CAPSULES notes and applications from BASi
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5-Fluorouracil

Determination of 5-Fluorouracil in Human Plasma Using LC/MS/MS

Introduction

BASi has developed a LC/MS/MS assay for 5-fluorouracil over the range of 10-10,000 ng/mL in human plasma. The method utilizes serial liquid-liquid extraction and column switching to provide good sample cleanup and adequate chromatographic retention. The result is a reliable, robust method that provides accurate results with quick turnaround.

5-Fluorouracil, a low molecular weight and highly polar compound, is an antineoplastic antimetabolite given intravenously in chemotherapy. It was first introduced in 1957, but is still widely used to treat several types of cancer including breast, colon, stomach, rectum, and pancreas. It is frequently co-administered with paclitaxel, docetaxel, leucovorin, or oxaliplatin. A survey of literature methods shows a limited number of LC/MS/MS assays for this compound.

The polar nature of the molecule presents several challenges for an analytical method. Extraction out of plasma can be difficult as 5-fluoruracil is not well retained on an SPE cartridge and liquid-liquid extraction can be inefficient with polar molecules. Without adequate sample cleanup the effects of the ion suppression regions at the beginning of an LC/MS/MS run can be troublesome. It becomes critical to have good retention to provide accurate, reproducible results. 5-Fluorouracil is not well retained by most conventional chromatographic conditions. The method developed and validated by BASi overcomes these challenges and has successfully been used to assay samples from human clinical trials.

Method Summary

Plasma samples (0.100 mL) are serially extracted with organic solvent after the addition of internal standard (5-bromouracil). After mixing and centrifugation the solvent is removed and evaporated to dryness. Samples are reconstituted in mobile phase and injected onto a 2.1 x 50 mm C18 column with a 4.6 x 12 mm precolumn which is backflushed with isopropanol (IPA) after every injection. Due to the polar nature of the molecule, a completely aqueous mobile phase is used. Detection is by MS/MS with electrospray in negative ion mode.

Method Performance Data

A method range of 10 – 10,000 ng/mL was achieved using 2 overlapping standard curves (10 – 600 ng/mL and 500 – 10,000 ng/mL). The validation data indicate excellent precision and accuracy of the standards as shown in **T1** and **T2**.

Quality control (QC) samples spiked with 5-fluorouracil were prepared at seven different levels over the range of the assay. QC samples (one low-level, two mid-range, and one high-level) were extracted (n=6) in each of the three validation runs. Excellent accuracy and precision were observed for these QC samples as shown in **T3**.

T1. Between-run (n=6) accuracy and precision for extracted standards.

Sample Name	5-Fluorouracil Mean	CV
SC-10 ng/mL	99.7%	4.1%
SC-25 ng/mL	101.0%	4.2%
SC-100 ng/mL	101.0%	3.5%
SC-600 ng/mL (low curve)	98.6%	3.0%
SC-600 ng/mL (high curve)	97.0%	3.7%
SC-4000 ng/mL	101.0%	1.2%
SC-10,000 ng/mL	107.0%	6.1%

T2. Coefficient of determination for standard curves.

	r^2 (low curve)	r^2 (high curve)
Run 1	0.999	0.996
Run 2	0.995	0.994
Run 3	0.999	0.997

T3. Between-run accuracy and precision for extracted QC samples.

Sample Name	5-Fluorouracil Mean	CV
QC-10 ng/mL	98.8%	12.0%
QC-25 ng/mL	105.0%	5.5%
QC-100 ng/mL	103.0%	3.5%
QC-600 ng/mL (low curve)	99.3%	5.4%
QC-600 ng/mL (high curve)	97.9%	6.2%
QC-2000 ng/mL	103.0%	3.1%
QC-8000 ng/mL	99.2%	7.3%
QC-9000 ng/mL	103.0%	8.3%

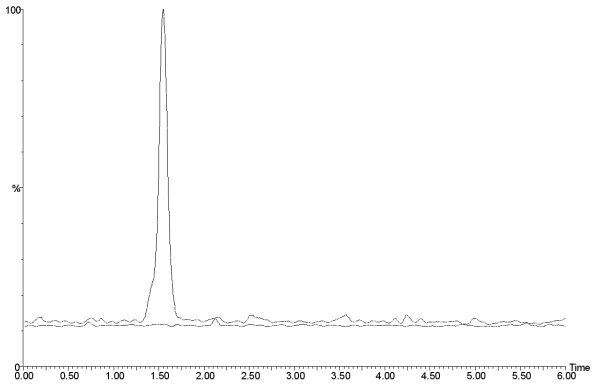
Ruggedness and Selectivity

The ruggedness of the 5-fluorouracil method was examined by subjecting spiked QC samples to normal sample handling challenges. Frozen, freeze-thaw, bench top, heat treatment (for deactivation of the HIV virus), and autosampler stability were examined. In all cases the method maintained the requisite precision and accuracy. Over-curve samples were diluted 100-fold into the range of the curve with excellent accuracy and precision.

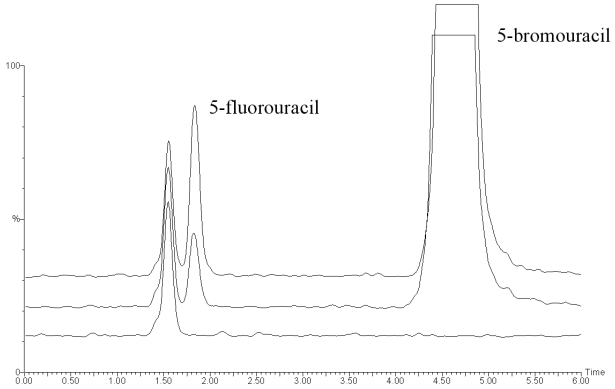
It is important to develop methods that are specific for the analyte of interest. Our method for 5-fluorouracil was examined for selectivity and possible interferences. No matrix interferences were observed in six different lots of blank human plasma. In addition a panel of 10 co-administered (oxaliplatin and leucovorin) and over-the-counter drugs did not interfere with the assay. Representative chromatograms are shown below in **F1**.

F1. Example MRM chromatograms

Extracted Blank Plasma



Overlay (Blank, LLOQ, 2.5X LLOQ)



Mid Level (500 ng/mL) Standard