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Simple and accurate quantitative analysis of 20 anti-tuberculosis drugs in human plasma using liquid chromatography–electrospray ionization–tandem mass spectrometry

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ABSTRACT

A simple and accurate liquid chromatography (LC)–tandem mass spectrometry (MS/MS) method for the quantitation of 20 anti-tuberculosis (anti-TB) drugs in human plasma, was developed as a tool for therapeutic drug monitoring. Two protein precipitation methods were adopted; one using methanol containing 0.13 N HCl, for precipitation of amikacin, kanamycin, streptomycin and pyrazinamide, and the other using acetonitrile, for precipitation of preamoxicillin, ciprofloxacin, clarithromycin, clofazimine, cycloserine, ethambutol, ethionamide, isoniazid, levofloxacin, linezolid, moxifloxacin, p-aminosalicylic acid (PAS), prothionamide, rifabutin, rifampin and roxithromycin. Separation was performed either on an HILIC silica column or a reversed-phase dC18 column, with a gradient elution. Detection was carried out in multiple reaction-monitoring (MRM) mode. The calibration curves were linear over a 50-fold concentration range, with correlation coefficients (r) greater than 0.9969 for all anti-TB drugs. The intra- and inter-day precision was less than 14.3%, and the accuracy ranged between 84.8 and 113.0%. The developed method was successfully applied to the identification and quantitation of anti-TB drugs in patients with multi-drug resistant TB.

1. Introduction

Therapeutic drug monitoring (TDM) is the process of obtaining the serum concentration of a medication and modifying the dose based on the results, with the intention of optimizing therapeutic benefits, while minimizing the risk of side effects or toxicity. TDM of anti-tuberculosis (anti-TB) drugs has drawn little attention from analysts and clinicians, because the benefits of drug monitoring are not well established. Recently, several medical institutes have developed protocols for monitoring anti-TB drugs and have applied them to the management of patients with TB [1,2]. However, TDM is not well established in the context of treatment of multi-drug resistant tuberculosis (MDR-TB), despite the low global treatment success rate of 60%, and a Korean treatment success rate of 45.3–62.6% [3–5].

The relatively low rate of treatment success in MDR-TB may be related to low serum concentrations of anti-MDR-TB drugs, because variation in anti-TB drug concentration has been associated with malabsorption, alcohol use, age, sex, hypoalbuminemia, patient weight and drug formulation [6–8]. TDM is more necessary for patients with drug-resistant TB or co-morbidities that complicate the clinical status, than patients who respond to the standard four-drug TB regimens [2]. Furthermore, treatment failure or drug toxicities are ongoing concerns in some MDR-TB patients. Adjusting the dosage using TDM has been shown to be a better treatment strategy than administering a standard fixed dose [1]. The need to monitor anti-TB drug levels to rapidly identify treatment failure has

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Table 1
Multiple reaction monitoring parameters, calibration range, correlation coefficient and LLOQ concentration for the tested 20 anti-TB drugs.

Compounds	Transition (*m/z*)	DP (V)	CE (eV)	Calibration range (μg/mL)	Correlation coefficient (*r*)	LLOQ (μg/mL)
Group 1						
Amikacin	586.2 → 425.3	60	30.0	1.0–50.0	0.9987	1.0
Kanamycin	485.5 → 163.3	60	30.0	1.0–50.0	0.9971	1.0
Streptomycin	582.2 → 263.3	140	42.5	1.0–50.0	0.9989	1.0
Pyrazinamide	124.0 → 81.0	60	20.0	2.0–100	0.9988	2.0
Group 2						
Amoxicillin	366.0 → 114.0	60	30.0	0.4–20.0	0.9996	0.4
Ciprofloxacin	332.0 → 231.2	60	52.0	0.2–10.0	0.9989	0.2
Clarithromycin	748.6 → 590.7	60	30.0	0.1–5.0	0.9988	0.1
Clofazimine	473.2 → 431.2	140	50.0	0.04–2.0	0.9987	0.04
Cycloserine	103.0 → 58.0	40	15.0	0.8–40.0	0.9989	0.8
Ethambutol	205.0 → 116.0	60	20.0	0.1–5.0	0.9973	0.1
Ethionamide	167.1 → 107.0	60	33.0	0.1–5.0	0.9984	0.1
Isoniazid	138.0 → 121.0	60	20.0	0.1–5.0	0.9982	0.1
Levofloxacin	362.0 → 318.0	60	30.0	0.2–10.0	0.9969	0.2
Linezolid	338.0 → 296.0	60	30.0	0.4–20.0	0.9982	0.4
Moxifloxacin	402.0 → 384.0	60	30.0	0.2–10.0	0.9996	0.2
PAS	154.0 → 119.0	60	30.0	1.0–50.0	0.9994	1.0
Prothionamide	181.0 → 154.3	60	30.0	0.2–10.0	0.9980	0.2
Rifabutin	847.6 → 815.5	60	30.0	0.04–2.0	0.9992	0.04
Rifampin	823.4 → 791.1	60	25.0	0.2–10.0	0.9992	0.2
Roxithromycin	837.6 → 679.6	60	30.0	0.2–10.0	0.9975	0.2

been widely discussed in the literature, because low serum concentrations of anti-TB drugs are closely related to treatment failure in human immunodeficiency virus (HIV)-infected [9] and non-HIV infected TB patients [10].

