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 method of choice of this study was developed based on the lack of sensitivity, selectivity, and accuracy, along with lengthy sample preparation and chromatography run times. We report here a liquid chromatography-tandem mass spectrometry (LC/MSMS) 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
Isocratic: A:B (45:55)
Flow rate: 0.8 mL/min
Injection Volume: 35 µL
Run time: 6.5 minutes

Extraction Procedure:

A 500 µL aliquot of each human plasma sample was mixed with 200 µL of internal standard solution prepared in 10% aqueous formic acid, and the mixture was processed using a SPEC PLUS™ 96-WELL C18 extraction plate.

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.

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 carbonylic 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 carbonylic 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.

Table I. Human plasma validation results

<table>
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<th>QC Conc. (ng/mL)</th>
<th>Bias%</th>
<th>CV%</th>
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Experimental data showed that the carbonylic acid forms of both 9-NC and 9-AC could be quickly and quantitatively converted to their respective lactones under acidic condition (Figure 4). Thus, all matrices were acidified prior to sample clean-up and total concentrations, including both lactone and carbonylic 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.

Results and Discussion