Analytical Validation, Separation and Stability Study of Multi Compound Formulations – Challenges and Approach

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1 ABSTRACT

Multi component formulations are useful as introducing multiple test articles compliment the effects and side-effects of one another and recently has shown to be an effective strategy, but often presents analytical and formulation challenges.

Optimization, validation and stability assessment of a two component unique formulation was carried out in a vehicle ensuring the formulations to be solutions for an IV route of dosing. A single analytical method was developed to quantitate two different test articles without any interference from the vehicle components. The test article G1 and G2 were formulated in a formulation containing 17mM $NaHC0_3$+ 0.3% HPMC (Hydroxypropyl Methylcellulose) in DI Water, pH 10±0.2. The most significant challenge was to optimize the order in which these formulation components were added and optimizing the acid/ base concentration used to reach a final pH where the formulation was a solution up to 50 mg/mL for each test article.

The three challenging aspects of the method was 1) establishing a short analytical method to separate the individual test articles, 2) detection of the individual test articles down to 1 µg/mL with reproducible response, and 3) ensuring no interference from the vehicle components, specifically HPMC. The assay was successfully validated with three sessions and the formulations were shown to be solutions at 50 mg/mL. Stability of the formulations was established for both test articles for up to 15 days of refrigerated storage.

This study shows strategies and general approaches for formulation optimization and analytical chemistry method development for multi component formulations.

2 FORMULATION PROCEDURE

The low formulation concentration for both G1 and G2 was at 0.1 mg/mL while the highest concentration was at 50 mg/mL. After much optimization the following procedures were implemented to get a clear formulation even at 50 mg/mL for both the test articles. The clear formulation was a visual indication of solubility which was also later proved analytically as a solution. The procedure for formulation preparation and the challenges faced are outlined below:

- After weighing out the necessary amount of G1 and G2, they were added into a 0.15N NaOH (2/3rd of the final formulation volume) solution in DI Water.
- The preparation was sonicated for approximately 5 minutes and subsequently mixed with a magnetic stirrer for approximately 15 minutes.
- The pH of the formulation was measured and adjusted, as necessary, to 12 using 1N NaOH.
- The sonication, stirring, and pH adjusted steps were repeated as necessary until all of the test article was dissolved. This was the most difficult step as optimization of starting NaOH concentration, initial pH and sonication time had to be done to ensure all material would go into the NaOH solution. The pH of the formulation was measured and adjusted, as necessary, to 10±0.1 using HCl or NaOH.
- An aliquot of the 50 mM $NaHCO_3$+ 1% HPMC vehicle (1/3rd of the final formulation volume and hence triple the concentration of its final value) was added and the formulation was mixed until uniform using a magnetic stirrer (approximately 10 minutes).
- The pH of the formulation was measured and adjusted, as necessary, to 10±0.1 using HCl or NaOH.

3 ANALYTICAL METHOD

- A single HPLC-UV method for both G1 and G2 were developed to simultaneously quantitate both.
- The diluent used was 10:90 (DMSO: Mobile Phase A)
- No interference of the vehicle was observed in the Quality control samples or Control group formulations
- All initial stocks were made in DMSO, followed by dilutions using the diluent
- Stock stability and process sample stability were obtained
- Highly reproducible chromatography and area counts were obtained

Instrument:	**Agilent 1100 liquid chromatograph equipped with a variable wavelength detector, autosampler, and Dionex Chromeleon® software version 6.8, or equivalent system**			
Column:	Symmetry Shield RP-18, 150 mm × 4.6 mm 5-µm particle-size			
Mobile Phase:	A: 1000:1 (v/v) DI water:Ortho-phosphoric acid B: 1000:1 (v/v) Methanol:Ortho-phosphoric acid			
Flow Rate:	1.00 mL/minute			
Gradient:	Time (minutes)	A (%)	B (%)	
	0	90	10	
	2.0	90	10	
	6.0	50	50	
	8.0	50	50	
	8.1	90	10	
	12	90	10	
Column Temperature:	40°C			
Autosampler Temperature:	Ambient			
Detector:	UV at 300 nm			
Injection Volume:	10 µL			
Retention Time:	Approximately 8.0 minutes for G1 and 5.6 minutes for G2			
Run Time:	12.0 minutes			

