ISONIAZID

Glenn A. Brewer

CONTENTS

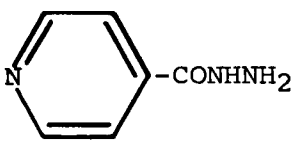
1. Description

1.1 Name, Formula, Molecular Weight

Generic names - Isoniazid[1], Isonicotinic Acid Hydrazide, INH, Isonicotinoylhydrazine, Isonicotinyl hydrazide, Isonicotinylhydrazine, Tubazid, Isoniazidum.

Chemical names - 4-Pyridinecarboxylic acid hydrazide, pyridine-4-carboxyhydrazide, pyridine- γ-carboxylic acid hydrazide.

Chemical Abstracts Registry No. 54-85-3[2]



$C_6H_7N_3O$ Mol.Wt.137.14

1.2 Appearance, Color, Odor, Taste

Colorless or white crystalline powder which is odorless and has at first a slightly sweet and then bitter taste[3].

2. Physical and Chemical Properties

2.1 Spectra

2.11 Infrared Spectrum

The infrared spectrum of isoniazid and other hydrazides of carboxylic acid have been recorded and band assignments were made[4]. Nagano et al[5] in a later paper made band assignments for isoniazid, metal complexes of isoniazid and related compounds.

The infrared spectra of isoniazid as a solid in a KBr pellet and as a mull in mineral oil are shown in Figures 1 and 2. The following assignments have been made by Mrs. Toeplitz[6].

Frequency(cm^{-1})	Assignment
3300-3000	Bonded NH and C-H
1670	C=O
1560	Amide II
1640	NH_2 deformation
1610 / 1500	ring C=C and C=N

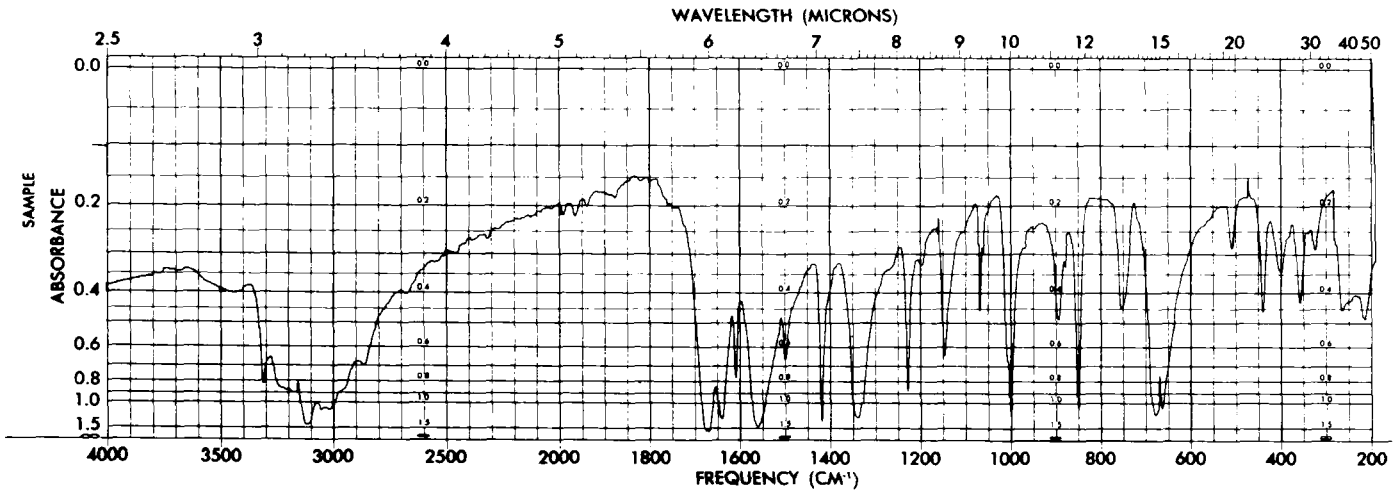


Figure 1:Infrared spectrum of isoniazid as a KBr pellet.

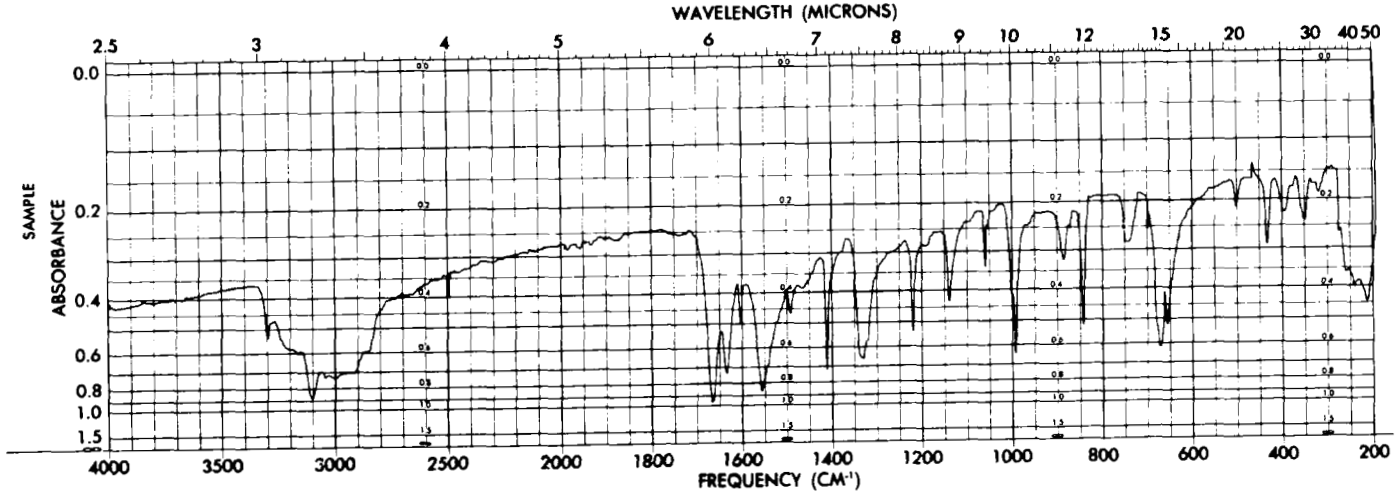


Figure 2:Infrared spectrum of isoniazid in mineral oil mull.

2.12 Ultraviolet Spectrum

Numerous authors have recorded the ultraviolet spectrum of isoniazid in a number of solvents[7,8,9,10,11,12]. The effect of the pH of the solution on the resulting spectrum has been noted. Zommer[13] has recorded the spectra of the hydrazones of isoniazid and acetone or p-hydroxybenzaldehyde.

The ultraviolet spectrum of isoniazid in dilute acid (0.01N aqueous HCl) shows two approximately equal maximima at 213 nm ($E^{1\%}_{1cm}$ 437) and 265 nm ($E^{1\%}_{1cm}$ 417). The minimum occurs at 233 nm.

The spectrum in distilled water shows a broad peak at 261 nm ($E^{1\%}_{1cm}$ 306) without a defined minimum. There is a shoulder at 208 nm. In dilute alkali (0.01N aqueous alkali) the spectrum taken immediately shows a shoulder at 266 nm ($E^{1\%}_{1cm}$ 293) and peaks at 272 nm ($E^{1\%}_{1cm}$ 298) and 295 nm ($E^{1\%}_{1cm}$ 284). On standing these peaks shift so that at 2 hours there are peaks at 256 nm ($E^{1\%}_{1cm}$ 173), 262 nm ($E^{1\%}_{1cm}$ 170) and 325 nm ($E^{1\%}_{1cm}$ 76). At 24 hours the 325 nm peak disappears. The same shift takes place with higher concentrations of alkali except that it occurs more rapidly.

The ultraviolet spectrum taken in methanolic rather than aqueous solvents are similar to those in water except that the absorption maximima generally occur at slightly lower wavelengths.

2.13 Chemiluminescence

Caen[15] has observed a weak chemiluminescence of isoniazid when solutions are oxidized with sodium hypochlorite. The luminescence increases with pH from 10.2 to 13. The maximum of the emission curve is at 0.552 μ corresponding to an energy of 51 Kcal. Two theories for the observed luminescence are offered, both of which depend on the presence of free OH and HO_2 radicals.

2.14 Fluorescence Spectrum

Isoniazid shows an intense fluorescence spectrum when oxidized with peroxide or after cleavage of the pyridine ring with cyanogen bromide. This fluorescence is the basis of several sensitive methods to determine isoniazid in biological materials (See Section 6.4). When a solution of isoniazid at pH 6.5 to 7.5 was treated with dilute peroxide at 100°C for 30 minutes we found the excitation maximum at 333 nm and the emission peak at 415 nm[14]. After isoniazid is reacted with cyanogen bromide reagent in 1.8N alkaline solution at room temperature we found an activation maximum at 312 nm and a fluorescence maximum at 392 nm[14].

Isoniazid also fluoresces when reacted with certain aromatic carbonyl compounds (Section 6.24).

2.15 N.M.R. Spectrum

Several authors have studied the nuclear magnetic resonance spectrum of the hydrazides of carboxylic acid including isoniazid[16,17,18]. Hillerbrand and co-workers[19] studied the N.M.R. spectrum of the copper salt.

The N.M.R. spectra of isoniazid and D_2O exchanged isoniazid are shown in Figures 3 and 4[20]. The 60 MHz NMR spectrum of isoniazid, in dimethyl sulfoxide-d_6 containing tetramethyl silane as internal reference shows the presence of hydrazino protons resonances at (ppm) 4.60 (broad, 2H, exchanged) and 10.15 (broad, 1H, exchanged). The aromatic protons resonances appear as multiplets at 7.73 (2H) and 8.70 (2H). (Figures 3 and 4). The complex pattern of the resonances, other than the expected doublets, suggests charge distribution in the pyridine ring. However, the hindered rotation around the N-C=O as well as C-aryl bonds can not be ruled out. The NMR spectrum in methanol -d_4 was similar to Figure 4, the hydrazino protons having been exchanged. The addition of deuterated HCl did not alter the spectrum except a downfield shift of the aromatic

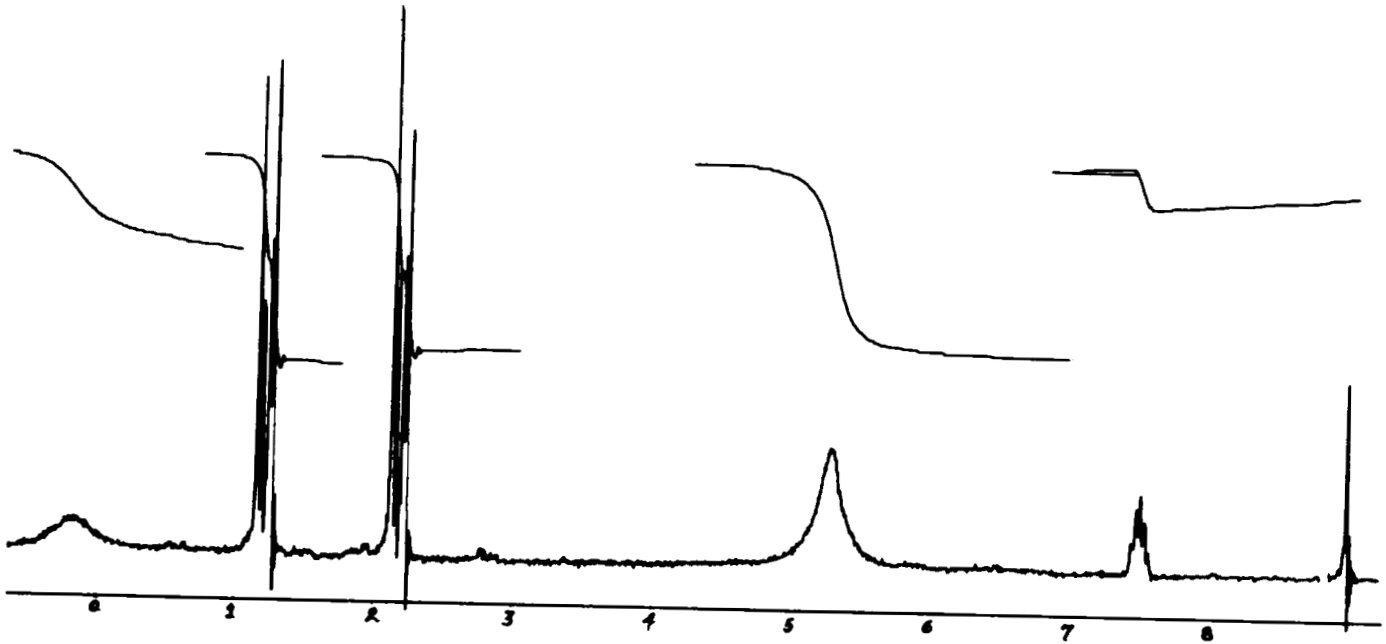


Figure 3:NMR spectrum of isoniazid in deuterodimethylsulfoxide.

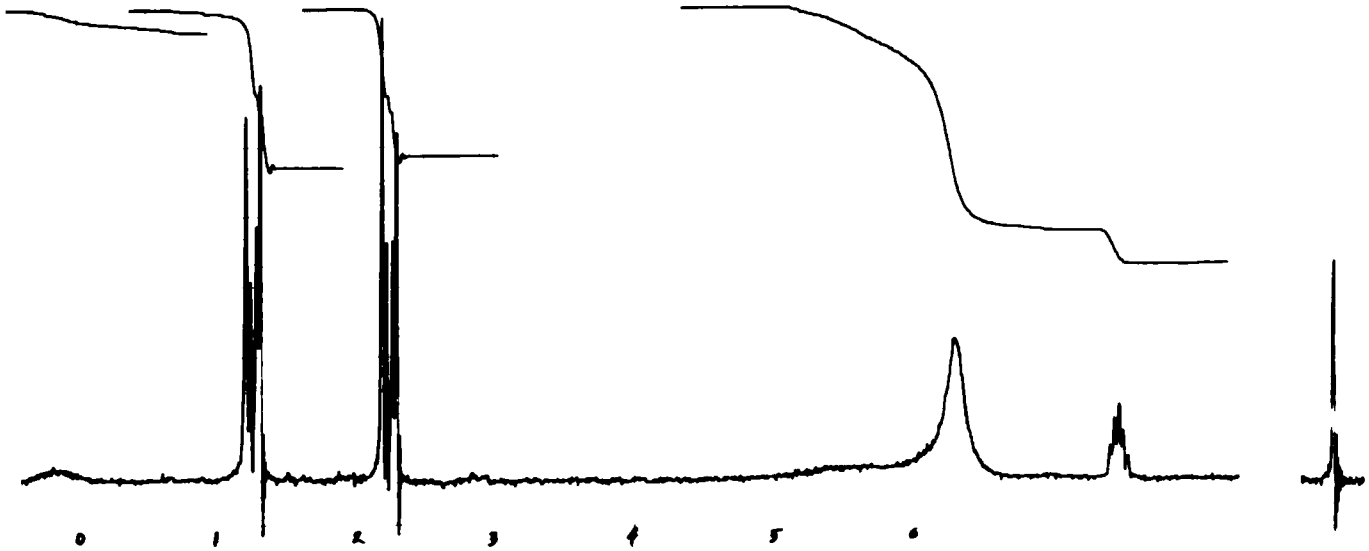


Figure 4:NMR spectrum of deuterium oxide exchanged isoniazid in deuterodimethylsulfoxide.

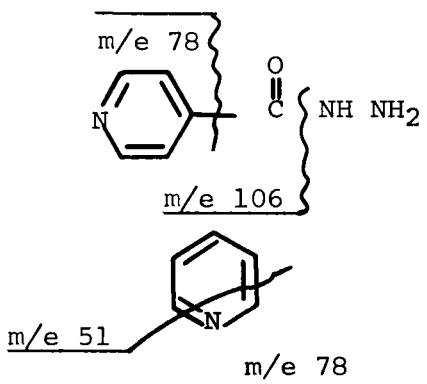
protons resonance by 0.1 ppm.

2.16 E.S.R. Spectrum

Hillerbrand and co-workers[19] used Electron Spin Resonance to study charge transfer interactions between isoniazid and copper ions.

2.17 Mass Spectrometry

Gillis[21] has discussed the fragmentation pattern for isoniazid and similar compounds. Figure 5 shows the electron-impact mass spectrum obtained on an AEI MS902 mass spectrometer equipped with a data acquisition system. The M+ occurs at m/e 137 and the fragment ions result from either direct bond cleavage (m/e 106, 78) or through the elimination of HCN from the pyridyl ring(m/e 51).



2.2 Physical Properties of the Solid

2.21 Melting Characteristics

The melting point of isoniazid is used as specification in the United States Pharmacopoeia[23] and European Pharmacopoeia[3]. The melting point occurs between 170 and 174°C.

2.22 D.T.A. and D.S.C.

Differential thermal analysis was used to study isoniazid before the technique gained its current popularity[24,25]. Pirisi[26] showed that isoniazid in the presence of zinc, copper and iron salts and mercuric oxide gives an abnormal D.T.A. pattern.

