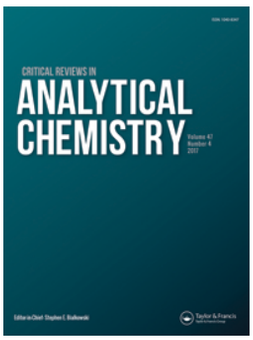

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Isoniazid: A Review of Characteristics, Properties and Analytical Methods

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ABSTRACT

Isoniazid is a synthetic antimicrobial and one of the most important first-line drugs used in the treatment of tuberculosis. Since it was introduced in the therapy in 1952, the drug remains at the front line of the antituberculosis treatment mainly due to its potency and high selectivity against *Mycobacterium tuberculosis*. Pharmaceutical analysis and therapeutic drug monitoring of isoniazid in both, pharmaceuticals and biological samples, plays an important role to comprehend aspects regarding to bioavailability, bioequivalence and therapeutic monitoring during patients following-up. In the last case, validated and simple methods are extremely useful for Public Healthy in order to guarantee the drug efficacy, safety and reduce the tuberculosis resistance. Among the available analytical tools, HPLC-based methods coupled to ultraviolet or mass spectroscopy are the most widely used techniques to quantify isoniazid. Therefore, this review highlights the main analytical methods reported in the literature for determination of isoniazid focusing in HPLC-based methods.

KEYWORDS

Analytical methods; high performance liquid chromatography; isoniazid; mass spectroscopy; ultraviolet

Introduction

Currently, tuberculosis (TB) is an infectious disease responsible for the largest number of deaths worldwide, exceeding even the deaths caused by human immunodeficiency virus (HIV) (World Health Organization, 2014). The last surveys conducted by the World Health Organization (WHO) have reported 10 million new TB cases and 1.3 million deaths worldwide (World Health Organization, 2015). In Brazil, data from the National Health Department reported 63.189 new cases of the disease in 2015 (BRASIL, 2016).

The drugs used to treat TB are generally classified as first- and second-line drugs. The first-line drugs used for treatment of drug-sensitive TB include isoniazid (INH), rifampicin (RIF), ethambutol (EMB) and pyrazinamide (PZA). The second-line drugs include aminosalicylic acid, thioamides, ethionamide, protionamide, rifabutin, rifapentine, cycloserine, capreomycin and several fluoroquinolones. These second-lines drugs are used for multidrug-resistant (MDR-TB) and extensively drug-resistant (XDR-TB) TB (Schito et al., 2015; Zumla et al., 2015).

Isoniazid is one of the most important drug used in the TB treatment. It has bactericidal effect against rapidly multiplying mycobacteria; however, it exhibits limited action in latent mycobacteria (Zhang, 2005). It was first synthesized in 1912, but its discovery as antitubercular agent was first reported simultaneously and independently in the early of 1950s by researchers from Hoffmann–La Roche, E. R. Squibb & Sons in the United States of America and from Bayer in Germany (Bernstein et al., 1952; Fox, 1952). After its discovery as anti-TB drug, isoniazid was introduced in the therapeutic regimen for TB treatment in 1952 (Marshall et al., 1952; Medical Research Council, 1954).

Recently, several Medicinal Chemistry groups have reported isoniazid derivatives with potent anti-TB activity; nonetheless, isoniazid keeps on top as one of the best options available for TB treatment even after 64 years of its discovery (Matei et al., 2013; Kumar et al., 2014; Martins et al., 2014; Fernandes et al., 2016). Indeed, isoniazid plays an important role in the current therapeutic regimen, and several clinical trials have demonstrated its importance in new therapeutic schemes under development (Rangaka et al., 2014; Villarino et al., 2015; Stennis et al., 2016).

In Brazil, isoniazid is available in tablet form at a concentration of 100 mg. In addition, the drug is also used in combination with rifampicin in soft capsules at two different concentrations: 100 mg + 150 mg and 200 mg + 300 mg, respectively. Both pharmaceutical forms, tablets and capsules are provided free of charge by the federal government, given the importance of the effective control of TB (BRASIL, 2011; Daher et al., 2015a). Around the world, isoniazid is marketed with the following brand names: Cotinazin (Pfizer); Dinacrin (Winthrop); Ditubin (Schering); Hycozid (Takeda); Iscotin (Daiichi); Isobicina (Maggioni); Isocid (CID); Isolyn (Abbott); Isonex (Dumex); Isonizida (Bial); Isozid (Fatol); Laniazid (Lannett); Mybasan (Antigen); Neoteben (Bayer); Nicizina (Pfizer); Niconyl (Parke-Davis); Nicotibina (Lapetit); Nydrazid (Bristol-Myers Squibb); Pycazide (Smith & Nephew); Pyricidin (Nepera); Rimifon (Roche); Tibinide (Ferrosan); Tubilysin (Orion) (Global Alliance for TB Drug Development, 2008).

