# OVERCOMING INTERFERENCE FROM VOLATILE BASES IN ASSAY OF MYDRIATIC DRUGS.

BY GEORGE E. ÉWE.

Effectual means of eliminating these volatile bases and so allowing a truer estimate of the actual alkaloidal content are described.

Mydriatic drugs may contain, in addition to alkaloids, various other bases. For instance, belladonna contains volatile bases of alkaloidal and of non-alkaloidal nature,[1] which can be divided into 3 groups: pyridines, pyrrols and diamines. In the first group pyridine has already been characterized, while in the second group have been found methylpyrrolline and methylpyrrollidine. The third group contains at least one diamine.

As ordinarily segregated from vegetable drugs and their preparations mydriatic alkaloidal residues may contain large proportions of these bases. Reference to the table following will show that these residues may contain sufficient volatile bases to increase the apparent alkaloidal content by as much as 77 per cent. The volatile base content of various lots of hyoscyamus increased the apparent alkaloidal content of the drug to the extent of 66, 77, 72 and 12 per cent, respectively; while that of a lot of belladonna leaves increased the apparent alkaloidal content to the extent of 8 per cent, and the volatile base content of a lot of stramonium leaves increased the apparent alkaloidal content of the leaves to the extent of 25 per cent.

The identity of these volatile bases was not determined.

Since certain of these bases exhibit acid-neutralizing power with the indicators used in titrating the alkaloids it is a matter of importance to seek a method of freeing the alkaloidal residue of these interfering bases so as to secure a more accurate estimation of the alkaloids themselves.

The concensus of opinion is that these interfering bases are generally quite volatile. Consequently, a method of volatilization which would rid the alkaloidal residue of these bases and leave the typical alkaloids unaffected would give a more accurate determination of the alkaloids.

In selecting a method of volatilizing these interfering bases recognition must be given to the susceptibility of mydriatic alkaloids to hydrolysis and overheating. The volatile bases are eliminated by means of heat, aeration or spontaneous volatilization. The use of heat is objectionable as overheating at water-bath temperatures as well as prolonged heating in the air at lower temperatures may cause destruction of a part of the alkaloids. A few experiments indicate that a 5-minute heating of a flask containing mydriatic alkaloidal residue on a covered water-bath appears to effect elimination of the volatile bases without material effect upon the alkaloids but such a heat treatment is not to be depended upon to yield comparable results in different hands. Aeration with ordinary atmospheric air is objectionable since the moisture in such air may hydrolyze the alkaloids to some extent. Aeration with dried air is inefficient in removing the volatile bases unless quite prolonged as the bases come off quite slowly, possibly because of the viscous condition often assumed by alkaloidal residues from vegetable drugs and their preparations. Aeration at not above 40° C. with dried air to apparent dryness followed by calcium

[1] *J. pharm. Belg.*, through *Pharm. Era*, 54 (1921), 274.

chloride desiccator treatment to constant weight (usually within 24 hours) appears to be a safe and efficient method of elimination of the volatile bases without danger of concurrent hydrolyzation of the alkaloids. The following procedure has been found to be practical and to give concordant results:—evaporate the chloroformic extracts to 2 cc. in an Erlenmeyer flask, then to apparent dryness at not above 40° C. by means of a current of air (dried by passing through a long column of granular anhydrous calcium chloride) drawn over the surface of the liquid in the flask, taking care to spread the alkaloidal solution in a thin layer around the sides of the flask as the drying is concluded; place flask on its side in a calcium chloride desiccator to constant weight, blow out flask with a current of dried air, immediately add 2 cc. of chloroform, a measured excess of standard sulphuric acid and 5 cc. of water, shake to dissolve the alkaloid, boil off the chloroform on a water-bath, cool and titrate immediately.

The results outlined below were obtained by different methods of preparing mydriatic alkaloids for titration and illustrate the effect of varying procedures upon assay results:

