Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2013, 5(10):102-107

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Kinetic spectrophotometric determination of acetylsalicylic acid in dosage form "ACELYSIN-KMP"

Mykola Ye. Blazheyevskiy[1] and Lyubomyr S. Kryskiw*[2]

[1]National University of Pharmacy, Physical and Colloid Chemistry Department,
[2]Toxicological Chemistry Department, 53, Pushkinskaya str., Kharkov, 61002, Ukraine

---

ABSTRACT

A kinetic spectrophotometric method has been developed for the determination of acetylsalicylic acid (ASA) in model solutions and dosage form "ACELYSIN-KMP". The proposed procedure is based on the indicator reaction of catalytic p-phenetidine oxidation by hydrogen peroxide in a weak alkaline medium. Compresent components and potential hydrolytic cleavage products do not interfere the ASA determination. Calibration graph for ASA has linear dependence in the range 12 - 180 μmol L^{-1}. The limit of quantitation is 12 μmol L^{-1}. For five determinations of 44 μmol L^{-1}, 88 μmol L^{-1} and 130 μmol L^{-1} ASA the reproducibility has a RSD of 1.18, 1.06 and 0.76% respectively. Hence the proposed method is more sensitive, simple and express in comparance with the well-known one. Dosage form "ACELYSIN-KMP" recovery is 98.95 ±1.32% of ASA.

Keywords: "ACELYSIN-KMP", acetylsalicylic acid, perhydrolysis, kinetic spectrophotometric determination, *p*-phenetidine**.**

INTRODUCTION

"ACELYSIN-KMP", Kyivmedpreparat - lysini acetylsalicylas is a mixture of DL-lysine acetylsalicylate and glycine with a ratio of 9:1. It has pharmacological properties of acetylsalicylic acid (ASA), but unlike the latter is easily soluble in water and can be used parenterally [1].

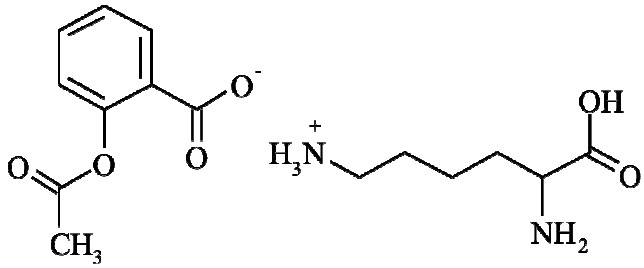

Fig. 1: Chemical structure of DL-lysine acetylsalicylate

SPhU and EPh recommend to determine content of ASA in pure ASA substance titrimetrically: after preliminary saponification, excess of alkali is titrated by hydrochloric acid with phenolphthalein as indicator [2-3]. According to the analytical normative documents DL-lysine acetylsalicylate ($C_{15}H_{22}N_2O_6$) assay is performed by reversed-phase HPLC with UV detection [4]. Dosage form "ACELYSIN-KMP" contains not less than 0.832g and not more than 0.968g of $C_{15}H_{22}N_2O_6$ per bottle.

In the scientific literature, determination of ASA is performed by spectrophotometric methods [5-8], fluorescence spectroscopy [9], potentiometry [10-13], liquid chromatography [14-16] and HPLC [17-19], differential scanning calorimetry [20], scattering of X-rays [21], microdialysis [22] etc. Gas chromatography and it combination with mass spectrometry and HPLC [23] is recommended in analytical toxicology for ASA quantitation in biological liquids.

In multicomponent dosage forms, the simplest and most widely used method of quantitative determination of ASA is a direct UV-spectrophotometry (when the absorption bands of the mixture components do not overlap) [24]. In the combined medicines "Kofitsyl plus" and "Askofen" for determination of ASA Firordt method was used [25]. For the simultaneous determination of ASA, phenacetin and caffeine in their mixtures derivative UV-absorption spectra relationship method was used [26].

Perhydrolysis with direct absorbance measurement of the reaction product - salycilate is known also [27]. Selective kinetic spectrophotometric method of aspirin assay has been previously proposed [28-29]. Two coupled reactions were used there: perhydrolysis of ASA as a process inducer and following hydroperoxide oxidation of chromogenic substrate.

