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Original Article

The Fast and Simple LC-MS/MS Method for Determination of Rifampicin in the Human Blood Plasma

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ABSTRACT

Background: Rifampicin is an important anti tuberculosis drug for the early infection and, more importantly, bactericidal activity against mycobacterium tuberculosis. LC-MS/MS, with the required molecular specificity, provides quantitative method to rapidly differentiate between the drugs and their metabolites. **Aim:** The current study represents the fast and simple LC-MS/MS method for determination of rifampicin in the human blood plasma. **Materials and Methods:** The plasma samples containing the rifampicin drug were cleaned up by using the protein precipitation method. The chromatographic division was performed by utilizing the ZORBAX Eclipse plus C_{18} Column (4.6 mm X 150 mm, 5µm). The versatile stage comprised of acetonitrile and 10 Mm ammonium acetic acid derivation in a proportion of 80:20% V/V, wash the column with the blend of solvents comprising of acetonitrile and water 80:20% V/V, maintaining column oven temperature at 30°C±1°C and the auto sampler temperature is kept up with at 10°C±1°C. The injection volume of the sample is 10.0 µL and the total run time of the experiment is 4 min with a flow rate of 1000 mL/min and with a split ratio of 50:50 for the entire experiment. **Results and Discussion:** An LC-MS/MS method for the rifampicin from the sample was performed. Sample volume of 0.300 ml, the injection volume is taken as 10.0 µL and total run time is 4 min, RT for rifampicin is 1.30±0.5 min and the reference drug roxithromycin RT is 3.00±0.5 min. **Conclusion:** The transient LC-MS/MS study permits the assessment of enormous quantities of blood tests in a brief timeframe with fast, simple and successful readiness, giving a quick, dependable and best device for RIF clinical observing and review.

Keywords: Rifampicin, LC-MS/MS, Protein precipitation, Acetonitrile.

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INTRODUCTION

Prediction of drugs in biological fluids is one of the main issues connected with bioavailability, bioequivalence, new medication improvement, drug use, pharmacokinetics and substance research is for the most part founded on the synthetic recipes, which are valuable in the expectation of medicines.[1] Rifampicin is the first-line drug in the treatment of pneumonic tuberculosis and extra pulmonary tuberculosis. It is used together with isoniazid or pyrazinamide as a secondary treatment for extra pulo-pulmonary tuberculosis.[2,3] Tuberculosis treatment has become a global problem because of the use of chemotherapy today is hampered by the high cost of the first vaccine, especially in the developing countries.[4] Rifampicin (Figure 1) is an important anti tuberculosis drug for the early infection and, more importantly, bactericidal activity against mycobacterium tuberculosis. Rifampicin is a group of macro cyclic antibiotics designed for mycobacterium Mediterranean and is one of the most important first-line antibiotics.[5] Pharmacological checking is the most common way of changing medication portion in light of Plasma drug fixation to accomplish the ideal clinical reaction while limiting the gamble of the aftereffects. A mass spectrometric technique for evaluating the rifampicin in human blood plasma utilizing ionization radiation is portrayed.[6,7] Since rifampicin is considered a macromolecule with an undifferentiated structure, the ESI interface may produce unstable ions. LC-MS/MS, with the required molecular specificity, provides quantitative method to rapidly differentiate between the drugs and their metabolites.[8,9]

Principle

In Analytical or HPLC in the liquid layer, the Separation of samples depends on the interaction between mobile and stationary phases. Once the chromatographic separation is complete, the compounds are eluted from the column and ionized at the ionization point. The ionized compounds are then fed into a mass spectrometer for accurate analysis. Test planning is one of the main strides of endlessly drug metabolite examination in bioanalytical

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research. These "terrible items" may associate with limited quantities of medications during investigation. The primary objective of the displaying system is to eliminate all unwanted species from the model without lessening the normal accuracy. These days, there are numerous strategies for the extraction of analytes from organic materials, like Solid phase Extraction (SSE), fluid Extraction (LLE) and Protein Precipitation (PP).[11,12] High throughput bioanalytical techniques involve well-defined tools; the same standard procedures; and replace manual processes with automation wherever possible. Auto samplers, HPLC systems, mass spectrometers and ionization sources that couple LC and MS are components of the LCMS system. In a perfect world, this ought to be constrained by a PC framework. Since HPLC is a generally utilized procedure, it won't be examined in this article. It ought to be noticed that there are restrictions in the stream rates and cell levels that can be utilized while joining HPLC with MS.[13] In most MS-coupled reversed-phase HPLC systems, the versatile stage is a combination of water and methanol or acetonitrile used as mobile phase Mobile stage cooling has some limitations; that is, they are generally indecisive. Chemicals called mobile phase modifiers are added.[14]

MATERIALS AND METHODS

Experiment

Drugs and reagents

Rifampicin is used as sample and roxithromycin is used as internal standards. HPLC-grade acetonitrile ammonium acetate and LCMS/MS-grade acetonitrile were purchased from relevant companies.

