IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 12, Issue 5 Ver. II (Sep. – Oct. 2017), PP 40-47
www.iosrjournals.org

Detection and Assay of Antimycobacterial AgentIsoniazid Utilizing Isocratic High PerformanceLiquid Chromatography

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Abstract: *Isoniazid was assayed by HPLC, utilizing a reversed-phase C-18 column with eluent solvent (5.3% ethanol, 93.7% water, 1% acetic acid). Samples for analysis were prepared in distilled water as the solvent. Detection of isoniazid was accomplished at 265 nm, which showed highest sensitivity for isoniazid . Column pump pressure approximately 2100 psig, rise time 0.1 with flow rate 1.0 mL/minute. Elution of isoniazid occurred at 2.2 minutes. Limit of detection (LOD) of isoniazid was 1.172 x 10^{-5} molar, but with detection going as high as 1.460 x 10^{-3} molar which provides a detection range of 125x. The limit of quantitation (LOQ) is 3.905 x 10^{-5} molar. Reverse phase isocratic conditions is shown to be effective for determination of isoniazid in aqueous based samples. The standard curve is highly linear reaching as high as 1.460 x 10^{-3} molar (y = 50570789.86 x), having coefficient of determination (R^2 = 0.9916) with very strong positive correlation coefficient (r= 0.9958). The percent recovery of drug ranged from 96% to 104%. Utilizing reversed phase column with isocratic solvent conditions (ethanol, acetic acid, and water in column solvent), is effective for determination of isoniazid.*

Keywords: *HPLC, isoniazid, isonicotinylhydrazide, tuberculosis, tuberculostat*

Date of Submission: 21-07-2017 Date of acceptance: 13-09-2017

I. Introduction

Tuberculosis (TB) is believed to be the most frequent and important bacterial disease causing both morbidity and mortality worldwide [1]. It is believed that as many as one-third of the world's population are infected with the *Mycobacterium tuberculosis* [1]. It is thought, there are nearly 9 million new cases of TB infection occur each year, with multidrug-resistant tuberculosis (MDR-TB) report in all regions of the world [2]. In the year 2009, Africa presented 14% of the global burden of MDR-TB cases [3]. MDR-TB and extensively drug resistant tuberculosis (XDR-TB) pose a substantial threat to the control of tuberculosis [4].

Isoniazid (INH) and rifampicin (RIF) are among the most effective anti-tuberculosis regimens that are used in many countries [5]. A serious problem recognized in TB treatment is patient non-compliance using the prescribed regimens [5]. Isoniazid, combined with rifampicin, have been applied to improve patient compliance and acceptance [5]. Methods used for determination of isoniazid include visible spectrophotometry at 475 nm and first-derivative ultraviolet spectrophotometry at 257 nm [5]. UV spectrophotometry is shown effective in determination of isoniazid from tablet dosage forms [6, 7].

HPLC analysis of rifampicin and isoniazid in pharmaceutical dosage forms utilizing reverse phase chromatography has been developed. A previously demonstrated method uses isocratic conditions with a octadecylsilane column and an aqueous mobile phase containing methanol (75%) and 0.02 M disodium hydrogen orthophosphate (25%) with pH at 4.5 (detection at 254 nm) [8]. An HPLC-diode array detector method for the simultaneous determination of rifampicin, isoniazid, pyrazinamide, and ethambutol in tablets was developed (isoniazid detected at 238 nm) [9].

Another HPLC technique of isoniazid detection was accomplished by adding docusate sodium surfactant to separate impurities [10]. Artificial neural network data modeling combined with HPLC has been used to detect isoniazid, and other first-line tuberculostats [11]. Another HPLC method achieved assay of isoniazid and other first-line tuberculostats after separation med on a Max-RP C(12) column, gradient elution, and mobile phase of methanol-acetonitrile-buffer [12].

The assay of tuberculostat isoniazid is important for pharmaceutical formulations applied for patient treatment of tuberculosis infection. HPLC has been shown to be a highly effective and sensitive methodology for dependable determination of drugs, including isoniazid. This study reports an effective and versatile approach for determination of isoniazid from common aqueous mixtures, mixtures of ampoule formulation, and solid/tablet specimens having various excipient combinations. HPLC detection of isoniazid is effective and valuable for quality control in industrial environments.