Therefore in the present paper, we describe a new kinetic spectrophotometric method of quantitative determination of ASA in the selected drug. It is based on the system of two coupled reaction: ASA perhydrolysis (reaction with excess of H_2O_2 in a weak alkaline medium with peroxyacetic acid (PAA) formation) and following *p*-phenetidine (*p*-Ph) oxidation by newly generate PAA to azodue (λ_{max} = 358 nm). Its increasing absorbance allows to determine ASA (**Fig. 2**). Kinetic spectrophotometric initial rate method was used for computing.

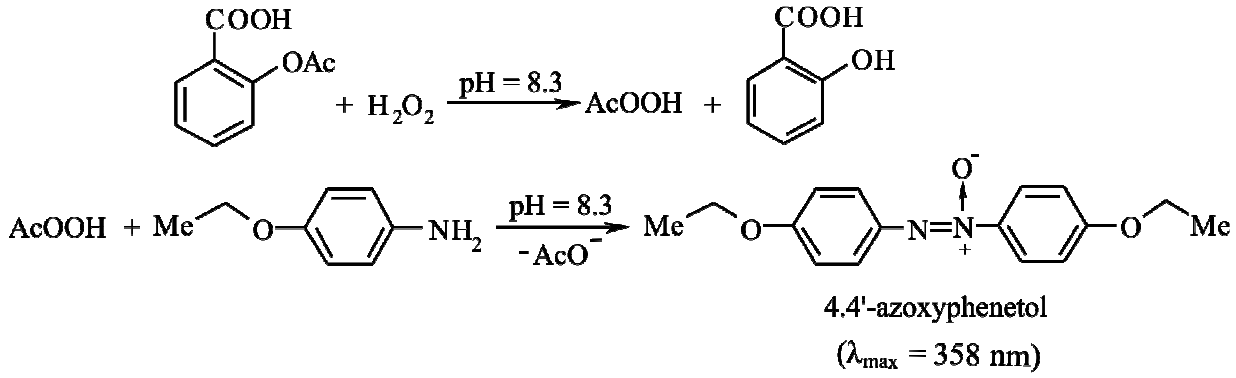

Fig. 2: Chemistry of ASA perhydrolysis and coupled *p*-phenetidine peroxyacid oxidation

Compresent glycine, lysine and potential hydrolytic cleavage products under these conditions of the reaction do not interfere the determination of ASA in dosage form "ACELYSIN-KMP".

EXPERIMENTAL SECTION

Chemicals and Reagents. *p*-Ph (4-etoxyaniline) 98%, (ALDRICH, Germany), double-distilled water (DDW), ethyl alcohol 96% (Dubovyazovskiy distillery, Ukraine), 30% solution of hydrogen peroxide was made of "Hydrogen peroxide" 50%, medical quality (LLC "Inter-Synthesis", Borislav, Ukraine). 0.2 M phosphate buffer solutions with a pH ranging from 7.7 - 9.5 [30] were used to create and maintain the desired pH. "ACELYSIN-KMP" powder for injection 1.0 g (JSC "Kyivmedpreparat", Kyiv, Ukraine), S/N 30412 from 02.04.2012 was used for the analysis.

Preparation of 1% *p*-Ph working solution. 1.00 g of *p*-Ph sample was dissolved in a 100 mL volumetric flask in 50 mL of 96% ethanol. The volume was completed to the mark with DDW at 20°C and shaken thoroughly.

Preparation of the Acelysin working standard solution (WSS), 0.1982 mg mL^{-1}. Contents of the vial "ACELYSIN-KMP" (Label claim: 1.0 g) was dissolved in DDW in a 250 mL volumetric flask, completed to the mark with DDW at 20°C and shaken thoroughly. 10 mL of the obtained solution was pipetted to a 100 mL volumetric flask and completed to the mark with DDW at 20°C and shaken thoroughly.

All the chemicals and reagents were analytical grade and the solutions were prepared freshly.

