academicJournals

Vol. 8(48), pp. 1228-1234, 29 December, 2014
DOI: 10.5897/AJPP2014. 4197
Article Number: 2D4CEC949555
ISSN 1996-0816

http://www.academicjournals.org/AJPP

African Journal of Pharmacy and Pharmacology

Full Length Research Paper

Sensitive liquid chromatography-mass spectrometry determination of isoniazid: Elimination of matrix effects

Janvier Engelbert Agbokponto[1,2], Chuting Gong[1], Assogba Gabin Assanhou[2,3], Desmond Omane Acheampong[3], Raphael Nammahime Alolga[3], Yvane Nova Mfono-Oke[3] and Li Ding[1]*

[1]Department of Pharmaceutical Analysis, China Pharmaceutical University, 24 Tongjiaxiang, Nanjing 210009, China.
[2]UFR/Pharmacie, Faculté des Sciences de la Santé, UAC, Cotonou, Benin.
[3]China Pharmaceutical University, 24 Tongjiaxiang, Nanjing 210009, China.

Received 10 October, 2014; Accepted 3 December, 2014

Small size and high polar basic compounds have always been challenging with heavy matrix effect due to their difficult complete separation from polar endogenous compounds contained in most biological matrix. In this study, a relevant design including column choice, mobile phase constituents, and liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) parameters optimization have been developed to remove matrix effect and chromatographic peak tail in LC-MS/MS determination of small weight and high polar basic compounds. The designed method was used to establish a rapid, selective and sensitive method for LC-MS/MS determination of Isoniazid (INH). The developed method was validated for the determination of INH in human plasma using a mere protein precipitation for sample preparation and 6-methyl nicotinic acid as internal standard (IS). The chromatographic separation was performed on a Sapphire C_{18} column under gradient elution program. The mobile phase consisted of methanol and aqueous ammonium acetate buffer at the flow rate of 0.3 ml/min. Electrospray ionization in positive ion mode and selective reaction monitoring were used for the quantification of INH with a monitored transitions m/z 138.1 → 121.0 for INH and m/z 138.0 → 92.1 for the IS. The validated method was linear over the range of 5 to 3000 ng/ml with a lower limit of quantification of 5 ng/ml. The correlation coefficient was r^2>0.998. The intra and inter-day precisions of the assay were 1.0 to 4.5 and 2.1 to 11.3%, respectively. In this study, the weak separation issue and matrix effect have been overcome, the chromatographic peak tailing circumvented and method sensitivity improved.

Key words: Isoniazid, liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS), matrix effect, high polar basic compounds.

INTRODUCTION

Pulmonary tuberculosis (TB) is one of the most serious public health problems worldwide, with an estimated 8.7 million new cases of TB in 2011 and 1.4 million deaths (WHO Global TB Control Report, 2013). Isoniazid (INH) (pyridine-4-carbohydrazide) is one of the oldest first-line medications for the prevention and treatment of TB. Individuals' variability in plasma concentrations of anti-tuberculosis drugs was associated with therapeutic failure

*Corresponding author. E-mail: dinglihg@sina.com

or drug toxicities during treatment and has been reported in patients with malabsorption, alcohol use, age, sex and hypoalbuminaemia (Peloquin, 2002; Tappero et al., 2005; Kimerling et al., 1998). These situations raised the need for accurate determination of INH plasma concentration for suitable therapeutic drug monitoring (TDM) and bioequivalence or pharmacokinetic studies. Despite the great importance of INH, its accurate control and determination by liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) has always been a challenge like many others basic drug compounds. For decades of chromatographic practice, the analysis of basic compounds has been problematic for analysts worldwide. In the early stages, two significant challenges were faced during the development of LC-MS/MS method for basic compounds were their weak retention time on chromatographic column and matrix effect issues (Taylor, 2005). Matrix effects occur when endogenous molecules are co-eluted with the analytes of interest and alter the ionization efficiency of the ionization source interface. They are caused by numerous factors, including, but not limited to endogenous phospholipids, dosing media, formulation agents and mobile phase modifiers (Larger et al., 2005; Hu et al., 2014). Endogenous phospholipids are present in high concentrations in biological matrices, such as plasma, tissue and bile (Simpemba et al., 2014; Bradamante et al., 1990) and have been known for causing ion suppression or enhancement in LC-MS/MS analyses. Previous studies had described these phenomena as the result of desolvation or competition for access to charges between the analytes droplets and endogenous phospholipids (King et al., 2000; Enke, 1997). Therefore, a good separation of the analytes of interest is required. Unfortunately, hydrophilic compounds such as INH and ephedrines present inherent challenges for separation under conventional reversed-phase chromatographic conditions. In addition, basic functional groups interact with residual silanol groups of the stationary phase to give a long chromatographic peak tailing (Gray et al., 2011). Previous methods employed to overcome this problem include the use of acidic mobile phases containing low amount of organic modifiers or organic additives in order to retain polar bases and achieve acceptable peak shape in reversed-phase chromatography (Deventer et al., 2009; Badoud et al., 2010).

