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APPLICATION OF ION PAIR RP–HPLC METHOD IN PHARMACEUTICAL QUALITY CONTROL DISSOLUTION TESTING FOR SIMULTANEOUS ESTIMATION OF COMBINED TABLET DOSAGE FORMS WITH ACETYLSALICYLIC ACID AND GLYCINE

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Abstract: The aim of the paper was to present the possibility of applying the novel method (RP-HPLC ion pair method) for the simultaneous dissolution determination of combined tablet dosage form containing acetylsalicylic acid and glycine in pharmaceutical industry. The samples were gradient eluted using a Pursuit XS Ultra C18 column (150 × 3.0 mm, with a particle size of 2.8 µm) with variable composition of mobile phase A (1-heptanesulfonic acid sodium salt aqueous solution (2.8 g/L), pH 2.2 ± 0.05 adjusted with orthophosphoric acid) and phase B (methanol). The detection was carried out at 210 nm with a constant flow rate of 0.4 mL/min. The method was validated by determining precision (repeatability and intermediate precision), accuracy, specificity, linearity, range, system suitability, robustness and stability in accordance with ICH guidelines. The method was accurate, precise and linear within the range of 0.03 – 0.18 mg/mL for acetylsalicylic acid and 0.016 – 0.096 mg/mL for glycine. The method is simple, convenient and suitable for analyzing acetylsalicylic acid and glycine in pharmaceutical formulations. The method could also be used for routine assay determination after small modification of sample preparation.

Keywords: method validation, dissolution, acetylsalicylic acid, glycine, quality control

The pharmaceutical industry is a special branch due to the type of supplied products. Quality of drugs should be shaped at each stage of manufacturing and functioning (1). The basis of quality control testing in pharmaceutical industry are properly developed and characterized research methods. Analytical method validation is necessary for ensuring quality and achieving acceptance of products, hence it allows to get safer product. The quality of analytical data is a key factor in the success of a drug and formulation development (2). Validation is the assessment of the performance of a defined test method (3). This process confirms that analytical procedure selected for a specific quality control test is acceptable for its intended use (4).

In case of pharmaceutical industry, method validation is required by drug registration agencies and law. Methods which are used in this specific industry have to be sufficiently sensitive, accurate, specific and precise to meet the regulatory requirements established in relevant guidelines of ICH – The International Conference on Harmonization of technical requirements for the registration of pharmaceuticals for human use (ICH Q2A and Q2B). These guidelines define 8 validation parameters and present the methodology (2). Method validation (before and during routine use) is also required by regulations such as Good Manufacturing Practice (GMP), Good Laboratory Practice (GLP), Food and Drugs Administration (FDA) and quality standards such as International Organization for Standardization (ISO 17025) (5) (see Fig. 1).

The major purpose of an oral solid pharmaceutical dosage form is to make available a defined and certain amount of active substances to human body through the gastrointestinal system (6). The pharma-

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ceutical laboratories and the regulatory agencies concentrates on the evaluation of the active ingredients release kinetics from dosage forms. For this purpose dissolution testing is used, which is one of the most common analytical test carried out in pharmaceutical R&D and Quality Control laboratories (3). This type of determination is usually a part of routine tests performed for each batch of solid dosage forms.

Acetylsalicylic acid (ASA) is an organic compound, classified as nonsteroidal anti-inflammatory drug (NSAID) (7) (Fig. 2). ASA mechanism of action is based on the inhibition of the cyclooxygenase activity which leads to the analgesic, antipyretic and anti-inflammatory function (8). Glycine (GLY) is endogenous aminoacid and neurotransmitter in the central nervous system (9). Combined ASA-GLY dosage forms with respect to long-term treatment compliance, show the improved gastrointestinal tolerability profile in relation to nonglycine-containing ASA alternatives (10, 11). Moreover aspirin is more soluble and more rapidly dissolved in glycine solution than water, which affects on the release kinetics of these kind of dosage forms (12). In this study, RP-HPLC ion pair method was used to determine the percentage drug release containing 75, 100 or 150 mg of acetylsalicylic acid (ASA) and 40 mg of glycine (GLY). HPLC is used to separate, identify and determine the concentration of a specific component in a sample. The aim of the study was to show the possibility of applying the presented method in routine QC laboratory analysis in the pharmaceutical industry. The validation was performed to meet the requirements of European regulatory filing.