Dr. Jacobson[27] has shown that the

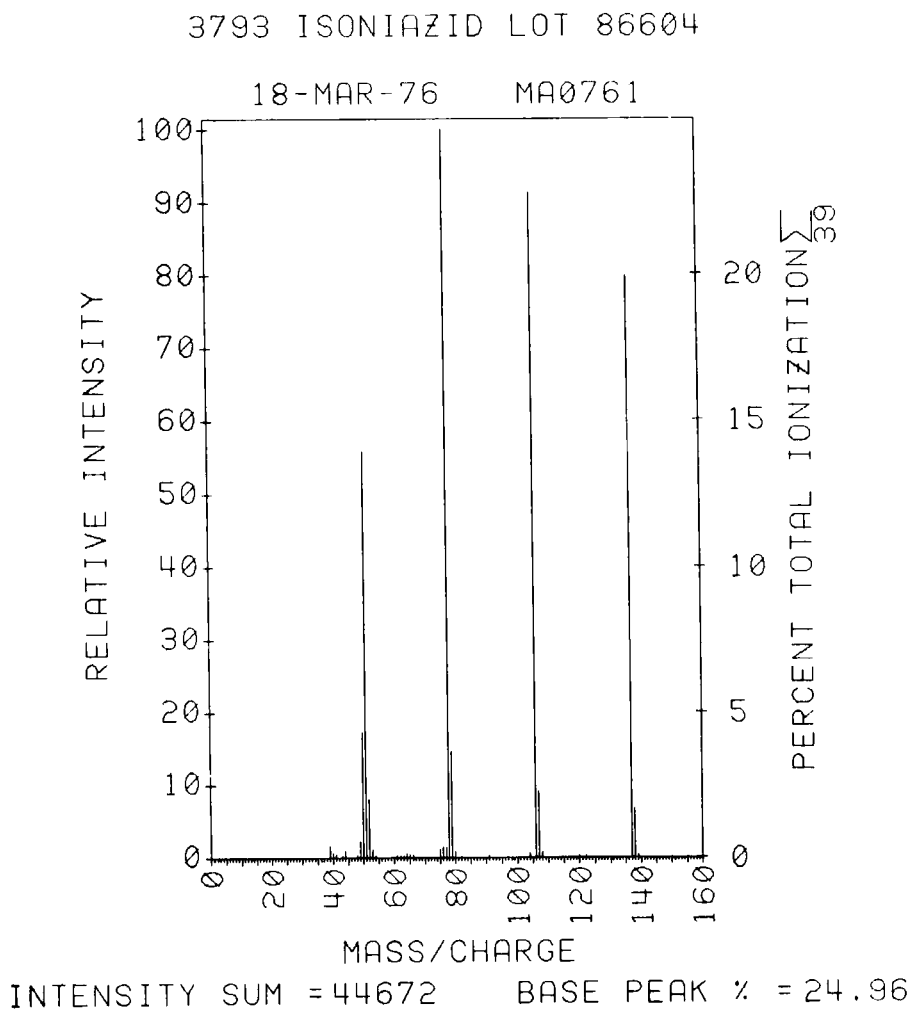


Figure 5:Low-resolution mass spectrum of isoniazid.

Squibb House Standard of isoniazid shows a sharp endotherm at 170°C using DuPont thermal analysis equipment.

The purity of this standard was determined to be 99.95 mole percent using a Perkin Elmer DSC-1B differential scanning colorimeter[27].

2.23 T.G.A.

Thermogravimetry can be used to determine moisture or residual solvents in isoniazid. When the Squibb House Standard was tested no loss on drying was recorded[27].

2.24 Electrical Moment

Lumbroso and Barassin[28] determined that the electrical moment of isoniazid was 2.92 μ.

2.25 Electrical Conductivity

The electrical conductivity of a compressed tablet of isoniazid was determined at temperatures between 50 and 150°C[29].

2.26 Crystal Characteristics

Bhat and co-workers[30] have reported that isoniazid crystals are orthorhombic, space group $P2_12_12_1$, with a, 14.915 (15) b, 11.400 (10) c, 3.835 (5)Å, d (measured) = 1.417 (7) d (calculated) = 1.395 and Z = 4.

2.27 X-Ray Diffraction

The powder x-ray diffraction curve for isoniazid is shown in Figure 6[31]. The relative intensities for the various peaks are given below:

Interplanar Distances d(ANGSTROMS)	Relative Intensities
8.84	0.098
7.30	0.408
6.10	0.398
5.64	0.451
5.25	1.000
4.49	0.502
3.69	0.296
3.51	0.398
3.42	0.102
3.36	0.068
3.27	0.197
3.10	0.235
3.04	0.060
3.01	0.058
2.80	0.170
2.63	0.076
2.47	0.168
2.42	0.115
2.33	0.187

2.3 Solubility

2.31 Water Solubility[32]

Fourteen grams of isoniazid are soluble in 100 ml of water at 25°C. Twenty-six grams are soluble in 100 ml of water at 40°C.

2.32 Solubility in Solvents[32,33]

Solvent	Solubility
ethanol (25°C)	2 g/100 ml
ethanol (boiling)	10 g/100 ml
chloroform	0.1 g/100 ml
ethyl ether	very slightly soluble
benzene	insoluble

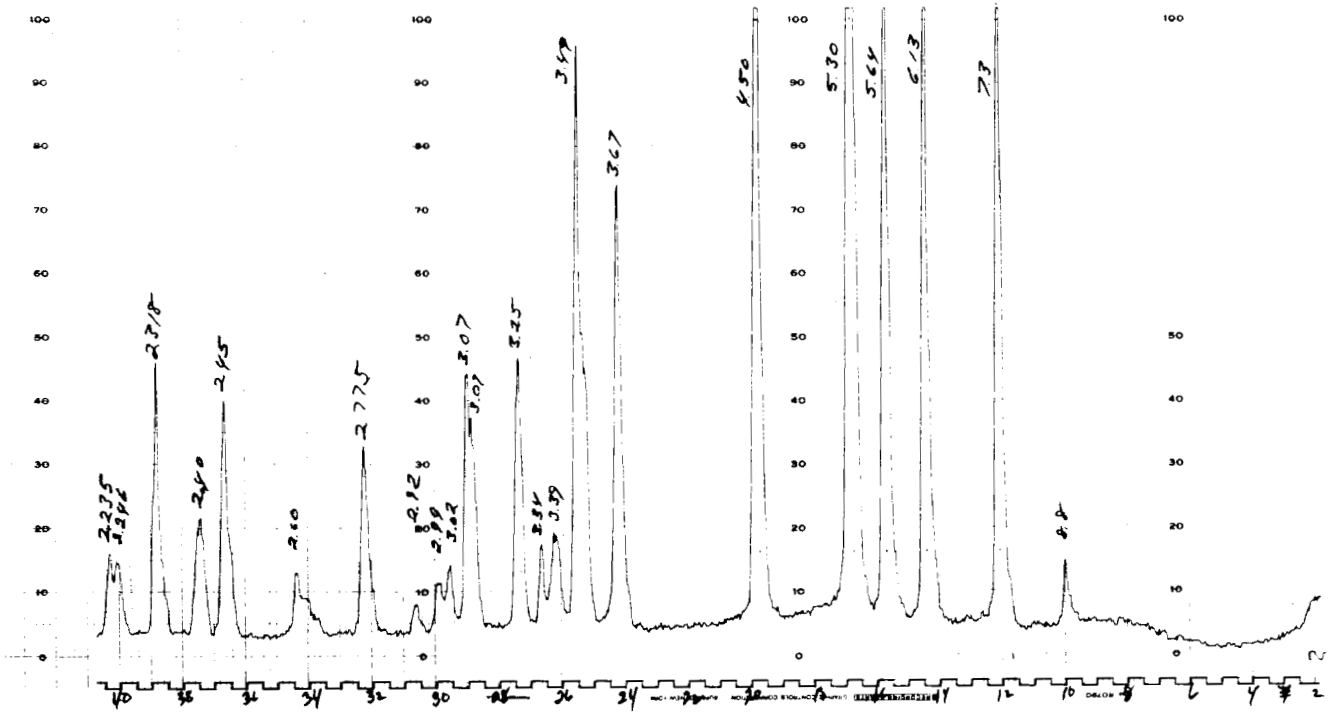


Figure 6:X-ray powder-diffraction pattern of isoniazid.

2.4 Physical Properties of Solution

2.41 pH

The pH of a solution (1 in 10) should be between 6.0 and 7.5[23].

2.42 Dissociation Constant

There is a discrepancy in the literature on the dissociation constants of isoniazid. This is in part due to the different methods of measurement employed.

Fallab[34] determined the basic dissociation constant as 3×10^{-11} measured conductometrically. Canić and Djordjević[35] established that the 1st basic constant should be ascribed to the pyridine nitrogen and the 2nd to the hydrazine group. This is contrary to previous work by Cingolani and Gaudiano[36].

Nagano and co-workers[37] determined the dissociation constants potentiometrically as $pK_1 = 2.13$, $pK_2 = 3.81$, $pK_3 = 11.03$.

Salvesen and Glendrange[38] determined the dissociation constants in 1.0M sodium chloride solution as $K_1 = 9.80 \times 10^{-3}$ and $K_2 = 1.42 \times 10^{-4}$.

Zommer and Szuszkiewicz[11] have established $pK_1 = 10.75$ and $pK_2 = 11.15$ and protonation constants of 3.57 for the pyridine N and 1.75 for the hydrazide N.

Rekker and Nauta[65] found that solutions of isoniazid became yellow at pH 10 and 2.7. The color is reversible on changing the pH. They explained this behavior on the basis of the existance of two positive ions, a monovalent yellow positive ion and a divalent colorless positive ion. The pK values are $pK' = 2.00$, $pK'' = 3.6$ and $pK''' = 10.8$.

2.43 Photolysis Constant

Salvesen and Eikill[39] established the photolysis constants for isoniazid at 20°C and 370 nm in M NaCl solution as $k_1 = 1.00 \times 10^{-2}$ and $k_2 = 1.45 \times 10^{-4}$.

2.44 Oxidation Potential

The oxidation potentials for isoniazid at various pH values were determined by Vulterin[40].

Solution in	Ef
1N HCl	0.78
0.025M $Na_2B_4O_7$	0.25
3N NaOH	-0.22

3. Metal Complexes

Isoniazid forms metal complexes with many divalent ions. These complexes have been used in the determination of isoniazid (see Sections 6.22, 6.25 and 6.29).

Tamura and Nagano[41] have determined the consecutive formation constants for the complex formed between isoniazid and Cd(II). The experiments were carried out at pH 7.2 (adjusted with NaOH) in M $NaNO_3$ using 0.001M $Cd(NO_3)_2$ at 25°C. The determination was made polarographically. The values determined were $k_1 = 35$, $k_2 = 0.57$, $k_3=52.5$. At high concentrations of isoniazid yellow crystals of Cd $(INH)_2$ $(NO_3)_2 \cdot H_2O$ precipated from solution indicating that contrary to the polarographic data that the 2:1 complex is more stable than the 3:1 complex. By pH titration the stepwise formation constants were $k_1 = 12.2$, $k_2 = 12.6$, and $k_3 = 3.4$.

The same authors[42] studied the formation constants of isoniazid and Cu(II), Zn, Ni(II), Co(II) and Mn(II).

The complexes of copper and isoniazid have been extensively studied by Ishidate[43].

4. History, Synthesis and Manufacturing

Isoniazid was first prepared by Meyer and Mally[500] in 1912 by heating a mixture of isonicotinic acid and hydrazine above 300°C. The activity of the compound against Mycobacterium sp. was first recognized by Chorine[501] and by Huant[502] in 1945. The drug was reported as a useful tuberculostatic agent by Farbenfabriken-Bayer, A.G., Hoffmann-LaRoche, Inc. and E. R. Squibb & Sons,Inc.

in 1952[503].

The basic method of manufacture of isoniazid is the condensation of hydrazine with a γ-substituted pyridine.

Hydrazine can be directly condensed with isonicotinic acid. The water formed in the reaction is usually removed by azeotropic distillation[44,45,46,47].

Esters of isonicotinyl acid can be hydrolyzed and the resulting acid condensed with hydrazine. Ammonia is usually employed for the hydrolysis[48].

γ-Picoline can be oxidized in 70% sulfuric acid with manganous dioxide to form isonicotinic acid. The corresponding acid chloride is made with thionyl chloride. The acid chloride is then reacted with hydrazine in anhydrous benzene to yield isoniazid[49]. In a modification of this procedure the acid chloride is reacted with ethanol to form the ethyl ester which is then reacted with hydrazine in ethanol to form isoniazid[50].

In a similar manner one can oxidize 2,4 dimethylpyridine with selenium and sulfuric acid.

The mixture is neutralized with ammonia. A mixture of isonicotinic acid, isonicotinamide and isonicotinic hydrazide is formed[51].

5. Stability

The stability of isoniazid has been studied extensively in solution and in various pharmaceutical preparations. Of particular interest is the reaction of the hydrazine group with naturally occuring aldehydes and ketones such as sugars or ketoacids and the complexation of isoniazid with metal ions.

Lewin and Hirsch[52] have shown that non-ionic chelating material can largely prevent the degradation of isoniazid when neutral and alkaline solutions are autoclaved. They noted that Cu(II) and Mn(II) ions accelerated the degradation of isoniazid in the presence of hydrogen peroxide.

Poole and Meyer[53] reported that isoniazid is unstable in human or rabbit plasma while it is

stable for several weeks in buffered aqueous solutions at pH values below 8. The instability in plasma is quite marked even at refrigerator temperatures.

Kakemi and co-workers[54] have studied the degradation of isoniazid in aqueous solution under anaerobic conditions. Alkaline hydrolysis under aerobic conditions yields a mixture of isonicotinic acid, isonicotinamide and 1,2 diisonicotinoyl hydrazine plus small amounts of unidentified products. Under anaerobic conditions isonicotinic acid and 1,2 diisonicotinoyl hydrazine were the principal products. When EDTA was added to the reaction mixture only isonicotinic acid was formed. First order kinetics were followed.

Inoue[55] found that at pH 3.1 under anaerobic conditions isoniazid hydrolyzes to form isonicotinic acid. Pseudo first order kinetics are followed. At lower pH values the effect of buffer type can be seen. Activation energies were calculated for the hydrolysis by different ionic species.

Horioka and co-workers[56] found that losses of isoniazid were encountered when the drug was blended with various antiacid preparations. The effect of temperature, humidity and pH on the stability was determined.

Hald[57] found that isoniazid underwent slow oxidation in aqueous solution, but in the presence of sucrose the isoniazid reacted with the aldohexoses formed on inversion. The reaction with sucrose could be inhibited by the addition of 0.3% sodium citrate.

Pawelczyk and co-workers[58] found that as long as conditions were kept anaerobic that the decomposition of isoniazid in the pH range 3 to 7 followed first order kinetics. They reported that a 1% solution of the drug was 37 times more stable at pH 6 than at pH 3. The effect of different buffer species on the rate of the reaction was noted.

Wu and co-workers[59] investigated the browning reaction between lactose and isoniazid in the

solid state with diffuse reflectance spectrophotometry. Thin-layer chromatography was used to demonstrate the presence of isonicotinoyl hydrazones of lactose and hydroxymethylfurfural.

Inoue[60] has established the effect of the presence of copper (II) ions on the rate of oxidation of isoniazid in solution. The reaction products were isonicotinic acid, isonicotinamide, 1,2-diisonicotinoylhydrazine, isonicotinecarboxaldehyde and isonicotinoyl hydrazone. The copper chelates of isoniazid are degraded by a first order reaction and the rate is determined by the ratio of the concentration of chelated species present.

Inoue and Ono[61] have established the kinetics of the degradation of isoniazid in the presence of Managenese (II). Shchukin[62] has studied the reaction of copper (II) with isoniazid.

Kakemi and co-workers[63] studied the stability of the sodium methanesulfonate salt of isoniazid from pH 3 to 9.

Rao and co-workers[64] have demonstrated that isoniazid in syrup formulations undergoes hydrazone formation with the free glucose that is present. Absorption of this hydrazone is reported to be impaired. The authors suggest the use of sorbitol as a replacement for sucrose.

6. Analytical Chemistry

6.1 Identity Tests

A large number of identity tests have been established for isoniazid. Most of these are colorimetric and are reported below in tabular form.

Reagent	Color	Reference
p-Dimethylaminobenzaldehyde	intense yellow	66,67,68,74,85,86
Alkaline $Na_2Fe(CN)_5NO$	intense orange	69,74
$K_4[Fe(CN)_6]$ + light	pink	70
Dinitrochlorobenzene	purple	71,74,86
o-Dinitrobenzene	violet	72
Reduction with Zn/HCl and phenylhydrazine	yellow	73
1,2 Naphthoquinone-4-Sulfonic acid + NaOH	bright red	74,75,76
1,2,4-Aminonaphtholsulfonic acid	orange to red	77
$SbCl_3$, $SbCl_5$ or $AsCl_3$	--	78
Epichlorohydrin	red	79
Dimethylglyoxime	red	80
Ethylenic dicarboxylic acids (fumaric, maleic acids, etc.)	yellow	81
Naphthoquinone-$HgCl_2$	--	82
3,5-Dinitrosalicylic acid	brown red	83
Ninhydrin	red orange	84,85
BrCN and NaOH	green-blue fluor.	85
Benzyl chloride NaOH	blue fluorescence	85
Dragendorff's Reagent	red	86,94

In addition to these color reactions a number of colored precipitates can be formed on the addition of metal salts or acids to isoniazid.

Reagent	Color of Precipitate	Reference
$AgNO_3$	White	74,94
SeO_2	Red	78,87,88
$HgCl_2$	White	74
$CuSO_4$	Blue	86
Hg_2Cl_2	White	86
KI	Amorphous mass	86,93,95
AuI	Dark crystals	86
Lead acetate + KI	Yellow acicular crystals	86
KBr	Effervescence followed by black and colorless crystals	86
$P_2O_5 \cdot I_2WO_3$	Precipitate	86
Picrolonic acid	Green-yellow	86,93
Tannic acid	ppt	86
Vitali's reagent	Yellow mass	86
Mecke's reagent	Rose-sienna	86
Fröhde's reagent	Blue	86
Mandelin's reagent	Red	86
Alloxan	White ppt	89
Disulfimides	--	90
Methyliodide	--	91
$K_2Cr_2O_7$	--	92
Phosphomolybdic acid	--	92
Picric Acid	Yellow needles	93,94
Reinecke's salt	--	94
Styphnic acid	--	94

Kay's reagent	--	94
Vaille's reagent	--	94
Na_2PtBr_6	--	94

Feigl and co-workers[96] have reported a spot test in which isoniazid is quaternized and pyrolyzed with $Na_2S_2O_3$ at 180°C. Acidified Fe $[Fe(CN)_6]$ is used for detection.

