New Method for Simultaneous Determination of Ascorbic and Acetylsalicylic Acids in Effervescent Tablets

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The determination of the real concentration in different pharmaceutical drugs represents a very important goal of the pharmaceutical industry. The main aim of the study was to set up a simple, cheap and rapid method in order to determine both ascorbic and acetylsalicylic acid content of effervescent tablets that could be found on the pharmaceutical market, without preliminary separation or extraction. For this purpose, a very simple and reliable clock method based on two Landolt-type systems of reactions, based on potentiometric techniques, has been developed. The method has been tested on different effervescent tablets which contain ascorbic acid and acetylsalicylic acid in different mixture ratio.

Key words: ascorbic acid, acetylsalicylic acid, potentiometric method, Landolt system, effervescent tablets

Ascorbic acid (AA) is a water-soluble vitamin widely used clinically. Because of its antioxidant and immunomodulatory activities, vitamin C is associated with a number of pharmaceutical substances in the treatment of some diseases, being in the researcher's attention in experimental pharmacological studies, in order to discover new associations of active substances. These associations imply an optimal benefic effect concerning the drug therapy of some diseases [1-5].

In some pharmaceuticals, vitamin C is associated with acetylsalicylic acid (aspirin, ASA), substance with anti-inflammatory and antipyretic properties. Most of these products are in the form of effervescent preparations (granules and tablets), used mainly to prevent and combat cold and flu symptoms [6]. From the pharmacological point of view, the combination of the two active ingredients in a single preparation is advantageous. From the point of view of drug quality control, in the case of medicines that contain two active substances, these associations have two big disadvantages as regards the separate identification: most analytical methods used to determine individual compounds are ineffective in the presence of both components and increased costs for necessary equipment [7].

In the literature there are many analytical methods used to determine both substances. Some of the methods could be used just for ascorbic acid determination as following: simple volumetric metod [8, 9], electrochemical [10], spectrophotometry [11-16] and chromatography, especially HPLC [17, 18]. Others methods could identify only acetylsalicylic acid when it is the only analyte in the sample as chromatographic techniques, electrochemical, spectrophotometric and kinetic [19-21]. Furthermore, there are some methods for simultaneous determination of AA and ASA: chromatographic techniques [22], derived spectrometry [23], electrochemical methods [24, 25], kinetic determinations [26], and electronic techniques [27].

This work brings a new way of tackling the kinetic methods, more precisely the application of the Landolt-type systems as regards their use in a less investigated field, namely the quantitative analytical chemistry of some substances of biochemical interest such as water-soluble vitamins, acids and amino acids [8-10, 28-32].

The aim of the study was to set up a simple, cheap and rapid method in order to determine both ascorbic and acetylsalicylic acid content of effervescent tablets that could be found on the pharmaceutical market, without preliminary separation or extraction.

Experimental part

Apparatus and reagents

The substances and reagents used in this study have been from Merck and had analytical purity.

All the potentiometric determinations were made using an electrochemical cell, by measuring the evolution of electrode potential with time. The oxidation-reduction electrode was platinum plate immersed in the reaction mixture, while the reference electrode was a saturated calomel. The cell was made by a double wall baker with circulating water and stirring. The curves potential vs. time were recorded using a Digital Multimeter with data logger (VA 18B PC link, USA).

The effervescent tablets used in this experiment were bought from local pharmacies. Five tablets (after weighing them) have been dissolved into 1 L deionised water. Furthermore successive dilutions have been done depending on the active ingredient concentration in order to fall in the range of our calibration curves.

Statistically analysis and data handling

The data obtained from the measurements potential *vs.* time have been processed using ORIGIN 8 (OriginLab corporation, MA, USA) program in order to obtain the first derivate of the curves. The experiments were replicated three times and the means were statistically compared using the ORIGIN 8 (OriginLab corporation, MA, USA).

