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Note

Purity evaluation of dipyridamole by high-performance liquid chromatography

F. FONTANI*, G. P. FINARDI, G. TARGA, G. P. BESANA and M. LIGORATI
Quality Control Department, Recordati s.p.a., Via Civitali 1, Milan (Italy)
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Dipyridamole (DPM) has been a widely used coronary vasodilating agent for many years and it is now an object of renewed interest since it has been ascertained that it also has an anti-platelet aggregation activity.

The synthetic route to DPM was described by Fischer *et al.*[1]. In the final steps of this procedure 2,4,6,8-tetrachloropyrimido[5,4-*d*]pyrimidine reacts with piperidine to afford the 2,6-dichloro-4,8-dipiperidine derivative, owing to the higher reactivity of the chlorine atoms in the 4- and 8-positions with respect to those in the 2- and 6-positions. More drastic conditions and use of diethanolamine allow the chlorine atoms in the 2- and 6-positions to be replaced to obtain DPM.

The degradation impurities of DPM were studied by Wasilewska and Wilczynska-Wojtulewicz[2,3] who looked at compounds with different substituents in the pyrimidopyrimidine ring; the impurities were separated by thin-layer chromatographic (TLC) procedures.

TLC tests have been described in 2.AB-7/DDR[4] and in the DPM specifications for the French Pharmacopoeia[5]. In these procedures 100 or 200 μg of DPM are applied on to a silica gel plate together with 0.5–2.0 μg of DPM (from a dilute sample solution) as reference spots to evaluate visually the intensity of the secondary spots in the sample. Spots were observed using a UV lamp or by spraying the plate with Dragendorff reagent. Unfortunately the colour of the fluorescent spot of DPM under a 366 nm UV lamp is very different from that of some potential impurities and this fact is a source of error in evaluating the quality of DPM by TLC.

On the other hand the responses of the impurity spots to Dragendorff reagent are very different from each other.

In our investigation we have considered three potential impurities which were available as pure compounds. These had been isolated by our Chemical Development Division from the residues of mother-liquors from syntheses or from crude DPM samples. The impurities were characterized through their nuclear magnetic resonance spectra and by other chemical and physical data: they are shown in Fig. 1.

As the quantitative analysis of DPM in biological fluids using HPLC had already been published[6–8], our aim was to study an HPLC procedure suitable for separating the impurities from DPM in order to develop a method for the evaluation of the purity of DPM which would not leave any such uncertainty as with the TLC procedure.

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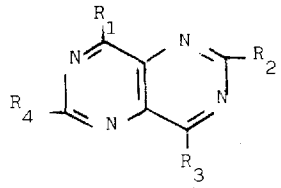

DPM: $R_1 = R_3$ = piperidine; $R_2 = R_4$ = diethanolamine

TP-MD: R_2 = diethanolamine; $R_1 = R_3 = R_4$ = piperidine

TD-MP: R_1 = piperidine; $R_2 = R_3 = R_4$ = diethanolamine

CL-DP-MD: $R_1 = R_3$ = piperidine; R_4 = diethanolamine; R_2 = chlorine

Fig. 1. Isolated potential impurities of dipyridamole (DPM).

EXPERIMENTAL

Materials and apparatus

DPM samples produced by Recordati and from foreign sources were used. All chemicals and reagents were of analytical-reagent grade and were used without further purification.

TLC was carried out with silica gel F_{254} (E. Merck) using precoated or in-house-coated plates. The mobile phase was *n*-butanol–acetic acid–water (77:12:35) and the plates were observed under 366- and 254-nm UV lamps immediately after having been dried by warm air.

HPLC analyses were carried out using a Perkin-Elmer Model 3B apparatus (with an LC75 detector and a 510 integrator) equipped with a LiChrosorb RP-18 (E. Merck) 10-μm column (250 × 4 mm) at 30°C. The mobile phase was a gradient of 0.015 *M* monosodium phosphate buffer (pH 7.6) (A) and acetonitrile (B), with B increasing from 60% to 90% in 15 min with a concave gradient (type 2 according to the Perkin-Elmer Model 3B apparatus). The flow-rate was 2 ml/min with an attenuation of 0.16 a.u.f.s. UV detection at 280 nm or fluorescence (measured through a Turner Model 111 fluorimeter connected to the apparatus), using an excitation wavelength of 285 nm in conjunction with a 470-nm emission filter, was used.

