



Simultaneous Quantitation of 9-Nitrocamptothecin an Anticancer Agent, and Its Metabolite, 9-Aminocamptothecin by LC/MS/MS in Rat, Rabbit, and Human Plasma and Human Urine

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Overview

A Liquid chromatography-tandem mass spectrometry (LC/MS/MS) method has been developed to improve specificity, sensitivity, and sample throughput for the quantitation of 9-nitrocamptothecin (9-NC) and 9-aminocamptothecin (9-AC) in human, rabbit, and rat plasma and human urine.

The addition of 10% HCOOH to plasma or urine quantitatively converts the carboxylic acid forms of both 9-NC and 9-AC to their respective lactones. Thus, analysis of the acidified or original (non-acidified) samples provides a method for the determination of the total (including carboxylic acid and lactone forms) or lactones only of 9-NC and 9-AC in animal and human plasma and urine.

The analytical method was successfully validated and employed to quantify 9-NC and 9-AC in human, rabbit, and rat plasma collected from Phase II and Phase III clinical studies.

Introduction

9-NC is an analogue of camptothecin. As a direct, highly potent inhibitor of the NDA-relegating activity of topoisomerase I, 9-NC shows a wide spectrum of activity in animal tumor models including slowly growing transplantable solid tumors and is now undergoing Phase III clinical evaluation in the USA and Europe for treatment of pancreatic cancer. 9-AC is a principal metabolite of 9-NC. The quantitation of 9-NC and 9-AC in biological fluids has been reported using high-performance liquid chromatography (HPLC) with fluorescence detection, however the method provided poor sensitivity, selectivity, and accuracy, along with lengthy sample preparation and chromatography run times. We report here a liquid chromatography-tandem mass spectrometry (LC/MS/MS) assay method for simultaneous quantitation of 9-NC and 9-AC in rat and rabbit plasma samples and human plasma and urine samples.

Experimental

Liquid chromatography:

LC System: Waters 2690 Separations Module
Analytical Column: Zorbax XDB-C18, 4.6 x 150 mm, 3.5 μm
Mobile Phase A: 1% HCOOH in H₂O
Mobile Phase B: 1% HCOOH in CH₃OH
Isocratic: A:B (43:57)
Flow rate: 0.8 mL/min
Injection Volume: 35 μL
Run time: 6.5 minutes

Mass Spectrometry:

MS System: PE Sciex API 365 mass spectrometer using APCI interface with multiple reaction monitoring (MRM) detection in positive ionization mode.

The mass spectrometer was set up for the following ion transitions:

9-NC: 394.2 → 350.2
9-AC: 364.2 → 320.1
12-NC (IS): 394.2 → 350.2

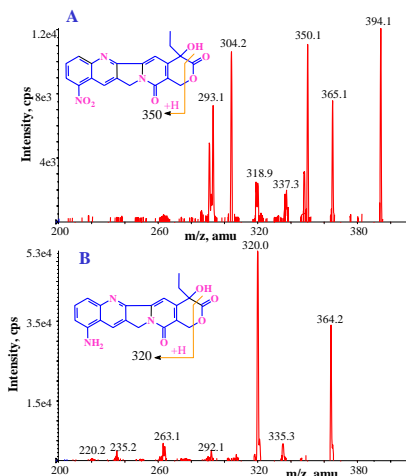


Figure 1. LC/ESI-MS/MS spectra of 9-NC (A) and 9-AC (B). The formation of the fragment ions at *m/z* 350 and 320 were proposed as shown in the attached structures.

Extraction Procedure:

A 500 μL aliquot of each human plasma sample was mixed with 200 μL of an internal standard solution prepared in 10% aqueous formic acid, and the mixture was processed using a SPEC PLUS™ 96-WELL C18 extraction plate. The desired eluates were evaporated to dryness under nitrogen and the residue was reconstituted in 200 μL of a mixture of methanol and 2% aqueous formic acid solution (50:50).

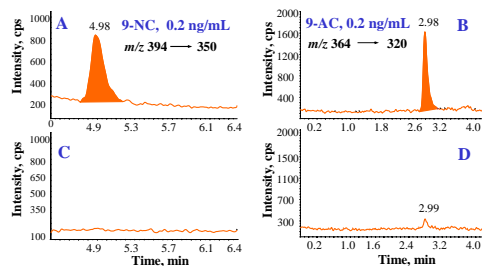


Figure 2. Mass Ion Chromatograms of 9-NC (A & C) and 9-AC (B & D) from a plasma sample at 0.2 ng/mL (A & B) or a blank plasma sample (C & D).

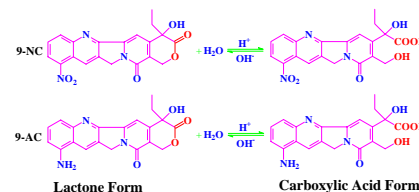


Figure 4. Equilibrium between lactone and carboxylic acid forms of 9-NC and 9-AC.

Results and Discussion

- Experimental data showed that the carboxylic acid forms of both 9-NC and 9-AC could be quickly and quantitatively converted to their respective lactones under acidic conditions (Figure 4). Thus, all matrices were acidified prior to sample clean-up and total concentrations, including both lactone and carboxylic acid forms, were determined.
- All methods were fully validated and determined to be selective, sensitive, accurate, precise, and reproducible with calibration ranges, for both 9-NC and 9-AC, of 0.5-50 ng/mL in rat and rabbit plasma and 0.2-20 ng/mL for human plasma and urine. Table I shows the validation data summary for human plasma method.

Conclusion

A liquid chromatography-tandem mass spectrometry assay method has been developed that substantially improves speed and sensitivity of assays for 9-NC and 9-AC in rat, rabbit, and human plasma, and human urine. The validated method has been applied to urine and plasma samples collected from Phase II and III clinical studies.

Table I. Human plasma validation results

Calibration Range		0.2 to 20 ng/mL				
Correlation coefficient (r, n=5)		9-NC:0.9990, 9-AC:0.9997				
Accuracy & Precision		9-NC		9-AC		
	QC Conc. (ng/mL)	Accuracy Bias%	Precision CV%	Accuracy Bias%	Precision CV%	
Inter-Batch (n=18)	LLOQ	0.2	5.83	11.79	0.64	9.95
	Low	0.4	3.13	7.51	-2.12	5.87
	Medium	8	2.00	5.83	-3.03	5.48
	High	16	2.68	4.74	-1.70	3.95
		Compared with Freshly Prepared QC (%)				
Storage Stability		9-NC		9-AC		
	Conditions					
Room Temperature	~ 24 hrs	97.92	to 100.6	101.1	to 101.4	
Freeze and Thaw	-20C, 3 cycles	101.6	to 103.1	101.4	to 103.4	
HPLC Autosampler	~ 4C, 2 days	97.11	to 100.0	90.38	to 93.13	
Long-Term Stability	~ 20C, 35 days	98.47	to 104.6	106.5	to 107.1	
Method Recovery		%				
9-NC		82.87	to	95.53		
9-AC		75.54	to	89.48		
12-NC (IS)		93.65	to	104.7		

Future Development

It was found that C18 cartridges can quantitatively retain 9-NC and 9-AC lactones while their acid forms elute with plasma proteins. The carboxylic acid forms of 9-NC and 9-AC can be quantitatively converted to their respective lactones under acidic conditions. Thus, introduction of a dual, tandem cartridge system, illustrated in the Figure to the right, to sample clean-up procedures, could result in separate recoveries of lactone and carboxylic acid forms of both components by LC/MS/MS analysis of the discrete eluates from the first (lactone forms) and second (carboxylic acids) C18 cartridges.

