Scientific paper

Quantitative Analysis of Acetylsalicylic Acid in Commercial Pharmaceutical Formulations and Human Control Serum Using Kinetic Spectrophotometry

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Abstract

The aim of this work is to develop a new kinetic spectrophotometric method for the determination of acetylsalicylic acid (ASA) in pharmaceutical formulations. In general, ASA analysis is not realised directly, and a previous quantitative hydrolysis in a basic medium is necessary, converting ASA to salicylate ions for its determination. The hydrolysis is carried out by sodium hydroxide solution. The method is based on a ligand-exchange reaction. The reaction was followed spectrophotometrically by monitoring the rate of disappearance of the cobalt(II)-1-nitroso-2-naphthol complex in alkaline medium at 410 nm. The optimum operating conditions regarding reagent concentrations and temperature were established. The initial-rate method is adopted for constructing the calibration curve, which was found to be linear over the concentration range 0.72–9.00 μg mL^{-1}. The optimized conditions yielded a theoretical detection limit of 0.35 μg mL^{-1} based on the $3.3S_0$ criterion. The interference effects of certain ingredients of powdery drugs, foreign ions and amino acids upon the reaction rate were studied in order to assess the selectivity of the method. The results are validated statistically and through recovery studies. The point hypothesis test have been performed which indicate that there is no significant difference between the proposed method and the reference method. The developed procedure was successfully applied to the rapid determination of acetylsalicylic acid in commercial pharmaceutical preparations and human control serum. The unique features of this procedure are that determination can be carried out at room temperature and analysis time is short. The newly developed method is simple, inexpensive and efficient for use in the analysis of a large number of samples.

Keywords: Acetylsalicylic acid; kinetic spectrophotometry; validation; pharmaceutical analysis

1. Introduction

Acetylsalicylic acid (ASA)-which is marketed in Bayer's familiar Over-the-Counter product range under the trade name Aspirin® and Bayer Aspirin®-has now enjoyed safe and effective use for 100 years, initially as a pain reliever, fever reducer and anti-inflammatory agent. It has proven effective as a general pain reliever and is routinely used in a wide range of pain indications, including headache, body and muscle ache, arthritis and many more. Low-dose acetylsalicylic acid also helps prevent life-threatening vascular complications connected with, such as retinopathy and nephropathy.[1,2]

The various analytical methods such as UV,[3,4,5] derivative UV spectrophotometry,[6] spectrofluorimetry,[7–9] solid-phase fluorimetry,[10,11] reflectance spectroscopy,[12–15] FT-Raman spectroscopy,[16,17] FT-IR/ATR spectrometry,[18,19] electroanalytical methods,[20–24] capillary electrophoresis,[25,26] FIA combined with electrochemical methods,[27–29] HPLC,[30,31] HPLC using on-line solid-phase extraction and on-line post-column photochemical derivatization,[32–34] LC-MS,[35,36] have been reported for the deter-

mination of acetylsalicylic acid. However, as far we know, there is no kinetic-spectrophotometric method for the determination of acetylsalicylic acid in the literature. Spectrophotometry is the technique of choice even today due to its inherent simplicity. It is frequently used in the laboratories of the developing countries to overcome a variety of analytical problems. There are certain advantages associated with kinetic methods such as:[37,38]

– simplicity owing to elimination of some experimental steps such as extraction, derivatization prior to absorbance measurements,
– high selectivity due to the measurements of the evaluation of the absorbance as a function of reaction time instead of measuring the concrete absorbance value,
– no interference from other active compounds present in the commercial dosage forms, if they resist the chemical reaction conditions
– free from background interferences
– because measurements are often made faster with kinetic methods than equilibrium methods, many very slow reactions become analytically useful.

The aim of this work is to develop a simple, rapid to be performed, reliable, selective kinetic method for the determination of acetylsalicylic acid in commercial pharmaceutical preparations. The method is based on a ligand-exchange reaction. The procedure is easier to execute and requires less sample handling than methods currently described in the literature. Using methods such as HPLC, insoluble additives should be removed to prevent the columns from becoming blocked. Also, some HPLC methods for ASA determination using on-line solid-phase extraction and post-column photochemical derivatization. For the GLC methods, chemical derivatization is essential. Fluorimetric methods are simple, accurate and precise, but these methods have not been widely used in practice because the species that can be detected are limited. Also, with this technique, insoluble excipients would cause an intense and time-dependent signal background. All these methods are time consuming, complex and require procedures they may increase the amount of the hydrolyzed product during manipulation of the sample.