Popkov[97] and Amelink[98] have reported on microcrystalline techniques for the detection of isoniazid.

6.2 Methods of Analysis

6.21 General Reviews

Deltombe[99], Slouf[100], Robles and Unzueta[101], Yalcindag[102], Brandys[103] and Garcia and co-workers[104] have all published reviews on the qualitative and quantitative determination of isoniazid.

6.22 Colorimetric methods

A number of authors have formed hydrazones of isoniazid with various aldehydes and ketones and used the highly colored products to determine the drug. Of the various aldehydes used, p-dimethylaminobenzaldehyde appears to be the most popular[105,106,107,108,109,110,111]. Benzaldehyde[57,160,161] and vanillin[113,197,199] have also been used. The official method of the AOAC is the reaction of isoniazid with benzaldehyde in sodium bicarbonate solution. The absorbance of the hydrazone is measured at 302 nm. The absorbance at 375 nm (background) is substracted as a correction[169].

Sodium 1,2-naphthoquinone-4-sulfonate reacts with the hydrazide portion of isoniazid in alkaline solution to produce an orange-red color with a maximum at 480 nm[114,115]. 2-3-Dichloro-1,4-naphthoquinone reacts

with isoniazid to give a blue color in alkaline solution[116,117]. The reaction is useful with pharmaceutical preparations which also contain sodium aminosalicylate[118]. An assay utilizing 1, 4-naphthoquinone has also been reported[119].

Isoniazid reduces phosphomolybdate in alkaline solution to molybdenum blue[120,121]. In a similar reaction molybdophosphotungstate gives a blue color[122]. An assay utilizing molybdic acid in alkaline acetone solution has also been reported[123].

Isoniazid reacts with cyanogen chloride[124,125,126], chlororhodanamine[127,128] or cyanogen bromide[129] to form glutaconic dialdehyde which can then be condensed with barbituric or 2-thiobarbituric acids to yield colored polymethine dyes.

Isoniazid reduces ferricyanide to ferrocyanide. The amount of ferrocyanide can be determined by the addition of ferric ion to yield a blue color[130,131].

Sodium pentacyanoaminoferroate reacts with isoniazid to give a yellow chromogen[132].

Isoniazid reacts with 1-chloro-2, 4-dinitrobenzene in alkaline solution to give a purple color[133,74,134]. 1-Fluoro-2,4 dinitrobenzene also reacts in a similar manner[135].

Isoniazid forms colored complexes with many metals which can be used in analytical methods. Complexes can be formed with ammonium vanadate[136,137,230], ferric chloride and 2,2' bipyridine[138], copper[139] and Nickel(II) and ferric ion[140].

Reinecke's salt forms a water insoluble precipitate with isoniazid. This precipitate dissolves in acetone and the concentration of isoniazid can be determined colorimetrically[141,142].

The following compounds have also been used in colorimetric assays for isoniazid.

Reagent	Reference
chloropicrin	504
epichlorohydrin	143
ninhydrin	144
triphenyltetrazolium chloride	145
9-chloroacridine	146
dinitrobenzoic acid	147
p-aminosalicylate-HVO_3	148
1,2,4-aminonaphtholsulfonic acid	77
p-nitrophenyldiazonium fluoroborate	149
7-chloro-4 nitrobenzo-2-oxa-1,3-diazole	150
acid chrome dark blue	151
2-bromo-1-acetonaphthone	112
N-(4-pyridyl)pyridinium chloride	152
picryl chloride	174

6.23 Spectrophotometric Methods

A number of authors have utilized the strong absorbance of isoniazid in the ultraviolet as a means of determining the concentration of the drug. In many methods the authors take the spectrum both in alkaline and acid solution as an identity test[153,154,155,156,157,158,159,165,237]. Isoniazid can be determined in the presence of p-aminosalicylate by an ultraviolet assay[162,163,164].

6.24 Fluorimetric Methods

Although isoniazid does not have any native fluorescence several sensitive fluorometric assays have been reported for the drug. Isoniazid is coupled with 2-hydroxy-1-naphthaldehyde to give a yellow-green fluorescence. The compound has an excitation maximum at 495 nm and an

emission maximum at 534 nm[166,167].

In another method the pyridine ring is cleaved with cyanogen bromide to form glutacondialdehyde. A Schiff's base is then formed with 4-aminobenzoic acid which has an excitation maximum at 336 nm[168].

6.25 Titrimetric Methods

A large variety of titrimetric methods have been employed for the determination of isoniazid in bulk and in formulated products.

A series of reviews have been written on titrimetric methods[170,171,172,173,202].

The official methods of analysis in the U.S.P.[23], B.P.[174] and European Pharmacopoeia[3] are titrimetric methods.

In the U.S.P.[23] a nitrite titration is utilized. In the B.P.[174] the isoniazid is reacted with bromine and the excess bromine is titrated with thiosulfate after the liberation of iodine by the addition of potassium iodide. In the European Pharmacopoeia[3] a direct titration with bromate is utilized with the addition of ethoxychysoidine as an indicator.

The various titrimetric methods are summarized in the Table.

Reagent	Titrant	Indicator	Reference
$KBr, KBrO_3, KI$	thiosulfate	starch	175,177,179,182
KBr	$KBrO_3$	ethoxychrysoidine	176,106,180,184
Br_2	alkali	phenolphthalein	178
-	$KBrO_3$	methyl orange	181,186
-	$KBrO_3$	potentiometric	181,183,185,187
I_2	thiosulfate	starch	74,188,192,196
-	KIO_3	ethoxychrysoidine	189
KIO_3, KI	thiosulfate	starch	190,193,195
$HI, K_2Cr_2O_7, KI$	thiosulfate	starch	191
$HClO_4$,	thiosulfate	starch	106,194
ICl, KI	thiosulfate	starch	198
-	I_2	thermometric	200
non-aqueous	$HClO_4$	crystal violet or methyl violet	217,211,210,209,208, 207,206,204,205,216, 213,201,74,57,203, 214
-	$NaNO_2$	-	233
non-aqueous	$HClO_4$	Sb electrode	212,276
non-aqueous	$HClO_4$	glass electrode	215
-	$NaClO_4$	potentiometric	218
non-aqueous	NaOMe	thymol blue	202
Cd^{++}	Complexon III	eriochrome Black T	219,221
Cd^{++}	$CaCl_2$	methylthymol Blue	220
Cu^{++}, NH_4SCN	$AgNO_3$	-	222
Cu^{++}, NH_4SCN	EDTA	murexide	223,224

$HCl, K_3Fe(CN)_6$	$ZnSO_4$	dead stop	225
$AgNO_3$	Complexon III	Eriochrome I	226
$AgNO_3, K_2[Ni(CN)_4]$	$MgSO_4$	Eriochrome Black T	227,228
Complexon III			
-	$CO(OAc)_3$	Pt electrode	229
$CuSO_4$	Na Versenate		230
Cu^{++}, NH_4SCN, KI	thiosulfate	starch	231
$AgNO_3$	NH_4SCN		232
-	$KMnO_4$	diphenylamine	234,235,236
-	$K_2Cr_2O_7$		235
-	$NaNO_2$	starch iodide paper	237
KBr	$NaNO_2$		238
Reinecke's salt, $AgNO_3$	NH_4SCN	Fe alum	239
Reinecke's salt, $AgNO_3$	NH_4SCN	Ag electrode	240
H_2SeO_3, KI	thiosulfate	starch	241,242
$NH_4OH, AgNO_3$	NH_4SCN	Fe alum	243
$Zn-Cu$ Couple, H_2SO_4	NaOH	methyl red	244,245
Chloramine T, KI	thiosulfate	starch	246,248
-	chloramine T	indigo carmine	247,249
$NaVO_3$	$Fe(NH_4)_2(SO_4)_2$	N-phenylanthranilic acid	250,254
$Hg(II)EDTA$	Pb^{++}	methylthymol blue	251
OsO_4	$NaVO_3$	diphenylamine	252,253
H_3PO_4	NH_4VO_3	photometric	255

Nessler's reagent	sodium diethyl-dithiocarbamate	$CuSO_4$	256
I_2	hydrazine	starch	257
-	ammonium hexanitro-cerate(IV)	α-naphtho-flavone	258
$Ce(SO_4)_2$	Mohr's salt	Pt electrode	259
-	$Ce(NO_3)_4$	Pt electrode	260
$K_2Cr_2O_7$	Mohr's salt	diphenylamine	261
isopropenyl tri-chloroacetate	NaOH	bromphenol blue	262
KOH	$K_3Fe(CN)_6$	Pt electrode	263,264
$K_3Fe(CN)_6$,KOH,H_2SO_4,KI	thiosulfate	starch	265

6.26 Electrochemical Methods

A number of authors have detailed polarographic methods for isoniazid. The reduction apparently occurs in two steps (total of 4 electrons) but the steps are not sufficiently well separated to be utilized analytically, so that the single wave is used. The half wave potential becomes more negative at higher pH values, but the height did not change greatly over the pH range studied[266,153,267,268,269,270,271,272,273,274,275,277,278,279,280,281].

A.C. Polarography has been used by Sato[282] and Vallon and co-workers[283] in the assay of isoniazid. Okuda and co-workers[284] reacted isoniazid with 1,2-naphthoquinone-4-sulfonic acid and have then used polarography to measure the reaction product.

Several authors have reported coulometric methods for the analysis of isoniazid with electrochemically generated chlorine[285,286] or bromine[286,287,288,289.290].

6.27 Gravimetric Methods

Relatively few gravimetric assays have been reported for isoniazid. This is probably because of the large number of colorimetric, titrimetric and electrochemical methods available which are faster and more convenient than the gravimetric methods.

Leal and Alves[234] have reported an assay using picric acid to form a water insoluble salt.

Akiyama and co-workers[291] precipitate isoniazide as the Cu(II) or Hg(II) salts. The salts are redissolved in hydrochloric acid and the metal is then reprecipitated as the sulfide which is determined gravimetrically.

The zinc[292] and cadmium[293] salts can be measured by direct gravimetry. The benzylidene derivative can be determined either gravimetrically or volumetrically[294]. Isoniazid can be quaternized and the salt can be then measured volumetrically or gravimetrically[295]. The phosphotungstate of isoniazid can be determined gravimetrically[164].

6.28 Microbiological and Enzymatic Methods

Several agar diffusion microbiological assays utilizing strains of Mycobacterium have been reported for isoniazid[296,297,298].

Isoniazid inhibits many enzyme systems and a number of these might be selected as the basis of enzymatic assays. Examples of enzyme systems which are inhibited are pea cotyledon amine oxidase, carrot root L-glutamic decarboxylase and wheat seedling transaminase[299]. The inhibition is reversed by the presence of keto acids.

6.29 Miscellaneous Methods

Oscillometric titrations have been used to determine isoniazid[300,301]. Isoniazid can be assayed gasometrically after oxidation with iodate[302] or ferricyanide[303,304].

Conductometric titrations with sodium hydroxide or hydrochloric acid have been

used to measure isoniazid content[305,306].

The copper chelate of isoniazid is soluble in methylisobutyl ketone. The copper content of the chelate is determined in the organic phase by atomic absorption spectrometry[307].

Isoniazid in pure solutions can be determined by refractometry[308].

6.3 Chromatographic Methods

6.31 Paper Chromatography

Numerous paper chromatographic systems have been used to separate isoniazid from intermediates used in the synthesis, degradation products and metabolic products. Since isoniazid absorbs strongly in the ultraviolet and gives a number of color reactions[309] there is no problem in detecting or quantitating the drug after the separation has been completed. A table of some paper chromatographic systems is given below:

Solvent System	Detection	Use	Ref.
Water saturated butanol	C^{14} labelled	Urine metabolites	310
Isoamyl alcohol-water-acetic acid(50:50:1.5)	CNBr,Microbiol.	Urine metabolites	311
Isopropanol-water(85:15)	--	Urine metabolites	312,313
Butanol-ammonia	--	Derivatives	314
1st Dimension sec.butanol-water (saturated)			
2nd Dimension isoamyl alcohol-acetone-acetic acid-water (56:24:6:14)	CNBr-o-phenyl-enediamine dimethyl-benzaldehyde	Urine quantitation	315

Butanol-10% NH_4OH(10:2) circular	Butanol sat.ammoniacal with silver nitrate	Impurities	316
Butanol-water(4:1) ascending	dimethylaminobenzaldehyde	Dosage forms	317
2,4-lutidine-isoamyl alcohol-water(5:100:9)	methanolic dinitro-chlorobenzene	Impurities	318
Butanol-HCl-pet.ether or Butanol-HCl-H_2O(paper sat. with KCl solution)	iodine-platinic iodide	other basic substances	319
(a)Butanol-ethanol-water (2:2:1) (b)Butanol-pyridine-water (16:4:3) (c)Ethanol-1.5N NH_4OH-water (17:1:2) (d)Phenol-isopropanol-water (16:1:5)	ultraviolet	metabolites	320
0.5 ammonium chloride	ultraviolet	metabolites	321
(a)Butanol saturated with water (b)Propanol-water(80:20)	--	metabolites in urine	322,323

(a) Isopropanol-25% NH_2OH(85:15) (b) Isopropanol-water(85:15) (c) Isopropanol-formic acid-water(80:10:10)	C^{14} and spray reagents	Metabolites	324
Pyridine-Water(65:35)	--	Metabolites	325
Isopropanol-NH_4OH-water(7:1:2)	$FeCl_3$ and	Metabolites	326
Butanol-acetic acid-water(5:1:4)	$K_3Fe(CN)_6$		
(a)Ethyl methyl ketone-acetone-formic acid water(40:2:1:6) (b)Ethyl methyl ketone-diethylamine-water(921:2:77) (c)Methyl isobutylketone-formic acid-water(ketone sat.with 4% formic acid) (d)Chloroform-methanol-formic acid-water($CHCl_3$ sat.with 1 part H_2O and 1 part 4% formic acid) (e)Benzene-ethylmethyl ketone-formic acid-water(9 parts benzene plus 1 part ketone sat.with 2% formic acid) (f)Benzene-formic acid-water (benzene sat. with 2% formic acid)			327

(a) Iso-propanol-water (17:3) (b) Butanol-acetic acid-water (4:1:5) (c) 1.4M potassium phosphate buffer pH 7.0	2,4,6 trinitro-benzene-sulfonic acid	328
Butanol-acetone-water (45:5:50)	chloranilic acid	329
Butanol-phosphoric acid-water (3:1:3)	--	330
(a) Butanol Sat. with water in atmosphere of NH_3 (b) 95% ethanol-M ammonium acetate (7:3) adjusted to pH 5	copper sulfate in ethanol then 0.1% benzidine in 50% aqueous ethanol	331

6.32 Thin-layer Chromatrogaphy

In recent years several authors have developed thin-layer chromatographic system for isoniazid. These are presented in tabular form.

System	Detection	Use	Ref
Chloroform-methanol (8:2) Chloroform-acetone-diethylamine (5:4:1) Cyclohexane-chloroform-diethyl-amine (4:5:1)	Folin-Ciocalteu or Phospho-molybdate	separation from other drugs	332
Butanol-phosphoric acid-water (3:1:3)	--	derivatives	330
Acetone-methanol-NH_4OH (50:50:1)	dimethylamino-benzaldehyde	identification	333
(a) Methanol (b) Chloroform (c) Ethanol	5:1 mixture 10% $CuSO_4$ and 10% NH_4OH	identity test	334

Isopropanol-acetone(6:4)	--	separation of hydrazine	335
Chloroform-methanol(6:4)	--	separation of isonicotinic acid	335
Chloroform-methanol(125:60)	UV iodine	hydrazone with lactose	59
(a) Ethyl acetate-cyclohexane-dioxane-methanol-water-NH_4OH(50:50:10:10:1.5:0.5) (b) same solvent but (50:50:10:10:0.5:1.5) (c) Ethyl acetate-cyclohexane-NH_4OH-methanol-water (70:15:2:8:0.5) (d) Ethyl acetate-cyclohexane-NH_4OH-methanol(56:40:0.4:0.8) (e) same but (70:15:5:10) (f) Ethyl acetate-cyclohexane-NH_4OH(50:40:0.1)	Ninhydrin or 0.5% H_2SO_4	separation from drugs of abuse	336
Methanol-NH_4OH-H_2O(100:1:4)	$KMnO_4$ bromothymol blue	other drugs	337

(a) Chloroform-methanol-13N ammonia (90:10:1) (b) Benzene-methanol-diethylamine (90:10:1) (c) Chloroform-hexanol-13N ammonia (90:10:0.2) (d) Chloroform-ethyl acetate 13N ammonia (50:50:1) (e) Chloroform-acetone-acetic acid (90:10:1) (f) Benzene-acetone-diethylamine (50:50:1) (g) Chloroform-acetone-acetic acid (50:50:1)	254 nm U.V. iron chloride-hexacyanoferrate, molybdophosphoric acid. Folin-Ciocalteu, potassium permanganate, ammoniacal silver nitrate, amminepentacyanoferrate, iodoplatinate, iodine, Dragendorff, cinnamaldehyde triphenyltetrazolium, dithiocarbamate or ammonium molybdate	338

Nishimoto and Toyoshima[339] found that isoniazid showed tailing on thin-layer chromatography due to trace metals in the silica gel. When the adsorbent was treated with EDTA the tailing was eliminated.

Wijnne and co-workers[340] found that isoniazid could be quantitated after thin-layer chromatography by coulometric titration. Schmidt[341] showed that isoniazid could be revealed on a thin-layer plate by exposure to iodine vapor. Kawale and co-workers[342] sprayed thin-layer plates with 1% mercurous nitrate to reveal isoniazid as black spots.

6.33 Ion exchange Chromatography

Tsuji and Sekiguchi[343] have shown that isoniazid is quantitatively adsorbed on Dowex 50 cation exchange resin in various metal forms. The strength of adsorption decreases in the following order: $Cu^{++} \geq Ni^{++} \geq Hg^{++} \geq H^{+} > Co^{++} > Cd^{++} \geq Zn^{++} > Fe^{++} > Pb^{++} > Mn^{++} > Al^{+++}$.

