THE QUANTITATIVE ESTIMATION OF DIGITALIS GLYCOSIDES BY MEANS OF KELLER-KILIANI AND PESEZ-DEQUEKER REAGENTS

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Previous work in this department[1] has established that the Pesez reaction[2,3,4] for the detection and estimation of gitoxin in commercial digitoxin may be employed for the quantitative estimation of digitoxose and glycosides containing digitoxose. The yellow colour produced has a maximum light-absorption at 4740 Å (Fig. 1) and we have shown that the reaction obeyed the Lambert–Beer law over the concentration range 2 to 4 μg./ml. of digitoxose (Fig. 2). The reaction is given by 2 only of the 3 molecules of digitoxose present in each of the primary glycosides

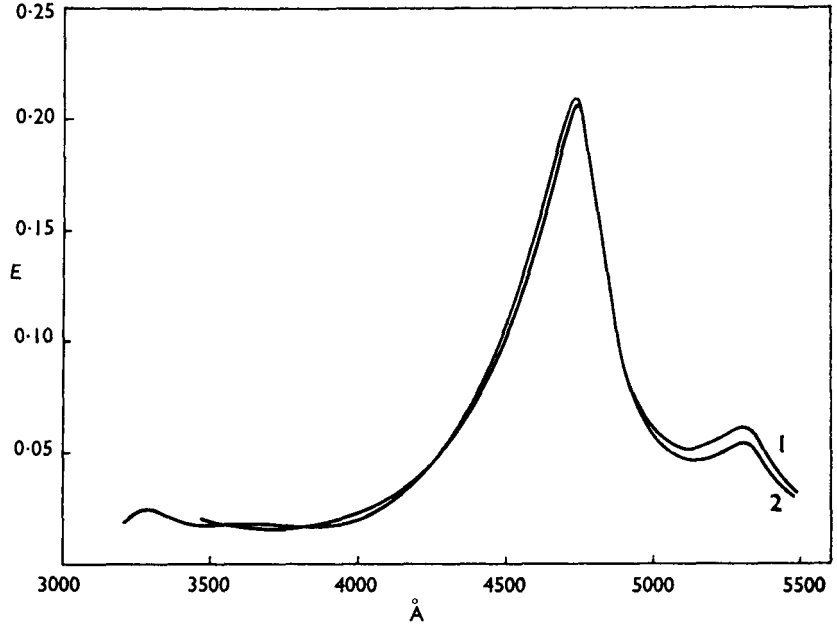

Fig. 1. Absorption spectra of digitoxose in the Pesez-Dequeker reaction. 1. 10·4 μg./ml. cell length 10 mm. 2. 20·8 μg./ml. cell length 5 mm.

desacetyldigilanids A and B and digilanids A, B and C. The sugars cymarose and sarmentose give the same absorption curve as digitoxose with the reagent; thus the glycoside cymarin may be estimated by this reaction.

The method of carrying out the Pesez–Dequeker reaction is as follows. Dissolve 10 to 20μg. of digitoxose or an equivalent quantity of glycoside in 1 ml. of dry acetone in a 12-mm. diameter hard glass test tube, add

phosphoric acid (density 1·70) to produce 5 ml., mix with a glass rod and immerse in a water bath at 35° C. for 15 minutes. Gitoxin or the dry residue derived from decolourised tincture of digitalis are incompletely soluble in 1 ml. of acetone and it is sufficient to suspend these materials in the acetone, a clear solution resulting upon the addition of phosphoric acid. Cool the mixture to 20° C. and determine the light absorption at 4740 Å in a 1-cm. cell by means of a spectrophotometer, using as a blank a mixture of acetone and phosphoric acid treated in the same manner as the assay process. This present work has been carried out on a Unicam S500 photoelectric absorptiometer.

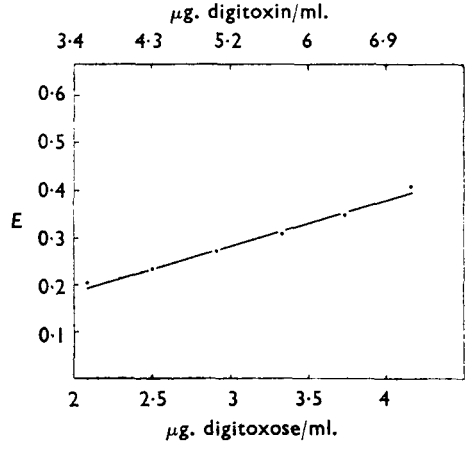

FIG. 2. Density concentration ratio of digitoxose/digitoxin in the Pesez-Dequeker reaction at 4740 Å.