2. Experimental Procedures

2. 1. Apparatus

The reaction rate was monitored spectrophotometrically. The absorbance of the solution was measured at the wavelength of 410 nm. The readings were performed on a Perkin-Elmer Lambda 15 UV/Vis spectrophotometer, connected to a thermostatic bath (operating in a temperature range 20.00–60.00 ± 0.02 °C).

A model 1200 Agilent Technologies was used for HPLC analysis. The analytical column was C_{18} (Zorbax, 5 μm, 250 × 4.6 mm).

The solutions were thermostated at 22.00 ± 0.02 °C before the beginning of the reaction.

2. 2. Reagents

A 1.0×10^{-3} mol L^{-1} stock solution of acetylsalicylic acid was prepared in ethanol (95%, V/V) from pharmaceutical 99.87% certified products, kindly provided by a pharmaceutical laboratory (Galenika, a.d., Belgrade, Serbia). Acatylsalicylic acid solution was stored at 4 °C. To prevent the possible decomposition of ASA to SA, solution was freshly prepared prior to experiments.

A 1.0 mol L^{-1} sodium hydroxide solution (Merck) was prepared in deionised water.

A 1.0×10^{-3} mol L^{-1} 1-nitroso-2-naphthol solution (Merck) was prepared by dissolving a known amount in ethanol (95%, V/V).

The cobalt(II) solution (1.7×10^{-3} mol L^{-1}) was prepared by dissolving $CoCl_2 \times 6H_2O$ (Merck) in water.

Ionic strength of reaction mixture was kept constant at 0.1 by adding an appropriate amount of NaCl solution (1.0 mol L^{-1}).

Analytical grade chemicals and deionised water (MicroMed high purity water system, TKA Wasseraufbereitungssysteme GmbH) were used for the preparation of all solutions. All the glassware used was washed with aqueous HCl (1:1) and then thoroughly rinsed with running, distilled water, and then finally with deionised water.

2. 3. Procedure

2. 3. 1. General Procedure

In order to obtain good mechanical and thermal stability, the instruments were run for 10 min before the first measurement. The reaction was carried out in the following way. In a special four-compartment vessel, the solution of 1-nitroso-2-naphthol was placed in the first, acetylsalicylic acid and sodium hydroxide in the second, cobalt(II) in the third, and electrolyte for the ionic strength and ethanol (95%, V/V) (total volume 10 mL) in the fourth compartment.

The vessel was thermostated at 22.00 ± 02 °C. The content was mixed well and than immediately transferred to the spectrophotometric cell with a path length of 10 cm. The change in absorbance was recorded at 410 nm as a function of time every 30 s for the first 5 min of the reaction. The rate of the reaction at different concentrations of each of the reactants was obtained by measuring the slope of the linear part of the kinetic curves to the absorbance-time plot (from Beer's law $A = \varepsilon \cdot l \cdot c$, $dA/dt = \varepsilon \cdot l \cdot dc/dt$, $dc/dt = \frac{dA/dt}{\varepsilon \cdot l}$, $slope = dA/dt$, $rate = dc/dt$). The calibration graph was constructed by plotting the slope of the linear part of the kinetic curve, versus concentration of ASA (c_{ASA}, μg mL^{-1})

2. 3. 2. Procedure for Tablets

A total of 20 tablets of each one of the different used pharmaceutical preparations containing ASA were weighed and finely powdered using a mortar and pestle. An accurately weighed quality of the resulting powder, equivalent to 50 mg (weight of the tablet) of ASA was dissolved in 25.0 mL of ethanol. Then the solution was centrifuged at 1800 rpm for 10 min and filtered through a 0.45 μm membrane filter (Millipore) directly in a 50.0 ml volumetric flask and filled up to a volume with ethanol to obtain a solution whose expected ASA concentration was 40 μg mL^{-1}. Aliquots of this solution were transferred into vessels covering the concentration range listed in Table 4.

In all cases it was assumed that the actual content of the tablet corresponds to that reported by the manufacturing laboratories.

