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LIFE SCIENCES

APPLICATION NOTE

Development and Validation of an MRM Assay for the Determination of Residual Tetracycline in a Protein Drug Substance and Process Intermediate

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ABSTRACT

Purpose: A UPLC-MRM-MS Assay was developed and validated to quantitate the residual Tetracycline inNGR-hTNF Drug Substance and Process Intermediates.

Methods: A Tetracycline Standard and pipemidic acid used as the Internal Standard were spiked intoNGR-hTNF Drug Substance and In-Process Intermediate Buffer containing 20 mM HEPES, 0.1 M NaCl, and 8M urea, pH 7. The samples were chromatographed on a Zorbax 300SB-C8 (4.6 x 50 mm, 3.5 µm) and eluted on an acetonitrile gradient in 0.1% formic acid. An MRM-MS Assay was developed on a Waters UPLC and AB Sciex 4000 QTRAP LC/MS/MS for both Drug Substance and Process Intermediates of NGR-hTNF.

Results: An MRM-MS Assay for quantitation of residual Tetracycline in both NGR-hTNF Drug Substance and In-Process Intermediate Buffer was developed and validated. The Tetracycline in these samples was analyzed directly in the Assay without prior clean-up of the protein sample. Linearity (r = 0.998 and 0.994 for Drug Substance and Process Buffer, respectively) of the Assay was demonstrated in this study. Accuracy by recovery of Tetracycline ranged from 109 to 114% in the Drug Substance, and 97 to 109% in the Process Buffer. The Intra Day and Intermediate Precisions were within 10%, and 15%, respectively in either matrix. The LOQ of the Assay was 0.8 ng/mL and 0.6 ng/mL of Tetracycline in the Drug Substance and Process Buffer, respectively. For Assay Specificity, the various buffers tested in this study did not interfere with the quantitation of Tetracycline. Small purposeful changes in gradient slope, column temperature, and formic acid in the mobile phase in the Assay resulted in less than 5% change in the concentration of Tetracycline indicating the method is robust.

Conclusions: A UPLC-MRM Assay was developed and validated for the determination of residual tetracycline in NGR-hTNF Drug Substance and Process Intermediate. The method determines the concentration of Tetracycline directly in protein samples without prior protein clean-up or precipitation.

BACKGROUND

MRM-based Assay has been a reliable standard test to quantitate small molecule Drug Substance in pharmacokinetic and clinical samples due to its exceptional sensitivity and specicity. Recently, the MRM platform method has been gaining much attention in supporting drug development in large molecule therapeutics and biologics. In the present study, Tetracycline, which is commonly used in the manufacture of genetically-expressed proteins, was quantified in the presence of the protein Drug Substance and Process Intermediates by MRM MS. The samples were directly analyzed without prior clean-up of the protein and bioprocessing components.

OBJECTIVE

The objective of the current study is to develop and validate a UPLC-MRM-MS Assay to quantitate the residual Tetracycline in NGR-hTNF Drug Substance and Process Intermediates.

METHODS

Materials

The main materials used in this project consist of a genetically expressed recombinant protein of NGR-hTNF and commercially-available Tetracycline and Pipemidic Acid Standards. The materials included puried Drug Substance in a drug formulation.

Equipment

The primary equipment used in this project included a Waters Acquity Binary UPLC and Sciex ABI 4000 QTRAP MS/MS system.

Sample Preparation

The general procedure consisted of spiking Tetracycline and Pipemidic Acid (Internal Standard) Standards into either the protein Drug Substance or In-Process Intermediate Buer. Following the preparation, the samples are directly analyzed by UPLC-MRM MS.

UPLC Conditions

The sample was chromatographed onto a Zorbax 300SB-C8 4.6 x 50 mm, 3.5 µm at 40°C. The analyte is eluted in an acetonitrile gradient containing 0.1% formic acid at 0.5 ml/min.

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QTRAP Parameters

Analysis was performed on MRM mode and positive ion detection using a Sciex ABI 4000 QTRAP MS/MS system. The transition ion pairs used were as follows:

	Precursor ion (m/z)	Product ion (m/z)	Dwell time (msec)
Tetracycline	445	410	200
Pipemidic Acid	304	286	200

RESULTS

Figure 1. (below, left) Tetracycline Linearity Plot in Drug Substance
Figure 2. (below, right) Tetracycline Linearity Plot in Process Buffer

