

Determination of Cytarabine (Cytosar-U[®]) and its metabolite Ara-U in Human and Dog Plasma and urine by LC/MS/MS

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Introduction

Cytarabine is an anti-cancer ("antineoplastic" or "cytotoxic") chemotherapy drug. Cytarabine belongs to the category of chemotherapy drugs called antimetabolites. Antimetabolites are very similar to normal substances within the cell. When the cells incorporate these substances into the cellular metabolism, they are unable to divide. Antimetabolites are cell-cycle specific. They attack cells at very specific phases in the cycle.

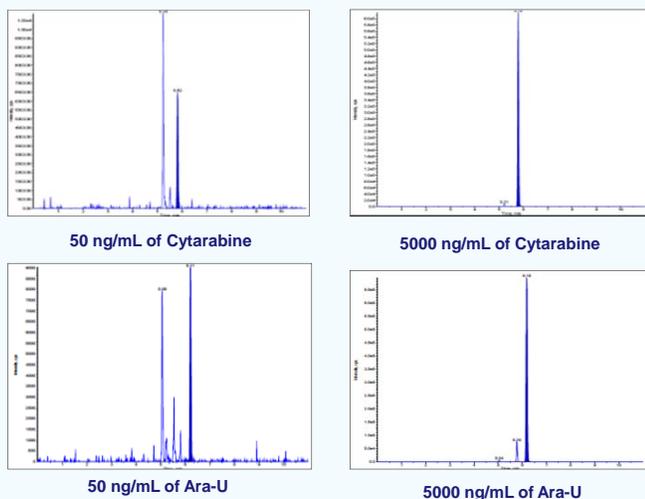
Purpose

Cytarabine is used to treat different forms of leukemia, including acute and chronic myelogenous (AML and CML) and acute lymphocytic leukemia (ALL). It is also used to treat lymphoma, meningeal leukemia and lymphoma (cancers found in the lining of the brain and spinal cord). (Chemocare.com, 2005, Cytarabine <http://www.chemocare.com/bio/cytarabine.asp>). Cytarabine is metabolized by deoxycytidine kinase and other nucleotide kinases to nucleotide triphosphate (active). The drug is inactivated by a pyrimidine nucleotide deaminase to nontoxic uracil derivative. (Drugs.com, 2010, Cytarabine <http://www.drugs.com/ppa/cytarabine.html>)

Method

A sensitive LC-MS/MS method was developed for the determination of Cytarabine and its metabolite Ara-U in dog and human plasma and urine. The samples were prepared through simple protein precipitation with Ara-C ¹³C₃ and Ara-U ¹³C ¹⁵N₂ as the internal standards. The final supernatant was analyzed on the API 4000 LC-MS/MS system by electrospray ionization (ESI) mass spectrometry with multiple reaction monitoring (MRM) of negative ions. The ions monitored were 242 → 109 for Cytarabine, 322 → 97 for Ara-U, 245 → 113 for Ara-C ¹³C₃ and 325 → 97 for Ara-U ¹³C ¹⁵N₂. The ratio of analyte product ion peak area to that of the internal standard were the responses used for quantitation.

Chromatographs

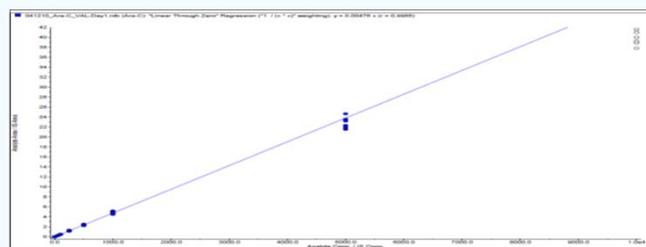
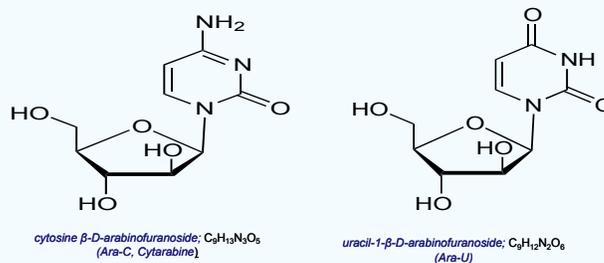


Results and Discussion

The validation showed that the method was linear ($r^2 = 0.99$) over the concentration range of 50 ng/mL to 5000 ng/mL for both Cytarabine and Ara-U. No significant interference was observed in blank plasma and urine.

The accuracy of the plasma and urine standards for Cytarabine and Ara-U was within 7.52% from the nominal concentration. The precision of the plasma and urine standards for Cytarabine and Ara-U did not exceed 11.27% at the LLOQ and was within 7.40% at all other levels.

The intra-day and inter-day accuracy for the determination of Cytarabine and Ara-U in plasma and urine samples did not exceed 13.12% for low QC and 12.20% for other QCs. The intra-day and inter-day precision for the determination of Cytarabine and Ara-U in plasma and urine samples did not exceed 12.47% CV for low QC and 10.69% for other QCs. Cytarabine and Ara-U extracted from plasma and urine was stable at 4°C (the temperature of the cooled auto-sampler) for at least 24 hours. Cytarabine and Ara-U were also stable in plasma and urine after 3 freeze-thaw cycles. The selectivity, sensitivity, linearity, accuracy, precision and robustness of the method are sufficient for analysis of Cytarabine and Ara-U in dog and human plasma and urine samples.



Typical Calibration curve

Conclusion

LC/MS/MS offers a specific and sensitive platform for determination of Cytarabine and its metabolite Ara-U for clinical diagnosis as well research and development which can include Safety, Efficacy, Toxicokinetics as well as Pharmacokinetics in pre-clinical and clinical studies.