Device

Ultrafast liquid chromatography (Shimadzu10 AD) was combined with the Thermo TSQ ultra (MS/MS) mass spectrometry system. These include a binary pump, an auto sampler, a column oven and appropriate controllers. The system is used with a thermos electrospray ion (HESI) source. Related software is used for management, data collection and processing. Electronic equipment, RIA vials, vortex mixers, vortex oscillators and high-speed cryogenic centrifuges are also used.

Chromatography conditions[15]

Utilize the ZORBAX Eclipse plus C_{18} column (4.6 mm X 150 mm, 5 µm) to accomplish chromatographic partition. Wash the chromatography section with a dissolvable combination of acetonitrile and water (80%:20% V/V). Keep up with the oven temperature at 30°C±1°C and the auto sampler temperature at 10°C±1°C.Then its injection volume rate was 10.0 µL, the absolute examination time was 4 min, the stream rate all through the whole investigation was 1,000 mL/min and the proportion was (50:50).

Preparation of solutions and samples

Sample preparation[16,17]

Remove the sample from the freezer and thaw at room temperature. RIF's (rifampicin) extraction from human plasma

Table 1: Parameters of MS/MS.

Compound	Rifampicin	Roxithromycin
Spray voltage(V)	5000	5000
Sheath gas pressure (Pa)	30	30
Ion sweep gas pressure (Pa)	0	0
Aux gas pressure (Pa)	10	10
Capillary temperature (°c)	250	250
Tube lens office	88	88
Skimmer offset	0	0
Collision pressure	1.0	1.0
Collision energy	13	13
Parent mass (m/z)	823.691	837.535
Product mass(m/z)	791.757	679.731

