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Titrimetric Determination of Ascorbic Acid and Isonicotinic Acid Hydrazide in Pharmaceutical Formulations with Dichromate as Oxidant

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ABSTRACT

Inspite of the beautiful red coloured oxidized product of O-anisidine, the studies on its application in analytical techniques are scanty. So, authors have taken up the investigation on the utility of O-anisidine as a new Analytical reagent in the dichrometric-Indicator reaction. The detailed reaction on the potassium dichromate and O-Anisidine has enabled the authors to utilize O-Anisidine in titration of Ascorbic Acid and Isonicitonic Acid Hydrazide. Suitable conditions have been established with different acids viz., hydrochloric acid, sulfuric acid, phosphoric acid, acetic acid to give sharp colour change at the equivalence point. The present method has been applied for the estimation of Ascorbic acid and also Isonicitonic Acid Hydrazide in pharmaceutical formulations and results obtained are in good agreement with the values obtained by standard methods.

Keywords: O-Anisidine, Ascorbic acid, Isonicitonic Acid Hydrazide, dichromate.

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INTRODUCTION

O-Anisidine is used as a reagent for the spectrophotometric determination of gold [**1**] in biological samples and also in some salts such as silver nitrate in Acid media. In this paper, authors have carried out titrimetric determination of Ascorbic Acid and Isonicitonic Acid Hydrazide, utilizing O-Anisidine as a new redox indicator in chromometric-titration. The observation about the oxidizing action of for the first time can be traced to Balard [**2**]. Subsequently, several workers have employed chromate as titrimetric reagent for the determination of various substances. Feit and Kubierschky [**3**] observed that the solution of dichromate in sulfuric acid media were stable for long periods of time and hence proposed dichromate as a reagent for titrimetric determination. Shulek.et.al [**4**] has formulated the theoretical principals underlying the use dichromate as a reagent. Details of different substance, O-dianisidine, Xanthane, Indigoid and dyes belonging to the classes of azo triphenyl, methane thiazine, oxazine and other substance proposed as Indicators in dichrometric titrations are summarized in **Table-1.** Even though O-dianisidine is used as indicators in the dichrometric titrations survey of literature has revealed that no attempts has been made in application of O-anisidine as indicator in titration with chromate. Literature reveals a number of methods [**5-21**] reported to determine ascorbic acid with chromate.

Table – 1: Indicators in Dichrometric Titrations

INDICATORS	ANALYTES	REFERENCE
Methyl orange	INH	Modrezejeweski and Zommer[30] Vulterin[24]
(p)-ethoxy chysodine	Ascorbic Acid, INH	Schlek et al [25] , Urbanji [26]
Naphtol blue Black and Variamine Blue	Ascorbic Acid	Sastry [28]
Phenothiazine derivatives	Hydroquinone and Ascorbic acid	Puzanowska Tarasiewicz [31]
Crystal Violet	INH	kuhni.et.al [29]
Nuetral Red, Azo Carmine G, Azo Carmine B Mercuric chloride Meldolas Blue and reszyl fast violet Acetate	Ascorbic Acid	Rao.et.al [32]
Azine dyes	Ascorbic Acid, INH	Rao and sastri [33]
O-dianisidine	Ascorbic acid, INH	Gowda and gurumurthy [34]

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MATERIALS AND METHODS

Reagents:

Dichromate

Potassium Dichromate is a standard substance and can be very easily obtained in the pure state by recrystallisation from water .Standard solution of potassium Dichromate can be prepared by dissolving the requisite amount of the substance in water. In view of primary standard nature of potassium Dichromate, standard solution of this substance can be dissolving an exactly weighted amount of substance in known volume of water. If potassium Dichromate used is of unknown purity solution have to be standardized by 1% Diphenyl Amine solution as Indicator.

O-Anisidine:

1% (or) O-anisidine in 2% Methanol is prepared from Aldrich chemical company, INC, USA. The Solution is diluted with triply distilled water and stored in amber-coloured bottle. O-Anisidine has been standardized by Spectrophotometric [**1**], chromatography [**22**] or TLC [**23**] methods.

Ascorbic acid:

0.1N Ascorbic acid solution is prepared by dissolving required quantity of reagent grade sample of the substance in triply distilled water and diluting to desired volume. A small quantity of EDTA (0.5g perL) is added as stabilizer. The ascorbic acid solution thus prepared is standardized against potassium Dichromate using (p)-ethoxy chrosoidine as Indicator. In order to establish optimum conditions for determinations of ascorbic acid the authors has carried out the following experiments in different acid media.

