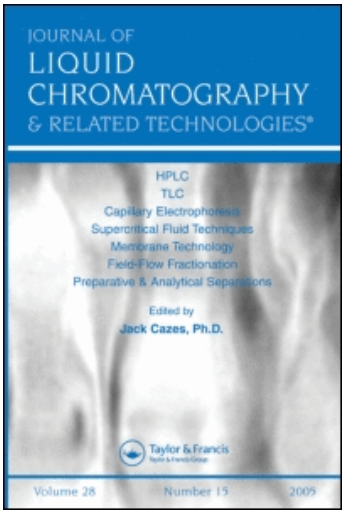

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VALIDATION OF A STABILITY-INDICATING HPLC METHOD FOR THE DETERMINATION OF DIPYRIDAMOLE IN DIPYRIDAMOLE INJECTION

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ABSTRACT

The validation of an isocratic high performance liquid chromatographic (HPLC) procedure employing ultraviolet (UV) detection for the analysis of dipyridamole in Dipyridamole Injection is reported. The method is simple, reproducible, accurate, and selective. The peak area versus dipyridamole concentration is linear over the range of 50-150% of its label claim of 5 mg/mL. The mean absolute recovery of dipyridamole using the described method is 101.0 ± 0.6%, (mean ± SD, n = 9). The precision (relative standard deviation, RSD) of label claim amongst five independent sample preparations, is not more than 1.4%. Intermediate precision, as determined from fifteen sample preparations, generated by two Analysts on different HPLC systems over three days, exhibits an RSD of 1.0%. The Standard and Assay Preparations are stable for up to 48 hours at room temperature.

The selectivity was evaluated by subjecting the finished product (Dipyridamole Injection) to thermal, acidic, basic, oxidative, and fluorescent radiation stress conditions. No interference in the analysis of dipyridamole was observed from degradation products, showing the method is stability-indicating.

INTRODUCTION

Dipyridamole is a coronary vasodilator used as an alternative to exercise in thallium myocardial perfusion imaging for the evaluation of coronary artery disease in patients who cannot exercise adequately.[1,2] Dipyridamole Injection is a parenteral solution consisting of dipyridamole (active), tartaric acid, and polyethylene glycol 600 in sterile water for injection.

The analysis of dipyridamole has been determined by spectrophotometry,[3] adsorptive stripping voltammetry,[4] and HPLC with UV[5-7] and electrochemical detection.[8,9] However, none of these methods are selective for potential degradation products in the finished product, although two methods[5,6] do demonstrate selectivity with the raw material. This manuscript describes the validation of a reverse phase HPLC method that is sensitive, accurate, and reproducible for the determination of dipyridamole in Dipyridamole Injection. Moreover, this method was determined to be stability-indicating.

According to the USP 23 <1225> guidelines, analytical methods for the quantitation of major components of bulk drug substance or preservatives in finished pharmaceutical products fall under Assay Category I.[10] Validation data elements required for Assay Category I include precision, accuracy, specificity, range, linearity, and ruggedness. The method for dipyridamole in Dipyridamole Injection satisfies all of these requirements.

EXPERIMENTAL

Chemical and Reagents

Dipyridamole Injection was formulated at Fujisawa USA, Inc. (Melrose Park, IL, USA). Dipyridamole was a USP reference standard. Polyethylene glycol 600 was purchased from Dow Chemical (Freeport, TX, USA). ACS reagent grade hydrochloric acid, acetic acid, sodium acetate, sodium hydroxide, hydrochloric acid, hydrogen peroxide, and NF grade tartaric acid were purchased from Mallinckrodt (Paris, KY, USA).

HPLC grade methanol was purchased from Baxter (Deerfield, IL, USA). The water was deionized and distilled. All reagents were used without further purification.

Apparatus

The chromatographic system consisted of a solvent delivery system, variable wavelength UV-visible detector set at 276 nm, variable volume injector (all HP Model 1050, Hewlett-Packard, Palo Alto, CA, USA). A Waters μBondapak C18 column (3.9 x 300 mm, 10 μm, Waters Associates, Milford, MA, USA) was maintained at ambient temperature. The flow rate was 1.0 mL/minute with a typical operating pressure of *ca.* 1300 psi. Under these conditions, the retention time of dipyridamole was 23 minutes.