II. METHODOLOGY

2.1 Chemical Reagents

All solvent reagents were analytical grade in quality and obtainedfrom Sigma-Aldrich (St. Louis MO 63178 USA).The isonicotinic acid hydrazide (isoniazid) drug for use as standards and preparationof samples can be obtained from Sigma-Aldrich (St. Louis MO 63178 USA).

2.2 Instrumentation

To accomplish high performance liquid chromatography (HPLC) analysis, an Alltech 426 HPLC Pump and LinearUVS 200 detector were utilized(Deerfield, Illinois 60015-1899). Reversed-phaseisocratic methodologyis utilized for analysis of all sample types. TheHPLC Column is of 5 µ packing, length of 150 millimeters and internal diameter of 4.6 millimeters. The Alltech instrumentation is controlled bycomputer interface.

2.3 Instrument Settings

In the analysis by HPLC, a reversed-phase C-18octadecylsilyl (C18H37) bonded phase column packing was utilized. The isoniazid analyte eluted at 2.2 minutes. Detection was accomplished byultraviolet detector set to 265 nm, rise time 0.1,range AUFS set to 1.0. The HPLC pump was setto 2100 psig and one milliliter per minute flowrate. Actual volume injected into thecolumn is about 20 microliters. The dead time ofeluting non-retained species is 1.5 minutes andcalculated based on relationship, dead time= to =volume/flow rate = 1.5 mL/1.0 mL/min [13].

2.4 Sample Types and Samples

Column solvent utilized throughout the projectwas made for a total volume of 1000 mL by adding 52.6 mL of 95% ethanol, 937.4 ml of distilledwater, and 10 mL of glacial acetic acid (stock at 17.4 molar). Therefore, the workingconcentrations:5% ethanol, 0.174 molaracetic acid, and 93.7% water (v/v). Sample solventwas used for solubilizing isoniazid in varioustest samples: distilled water.Stock standard of isoniazid was prepared bydissolving 1.509 grams of isoniazid into 250 mL volumetric flask of distilled water,making a mixture of 6.000×10^{-2} molar. If any sample requiredclarificationprior to HPLC analysis, then where necessary, this is accomplishedby Whatman 6900-2502 GD/X 25Sterile Syringe Filter, 25 mm, 0.2 Micron, PVDFFiltration Medium, with a suitable plastic syringe.Samples for HPLC analysis were indistilled water as solvent, which works very well for the very water soluble isoniazid. Ampoule type samples, used in clinical application, were isoniazid drug prepared in dilute HCl aqueous solvent with HCl concentration at 1.00×10^{-6} molar. Tablet/solid samples were the drug isoniazid prepared in various known percentage of combinations of excipients lactose, cellulose, starch, and/or dextrin. After thorough mixing of isoniazid with excipient(s), the tablets were prepared utizling a standard Parr Pellet Press (Parr Instrument Company, 211 Fifty Third Street, Moline Illinois 612265 USA). The pressed tablets were then carefully weighed by digital balance, the isoniazid determined by mass percentage present, then followed by solubilization in distilled water utilizing 100 mL volumetric flasks. Clarification of solution can be accomplished by syringe filter. Samples were then analyzed after 48 hours of mixing/settling and harvesting of supernatant.

2.5 Statistical Analysis, Properties Determination, Molecular Modeling

Where indicated the numerical analysis utilizingPaired tests, one-way ANOVA, Kruskal-Wallis test, Mann-Whitney test, andcorrelation between sets of data was performedby PAST version 2.06 (copyright Hammer andHarper 1999-2011). Summary statistical analysiswas also performed by Microsoft EXCEL(copyright 2010 Microsoft Corporation, MicrosoftOffice Professional Plus 2010) and PAST v. 2.06.The Grubb's test for outliers (extremestudentized deviate) was performed by GraphPad InStat version 3.00 (Copyright 1992-1998Graph Pad Software Inc. (www.graphpad.com)for Windows 95, San Diego California USA).Determination of 95% confidence intervals wasaccomplished by Method Validator version 1.1(copyright Philippe Marquis).Linear regression was accomplished by EXCEL and Method Validator version 1.1. Molecular properties of isoniazidwere determined utilizing Molinspirationcheminformatics http://www.molinspiration.com/(Molinspiration Cheminformatics, Nova ulica, SK-900 26 Slovensky Grob, Slovak Republic).Passing-Bablok regression analysis was performed by ACOMED statistik: www.acomed-statistik.de (copyright Dr. Thomas Keller).

