

## PHARMACEUTICAL SCIENCES | ФАРМАЦЕВТИЧЕСКИЕ НАУКИ

### DIPEROXYAZELAIC ACID AS REDOX TITRANT. PART I. POTENTIOMETRIC DETERMINATION OF N-ACETYLCYSTEINE AND ASCORBIC ACID

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#### ABSTRACT

A new peroxyacidmetric method for the quantitative determination of N-acetylcysteine (ACC) was developed. Titration is directly generated in the sample solution of iodine using as titrant of diluted solution diperoxyazelaic acid (DPAA), achieves the higher sensitivity and determination of ACC simplify temperature conditions, which is impossible to be achieved in the classical method iodine macrodetermination. The RSD was 3.19-1.60% ( $\delta = -0.3 \dots 0\%$ ) for 1.63-3.26 mg of ACC in 20 mL.

A new approach for quantitative determination of ascorbic acid in substance and a solution for injection by method of redox potentiometric titration by means in situ generated iodine in the potassium iodide solution using as titrant DPAA was proposed. The validation of the proposed analytical method by such characteristics as accuracy, convergence, limit of detection (LOD), limit of quantitative detection (LOQ) was carried. The resulting metrological characteristics methods do not exceed the eligibility criteria for SPU. The technique is characterized by simplicity and speed performance, high sensitivity and selectivity, reproducibility and accuracy of satisfactory results. The proposed method was successfully applied to commercial substance and 5 % injection solution of ascorbic acid. The average percentage recovery ( $n=7$ ) was 100.31 % and 98.32%; RSD 1.30 % and 1.31 % for accuracy,  $\delta +0.71 \dots +0.28\%$  and  $-0.28\%$  for substance and 5 % solution for injection respectively. The limit of quantification (LOQ) of ACC and ascorbic acid was 0.03 mg and 0.06 mg to 20 mL final volume respectively

**Keywords:** Ascorbic acid, N-Acetylcysteine, Potentiometric method, Diperoxyazelaic acid.

#### INTRODUCTION

N-acetyl-L-cysteine (ACC) - (2R)-2-acetylamino-3-sulfanylpropanoic acid - a chemical structure is a derivative of cysteine with an acetyl group attached to the amino group of cysteine. It is a famous drug (Acetadote, Fluimucil, Mucomyst, Parvolex): in clinical practice, it is traditionally used as an expectorant and anti-inflammatory. ACC promotes the synthesis of glutathione in vivo, and then apply it as an antidote in cases of acute poisoning with paracetamol, aldehydes, phenols, etc. [1].

L-(+)-Ascorbic (AA) or (vitamin C, (R)-3,4-dihydroxy-5-((S)-1,2-dihydroxyethyl) furan-2(5H)-one, E 300) related glucose organic compound, which is one of the main substances in the human diet - necessary for the proper functioning of bone and connective tissue. The biological function and reducing coenzyme some metabolic processes, is a powerful antioxidant. Naturally, ascorbic acid is found in many fresh fruits and vegetables [2].

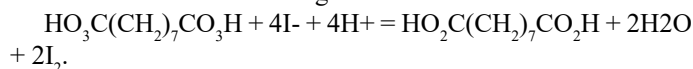
They are produced in the form of individual and combined drugs, in which ascorbic acid acts as a stabilizer. Thus, in a preparation ACC®100 production enterprise «Salutas Pharma GmbH» company «Hexal AG» (Germany), ascorbic acid content of 12.5 mg to 100 mg of ACC. It includes as saccharine, sucrose, orange flavoring as excipients to 3.0 grams per one bag [1].

For the quantitative determination of ascorbic acid in samples with complex multi foundation should be applied some chromatographic separation [3, c. 214] and / or selected physicochemical methods [4, c. 39; 5, c. 670; 6, c.1; 7, c. 49]. However, any instrumental method, for example, such as HPLC, provides for certain material costs for the purchase of reference materials and device, so not always available. For the

quantitative determination of ascorbic acid and ACC, which contained medicines in relatively high amounts, it is advisable to use a cheaper and yet affordable titrimetric method.

The disadvantages of the recommended methods in State Pharmacopoeia of Ukraine (SPHU) of the iodimetric determination of ACC in individual preparations include inconvenience is the need of deep cooling solution sample before titration ( $+10^\circ\text{C}$ ) and relatively low sensitivity and accuracy, due to the use of a relatively high concentration of titrant (0.05 mol/L) [8].