Equipment. Reaction rate was monitoring by the increasing of absorbtion of the 4.4'-azoxyphenetole (λ_{max} = 358 nm), which is the product of perhydrolysis of ASA and conjugate peroxyacid oxidation of *p*-Ph on the SF-46

spectrophotometer (LOMO, USSR) with 1 cm matched quartz cells. pH of the solutions was monitored by a glass electrode ESL 43-07 type (reference electrode - a silver/silver chloride electrode EVL-1M3.1 type) on the laboratory ionometer I-130 (NPO "Analitpribor").

Obtaining of calibration curve. From 1 to 5 mL of Acelysin WSS was transferred into the 25 mL volumetric flask with consecutive addition of 10 mL of buffer solution (pH = 8.3), 2.0 mL of *p*-Ph solution, 2.5 mL H_2O_2, completed to the mark with DDW at 20°C and shaken thoroughly during 30 sec. Absorbance of obtained solution was measured at 358 nm vs. blank solution (without examined substance). Time was controlled from the moment of solution mixing with the stop-watch.

Obtained results estimated by the reference of International Union of Pure and Applied Chemistry (IUPAC) [31] and State Pharmacopeia of Ukraine (SPhU) [2] with mathematical statistics methods. Limit of quantitation (LOQ) was calculated as recommended in work [32]. Accuracy verification realized by “input-output” analysis of model solution. ASA content in model solutions was found by the method of standard.

RESULTS AND DISCUSSION

Kinetic curve of 4.4'-azoxyphenetol accumulation in *p*-Ph - H_2O_2 - ASA system (pH = 8.3) was obtained (**Fig. 3**). Sites from 5 to 10 min has linear dependence and specify initial reaction rate (A vs. τ dependence tangent angle).

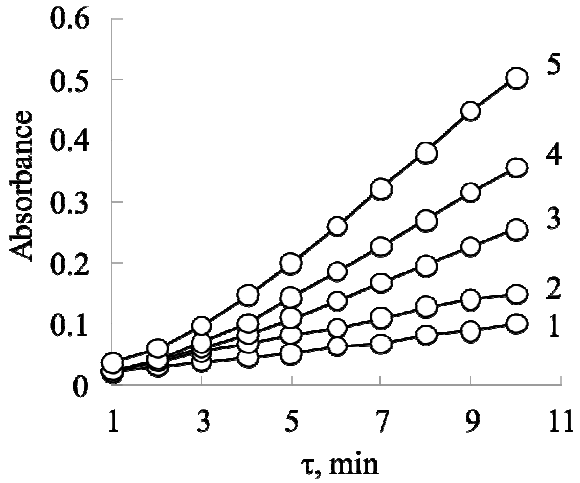

Fig. 3: Kinetic curve of 4.4’-azoxyphenetol accumulation in *p*-Ph- H_2O_2-ASA system
c (p-Ph) = 5.84 × 10^{-3} mol L^{-1}; c (H_2O_2)=0.224 mol L^{-1}; c (ASA), μmol L^{-1}: 1 - 22; 2 - 44; 3 - 88; 4 - 130; 5 - 180; pH = 8.3.

Ascertain that the reaction rate amount to the maximal in pH range 8.2 - 8.5 (**Fig. 4**).

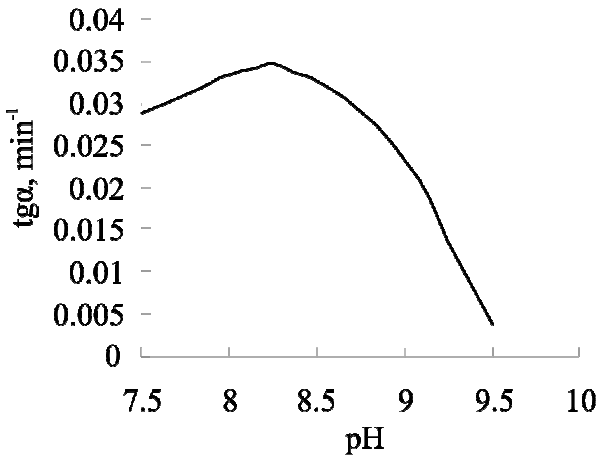

Fig. 4: Dependence of 4.4'-azoxyphenetol formation rate on pH in *p*-Ph - H_2O_2 - ASA system
c (p-Ph) = 5.84 × 10^{-3} mol L^{-1}; c (H_2O_2) = 0.224 mol L^{-1}; c (ASA) = 88 μmol L^{-1}.