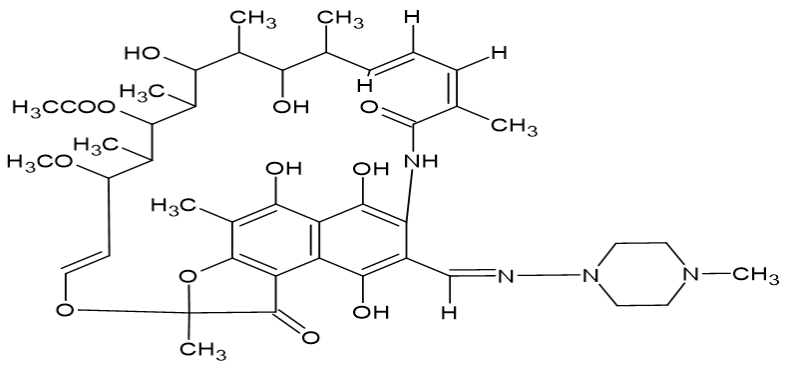


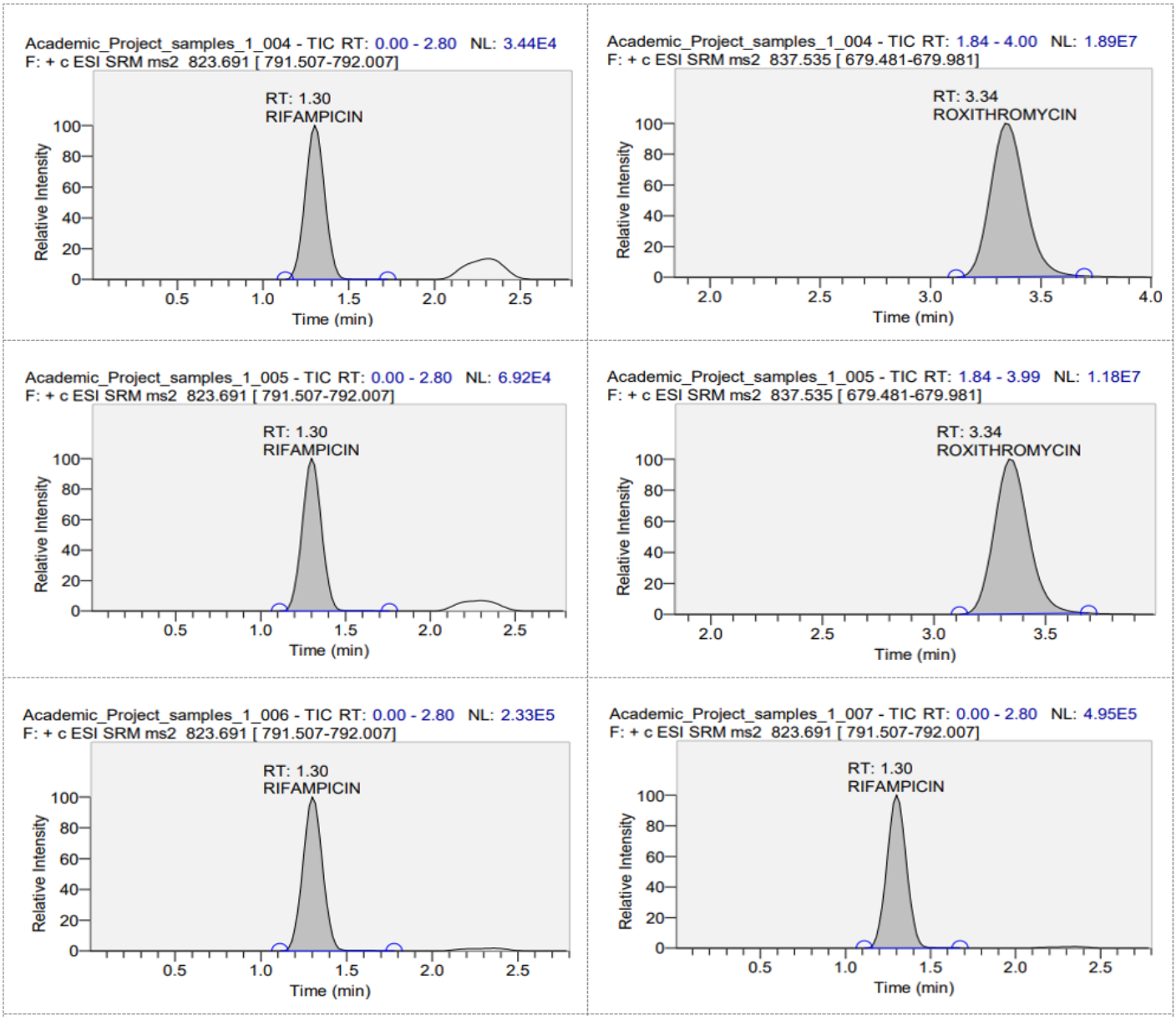
Figure 1: Structure of rifampicin.[10]

