A Rapid High-Performance Liquid Chromatographic Method for the Simultaneous Quantitation of Aspirin, Salicylic Acid, and Caffeine in Effervescent Tablets

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Abstract

A rapid reversed-phase high-performance liquid chromatographic procedure is developed and validated for the simultaneous quantitation of aspirin, salicylic acid, and caffeine extracted from an effervescent tablet. The method uses a Hypersil C_{18} column (5 µm, 15 cm × 4.6 mm) for an isocratic elution in a water–methanol–acetic acid mobile phase at a wavelength of 275 nm. The tablets' buffering effects and acid neutralizing capacity require an extraction solvent of methanol–formic acid. The range of linearity for aspirin is 0.5–1.25 mg/mL, caffeine 0.065–0.195 mg/mL, and salicylic acid 0.4–6.0% of aspirin. The overall recovery is 100.2%, 100.7%, and 99.2% for aspirin, caffeine, and salicylic acid, respectively. Under the conditions of the method, aspirin, caffeine, and salicylic acid are adequately resolved with proper peak symmetry in less than 7 min.

Introduction

Aspirin (acetylsalicylic acid) is extensively used in the treatment of mild to moderate pain, fever, and inflammatory diseases. Aspirin belongs to the groups of medicines known as salicylates and anti-inflammatory analgesics and is a nonsteroidal anti-inflammatory agent (1,2). Salicylic acid is the hydrolysis product of aspirin and is usually present in small amounts of aspirin–caffeine effervescent tablets. Salicylates are not as therapeutically effective as aspirin (1,2). Caffeine, like theobromine and theophylline, is a xanthine derivative. Caffeine belongs to the group of medicines called central nervous system stimulants. It is used to help restore mental alertness when unusual tiredness, weakness, or drowsiness occurs (1,2). Both of these drugs are classified as "Generally Regarded as Safe" and are widely used throughout the over-the-counter medicines (3,4).

At present, there is not a United States Pharmacopeia (USP) compendial monograph specific for the simultaneous assay of aspirin, salicylic acid, and caffeine (Figure 1) (5). A literature search revealed that a number of high-performance liquid chromatographic (HPLC) methods have been developed to determine aspirin, salicylic acid, and caffeine simultaneously in dosage forms or in biological materials (6–8).

The various methods found in the literature describe more complex mobile phases that are either pH dependent, use a buffer, or require an ion-pairing reagent (9,10). Often, the peak symmetry and resolution from excipients for either aspirin or caffeine have shown to be of poor quality. Therefore, a method has been developed using a diluent of methanol and formic acid (95:5), which provides excellent separation from tablet excipients with gaussian peaks. A water–methanol–acetic acid mobile phase, which does not need further modifications, was used. The method was validated in accordance with International Conference on Harmonization guidelines on the Validation of Analytical Procedures (11).

Experimental

Apparatus

Liquid chromatograph

The liquid chromatograph used in this study was a PerkinElmer system (Norwalk, CT). The PerkinElmer system consisted of a Series 200 LC pump, Series 200 Autosampler equipped with a 150-µL loop and PerkinElmer Series 200 Peltier sample tray, Series 245C diode-array detector with a 600 Series link interface, Turbochrom software, and HP LaserJet 5000 printer (Hewlett-Packard, Palo Alto, CA).

Column

The columns used were commercially available stainless steel HPLC with a column length of 15 cm and inside diameter of 4.6 mm packed with Hypersil C_{18} (5 µm) or Keystone BDS C_{18} (Thermo Hypersil-Keystone, Cheshire, U.K.).

Mobile phase

The mobile phase was a mixture of water–methanol–glacial acetic acid (690:280:30). This mobile phase maintained an

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apparent pH of 4.7 for several weeks. The mixture was degassed using helium sparge. The flow rate was 1.5 mL/min, autosampler was chilled to 4°C, and column temperature was ambient.

Chemicals

HPLC-grade methanol and glacial acetic acid were purchased from VWR Scientific Products (South Plainfield, NJ) and used without further purification. Water was purified using a Milli-Q filter system (Millipore, Milford, MA). Formic acid was purchased from Fisher Scientific (Springfield, MA). Caffeine and salicylic acid standards were purchased from USP Convention (Rockville, MD). Aspirin reference standard was prepared as a pharmaceutical active ingredient (Bayer, Spain), and qualified for use as an in-house reference standard.

