifuge at 4000 rpm for 5 min.

Plasma samples should be stored at -20° C for no longer than 4 weeks

PREANALYTICAL PROCEDURE

Dispense in a tube :

- 850 µl of H₂O HPLC grade
- 100 µl of **Reagent H - Test Solution**
- 50 µl of **Reagent B - Internal Standard Solution**

NOTE : Before start the analytical session inject 20 µl of this solution in HPLC for verify that the Test Solution has retention time similar to fig. 1. 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 : Prepare Clean up columns per each Sample, Calibrator and Controls.

- Condition clean up columns by fluxing 1000 µl of **Reagent C – Conditioning Sol. 1**
don't let the columns be dry
- Condition clean up columns by fluxing 1000 µl of **Reagent D – Conditioning Sol. 2**
don't let the columns be dry

STEP 2 : Dispense in plastic tubes 0,5 ml of Sample/Calibrator/Controls and add 1,5 ml of **Reagent A – Diluting Solution** + 50 µl of **Reagent B – Internal Standard**

Vortex for 10 sec.

STEP 3 : Dispense 2,05 ml of Sample/Calibrator/Controls prepared in STEP 2 in Clean up Columns

Don't let the columns be dry!

STEP 4 : Wash Clean-up Columns with 1 ml of **Reagent E – Wash Solution** (Let dry with a light flux of air)

STEP 5 : Elute with 300 µl of **Reagent F – Eluting Solution**

STEP 6 : Add 300 µl of **Reagent G – Stabilizing Sol.**

Vortex for 10 sec.

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

INJECTION :

- Inject 20 µl into the chromatographic system.

ANTIARRHYTHMICS - Warnings

REAGENT H : TEST SOLUTION

ANTIARRHYTHMICS:	
Verapamil	10 µg/ml
Propafenone	10 µg/ml
Flecainide	10 µg/ml

PARAMETERS OF THE SPECTROFOTOMETRIC DETECTOR

λ	207 nm
GAIN	0,001 AUFS
INTEGRATION TIME	10 secondi

HPLC COLUMN PROTECTION

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

Install a <u>new</u> analytical C ₁₈ Reversed-Phase column POROSHELL 120 EC-C18 (50 x 4,6 mm, 2,7 µ). Disconnect the detector and filter 30 ml of H ₂ O : Methanol (20 : 80 v / v) set flow of 1,0 ml/minute. Don't recycle the washing solutions. Flux 30 ml of a solution of H ₂ O : Methanol (20 : 80 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 ml/minute and discharge the first 20 ml. Finally inject the Chemical Standard and verify the quality of the HPLC run. 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.
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COLUMN CLEANING

Disconnect the detector. Flux 15 ml of H ₂ O : Acetonitrile (80 : 20 v / v) set flow of 1,0 ml/minute and discharge. Flux a solution of H ₂ O : Acetonitrile (30 : 70 v / v) for 30 minutes and 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 H ₂ O : Acetonitrile or Methanol (30 : 70 v / v).
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INJECTION NEEDLE WASHING

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

LOOP	20 µl
Recommended Flow rate	1,0 ml/min
Pressure	About 110 bar

ACCESSORIES AND CONSUMABLES

CODE	DESCRIPTION	PACKAGING
Z78016	Calibrator lyophil. In plasma for Antiarrhythmics	4 x 2 ml
Z78019	Control lyophil. In plasma for Antiarrhythmics – 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
S29057U	Standard glass vials of 2 ml with screw cap	1 x 100 Pcs

ANTIARRHYTHMICS IN PLASMA (Reference Chromatograms)