No adsorption occurs on resin in the Ba^{++}, Mg^{++}, Ca^{++} or Na^{+} forms.

Heller and co-workers[344] separated acetylisoniazid from isoniazid on a column of Dowex 1-X8 in the pyruvate form.

Kakemi et al,[345] developed a chromatographic method for the separation of isoniazid from some degradation products. Isoniazid is adsorbed on a weak cation exchanger such as Amberlite CG-50 in the hydrogen form. Isonicotinic acid is not adsorbed and is determined colorimetrically using cyanogen bromide. To determine isonicotinamide the sample solution is oxidized with alkaline ferricyanide and then passes through a column of strong anion exchanger such as Dowex 1-X8 in the chloride form. The amide is unchanged and is not adsorbed on the resin. Another degradation product, 1,2-diisonicotinoyl hydrazide is determined by adjusting the sample to pH 8.9 with borate buffer and determining the absorbance at 329 nm.

Peters and co-workers[346,347] were able to separate and quantitate a large number of metabolites of isoniazid using Dowex AG-50-X4 resin in the hydrogen and ammonium forms. Selective color reactions were used to differentiate the groups of metabolites.

Fan and Wald[348] separated p-aminosalicylic acid from isoniazid using a Dowex 2-X8 column. Inoue and co-workers[349] used a system similar to that of Kakemi et al[345] to separate isoniazid from its degradation products.

Lewandowski and Sybirska[350] separated isoniazid from isonicotinic acid by paper chromatography using butanol saturated with water. The paper was connected with an ion exchange paper in the acid form. The spots were eluted with dioxane. The sharp zones on the ion exchange paper were visualized with picryl chloride.

Darawy and Mobarak[351] chromatographed several drugs on CM-82 carboxymethyl cellulose cation exchange paper using a water-

acetone-foramide(10:1:1) solvent system.

6.34 Other Chromatographic Methods

Barreto and Sabino[352] used a anhydrous sodium sulfate column eluted with chloroform-diethylamine(9:1) to concentrate metabolites of isoniazid from serum or urine.

Smolarek and Dlugosch[353] separated isoniazid and p-aminosalicylic acid by paper electrophoresis in barbital buffer, pH 8.5. Barreto[354] used two dimensional electrophoresis to separate the metabolites of isoniazid. Russell[355] also used paper electrophoresis to separate several acyl hydrazides. A pH 2.0 acetate buffer was used.

Isoniazid was separated from several antituberculosis drugs by gas chromatography[356,357]. A silanized chromosorb G coated with 6% QF1 was used. Gas chromatography was used to separate the products of oxidation of hydrazides with Fehling's solution[358].

6.4 Determination of Isoniazid and its Metabolites in Body Fluids and Tissues

The methods described in this section were specifically developed for the determination of isoniazid in body fluids and tissues. Many of the methods are similar to other general analytical methods described in Section 6.2 perhaps differing only in the extraction procedure.

6.41 General Reviews

Terze and Dadiotou[359] studied a number of color reactions to determine their application to blood level assays. Ginoulhiac[360] also made a literature review of blood level methods. A critical review of methods for isoniazid determination has been written[364].

6.42 Colorimetric Methods

Colorimetric methods are most popular for the determination of isoniazid in biological samples. The methods are listed in tabular form.

Reagent	Pretreatment of sample	Type of specimen	Ref.
Dimethylaminobenzaldehyde	acid hydrolysis	serum & urine	361,370, 375
Dimethylaminobenzaldehyde	none	urine	362,363
Dimethylaminobenzaldehyde	extraction into isoamyl alcohol-ether from alkaline solution	plasma and urine	365,366, 367,368, 369.
Dimethylaminobenzaldehyde	deproteinization with $HClO_4$	serum	371
Dimethylaminobenzaldehyde	deproteinization with trichloroacetic acid	serum and tissues	372
Vanillin	none	serum	373,376, 377
Vanillin	deproteinization with trichloroacetic acid	serum	374,432, 433,434
Vanillin	extraction with organic solvent	serum	375
Vanillin	extraction with propanol	milk	378
Cinnamaldehyde	deproteinization with trichloroacetic acid	serum	379,380, 429,430
Cinnamaldehyde	extraction with butanol-chloroform	serum	381

o-Nitrobenzaldehyde	deproteinization with trichloroacetic acid	serum	382
Salicylaldehyde-$FeCl_3$	extraction into isoamyl alcohol-ether from alkaline solution	serum	383
Salicylaldehyde	none	biological fluids	384
Salicylaldehyde	extraction with acetone	cadavers	385
Glutaconic aldehyde	deproteinization with trichloroacetic acid	plasma	386
β-diketone	none	biological materials	387
Catechol	deproteinization with trichloroacetic acid	citrated blood	388
Catechol	automated method	serum	389
H_2O_2-CNBr	deproteinized serum	serum & urine	390
CNBr	deproteinized trichloroacetic acid	biological fluids	391,404, 411,412
Alkaline hydrolysis-CNBr	deproteinized trichloroacetic acid	urine	392
NH_4VO_3-H_2SO_4	acid hydrolysis	urine	393,394,395, 396,397,398, 399,370,435
KCN, Chloramine T-barbituric acid	--	plasma, urine tissues, serum	400,401

1-amino-2-naphthol-4-sulfonic acid	--	urine biol. fluids	402
Naphthoquinone-4-sulfonic acid	--	urine	403,404,405, 406,407
Naphthoquinone-4-sulfonic acid	deproteinization $Zn(OH)_2$	urine	408
2,4,6-trinitrobenzene-sulfonic acid	extraction methyl isobutyl ketone	whole blood	409,410
Dinitrochlorobenzene	--	urine	411
Dinitrochlorobenzene	deproteinized serum	serum	412,413
$K_3Fe(CN)_6$	--	serum	414
Sodium pentacyanoamino-ferroate	deproteinized tissue	tissue, urine	415,416,417
$K_3Fe(CN)_6$	deproteinized with sodium tungstate	spinal fluid	418
Nitropentacyano-ferroate	deproteinized with phosphoric acid	serum	419
Naphthoquinone	--	spinal fluid urine	420
Naphthoqinone	--	blood	421
H_2O_2,CNBr, aniline	trichloracetic acid	blood urine	422
Picryl chloride	tungstic acid extraction BuOH,Et_2O	plasma urine spinal fluid	423,424
$KMnO_4$,BrCN,NH_3	Protein-free filtrate	plasma	425

4-pyridylpyridinium dichloride,NaOH,HCl	trichloracetic acid filtrate	plasma	426
$KBrO_3$+ methyl orange	acid tungstate	blood	427
Zn powder + heat	--	urine	428

6.43 Turbidimetric Method

Isoniazid reduces K_2HgI_4 to form HgI which is insoluble. The resulting turbidimetry can be measured to determine the amount of isoniazid present. Wagner and co-workers[431] have applied this method to blood following deproteinization with barium hydroxide and zinc sulfate.

6.44 Fluorimetric Methods

A number of fluorimetric methods for isoniazid have been reported. Hedrick and co-workers[436] absorbed a protein free filtrate of serum on pH 6.5 Amberlite XE-64 ion exchange resin. The isoniazid was eluted with dilute acid and then reacted with hydrogen peroxide in pH 8.7 buffer. The oxidation product fluoresces at 415 nm when activated by ultraviolet light at 320 nm. As little as 0.05 γ/ml of serum can be determined.

Scott and Wright[437] reacted salicylaldehyde with isoniazid and reduced the resulting hydrazone. The resulting compound was highly fluorescent. Reiss, Morse and Putsch[438] assayed isoniazid fluorimetrically after absorption and elution from ion exchange resin and treatment with alkaline cyanogen bromide. Wilson, Lever and Small[439] utilized the fluorescence of the zinc chelate of the hydrazone of isoniazid with pentane-2,4-dione in an assay in serum.

Ellard, Gammon and Wallace[440] have developed specific fluorimetric assays for isoniazid, acetylisoniazid, mono-and diacetylhydrazine, isonicotinic acid and isonicotinylglycine in serum and urine. Boxenbaum and Riegelman[441] have also developed assays for isoniazid and its metabolites in whole blood.

Miceli, Olson and Weber[442] have established a micro method for

the fluorimetric determination of isoniazid in serum. As little as 25 µl of serum can be used in the assay.

Peters, Morse and Schmidt[443] and O'Barr, Keith and Blair[444] have compared fluorimetric and microbiological assays for isoniazid in serum.

6.45 Electrochemical Methods

Lauermann and Otto[445] hydrolyzed isonicotinic acid hydrazide and its metabolites to isonicotinic acid with alkali. The hydrolysis product was determined polarographically. The authors found that the results obtained by this method in the analysis of cadaveric fractions was comparable to those obtained when the method of Nielsch and Giefer[401] was used. The polarographic method was less time consuming.

Kane [446] determined isoniazid in biological fluids without prior separation.

6.46 Gasometric Methods

The hydrazine group in isoniazid can be readily decomposed into nitrogen gas. Several authors have utilized this relatively selective finish for blood and urine level assays.

Strickland and Hentel[447] reacted isoniazid with sodium iodate in alkaline solution. The assay is not effected by the presence of p-aminosalicylic acid which is often given in conjunction with isoniazid. Harting and Gerzanits [448] used alkaline ferricyanide to liberate the nitrogen gas.

Ito and co-workers[449,450] were able to selectively use copper, iron and chromium azometry to determine isoniazid and its various metabolites in urine.

6.47 Miscellaneous Chemical Assays

Verrotti and Bardelli[451] determined isonizid in cerebrospinal fluid by iodometric titration.

Schwenk and co-workers[452] employed a radioimmunoassay for the determination of isoniazid in biological fluids.

6.48 Microbiological Assays

Although isoniazid is readily measured in biological fluids and tissues by chemical assays, as with many antibacterial substances a number of microbiological assays for this substance have been proposed.

Microorganism	Type of assay	Sensitivity	Ref.
Mycobacterium phlei	agar diffusion	2.5-30 γ/ml	453
Koch bacilli	turbidimetric	--	454
tubercle bacteria	cord formation	--	455
bacilli	vertical diffusion	--	456
Mycobacterium tuberculosis HV37	vertical diffusion	--	457
Mycobacterium tuberculosis	agar diffusion	--	458
Mycobacterium tuberculosis	vertical diffusion	> 0.49 γ/ml	459
$H_{37}Rv$ and $H_{37}Ra$ BGG	assay of isoniazid in milk-agar diffusion		378
--	vertical diffusion		460
Mycobacterium tuberculosis	vertical diffusion		461
--	vertical diffusion tube dilution		462
--	vertical diffusion for urine		463,464

Bartmann and Freise[465] studied the tissue binding of isoniazid with the microbiological assay. They found 40% binding with human tissue while mice and guinea pig tissue gave 80% binding. Incubating the tissue at an elevated temperature did not raise the recovery.

Nishi[466], Poole and Meyer[467], Tansini and co-workers[376], Peters and co-workers[433] and O'Barr et al[444], all compared various chemical assays and microbiological assays. All workers conclude that the two methods gave comparable results.

6.49 Chromatographic Assays

The metabolism of isoniazid is complex and many workers have selected chromatographic assays to measure the drug in tissue and biological fluids. These methods provide the specificity that are not given by many chemical methods.

A large number of chromatographic systems are given in section 6.3. Many of these methods could probably be used to measure isoniazid in tissues and biological fluids. The methods given in this section have been developed just for this purpose.

Makino and co-workers[468] followed the metabolism of isoniazid in liver and in urine by paper chromatography (water saturated butanol, 1% ammonia-isopropanol(3:20), butanol saturated with 0.02M phosphate buffer pH 7.4, 1% ammonia saturated butanol and butanol-acetic acid-water (4:1:5)).

Leuschner[469] used sec-butanol saturated with water and isoamyl alcohol as developing solvents.

Iwainsky[313] separated the hydrazones of isoniazid and pyruvic and α-ketoglutaric acid from isoniazid with paper chromatography.

Sezaki[470] separated isoniazid from pyrazinamide in urine by means of Amberlite IRA-400. Belles and Littleman[471] used Dowex 50-X8 to separate isoniazid from acetylisoniazid. Abiko

and co-workers[472] use Dowex 1-X10 to separate these as well as the hydrazone of glucuronic acid.

Peters, Miller and Brown[346] utilized ion exclusion chromatography to separate metabolites of isoniazid into ionized, slightly ionized and unionized groups of compounds. The individual metabolites were measured with specific colorimetric assays.

Okudaira and co-workers[473] used Dowex 1 and Dowex 50 columns in tandem to separate the various metabolites of isoniazid.

Paper chromatographic systems have been used to isolate the various metabolites of isoniazid[474,475,352].

Barreto and Sabino[476] have described a two dimension separation of isoniazid metabolites using paper chromatography and paper electrophoresis. The same authors[352] have also used a sodium sulfate column developed with chloroform-diethylamine(90:10) to separate the metabolites of isoniazid.

Fartushnyi and Sukhin[477] have used TLC to determine isoniazid and other drugs in cadavers.

Cattaneo, Fantoli and Ferrari[478] claim that their chromatographic studies indicate that the tumorogenic effect of isoniazid in mouse lung is due to the large amount of isonicotinic acid produced in that organ.

Hughes[479] separated acetylisoniazid from isoniazid by counter-current distribution (butanol-ethylene dichloride- 9:1 - 2M phosphate buffer pH 5.1).

Ozawa and Kiyomoto[480] isolated three conjugated metabolites of isoniazid by paper chromatography. Cuthbertson *et al*[481] used paper chromatography to determine isonicotinoylglycine. They used the following systems:

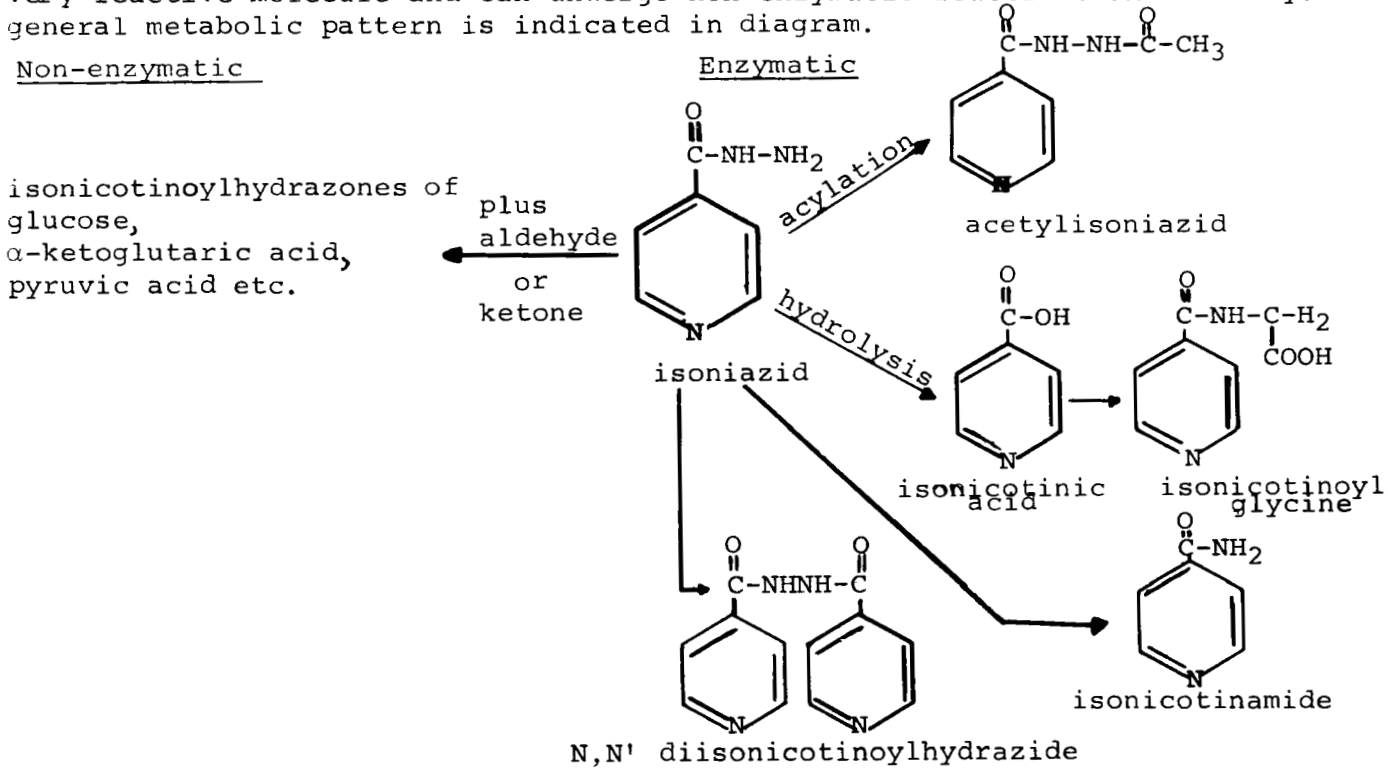
Water saturated butanol
Methylethylketone:acetic acid:water(49:1:50)
Propanol:water(4:1)

Zamboni and Defranceschi[482] used a isopropanol:water(85:15) system to separate the hydrazones of pyruvic and α-ketoglutaric acid from

isoniazid.

7. Drug Metabolism

The drug metabolism of isoniazid is unusually complicated in that it is a very reactive molecule and can undergo non-enzymatic reactions in the body. A general metabolic pattern is indicated in diagram.



The major metabolite of isoniazid is N-acetylisoniazid. The rate of acetylation is genetically controlled[483,484 485]. It has been established that the slow acetylation is a autosomal recessive trait. The acylation occurs by N-acetyl transferase. Six hours after the oral administration of 4 mg/Kg of isoniazid fast acetylators have plasma concentrations of 0.2 μg/ml or less while slow acetylators have plasma levels higher that 0.4 μg/ml[486].