2. 3. 3. Serum Sample Preparation

Human lyophilised control serum (Lyotrol N, bioMérieux® sa, France) was used. ASA was added covering the concentration range listed in Table 5. The concentration of ASA was chosen to match its normal therapeutic concentration in human serum.[39] To 0.5 mL of serum, appropriate amount of the stock solution of ASA (10 mg mL^{-1}) and 15 mL of ethanol was added and after brief vortex mixing it was centrifuged for 5 min at 3000 rpm to deposit the protein precipitate. The separeted supernatant was collected in a 25.00 mL standard volumetric flask and filled up to the mark with the same solvent. Serum sample was contained 100.0 μg mL^{-1} of ASA. Aliquots of this solution were transferred into vessels covering the concentration range listed in Table 5. For kinetic determination, Fe^{3+} ions were masking by adding appropriate amount of F^- ions (1×10^{-4} g mL^{-1}). For HPLC determination, aliquots of ASA solution were transferred in a 10.00 mL volumetric flask, evaporated to dryness in a water bath, the residue was reconstituted with mobile phase and 10 μL was transferred into glass vial for automatic injection into the HPLC system.

2. 3. 4. Comparative Method

The procedures for comparative methods (HPLC and titrimetric method with visual indication) have been described in the British Pharmacopoeia[40] and US Pharmacopoeia.[41] Acetylsalicylic acid was detected and quantified on a 250 × 4.6 mm Zorbax C_{18} (5 μm) analytical column operating at room temperature. The mobile phase was a mix of phosphoric acid-acetonitrile-water, 2:400:600 (by vol). The eluate was monitored at 237 nm. Injection of the samples (10 μL) was performed using an autosampler. Flow rate was 1 mL min^{-1}.

3. Results and Discussions

3. 1. Mechanism of the Reaction

According to Kolthoff and Jacobsen[42] divalent cobalt coordinates with two ligands (1-nitroso-2-naphthol, R(NO)OH), liberating two hydrogen ions for each cobalt ion present.

$$Co^{2+} + 2R(NO)OH \rightarrow Co[R(NO)O]_2 + 2H^+$$

Analyses of the Co(II) complex indicated six coordination (an octahedral geometry), actually Co(II) complex corresponded to $Co[R(NO)O]_2 \cdot 2H_2O$.

In other case, SA also shows complexing ability with Co(II).[43] The complex agrees with the empiric formula $[Co(SA)(H_2O)_2]Cl_2$. The IR and electronic spectra and microanalytical data confirmed the coordination via

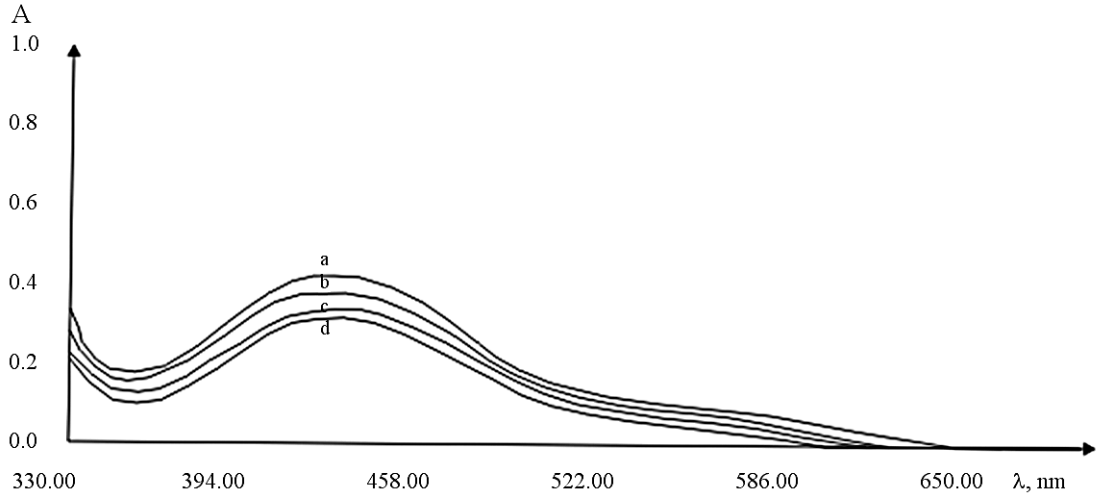

Fig. 1. Absorption spectra (a–d) of the reaction at 2 min interval. Conditions: $c_{R(NO)OH} = 1.0 \times ^{-5}$ mol L^{-1}, $c_{NaOH} = 1.0 \times ^{-2}$ mol L^{-1}, $c_{Co^{2+}} = 1.7 \times ^{-5}$ mol L^{-1}, $c_{ASA} = 5.1 \times ^{-5}$ mol L^{-1}, t = 22.00 ± 02 °C