According to WHO definition, drug quality control comprises the set of procedures undertaken to ensure the identity and purity of a particular pharmaceutical. These procedures may be performed mainly through chromatographic methods

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(e.g. high performance liquid chromatography (HPLC), gas chromatography (GC), and thin layer chromatography (TLC)) (World Health Organization, 2007). Additionally, several analytical techniques are used to determine drug quality, such as infrared spectroscopy (IR), ultraviolet spectroscopy (UV), mass spectroscopy (MS) and electrochemical methods (Tzanavaras and Themelis, 2007; Vogt and Kord, 2011; Bleye et al., 2012; Wen and Zhu, 2015). Therefore, the pharmaceutical analysis, specifically the development and application of analytical methods plays an important role to ensure the quality of medicines and also quantification of specific drugs in biological samples (Bridwell et al., 2010; Tiwari and Tiwari, 2010; Johnston and Holt, 2014).

Considering the importance that isoniazid presents for current TB treatment, in this review, we describe the characteristics, properties and highlight the HPLC-based analytical methods for quantification and determination of isoniazid in biological samples and pharmaceuticals reported in literature so far. For this purpose, we have focused the search in the following databases: PubMed, Scopus and Web of Science whose period ranged from 1977 to 2016.

Isoniazid

Synthesis

Isoniazid, also known as isonicotinic acid hydrazide, is a relatively simple chemical structure that consists of pyridine ring and a hydrazine group attached at *para* position to the pyridine nitrogen. Isoniazid is prepared through the reaction of 4-cyanopyridine and hydrazine hydrate in an aqueous alkaline medium at 100°C under reflux for 7 hours with subsequently crystallization in ethanol, thereby leading the desired compound with 62% of yield (Sycheva et al., 1972; Sittig, 1988) (Figure 1).

Mechanism of action and microbiological spectrum

Isoniazid has bactericidal action exclusively against mycobacteria especially those in fast-growing phase; however, is bacteriostatic on slow-growth mycobacteria or even in a latent state. The microbiological spectrum of isoniazid comprises several species of *Mycobacterium* genus, including *M. tuberculosis, M. bovis* and *M. kansaii* being a highly specific antimicrobial to this genus; nevertheless, it is largely ineffective against other species of microorganisms (Global Alliance for TB Drug Development, 2008). Isoniazid remains on the front-line drug against TB due in part to its outstanding minimal inhibitory concentration (MIC) of 0.1–0.7 μM against *M. tuberculosis* (Scior et al., 2002). Although isoniazid is frequently co-administered with other antitubercular drugs, it has also been given alone as a prophylactic agent.

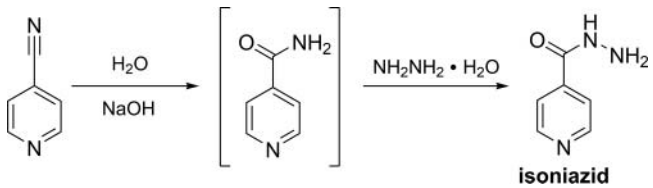

Figure 1. Synthesis of isoniazid.

Isoniazid enters the mycobacterial cell by passive diffusion through the cell wall (Bardou et al., 1998). Once inside the mycobacterial cell, isoniazid itself is non-toxic; however, acts as a prodrug and is activated by a mycobacterial enzyme, namely *KatG*. Subsequently, its activation, the generated metabolite produces reactive oxygen species (ROS), such as superoxide, hydrogen peroxide, peroxynitrite and the isonicotinoyl radical (Timmins and Deretic, 2006). The produced isonicotinoyl radical binds covalently to a NAD molecule and the resulting adduct acts inhibiting the FASII enoyl-ACP reductase InhA (Vilchèze and Jacobs, 2007). This enzymatic complex plays a vital role in the mycolic acid biosynthesis pathway, and its inhibition leads to an accumulation of long-chain fatty acids, thereby inhibiting the cell wall synthesis and subsequent leading to the cell death (Marrakchi et al., 2000). Furthermore, the generated ROS may cause damage to a wide range of important cellular components, including DNA, lipids and proteins and therefore may contribute to the powerful bactericidal activity of the drug (Deretic et al., 1996; Wengenack and Rusnak, 2001; Timmins et al., 2004).

