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The Assay of Alkaloidal Salts

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IN the control of the purity of alkaloidal salts and similar compounds the question arises whether it is necessary to estimate the physiologically active base, or if it is sufficient to determine the other part of the compound molecule. In general, the estimation of the active base gives the best control of purity, but the methods are often laborious and troublesome. It is often difficult to find a good method for the estimation of the active substance, whereas a simple and quick method for the estimation of the other part of the molecule is readily available.

The British Pharmacopœia, 1948, selects the estimation of the active base, and it must be said that this standpoint is scientifically unassailable. For many substances the Pharmacopœia describes assays on this principle, including Amethocainæ Hydrochloridum, Amphetaminæ Sulphas, Cinchocainæ Hydrochloridum, Codeinæ Phosphas, Emetinæ Hydrochloridum, Homatropinæ Hydrochloridum, Mepacrinæ Hydrochloridum, Mepacrinæ Methanosulphonas, Morphinæ Hydrochloridum, Morphinæ Sulphas, Quinidinæ Sulphas, Quininæ Bisulphas, Quininæ Dihydrochloridum Quininæ Hydrochloridum, Quininæ et Æthylis Carbonas, Quininæ Sulphas and Strychninæ Hydrochloridum. The principle of estimation of the active part of the molecule, however, is not introduced in the monographs on Apomorphinæ Hydrochloridum, Atropinæ Sulphas, Butacainæ Sulphas, Cocainæ Hydrochloridum, Diamorphinæ Hydrochloridum, Ephedrinæ Hydrochloridum, Hyoscinæ Hydrobromidum, Papaverinæ Hydrochloridum, Physostigminæ Salicylas, Pilocarpinæ Nitras and Procainæ Hydrochloridum. Possibly the assay is not introduced in these monographs, because some of the alkaloidal salts have a sharp melting-point or a sufficiently narrow melting-range to guarantee the purity of the drug.

Four methods for the determination of the purity of such compounds may be discussed.

1. *The Method of the British Pharmacopœia,* 1948. In this the base is liberated by alkali from a solution of the salt and extracted by shaking several times with a suitable solvent, the solvent is evaporated, and the residue is dried and weighed or titrated. Such assays involve much work, and cannot be indicated as simple pharmacopœia methods.

2. *Chromatographic determination.* This method was described by Reimers, Gottlieb and Christensen.[1]. I have used it for the salts of cinchocaine, cocaine, emetine, diethylmorphine physostigmine, pilocarpine, procaine, scopolamine and tetracaine, and find that it gives very good results. If the apparatus is kept ready for use and the worker has some experience, the chromatographic method requires less time and material than the extraction of the active base by a solvent. It is a condition for good results, that the aluminium oxide must be completely free from alkali and must give a good adsorption test.

3. *Titration of the acid.* This gives the correct percentage of the alkaloid

if it is correctly neutralised by its equivalent quantity of acid, and it is possible, by determining the acid part of the molecule, to get good control of the purity. The method is simply and quickly performed by titrating the alkaloidal salt solution with 0·1N sodium hydroxide, using phenolphthalein as indicator. If the alkaloid precipitates on adding sodium hydroxide solution, alcohol or chloroform is added to dissolve it. The method is used in the Netherlands Pharmacopœia for all the alkaloidal salts. If the alkaloid is not correctly neutralised in the salt, the titration will show a high percentage of alkaloid if there is too much acid, and a low percentage if there is too much base. It is possible to show that the alkaloidal salt has the correct composition by determination of the *p*H of an 0·1N solution. This can be calculated from the dissociation exponent of the base or estimated colorimetrically or electrometrically. Kolthoff[2] and Schoorl[3] have recorded the *p*H values of several alkaloidal salt solutions. Table I gives figures, determined in my laboratory, for the *p*H values of alkaloidal solutions of various concentrations.