the carboxyl (–COOH) and phenolic (–OH) oxygenes with displacement of the protons from both groups.[43,44] In the present study, kinetic method was described based on ligand-exchange reaction. Determination of ASA involved its quantitative alkaline hydrolysis which converted ASA to salicylate ions and its complexation with cobalt(II) ($C_9H_8O_4 + 2OH^- \rightarrow C_7H_5O_3^- + CH_3COO^- + H_2O$). Its chelate cobalt complex is more stable than that formed with R(NO)OH. The reaction moves to the right and SA was determined by monitoring the rate of disappearance of the cobalt(II)-1-nitroso-2-naphthol complex at 410 nm (Fig. 1). At can be seen, reaction can be monitored spectrophotometrically by measuring the decrease in absorbance versus time.

$$Co[R(NO)O]_2 \cdot 2\,H_2O + SA \rightarrow$$
$$[Co(SA)(H_2O)_2]Cl_2 + 2R(NO)OH$$

3. 2. Kinetic studies

3. 2. 1. Optimization of Variables

A tangent method was used to process the kinetic data. The rate of the reaction was obtained by measuring the slope of the linear part of the kinetic curves to the absorbance-time plot (slope = dA/dt).

In order to achieve the best sensitivity, the working conditions needed to be optimized. Therefore, the dependence of the rate of reactions on the concentration of each of the reactants and tempereture was studied.

Effect of the concentration of NaOH. The effect of the concentration of sodium hydroxide (Fig. 2) was studied in the range 0.01–0.12 mol L^{-1}. It can be seen that the reaction rate was increased with an increasing concentration of NaOH up to 0.09 mol L^{-1}; beyond this concentration the rate of the reaction remained constant. For further work a concentration of 0.10 mol L^{-1} was used.

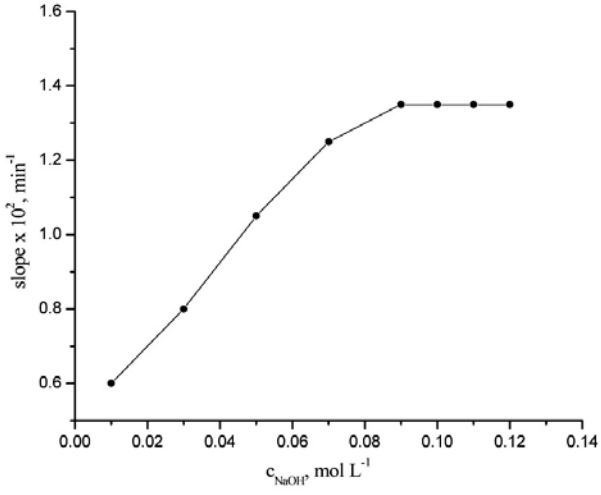

Fig. 2. Effect of sodium hydroxide concentration on the slope of the absorbance-time curve. Initial concentrations: $c_{R(NO)OH}$ = 1.0 × $^{-5}$ mol L^{-1}, $c_{Co^{2+}}$ = 1.7 × $^{-5}$ mol L^{-1}, c_{ASA} = 1.0 × $^{-5}$ mol L^{-1}, t = 22.00 ± 02 °C

Effect of the concentration of 1-nitroso-2-naphthol. The effect of the concentration of 1-nitroso-2-naphthol on the rate of reaction (Fig. 3) was studied in the range 0.4–2.0 × 10^{-5} mol L^{-1}. It can be seen that the reaction rate increases with increasing of the1-nitroso-2-naphthol concentration from 0.40–1.25 × 10^{-5} mol L^{-1} and become constant at 1.25 × 10^{-5} mol L^{-1}. Thus, a concentration of 1.50 × 10^{-5} mol L^{-1} was chosen as the optimum concentration.