Procedure

Solutions of DPM in methanol were used for the analyses.

When the DPM sample contained significant amounts of impurities, 10 μl of a 0.1–0.2% solution were injected, the peaks were integrated and the relative percentages were obtained directly from the instrument. Taking into account the detector response factors the purity of the DPM was then calculated.

When the DPM sample contained very small amounts of impurities, 10 μl of a 0.3% solution were injected; because at this concentration there was not much correspondence between UV response and DPM concentration (because of the saturation of the detector), the areas of the impurity peaks were compared with the

areas of the peaks obtained by injecting separately 10 μl of a methanol solution containing known amounts of each impurity (from 3 to 12 μg/ml, equivalent to a percentage between 0.1 and 0.4% with respect to the DPM sample).

RESULTS AND DISCUSSION

By operating under the above conditions it is possible to separate DPM from the three impurities in question, as shown in Fig. 2. In the analysis of standard solutions containing each component in equal concentration, the detector response factors of the three impurities with respect to DPM were calculated. These are reported in Table I, together with the minimum amount of each impurity which can be detected.

Fig. 3 shows the chromatogram obtained from a practically pure DPM sample

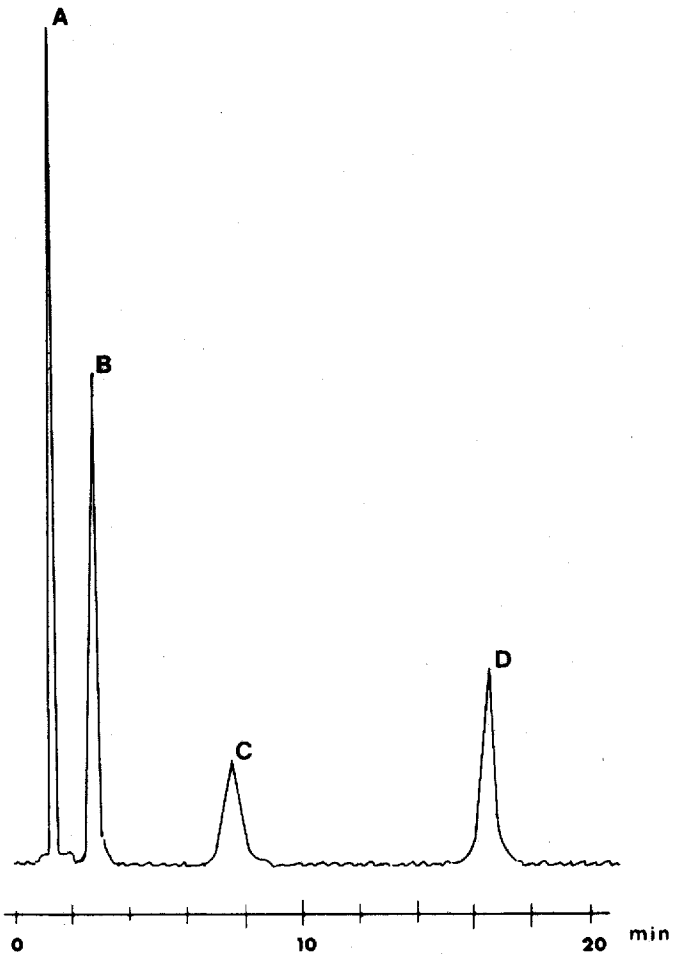

Fig. 2. HPLC separation of a mixture of dipyridamole (B) and potential impurities. Peaks: A = TD-MP; C = Cl-DP-MD; D = TP-MD (see Fig. 1).