The Beer's law was verified from the calibration curve by plotting a graph of concentration vs. increasing of absorbance from the series of ASA concentrations ranging from 22 - 180 μmol L^{-1} (**Fig. 5**).

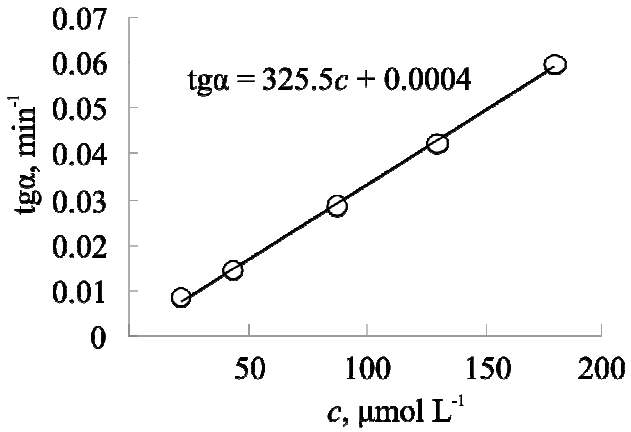

Fig 5: Calibration curve of ASA determination (λ_{max} 358 nm) in *p*-Ph - H_2O_2 - ASA system
c (p-Ph) = 5.84 × 10^{-3} mol L^{-1}; c (H_2O_2) = 0.224 mol L^{-1}; pH = 8.3; T = 295 K.

The optical characteristics and the analytical data of the calibration curve including standard deviations for the slope and intercept (S_a, S_b) are summarized in **Table 1.**

Table 1: Optical characteristics and regression output

Parameter	Value
Wavelength of detection (nm)	358
LOQ (μmol L^{-1})	12
Beer's Law Limit (μmol L^{-1})	22 - 180
Regression equation*	tgα = 325.5*c* + 0.0004
Slope (a)	325.5
Intercept (b)	0.0004
S_a	21.386
S_b	0.0023
Correlation coefficient (r)	0.9987

** tgα = ac + b, where c is the concentration of analyte, mol L^{-1}, and tgα is the initial reaction rate*

It is shown experimentally that the perhydrolysis reaction is the limitative stage of *p*-Ph oxidation in *p*-Ph - H_2O_2 - ASA system. Stated kinetic feature of the passing reactions and sufficiently high selectivity of indicator reaction of *p*-Ph oxidation by newly generate PAA in the presence of relatively large excess of H_2O_2 is the basis of developed selective kinetic spectrophotometric tangent method of quantitative assay of ASA in dosage form «ACELYSIN-KMP».

Procedure of assay. Contents of the vial «ACELYSIN-KMP» was taken in a 250 mL volumetric flask and dissolve in a few mL of DDW. After dissolution, the solution was completed to the mark with the same solvent at 20°C and shake thoroughly. 10 mL of the obtained solution was pipetted to a 100 mL volumetric flask and completed to the mark with DDW at 20°C and shaken thoroughly.

2 mL of tested Acelysin solution was transferred into the 25 mL volumetric flask with consecutively addition of 10.0 mL of buffer solution (pH = 8.3), 2.0 mL of *p*-Ph solution, 2.5 mL H_2O_2, completed to the mark with DDW at 20°C and shaked thoroughly during 30 sec. Absorbance of the obtained solution was measured at 358 nm vs. blank solution (without tested substance). Time was controlled from the moment of solution mixing with the stop-watch. Test with the WSS of Acelysin was performed analogously.

