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Development of HPLC and LC–MS/MS methods for the analysis of ivacaftor, its major metabolites and lumacaftor in plasma and sputum of cystic fibrosis patients treated with ORKAMBI or KALYDECO

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ABSTRACT

ORKAMBI (ivacaftor-lumacaftor [LUMA]) and KALYDECO (ivacaftor; IVA) are two new breakthrough cystic fibrosis (CF) drugs that directly modulate the activity and trafficking of the defective CFTR underlying the CF disease state. Currently, no therapeutic drug monitoring assays exist for these very expensive, albeit, important drugs. In this study, for the first time HPLC and LC–MS methods were developed and validated for rapid detection and quantification of IVA and its major metabolites hydroxymethyl-IVA M1 (active) and IVA-carboxylate M6 (inactive); and LUMA in the plasma and sputum of CF patients. With a mobile phase consisting of acetonitrile/water:0.1% formic acid (60:40 v/v) at a flow rate of 1 mL/min, a linear correlation was observed over a concentration range from 0.01 to 10 μg/mL in human plasma (IVA $R^2 > 0.999$, IVA M1 $R^2 > 0.9961$, IVA M6 $R^2 > 0.9898$, LUMA $R^2 > 0.9954$). The assay was successfully utilized to quantify the concentration of LUMA, IVA, M1 and M6 in the plasma and sputum of CF patients undergoing therapy with KALYDECO (IVA 150 mg/q12 h) or ORKAMBI (200 mg/q12 h LUMA-125 mg/q12 h IVA). The KALYDECO patient exhibited an IVA plasma concentration of 0.97 μg/mL at 2.5 h post dosage. M1 and M6 plasma concentrations were 0.50 μg/mL and 0.16 μg/mL, respectively. Surprisingly, the ORKAMBI patient displayed very low plasma concentrations of IVA (0.06 μg/mL) and M1 (0.07 μg/mL). The M6 concentrations (0.15 μg/mL) were comparable to those of the KALYDECO patient. However, we observed a relatively high plasma concentration of LUMA (4.42 μg/mL). This reliable and novel method offers a simple and sensitive approach for therapeutic drug monitoring of KALYDECO and ORKAMBI in plasma and sputum. The introduction of the assay into the clinical setting will facilitate pharmacokinetics/pharmacodynamic analysis and assist clinicians to develop more cost effective and efficacious dosage regimens for these breakthrough CF drugs.

1. Introduction

Cystic fibrosis is an autosomal recessive life limiting disease that is caused by defective or deficient cystic fibrosis transmembrane conductance regulator (CFTR) protein [1]. The disease affects the exocrine mucus glands in the lung, liver, pancreas, and intestines causing progressive multi-system failure such as loss of lung function and pancreatic insufficiency [2–6]. KALYDECO (ivacaftor, IVA) is the first FDA-approved CFTR modulator, with evidenced clinical efficacy producing a significant improvement in the lung function of CF patients with the G551D-CFTR mutation (found in 4–5% of CF cases) [7]. More specifically, IVA functions as a CFTR potentiator producing an increased CFTR channel open probability to enhance chloride influx [8–10]. ORKAMBI is a novel combination treatment that combines the potentiator function of IVA with lumacaftor (LUMA), a CFTR corrector which improves the surface trafficking of CFTR in CF patients bearing a homozygous F508del mutation, expanding the therapeutic window to the broader CF patient collective (~28%) [11]. The annual cost per patient for both drugs is high ($259,000-$311,000 USD), and presently their manufacturer Vertex has a market monopoly as these are the only drugs on the market available for their indication [12]. The clinical effi-

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cacy of ORKAMBI *versus* the cost of therapy has been brought into question due to potential antagonistic drug–drug interactions between IVA and LUMA that potentially limit the clinical efficacy of ORKAMBI [13,14]. Additional factors that may limit ORKAMBI's clinical efficacy come from its less than ideal pharmacokinetic properties [15,16]. IVA is extensively metabolized by cytochrome P450 enzymes, primarily to an active metabolite hydroxymethyl-IVA (M1) and an inactive form IVA-carboxylate (M6) [15,17,18]. LUMA on the other hand is not extensively metabolized and is largely excreted unchanged in the faeces [16]. Both IVA and LUMA are very hydrophobic molecules and are ~99% bound to plasma proteins, which significantly limits the free (active) drug concentration [2,14]. Presently, very little information is available concerning the steady-state plasma concentrations of IVA, M1, M6 and LUMA achievable in patients under the current recommended dosage regimen no methods for therapeutic drug monitoring have been reported so far. In this study, we have developed a reliable and simple LC–MS method for the quantification of IVA, its major metabolites M1, M6 and LUMA in plasma and sputum for therapeutic drug monitoring of CF patients receiving KALYDECO or ORKAMBI therapy. The implementation of the assay in clinical centers should assist clinicians to develop exposure-response relationships to maximize drug efficacy as well as to contain healthcare costs.