Preparation of Solutions

Mobile phase

Prepare an acetate buffer by dissolving 2.38 g of sodium acetate in 350 mL water, adjust to pH 5.1 ± 0.1 with 36% acetic acid and mix. Add 650 mL methanol to the acetate buffer, mix and filter through a 0.5 μm filter, and degas.

Standard preparation

Accurately weigh Dipyridamole, USP reference standard and dilute to volume with Mobile Phase and mix to yield a concentration of 1.0 mg/mL.

Assay preparation

Accurately transfer Dipyridamole Injection (label claim 5.0 mg/mL dipyridamole) and dilute to volume with Mobile Phase and mix to yield a dipyridamole concentration of 1.0 mg/mL.

System Suitability

The system suitability results were calculated according to the USP 23 <621> from typical chromatograms.[11] The instrument precision, as determined by five successive injections of the Standard Preparation, should provide an

RSD not more than (NMT) 2.0%. The column efficiency should be greater than 1000 theoretical plates. The tailing factor should not exceed 1.5 at 5% peak height.

Specificity

The specificity of the method was studied through the analysis of stressed Dipyridamole Injection (finished product) and stressed Placebo Solutions (finished product without dipyridamole). The finished product was subjected to thermal, acidic, basic, oxidative, and fluorescent light environments for set periods of time or until dipyridamole degradation of 10-30%, as determined by peak area percent, was obtained.

Five mL aliquots of the finished product and Placebo Solution were sealed in transparent glass containers with equal head space and exposed to various stress conditions. Thermal stressed samples were stored at 70°C. Acid stressed samples were adjusted to pH 2 with concentrated HCl. Base stressed samples were adjusted to pH 12 with 50% NaOH. Oxidative stressed samples were subjected to 30% H_2O_2. Fluorescent stressed samples were subjected to 500-700 foot-candles of radiation.

Data Acquisition

The peak areas of dipyridamole were measured using HP Chemserver Model 4930 (Hewlett-Packard, Palo Alto, CA, USA). The chromatographic data was automatically processed for peak area followed by an unweighted linear regression analysis.

RESULTS AND DISCUSSION

Chromatography

Typical chromatograms obtained from a 15 μL injection of a Standard Preparation, Assay Preparation, and Placebo Solution are illustrated in Figures 1 (a-c), respectively. The retention time of dipyridamole was 23 minutes. The overall chromatographic run time was 30 minutes.