Our previous publication[1] has shown comparative results for the estimation of digitoxose-containing glycosides present in tincture of digitalis by means of the Pesez–Dequeker method and by the Keller–Kiliani method as described by Lindewald and Soos[5,6]. The purpose of this present paper is to compare the sensitivity and the accuracy of the Pesez–Dequeker method and the Keller–Kiliani method as described by Rowson[7] when applied to pure digitalis glycosides and to mixtures of such glycosides with known digitoxose contents.

EXPERIMENTAL

The Pesez–Dequeker reagent and the Keller–Kiliani reagent (Rowson[7]) were initially standardised against a sample of pure digitoxin (Roche), shown to be free from gitoxin by the methods of Pesez[2,3] and Tattje[8]. Aliquot portions of a standard solution of the glycoside containing 0·140 g./l. were employed and the density readings for both reagents are recorded in Table I. The straight-line graph of colour density plotted against digitoxin concentration is shown in Figure 3.

TABLE I

COLOUR DENSITY OF DIGITOXIN WITH KELLER–KILIANI AND PESEZ–DEQUEKER REAGENTS

Method	Digitoxin used (μg.)	Digitoxose equivalent (μg.) $f = 0{\cdot}58108$	Density readings
Keller–Kiliani	350	203	0·325
	420	244	0·392
	490	285	0·465
Pesez–Dequeker	28	16·3	0·222
	35	20·3	0·284
	42	24·4	0·348

TABLE II

THE STANDARD SOLUTIONS USED IN THE COMPARATIVE ASSAYS

Test Substances	Concentration (g. l.)	Solvent
Digitoxin	0·140	Ethanol (94 per cent.)
Digitoxigenin (Roche)	0·030	Ethanol (94 per cent.)
Desacetyldigilanid A (Sandoz)	0·070	Ethanol ,,
Gitoxin (Roche)	0·070	Ethanol ,, — chloroform in equal volume
Gitoxigenin (Roche)	0·015	Ethanol (94 per cent.)
Desacetyldigilanid B (Sandoz)	0·035	Ethanol ,,
Digitalinum verum (Roche)	0·070	Ethanol ,,

Standard solutions of 6 other digitalis glycosides or genins were used for the further comparison of both methods of estimation and they are indicated in Table II. The genins and a digitoxose-free glycoside (digitalinum verum) were selected since they are probably normal constituents of digitalis leaf and, although they give no reaction with either reagents under investigation, it was thought desirable to determine if their presence influences the behaviour of digitoxose-containing glycosides with the reagents. By taking appropriate volumes of one or more of the standard solutions, the individual glycosides or their mixtures were subjected to estimation by the Keller–Kiliani or the Pesez–Dequeker reagents; the solution being evaporated and the residue dissolved in 10 ml. of Keller–Kiliani reagent or in 1 ml. of acetone for the Pesez–Dequeker reaction. Only the pure gitoxin was suspended in the acetone and phosphoric acid added to the suspension.

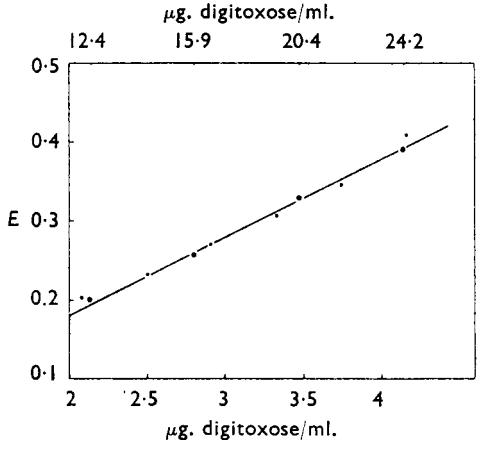

FIG. 3. Curve of density-digitoxin concentration, expressed as digitoxose in the Keller-Kiliani (Rowson) (•) and Pesez-Dequeker reaction (•).