TABLE I

DETECTOR RESPONSE FACTORS (DRF) AND MINIMUM AMOUNT DETECTABLE (MAD) OF THE THREE IMPURITIES

Product	*DRF*		*MAD (in ng)*	
	UV	*Fluorescence*	*UV*	*Fluorescence*
TP-MD	0.954	0.730	2	0.2
TD-MP	0.917	1.025	5	0.5
Cl-DP-MD	0.510	0.132	10	1

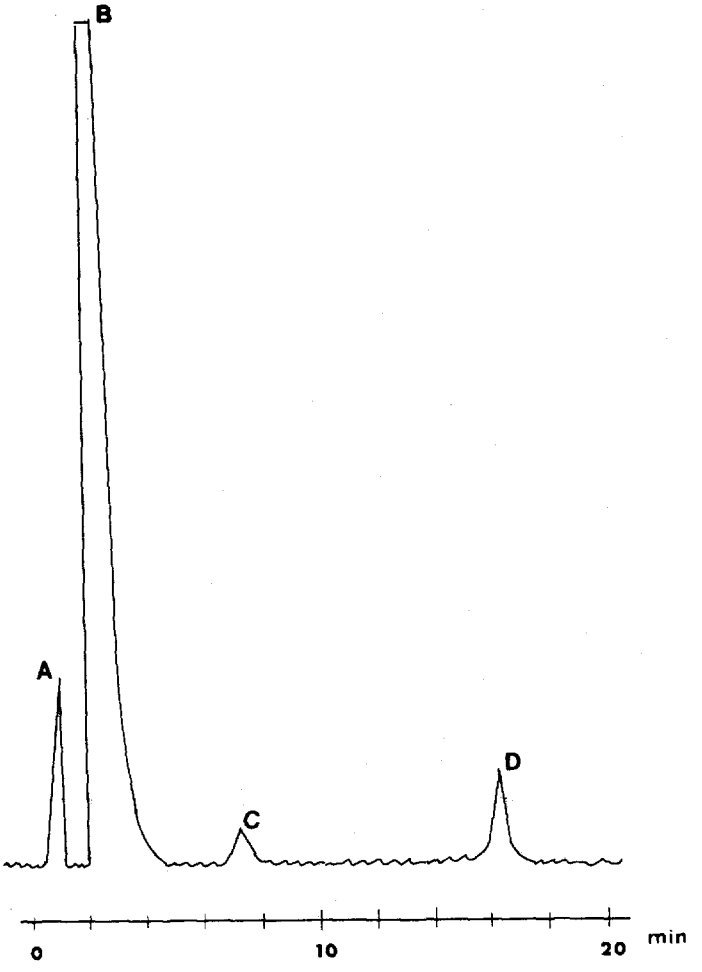

Fig. 3. HPLC separation of a practically pure sample of dipyridamole to which had been added 0.3% of each impurity. Peaks as in Fig. 2.

to which was added 0.3% of each impurity in question (by using 280-nm UV light as detector) to evidence the high sensitivity of the procedure.

To demonstrate the specificity of the method, preparative TLC was performed on a residue obtained from the evaporation of the mother-liquors from the synthesis that contained all possible impurities. The resulting plate was divided into three zones, the first containing those spots having R_F values greater than that of DPM, the second containing the DPM spot (R_F *ca.* 0.5) and the third containing those spots with R_F values lower than that of DPM. The silica gel from the first and the third zones was removed, eluted with methanol and the resulting solutions were assayed through the HPLC procedure. No peak from the TLC secondary spots showed a retention time that might have interfered with the DPM peak.

By the same procedure we analysed the second zone when the DPM peak together with the peak of an "unknown" impurity was obtained. This fact shows the higher selectivity of the HPLC method compared to TLC. This "unknown" impurity

TABLE II

ACCURACY AND LINEARITY IN ASSAY OF IMPURITIES

Impurity	*μg in 10 μl**	*Peak area***		*Correlation factor (r)*
		Average	*CV*	
TP-MD	0.03	1.1755	15.2	
	0.06	3.0606	8.4	
	0.12	5.4008	5.1	0.9918
TD-MP	0.03	1.4113	10.7	
	0.06	2.8508	5.2	
	0.12	5.6943	0.9	0.9999
Cl-DP-MD	0.03	0.7350	6.5	
	0.06	1.5864	8.4	
	0.12	2.8548	1.8	0.9970

* Amounts equivalent to 0.1, 0.2 and 0.3% of impurity with respect to 10 μl of a 0.3% sample solution.