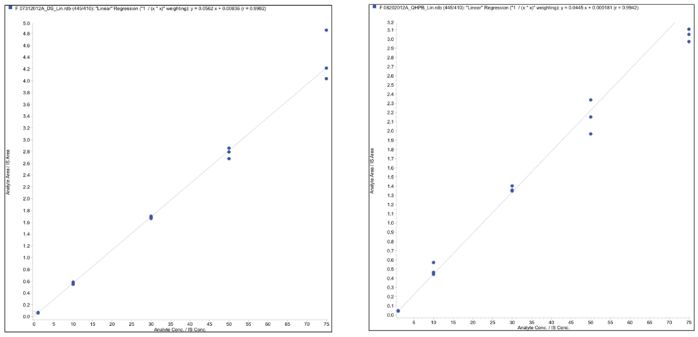


Figure 3. (below) Representative Chromatograms for Specificity: Drug Substance and Process Buffer

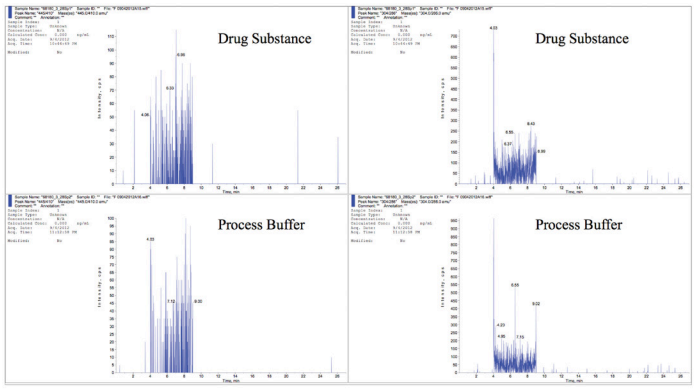


Figure 4. (below) Representative Chromatograms for Specificity: Citrate Buffer and CMS

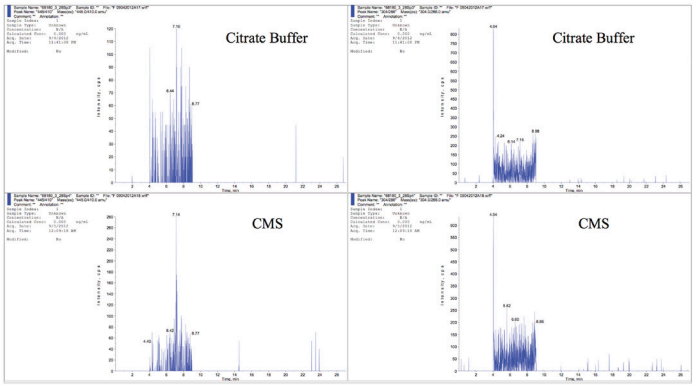


Figure 5. (below) Representative Chromatograms for Solution Stability: Freeze-Thaw

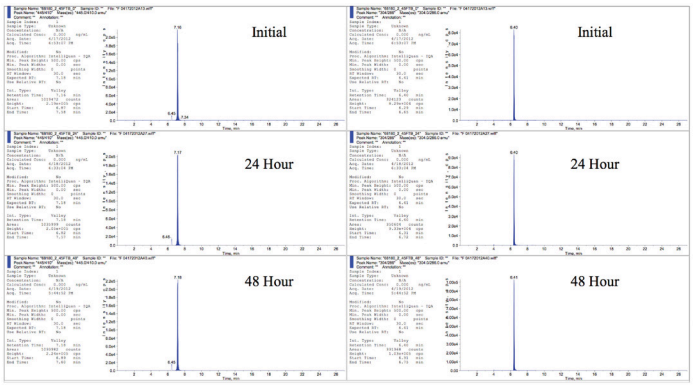


Figure 6. (below) Representative Chromatograms for Robustness: Gradient Slope

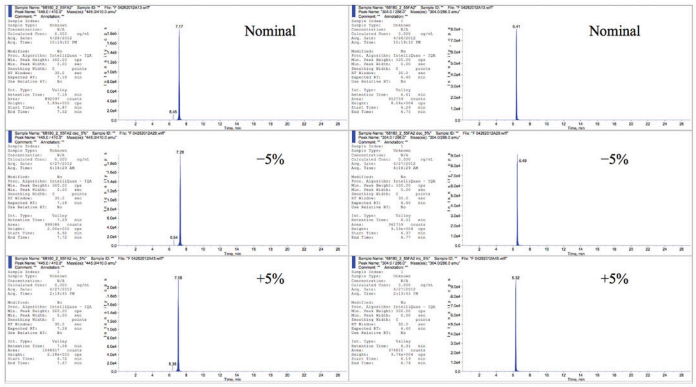
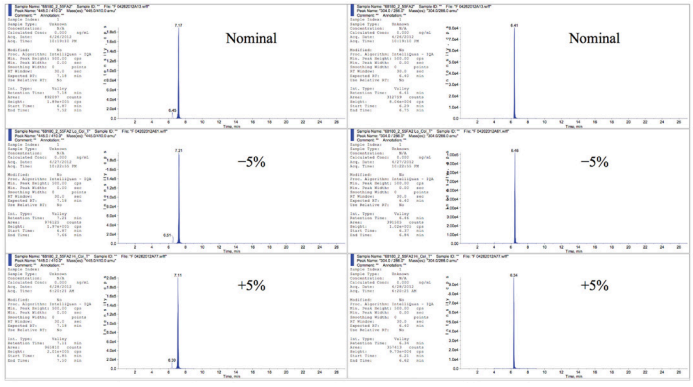


