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Short communication

Liquid chromatography–mass spectrometry analysis of diethylcarbamazine in human plasma for clinical pharmacokinetic studies

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ABSTRACT

A sensitive and selective liquid chromatographic method using mass spectrometric detection was developed for the determination of diethylcarbamazine (DEC) in human plasma. DEC and its stable isotope internal standard d_3-DEC were extracted from 0.25 mL of human plasma using solid phase extraction. Chromatography was performed using a Phenomenex Synergi 4μ Fusion-RP column (2 mm × 250 mm) with gradient elution. The retention time was approximately 4.8 min. The assay was linear from 4 to 2200 ng/mL. Analysis of quality control samples at 12, 300, and 1700 ng/mL (N = 15) had interday coefficients of variation of 8.4%, 5.4%, and 6.2%, respectively (N = 15). Interday bias results were −2.2%, 6.0%, and 0.8%, respectively. Recovery of DEC from plasma ranged from 84.2% to 90.1%. The method was successfully applied to clinical samples from patients with lymphatic filariasis from a drug–drug interaction study between DEC and albendazole and/or ivermectin.

1. Introduction

Diethylcarbamazine (DEC, Fig. 1) is an anti-parasitic agent utilized in the treatment of lymphatic filariasis (LF). Diethylcarbamazine not only kills the microfilaria, but is the most potent available drug against adult *Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori*, the three lymphatic dwelling parasites that infect humans [1]. In areas where onchocerciasis and loaisis are not co-endemic with LF (endemic areas outside of sub-Saharan Africa), DEC combined with albendazole are the primary drugs used for annual mass drug administration (MDA) as the primary tool to elimination of LF in certain geographic areas [2]. The World Health Organization has set a goal to eradicate LF by 2020, using this MDA program with antifilarial medications with the aim to interrupt transmission and stop the spread of infection. The recommended regimens must be administered once a year for at least 5 years. Although numerous clinical trials for the treatment of lymphatic filariasis have been conducted, pharmacokinetic studies of DEC in body fluids have been hampered by the lack of analytical methods with low quantitation limits and low required sample volumes. Chromatographic methods with UV detection have been published for determining DEC in medicated salts [3] or tablet formulations [4]. However, these UV methods had lower limits of quantitation (LLOQ) ≥100 ng/mL. Several gas chromatographic methods have been used to analyze DEC in plasma [5–10]. The most sensitive gas chromatographic method has a LLOQ of 10 ng/mL with a required sample volume of 1 mL of plasma using a NPD detector [9]. However, the range of the method was limited, with an upper limit of quantitation of 200 ng/mL, which necessitates many re-runs for over-range samples. A modification of the method used a FID detector and 0.5 mL of plasma has also been published; the range of the calibration curve in this approach ran from 100 to 2000 ng/mL [10].

To better support pharmacokinetic analyses for drug–drug interaction studies, a method is needed that reduces the amount of plasma needed and achieves a sensitivity of ≤10 ng/mL. DEC

Abbreviations: DEC, diethylcarbamazine; LF, lymphatic filariasis; LLOQ, lower limit of quantitation; SPE, solid phase extraction.

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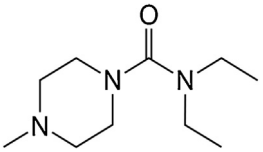

Fig. 1. Structure of diethylcarbamazine.

is a weak base that would be expected to readily form a protonated species under typical LC–MS conditions, the detection of which should be quite sensitive relative to UV detection. Previously, gas chromatographic methods for DEC were developed because of the lack of suitable stationary phases to retain small polar compounds on liquid chromatography columns [9,10]. However, the number and quality of available stationary phases has rapidly evolved since that time, making liquid chromatography feasible for bioanalytical work with DEC. For the present work, a column/mobile phase combination was readily found with a retention factor k' of 1.5, which provided adequate resolution from endogenous substances not removed in the extraction from plasma.

2. Experimental

2.1. Solvents and chemicals

Methanol and acetonitrile were Optima grade purchased from Fisher Scientific (Fair Lawn, NJ, USA). Dibasic sodium phosphate was ACS grade purchased from Fisher Scientific. Tribasic sodium phosphate (ACS grade), formic acid (≥88%) and diethylcarbamazine citrate (Vetranal analytical standard) were purchased from Sigma–Aldrich (Saint Louis, MO, USA). The internal standard d_3-diethylcarbamazine citrate was purchased from Toronto Research Chemicals (Toronto, Ontario, Canada). Phosphate buffer, pH 10.3 was prepared from the sodium phosphates. A solution of 0.1% formic acid in methanol was prepared to elute the solid phase extraction (SPE) cartridge. Ultrapure analytical grade Type I water was produced by a MilliQ Plus water system (Millipore Corporation, Bedford, MA, USA).

