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A STABILITY INDICATING METHOD FOR DIPYRIDAMOLE

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ABSTRACT

An HPLC method for the assay and chromatographic purity assessment of dipyridamole raw material and capsule product was developed. A mobile phase composing of methanol: aq 200 mM pentane sulphonic acid, sodium salt, [70:30 v/v], had triethylamine added [2ml/L] and was then adjusted to pH 3.0 with phosphoric acid. The mobile phase was pumped through an octadecylsilated-silica column, being held at 60° C, at a flow rate of 1.5ml/min. Detection was made at 288nm.

The resolution of degradation products, along with precision and accuracy were investigated for the system.

INTRODUCTION

During analysis of a dipyridamole raw material sample an inconsistency was discovered between the British Pharmacopoeia(1) tlc related substance test for dipyridamole [I, Fig 1] and the United States Pharmacopoeia(2) HPLC chromatographic purity test.

By the tlc method a secondary spot was observed [greater Rf than dipyridamole] with an intensity larger than the specified limit. By the USP HPLC conditions, no secondary peak was observed before the specified run time of ten minutes. Therefore, the excessive elution time of impurity II meant that the sample complied with the USP but failed the BP specifications for related substances.

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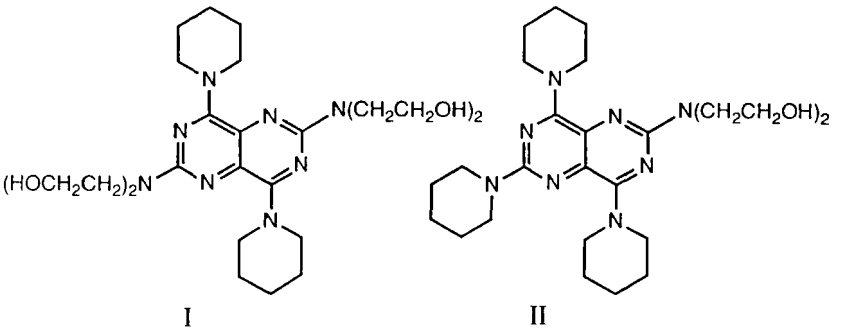

I II

FIGURE I

Consultation with the raw material supplier indicated that the observed impurity was a by-product of the synthetic pathway for dipyridamole [II, Fig I]. Subsequently it was confirmed that the USP test method was not developed to detect synthetic impurities (3).

Varying the organic component of the mobile phase employed by the USP chromatographic purity method did not appear to reduce the run time of the method sufficiently.

Therefore, the objective became to develop a stability indicating assay for dipyridamole which also resolved the synthetic by-product (II), within a run time of less than 20 minutes.

It was found that the addition of pentane sulphonic acid sodium salt, (PSA), to the mobile phase reduced the retention time for the impurity II. The resultant method is reported in this manuscript.

EXPERIMENTAL

Instrumentation

The chromatographic system consisted of a Waters 490E Programmable Multiwavelength Detector, with a Waters 712 WISP sample delivery system, attached to a Waters 600E System Controller. The system was operated remotely using the Maxima 825 Chromatography Workstation Version 3.3 upon which data handling was also performed.

Chemicals and Reagents

Dipyridamole USP reference standard was purchased from USP [Rockville, MD]. The Dipyridamole (I) raw material and the synthetic

impurity (II) were supplied by an Italian manufacturer. HPLC grade pentane sulphonic acid sodium salt (PSA) and methanol were obtained from BDH Chemicals, Poole. HPLC grade water was obtained by passing water through a reverse osmosis system, and dispensing via a NANOpure cartridge system.

Chromatographic Conditions

The column was 250 x 4.6mm id 5 μm ODS I [Phenomenex,]. The mobile phase was composed of methanol:200mM aq. PSA [70:30 v/v]. Triethylamine was added to the mobile phase [2ml/L] before it was adjusted to pH 3.0 with phosphoric acid. The eluant flow rate was 1.5ml/min while the column was maintained at a temperature of 60 ° C. UV detection was at 288nm [identical to that used in the USP] with a 20 μl sample injection volume.

Methods*

The dipyridamole standard was prepared by accurately weighing 30mg of dried USP standard into a 100ml volumetric flask and diluting to volume with methanol. A dilution of 5ml to 200ml of the solution was made with methanol, and the resultant solution [7.5mg/L] was filtered through a 0.45μ filter.