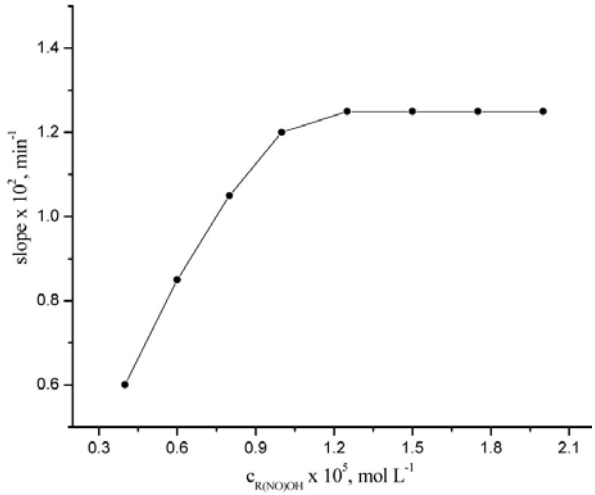

Fig. 3. Effect of 1-nitroso-2-naphthol concentration on the slope of the absorbance-time curve. Initial concentrations: c_{NaOH} = 0.1 mol L^{-1}, $c_{Co^{2+}}$ = 1.7 × $^{-5}$ mol L^{-1}, c_{ASA} = 1.0 × $^{-5}$ mol L^{-1}, t = 22.00 ± 02 °C

Effect of the concentration of Co(II). A correlation between slope and the Co(II) concentration is given on Fig. 4. The influence of the concentration of Co(II) on the rate of reaction examined in the range of 0.17–5.90 × 10^{-5} mol L^{-1}. The reaction rate increased with increasing the

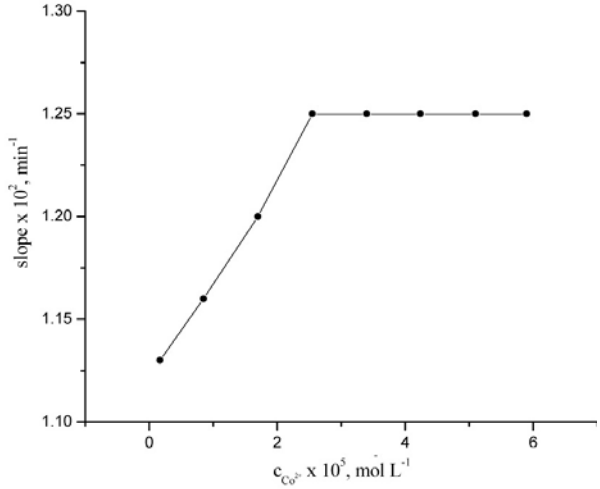

Fig. 4. Effect of cobalt(II) concentration on the slope of the absorbance-time curve. Initial concentrations: c_{NaOH} = 0.1 mol L^{-1}, $c_{R(NO)OH}$ = 1.5 × $^{-5}$ mol L^{-1}, c_{ASA} = 1.0 × $^{-5}$ mol L^{-1}, t = 22.00 ± 02 °C

concentration of Co(II) from 0.17–2.55 × 10^{-5} mol L^{-1} and become constant at 2.55 × 10^{-5} mol L^{-1}. Thus a concentration of 3.40 × 10^{-5} mol L^{-1} in the final solution was used throughout the experiment.

Effect of the temperature. The effect of temperature (Fig. 5) on the reaction rate was studied at 292, 295, 298, 301 and 304 K. The absorbance-time curves obtained at these temperatures indicated the temperature dependence of the reaction rate. The rate for different concentrations of ASA at each temperature was calculated and utilized for plotting the calibration curve. At temperature > 298 K, the linear dynamic range of determination decreases. It was found that the calibration graph obtained at 295 K possessed good linearity (r^2 = 0.9986) and it is recommended that the determination can be carried out at 295 K.

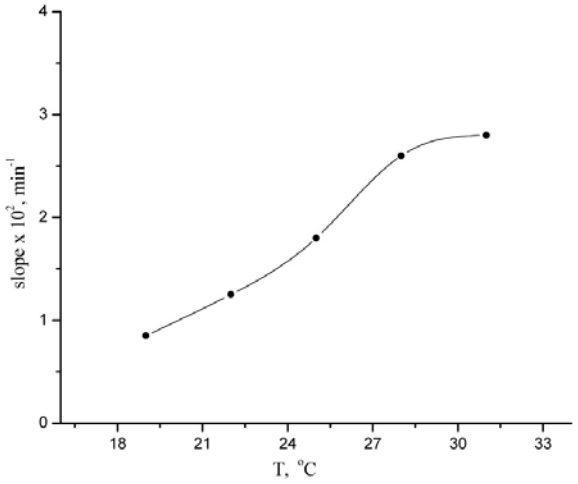

Fig. 5. Effect of the temperature on the slope of the absorbance-time curve. Initial concentrations: c_{NaOH} = 0.1 mol L^{-1}, $c_{R(NO)OH}$ = 1.5 × 10^{-5} mol L^{-1}, $c_{Co^{2+}}$ = 3.4 × 10^{-5} mol L^{-1}, c_{ASA} = 3.0 × 10^{-5} mol L^{-1}