However, these approaches may not be sufficient to strongly retain basic compounds and remove the matrix effect in sensitive analysis. Previous studies on INH determination in plasma used complex sample treatment and long run time with post-run column washing in order to remove matrix effect (Ng et al., 2007; Huang et al., 2009; Li et al., 2004). Some used protein precipitation (PPT) followed by dryness and reconstitution in mobile phase (Ng et al., 2007; Chen et al., 2005). This sample preparation technique showed many disadvantages such as loss of analyte during extraction and dry process due to the small weight of INH, low extraction recovery and time consumption. The preview studies which used PPT lacked in sensitivity (LLOQ 50 ng/ml) and needed long post-run column washing (Huang et al., 2009), whereas, the increasing focus on high throughput sample analysis has led to the possible simplest and fastest method. This study therefore aimed at investigating the use of weak acidic aqueous buffer and a specific gradient elution program as an alternative option for matrix effects elimination and quantification of INH in human plasma by LC-MS/MS using a simple PPT for sample preparation.

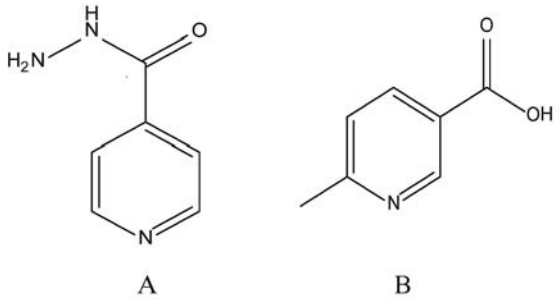

Figure 1. The chemical structures of Isoniazid (INH) and 6-methyl nicotinic acid (IS).

EXPERIMENTAL

Chemicals and reagents

The reference standards of INH (99.9%) and 6-methyl nicotinic acid (99.2%), used as internal standard (IS), were purchased from the National Institute for the Control of Pharmaceutical and Biological Products of China, Bejing, China (Figure 1). Acetonitrile and methanol used for chromatography were of HPLC grade and were purchased from Merck, Germany. Formic acid, acetic acid and ammonium acetate were of analytical grade purity and were purchased from Nanjing Chemical Reagent Co. Ltd (Nanjing, China). Distilled water was used throughout the experiment.

LC-MS/MS instrumentation and conditions

Liquid chromatography was performed on an Agilent 1200 Series liquid chromatography instrument (Agilent Technologies, Palo Alto, CA, USA), which included an Agilent 1200 binary pump (model G1312B), vacuum degasser (model G1322A), Agilent 1200 autosampler (model G1367C), temperature controlled column compartment (model G1330B). The chromatographic separation was achieved on a Sapphire C_{18} column (150 × 2.1 mm ID, 5 µm PD, Sepax Technology) protected by a security guard C_{18} column (4 × 2.0 mm ID, 5 µm PD, Phenomenex, Torrance, CA, USA). The column temperature and auto-sampler temperature were maintained at 35 and 10°C, respectively. The chromatographic separation was achieved using a gradient elution method with a binary mobile phase made of aqueous buffer containing 5 mM ammonium acetate and 0.01% acetic acid (solvent A), and methanol containing 0.01% formic acid (solvent B). The flow rate was set at 0.3 ml/min and the injection volume was 8 µl. Gradient conditions were as follows: 0 to 0.20 min, linear from 4 to 35% B; 0.2 to 1.2 min, isocratic 35% B; 1.2 to 1.4 min, linear back to 4% B and 1.4 to 7 min, isocratic 4% B. The LC system was coupled with an Agilent 6410B triple quadrupole mass spectrometer (Agilent Technologies, Palo Alto, CA, USA) equipped with an electrospray ionization source (model G1956B). The electrospray ionization source (ESI) in positive mode was optimized as follow: an

