



Analytical Challenges in the Development of an Assay for Capecitabine and Three of its Metabolites

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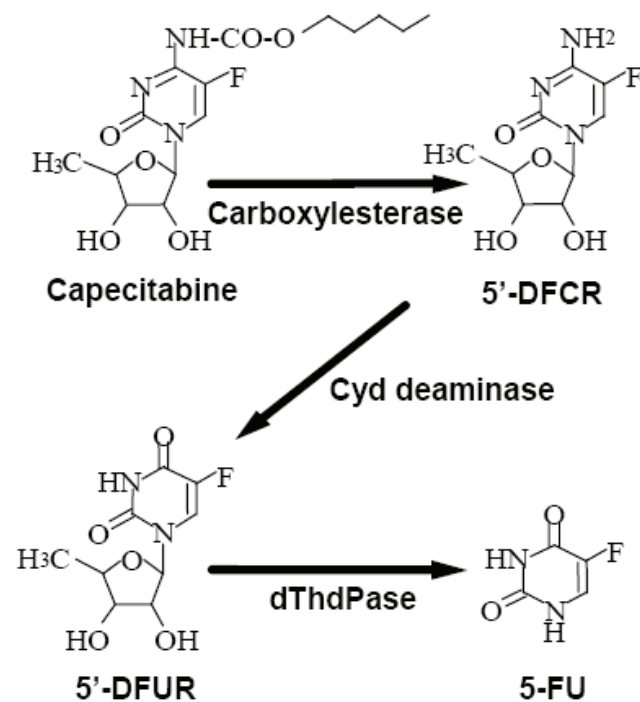
Objective

To develop a robust bioanalytical assay for capecitabine and three of its metabolites in human plasma to support clinical oncology studies.

Abbreviations

Cape = capecitabine (MW 359)
 5DFC/5'-DFCR = 5'-deoxy-5-fluorocytidine (MW 245)
 5FUR/5'-DFUR = 5'-deoxy-5-fluorouridine (MW 246)
 5FU = 5-fluorouracil (MW 128)

Metabolic Pathway: Capecitabine to 5-FU



Analytical Challenges

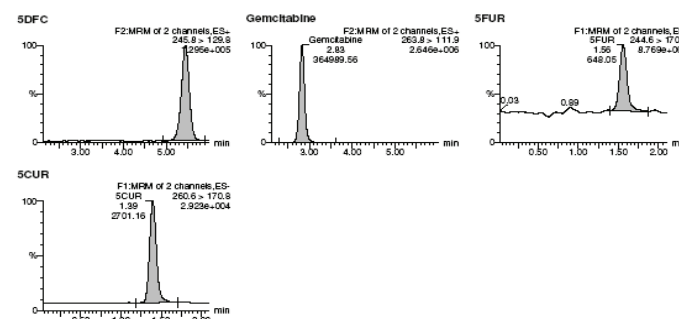
- Multiple analytes
- Metabolites are highly water soluble (poorly retained)
- Low molecular weight for 5FU (interferences – clean up required)
- Both positive and negative ionization mode required
- Dimerization seen at high concentrations
- Stable label internal standard available only for parent
- Capecitabine unstable in plasma!

Step 1: Determine MS Conditions Challenge: Positive/Negative Ionization

Compound	Mode	
Capecitabine	Positive	✓
	Negative	✓
5-deoxy-5-fluorocytidine	Positive	✓
	Negative	X
5-deoxy-5-fluorouridine	Positive	X
	Negative	✓

Step 2: DryLab Experiments/Chromatography Challenge: Poor retention.

- Poor retention on RP-18 except for capecitabine
- Low organic gradient required for the three analytes
- Metabolite retention order switch with NH₄ formate to formic acid
- Good peak shape
- Polarity change required to capture parent and metabolites; minor drifts in retention times could be problematic
- MPA: formic acid, NH₄ formate and NH₄ carbonate
- MeOH and ACN as MPB



Step 3: Internal Standard Candidates based on Structural Similarity Challenge 1: Positive/Negative Ionization Challenge 2: No good candidates

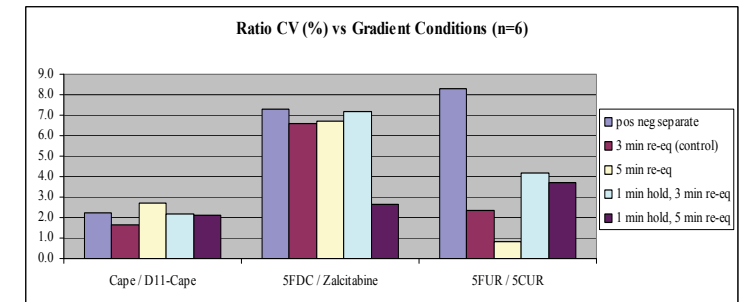
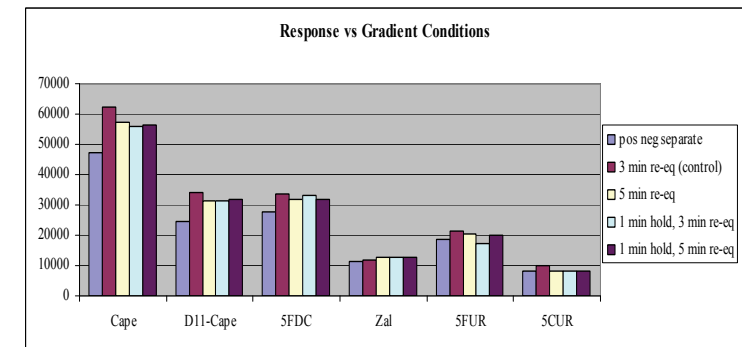
Compound	m/z	Mode	
Stavudine	225	positive	X
	223	negative	✓
2-deoxycytidine	228	positive	✓ (dimers)
	227	negative	✓
Zalcitabine	212	positive	✓ (dimers)
	210	negative	X
Cytarabine	244	positive	✓
	242	negative	X
5-ethyl-2-deoxyuridine	257	positive	✓
	255	negative	✓

- Screen more IS candidates
- Gemcitabine (known to be unstable)
 - 5-chloro-2-deoxy-uridine
 - 2-deoxy-uridine

Step 5: Optimize Chromatography Challenge 1: Short Column Life Challenge 2: Optimize Chromatography Conditions for all Analytes Challenge 3: Internal Standard Variability

- Better peak shape on new column
- ↑ CV as column aged
- Continued variability of IS and 5FUR (earliest eluting peak) response with time
- Isocratic hold at beginning
- pH of carbonate buffer
- Concentration of carbonate buffer
- Gradient (linear, step, final)
- Re-equilibration time
- Isocratic hold at end

Introduction of New Concept: Mass Spec Re-equilibration Time



Two isocratic runs (two injections/one extraction), one for cape (4 min) and one for metabolites (6 min) instituted. Overall run time the same.

Conclusions

- ↑ analytes ↑ chance of failure statistically
- Concept of "mass spec" re-equilibration beyond column re-equilibration for early eluting compounds
- Sometimes two isocratic runs = the time of a gradient
- Capecitabine and metabolites assay successfully validated and used for oncology clinical trials.

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