Stability of an aspirin in the aspirin+curcumin admixture at different storage conditions

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ABSTRACT

Aims: The pure stability of aspirin in the aspirin (100 μg/mL) only and aspirin (100 μg/mL) +curcumin(600 μg/mL) admixture without any ingredient under two solvents (distilled water, DW and normal saline, NS), three storage temperatures(25˚C, 4˚C and -20˚C) and periods (1st, 3rdand 7thdays) was evaluated.
Study Design: The injectable DW- and NS-aspirin containing solutions in the laboratory cap polyethylene bottle were stored and evaluated at controlled temperature (25˚C, 4˚C and -20˚C) during 7 days.
Methodology: Effects of admixture compounds, periods of storage and temperature of storage on the concentrations of active compound (aspirin) were analyzed. The concentration of aspirin in each solution was determined by stability-indicating high-performance liquid chromatography (HPLC)-ultraviolet (UV) detection. A 1.0 mL volume of each sample was withdrawn and reconstituted with 3.0 mL of ethanol and directly injected into HPLC system immediately after filtration at 1st, 3rdand 7th days for analysis. The stability of the solutions was determined by calculating the percentage of the initial aspirin concentrations remaining at each test condition and periods. Stability was defined as the retention of at least 90% of the initial aspirin concentration.
Results: The concentration of aspirin of the aspirin only and aspirin+curcumin admixture solutions remained at least 90% of original without any color change or precipitation in the DW and NS solution at 4˚C and -20˚C throughout 7 day period and showed instability that decreased gradually below 90% of original concentrations after 1 day at 25˚C in the two solutions.
Conclusion: Two kinds of solutions of only aspirin and aspirin+curcumin admixture, in DW and NS, showed different stability dependence on temperature of storage that means maintained stability at 4˚C and -20˚C and did not show effect of admixture of curcumin on aspirin stability during 7 days except 25˚C.

Keywords: Aspirin; Stability; Curcumin admixture; HPLC.

1. INTRODUCTION

The chemical stability of a drug is of great importance since it becomes less effective as it undergoes degradation. Also, drug decomposition may yield toxic by-products that are harmful to the patient. Acetylsalicylic acid (ASA) decomposes rapidly in solutions of ammonium acetate or of the acetates, carbonates, citrates or hydroxides of the alkali metals. ASA is stable in dry air, but gradually hydrolyses in contact with moisture to acetic and salicylic acids. In solution with alkalis, the hydrolysis proceeds rapidly and the clear solutions formed may consist entirely of acetate and salicylate [1]. Daily Aspirin's effect on cancer has been widely studied, particularly its effect on colorectal cancer. Multiple meta-analyses and reviews have concluded that regular use of aspirin reduces the long-term risk of CRC incidence and mortality [2-5]. In addition, experiments were carried out to measure the synergistic effects of a combination of aspirin with other chemopreventive agents for the anticancer benefit [6-10]. Therefore, it is necessary to evaluate the physical and chemical stabilities of aspirin in order to determine the formulation of optimal dosage form that balances chemopreventive efficacy with safety of aspirin in combination with other agents. The purpose of this study is to provide information about the physical and chemical stability of aspirin in aspirin and curcumin of admixture

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under two solvents, various storage temperatures and periods. Thus, the study evaluated the stability of aspirin in the aspirin (100 μg/mL) only and aspirin (100 μg/mL) + curcumin (600 μg /mL) admixture without any ingredient under two solvents (distilled water, DW and normal saline, NS), three storage temperatures (25˚C, 4˚C and -20˚C) and periods (1st, 3rd and 7th days)

2. MATERIALS AND METHODS

2.1 Chemicals and reagents

Aspirin was purchased from Sigma-Aldrich Co. (St. Louis, MO, USA) and curcumin was purchased from Now Foods (Bloomingdale, USA), respectively. Acetonitrile, formic acid and ethanol were all HPLC-grade and purchased from Sigma Co. (St. Louis, MO, USA) while other reagents and solvents used were of analytical grade. All aqueous solutions including the buffer for the HPLC mobile phase was prepared with water that purified by Milli-Q water purification system (Millipore, Milford, MA, USA). The filter with pore size of 0.22 μm (Millipore, Milford, MA, USA) was used for filtration of mobile phase and 0.45 μm (PALL, USA) for filtration of standard and sample solution.

