

Quantitation of Humalog Insulin by Reversed-Phase High-Performance Liquid Chromatography

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Abstract

Introduction:

The methodology for *in vitro* testing of insulin delivery systems for long-term infusion of lispro insulin requires insulin flow studies over time, with the measurement of lispro concentrations in wells or other laboratory fluid collection systems. We postulated that the efficiency of the insulin assay could be improved if the insulin collected in the wells could be measured without having to perform dilutions of insulin samples. The manufacturer's method for the identification of Humalog[®] insulin by high-performance liquid chromatography (HPLC) does not provide a detailed method for the quantitation of the clinical formulation. No reports could be found in the literature describing a method to quantitate lispro insulin's entire concentration range, nor could we find detailed information on pH effects. The purpose of this study was to investigate the use of a reversed-phase HPLC method to quantitate Humalog insulin at three levels of pH.

Methods:

Serial dilutions of Humalog insulin stock solution were prepared at pH levels of 2.6 and 7.4 (concentrations 0.20–100 IU/ml) to construct calibration curves. Samples were also prepared at lower concentrations (0.23–15.0 IU/ml) at pH levels of 4.0 and 7.4. Areas under curve data were plotted against known concentrations, and the limit of quantitation and precision of the assay were determined.

Results:

Calibration curves for the 0.20–100 IU/ml concentration (pH 7.4 and 2.6) revealed no significant differences ($p < 0.01$) between those curves. pH and concentration did not have an effect on the overall precision of the method. Linearity was not conserved past the 15-IU/ml concentration. Coefficient of variation (CV) values were generally <15% except at the lower concentrations where the largest CVs were observed. Mean known concentrations plotted on a logarithmic scale demonstrated linearity from 12.5 to 100 IU/ml.

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Abbreviations: (AUC) area under the curve, (IP) isoelectric point, (LOQ) limit of quantitation, (PBS) phosphate-buffered saline, (RP-HPLC) reversed-phase high-performance liquid chromatography, (TFA) trifluoroacetic acid

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Abstract cont.**Conclusions:**

Humalog insulin clinical formulation can be quantitated over the entire concentration range by a reversed-phase HPLC method using nonlinear regression analysis. The method is reproducible at lower (<15 IU/ml) and higher insulin concentrations. Linear regression analysis may be used when the concentrations of interest are in the 0- to 15-IU range. Preparation of insulin solutions at pH 2.6, 4.0, and 7.4 did not significantly affect the reproducibility of the assay.

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