Journal of Chromatography, 189 (1980) 241–247

CHROM. 12,470

REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC ANALYSIS OF CINCHONA ALKALOIDS IN PHARMACEUTICALS

M. A. JOHNSTON, W. J. SMITH, J. M. KENNEDY, A. R. LEA and D. M. HAILEY
National Biological Standards Laboratory, P.O. Box 462, Canberra City, A.C.T. 2601 (Australia)
(Received October 9th, 1979)

SUMMARY

An high-performance liquid chromatographic assay for quinidine and dihydroquinidine sulphates in pharmaceutical dosage forms has been developed. The method makes use of a reversed-phase system with a C_{18} bonded column and theophylline as an internal standard. Recovery of both compounds is quantitative and the method compares favourably with that of the United States Pharmacopoeia with regard to speed and precision. The method should also be suitable for the control of quinine salts in pharmaceuticals.

INTRODUCTION

The cinchona alkaloids quinidine and quinine are in widespread therapeutic use throughout the world and are most commonly used in solid dosage forms as the sulphates and bisulphates. The possibility of impurities in these drugs has been recognised for many years and official compendia such as the British Pharmacopoeia (B.P.) and the United States Pharmacopoeia (U.S.P.) previously controlled these impurities by means of semiquantitative thin-layer chromatographic (TLC) tests for "other cinchona alkaloids" (primarly cinchonine and cinchonidine). Cinchonine and cinchonidine do not normally occur in commercially available quinine and quinidine salts for therapeutic use. More recent pharmacopoeial monographs have included tests for the dihydro impurities which are commonly present in quinine and quinidine and are formed by saturation of the 5-vinyl function in the parent compounds. The European Pharmacopoeia (E.P.) monographs for quinine hydrochloride, quinine sulphate and quinidine sulphate substances include an assay for the dihydro impurities which is based on the bromination of the vinyl functions. This method, while suitable for the drug substances, is of rather low specificity and cannot be applied to formulations containing salts of quinine and quinidine. The U.S.P. test for dihydro impurities, which is applied both to the drug substances and the tablet and capsule formulations, involves a TLC separation followed by removal of the appropriate spots and subsequent spectrofluorimetric analysis. This procedure, which follows the work of Smith *et al.*[1], has suitable specificity with regard to the TLC system, but in our hands

suffers from a number of disadvantages. Besides possible problems inherent in removal of the spots, the assay is subject to interference from substances in the TLC adsorbants available in this country. This necessitates a prior washing procedure which increases the length and tedium of the assay. Improvement of the method by on-plate scanning is a possibility[2] but is not feasible for an official method because of lack of availability of equipment and difficulties in suitable specification of the apparatus.

From the point of view of official requirements for therapeutic goods, it is desirable to have a rapid, specific method for determination of impurities in quinine and quinidine salts. It is also appropriate to have the same general impurity limits for all quinidine salts and also separate common limits for all quinine salts. The situation with respect to pharmacopoeial standards is somewhat inconsistent as regards limits for dihydro impurities. The B.P. at present has no limit for the contents of dihydroquinidine or dihydroquinine, while the limits of the E.P. are 15 and 10%, respectively. The U.S.P. limit for dihydroquinidine in quinidine is 20% of the stated content while the corresponding value for dihydroquinine is 10%.

In addition, there are at present no official standards in Australia for some quinine and quinidine salts and formulations. Levels of dihydro impurities in products available in this country are typically between 1 and 7% in quinine salts and 3 and 21% for quinidine salts[3] although a dihydroquinidine content as high as 35% has been found[4]. In general, the impurity levels in quinidine salts resemble those reported by Smith *et al.*[1] and by Harvey *et al.*[5].

In the development of a method for control of dihydro impurities in preparations containing quinidine and quinine salts consideration was given to the use of nuclear magnetic resonance (NMR) following Huynh-Ngoc and Sirois[6] and to gas–liquid chromatography (GLC), following Smith *et al.*[1]. The use of NMR was not feasible because of sensitivity and precision difficulties with the apparatus available and because of the minor usage of this technique by pharmaceutical industry in Australia. Gas chromatography using 3% OV-225 met with some success, but gave inadequate resolution and reliability for an official method. It was therefore decided to proceed with the development of an high-performance liquid chromatographic (HPLC) method. A number of HPLC procedures for quinidine, quinine and their impurities have been described. Pound and Sears[7] used a silica gel column with a tetrahydrofuran (THF)–ammonium hydroxide mobile phase to analyse these compounds in commercial formulations. HPLC analyses of quinidine and dihydroquinidine in plasma samples have in general been carried out using reversed-phase systems, such as those used by Crouthamel *et al.*[8], Kates *et al.*[9] and Powers and Sadee[10]. Low and Kennedy[3] used ion-pair reversed-phase chromatography in surveying the quinine and quinidine products available in Australia. The actual resolution of the components and the time required for each analysis however limit the general application of the procedure. The procedure reported here is an ion-pair reversed-phase method which has been found to be of use in overcoming some of the problems mentioned above.