2.2 Sample preparation

Solutions of the designated samples (aspirin 2.5 mg, aspirin 2.5 mg and curcumin 15 mg) and diluents (to final volume of 25 mL with normal saline and distilled water) were prepared (Table 1). At the time of preparation of each test solutions, 1 mL of samples were collected at specified time intervals; 0, 1st, 3rd and 7th day, and added in 15 mL falcon tubes and stored in RT(25±2℃), CT(4±2℃), and FT (-20±2℃) until analyzed. A 1.0 mL volume of each sample was withdrawn and reconstituted with 3.0 mL of ethanol and directly injected into HPLC system immediately after filtration at 1, 3 and 7 days for analysis.

Table 1. Study protocol

Compound (μg/mL)	Period(day)	Temperature(℃)	Diluent [a]
Aspirin (100) only	1, 3, 7	-20, 4, 25	DW, NS
Aspirin (100)+Curcumin (600) admixture	1, 3, 7	-20, 4, 25	DW, NS

[a] *DW = distilled water, NS = 0.9% sodium chloride solution.*

2.3 HPLC method

The concentration of aspirin in each sample solution was determined using a stability-indicating HPLC assay method based on several references [11-16]. The chromatographic system used in the analysis consisted of an isocratic solvent delivery pump (Model 515 pump, Waters Scientific Co., USA), an autosampler(Model Nanospace SI-2 Autosampler, Shiseido Co., Japan) and analytical column Cadenza CD-C18 (250 x 4.6 mm, 3 μm) and guard column Unison US-C18, (5 x 2 mm, 5 μm) (Imtakt Corporation, Kyoto, Japan). A variable wavelength ultraviolet detector (Model 486 Tunable Absorbance Detector, Waters Scientific Co., USA) set at 220 nm and the integration of the chromatograms (Model dsCHROM® Data module, Do-Nam instrumental Co., Seoul, Korea) were used. The mobile phase consisted of distilled water, acetonitrile and formic acid. (v/v/v, 45/55/0.05, pH 3.3). The flow rate of mobile phase was 0.8ml/min. The method was validated for linearity, precision (inter-day and intra-day), accuracy and selectivity [17]. The experiment was repeated five times on the same day and five consecutive days to determine inter- and intra-day precisions [18]. Linearity, accuracy and precision were evaluated by determining five concentrations of aspirin in range of 6.25~100 μg/mL. Linear regression analysis of peak area and concentration yielded a good correlation coefficient ≥0.999. Inter-and intra-day precision were expressed as the percent relative standard deviation (% RSD). The accuracy was expressed as the percent ratio between the experimental and nominal concentrations for each sample.

2.4 Standard solutions

A 1 mg/mL of stock solution of aspirin was prepared by dissolving in 75% (V/V) ethanol/DW. Standard samples of aspirin were prepared by diluting the stock solution with 75% (V/V) ethanol/DW to concentrations of 6.25, 12.5, 25, 50 and 100 μg/mL. The injection volume was 5 μL. The standard samples were assayed to repeat five times on the same day as an external standard method.

2.5 Sample analysis

Each aspirin sample solution was prepared as described. A 5 μL of sample was injected into the HPLC system, and each sample was assayed in three times a day. Effects of admixture compounds, periods of storage and temperature of storage on the concentrations of active compound (aspirin) were analyzed. The samples were visually inspected for color change and precipitate formation was evaluated on each day of analysis.

2.6 Data analysis

The stability of aspirin in the aspirin only and aspirin+curcumin admixture solutions was determined by calculating the percentage of the initial aspirin concentrations remaining at each test condition and hours. Stability was defined as the retention of at least 90% of the initial aspirin concentration.

2.7 Statistical analysis

Experimental data were expressed as the mean ± standard deviation (S.D.). Differences in the concentrations of aspirin for 7 days investigated at the three different temperatures were performed by one-way ANOVA with scheffe test as a post hoc test using SPSS version 18.0. Statistical significance was set at $p<0.05$.

3. RESULTS AND DISCUSSION

The linearity could be established for aspirin in the concentration range of 6.25~100 μg/mL (r^2= 0.9998, Fig.1A). Table 2 lists the percent relative standard deviation (% RSD) data obtained on analysis of the samples (n=5) on the same day and on consecutive days (n=5). The results were within limits (%RSD <10) shown in Table 2. As evident, the %RSD values were <9.84% and <2.10% for inter-day and intra-day results, respectively. Accuracies were 96.6~102.2% and 98.6~101.0%for inter-day and intra-day, respectively, meaning that the method was sufficiently precise and accurate.

