

the absence of chlorides in a number of residues tested after titration, although if the chloroform solution is allowed to stand for some time and then evaporated spontaneously, chlorides will be found in the residue. At a temperature of 100° C., the alkaloid is almost completely volatile; 20 cc. of a chloroform solution containing 0.87 Gm. alkaloid by volumetric estimation, after evaporating and drying for one hour at 100° C. weighed 0.0365 Gm., and after 5 hours 0.0045 Gm., and when titrated was found to equal only 0.1 cc. *N*/50 acid = 0.00033 Gm. ephedrine.

The use of ether for the extraction of the drug gives lower results than when ether-chloroform mixture (3-1) is used, and the final extraction of the aqueous solution with ether is much slower than with chloroform, although complete extraction may be obtained with seven or eight 25-cc. portions. The maceration of the drug in the ether-chloroform and ammonia mixture for two hours gives practically the same results as macerating for a longer period (12-48 hrs.). No trouble whatever with emulsions has been experienced.

To overcome the loss through volatility of the alkaloid the following modification of the U. S. P. X method for belladonna is suggested:

Use sodium hydroxide solution instead of ammonia in the extraction of the drug, ether and sodium hydroxide solution in extraction of the aqueous solution and add the volumetric acid solution to the ether solution before evaporating. Owing to the solubility of ephedrine in water it is not advisable to wash the ether solution, so care must be taken that none of the alkaline solution passes with the ether into the flask containing the volumetric acid; but repeated trials have shown that by allowing a thorough separation of the ether and then passing it through absorbent cotton well wet with ether this can be entirely avoided.

A number of other assays using various modifications were made and the results of these and of other work now under way will, it is hoped, appear in a later report.

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EPHEDRINE ASSAYS BY TITRATION.

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The standards of the American Medical Association for ephedrine salts, include a gravimetric assay for the alkaloidal content. The only other published information about the assay of ephedrine applies to its determination in the crude drug. The U. S. P. IX assay for Belladonna Root was used in these instances.¹

More than twelve hours are required for drying the base for the gravimetric determination by the A. M. A. method. It continues to lose weight for a time after that. A white sublimate either the alkaloid itself or a decomposition product continues to settle on the insides of desiccators in which it is dried. It cannot be heated or warmed without loss after the evaporation of the solvents with which it is extracted. It melts at about 40° C.

¹ JOUR. A. PH. A., 15 (1926), 9, 748; also 15 (1926), 12, 1070.

Since the base is titratable like other alkaloids, investigation of the following points was undertaken to apply a dependable rapid procedure for its estimation by titration:

1. Choice of solvents and technic of extraction manipulations.
2. Avoiding decomposition or loss by volatilization during evaporation of solvent.
3. Possibility of error in the titration due to ammonia in the solvent.

Since the A. M. A. laboratory has shown that the hydrochloride is formed when ephedrine alkaloid is treated with chloroform it is necessary to use ether or some other solvent. Losses due either to decomposition or volatilization, occurring when solutions of the extracted ephedrine are evaporated to dryness (shown in Column 1, Table II) necessitates treating the solution of the drug with excess of standard acid before all the solvent is evaporated off. The practicability of this was suggested by the work of Palkin and Watkins¹ on the stability of atropine and the practice proved to be equally as dependable as they found it to be, using other solvents on other products. A number of determinations on known quantities of ephedrine base were conducted to determine if the accuracy of the determination was affected by the possible presence of ammonia dissolved in or carried over in the water with the ether. These results are shown in Table I.

TABLE I.
ELIMINATION OF AMMONIA IN ETHER EXTRACT.

Expt. no.	Ephedrine taken. Mg.	10% NH ₄ OH used. Cc.	Treatment.	N/50 acid consumed by solvent.	
				Cc.	Mg. Per cent.
1	None	7	Fractional Extraction with about 160 cc. of ether. Evaporated to about 10 cc. Then added the standard acid.	None	
4	None	7	Fractional Extraction with about 160 cc. of ether. Evaporated to about 10 cc. Then added the standard acid.	0.04	
5	None	7	Fractional Extraction with about 160 cc. of ether. Evaporated to about 10 cc. Then added the standard acid.	0.04	
7	None	7	Fractional Extraction with about 160 cc. of ether. Evaporated to about 10 cc. Then added the standard acid.	0.14	
8	None	7	Fractional Extraction with about 160 cc. of ether. Evaporated to about 10 cc. Then added the standard acid.	0.16	
3	None	None	Blank on about 160 cc. of ether. Evaporated to about 10 cc. Standard acid then added.	0.16	
6	None	None	Blank on about 160 cc. of ether. Evaporated to about 10 cc. Standard acid then added.	0.04	
9	None	None	Blank on about 160 cc. of ether. Evaporated to about 10 cc. Standard acid then added.	0.04	

¹ JOUR. A. PH. A., 16 (1927), 1.