The most common mechanisms of resistance related to isoniazid include mutations in the *M. tuberculosis inhA* gene and *katG* enzyme. The *inhA* gene is responsible for mycolic acid biosynthesis and the *katG* enzyme by the activation of isoniazid, which avoid the isoniazid to interact with the binding site in the *inhA* complex. Additionally, mutations in other genes seems to be associated with resistance to isoniazid, such as *ahpC, KasA* and *ndh* (Blanchard, 1996; Jagielski et al., 2014; Shekar et al., 2014).

Pharmacokinetics

Oral, intravenous and intramuscular are routes of administration for isoniazid, but the most common is the oral route. Isoniazid is promptly absorbed after oral administration and reaches the peak of seric concentrations in 0.5–2 hours within 100% of bioavailability in the most of the cases (Weber and Hein, 1979). Co-administration of isoniazid with food significantly reduces its bioavailability. Moreover, there is evidence that suggest that HIV-positive patients may exhibit poor absorption of isoniazid (Sahai et al., 1997; McIlleron et al., 2012).

Isoniazid is widely distributed throughout body fluids and tissues, with a volume of distribution of approximately 61% of body weight and its plasma protein binding is very low (Weber and Hein, 1979). Acetylation is the main metabolic transformation that occurs with isoniazid. Specifically, this metabolic process is strongly influenced by genetic aspects. The predominant liver metabolism justify the high risk of hepatotoxicity, especially for those TB-patients using an association with rifampicin (Durand et al., 1996; Fountain et al., 2005; Senousy et al., 2010). The elimination half-life of isoniazid and its metabolites is 0.5 to 4 hours and their main elimination route is through the kidney, with 75% to 96% of the drug and metabolites excreted in urine within 24 hours (Ellard and Gammon, 1976).

Physicochemical properties

Isoniazid, pyridine-4-carbohydrazide, is a synthetic antimicrobial, colorless or white crystalline powder. The molecular

formula is $C_6H_7N_3O$, molecular weight is 137.14 g/mol, melting point of 171.4°C and LogP of −0.64. Isoniazid presents slightly solubility in water (∼14% at 25°C, ∼26% at 40°C), low solubility in ethanol (∼2% at 25°C) and chloroform (∼0.1% at 25°C). The drug is practically insoluble in non-polar organic solvents, such as benzene, toluene and ethyl ether (Global Alliance for TB Drug Development, 2008). Isoniazid is stable at room temperature; however, may produce toxic by-products of nitrogen oxides when heated to decomposition. The pH of an aqueous solution (1%) is 5.5– 6.5 and its pKa at 20°C is 1.82. The wavelength of maximum absorbance observed using ethanol as solvent is 263 nm; while in water this value is found to be 266 nm. The intense mass spectral peaks is 78 m/z, 106 m/z and 137 m/z (O'Neil, 2001; National Center for Biotechnology Information, 2016).

Analytical methods

Drug analysis plays an important role in the pharmaceutical field, being present since the stages of initial drug discovery until post-marketing stages (Lee and Webb, 2003; Hanna-Brown, 2012). The analytical techniques contribute to important information concerning drugs, including bioavailability and bioequivalence studies, physical and chemical stability of the drug molecule, design of the dosage form, quantification and identification of impurities and quantification of drug content in the marketed products. Furthermore, pharmacokinetic parameters for therapeutic drug monitoring are also assessed through analytical techniques (Bonfilio et al., 2010; Siddiqui et al., 2013).

Chromatographic techniques, especially those involving HPLC are widely used in pharmaceutical analysis (Corrêa and Salgado, 2011; Bonfilio et al., 2012; Chierentin and Salgado, 2016; Curbete and Salgado, 2016; Fernandes and Salgado, 2016; Pedroso and Salgado, 2016). A number of detectors coupled with HPLC systems are currently used, such as ultraviolet-visible (UV) spectrophotometry, fluorescence methods, MS, and electrochemical methods (Bonfilio et al., 2010). For several years, HPLC methods with UV detector was the main detection technique used in drug analysis for both, pharmaceuticals and biological samples (Roškar and Lušin, 2012). Nevertheless, in the last years, it has been observed an increased number of methods involving HPLC coupled with mass spectrometry. Currently, this technique has become the most powerful analytical tool for drug quantification and identification (Berchtold et al., 2014; Adaway et al., 2015; Garg and Zhang, 2016).

Therefore, in this review, we highlight the analytical methods for quantification and identification of isoniazid and its metabolites in pharmaceuticals and biological samples, focusing on HPLC-based methods.

The analytical methods reported in the literature for determination and quantification of isoniazid in both, pharmaceuticals and biological samples are presented in Table 1.