We set a goal to work out unified methods of microdetermination of ACC or AA small amounts by means of diperoxyazelaic acid as redox titrant by the potentiometric titration method with iodine generated in situ:



Implementation of the determination in this way can simplify of the technique, to avoid the inherent of method iodimetry negative phenomenon and prevent losses of iodine and influence of oxygen on the analysis result.

#### EXPERIMENTAL PART

Ascorbic acid, a substance production by Northeast Pharmaceutical Group Co, Ltd (China), a series DY0261520104 (26.01.2015). N-acetylcysteine substance, manufacturer Moehs Catalana S.L., Spain, 091 108 series. Ascorbic acid -Darnitsa, injection, 50 mg/mL and 2 mL ampoules (Kyiv, Ukraine), series 20213; The content of the active ingredient for the certificate of 49.3 mg/mL. Excipients: sodium bicarbonate, sodium

metabisulphate (E 223), disodium EDTA, water for injections. Potassium iodide, «chemical grade».

Production of a solution of formaldehyde: formalin solution (37%) diluted with water to 1%.

As titrant was used diperoxyazelaic acid (DPAA), which is obtained acylation of hydrogen peroxide, azelaic acid by a known method [9, p. 1929]. DPAA solution with a concentration of 0.01 mol/L produced by accurate sample weight-to-volume method, which is standardized by iodometric titration [10, p. 352].

Titration was performed using the electrode pair of platinum indicator electrode spot «EPV-1» - saturated potassium chloride reference electrode chloride type «EVL - 1M3.1» (both produced by «Factory of devices measuring» Belarus, Gomel g ) and 10 ml microburette measured volume of titrant to within  $\pm 0.01$  ml. Electromotive force range without transferring ions recorded digital laboratory ionometric I-130 «Factory of devices measuring» Belarus, Gomel g ) with an accuracy of  $\pm 0.1$  mV. Titration was performed at  $+18-20^\circ\text{C}$ .

## RESULTS AND DISCUSSION

Experimentally, it was found that ACC by iodine and / or triiodide in terms of titration (excess ACC regarding titrant) at room temperature is S-oxidized to different products, depending on the pH, namely at pH 1.1 (0.1 mol / L HCl) oxidized of ACC is likely to thiosulfate ester ACC interacting with iodine in molar ratios of 1:1 (ACC 0.01mmol consumed 0.005 mmol DPAA), while at pH 4.7 (0.1 mol / L  $\text{KH}_2\text{PO}_4$ ) ACC by iodine is S-oxidized more deeply - probably to N-acetylsulfate acid and / or the corresponding tiosulfonate interacting with iodine in a molar ratio of 2:1 (0.01 mmol ACC spent 0.01 mmol DPAA).

AA under similar conditions always form dehydroascorbic acid interacting with iodine in a molar ratio of 1:1 and / or own DPAA - in a molar ratio of 2:1. The typical titration curves in Fig. 1 and 2 are titration of 0.01 mmol ACC and AA 0.01 mmol diperoxyazelaic acid in the presence of KI.

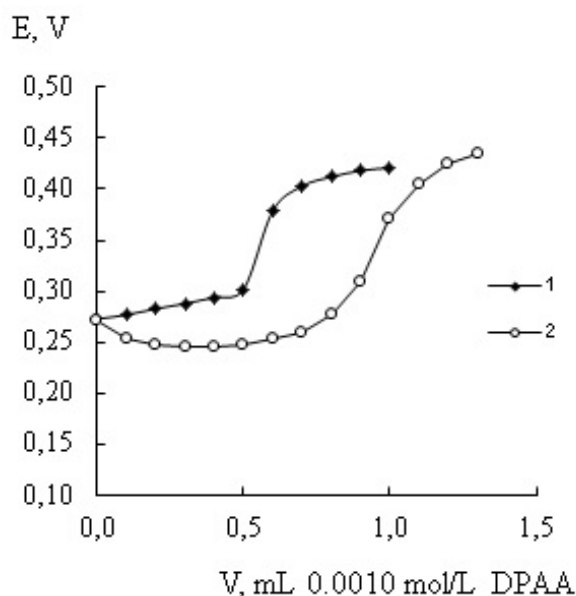


Figure 1. Potentiometric titration of 0.01 mM N- ACC diperoxyazelaic acid in the presence of KI. 1 - 0.1 mol / l HCl ; 2 - pH 4.7 (0.2 M  $\text{KH}_2\text{PO}_4$ ); 0.25% KI

Procedure for the determination of ACC (method 1). Weigh accurately 0.16 g of ACC and dissolve in 100.00 mL of a freshly double distilled water. Aliquot portion (1.00 or 2.00 mL) of the test solution (0.16 g was transferred to a beaker of 50 mL, add 18-19 mL 0.1 mol / L HCl, 1.0 ml of 5 % solution of KI and titrated 0.01 mol / L solution DPAA using 10 mL microburet.

