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Rapid Determination of Clarithromycin in Human Plasma by LCMS/MS Assay

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A rapid liquid chromatographic tandem mass spectrometric (LC-MS/MS) assay for the measurement of clarithromycin level in human plasma was developed and validated using erythromycin as internal standard (IS). Analysis was performed at room temperature using a reversed phase Atlantis dC18 (2.1 × 100 mm, 3 µm) column. The components of interest were detected in the positive ion mode of electrospray ionization using transition 749 → 158.4 and 719.3 → 158.2 for clarithromycin and the IS, respectively. Quantification and detection limits were 5 and 2 ng/ml, respectively. Mean extraction recovery was ≥ 86% for clarithromycin and 99% for the IS. Relationship between clarithromycin concentration and peak height ratio of clarithromycin to the IS was linear (R2 ≥ 0.9833) in the range of 0.005-4.0 µg/ml, and the intra- and inter-day coefficient of variations were 2.9% to 13.1% and 2.5% to 9.6%, respectively. Clarithromycin in human plasma was stable for at least 24 hours at room temperature (≥ 83%) or 14 weeks at -20°C (≥ 93%), and after three freeze-thaw cycles (≥ 83%). The method was successfully used to determine clarithromycin levels in human plasma samples obtained from a healthy volunteer. Atmospheric Pressure Ionization; CAS: Chemical Abstract Number; CV: Coefficient of Variation; eV: Electron Voltage; FT: Freeze-Thaw; HPLC: High Performance Liquid Chromatography; IS: Internal Standard; kV: Kilo Voltage; L/hr: Liters/ Hour; LCMS/MS: Liquid Chromatography-Tandem Mass Spectrometry; m/z: Mass to Charge Ratio; ng/ml: Nanogram/Milliliter; QC: Quality Control; RT: Room Temperature; SD: Standard Deviation; UV: UltraViolet; USP: United State Pharmacopeia; µg/L: Microgram/Liter. Clarithromycin (CAS: 81103-11-9) is a broad-spectrum semisynthetic macrolide antibiotic used in the treatment of various bacterial infections. Its absolute bioavailability is about 55%, with a peak plasma concentration of 2.41 to 2.85 µg/ml at 2-3 hours after the ingestion of a 500 mg therapeutic dosage. A thorough literature review of clarithromycin assays revealed a number of high-performance liquid chromatography (HPLC) methods using ultraviolet (UV) fluorescence, electrochemical, or liquid chromatography-mass spectrometry (LC-MS/MS) detection. Some of these methods require laborious multi-step, extraction procedures 6rmination of clarithromycin in biological matrix employed electrochemical detection, since the molecule lacks a suitable], derivatization, or require large sample volumes. Most of the reported assays for the dete chromophore to be detected by UV. Some HPLC-UV assays used a wavelength of ≤ 210 nm, where interference from sample matrix usually occurs. Clarithromycin was also measured by fluorescence detection after sample treatment with 9-fluorenylmethyl chloroformate or 9-fluorenylmethyloxycarbonyl chloride. The reported LC-MS/MS methods used various compounds as internal standard including stable isotope-labelled clarithromycin. All chemicals were of analytical grade unless stated otherwise. Clarithromycin and erythromycin were purchased from USP reference standard Rockville, MD, USA. Triethylamine, phosphoric acid, tert. butyl methyl ether, and acetonitrile (HPLC grade) were purchased from Fisher Scientific, NJ, USA. Water for HPLC was prepared by reverse osmosis and further purified by using synergy water purification system (Millipore, Bedford, MA, USA). The study was approved by Analysis was performed on a reversed phase Atlantis dC18 (2.1× 100 mm, 3 µm) column proceeded by Symmetry C18 (3.9 × 20 mm, 5 µm) guard column. The mobile phase, containing 0.05% triethylamine (pH=4.0, adjusted with phosphoric acid) and acetonitrile (65:35, v: v), was filtered through a 0.22 µm membrane filter (Millipore Corporation, Bedford, MA, USA), degassed, and delivered at a flow rate of 0.25 ml/ min. Mass Lynx software working under Microsoft Windows XP professional environment was used to control the instruments, data acquisition, peak integration, peak smoothing, and signal-to-noise ratio measurements. The electrospray ionization source was operated in the positive-ion mode at a capillary voltage of 4.0 kV and a cone voltage of 30 V. Nitrogen was used as nebulizing and desolvation gas at a flow rate of 60 and 600 L/hr, respectively. Argon was used as the collision gas at a pressure of 1.28 × 10-3 mbar. The optimum collision energy for clarithromycin and erythromycin (internal standard, IS) was 25 eV. The ion source and the desolvation temperatures were maintained at 125 and 350°C, respectively. Clarithromycin and IS stock solutions were prepared in methanol (100 µg/ml). Calibration standards at nine different concentrations (0.005-4.0 µg/ml) and quality controls at four concentrations: 0.005, 0.015, 2.0, and 3.8 µg/ml were prepared in human plasma. IS working solution was prepared in methanol (1.0 µg/ml). Standard and control solutions were vortexed for one minute, and 200 µl aliquots were transferred into 7 ml glass culture tubes and stored at -20°C until used. 50 µl of the IS working solution was added to 200 µl plasma sample, calibration standard, or quality control (QC) samples in a 7 mL culture tubes and vortexed. 4.0 ml tert. butyl methyl ether was added, vortexed for one minute, and centrifuged at 6000 rpm for 10 minutes at room temperature. The clear supernatant layer was transferred to a clean culture tube and dried under gentle steam of nitrogen at 40°C. The residue was reconstituted in 100 µl mobile phase. 5 µl of the clear solution was injected into the LC-MS/MS system. Although mass spectrometry has been recognized as a technique for quantification since its inception, the greatest impetus to its use in the field of quantitative measurement of organic compound has come from its coupling with liquid chromatography (LC). In order to optimize LC conditions, we initially used ammonium acetate buffer in combination with acetonitrile in range of 40-60%. No satisfactory results were obtained. However, after replacing ammonium acetate with triethylamine, we found consistently satisfactory results. Triethylamine in the mobile phase facilitated the generation of ion in the electrospray

ionization mode and allowed completing the analysis within 3.0 minutes. Therefore, detection and quantification of clarithromycin were optimized using a mobile phase composed of 0.05% triethylamine (pH=4.0, adjusted with phosphoric acid) and acetonitrile (65:35, v: v) at a flow rate 0.25 ml/min. Although mass spectrometry has been recognized as a technique for quantification since its inception, the greatest impetus to its use in the field of quantitative measurement of organic compound has come from its coupling with liquid chromatography (LC). In order to optimize LC conditions, we initially used ammonium acetate buffer in combination with acetonitrile in range of 40-60%. No satisfactory results were obtained. However, after replacing ammonium acetate with triethylamine, we found consistently satisfactory results. Triethylamine in the mobile phase facilitated the generation of ion in the electrospray ionization mode and allowed completing the analysis within 3.0 minutes. Therefore, detection and quantification of clarithromycin were optimized using a mobile phase composed of 0.05% triethylamine (pH=4.0, adjusted with phosphoric acid) and acetonitrile (65:35, v:v) at a flow rate 0.25 ml/min. The absolute recovery of clarithromycin was assessed by comparing absolute peak height of spiked plasma and mobile phase samples, using five replicates of four QC samples (0.005, 0.015, 2.0 and 3.6 µg/ml). Similarly, the recovery of the IS was determined by comparing the peak height of the IS in 5 aliquots of human plasma spiked with 1.0 ng/ml IS with the peak height of equivalent samples prepared in mobile phase. The extraction recoveries were 86-101% for clarithromycin and 99% for the IS. 50 µl of the IS working solution was added to 200 µl plasma sample, calibration standard, or quality control (QC) samples in a 7 mL culture tubes and vortexed. 4.0 ml tert. butyl methyl ether was added, vortexed for one minute, and centrifuged at 6000 rpm for 10 minutes at room temperature. The clear supernatant layer was transferred to a clean culture tube and dried under gentle steam of nitrogen at 40°C. The residue was reconstituted in 100 µl mobile phase. 5 µl of the clear solution was injected into the LC-MS/MS system. Although mass spectrometry has been recognized as a technique for quantification since its inception, the greatest impetus to its use in the field of quantitative measurement of organic compound has come from its coupling with liquid chromatography (LC). In order to optimize LC conditions, we initially used ammonium acetate buffer in combination with acetonitrile in range of 40-60%. 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