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Development and validation of a dissolution method for warfarin sodium and aspirin combination tablets

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Abstract

A dissolution method for warfarin sodium-aspirin combination tablets was developed which utilizes USP Apparatus 1 (baskets) at 50 rpm with 900 ml of phosphate buffer (pH 6.8; 0.05 M) medium at 37°C. A reversed-phase liquid chromatographic method was also developed for the simultaneous determination of warfarin sodium, aspirin and salicylic acid on an octadecylsilica column using acetonitrile–tetrahydrofuran–glacial acetic acid–water (23:5:5:67, v/v/v/v) as the mobile phase with UV detection at 282 nm. Validation data were obtained which demonstrate that the dissolution methodology is accurate, precise, linear and rugged for the combination tablets. © 1997 Elsevier Science B.V.

Keywords: Warfarin sodium; Aspirin; Dissolution; Validation ; Reversed-phase liquid chromatography

1. Introduction

A combination warfarin sodium and aspirin tablet was under development at DuPont Merck which necessitated a suitable dissolution methodology, The United States Pharmacopoeia (USP) contained dissolution methods for each of the individual components, warfarin sodium and aspirin [1,2], but did not contain a dissolution method for combination product. A review of the literature indicated there were no reported dissolution methods for a combination warfarin sodium–aspirin product. Although several dissolution studies have been reported for both aspirin single-entity and multi-component tablets [3–6], they typically employed the USP methodology. The USP single-entity dissolution media were unsuitable for the combination product because of the poor solubility of warfarin sodium as discussed further below. Consequently, the in-house development of a dissolution method for the combination tablet was necessary in order to support the product development effort.

In addition, the USP dissolution procedures for the single-entity products utilized UV spectrophotometry (aspirin) and reversed-phase liquid chromatography (RPLC) (warfarin sodium) for the quantitative determination of the active drug components. Spectrophotometry was unsuitable for the combination product owing to the lack of

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Table 1
Recovery of warfarin sodium (1 mg) in various dissolution media

Component	Medium (900 ml)	Recovery(%)[a] ($n = 2$)
Warfarin alone	Water	101.8
Warfarin in presence of aspirin	Water	77.7
Warfarin alone	pH 4.5 acetate buffer	57.9
Warfarin in presence of aspirin	pH 4.5 acetate buffer	65.5
Warfarin alone	pH 6.8 phosphate buffer	98.8
Warfarin in presence of aspirin	pH 6.8 phosphate buffer	99.1

[a]USP Apparatus 2 (paddles) at 50 rpm.

specificity and insufficient sensitivity. Also, the warfarin sodium single-entity RPLC procedure was unsuitable for the combination product because aspirin did not have sufficient retention. Many LC methods have been reported for the determination of both aspirin and warfarin sodium in pharmaceutical products [7–15]; however, at the time the dissolution method was being developed, none of these methods offered simultaneous determination of both components. Therefore, an RPLC method was developed for the simultaneous determination of warfarin sodium and aspirin in the dissolution sample. This paper describes the development and validation of the dissolution methodology for warfarin sodium–aspirin combination tablets.

1.1. Method development history

The dissolution methodology was developed over the course of several years and therefore only the most important aspects of the method development will be presented. The dissolution media listed in the USP dissolution methods for single-entity warfarin sodium and aspirin tablets were water and acetate buffer (pH 4.5; 0.05 M), respectively. However, the data in Table 1 show that water was not a suitable medium for the combination tablets because of the low recovery of warfarin sodium in the presence of aspirin. This is because aspirin lowers the pH of the medium to about 3.6, where the solubility of warfarin sodium is poor (note: the pK_a of warfarin is 5.0 [16]). Likewise, acetate buffer medium was unsuitable because of the low recovery of warfarin sodium alone and in the presence of aspirin. This is because the pH of the acetate buffer medium is in a region where the solubility of warfarin sodium is poor. Several phosphate buffer media in the pH range 5.6–6.8 were investigated and it was found that pH 6.8 phosphate buffer medium gave accurate recovery results for warfarin sodium both alone and in the presence of aspirin.