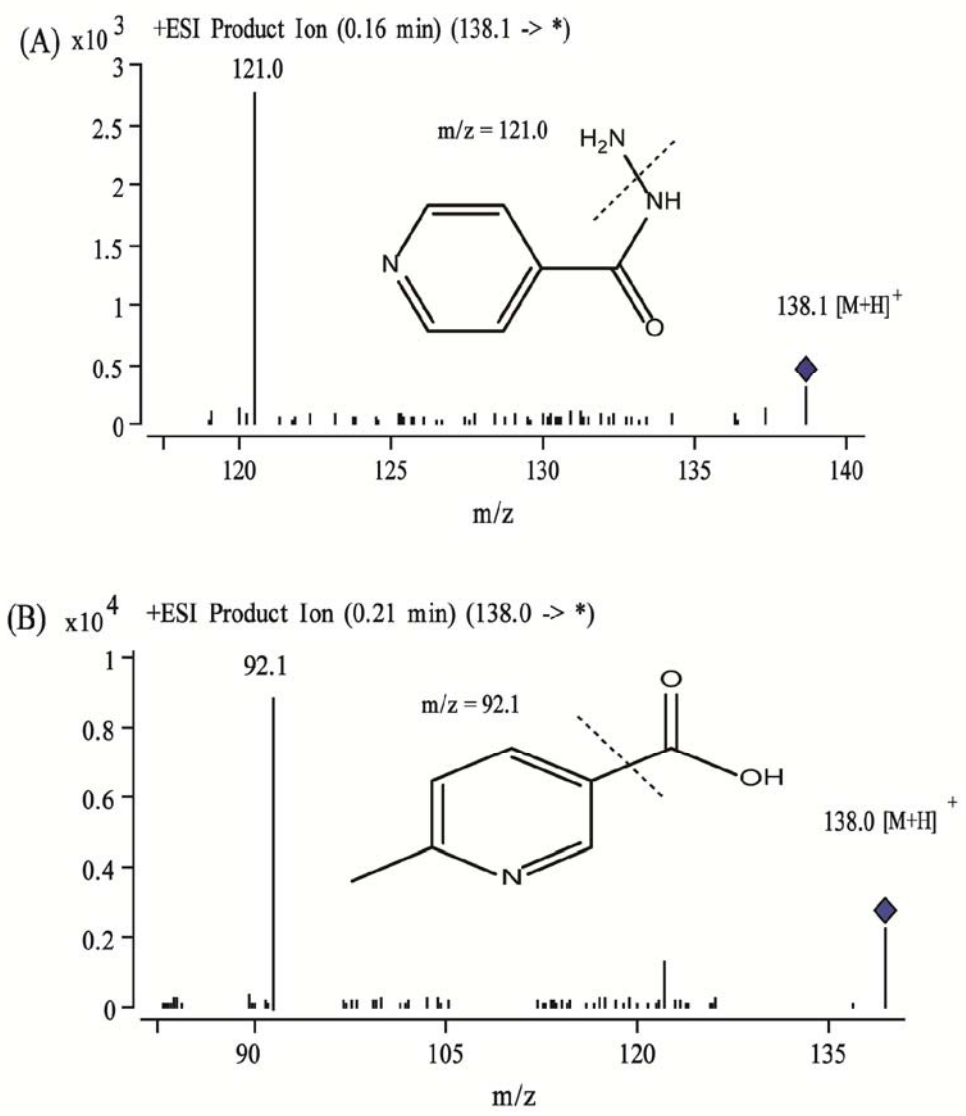

Figure 2. Positive product ion mass spectra of INH (A) and 6-methyl nicotinic acid (B).

electrospray ionization source (model G1956B). The electrospray ionization source (ESI) in positive mode was optimized as follow: a drying gas (N_2) flow of 12 L/min, nebulizer pressure of 50 psig, drying gas temperature of 350°C, capillary voltage of 4.5 kV. The fragmentation transitions for the multiple reaction monitoring (MRM) were m/z 138.1 → 121.0 for INH and m/z 138.0 → 92.1 for the internal standard (I.S) (Figure 2). The fragmentor voltage for both INH and I.S was 100 V while theirs collision energy (CE) were 12 and 25 eV, respectively. The mass spectrometry valve was diverted to waste between 0 and 3 min.