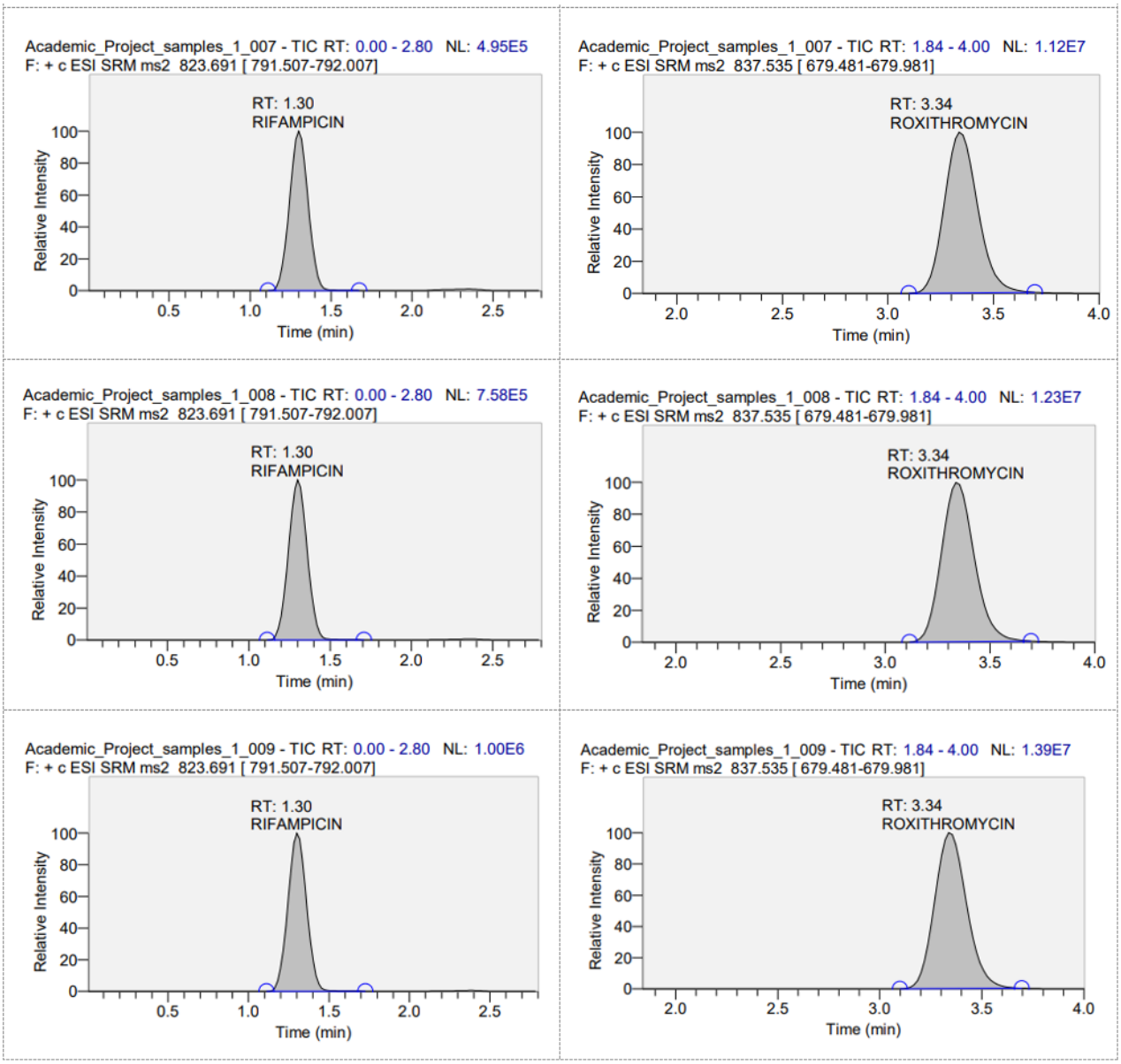
using the protein precipitation method. Prepare100 µL aliquots of the sample in pre-labelled RIA vials, then, at that point, add 50 µL which is used as internal standard (roxithromycin 1,000 µg/mL) and vortex equally utilizing a mixer. Add 0.400 mL of weighty precipitation (acetonitrile 100%) to the solution and cap the vial, then vortex the sample at 1000 rpm on vibramax for 10 min and centrifuge the sample at a constant 4500 rpm for 10 min to reach 4°C. Move 0.300 mL of the supernatant to a pre-labelled injection vial, move the reconstituted test to named auto samplers and inject it into the LC-MS/MS instrument for recognition (Table 1). In the examination, the internal standard roxithromycin was ready and utilized at a concentration of 1,000 µg/mL.

MS/MS parameters

Chromatograms

Standard

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RT: 3.34
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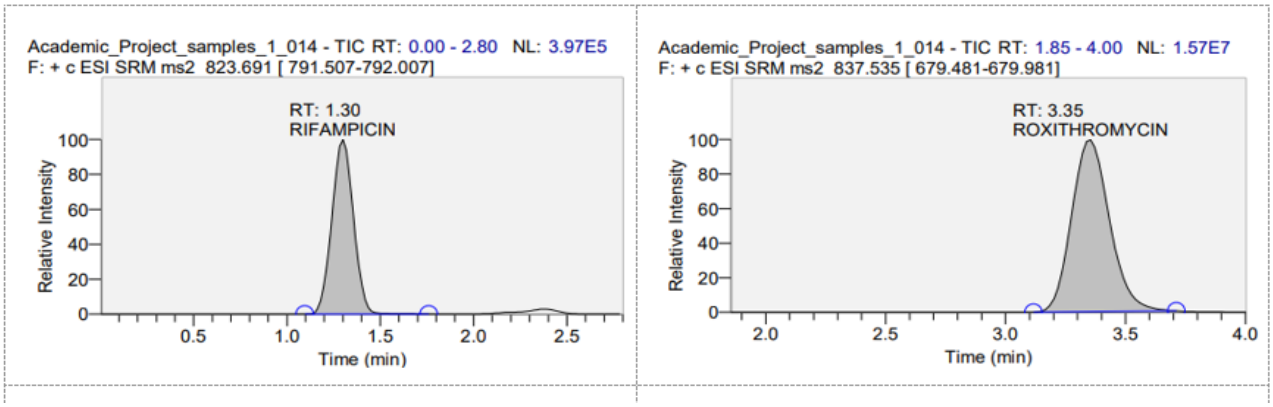
ROXITHROMYCIN
Relative Intensity
Time (min)