2. Material and methods

2.1. Materials

IVA and LUMA were purchased from SelleckChem, USA. IVA-carboxylate (Catalogue number 510242247CS) and hydroxymethyl-IVA (Catalogue number 510240849CS) were from Clearsynth (Canada) and were used as internal standards (IS) in methanol at 10 μg/mL. Methanol (MeOH, LC–MS grade), acetonitrile (ACN, LC–MS grade) and formic acid were purchased from Sigma-Aldrich (Australia). Experiments were performed on a triple-quadrupole Shimadzu 8030 LC–MS and a Nexera X2 Shimadzu HPLC (SPD-M30A detector) systems using a Waters C8 column (5 μm, 3.9 × 50 mm i.d.). Plasma was obtained from the Australian Red Cross. Plasma and sputum samples were collected from two compliant volunteers receiving ORKAMBI or KALYDECO therapy.

2.2. Preparation of stock solutions and sample standards

Two independent stock solutions of each analyte (IVA, M1, M6 and LUMA) were freshly prepared in methanol for each analytical run at 100 μg/mL. Stock solutions of the internal standards (IS) were prepared in methanol at 10 μg/mL. The IS working solutions were prepared by a further dilution of each IS stock solution with methanol. Eight calibration standards stock solutions of each analyte at 1, 2.5, 5, 10, 25, 40, 65 and 80 μg/mL were prepared in methanol by serial dilution of the 100 μg/mL stock solutions. The LC–MS calibration standards were freshly prepared at the beginning of each analytical run by dilution of the calibration standards stock solutions into human plasma to achieve following concentrations: 0.01, 0.025, 0.05, 0.1, 0.2, 0.5, 1, 2.5, 5 and 10 μg/mL.

2.3. Sample pre-treatment

Plasma or sputum samples were vortex-mixed before sampling and an aliquot of 200 μL was transferred into a 1.5 mL polypropylene microcentrifuge tube (VWR). Protein precipitation was achieved by an addition of 200 μL of a mixture of ACN/0.1% formic acid. The mixture was vortexed vigorously, then centrifuged at 10,000 × *g* for 10 min (Eppendorf Centrifuge 5430) at room temperature. A 200 μL aliquot of the supernatant was filtered through a 13-mm syringe filter (0.45 μm nylon, GRACE, USA) into an HPLC 1.5 mL vial [Phenomenex VEREX, 9 mm, PP, 300 μL, PTFE/Silicone septa]. An aliquot of 5 μL was injected onto the HPLC column. The method validation procedure for the IVA assay was based on the FDA guidelines for bioanalytical method validation [19]. Partially method validation was performed including parameters listed in Table 1 as the aim was to develop a method that is easily accessible for hospital laboratories measuring all 3 or 4 compounds in one single assay.

2.4. HPLC analysis

Initial HPLC method development for the analysis of IVA and LUMA in biological matrices was performed on a Shimadzu Nexera X2 HPLC system. Analytes were separated on Waters C8 column (3.9 × 150 mm id, 5 μm) under isocratic conditions at 1 mL/min. The mobile phase consisted of ACN/H_2O:0.1% formic acid mixture (60:40 v/v). Column temperature was set to 30 °C and sample compartment temperature was set to 4 °C. The injection volume was 5 μL. Total run time was 13 min including the wash and equilibration steps. Quantification of IVA (also M1 and M6) and LUMA was performed at 309 nm and 295 nm, respectively.

2.5. LC–MS/MS analysis

The LC–MS/MS analysis of patient samples was performed on a Shimadzu 8030 LC–MS system coupled with the 8030 triple quadrupole mass spectrometer. The mass spectrometer operated in a positive electrospray ionization mode. LC–MS settings were: ion spray voltage 4.5 kV, collision energy 295.9 V, nebulizing gas: nitrogen at 3 L/min; collision gas: argon; drying gas flow 20 L/min, lens voltage Q3: −22 V, desolvation temperature 250 °C with a heat block temperature of 400 °C. The mobile phase flow was split before entering the mass spectrometer in ratio 2:1 (waste:MS inlet). Analytes were detected using multiple reaction monitoring (MRM). The ion transitions of m/z 392.49 → 393, m/z 408.49 → 409, m/z 422.47 → 423 and m/z 452.40 → 453 were monitored for IVA, M1, M6 and LUMA, respectively.

2.6. Calibration curves

The LC–MS calibration curves were constructed before each analytical run using the relationship between the peak area ratios of IVA to IS and the calibration standard nominal concentrations of IVA (M1, M6 or LUMA). Linear least-squares regression analysis with weighting 1/C^2 was performed according to the reciprocal of concentrations (Shimadzu).

2.7. Accuracy and precision

The intra-day accuracy of each analyte was assessed with six independently prepared quality control (QC) samples on the same day at concentrations of 0.05, 0.5 and 8 μg/mL. The inter-day accuracy was assessed with six independently prepared QC samples on three consecutive days. Accuracy and precision were calculated via relative standard deviation (RSD). For each QCs, the RSD values should be less than 15% [20]. The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility. In this study, the precision of our analytical procedure was expressed as relative standard deviation or coefficient of variation of a series of measurements. The relative standard