III. Results And Discussion

Isoniazid is an important first-line tuberculostat for the clinical treatment of tuberculosis infection. The molecular structure of isoniazid is shown in Fig. 1. It consists of apyridine ring (a heterocyclic structure)with a hydrazide substituent ($-C(=O)NHNH_2$). The Log P value of -0.97 indicates the compound is more hydrophilic (less lipophilic is higher values of Log P which indicate more lipophilictrait). Isoniazid readily dissolved intothe

test samples solvent of distilled water for standards as well oraqueous samples for tablet/solid preparations. The entire study was accomplished utilizing isocratic conditions. In isocratic conditions the mobile phase composition remainsconstant. An advantage of isocratic systeminclude the column being equilibrated all the timeand does not suffer from fast chemical changes[14]. Other considerations that make isocraticelution conditions more preferable include [14]: 1) When samples for testing contain less than 10weakly retained components; and 2) the gradientbaseline would impede trace analysis.

The mobile phase in reversed phase HPLC will consist of water as aqueous solution and an organic modifier. In reversed phase HPLC then, water is the "weakest" solvent. Since water is most polar, it repels the hydrophobic analytes into the stationary phase more than any other solvent, and hence retention times are long (making it chromatographically "weak") [15]. The organic modifier is added (usually only one modifier type at a time), then the (hydrophobic) analyte is no longer as strongly repelled into the stationary phase, will spend less time in the stationary phase, and therefore elute earlier [15]. By definition, this makes the modifier chromatographically "strong" as it speeds up the elution (reduces retention).As more organic modifier is added to the mobile phase, the analyte retention time will continue to decrease. Common organic modifiers include alcohols; with ethanol utilized in this study.

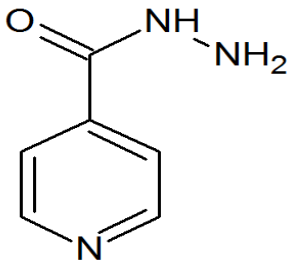

Figure 1. The molecular structure of isoniazid having the hydrazide group (-C(=O)NHNH$_2$). The IUPAC name is pyridine-4-carbohydrazide, with SMILES notation (simplified molecular-input line-entry system) as O=C(NN)c1ccncc1. This drug has a molecular formula $C_6H_7N_3O$, polar surface area of 68.01 Angstroms^2, Log P = -0.97, and formula weight 137.13 grams/mole.

It is important to determine thewavelength setting of the UV-Vis detector tosignal the elution of isoniazid from the HPLC instrument. Practically the wavelength of detectionis set to a wavelength where the maximumabsorbance of the analyte occurs in thesolvent mixture used for HPLC determination.To identify the maximum wavelength of detection for isoniazid, mixtures having identical molar concentration of isoniazid in distilled water were injected using constant column and instrument settings. Only thewavelength of detection is varied, the wavelengthshowing maximum absorbance was selected for following analysis.The wavelengths examined were (see Fig. 2): 240 nm, 250 nm, 260nm, 265 nm, 270 nm, 280 nm, 290 nm, 300 nm, and 310 nm. Maximum absorbance for isoniazid occurred at265 nm and this wavelength was utilized for assay in this study.

Thisresult is plotted andshown in Fig. 2. The maximum absorbance isat 265 nm. This is thewavelength setting for the detector in thisdetermination of isoniazid. Absorbance atwavelengths above and below 265 nm quickly drop lower. Formation of the standard curve and other analysis of test samples wasconducted at detection wavelength of 265 nm.