The least squares' equation[45] (y = bx + a, where b and a are, respectively, its slope and intercept) for the calibration graph and correlation coefficient (r^2) for the determination of acetylsalicylic acid in the interval 0.72 to 9.00 μg ml^{-1} under the optimal reaction conditions ($c_{R(NO)OH}$ = 1.5 × 10^{-5} mol L^{-1}, c_{NAOH} = 0.1 mol L^{-1}, $c_{Co^{2+}}$ = 3.4 × 10^{-5} mol L^{-1}, t = 22.00 ± 0.02C) were calculated:

$$slope \times 10^2 = 0.053 \times c_{ASA} + 1.142 \qquad r^2 = 0.9986$$

where *slope* is the slope of the linear part of the kinetic curve to the absorbance-time plot ($slope = dA/dt = \varepsilon \cdot l \cdot dc/dt$, Beer`s law) and c_{ASA} is the acetylsalicylic acid concentration expressed in μg mL^{-1}.

The variance (S_0^2) of the calibration line was evaluated to be 3.0 × 10^{-9} (μg mL^{-1})^2. The low value of variance indicated negligible scattering of the experimental data points around the line of regression. Quantitative parameters of the analysis are given in Table 1.

Table 1 Quantitative parameters of the analysis

Parameters	
Calibration range / μg mL^{-1}	0.72 – 9.00 , n = 6
Regression equation	slope × 10^2 = 0.053 × c_{ASA} + 1.142
Slope ± SD	(0.053 ± 0.001) × 10^{-2}
Intercept ± SD	(1.142 ± 0.004) × 10^{-2}
Correlation coefficient, r^2	0.9986
Variance (S_0^2) / (μg mL^{-1})^2	3.0 × 10^{-9}
Detection limit / μg mL^{-1}	0.35

The following kinetic equation for the reaction was deduced on the basis of the graphic correlations obtained.

$$rate = kc_{ASA}$$

k– constant proportional to the rate constant of the reaction

The equation is valid for the following concentrations: R(NO)OH (1.25–2.00) × 10^{-5}mol L^{-1}, NaOH 0.09–1.20 mol L^{-1}, Co(II) (2.55–5.90) × 10^{-5} mol L^{-1}, ASA 0.72–9.00 μg mL^{-1}.

The limits of detection (LOD) was evaluated using the following equation:[46–48] LOD = 3.3 × S_0/b where S_0 is the standrad deviation of the calibration line and *b* is the slope and found to be 0.35 μg mL^{-1}.

The precision and accuracy of the above system were studied by performing the experiment 5 times for different concentrations of acetylsalicylic acid. Table 2 shows the results of accuracy and precision of the recommended procedure.

Table 2 Accuracy and precision of the determination of acetylsalicylic acid

Taken μg mL^{-1}	**Found**[a] $\bar{x} \pm SD$, μg mL^{-1}	**RSD**[b] (%)	$(\bar{x} - \mu) / \mu \cdot 100$[c]
0.72	0.74 ± 0.03	3.63	2.78
5.40	5.27 ± 0.11	2.08	2.41
9.00	9.11 ± 0.1	1.14	1.22

[a] Mean and standard deviation of five determinations and 95 % confidence interval,
[b] relative standard deviation,
[c] accuracy of the method

The effect of temperature on reaction rate is well known and important in understanding the various activation parameters of the reaction products. In order to evaluate the apparent activation parameters, the reaction rate was studied at 292, 295, 298, 301 and 304 K at c_{NaOH} = 0.1 mol L^{-1}, $c_{R(BO)OH}$ = 1.5 × 10^{-5} mol L^{-1}, $c_{Co^{2+}}$ = 3.4 × 10^{-5} mol L^{-1}, c_{ASA} = 3.0 × 10^{-5} mol L^{-1}. Arrhenius curve was constructed by plotting log k versus 1/T and found to be linear with coefficient of correlation, r = 0.9997. Activation energy (Ea) can be calculated from the slope (-Ea/2.303R) and found to be 79.81 ± 0.41 kJ mol^{-1}. The ability to predict changes in the rate of the reaction is based on a knowladge of the value

of the activation energy. According to Hammond[49] and Smith[50], most reactions which have observable rates at ordinary temperatures have activation energies of 15–30 kcal mol^{-1} (62.76–125.52 kJ mol^{-1}). A value of 79.81 kJ mol^{-1} is in accordance with this postulate, indicated that the reaction is feasible.