In a metabolic scheme,such as the one indicated earlier,relative amounts of the various metabolites found in the urine will differ for each individual and will depend on genetic factors, previous drug history (enzyme induction) and general nutrition (availability of ketoacids).

Reviews on the drug metabolism of isoniazid have been prepared by a number of authors[487,488, 489,490,491,492,493,494,495,496,497].

Toth and Shimizu have reported that the continuous administration of N-acetylisoniazid in rats has markedly increased the incidence of lung tumors in this species. Since the N-acetyl derivative is a major metabolite in man this poses some questions on the long term administration of the compound[497].

8. Biopharmaceutics

Kakemi and co-workers[498] determined the rate of absorption of derivatives of isoniazid in the stomach and intestine. The authors report a rough correlation between degree of absorption and lipid-water partition coefficient.

9. References

1. Griffiths, M.C.; Dickerman, M.J. and Miller, L.C.; USAN and the USP Dictionary of Drug Names page 152(1975).
2. Anon; Chemical Abstracts Service, Registry Handbook-Number Section (1974).
3. Anon; European Pharmacopoeia Volume I,page 310 (1969).
4. Prevoršek, D.;Bull.soc.chim.France,795-801 (1958);C.A. 53 21160b(1959).
5. Nagano, K.; Kinoshita, H. and Hirakawa,A.; Chem.Pharm.Bull. 12,1198-1206(1964);C.A. 62 2759a (1965).
6. Toeplitz, B.; E. R. Squibb and Sons; Personal Communication.
7. Lapp,C.; Bull.soc.pharm.Nancy 34, 14-17(1957); C.A. 53 21145d (1959).
8. Grebennik, L.I.;Farmakol. i Toksikol. 24, 233-7(1961);C.A. 55 26263a(1961).
9. Pinyazhko,R.M.;Farmatsevt.Zh. 20, 18-22(1965); C.A. 64 7969e (1966).
10. Kracmar, J.; Kracmarova, J. and Zyka, J.; Pharmazie 23, 567-73 (1968);C.A.70 31719r (1969).
11. Zommer, S. and Szuszkiewicz, J.;Chem.Anal. 14, 1075-83(1969); C.A. 72 89616n(1970).
12. Celechovsky, J.; Greksakova, O. and Polasek,E.; Acta Fac.Pharm. 17,51-5(1969);C.A.73 112880a (1970).
13. Zommer, S.; Acta Pol.Pharm. 29,533-5(1972); C.A. 78 158502k (1973).
14. Dunham, J.; E. R. Squibb and Sons;Personal Communication.
15. Caen, G.; J. Chim.phys. 51,60-3(1954);C.A. 48 10438e (1954).
16. Titov, E.V.;Kapkan, L.M.;Rybachenko,V.I. and Korzhenevskaya, N.G.; Reakts Sposobnost Org. Soedin 5, 673-81 (1968); C.A. 70 67448r(1969).
17. Titov, E.V. and Kapkan, L.M.; Dokl.akad.Nauk SSSR 184,1342-5(1969);C.A. 70 114411k (1969).

18. Titov, E.V.;Kapkan, L.M. and Chervinskii,A.Yu.; Teor.Eksp.Khim. 8, 202-9(1972);C.A.77 74367m (1973).
19. Hillerbrand M.;Lohmann, W.; Penka,V. and Ehling, U.; Z.Naturforsch, Teil C 30C,33-6 (1975);C.A. 82 169663x (1975).
20. Puar, M.S.; E. R. Squibb and Sons; Personal Communication.
21. Gillis, R.G.; Org.Mass Spectrom. 5,1107-11 (1971); C.A. 75 150910j (1971).
22. Funke, P.T.; E. R. Squibb and Sons;Personal Communications.
23. Anon; United States Pharmacopoeia XIX page 272 (1975).
24. Mattu, F. and Pirisi, R.; Chimica 8, 188-9 (1953); C.A. 48 1897b (1954).
25. Mattu, F. and Pirisi, R.; Rend.seminar.fac.sci. univ.Cagliari 25,96-117(1955);C.A.50 7672h (1956).
26. Pirisi, R.; Rend.seminar.fac.sci.univ.Cagliari 25,88-95(1955);C.A. 50 7668c (1956).
27. Jacobson, H.and Valenti, V.; E. R. Squibb and Sons;Personal Communication.
28. Lumbroso, H. and Barassin,J.; Bull.Soc.Chim. France, 3190-7(1964);C.A. 62 10322e(1965).
29. Ziolkowski, J. and Guminiski, K.; Roczniki Chem. 38, 1807-13(1965);C.A. 63 9168b(1965).
30. Bhat, T.N.; Singh, T.P. and Vijayan, M.; Acta Crystallogr.,Sect.B B30, 2921-2(1974); C.A. 82 50126j (1975).
31. Jacobson, H. and Ochs, Q.; E. R. Squibb and Sons; Personal Communication.
32. Dèl Río-Estrada, C. and Dougherty, H.W.; Kirk-Othmer Encyclopedia of Chemical Technology 2nd Vol 21 Page 528-533 John Wiley & Sons,Inc. (1970).
33. Clarke, E.G.C.; Isolation and Identification of Drugs page 383, The Pharmaceutical Press London (1969).
34. Fallab, S.; Helv.Chim.Acta 36, 3-5(1953); C.A. 47 7296c (1953).
35. Canić,V.D. and Djordjević,R.Dj.;Beograd 21, 193-9(1956);C.A.52 16026f (1958).

36. Cingolani and Gaudiano; Rend.ist.super. sanità 17, 601 (1954).
37. Nagano, K.;Tsukahara, H.;Kinoshita, H. and Tamura, Z.; Chem.Pharm.Bull. II,797-805(1963); C.A.59 4867h (1963).
38. Salvesen, B. and Glendrange,J.H.; Medd.Norsk. Farm.Selsk 28 272-8(1966);C.A. 66 68866e(1967).
39. Salvesen, B. and Eikill, S.A.; Medd. Norsk. Farm.Selskap 27,135-44(1965);C.A.64 9507f (1966).
40. Vulterin, J.; Talanta 10 891-8(1963); C.A. 59 12142i (1963).
41. Tamura, Z. and Nagano, K.;Chem.Pharm.Bull. 11, 793-6(1963);C.A.59 14867f(1963).
42. Tamura, Z. and Nagano, K.; ibid 999-1013(1963); C.A. 59 14868a (1963).
43. Ishidate, M.; Anal.Chem.Proc.Intern.Symp., Birmingham Univ.,Birmingham,Engl. 178-82 (1962);C.A. 59 10967b(1963).
44. Thomassen, H.B.;Dan Pat. 87,228(July 7,1959); C.A. 54 7742 (1960).
45. Pavlov, L.N.; Gangrskii, P.A. and Polyachenko, V.M.;U.S.S.R. Pat 106,220 (July 25, 1957);C.A. 51 16563 (1957).
46. Pasedach, H. and Seefelder,M.;Ger.Pat 1.116,667 (Nov. 19, 1961);C.A.56 14247(1962).
47. Frehden, O. and Lazarescu, G.;Rev.Chim. 13 91-5(1961);C.A.57 11158i (1962).
48. Takizawa, S.; Jap.Pat. 7472(Nov.15,1954); C.A. 50 9447 (1956).
49. Magrane, D.; Span.Pat. 202,450(May 28,1952); C.A. 50 17323 (1956).
50. Lock, G.; Pharm.Ind. 14,366-8(1952);C.A.47 10531 (1953).
51. Ishikawa, M.;Japan.Pat. 2629(April 21,1955); C.A. 51 14832(1957).
52. Lewin,E. and Hirsch, J.G.;Am.Rev.Tuberc.71 732-42(1955);C.A.50 2918e(1956).
53. Poole,N.F. and Meyer,A.E.,;Proc.Soc.Exptl.Biol. Med. 104,560-2(1960);C.A.55 661b(1961).
54. Kakemi, K.; Sezaki,H. and Inoue,S.;Yakugaku Zasshi 86 163-8(1966);C.A.64 17362c(1966).

55. Inoue,S.; Yakugaku Zasshi 87,883(1967); C.A. 68 21441p (1968).
56. Horioka, M.;Tsuruoka, Michio and Shinmura,Y.; Kyushu Yakugakkai Kaiho 23 31-6(1969);C.A.72 93307e (1970).
57. Hald, J.G.; Dan.Tidsskr. Farm. 43,156-9(1969); C.A. 72 157196 (1970).
58. Pawelczyk, E; Herman, T. and Sukowski, R.; Diss Pharm. Pharmacol 21 481-9(1969);C.A.72 35729h (1970).
59. Wu, Wen-Hung;Chin,Ting-Fong and Lach, J.Pharm. Sci. 59 1234-42 (1970);C.A. 73 101974h(1970).
60. Inoue,S.;Yakugaku Zasshi 91 81-7(1971);C.A.74 111385b(1971).
61. Inoue,S. and Ono,Y.; ibid 91 88-94(1971); C.A.74 111386c(1971).
62. Shchukin, V.A.; Sb.Aspir.Rab.,Kazav.Univ., Estestr.Nauki.Giol.,Khim.;171-80(1972); C.A. 81 6776b(1974).
63. Kakemi, K.; Sezaki, H., Nudai, T.; Nishikiori, K.; Inoue, S. and Ono,Y.; Yakugaku Zasshi 91 95-100 (1971);C.A. 74 111387d(1971).
64. Rao,K.V.N.; Kailasam, S.; Menon, N.K. and Radhakrishna, S.; Bull WHO 45,625-32(1971); C.A. 76 117460k (1972).
65. Rekker, R.F. and Nauta, W. Th;Pharm Weekblad 99,1157-65(1964),C.A.63 435c (1965).
66. Feigl, F. and Mannheimer, W.A.; Microchemie ver.Mikrochim Acta 40,355-8(1953);C.A.47 7376b(1953).
67. Lucas,V.;Rev.brasil.farm. 33,471-3(1952); C.A. 47 9216e(1953).
68. Cooper, P.; Pharm.J. 177, 495-6(1956); C.A. 51 6945g (1957).
69. Laubie,H.; Bull.soc.pharm.Bordeaux 90,106-8 (1952); C.A.46 9019g (1952).
70. Murti, P.S.; Rao, G.;Bala, B. and Rao,P.V. Krishna; Anal.Chim.Acta 71,202-4(1974); C.A. 81 85757x(1974).
71. Garattini,S.; Atti soc.lombarda sci.med. biol. 8, 107-9(1953);C.A. 48 1895b (1954).

72. Yavors'kii,N.P.;Farmalsevi Zh. 17,9-12(1962); C.A. 58 13722d (1963).
73. Sánchez, J.A.; Rev.Asoc.bioquim.Argentina 17, 321-3(1952);C.A. 47 6822a (1953).
74. Scott, P.G.W.; J.Pharm. and Pharmacol. 4, 681-6(1952);C.A. 47 1334d(1953).
75. Yarvars'kii,N.P.;Farmatsevt.Zh. 20,29-33(1965); C.A. 64 7971a (1966).
76. Cattaneo, C.; Fantoli, V. and Belasio, L.; Ann.ist. "Carlo Forlanini" 20,59-67(1960); C.A. 55 12520d (1961).
77. Gomez, S.A.; Arch.bioquim.quim.y farm., Tucumán. 8, 69-95(1957);C.A.55 908c(1961).
78. Poggi, A.R.; Mattu, F.;Pirisi,R. and Milletti, M.; Chimica 8,238-41(1953);C.A.48 1880b (1954).
79. Waksmundzki, A. and Romanowski, H.; Ann.Univ. Mariae Curie-Sklodowska,Lublin-Polonia,Sect.AA, 8,71-6(1953);C.A. 51 8374b(1957).
80. Ohkuma, S. and Yasumasa, K.; J.Pharm.Soc. Japan 76,894-6 (1956); C.A. 51 953b (1957).
81. Neuzel, E. and LeDuc, Y.;Bull.Soc.Pharm. Bordeaux 100 159-79(1961); C.A. 56 8682a(1962).
82. D'Amico,E. and Russo,R.; Aggiorn.Pediat.17 227-32(1966); C.A. 65 9334e(1968).
83. Dospeux,P.F.; Chim.Analyt.53 251-3(1971); A.A. 22 1129 (1972).
84. Yavorskii,N.P.; Aptechn. Delo 14,48-50(1965); C.A. 63 5452h (1965).
85. Leuschner, F.; Naturwissenschaften 40,554 (1954); C.A. 48 8698b (1954).
86. Calabrug, J.A.G. and Fresneda, M.F.;Galenica Acta 6 221-37(1953) C.A. 48 7499b (1954).
87. Castel, P.; Orzalesi, H. and Dubois, A.; Trav.soc.pharm.Montpellier 12,73-4(1952); C.A. 48 10297e (1954).
88. Watanabe, T.; Eisei Shikenjo Hôkoku 74 111-12 (1956);C.A. 51 15339f (1957).
89. Neuzil,E.; Bull.soc.pharm.Bordeaux 91 122-30 (1953); C.A. 48 329a (1954).
90. Runge, F.;Engelbrecht, H.J. and Franke, H.; Pharmazie 12 8-13 (1957); C.A. 52 15830a(1958).

91. Mladenović, M.;Blagojević,Z. and Zivanov,C.; J.pharm.Belg. 13 559-63 (1958);C.A. 53 13504c (1959).
92. Shilov,Yu. M and Chichiro,V.E.;Aptechn.Delo 12,65-8(1963);C.A. 61 10534b (1964).
93. Il'yasov,Ya.Z.; Sudebno-Med.Ekspertiza,Min. Zdravookhr. SSSR 9,43-5(1965);C.A.67 29824r (1967).
94. Stebletsova,Zh.D.;Farm.Zh.(Kiev)24 31-4(1969); C.A. 70 99672a(1969).
95. Gul'ko,R.N.; Yavorskii,N.P.;Garasevich,S.N. and Pershina,G.I.; Farmatsiya(Moscow) 22,29-32 (1973); C.A. 80 19649s(1974).
96. Feigl,F., Anger, V. and Goldstein, D.; Helv. Chim.Acta 43,2139-42(1960); C.A. 55 13178i (1961).
97. Popkov,V.A.; Farmatsiya(Moscow) 16,77(1967); C.A. 66 98547u (1967).
98. Amelink,F.;Pharm.Weekblad 87,821-36(1952); C.A. 49 5773d (1955).
99. Deltombe,J.; J.pharm.Belg. 8 59-75(1953); C.A. 47 9559f (1953).
100. Slouf,A.; Ceskoslov.farm.2 168-71(1953); C.A. 49 2032e (1955).
101. Robles G. G. and Unzueta,L.; Actas y tabajos congr.peruano quim., 403-6(1953);C.A.51 4653a(1957).
102. Yalcindag O.N.; Kim.Sanayi 14,47-55(1966); C.A. 66 111301f(1967).
103. Brandys,J.;Farm.Pol.23 430-4(1967);C.A.69 12934v (1968).
104. Garcia,M.T.;Castello,J.M. and Colome,J.; Cienc.Ind.Farm. 5,311-15(1973);C.A. 82 160277s (1975).
105. Gemeinhardt, K. and Rangnick, G.F.; Arzneimittel-Forsch. 3,45-7(1953);C.A.47 5479d(1953).
106. Battistessa,M.J. and Pross, H.;Rev. farm 95, 113-16(1953);C.A.49 9227i (1955).
107. Kidani,I.; Nakashima,T.;Kochi,Y and Kasahara, S.; Bull.Natl. Hyg.Lab. 72, 95-7(1954); C.A. 49 6035c(1955).

108. Machek,G.;Scientia Pharm. 24, 11-17(1956); C.A. 50 9690h(1956).
109. Inamdar, M.C.; Padlia, S. and Shah, V.N.; Indian J. Pharm. 32,62-3(1970);C.A.73 38599x (1970).
110. Spinkova, V.; Pharm.Acta Helv 46,643-8 (1971);C.A. 76 37469e(1972).
111. Deodhar, R.D.; Shastri, M.R. and Ganatra, J.P.; Indian.J.Pharm. 32,99-101 (1970); C.A. 73 91302w (1970).
112. Hirose, S. and Uno, T.; Yakugaku Zasshi 81, 1623-5(1961);C.A. 56 10286h(1962).
113. Deeb,E.N.;Drug Standards 22, 194-9(1954); C.A. 49 2027d (1955).
114. Pratt, E.L.; Anal.Chem.25,814-16(1953);C.A.47 9391f(1953).
115. Nielsch,W. and Giefer,L.;Mikrochim.Acta,17-26 (1960);C.A.59 1097b 1973).
116. Akatsuka,M.;Yakugaku Zasshi 83,227-33(1963); C.A. 59 7475f (1963).
117. Abou-Ouf,A.A.; Taha, A.M. and Saidhom,M.B.; J. Pharm.Sci. 62 1700-2(1973);C.A.80 7009v (1974).
118. Elliston, S.C. and Hammond, M.D.; Analyst 90, 298-300(1965); C.A. 63 5454c(1965).
119. Pavlyuchenkova, L.P. and Veksler, M.A.; Farmatsiya (Moscow) 23,29-33(1974);C.A.81 54487e(1974).
120. Ozawa, H. and Kiyomoto,A.;J.Pharm.Soc.Japan 72,1059-60(1952);C.A.46 11047a(1952).
121. Akiyama,T.;Yabuuchi,T. and Shiono,K.; Kyoto Yakka Daigaku Gakuho 7, 48-56(1959); C.A.54 6031e(1960).
122. Bose,B.C. and Vijayvargiya,R.;Indian J.Pharm. 28,328-30(1966);C.A. 66 108307g(1967).
123. Colarusso,R.J.;Schmall,M.; Wollish,E.G. and Shafer, E.G.E.;Anal.Chem. 30,62-5(1958); C.A. 52 4931b(1958).
124. Nielsch,W. and Giefer, L.; Z. anal.Chem. 171, 401-10(1960);C.A. 54 8428f(1960).
125. Fried, R.;Mitt.Deut.Pharm.Ges. 32,157-9(1962); C.A. 58 2323b(1963).