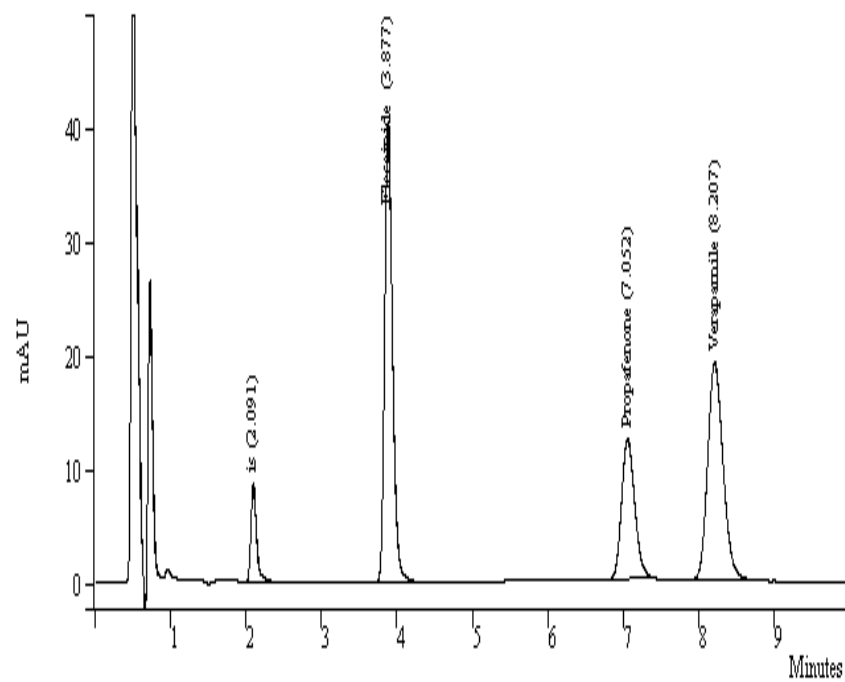


Fig. 1 :	Test Solution	
R.T. 2.09	Internal Standard	
R.T. 3.87	Flecainide	
R.T. 7.05	Propafenone	
R.T. 8.20	Verapamil	

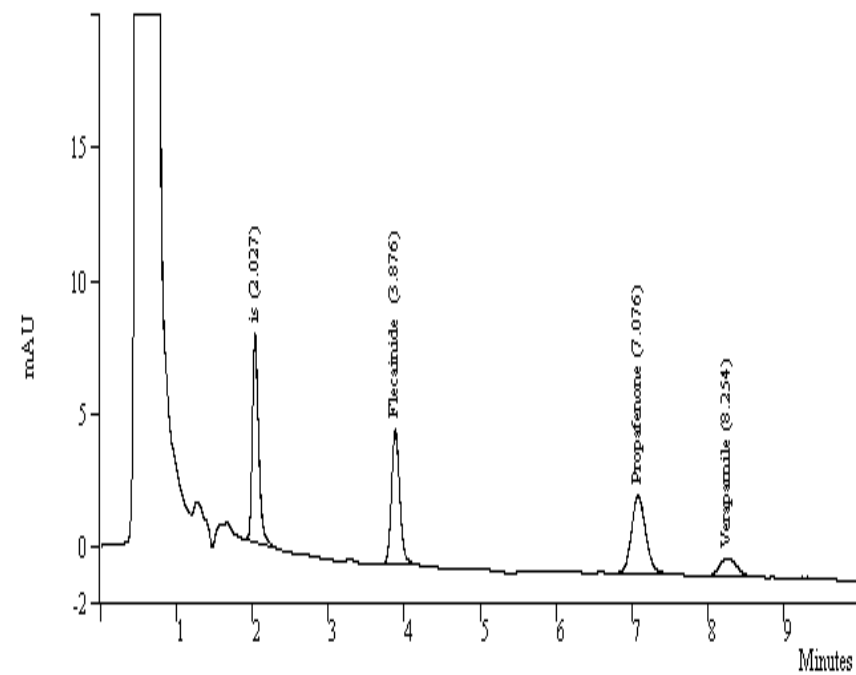


Fig. 2 :	Plasma Calibrator		
R.T. 2.02	Internal Standard		
R.T. 3.87	Flecainide	0,7 µg/ml	
R.T. 7.07	Propafenone	1,5 µg/ml	
R.T. 8.25	Verapamil	0,2 µg/ml	

ANTIARRHYTHMICS IN PLASMA

(Reference Chromatograms)