4. Interference Studies

To assess the selectivity of the method, the interference of those species accompanying ASA in pharmaceuticals and serum was studied. The tolerance limits (expressed as w/w ratio), for the species studied on the determination of 5.40 μg mL^{-1} of ASA are given in Table 3. As can be seen, the usual ingredients of powdery drugs (mannitol, sorbitol, fructose, glucose, lactose) and some of the amino acids (Ala, Phe, Asp, Met, Tyr, Trp), will not interfere with the method, because the amounts tolerated are much higher than those usualy present in pharamceuticals. Binder such as gelatin and filler such as talc are insoluble in ethanol which was used for dissolving pharmaceutical preparations. Silicon dioxide is also insoluble in ethanol. It also should be noted that a higher tolerance level exists for the presence of vitamins B_1, B_6 and B_{12}. Amino acids (His, Arg, Lys, Ser, Gly) and ions Ca^{2+}, Zn^{2+}, Mg^{2+} interfere with the method. More severe interference was observed for Fe^{3+}(masked with F^- ions) and Cu^{2+}. Amino acids and investigated ions are not significant interfering species because they are not present in the drug formulation of ASA. No interference was found when up to 100-fold concentrations of stearic acid, nicotinic acid, citric acid, Si^{4+}, Mn^{2+}, Cd^{2+} and 1000-fold concentrations of Li^+, K^+, F^- and $C_2O_4^{2-}$ ions were present.

5. Applicability of the Proposed Method

The proposed method was applied to the determination of acetylsalicylic acid in three pharmaceutical formulations and human control serum using the direct calibration curve. They were treated as described in the Experimental section. As can be seen in Table 4, the results obtained by this method are in accordance with the official HPLC method. Also, good recovery was observed in the case of serum sample (Table 5), indicating that the constituents of the human control serum do not interfere (Fe^{3+} ions were masked with F^- and the protein precipitate was deposited) in any way with the detection of acetylsalicylic

Table 3 Effect of foreign species on the determination of 5.40 μg mL^{-1} of acetylsalicylic acid

Foreign species	I[a] (%)	Tolerance level (μg mL^{-1} interfering substance/μg ml^{-1} ASA)
fructose, glucose, lactose	5–10	10^3
Li^+, K^+, mannitol, sorbitol, F^-, $C_2O_4^{2-}$	< 5	
Si^{4+}, Mn^{2+}, B_1, B_6, B_{12}	5–10	10^2
Ala, Phe, Asp, Met, Tyr, Trp, stearic acid, nicotinic acid, citric acid, Cd^{2+}	< 5	
His, Arg, Lys, Ser, Gly, Ca^{2+}, Zn^{2+}, Mg^{2+}	5–10	10
Fe^{3+}, Cu^{2+}	Interference	1

[a] Interference coefficient, $I = (c^\circ_{ASA} - c_{ASA}) / c^\circ_{ASA}\, c^\circ_{ASA}$ and c_{ASA} are measured concentrations of acetylsalicylic acid without and with the interfering substance

Table 4 Determination of acetylsalicylic acid by the kinetic and official methods (HPLC and titrimetric method)

Pharmaceutical Preparation	Taken μg mL^{-1}	ASA found by the proposed method[a] $\bar{x} \pm SD$, μg mL^{-1}	RSD[a] %	Recovery[a] %	HPLC[a] $\bar{x} \pm SD$, μg mL^{-1}	F-value[b]	t-value[b]	Titrimetric method[a] $\bar{x} \pm SD$, μg mL^{-1}
Aspirin®[c]	1.80	1.76 ± 0.09	4.87	97.78	1.72 ± 0.07	1.653	0.781	1.70 ± 0.08
Cardiopirin®[d]	4.50	4.58 ± 0.11	2.41	101.77	4.54 ± 0.09	1.494	0.632	4.59 ± 0.1
Andol®[e]	6.31	6.27 ± 0.16	2.62	99.37	6.23 ± 0.14	1.306	0.422	6.21 ± 0.18

[a] Data are based on the average obtained from five determinations
[b] Theoretical F-value ($\nu_1 = 4$, $\nu_2 = 4$) and t-value ($\nu = 8$) at 95 % confidence level are 6.39 and 2.306, respectively
[c] Tablets (from Bayer, d.o.o., Belgrade, Serbia) containing acetylsalicylic acid 100 mg and excip.
[d] Tablets (from Pharmaswiss d.o.o., Belgrade, Serbia) containing acetylsalicylic acid 100 mg and excip.
[e] Tablets (from Pliva Hrvatska d.o.o, Zagreb, Croatia) containing acetylsalicylic acid 100 mg and excip.

