# THE THERMOMETRIC DETERMINATION OF ISONICOTINIC ACID HYDRAZIDE IN ISONIAZID TABLETS

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The determination of isonicotinic acid hydrazide in the dosage form of isoniazid tablets is done using thermometric titrimetry. The hydrazide is oxidized by hexacyanoferrate(III) ions. It is not necessary to separate the active ingredients from the powdered sample. The results from the proposed method are compared with those obtained using the standard B.P. method.

Changes in attitudes towards absolute tolerances in the amounts of active constituents in tablets have led to an interest in methods that are capable of assaying single dosage forms. The high labour cost of analysis has made it advisable to attempt to use automatic methods, especially for routine assays. Several methods are available for this type of analysis, including thermometric determinations, for the assay of pharmaceuticals that contain an active ingredient mixed with several inactive components, *viz.* the matrix of the excipients.

This paper describes a method for the determination of the active ingredient, isonicotinic acid hydrazide, in isoniazid tablets. The tablets are used in the treatment of tuberculosis. The thermometric method described has proved successful and it has been shown that the matrix materials are thermally neutral, i.e. do not take part in the proposed reaction. An enthalpimetric method was used to determine the heat of the reaction.

Previous work on the reaction between isonicotinic acid hydrazide and potassium hexacyanoferrate(III) has been studied by potentiometric and volumetric titrimetry [1]. The reaction is said to proceed instantaneously and quantitatively in 10–25% w/v potassium hydroxide.

The use of hexacyanoferrate(III) as an oxidimetric titrant is well reported in the literature. It has the advantage that it maintains its electrode potential over a range of alkaline concentrations (1-2*M* potassium hydroxide).

## Experimental

*Thermometric system*

Details of the apparatus have been previously described [2]. The reaction vessel was surrounded by a block of polystyrene and all the work carried out in a constant temperature laboratory at 23° (±0.2°).

The basic circuit of the electrical bridge system has been previously reported [3], and the temperature sensor used was a thermistor with a nominal resistance of 10k Wat 25°.

The titrant was delivered by an LKB peristaltic pump, delivering titrant at a rate of about 0.28 $cm^3$ $min^{-1}$. The actual rate was determined by gravimetry. The mixtures were stirred magnetically.

*Enthalpimetric system*

Details of the apparatus have again been previously described [4]. The same bridge circuit was used but stirring was by microstirrer. The titrant was injected rapidly using a simple hypodermic syringe with a capacity of 2 $cm^3$ and the pipettes were designed to facilitate adequate stirring and the rapid attainment of thermal equilibrium throughout the reaction cell.

*Reagents*

Potassium hexacyanoferrate(III)

Approximately 0.6*M* aqueous solutions of potassium hexacyanoferrate(III) ($K_3Fe(CN)_6$), were accurately prepared by dissolution of the appropriate amount of solid in water. During the investigation, fresh solutions were prepared daily.

Isonicotinic acid hydrazide (Isoniazid)

General reagent grade "Isoniazid" was recrystallized from water. The crystals were then dried in an air oven at 45 °C for several hours.

Approximately 0.1*M* aqueous solutions were prepared by dissolving the appropriate amount of the recrystallized solid in water.

Potassium hydroxide

A 20% w/v aqueous solution of potassium hydroxide was prepared.

Assay of recrystallized isoniazid

1 $cm^3$ of isoniazid solution was pipetted into a plastic reagent bottle and 14 $cm^3$ of 20% w/v potassium hydroxide added. When thermal equilibrium had been attained the solution was titrated with the potassium hexacyanoferrate(III) solution delivered by the automatic pump. The curve was recorded on a Servoscribe 1S strip recorder (f.s.d. = 50 mV, chart speed = 60 mm $min^{-1}$).

The result was that 100 mm of chart corresponded to $0.81(5) \times 10^{-4}$ moles of isoniazid, i.e. 11.16 mg of isoniazid.

Assay of isoniazid tablets

6 isoniazid tablets, nominally containing 0.1 g of isoniazid per 0.3 g tablet, were crushed and weighed.

(i) *Thermometric method of assay*

Known amounts of the powdered tablet were stirred with 1 $cm^3$ of distilled water and 14 $cm^3$ of 20% w/v potassium hydroxide and assayed by the method used for recrystallized isoniazid.

(ii) *Volumetric B.P. method of assay*

A known amount of the powdered tablet (*ca.* 1.2 g) was dissolved as completely as possible in water. The solution was filtered, the residue was washed and the combined filtrate and washings were diluted to 250 $cm^3$. The solution was assayed by the recommended B.P. method [5]. This involved reacting the isoniazid with an excess of bromine under acidic conditions. Potassium iodide was then added (the excess of the bromine oxidizing the iodide to iodine),and the iodine produced was titrated against standardized sodium thiosulphate solution using freshly prepared starch as the indicator. The titration was repeated without isoniazid in order to obtain the requisite blank value.

*Enthalpimetric method for the determination of the heat of the reaction*

This was carried out on the recrystallized isoniazid (the inactive components of the tablets have been shown to have no effect on the heat of the reaction, i.e. thermally neutral).