Assay solutions for dipyridamole raw material samples were prepared by weighing accurately 30mg of sample into a 100ml volumetric flask. Methanol [75ml] was added and the resultant solution mechanically shaken for 30 minutes before diluting to volume with methanol [solution 1]. A dilution of 5 to 200ml [as for the standard] was then made to obtain solution 2. To assay capsule products a weight of capsule contents equivalent to 30mg of dipyridamole was used, with extraction and dilution in the same manner as for a raw material.

For assessing chromatographic purity the secondary peak areas obtained from a 50 μl injection of solution[1] were compared with the dipyridamole peak area obtained from a 50 μl injection of a 1:5 methanol dilution of solution 2. No secondary peak area is to have a greater area than the area calculated for the dipyridamole peak.

The dipyridamole peak should meet the specifications reported in Table I.

RESULTS AND DISCUSSION

Preliminary investigations indicated that methanol and aqeuous PSA in the ratio 70 : 30 [respectively] resulted in an acceptable k' value

* Low-actinic glassware was employed throughout.

TABLE I

Parameter	Limit
Retention Time	2.9 - 3.1 min
% RSD [n=6]	**NMT 2.0%
Tailing	NMT 1.5 *

* Refer (4)
** NMT: not more than

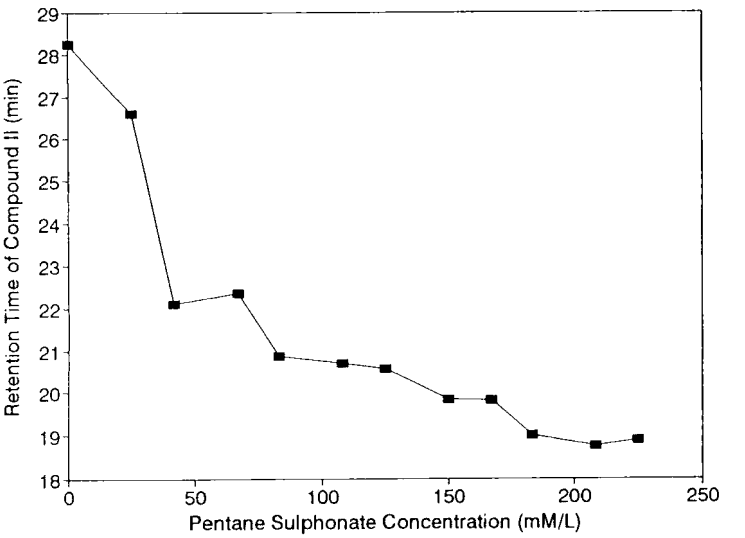

FIGURE II

The effect of the concentration of counter ion in the mobile phase on the retention time of impurity II.

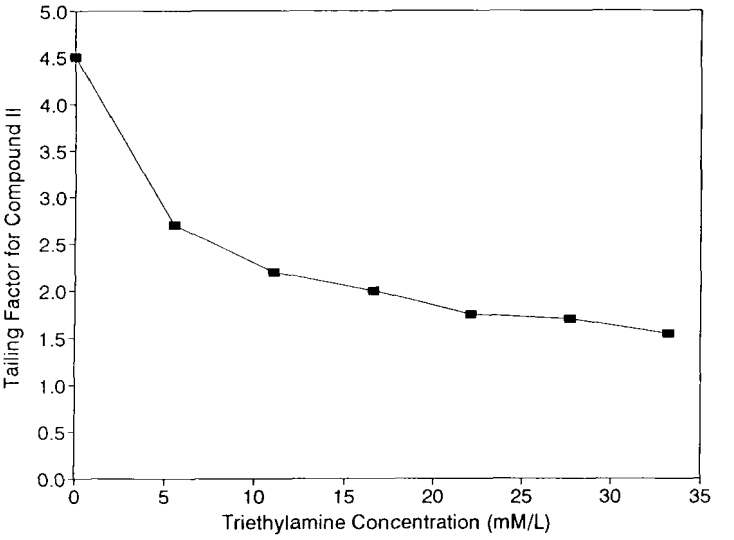

FIGURE III

The effect of increasing concentration of amine modifier in the mobile phase on the tailing factor observed for impurity II.

for dipyridamole. An experiment was performed to optimize the concentration of aqueous PSA. Figure II illustrates the trend of reduction in the retention time for compound II as the concentration of PSA in the mobile phase is increased. From the data it was determined that an aqueous PSA concentration of 200mM/L gave optimal reduction in the retention time of impurity II.