Isonicitonic acid Hydrazide solution (INH)

0.1N solution of INH is prepared by dissolving the required amount of substance (fluka) in triply distilled water and solution is standardized as per method of Vulterin [**24**]**.**

RESULTS

Part -I
Titration of Ascorbic Acid

Schulek et al [**25**] determined ascorbic acid in pure form as well as in medicinal preparations and injections by titration with potassium Dichromate in the medium of Hydrochloric acid (HCl) and in the presence of potassium Dichromate using (p)-ethoxychrysoidine as indicator. Ruzicka [**27]** observed nine oxazones resofin, o-acetyl-

resorufin, rezazuran, o-acetylrezusin seazarin, 3-aminophononzaone, 7-amino-2-phenoxazone and gallacyanine as indicators. Sastry [**28**] described ascorbic acid –Dichromate titration in 0.7-1.0 M HCl media using Naphthol-blue black and Variamine blue respectively as indicators. The present authors has investigated the use of O-anisidine as the indicator in the titration of ascorbic acid with potassium Dichromate in Hydrochloric, sulfuric, acetic and phosphoric acid medium and established suitable conditions for satisfactory titrations.

Table – 2: Titration of Ascorbic Acid with Dichromate

Overall strength of Acid Hydrochloric Acid (N)	Volume of consumed Dichromate (ml)	Observations
0.5	5.0	Indicator transition is not sharp
1.0	5.0	Indicator transition is not sharp
2.0	5.0	Indicator transition is not sharp
4.0	5.0	sluggish colour change not sharp
5.0	4.98	sluggish colour change not sharp
Sulfuric Acid(N)		
1.0	4.90	Indicator transition is not sharp
2.0	4.80	Colourless to reddish white colour waiting for 15 sec
4.0	4.80	Colourless to reddish white colour waiting for 15 sec
6.0	4.80	Colourless to reddish white colour waiting for 15 sec
Acetic Acid (N)		
2.0	4.90	Colour transitions is not found
4.0	4.80	Colourless to red for 20sec
6.0	4.80	Colour transition is sharp
8.0	4.80	Colour transition is sharp
10.0	4.80	Colour transition is sharp
Phosphoric acid(N)		
2.0	4.90	turbidity formed, not sharp
4.0	4.85	turbidity formed, not sharp
6.0	4.90	Indicator colour change is at highest value
8.0	4.90	Indicator colour change is at highest value
10.0	4.90	Indicator colour change is at highest value
12.0	4.90	Indicator colour change is at highest value

Procedure:

5.0 ml 0.1030 N ascorbic acid is taken in titration vessel, 0.1ml of 1% O-anisidine indicator is added, made up to volume with distilled water in 50ml volumetric flask, and titrated

with hydrochloric, sulfuric, acetic, phosphoric acids respectively, to obtain a colour change from colourless to reddish yellow, results are summarized in **Table 2.** .

From the experimental observation it is found that between 4 -12 N acetic medium, colour change is observed from colourless to red and waiting for 20 seconds is necessary at equivalent point .In other experimental observation it is found that the indicator is not functioning well at any Hydrochloric and phosphoric acid concentrations. No improvement is observed even on heating. So authors recommended 2.0N sulfuric acid and 6.0N acetic acid concentration for titration of ascorbic acid with Dichromate.

Effect of Indicator:

The effect of Indicator concentration of O-anisidine is also studied using different volume of Indicator at 2.0N sulfuric acid and 6.0 N acetic acid .The colour change of indicator is also sharp in between 0.1-0.5ml of 1% O-anisidine. So, 0.2ml of indicator is recommended for titration of ascorbic acid with Dichromate. Some typical results are in **Table 3**.