EXPERIMENTAL

Chemicals and reagents

Acetylsalicylic acid and glycine standards were obtained from Fluka. Talc was produced by Imifabi, potato starch was produced by Pepees. 1-Heptanesulfonic Acid Sodium Salt (HPLC grade) was obtained from Fisher Chemical. Orthophosphoric Acid, sodium acetate trihydrate, acetic acid, sodium chloride, hydrochloric acid, potassium dihydrogen phosphate and sodium hydroxide were analytical grade and were purchased from Chempur. Methanol (HPLC grade) was obtained from Sigma-Aldrich. High purity deionized water was obtained from ELGA Purelab UHQ PS (High Wycombe, Bucks, UK) purification system. The 0.45 μm nylon syringe filters were purchased from Agilent Technologies.

Instruments

Chromatographic analysis was performed using the Agilent HPLC system (Santa Clara, CA, USA) and Shimadzu HPLC system (Kyoto, Japan) equipped with quaternary pump as a solvent delivery system and variable wavelength detector. Chromatographic data were recorded and processed using Chem Station and LC solution software. All weights were taken on electronic balance (Mettler Toledo). All dissolution experiments were carried out using a dissolution instrument (Erweka GmbH, Heusenstamm, Germany).

Chromatographic conditions

Separation was performed using the Pursuit XS Ultra C18 150 × 3.0 mm, d_p = 2.8 μm as stationary

Figure 1. Method validation – regulations

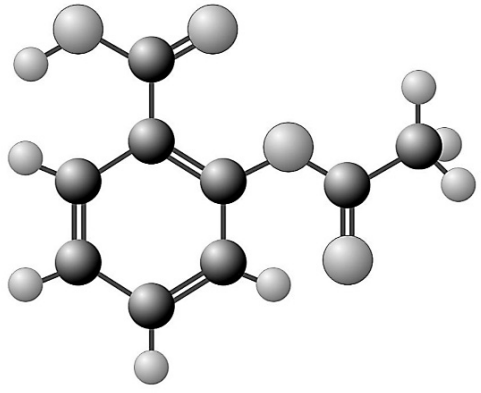

Figure 2. Acetylsalicylic acid model

Table 1. Mobile phase program for gradient elution.

Time [min]	Flow [mL/min]	Solvent A [%]	Solvent B [%]
0.0	0.4	98.5	1.5
4.0	0.4	98.0	2.0
12.0	0.4	40.0	60.0
20.0	0.4	40.0	60.0
20.1	0.4	98.5	1.5
35.0	0.4	98.5	1.5

Table 2. Linearity study parameters (regression analysis of calibration curves, n = 3).

Parameter	GLY	ASA
Linearity range (mg/mL)	0.016 - 0.096	0.03 - 0.18
Slope	720.75	122344432.40
Intercept	0.10	91692.84
Correlation coefficient (r^2)	0.999	0.999

phase maintained at 30°C. Gradient elution with the mobile phase involved a variable composition of solvent A (1-heptanesulfonic acid sodium salt aqueous solution (2.8 g/L), pH 2.2 ± 0.05 adjusted with orthophosphoric acid) and solvent B (methanol). The mobile phase was pumped through the column with flow rate of 0.4 mL/min (Table 1). Injection volume was 20 µL, detection wavelength – 210 nm. Samples during analysis were stored at thermostated autosampler at 10°C. The run time was 25 min + 10 min of "post time".

Buffer solutions preparation

Preparation of hydrochloric acid media with pH = 1.2

250.0 mL of 0.2 M sodium chloride and 425.0 mL of 0.2 M hydrochloric acid were mixed and diluted to 1000.0 mL with water. Therefore medium was degassed.