The peak corresponding to compound II exhibited tailing under these chromatographic conditions. In order to reduce this effect, triethylamine was added to the mobile phase. Figure III illustrates the effect of increasing the concentration of triethylamine [mM/L] on the tailing factor observed for the peak corresponding to compound II. An optimum concentration of 30 mM/L in the eluant was determined, resulting in a tailing factor of 1.8. This is consistent with the recommended level of amine modifier in a mobile phase (5).

A limited investigation of the effect of mobile phase pH indicated that adjustment to pH 3.0 maintained peak shape and resolution.

Figure IV shows a typical chromatogram of I and II under the conditions employed. The peak obtained for dipyridamole is symmetrical and is well resolved from II.

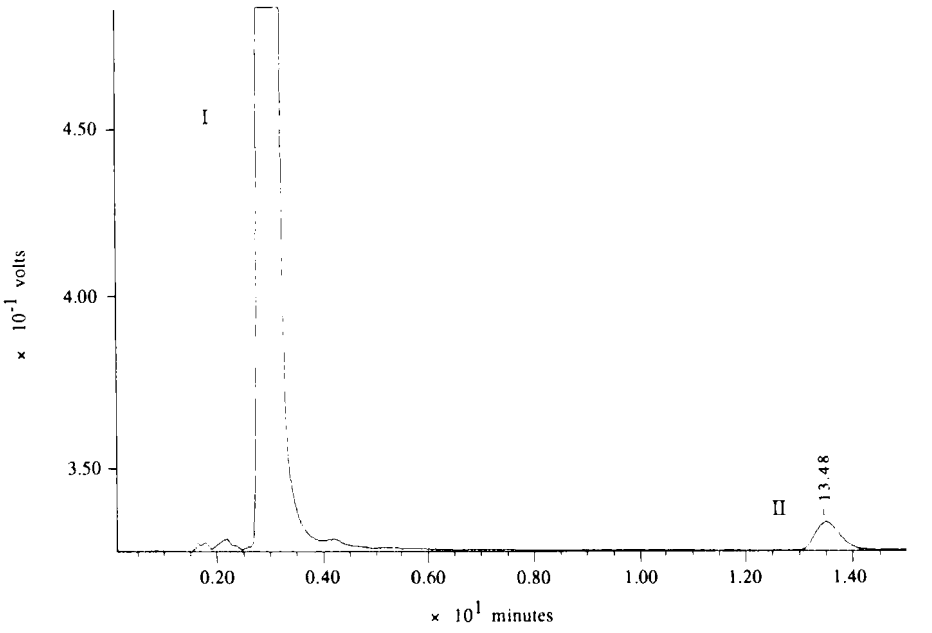

FIGURE IV

A typical chromatogram of dipyridamole I and the synthetic impurity II obtained with the proposed method.

Figure V shows the chromatograms obtained from degrading dipyridamole under the following conditions:

1.	Alkaline: 0.1M sodium hydroxide	(a)
2.	Acid: 01.M hydrochloric acid	(c)
3.	Neutral: water	(e)
4.	Hydrogen Peroxide 1%	(g)

The solutions were then stored at 40° C for 1 week and re-analysed resulting in chromatograms b, d, f and h respectively.

Under alkaline conditions (a) a degradation product is observed adjacent to dipyridamole; highlighted by the arrow. The resolution of this component was, however, deemed acceptable for the purposes of this method. Degradation products observed under the other conditions appear well resolved [Resolution NLT 2] from the peak due to dipyridamole.

