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Potentiotitrimetric Determination of Isoniazid Using Twin Copper Based Mercury Film Electrode

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This study deals with the quantitative determination of isoniazid by employing twin copper based mercury film electrode of different lengths to improve the analytical power of detection and the reliability of the results gained and to improve the economy of the method employed for the routine determination of isoniazid in pharmaceutical formulations. The electrode is specialy fabricated for this titration.

Key Words: Isoniazid, Potentiotitrimetric, Micro electrode.

INTRODUCTION

In the early sixties, potentiometric titrations using dissimilar electrode pairs was applied to produce first derivative type curves. The voltage peak coincides with the equivalence point and is suitable for the quantitative determination of substance studied. Yakubik *et al.*[1] used glass silver electrodes for the quantitative determination of acids. Silver nitrate has been used extrensively as a titrant for the titrimetric determination of inorganic ions/compounds such as halides, cyanide, iodate, sulphide, phosphate and organic compounds such as tetraphenyl borate, thiourea and isoniazid. Vulterin and Zyka[2] proposed a method for the determination of silver ion with isoniazid as a reducing titrant in ammonia and alkali hydroxide medium by using metallic silver and saturated calomel electrodes. This method is quantitative and Ag^+ ion is reduced to metallic silver as follows.

$$4Ag[NH_3]_2^+ + C_5H_4N\cdot CONHNH_2 + 4OH^- \rightarrow 4Ag + C_5H_4NCOOH + N_2 + 8NH_3 + 3H_2O$$

Hanselman *et al.*[3] proposed a rapid and sensitive method for the micro determination of silver ion with iodide. They used two platinum microelectrodes of different sizes electroplated with silver, when the emf was plotted against volume of titrant, a curve similar to first derivative curve was obtained. They have given various reasons to explain the electrode response which are rather empirical and not very much convincing. According to them, the main reasons are the contractile stress corrosion and expansive stress corrosion produced during the electroplating of micro electrodes. No further explanation of contractile and expansive stress corrosion is given. Besides they found that the reversal of leads to pH meter had no effect on the electrode response.

The literature survey reveals that no work has been reported for the quantitative determination of isoniazid by employing twin copper based mercury film electrode of different lengths. Hence it was thought that it would be worth investigating the behaviour of such electrodes in the argentometric determination of isoniazid. In present studies a pair of copper based mercury film micro electrodes of different sizes have been tried for the potentiotitrimetric determination of isoniazid based on the reaction proposed by Vulterin and Zyka[2].

EXPERIMENTAL

0.1 M Silver nitrate solution: 0.1 M Silver nitrate solution was prepared and standardized against sodium chloride solution[4].

0.02 M Isonictonic acid hydrazide (isoniazid) solution: The 0.02 M solution of isoniazid was prepared in distilled water and standardized with potassium dichromate solution by the method recommended by Vulterin and Zyka[2].

Ammonia solution: 1:1 Ammonia solution (Merck) was prepared by diluting liquor ammonia solution (25 % v/v).

75 % Sodium hydroxide solution.

Concentrated hydrochloric acid (11.3 M).

0.02 M Mercuric nitrate: The 0.02 M solution of mercuric nitrate was prepared in distilled water by dissolving 1.7 g mercuric nitrate monohydrate in 400 mL water containing 10 mL nitric acid (2.00 M) and diluting to 500 mL.

Fabrication of mercury coated twin copper microelectrodes: Copper wire is cleaned by aberration and treated with dilute nitric acid (1 M) for 2 min. It is then washed several times in distilled water, air dried and cut into two pieces of 1.0 and 2.3 cm lengths. The mercury coated twin copper electrode assembly was fabricated by twisting two plastic sleeved copper wire (99 % purity) of 15 cm length and 1 mm thickness. The plastic sleeve was removed at one end to expose about 1.0 and 2.3 cm. Araldite was applied at the junction of the copper wire and the plastic sleeve to prevent the entry of solution into the sleeve. The length of mercury coated copper wire electrodes thus fabricated was 2.0 and 0.7 cm. The fabricated twin mercury coated copper electrode assembly is dipped in 0.02 M acidified mercuric nitrate solution for 10 min for uniform coating of mercury on the twin copper electrodes. Electrical contact was made to a pH meter through the free ends of the copper wire (Aligator clip). The electrode assembly is shown in Fig. 1.

