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Note

Inter-laboratory study on the separation of cinchona alkaloids by high-performance liquid chromatography

M. A. JOHNSTON, A. R. LEA* and D. M. HAILEY
National Biological Standards Laboratory, P.O. Box 462, Canberra City, A.C.T. 2601 (Australia)
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The present Australian official method for determining dihydroquinidine and dihydroquinine in quinidine and quinine salts is a titrimetric assay specified in the *British Pharmacopoeia*[1]. The titrimetric method is only applicable to the drug substance and is not suitable for formulations. To overcome this shortcoming, a high-performance liquid chromatographic (HPLC) method was developed[2]. The method is a reversed-phase method utilising a C_{18} column with methanol, water and acetic acid as the mobile phase. The method has been used routinely by this laboratory for the past two years. In developing the method and other HPLC methods intended for use as referee procedures, use has been made of commercially available columns which are used routinely by industry and official testing laboratories.

In our hands the μBondapak C_{18} column produced adequate separation of cinchona alkaloids including cinchonine and cinchonidine and gave quantitative recovery of quinidine and dihydroquinidine. The column was also used successfully in a study of the absorption of quinidine and dihydroquinidine in humans[3].

INTER-LABORATORY STUDY

As the method was developed with a view to incorporating it in standards for therapeutic goods, it was decided to evaluate the procedure by inter-laboratory study. All participating laboratories were supplied with quinidine sulphate, dihydroquinidine, and theophylline reference substances and quinidine sulphate tablets. The trial design required the participants to meet column performance criteria to ensure that all columns used for the assay were operating in an efficient and reproducible fashion before proceeding to analysis of the tablets. Limits were placed on the resolution factors R_1 and R_2 and the coefficients of variation on these factors for six injections of the reference substances provided. The substances eluted from the column in the order theophylline, quinidine and dihydroquinidine. In assessing the data the methods adopted were those described by Youden and Steiner[4] and in ref. 5.

Column performance

Seven laboratories were able to gain the required resolution while two were unable to obtain a workable separation as quinidine and dihydroquinidine failed to elute from the column.

The participating laboratories used predominantly Waters μBondapak C_{18} columns although other successful separations were achieved with Whatman ODS 10-μm and Partisil ODS columns. The unsuccessful laboratories used Waters Radpak ODS and Varian CH-10 columns.

Sample assays

The assay results for total alkaloids and individual alkaloids are given in Tables I and II respectively. The reliability of the method has been assessed by the determination of the content of total alkaloids, content of quinidine and content of dihydroquinidine. Two sample charts for each of these factors for the seven laboratories are shown in Figs. 1–3.

TABLE I

ESTIMATED CONTENTS OF ALKALOIDS (% NOMINAL CONTENT)

Lab. No.	*A*	*B*
1	100.0	100.0
2	91.5	79.0
3	97.7	81.8
4	96.3	81.1
5	98.2	86.1
6	98.6	84.3
7	96.0	84.4

TABLE II

ESTIMATED CONTENT OF INDIVIDUAL ALKALOIDS (% TOTAL NOMINAL CONTENT)

Lab. No.	*Quinidine assay*		*Dihydroquinidine assay*	
	A	*B*	*A*	*B*
1	82.0	83.1	18.0	16.9
2	73.9	63.9	17.6	15.1
3	79.9	66.9	17.8	14.9
4	79.5	68.0	16.8	13.2
5	78.8	69.8	19.4	16.3
6	79.6	66.0	19.0	18.3
7	79.2	68.2	16.8	16.2

The wide range of results obtained for the total alkaloids and quinidine assays can in large part be attributed to one set of results, from laboratory 1, which is an extreme outlier. The other results fall about the mean along the 45° line. The results for quinidine and total alkaloids obtained by laboratory 1 fail Dixon's rejection test for outliers[4] and if these results are rejected the mean assay values shown in Table III are obtained.

These results are considered to be encouraging. However, additional data for the sample pairs are required before a complete assessment of the method can be made.