Preparation of standard solution and calibration curve

Stock solution of INH was prepared in acetonitrile to yield a concentration of 1 mg/ml while the IS was dissolved in methanol to reach the same concentration, and both were stored at -20°C. From the stock solution of INH, serial concentrations of working solutions of 0.05, 0.15, 0.5, 1.5, 5, 15 and 30 µg/ml were obtained by dilution with water. Working solution of the IS (10 µg/ml) was obtained by appropriate dilution from its stock solution of 1 mg/ml in water. These working standard solutions were used to prepare the calibration standard and quality control (QC) samples. Seven non-zero samples were prepared by spiking blank plasma with appropriate amount of working solutions to obtain 5, 15, 50, 150, 500, 1500 and 3000 ng/ml of calibration standard concentrations. The QC samples were prepared at 5 ng/ml for low limit of quantification (LLOQ), 10 ng/ml for low QC (QC-L), 200 ng/ml for middle QC (QC-M) and 2400 ng/ml for high QC (QC-H).

Plasma sample preparation

After thawing at ambient temperature (<30°C) and vortex mixed, 200 µl aliquots of plasma samples were put in 1.5 ml plastic centrifuge tube and added 20 µl of IS (10 µg/ml) and vortex-mixed. Thereafter, 400 µl of methanol was added and vortex-mixed for 5 min, and centrifuged at 15,600 rpm for 8 min. Then, 100 µl of the supernatant was transferred into a capped tube and further added with 100 µl of 5 mM ammonium acetate buffer solution containing 0.01% of acetic acid. Eventually, 8 µl of the latter mixed solution was injected into the LC-MS/MS system for analysis.

Method validation

The method was validated to meet the acceptance criteria of industrial guidance for the bioanalytical method validation (ICH, Guidance for Industry, 2001). The method was validated for selectivity, linearity, precision, accuracy, recovery, and stability. Selectivity was assessed, by comparing the chromatograms of six different batches of blank plasma obtained from six different sources with those of corresponding spiked plasma. Each blank sample was tested for no endogenous interferences. Method sensitivity was determined by the limit of detection (LOD) and LLOQ, which were 3 and 10 times the signal-to-noise, respectively. To assess carryover effects, blanks were injected immediately after the highest calibration standard, and the response of any interfering peak had to be less than 10% of the response of an LLOQ sample. All validation experiments were carried out at four QC levels (LLOQ 5 ng/ml, QC-L 10 ng/ml, QC-M 200 ng/ml, and QC-H 2400 ng/ml). The intra-day and inter-day precision were assessed by three run batches on different days. The precision was expressed as the relative standard deviation (RSD) and the accuracy as the relative error (RE). The mean value should be within 15% of the actual value except for LLOQ, which should not deviate by more than 20%.

The matrix effect of plasma components on the INH and IS were determined by comparing the post-extracted QC samples with the neat samples at equal concentrations. The recovery of INH and IS were determined by comparing the responses of the analytes extracted with the response of analytes spiked in post-extracted plasma sample at equivalent concentrations. Recovery was determined at low, middle and high quality control concentrations. Dilution integrity was performed to extend the upper concentration limit with acceptable precision and accuracy.

Auto-sampler stability, bench top stability, freeze-thaw stability and long-term stability of the analytes were assessed. The resulting values for these samples were compared to nominal concentration and expressed as a percentage of the nominal concentration. All stability evaluations were based on back-calculated concentrations. Samples were considered to be stable when their RE was less than 15%.