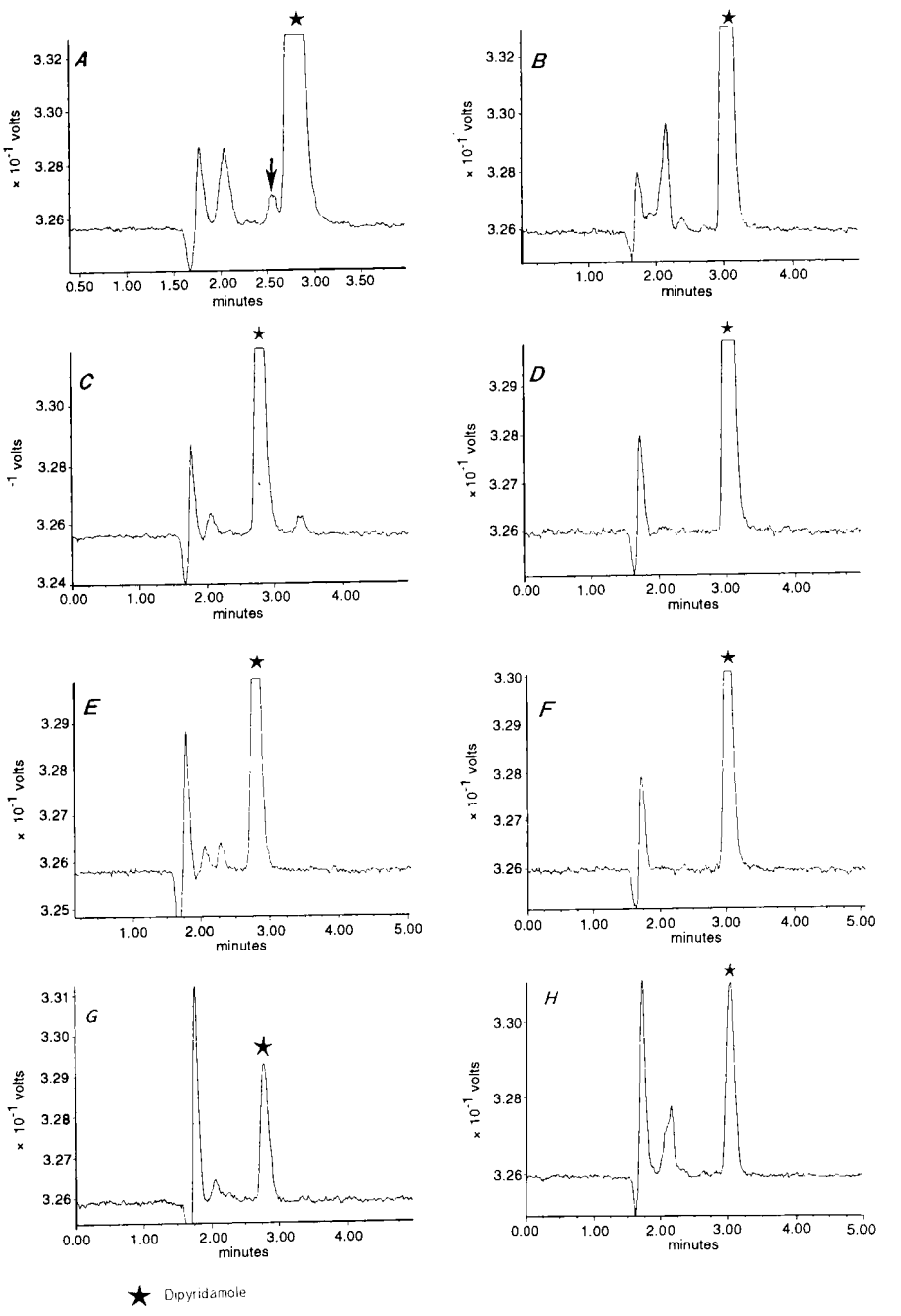

FIGURE V

Chromatograms obtained from degradation of dipyridamole under the following conditions:

Alkaline (a), acid (c), water (e), peroxide (g) and the corresponding chromatograms after storage at 40° C for one week: (b), (d), (f), (h) respectively.

TABLE II

Peak Areas And Retention Times Observed from Six Replicate Injections of Three Independently Prepared Dipyridamole Standards.

	Dipyridamole Concentration [mg/L]					
	4.26 [50%]		8.73 [100%]		10.72 [150%]	
Run No	RT [min]	Area	RT [min]	Area	RT	Area
1	3.033	197519	3.025	412702	3.025	508151
2	3.033	196867	3.025	415982	3.025	508469
3	3.025	197927	3.025	412428	3.025	509988
4	3.033	199735	3.025	413240	3.025	508475
5	3.033	198763	3.025	414225	3.025	508327
6	3.033	197797	3.025	412969	3.033	508666
Mean	3.032	198101	3.025	413591	3.026	508679
% RSD	0.11	0.51	0	0.32	0.11	0.13

TABLE III

Data obtained for the analysis of a dipyridamole raw material sample.