126. Mesnard, P.;Devaux,G. and Fauquet, J.; Sci.Pharm.Proc 25th 2,77-86 (1965);C.A.69 109870j(1968).
127. Aliev,A.M. and Beisenbekov,A.S.; Azerb.Med. Zh. 48,85-8 (1971);C.A.77 39337e (1972).
128. Aliev,A.M. and Beisenbekov, A.S.; Farmatsiya (Moscow) 22,35-8(1973);C.A.79 129120z (1973).
129. Mesnard, P.; Arch.Pharm. 22,105-21(1966); C.A. 66 118877g(1967).
130. Jacobs, M.B.; J.Am.Pharm.Assoc. 42 346-8 (1953);C.A.47 9569g(1953).
131. Blanc,P.;Bertrand, P. and Liandier,L.; Lyon pharm.Spec.161-4(1956);C.A.53 22185c (1959).
132. Naito,T.; Shirai, H. and Oda N.;Bull. Nagoya City Univ. Pharm.School, 34-7 (1955); C.A. 50 17331f (1956).
133. Ballard, C.W. and Scott, P.G.W.;Chemistry and Industry, 715(1952); C.A. 47 823b(1953).
134. Schoog, M.; Münch. med.Wochschr.94,2135-7 (1952);C.A. 47 2645i (1953).
135. Sil,J.;Agrawal, K.C. and Dutta, B.N.;Sci. Cult. 28 472-4(1962);C.A. 61 6868b(1964).
136. Alessandro, A.;Giorn.med.militare 104,195-7 (1954);C.A. 48 13790d (1954).
137. Rao,P.V.; Rao,G.B.B. and Murti, P.S.; Microchim Acta, 979-84(1974);C.A. 82 64615e (1975).
138. Avanza, H.L.; Jornada Med. 9, 673-4(1955); C.A. 50 7003f (1956).
139. Zommer, S. and Lipiec, T.; Acta Polon.Pharm. 20,229-32(1963);C.A. 62 1517g (1965).
140. Munson, J.W. and Connors, K.A.;J.Pharm.Sci. 61 211-13 (1972); C.A.76 103829a(1972).
141. Danek, A.; Dissertationes Pharm.11,237-42 (1959);C.A. 54 13555h(1960).
142. Teodorescu,Gr. and Dinescu, A.;Bul.Inst. Politeh. Bucuresti 26,49-52(1964);C.A.63 14045e(1965).
143. Roth, H.J. and Schrimpf; Arch.Pharm. 293, 22-8(1960);C.A. 54 16286d(1960).

144. Tsuji, A. and Hojo, M.;Bunseki Kagaku 11, 1255-62 (1962);C.A. 58 8852h (1963).
145. Vasileva-Aleksandrova, P.; Aleksandrov, A. and Kovacheva, E.; Nauch.Tr. Vissh.Pedagog. Inst. Plovdiv, Mat.,Fiz.,Khim.,Biol. 6, 111-16(1968); C.A. 71 74096y(1969).
146. Stewart, J. T. and Settle, D.A.; J.Pharm.Sci. 64,1403-5(1975);C.A. 83 168545u(1975).
147. Akiyama, T. and Fujimoto, K.; Kyoto Yakka Daigaku Kakuho 14,32-6(1966);C.A. 68 34582w(1968).
148. Deltombe, J. and Stainier, R.; J.Pharm.Belg. 17,239-40 (1962); C.A. 61 5461a(1964).
149. Chekryshkina, L.A.; Farmatsiya(Moscow) 23 44-6 (1974); C.A. 81 126821j (1974).
150. Tan, H.S.I.; J.Pharm.Sci. 62 993-7(1973); C.A. 79 45897v (1973).
151. Kydymor, G.I. and Sidorova, E.F.; Nauch.Tr., Perm. Farm. Inst. 4 64-7(1971); C.A. 79 149340z(1973).
152. Teodorescu N. and Bebesel, E.;Farmacia 14 743-750(1966); C.A. 67 102843c (1967).
153. Neuss, J.D.; Seagers, W.J., and Mader W.J.; J.Am.Pharm.Assoc. 41, 670-73(1952); C.A. 47 1545b (1953).
154. Carol, J.; J.Assoc.Offic Agr.Chemists 36, 722-5(1953); C.A. 48 10300i (1954).
155. Goldman, D.S.; Science 120, 315-16(1954).
156. Pinyazhko, I.R.M.; Farmatsevt.Zh. 20, 17-21 (1965); C.A. 64 7969h (1966).
157. Kracmar, J.; Kracmarova, J. and Zyka, J.; Cesk.Farm. 17, 68-69(1968);C.A. 69 5266a (1968).
158. Ghe A.M. and Peretti, A.; Ric.Sci. 38,937-41 (1968);C.A. 71 3951n (1969).
159. Tiess, D.; Wiss. Beitr.,Martin-Luther-Univ. Halle-Wittenberg 12, 152-4(1968);C.A. 74 74412k (1971).
160. Welsh, L.H.; J.Assoc.Offic.Agr.Chemists 40, 807-14(1957); C.A. 51 17097i (1957).
161. Welsh, L.H.; ibid 41, 496-8 (1958); C.A. 52 20894i(1958).

162. Dutt, M.C., and Chua, T.H.; J.Pharm.Pharmacol. 16,696-9 (1964);C.A. 61 15936a (1964).
163. Sattler, H.; Pharm.Ztg. 111,1395-1405(1966); C.A. 66 40761t (1967).
164. Sarkar, B. N.; Indian J. Pharm. 34, 58-61 (1972); C.A. 77 130658w (1972).
165. Steinke, G. and Schmidt, F.;Krankenhaus-Apoth. 24,20-2(1974);C.A. 81 96483f (1974).
166. Momose, T.; Ueda, Y.; Mukai,Y. and Watanabe, K.; Yakugaku Zasshi 80, 225-8 (1960); C.A. 54 11861d(1960).
167. Uno, T. and Taniguchi, H.; Japan Analyst 20, 997-1002(1971); A.A. 25 447(1973).
168. Bartos, J.; Annls. pharm. fr. 29, 71-74(1971); A.A. 21 3502 (1971).
169. Anon.; Official Methods of Analysis of the Association of Official Agricultural Chemists 9th Ed. page 526 32.289 (1960).
170. Kühni, E.; Jacob, M. and Grossglauser, H.; Pharm. Acta Helv. 29,233-50(1954);C.A.49 3469b(1955).
171. Berka, A. and Zýka,J.;Pharmazie 13,81-92 (1958); C.A. 53 1634f(1959).
172. Duda, H. and Elste, U.; Deut.Apoth.Ztg. 110,593-8(1970);C.A.73 91265m(1970).
173. Blake, M.I.; Bode, D. and Rhodes, H.J.; J.Pharm.Sci. 63,1303-6(1974);C.A. 82 35088e(1975).
174. Anon.;British Pharmacopoeia page 256 (1973).
175. Haugas, E.A. and Mitchell, B.W.;J.Pharm.and Pharmacol. 4 687-92(1952);C.A. 47 1010a(1953).
176. Wojahn, H.;Arzneimittel-Forsch. 2, 324-6 (1952);C.A. 46 10052a(1952).
177. Coppini,D.;Cameroni,R. and Monzani,A.; Ricerca sci. 22,1783-4(1952);C.A.47 5310b (1953).
178. Rosenthaler,L.;Pharm.Acta.Helv. 30,69-72 (1955); C.A. 49 10123a(1955).
179. Kochi, Z.;J.Pharm.Soc.Japan 75,748-50(1955); C.A. 49 13596g(1955).
180. Horn, D.;Pharmazie 8, 646-7(1953);C.A.51 4649b (1957).

181. Modrzejewski, B. and Zommer, S.;Chem.Anal. 7,659-66(1962); C.A. 57 13889e(1957).
182. Miszczuk, B. and Taborska, H.;Przemyst Chem. 11,706-10(1955);C.A. 52 20895i(1958).
183. Jančik,F.; Činkova, O. and Körbl, J.; Collection Czechoslov.Chem.Commns. 24, 2695-8(1959);C.A. 54 8429g(1960).
184. Laszlovszky, J.;Acta Pharm.Hung. 30,101-9 (1960); C.A. 54 19268c(1960).
185. Vulterin, J.; Collection Czech.Chem.Commun.28, 1393-1400(1963);C.A.59 10767h (1963).
186. Vulterin, J.;Cesk.Farm. 12,391-3(1963); C.A. 62 15994c (1965).
187. Wihchurch,R.; J.Assoc.Off.Anal.Chem. 56, 1464-6(1973);C.A. 80 87544d(1974).
188. Canbäck, T.; J.Pharm.Pharmacol.4 407(1952); C.A. 46 8325i(1952).
189. Domleo, A.P.; J.Pharm.and Pharmacol. 5, 117-18(1953);C.A. 47 4251d(1953).
190. Struszyński, M. and Bellen, Z. Przemyst.Chem. 32,40-1(1953);C.A.47 8586g (1953).
191. Montequi, F.; Farm.nueva 18,10-14(1953); C.A. 47 6603d(1953).
192. Noronha da Costa, A.; Rev.brasil.farm.33, 343-50(1952); C.A. 47 7736d(1953).
193. Kattionis,A.; Chim.Chronika 17,78-80(1952); C.A. 47 11665i(1953).
194. Berka, A. and Zijka, J.;Chem.Listy 50,314-16 (1956);C.A. 50 7651a(1956).
195. Spacu, P. and Teodorescu, G.;Bul.inst. politeh Bucuresti 18,47-50(1956);C.A.51 17607h (1957).
196. Tsupikov, M.T.; Aptechn.Delo.13,55-7(1964); C.A. 61 9361c(1964).
197. Laipanov, A. Kh. and Lobanov,V.I.; Farm. Zh. 28,44-8(1973);C.A.78 140435f(1973).
198. Nambisan, P.N.K. and Nair, C.G.R. Indian J. Chem. 10,665-6(1972);C.A.78 37750f(1973).
199. Laipanov,A.Kh. and Lobanov,V.I.;Farmatsiya 22, 48-9(1972);C.A.79 23619d(1973).
200. Greenhow,E.J.;Chem.Ind.14,697-8(1973);C.A.79 121642a(1973).

201. Alicino, J.F.; J.Am.Pharm.Assoc.41,401-2 (1972);C.A.46 9786i(1946).
202. Anastasi,A.; Mecarelli,E. and Novacic,L.; Mikrochemie Ver.Mikrochim Acta 40,53-9 (1952);C.A.47 8317h(1953).
203. Pilar de los Rios de Bascones; Rev.sanidad y asistencia social. 21,221-30(1956);C.A. 51 4652e(1957).
204. Kahane,E. and Sackur,O.;Ann.pharm.franç.11 175-82(1953);C.A.47 7375d(1953).
205. Kashima,T.;Bull.Natl.Hyg.Lab.72 145-50 (1954);C.A.49 7186h(1955).
206. Sellés, E. and Flores, E.S.;Galenica Acta 8, 291-349(1955);C.A.51 2234i(1957).
207. Mizukami,S. and Hirai,E.;Yakugaku Zasshi 79, 454-7(1959);C.A. 53 16471b(1959).
208. Keller, W. and Weiss, F.;Pharmazie 12,462-71 (1957);C.A.52 3260b(1958).
209. Ko.I.S.; Kim,J.B. and Choi,B.K. Repts.Natl. Chem.Lab. 3,50(1959);C.A.54 11385a(1960).
210. Tao,T. and Yu,H.Y.;Yao Hsueh Hsueh Pao 8, 206-16(1960);C.A.56 7427f(1962).
211. Solomon-Ionescu,I.;Popescu,D. and Enache,St.; Rev.Chim.14,532(1963),C.A.60 3950c(1964).
212. Vajgand,V. and Pastor, T.;Glasnik,Hem.Drustva, Beograd 28,1-7(1963);C.A.60 15118g(1964).
213. Boichinov,K. and Karatodorov,K.Farmatsiya 14, 12-17(1964);C.A.61 6868c(1964).
214. Nickel,P. Pharm.Ztg.113,1609-12(1968);C.A.70 31693c(1969).
215. Devani,M.B. and Shishoo,C.J.;J.Pharm.Sci. 59 90-2(1970).
216. Shishov,C.J. and Devani,M.B.;J.Pharm.Sci.59, 92-3(1970);C.A.72 47410k(1970).
217. Sell,E.; Farm.Pol.26,761-6(1970);C.A.74 130450k(1971).
218. Vajgand,V.J.;Pastor,T.J. and Bjelica,L.J.; Glasn.hem.Drust.,Beogr.35,345-352(1970); A.A. 21 4158(1971).
219. Budějinský,B.;Pharmazie 10,567-9(1955);C.A.50 5985f(1956).
220. Korbl,J.;Collection Czech.Chem.Communs. 25 76-84(1960);C.A.54 13988d(1960).

221. Adamová,E. and Zýka,J.;Ceskoslov.farm. 4, 9-10(1955);C.A.54 3858a(1960).
222. Grecu,I. and Curea, E.;Farmacia 8,503-6(1960); C.A.55 19136d(1961).
223. Tatsuzawa,M.;Bunseki Kagaku 11,1055-9(1962); C.A. 57 16748f(1962).
224. Ciogolea,G.;Morait,G.;Teodorescu,N. and Petroniu,L.;Farmacia 12 401-8(1964);C.A.61 13131e(1964).
225. van Pinxteren,J.A.C. and Verloop,M.E.;Pharm. Weekblad 99,1125-33(1964);C.A.63 1659f(1965).
226. Blagojevic,Z. and Radosavljevic,J.;Acta Pharm. Jugoslav. 16,27-30(1963);C.A.65 571b(1966).
227. Blagojevic,Z. and Radosavljevic,J.;Acta Pharm. Jugoslav. 17,131-6(1969);C.A.70 60882u(1969).
228. Blagojevic,Z. and Radosavljevic, J.; Gyogyszereszet 13,121-4(1969);C.A.71 33486h(1969).
229. Pszonicka,M. and Skwara,W.;Chem.Anal.15, 175-82(1970); C.A. 73 10502m (1970).
230. Barrosa,M.T.;Rev.Port.Farm.19,206-9(1969); C.A. 74 15757d(1971).
231. Kassem,A.A. and El-Marakby,M.M.;J.Drug Res 2 79-87(1969);C.A.74 67753u(1971).
232. Soliman,R. and Belal,S.A.;Pharmazie 29,204 (1974);C.A.81 111504j(1974).
233. Armestar,A.M.;Congr.farm.bioquim.peruano y convención farm. Norte,Actas y trabajos 315-16(1953);C.A.49 7193h(1955).
234. Leal,A.M. and Alves, M.A.;Rev.port.farm.2, 175-9(1952);C.A.47 5635f(1953).
235. Merz,K.W. and Schirm,M.;Naturwissenschaften 39,570-1(1952);C.A. 48 1629d(1954).
236. Rao,R.S.;Rao,G.B.B. and Rao, P.V.K.; Chem.Anal. 19,927-30(1974);C.A.82 51131u(1975).
237. Businelli,M. and Rocchi,B.;Farm.sci.1 tec. 7,153-60(1952);C.A.46 9019i(1952).
238. Deys,H.P.;Pharm.Tijdschi.Belgie 36,117-20 (1959);C.A.54 7067g(1960).
239. Spacu,P. and Teodorescu,Gr.;Rev.chim.8, 42-3(1957); C.A.52 1552a(1958)
240. Danek,A.;Acta Polon.Pharm. 18,229-34(1961); C.A. 56 8842d(1962).

241. Franchi,G.;Farm.sci.e tec.7,640-4(1952); C.A.47 4044b(1953).
242. Akiyama,T.;Fujiwara,M. and Ichida H.; Bull. Kyoto Coll. Pharm. 4,18-20(1956); C.A. 51 4650a(1957).
243. Sánchez, J. A.; Rev.Asoc.bioquim.Argentina 17,324-6(1952);C.A.47 6822b(1953).
244. Mitchell,B.W.; Haugas,E.A. and McRoe,C.S.; J.Pharm.and Pharmacol.9,42-5(1957);C.A.51 6087b(1957).
245. Kum-Tatt,L. and Yan-Hon,H.;J.Pharm.Pharmacol. 14,123-4(1962);C.A.57 2337b(1962).
246. Spacu,P.;Teodorescu,Gr. and Găvănescu,D.; Bul.inst.politch.Bucuresti 18,51-4(1956); C.A.51 17607i(1957).
247. Ibadov,A. Yu. and Drebentsova,N.F.;Dokl. Akad.Nauk Uz.SSR 21 39-41(1964);C.A. 61 10538a(1964).
248. Nair,V.R. and Nair, C.G.R.;Analytica chim. Acta 57,429-34(1971);A.A.23 799(1972).
249. Pinzauti,S.;Dal Piaz,V.and LaPorta, E.; J.Pharm.Sci. 63,1446-8(1974);C.A.82 7701f(1975).
250. Gowda,H.S. and Rao,G.G.;Z.Anal.Chem.165, 36-8(1959);C.A.53 11765h(1959).
251. Buděšínský,B.;Collection Czechoslov.Chem. Communs.26,781-7(1961);C.A.55 14174b(1961).
252. Rao,P.V.K. and Rao, G.B.B.;Analyst 96, 712-15(1971);C.A.76 6762r(1972).
253. Rao,P.V.K. and Rao,G.B.B.;Fresenius Z.Anal. Chem. 256,360-1(1971);C.A.76 37475d(1974).
254. Eremina,Z.I. and Antonchik,I.A.;Farm.Zh.(Kiev) 27,79-81(1972);C.A.77 92916u(1972).
255. Rao,P.V.K.;Rao,G.B.B. and Rao,R.S.;Anal.Chim. Acta 65,227-30(1973);C.A.79 57733b(1973).
256. Tatsuzawa,M.;Bunseki Kagaku 10,129-33(1961); C.A.55 23931e(1961).
257. Panwar,K.S.;Rao,S.P.and Gaur,J.N.;Anal.Chim. Acta 25,218-21(1961);C.A.55 26865d(1961).
258. Rao,G.G. and Rao,P.V.K.;Talanta 11,1489-96 (1964);C.A.62 1239h(1965)
259. Gein,L.G. and Sumbaikina,Z.A.;Farmatsiya 16, 41-3(1967);C.A.67 84919a(1967).

