# Método cuantitativo espectrofotométrico para determinación de Isoniazida en orina. Aplicación en atención farmacéutica

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**Resumen**

**Objetivo:** Este trabajo consiste en la determinación de isoniazida en orina por un método espectrofotométrico, rápido y sencillo susceptible de ser realizado en Atención Farmacéutica o enfermos con tuberculosis.
**Métodos y resultados:** Inicialmente se ha estudiado si el color rojo-violáceo que se produce en el Test de Eidius-Hamilton, cumple la ley de Lambert-beer, en que intervalos y que condiciones son las más idóneas para su realización. Se ha realizado una recta de regresión desde 5 hasta 200 gammas de n-acetilisoniazida obtenida según Fox HH y Gibas JT, estudiándose en disolventes agua y orina de individuo sano, obteniéndose valores similares. Se realizo también un análisis de una serie de muestras de n-acetilisoniazida para medir el error que resulta de 0.012.
**Conclusiones:** Del estudio concluimos que se puede realizar el análisis cuantitativo espectrofotométrico preferentemente en intervalos de 30 a 60 gammas de n-acetilisonoazida, siendo el limite máximo de 100 gammas.

**Palabras claves:** Isoniazida. N-Acetilisoniazida. Método Espectrofotométrico.

**Summary**

**Objective:** This work consists of the quantitative determination of Isoniazid in urine by a spectrophotometer method, quickly, simply and capable of being realized to patients that have tuberculosis for the pharmaceutical attention.
**Methods and Results:** Initially it has been studied if the red-purplish colour that takes place in the Eidus-Hamilton test complies with the law of Lambert-Beer, which intervals and which conditions are the most suitable for its accomplishment. A straight line of regression has been realized from 5 to 200 gammas of N-Acetilisoniazid obtained according to Fox HH. and Gibas JT, being studied using as dissolvers water and urine of healthy persons, and the values obtained are similar. There was realized also an analysis of a series of samples of N-Acetilisoniazid to measure the error, which results 0.012.
**Conclusions:** Of the realized study we conclude that is possible to realize the quantitative spectrophotometer analysis, preferably in the interval from 30 to 60 gammas of N-Acetilisoniazid, being the maximum limit of 100 gammas.

**Key words:** Isoniazid. N-acetilisoniazid. Spectophotometer method.

## Introduction

The directly observed treatment (T.O.D.) consists in giving to the patients the medicines that they need, while the capture is observed "in situ" by the corresponding sanitary professional[1,2]. This system that in case of the Comunidad Valenciana is realized institutionally (for patients with tuberculosis) thanks to agreement between the Coselleria of Health and Pharmacist's College, spreads as part of the Pharmaceutical Attention. Nevertheless there are numerous cases in which, because of reasons of work, calendar, etc.. of the patient, the capture can not be directly observed, then the medication is prepared for them in order that these patients take it in their domicile, daily or twice-weekly, in these cases a follow-up is carried out to verify the presence of Isoniazid in the urine (this medicine is common in all the treatments of tuberculosis) that the patients brings to the laboratory and that it is analysed following the method proposed by Eidus-Hamilton[3] and modified by Horacio Jiménez[4]. This qualitative, quickly and simple test might be applied by the pharmacy that realize pharmaceutical attention with patients with tuberculosis for cases that is not possible to do the T.O.D. in the strict sense.

Nevertheless, we believe that we should not remain only in the qualitative aspects of the test, but advance in quantitative aspects, in order to determinate the Isoniazid in urine by a spectrophotometer and quickly method that could be carried out in small laboratories and pharmacy, since the bibliography only gathers determinations by means chromatography serum and urine[5-10] analysis of more difficult accomplishment in

the pharmacy, or qualitative methods based on the Eidus-Hamilton test as the Arkansas[11-16] method which consists of adding to the pipes of analysis, a few drops of barbiturate acid , giving, when positive result, a blue-greenish or turquoise coloration, another method is the Ellard and Greenfield[17].