RESULTS AND DISCUSSION

Matrix effects

Evaluating matrix effects is of utmost importance when developing a quantitative LC-MS/MS method. Endogenous phospholipids are present in high concentrations in biological matrices, such as plasma (Larger et al., 2005) and have been implicated in causing ion suppression or enhancement in LC/MS/MS analyses. Researchers have described this phenomenon as the effect of endogenous phospholipids on desolvation of the chromatographic eluent droplets in electrospray MS analysis (King et al., 2000) or as a result of competition for excess charges on the droplet surface (Enke, 1997). Phospholipids contain both polar head group, which contains a negatively charged phosphate group and a positively charged quaternary amine group, and one or two long alkyl chain(s). The polar head group imparts strong ionic character to the phospholipids, while the long alkyl chains make them extremely hydrophobic and often require nearly 100% organic solvent to be eluted from a reversed-phase chromatographic column. Moreover, when residual phospholipids were not fully eluted from the chromatographic column, they built up on the column and were unexpectedly eluted in subsequent analytical runs. The late elution time of 3.76 min achieved in this study prevented INH from those matrix effects as it was not expected to co-elute with the phospholipids.

Instruments optimization

Chromatography

Chromatographic conditions were optimized through several trials to achieve good resolution and high analyte response, as well as reduce analytical run time. Several columns have been tested, (such as Hedera ODS-2 C_{18} column (150 × 2.1 mm ID, 5 µm PD), Hedera CN column (150 × 2.0 mm ID, 5 µm Pd), Amethyst C_{18}-p column (150 × 2.1 mm ID, 5 µm PD) and Zorbax eclipse plus C_{18} (150 × 2.1 mm ID, 3.5 µm PD), to achieve good retention, symmetrical peak shape and good separation, but unfortunately most of them failed to achieve those criteria. Although sapphire C_{18} column showed a slight peak tail, it provided better retention and separation of the analytes compared to other columns. Therefore, sapphire C_{18} column was chosen for further optimization.

The separation and ionization of INH and IS were highly affected by the composition of the mobile phase. Various combinations of acetonitrile or methanol as organic modifiers and aqueous buffer were investigated to identify which mobile phase composition provided the optimal separation, highest responses and good peak shape for INH and IS. In the present study, methanol was preferred to acetonitrile because it gave better mass spectrometric response and strong column flushing. The settled gradient elution allowed good retention, good separation, good peak shape, and better elimination of matrix effect with increased sensitivity. First of all, high aqueous eluent (96% water) strengthened the retention of INH and IS on the column and allowed complete separation of the analytes from endogenous compounds. The relatively low concentration of acid modifiers in the aqueous buffer is relevant for reducing the ionization of endogenous components, but it is enough for effective ionization of the analytes of interest. The 0.01% formic acid in the methanol produced a strong elution power on the analytes, increased the response of the analytes, eliminated peak tailing and yielded symmetrical peaks. The gradient elution was able to flush the column after every injection, which could prevent the next injections from matrix effect.

Conditions for ESI-MS/MS

The high polarity and small size of INH permanently confined it to the inside of the spraying droplets which limited it ionization. Thus, high ionization would need high drying gas temperature, nebulizer and organic solvent during the analyte desolvation and protonation. Therefore, the ESI ionization source was optimized with a drying gas (N_2) flow of 12 L/min, nebulizer pressure of 50 psi, drying gas temperature of 350°C. ESI in positive ion

mode was used. Various fragmentor voltages (50, 70, 80, 90, 100, 110, 130 and 150 V) were optimized. The result showed that the highest intensity was obtained with a fragmentor voltage of 80 V. Unfortunately, at that fragmentor voltage impurities appeared near the analytes' peaks; to avoid all interferences, 100 V was chosen as fragmentor voltage to carry out LC–ESI-MS/MS as it showed higher sensitivity and clean chromatograms. The collision energy (CE) was also optimized to 12 eV.

Preparation of plasma sample

Sample preparation is a critical step for accurate and reliable LC-MS/MS analysis. The current widely employed methods for biological sample preparation are liquid-liquid extraction (LLE), protein precipitation (PPT) and solid-phase extraction (SPE). INH is a highly polar compound difficult to be extracted from plasma by LLE. SPE fails in better extraction of INH because ordinary SPE columns lack in retaining the analyte, and those which provide clean and better sample extraction are very expensive and are not suitable for routine analyses (Huang et al., 2009). Studies elsewhere made use of protein precipitation followed by dryness and reconstitution in mobile phase (Ng et al., 2007; Chen et al., 2005), but this sample preparation technique showed many disadvantages like long time consumption, loss of analyte during extraction process causing a very low extraction recovery. Therefore, a simple methanol PPT used in this study was more suitable for high throughput LC-MS/MS analysis of INH.