260. Pinzauti,S.;Dal Piaz,V. and La Porta, E.; Farmaco Ed.Prat. 29,136-42(1974);C.A.81 29576a(1974).
261. Rao,P.V.K. and Rao,G.B.B.;Res.Ind.17,143-4 (1972);C.A.80 19640g(1974).
262. Volodina,M.A. and Karandi,I.V.;Vestn.Mosk. Univ.,Khim 13,357-8(1972);C.A.77 147343n(1972).
263. Vulterin,J. and Zýka,J.;Chem.Listy 48 839-42 (1954);C.A.48 13521a(1954).
264. Sakurai,H.;Kimura,T. and Senoo,S.; Yakuzaigaku 16 39-41(1956);C.A.51 9089a(1957).
265. Akiyama,T.;Fujiwara,M. and Ichida,H.; Kyoto Yakka Daigaku Gakuhó 5,42-4(1957);C.A.52 3262i(1958).
266. Tachi,I. and Nagata,V.;Kagaku no Ryóiki 6, 490-1(1952); C.A.46 11045f(1952).
267. Anastasi,A.;Mecarelli,E. and Novacic,L.; Mikrochemie ver Mikrochim.Acta 40,113-20 (1952);C.A.47 3185a(1953).
268. Maruyama,M.;J.Pharm.Soc.,Japan 72,1213-4 (1952);C.A.47 417g(1953).
269. Varela,G.;Anales.real soc.espaň.fís.y.quím 48B 713-15(1952);C.A.47 8323c(1953).
270. Liberti,A.;Cervone,E. and Cattaneo,C.; Giorn.biochim.1,440-6(1952);C.A.49 9888i (1955).
271. Tsao,King-Hung;Loo,Yun-Chieng and Tang, Teng-Han.;Yao Hsüeh Hsüeh Pao 4,121-6(1956); C.A.52 6716a(1958).
272. Maruyama,M.; Ann.Rept.Takamine Lab.8,122-7 (1956);C.A.54 19310a(1960).
273. Sato,H.;Eisei Shikenjo Hôkoku 77, 39-44 (1959);C.A.55 14281i(1961).
274. Lund,H.;Acta Chem.Scand.17,972-8 and 1077-86 (1963);C.A.59 13598g(1963).
275. Vajgand,V. and Pastor,T.;Glasnik Hem.Drustva, Beograd 27,263-70 (1962);C.A.59 13326c(1963).
276. Vajgand,V. and Pastor T.;J.Electroanal.Chem. 8 40-8(1964);C.A.62 4619f(1965).
277. Lund,H.;Abhandl.Deut.Akad.Wiss.Berlin Kl. Chem.,Geol.Biol. 434-42(1964);C.A.62 8674d(1965).
278. Brandys,J.;Diss.Pharm.Pharmacol. 18,319 (1966);C.A. 66 22276j(1967).

279. Schlitt,L.;Rink,M. and V. Stackelberg, M.; J. Electroanal.Chem. 13, 10-20(1967), C.A. 66 5798p(1967).
280. Turczan, J.W.; J.Ass.Offic.Anal.Chem.50, 652-4(1967);C.A.67 67658x(1967).
281. Kitaev,Yu.P. and Budnikov,G.X.; Izv.Akad. Nauk SSSR, Ser.Khim. 554-61(1967)C.A.67 90282d(1967).
282. Sato, H.;Eisei Shikenjo Hokoku 78,21-4 (1960);C.A. 55 24395a(1961).
283. Vallon, J.J.;Badinand,A. and Bichon,C.; Anal.Chim.Acta 78,93-8(1975);C.A.83 152435x (1975).
284. Okuda,Y.;Ohara,M.;Horino,S.;Masak,H.; Furukawa, Y. and Yamasaki, M.; Rev.Polarog. 9,112-15(1961);C.A.60 7307e(1964).
285. Kalinowski,K.;Acta Polon.Pharm.11,113-16 (1954);C.A.48 14123b(1954).
286. Kalinowski,K. and Zwierzchowski,Z.; Acta Polon.Pharm. 20,309-13(1963);C.A.62 1517h (1965).
287. Stoicescu,V.;Ivan,C. and Beral,H.;Rev.Chim. 19,484(1968);C.A.70 6568h(1969).
288. Brandys,J.;Diss.Pharm.Pharmacol.21,87-92 (1969);C.A.72 38257p (1970).
289. Curran, D.J. and Curley, J.E.;Analyt.Chem. 42,373-7(1970);A.A. 20 2497(1971).
290. Jennings, V.J.; Dodson, A. and Harrison,A.; Analyst 99 145-8(1974);C.A.81 57876s(1974).
291. Akiyama, T.;Fujita,M.; Fujiwara,M. and Kawashima, K.;Bunseki Kagaku 5, 701-3(1956); C.A. 52 155f(1958).
292. Grecu,I. and Curea, E.;Rev.Chim.13, 162-3 (1962);C.A.57 11856i(1962).
293. Grecu, I.; Curea, E. and Pitis, M.; Acad.Rep. Populare Romine,Filiala Cluj, Studii Cercetari Chem. 13 213-23(1962);C.A.61 10027g(1964).
294. van Pinxteren J. A.C. and Verloop, M.E.; Pharm.Weekblad 100, 189-95(1965);C.A. 63 5454a(1965).
295. Zivanov, D.; Blagojevic, Z. and Mladenovic,M.; Arhiv.Farm. 14,273-8(1964);

C.A. 62 2671h(1965).
296. Tabenkin,B.; Dolan,B. and Johnson, M.G.; Proc.Soc.Exptl.Biol. Med. 80,613-15(1952); C.A. 46 10254b(1952).
297. Cerotti,G.; Atti soc lombarda sci med biol. 7,76(1952);C.A.47 2248d(1953).
298. Ifrim,A. and Coniver, L.;Lucrările prezentate conf. natl. farm.,Bucharest 674-8(1958);C.A.53 9354h(1959).
299. Suzuki,Y.;Physiol.Plantarum 19,257-63(1966); C.A.64 20211c(1966).
300. Vajgand,V.J. and Pastor, T.J.;Glas.Hem.Drus., Beograd. 31,9-17(1966);C.A.67 39955y(1967).
301. Dusinsky,G. and Faith,L.;Pharmazie 22, 475-82(1967);C.A.68 53280b(1968).
302. McKennis J.H. and Yard, A.S.; U.S. Dept.Com., Office Tech.Serv. PB Rept. 143,914(1957); C.A. 55 17375i(1961).
303. Harting, H. J.Am.Pharm.Assoc.42,323-4(1953); C.A. 47 7163f(1953).
304. Kazuo,T.;Yakuzaiguku 32,150-4(1972);C.A.79 149305s(1973).
305. Artamonov,B.P. and Kupina,N.A.;Med.Prom. SSSR 19,61-3(1965);C.A.64 4871f(1966).
306. Nikolic,K. and Cupic,Z.;Ark.Farm.(Belgrade) 17,201-4(1967);C.A.69 54298w(1968).
307. Kidani,Y.;Inagaki,K.;Saotome,T. and Koike,H.; Buneski Kayaku 22 896-9(1973);C.A.79 139672v (1973).
308. Belilovskii,Ya.E.;Farmatsiya 21,62-4(1972); C.A.77 130636n(1972).
309. Mattu,F.;Rend.seminar.fac.sci.univ.Cagliari 22,92-106(1952);C.A.48 4179f(1954).
310. Roth,L.J. and Manthei,R.W.;Proc.Soc.Exp. Biol.Med. 81,566(1962); C.A.48 9438f(1954).
311. Ceriotti,G.;Defranceschi,A.;DeCarneri,I. and Zamboni,V.;Brit.J.Pharmacol.8 356 (1953); C.A. 48 885g(1954).
312. Defranceschi,A. and Zamboni, V.; Biochim. Biophys.Acta 13 304(1954);C.A.49 9082h(1955).
313. Iwainsky,H.;Arzneimitt,Forsch.7,745(1957); C.A. 52 5665c(1958).

314. Savoia,F.;Boll.soc.ital.biol.sper.,30,29-30 (1954);C.A.48 9438f(1954).
315. Leuschner,F.;Naunyn-Schmiedebergs Arch.exptl. Pathol.Pharmakol. 221,323-7(1954);C.A.48 8853g(1954).
316. Itai,T.;Oba,T. and Kamiya,S.;Bull.Natl.Hyg. Lab. 72,87-90(1954);C.A.49 6350h(1955).
317. Unverricht,W.;Schattmann,K. and Senft,G.; Ärztl.Wochschr.9 838(1954);C.A.49 1280h(1955).
318. Ishikawa,M. and Kikkawa,I.;Ann.Repts.Shionogi Research Lab. 4, 39-42 (1954);C.A.50 15629g (1956).
319. Fischer,P. and Bürgen, A.;Pharm.Acta.Helv. 31,518-42(1956);C.A.51 6945b(1957).
320. Abe,M.;Sci.Rept.Res.Inst.Tohoku.Univ.,Ser.C. 8,1(1958);C.A.52 17529i(1958).
321. Albert,A. and Rees,C.W.; Biochem.J. 61, 128 (1955);C.A.49 16197c(1955).
322. Boone,I.U.;Magee,M. and Turney,D.F.;J.Biol. Chem.221,781(1956);C.A.50 15784h(1956).
323. Boone,I.U.;Strang,V.G. and Rogers,B.S.; Am.Rev.Tuberc.76 568(1957);C.A.53 3361c(1959).
324. Diller,W.;Krüger-Thiemer,E. and Wempe,E.; Arzneimittel-Forsch.9 423-9(1959);C.A.53 21363b(1959).
325. Barreto, R.C.R.;J.Chromatog.11,344(1963); C.A. 57 6247a(1962).
326. Greulach,V.A. and Haesloop, J.G.;Anal.Chem. 33 1446-7(1961);C.A.55 25577f(1961).
327. Reio,L;J.Chromatog.47,60-85(1970);C.A.73 21216x(1970).
328. LaRue,T.A.;J.Chromatogr.32,784-5(1968); C.A. 68 74892t(1968)
329. Barreto,R.C.R.;J.Chromatog.6,416-19(1961); C.A. 56 12295f(1962).
330. Gonnard,P.;Camier,M. and Boigne,N.; Boll. Soc.Chem.Biol.46 407-11(1964);C.A.61 6867d(1963).
331. Pallini,V.;Vasconetto,C. and Ricci, C.; Boll. Soc.Ital.Biol.Sper. 41 673-5(1965);C.A.64 2401f(1966).
332. Schmid,E.;Hoppe,E.;Meythaler,Chr.and Zicha,L.;Arzneimittel-Forsch.13,969-72(1963);

C.A.60 7351c(1962).
333. Thomas, J.J. and Dryon, L.;J.Pharm.Belg. 19, 481-504(1965);C.A.63 1660e(1965).
334. Guven, K.C.;Eczacilik Bul. 9, 186-9(1967); C.A. 69 5256x(1968).
335. Spinkova, V.;Cesk.Farm. 16,138-42(1967); C.A. 67 47125t(1967).
336. Kaistha,K.K. and Jaffe,J.H.;J.Pharm.Sci. 61, 679-89(1972);C.A.77 57236a(1972).
337. Garcia Carro,A.J.; An.Real Acad.Farm. 39, 211-36(1973);C.A.80 74377b (1974).
338. DeSagher, R.M.;De Leenheer, A.P. and Claeys, A.E.; J. Chromatogr. 106 357-62(1975);C.A.83 157v(1975).
339. Nishimoto,Y. and Toyoshima, S.; Yakugaku Zasshi 87,27-32(1967);C.A.66 98544r(1967).
340. Wijnne,H.J.A.; Bletz,E. and Frijns,J.M.; Pharm. Weekbl. 102,959-70(1967);C.A.67 102844d(1967).
341. Schmidt,F.;Krankenhaus-Apoth.23,10-12(1973); C.A.79 45866j(1973).
342. Kawale,G.B.;Joglekar,V.D.;Barve,V.P. and Mahal, H.S.;Sci.Cult.38,373-376(1972); A.A. 25 3438(1973).
343. Tsuji,A. and Sekiguchi, K.;Nippon Kagaku Zasshi 81,847-52(1960);C.A.55 10015d(1961).
344. Heller,A.;Kasik, J.E.;Clark, L. and Roth,L.J.; Anal.Chem. 33,1755(1961).
345. Kakemi,K., Sezaki,H. and Inoue,S.; Yakugaku Zasshi 85,674-9(1965);C.A.63 12974a(1965).
346. Peters, J.H., Miller, K.S. and Brown, P.; Anal.Biochem 12,379(1965);C.A.63 8896a(1965).
347. Peters, J.H., Miller, K.S. and Brown, P.; J. Pharm.Exp.Ther. 150 298(1965);C.A. 63 8896a(1965).
348. Fan, M.C. and Wald, W.G.;J.Assoc.Offic.Agr. Chemists 48,1148-50(1965);C.A.64 6407d(1966).
349. Inoue, S.;Ogino,A. and Ono,Y.;Yakuraigaku 26,302-7(1966);C.A.69 99472g(1968).
350. Lewandowski,A. and Sybirska,H.;Chem.Anal.13, 319-24(1968);C.A.69 73784t(1968).

351. Darawy,Z.I. and Mobarak,Z.M.;Pharmazie 28, 37-39(1973);A.A. 25 1155(1973).
352. Barreto,R.C.R. and Sabino,S.O.;J.Chromatog. 13,435(1964);C.A.60 13542e(1964).
353. Smolarek,W. and Dlugosch,G.; Naturwissenschaften 41,18(1954);C.A.48 10996i(1954).
354. Barreto,R.C.R.;J.Chromatog.9,180(1962);C.A. 57 6247a(1962).
355. Russell,D.W.;J.Chromatog. 19,199-201(1965); C.A.63 10652h(1965).
356. Calo,A.;Cardini,C. and Quercia,V.;Boll.Chim. Farm. 107,296-9(1968);C.A.69 80244v(1968).
357. Calo,A.; Cardini,C. and Quercia,V.;J.Chromatogr. 37, 194-6(1968);C.A.70 6564d(1969).
358. Gelbicova-Ruzickova,J.;Novak,J. and Chundela,B.;Proc.Conf.Appl.Phys.Chem. 295-300(1971);C.A.76 90104h(1972).
359. Terze,B. and Dadiotou,M.T.;Chim.Chronika 17, 45-8(1952);C.A.47 9862f(1953).
360. Ginoulhiac,E.;Rass.med.29,86-9(1952);C.A.48 13791b(1954).
361. zuSchwabedissen,O.M.;Deut.med.Wochschr.78, 104-5(1954);C.A.47 4406d(1953).
362. Smolarek,W. and Stahl,R.;Deut.med.Wochschr. 78,273-4(1953);C.A.47 5978f(1953).
363. Schattmann,K.;Deut.med.Wochschr.79,758-9 (1954);C.A.48 8851b(1954).
364. zuSchwabedissen,O.M.;Deut.med.Wochschr.79, 759-60(1954);C.A.48 8851c(1954).
365. Kelly,J.M. and Poet,R.B.;Am.Rev.Tuberc.65, 484-5(1952);C.A.47 12478g(1953).
366. Wojahn,H. and Wempe,E.;Arzneimittel-Forsch. 3 191-2(1953);C.A.47 5983d(1953).
367. Marenzi,A.D. and Gomez, C.J.;Rev.asoc.méd. argentina 66,379-80(1952);C.A.47 7013b(1953).
368. Wojahn,H. and Wempe,E.;Arzneimittel-Forsch.4, 294-5(1954);C.A.48 8856e(1954).
369. Malosetti,H.;Balea,E. and Addiego,A.;Anales. fac.med.Montevideo 40,13-17(1955);C.A.50 16924g(1956).
370. Peukert,D.;Arzneimittel Forsch.7,304-6(1957); C.A. 51 13047b(1957).

371. Waldron-Edward, D.M.;Irish J. Med.Sci.363, 130-6(1956);C.A.54 13228e(1960).
372. Litman,I.I.;Tr.Leningrad.Inst.Usoversh. Vrachoi 49 165(1966);C.A.69 34297s(1968).
373. Deeb,E.N. and Vitagliano,G.R.;J.Am.Pharm. Assoc. 44,182-5(1955);C.A.49 7633b(1955).
374. Yantchitch,M.;Ann.biol.clin. 12,328-9(1954); C.A. 49 1126f(1955).
375. Leifheit,H.C. and Smith,E.R.B.;Am.J.Clin. Pathol. 31,142-7(1959);C.A.53 9349d(1959).
376. Tansini,G.;Perna,G. and Trotta,E.;Atti.soc. lombarda sci.med.biol. 13,258-62(1958); C.A.53 13251e(1959).
377. Pedenko,E.P. and Kozlovskii,I.Z.;Lab.Delo 249(1974);C.A.81 114327g(1974).
378. Ruffo,G.;Arch.Vet.Ital.16,245-55(1965);C.A.65 11143b(1966).
379. Jessamine,A.G.;Hamilton,E.J. and Eidus,L.; Can.Med.Assoc.J. 89,1214-17(1963);C.A.60 8536b(1964).
380. Eidus,L. and Hamilton, E.J.;Clin.Chem. 10, 581-8(1964);C.A.61 12307g(1964).
381. Eidus,L. and Harnanansingh,A.M.T.;Clin.Chem. 17,492-4(1971);C.A.75 74372t(1971).
382. Rinaldi,M.R. and Cragnolino,N.A.;Rev.asoc. bioquím.argentina 18,162-3(1953);C.A.47 12479f(1953).
383. Aoki,S.;Terai,I. and Mori,K.;Iryo 6,33-5 (1952);C.A.47 4941h(1953).
384. Guillaume,J.;Tacquet,A. and Berthelot,J.Y.; Rev.franç.études clin.et biol. 5,729-33 (1960);C.A.55 7531i(1961).
385. Fartushnyi,A.F.;Sud.-Med.Ekspertiza 11,26-31 (1968);C.A.70 18570r(1969).
386. Prescott,B.;Kauffmann,G. and James,W.D.; Proc.Soc.Exptl.Biol.Med. 84,704-6(1963); C.A.48 4620g(1954).
387. Lever,M.;Biochem.Med.6,65-71(1972);C.A.76 123789r(1972).
388. Hashmi,M.H.;Adil,A.S.;Malik,F.R.and Ajmal,A.I. Mikrochim.Acta 772-7(1969);C.A.71 59223u(1969)
389. Bartels, H. and Spring,P.;Chemotherapy 21, 1-10(1974);C.A. 82 92852y(1975).