Results and discussions

The combination of ascorbic and acetylsalicylic acids is used commonly in the case of flue, cold or headache. On the pharmaceutical market there are different tablets

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which associate both compounds. The effervescent tablets are the most used ones. The main purpose of the study is to set up a simple, cheap and rapid method in order to determine both ascorbic and acetylsalicylic acid content in the effervescent tablets. Our method is based on two Landolt type reaction systems.

In this type of oscillating reactions system, as long as the trapping agent exists in the system (in this case ascorbic acid and acetylsalicylic acid), there is no change in the evolution of the potential and the first derivative of the curve.

The concentration of species: bromate, bromine, bromide and hydrogen ion (in one Landolt system), respectively hydrogen peroxide and iodide (in the other Landolt system) do not change significantly.

When the trapping agent, namely analyte, is completely consumed, the bromine is liberated and Landolt effect appears. The ratio bromate-bromide is modified and consequently the potential. The dependence of the potential *vs.* time presents an inflexion point that represents the end of the reaction. Actually, the total consumption of the analyte corresponds to the moment of steep increase of potential. It was considered that is more convenient to take that as the end point of the process (operationally defined), because it can be obtained more precisely. The bromine generation with a blank probe follows the same behaviour.

When ascorbic acid (vitamin C) plays the role of a trapping agent for bromine, first ascorbic acid is completely consumed, bromine accumulates suddenly in the mixture, and the end point could be visualised by using potentiometric measurements [8, 9, 26, 28].

Choosing the concentration of BrO_3^-, Br^-, H^+ large enough, bromide being re-formed back by ascorbic acid reduction of bromine, the reaction rate is practically constant at small degree of consumption. It means that, within equal time intervals, the same amount of bromine is generated and the same concentration of ascorbic acid is oxidised. Therefore:

$$Rate = \frac{-d[C_6H_8O_6]}{dt} = \frac{-\Delta[C_6H_8O_6]}{\Delta t} = \frac{[C_6H_8O_6]_t}{\tau} \quad (1)$$

If acetylsalicylic acid is also present in the mixture, then its rapid bromination at the aromatic ring takes place in parallel with the oxidation of ascorbic acid. A supplementary consumption of bromate is needed. Consequently, more time delay until the steep increase in bromine concentration is required.

The reaction conditions and different concentrations of the reagent($[KBrO_3^-]_0 = 3\times10^{-3}$M, $[KBr] = 0.2$M, $[H^+] = 2\times10^{-2}$ M, T = 293 K) have been optimised in one of our previous work [8, 9, 26]. As any clock- reaction, it requires several restrictions, imposed by the system itself: as the Landolt effect should appear at a value of time long enough we have to avoid relative errors being too large, to avoid hydrogen peroxide self-decomposition, and also the oxidation of ascorbic acid by hydrogen peroxide.

Furthermore, ascorbic acid could be determined also using the second Landolt type system, based on I^--H_2O_2, as has been shown in the other work [8, 9, 26, 28]

We used both systems in order to determine the ascorbic and acetylsalicylic acids in the mixture. With the first one, the sum of both acids has been determined, while with the second one we determined the concentration of ascorbic acid.

Calibration curves

All calibration curves were obtained by plotting the time for Landolt-effect apparition function of the analyte (the trapping agent). Due to the fact that our aim has been to determine the concentration of both analytes (ascorbic acid and acetylsalicylic acid), two calibration lines have been obtained. First one was obtained just for ascorbic acid and the second one was made with ascorbic acid in the presence of acetylsalicylic acid, both substances being together in solutions in the same proportion of 1:1.66, just like in the most frequent ratio that exists in the pharmaceuticals (fig. 1).