TABLE I

	0·1 N	0·01 N	1 per cent.
	*p*H	*p*H	*p*H
Cinchocainæ Hydrochloridum	4·75	—	—
Pilocarpinæ Hydrochloridum	4·58	4·73	—
Procaine Hydrochloridum	5·72	—	5·82
Ethylmorphinæ Hydrochloridum	4·58	—	5·10
Emetinæ Hydrochloridum	5·23	—	5·25
Hyoscinæ Hydrochloridum	—	—	4·93
Homatropinæ Hydrochloridum	5·79	—	—
Codeinæ Phosphas	4·24	—	—
Dihydrocodeinonæ Bitartras	3·32	—	—
Dihydro-oxycodeinonæ Hydrochloridum	—	6·12	—
Acetyldimethyldihydrothebainæ Hydrochloridum	—	5·85	—

The following experiment proves that the determination of the *p*H of an alkaloidal salt solution is a very good method for the control of purity and correct composition.

To a sample of atropine sulphate a small quantity of acid or of alkaloidal base was added to ascertain if the *p*H change of the solution was practically measurable. Both substances caused a considerable change in the *p*H of the solution. Figures are given in Tables II and III.

TABLE II

THE EFFECT ON *p*H OF THE ADDITION OF ACID TO A SOLUTION OF ATROPINE SULPHATE

Concentration	*p*H	*p*H after addition of 0·05 ml. of N/1 acid.	Difference	*p*H after addition of 0·1 ml. of N/1 acid.	Difference
712 mg. of atropine sulphate in 20 ml. of water	5·10	4·20	−0·90	3·78	−1·32
	5·09	4·18	−0·91	3·76	−1·33
	5·10	4·18	−0·92	3·76	−1·35

The conclusion is that the *p*H determination is a very good test for the correct neutralisation of the base by the acid.

4. *Double Titration.* This method[4] consists of a titration of the acid and basic parts of the molecule. A milli-equivalent of the alkaloidal salt is dissolved in 5 ml. of water, this solution is mixed with 15 ml. of alcohol (96 per cent.) and titrated with 0·1 N sodium hydroxide with phenolphthalein

as indicator. Theoretically 10 ml. should be required. Then bromothymol blue is added and the liberated base is titrated by 0·1 N acid, of which also 10 ml. should be required. In the first titration, the first pink colour gives the end of the titration. Strong bases (atropine, emetine, etc.) which are

TABLE III

THE EFFECT ON *p*H OF THE ADDITION OF ATROPINE BASE TO A SOLUTION OF ATROPINE SULPHATE

Concentration	*p*H	*p*H after addition of 10 mg. of base	Difference
712 mg. atropine sulphate in 20 ml. of water	4·90	7·90	+3·00
	4·91	7·94	+3·03
	4·93	7·94	+3·03

alkaline to phenolphthalein must be extracted by means of an organic solvent such as chloroform. For the second titration, the alkaloidal base must be dissolved in as little alcohol as possible. If the alkaloidal base is extracted and dissolved in chloroform, it is possible to : (a) add an excess of acid and extract the base in the aqueous layer, after which the free acid is titrated with sodium hydroxide: (b) evaporate the solution and dry the alkaloid (if not volatile) dissolve it in an excess of 0·1N acid and titrate with 0·1N sodium hydroxide. The double titration can also be done with microburettes, and only a small quantity of material is then required. The method gives good control of the correct neutralisation of the alkaloid by the acid and also of the correct composition of the drug and directly the percentage of the active principle.

The conclusions of my experience are: 1. The direct determination of the active principle is scientifically unassailable but often requires complicated assays; 2. The direct determination by extraction with a solvent can be replaced by a chromatographic determination if a pure aluminium oxide is available; 3. The active principle can be determined indirectly by a simple titration of the acid part of the molecule and the determination of the *p*H of a dilute solution of the alkaloidal salt; 4. The same result can be obtained by the double titration of the acid part and alkaloid.

Methods (3) and (4) can be recommended for the control of purity and correct composition for all alkaloidal salts mentioned in the Pharmacopœia where there is no assay prescribed.

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3. Schoorl, *ibid.*, 1939, **76,** 1496.
4. Schoorl, Dijkstra and Donkers, *ibid.*, 1941, **78,** 4.