Column	1	2	3	4
Run No	Titri-metric	HPLC [Day 1]	Day 2	Recoveries
1	98.76	100.09	95.23	100.63
2	100.70	98.5	94.09	102.15
3	100.77	97.99	94.93	100.31
4	100.77	98.44	95.05	101.35
5	100.76	98.46	95.77	101.17
6	N/A	98.84	95.78	99.80
Mean	100.35	98.66	95.14	100.90
% RSD	0.89	0.77	0.71	0.83

The BP specification for related substances, that is that no secondary product is to be present at a level of greater than 0.5% of the dipyridamole input, was used to develop the chromatographic purity test, see method section. For impurity II, the minimum concentration at which five replicate injections [50 μl] gave a % RSD of less than 10 was 0.31mg/L. This is equivalent to a 0.1% impurity level.

Table II illustrates the repeatability of retention time and peak area for three independently prepared dipyridamole standard solutions of differing concentrations. Linear regression of the data results in a percent intercept of 1.6 and an r value greater than 0.9999.

Variable injection volumes of the dipyridamole standard were analyzed to investigate linearity of the peak area versus concentration curve. The curve is linear over the concentration range [1.9-9.5mg/L] with an r value 0.9999 and an insignificant percent intercept [0.4%].

A raw material sample of dipyridamole was assayed by titration with perchloric acid, [Table III, Column 1]. Statistical comparison of these data with the results obtained by the chromatographic method [Column 2] was undertaken using a null hypothesis at a 5 percent level of significance (6). The comparison suggests that there is no

TABLE IV

Data obtained for the analysis of dipyridamole capsules

Column	1	2	3
Run No	% Assay	Assay [Day 2]	Recovery [%]
1	93.82	94.80	100.24
2	95.72	96.05	*94.34
3	95.47	93.05	101.49
4	94.99	93.40	101.71
5	95.94	94.43	101.46
6	93.75	96.28	101.53
Mean	94.95	94.67	101.29
% RSD	0.69	1.4	0.59

* Result rejected as spurious

convincing evidence of a tendency for results from either method to be significantly different.

The same raw material solutions were refrigerated overnight, then assayed on day two with a fresh standard, [Table III, Column 3]. A similar statistical comparison of these data with those from day one suggest a significant difference, as a consequence standards and sample solutions should be freshly prepared daily.

Recovery experiments were undertaken by spiking a 30mg of raw material with 1.8mg of dipyridamole standard. The percent recoveries obtained are reported in Table III, [Column 4]. These data indicate the assay precision is excellent and that the extraction procedure [methanol with 30 minutes of mechanical shaking] employed is adequate.

The proposed method was also applied to the assay of a commercial brand of slow release capsule, results of which are presented in Table IV [Column 1]. These data indicate excellent

repeatability. Standard and assay sample solutions were prepared freshly on the following day and reassayed [Column 2]. The overall %RSD for these results [column 1 and 2] was 1.17 which indicates excellent reproducibility. Column 3 records the percent recovery obtained by spiking an amount of capsule contents [equivalent to 30mg of dipyridamole] with 1.8mg of dipyridamole standard.

CONCLUSION

The chromatographic method reported in this paper may be applied to the stability assay of dipyridamole raw material samples and capsule products. The method may also be employed to determine the chromatographic purity of samples.

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REFERENCES

1. The British Pharmacopoeia, British Pharmacopoeia Commission, London, 1988.

2. The United States Pharmacopeia. The National Formulary. United States Pharmacopeial Convention, Inc. Rockville, MD, XXII, 1990.

3. Personal Communication: From W. Larry Paul, USP. to J.H. Bridle, Feb, 1990.

4. L.R. Snyder, J.L. Glajch, J.J. Kirkland, "Practical HPLC Method Development", Wiley, New York, 1988.

5. J.W. Dolan, LC.GC, 7(16), 476, (1989)

6. C. Chatfield, "Statistics for Technology A Course in Applied Statistics", Chapman and Hall, London, 1970