Upon absorption, isoniazid is metabolized *in vivo* and leads to the formation of several metabolites, namely acetylisoniazid, isonicotinic acid, isonicotinamide, monoacetylhydrazine and diacetylhydrazine (Ellard and Gammon, 1976; Weber and Hein, 1979). Therefore, it is important the simultaneous detection of these metabolites through the analytical methods. Indeed, the analytical methods presented herein not only cover isoniazid, but also most of its metabolites (Table 1), especially acetylisoniazid and isonicotinic acid, which are the acetylated and hydrolyzed metabolites of isoniazid, respectively (Figure 2) (Ellard et al., 1972; Preziosi, 2007).

Isoniazid is frequently co-administered with others first-line antitubercular drugs in fixed-dose combination, including pyrazinamide, ethambutol and rifampicin (World Health Organization, 2010). Thus, the simultaneous determination of isoniazid and these anti-TB drugs plays an important role in the quality control of pharmaceuticals, therapeutic drug monitoring and bioequivalence studies in patients with TB. Several analytical methods described herein (Table 1) were designed to detect and quantify isoniazid but also pyrazinamide, ethambutol and rifampicin. Among the methods involving UV detectors, the switch of wavelength for each drug analyzed were frequently accomplished by using diode array detector (DAD). Regarding the HPLC-MS methods, the m/z of each drug is analyzed according to the generated fragment. For isoniazid, the main fragments generated upon ionization presents m/z of 121 and 79 (Figure 3).

Currently, there is a huge concern about the development of non-aggressive methods to the environment and with inferior toxicity to the human health (Alfonsi et al., 2008; Płotka et al., 2013). Regarding the analytical conditions, most of the methods reported in this review have used water, phosphate buffer, methanol and acetonitrile as mobile phase in isocratic or gradient elution. Methanol is classified as a green solvent by several authors, since it presents low environmental hazards, while other authors are more cautious in this classification, given its relative health hazards (Curzons et al., 1999; Capello et al., 2007; Henderson et al., 2011; Byrne et al., 2016). Nevertheless, methanol is much greener than acetonitrile, considering that the latter is not recommendable from an environmental perspective and present moderate toxicity to human health (Capello et al., 2007; Ghosh, 2012). Moreover, several methods have utilized others non-green or toxic solvents, including chloroform, dichloromethane, isopropanol and tetrahydrofuran (Dunn, 2012).

Some methods have presented particular characteristics compared to the majority reported in the literature. For example, Liu and coworkers have reported an HPLC-based method for determination of isoniazid in rabbit vertebrae through an isotope tracing technique. Isoniazid is frequently used in surgical sites to locally kill residual mycobacteria in the post-operative. Therefore, this method was developed to clarify the real distribution and elimination of isoniazid in the vertebrae *in vivo* (Liu et al., 2013). Koga also reported a particular method for determination of isoniazid and several others antimicrobial drugs in human polymorphonuclear leukocytes (Koga, 1987). Some bacteria are able to remain viable within these immune cells even after being phagocytized and the knowledge whether the drugs can penetrate into the cells plays an important role (Prokesch and Hand, 1982; Jacobs and Wilson, 1983). However, the authors concluded that isoniazid has a very low concentration within these cells (Koga, 1987).

Ng and colleagues also reported an HPLC-MS/MS method to determination of isoniazid and acetylisoniazid in rat alveolar macrophages. This determination plays an important role

Table 1. HPLC-based methods described for analysis of isoniazid in pharmaceuticals and biological samples.