Procedure for the potentiometric determination of isoniazid: An aliquot of silver nitrate (0.01 M) was transferred into the titration vessel followed by 5 mL each of ammonia (1:1) and sodium hydroxide (75 %) solutions and diluted to about 75 mL. The twin microelectrode assembly was immersed into the stirred solution and titrated potentiometrically with approximately diluted isoniazid solution taken in a semi-microburette. Potential *versus* volume of titrant was plotted to locate the equivalence point.

Determination of isoniazid in pharmaceutical preparations: The commercially available isoniazid tablets such as Isokin-300, isocaldin, isonex, isocaldin syrup have been analyzed by the following procedure. Five tablets of each variety was taken and weighed accurately. A known weight of the finely powdered sample was taken and diluted with water to a known volume. The general procedure given above was followed.

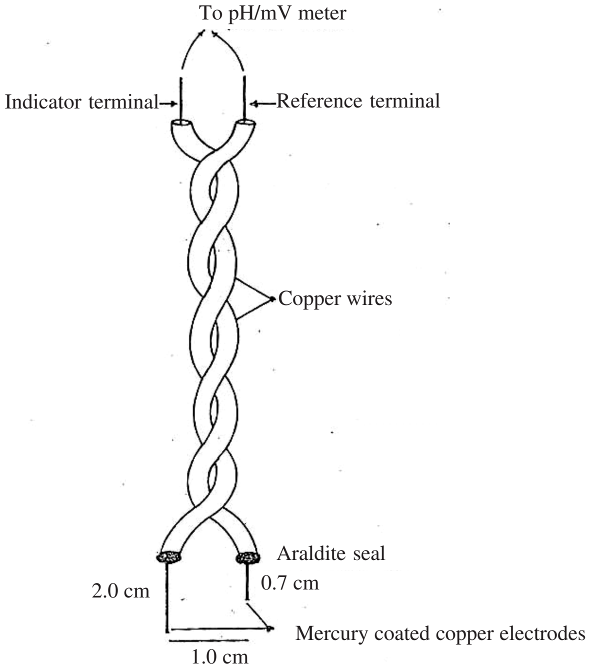

Fig. 1. Twin copper based mercury film electrode assembly for potentiotitrimetry

The amount of isoniazid present in each tablet is given by the formulae.

$$\frac{1}{4}\times 137.14\times M\times V\times\frac{\overline{B}}{B}\frac{A}{a}$$

M = Molarity of silver nitrate solution, V = volume of silver nitrate solution, $\overline{B}$ = average weight of the tablet, B = weight of the sample taken for analysis, A = total volume of the made up solution, a = volume of aliquot taken for analysis.

Isoniazid preparations were also determined by British pharmacopoeia method (1988)[5] and compared with the proposed methods.

RESULTS AND DISCUSSION

Fig. 2 shows the potentiometric curve of iodide with Ag^+. While the short electrode was connected to indicator terminal and the long electrode to reference terminal of the pH meter, the curve resembled the usual potentiometric curve. The same experiment was repeated by titrating iodide with silver nitrate taken in burette.

