Determination of Plant Acids by Ion Exchange Chromatography. I. Percuprimetric Determination of Tartaric Acid in Presence of Oxalic Acid

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A method is described for oxidimetric determination of tartaric acid or tartrates with potassium cupri-3-periodate in alkaline medium. The oxidation is completed in 5 minutes at room temperature, and the unreacted reagent is retitrated with standard arsenite solution. Oxalic acid is not oxidized under these conditions. This method could be used for determination of tartaric acid in presence of other plant acids (e. g. in fruits and fruit juices) after separation accomplished by a method developed by Schenker and Rieman III[1], using ion exchanger Dowex 1 - X 10.

This method offers several advantages over methods described earlier. The necessity of termostating and the interference of oxalic acid is avoided. Results are quoted to show the accuracy of the proposed method. A reproducibility of ± 0,02 mg. has been attained in the range from 0.188 to 3.20 mg. of tartaric acid in 10 ml. of aqueous solution.

Schenker and Rieman III[1] have developed a method for ion exchange chromatographic determination of malic, tartaric and citric acids that is applicable to fruits, fruit juices and fruit products.

In this method oxalic acid interfers with tartaric acid because these two acids are found together in the third fraction, and both are oxidized with permanganate in acid medium.

Oxalic acid and oxalates may be found in considerable amounts in plant tissues as well as in fruits. For example, oxalic acid is the main acid constituent of fruits of *Citrus decumana L.* and *Phyllanthus simplex Retz.*[2] In somewhat lower amount oxalic acid occurs with other plant acids e. g. in fruits of *Citrus limonum, Citrus medica L., Phyllanthus emblica L., Punica granatum L. and Zizyphus jujuba Lamk.*[2]. A considerable amount of oxalic acid is often found in leaves and stems of plants, in some cases even up to 10 per cent or more of the dry matter. Thus in attempting to apply the method of Schenker and Rieman III[1] to the determination of plant acids in these fruits and in other plant tissues, it was necessary to eliminate the interferences of oxalic acid.

There are several methods for oxidimetric determination of tartaric acid with different oxidants in acid or alkaline medium. Schenker and Rieman III[1] used potassium permanganate in acid medium. Under this condition for quantitative oxidation of tartaric acid to CO_2, a large excess of permanganate is required, and it is necessary to termostate the mixture for 105 minutes in a 30°C water bath. Wilard and Young[3] have examined in detail the determi-

nation of tartaric acid with ceric sulfate in sulfuric acid medium. The relationship between the number of molecules of tartaric acid an the number atoms of oxygen consumed is 3.6. Gopala Rao and Sankegowda[4] proposed the use of sodium vanadate in acid medium. With all the above mentioned methods, oxalic acid is also completely oxidized. After Singh and coworkers[5] tartaric acid is oxidized with an excess of permanganate in strongly alkaline medium to two mols of CO_2 and one mol of oxalate. Only the tartaric acid should be determined by this method. But all methods using permanganate in alkaline or acid medium have the disadvantage of spontaneous decomposition of permanganate at elevated temperatures and a prolonged time of action.

G. Beck[6] titrated potassium cupri-3-periodate with K-Na-tartrate solution at room temperature, and stated that one mol of tartrate consumes 6 equivalents of Cu^{III} which are equivalent to 3 atoms of oxygen. The tartrate is not completely decomposed to CO_2 by this method. Beck[6] used the above mentioned reagent for oxidation of various organic and inorganic compounds, and introduced the term »Percuprimerty«.

According to our experience the reduction of potassium cupri-3-periodate with K-Na-tartrate proceeds toward the end of the titration very slowly, so that the titration lasts long and it is very difficult to determine the titration endpoint. The reduction of potassium cupri-3-periodate with arsenite solution proceeds faster, and we tried to oxidize tartrate with an excess of potassium cupri-3-periodate and to retitrate with arsenite solution. The oxidation of tartrate is completed, at room temperature, in approximately 3 to 5 minutes. Results obtained in this manner showed a good reproducibility in oxidation time of 5 to 15 minutes, but they were 7.5 per cent to high when as equivalent weigh 1/6 of the molecular weight of tartaric acid was employed, as stated by Beck[6]. From this observation it can be deducted that the molar relationship between tartrate and Cu^{III} is 1/6.4 and consequently the equivalent weight for the tartaric acid is 23.5.

EXPERIMENTAL

Apparatus

Columns made of glas tubes of 16 mm. internal diameter were filled to a depth of 25 cm, with Dowex 1 - X 10 (100—200 mesh) and were prepared for use as directed by Schenker and Rieman III[1]. The desired flow rate through the column was controled by applied presure (100 ml. per hour).

A Trénel pH-meter was adapted for potentiometric titrations. As indicator electrode a platinum electrode was used, and a saturated calomel electrode as reference electrode.