Table 1. Analytical Method details for separation of G1 and G2

4 VALIDATION RESULTS

- For the validation session, triplicate standards at 5 different concentration levels and triplicate QCs at 3 different concentration levels were ran.
- Precision and Accuracy was established with QC samples
- All peaks had an asymmetry factor within 1.02-1.14
- A Linear fit function was applied to both calibration curves
- All results for % relative Error (%RE) and Relative Standard Deviation (RSD) met acceptance criterion

Test Article	Calibration Conc. Range (µg/mL)	RSD Range of Values (%)	%RE Range of Values (%)
G1	1-25	0.076 to 6.8	-6.0 to 4.2
G2	1-25	0.31 to 1.4	-5.2 to 4.7

Table 2. Calibration Reproducibility and Validation

Test Article	Validated Conc. Range (mg/mL)	RSD Range of Values (%)	%RE Range of Values (%)
G1	0.1-50	0.13 to 2.0	-3.7 to 1.8
G2	0.1-50	1.8	0.60

Table 3. Precision and Accuracy

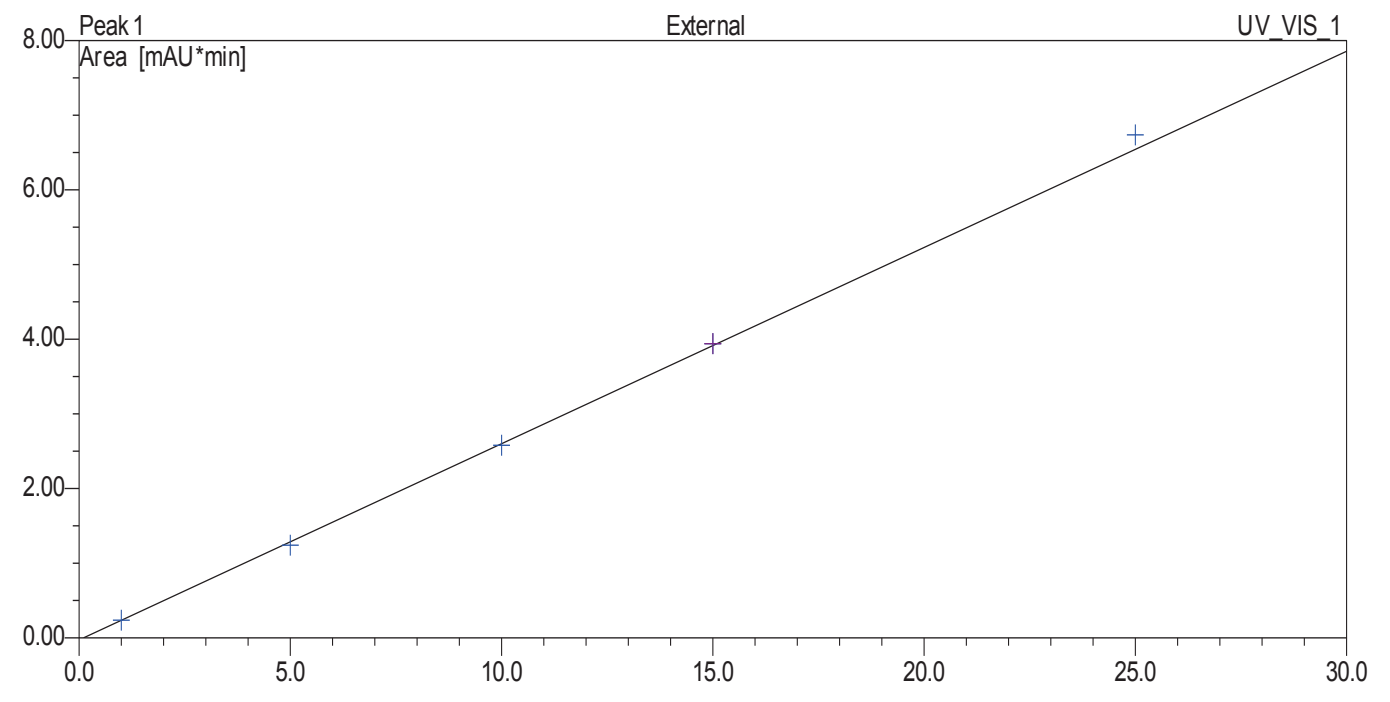

Fig 1. Calibration Curve for G1, 1-25 µg/mL

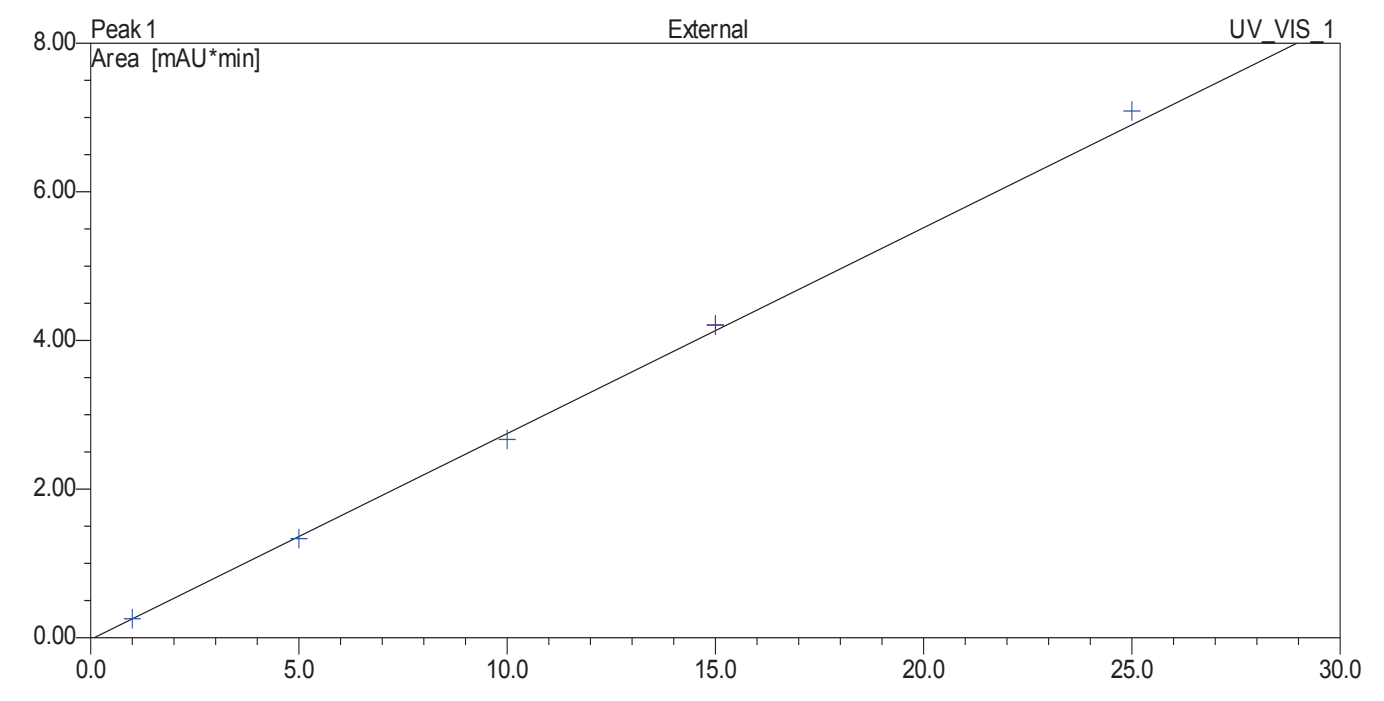

Fig 2. Calibration Curve for G2, 1-25 µg/mL

5 FORMULATION SOLUBILITY AND STABILITY

- Solubility at 50 mg/mL was established by comparing the mean concentration of the pre-filtered and post-filtered formulations.
- Formulation stability was established under multiple storage conditions and durations at 0.1 and 50 mg/mL