Table 2. Validation data and retention time (RT) under conditions of HPLC Assays

Range (μg/mL)		Validation (n=5)			RT (min)	
			Inter-day	Intra-day	Aspirin	Degradation Products
Aspirin	6.25-100[a]	Precision (% RSD)	0.76~9.84	0.70~2.10	4.6	5.3
		Accuracy (%)	96.6~102.2	98.6~101.0		

[a] *Samples were diluted with 75% ethanol*

The retention time of the intact aspirin and the degradation product were about 4.6 and 5.3 minutes (Fig.1B, Fig. 1C).The aspirin undergoes hydrolysis in solutions at room temperature with the resultant degradation products being salicylic acid and acetic acid [13].

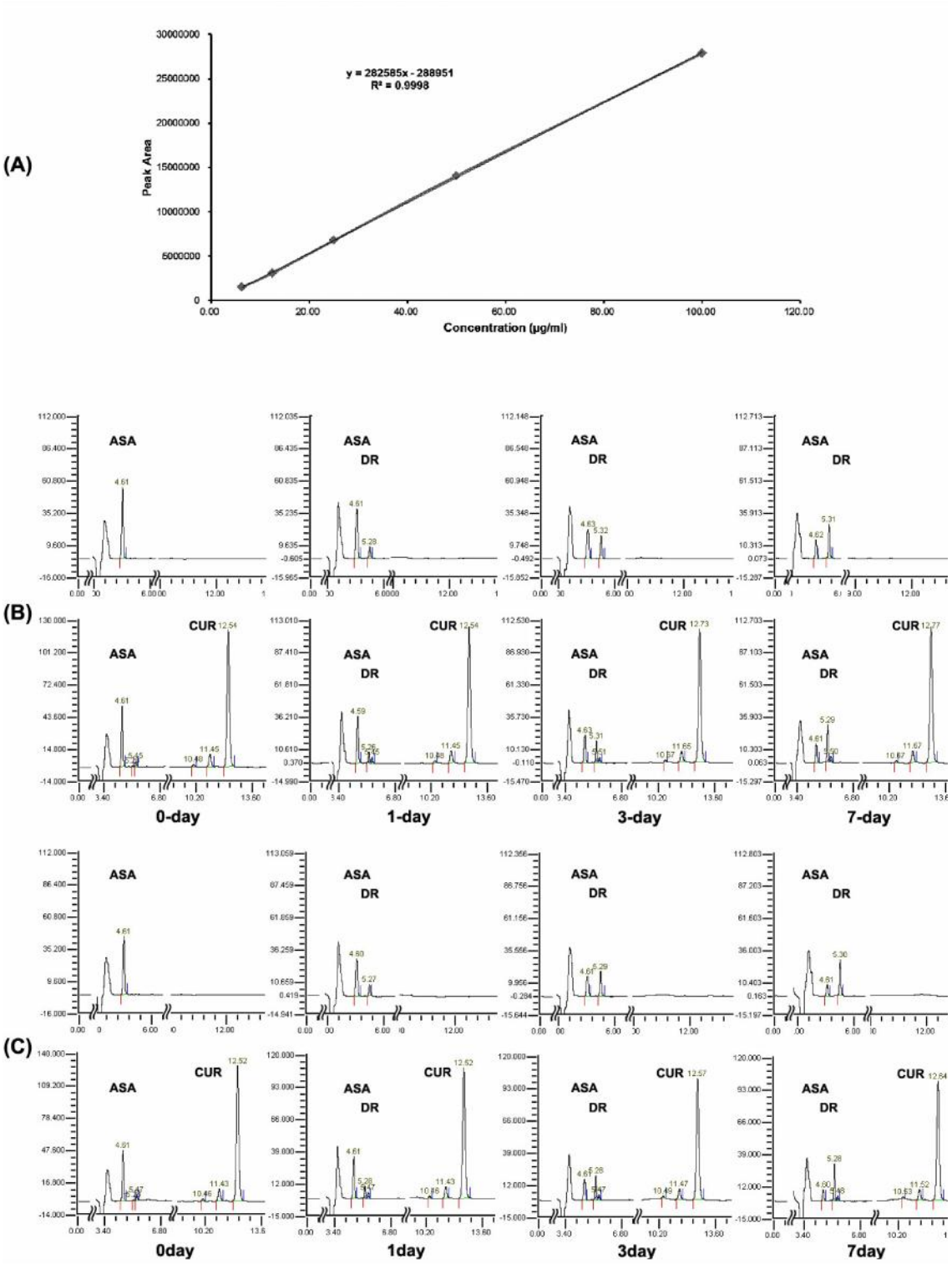

Fig. 1. (A) Calibration curve for the determination of aspirin concentrations (6.25~100 μg/mL). (B) Chromatogram of aspirin(25 μg/mL in DW) and asprin+curcumin admixture (25μg/mL+150μg/mL in DW) and (C) Chromatogram of aspirin(25 μg/mL in NS) and asprin+curcumin admixture (25μg/mL+150μg/mL in NS) after 0, 1st, 3rd and 7th days of storage at 25℃.

ASA=aspirin, DR= degradation product and CUR= curcumin.

The concentrations of aspirin which were observed at analytic time of each day during 7 days for each

of the designated samples (aspirin only and aspirin+curcumin admixture), solution (DW and NS) and storage conditions are listed in Table 3.

In the aspirin in DW solution, the mean values of concentration were statistically ($p < 0.05$) different from 1st to 7th day among temperatures. The difference mean values of 7th day were higher than the other day. These were values 19.14±0.41 (in 25℃), 3.38±2.54(in 4℃), and 0.55±0.51 (in -20℃). In the aspirin in NS solution, the mean values were statistically ($p < 0.05$) different according to the three temperature of storage except 1st day. But the difference mean values of 1st day among temperatures tended to be slightly high in 25℃. The difference mean values of 7th day were 16.29±0.75 (in 25℃), 1.93±0.49(in 4℃), and 1.63±1.09 (in -20℃). In the aspirin+curcumin admixture in DW solution, the mean values of concentration were statistically ($p < 0.05$) different from 1st to 7th day among temperatures. The