Method	Analytes	Mobile phase	Column	Detection	Flow Rate (mL/min)	Matrices	Reference
HPLC-UV	Isoniazid	Isocratic: phosphate buffer pH 6.9 and methanol (95:5, v/v)	Column C_{18} (250×4.6 mm; 5 μm)	UV at 254 nm	1.5	Standard	US Pharmacopoeia, 2016 Brazilian Pharmacopoeia, 2010
HPLC-UV	Isoniazid	Isocratic: chloroform, methanol, 2-propanol and water (85:5:10:0.5, v/v/v/v)	Column Whatman HC Pellosil (1 m × 2.1 mm)	UV at 254 nm	1.2	Tablets	(Bailey and Abdou, 1977)
HPLC-UV	Isoniazid and acetylisoniazid	Isocratic: methanol and water (60:40, v/v; pH 2.5 adjusted with 2 M sulfuric acid)	Column C_{18} Micro-Bondapak (300×4.0 mm; 10 μm)	UV at 266 nm	2.0	Human plasma and urine	(Hutchings et al., 1977)
HPLC-UV	Isoniazid and 1-isoniconinyl-2-lactosylhydrazine	Isocratic: acetonitrile and aqueous acetate buffer (0.01 M; pH 3.5) (95:5, v/v)	Column Cyanopropyl (250×3.2 mm; 5 μm)	UV at 254 nm	1.5	Tablets	(Butterfield et al., 1980)
HPLC-UV	Isoniazid and acetylisoniazid	Isocratic: water, acetonitrile (80:20, v/v) and 0.01 M phosphoric acid	Column Nitrile Spherisorb (250×4.5 mm; 5 μm)	UV at 266 nm	2.0	Human plasma	(Hutchings et al., 1983)
HPLC-UV	Isoniazid and acetylisoniazid	Isocratic: 10 mM sodium dihydrogen phosphate buffer pH 3.0 adjusted with phosphoric acid, 1 mM dodecyl sulphate and 25% of acetonitrile	Column C_{18} Ultrasphere ion-pair (150×4.6 mm; 5 μm)	UV at 270 nm	1.5	Human plasma and urine	(Svensson et al., 1985)
HPLC-UV	Isoniazid, acetylisoniazid, acetylhydrazine, and diacetylhydrazine	Isocratic: 10 mM phosphate buffer pH 5.5, methanol, acetonitrile and dichloromethane (73:17:9:1, v/v/v/v)	Column C_{18} Nucleosil RP-18 (300×4.5 mm; 10 μm)	UV at 220 nm	2.0	Human plasma and urine	(Von Sassen et al., 1985)
HPLC-UV	Isoniazid and others antimicrobial drugs	Isocratic: 0.05 M phosphate buffer pH 6.0 and methanol 3%	Column C_{18} μBondapak (150×3.9 mm; 5 μm)	UV at 254 nm	3.0	Human polymorphonuclear leukocytes	(Koga, 1987)
HPLC-UV	Isoniazid and hydrazine	Isocratic: acetonitrile, water, and triethylamine (70:30:0.4, v/v/v)	Column C_{18} μBondapak (300×3.9 mm; 10 μm)	UV at 320 nm	1.0	Rabbit plasma and cerebrospinal fluid	(Walubo et al., 1991)
HPLC-UV	Isoniazid	Gradient: (A) 0.05 M potassium dihydrogen phosphate buffer (pH 4.5); (B) acetonitrile and isopropanol (4:1, v/v) 90% A: 10% B (v/v) 1.0 min 30% A: 70% B (v/v) 15.0 min 30% A: 70% B (v/v) 4.0 min	Column C_8 Whatman Partisil (250×4.6 mm; 10 μm)	UV at 280 nm	1.5	Human plasma, serum and cerebrospinal fluid	(Seifart et al., 1993)
HPLC-UV	Isoniazid, acetylisoniazid, and hydrazine	Gradient: (A) potassium dihydrogen phosphate buffer 50 mM; (B) acetonitrile and isopropanol (4:1, v/v) 60% A: 40% B (v/v) 1.0 min 30% A: 70% B (v/v) 13.5 min	Column C_8 Whatman Partisil (250×4.6 mm; 10 μm)	UV at 340 nm	1.0	Human plasma	(Seifart et al., 1995)

(*Continued on next page*)

Table 1. (*Continued*)