1 $cm^3$ of isoniazid solution was mixed with 14 $cm^3$ 20% w/v potassium hydroxide solution and the mixture was stirred until thermal equilibrium was attained. Approximately 1 $cm^3$ of about 0.6*M* potassium hexacyanoferrate(III) was injected into the solution and the heat pulse recorded. To eliminate dilution effects, the experiment was repeated without isoniazid solution, i.e. 1 $cm^3$ of distilled water replaced the 1 $cm^3$ of isoniazid solution. The system was calibrated by injecting about 1 $cm^3$ of 2*M* hydrochloric acid into 15 $cm^3$ of a TRIS solution of known concentration. The heat of the reaction between TRIS and hydrochloric acid has been previously established [6].

*Reproducibility of results*

Two series, each of 15 identical samples of isoniazid solution, were assayed by the proposed thermometric method. The nominal weights in each series were 14 mg and 5.0 mg, respectively. The sensitivity of the method was adjusted by altering the speed of the chart from 60 mm $min^{-1}$ to 120 mm $min^{-1}$ as appropriate.

## Results

(i) *Thermometric method of assay*

The results are presented in Table 1. The average mass of the tablet was 300.1 $\pm$ 0.1 mg. The calculated average amount of isoniazid in each tablet was 96.65 mg. The calculation graph is linear and passes through the origin.

Table 1

| Wt. of tablet used, mg | Chart dist., mm | Wt. of isoniazid in sample, mg | Wt. of isoniazid per g of tablet, mg |
|---|---|---|---|
| 20.4 | 58.3 | 6.50 | 95.7 |
| 30.4 | 88.0 | 9.82 | 96.9 |
| 42.9 | 124.3 | 13.87 | 96.9 |
| 50.8 | 146.1 | 16.34 | 96.6 |
| 60.6 | 175.5 | 19.59 | 96.9 |
| 70.0 | 203.0 | 22.66 | 97.2 |

(ii) *B.P. method of assay*

Aliquots of the sample were assayed and the average amount of isoniazid present in each tablet was calculated to be 96.7 mg.

(iii) *Determination of the heat of reaction*

$1.487 \times 10^{-3}$ moles of TRIS, when reacted with HCl, produced a heat pulse corresponding to 38.35 mm of chart. $1.024 \times 10^{-4}$ moles of isoniazid, when reacted with potassium hexacyanoferrate(III) under identical conditions, produced a heat pulse corresponding to 38.69 mm.

The heat of the TRIS reaction is reported as 47.44 kJ $mol^{-1}$ (evolved) and hence the heat of reaction between the isoniazid and the potassium hexacyanoferrate(III) under the same conditions is calculated as 695 kJ $mol^{-1}$ (isoniazid) (evolved).

(iv) *Tests of reproducibility*

The results are shown in Table 2. The standard deviation is 0.59 mm for 5 mg amounts of isoniazid, which corresponds to a deviation of 0.006 mg.

The standard deviation for 13.99 mg amounts of isoniazid is 0.40 mm, which corresponds to 0.005 mg. These are considered acceptable for a routine method.

Table 2

Reproducibility
Amount of isoniazid:

| Run No. | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| Chart distance *A* | 85.5 | 86.2 | 85.5 | 86.7 | 86.5 | 86.8 |
| Chart distance *B* | 123.3 | 123.8 | 123.5 | 123.4 | 123.1 | 123.2 |

The chart speed in series *A* was 120 mm per min; in series *B* it was 60 mm per min

of the method
$A = 5.0$ mg; $B = 13.99$ mg

| 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
|---|---|---|---|---|---|---|---|---|
| 86.4 | 86.5 | 85.6 | 86.5 | 86.5 | 86.5 | 85.8 | 86.1 | 86.2 |
| 123.2 | 123.4 | 123.2 | 122.7 | 122.0 | 123.0 | 122.8 | 122.6 | 123.0 |

## Discussion of results

Since the calibration graph passes through the origin it indicates that no heat is produced from the tablet matrix on injection of the reagent. Although the work was done using sample weights below that of a single tablet, it is easily possible to determine the active ingredient of a single tablet by this method. It is only necessary to decrease the sensitivity of the recorder and/or to increase the concentration of the hexacyanoferrate used.

The advantages of the thermometric method is that it is not necessary to separate the isoniazid from the matrix. The method compares favourably both in accuracy and precision with the standard B.P. method and is more advantageous with respect to time of analysis and to the assay of single tablets. The B.P. titrimetric method involves the use of bromine solutions and these require frequent standardization.

Since the heat of reaction is relatively large, 695 kJ $mol^{-1}$ (cfd. to $\Delta H$ for HCl/NaOH of 55.5 kJ $mol^{-1}$), the method is extremely sensitive and can be used for small amounts with acceptable precision and accuracy.

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## References

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ZUSAMMENFASSUNG — Die Bestimmung von Isonicotinsäurehydrazid in der Dosierungsform von Isoniazid-Tabletten wird durch Anwendung der thermometrischen Titration durchgeführt. Das Hydrazid wird durch Hexacyanoferrat(III)-Ionen oxidiert. Die Trennung der aktiven Inhaltsstoffe von der pulverförmigen Probe ist nicht erforderlich. Die Ergebnisse der vorgeschlagenen Methode werden mit den der Standard. — B. P.-Methode verglichen.

Резюме — Используя термометрическую титриметрию, выполнено определение гидразида изоникотиновой кислоты, находящегося в дозированной форме в таблетках изониазида. Гидразид окисляли гексацианоферрат(III) ионами. Не было необходимости выделять активные компоненты из порошкового образца. Результаты, полученные на основе этого метода, сопоставлены с результатами, полученные на основе этого метода, сопоставлены с результатами, полученными на основе стандартного БП метода.