Table III gives the results of the comparative assays using both reagents for the estimation of the individual glycosides containing digitoxose and of their mixtures. It will be seen that the quantitative estimations are closely comparable when using the 2 different reagents; the Pesez–Dequeker method is more sensitive and it is easier to carry out. The presence of genins or of digitalinum verum does not exert any influence upon the results.

CONCLUSIONS AND SUMMARY

1. The Pesez–Dequeker reaction and the Keller–Kiliani reagent of Rowson have been used to estimate desacetyldigilanid A, digitoxin,

TABLE III

COMPARATIVE RESULTS OF THE KELLER–KILIANI AND PESEZ–DEQUEKER TEST ON DIGITOXOSE GLYCOSIDES AND MIXTURES

Concentrations and volumes of the test substances (see Table II)		Digitoxose				
			Keller–Kiliani		Pesez–Dequeker	
Test substances	Standard solution (ml.) see Table II	Content (μg.)	found (μg.)	deviation (per cent.)	found (μg.)	deviation (per cent.)
Gitoxin	2·00	80·0	81	+1·25		
	2·50	100·0	100	0·0		
	3·00	120·0	120	0·0		
	0·40	15·9			16·2	+1·9
	0·50	19·9			20·2	+1·5
	0·60	23·9			24·0	+0·4
Desacetyldigilanid A	7·00	157·0	172	+8·7		
	8·00	179·0	196	+8·7		
	9·00	201·0	223	+11·0		
	0·60	13·4			14·0	+4·0
	0·70	15·7			16·5	+4·8
	0·80	17·9			19·0	+5·7
Desacetyldigilanid B	10·00	110·0	113	+2·7		
	13·00	143·0	147	+2·8		
	16·00	176·0	181	+2·8		
	1·20	13·2			13·2	0·0
	1·40	15·4			15·1	−2·0
	1·60	17·6			17·3	−1·7
Mixture A:—digitoxin + digitoxigenin	2·50*	203·0	201	−1·0		
	3·00*	244·0	241	−1·2		
	3·50*	285·0	280	−1·7		
	0·20*	16·3			15·8	−3·0
	0·25*	20·3			20·3	0·0
	0·30*	24·4			23·8	−2·5
Mixture B:—as mixture A + desacetyldigilanid A	1·70*	176·0	181	+2·8		
	2·00*	207·0	209	+1·0		
	2·30*	238·0	238	0·0		
	0·15*	16·0			17·1	+6·8
	0·20*	21·0			22·1	+5·2
	0·25*	25·6			27·5	+7·4
Mixture C:—as mixture B + gitoxin	1·30*	186·0	187	+0·5		
	1·50*	215·0	218	+1·5		
	1·70*	244·0	246	+0·8		
	0·10*	14·4			14·5	+0·7
	0·15*	21·5			21·9	+1·9
	0·20*	28·7			26·2	−8·7
Mixture D:—as mixture C + gitoxigenin	1·30*	186·0	187	+0·5		
	1·50*	215·0	218	+1·5		
	1·70*	244·0	246	+0·8		
	0·10*	14·4			14·5	+0·7
	0·15*	21·5			21·1	−1·9
	0·20*	28·7			28·8	+0·3
Mixture E:—as mixture D + desacetyldigilanid B	1·10*	170·0	174	+2·4		
	1·30*	200·0	207	+3·5		
	1·50*	231·0	240	+3·5		
	0·10*	15·4			15·7	+2·0
	0·15*	23·2			23·3	+0·5
	0·20*	30·9			30·8	−0·3
Mixture F:—as mixture E + digitalinum verum	1·10*	170·0	177	+4·0		
	1·30*	200·0	208	+4·0		
	1·50*	231·0	242	+4·7		
	0·10*	15·4			16·3	+7·0
	0·15*	23·2			23·6	+1·7
	0·20*	30·9			31·1	+0·6
Number of assays = 27						
Average deviation, per cent.				2·72		2·71
Standard deviation				3·93		3·76

* ml. standard solution of each of the test substances.

desacetyldigilanid B and gitoxin as individual glycosides or in mixtures, also when in association with digitoxigenin, gitoxigenin and digitalinum verum.

2. Both reactions give the same accuracy of results in each estimation.

3. The presence of the genins and of digitalinum verum does not influence the results.

4. The Pesez–Dequeker reaction is the more sensitive and is more convenient to employ; it is thus recommended as the method of choice for the estimation of digitoxose-containing glycosides.

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