Method	Analytes	Mobile phase	Column	Detection	Flow Rate (mL/min)	Matrices	Reference
HPLC-UV	Isoniazid	Isocratic: water and methanol (60:40, v/v) containing 5 g/L of ammonium formate	Column C_{18} Supelco (250×4.6 mm; 5.0 μm)	UV at 313 nm	1.0	Rat plasma	(Hanser-Jr et al., 1995)
HPLC-UV	Isoniazid coupled to *trans*-cinnamaldehyde	Isocratic: water, acetonitrile, triethylamine and acetic acid (600:400:2:1, v/v/v/v; pH 5.0)	Column C_{18} Nova-pak (125×3.9 mm; 4 μm)	UV at 340 nm	1.3	Human serum	(Sadeg et al., 1996)
HPLC-UV	Isoniazid and pyrazinamide	Isocratic: 0.025 M tetrabutylamonium hydrogensulphate, methanol and acetonitrile (96:2:2, v/v/v; pH 3.0 adjusted with triethylamine)	Column C_{18} Metaphase-CrestPak ODS (250×4.0 mm; 5 μm)	UV at 248 nm	1.5	Standard	(Patel et al., 1998)
HPLC-UV	Isoniazid and acetylisoniazid	Isocratic: ammonium acetate buffer (0.05 M; pH 6) and acetonitrile (99:1, v/v)	Column C_{18} μBondapak (300×3.9 mm; 10 μm)	UV at 275 nm	1.2	Human plasma	(Moussa et al., 2001)
HPLC-UV	Isoniazid and pyrazinamide	Isocratic: methanol, water, perchloric acid 70% and tetrabutylamonium hydroxide 40% (2:8:0.005:0.0025, v/v/v/v)	Column C_8 Spherisorb (250×4.6 mm; 4 μm)	UV at 267 nm	1.0	Human plasma	(Agrawal et al., 2002)
HPLC-UV	Isoniazid and acetylisoniazid	Isocratic: 0.01 M phosphate buffer pH 2.5 and methanol containing 0.01 M sodium dodecyl sulfate (60:40, v/v)	Column C_{18} L-Column ODS (150×4.6 mm; 5.0 μm)	UV at 267 nm	1.0	Human urine	(Hashiguchi et al., 2002)
HPLC-UV	Isoniazid, pyrazinamide, and rifampicin	Isocratic: ethanol, water, chloroform and acetonitrile (55:40:4:1, v/v/v/v)	Column C_{18} YMC-ODS (150×4.6 mm; 5.0 μm)	UV at 337 nm	1.4	Tablets and human serum	(Khuhawar and Rind, 2002)
HPLC-UV	Isoniazid, pyrazinamide, and rifampicin	Gradient: (A) acetonitrile; (B) 50 mM phosphate buffer pH 3.5 3% A: 97% B (v/v) 5.0 min 50% A: 50% B (v/v) 25 min 3% A: 97% B (v/v) 10 min	Column C_{18} Lichrospher 100 RP-18 (250×4.0 mm; 5 μm)	UV at 261 nm	1.0	Tablets	(Calleri et al., 2002)
HPLC-UV	Isoniazid	Isocratic: acetonitrile and 10 mM ammonium acetate pH 4.5 (30:70, v/v)	Column C_{18} Kromasil (250×4.6 mm; 5 μm)	UV at 323 nm	1.0	Mice plasma	(Jayaram et al., 2004)
HPLC-UV	Isoniazid, pyrazinamide, and indomethacin	Isocratic: water, methanol and tetrahydrofuran (59:39:2, v/v/v)	Column C_{18} YMC-ODS (150×4.6 mm; 3.5 μm)	UV at 328 nm	2.0	Tablets	(Khuhawar et al., 2005)
HPLC-UV	Isoniazid, pyrazinamide and rifampicin	Isocratic: acetonitrile and tetrabutylammonium hydroxide 0.0002 M (42.5:57.5, v/v; pH 3.10 adjusted with H_3PO_4)	Column C_{18} μBondapak (250×4.6 mm; 10 μm)	UV at 260 nm	1.0	Tablets	(Glass et al., 2007)
HPLC-UV	Isoniazid, pyridoxine, pyrazinamide and rifampicin	Gradient: (A) acetonitrile; (B) potassium dihydrogen phosphate buffer 15 mmol/L of pH adjusted to 4.0 with orthophosphoric acid 11% A: 89% B (v/v) 4.5 min 50% A: 50% B (v/v) 15.5 min	Column C_{18} Phenomenex Luna (250×4.6 mm; 5 μm)	UV at 235 nm	1.0	Tablets	(Dhal and Sharma, 2009)