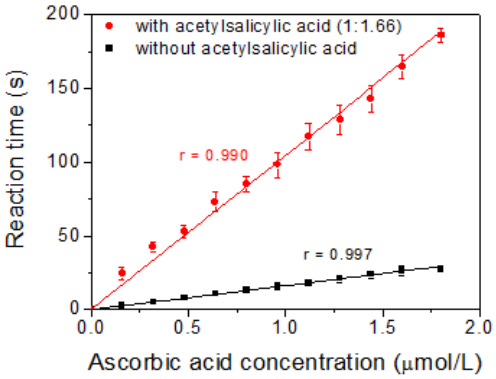

Fig. 1. The calibration curves for ascorbic acid with and without acetylsalicylic acid in a pre-established ratio of 1:1.66

To ensure that the effect of acetylsalicylic acid delayed reaction time is additive to that of ascorbic acid, the calibration lines were drawn for each substance alone, and with the increasing of the concentration of one at a constant amount (0.8 μmol/L) of the other [8]. As table 1 shows, the lines are almost parallel, because two different processes occur simultaneously in the mixture: the oxidation of the ascorbic acid and acetylsalicylic acid bromination.

It could be seen that in both cases the curves have almost the same slopes, fact that demonstrates the cumulative effect of the ascorbic acid and acetylsalicylic acid; this cumulative effect can be used to determine the sum of both acids in the samples.

Application of the method on the real samples

The method has been applied for the determination of ascorbic acid and acetylsalicylic acid in the mixture from different medicines (which not contain additives and / or active substances with reducing character that can perturb the two Landolt type reactions systems). The results are presented in table 2.

Table 1

THE CALIBRATION LINES FOR EACH SUBSTANCE ALONE AND WITH THE INCREASING OF THE CONCENTRATION OF ONE SUBSTANCE AT A CONSTANT AMOUNT OF THE OTHER (UNDER THE REACTION CONDITIONS: $[KBrO_3^-]_0 = 3\times10^{-3}$M, $[KBr] = 0.2$ M, $[H^+] = 2\times10^{-2}$ M, T = 293 K)

Equation for each substance alone	
ASA	**AA**
$t = (6.7\pm2.1) + (5.89\pm0.13)10^5\,[ASA]sec$	$t = (0.2\pm0.9) + (7.40\pm0.09)10^5\,[AA]sec$
Equation for each substance in the presence of a constant amount of other substance	
ASA in the presence of a constant amount of **AA**	**AA** in the presence of a constant amount of **ASA**
$t = (59.2\pm0.6) + (15.55\pm0.07)10^5\,[ASA]sec$ with r=0.9992; s_0=1.2 sec.; N=11	$t = (30.0\pm0.7) + (7.51\pm0.08)10^5\,[AA]sec$ with r=0.9995; s_0=1.28 sec.; N=11

Table 2

PHARMACEUTICALS IN EFFERVESCENT TABLETS FORM, WITH ASCORBIC ACID AND ACETYLSALICYLIC ACID WHICH ARE SUITABLE FOR THE PROPOSED METHOD

Pharmaceuticals	Producer	Active substance [mg/tablet]		Ratio of active substances AA/ASA
		AA	ASA	
UPSARIN VITAMINE C	UPSA Laboratoires France	200	330	1:1.65
ASPIRINE VITAMINE C	UPSA Laboratoires France	200	500	1:2.5
ASPIRIN PLUS C	BAYER BITTERFELD GmbH, Germany	240	400	1:1.66
ASPIRIN 400MG C*	BAYER BITTERFELD GmbH, Germany	240**	400**	1:1.66
ASPIRIN PLUS C Forte	BAYER BITTERFELD GmbH, Germany	480	800	1:1.66
ASPRO 500 mg VITAMINE C	BAYER BITTERFELD GmbH, Germany	300	500	1:1.66
ACIDO ACETILSALICILICO E VITAMINA C	MYLAN GENERICS Italy	200	330	1:1.65
ASPIRINA + VITAMINA C	VENT3 República Argentine	200	500	1:2.5

*drug present in effervescent granules form;
** active substance in mg/envelope.