The combination tablets were being developed in two dosage strengths, 1/80 and 3/80 mg of warfarin sodium/aspirin, respectively. For the

Table 2
Dissolution of warfarin sodium–aspirin tablets (3/80 mg) using apparatus 2 (paddle) at 50 rpm and 900 ml of phosphate buffer (pH 6.8; 0.05 M)

	Time (min)						
	5	10	15	30	45	60	Fast Stir
Aspirin dissolved (%)							
Mean ($n = 3$)	30.7	50.5	66.9	93.6	99.5	101.3	110.0
R.S.D. (%)	21.6	12.9	8.4	1.4	1.2	1.8	0.8
Range	26.0–38.3	45.0–57.7	61.8–72.9	92.2–94.7	98.2–100.6	99.8–103.3	109.3–110.9
Warfarin sodium dissolved (%)							
Mean ($n = 3$)	23.7	52.1	79.1	88.7	90.5	92.5	101.6
R.S.D. (%)	16.8	17.5	5.6	2.0	2.1	2.4	0.9
Range	20.6–28.2	45.8–62.5	76.4–84.2	86.6–89.7	88.3–91.6	90.0–94.0	100.6–101.9

Table 3
Dissolution of warfarin sodium-aspirin tablets (3/80 mg) using Apparatus 1 (basket) at 100 rpm and 900 ml of phosphate buffer (pH 6.8; 0.05 M)

	Time (min)						
	5	10	15	30	45	60	Fast Stir
Aspirin dissolved (%)							
Mean ($n = 3$)	63.7	88.6	103.5	108.1	109.0	109.5	110.2
R.S.D. (%)	0.5	1.4	0.3	1.8	1.6	1.7	2.5
Range	63.3–63.9	87.4–89.9	103.2–103.7	106.4–110.2	107.6–110.9	108.3–111.7	107.6–113.1
Warfarin sodium dissolved (%)							
Mean ($n = 3$)	36.8	97.9	99.4	100.0	100.5	100.2	100.4
R.S.D. (%)	3.8	2.9	1.2	0.8	1.2	1.2	1.4
Range	35.6–38.3	94.6–99.8	98.9–100.8	99.1–100.6	99.6–101.9	98.9–101.3	99.0–101.9

higher dose tablets sink conditions (i.e. dissolution concentration not more than one-third of saturation concentration) were met for both active ingredients in 900 ml of pH 6.8 medium. The aqueous solubility of warfarin sodium is 0.328 mg ml^{-1} at pH 6.87 and 25°C (in-house data). The Merck Index [17] indicates that 1 g of aspirin dissolves in 300 ml of water at 25°C and in 100 ml of water at 37°C.

The combination product was being developed as a trilayer tablet formulation. The paddle apparatus at 50 rpm was found to be unsuitable for the trilayer tablets because the warfarin sodium profile plateaued between 30 and 60 min with incomplete release in 60 min (Table 2). Therefore, a different apparatus with various speeds was tried in order to optimize the dissolution parameters for the trilayer tablets. The basket apparatus at 100 rpm was not suitable since the release rate was too rapid (Table 3). The basket apparatus at 50 rpm was found to be acceptable since the release rate was not too rapid and complete release of warfarin sodium was obtained (Table 4).

It is well known that aspirin hydrolyses to salicylic acid in aqueous medium [18]. The hydrolysis is rapid and occurs as the dissolution test is being conducted such that a significant amount of the aspirin in solution is hydrolysed to salicylic acid before the end of the dissolution test. In addition, the sample solution continues to degrade during the course of the HPLC analysis. Therefore, in order to quantitate the amount of aspirin that was originally present in the sample solution, the amount of salicylic acid found in the sample is converted to its aspirin equivalent and added to the amount of aspirin found remaining in the sample to obtain the total amount of aspirin contained in the sample. This is possible since the combination aspirin–warfarin standard solution contains approximately 25% dilution medium (acetonitrile-formic acid, 99:1, v/v) which reduces the rate of aspirin hydrolysis considerably. Consequently, the aspirin component of the standard is stable throughout the HPLC run. This provides an accurate aspirin calibration curve for quantitation of aspirin in the samples.

The dissolution methodology for warfarin sodium–aspirin combination tablets utilizes USP Apparatus 1 (baskets) at 50 rpm and 900 ml of deaerated phosphate buffer (pH 6.8; 0.05 M) at 37.0 ± 0.5°C. Sample analysis for both active components is performed simultaneously by RP-HPLC. This dissolution method was being applied to the trilayer tablet formulation. The remainder of this paper describes the validation of this dissolution method. Data are presented which demonstrate that the dissolution method is accurate, precise, linear and rugged for the trilayer tablet formulation.