EXPERIMENTAL

Apparatus

All HPLC work was carried out on a Waters Assoc. μBondapak C_{18} 30 cm × 4 mm column using an Altex 110A pump and 20-μl loop injector. The detector was a

Chromatronix Model 230 fitted with an 8-μl flow cell. Peak areas were measured using a Hewlett-Packard Model 3380A reporting integrator.

Fluorescence measurements were made with a Perkin-Elmer Model MPF 44A spectrofluorimeter. TLC plates were prepared from Kieselgel H (Merck, Darmstadt, G.F.R.).

Materials

Reference materials used in this work were obtained from the following sources: quinidine sulphate, Sigma (St. Louis, Mo., U.S.A.); dihydroquinidine, I.C.N. Pharmaceuticals (New York, N.Y., U.S.A.); quinine, BDH (Poole, Great Britain); cinchonine and cinchonidine, Aldrich (Milwaukee, U.S.A.); theophylline, R.P. Scherer (Tempe, N.S.W., Australia). Dihydroquinine was prepared from quinine by hydrogenation[11].

All solvents used were analytical-reagent grade (Merck).

Chromatography conditions

Mobile phase: methanol–water–acetic acid (25:75:1). Separation of cinchonine–cinchonidine was carried out using methanol–water–acetic acid (20:80:1). Pressure: 3000 p.s.i. Flow-rate: 1.5 ml/min. Detector: 254 nm, 0.08 a.u.f.s. attenuation with 16 on the integrator.

Internal standard solution

A 100-mg amount of theophylline was dissolved in 100 ml of the solvent mixture used for the mobile phase.

Recovery experiments

Samples for recovery experiments were made as single tablets using the following formulation: quinidine sulphate, 100 mg; dihydroquinidine, 83.5 mg (equivalent to 100 mg sulphate); maize starch, 19 mg; lactose, 148 mg; alginic acid, 2 mg; magnesium stearate, 8 mg; povidone, 3 mg.

Each tablet was prepared by shaking the weighed mixture of excipients and active substances in a sealed vial and compressing the mixture in a hydraulic press by application of 23.1 MPa. The tablet was weighed and the amount of available active substances calculated.

HPLC assay procedure for tablet samples

Twenty tablets were weighed accurately and ground to a fine powder. A quantity of this powder equivalent to approximately 50 mg of quinidine sulphate was weighed into a 100-ml volumetric flask, shaken for 15 min with a quantity of methanol–water–acetic acid (80:20:1) and then made up to volume. A 10.0-ml volume of this solution was transferred to a 50-ml volumetric flask, 5.0 ml of the internal standard solution added, and the solution made up to the mark with the aforementioned solvent. This solution was then chromatographed using the HPLC conditions described above.

Analyses carried out

Three sets of comparative data were obtained. In the first instance, a series of solutions of quinidine sulphate reference substance in mobile phase were prepared and

analysed both by the HPLC procedure and the method of the U.S.P. to provide a direct comparison between the methods. Samples from three recently imported batches of quinidine sulphate were then assayed by the HPLC procedure and those of the U.S.P. and E.P. to provide a further comparison. In the second stage of the work, the HPLC method was applied to three commercially available samples of quinidine sulphate tablets, 250 mg, which had been previously tested using the procedures of the B.P. 1973 as part of the official sampling program. Finally, the HPLC method was used to check the recovery of the extraction/work up procedure. The extracts from the single-tablet assays were also analysed by the method of the U.S.P.

RESULTS AND DISCUSSION

A chromatogram of a mixture of quinine, dihydroquinine, cinchonine and cinchonidine is shown in Fig. 1 while Fig. 2 shows a chromatogram of a mixture containing the above compounds plus quinidine and dihydroquinidine. It can be seen that the chromatographic system used successfully resolves all components except dihydroquinidine and quinine, which would not be expected to be present together in a formulation. Theophylline has a convenient retention volume for internal standard purposes. From the point of view of selectivity, therefore, the HPLC procedure is considered suitable for control of pharmaceutical dosage forms. Fig. 3 shows a chromatogram from one of the commercial samples of quinidine sulphate tablets and shows the adequate separation of quinidine and dihydroquinidine and the absence of other cinchona alkaloids.

