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Development of a Liquid Chromatography-Mass Spectrometry (LC-MS) Assay for the Measurement of Ivacaftor in Plasma

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Here we report the development of an LC-MS assay for the measurement of Ivacaftor in plasma. Ivacaftor is the first disease-modifying agent available for the treatment of cystic fibrosis that targets the defective chloride channel that leads to the characteristic thick secretions and organ damage associated with the condition. The clinical response to this drug is variable; some patients show a dramatic improvement in FEV_1 (forced expiratory volume in 1 second) of up to 35% while others show no change.(1) As Ivacaftor is metabolized through cytochrome P450 3A4 the rate at which it is eliminated is likely subject to great inter-individual variability.(2) It is currently unknown what influence this variability has on the clinical response to the drug and the development of this assay is the first step in investigating this important plasma concentration- clinical response relationship.

Plasma samples were diluted with 1:1 phosphate buffered saline and 20µL of internal standard added. Samples were loaded onto Biotage ISOLUTE® SLE+ supported liquid extraction columns and washed through with methyl tert-butyl ether. Samples were blown to dryness and reconstituted with 300µL of acetonitrile:water 1:1 with 0.1% formic acid and then analysed by LC-MS.

The method was linear in the range 0.125µg/mL to 8µg/mL. Weighting of $1/x^2$ was the simplest model that adequately described the concentration-response relationship with minimum absolute sums of relative error.

The intraday and interday accuracy and precision were within the recommended ±15% interval (± 20% for lower limit of quantification [LLOQ]). (3)

Table 1: Interday accuracy and precision

Ivacaftor Concentration (µg/mL)	Ivacaftor concentration found (µg/mL) (mean ± SD)			% of Nominal Concentration	Coefficient of variation
	Run 1	Run 2	Run 3		
6	5.866 ±0.188	5.897 ±0.139	5.952 ±0.467	98.42 ±4.692	4.77%
4	3.796 ±0.24	4.598 ±0.185	4.326 ±0.281	106.0 ±10.24	9.66%
0.375	0.425 ±0.02	0.358 ±0.019	0.409 ±0.019	105.8 ±9.391	8.88%

0.125	0.108 ±0.012	0.099 ±0.005	0.127 ±0.008	89.03 ±11.53	12.95%

There was no significant interference at the retention times of either the analyte at the LLOQ of 0.125μg/mL, or the internal standard. This LLOQ was chosen as it is suitable to allow accurate measurement of Ivacaftor at the established EC_{90} of 0.405 μg/mL.(2)

The analyte was shown to be stable stored at -80 ℃ for 1 week. Recovery at a concentration of 6μg/mL and 0.375 μg/ml was 107.35% and 87.53% respectively. Plasma samples are stored for analysis at 1, 3 and 6 months.

Establishment of this reliable method to measure plasma levels of ivacaftor will facilitate studies on the variation of the pharmacokinetics and effectiveness of the drug in the real-world setting.

(1) Ramsey BW et al (2011) N Engl J Med;**365**: 1663-1672

(2) Ivacaftor Summary of Product Characteristics, http://ec.europa.eu/health/documents/community-register/2012/20120723123715/anx_123715_en.pdf

(3) European Medicines Agency Guideline for Bioanalytical Method Validation, www.**ema**.europa.eu/docs/en_GB/document.../WC500109686.pdf