Procedure for the determination of ACC (method 2). Weigh accurately 0.16 g of ACC and dissolve in 100.00 mL of a freshly double distilled water. Aliquot portion (1.00 or 2.00 mL) of the test solution was transferred to a beaker of 50 mL, add 18-19 mL 0.2 mol/L solution  $\text{KH}_2\text{PO}_4$  pH 4.7, 1.0 ml of 5% solution of KI and titrated 0.01 mol/L solution DPAA using 10 mL microburet. ACC content in mg under different conditions calculated by the formula:

According to a pH of 1.1:

$$X(\text{ACC}) = 2M(\text{ACC}) \cdot V_m \cdot c(\text{DPAA}) \cdot (V / V_1)$$

where  $M(\text{ACC})$  - the molar mass of the ACC (163.2 g/mol);

$V_m$  - volume of titrant consumed for titration, mL;  
 $V_1$  - volume of solution aliquots ACC, taken for titration, mL;

$V$  - total volume of solution ACC;  
 $c$  - (DPAA) molar concentration of standard solution of diperoxyazelaic acid, mol/L;

According to a pH of 4.7:

$$X(\text{ACC}) = M(\text{ACC}) \cdot V_m \cdot c(\text{DPAA}) \cdot (V / V_1)$$

where  $M(\text{ACC})$  - the molar mass of the ACC (163.2 g/mol);

$V_m$  - volume of titrant consumed for titration, mL;

$V_1$  - volume of solution aliquots ACC, taken for titration, mL;

$V$  - total volume of solution ACC;

$c(\text{DPAA})$  - molar concentration of standard solution diperoxyazelaic acid, mol/L.

Results potentiometric determination of N- acetylcysteine in sample solutions are presented in Table. 1.

Table 1

Results potentiometric determination of N- acetylcysteine in model solution

Was taken N-ACC, mg	Was found, mg	Metrological characteristics
(approaching 1) pH = 1.1		
1.63	1.57 1.63 1.70 1.60 1.67	X' = 1.63 mg S = 0.052 S <sub>X'</sub> = 0.023 ΔX' = 0.065 RSD = 3.19 (δ = 0%)
3,26	3.25 3.20 3.35 3.30 3.30	X' = 3.28 mg S = 0.057 S <sub>X'</sub> = 0.,025 ΔX' = 0.071 RSD = 1.74 (δ = +0,6%)
(approaching 2) pH = 4.7		
1.63	1.60 1.63 1.62 1.67 1.65	X' = 1.63 mg S = 0.027 S <sub>X'</sub> = 0.012 ΔX' = 0.034 RSD = 1.65 (δ=0%)
3.26	3.18 3.22 3.30 3.25 3.30	X' = 3.25 mg S = 0.052 S <sub>X'</sub> = 0.023 ΔX' = 0.065 RSD = 1.60% (δ = -0.3%)

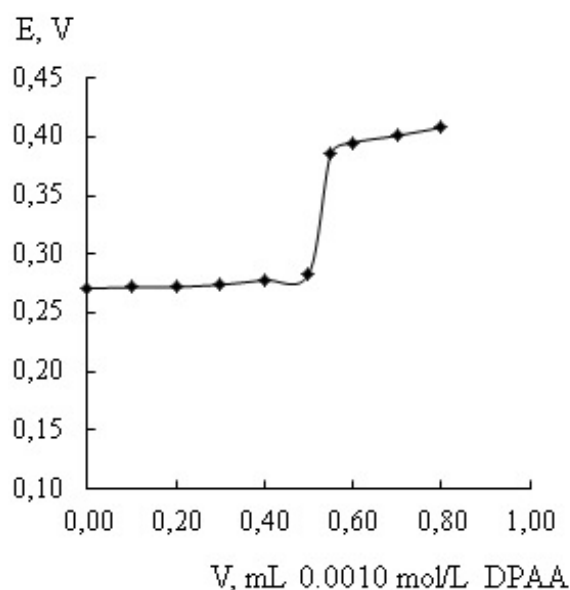


Figure 2. Potentiometric titration curver of 0.01 mmol AA with diperoxyazelaic acid in the presence of KI at pH 1.1. 0.1 mol / L HCl, 0.25% KI