Preparation of acetate buffer with pH = 4.5

29.9 g sodium acetate and 16.6 mL of glacial acetic acid were mixed and filled with water to obtain 10 liters of solution. pH of the solution was measured and if necessary, pH was adjusted with glacial acetic acid to pH = 4.5 ± 0.05. Therefore medium was degassed.

Preparation of phosphate buffer with pH = 6.8

250.0 mL of 0.2 M potassium dihydrogen phosphate and 112.0 mL of 0.2 M sodium hydroxide were mixed and diluted to 1000.0 mL with water. Therefore medium was degassed.

Analysis determination

QC dissolution test conditions

The dissolution was carried out with a paddle method (75 rpm) at 37 ± 0.5°C. The volume of the medium – 500 mL. After 30 min 10 mL of the sample was taken and filtered through a syringe filter to a HPLC vial.

Due to nonlinearity of the method above the specified range, tablets containing 100mg and 150 mg of acetylsalicylic acid finally were diluted 1 : 2 in dissolution medium.

Preparation of standard solution of acetylsalicylic acid and glycine

The procedure of standard solution preparation for the dosage 75 mg + 40 mg (ASA + GLY) was as follows – 75 mg of ASA and 40 mg of GLY was weighed and placed into the 500 mL measuring flask. The standards were dissolved in 10 mL of methanol and the measuring flask was filled up to to the volume with the appropriate pH medium, in which studies were conducted.

For remaining dosage forms the preparation was analogous, standards were weighed respectively, 50 mg of ASA and 20 mg of GLY for dosage 100 mg + 40 mg and 75 mg of ASA and 20 mg of GLY for dosage 150 mg + 40 mg.

Analytical method validation

In this study, RP-HPLC ion pair method was used to determine the percentage drug release containing 75, 100 or 150 mg of acetylsalicylic acid and 40 mg of glycine. HPLC is used to separate, identify and determine the concentration of a specific component in a sample. Presented method is fast, reproducible and easy to operate. The validation was performed to meet the requirements of European regulatory filing.

Linearity

The linearity of an analytical method express its ability to obtain test results which are directly proportional to the analite concentration in the sample (2).

A stock solutions of glycine (0.8 mg/mL) and acetylsalicylic acid (2.0 mg/mL) were prepared with the dissolution medium – acetate buffer with pH = 4.5. Various working standard solutions were prepared from the stock solutions in the range of 0.016 to 0.096 mg/mL for glycine and 0.03 to 0.18 mg/mL for acetylsalicylic acid. The working standards solutions were injected into the HPLC system.

The linearity experiment was performed at five concentrations in three replications (for both components) to ensure that the detector's response was linear for various drug concentrations. Both calibration curves were constructed by plotting the peak area versus the concentrations of acetylsalicylic acid and glycine and the regression equations were determined.

Accuracy (% recovery)

The accuracy expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found (13). In this study accuracy was determined by application of the procedure to the same excipients as those in the commercial formulation to which an addition of the active substances (certified reference materials of known purity) have been added. The determination was performed at three concentrations in three replications (for both components) according to the minimum recommendation of ICH, in the range of 0.016 to 0.096 mg/mL for glycine and 0.03 to 0.18 mg/mL for acetylsalicylic acid.

Precision

The precision of an analytical procedure illustrates information on the random errors. The precision of an analytical procedure expresses the closeness of the agreement (degree of scatter) between a series of measurements obtained from the multiple samples of the same homogeneous sample under the prescribed conditions. It can be presented as repeatability (intra-day precision) and intermediate precision (inter-day precision) (13, 14). According to the ICH guideline it may be expressed as the variance, standard deviation or coefficient of variation (CV) of a series of measurements.