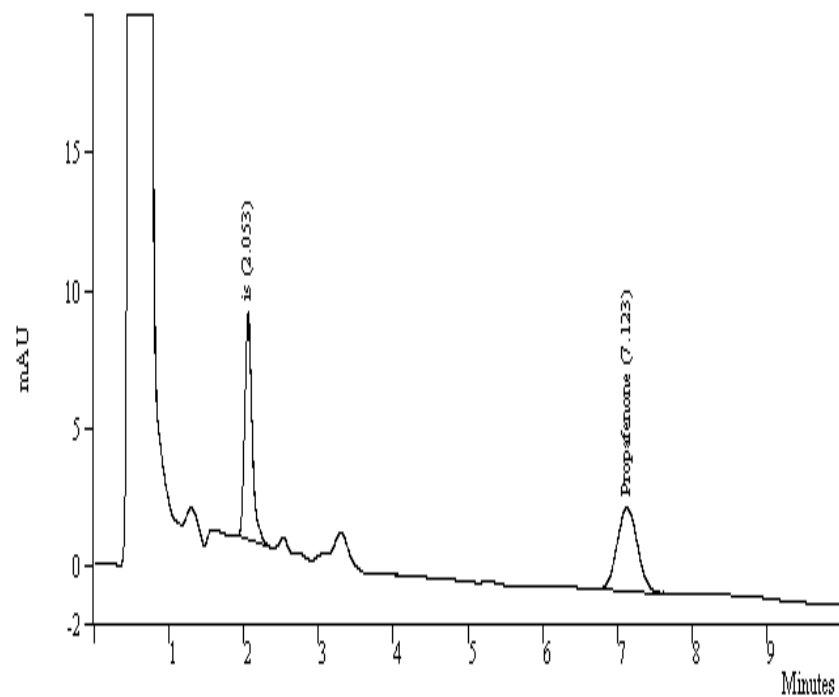


Fig. 3 : Sample of Propafenone			
R.T.	2.05	Internal Standard	
R.T.	7.12	Propafenone	3,0 µg/ml

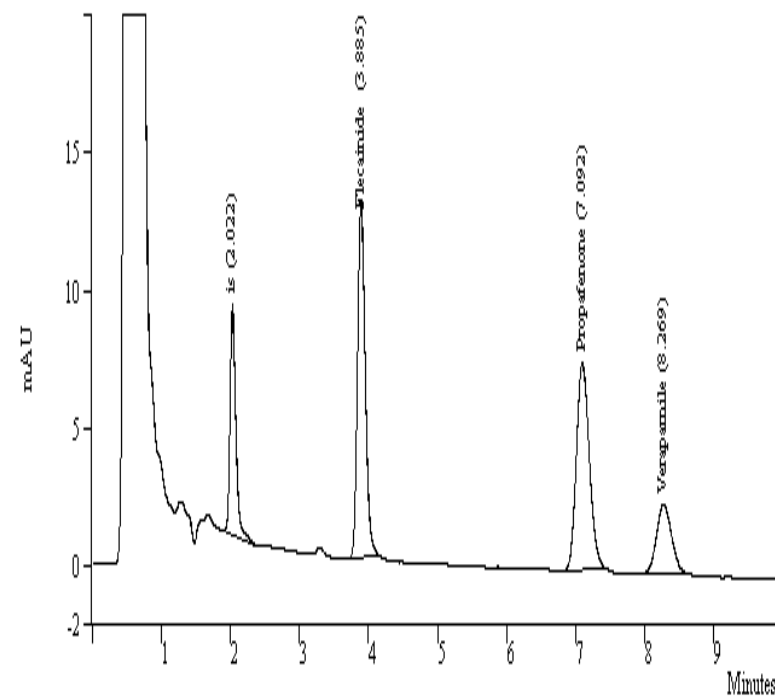


Fig. 4 : High Plasma Control			
R.T.	2.02	Internal Standard	
R.T.	3.88	Flecainide	2,5 µg/ml
R.T.	7.09	Propafenone	3,0 µg/ml
R.T.	8.26	Verapamil	1,5 µg/ml