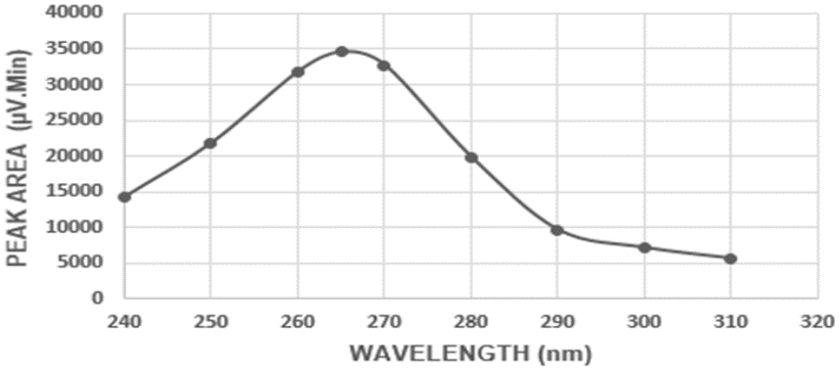

Figure 2. Comparison of peak area to wavelength in nanometers (nm) for nine injected samples. Concentration of all samples at 6.000 x 10^{-4}molar in aqueous solvent. The absorbance maximum occurred at 265 nm, which is the wavelength for detection of isoniazid by HPLC detector.

The standard curve obtained for the assay of isoniazid is shown in Fig. 3. The result is very highly linear with a correlation coefficient of0.9958, indicating a very strong positive inter-relationship of concentration and area of peak (uV.Min). The range of detection runs from 1.172 x 10^{-5} molar to 1.460 x 10^{-3}molar. Theequation of the line is calculated to be: (y = 50570789.86 x). The limit of detection(LOD) is calculated to be here at 1.172 x 10^{-5} molar, which is the concentration having a signal to noise ratio of three [16, 17]. The limit of quantification (LOQ) is 3.905 x 10^{-5} molar (calculated from signal to noise ratio at 10). The coefficient of determination becomes R^2 =0.9916, or 99.16% of the variance in thedependent variable (area) is predictable fromthe independent variable (concentration) [16, 17].

The drug isoniazid eluted at 2.2 minutes.The 95% confidence interval for the slope is from4.6371 x 10^7 to 5.0775 x 10^7. The standard curve is highly linear with valuesfound to be contained within a 95% ellipses (seeFig. 4). The confidence region is determined sothat should a set of measurements to berepeated many times and a confidence regioncalculated, then on average (95%) theconfidence region will include the true values ofthe set of variables [18, 19, 20,21]. This outcomeindicating consistent determination.

An example chromatogram showing isoniazid elution is presented in Fig. 5. Note that the peak is sharp and well defined. This was a consistent attribute for the isoniazid analysis performed in this study.

Recovery of the analyte need not to be 100%,but the extent of recovery of the analyte shouldbe consistent, precise and reproducible [16, 17]. Thiswas accomplished in this study. The record ofrecovery rate for isoniazid is presented inTable 1. The values of calculated molar values isdetermined from the molarity of the stocksolution of a known amount of isoniaziddissolved in aqueous solution and are compared to values following HPLC analysis.

The meanvalue for the percent recoveries shown in Table 1 of analyte is100 %, with a standard deviation of 1.8 %.Grubb's test showed no outliers detected in actual percent recovery values [18, 19]. Statistical analysis ofcalculated molar values compared toHPLC measured molarvalues gives correlation r = 0.9987, with one-way ANOVA test indicating the twosets of values have equal means (*P = .96*). The Paired Sample test showed equal means (*P = .20*) and equal medians (*P = .087*) [18, 19, 20]. Mann-Whitney analysis indicated that calculated and HPLC molar values have equal medians (*P = .90*), as does the Kruskal-Wallis test showing equal medians (*P = .89*).