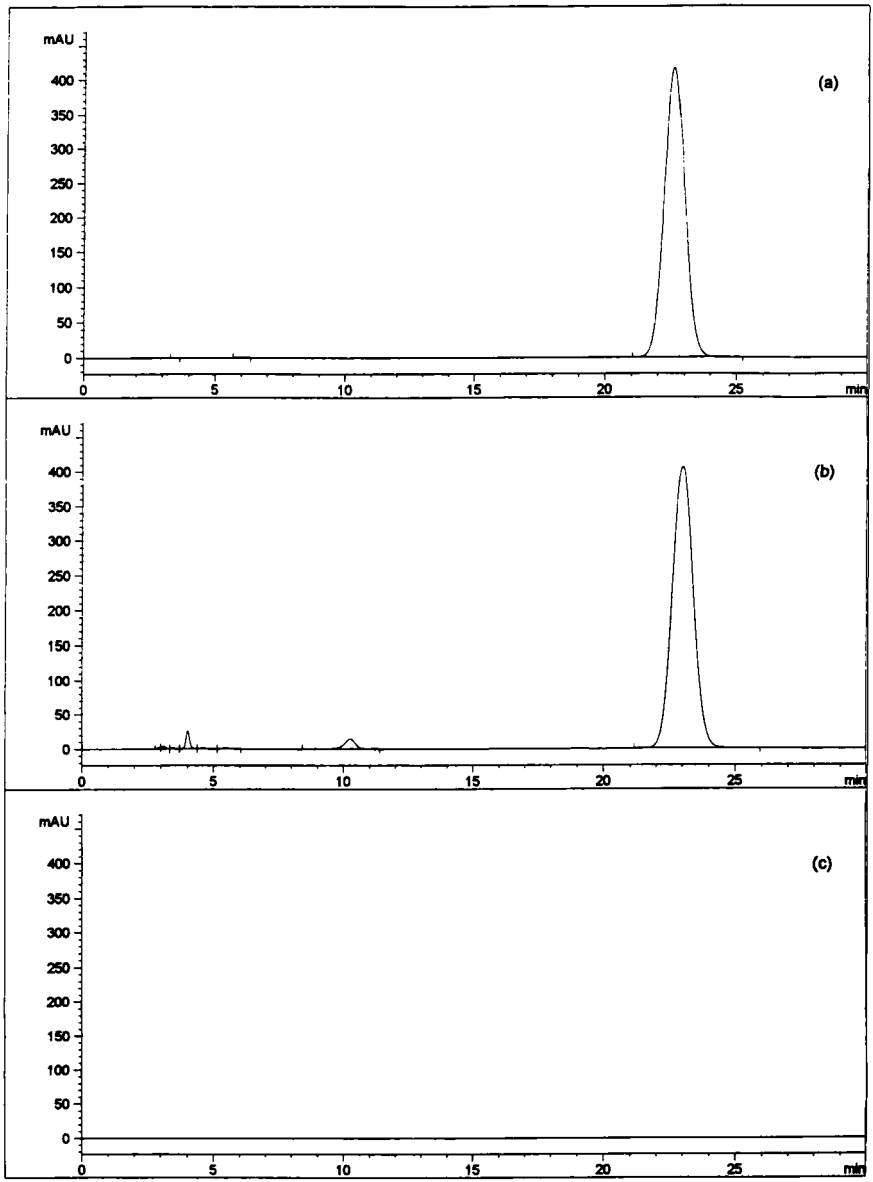

Figure 1. Typical chromatograms of (a) a Standard Preparation, (b) Assay Preparation and (c) Placebo Solution (Dipyridamole Injection not containing dipyridamole). The retention time of dipyridamole is 23 minutes.

Table 1

System Suitability*

Run	% RSD (n=5)	Tailing Factor Limit: NMT 2.0	Column Efficiency Limit: NLT 1000
1	0.3	1.3	2857
2	0.4	1.1	2704
3	0.1	1.2	3407
4	0.1	1.2	3079
5	0.2	1.0	3352
6	0.3	1.1	3021
7	0.1	1.0	3015
8	0.7	1.1	3202
9	0.2	1.1	2655
10	0.4	1.0	3480

* Tailing factor and column efficiency are calculated from the dipyridamole peak.

System Suitability

In all cases, the column efficiency for dipyridamole was greater than 2650 theoretical plates. The tailing factors of dipyridamole were not more than 1.3. The instrument precision, determined by 5 replicate injections of the Standard Preparation, exhibited a maximum RSD of 0.7%. Table 1 illustrates the system suitability results obtained over 10 independent runs spanning 3 months.

Precision

The precision (repeatability and intermediate precision) of the method was determined from one lot of finished product.

Repeatability

Five Assay Preparations were analyzed in a single session by Chemist I with HPLC System I. The RSD of the five results were within the 2% limit (Table 2).

Table 2

Assay Precision*

Run	Assay Value (%)	Average Assay (%)	RSD (%)
1 Chemist I HPLC System I	99.8 100.3 100.3 100.4 97.2	99.6	1.4
2 Chemist I HPLC System I	100.9 100.8 100.7 100.7 100.0	100.6	0.4
3 Chemist II HPLC System II	101.4 101.3 101.4 100.1 100.5	100.9	0.6
Intermediate Precision (n=15)		100.4	1.0

*Repeatability acceptance criteria: RSD NMT 2%.