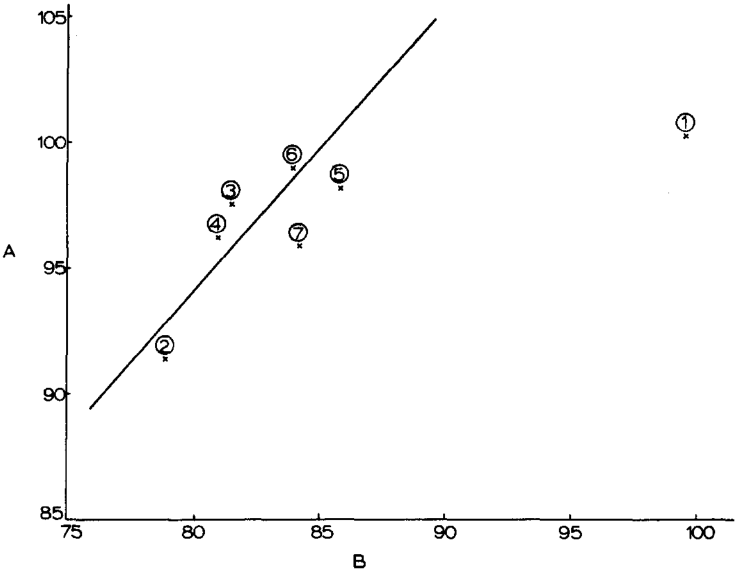

Fig. 1. Two sample chart for the determination of total alkaloids.

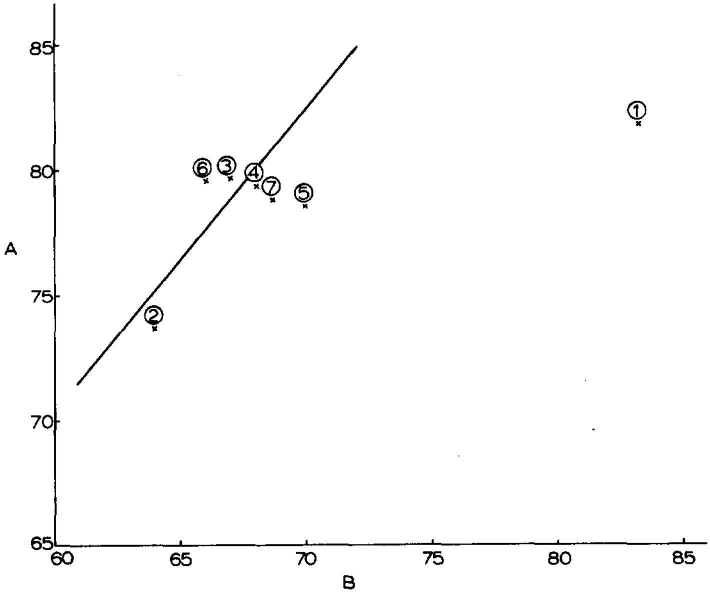

Fig. 2. Two sample chart for the determination of quinidine.

DISCUSSION

Off the nine laboratories that agreed to participate in the trial seven successfully completed the assays. The failure of the other two laboratories to achieve the sepa-