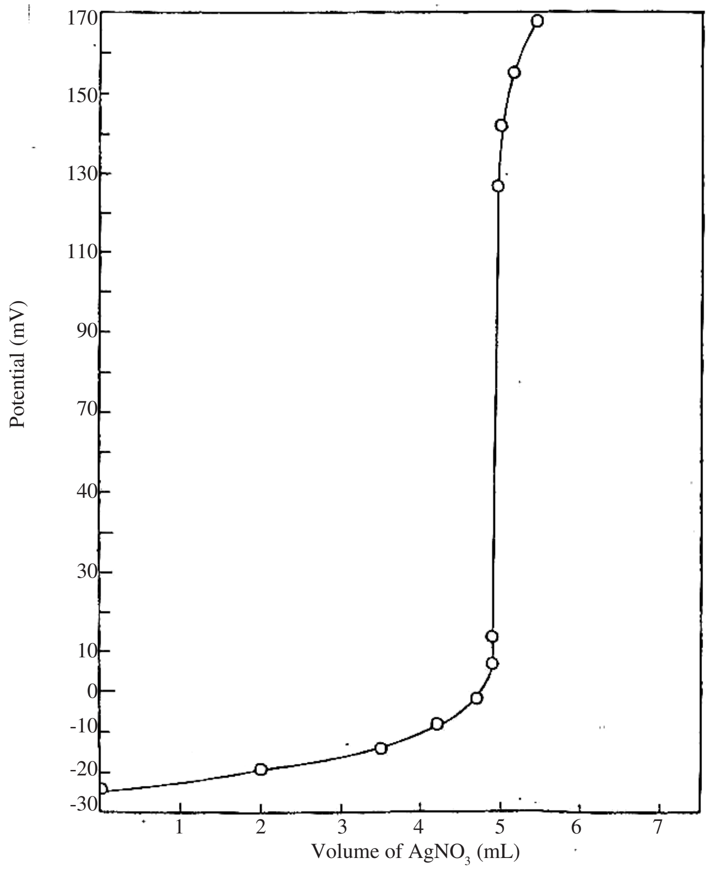

Fig. 2. Titration curve obtain with twin copper based mercury film microelectrodes (Ref.: long electrode, Ind: short electrode) for the titration of silver nitrate with iodide

Fig. 3 shows the titrimetric curve which resembles a first derivative curve in conventional potentiometry. Since the aim of the present work is to develop a simple method for routine estimation of isoniazid in pharmacentical preparations, various preliminary investigations were carried out. For example, silver nitrate mixed with NaOH and NH_3 was titrated with isoniazid solution, using twin copper based mercury film micro electrodes of equal lengths (1 cm each). The curve is shown in Fig. 4 although the results were accurate and precise, the stabilization of potential was slow throughout the titration.

The same experiment was repeated using twin copper based mercury film micro electrodes of different lengths. The short electrode was connected to indicator and long electrode was connected to reference terminals, respectively. The resultant curve is shown in Fig. 5. In this case too, the reproducibility was poor. In the next attempt, the experiment was repeated with the short electrode connected to reference terminal and long electrode to the indicator terminal. The resulting curve was similar to first derivative curve and is shown in Fig. 6. In this case the reproducibility was good

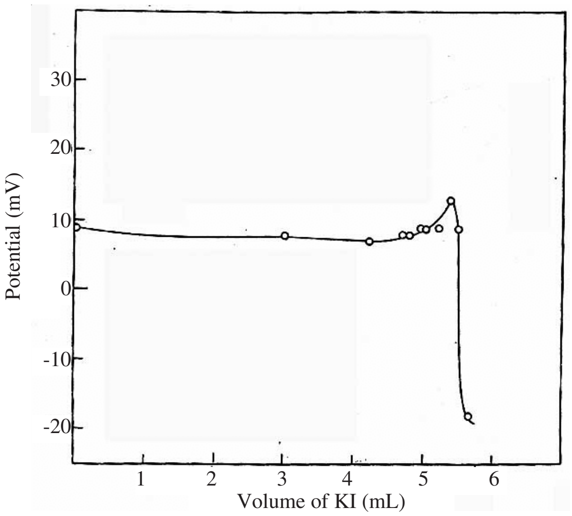

Fig. 3. Titration curve obtained with twin copper based mercury film electrodes of different lengths (Ref.: short electrode, Ind: long electrode) for the titration of silver nitrate with iodide

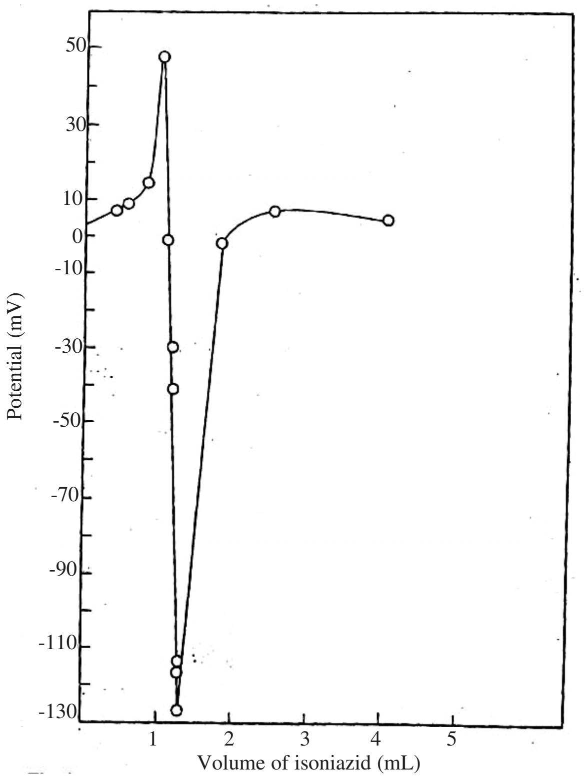

Fig. 4. Titration curve obtained with twin copper based mercury film microelectrodes of equal lengths for the titration of silver nitrate with isoniazid