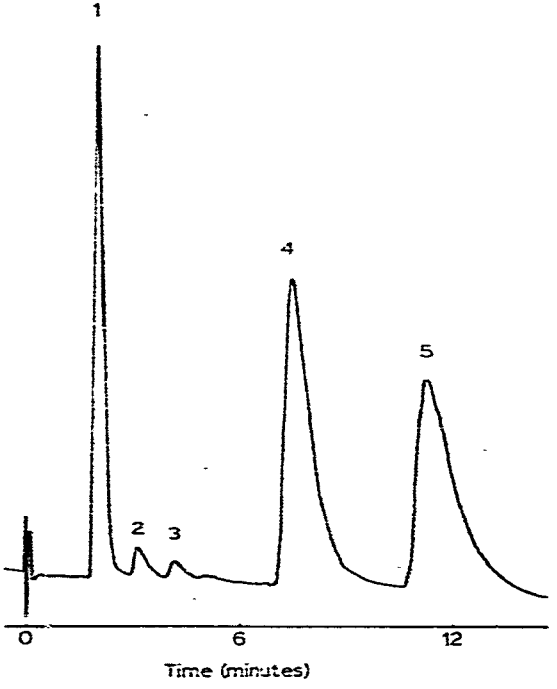

Fig. 1. Separation of a mixture of cinchona alkaloids. 1 = theophylline; 2 = cinchonine; 3 = cinchonidine; 4 = quinine; 5 = dihydroquinine.

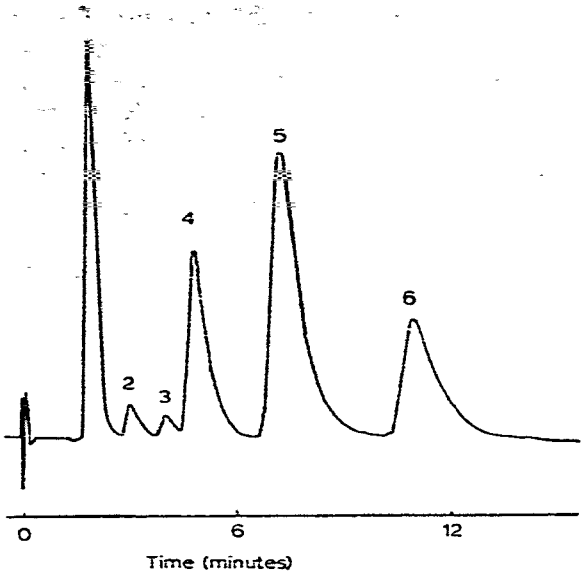

Fig. 2. Separation of a mixture of cinchona alkaloids containing both quinine and quinidine. 1 = Theophylline; 2 = cinchonine; 3 = cinchonidine; 4 = quinidine; 5 = quinidine and dihydroquinidine; 6 = dihydroquinine.

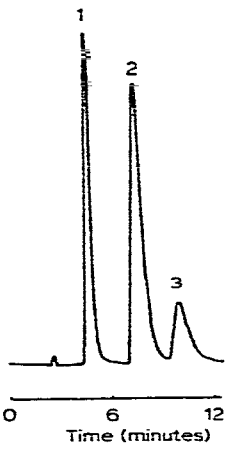

Fig. 3. Chromatogram from the analysis of a tablet containing quinidine sulphate. 1 = Theophylline; 2 = quinidine; 3 = dihydroquinidine.

TABLE I

ANALYSIS OF QUINIDINE SULPHATE–DIHYDROQUINIDINE SUBSTANCE

(a) Known solutions of quinidine sulphate

Concentration (mg/ml)	*Dihydroquinidine: quinidine ratio (× 100)*	
	HPLC	*U.S.P.*
0.1	25.3, 25.4, 26.0	
0.2	25.9, 23.9, 23.2	28.0, 30.4
0.3	23.9, 24.4, 23.7, 25.5	24.3, 26.9, 30.7
0.4	22.6, 22.7	23.1, 26.5, 23.0
0.5	23.1, 23.6, 22.7	22.0, 30.3, 30.0
All results mean ± S.D.	24.1 ± 1.2 (n = 15)	26.8 ± 3.3 (n = 11)