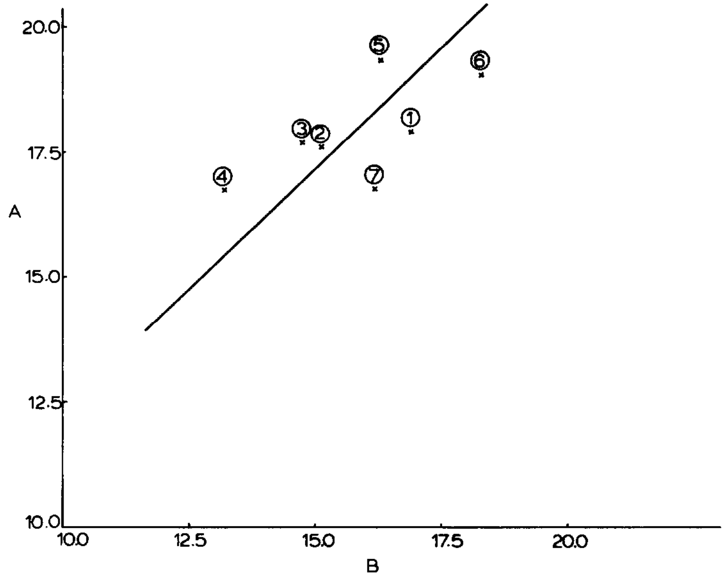

Fig. 3. Two sample chart for the determination of dihydroquinidine.

TABLE III

MEAN ASSAY VALUES AND STANDARD DEVIATIONS FOR TOTAL ALKALOIDS, QUINIDINE AND DIHYDROQUINIDINE

	Total alkaloids (%)	*Quinidine (%)*	*Dihydroquinidine (%)*
Results			
Sample A	96.9	79.0	17.9
Sample B	82.8	67.1	15.8
*Standard deviations**			
S_D	3.46	2.8	1.7
S_R	1.26	1.3	0.9
S_B	2.27	1.8	1.0

* According to the procedure of Youden (see ref. 4). S_D, total error; S_R, repeatability; S_B, bias.

ration is thought to be due to irreversible adsorption of the alkaloids to active silanol groups on the column packing. The problems encountered by these two laboratories highlight the need to specify a suitable column when drawing up a method for official testing purposes.

Hobson-Frohock and Edwards[6] described a HPLC method for cinchona alkaloids using a bonded cyanopropyl column in the reversed-phase mode. These authors were unable to produce an adequate separation using the method developed in this laboratory even though three different columns were tested.

The work described by Hobson-Frohock and Edwards[6] does not refer to the separation of the dihydro compounds but rather to the resolution of quinine, quinidine, cinchonidine and cinchonine. The method appears to be a useful addition of the literature on analysis of cinchona alkaloids although optimum separation required more than 25 min and no data were given on the reproducibility.

The difficulties of two laboratories in the present study and those experienced by Hobson-Frohock and Edwards[6] illustrate the problems of defining standard conditions for an HPLC method. This subject has received much attention in the literature but practical difficulties remain. Differences in performance are encountered between columns from a single manufacturer but more particularly between columns, nominally with the same type of packing, but prepared by different manufacturers.

Majors[7] has listed over 30 octadecylsilane bonded packings, all of which differ in percentage of phase loading, pore size and proportion of residual silanol groups. The presence of residual silanol groupos often strongly influences the chromatographic behaviour of bases and variations in the proportion of residual silanol groups may therefore explain the difficulties experienced with the separation of cinchona alkaloids. As there is understandable reluctance by manufacturers to specify closely all physical and chemical characteristics of the columns they produce, the use of commercially available columns may continue to present difficulties and contribute to the proliferation of HPLC methods described in the literature.

For the time being, the approach taken by this laboratory will be to use relatively well defined commercial columns and include criteria for column performance in any official standards.

REFERENCES

1 *British Pharmacopoeia*, Her Majesty's Stationery Office, London, pp. 522, 5231.

2 M. A. Johnston, W. J. Smith, J. M. Kennedy, A. R. Lea and D. M. Hailey, *J. Chromatogr.*, 189 (1980) 241.

3 D. M. Hailey, A. R. Lea, D. M. Coles, P. E. Heaume and W. J. Smith, *Eur. J. Clin. Pharmacol.*, 21 (1981) 195–119.

4 W. J. Youden and E. H. Steiner, *Statistical Manual of the AOAC*, Association of Official Analytical Chemists, Washington, DC, 1975.

5 *I.S.O. International Standard 5725, Precision of Test Methods —Determination of Repeatability and Reproducibility*, January 1978.

6 A. Hobson-Frohock and W. T. E. Edwards, *J. Chromatogr.*, 249 (1982) 369–372.

7 R. E. Majors, *J. Chromatogr. Sci.*, 18 (1980) 488.