For precision evaluation, the intra-day and inter-day precision were determined for the concentration of analites corresponding to 100% of disso-

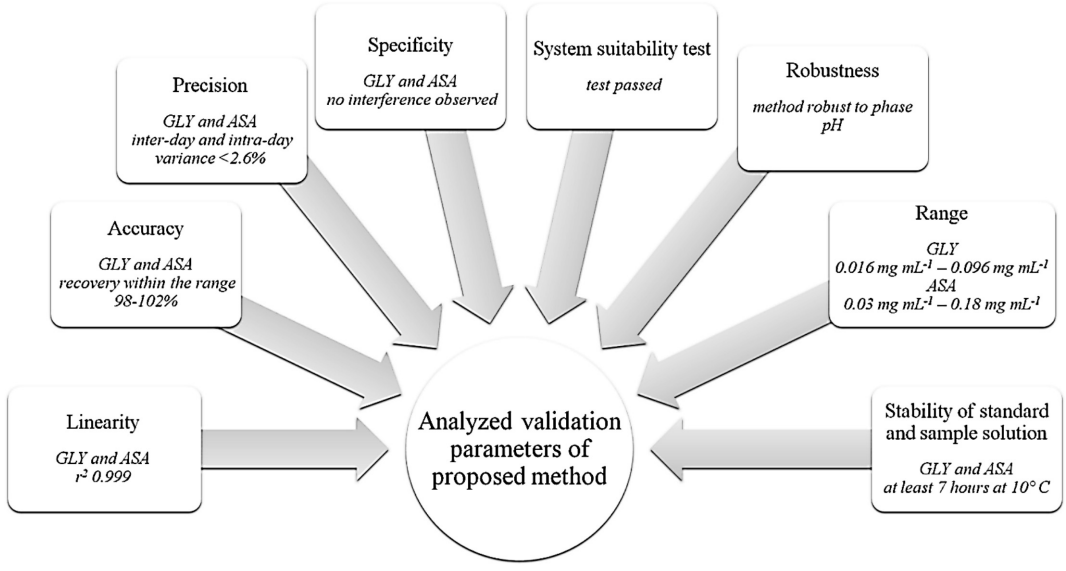

Figure 3. Analyzed validation parameters of proposed method

Table 3. Accuracy.

Dosage ASA/GLY [mg/mg]	Analite	Theoretical concentration [mg/mL]	Found concentration [mg/mL] (mean [a] ± SD)	% Recovery (mean [a] ± SD)	% CV	Average recovery [%] (mean ± confidence interval)
75/40	GLY	0.016 (20%)	0.0159 ± 0.0001	99.13 ± 0.78	0.79	100.12 ± 0.87
		0.080 (100%)	0.0803 ± 0.0009	100.41 ± 1.17	1.17	
		0.096 (120%)	0.0968 ± 0.0007	100.83 ± 0.73	0.72	
75/40	ASA	0.03 (20%)	0.030 ± 0.0004	100.07 ± 1.49	1.49	99.67 ± 0.89
		0.15 (100%)	0.149 ± 0.0013	99.07 ± 0.84	0.84	
		0.18 (120%)	0.180 ± 0.0021	99.87 ± 1.15	1.15	
100/40	GLY	0.016 (40%)	0.0160 ± 0.0003	99.79 ± 1.68	1.69	100.06 ± 0.73
		0.040 (100%)	0.0400 ± 0.0001	100.06 ± 0.18	0.18	
		0.096 (240%)	0.0963 ± 0.0005	100.33 ± 0.56	0.56	
100/40	ASA	0.03 (30%)	0.030 ± 0.0001	100.48 ± 0.35	0.35	99.89 ± 0.61
		0.10 (100%)	0.100 ± 0.0010	99.60 ± 1.03	1.03	
		0.18 (180%)	0.179 ± 0.0011	99.58 ± 0.62	0.62	
150/40	GLY	0.016 (40%)	0.0160 ± 0.0001	100.19 ± 0.58	0.58	100.21 ± 0.49
		0.040 (100%)	0.0402 ± 0.0004	100.40 ± 0.92	0.91	
		0.096 (240%)	0.0960 ± 0.0005	100.05 ± 0.55	0.55	
150/40	ASA	0.03 (20%)	0.030 ± 0.0002	99.49 ± 0.51	0.51	99.76 ± 0.40
		0.15 (100%)	0.149 ± 0.0003	99.57 ± 0.23	0.23	
		0.18 (120%)	0.180 ± 0.0009	100.23 ± 0.49	0.49	

[a] Average of three determinations

lution of each dosage. The intra-day precision was performed by one analyst at the same day. The inter-day precision was performed by the second analyst a few days later, using different HPLC instrument. Six different measurements were performed in each group. Standard deviations in groups of results, % coefficient of variation, intra-day and inter-day variance were calculated.