difference mean values of 7th day were 18.71±2.13 (in 25℃), 0.91±0.58 (in 4℃), and -0.83±1.18 (in -20℃). In the aspirin+curcumin admixture in NS solution, the mean values of concentration were statistically ($p < 0.05$) different from 1st to 7th day among temperatures. The difference mean values of 7th day were 17.05±4.42 (in 25℃), 0.93±1.32 (in 4℃), and 0.26±0.61 (in -20℃).

The differences mean values showed that the concentration of aspirin decreased. Therefore, the concentration of aspirin reduced according to change of temperature and time, regardless of solution type and the admixture of curcumin.

The concentration of Aspirin remained by a minimum of 22% of the 0 day concentration under RT (25 ± 2℃), 90% of the 0 day concentration under CT(4℃ ± 2℃) and 94% of the 0 day concentration under FT (-20℃ ± 2℃).

Table 3. Stability of aspirin in the aspirin only and aspirin+curcumin admixture solutions at 25, 4 and -20℃

Nominal concentration (μg/mL), Diluent [a]	Time (day)	Temperature (℃)	Concentration (μg/mL)	% Remaining	Difference[b] (μg/mL)	*p*-Value
Aspirin, (25), DW	0	25	26.44±0.53	100±0.00	0.00±0.00	
		4	26.84±0.97	100±0.00	0.00±0.00	
		-20	31.14±1.51	100±0.00	0.00±0.00	
	1st	25	21.57±0.93	81.6±3.51	4.88±1.04^{A}	0.000
		4	24.37±0.85	90.8±2.13	2.48±1.49^{B}	
		-20	31.02±0.85	99.6±2.74	0.13±0.94^{C}	
	3rd	25	14.94±0.44	56.5±1.66	11.5±0.85^{A}	0.000
		4	24.44±1.38	91.0±5.14	2.4±2.14^{B}	
		-20	32.98±3.53	105.9±11.34	-1.84±2.53^{C}	
	7th	25	7.30±0.24	27.6±0.89	19.14±0.41^{A}	0.000
		4	23.46±1.68	93.9±5.69	3.38±2.54^{B}	
		-20	30.59±1.19	98.2±3.82	0.55±0.51^{C}	
Aspirin, (25), NS	0	25	21.11±0.76	100±0.00	0.00±0.00	
		4	20.85±0.55	100±0.00	0.00±0.00	
		-20	29.72±1.32	100±0.00	0.00±0.00	
	1st	25	18.73±2.12	88.7±10.04	2.39±2.29^{A}	0.140
		4	20.26±0.97	97.1±4.65	0.6±0.63^{A}	
		-20	27.99±0.19	94.2±0.64	1.73±1.48^{A}	
	3rd	25	10.68±0.27	50.6±1.26	10.43±0.79^{A}	0.000
		4	19.56±0.54	93.8±2.61	1.3±0.39^{B}	
		-20	28.10±0.92	94.5±3.10	1.62±0.48^{B}	
	7th	25	4.82±0.18	22.8±0.84	16.29±0.75^{A}	0.000
		4	18.92±0.31	90.7±1.48	1.93±0.49^{B}	
		-20	28.10±0.36	94.5±1.23	1.63±1.09^{B}	