Table 4
Dissolution of warfarin sodium–aspirin tablets (3/80 mg) using Apparatus 1 (basket) at 50 rpm and 900 ml of phosphate buffer (pH 6.8; 0.05 M)

	Time (min)						
	5	10	15	30	45	60	Fast Stir
Aspirin dissolved (%)							
Mean ($n=3$)	26.2	50.5	70.3	99.8	105.3	106.0	106.5
R.S.D. (%)	17.9	15.7	7.2	1.8	0.9	0.8	3.7
Range	22.2–31.4	45.9–59.6	64.8–74.7	98.3–101.8	104.5–106.4	105.0–106.6	102.8–110.7
Warfarin sodium dissolved (%)							
Mean ($n=3$)	21.0	45.9	91.2	99.5	100.4	100.1	100.6
R.S.D. (%)	15.9	4.3	5.6	1.9	0.4	0.8	2.4
Range	17.3–23.8	44.3–48.1	85.8–96.0	98.0–101.7	100.0–100.8	99.6–101.0	98.7–103.4

2. Experimental

2.1. Apparatus

The recovery tests and ruggedness studies were conducted on a Distek 2100 dissolution apparatus (Distek, Monmouth Junction, NJ, USA) using USP Apparatus 1 (basket) at 50 rpm. The dissolution medium was deaerated by the heat and vacuum technique using the Dissofill apparatus (Erweka Instruments, Milford, CT, USA). An Instrutex Model DS500UP Autosampler (Instrutex International, Hoosick Falls, NY, USA) was used for the additional filter studies. The HPLC system used consisted of a Waters WISP 712B autoinjector (Waters, Milford, MA, USA), a Waters 510 pump, an Applied Biosystems 783 variable-wavelength detector (Applied Biosystems, Ramsey, NJ, USA) and a Waters column temperature control module. Chromatograms were recorded and analyzed using a Multichrom chromatography data system (Fisons Instruments, Beverly, MA, USA).

Table 5
Accuracy and precision data for the warfarin sodium–aspirin combination tablets dissolution method

Component	Recovery (%) (grand mean ± R.S.D. $n=9$)	
	1/80 mg tablets	3/80 mg tablets
Aspirin	100.3 ± 0.4	101.0 ± 0.4
Warfarin sodium	98.7 ± 0.7	101.0 ± 0.6

2.2. Materials

Aspirin reference standard was used in the recovery tests and ruggedness studies and was obtained from the United States Pharmacopoeial Convention (Rockville, MD, USA). The warfarin sodium drug substance and placebo tablets used for the recovery tests and the warfarin (free acid) reference standard and combination tablets used for the ruggedness studies were obtained from DuPont Merck (Wilmington, DE, USA). HPLC-grade acetonitrile (EM Science, Gibbstown, NJ, USA) and reagent-grade formic acid (Eastman Kodak, Rochester, NY, USA) were used in the preparation of dilution medium for the standard solutions. Whatman, (Clifton, NJ, USA) 0.45 μm PVDF autovial syringeless filters were used for sample filtration. Additional filter studies were conducted with Instrutex 45 μm filter pipettes. Reagent-grade sodium phosphate, dibasic, anhydrous and sodium phosphate, monobasic, monohydrate (EM Science) were used to prepare the dissolution medium. Milli-Q water (Millipore, Milford, MA, USA) was used for both the dissolution medium and the HPLC mobile phase. HPLC-grade acetonitrile and tetrahydrofuran (EM Science) and reagent-grade glacial acetic acid (J.T. Baker, Phillipsburg, NJ, USA) were also