There are also described the tests called "Test of house" in which the reagent is impregnated in strips of paper, though they are merely qualitative[15,18]. In this respect is in which this work is realized (and which forms part of a doctoral thesis), and it tries to study if the red-purplish colour that takes place in the Eidus-Hamilton test complies with the law of Lambert-Beer and in what intervals is possible to measure quantitatively. A statistical study has been realized finally to support the measurements and the used method.

## Material and methods

Spectrophotometer Photometer 4010. Spinner Centromix. Pipette Capilettor de 0.01 a 5 ml. Absorption cells 1 $cm^2$. Isoniazid BP 93. Acetic Anhydride pure PANREAC.

The obtaining of N-Acetilisoniazid is realized following the skill described by Fox H.H. and Gibas TJ.[19] using the Isoniazid and the Acetic Anhydride in stequiometric quantities and dioxano as dissolver , the precipitate appears in 30-40 days, it is placed in a glass of broad mouth, it is washed with dioxano and it is left to dry up to constant weight, remaining it in a weight substances of silica gel.

A spectrophotometer sweep is realized to the red-purplish liquid obtained from the Eidus-Hamilton modified reaction to determinate the maximum of absorbance.

For the study of the Lambert-Beer law[20], watery dissolutions are prepared, in concentrations from 5 to 200 gammas (in intervals of 5 gammas) from dissolutions of 2 mgr/ml of N-Acetilisoniazid, and doing in all of them the Eidus-Hamilton modified reaction[4]. Several measurements have been realized to obtain the straight line regression, using as dissolvent water or urine of a healthy individual, in the latter case the urine is used as a target.

The problem dissolutions of N-Acetilisoniazid are prepared in concentrations of 20 to 50 gammas of N-Acetilisoniazid, and using as target the same dissolver.

## Results

In the spectrophotometer scanning at different wavelength, the maximum of absorbance is obtained at 546 nm as it is showed in the Figure 1, and all the spectrophotometer measurements are realized using this wavelength.

In the Figure 2 are represented the datum of absorbance gathered in the Table 1. The straight

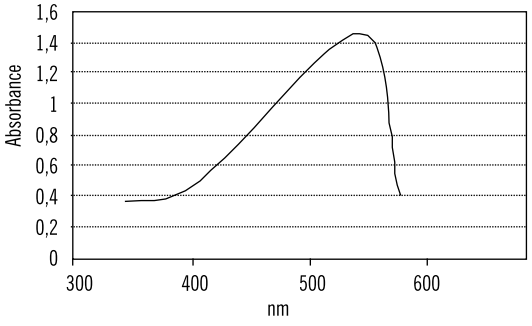


*Figure 1. Absorbance of the red-purplish color al different wavenenght*

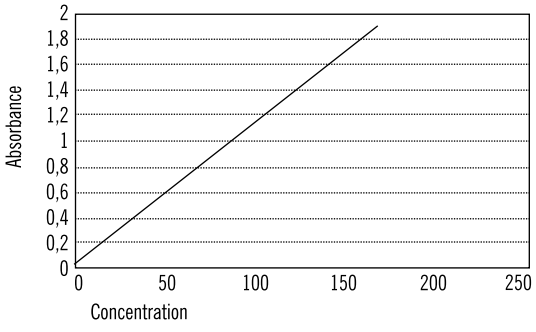


*Figure 2. Graphical representation of Table 2*

| C | 5 | 10 | 15 | 20 | 25 | 30 | 35 | 40 | 50 | 60 | 70 | 80 | 100 | 150 | 200 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 0.067 | 0.123 | 0.185 | 0.215 | 0.291 | 0.348 | 0.408 | 0.469 | 0.569 | 0.662 | 0.767 | 0.867 | 1.107 | 1.507 | 1.720 |

C: concentration gamma/ ml.. A: absorbance at 546 nm.

*Table 1. Absorbance obtained to different concentrations of N-Acetilisoniazid in a watery field.*

line of the linear regression is obtained by means the Microsoft Excel 2000 method.