Unknown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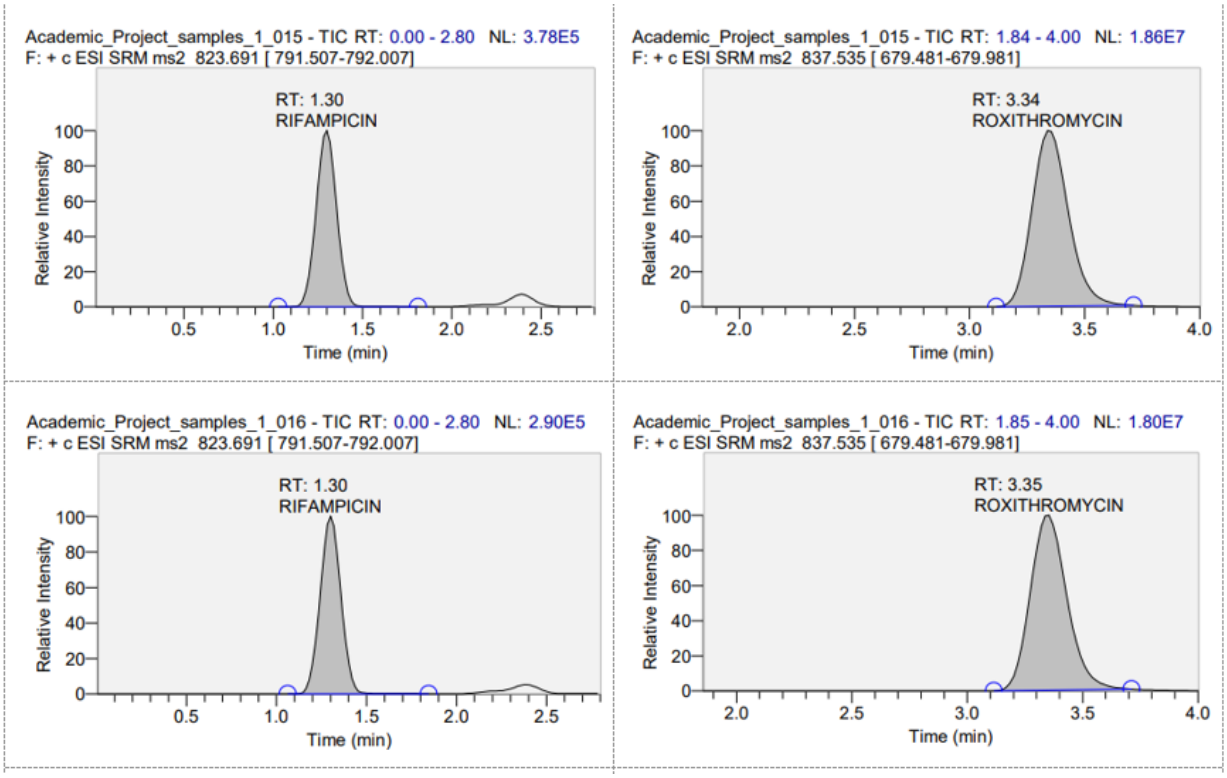
Time (min)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