Aspirin + Curcumin, (25+150),DW	0	25	26.66±2.32	100±0.00	0.00±0.00	
		4	23.41±1.21	100±0.00	0.00±0.00	
		-20	30.23±4.04	100±0.00	0.00±0.00	
	1st	25	20.78±1.89	78.0±7.07	5.88±1.37^{A}	0.000
		4	23.60±2.43	100.8±10.38	-0.2±1.26^{B}	
		-20	30.11±4.02	99.6±13.30	0.12±0.40^{B}	
	3rd	25	15.07±1.87	56.5±7.03	11.58±1.37^{A}	0.000
		4	22.57±0.66	96.4±2.84	0.84±0.70^{B}	
		-20	29.70±3.57	98.2±11.81	0.53±0.82^{B}	
	7th	25	7.95±0.24	29.8±0.90	18.71±2.13^{A}	0.000
		4	22.49±1.51	96.1±6.45	0.91±0.58^{B}	
		-20	31.06±2.92	102.8±9.65	-0.83±1.18^{B}	
Aspirin + Curcumin, (25+150),NS	0	25	23.19±5.16	100±0.00	0.00±0.00	
		4	19.64±1.89	100±0.00	0.00±0.00	
		-20	29.23±2.10	100±0.00	0.00±0.00	
	1st	25	20.18±2.69	87.0±11.58	3.01±2.66^{A}	0.000
		4	19.09±3.19	97.2±16.23	0.55±1.50^{A}	
		-20	31.42±2.76	107.5±9.43	-2.19±1.13^{B}	
	3rd	25	12.87±2.07	55.5±8.93	10.31±3.20^{A}	0.000
		4	18.40±2.28	93.7±11.60	1.25±0.74^{B}	
		-20	28.01±2.62	95.8±8.95	1.22±1.30^{B}	
	7th	25	6.14±0.77	26.5±3.33	17.05±4.42^{A}	0.000
		4	18.72±3.01	95.3±15.34	0.93±1.32^{B}	
		-20	28.97±1.91	99.1±6.53	0.26±0.61^{B}	

Mean ± S.D.(n=7)

[a]DW = distilled water, NS = 0.9% sodium chloride injection.

[b]Difference = concentration differences from 0 day at each temperature

**P<0.05 as compared the difference mean values among the three temperatures at each time (1st,3rd and 7th days) by one-way ANOVA.*

A,B and C =different alphabetical superscript means that each group is significantly different

The results of this study indicate the chemical stability of aspirin solution. Therefore, the concentration of aspirin of the aspirin only and aspirin+curcumin admixture solutions remained at least 90% of original without any color change or precipitation in the DW and NS solution at 4˚C and -20˚C throughout 7th day period and showed instability that decreased gradually below 90% of original concentrations after 1st day at 25˚C in the two solutions.

4. CONCLUSION

The objective of this study was to provide information about the physical and chemical stability of aspirin only and aspirin+curcumin admixture under two solvents, three storage temperatures and periods by HPLC assay method.