Procedure for determination of ascorbic acid. Aliquot portion (1.00 or 2.00 mL) of the test solution (0.176 g (precise weights) AA at 100.00 mL double distilled water) was transferred to a beaker of 50 ml, add 18-19 ml of 0.2 mol / l  $\text{KH}_2\text{PO}_4$  solution at pH 4.7, 1.0 mL of 5 % solution of KI and titrated 0,01 mol / L solution DPAA using 10 ml microburette.

AA content in mg under different conditions calculated by the formula:

$$X(\text{AA}) = 2M(\text{AA}) \cdot V_m \cdot c(\text{DPAA}) \cdot (V / V_1)$$

where M (AA) molar mass of ACC (176.13 g/mol);  
 $V_m$  - volume of titrant consumed for titration, mL;  
 $V_1$  - AA aliquot volume of the solution taken for titration, mL;  
V - total volume of solution AA mL;  
c - (DPAA) molar concentration of standard solution diperoxyazelaic acid mol/L;

The results of determination of ascorbic acid in model solutions and substance listed in the table 2 and 3, respectively.

Table 2.

The results of the quantitative determination of ascorbic acid in the sample solutions

Ascorbic acid was taken mg	Was found, mg	Metrological characteristics
1.76	1.80 1.83 1.70 1.73 1.75	X' = 1.76 mg S = 0.053 S <sub>X</sub> ' = 0.0235 ΔX' = 0.065 RSD = 3.30 (δ = 0%)
3.52	3.57 3.59 3.52 3.45 3.49	X' = 3.52 mg S = 0.057 S <sub>X</sub> ' = 0.026 ΔX' = 0.071 RSD = 1.63 (δ = 0%)

Procedure for determining the content of the basic substance in a substance ascorbic acid. 0.09 g (exact sample) tested dissolve powder in 40 ml of double- distilled water in a volumetric flask with 50 ml of water and dilute to the mark . In a beaker 50 mL using a pipette make 1.00 ml of solution manufactured , add 18 ml of 0.2 mol/L solution KH<sub>2</sub>PO<sub>4</sub> pH 4.7, 1.0 ml of 5 % solution of K i and titrated by 0.01 mol/L DPAA solution with vigorous stirring using 10 ml mikroburet.

The content of ascorbic acid w (AA), in %, calculated by the formula:

$$w(\text{AA}) = 2M(\text{AA}) \cdot c \cdot V_m(\text{DPAA}) \cdot V / V_1 \cdot K \cdot 1000 / ms \cdot 100\%$$

where M (AA) – the molar mass of AA (176.13 g/mol);  
V<sub>m</sub> - volume of titrant consumed for titration, ml;  
V<sub>1</sub> - AA aliquot volume of the solution taken for titration, mL;  
V - total volume of solution AA ml;  
c - (DPAA) molar concentration of standard solution diperoxyazelaic acid mol/L;  
K - coefficient amendments concentrations costly to 0.0100 mol/L;  
1000 – recalculation of mg to g;  
ms - mass (weight) powder sample tested drug substance, g .

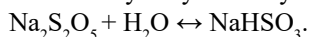
Table 3

The results of determination of the basic substance in a substance ascorbic acid.

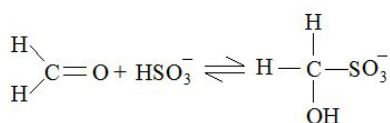
Content of basic substance %	Was found, %	Metrological characteristics (n=7; P=95)
99.6%*	101.42	X' = 100.31 mg
100.02%**	101.99	S = 1.30
	100.01	S <sub>X</sub> ' = 0.49
	99.05	ΔX' = 0.71
	99.15	RSD = 1.30 (δ = +0.71%...+0.28%)
	101.50	
	99.02	

Note. \* Declared in the certificate manufacturer \*\* Established us pharmacopeia method (DFUkraine)

