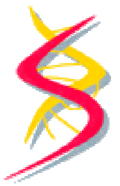
BioStrands



EVALUATION OF AN ELISA METHOD FOR MEASURING AMPRENAVIR IN PLASMA

Montagna M.[1], Bastiani E.[2], Cusato M.[1], Benedetti F.[3], Rinaldi S.[2], Donadel E.[2], Berti F.[3], Meroni V.[4], and Regazzi M.[1]

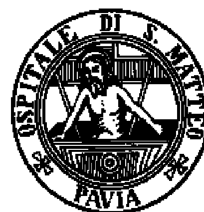
[1]Dept. of Pharmacology, [4]Div of Infectious Diseases, IRCCS Policlinico “S. Matteo”, Pavia, Italy .

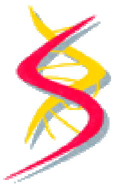
[2]BioStrands Srl, Area Science Park, Trieste, Italy,

[3]Dept. Of Chemical Science, University of Trieste, Italy.

[1]Address: IRCCS Policlinico “S. Matteo”, P.le Golgi, 2, I–27100 Pavia (ITALY)

E–mail: regazzim@smatteo.pv.it
tel+39 0382 503471





Introduction

Amprenavir is a sulphonamide compound which inhibits the HIV protease enzyme, preventing post-translational processing of viral proteins and formation of mature infectious virions. The HIV-1 protease enzyme was shown to be responsible for processing the HIV-1 polyprotein precursor into functionally active viral protein. Without protease, immature, noninfectious virions are produced.

Amprenavir acts by preventing the virally encoded HIV-1 protease from processing the cleavage of its natural substrates.

Then, APV and other HIV protease inhibitors (PIs) exert their antiretroviral effect late in the HIV cycle by interfering with virion maturation. Pharmacodynamic data also provide evidence for exposure-response relationships between APV concentrations and the emergence of viral resistance. Headache and perioral numbness were significantly associated with $Css,_{max}$ and perioral numbness with $Css,_{ave}$. Amprenavir dosage should be reduced in subjects with liver disease.

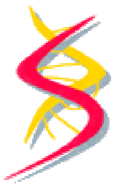
Therapeutic Drug Monitoring (TDM) may have a role in optimising antiretroviral therapy.

Aim of the study

To validate a sensitive ELISA assay to measure Amprenavir plasma concentrations, we investigated precision, accuracy, linearity and compared ELISA against a conventional HPLC assay

BioStrands

HPLC assay



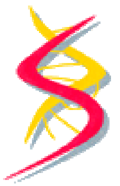
Briefly, 0.25 mL of plasma was vortexed for 10 min with IS, 0.5 mL of borate buffer and 2.5 mL of hexane-ethylacetate (50/50) and then centrifuged for 5 min at 1500 rpm.

After centrifugation the organic phase was separated by dry ice and then evaporated to dryness under a stream of dry N_2.
The dried extract was reconstituted in 0.5 mL of FM, and vortexed.

Hexane (1 mL) was added, and the mixture was vortexed briefly.
After centrifugation, the mobile phase was aspired and injected into HPLC.

Separation of APV and internal standard from other components in the sample was achieved on a Hypersyl C18 with use of a 0.1% H_3PO_4 aqueous:acetonitrile buffer.
Eluting peaks were detected by their ultraviolet absorbance at 220 nm.

The concentration of APV in patient samples was interpolated from a plot of peak height ratio (APV:I.S.) versus APV concentration for the standards.



ELISA assay

The ELISA Amprenavir test is based on the competition between the drug in the plasma sample and the drug conjugate to the detection enzyme: they compete for the same binding sites of the polyclonal antiserum.

The detection of the binding between the enzyme-conjugated drug and the specific antibody is done by the addiction of a chromogenic substrate.

The enzymatic activity produces a colored solution whose adsorbance can be read by a microplate reader at 450 nm.

BioStrands

The adsorbance value is inversely proportional to the drug concentration.
Reagents for this polyclonal antibody-based assay were kindly provided by BioStrands Srl.

To the plates we added: blank, standard solution, quality control and unknown samples appropriately diluted (1:100) in buffer.

The standard concentrations were:
0, 2.5, 5, 10, 25, 50 and 100 ng/mL.

The standard 0 was TB (total binding).

Then, APV enzyme was added to all wells and APV anti-serum to all wells except the blank.

After 1-hour incubation at RT followed by washings, chromogenic substrate was added to each well and then incubated for 30 min.

The colour reaction was stopped by adding Stop Solution to each well and then reading at 450 nm.
APV serum levels were interpolated from standard curve by measuring concentration standard vs $B/B^{\circ} \times 100$.

(B°= adsorbance of TB and B= adsorbance of single standards).

BioStrands

ELISA Assay Parameters

Validation Requirements

The following studies were undertaken to validate our methods.

During analysis of unknown clinical samples, a standard curve and three sets of QC samples were assayed during each run and run in duplicate.

The acceptance criterion was that the QC samples must fall within either ? 2 DS or ? 20% of the established mean from the pre-study validation.