** As integration values in five replicated injections.

TABLE III

RESULTS OBTAINED IN THE ASSAY OF SOME SAMPLES OF DIPYRIDAMOLE FOR PHARMACEUTICAL USE

Lot	*Purity (%)*	*Main impurities (%)*		
		TD-MP	*TP-MD*	*"Unknown"*
8282*	99.7	traces	traces	–
8250*	99.9	traces	traces	–
8223*	99.4	0.3	0.3	–
A**	99.3	0.4	0.3	–
B**	98.4	0.6	0.5	0.5

* Recordati production.

** Foreign production.

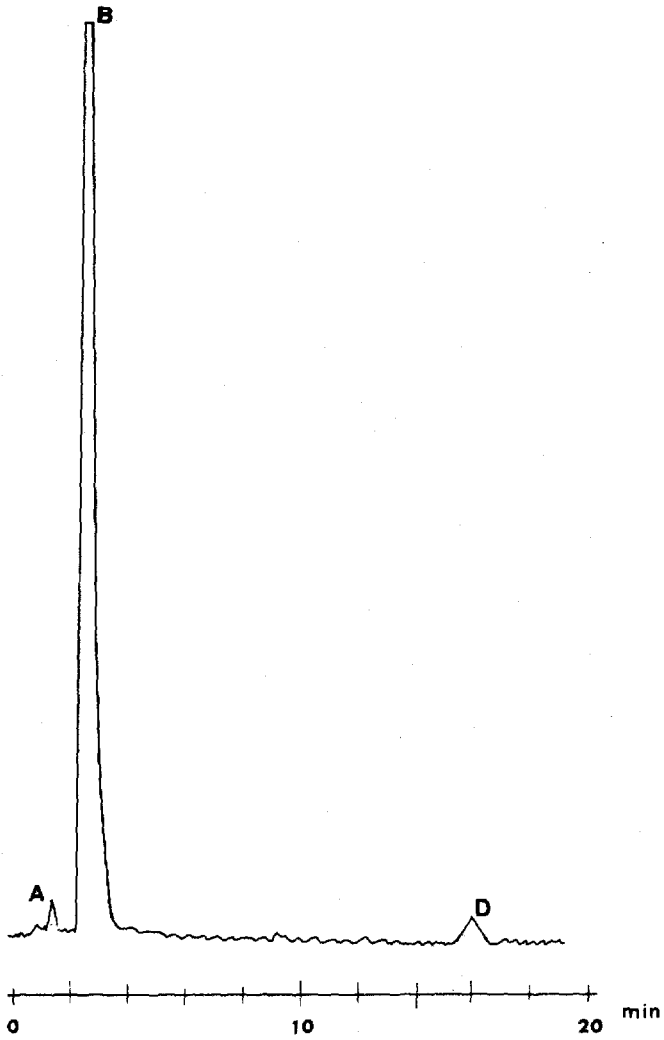

Fig. 4. HPLC assay of a pharmaceutical grade dipyridamole with more than 99.6% purity. Peaks as in Fig. 2.

has a retention time between those of DPM and Cl-DP-MD and was occasionally found when analysing "crude" or "pharmaceutical grade" DPM. To study the accuracy and linearity of the responses of the impurities in the HPLC assay, three standard solutions in methanol containing 3.0, 6.0 and 12.0 μg/ml of each impurity were injected (10 μl) and the peak areas were obtained by integration. The results of five replications are reported in Table II and they show the reliability of the procedure. Fig. 4 shows the chromatogram of a pharmaceutical-grade DPM. In this sample the impurities (TP-MD and TD-MP) are present in 0.1–0.2% amounts and the purity of the DPM is to be greater than 99.6%.

Finally, some results obtained by analysis of batches from our own manufac-

ture or from other producers are reported in Table III. The proposed HPLC procedure allows us to control the chromatographic purity of DPM for pharmaceutical uses.

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