(b) Samples from imported batches of quinidine sulphate

Sample	*Dihydroquinidine: quinidine ratio (× 100)*		
	HPLC	*U.S.P.*	*E.P.*
1	23.2	22.0	13.0
2	4.7	9.0	7.0
3	22.8	27.3	17.2

The results from the analysis of samples of quinidine sulphate substance are given in Table I, and are reported in terms of the ratios of the content of dihydroquinidine to the content of quinidine. Agreement between the HPLC and U.S.P. methods is reasonable overall, but the total HPLC procedure is complete within 30 min, while the TLC–fluorimetric assay takes about 3 h. In the analysis of the three samples of imported material, agreement between the HPLC method and those of the U.S.P. and E.P. was not very close. The E.P. method tended to underestimate the dihydroquinidine content when this was appreciable.

Results from the analysis of commercially available tablets are given in Table II. The results using the B.P. assay, which makes use of a non-aqueous titration, are of the same order as the HPLC assay results but are consistently higher.

TABLE II

ANALYSIS OF COMMERCIALLY AVAILABLE QUINIDINE SULPHATE TABLETS

All results are expressed as a percentage of the stated content of quinidine sulphate.

Tablet brand	*Dihydroquinidine sulphate*	*Quinidine sulphate*	*Total*	*Total by B.P. assay*
A	2.8	95.9	98.7	102.1
B	14.0	80.5	104.5	102.5
C	8.7	89.8	98.5	103.6

The results of the recovery experiments are given in Table III and indicate that quinidine and dihydroquinidine are quantitatively extracted by the procedure described above. The precision achieved with the U.S.P. method in this experiment was closely comparable to that of the HPLC procedure. However, the result reported for tablet 4 is that of a repeat determination after a previous value of 0.746 obtained by the U.S.P. method had been rejected.

TABLE III

RESULTS OF RECOVERY EXPERIMENTS USING SINGLE-TABLET ASSAYS

Tablet No.	*Recovery of quinidine (%)*	*Recovery of dihydroquinidine (%)*	*Ratio of dihydroquinidine to quinidine*	
			HPLC	*U.S.P.*
1	96.4	99.9	1.010	1.043
2	99.2	104.6	1.024	1.007
3	99.4	103.9	1.151	0.990
4	100.1	105.7	1.107	1.117
5	100.1	101.0	1.020	0.964
6	98.1	98.0	0.994	1.113
Mean ± S.D.	98.9 ± 1.4	102.2 ± 3.0	1.051 ± 0.063	1.039 ± 0.064

Detailed recovery experiments have not, as yet, been conducted with quinine salts or with quinidine bisulphate. However, in view of the nature of the extracting solvent, it is anticipated that the solubilities of these other compounds will be high and that recoveries from the dosage forms will be quantitative.

CONCLUSION

An HPLC method has been developed for the analysis of quinidine, dihydroquinidine and cinchonine in formulations containing quinidine salts. The method is specific and compares favourably with that of the U.S.P. with regard to speed and precision. The method should also be suitable for the analysis of formulations containing quinine salts.

REFERENCES

1 E. Smith, S. Barkan, B. Ross, M. Maienthal and J. Levine, *J. Pharm. Sci.*, 62 (1973) 1151.
2 B. Wesley-Hadzija and A. M. Mattocks, *J. Chromatogr.*, 144 (1977) 223.
3 G. K.-C. Low and J. M. Kennedy, unpublished results.
4 T. Burgess, personal communication.
5 H. E. Harvey, M. P. Heenan and W. J. Passl, *Report No. C.D. 2229*, Department of Scientific and Industrial Research, New Zealand, 1976.
6 T. Huynh-Ngoc and G. Sirois, *Pharm. Acta. Helv.*, 49 (1974) 37.
7 N. J. Pound and R. W. Sears, *Can. J. Pharm. Sci.*, 10 (1975) 122.
8 W. G. Crouthamel, B. Kowarski and P. K. Narang, *Clin. Chem.*, 23 (1977) 2030.
9 R. E. Kates, D. W. McKennon and T. J. Comstock, *J. Pharm. Sci.*, 67 (1978) 269.
10 J. L. Powers and W. Sadee, *Clin. Chem.*, 24 (1978) 299.
11 M. Heidelberger and W. A. Jacobs, *J. Amer. Chem. Soc.*, 51 (1919) 819.