Method validation

Selectivity and carry-over

The selectivity of the method was determined by analyzing blank controls from six different individuals. There was no endogenous interference observed at the retention times of INH and the IS which were 3.76 and 3.98 min, respectively. No carry-over peaks were observed at the retention times of INH and the IS. Figure 3 shows typical chromatograms of blank plasma, blank plasma spiked with 5 ng/ml and blank plasma spiked with 150 ng/ml of INH. The peaks' shapes were symmetrical.

Matrix effect, recovery and dilution integrity

The matrix effect evaluated for the QC standards at three concentration levels ranged from 92.8 to 99.89%, suggesting no ion suppression or enhancement from the plasma matrix to the analytes (Table 1). Matrix effects on the ionization of the analyte were not obvious under these conditions. The overall mean recovery for INH at three QC concentration levels (10, 200 and 2400 ng/ml)

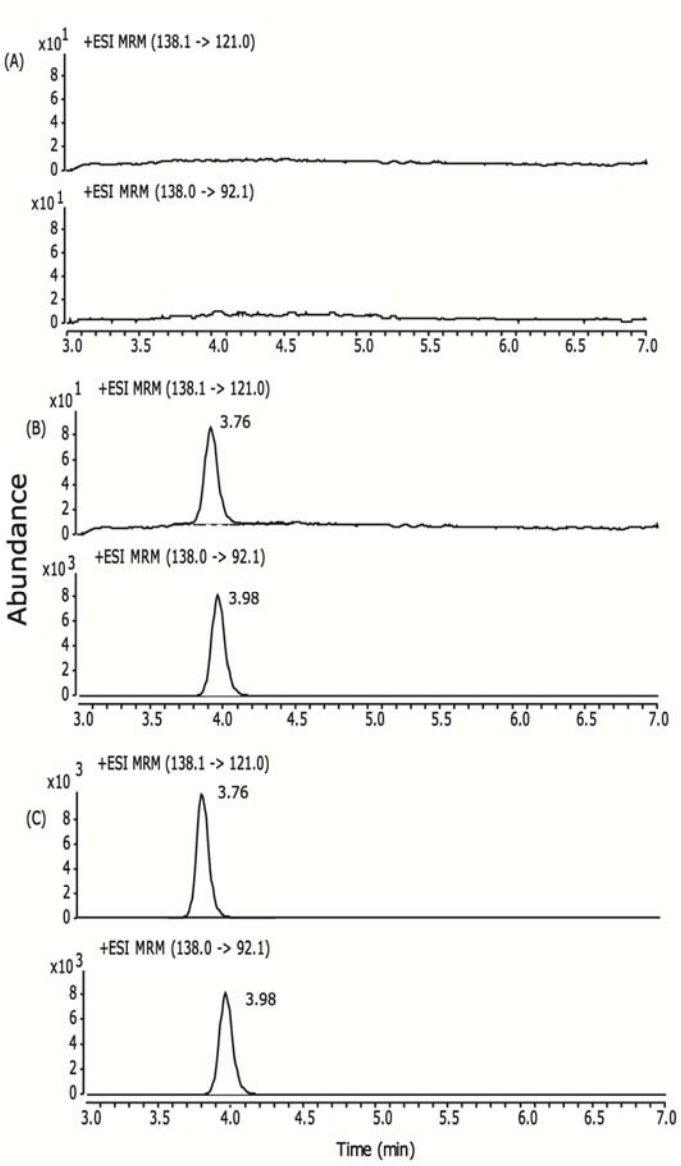

Figure 3. Typical MRM chromatograms of INH.
Blank plasma sample (A), blank plasma sample spiked with INH 5 ng/Ml (B), and blank plasma sample spiked with INH 150 ng/mL (C).

were 94.6, 91.2 and 87.4%, respectively, with RSD of less than 4.9%. The mean matrix effect and recovery of the IS were 94.0 ± 5.9 and 82.8 ± 3.7%, respectively. Thus, the consistency in recoveries of INH and IS supported protein precipitation as the suitable sample treatment procedure applicable for routine analysis of INH. The dilution integrity test extends the upper limit of quantification up to 10 µg/ml, and demonstrated an accuracy of 8.6% for the diluted plasma samples.