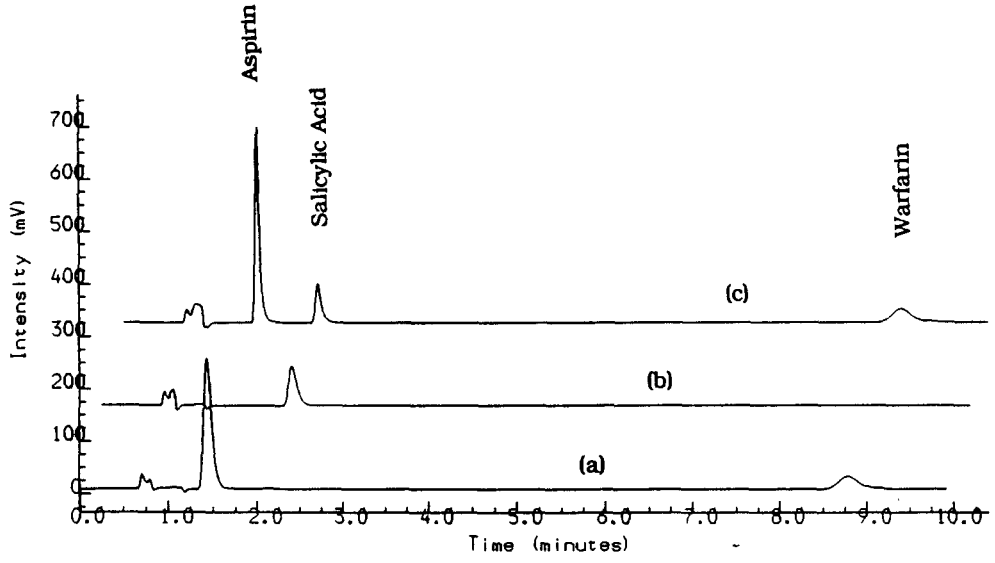

Fig. 1. Representative standard and sample chromatograms for the 3/80 mg strength tablets: (a) warfarin sodium–aspirin combination standard: (b) salicylic acid standard; (c) sample.

used for the preparation of the mobile phase for HPLC.

2.3. Reagents

The dissolution medium, phosphate buffer (pH 6.8; 0.05 M), was prepared by dissolving, per l of water, 3.478 g of sodium phosphate, dibasic, anhydrous, and 3.519 g of sodium phosphate, monobasic, monohydrate. The pH was adjusted, if necessary, to 6.80 ± 0.05 with 0.1 N sodium hydroxide or phosphoric acid (85%). Dilution medium was used for the preparation of the standard solutions and consisted of acetonitrile-formic acid (99:1, v/v).

Table 6
Additional filter data for the warfarin sodium–aspirin combination tablets dissolution method using Instrutex filters

Component	Recovery (%) (Mean ± R.S.D. $n = 3$)		
	5 min	10 min	15 min
Aspirin	100.1 ± 0.2	100.1 ± 0.1	100.1 ± 0.1
Warfarin sodium	99.9 ± 0.3	99.7 ± 0.2	100.1 ± 0.1

2.4. Preparation of standard solutions

Warfarin (free acid) stock standard solution was prepared at a concentration of approximately 0.4 mg ml^{-1} in phosphate buffer. To dissolve the warfarin, 10% (v/v) of 0.1 N sodium hydroxide was used in the warfarin stock standard solution. The warfarin stock standard solution was diluted to approximately 0.014 and 0.04 mg ml^{-1} for the 1/80 and 3/80 mg strengths, respectively, with dilution medium–phosphate buffer (25:75, v/v) to prepare the warfarin intermediate standard solutions.

Table 7
Sample solution stability of aspirin and warfarin sodium in phosphate buffer (pH 6.8; 0.05 M)

Time (h)	% of Initial		
	Aspirin	Total aspirin equivalent[a]	Warfarin sodium
24	72.6	98.6	99.9
48	57.3	98.8	100.3

[a]Aspirin content plus the aspirin equivalent amount from the salicylic acid.

Table 8
Stock standard solution stability of aspirin, salicylic acid and warfarin sodium: Standard solutions prepared from stock standard solutions stored for 96 h

Standard	% of Theoretical		
	Aspirin	Salicylic acid	Warfarin sodium
High	98.0	99.3	99.9
Mid	97.7	99.3	99.5
Low	97.4	98.3	98.6
Mean	97.7	99.0	99.3
R.S.D. (%)	0.3	0.6	0.7

Aspirin stock standard solution was prepared at a concentration of approximately 0.6 mg ml^{-1} in dilution medium. The aspirin stock standard solution was diluted to approximately 0.18 mg ml^{-1} with dilution medium-phosphate buffer (25:75, v/v) to prepare the aspirin intermediate standard solution.