Table I. System Precision Parameters (Standard Solutions)

Component	%Coefficient of variation (N = 6)	Resolution	Tailing factor	Relative retention time (against void time)
Caffeine	0.3	–	1.9	2.0
Aspirin	0.2	10	1.1	3.6
Salicylic acid	1.3	10	1.1	6.4

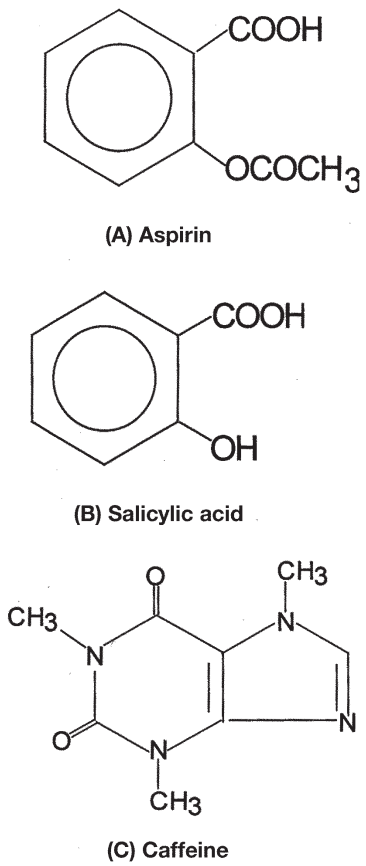

Figure 1. Structures of compounds studied.

Analytical sample

The sample tablets were provided by Bayer Consumer Care Manufacturing (Elkhart, IN). The effervescent tablets were prepared to contain 500 mg aspirin and 60 mg caffeine. The analytical effervescent placebo was provided by Bayer Consumer Care Formulation Development (Morristown, NJ).

Standard preparation

Salicylic acid reference standard solution (free salicyclic acid)
Approximately 50 mg of salicylic acid (accurately weighed) was transferred to a 50-mL volumetric flask, dissolved in and diluted to volume with methanol–formic acid (95:5), and mixed well.

Working reference standard solution

Approximately 250 mg of aspirin and 30 mg of caffeine (accurately weighed) were transferred to the same 250-mL volumetric flask. Approximately 100 mL of methanol–formic acid (95:5) was added and sonicated for 5 min to completely dissolve. A free salicyclic acid (FSA) reference standard solution (10.0 mL) was transferred to the same 250-mL volumetric flask. The flask was diluted to volume with methanol–formic acid (95:5) and mixed well. An aliquot was filtered through a 0.45-µm nylon filter directly into an HPLC vial and sealed. The vial was placed immediately into an autosampler previously chilled to 4°C.

Preparation of samples

Assay

Ten tablets were ground to a fine powder using a glass mortar and pestle. An accurately weighed portion equivalent to 250 mg aspirin and 30 mg caffeine was quantitatively transferred to a 250-mL volumetric flask. Approximately 150 mL of methanol–formic acid (95:5) was added to the flask and sonicated (at least 5 min) to

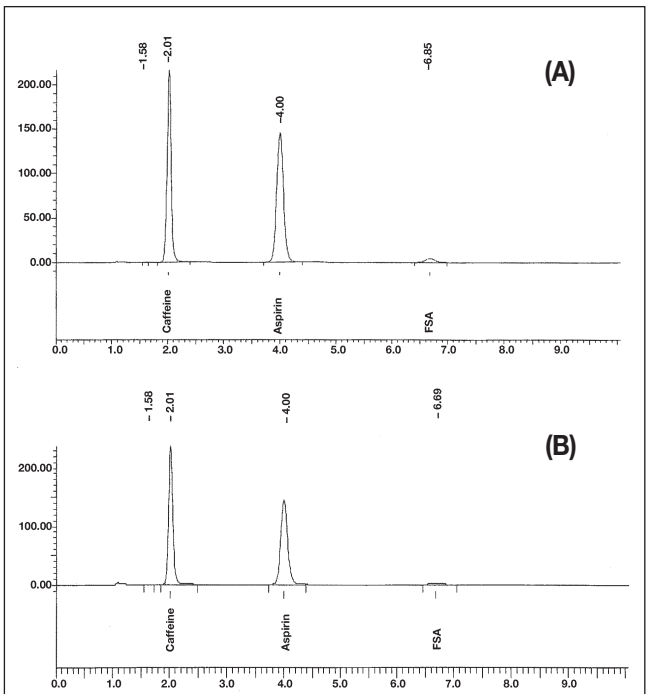

Figure 2. Representative chromatograms: (A) working standard solution and (B) authentic sample preparation.

dissolve. The flask was brought to volume with methanol–formic acid (95:5) and mixed well. An aliquot of the sample was then filtered through a 0.45-µm nylon filter directly into an HPLC vial and sealed. The HPLC vial was placed immediately into an autosampler previously chilled to 4°C.