Table 3

COMPARISON BETWEEN DIFFERENT METHODS FOR ASCORBIC ACID (AA) AND ACETYLSALICYLIC ACID (ASA) DETERMINATION (IN MG/TABLET)

Pharmaceuticals	Prospectus		Landolt method		HPLC method		Titration method	Spectrophotometric method
	AA	ASA	AA	ASA	AA	ASA	AA	AA
UPSARIN VITAMINE C	200	330	202	331	201	328	197	198
ASPIRINE VITAMINE C	200	500	203	497	201	502	196	195
ASPIRIN PLUS C	240	400	243	401	240	399	242	241
ASPIRIN 400MG C*	240	400	240	399	242	402	245	244
ASPIRIN PLUS C Forte	480	800	475	798	482	803	475	477
ASPRO 500 mg VITAMINE C	300	500	298	504	300	502	295	307
ACIDO ACETILSALICILICO E VITAMINA C	200	330	201	333	202	329	195	196
ASPIRINA + VITAMINA C	200	500	197	502	201	500	198	198

*drug present in effervescent granules form; active substance in [mg/envelope].

Even more, the results obtained by using the kinetic method based on Landolt-type redox systems were compared with those obtained by using some non-kinetic standardized method such as: the classical iodometric titration method or the titration method that uses 2,6-dichlorophenolindophenol (for ascorbic acid); the spectrophotometric determination of ascorbic acid with 2,6-dichlorophenolindophenol, after the extraction with xylene; HPLC determination of ascorbic acid and acetylsalicylic acid from effervescent tablets [16]. Furthermore, we used also for comparison the prospectus of the pharmaceutical form.

The results have shown that our determination using the Landolt method is reliable with the prospectus and other classical and more expensive methods (table 3).

Conclusions

In order to develope a sensitive, simple, selective and cheap method for the determination of ascrobic acid and acetysalycilic acid in effervescent tablet/granule pharmaceutical form, two Landolt-type systems: hydrogen peroxide – iodide and bromate – bromide have been used. The system evolution was monitored potentiometrically, under the strict control of the reaction conditions (temperature, concentration of reactants, interferents). Using the hydrogen peroxide – iodide Landolt system, only ascorbic acid can be determined, and using the bromate – bromide system, the sum of the two substances can be determined. By subtraction, the acetylsalicylic acid content has been obtained. The results thus acquired were compared to experimental data obtained by the use of other non-kinetic (equilibrium) methods and are quite similar.

References

1.ABDEL-DAIM, M. M., Cytotechnol, 68, p. 279, 2016.
2.ABDEL-DAIM, M. M., GHAZY, E. W, FAYEZ, M., Can. J. Physiol. Pharmacol., 93, p. 45, 2015.