Calculation of ASA content (*w)* in converting to the dried substance in dosage form "ACELYSIN-KMP" in %, was made by the formula: $w = \frac{C_{st} \cdot tg\alpha \cdot 250 \cdot 100 \cdot 100 \cdot 100\%}{tg\alpha_{st} \cdot 1000 \cdot 10 \cdot m_s \cdot (100 - w_{H_2O})}$, where

C_{st} - ASA concentration in WSS solution, mg mL^{-1};
tgα - tangent of angle slope in the test with the examined solution of Acelysin, min^{-1};
$tg\alpha_{st}$ - tangent of angle slope in the test with the WSS solution, min^{-1};
10 - volume of the solution taken for analysis, mL;

250 - volume of the volumetric flask, mL;
m_s - mass of ASA in the vial "ACELYSIN-KMP", g;
W_{H_2O} - water content, taken from the Quality Certificate, %.

ASA concentration in model solution *c*, mol L^{-1}, was computed by the calibration curve equation. The results of the analysis of dosage form "ACELYSIN-KMP" in model solutions and in drug are shown in **Table 2** and **Table 3** respectively.

Table 2: Metrological characteristics of the results of kinetic ASA determination in model solutions of dosage form "ACELYSIN-KMP" (*n* = 5, *P* = 0.95)

Metrological characteristics	Amount taken, mol L^{-1}		
	4.40×10^{-5}	8.80×10^{-5}	1.30×10^{-4}
$\overline{X}$	4.38×10^{-5}	8.76×10^{-5}	1.29×10^{-4}
s	5.14×10^{-7}	9.32×10^{-7}	9.86×10^{-7}
$s_{\overline{X}}$	2.30×10^{-7}	4.17×10^{-7}	4.41×10^{-7}
$\Delta\overline{X}$	6.39×10^{-7}	1.16×10^{-6}	1.23×10^{-6}
RSD, %	1.18	1.06	0.76
ε, %	1.46	1.32	0.95
δ, %	-0.55	-0.41	-0.39

Table 3: Metrological characteristics of the results of kinetic ASA determination in dosage form "ACELYSIN-KMP" (*n*=5, *P*=0.95)

ASA content, g*	$\overline{X} \pm \Delta\overline{X}$	s	$s_{\overline{X}}$	*RSD, %*	*δ*	Recovery ± *ε*, %
0.5012	0.4959 ± 0.0066	5.28×10^{-3}	2.36×10^{-3}	1.06%	-1.05	98.95 ± 1.32

**Claimed in the Quality Certificate*

CONCLUSION

Thus, a highly selective and sensitive kinetic spectrophotometric method has been developed for the determination of ASA in dosage form "ACELYSIN-KMP" using the indicator reaction of catalytic *p*-Ph oxidation by hydrogen peroxide in water medium. Evidently, that the obtained results show the possibility of determination of significantly lower amounts of analyte in comparance with Pharmacopeia's method [2-3]. Method has satisfactory reproducibility and accuracy (RSD ≤ 1.18%, δ ≤ -0.55) with a limit of quantitation LOQ = 12 μmol L^{-1} of ASA. It does not require the usage of toxic solvents or reagents and sophisticated equipment. Dosage form "ACELYSIN-KMP" recovery is 98.95 ± 1.32% of ASA.