Specificity

The specificity was verified by comparing the following chromatograms:

- tablet powder without glycine and acetylsalicylic acid prepared with the same excipients as those in the commercial formulation – in the amount of 100% of tablet content and 300%,
- tablet powder with glycine and acetylsalicylic acid prepared with the same excipients as those in the commercial formulation,
- standard solutions.

All mentioned above solutions were prepared for each dosage form. To assess specificity of the method for dissolution profiles, samples were prepared using as the sample solvents following Ph. Eur. buffers: medium with pH 1.2 (NaCl – HCl); acetate buffer with pH 4.5; phosphate buffer with pH 6.8). Above solutions, mobile phase and sample solvents were injected into the HPLC system following test conditions, the chromatograms were recorded and the responses of the peaks if any measured.

System suitability test

The system suitability was verified by comparing the surface area of the peaks for 6 replicates of one standard – 6 injections from the same vial. The study was performed separately for each dose.

Robustness

To assess method robustness, it was tested if slight changes in mobile phase pH would affect the determination of the active substances amount. Six independent samples from reconstituted tablets were injected into the system for each dose, at the concentrations equivalent to 100% of dissolution. Samples were analyzed in 3 replications with vari-

able pH of component A (2.1; 2.2; 2.3). Groups of the results were compared using the variance analysis ($\alpha = 0.05$).

Range

The analytical method provides an acceptable degree of linearity, accuracy and precision (% CV does not exceed 2.7%) (15).

Stability of standard and sample solutions

The stability of the samples and standards solutions was evaluated by preparing the solutions as per the proposed method and analyzed initially and after 7 to 11 h by keeping the solutions in autosampler at 10°C. The study was performed separately for each dose. Calculations were based on the peak areas of 6 injections at the start of analysis and after predetermined time. The mean values obtained were compared using the t and F tests.

Statistics

Statistics were performed using Excel software (Microsoft Inc, Redmond, Wash).

RESULTS AND DISCUSSION

Obtained results for all eight determined validation parameters, which allowed to positively assess presented method are shown in Figure 3.

A linear correlation was obtained between the peak area obtained versus concentrations of both components; the calibration curves were linear for concentrations within the range of 0.016 to 0.096 mg/mL for glycine and 0.03 to 0.18 mg/mL for acetylsalicylic acid. In case of acetylsalicylic acid – this method is non linear above specified range. All the validation parameters for linearity are listed in Table 2.

The accuracy experiments were performed for glycine and acetylsalicylic acid for three dosage