Table – 3: Effect of Indicator Concentration

Volume of Indicator (mL)	0.05	0.10	0.20	0.30	0.40	0.50
Volume of dichromate (mL) In Sulfuric Acid 2N	4.90	4.80	4.80	4.80	4.80	4.80
Acetic Acid 6N	4.90	4.80	4.80	4.80	4.80	4.80

Recommended procedure:

An aliquot of 5.0ml 0.1030N ascorbic acid is taken in titration vessel an overall acidity of 2N sulfuric(or) acetic acid is maintained in the total volume of 50ml , 0.20ml indicator is added and titration is carried out with 0.1072 N Dichromate to a colour change from colourless to red. The typical results are in **Table-4**, Reverse titration is carried out for the estimation of Dichromate with ascorbic acid by adopting similar procedure in different hydrochloric, sulfuric, acetic and phosphoric acid concentrations. It is found that indicator is not functioning well at any concentrations so reverse titration is not recommended for the titration of Dichromate with ascorbic acid.

Application of the Developed Method:

The Indicator method developed can be applied successfully for determination of ascorbic acid contents in vitamin C tablets as per recommended procedure, One tablet is ground to a free powder and dissolved in double distilled water and the solution filtered through G-4 sintered glass funnel and diluted to a known volume. An aliquot of this solution is titrated with 0.10N dichromate solution in 2.0 N sulfuric acid medium using O-anisidine as indicator. A similar aliquot is titrated with potassium Dichromate using (p)-ethoxy chrysodine as indicator. The samples of Vitamin C tablets and typical results obtained while employing both

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the methods are present in the **Table -5**. It is observed that these results are in good agreement with standard methods.

Table – 4: Estimation of Ascorbic Acid with Dichromate

Taken	Amount of Ascorbic acid (mg)	
	Found	Relative error (%)
Sulfuric Acid, 2N and Acetic Acid, 6N		
5.032	5.029	0.05
7.504	7.489	0.19
10.026	10.015	0.109
20.082	20.082	Nil
30.051	30.051	Nil
40.062	40.184	-0.304

Table – 5: Determination of Ascorbic Acid Contents of Commercial Vitamin C Tablets

Trade Name and Manufacturer	Indicator and amount of ascorbic acid found, g	
	(p)-ethoxy chrydoidine	O-anisidine
Celin / Glaxo	0.50 ± 0.01	0.50 ± 0.01
Sorvosin /EIPW	0.49± 0.01	0.49 ± 0.01
Sukcee / IDPL	0.49± 0.01	0.49 ± 0.01
Redoxon/ Roche	0.50± 0.01	0.50 ± 0.01

Part -II

Titration of Isonicitonic Acid Hydrazide (INH)

Kuhni **[29]** used crystal violet as indicator in the titration of INH and reported that results obtained were 0.5% high. Solution of medically pure INH tablets can be directly titrated with potassium Dichromate solution either potentiometrically or visually in Hydrochloric or Phosphoric acid medium using Methyl red or Methyl orange as Indicator Urbanji.et.al[**26**] dissolving the sample in 2N Hydrochloric acid and titrated with mixture of potassium dichromate- potassium bromide(0.1N) using (p)-ethoxy chrosodine as Indicator for the first disappearance of red colour.The authors of this paper has undertaken a study on the feasibility of INH-Dichromate titration using O-anisidine, as an indicator.

Procedure:

5.0ml 0.1040 INH is taken in the titration vessel required amounts of hydrochloric, sulfuric, acetic and phosphoric acids are added to give desired concentration and 0.1ml of 1%

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O-anisidine indicator is added and made up to volume with distilled water in 50ml volumetric flask .The titration is carried out with 0.1072 N dichromate to a colour change from colourless to yellowish red. The results are given the **Table-6**, from the experimental study, it is found that the Indicator is not functioning well in phosphoric acid, sulfuric acid medium no improvement is observed even at higher temperature, but in hydrochloric acid between 2.0N-6.0N the indicator is functioning well, and colour change is from yellow to colourless. In case of acetic acid medium between 2.0N-10N, Indicator functioned well and stoichiometric results are obtained The colour change is observed from yellowish red to colourless and waiting for about 30sec is necessary for equivalent point.