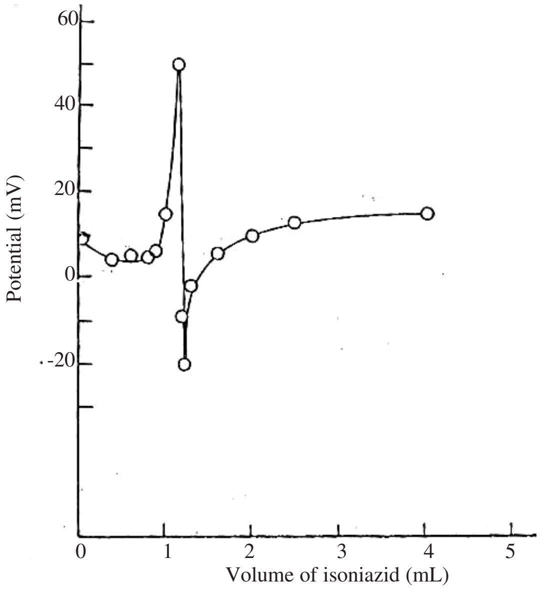

Fig. 5. Titration curve obtained with twin copper based mercury film microelectrodes of different lengths (Ref.: long electrode; Ind: short electrode) for the titration of silver nitrate with isoniazid

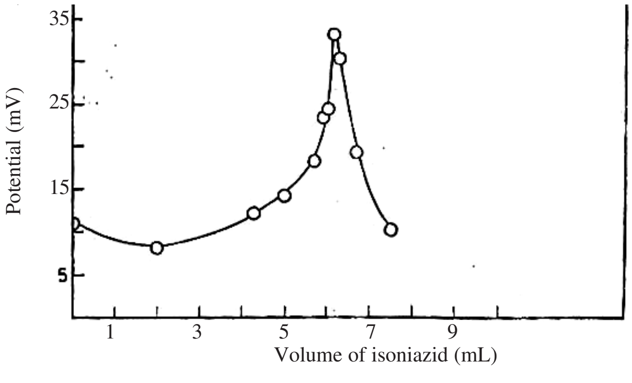

Fig. 6. Titration curve obtained with twin copper based mercury film electrodes of different lengths (Ref.: short electrode, Ind: long electrode) for the titration of silver nitrate with isoniazid

and hence further investigation was done with short electrode connected to reference terminal and long electrode connected to indicator terminal, respectively. The reverse titration, that is isoniazid titrating against silver nitrate was attempted because of the instability of isoniazid in alkaline medium. The available data is not sufficient at present to give an unambiguous explanation of electrode response. We are using two copper based mercury film electrodes of different lengths, it is possible that the difference in surface area is responsible for a first derivative type curve.

Effect of ammonia and sodium hydroxide: The concentration of ammonia should be more than 1-2 % in the total volume of about 75 mL of the titrant solution for the effective formatin of amine complex with silver ions. A media of ammonia, however is not sufficient for the quantitative reduction of silver so that solutions must be used containing besides ammonia, 20-35 % of an alkali metal hydroxide. At lower concentrations of ammonia and sodium hydroxide solutions the peak of the curve was not well defined. Under the optimum experimental conditions, a series of titrations were performed at different concentrations of isoniazid. 10-70 mg of isoniazid could be quantitatively determined by this method.

Precision and accuracy: In order to study the precision and accuracy of the proposed method, standard solutions containing four different concentrations of isoniazid were prepared and five replicate analyses were made on each standard isoniazid solution. The overall relative standard deviation (RSD) for 5 determination was 0.116 %, while the standard analytical error (SD/√n) did not exceed 0.01. The results of this study are given in Table-1.