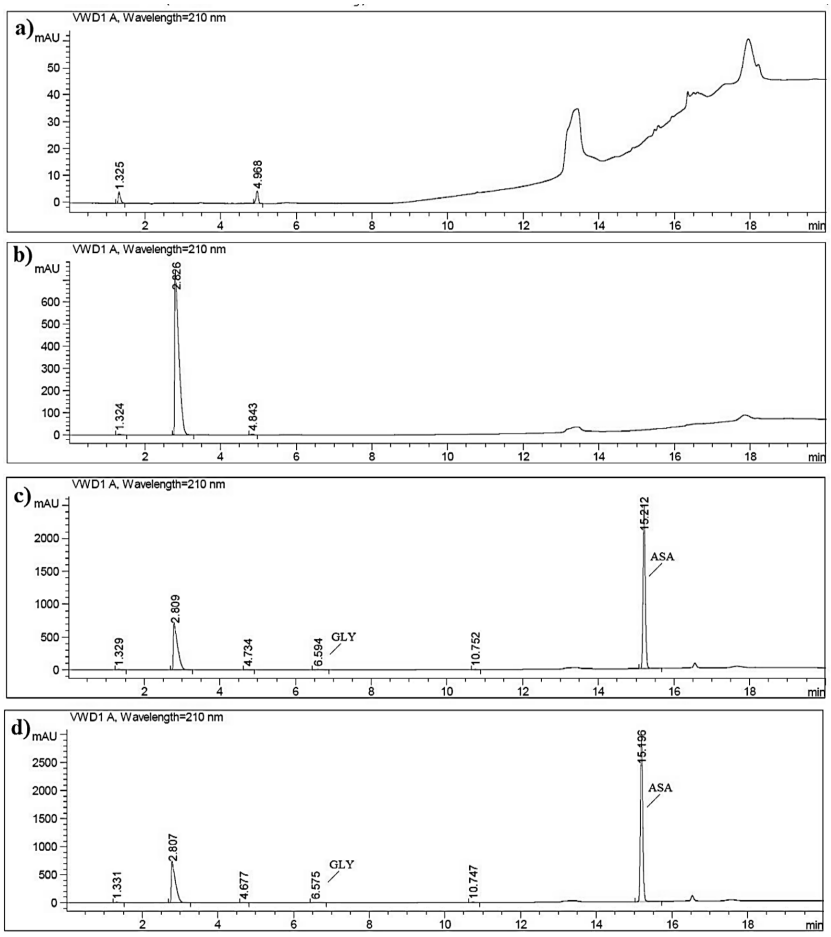

Figure 4. Exemplary chromatograms of analysis using acetate buffer pH 4.5 a) blank, b) placebo chromatogram, c) standard chromatogram and d) sample chromatogram

Table 4. Precision.

Dosage ASA/GLY [mg/mL]	Analite	Measured concentration [a] [mg/mg]		Precision values	
		GLY	ASA	GLY	ASA
75/40	Theoretical concentration [mg/mL]	0.080	0.15	0.080	0.15
					Intra-day variance[b] (n = 12)
	Intra-day found concentration 1st measurement [mg/mL] (mean ± SD)	0.083 ± 0.002 % CV = 2.04	0.151 ± 0.003 % CV = 1.87	1.94	1.53
	Intra-day found concentration 2nd measurement [mg/mL] (mean ± SD)	0.084 ± 0.002 % CV = 2.12	0.152 ± 0.003 % CV = 2.01		
					Inter-day variance[b] (n = 6×3)
	Inter-day found concentration [mg/mL] (mean ± SD)	0.081 ± 0.001 % CV = 1.67	0.152 ± 0.001 % CV = 0.70	2.46	1.58
100/40	Theoretical concentration [mg/mL]	0.040	0.100	0.040	0.100
					Intra-day variance[b] (n = 12)
	Intra-day found concentration 1st measurement [mg/mL] (mean ± SD)	0.043 ± 0.001 % CV = 2.07	0.098 ± 0.002 % CV = 2.16	2.25	1.64
	Intra-day found concentration 2nd measurement [mg/mL] (mean ± SD)	0.042 ± 0.001 % CV = 2.49	0.097 ± 0.001 % CV = 1.39		
					Inter-day variance[b] (n = 6×3)
	Inter-day found concentration [mg/mL] (mean ± SD)	0.042 ± 0.001 % CV = 2.21	0.102 ± 0.001 % CV = 1.36	2.29	2.47
150/40	Theoretical concentration [mg/mL]	0.040	0.15	0.040	0.15
					Intra-day variance[b] (n = 12)
	Intra-day found concentration 1st measurement [mg/mL] (mean ± SD)	0.041 ± 0.001 % CV = 2.24	0.152 ± 0.001 % CV = 0.49	2.08	0.95
	Intra-day found concentration 2nd measurement [mg/mL] (mean ± SD)	0.041 ± 0.001 % CV = 2.31 %	0.151 ± 0.002 CV = 1.06		
					Inter-day variance[b] (n = 6×3)
	Inter-day found concentration [mg/mL] (mean ± SD)	0.042 ± 0.042 % CV = 1.70	0.150 ± 0.002 % CV = 1.29	2.50	1.06

[a] Mean value of six different determinations. [b] Each value shows %CV

forms with a presence of suitable amount of excipients for each dose. The average recoveries with confidence intervals were in the range of 98% – 102%. The determination involved measurement in triplicate at each level with % coefficient of variation in range of 0.23% to 1.49% for acetylsalicylic acid and 0.18% to 1.69% for glycine. Analyzing the results of the accuracy test, it was concluded that the parameter values of regression line of relationship of theoretical value and determined value are close to 1 for the slope coefficient and 0 for the Y-intercept, thus acceptance criteria were met. The values of the recovery (%) with confidence intervals and % CV are shown in Table 3.