HPLC-UV	Isoniazid, acetylisoniazid, pyrazinamide, and rifampicin	Gradient: (A) methanol; (B) acetonitrile; (C) 20 mM 1-heptanesulfonic acid sodium pH 2.5 10% A: 8% B: 82% C (v/v) 6 min 0% A: 65% B: 35% C (v/v) 13 min 10% A: 8% B: 82% C (v/v) 5.5 min	Column C_{12} Synergi Max-RP (250×4.6 mm; 4 μm)	UV at 264 nm	0.8–1.2	Human plasma	(Zhou et al., 2010)
HPLC-UV	Isoniazid, isonicotinic acid, isonicotinamide, and ethambutol	Isocratic: potassium dihydrogen orthophosphate buffer with pH 6.9 (100%)	Column C_{18} Inertsil ODS 3V (250×4.6 mm; 5 μm)	UV at 254 nm	1.5	Tablets	(Ayyappan et al., 2011)
HPLC-UV	Isoniazid, ethambutol, pyrazinamide and rifampicin	Gradient: (A) acetonitrile; (B) potassium dihydrogen phosphate buffer (8 mM; pH 6.8) 10% A: 90% B (v/v) 0 min 60% A: 40% B (v/v) 18 min 60% A: 40% B (v/v) 6.0 min	Column C_{18} Phenomenex Luna (250×4.6 mm; 5 μm)	UV at 210 nm	1.0	Tablets	(Wang et al., 2012)
HPLC-UV	Isoniazid	Isocratic: 0.1 M phosphate buffer pH 3.0 and methanol (97.5:2.5, v/v)	Column C_{18} Eclipse XDB (150×4.6 mm; 5 μm)	UV at 264 nm	0.8	Rabbit vertebrae	(Liu et al., 2013)
HPLC-UV	Isoniazid, ethambutol, pyrazinamide and rifampicin	Gradient: (A) 20 mM monobasic sodium phosphate buffer with 0.2% triethylamine (pH 7.0) and acetonitrile (96:4, v/v); (B) acetonitrile 100% A: 0% B (v/v) 5 min 48% A: 52% B (v/v) 7 min 100% A: 0% B (v/v) 5 min	Column C_{18} Purosphere Star RP-18 (250×4.6 mm; 5 μm)	UV at 238 nm	1.5	Tablets	(Chellini et al., 2015)
HPLC-UV	Isoniazid, pyrazinamide and rifampicin	Isocratic: 50 mM phosphate buffer pH 4.2 and acetonitrile 0.25% (v/v)	Column C_{18} Purosphere LichroCart RP-18 (125×4.6 mm; 5 μm)	UV at 263 nm	1.0	Human plasma and cerebrospinal fluid	(Pouplin et al., 2016)
HPLC-MS/MS	Isoniazid and ethambutol	Isocratic: methanol, water and formic acid (10:90:0.3, v/v/v)	Column C_{18} Atlantis (150×2.1 mm; 3 μm)	MS using *m/z* 79.0	0.2	Human plasma	(Chen et al., 2005)
HPLC-MS/MS	Isoniazid and acetylisoniazid	Gradient: (A) methanol; (B) 0.1% formic acid in water 8% A: 92% B (v/v) 6 min 98% A: 2% B (v/v) 3 min 98% A: 2% B (v/v) 7 min 8% A: 92% B (v/v) 2 min	Column C_{18} Aquasil (300×3.0 mm; 3 μm)	MS using *m/z* 121.2	0.2	Rat plasma and alveolar macrophages	(Ng et al., 2007)
HPLC-MS/MS	Isoniazid, ethambutol, pyrazinamide and rifampicin	Gradient: (A) methanol and formic acid 0.3%; (B) water and formic acid 0.3% 60% A: 40% B (v/v) 1.8 min 80% A: 20% B (v/v) 0.2 min 60% A: 40% B (v/v) 2.0 min	Column C_{18} Hydrosphere (50×2.0 mm; 3 μm)	MS using *m/z* 121.0	0.15 – 0.4	Human serum	(Song et al., 2007)

(*Continued on next page*)

Table 1. (*Continued*)

Method	Analytes	Mobile phase	Column	Detection	Flow Rate (mL/min)	Matrices	Reference
HPLC-MS/MS	Isoniazid, rifampicin and levofloxacin	Gradient: (A) water and formic acid 0.05%; (B) methanol 93% A: 7% B (v/v) 4.5 min 88% A: 12% B (v/v) 4.5 min 10% A: 90% B (v/v) 4 min 93% A: 7% B (v/v) 3.5 min	Column C_4 Hydrosphere (250×4.6 mm; 5 μm)	MS using *m/z* 121.0	1.0	Mouse plasma and tissues	(Fang et al., 2010)
HPLC-MS	Isoniazid, acetylisoniazid and isoniazid-vitamin B6 forms	Isocratic: 1 mM ammonium formate pH 6 and acetonitrile (20:80, v/v)	Column C_{18} Agilent Zorbax SB-Aq (150×3.0 mm; 3.5 μm)	MS using *m/z* 138.0	0.1 – 0.2	Standard	(Pasáková et al., 2011)
HPLC-MS/MS	Isoniazid, ethambutol, pyrazinamide and rifampicin	Isocratic: acetonitrile and water containing 0.1% formic acid (60:40, v/v)	Column C_{18} Agilent Zorbax SB (50×2.1 mm; 1.8 μm)	MS using *m/z* 121.0	0.3	Human plasma	(Xu et al., 2013)
HPLC-MS/MS	Isoniazid	Isocratic: isopropanol-acetonitrile and water containing 0.046% formic acid (80:20, v/v)	Column C_{18} Inertsil Sil 100A (50×2.1 mm; 5 μm)	MS using *m/z* 121.3	0.3	Human plasma	(Daher et al., 2015b)
HPLC-MS/MS	Isoniazid, acetylisoniazid, isonicotinic acid and rifampicin	Gradient: (A) 5 mM ammonium acetate pH 6.7; (B) 90% acetonitrile in water containing 0.1% formic acid 100% A: % B (v/v) 1 min 97% A: 3% B (v/v) 2 min 50% A: 50% B (v/v) 0.1 min 30% A: 70% B (v/v) 1.9 min 5% A: 95% B (v/v) 1 min	Column C_{18} Agilent Zorbax SB-Aq (50×4.6 mm; 5 μm)	MS using *m/z* 121.1	0.75	Human plasma	(Hee et al., 2015)