TABLE-1
RESULTS OF FIVE REPLICATE DETERMINATION OF ISONIAZID AND STATISTICAL ANALYSIS OF THE DATA

Amount taken (mg)	Amount found (mg)	Mean (x) ± SD	Mean recovery (%)	Standard analytical error (s/√n)
10	9.88, 9.94 9.94, 9.92, 9.92	9.92 ± 0.0282	99.2	0.0126
20	20.15, 20.15, 20.12, 20.18, 20.18	20.16 ± 0.0251	100.8	0.0112
36	35.88, 35.88, 35.92, 35.92, 35.94	35.91 ± 0.0268	99.8	0.0119
48	48.11, 48.11, 48.06, 48.06, 48.04	48.08 ± 0.0321	100.2	0.0143

Linear regression: Linear regression (also known as method of least squares) is usually regarded as a mathematical method of fitting 'the best straight line' through bivariate data points. One axis of regression graph is used for the results obtained (measured value) and the other axis for true concentration. If each measured value (y) is identical with true concentration (x) the regression line will have a zero intercept (a) and slope (b) of unity and straight line plot takes the algebric form y = bx + a. The line thus drawn or calculated is known as line of regression of y on x, *i.e.*, the line indicating how varies, when x is set to chosen values. In analytical practice, calculation of regression line is important because it helps to recognise the fixed bias or relative bias of a newly developed method. If the values of the intercept differs from zero, there is a "constant systematic error", while the value of slope different from unity indicates a "proportional systematic error". These observations are useful especially to minimise the error by modification of the experimental conditions.

The appropriate formulae for calculating a, b and their standard deviations are given below, regression calculations are generally performed on a programmed calculator or a personal computer. The maximum available number of significant figures are used in such calculations rounding only in the end.

In Table-2 analytical data for the potentiometric determination of isoniazid using twin copper based mercury film electrodes of different lengths are given. Applying regression analysis to the data presented below, -0.0017 and 1.002 are obtained for a and b, respectively. The regression equation y = 1.002x - 0.0017 suggest that the method has fixed bias of 0.0017 and relative bias of 0.10 % (*i.e.*, 1.002 is 100.2 %). In practice, the analytical chemist commonly wishes to test for an intercept differing from zero and slopes differing from unity. Such tests performed by determining the estimates of standard deviation of slopes S_b, intercept S_a and of the scatter around the regression line $S_{y/x}$ generally 95 % confidence limit. Using a = 0.0018, t = 2.57, (five degrees of freedom, t value obtained from t-table, n = 7), at 95 % confidence interval, one can calculate the true intercept.

TABLE-2
LINEAR REGRESSION ANALYSIS OF POTENTIOMETRIC DETERMINATION OF ISONIAZID

Amount of isoniazid taken (mg)	Amount found (mg)	Recovery (%)
10	9.92	99.2
16	15.94	99.6
20	20.14	100.7
25	25.22	100.9
36	35.94	99.8
48	47.89	99.8
56	55.91	99.8

Formulae for linear regression analysis

$$a = \bar{y} - b\bar{x} \tag{1}$$

$$b = \frac{\Sigma_i\{(x_i - \bar{x})(y_i - \bar{y})\}}{\Sigma_i(x_i - \bar{x})^2} \tag{2}$$

$$r = \frac{\Sigma_i\{(x_i - \bar{x})(y_i - \bar{y})\}}{\{[\Sigma_i(x_i - \bar{x})^2][\Sigma_i(y_i - \bar{y})^2]\}^{1/2}} \tag{3}$$

Standard deviations for the slope S_b and the scatter around the regression line $S_{y/x}$

$$S_{y/x} = \left\{\frac{\Sigma_i(y_i - \bar{y})^2}{(n-2)}\right\}^{1/2}$$

$$S_a = S_{y/x}\left\{\frac{i x_i^2}{n\Sigma(x_i - \bar{x})^2}\right\}$$

$$S_b = \frac{S_{y/x}}{\{\Sigma(x_i - \bar{x})^2\}^{1/2}}$$

Confidence limit for slope and intercept = b ± tS_b, a ± tS_a, where x_i = individual true value, y_i = individual measured value, $\bar{x}, \bar{y}$ = mean of set of data, n = number of repetitions, t = students t (from table for 95 % confidence limit), $y_i - \hat{y}$ = (y: residuals) = (measured value - predicted value), a ± $t(S_a)$ = -0.0017 ± 0.31575 = 0.31405 to -0.31745.

