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Letters to the Editor

The influence of spectral slit width on the absorption of visible or ultra-violet light by Pharmacopoeial substances

SIR,—For accurate results in spectrophotometric assays, the spectral slit width of the spectrophotometer must be small in comparison with the half width of the absorption band, and for a number of substances official in the B.P. 1958 particular care is needed to avoid spuriously low results (Rogers, 1959). Andersen (1964) has measured the slit-width effect at wavelengths of maximum

TABLE 1. EFFECT OF CHANGE OF SPECTRAL SLIT WIDTH ON THE SPECTROPHOTOMETRIC DETERMINATION OF EXTINCTION

Substance	λmax (mμ)	Max *h* (mμ) for extinction error of < 0·2 %	1 %	2
Acetazolamide	265	1·9		
Apomorphine hydrochloride	273	0·8	1·6	
Benztropine methanesulphonate	258	0·5	0·8	1·1
Carbimazole	291	4·0		
Chloramphenicol	278	2·3		
Chlorothiazide	292	1·1	1·6	2·3
Chlorpheniramine	262*	0·5	0·9	1·3
	265†	1·1	1·6	
Chlorpromazine hydrochloride	254	1·2		
Colchicine	350	1·7	4·0	
Cyanocobalamin	361	1·5	1·8	2·3
Cyclomethycaine sulphate	261	1·4		
Cycloserine	219	0·8		
Deoxycortone trimethylacetate	240	1·3		
Dichlorphenamide	285	0·7	1·1	1·4
Dimethisterone	240	1·3		
Diphenhydramine hydrochloride	258	0·5	0·9	1·1
Ethinyloestradiol	280	1·1	1·8	2·4
Fluoxymesterone	240	1·3		
Griseofulvin	291	0·9	1·6	2·3
Hydrochlorothiazide	273·5	1·5		
Hydrocortisone esters	240	1·4		
Hydroxocobalamin	351	1·3	1·7	2·2
Levorphanol tartrate	279	0·7	1·5	2·1
Mepyramine maleate	316	0·9	2·2	
Methandienone	245	1·4		
Methyltestosterone	240	1·3		
Nandrolone phenylpropionate	240	1·3		
Nitrofurantoin	367	1·7		
Norethandrolone	240	1·3		
Norethisterone	240	1·3		
Oxytetracycline dihydrate and hydrochloride	353	3·0		
Paracetamol	249	1·5		
Perphenazine	254	1·0		
Phenindione	278	0·9	2·3	
Phenoxybenzamine hydrochloride	272	0·8	1·2	2·0
Phenoxymethylpenicillin and salts	268	0·7	0·9	1·1
Phytomenadione	249	0·7	0·9	1·1
Probenecid	248	1·5		
Prochlorperazine salts	258	1·2		
Progesterone	240	1·2		
Promazine hydrochloride	251	1·3		
Promethazine hydrochloride	249	1·2		
Pyridostigmine bromide	269·5	0·9		
Riboflavine	444	0·9		
Sodium anoxynaphthonate	570	1·0		
Testosterone and esters	240	1·2		
Thioridazine hydrochloride	310	1·4	2·5	
Trifluoperazine hydrochloride	256	1·1	1·7	
Tripelennamine hydrochloride	245	1·3		
Tubocurarine chloride	280	1·1	1·7	2·3
Warfarin sodium	308	1·0	2·1	

* Solvent water.
† Solvent 0·5 N sulphuric acid.
Solutions were prepared as directed by the B.P. 1963.

and minimum ultra-violet absorption of potassium phenoxymethylpenicillin, prednisolone and yohimbine hydrochloride, and confirmed that the effect is greater, the narrower the absorption maximum.

The earlier survey has now been extended to those substances that are subject to spectrophotometric assay in the B.P. 1963. The same experimental procedure has been used as before (Rogers, 1959), except that the spectrophotometers on this occasion were a Hilger and Watts Uvispek H.700 Mark VII and a Unicam SP.500. Table 1 lists the drugs examined and shows in the third column the widest half-intensity spectral slit width h that may safely be used. For convenience, substances that are subject to spectrophotometric assay in both the 1958 and 1963 editions of the B.P. have been included in the Table, as well as the newer substances, and the opportunity has been taken to give revised, more accurate values for one or two drugs.

Examination of the Table shows that the greatest care should be taken with those substances that show the vibrational structure of the benzenoid absorption near 255 mμ, namely apomorphine hydrochloride, benztropine methanesulphonate, chlorpheniramine maleate, dichlorphenamide, diphenhydramine hydrochloride, levorphanol tartrate, phenindione, phenoxybenzamine hydrochloride, phenoxymethylpenicillin and its salts, and phytomenadione.

The chlorpheniramine assays show a feature of some interest. With the tablets, the final solution is acid; there is little vibrational structure in the spectrum, and the slits can be opened quite widely before low results will be obtained. With the injection, however, the solution is near neutrality, and because the vibrational structure is marked in the spectrum, the slit-width setting of the spectrophotometer is critical. It would seem desirable to use an acid solvent for this assay also.

The avoidance of slit-width errors in assays may be secured (*a*) by specification of a maximum permitted half-intensity spectral slit width h (Rogers, 1959), (*b*) by requiring that "the instrumental slit width used should always be such that a further reduction does not result in an increased extinction reading," as in Appendix IV.H of the B.P. 1963, or (*c*) by adoption of a procedure in which the extinction of the sample is compared with that of a reference substance under the same conditions (Anderson, 1964). A combination of (*b*) and (*c*) would seem to be the most reliable.

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