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A Sensitive Quantification of Unbound Vemurafenib in Human Plasma Using Equilibrium Dialysis Followed By Ultra-performance Liquid Chromatography Coupled to a Tandem Mass Spectrometry

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INTRODUCTION

Vemurafenib is a BRAF kinase inhibitor used for the treatment of metastatic melanoma with the BRAF V600E mutation. Recent pharmacokinetic data indicated that having a low plasma vemurafenib concentration was significantly associated with tumor progression, and the unbound fraction of vemurafenib was a sensitive biomarker in pharmacodynamics studies. These observations are consistent with the fact that the unbound or free drug fraction is pharmacologically active.

Methods using liquid chromatography coupled to a tandem mass spectrometry are available, with the reported limits of detection between 0.02 µM to 0.4 µM (or 10-200 ng/mL). However, Vemurafenib is a highly protein bound (>99%) compound, and quantification of unbound vemurafenib remains a challenge. This paper describes a sensitive LC-MS/MS assay for the quantification of unbound vemurafenib in human plasma using equilibrium dialysis.

OBJECTIVE

To develop a sensitive LC-MS/MS assay suitable for the quantification of unbound vemurafenib in human plasma using equilibrium dialysis.

METHODS

Vemurafenib was spiked into human plasma at concentrations 5, 150, 350 and 500 µM. Each 0.1-mL aliquot of spiked plasma, and 0.35 mL of PBS buffer were then added to their respective compartments of a Thermo Scientific rapid equilibrium dialysis plate and incubated at 37°C for 4 hours. After incubation, samples from the plasma compartment containing bound drug fraction were extracted and diluted with the internal standard solution; The PBS buffer containing free-drug fraction was diluted 1:1 with the internal standard solution prior to LC-MS/MS analysis. The internal standard solution consisted of stable isotope labeled Vemurafenib-d7 in methanol.

The UHPLC mobile phases consist of 1.6 mL of HFBA in 1 L of water (A) and 1.6 mL of HFBA in 1 L of acetonitrile (B). The vemurafenib were chromatographically separated on an ACQUITY UPLC HSS T3 1.8 µm, 100 × 2.1 mm column through a gradient elution by increasing mobile phase B from 60% to 90% in 2 minutes, and holding at 90% for 1.5 minute, then re-equilibrating the column at 60% mobile phase B for 1.5 minutes. The flow rate was 0.35 mL/min.

The vemurafenib and vemurafenib d7 were quantified by monitoring m/z 490 > 255 and 497 > 255 on a Thermo Scientific TSQ Vantage triple quadruple mass spectrometer equipped with Ultimate 3000 UHPLC system. The LC-MS/MS system was operated in positive ion mode.

RESULTS

Figure 1: Graphic representation of calibration curve for Vemurafenib across a range of 25–50,000 pM (0.012-24.5 ng/ml).

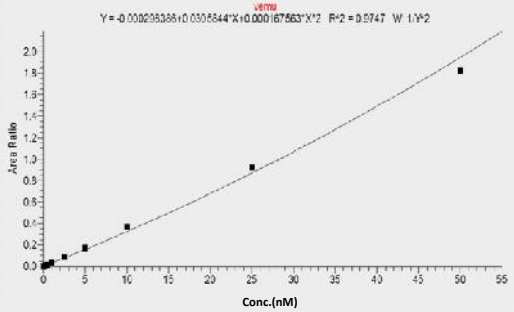
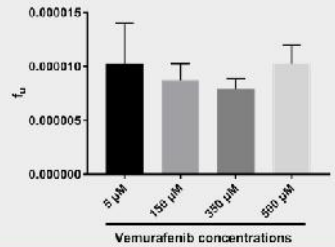


Table 1: Accuracy and reproducibility of Vemurafenib LC-MS/MS assay

Conc. (pM)	QC Samples	Calculated Conc. (pM)	% Error	%RSD
25	Replicate 1	25.7	3%	12%
	Replicate 2	24.5	-2%	
	Replicate 3	20.2	-19%	
	Replicate 4	21.3	-15%	
	Replicate 5	26.5	6%	
50	Replicate 1	55.3	11%	11%
	Replicate 2	43.4	-13%	
	Replicate 3	57.3	15%	
	Replicate 4	49.8	0%	
	Replicate 5	50.1	0%	
500	Replicate 1	539.9	8%	8%
	Replicate 2	564.8	13%	
	Replicate 3	474.5	-5%	
	Replicate 4	493.3	-1%	
	Replicate 5	571.7	14%	
5000	Replicate 1	5499.5	10%	1%
	Replicate 2	5605.8	12%	
	Replicate 3	5430.4	9%	
	Replicate 4	5405.3	8%	
	Replicate 5	5439.3	9%	

RESULTS

Figure 2: The unbound concentrations were 0.045, 0.98, 2.08, and 3.83 nM in the buffer chambers of the RED plate after 4-h equilibrium dialysis of human plasma originally spiked with 5, 150, 350 and 500 µM of vemurafenib, respectively.



CONCLUSION

A highly sensitive LC-MS/MS assay for unbound vemurafenib in human plasma was developed. More than 100 fold improvement on sensitivity was achieved. The unbound concentration and fraction of vemurafenib in human plasma at different concentration was successfully determined.

FUNDING

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REFERENCES

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