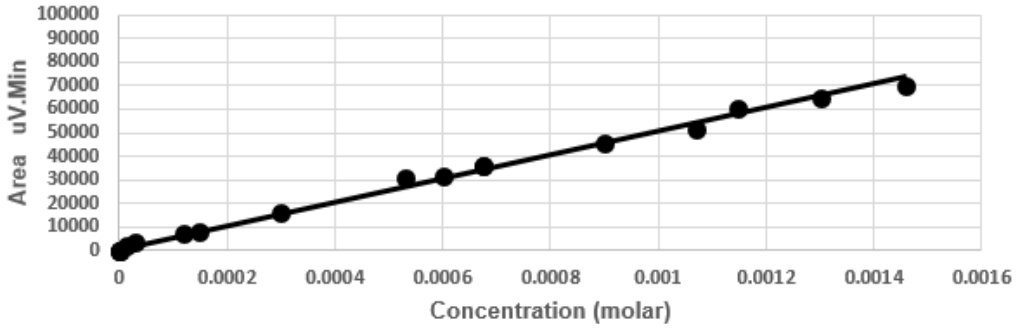

Figure 3. The standard curve for determination of concentration of drug in mixtures analyzed in terms of molar amount. The equation of the line is y = 50,570,789.86x with coefficient of determination of R^2 = 0.9916 (accounting for 99.16% or variance in the model). The correlation coefficient r = 0.9958. The Runs Test showed result of *P=.086* indicating no departure from linearity for this standard curve (standard deviation of residuals from line is 1947.1).

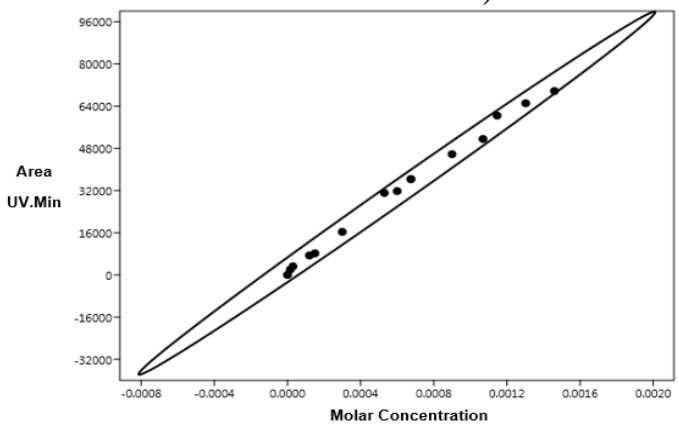

Figure 4. 95% ellipses result for standard curve shows all points of line within 95% inclusion. So it follows, this is smallest ellipse that will cover 95% of the points of the diagram.

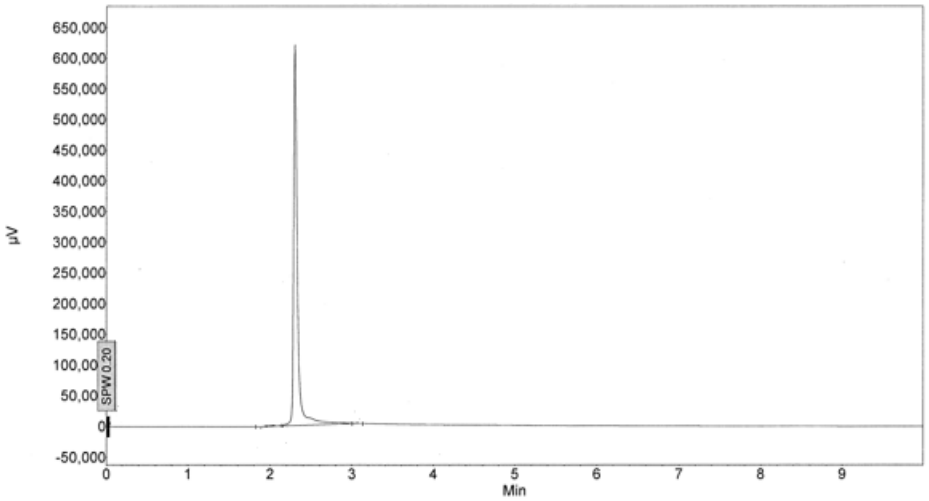

Figure 5. Example HPLC chromatogram showing elution of isoniazid 2.2 minutes under conditions described in Methodology. The isoniazid concentration is 6.750×10^{-4} molar.

Table 1. Percent recovery of drug, comparing calculated molarity to values from HPLC analysis.