The warfarin and aspirin intermediate standard solutions were combined and diluted to prepare combination warfarin sodium–aspirin standard solutions at concentrations of approximately 1.20/90.0, 0.90/72.0 and 0.60/54.0 μg ml^{-1} and 3.43/90.0, 2.57/72.0 and 1.71/54.0 μg ml^{-1} for the 1/80 and 3/80 mg strengths, respectively. The combination warfarin sodium–aspirin standard solutions were diluted with dilution medium–phosphate buffer (25:75, v/v). The warfarin sodium concentration in the combination warfarin sodium–aspirin standard solutions was determined by multiplying the warfarin concentration by 1.071, which is the ratio of the molecular weight of warfarin sodium to that of warfarin.

Salicylic acid stock standard solution was prepared at a concentration of approximately 0.8 mg ml^{-1} in dilution medium. The salicylic acid stock standard solution was diluted to approximately 0.08 mg ml^{-1} in dilution medium–phosphate buffer (25:75, v/v) to prepare the salicylic acid intermediate standard solution. The salicylic acid intermediate standard solution was diluted to concentrations of approximately 16.0, 12.8 and 9.6 μg ml^{-1} in dilution medium–phosphate buffer (25:75, v/v) to prepare the salicylic acid standard solutions.

2.5. Dissolution and analysis procedure

The dissolution medium used was 900 ml of phosphate buffer (pH 6.8, 0.05 M) at 37.0 ± 0.5°C. Agitation was accomplished via the USP Apparatus 1 (baskets) at 50 rpm. Sample aliquots of 4 ml were taken, filtered through a 0.45 μm. Whatman PVDF autovial syringeless filter and collected for analysis. For tablet testing, samples were taken at 5, 10, 15, 30 and 45 min timepoints.

The combination warfarin sodium–aspirin and salicylic acid standard solutions and the samples were injected on to a Waters C_{18}, Novapak 15 cm × 3.9 mm i.d. column (Millipore) using a mobile phase containing acetonitrile–tetrahydrofuran–glacial acetic acid–water (23:5:5:67 v/v/v/v). The flow rate was 1.5 ml min^{-1} and the column temperature was maintained at 35°C. The injection volume was 100 μl and UV detection at 282 nm was applied.

The amount of salicylic acid found in the samples was converted to its aspirin equivalent by multiplying the salicylic acid concentration by 1.304, which is the ratio of the molecular weight of aspirin to that of salicylic acid. This amount of aspirin equivalent found from the salicylic acid was added to the amount of undegraded aspirin found remaining in the sample to obtain the total amount of aspirin that was originally present in the sample solution.

3. Results and discussion

3.1. Accuracy and precision

Recovery studies were conducted by placing a placebo tablet in 900 ml of phosphate buffer (pH 6.8; 0.05 M) at 37°C and spiking with 50, 75 and 100% of the label claim of each active drug substance (i.e. warfarin sodium and aspirin). The samples were stirred with the basket at 50 rpm for 45 min and 4 ml aliquots were taken, filtered through 0.45 μm Whatman PVDF autovial syringeless filters and analyzed by HPLC. The placebo samples were prepared using the same procedure, but no active drug substance was spiked into the sample. The recovery results

Table 9
Effect of deaeration on the dissolution rate of warfarin sodium–aspirin 1/80 mg tablets

Medium	Component	Amount dissolved (%)	Time (min)				
			5	10	15	30	45
Deaerated medium Dissofill (Analyst 1. Distek Bath H)	Aspirin	Mean ($n = 6$)	48.4	76.7	89.2	100.6	101.2
		R.S.D. (%)	17.6	14.6	11.5	3.2	3.0
		Range	35.6–60.3	57.8–88.4	71.5–99.0	95.5–105.1	96.9–105.4
	Warfarin Sodium	Mean ($n = 6$)	21.8	67.2	98.5	103.9	104.0
		R.S.D. (%)	24.1	11.2	8.9	2.0	1.9
		Range	16.5–30.0	57.0–78.0	85.3–106.5	101.5–106.8	101.4–107.1
Non-Deaerated Medium (Analyst 1. Distek Bath H)	Aspirin	Mean ($n = 6$)	44.8	65.8	80.4	99.4	102.5
		R.S.D. (%)	34.6	20.2	10.8	4.4	4.6
		Range	26.4–67.6	51.4–86.0	71.6–93.7	95.2–106.6	98.0–109.7
	Warfarin Sodium	Mean ($n = 6$)	28.3	66.7	98.2	102.4	102.7
		R.S.D. (%)	9.2	15.3	6.1	2.0	2.3
		Range	25.3–32.5	52.2–75.5	86.4–103.1	99.4–105.2	99.5–106.0