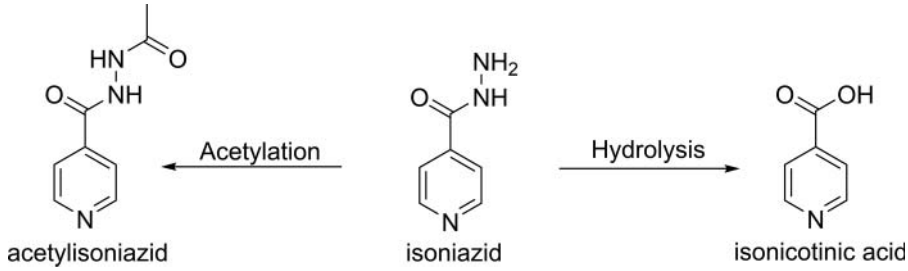

Figure 2. Principal metabolites of isoniazid detected by analytical methods.

because the macrophages are the main immune cell that phagocyte the *Mycobacterium tuberculosis* (Ng et al., 2007). In another study, an HPLC-MS/MS technique was used for the determination of isoniazid and others antimicrobials in mouse tissues including brain, lung, liver, kidney and small intestine (Fang et al., 2010).

Although some methods presented specific applications as described above, most HPLC-based methods highlighted herein were developed for determination and quantification of isoniazid in human plasma, urine or tablets.

The use of ultraviolet detectors coupled with HPLC systems was certainly the most used detection technique in pharmaceutical analysis and is still widely used (Roškar and Lušin, 2012). Nonetheless, the increased number of methods using mass spectrometry has been increasing in recent years. For instance, in this review were reported 28 HPLC-based methods using UV detection from the period ranging from 1977 until 2016. Analytical methods using mass detectors have emerged during the 2000s and few methods were still reported until now. Indeed, mass detectors present high sensitive and selectivity over UV detectors (Baldrey et al., 2002; Suneetha and Raja, 2016); however, the high cost of mass detectors is an obstacle for many laboratories worldwide that become this method not practicable to developing countries.

The analytical methods described in this review cover a number of HPLC-based methods coupled to UV detector. Nevertheless, they differ from each other in certain parameters, including mobile phase, flow rate and wavelength. Indeed, the mobile phase is the parameter that presents more variation between the described methods. Among these methods, a number of solvents and buffers have been reported, including chloroform, methanol, water, acetonitrile, dichloromethane, isopropanol, tetrahydrofuran, ammonium acetate buffer and phosphate buffer. Additionally, the wavelength used for each other also differs. Regarding the stationary phase, most of the methods have utilized reversed-phase C_{18} columns (Table 1).

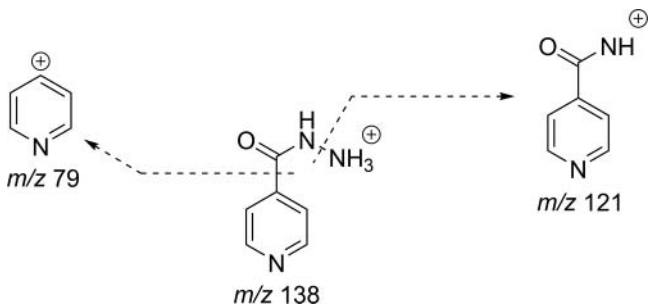

Figure 3. Fragments generated by isoniazid in mass spectrometry.

Conclusions

Isoniazid remains as one of the most import drug for TB treatment, even after its discovery more than 60 years ago. Its high selectivity and potency against mycobacteria contribute to its success. Furthermore, its relative simple chemical structure, the easy method of preparation of only one synthetic step and the good yield achieved in the synthesis are also factors that make isoniazid so important. Additionally, isoniazid has good pharmacokinetic properties. The analytical methods available in the scientific literature for identification and quantification of isoniazid cover different techniques. Nevertheless, HPLC-based methods coupled with UV or MS represent the principal analytical technique for determination of isoniazid in both, pharmaceutical and biological matrices. The advantages of performing analytical studies through HPLC-based methods are related to the high specificity, speed of analysis, accuracy and sensitive. These large numbers of advantages are directly related to the detection technique coupled. Most of the HPLC methods highlighted herein were developed for simultaneous quantification of isoniazid and other antitubercular drugs, including rifampicin, ethambutol and pyrazinamide. Furthermore, several methods were also designed to determine the main metabolites of isoniazid, such as acetylisoniazid and isonicotinic acid. This review aimed to present an overview of the current state of the art of analytical methods for determination of isoniazid.

Conflict of interest

The authors declare no conflicts of interest.

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