TEST	CALCULATED MOLARITY	HPLC DETERMINED MOLARITY	PERCENT RECOVERY
1	9.750×10^{-4}	1.021×10^{-3}	104
2	1.125×10^{-3}	1.150×10^{-3}	102
3	1.275×10^{-3}	1.231×10^{-3}	96.6
4	6.450×10^{-4}	6.554×10^{-4}	101
5	7.050×10^{-4}	7.117×10^{-4}	101
6	8.550×10^{-4}	8.455×10^{-4}	98.9
7	4.050×10^{-4}	4.084×10^{-4}	101
8	5.100×10^{-4}	5.126×10^{-4}	101
9	6.150×10^{-4}	6.105×10^{-4}	99.3
10	7.350×10^{-4}	7.278×10^{-4}	99
11	8.400×10^{-4}	8.312×10^{-4}	99
12	7.500×10^{-5}	7.920×10^{-5}	104
13	2.700×10^{-4}	2.806×10^{-4}	103
14	5.550×10^{-4}	5.581×10^{-4}	101
15	7.950×10^{-4}	8.021×10^{-4}	101
16	8.850×10^{-4}	8.750×10^{-4}	98.9
17	2.100×10^{-4}	2.154×10^{-4}	103
18	2.850×10^{-4}	2.909×10^{-4}	102
19	3.900×10^{-4}	3.991×10^{-4}	102
20	6.150×10^{-4}	6.296×10^{-4}	102
21	7.800×10^{-4}	7.799×10^{-4}	100
22	8.100×10^{-4}	8.104×10^{-4}	100
23	3.300×10^{-4}	3.348×10^{-4}	101
24	3.600×10^{-4}	3.685×10^{-4}	102
25	6.900×10^{-4}	6.901×10^{-4}	100
26	9.900×10^{-4}	9.932×10^{-4}	100
27	1.035×10^{-3}	1.038×10^{-3}	100
28	4.050×10^{-4}	4.184×10^{-4}	103
29	5.100×10^{-4}	5.185×10^{-4}	101
30	6.150×10^{-4}	6.313×10^{-4}	102
31	2.400×10^{-4}	2.468×10^{-4}	103
32	3.450×10^{-4}	3.479×10^{-4}	101
33	4.350×10^{-4}	4.333×10^{-4}	99.6
34	5.700×10^{-4}	5.669×10^{-4}	99.4
35	7.050×10^{-4}	6.945×10^{-4}	98.5
36	9.150×10^{-4}	8.898×10^{-4}	97.2
37	4.050×10^{-4}	4.017×10^{-4}	99.2
38	4.950×10^{-4}	4.883×10^{-4}	98.7
39	6.150×10^{-4}	6.154×10^{-4}	100
40	7.650×10^{-4}	7.608×10^{-4}	99.4
41	9.600×10^{-4}	9.935×10^{-4}	103

Further comparison of percent recovery in Table 1 by Passing-Bablok analysis further showed consistent and efficient recovery of the drug isoniazid. The Passing-Bablok regression analysis isparticularly suitable for method comparisonstudies and allows comparing two measurementmethods [20, 21]. It is a statistical procedure thatallows estimation of an analytical method'sagreement and possible systematic bias betweenthem [21].Analysis of Table 1 recovery of isoniazid comparing calculated molar values to HPLC measured molar values, shows anexcellent relationship by Passing-Bablok regression (slope = 0.9896, y-axis intercept = 0.00). The 95% confidenceinterval for the y-axis intercept includes zero (0.000 to 0.000) andthe 95% confidence interval for the slopeincludes 1 (0.9772 to 1.003). Therefore, there are no constantdifferences between the calculated andHPLC measured values, and these values can be usedinterchangeably. Therefore, the calculated molarvalues are representative of the HPLC measured molarvalues.

The molar concentration of isoniazid found in solid/tablet formulation was also determined with results of percent recovery presented in Table 2. Common excipients that are incorporated in oral dosages of isoniazid tablets can include starch, dextrin, lactose, and/or cellulose. These excipients are utilized according to manufacturer preference and particular clinical application. All solid forms of drug carrier shown in Table 2 were analyzed by HPLC, and showed an excellent level of percent recovery when comparing expected molarity from tablet/solid mass to the molarity obtained following HPLC analysis.