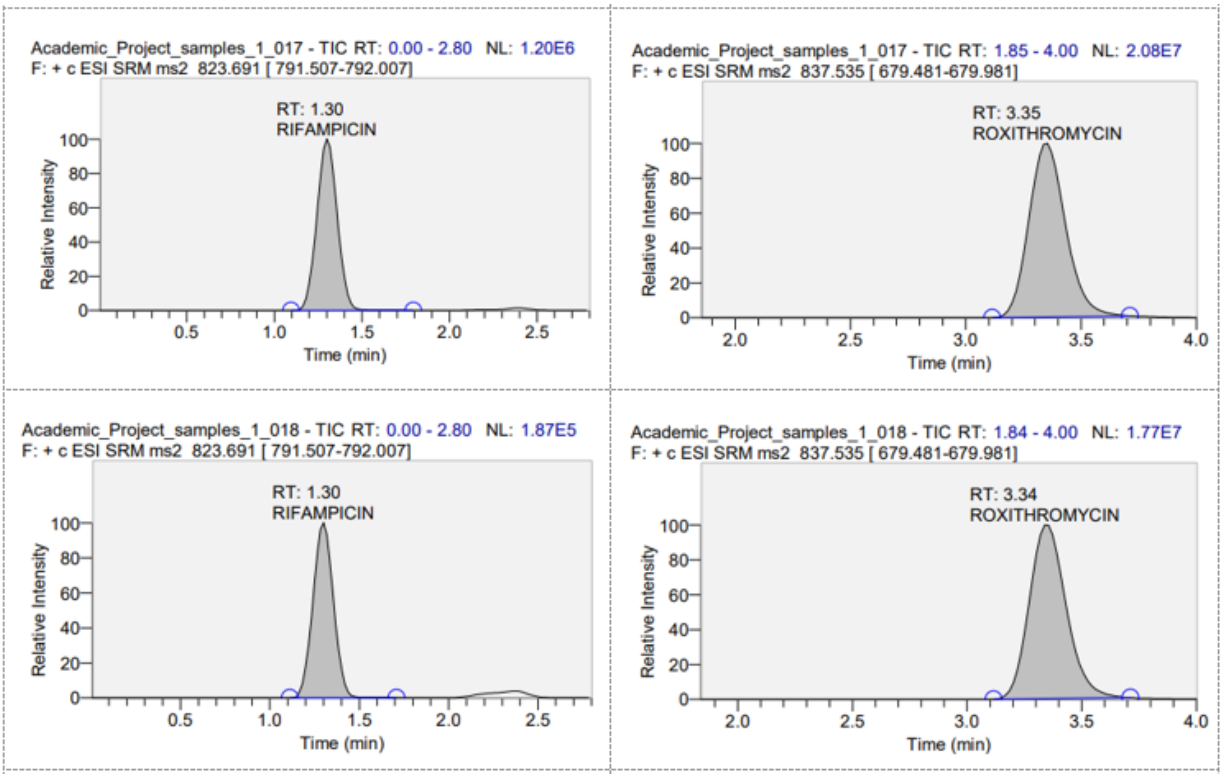
Time (min)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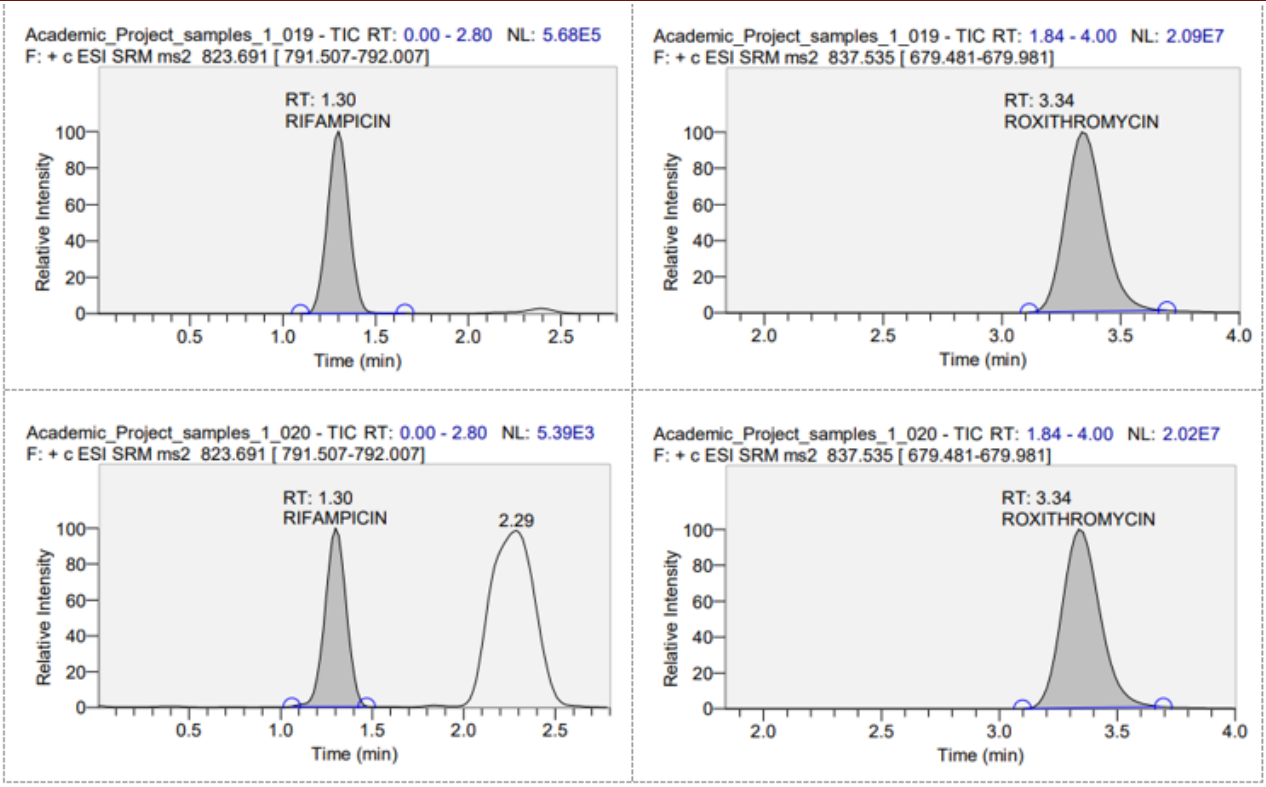
Relative Intensity
Time (min)