Figure 7. (below) Representative Chromatograms for Robustness: Column Temperature



APPLICATION NOTE

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Table 1. (below) Summary of Results: Linearity, Accuracy, and Repeatability

Test	Acceptance Criteria	Results			
Linearity	• Correlation Coefficient must be r ≥ 0.98	Sample		Correlation Coefficient (r)	
		Drug Substance		1.00	
		Process Buffer		1.00	
Accuracy by Recovery	• Recovery for the DS and the 30 ng/mL spiked representative Process Intermediate samples must be between 75 to 125% of the theoretical value • Recovery for the 10 ng/mL Process Intermediate samples will be reported only	Sample	Level	%Recovery	Mean %Recovery
		Drug Substance	30 ng/mL	111	109
				109	
				108	
			50 ng/mL	104	108
				109	
				111	
			75 ng/mL	126	114
				109	
				105	
		Process Buffer	30 ng/mL	112	109
				108	
				107	
			50 ng/mL	103	103
				112	
				94	
			75 ng/mL	95	97
				99	
				97	
		Process Intermediate	10 ng/mL		100
			30 ng/mL		100
Repeatability	• %RSD ≤ 10% of the determinations	%RSD = 2			

Table 2. (above) Summary of Results: LOQ Determination, Solution Stability, and Robustness

LOQ Determination	• Concentration obtained from the calculation, $LOQ = 10\left(\frac{SD}{S}\right)$ will be reported. • Concentration and %RSD obtained with the LOQ-spiked samples will be reported	Component	Concentration (ng/mL)	%RSD
		Drug Substance	0.76	
		Process Buffer	0.56	
		LOQ Confirmation	0.9	5
Solution Stability	• Change of results ≥ 20% RD vs. Time 0 (or initial time) indicates the samples are not stable • Report all results and provide representative chromatograms	Time Point	Condition	%RD
		Initial	Freeze/Thaw	
			Refrigerated	
		24 Hour	Freeze/Thaw	−7
			Refrigerated	−12
		48 Hour	Freeze/Thaw	−14
			Refrigerated	−16
Robustness	• Change of results ≥ 20% RD vs. nominal conditions indicates a critical method parameter • Report all results and provide representative chromatograms	Parameter	Change	% RD
		Nominal	NA	NA
		Gradient slope	−5%	−2
			+5%	−3
		Column temperature	−5%	−4
			+5%	−1
		Formic acid concentration	−5%	0
			+5%	1

DISCUSSIONS

An MRM-MS Assay was validated to quantitate residual Tetracycline in both NGR-hTNF Drug Substance and in Process Intermediate Buffer. Linearity was demonstrated from 1 ng/mL to 75 ng/mL for both sample matrices. In addition to spiked Drug Substance and Process Intermediate Buffer, Accuracy of the Tetracycline determination was also confirmed in actual Process Intermediate Sample spiked with the Tetracycline Standard. The LOQ of the Assay was 0.8 ng/mL for the Drug Substance and 0.6 ng/mL for the Process Intermediate Buffer.

For Specificity, the various buffer and protein samples tested in this study did not interfere with the quantitation of Tetracycline in these samples. The planned changes in gradient slope, column temperature, and Formic Acid in the Mobile Phase in the Assay resulted in less than 5% change in Tetracycline concentration. Hence, none of these Assay parameters were considered critical elements of the assay for quantitation of Tetracycline.

In conclusion, the results obtained from this study met the acceptance criteria for the evaluation of System Suitability, Accuracy by Recovery, Repeatability, Intermediate Precision, Specificity, LOD Determination, LOQ Determination, Solution Stability, and Robustness. The totality of the validation study included determination of Tetracycline in test materials containing various levels of protein concentrations. Furthermore, the Process Intermediate Buffer used in this study is a representative of the Process Intermediates typically encountered in the manufacture of the NGR-hTNF Drug Substance.

CONCLUSION

A UPLC-MRM Assay was developed and validated for the determination of residual Tetracycline in protein Drug Substance and Process Intermediates. The method passed all validation criteria and is deemed validated and suitable for its intended use.

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