Table –6: Titration of INH with Dichromate

Overall strength of Acid	Volume of consumed Dichromate, ml	Observations
Hcl acid, N		
0.5	4.90	Indicator transition is not sharp
2.0	4.85	yellow to colorless is sharp
4.0	4.85	yellow to colorless is sharp
6.0	4.85	yellow to colorless is sharp
Sulfuric Acid, N		
0.5	4.90	Indicator transition is not sharp
2.0	4.90	Indicator transition is not sharp
4.0	4.90	Indicator transition is not sharp
6.0	4.90	Indicator transition is not sharp
8.0	4.90	Indicator transition is not sharp
Acetic Acid, N		
2.0	4.85	yellow to colourless and waiting 15 sec
4.0	4.85	yellow to colourless and waiting 15 sec
6.0	4.85	colour transition is sharp
8.0	4.85	colour transition is sharp
10.0	4.85	colour transition is sharp
Phosphoric acid, N		
4.0	4.80	sluggish colour change and not sharp
6.0	4.70	sluggish colour change and not sharp
8.0	4.70	sluggish colour change and not sharp
10.0	4.70	sluggish colour change and not sharp
12.0	4.70	sluggish colour change and not sharp

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Effect of indicator concentration:

The colour change of indicator is sharp using 0.10ml indicator so; 0.1ml of Indicator is used for titration of INH with dichromate. Results of concentration of Indicator are in **Table-7.**

Table – 7: Effect of Indicator Concentration

Volume of Indicator, mL	Volume of dichromate (ml)	
	Hcl acid, 2N	Acetic acid, 6N
0.05	4.70	4.70
0.10	4.85	4.85
0.20	4.85	4.85
0.30	4.85	4.85
0.40	4.85	4.85
0.50	4.85	4.85

Recommended procedure:

An aliquot of 5.0ml 0.1040N INH is taken in titration vessel an overall acidity of 2N or 5N acetic acid is maintained an the total volume of 50ml 0.1ml of indicator added and titration is carried out with 0.1072 N dichromate some typical results of estimation of INH in **Table-8.** Reverse titration is carried out for the dichromate with INH by adopting same procedure in different acids media .It is found that the indicator is not functioned well at any acid concentrations .After addition of excess oxidant, slowly colour developed . Hence it is not recommended to estimate dichromate with INH.

Table – 8: Estimation of INH with Dichromate

Taken	Amount of INH, mg	
	Found	Relative error (%)
HCl, 2N		
1.090	1.090	Nil
1.590	1.598	-0.50
2.693	2.696	-0.11
10.801	10.882	-0.74
15.604	15.604	Nil
20.806	20.868	-0.29
Acetic Acid,5N		
1.090	1.085	0.458
1.590	1.587	0.188
2.693	2.688	0.155
10.801	10.801	Nil

15.604 20.806	15.625 20.825	-0.145 -0.091

Application of the Developed Method:

The indicator method now developed can be successfully adopted for the determination of INH contents in commercial tablets as per the following procedure, one tablet is ground to fine powder dissolved in deionised water the solution is filtered through G-4 sintered funnel and dilute to suitable known volume Aliquot of this solution are titrated with 1.0M Hydrochloric acid medium with Potassium dichromate solution (0.1M) using O-anisidine as indicator. Similarly aliquots is titrated with methyl red as indicator. As per vulterin, some typical results obtained in this method are in the **Table -9.** It is evident from these results that there is an excellent agreement between the above stated methods.

Table – 9: Determination of INH in Commercial Tablets

Trade name and Manufacturing	Indicator and amount of INH found, g	
	Methyl red	**O-anisidne**
Isonex-Dumex Pharmaceuticals	0.30 ± 0.01	0.30 ± 0.01
Isokin Davis-warner Lambert (U.S.A)	0.29 ± 0.01	0.29 ± 0.01
Docina-306	0.30 ± 0.01	0.30 ± 0.01
Nydrazid-squibb	0.30 ± 0.01	0.30 ± 0.01

DISCUSSION

The – authors have studied the application of o-anisidine as indicator in titrations of ascorbic acid and INH with Dichromate revealed the following observations. 0.1 ml of 1% of the indicator in a total volume of 50 ml of the titrant mixture resulted in a very sharp colour change from light yellow to red for a fraction of a drop of 0.1N potassium dichromate solution exactly at the equivalence point in titrations of different reductants. The titrations are possible in sulphuric acid, phosphoric acid and acetic acid media. The titrant systems chosen in the investigation are Ascorbic acid and Isonicotinic acid hydrazide and the calculated amounts of the titrants coincided with in a relative error of 0.1% with respect to the standard method. This method is for the assay of Ascorbic acid and Isonicotinic acid hydrazide in pharmaceutical formulations. The sharp colour change obtained with a very small quantity of o-anisidine as indicator in the titrations of different reductants, its easy availability and low cost, the authors feels, o-anisidine can be used as one of the best indicator in these titrations.

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