Conc (mg/mL)	Analyzed Conc. (mg/mL)	Percent of Target (%)	Mean Conc. (mg/mL)	SD	RSD (%)	Mean Conc % of Target (%)	Percent of Pre-Filtered (%)
50	50.9 48.9	102 97.8	49.9	1.4	2.9	99.8	103
				Pre-Filtered Conc = 48.3 mg/mL			

Table 4. Formulation Solubility at 50 mg/mL

Test Article	Storage Condition	Storage Duration	Mean Concentration, mg/mL (% of Time-Zero)	
			Low Group (0.1 mg/mL)	High Group (50 mg/mL)
G1	Room Temperature	16 Hours	0.097 (98.4)	49.9 (100)
	Refrigerated	8 Days	0.096 (97.6)	49.9 (100)
		15 Days	0.094 (95.1)	48.0 (97.2)
G2	Room Temperature	16 Hours	0.101 (106)	52.1 (102)
	Refrigerated	9 Days	0.087 (92.5)	47.8 (93.7)
		15 Days	0.097 (103)	48.2 (94.7)

Table 5. Formulation Stability in Vehicle

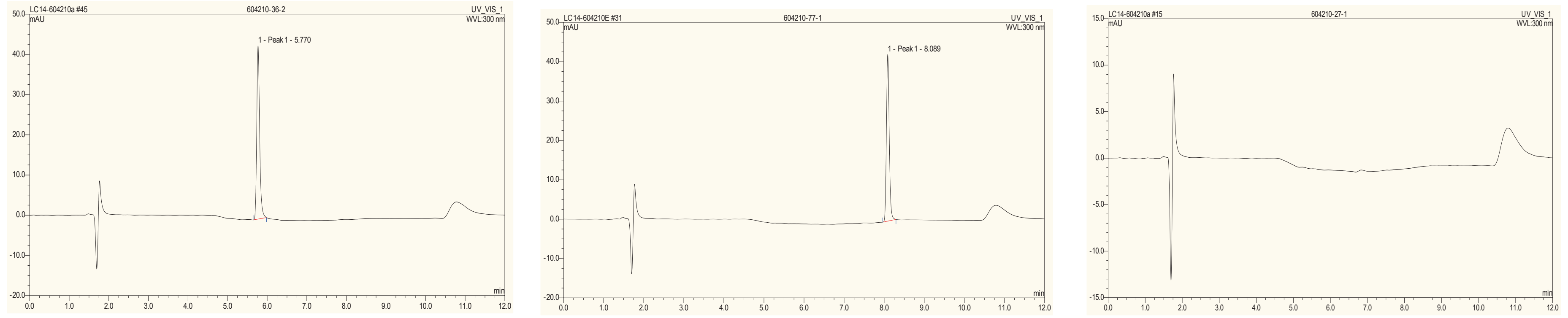

Fig 3, 4 and 5. Chromatogram of a processed formulation sample for G1, G2 and for the control group respectively

6 CONCLUSION AND ACKNOWLEDGEMENT

- Successful development of a two component formulation which is soluble up to 50 mg/mL.
- Validation of a single method which can measure both the components(test articles) of the formulation.
- Established solubility at 50 mg/mL for both the test articles.
- Established process-sample stability for up to 12 days under room temperature for calibration standards and Quality Control samples
- No interference from the vehicle components for the method.
- Linear calibration curve for both test articles.
- Stability of both the test articles for up to 15 days of refrigerated storage and short term room temperature storage.

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