2	None	None	Blank on about 160 cc. of ether. Evaporated to about 10 cc. Standard acid then added.	0.13		
11	0.1072	7	Fractional extraction with about 160 cc. of ether. Alkaloid added to the combined ether but not extracted by the ether. Evaporated to about 10 cc. Then added standard acid in excess.	32.48	0.1073	100.1
12	0.1156	7	Fractional extraction with about 160 cc. of ether. Alkaloid added to the combined ether but not extracted by the ether. Evaporated to about 10 cc. Then added standard acid in excess.	34.87	0.1152	99.7
22	0.1074	7	Fractional extraction with about 160 cc. of ether. Alkaloid added to the combined ether but not extracted by the ether. Evaporated to about 10 cc. Then added standard acid in excess.	32.33	0.1068	99.6
21	0.0989	7	Fractional extraction with about 160 cc. of ether. Alkaloid added to the combined ether but not extracted by the ether. Evaporated to about 10 cc. Then added standard acid in excess.	29.72	0.0982	99.3
23	0.1037	7	Fractional extraction with about 160 cc. of ether. Alkaloid added to the combined ether but not extracted by the ether. Evaporated to about 10 cc. Then added standard acid in excess.	31.46	0.1039	100.2

COMMENTS ON TABLE I.

1. The apparent consumption of acid by the ether in Experiments 1 to 8 inclusive is believed to be due to an effect of traces of unexpelled ether on the end-point and not to actual consumption of acid.

2. The quantity of *N*/50 acid consumed by the ether is the same, whether the ether is shaken with ammonia or used untreated.

3. This apparent consumption of acid is equivalent to an average of 0.1 cc. which would not cause an error greater than 0.2% in the result.

4. Results of titrating ephedrine in presence of ether which has been treated with ammonia, shown by Experiments 11, 12, 21, 22 and 23, show that the titration can be performed by adding the standard acid to the last 10 cc. of ether solution of the ephedrine with a limit of accuracy of 1%.

5. No effect of ammonia on the titration into ether treated with ammonia and evaporated to about 10 cc. could be detected.

METHOD.

Dissolve about 0.2 Gm. of the ephedrine salt accurately weighed, in 10 cc. of water in a separatory funnel and add 5 cc. of ammonia water. Shake the mixture vigorously with ether using 30 cc., 25 cc., 20 cc., 15 cc., 15 cc., 15 cc., respectively, or more if necessary until extraction is complete. The aqueous layer should be separated completely by rotating the funnel after withdrawing the main bulk of this layer and allowing the funnel to stand, then withdrawing the aqueous layer again before adding the next portion of ether. Combine the ether extractions in a second separatory funnel and allow to stand until any aqueous solution has settled. This

is withdrawn, if present, and combined with that in the first funnel for testing for complete extraction. Transfer the ether solution to a 250-cc. beaker, set on a steam-bath before a fan and evaporate following the usual precaution for ether, to avoid mechanical loss by heating too hot. When the volume is reduced to about 10 cc. add an excess of 0.05 *N* sulphuric acid and 0.5 cc. of brom-thymol blue indicator. Warm gently on a steam-bath while evaporating the balance of the ether layer by aid of a fan. Add 5 cc. of neutral alcohol. Cover the beaker with a watch glass, heat the contents sufficient to cause the alcohol to reflux the sides of the beaker. Titrate the excess acid with 0.02 *N* alkali. One cc. of 0.05 *N* acid equals 8.26 mg. of ephedrine base or 10.8 mg. of ephedrine hydrochlorides or 10.71 mg. of ephedrine sulphate.

TABLE II.
RESULTS OF EPHEDRINE DETERMINATION IN EPHEDRINE HYDROCHLORIDE.

