

A simple and rapid UHPLC-MS/MS method for measurement of hydroxychloroquine in saliva Seraphim JC¹, Amaral ACS¹, Kupa LVK¹, Romano P^{2,3}, Chalom MY², Almeida REP^{2,3}, Duarte NJC^{2,3}, Carvalho VM⁴, Silva CA¹, Bonfa E¹Rheumatology Division Hospital das Clínicas HCFMUSP², Central Laboratory Division, ³Laboratório de Investigação Médica (LIM 03), Hospital das Clínicas HCFMUSP, Faculdade de Medicina, Universidade de São Paulo ⁴Grupo Fleury, São Paulo, SP, BR.

Background

Liquid chromatography-tandem mass spectrometry (LC-MS/MS) is considered the gold standard for therapeutic drug monitoring (TDM) in several clinical settings to guarantee efficacy while avoiding toxicity. Hydroxychloroquine (HCQ) blood levels and clinical efficacy has been correlated in Systemic Lupus Erythematosus. Saliva is emerging as an alternative matrix to overcome the limitations of blood sample in TDM. Therefore, we developed and validated a LC-MS/MS method to measure HCQ in saliva.

Methods

Positive Electrospray Ionization was used in a triple quadrupole LC-MS/MS system at SRM Mode and yields consistent signals with selective transitions of m/z 336.2 \rightarrow (247.0; 158.0) and 340.2 \rightarrow (251.0; 162.0) for HCQ and internal standard (HCQ-*d4*), respectively. To each 90 μ L of saliva, 10 μ L (500ng/mL) of internal standard and 100 μ L of water were added, centrifuged at 8000 rpm for 10 min and 40 μ L of the supernatant were injected into an on-line extraction column, SolExTM RSLC HPD, 2.1 x 20 mm eluted by a binary gradient with water [A] and methanol, acetonitrile, water, formic acid (50:40:9.5:0.5) [B] at 1 mL/min. Analytes were transferred by forward flush to an analytical column Accucore PFP 80 A, 50 x 2.1 mm, 2.6 μ m) eluted by a binary gradient with ammonium acetate 25 mM [A] and methanol, acetonitrile (70:30) [B] at 0.4 mL/min. Validation studies were performed based on international standards and included precision, accuracy, linearity, quantification limits and recovery.

Results

HCQ and HCQ-*d4* eluted consistently at 5.1 min. The calibration curve of peak area ratio (HCQ/ HCQ-*d4*) versus concentrations was linear from 0.5 to 100 ng/mL ($r^2 > 0.99$). The method had a detection limit of 0.045 ng/mL and lower limit of quantification of 1.0 ng/mL. Intra-day precision yields a coefficient of variation of 1.1%, 1.5%, and 1.9%, respectively for 80.0 ng/mL, 15.0 ng/mL, and 2.0 ng/mL, and inter-assay had 14.4%, 5.1%, and 15.1%. The accuracy was < 20% as recommended for relative error and the recovery was 96.0%, 100.3%, and 103.0%, respectively for high, medium and low HCQ.

Conclusions

The UHPL-MS/MS method for measurement of HCQ in saliva was successfully developed, validated and can be applied for TDM and pharmacokinetic studies.

Key words: Hydroxychloroquine; LC-MS/MS; saliva levels; Therapeutic drug monitoring