The mean percent recovery of analyte isoniazid from solid formulation is 100 % with a standard deviation of 3.0 %. No outliers were detected in the percent recovery values, by Grubb's test [18, 19]. Comparison of molarity values in solid formulation to HPLC molarity values were shown to have the same mean by one-way ANOVA analysis (*P = .97*). The Paired test performed on molarity values also indicated the two sets of values have equal means (*P = .79*) and equal medians (*P = .82*) [18, 19, 20]. Mann-Whitney analysis indicated that calculated and HPLC molar values have equal medians (*P = .92*), as does the Kruskal-Wallis test showing equal medians (*P = .90*). The Pearson correlation r between the two sets of values is 0.9852. This is a highly reproducible recovery analysis for isoniazid from various types of solid/tablet formulation types. Analysis of calculated molar values to compare to HPLC measured molar values shown in Table 2, has an excellent relationship by Passing-Bablok regression (slope = 0.9511, y-axis intercept = 0.000). The 95% confidence interval for the y-axis intercept includes zero (-0.0001 to 0.0001) and the 95% confidence interval for the slope includes 1 (0.8777 to 1.1575). Therefore there are no constant differences between the calculated and HPLC measured values, and these values can be used interchangeably. Therefore, the calculated molar values are representative of the HPLC measured molar values.

Table 2. Percent recovery of drug following HPLC analysis of solid/tablet forms.

Sample	Component by Percent of Total Mass				Molarity from Solid Recovered by Mass	Molarity Analysis by HPLC	Percent Recovery
	Lactose	Cellulose	Starch	Dextrin			
1	-	-	89.9	-	8.583×10^{-4}	8.348×10^{-4}	97.3
2	-	76.8	-	-	1.108×10^{-3}	1.101×10^{-3}	99.4
3	-	-	-	88.1	9.515×10^{-4}	9.993×10^{-4}	105
4	-	33.0	28.5	21.6	1.101×10^{-3}	1.080×10^{-3}	98.1
5	-	10.4	26.4	47.2	1.133×10^{-3}	1.178×10^{-3}	104
6	-	30.3	47.1	-	1.062×10^{-3}	1.034×10^{-3}	97.4
7	33.0	45.5	-	-	1.257×10^{-3}	1.214×10^{-3}	96.0
8	63.3	19.1	-	-	7.294×10^{-4}	7.356×10^{-4}	101
9	48.3	40.2	-	-	8.118×10^{-4}	8.144×10^{-4}	100
10	29.4	43.9	-	-	8.169×10^{-4}	8.435×10^{-4}	103
11	23.7	53.7	-	-	8.096×10^{-4}	8.307×10^{-4}	103

Analysis of isoniazid content was also effectively accomplished from ampoule type formulations the outcome presented in Table 3. The ampoule formulations are still in aqueous solvents but containing 1.00×10^{-6} molar HCl for preservation and stability. Ampoule formulations are utilized in the cases where patients are too ill to receive the oral dosage regimen [1, 5]. Various concentrations of isoniazid were prepared in this dilute HCl solvent system and analyzed by HPLC. The results with expected molarity and HPLC determined molarity values are shown in Table 3. The percent recovery of isoniazid s was effective. The mean percent recovery of analyte isoniazid from ampoule formulation is 99.3 % with a standard deviation of 1.4 %. No outliers were detected in the percent recovery values, by Grubb's test [18, 19]. The statistical comparison of calculated molarity concentrations to HPLC molarity values were shown to have the same mean by one-way ANOVA analysis (*P = .92*). The paired test of molarity values also indicated the presence of equal medians (*P = .12*) [18, 19, 20]. Mann-Whitney analysis indicated that calculated and HPLC molar values have equal medians (*P = .92*), as does the Kruskal-Wallis test showing equal medians (P = .91). The Pearson correlation r between the two sets of values is 0.9984. This is a highly reproducible recovery analysis for isoniazid from various types of solid/tablet formulation types.