RESULTS AND DISCUSSION

The mass spectrometer ion source was optimized to achieve maximum response to the control drugs rifampicin and roxithromycin. Chromatographic separation is achieved by adding "ZORBAX Eclipse plus C_{18} Column". This sample is prepared for the quantitative measurement of rifampicin and further optimized for reduced time; when huge samples need to be evaluated, this is particularly crucial. Acetonitrile was determined to be the most commonly used chemical molecule based on knowledge of plasma protein precipitation. Centrifugation is used to remove the precipitate after intense vortexing to precipitate proteins from the plasma sample. The process of turning the protein into a fine protein precipitate is the conventional and most time-consuming step in these bioassays. An LC-MS/MS method on rifampicin sample volume 0.300 mL, volume 10.0 µL, total run time 4 min, RT 1.30±0.5 min for rifampicin and RT 1.30±0.5 min for roxithromycin the duration of RT Control drug was 3.00±0.5 min. Rifampicin has a mass/concentration ratio of 823.691 for the parent molecule and 791.757 for the product. The typical roxithromycin molecule has an m/z of 837.535 for the parent molecule and 679.731 for the product molecule. Fast, simple, high-throughput sample preparation and rapid processing of large blood samples in a short amount of time is made possible by short LC-MS/MS run times, which also make RIF's clinical evaluation and analysis quick, accurate and affordable.

CONCLUSION

A simple method was developed to determine RIF in human blood using LC-MS/MS. Purification of rifampicin-containing plasma samples by protein precipitation. Chromatographic division was performed utilizing a ZORBAX Eclipse and C_{18} column (4.6 mm x 150 mm, 5 µm). The mobile phase contains acetonitrile and 10Mm ammonium acetic acid derivation, the proportion is 80:20% V/V, the total working time is 4 min, rifampicin RT is 1.30±0.5 min and the drug used is roxithromycin RT 3.00±0.5 min. The m/z of the parent particle of rifampicin is 823.691 and the m/z of the item is 791.757. The *m/z* of the parent atom of standard roxithromycin is 837.535; also, the *m/z* of the thing molecule is 679.731. The transient LC-MS/MS study permits the assessment of enormous quantities of blood tests in a brief timeframe with fast, simple and successful readiness, giving a quick, dependable and best device for RIF clinical observing and review.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

PTP: Pulo-Pulmonary Tuberculosis; **RIF:** Rifampicin; **LC-MS/MS:** Liquid chromatography-mass spectroscopy/mass spectroscopy; **SSE:** Solid phase extraction; **LLE:** Liquid Liquid Extraction; **PP:** Protein Precipitation.

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