Table 5 Determination of acetylsalicylic acid in human control serum by standard addition method

Proposed method µg mL^{-1}		RSD[a]	Recovery[a]	HPLC[a]	F-	t-	Titrimetric method[a]
Added	Found[a] $\bar{x} \pm SD$	%	%	$\bar{x} \pm SD$, µg mL^{-1}	value[b]	value[b]	$\bar{x} \pm SD$, µg mL^{-1}
7.20	7.08 ± 0.24	3.38	98.33	7.11 ± 0.35	2.127	0.158	7.05 ± 0.28
9.00	8.93 ± 0.29	3.23	99.22	8.90 ± 0.4	1.902	0.136	8.87 ± 0.31

[a]Data are based on the average obtained from five determinations
[b]Theoretical F-value ($\nu_1 = 4$, $\nu_2 = 4$) and t-value ($\nu = 8$) at 95 % confidence level are 6.39 and 2.306, respectively

acid. Therefore, the proposed method could be used for the determination of acetylsalicylic acid in serum samples. The results of the proposed method were statistically compared with those of the official method using a point hypothesis test.[51,52] Statistical analysis of the results (Tables 4 and 5) showed that calculated F– and t– values at 95% confidence levels are less than the theoretical ones, confirming no significant differences between the performance of the proposed and the official method.

6. Conclusion

Despite the great number of methods described in the literature fot the analysis of ASA, the proposed kinetic-spectrophotometric method for the determination of acetylsalicylic acid in pharmaceutical samples reported in this paper is simple, rapid, inexpensive, and thus is very appropriate for routine quality control analyses of active drug in the laboratories of hospitals, pharmaceutical industries and research institutions. The procedure is easier to execute and requires less sample handling than methods currently described in the literature. Statistical comparison of the results with the official method showed good agreement and indicates no significant difference in accuracy and precision. So, a detection limit of 0.35 µg mL^{-1} can be achieved, much better than those provided by spectrophotometric,[3,4,5,6] or by the conventional spectrofluorimetric[8,11], or even by HPLC[30,33] methods. Also, the precision of the proposed method of analysis was found to be good (for drug samples and serum RSD < 5%).

7. Acknowledgement

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Abstract

Namen našega dela je bil razvoj nove kinetične spektrofotometrične metode za določevanje acetilsalicilne kisline (ASA) v farmacevtskih pripravkih. V splošnem je za določevanje ASA potrebna predhodna kvantitativna hidroliza v alkalnem, s katero pretvorimo ASA v salicilatni anion, ki ga določujemo. Hidrolizo izvedemo v raztopini natrijevega hidroksida. Metoda je osnovana na reakciji izmenjave ligandov in spektrofotometričnim merjenjem razpada kobaltovega(II)-1-nitrozo-2-naftolnega kompleksa pri 410 nm. Določili smo optimalne pogoje reakcije glede koncentracije reagentov in temperature. Za pripravo umeritvene krivulje smo uporabili postopek začetne reakcijske hitrosti, ki je dal linearen odziv v koncentracijskem območju 0,72–9,00 μg mL^{-1} in teoretično mejo detekcije 0,35 g mL^{-1}. Selektivnost metode smo ugotavljali na osnovi proučevanja vplivov motečih ionov, amino kislin in drugih sestavin v praškastih zdravilih na hitrost reakcije. Statistična obdelava rezultatov in dobljeni izkoristki so pokazali, da med predlagano in referenčno metodo ni statistično pomembnih razlik. Razvito metodo smo uspešno uporabili za določevanje acetilsalicilne kisline v komercialnih farmacevtskih pripravkih in humanem serumu. Pomembne prednosti metode so mo nost izvedbe analize pri sobni temperaturi in kratek čas celotnega postopka. Nova metoda je enostavna, poceni in učinkovita za analizo velikega števila vzorcev.