Reagents

Potassium cupri-3-periodate 0.01 N $K_7[Cu(IO_6)_2]$ was prepared according to Beck[7].

Potassium hydroxide solution. 40 g. of KOH was dissolved in 100 ml. of water.

Precupri reagent. Equal volumens of above solution of potassium cupri-3-periodate and of potassium hydroxide were mixed together one day before use.

Standard arsenite solution 0.02 N. 0.989 g of arsenious oxide and 1.5 g. of KOH were dissolved in 20 ml. of water. After dissolving the solution was brought to 1 litre with water. The normality was from time to time cheched against standard potassium permanganate solution.

Eluants A and B were prepared as directed by Schenker and Rieman III[1].

Tartaric acid and K-Na-tartrate were commercial products of analytical purity and were used in these investigation without any further purification. All other chemicals used were also of analytical purity.

Procedure

20.0 to 40.0 ml. of percupri reagent is placed in a 100 ml Erlenmeyer flask, and an appropriate aliquot of tartrate solution (5.0 to 15.0 ml. containing ap to 3.0 mg of tartaric acid) is added. The solutions are thoroughly mixed and allowed to stand for 5 to 10 minutes at room temperature. The unreacted potassium cupri-3-periodate is titrated with standard arsenite solution, and marked as *D*. (The endpoint of the titration is indicated by change of colour from green to blue. Towards the end of the titration, when the colour becomes emerald green, it is necessary to wait 20 to 30 seconds after each addition of arsenite solution to be sure that the colour does not change any more.)

At the same time a blank titration is run with the same amount of percupri reagent, and is marked as *C*.

$$(C - D) \cdot N \cdot F = \text{mg. of tartaric acid.}$$

N is the normality of standard arsenite solution, and F is

$$\frac{\text{mols of tartaric acid}}{6.4} = 23.5$$

Tartaric acid can be separated from interfering materials such as sugars, colouring mater and other plant acids, by the ion exchange chromatographic method described by Schenker and Rieman III[1]. In the third fraction of the effluent that contains all tartrate and oxalate ions present in the sample, tartrate is determined as described above.

TABLE I.

Analyses of known amounts of tartaric acid dissolved in water or in eluants A or B

Experiment No.	Dissolved in	Tartaric acid mg.		Error	
		Taken	Found	mg	per cent.
1.	10 ml of water	1.60	1.62	+0.02	+1.2
2.	,,	0.80	0.83	+0.03	+3.7
3.	,,	0.32	0.31	—0.01	—3.3
4.	,,	2.10	2.11	+0.01	+0.5
5.	,,	3.20	3.18	—0.02	—0.6
6.	10 ml of eluant A	1.50	1.49	—0.01	—0.7
7.	,,	1.60	1.58	—0.02	—1.2
8.	,,	2.10	2.10	± 0.0	± 0.0
9.	,,	3.20	3.18	—0.02	—0.6
10.	5 ml ,,	0.75	0.75	± 0.0	± 0.0
11.	10 ml of eluant B	1.50	1.51	+0.01	+0.7
12.	,,	2.10	2.09	—0.01	—0.5
13.	,,	0.80	0.81	+0.01	+1.2
14.	,,	0.32	0.32	± 0.0	± 0.0

RESULTS

Tables I. and II. sumarize the results obtained with samples of tartaric acid and K-Na-tartrate dissolved in water, or in eluants A or B. The solutions containing the required amount of tartrate were prepared from stock solutions

TABLE II.

Analyses of known amounts of K-Na-tartrate dissolved in 10 ml. of eluant B.

Experiment No.	K-Na-tartrate mg.		Error	
	Taken	Found	mg.	per cent
1.	3.60	3.59	—0.01	—0.3
2.	2.53	2.54	+0.01	+0.4
3.	2.53	2.53	± 0.0	± 0.0
4.	1.26	1.28	+0.02	+1.6
5.	1.26	1.25	—0.01	—0.8
6.	0.50	0.50	± 0.0	± 0.0

TABLE III.

***Analyses** of known amounts of tartaric acid in presence varied amounts of oxalic acid.*

Experiment No.	Dissolved in eluant B ml.	Oxalic acid taken mg.	Tartaric acid mg.		Error	
			taken	found	mg.	per cent
1.	10.0	0.4	1.28	1.27	—0.01	—0.8
2.	10.0	2.0	1.28	1.27	—0.01	—0.8
3.	10.0	4.0	1.28	1.26	—0.02	—1.6
4.	10.0	1.0	0.75	0.75	± 0.0	± 0.0
5.	10.0	1.0	0.375	0.374	—0.001	—0.3
6.	5.0	0.5	0.188	0.191	+0.003	+1.6

TABLE IV.

Analyses of tartaric acid in presence of malic, citric, and oxalic acid, after separation on ion exchange columns of Dovex 1 - X 10.

