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COULOMETRIC DETERMINATION OF L-CYSTINE BY OXIDATION REACTION WITH ELECTROGENERATED CHLORINE

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L-Cystine is one of the well-known substitutable alpha-amino acids, a stable (oxidized) form of the amino acid of cysteine. The amino acids of L-Cystine and L-Cysteine are involved in the formation of peptides (insulin and immunoglobulins) and proteins in the formation of their structure. The body easily converts them into one another, in the metabolism they are equivalent. Most of these two amino acids are contained in the proteins of the human and animal's covering tissues: hair (up to 14%), horns (up to 7%), and skin. L-Cystine provides elasticity of keratin. Therefore, this substance is included in the vitamin complexes to improve the appearance (skin and hair), biologically active additives and shampoos. But this is not the only application of cystine. The spectrum of drugs is wide and encompasses not only illnesses associated with worsening of the skin, but also cases of intoxication with heavy metals (due to the ability to form complexes with metal ions, L-Cystine and Cysteine, were effective in poisoning with copper and other metal salts, and therefore help to deduce them from an organism). As a food additive (E921) for the improvement of flour products, sodium and potassium salts of L-cystine are used. A drug with the same name L-Cystine exhibits antioxidant; hepatoprotective; detoxification immunomodulatory wound healing; mucolytic and expectorant actions. Often, cystine is used to treat diabetes, Alzheimer's disease, bronchitis and protein deficiency. This substance is involved in the metabolism and helps with violations of connective tissues; it is also prescribed during the period of rehabilitation after operations and in diseases of the joints. The drug has two forms of release: capsules and ampoules. L-Cystine is also part of other combined medications. Thus, one hypoxic tablet of the drug "Eltacin" contains as active substances L-Cystine 70 mg, glycine 70 ml, L-glutamic acid; Excipients: Methylcellulose 7.8 mg, Magnesium stearate 2.2 mg. A mixture of these substitutable amino acids is metabolic regulators: increase the intracellular concentration of glutathione and the activity of glutathione dependent enzymes, normalize oxidation-reduction processes and utilization of oxygen in tissues, and therefore the drug exhibits antioxidant, antihypoxic, increasing the processes of ATP synthesis, increases efficiency, etc. The European Pharmacopoeia 8.0 [1] for the quantitative determination of L-Cystine recommends the method of inverse bromometry with visual fixation of cl. T. However, the interaction with bromine runs long, the interaction time should be no less than 10 minutes, and there is also a very large spread of the values obtained. The need for prior standardization of titrant (sodium thiosulfate) leads to an additional increase in analysis time. In addition, the sensitivity of the recommended method is limited by a relatively high concentration of titrant (0.05 mol/L). Therefore, a decision was taken to determine the L-Cystine by electrically generated free chlorine used the procedure described in [2].

It was established that L-Cystine interacts with electrically generated Chlorine rapidly and in a ratio of 1 to 5. L-Cystine spend 10 electrons during oxidation. The preliminary results of the measurements show that optimum determination conditions are the following: 2 mol/L NaCl in 0.5 mol/L sulfate acid solution. The results obtained are in agreement with the literature data [3]. The electrogeneration of chlorine was performed on a platinum electrode under a constant current of 2.5 mA. The end-point of titration was established potentiometrically with platinum and silver chloride electrodes [2]. The coefficients of the regression equation, evaluation of precision and accuracy, *LOD* and *LOQ* of the proposed method are given in the Tables 1–3. The proposed method was applied for the analysis of of L-Cystine in tablets "Eltacin" (Table 4).

Table 1. Regression output (Y=a + b·X)

Таблиця 1. Коефіцієнти рівняння регресії Y=a + b·X

Parameter	*Data*
Range, μg/g	0,8-87,9
Regression equation:	*Y=a+bX*
Slope, b·	0,0402
Intercept, a	0,0005
Regression coefficient, r	0,999
Standard deviation of the analytical signal, Sa	0,0031
LOD, μg/g	0,25
LOQ, μg/g	0,76

Table 2. Results of analysis of model solutions of L-Cystine (n = 4, P = 0.95)

Таблиця 2. Результати аналізу модельних розчинів L-Цистину (n = 4, P = 0.95)

Amount taken, μg/g	*Amount found, $x \pm t_a \cdot S$,* μg/g	*Recovery±RSD*, %	δ, %
1,99	2,01±0,08	101,0±1,25	+1,00
15,9	16,0±0,8	100,4±1,57	+0,63
59,2	59,7±0,6	100,9±0,32	+0,84
65,9	65,5±0,6	99,5±0,29	-0,61
87,9	87,2±0,7	99,2±0,25	-0,80

Table 3. Evaluation of systematic error by the method of "additives"

Таблиця 3. Оцінювання систематичної похибки методом «добавок»

Amount founded L-Cystine in *Eltacine*, μg	Amount added L-Cystine, μg	Calculated amount L-Cystine, μg	Total amount found L-Cystine, μg	Recovery ±RSD, %	δ, %
165,1	46,2	211,3	209,7	99,87±0,51	- 0,13%
99,2	46,6	145,8	145,8		
33,3	47,9	81,2	81,4		

Table 4. Results of analysis of L-Cystine in tablets "Eltacin" by the proposed method (n = 4, P = 0.95)
Таблиця 4. Результати кількісного визначення L-Цистину в пігулках «Елтацин» запропонованим методом (n = 4, P = 0.95)

Labelled amount, mg	*Permissible interval*, mg	*Amount found*, mg	*Recovery* ± *RSD*, %
70,0	64,7–75,3	71,2±0,4	101,7±0,35

Thus, the method of quantitative determination of L-Cystine in a substance and tablets "Eltacin" is proposed with the help of coulometric titration, which allows obtaining accurate results with RSD ≤ 1.6%.

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КУЛОНОМЕТРИЧНЕ ВИЗНАЧЕННЯ L-ЦИСТИНУ ЗА РЕАКЦІЄЮ ОКИСНЕННЯ ЕЛЕКТРОГЕНЕРОВАНИМ ХЛОРОМ

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Запропонований новий метод кількісного визначення вмісту основної речовини у субстанції L-Цистину та препараті «Елтацин», котрий ґрунтується на кулонометричному титруванні L-Цистину електрогенерованим хлором у кислому середовищі. Оптимізовано умови електрогенерації титранта з мінімальної похибкою визначення: фоновий електроліт - 0,5 моль/л розчин сульфатної кислоти та 2,0 моль/л натрій хлориду, силу струму I=2-3 мА. Здійснена процедура валідації опрацьованої методики за такими параметрами: робочий діапазон з межами визначення, правильність та відтворюваність, межа виявлення та межа кількісного визначення. Показано, що запропонована методика дозволяє отримувати достатньо точні (правильні та відтворювані) результати визначення вмісту L-Цистину як у субстанції L-Цистину, так і у пігулках «Елтацин», з RSD ≤ 1.6%.

Ключові слова: кулонометрія, L-Цистин, хлор, Елтацин