It has been found that for calculated values the obtained intra-day and inter-day variance did not

exceed predetermined value (3.7%). Maximum value of intra-day variance (% CV = 2.25%) was obtained for glycine in a dosage of 100 mg + 40 mg, while maximum value of inter-day variance (% CV = 2.50%) was obtained also for glycine in a dosage of 150 mg + 40 mg. Maximum % coefficient of variation in a group of results was noted for glycine in a dosage 100 mg + 40 mg – 2.49%. Due to the fact that concentration of glycine is significantly lower than acetylsalicylic acid concentration, greater variations are obtained for this analite. Mean values of concentrations, standard deviations in groups of results, % coefficient of variation, intra-day and inter-day variance were presented in Table 4.

Chromatogram of the placebo has not shown any interference at the retention time of both glycine and acetylsalicylic acid. Figure 4 shows exemplary

Table 5. System suitability tests.

Dosage ASA/GLY [mg/mg]	Analite	Standard concentration [mg/mL]	% CV [a]
75/40	GLY	0.080	0.33
75/40	ASA	0.150	0.22
100/40	GLY	0.040	0.41
100/40	ASA	0.100	0.28
150/40	GLY	0.040	0.36
150/40	ASA	0.150	0.40

[a]% CV of six injections

Table 6. Robustness.

Dosage ASA/GLY [mg/mg]		Measured concentration [a] [mg/mL]	
	Analite	GLY	ASA
75/40	Theoretical concentration [mg/mL]	0.080	0.15
	pH 2.1 [mg/mL] (mean ± SD)	0.080 ± 0.001 % CV = 1.72	0.150 ± 0.002 % CV = 1.09
	pH 2.2 [mg/mL] (mean ± SD)	0.081 ± 0.001 % CV = 1.67	0.152 ± 0.001 % CV = 0.70
	pH 2.3 [mg/mL] (mean ± SD)	0.081 ± 0.001 CV = 1.49	0.150 ± 0.001 % CV = 0.78
100/40	Theoretical concentration [mg/mL]	0.040	0.100
	pH 2.1 [mg/mL] (mean ± SD)	0.043 ± 0.001 % CV = 2.39	0.102 ± 0.001 % CV = 1.35
	pH 2.2 [mg/mL] (mean ± SD)	0.042 ± 0.001 % CV = 2.21	0.102 ± 0.001 % CV = 1.36
	pH 2.3 [mg/mL] (mean ± SD)	0.042 ± 0.001 % CV = 1.56	0.103 ± 0.002 % CV = 1.43
150/40	Theoretical concentration [mg/mL]	0.040	0.15
	pH 2.1 [mg/mL] (mean ± SD)	0.042 ± 0.001 % CV = 2.66	0.151 ± 0.002 % CV = 1.33
	pH 2.2 [mg/mL] (mean ± SD)	0.042 ± 0.001 % CV = 1.70	0.150 ± 0.002 % CV = 1.29
	pH 2.3 [mg/mL] (mean ± SD)	0.042 ± 0.001 % CV = 2.42	0.151 ± 0.002 % CV = 1.27

[a] Mean value of six different determinations

Table 7. Stability of sample and standard solutions.

Dosage ASA/GLY [mg/mg]	Analite	Type	% CV [a] initial	% CV [a] after time
75/40	GLY	Standard	0.33	0.33
	GLY	Sample	0.23	0.11
	ASA	Standard	0.22	0.33
	ASA	Sample	0.18	0.15
100/40	GLY	Standard	0.41	0.88
	GLY	Sample	0.12	0.20
	ASA	Standard	0.28	0.26
	ASA	Sample	0.12	0.20
150/40	GLY	Standard	0.36	0.53
	GLY	Sample	0.57	0.45
	ASA	Standard	0.40	0.24
	ASA	Sample	0.42	0.24

[a] %CV of six injections

chromatograms for 75 mg + 40 mg dosage in acetate buffer with pH 4.5.

