Development and Validation of an LC/MS/MS Bioanalytical Method for Determination of Aprepitant in the Presence of Its Prodrug Fosaprepitant in Human Plasma

C. Nattrass¹, S. Burke¹, B. Hoffman¹, Z. Shen², D. E. Mulvana¹
¹Q2 Solutions, ²Pfizer Inc.

**Purpose**

Aprepitant (APR), is a substance P/neurokinin 1 (NK1) receptor antagonist for the treatment of nausea and vomiting associated with emetogenic cancer chemotherapy. APR is often administered intravenously as its phosphorylated prodrug fosaprepitant (FAPR) as this is often more convenient in the therapeutic setting. FAPR is rapidly converted to APR in the body and thus determination of pharmacokinetics of APR is most relevant in clinical studies. To allow for pharmacokinetic analysis in clinical studies a bioanalytical method for APR in human plasma was required that could be used for trials in which either APR or FAPR are dosed. This posed some analytical challenges in method development due to the presence of high levels of FAPR as well as the conversion of FAPR to APR.

**Methods**

The method was validated in human plasma (K2EDTA) over a concentration range of 10-10,000 ng/mL APR. Accuracy of dilution of samples was demonstrated up to 150,000 ng/mL of APR. All plasma samples were maintained in an ice-water bath during processing. After addition of the internal standard (aprepitant-d4) to plasma samples (0.05 mL) the plasma was deproteinized with 0.3 mL of acetonitrile. The supernatant of the precipitated sample was diluted 1:2 (v:v) with 0.1% ammonium hydroxide and injected onto a Waters XBridge C8 (5 µm 2.1x30 mm) column. The analytes were eluted isocratically with an eluent consisting of acetonitrile, ammonium formate and formic acid and detected using an AB-Sciex API-4000 in positive ionization mode.

**Results**

APR is highly protein bound (approximately 98%) with low aqueous solubility. Among the challenges encountered in method development was instability of APR in aqueous solutions but this was resolved by use of dimethylformamide as solvent for stock solutions. Additional challenges were encountered due to instability of FAPR in whole blood and plasma. This was addressed by incorporation of the use of an ice-water bath during all plasma sample handling steps and the use of wet ice and refrigerated centrifugation during clinical blood sample collection and processing. The final sample preparation method consisted of a simple protein precipitation with acetonitrile followed by dilution in basic water. Previously, instability had been encountered as a result of the pH of the sample extracts. The initially developed method used 0.1% formic acid for dilution of the supernatant to match the chromatographic mobile phase. However, through use of clinically relevant stability QCs in the presence of FAPR, it was found that FAPR was converting to APR at an unacceptable rate, even over the course of an analytical run. Thus the method was modified to use basic (0.1% ammonium hydroxide) for dilution of the supernatant in the final injection solvent which stabilized the prodrug. Since initial FAPR plasma levels can reach 10,000 ng/mL, it was important to demonstrate stability of the prodrug especially when very low levels of APR are present. A low level of APR impurity observed in the FAPR reference standard exacerbated this challenge. It was important to measure this impurity to properly characterize the stability QCs and this was done over three analytical runs during method validation. The method was validated according to current regulatory guidelines. Analytical QCs were prepared that contained only APR and additional stability QCs were prepared that contained either FAPR, APR or both at various levels. Accuracy and precision of APR QCs was acceptable at all levels (10, 30, 300, 3000, 7500 and 150,000 ng/mL). Accurate quantitation was observed in multiple plasma lots, including hemolyzed and lipemic plasma. APR was stable in plasma (in an ice-water bath) for up to 24 Hr and after 5 freeze-thaw cycles while FAPR was only stable in plasma (in an ice-water bath) for up to 4 Hr and after 5 freeze-thaw cycles at all levels. APR QCs (including the dilution QC at 150,000 ng/mL) were stable at -20°C and -70°C for up to 204 days. However, APR QCs containing high levels of FAPR were stable at -70°C for up to 49 days but stability was not acceptable at -20°C. Stability in whole blood was acceptable for APR for up to 1 Hr at room temperature and in an ice-water bath but FAPR was only stable in an ice-water bath for 1 Hr but not at room temperature. APR was stable in DMF solutions for up to 110 days when stored at 4°C.

**Conclusion**

The method was fully validated and is suitable for bioanalysis of APR in the presence of its prodrug FAPR in human clinical trials.