Experiment No.	Acids taken mg.				Found mg. of Tartaric acid	Error	
	Malic	Citric	Oxalic	Tartaric		mg.	per cent
1.	10.0	10.7	—	25.0	24.9	—0.1	—0.4
2.	5.0	9.8	10.0	15.5	15.6	+0.1	+0.6
3.	10.0	4.0	7.5	10.5	10.4	—0.1	—1.0
4.	11.0	10.7	—	10.0	10.0	± 0.0	± 0.0
5.	7.5	10.7	7.5	5.0	5.0	± 0.0	± 0.0

by appropriate dilution. Table III. gives the results of determinations of tartaric acid in presence of various amounts of oxalic acid, dissolved in eluant B. From these results it is seen that boric acid, sodium tetraborate, and sodium nitrate, the ingredients of eluants A and B, and oxalic acid do not interfere with the determination of tartaric acid.

To check the method with regard to ion exchange columns, solutions of known amounts of malic, tartaric, citric and oxalic acids were prepared; 5.0 ml of solution was taken in an ion exchange column, and eluted first with eluant A and than with eluant B. In an aliquot (10 ml.) of the third fraction tartaric acid was determined as described before. The results of these experiments are shown in table IV., and are in good agreement with the amount of tartaric acid taken.

DISCUSSION

In preliminary experiments potassium cupri-3-periodate was mixed with an equal volum of a solution of potassium hydroxide (40 grams dissolved in 100 ml. of water) immediately before the addition of tartrate solution as the oxidation proceeds in strongly alkaline medium. It was observed that the titre of this mixture against standard arsenite solution decreased rapidly at first, so that after 15 minutes it showed 95 per cent of its original value. After standing for one day the titre fell to 75—80 per cent of its original value, depending on the temperature ,and then decresed very slowly. In 10 hours the titre fell only 1.5 per cent. When the mixture was boiled for 10 to 15 minutes it became green. All that indicates that Cu^{III} turnes to Cu^{II} in strongly alkaline medium,

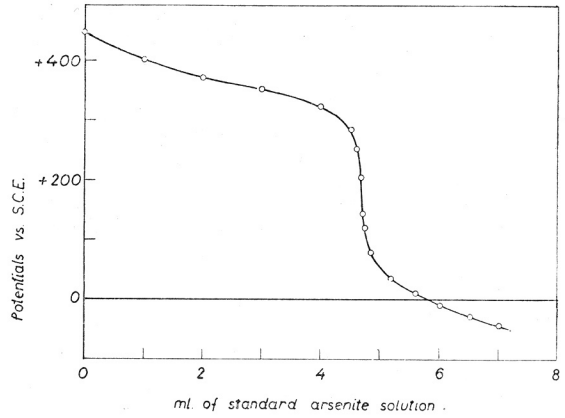

Figure 1. Titration curve of potassium cupri-3-periodate with standard arsenite solution in alkaline medium. Potentials are given vs. saturated calomel electrode (S. C. E.).

and that this change is faster at elevated temperature. It is noteworthy that the titre of potassium cupri-3-periodate prepared in the proposed maner without mixing with KOH solution was stable for a longer time.

Opposite behavior could be observer when the mixture of potassium cupri-3-periodate and KOH solution was titrated with arsenite solution to the blue

colour. With time this solution became green, then more deeply green and finaly brown. With boiling the change of the colour proceeded more rapidly. Apparently Cu^{II} was oxidized to Cu^{III}. The green or brown coloured solution could be again titrated to to the blue colour with arsenite solution.

The titration of potassium cupri-3-periodate with arsenite could be folowed also potentiometricaly using a platinum electrode as indicator. Figure 1. shows a curve obtained by titrating of 20 ml. of percupri reagent with standard arsenite solution. The curve shows a tipical inflection point where the slope $\Delta E/\Delta V$ is maximal and indicates the equivalence point. The endpoint of the titration determined potantiometrically coincided with that obtained through vicual observation of colour changes.

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IZVOD

Određivanje biljnih kiselina kromatografijom na ionskim izmjenjivačima. I. Perkuprimetrijsko određivanje vinske kiseline u prisutnosti oksalne kiseline

N. Velikonja

Razrađena je metodika za oksidimetrijsko određivanje vinske kiseline, odnosno tartarata, s pomoću kalijeva kupri-3-periodata u alkaličnoj sredini. Oksidacija tartarata dovršena je za 5 minuta kod sobne temperature, a suvišak reagensa titriramo standardnom otopinom arsenita. Pod ovim uvjetima oksalna kiseline ne reagira s reagensom. Tu metodu možemo primijeniti za određivanje vinske kiseline i u prisutnosti drugih kiselina (na pr. u voću, voćnim sokovima i voćnim prerađevinama), pošto je izvršeno odjeljivanje tih kiselina na ionskom izmjenjivaču Dowex 1 - X 10, po Schenker-Riemanovoj metodi.[1]

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