Analysis of Table 3 calculated molar values in comparison to HPLC measured molar values shows an excellent relationship by Passing-Bablok regression (slope = 0.9725, y-axis intercept = 0.000). The 95% confidence interval for the y-axis intercept includes zero (0.00 to 0.00) and the 95% confidence interval for the slope includes 1 (0.9488to 1.000). Therefore, there are no constant differences between the calculated and HPLC measured values, and these values can be used interchangeably. Therefore, the calculated molar values are representative of the HPLC measured molar values. Therefore, this HPLC methodology presented in this study can effectively ascertain the concentration of isoniazid from standard aqueous preparations, solid/tablet formulations, and ampoule formulations.

Table 3. Percent recovery of drug following HPLC analysis of ampoule formulations.

Sample Run	Calculated Molarity	Molarity Analysis By HPLC	Percent Recovery
1	$4.350x10^{-4}$	$4.319 x10^{-4}$	99.3
2	$5.250 x10^{-4}$	$5.233 x10^{-4}$	99.7
3	$6.000 x10^{-4}$	$6.074 x10^{-4}$	101
4	$7.500 x10^{-4}$	$7.515 x10^{-4}$	100
5	$8.250 x10^{-4}$	$8.248 x10^{-4}$	100
6	$4.500 x10^{-4}$	$4.666 x10^{-4}$	103
7	$5.100 x10^{-4}$	$5.038 x10^{-4}$	98.8
8	$5.700 x10^{-4}$	$5.581 x10^{-4}$	98
9	$6.150 x10^{-4}$	$6.096 x10^{-4}$	99.1
10	$8.550 x10^{-4}$	$8.363 x10^{-4}$	98
11	$9.300 x10^{-4}$	$9.121 x10^{-4}$	98.1
12	$6.450 x10^{-4}$	$6.536 x10^{-4}$	101
13	$7.800 x10^{-4}$	$7.835 x10^{-4}$	100
14	$8.850 x10^{-4}$	$8.926 x10^{-4}$	101
15	$6.750 x10^{-4}$	$6.609 x10^{-4}$	97.9
16	$7.200 x10^{-4}$	$7.081 x10^{-4}$	98.3
17	$8.100 x10^{-4}$	$7.937 x10^{-4}$	98
18	$8.850 x10^{-4}$	$8.680 x10^{-4}$	98.1
19	$9.000 x10^{-4}$	$8.872 x10^{-4}$	98.6
20	$9.600 x10^{-4}$	$9.465 x10^{-4}$	98.6

The instrumental analysis of pharmaceutical products meant for human consumption is a vital function of analytical chemistry. Thevarious methodologies developed have becometools for identifying adulterants(substitutes in products purposefully introducedby manufacturer), contaminants, and qualitycontrol management during manufacturing. Types ofinstrumentation applied in the analysis of pharmaceutical products and the numerousmethodologies available allow investigators abroad range of choices. The analysis of pharmaceutical products by HPLC will continue to be avigorous and successful area of application, due to highspecificity and sensitivity of detection. Analysisby HPLC will continue to permit confidence in the quality of pharmaceutical products availableto medical facilities.

IV. Conclusion

The first-line tuberculostat isoniazid is assayed effectively by isocratic reversed phase high performance liquid chromatography. Detection of isoniazid utilizing UV absorbance was accomplished at 265 nm. A standard curve enabled detection of amounts from 1.172×10^{-5} molar to more than 1.460×10^{-3} molar, a detection span of 125-fold. The coefficient of determination obtained as $R^2 = 0.9916$ accounts for 99.16% of variance in the dependent variable. Percent recovery of isoniazid from aqueous mixtures averaged 101%, with a standard deviation of 1.8%. Isoniazid was effectively assayed from aqueous samples, ampoule solution preparations, and tablets/solid samples. The methodology presented in this study will beuseful for quality control analysis as part of commercial production. Analysis methods for isoniazid determinationare a necessary objective to ensure quality control of commercial products and pharmaceutical medicaments applied in the clinical treatment of tuberculosis infection.

Acknowledgements

This project was supported by the College of Arts & Sciences, Durham Science Center, University of Nebraska at Omaha, Omaha NE 68182 USA.

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Ronald Bartzatt. "Detection and Assay of Antimycobacterial AgentIsoniazid Utilizing Isocratic High PerformanceLiquid Chromatography." IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS) , vol. 12, no. 5, 2017, pp. 40–47.