Linearity, accuracy and precision

Four calibration analyses were performed in 4 batches.

Table 1. Matrix effect data from the analysis of INH in human plasma (n = 6).

Concentration of INH (ng/ml)	ME (mean ± SD, %)
10.03	99.8 ± 3.4
200.6	98.7 ± 1.4
2407	92.8 ± 2.6

SD: Standard deviation.

Table 2. Precision and accuracy data for the analysis of INH in human plasma (n = 5).

Analyte	Concentration (ng/ml)		RSD (%)		RE (%)
	Added	Measured (M ± SD)	Intra-day precision	Inter-day precision	Accuracy
Isoniazid (INH)	5.014*	4.607 ± 0.667	4.5	15.7	-8.1
	10.03	9.252 ± 1.002	3.6	11.3	-7.7
	200.6	200.0 ± 4.926	2.5	2.1	-0.3
	2407	2384 ± 51.63	1.0	5.2	-1.0

*LLOQ concentration level. RSD and RE acceptance range are within ±20%. M ± SD: Mean ± Standard deviation.

Table 3. Stability of INH in human plasma under various storage conditions (n = 3).

Storage condition	Concentration (ng/ml)		RSD (%)	RE (%)
	Added	Measured (M ± SD)		
Room temperature for 6 h	10.03	9.244 ± 0.1	1.0	-7.8
	2407	2288 ± 14.6	0.6	-4.9
Auto-sampler stability at 10°C for 14 h	10.03	9.35 ± 0.2	2.2	-6.7
	2407	2318 ± 26.1	1.1	-3.7
Three freeze/thaw cycles	10.03	10.91 ± 0.44	4.1	8.8
	2407	2528 ± 35.61	1.4	5.1
Long term stability at -20°C for three weeks	10.03	9.50 ± 0.2	2.1	-5.2
	2407	2368 ± 25.1	1.1	-1.6

The typical equation of the calibration curves was $f = 0.03530 \times C + 0.003107$ ($r^2 > 0.998$), and linear over the concentration range of 5 to 3000 ng/ml. The back-calculated results showed good day-to-day accuracy and precision. Validation samples of five replicates of the LLOQ and QC samples were prepared and analyzed in four separate analytical batches to evaluate the accuracy and intra-day and inter-day precision of the methods. The precision and accuracy for quantification of INH in human plasma are summarized in Table 2. The results showed that both intra-day and inter-day values were all within the limits of acceptance. The method was accurate and precise

Stability

The stability results are summarized as shown in Table 3. INH was found to be stable in plasma sample for a minimum period of 6 h at room temperature and after at least three freeze-thaw cycles. The analytes were stable in processed plasma samples for 14 h at 10°C. The spiked plasma samples of INH stored at -70°C for long-term stability were found to be stable for a minimum period of three weeks. The accuracy results for INH at the levels of 10 and 2400 ng/ml in all the stability studies fell in the range of 92.8 to 108.8%.

Conclusion

Matrix effect is one of the main challenges during LC-MS/MS analysis of compounds in biological fluids. It affects the sensitivity, accuracy and robustness of the method. In this work, the matrix effect was eliminated and

a sensitive LC-MS/MS method was developed for the quantification of INH in human spiked plasma samples.

Conflict of interest

The authors have declared no conflict of interest

REFERENCES

Badoud F, Grata E, Perrenoud L, Saugy M, Rudaz S, Veuthey JL (2010). Fast analysis of doping agents in urine by ultra-high-pressure liquid chromatography-quadrupole time-of-flight mass spectrometry. II: Confirmatory analysis. J. Chromatogr. A. 1217(25):4109-4119.

Bradamante S, Barchiesi E, Barenghi L, Zoppi F (1990). An alternative expeditious analysis of phospholipid composition in human blood plasma by 31P NMR spectroscopy. Anal. Biochem. 185 (2): 299-303.