Several analytical methods have been developed to measure plasma concentrations of anti-TB agents, including high-performance liquid chromatography (HPLC)-ultraviolet detection [11], HPLC-fluorescence detection [12], and HPLC–tandem mass spectrometry (MS/MS) [13,14]. However, these methods were designed for single drug analysis or for simultaneous evaluation of first-line anti-TB drugs. In the treatment of patients with MDR-TB, at least five anti-TB drugs are routinely used in the intensive phase. The choice of anti-TB drugs is dependent on a drug susceptibility test and the patient's condition. Therefore, a more convenient analytical tool for TDM in patients with MDR-TB would be compatible with all prescribed anti-TB drugs. However, no method that is applicable simultaneously to both first-line and second-line antituberculosis drugs has been developed.

In the present study, we developed a simple and rapid analytical method for quantitative determination of plasma concentrations of 20 anti-tuberculosis drugs, including the first line anti-TB drugs, using LC–MS/MS. The developed method could be successfully applied to therapeutic monitoring of anti-TB drugs in patients.

2. Experimental

2.1. Chemicals

Amoxicillin, apramycin, *D*-cycloserine, ciprofloxacin, clarithromycin, clofazimine, ethionamide, isoniazid, kanamycin, levofloxacin, p-aminosalicylic acid (PAS), rifampicin, roxithromycin and streptomycin sulfate were purchased from Sigma–Aldrich (St. Louis, MO). Amikacin sulfate, ethambutol dihydrochloride, linezoid, moxifloxacin hydrochloride, moxifloxacin-d_4, prothionamide, pyrazinamide, rifabutin, and rifampin-d_3 were obtained from Toronto Research Chemicals (North York, ON). HPLC-grade acetonitrile and methanol were purchased from Burdick & Jackson (Muskegon, MI). Drug-free human heparinized plasma was provided by Busan Paik Hospital (Busan, South Korea). All other chemicals and solvents were of the highest analytical grade available.

2.2. Preparation of standards and quality control samples

Stock solutions of amikacin, apramycin, kanamycin, streptomycin, cycloserine, and ciprofloxacin HCl were prepared in distilled water. Stock solutions of ethambutol, isoniazid, levofloxacin, moxifloxacin, moxifloxacin-d_4, PAS, prothionamide, rifabutin, rifampin, and roxithromycin were prepared in methanol. Stock solutions of amoxicillin, clarithromycin, clofazimine, ethionamide, linezolid, pyrazinamide, and rifampin-d_3 were prepared in dimethyl sulfoxide (DMSO). All stock solutions were stored at −20 °C prior to use. Two separate preparation methods were used to extract drugs, which were divided into two groups based on their chemical properties. Working solutions of group one and group two drugs were prepared by mixing four (amikacin, kanamycin, streptomycin, and pyrazinamide) and 16 compounds (amoxicillin, ciprofloxacin, clarithromycin, clofazimine, cycloserin, ethambutol, ethionamide, isoniazid, levofloxacin, linezolid, moxifloxacin, PAS, prothionamide, pyrazinamide, rifabutin, rifampin and roxithromycin), respectively. Working solutions of group one and group two compounds were serially diluted with distilled water and acetonitrile, respectively. Calibration standard samples were prepared in blank plasma by spiking with an appropriate volume of serially diluted stock solution to generate the six concentrations for the calibration curve. Quality control (QC) samples were prepared daily at low, medium and high concentrations.

2.3. Sample preparation

Sample preparation was performed by protein precipitation with methanol or acetonitrile. In the case of group one compounds, 200 μL of methanol containing 5 μg/mL apramycin, as an internal standard, were added to 100 μL of human plasma acidified with 10 μL of 4 N HCl. After vortex mixing and centrifugation at 9000 × *g* for 10 min at 4 °C, 1 μL of the supernatant was injected into the LC–MS/MS system. For the group two compounds, 100 μL of acetonitrile containing 1 μg/mL of moxifloxacin-d_4 and rifampin-d_3, as internal standards, was added to 50 μL of plasma. Rifampin-d_3 was used for the quantitation for rifampin and moxifloxacin-d_4 was used for the quantitation of the rest of 15 anti-TB drugs. After vortex mixing and centrifugation at 9000× *g* for 10 min at 4 °C, 50 μL of the supernatant were removed and further diluted with three