Recovery

Aliquots of spiking solutions (containing both caffeine and aspirin) were pipetted in triplicate into 250-mL volumetric flasks containing placebo at levels corresponding to approximately 50%, 75%, 100%, 125%, and 150% of the label claim for one tablet. The samples were then prepared according to the assay procedure.

Because the presence of aspirin in the placebo would create an immediate bias in the salicylic acid levels, separate recovery samples were made for salicylic acid. The samples were prepared in triplicate by adding salicylic acid spiking solution volumetrically to 250-mL volumetric flasks containing placebo. The samples were then prepared according to the assay procedure.

The final concentrations for aspirin ranged from 0.5 to 1.5 mg/mL. The final concentration for caffeine ranged from 0.06 to 0.18 mg/mL. The salicylic acid ranged from 4 to 1.5 µg/mL.

Chromatographic conditions

The diode-array detector was set to a wavelength of 275 nm. The injection volume was 5 µL. The standards and samples were cooled in order to minimize any degradation that might occur during the analysis time.

System suitability tests

System suitability was evaluated using the percent relative standard deviations (%RSD) of the peak areas of six replicate injections of the working standard solution. Tailing factors and the resolutions were determined.

Table II. Repeatability

Component	%Coefficient of variation (*N* = 6)	%Average recovery (w/w)
Caffeine	1.2	100.1
Aspirin	0.3	99.6
Salicylic acid	2.6	2.0

Table III. Limit of Quantitation for Salicylic Acid

Trial	Amount added (mg/mL)	Amount found (mg/mL)	Recovery (%)
1	0.00420	0.00426	101.4
2	0.00420	0.00423	100.7
3	0.00420	0.00415	98.8
Mean recovery	Overall = 99.2%		
%RSD	Overall = 3.4%		

Results and Discussion

Precision

The system precision (system suitability) was determined by chromatographing six replicate injections of the working standard solution at the 100% level. Calculated were the relative retention times (void time = 1), coefficient of variation (%RSD) of the peak area responses, the tailing factors, and the resolution. The range for the coefficient of variation was from 0.2% to 1.3%. The tailing factors were less than or equal to 2.0, and the resolution between peaks was greater than 4 (Table I, Figure 2A).

Repeatability

The repeatability was determined by chromatographing six replicate solution preparations of the authentic samples, which had been aged for one year at 25°C and 60% relative humidity. The coefficient of variation (%RSD) of the peak area responses and the percent recovery by weight for aspirin, salicylic acid, and caffeine were calculated. The range for the coefficient of variation

Table IV. Linearity for Aspirin

	Nominal percent of 500 mg/tablet				
	50	75	100	125	150
Amount added (mg/mL)	0.5	0.75	1.0	1.25	1.5
Peak area response	635729	944949	1260153	1571021	1833233
Peak area response	636494	950120	1242188	1553390	1816667
Peak area response	633244	946618	1260058	1571866	1818389
Correlation coefficient = 0.99985					
R^2 = 99.97%					

Table V. Linearity for Caffeine

	Nominal percent of 60 mg/tablet				
	50	75	100	125	150
Amount added (mg/mL)	0.065	0.098	0.130	0.163	0.195
Peak area response	576637	870208	1156438	1440095	1728070
Peak area response	573497	853771	1152395	1440978	1738432
Peak area response	576420	861668	1143538	1431375	1714439
Correlation coefficient = 0.9998					
R^2 = 99.98%					

Table VI. Linearity for Salicylic Acid

	Nominal percent at 4% of aspirin (500 mg/tablet)			
	LOQ	4 (100%)	5 (125%)	6 (150%)
Amount added (mg)	0.004	0.100	0.125	0.150
1. Peak area response	5064	50999	63121	74063
2. Peak area response	5029	54007	59775	72196
2. Peak area response	4927	54399	65855	77466
Correlation coefficient = 0.95				