We also have explored the possibility of determination by potentiometric titration using in situ generated free iodine in the reaction of iodide with DPAA diluted standard solution of ascorbic acid in pharmaceuticals factory production solution for injection 5%. As the excipient of the pharmaceutical form of ascorbic acid in the solution for injection is sodium metabisulfite that water is hydrolyzed to hydrogen sulfite:



Possible impact of NaHSO<sub>3</sub> preventing the determination of ascorbic acid removed by masking it with formaldehyde. When adding formaldehyde to a working solution dosage form of ascorbic acid is a reaction that is:



The resultant formaldehyde-bisulfite complex analysis of the conditions does not react with DPAA, and therefore does not interfere with the determination of ascorbic acid.

Procedure of quantitative determination of ascorbic acid in solution for injection by redox potentiometric titration standard 0.01 mol/L solution diperoxyazelaic acid. 2.00 mL solution for injection ascorbic acid transferred to a volumetric flask of 10 mL of water and brought to the mark. With selected pipette 2.00 mL working solution manufactured dosage form of ascorbic acid and transferred to a beaker of 50 ml, 20 mL was added of 0.2 mol/L phosphate buffer solution with pH 4.7, 0.15 mL of 1% solution of formaldehyde and 0.2 mL of 0.01 mol/L potassium iodide and turned on a magnetic mixer. Immersed electrodes every 60 seconds using mikroburette portions of 0.1 ml titrant flows 0.01 mol/L solution DPAA. Near end point titration (ept) titrant was added portions of 0.02 mL. At the ept recorded by a sharp change in the cell voltage (emf). Volume of consumed on titration solution which was responsible ept was determined graphically by the break point of the potentiometric titration curve. The content of ascorbic acid in the solution for injection X in mg/mL was calculated using the formula:

$$X = \frac{T \cdot V_m \cdot 5 \cdot 1000}{V_a}$$

where T – titer value, each mL of 0.01 mol/L diperoxyazelaic acid VS 0.0035224 g of  $C_6H_8O_6$ ;

$V_m$  volume of solution titrant consumed for titration, mL;

5 dilution factor ; 1000 - recalculation in mg ;

$V_a$  volume working solution dosage form of ascorbic acid taken for titration, mL.

Table 4.

The results of the quantitative determination of ascorbic acid in the solution for injection 5 % (n = 7 ; P = 0.95)

Solution taken for analysis, mL	Ascorbic acid was found, mg/mL	Metrological characteristics
2.00	49.50	$\bar{X}' = 49.16\text{mg}$ $S = 0.64$ $S_{\bar{X}'} = 0.24$ $\Delta\bar{X}' = 0.60$ $RSD = 1.31\%$ ( $\delta^* = -0.28\%$ )
2.00	48.05	
2.00	49.05	
2.00	48.67	
2.00	49.54	
2.00	49.30	
2.00	50.01	

Note. \* To calculate the used value content of ascorbic acid ( $\mu$ ), indicated in the certificate of quality.  $\delta = (-\mu) \cdot 100\% / \mu$ .

## CONCLUSIONS

Titration by directly generated in the sample solution in situ iodine using diluted solution DPAA as titrant achieves higher sensitivity and determination ACC simplify temperature conditions, which is impossible in the classical iodimetry method. RSD was 3.19-1.60% ( $\delta = 0.3 \dots 0\%$ ) for 1.63-3.26 mg of ACC in 20 mL.

A new method of quantitative determination of ascorbic acid substance and a solution for injection by redox potentiometric titration using a titrant DPAA. Carried validation of the proposed analytical method by such characteristics as linearity, accuracy, convergence, limit of detection (LOD), limit of quantitative detection (LOQ). The resulting metrological characteristics methods do not exceed the eligibility criteria for SPU. The technique is characterized by simplicity and speed performance, high sensitivity and selectivity, reproducibility and accuracy of satisfactory results. The proposed method was successfully applied to commercial substance and 5 % injection solution of ascorbic acid. The average percentage recovery (n=7) was 100.31% and 98.32%; RSD 1.30% and 1.31% for accuracy,  $\delta +0.71\% \dots +0.28\%$  and  $-0.28\%$  for substance and 5% solution for injection respectively. The limit of quantification (LOQ) of ACC and ascorbic acid was 0.03 mg and 0.06 mg to 20 mL final volume respectively.

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