In the Table 2 figure the values of absorbance of different problem samples studied in watery field, as well as the values of the theoretical prepared concentrations , also figure the values obtained from the extrapolation of absorbance in the straight line of concentrations (Figure 2), as well as the % of diversion of every measurement.

*Table 2. Calculated concentrations for several samples of watery solutions of N-Acetilisoniazid.*

| Sample | Real concentration (gamma) | Absorbance | Calculated concentration (gamma) | % of error |
|---|---|---|---|---|
| 1 | 20 | 0.220 | 19.6 | -2 |
| 2 | 20 | 0.239 | 21.1 | +5.5 |
| 3 | 20 | 0.229 | 20.3 | +1.5 |
| 4 | 20 | 0.255 | 22.5 | +12.5 |
| 5 | 20 | 0.245 | 21.6 | +8.0 |
| 6 | 25 | 0.326 | 27.9 | +11.6 |
| 7 | 25 | 0.264 | 22.5 | -1.0 |
| 8 | 25 | 0.281 | 24.9 | -0.4 |
| 9 | 25 | 0.282 | 24.9 | -0.4 |
| 10 | 25 | 0.283 | 24.9 | -0.4 |
| 11 | 25 | 0.320 | 27.8 | +11.2 |
| 12 | 30 | 0.313 | 28.8 | -4.0 |
| 13 | 30 | 0.315 | 29.6 | -1.2 |
| 14 | 30 | 0.332 | 30.8 | +2.4 |
| 15 | 30 | 0.359 | 32.5 | +8.3 |
| 16 | 30 | 0.315 | 29.6 | -1.2 |
| 17 | 30 | 0.328 | 30.3 | +1.0 |
| 18 | 30 | 0.317 | 29.7 | -1.0 |
| 19 | 35 | 0.411 | 36.1 | +3.1 |
| 20 | 35 | 0.379 | 33.8 | -8.3 |
| 21 | 35 | 0.400 | 35.5 | +1.4 |
| 22 | 35 | 0.382 | 33.9 | -3.1 |
| 23 | 35 | 0.412 | 36.2 | +8.3 |
| 24 | 35 | 0.412 | 36.2 | +8.3 |
| 25 | 35 | 0.434 | 37.6 | +7.3 |
| 26 | 35 | 0.393 | 34.5 | -1.4 |
| 27 | 40 | 0.462 | 39.1 | -2.2 |
| 28 | 40 | 0.457 | 38.7 | -3.2 |
| 29 | 40 | 0.477 | 39.8 | -1.2 |
| 30 | 40 | 0.463 | 39.2 | -2.0 |
| 31 | 40 | 0.491 | 41.1 | +2.7 |
| 32 | 40 | 0.540 | 43.8 | +9.3 |
| 33 | 40 | 0.493 | 41.1 | +1.8 |
| 34 | 40 | 0.435 | 37.3 | -6.8 |
| 35 | 50 | 0.585 | 50.5 | +1.0 |
| 36 | 50 | 0.608 | 52.1 | +4.2 |
| 37 | 50 | 0.559 | 48.5 | -3.0 |
| 38 | 50 | 0.579 | 50.0 | 0.0 |
| 39 | 50 | 0.559 | 48.5 | -3.0 |
| 40 | 50 | 0.594 | 51.2 | +1.4 |
| 41 | 50 | 0.549 | 47.7 | -4.6 |

For the accomplishment of the statistical study it has been taken as the Y value of the concentrations of N-Acetilisoniazid of the real samples, and as the X values the measured absorbance that figure in the Table 2, in the first and in the second column respectively.

We accept as valid the linear regression X/Y simply on having visualized the graph of dispersion (Figure 3), which is the following one:

$Y^* = a + bX = 1.836 + 81.889X$.

Y shows a coefficient of determination R2 = 0.957, which supports the kindness of the regression.

The value t associated with the slope b of the straight line, presents a signification of 0.000, confirming the existence of the linear relation between X and Y.