Table 10
Effect of deaeration on the dissolution rate of warfarin sodium–aspirin 3/80 mg tablets

Medium	Component	Amount dissolved (%)	Time (min)				
			5	10	15	30	45
Deaerated medium Dissofill (Analyst 1. Distek Bath H)	Aspirin	Mean ($n = 6$)	41.2	70.9	85.8	101.9	102.9
		R.S.D. (%)	12.3	9.1	5.6	2.5	2.8
		Range	34.9–47.3	59.7–77.3	79.8–91.6	99.3–106.0	100.1–107.6
	Warfarin Sodium	Mean ($n = 6$)	17.9	45.5	85.2	102.5	103.1
		R.S.D. (%)	24.4	6.8	18.9	1.7	1.7
		Range	12.7–23.3	40.9–48.8	66.5–100.8	100.3–104.6	100.5–104.9
Non-deaerated medium (Analyst 1. Distek Bath H)	Aspirin	Mean ($n = 6$)	29.9	50.7	66.0	95.7	101.6
		R.S.D. (%)	18.5	17.5	12.6	6.0	5.4
		Range	21.9–35.8	37.2–59.7	55.8–77.1	86.0–101.6	93.7–109.0
	Warfarin Sodium	Mean ($n = 6$)	14.6	31.3	57.2	97.2	100.9
		R.S.D. (%)	11.7	10.5	18.2	5.9	2.9
		Range	12.8–17.8	26.8–36.0	43.0–69.8	86.7–101.4	96.5–103.5

Table 11
Effect of various analysts and instruments on the dissolution rate of warfarin sodium–aspirin 1/80 mg tablets

Conditions	Component	Amount dissolved (%)	Time (min)				
			5	10	15	30	45
Analyst 1, Distek Bath, H HPLC 66	Aspirin	Mean ($n=6$)	48.4	76.7	89.2	100.6	101.2
		R.S.D. (%)	17.6	14.6	11.5	3.2	3.0
		Range	35.6–60.3	57.8–88.4	71.5–99.0	95.5–105.1	96.9–105.4
	Warfarin Sodium	Mean ($n=6$)	21.8	67.2	98.5	103.9	104.0
		R.S.D. (%)	24.1	11.2	8.9	2.0	1.9
		Range	16.5–30.0	57.0–78.0	85.3–106.5	101.5–106.8	101.4–107.1
Analyst 2, Distek Bath, 3 HPLC 37	Aspirin	Mean ($n=6$)	44.6	67.2	82.5	101.1	102.3
		R.S.D. (%)	26.9	24.5	17.0	3.9	3.4
		Range	33.2–65.2	50.2–95.3	61.7–103.3	96.4–106.5	97.2–106.9
	Warfarin Sodium	Mean ($n=6$)	28.6	67.8	95.9	101.9	101.8
		R.S.D. (%)	12.8	13.2	8.5	2.3	2.4
		Range	25.2–35.1	52.3–76.9	80.6–104.2	99.4–104.5	99.0–104.5

(Table 5) show that the dissolution method is accurate and precise. Also the method was found to be free of placebo interference. Representative standard and sample chromatograms are shown in Fig. 1.

3.2. Additional filter studies

Additional filter studies, using Instrutex automated equipment, were performed by preparing a combination warfarin sodium–aspirin solution in phosphate buffer (pH 6.8; 0.05 M) at a concentration of approximately 0.58/45.5 μg ml^{-1} (i.e. 50% of the label claim of the 1/80 mg strength in 900 ml of dissolution medium) and passing this solution through Instrutex filters. A 5 ml volume was passed through the filter using the Instrutex (Model DS500UP) Autosampler with the first 2 ml being discarded. This procedure was repeated every 5 min for 15 min using the same filter. The filtrates were then analyzed by HPLC versus the 100% standard solutions prepared as described above to determine the percentage recovered. The results (Table 6) show that the Instrutex filters are acceptable alternatives to use in the dissolution method since there is no adsorptive loss of either active drug on these filters. In addition, the Instrutex filter recovery data are consistent over several time intervals.