Sample.	Council of A. M. A. requirements. Per cent.	By Council gravimetric method. Per cent.	By titration of portion weighed in Column 3. Per cent.	By the proposed titration method on same samples as used in Column 3. Per cent.
A	80-82.5 ¹	81.5	64.5	80.3
B		81.6	75.9	81.2
C		81.7	78.4	80.8
		81.0	77.7	80.9
D		81.1	75.8	81.5
		82.4	78.1	80.8
		81.6	69.0	81.8
		80.9	72.6	81.6
		80.0	74.5	

¹ Theoretical 81.9%.

COMMENTS ON TABLE II.

The results in the fourth column of the above tabulation showing the per cent of base by titration of the portion used for the gravimetric determination are all in cases lower than the minimum per cent of base found by the Council on Pharmacy gravimetric method or the proposed titration method. This has not been accounted for to date.

OTHER APPLICATIONS OF THE METHOD.

The alkaloid may be extracted from hypodermic tablets of ephedrine salts and titrated by this method without modification and from syrup of ephedrine salts by varying the quantity of solvent as required.

Ephedrine Inhalent may be titrated by adding to an accurately weighed quantity of the oil solution of the alkaloid about 30 cc. of petroleum ether, 25 cc. of water, an excess of standard acid and 0.5 cc. of brom-thymol blue indicator. The mixture is shaken thoroughly until all the alkaloid has been extracted into and neutralized by the acid solution and the excess acid titrated in the usual manner.

CONCLUSIONS.

1. When ammonia solution, equivalent in volume and strength to that used for the assay of ephedrine, is extracted fractionally by ether, as directed in the method submitted, the latter evaporated to about 10 cc. and the alkalinity of the residue titrated, the titration shows no effect of ammonia in the residue.

2. A method for the extraction and titration of ephedrine in its salts has been developed, by refining well-known procedures and showing that ammonia if carried over in the solvent has no effect on the titrations by this method.

3. The limits of accuracy of the method are within one per cent, which is considered acceptable for alkaloidal extraction assays.

4. It can be extracted from its salts and titrated by the method submitted herewith in a period of one hour.

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CARE OF ANIMALS FOR BIOLOGIC ASSAYS.

(Continued from p. 359, April JOUR. A. PH. A.)

RABBITS.

The rabbit is indigenous to virtually every part of Europe and America. Australia has also a species of rabbit somewhat akin in type and habits to the American "Jack."

The rabbit is a different species from that of the hare. The rabbit is of the species *lepus cuniculus* while the hare is of the species *Lepus timidus*. Both belong to the family *rodentiae*—a creature with long rat-like front gnawing teeth.

In its gregarious habits the rabbit also differs widely from those of the hare, and in the wild state is said to be monogamous. This latter trait, however, ceases with domestication, and either sex becomes altogether polygamous. It also differs from the hare in that its young are born immature, with eyes closed and the body nude of hair, in a nest lined with fur pulled from the mother, burrowed in the ground whenever possible; while those of the former (hare) are born with eyes open, and body nicely covered, in a "form" on top of the ground.¹

Varieties.—There are many varieties of domestic rabbits the more common of which are the American, Dutch, Lop, New Zealand, Polish, English, Angora, Spotted, Flemish Giant, Belgian Hare, the Tan in Black, the Silver in Brown, Fawn, Grey and Blue, the Havana, Imperial and Sells.

Housing.—The cages shown in Figs. 3, 5 and 7 are very satisfactory for housing rabbits. The bottom of the trays should be covered with sawdust on top of which is placed three or four inches of hay. If the rabbits are to be kept outdoors wooden hutches are preferable as they are warmer in winter. The character and size of the hutches depends upon the number of rabbits to be housed. Any style or shape box is apparently satisfactory. Five sides of the hutch should be constructed of wood. The sixth side should contain the door made of a wooden or metal frame covered with wire screen. For breeding purposes one end of the hutch should contain a nesting compartment, separated from the rest of the hutch by a wooden partition containing a hole sufficiently large to permit the rabbits to pass from one compartment to the other. Each compartment should contain a removable metal tray to facilitate cleaning. It is not necessary to heat outdoor hutches as rabbits can stand cold weather. The hutches should, however, be protected from drafts and storms in winter and excessive heat and

¹ Deardorff, Rabbit Culture and Standard.