From the previous values, the concentrations estimated for the available absorbance (V) , together with the residues in absolute value (W) and percentages (%), remain reflected in the Table 3 and 4.

Studying the residues in percentages - variables % - we appreciate again the good linear adjustment between the variables X and Y. The statistician of Burbin-Watson which takes the value 1.612, assures the independence that demands the specification of the model.

The percentiles for the variable % are reflected in the Table 3, and the principal statistician for the 41 observations of the variable % are reflected in the Table 4.

## Discussion

Inside the programs of pharmaceutical attention that are followed in the Comunidad Valenciana for the agreement between the Generalitat and the pharmacists's official college, we find the T.O.D. (Directly observed treatment) for patients of pulmonary tuberculosis, so much in its curative character, as preventive (T.I.L.), which consists of that the patient takes the treatment in front of the sanitary professional, so much if it is daily or twice-weekly. Nevertheless in many occasions this direct observation is not possible, for reasons of work, distance, etc, and in order that the prevalence is effective, the treatment is given to take it in the domicile (with a guideline in writing that explains it to them) in this case the patient has to bring to the clinic or the pharmacy the urine gathered in a few suitable conditions (Jimenez, *et al.*), where there carries out a qualitative analysis of the Isoniazid (present in all the treatments), according to Eidus-Hamilton test, to know if the takings have been realized.

Due to the hepatotoxicity of the Isoniazid, the clinical professional has to suspend or to decrease the treatment in case that the hepatic markers or the concentration of Isoniazid increase to much, that is the reason why sometimes is suitable to measure quantitatively the concentration in urine. The methods of measurement of Isoniazid in urine are usually very sophisticated and are difficult to accomplish in small laboratories or pharmacys.

If we observe the Figure 3 we can appreciate that for concentrations from 5 to 100 gammas of N-Acetilisoniazid there is obtained a regression straight line of R2 =0.9987, and from this concentration it does not follow a linear correlation; so we can deduce that the test complies with the law of the Lambert-Beer for concentrations not superior to 100 gammas of N-Acetilisoniazid, and not for superiors concentrations. So it is advised to dilute the samples in case the concentrations are superior to 100 gammas, being advisable that these concentrations should be include in the interval from 30 to 660 gammas as is demonstrated in the information gathered in the Table 4.

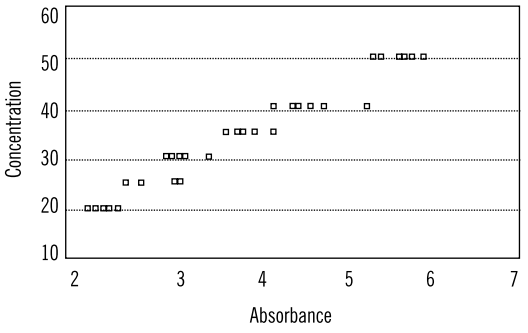


*Figure 3.*
*Graph of dispersion*

| | | | | | | |
|---|---|---|---|---|---|---|
| 5 | 10 | 25 | 50 | 75 | 90 | 95 |
| -12.5 | -10.4 | -3.6 | 0.6 | 5.0 | 7.7 | 8.6 |

*Table 3.*

| Datum | Rank | Minimum | Maximum | Average | Standard deviation |
|---|---|---|---|---|---|
| 41 | 22.4 | -13.2 | 9.2 | -0.06249 | 6.018 |

*Table 4.*

Analysing the information of the statistical study present at Table 5, we observe that the 80% of the effected valuations using the regression straight line, present mistakes -expressed in percentages- fitted inside the interval (-10.4, 7.7), so that only a 10% of the observations present mistakes that overcomes the above mentioned interval with positive sing, and the other 10% overcomes it with negative sign. The maximum mistake committed by excess is of 9.2% and for fault of 13.2%, acceptable values for a quantitative analysis.

