O-Anisidine as Indicator in Titrimetric Determination of Ascorbic Acid and Isonicotinic Acid Hydrazide in Pharmaceutical Formulations

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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 bromatometric-Indicator reaction. The detailed reaction on the potassium bromate and O-Anisidine has enabled the authors to utilize O-Anisidine in titration of Ascorbic Acid and Isonicitonic Acid Hydrazide. Suitable conditions has 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.

Key Words: O-Anisidine, Ascorbic acid, Isonicitonic Acid Hydrazide, Bromate.

INTRODUCTION:

O-Anisidine is used as a reagent for the spectrophotmetric determination of gold [Jenic Joseph.et.al 1876] 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 Bromatometric-titration. The observation about the oxidizing action of bromate for the first time can be traced [Balard 1826]. Subsequently, several workers have employed bromate as titrimetric reagent for the determination of various substances. [W.Feit and K.Kubierschky; 1891] observed that the solution of potassium bromate in sulfuric acid media were stable for long periods of time and hence proposed potassium bromate as a reagent for titrimetric determination. [E.Suchulek. et.al 1960] has formulated the theoretical principals underlying the use potassium bromate 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 bromotometric titrations are summarized in Table-1.

Table – 1 Indicators in Bromatometric Titrations

INDICATORS	ANALYTES	REFERENCE
Methyl orange	INH	[ModrezejeweskiandZommer1962] [Vulterin 1963]
(p)-ethoxy chysodine	Ascorbic Acid, INH	[Schlek et al 1941], Urbanji[1973]
Naphtol blue Black and Variamine Blue	Ascorbic Acid	[Sastry 1965]
Phenothiazine derivatives	Hydroquinone and Ascorbic acid	Puzanowska Tarasiewicz[1954]
Crystal Violet	INH	[kuhni.et.al]
Nuetral Red, Azo Carmine G, Azo Carmine B Mercuric chloride Meldolas Blue and reszyl fast violet Acetate	Ascorbic Acid	[Rao.et.al 1982]
Azine dyes	Ascorbic Acid, INH	[Rao and sastri 1987]
O-dianisidine	Ascorbic acid, INH	[Gowda and gurumurthy 1982]

Even though O-dianisidine is used as indicators in the bromatometric titrations survey of literature has revealed that no attempts has been made in application of O-anisidine as indicator in titration with bromate. Literature reveals a number of methods [S.P. Arya. et.al 2000, R.P.S. Chauhan and U.B. Singh, 1995, P.T. Kissinger and W.R. Heineman, 1996, P. Tomcik.et.al. 2001, R. Sandulescu. .et.al 1997, J.J. Sun .et.al. 1998, L.E. Leon, 1996, M. Cheregi and A.F. Danet1997,X. Hu, Z. Leng1995, P. Jandu, .et.al 1996, H. Chem. .et.al1997,A.A. Ensafi and B. Rezai, 1998, R. Koncki. .et.al 1999, Ensafi.A.A. .et.al 2002, István Szalai and Endre Körös1998,. Gao J et.al 2001,Stella Barrett. .et.al, 2006] reported to determine ascorbic acid with potassium bromate.

MATERIALS AND METHODS

Potassium bromate

Potassium bromate is a standard substance and can be very easily obtained in the pure state by recrystallisation from water .Standard solution of potassium bromate can be prepared by dissolving the requisite amount of the substance, recrystallised and dried at 180 ^{O}C in water. In view of primary standard nature of potassium bromate, standard solution of this substance can be dissolving an exactly weighted amount of substance in known volume of water. If potassium bromate used is of unknown purity solution have to be standardized by direct and indirect method.

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 [Erdy. Et.al.1952] or TLC [N.V.Rao and C.K.Shastri, 1987]

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 bromate 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 1963]

RESULTS

Titration of Ascorbic Acid

[Schulek et al.1941] determined ascorbic acid in pure form as well as in medicinal preparations and injections by titration with potassium bromate in the medium of Hydrochloric acid (HCl) and in the presence of potassium bromide using (p)-ethoxychrysoidine as indicator. [Ruzicka E.Ruzicka 1964] bserved nine oxazones resofin, o-acetyl-resorufin, rezazuran, o-acetylrezusin seazarin, 3-aminophononzaone, 7-amino-2-phenoxazone and gallacyanine as indicators. [Sastry 1965] described ascorbic acid – bromate 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 bromate in Hydrochloric, sulfuric, acetic and phosphoric acid medium and established suitable conditions for satisfactory titrations.

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.

Table – 2 Titration of Ascorbic Acid with Bromate

Overall strength of Acid Hydrochloric Acid (N)	Volume of consumed Bromate (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*

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 bromate.

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 bromate. 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 bromate (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 bromate to a colour change from colourless to red. The typical results are in Table-4, Reverse titration is carried out for the estimation of bromate 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 bromate with ascorbic acid.

Table – 4 Estimation of Ascorbic Acid with Bromate

Taken	*Amount of Ascorbic acid (mg)*	
Sulfuric Acid, 2N and Acetic Acid, 6N	Found	*Relative error (%)*
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*

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 bromate solution in 2.0 N sulfuric acid medium using O-anisidine as indicator. A similar aliquot is titrated with potassium bromate using (p)-ethoxy chrysodine as indicator. The samples of Vitamin C tablets and typical results obtained while employing both the methods are present in the Table -5. It is observd that these results are in good agreement with standard methods.

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*

Titration of Isonicitonic Acid Hydrazide (INH)

[Kuhni.et.al 1973] 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 bromate solution either potentiometrically or visually in Hydrochloric or Phosphoric acid medium using Methyl red or Methyl orange as Indicator [Urbanji.et.al 1973] dissolving the sample in 2N Hydrochloric acid and titrated with mixture of potassium bromate- potassium bromide(0.1N) using (p)-ethoxy chrosodine as Indicator for the first diasappereance of red colour.The authors of this paper has undertaken a study on the feasibility of INH-Bromate 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% 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 bromate 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, colour change is from yellow to colourless. In case of aceticacid 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 Bromate

Overall strength of Acid	Volume of consumed Bromate, 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*

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 bromate. Results of concentration of Indicator is in Table-7**.**

Table – 7 Effect of Indicator Concentration

Volume of Indicator, mL	*Volume of bromate (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 bromate some typical results of estimation of INH in Table-8. Reverse titration is carried out for the bromate 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 bromate with INH.

Table – 8 Estimation of INH with Bromate

Taken	Amount of 1NH, 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	15.625	-0.145
20.806	20.825	-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 bromate 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 these 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 Bromate revealed the following observations. 0.1 ml of 1% of the indicator in a total volume of 50 ml of the titrand mixture resulted in a very sharp colour change from light yellow to red for a fraction of a drop of 0.1N potassium bromate 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 titrand systems chosen in the investigation are Ascorbic acid and Isonicotinic acid hydrazide and the calculated amounts of the titrands 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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