Chen X, Song B, Jiang H, Yu K, Zhong D (2005). A liquid chromatography/tandem mass spectrometry method for the simultaneous quantification of isoniazid and ethambutol in human plasma. Rapid Commun Mass Spectrom, 19(18):2591-2596.

Deventer K, Pozo OJ, Van Eenoo P, Delbeke FT (2009). Development and validation of an LC-MS/MS method for the quantification of ephedrines in urine. J. Chromatogr. B. 877(4):369-377.

Enke C (1997). A predictive model for matrix and analyte effects in electrospray ionization of singly-charged ionic analytes. Anal. Chem. 69(23): 4885-4893.

Gray N, Musenga A, Cowan DA, Plumb R, Smith NW (2011). A simple high pH liquid chromatography-tandem mass spectrometry method for basic compounds: application to ephedrines in doping control analysis. J. Chromatogr. A. 1218: 2098–2105.

Hu L, Agbokponto JE, Li X, Ding L, Liu B, Zhong S, Zhang X, Du Y (2014). In vivo and in vitro evidence of the sex-dependent pharmacokinetics and disposition of G004, a potential hypoglycemic agent, in rats. Eur. J. Drug Metab. Pharmacokinet.,

Huang L, Marzan F, Jayewardene AL, Lizak PS, Li X, Aweeka FT (2009). Development and validation of a hydrophilic interaction liquid chromatography-tandem mass spectrometry method for determination of isoniazid in human plasma. J. Chromatogr. B. 877(3):285-290.

International Conference on Harmonization (ICH) (2001). Guidance for Industry: Bioanalytical Method Validation, US FDA Federal Register, Center for Drug Evaluation and Research (CDER).

Kimerling ME, Phillips P, Patterson P, Hall M, Robinson CA (1998). Low serum antimycobacterial drug levels in non-HIV-infected tuberculosis patients. Chest 113(5):1178–1183.

King R, Bonfiglio R, Fernandez-Metzler C, Miller-Stein C, Olah T (2000). Mechanistic investigation of ionization suppression in electrospray ionization. J. Am. Soc. Mass Spectrom. 11 (11): 942-950.

Larger P J, Breda M, Fraier D, Hughes H, James CA. (2005). Ion-suppression effects in liquid chromatography-tandem mass spectrometry due to a formulation agent, a case study in drug discovery bioanalysis. J. Pharm. Biomed. Anal. 39(1-2):206-216.

Li AC, Junga H, Shou WZ, Bryant MS, Jiang XY, Naidong W (2004). Direct injection of solid-phase extraction eluents onto silica columns for the analysis of polar compounds isoniazid and cetirizine in plasma using hydrophilic interaction chromatography with tandem mass spectrometry. Rapid Commun. Mass Spectrom. 18(19):2343-2350.

Ng KY, Zhou H, Zhang YL, Hybertson B, Randolph T, Christians U (2007). Quantification of isoniazid and acetylisoniazid in rat plasma and alveolar macrophages by liquid chromatography-tandem mass spectrometry with on-line extraction. J Chromatogr B. 847(2):188-198.

Peloquin CA (2002). Therapeutic drug monitoring in the treatment of tuberculosis. Drugs 62(15): 2169–2183.

Simpemba, E., Liu, RJ, Sun, CL., Agbokponto, J.E., Ding L (2014). Simultaneous determination of rabeprazole and its two active metabolites in human urine by liquid chromatography with tandem mass spectrometry and its application in a urinary excretion study. J. Sep. Sci. 37(15):1951-1956.

Tappero JW, Bradford WZ, Agerton TB, Hopewell P, Reingold AL, Lockman S, Oyewo A, Talbot EA, Kenyon TA, Moeti TL, Moffat HJ, Peloquin CA. (2005). Serum concentrations of antimycobacterial drugs in patients with pulmonary tuberculosis in Botswana. Clin. Infect. Dis. 41(4):461–469.

Taylor PJ (2005). Matrix effects: the Achilles heel of quantitative high-performance liquid chromatography-electrospray-tandem mass spectrometry. Clin. Biochem. 38(4):328–334.

World Health Organization (WHO) (2013). Global TB Control Report 2013.
WHO/HTM/TB/2013.11http://apps.who.int/iris/bitstream/10665/91355/1/9789241564656_eng.pdf