The resulting chromatograms confirm the specificity of the method: there is no interfering peaks derived from the placebo, mobile phase, solvents with the peaks of the active substances (in 3 different dissolution media: pH 1.2; pH 4.5; pH 6.8). System suitability is an important parameter to ensure whether the used method was valid or not. According to the USP, the HPLC method is considered suitable when the CV of peak area < 1%, the tailing factor < 2 and the theoretical plates > 2000 (16). The system suitability was verified by comparing the peak areas for 6 injections of one standard solution.

All the standards peaks revealed theoretical plates were above = 1500. Moreover the coefficient of variation results from six injections also showed accordance to the limits. These results testify that proposed method is valid and can be easily used for routine analysis. System suitability parameters and calculations were presented in Table 5.

Analyzing the results of variance analysis (α = 0.05) it can be assumed that obtained mean values (n = 6) are not significantly different ($F < F_{cr}$). The parameter used to define robustness was calculated concentration in the samples. The method has shown to be robust to slight changes in mobile phase component A pH (Table 6). Additionally robustness of the method was also confirmed by intermediate precision.

Taking into account that the method in the concentration range of acetylsalicylic acid 0.03 – 0.18 mg/mL and glycine 0.016 – 0.096 mg/mL is linear, accurate, robust to small variation of component A pH and precise, it can be considered that this is the range of the method.

Stability of standards and samples solutions was confirmed for at least 7 h at 10^{o}C in the autosampler, by comparing mean peak areas of the six injections at the beginning of the analysis and after predetermined time. % CV of peak areas falls within the limits of acceptance (Table 7). Means did not differ significantly (t test and F met).

Presence of the two compounds (acetylsalicylic acid and glycine) in the pharmaceutical formulation demonstrates synergism of action. According to Authors (17, 18, 19) glycine increase aspirin solubility, which probably is caused by forming "glycine capsule" around the aspirin molecules and at the same time cause decrease of irritating properties of aspirin on the mucosal membrane. Therefore, it is correct to use these substances together, however in formulation with low doses of acetylsalicylic acid (17). The literature survey showed lack of publications concerning simultaneous determination of acetylsalicylic acid and glycine in dosage forms by ion-pair RP-HPLC, despite the presence of a wide range of medicinal products containing these both substances at the pharmaceutical market. In this case, the biggest challenge is the determination of aminoacid – glycine. The Authors (20) used classical sample derivatization with ninhydrin and ion-exchange separation to obtain more precise results. Bartolomeo and Maisano (21) proposed semi-auto-

mated derivatization, which occurs immediately before sample injection in autosampler, adapting the Agilent application note chromatographic conditions (22) – RP-HPLC method with UV-Vis detection. Taking into account results obtained in the study, it is possible to determine the dissolution of the tablets containing 40 mg of glycine with appropriate accuracy and precision without sample derivatization by using the proposed method. Additional advantage is simultaneous determination of two components, which makes analysis of the complex preparation much faster for quality control laboratory.

CONCLUSIONS

A simple ion-pair reverse phase liquid chromatographic method was developed for the simultaneous determination of dissolution of combined tablet dosage form containing acetylsalicylic acid and glycine. The method was validated by determining precision (repeatability and intermediate precision), accuracy, specificity, linearity, range, system suitability, robustness and stability in accordance with ICH guidelines. The method was accurate, precise and linear within the range of 0.03 – 0.18 mg/mL for acetylsalicylic acid and 0.016 – 0.096 mg/mL for glycine.

The method is simple, convenient and suitable for analyzing acetylsalicylic acid and glycine in pharmaceutical formulations. The possibility of presented method application in routine QC laboratory analysis in the pharmaceutical industry was confirmed. The method could also be used for routine assay determination after small modification of sample preparation.

Conflict of interest

The authors are not aware of any conflict of interest.

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