REFERENCES

[1] Compendium **2011** - Pharmaceutical preparations, ed. by VM Kovalenko; AP Viktorova, Moryon, Kyiv, **2011**; 181.
[2] State Pharmacopoeia of Ukraine, State Enterprise "Scientific and Expert Pharmacopoeial Centre", 1st Edition, RIREH, Kharkiv, **2001**; 392.
[3] European Pharmacopea, 5th Edition, European department for the Quality of Medicines, Strasbourg, **2005**; 1104.
[4] State Pharmacopoeia of Ukraine, State Enterprise "Scientific and Expert Pharmacopoeial Centre", 1st Edition, Suppl. 2, Kharkiv, **2008**; 78-84.
[5] SZ Bathaie; L Nikfarjam; R Rahmanpour; AA Moosavi-Movahedi, *Spectrochim. Acta A Mol. Biomol. Spectrosc*., **2010**, 77(5), 1077-108.
[6] Y Wanga; PP Xua; XX Lia; K Niea; MF Tuoa; B Kongb; J Chena, *J. Pharm. Anal.*, **2012**, 2(5), 386-389.
[7] LX Quiao; ZG Gao; Q Xing; R Qiu, *J. Hebei Norm. Univ.*, **2001,** 25(2), 219-220.
[8] S Ghajar; MR Sohrabi, *J Chem Pharm Res*., **2012**, 4(1), 814-821.
[9] JC Alves; RJ Poppi, *Anal. Chim. Acta*, **2009**, 642(1), 212-216.
[10] RN Goyal; S Bishnoi; B Agrawal, *J. Electroanal. Chem.*, **2011**, 655(2), 97-102.
[11] Z Wang; Li H; J Chen; Z Xue; B Wu; X Lu, *Talanta*, **2011**, 85(3), 1672-1679.
[12] M Yousef Elahi; SZ Bathaie; SH Kazemi; MF Mousavi, *Anal. Biochem.*, **2011**, 411(2), 176-184.
[13] BJ Sanghavi; AK Srivastava, *Electrochim. Acta*, 55(28), **2010**, 8638-8648.
[14] G Kahsay; A Van Schepdael; E Adams, J. Pharm. *Biomed. Anal.*, **2012**, 61, 271-276.
[15] E Yamamoto; S Takakuwa; T Kato; N Asakawa, *J. Chromatogr. B*, **2007**, 846(1-2), 132-138.
[16] YRK Reddy; S Reddy; MRP Reddy; K Mukkanti, *J Chem Pharm Res*., **2013**, 5(4), 181-187.
[17] SR Polagania; NR Pillib; Ganduc, *J. Pharm. Anal.*, 2(3), 206-213.

[18] MS Elmasry; IS Blagbrough; MG Rowan; HM Saleh; AA Kheir; PJ Rogers, *J. Pharm. Biomed. Anal.*, **2011**, 54(4), 646-652.
[19] V Krishnaiaha; YVR Reddy, *J Chem Pharm Res*., **2012**, 4(3), 2349-2353.
[20] L Campanella; V Micieli; M Tomassetti; S. Vecchio, *Thermochim. Acta*, **2011**, 526(1-2), 151-156.
[21] A Hodzic; M Llusa; SD Fraser; O Scheibelhofer; DM Koller; F Reiter; P Laggner; JG Khinast, *Int. J. Pharm.*, **2012**, 428(1-2), 91-95.
[22] LH Shaw; TH Tsai, *J. Chromatogr. B.*, **2012**, 895-896, 31-38.
[23] LY Galichet. Clarke's Analysis of Drugs and Poisons, 3-rd Edition, Pharmaceutical Press, London, Electronic version, **2005**.
[24] IV Vlasova; V Shilov; YuS Fokin *Zav. Labor.*, **2011**, 77(1), 21-27.
[25] IV Vlasova; SA Kulakova; AV Pomortseva, *Zav. Labor.*, **2005**, 71(9), 18-20.
[26] Y Wei; D Wang; H Yan, *Spectrosc. Spectral Anal.*, **2001**, 21(4), 534-537.
[27] MYe Blazheyevskiy, LS Kryskiw. Actual questions of development of new drugs, Abstracts of XX international scientific and practical conference of young scientists and students, Publishing Office, Kharkiv, **2013**; 73.
[28] MYe Blazheyevskiy, *Pharmac. J.*, **2004**, 3, 65-72.
[29] MYe Blazheyevskiy, NY Bondarenko. Achievements and prospects of the pharmaceutical industry in Ukraine, Mater. VI Nat. Congress of Pharmacists of Ukraine, Kharkiv, **2005**; 134.
[30] AI Lazarev, IP Kharlamov, PY Yakovlev, EF Yakovleva. Handbook of chemical-analytics, Metallurgy, Moscow, **1976**; 184.
[31] K Doerffel. Statistics in analytical chemistry, Mir, Moscow, **1994**; 267 p.
[32] LP Eksperiandova; KN Belikov; SV Khimchenko; TA Blank, *J. Anal. Chem.*, **2010**, 65(3), 229-234.