2.2. Blank plasma, plasma standards and controls, patient samples

The citrate salts of DEC were used to make stock solutions. DEC citrate and d_3-DEC citrate were weighed on a Mettler-Toledo AG104 analytical balance (Mettler-Toledo Inc., Hightstown, NJ, USA). The weights were corrected for the salt to determine the amount of free base. The weighed amount was dissolved in methanol in a volumetric flask to make 1.0 mg/mL stock solutions, which were stored at −20 °C. DEC stock solution was prepared in duplicate. Working solutions of DEC were prepared daily at 10.0, 1.0, 0.10, and 0.010 μg/mL by serial dilution of each stock solution with water. A 0.8 μg/mL working solution of d_3-DEC was prepared by diluting the stock solution with water in a volumetric flask, which was stored at −20 °C and used for 1 week. Calibration standards were prepared by adding between 10 and 75 μL of the appropriate working solution to a 1.5 mL microcentrifuge tube containing 20 μL of internal standard, 250 μL of plasma, and 700 μL of phosphate buffer. Control samples were prepared similarly using the duplicate set of DEC working solutions. The working curve consisted of samples containing 4, 8, 16, 64, 256, 520, 1400, and 2200 ng DEC/mL plasma. Controls were 12, 300, and 1700 ng DEC/mL plasma. Patient samples, blanks, and blank zeros were prepared by adding 20 μL of internal standard and 700 μL of phosphate buffer to 250 μL of patient plasma. All solutions were vortex mixed and subjected to SPE prior to instrumental analysis. The lowest calibrant of the calibration curve was chosen because it was deemed more than sufficient for pharmacokinetic analysis and to minimize difficulties in achieving the regulatory guidelines for precision. The signal-to-noise ratio was >50 at the LLOQ, leaving some capacity to lower either the quantitation limit or the volume of plasma required for analysis if needed for specialized studies in young children or critically-ill patients.

2.3. Solid phase extraction

Agilent C18 (50 mg/1 mL) SPE Cartridges (Agilent Corp., Santa Clara, CA, USA) were used for sample preparation. A Cerex positive pressure SPE processor was used with nitrogen to modulate flow. All flow rates were approximately 1 mL/min. The cartridges were conditioned with 1 mL of methanol followed by 1 mL of water. After loading the prepared sample, the cartridge was washed with 2 mL × 1 mL of water and eluted with 2 mL × 1 mL of 1% formic acid in methanol. Solvent was removed under flowing nitrogen at 30 °C. The residue was reconstituted in 100 μL of 5% acetonitrile in water and transferred to an autosampler vial with glass insert for analysis. The recovery of DEC from plasma was determined by spiking triplicate plasma samples with DEC at the three control concentrations and comparing the average peak area ratio of the spiked sample to the average peak area ratio of triplicate plasma samples with analyte added post extraction, followed by drying and reconstitution. Internal standard was added post extraction in all recovery samples.

2.4. Instrumentation

The instrumentation system consisted of a Shimadzu 2010A LC–MS platform in APCI positive mode operating under LCMSSolution (Version 3.01) software (Shimadzu, Columbia, MD, USA). The analytical column was a Phenomenex Fusion-RP (4 μm, 80 Å, 2.0 mm × 250 mm) preceded by a Phenomenex Fusion-RP SecurityGuard guard (2.0 mm × 4 mm) column. Separation conditions were: sample temperature, 23 (±3) °C; column temperature, 23 (±3) °C; sample injection volume, 25 μL. Solvent A (46%) was 0.05% formic acid; solvent B was acetonitrile with 0.05% formic acid. The analysis was run on a gradient between 5% and 57% B over 7.5 min at a flow rate of 0.25 mL/min. The total run time for a LC–MS analysis was 10 min.

The mass spectrometer was tuned using a polyethylene glycol solution following the manufacturer's protocol; the interface, CDL, and Q-array voltages optimized in the tune were used for the analysis. The scan interval was 1.0 s, microscan 0.15 amu, the APCI probe temperature was 375 °C, and the CDL (curved desolvation line) and block temperatures 200 °C. Nitrogen flow through the probe was 2.5 L/min. DEC and d_3-DEC yielded [M+1] ions with no significant adduct formation or in-probe fragmentation. The use of the stable isotope internal standard greatly lessens the likelihood of significant matrix effects.

2.5. Calculations and precision

The DEC concentrations were calculated from the peak-area ratio of DEC to d_3-DEC for standards, controls, and samples. The linear least squares equation was calculated with $1/Conc^2$ weighting. Coefficients of determination (r^2) were 0.993 or better. During validation, series of plasma controls spiked with DEC at 4, 12, 300, and 1700 ng/mL were analyzed for accuracy and precision in pentuplicate on three days (Table 1). The lower limit of quantitation was 4 ng/mL; S/N at was >50 at the LLOQ. During sample analysis,