Intermediate Precision

Intermediate precision was evaluated using Chemist I/HPLC System I to independently analyze five Assay Preparations from the same lot of finished product, and to have another analyst using a different chromatographic system (Chemist II/HPLC System II) analyze five Assay Preparations from the same lot. The RSD of each individual precision run was not more than 2% (Table 2).

Table 3

Assay Accuracy*

Approximate % Claim of Sample	Amount Determined (mg/mL)	Theoretical Amount (mg/mL)	Amount Recovered (%)	Average Recovery (%) (n=3)	RSD (%)
50	0.5104	0.5000	102.1	101.5	0.6
	0.5065	0.5000	101.3		
	0.5049	0.5000	101.0		
100	1.008	1.000	100.8	101.0	0.4
	1.007	1.000	100.7		
	1.014	1.000	101.4		
150	1.520	1.500	101.3	100.6	0.7
	1.500	1.500	100.0		
	1.506	1.500	100.4		
	Overall Recovery (n=9)			101.0	0.6

*Accuracy acceptance criteria: 97.0 to 103.0%. Precision acceptance criteria: 2% within each level.

Table 4

Linearity of Dipyridamole*

% Label Claim	Final Concentration (mg/mL)	Average Peak Area Response
50	0.50	12891651
80	0.80	20654643
100	1.00	25685373
120	1.20	30784865
160	1.61	40631288
	slope, m = 2.50×10^7	
	y-intercept, b = 5.81×10^5	
	correlation, r = 1.000	
	bias = 0.02%	

* Coefficient of Correlation Acceptance Criteria: NLT 0.999. Bias acceptance criteria: ± 3.0%.

Table 5

Stability of Analytical Solutions*

Time (Hours)	Peak Area Response and % Change			
	Standard Preparation	% Change	Assay Preparation	% Change
Zero Time	25622948	NA	25795058	NA
25	25480425	-0.6	25677676	-0.5
48	25606819	-0.1	25803515	0.0

* Stability Criteria: stable over the interval where the % change from zero time is within 2%.

Furthermore, the average percent assay values obtained were 99.6, 100.6, and 100.9% for runs 1, 2, and 3, respectively (Table 2). This yields an intermediate precision RSD value of 1.0% (mean=100.4% dipyridamole, n=15) amongst the three runs. The low scatter in the data supports the high degree of ruggedness of the analytical method.

Accuracy

The accuracy of the method was shown by analyzing Placebo Solutions spiked with known amounts of dipyridamole and comparing the analytical result to the known added value. The average percent recovery was calculated at each concentration level. The average amounts recovered were 101.5, 101.0, and 100.6% for concentrations of about 50, 100, and 150% of label claim, respectively.

This yields an overall average recovery of 101.0% (n=9) for the analytical method (Table 3). Since the results obtained are within the acceptable range of 97.0 to 103.0%, the method is deemed to be accurate.

Linearity

A linear response in peak area for dipyridamole over the range of 50-160% of its label claim was observed. The correlation coefficient was 1.000 and the bias was 0.02% (Table 4).

Table 6

Specificity Results

Stress Condition of Finished Product	% Degradation	Peak Homogeneity Limit: NLT 990
Thermal (70°C, 110 hrs)	16.0	999.99
Acid (2 weeks)	0.2	999.95
Base (2 weeks)	1.1	999.98
Oxidation (110 hrs)	11.0	999.98
Fluorescence (500-700 ft-candles, 72 hrs)	6.0	999.99

Range

The range of the assay method has been set at 50 t0 150% of the finished product label claim (5mgmL dipyridamile), since the method has been shown to be precise, accurate, and linear within this range.

Stability of Analytical Solutions

The stability of the analytical solutions was determined from the Standard Preparation (prepared from USP Reference Standard) and Assay Preparation (prepared from finished product) at room temperature. These solutions were analyzed at 0, 24, and 48 hours and analyzed against a freshly prepared standard at each time interval. The dipyridamole concentrations were examined as a function of time (Table 5). These data were evaluated for percent change from time zero. The Standard Preparation and Assay Preparation were found to be stable for 48 hours, respectively. Since the percent change is within ± 2%, the solutions are considered stable at room temperature.