3.ABDEL-DAIM, M. M., GHAZY E. W., Iran. J. Basic. Med. Sci., 18, 2015, p. 221.
4.ABDEL-DAIM, M. M., EL-GHONEIMY A., Ren. Fail., 37, 2015, p. 297.
5.CANDELARIO-JALIL., E., AKUNDI, R. S., BHATIA, H. S., LIEB, K., APPEL, K., MUNOZ, E., HULL, M., FIEBICH, B. L., J. Neuroimmunol.,174, 2006, p.39.
6.DE MIRANDA, J. A., CUNHA, R. R., GIMENES, D.T., MUNOZ, R. A. A, RICHTER, E. M., Quim. Nova, 35, 2012, p. 1459.
7.SUPALKOVA, V., PETREK, J., HAVEL, L., KRIZKOVA, S., PETRLOVA, J., ADAM, V., POTESIL, D., BABULA, P., BEKLOVA, M., HORNA, A., KIZEK, R., Sensors, 6, 2006, p.1483.
8.BUNGAU, S., Vitamins and amino acids determination using kinetic methods, Colecþia Science&Technology, Ed. Italian Academic Publishing, Bucuresti, 2014, pp.125-167.
9.BUNGAU, S., BALDEA, I., COPOLOVICI, L., Rev. Chim.(Bucharest), **54**, no. 3, 2003, p. 213.
10. YILMAZ, S., SADIKOGLU, M., SAGLIKOGLU, G., YAGMUR. S., ASKIN, G., Int. J. Electrochem. Sci., 3, 2008, p. 1534.
11. HE, B., ZHANG, J., Int. J. Electrochem. Sci., 10, 2015, p. 9621.
12.KLEPO, L., COPRA-JANICIJEVIC, A., KUKOC-MODUN L., Molecules, 21, nr.1, 2016, p. 101.
13.SZPIKOWSKA SROKA B., POLEDNIOK J., J. Anal. Chem, 66, 2011, p. 941.
14.SALKIC, M., KERAN, H., JASIC, M., Agric. Conspec. Sci, 74, nr.3, 2009, p. 63.
15.SALKIC, M., SELIMOVIC, A., Croat. Chem. Acta, 88, nr.1, 2015, p. 73.
16. WABAIDUR, S. M, ALOTHMAN, Z. A., Khan, M. R., Spectrochim. Acta A Mol. Biomol Spectrosc., 108, 2013, p. 20.
17.MITIC, S. S., KOSTIC, D. A., NASKOVIC- DOKIC, D. C., MITIC, M. N., Trop J Pharm Res, 10, 2011, p.105.
18.GAZDIK, Z., ZITKA, O., PETRLOVA, J., ADAM, V., ZEHNALEK J., HORNA, A., REZNICEK, V., BEKLOVA M., KIZEK, R., Sensors, 8, nr.11, 2008, p.7097.
19.BOCHENSKA, P., PYKA, A., J. Liq. Chrom. & Relat. Technol., 35, nr.10, 2012, p. 1346.
20.TORRIERO A. J., LUCO, J. M., SERENO L., RABA J., Talanta, 62, nr.2, 2004, p.247.
21. COPOLOVICI, L., BUNGAU, S.,DRAGAN,F., Rev. Chim.(Bucharest), **56**, no. 4, 2005, p. 374.
22.KHAN, M. R., ALOTHMAN, Z. A., NAUSHAD, M. U., GHFAR, A. A., WABAIDUR, S. M., J. Liq. Chrom. & Relat. Technol.,Vol. 35, nr.17, 2012, p. 2454.
23.TORAL, M. I.,RICHTER, L. N., TASSARA, A., TAPIA, A. E., RODRIGUEZ C., J AOAC Int., 84, nr.1, 2001 p. 37.
24.DE MIRANDA, J. A. T., CUNHA, R. R.,. GIMENES, D. T., MUNOZ, A. R., RICHTER, E. M.**,** Quim. Nova, 35, 2012, p. 1459.
25.PUANGJAN, A., CHAIYASITH, S., WICHITPANYA, S., DAENGDUANG, S., PUTTOTA, S., J Electroanal. Chem., 782, 2016, p.192.
26.BUNGAU, S., COPOLOVICI, L., BALDEA, I., Farmacia, nr. 4, 2004, p. 60.
27. PEREIRA, O. R., GOMES, C., GONÇALVES, C., BORLIDO, D., BATISTA, M., TEIXEIRA, T., DIAS, L. G., Atencion Primaria, 46, nr.1, 2014, p. 85.
28. BUNGAU, S., FODOR, A., TIT, D. M., SZABO, I., Anals Oradea Univ., Fasc. Environ. Prot., XVI/A, 2011, p. 212.
29. BUNGAU, S., SZABO, I., BADEA, G. E., FODOR, A., Rev. Rou. Chim.(Bucharest), **57**, no. 2, 2012, p. 101.
30. BUNGAU, S., COPOLOVICI, L., BALDEA, I., MERCA, V., Rev. Chim., (Bucharest), **55**, no. 12, 2004, p. 1009.
31. BUNGAU, S., COPOLOVICI, L., BALDEA, I., SZABO, I., Rev. Chim.(Bucharest), **55**, no. 11, 2004, p. 886.
32. CARABAN, A., BUNGAU, S., FODOR, A., STANASEL, O., Rev. Chim.(Bucharest), **57**, no. 6, 2006, p. 607.

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