*Table 5. Concentrations of N-Acetilisoniazid, absorbance and errors of the taken measures*

| X | Y | V | W | % |
|---|---|---|---|---|
| 20 | 0.220 | 19.9 | 0.1 | 0.7 |
| 20 | 0.239 | 21.4 | -1.4 | -6.6 |
| 20 | 0.229 | 20.6 | -0.6 | -2.9 |
| 20 | 0.255 | 22.7 | -2.7 | -12.0 |
| 20 | 0.245 | 21.9 | -1.9 | -8.7 |
| 25 | 0.326 | 28.5 | -3.5 | -12.4 |
| 25 | 0.264 | 23.5 | 1.5 | 6.6 |
| 25 | 0.281 | 24.9 | 0.1 | 0.6 |
| 25 | 0.282 | 24.9 | 0.1 | 0.3 |
| 25 | 0.283 | 25.0 | 0.0 | -0.1 |
| 25 | 0.320 | 28.0 | -3.0 | -10.9 |
| 30 | 0.313 | 27.5 | 2.5 | 9.2 |
| 30 | 0.315 | 27.6 | 2.4 | 8.6 |
| 30 | 0.332 | 29.0 | 1.0 | 3.4 |
| 30 | 0.359 | 31.2 | -1.2 | -4.0 |
| 30 | 0.315 | 27.6 | 2.4 | 8.6 |
| 30 | 0.328 | 28.7 | 1.3 | 4.5 |
| 30 | 0.317 | 27.8 | 2.2 | 7.9 |
| 35 | 0.411 | 35.5 | -0.5 | -1.4 |
| 35 | 0.379 | 32.9 | 2.1 | 6.5 |
| 35 | 0.400 | 34.6 | 0.4 | 1.2 |
| 35 | 0.382 | 33.1 | 1.9 | 5.7 |
| 35 | 0.412 | 35.6 | -0.6 | -1.6 |
| 35 | 0.412 | 35.6 | -0.6 | -1.6 |
| 35 | 0.434 | 37.4 | -2.4 | -6.4 |
| 35 | 0.393 | 34.0 | 1.0 | 2.9 |
| 40 | 0.462 | 39.7 | 0.3 | 0.8 |
| 40 | 0.457 | 39.3 | 0.7 | 1.9 |
| 40 | 0.477 | 40.9 | -0.9 | -2.2 |
| 40 | 0.463 | 39.8 | 0.2 | 0.6 |
| 40 | 0.491 | 42.0 | -2.0 | -4.9 |
| 40 | 0.540 | 46.1 | -6.1 | -13.2 |
| 40 | 0.493 | 42.2 | -2.2 | -5.2 |
| 40 | 0.435 | 37.5 | 2.5 | 6.8 |
| 50 | 0.585 | 49.7 | 0.3 | 0.5 |
| 50 | 0.608 | 51.6 | -1.6 | -3.2 |
| 50 | 0.559 | 47.6 | 2.4 | 5.0 |
| 50 | 0.579 | 49.3 | 0.7 | 1.5 |
| 50 | 0.559 | 47.6 | 2.4 | 5.0 |
| 50 | 0.594 | 50.5 | -0.5 | -1.0 |
| 50 | 0.549 | 46.8 | 3.2 | 6.8 |

Using urine of a healthy person as dissolver of the N-Acetilisoniazid and as a target, practically equal results have been obtained that in watery field.

We can conclude that the quantitative analysis of Isoniazid in urine, in patients with pulmonary tuberculosis who take this medicine, it is possible to realize by a simple, spectrophotometer, cheap and capable of being able to be realized in a pharmacy that can do Pharmaceutical Attention to tubercular patients , since in cases of a concentration excessively raised of Isoniazid in urine it might warn the doctor in order that him/her diminishes or withdraw the dose, since it can produce hepatotoxicity in some patients[21]. The concentrations more adapted for the accomplishment of the test are included in the interval from 30 to 60 gammas of N-Acetilisoniazid, that is when the mistakes are minor, therefore it is advised to dilute or to concentrate in the cases of major or minor concentration respectively, although the latter case is less probable.

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