It can be 95 % confident that the true intercept lies between 0.31405 to -0.31745. Similarly, the estimate error can calculated at 95 % confidence interval for the slope, b ± (tS_b) = 1.002 ± 8.215 × 10^{-3} = 1.0102 - 0.9937. The true slope lies between 1.0102-0.9937. In other words, the relative bias of the method lies between a systematic increase of 1.02 % and systematic decrease of 0.63 %.

Test of linearity: Another important charactersitics usually associated with the fitting of the best curve is the Pearson product-moment coefficient (r). It is used as a measure of correlation between two variables rather being functionally related (that is, not directly dependent on one another). It can achieve values in the range $-1 \leq r \leq +1$. The +1 value describes perfect correlation, all the points lying exactly on a straight line of a positive slope. Similarly negative sign describes negative correlation, that is straight line of a negative slope. As a general rule $0.90 < r < 0.95$ indicates a fair linearity, $r > 0.99$ indicates excellent linearity. However sometimes eqn. 3 can give rise to a 'r' value, even if the data are non-linear in character. To avoid using wrong deduction, the numerical data must be physically plotted (on a graph paper or a computer monitor). Application of eqn. 3 results in a value for 'r' = 0.999 implying that there is excellent linear association between measured and true concentration.

Limitations of linear regression: In linear regression, one implicity assumes that the magnitude of measurement error will be the same at all concentrations (without this there will be rationale for ordinary least squares). However this is an unrealistic assumption in application to analytical chemistry, where it is recognised that the standard deviation increases with concentration. Another assumption used in deriving the simple linear regression is that the dependent variable 'y' value contains the random errors in measurement, while the independent variable 'x' value is free from random errors because it is predetermined by analysts. This is appropriate in some analytical contexts, for example calibration, but it is not true in the comparison of analytical techniques, where both variables contain random errors of measurement and there is no real distinction in status between them. Despite these theoretical objections, simple linear regression is used widely. To overcome these shortcomings other methods of regression analysis have been proposed which includes weighted regression and curvilinear regression which involve complicated and time consuming calculations.

Effect of diverse compounds: To establish the effect of diverse compounds in the determiation of isoniazid, commercially available tablets/syrup such as Isokin-300, Isonex-300, Isocaldin tablets and Isocaldin syrup were analyzed by the proposed method and results were compared with B.P. method[5]. From the results, it is infered that large amounts of diverse compounds/ions (sucrose, starch, gumacacia, vitamin-B_6) do not interfere in a determination.

Comparison with a standard method: Isoniazid tablets/syrup of four different makes were each analysed using the proposed procedure and simultaneously by the B.P. method[5]. The results are give in Table-3.

TABLE-3
DETERMINATION OF ISONIAZID IN PHARMACEUTICAL PREPARATIONS

Trade name	Isoniazid found mean ± SD	
	Proposed method	B.P. method
Isonex (Pfizer)	298.28 ± 1.80	300.00 ± 2.24
Isokin-300 (Warner)	298.71 ± 2.06	301.00 ± 1.63
Isocaldin tablet (Retort Laboratories)	305.00 ± 2.45	317.86 ± 2.19
Isocaldin (Syrup) (Retort Laboratories)	306.57 ± 3.21	306.29 ± 2.69

For three out of four tablets/syrups (Namely isokin-300, isonex and isocaldin syrup) the calculated t-values was less than the tabulated t-value. This indicates that there is no significant difference between the proposed procedure and the B.P. method[5]. However for isocaldin tablets the results obtained by the BP method is higher than the proposed method. This is probably due to the reaction between the excipients and the titrant ($KBrO_3$) or the bromine generated during the reaction.

Conclusion

The proposed method with twin copper based mercury film micro electrodes, is simple, rapid and quantitative for the determination of isoniazid. The special features of the methods are as follows (i) The proposed method is an accurate alternative to the traditional analytical procedures for isoniazid especially in coloured pharmaceutical formualtions. (ii) There is no need to locate the equivalence point by graph because of the first derivative type of curve at the equivalence point. (iii) Simple equipment/procedure may propose this method to be adopted for the routine analysis of isonizid in pharmaceutical industries.

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