Table VII. Percent Recovery for Aspirin

	Nominal percent of 500 mg/tablet				
	50	75	100	125	150
Amount added (mg/mL)	0.5	0.75	1.0	1.25	1.5
%Recovery	101.4	100.4	101.3	101.0	98.3
%Recovery	101.5	101.0	99.9	99.9	97.4
%Recovery	101.0	100.6	101.3	101.1	97.5
%Mean recovery Overall = 100.2	101.3	100.7	100.8	100.7	97.7
%RSD Overall = 1.4	0.3	0.3	0.8	0.7	0.5

Table VIII. Recovery for Caffeine

	Nominal percent of 60 mg/tablet				
	50	75	100	125	150
Amount added (mg)	0.5	0.75	1.0	1.25	1.5
%Recovery	100.7	101.3	101.0	100.6	101.2
%Recovery	100.2	99.4	100.7	101.3	101.8
%Recovery	100.7	100.4	99.9	100.6	100.4
%Mean recovery Overall = 100.7	100.5	100.4	100.5	100.8	101.1
%RSD Overall = 0.6	0.3	0.9	0.6	0.4	0.7

Table IX. Recovery for Salicylic Acid

	Nominal percent at 4% of aspirin (500 mg/tablet)			
	10	100	125	150
Amount added (mg)	0.004	0.100	0.125	0.150
%Recovery	101.4	100.2	99.3	97.4
%Recovery	100.7	106.2	94.0	94.3
%Recovery	98.7	106.9	103.6	101.7
%Mean recovery Overall = 99.2	100.3	104.4	98.9	97.8
%RSD Overall = 3.4	1.4	2.2	4.8	3.8

was from 0.3% to 1.2%. The average recoveries were 99.6%, 100.1%, and 2.6% for aspirin, caffeine, and salicylic acid respectively (Table II, Figure 2B).

Limit of quantitation

The limit of quantitation (LOQ) for the active pharmaceutical ingredients, aspirin and caffeine, was not determined. The aspirin degradation product, salicylic acid, was independently spiked in the presence of placebo. At a wavelength of 275 nm, the lowest amount of FSA that could be quantitatively determined with suitable precision and accuracy was 0.004 mg/mL, equivalent to 0.4% FSA in 500 mg of aspirin (Table III).

Range of linearity

The linearity of peak area responses versus concentration was studied from approximately 0.5 to 1.25 mg/mL, or 50–125% of the formula amount for aspirin. The peak area response versus concentration was studied from approximately 0.065 to 0.195 mg/mL, or 50–150% of the formula amount for caffeine. For FSA, peak area response versus concentration was studied in the concentration range of 0.4% to 6.0% of the formula amount of aspirin. The allowable maximum limit for FSA in the product is 4.0%, with respect to aspirin. Thus, the range studied represents 10% (LOQ) to 150% of the FSA limit. The correlation coefficient was 0.99 for aspirin and caffeine. The correlation coefficient for FSA was found acceptable at 0.95 (Tables IV–VI).

Recovery

By spiking the placebo with stock solutions of the reference standards, the recoveries of aspirin, caffeine, and salicylic acid were assessed. The spiking was done at five levels in triplicate. The overall recovery was 100.2%, 100.7%, and 99.2% for aspirin, caffeine, and salicylic acid, respectively (Tables VII–IX).

Stability of analytical solutions

Aspirin is known to rapidly hydrolyze in solution at room temperature, as well as in the presence of effervescent buffers. Therefore, the stability of the standard and sample solutions was evaluated by analyzing the solutions initially at 6 and 24 h. Because of the hydrolysis of aspirin, the solutions should optimally be stored at 4°C and tested within 6 h of preparation (Table X).

Table X. Stability of Standard and Sample Solutions (%Potency)

Injection	Initial freshly prepared	6 h Room temperature	6 h Chilled	24 h Room temperature	24 h Chilled
Caffeine in standard		100.6%	100.3%	99.3%	99.2%
Caffeine in sample (*N* = 3)	97.2%	96.1%	96.8%	97.0%	96.6%
Aspirin in standard		100.6%	100.3%	99.3%	99.2%
Aspirin in sample (*N* = 3)	100.6%	93.6%	99.7%	70.0%	92.3%
FSA in standard		100.1%	97.7%	97.4%	97.4%
FSA in sample (*N* = 3)	1.7%	7.3%	3.1%	24.5%	7.2%

Conclusion

The described method is found to be rapid, linear, accurate, reproducible, and capable of simultaneously quantitating aspirin, caffeine, and salicylic acid in effervescent tablets. Thus, the method can be used for the routine analysis of stability samples and the quality control of products containing aspirin and caffeine.

Acknowledgments

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