390. Ginoulhiac,E.;Russ.med.29,86-9(1952); C.A.46 9646f(1952).
391. Defranceschi,A. and Zamboni,V.;Giorn.biochim. 1,405-16 (1952);C.A.49 9082h(1955).
392. Hölscher,F.M.;Minerva pediat.7,160-1(1955) C.A.49 14867f(1955).
393. Wollenberg,O.;Klin.Wochschr.30,906-7(1952); C.A. 47 2087i(1953).
394. Kellner,H. and Südhof,H.;Klin.Wochschr.31, 287-8(1953);C.A.47 7014g(1953).
395. Roberts,R.W. and Deuschle,K.W.; Am.Rev. Respirat.Diseases 80,904-8(1959);C.A.55 10551d(1961).
396. Paunescu,E.;Acad.Rep.Polulare Romine,Inst. Biochim.;Studii Cercetari Biochim. 4 117-22 (1961);C.A.56 16081a(1962).
397. Paunescu,E. and Buzescu,M.;Rumanian Med.Rev. 5,38-40(1961);C.A.57 10502g(1962).
398. Smirnov,G.A. and Kozulitsyna,T.I.;Lab.Delo 48-52(1964);C.A.60 11039f(1964).
399. Maier,N. and Moisescu,V.;Beitr.Klin.Tuberk. 128,213-17(1964);C.A.61 8773a(1964).
400. Nielsch,W.;Chemiker-Ztg. 82 494-9(1958); C.A. 53 6913d(1959).
401. Nielsch,W. and Giefer,L.;Arzneimittel-Forsch. 9 636-41,700-07(1959);C.A.54 5803c(1960).
402. Rapi,G.;Sperimentale,Sez.chim.biol.4,11-22 (1953); C.A.47 10604a(1953).
403. Rubino,G.F. and Bracco,M.;Minerva med. 1136-7(1952);C.A.48 12212g(1954).
404. Nardini,F.B. and Periti,P.;Boll.soc.ital. biol.sper. 29,356-7(1953);C.A.48 12317h(1954).
405. Short,E.A.;Lancet266,656-7(1954);C.A.49 13598d(1955).
406. Brettoni,B.;Arch.ital.sci.farmacol.2,227-8 (1955);C.A.50 1971b(1956).
407. Shirai,Y.;Ohishi,K.;Suchiro,K.;Motoike,H. and Adachi,R.;Seikagaku 29,557-62(1957);C.A.55 10551a(1961).
408. Short,E.I.;Tubercle 42 218-26(1961);C.A. 62 11001a(1965).
409. Dymond,L.C. and Russell,D.W.; Clin.Chim.Acta 27,513-20(1970);C.A.72 119665x(1970).

410. Russell,D.W.;Clin.Chim.Acta 31,367-73(1971); C.A. 74 97428j(1971).
411. Morvillo,V. and Garattini,S.;Boll.soc.ital. biol.sper.28,388-90(1952);C.A.48 217c(1954).
412. Porcellati,G. and Preziosi,P.;Boll.soc.ital. biol.sper.29,269-71(1953);C.A.48 1577d(1954).
413. Ioffe,R.A.;Lab.Delo 47(1967);C.A.66 84348t (1967).
414. Jacobs,M.B.; Science 118,142-3(1953);C.A.47 11302d(1953).
415. Scardi,V. and Bonavita,V.;Arch.ital.sci. farmacol. 7,206-11(1957);C.A.51 16647d(1957).
416. Scardi,V. and Bonavita,V. Clin.Chem.3, 728-31(1957);C.A.52 4738c(1958).
417. Holecek,V. and Herlik,J.;Rozhledy Tuberk. 24,3-6(1964);C.A.60 16400h(1964).
418. Castel,P.;Carli,G.;Gras,G. and Cambou,P.; Trav.soc.pharm.Montpellier 15,27-36(1955); C.A.49 14080e(1958).
419. Bjornesjo,K.B. and Jarnulf,B.; Scand.J.Clin. Lab.Invest.20 39-40(1967);C.A.67 107109c (1967).
420. Brettoni,B.;Boll.soc.ital.biol.sper.28 939-42(1952);C.A.47 8809b(1953).
421. Oba,Y.;Igaku Kenkyu 31,124-42(1961)C.A.55 27505i(1961).
422. Ginoulhiac,E. and Tenconi,L.;Giorn.mal. infettive e parassitar 4 63-7(1952);C.A.47 9400a(1952).
423. Cuthbertson,W.F.J.;Ireland,D.M.;Wolff,W. and Kuper,S.W.A.;Brit.Med.J. I 609-11(1954); C.A. 48 13777d(1954).
424. Hunter,G.;Brit.Med.J. I 585(1955);C.A.49 10407e(1955).
425. Rubin,S.H.;Drekter,L.;Scheiner,J. and DeRitter,E.;Diseases of the Chest 21 439-49 (1952);C.A.46 6686i(1952).
426. Prescott,B.;Katz,S. and Kauffman,G.; J.Lab.Clin.Med. 44, 600-3(1954);C.A.49 1131c(1955).
427. Kinoshita,Y. and Moriyama,S.;Bull.Nagoya City Univ.Pharm.School 1,37-9(1953);C.A.50 9489h(1956).

428. Movrin, M.; Bull. Sci. Cons. Acad. Sci. Arts RSF Yougoslavie, Sect. A. 14, 141-2 (1969); C.A. 71 69029z (1969).
429. Hodgkin, M.M.; Hsu, A.H.E.; Varughese, P. and Eidus, L.; Int. J. Clin. Pharmacol., Ther. Toxicol. 7, 355-62 (1973); C.A. 79 112860t (1973).
430. Varughese, P.; Hamilton, E.J. and Eidus, L.; Clin. Chem. 20 639-41 (1974); C.A. 81 99149n (1974).
431. Wagner, J.; Kraus, P. and Večeřek, B.; Rozhledy Tuberk 16, 211-13 (1956); C.A. 50 9498a (1956).
432. Maher, J.R.; Whitney, J.M.; Chambers, J.S. and Stanonis, D.J.; Am. Rev. Tuberc. Pulmonary Diseases 76 852-61 (1957); C.A. 53 3345a (1959).
433. Goedde, H.W.; Schloot, W. and Benkmann, H.G.; Chemotherapia 12, 61-7 (1967); C.A. 67 61487a (1967).
434. Bracco, M. and Savio, E.; Ann. Med. Sondalo 9 201-5 (1961); C.A. 56 14560f (1962).
435. Wareska, W.; Polski Tygodnik Lekarski 8, 1736 (1953); C.A. 48 14082c (1955).
436. Hedrick, M.T.; Rippon, J.W.; Decker, L.E. and Bernsohn, J.; Anal. Biochem. 4 85-98 (1962); C.A. 58 743e (1963).
437. Scott, E.M. and Wright, R.C.; J. Lab. Clin. Med. 70 355-60 (1967); C.A. 67 72217g (1967).
438. Reiss, O.K.; Morse, W.C. and Putsch, R.W.; Amer. Rev. Resp. Dis. 96 111-14 (1967); C.A. 67 80847c (1967).
439. Wilson, D.M.; Lever, M. and Small, C.W.; Amer. J. Med. Technol. 39 451-3 (1973); C.A. 81 34m (1974).
440. Ellard, G.A.; Gammon, P.T. and Wallace, S.M.; Biochem. J. 126 449-58 (1972); C.A. 76 135487d (1972).
441. Boxenbaum, H.G. and Riegelman, S.; J. Pharm. Sci. 63 1191-7 (1974); C.A. 82 38374f (1975).
442. Miceli, J.N.; Olson, W.A. and Weber, W.W.; Biochem. Med. 12 348-55 (1975); C.A. 83 157655d (1975).
443. Peters, J.H.; Morse, C.W. and Schmidt, L.H.; Am. Rev. Respirat. Diseases 91, 225-31 (1965); C.A. 63 2245b (1965).

444. O'Barr, T.P.;Keith, D.J. and Blair, E.B.; Amer.Rev.Resp.Dis. 107 472-4(1973);C.A.79 27020z(1973).
445. Lauermann,I. and Otto, L.; Wiss.Z.Martin-Luther Univ.,Halle-Wittenberg,Math.-Naturwiss.Reihe 17,561-7(1968);C.A.71 28801b(1969).
446. Kane,P.O.; Nature 183,1674(1959);C.A. 55 7528i(1961).
447. Strickland,R.D. and Hentel, W.; Am.J.Clin. Pathol. 24,988-92(1954) and Tech.Bull Registry Med. Technologists 24,168-72 (1954);C.A.48 12376e(1954).
448. Harting, H. and v. Gerzanits, P.; Acta Med. et Biol. 2,643-48(1954);C.A.50 9484a(1956).
449. Ito,F.;Nasu,Y.;Mizobata,H.;Yuasu, M. and Nishi, K.; Kekkaku 33 345-7(1958);C.A.52 20351h(1958).
450. Ito, F.; Nasu,Y. and Nishi, K.; Med.J.Osaka Univ. 9 567-71(1958);C.A.53 13253h(1959).
451. Verrotti,M. and Bardelli,N.; Rass. studi psichiat.41,472-5(1952)C.A. 47 4410h(1953).
452. Schwenk,R.;Kelly,K.;Tse,K.S.and Sehon,A.H.; Clin.Chem.21 1059-62(1975);C.A.83 125974r (1975).
453. Bönicke,R.;Naunyn-Schmiedeberg's Arch.exptl. Pathol.u.Pharmakol. 216,490-3(1952);C.A.47 4937i(1953).
454. Cascio,G. and Purpura,R.;Bull.soc.ital.biol. sper. 28,1947-8(1952);C.A.49 6361c(1955).
455. Grosset, J.; Grumbach,F. and Canetti,G.; Ann.inst.Pasteur 92,752-9(1957);C.A.51 15678i(1957).
456. Strupczewska-Januszowa, H.; Gruzlica 29, 481-4(1961);C.A.56 15768i(1962).
457. Pregowski,W.; Zukowska, H. and Szymska,E.; Przeglad Lekar. 16,310-13(1960);C.A.57 4974a (1962).
458. Ginsburg,T.S. and Poddubny, A.F.;Lab Delo 9, 37-40(1963);C.A.62 6795g(1965).
459. Finn,E.R. and Vil'derman, A.M. Zdravookhranenie 39-43(1964);C.A.63 1077f (1965).

460. Tagawa,C.;Nippon Kagaku Rhohogakukai Zasshi 13,156-64(1965);C.A.64 14776g(1966).
461. Vil derman,A.M.; Finn, E.R. and Evgrafova, Z.A.;Vopr.Bor'by s Tuberkulezom,Minsk,Sb 119-22(1964)C.A. 64 4104f(1966).
462. Sen,P.K.;Saha,J.R. and Chatterjee,R.; Indian J. Med.Res. 60,19-27(1972);C.A.77 42992v(1972).
463. Kharizanova,T. and Simova,V.;Pr. Nauchnoizsled.Khim.-Farm.Inst. 8 353-8 (1972);C.A.79 38384x(1973).
464. Kharizanova,T. and Simova,V.;Advan. Antimicrob.Antineoplastic Chemother.; Proc.Int.Congr.Chemother., 7th; 1971 Part 2 697-9;C.A.79 73417g(1973).
465. Bartmann,K. and Freise,G.;Beitr.Klin. Tuberk 127 546-60(1963);C.A.60 5880g(1964).
466. Nishi,K.;Kekkaku 33 272-3(1958);C.A.52 16463i(1958).
467. Poole,N.F. and Meyer, A.E.;Proc.Soc.Exptl. Biol.Med. 98 375-7(1958);C.A.52 20340i(1958).
468. Makino,K.;Kinoshita,T. and Itoh,T.;Nature 173, 36(1954);C.A.48 5367f(1954).
469. Leuschner,F.;Arzneimittel-Forsch.4,686 (1954);C.A.49 2555g(1955).
470. Sezaki,H.;Yakugaku Zasshi 78,1211-15 (1958);C.A.53 8259e(1959).
471. Belles,Q.C. and Littleman,M.L.; Am.Rev. Respirat.Diseases 81,364-72(1960);C.A.55 11519c(1961).
472. Abiko,Y.;Onoue,K.;Yamamura,Y.; Nakazono,I. and Yoshida,T.; J. Biochem. (Tokyo)48, 838-45(1960);C.A.55 14691d(1961).
473. Okudaira,M.;Kuchii,M. and Hasaki,K.;Iryo 23, 1283-90(1969); C.A.73 23657r(1970).
474. Siegel,D.;Z.ges.inn.Med.u.ihre Grenzgebiete 13 66-68(1958);C.A.54 23032e(1960).
475. Barreto,R.C.R.;J.Chromatog.7 82-5(1962); C.A. 57 6246i(1962).
476. Barreto,R.C.R. and Sabino,S.O.;J.Chromatog. 9 180-6(1962);C.A.58 9395f(1963).
477. Fartushnyi,A.F. and Sukhin,A.P.;Sud.-Med. Ekspert. 16,39-43(1973);C.A.80 78879q(1974).

478. Cattaneo,C.;Fantoli,U. and Ferrari, S.; Ann.Ist. "Carlo Forlanini" 27, 78-93 (1967);C.A.68 11373d(1968).
479. Hughes,H.B.;J.Pharm.Exp.Ther. 109, 444(1953); C.A. 48 2241i(1954).
480. Ozawa,H. and Kujomoto,A.;Igaku To Seibutsaugaku 27,110(1953);C.A.48 1580f (1954).
481. Cuthbertson,W.F.J.;Ireland,D.M. and Wolff,W.; Biochem.J. 55,669(1953);C.A.48 1462i(1954).
482. Zamboni,V. and Defranceschi; Biochim. Biophys.Acta.14,430(1954);C.A.48 12218d(1954).
483. Mandel,W.;Heaton,A.D.;Russele,W.F. and Middlebrook, G.; J.Clin.Invest. 38,1356-1365 (1959);C.A.54 2590e(1960).
484. Evans,D.A.P.;Manley,K.A. and McKusick,V.A.; Br.med.J. 485-491(1960);C.A.55 11636g(1961).
485. Sunahara,S.;Urano,M. and Ogawa,M.;Science 134, 1530(1961).
486. Goodman,L.S. and Gilman, A.; The Pharmacological Basis of Therapeutics 5th Ed page 1205 MacMillan Publishing Co.Inc. New York.
487. Goldstein,A.;Aronow, L.and Kalman, S.M.; Principles of Drug Action: The Basis of Pharmacology 2nd Ed. page 452, John Wiley and Sons, New York.
488. Venkataraman,P.; Eidus, L.; Ramachandian, K. and Tripathy, S.P.; Tubercle (G.-B.) 46, 262(1965).
489. Hirtz,J.L.;Analytical Metabolic Chemistry of Drugs pages 191-198(1971),Marcel Dekker Inc.,New York.
490. Grebennik,L.I.;Sov.Med. 31,47-51(1968); C.A.69 34361h(1968).
491. Russell,D.W.; Clin.Chem.Acta. 41,163-8 (1972);C.A.78 52461f(1973).
492. Ritter,W.; Prax.Pneumol.27 139-45(1973); C.A. 79 61303d(1973).
493. Ellard,G.A. and Gammon,P.T.;Advan.Antimicrob. Antineoplastic Chemother.,Proc.Int.Congr. Chemother.7th 45-6 (1971);C.A.79 100376b (1973).

494. Mitchell,J.R. and Jollow,D.J.;Drug Interact. 65-79(1974);C.A.82 92730g(1975).
495. Iwamoto,T.;J.Pharm.Soc.Japan 74 36-9(1954); C.A. 48 5362b(1954).
496. Nakazono,I. and Hirata,H.;Kekkaku 34 101-6(1959);C.A.54 25288e(1960).
497. Toth,B. and Shimizu,H.;Eur.J.Cancer 9, 285-9(1973);C.A.79 101439m(1973).
498. Kakemi,K.;Arita,T.;Sezaki,H. and Takasugi, N.;Chem.Pharm.Bull. 13 551-7 (1965);C.A.63 7511c(1965).
499. Juchau,M.R. and Horita,A.;Drug Metabolism Reviews, DiCarlo F.J. Editor, Vol.1 pages 71-86, Marcel Dekker Inc. New York, 1973.
500. Meyer,H. and Mally,J.;Monatsh.Chem.33, 393(1912);C.A.6 2073(1912).
501. Chorine,V.; Compt.rend.acad.sci.220,150 (1945).
502. Huant,E.;Gazette des Hopitaux Aug.15(1945).
503. Long,E.S.;The Chemistry and Chemotherapy of Tuberculosis,3rd ed.,The Williams & Wilkins C Co.,Baltimore,1958.
504. Kirschbaum,A.;Pharm.Acta Helv.27 229-33 (1952);C.A. 47 2429b(1953).