Specificity

Dipyridamole Injection was stressed by thermal, acidic, basic, oxidative, and fluorescent radiation for up to 2 weeks or until approximately 10-30% degradation of dipyridamole was achieved, as determined by peak area percent. The results of the stress studies are presented in Table 6.

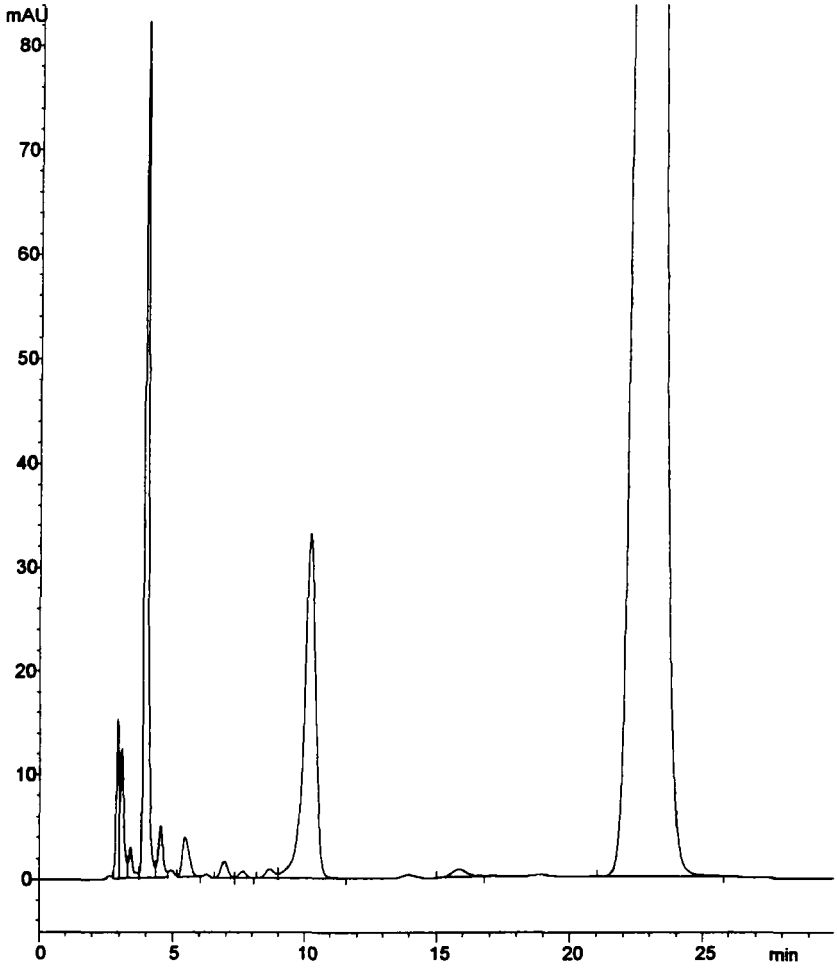

Figure 2. Specificity chromatogram of stressed Dipyridamole Injection. The retention time of dipyridamole is 23 minutes.

No interfering peaks at the retention time of dipyridamole were observed in any of the stressed samples. A chromatogram illustrating the specificity from a combination of thermal, oxidation and light stress is provided in Figure 2.

Peak Homogeneity

The control sample, stress samples, and placebo samples were analyzed using an HPLC equipped with a photodiode array detector. The dipyridamole peak was determined to be homogeneous since a purity value ≥ 990 was obtained in all cases (Table 6).

CONCLUSION

The described isocratic HPLC method for the analysis of dipyridamole has been evaluated for system suitability, linearity, precision, accuracy, stability of solutions, and specificity. The dipyridamole peak response has been shown to be precise, accurate, and linear, in the range of 50 to 150% label claim. Precision between two chemists on two different chromatographic systems was demonstrated to be within 1.0%. The Standard and Assay Preparations were found to be stable for 48 hours, at room temperature. Finally, the method has proven to be specific under a variety of stress conditions, while maintaining peak homogeneity. Consequently, the validated method for the determination of dipyridamole in Dipyridamole Injection is regarded as stability-indicating.

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