3.3. Linearity

Five combination warfarin sodium–aspirin standard solutions ranging from 25 to 124% of the label claim for aspirin and from 26 to 130% for warfarin sodium were prepared for each tablet strength and analyzed by HPLC. Also five salicylic acid standard solutions ranging from 6 to 33% of the label claim of aspirin were prepared and analyzed by HPLC. Linear regression of the peak area versus the concentration of each component was performed and correlation coefficients of 0.9999 were obtained for each component, which demonstrates that the method is linear.

3.4. Injection precision

The injection precision for each tablet strength was determined by injecting a 100% combination warfarin sodium–aspirin standard solution ten times for each tablet strength. Excellent injection precision was obtained with R.S.D.s of $\leqslant 0.4$ for each component.

3.5. Sample and standard solution stability

A sample solution (100% spike) from the 3/80 mg strength recovery study was reanalysed after

24 and 48 h versus freshly prepared combination warfarin sodium–aspirin and salicylic acid standard solutions. The results (Table 7) show that aspirin is unstable in phosphate buffer medium (pH 6.8; 0.05 M), as expected, with 72.6% remaining after 24 h and 57.3% remaining after 48 h, whereas warfarin sodium is stable in phosphate buffer (pH 6.8; 0.05 M) for at least 48 h under ambient laboratory conditions. Using the procedure described previously, the total amount of aspirin in the sample was 98.6% at 24 h and 98.8% at 48 h after conversion of the salicylic acid to its aspirin equivalent. Therefore, sample solutions can be held under ambient laboratory conditions for up to 48 h and the total aspirin content can be accurately quantitated using the above procedure provided that standard solutions are prepared fresh or fresh dilutions from the stock standard solutions are made before the HPLC analysis is conducted (since the standard solutions degrade after 24 h).

The intermediate and working standard solutions were found to be unsuitable for use after 24 h owing to the degradation of both aspirin and salicylic acid and, therefore, must be prepared fresh for each run. However, the salicylic acid and aspirin stock standard solutions are prepared in 100% dilution medium and their stability was investigated since it was expected that they would remain stable for some time. Also, the warfarin stock was expected to remain stable in phosphate buffer (pH 6.8; 0.05 M) medium. Therefore, stock standard solutions were prepared for aspirin and salicylic acid in dilution medium and for warfarin sodium in phosphate buffer (pH 6.8; 0.05 M). The stock standard solutions were stored under ambient laboratory conditions for 96 h, and then the combination warfarin sodium–aspirin and salicylic acid standard solutions were prepared from the stock solutions and analyzed by HPLC versus freshly prepared standard solutions. The data (Table 8) show that the aspirin, salicylic acid and warfarin stock standard solutions can be stored under ambient laboratory conditions for 96 h prior to preparation of the combination warfarin sodium–aspirin and salicylic acid standard solutions.

3.6. Ruggedness

The effect of deaeration on the dissolution rate of 1/80 and 3/80 mg tablets was investigated. The Dissofill apparatus was used to deaerate the dissolution medium. The data (Table 9 Table 10) show that the method is sensitive to dissolved air in the medium. Dissolved air in the medium (i.e. non-deaerated medium) appears to cause a decrease in the dissolution rate, although the effect is not as pronounced with the 1/80 mg strength tablets. Therefore, deaerated medium is necessary for this method as required by the USP [19].

The effects of various analysts and instruments on the results for the 1/80 mg tablets were also evaluated using deaerated medium. The results (Table 11) show good agreement, within 2% or less at the 30 min timepoint, between various analysts and equipment (dissolution baths and HPLC systems).

4. Conclusion

A method had been developed and validated for warfarin sodium–aspirin combination tablets which determines the dissolution profile of both components simultaneously using phosphate buffer (pH 6.8; 0.05 M) and USP Apparatus 1 (baskets) at 50 rpm. This circumvents the problem previously described with the USP single-entity dissolution media, and provides the optimum dissolution conditions for this product. Also, an HPLC method was developed to measure concomitantly, aspirin, salicylic acid and warfarin sodium in the dissolution samples, thus eliminating the need for separate HPLC methods for the warfarin sodium and aspirin components.

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