This study showed different stability of aspirin that depended on temperature of storage and period. On the hand admixture of curcumin on aspirin stability did not show any effect during 7 days except for 25˚C. However, this study was carried out for only one week. Therefore, further study is needed for the evaluation of chemical stability during a longer period of time.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

AUTHOR'S CONTRIBUTIONS

This work was carried out in collaboration between all authors. Authors JSK and HJK designed the study and wrote the protocol. Author JSK, HJK and MAK carried out the Aspirin stability testing, performed the statistical analysis and wrote the first draft of the manuscript. Authors YSP and SHK managed the literature searches and overall revision and submission. Authors read and approved the final manuscript.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

REFERENCE

1. Reynolds EF, editors. Aspirin and similar analgesic and anti-inflammatory agents. Martindale: The Extra Pharmacopoeia. 28th ed. 1982; 234–282.
2. Algra AM, Rothwell PM. Effects of regular aspirin on long-term cancer incidence and metastasis: a systematic comparison of evidence from observational studies versus randomised trials. The lancet oncology. 2012;13(5):518–527.
3. Manzano A, Pérez-Segura P. Colorectal cancer chemoprevention: is this the future of colorectal cancer prevention?. The Scientific World Journal. 2012;2012:1-8.
4. Chan AT, Arber N, Burn J, Chia WK, Elwood P, Hull MA, et al. Aspirin in the chemoprevention of colorectal neoplasia: an overview. Cancer prevention research. 2012;5(2):164–178.
5. Thun MJ, Jacobs EJ, Patrono C. The role of aspirin in cancer prevention. Nature reviews. Clinical oncology. 2012;9(5):259–267.
6. Sutaria D, Grandhi BK, Thakkar A, Wang J, Prabhu S. Chemoprevention of pancreatic cancer using solid-lipid nanoparticulate delivery of a novel aspirin, curcumin and sulforaphane drug combination regimen. Int J Oncol. 2012;41(6):2260-2268.
7. al-Gohary OM, al-Kassas RS. Stability studies of aspirin-magaldrate double layer tablets. Pharm Acta Helv. 2000;74(4):351-360.
8. Perkins S, Clarke AR, Steward W, Gescher A. Age-related difference in susceptibility of Apc(Min/+) mice towards the chemopreventive efficacy of dietary aspirin and curcumin. Br J Cancer. 2003;88(9):1480-1483.
9. Montgomery ER, Taylor S, Segretario J, Engler E, Sebastian D. Development and validation of a reversed-phase liquid chromatographic method for analysis of aspirin and warfarin in a combination tablet formulation. J Pharm Biomed Anal. 1996;15(1):73-82.
10. Rajput AP, Sonanis MC. Development and validation of a rapid RP-UPLC method for the determination of aspirin and dipyridamole in combined capsule formulation. Int J Pharm Pharm Sci. 2011;3(2):156-160.
11. Sultana N, Arayne MS, Ali KA, Nawaz M. Simultaneous determination of clopidogrel and aspirin by RP-HPLC from bulk material and dosage formulations using multivariate calibration technique. J Chromatogr Sci. 2011;49(2):165-169.
12. Franeta JT, Agbaba D, Eric S, Pavkov S, Aleksic M, Vladimirov S. HPLC assay of acetyl salicylic acid, paracetamol, caffeine and phenobarbital in tablets. Farmaco. 2002;57(9):709-713.
13. Sawyer M, Kumar V. A rapid high-performance liquid chromatographic method for the simultaneous quantitation of aspirin, salicylic acid, and caffeine in effervescent tablets. J Chromatogr Sci. 2003;41(8):393-397.
14. Chaudhary A, Wang J, Prabhu S. Development and validation of a high-performance liquid chromatography method for the simultaneous determination of aspirin and folic acid from nano-

particulate systems. Biomed Chromatogr. 2010;24(9):919-925.
15. Yamamoto E, Takakuwa S, Kato T, Asakawa N.Sensitive determination of aspirin and its metabolites in plasma by LC-UV using on-line solid-phase extraction with methylcellulose-immobilized anion-exchange restricted access media. J Chromatogr B Analyt Technol Biomed Life Sci. 2007;846(1-2):132-138.
16. Bae SK, Seo KA, Jung EJ, Kim HS, Yeo CW, Shon JH et al. Determination of acetylsalicylic acid and its majormetabolite, salicylic acid, in humanplasma using liquid chromatography-tandem mass spectrometry: application to pharmacokinetic study of Astrix in Korean healthy volunteers. Biomed Chromatogr. 2008;22(6):590-595.
17. International Conference on Harmonization (ICH). Guidance to industry: Q2B Validation of analytical procedure: Methodology. Center for Drug Evaluation and Research (CDER). Rockville, MD. November.1996;1-10.
18. Guidance for industry: Bioanalytical Method Validation. US Department of Health and Human Services, Food and Drug Administration. Center for Drug Evaluation and Research (CDER) & Center for Veterinary Medicine (CVM). Rockville, MD. 2001.