| Procedure No. | Hyoscyamus No. 1, Per Cent. | Hyoscyamus No. 2, Per Cent. | Hyoscyamus No. 3, Per Cent. | Hyoscyamus No. 4, Per Cent. | Hyoscyamus No. 5, Per Cent. | Hyoscyamus No. 6, Per Cent. | Belladonna, Per Cent. | Stramonium, Per Cent. |
|---|---|---|---|---|---|---|---|---|
| 1 | 0.115, 0.116 } 0.1155 | | | 0.131 | 0.125 | 0.155 | 0.703, 0.708 } 0.705 | 0.323, 0.328 } 0.326 |
| 2 | 0.0998, 0.0875 } 0.0937 | | | | | | 0.693, 0.688 } 0.690 | 0.299, 0.289 } 0.294 |
| 3 | 0.0623, 0.0654 } 0.0638 | 0.0702, 0.0702 } 0.0702 | 0.094, 0.096 } 0.095 | | | | 0.639, 0.654 } 0.647 | 0.260, 0.252 } 0.256 |
| 4 | 0.0654, 0.0534 } 0.0594 | | | | | | | |
| 5 | 0.0718, 0.0671 } 0.0695 | | | | | | | |
| 6 | | 0.0561, 0.0532, 0.0505, 0.0561 } 0.0540 | | | | | | |
| 7 | | 0.0479, 0.0434 } 0.0457 | | | | | | |
| 8 | | | 0.095, 0.095 } 0.095 | | | | 0.649, 0.654 } 0.651 | 0.260, 0.269 } 0.264 |
| 9 | | | 0.111, 0.106 } 0.1085 | | | | | |
| 10 | | | 0.095, 0.096 } 0.0955 | | | | | |
| 11 | | | 0.104, 0.109 } 0.1065 | | | | 0.688, 0.683 } 0.685 | 0.294, 0.304 } 0.299 |
| 12 | | | | 0.074 | 0.0723 | 0.139, 0.138 } 0.138 | 0.644, 0.649 } 0.647 | 0.252, 0.269 } 0.260 |
| 13 | | | | | 0.0649 | 0.131 | | |

NOTE: The various results in a given series were obtained on aliquots of the final chloroformic extracts containing the alkaloids, thus limiting the differences in results solely to the different methods of preparing the alkaloids for titration.

The procedures mentioned in the foregoing tables are as follows:

1. Evaporate to 2 cc., add standard acid, boil off chloroform.
2. Evaporate just to dryness on covered water-bath.
3. Evaporate on water-bath, then heated on covered water-bath for 5 minutes.
4. Same as preceding test but heated for 10 minutes.
5. Evaporate just to dryness; flask kept on its side in calcium chloride desiccator over night.

6. Evaporate to 5 cc., dried at 40–50° C. in air to constant weight.

7. Same as preceding test but dried at 60–70° C.

8. Evaporate to 2 cc. on water-bath; to dryness in current of dry air at 40° C.; flask kept on its side in calcium chloride desiccator over night (constant weight), chloroform and standard acid added; chloroform boiled off.

9. Evaporate to 5 cc. on water-bath; to dryness in current of dry air at 40° C.; dissolved in neutral alcohol; standard acid added.

10. Same as preceding test but flask kept on its side in calcium chloride desiccator over night (constant weight) before adding alcohol.

11. Evaporate to 5 cc. on water-bath; 5 cc. neutral alcohol added; to dryness in current of dry air at 40° C.; dissolved in chloroform; standard acid added; chloroform boiled off.

12. Same as preceding test but flask kept on its side in calcium chloride desiccator to constant weight before dissolving in chloroform and titrating.

13. Same as preceding, except flask kept in desiccator 24 hours after constant weight.

In the JOURNAL, A. PH. A., 16 (1927) 1039–1044, Watkins and Palkin described a method of assaying hyoscyamus which gave yields of alkaloids more than 3 times as great, in some cases, as those obtained by the U. S. P. IX and X procedures. In Watkins and Palkin's method the alkaloids are prepared for titration by evaporating the final chloroformic solution of the alkaloids to about 5 cc., adding a measured excess of standard sulphuric acid, evaporating off the chloroform, cooling and titrating. Their method thus includes the larger part of the volatile bases which may be present in the alkaloidal residue but avoids overheating and exposure to the moisture of the air. The U. S. P. IX prescribed that the alkaloids of hyoscyamus be prepared for titration by evaporating the final chloroformic solution of the alkaloids to dryness, treating the residue twice with 5 mils of ether and evaporating to dryness each time, followed by dissolving the alkaloids in a measured excess of standard sulphuric acid and titrating. This method thus eliminated more or less of the volatile bases that may have been present but subjected the alkaloids to possible overheating and hydrolyzation. The U. S. P. X states that a solution of the alkaloids of hyoscyamus should not be evaporated to dryness on the water-bath because these alkaloids "are very sensitive to heat" and that "after the last trace of chloroform has evaporated spontaneously, 1 or 2 cc. of neutral alcohol should be added and warmed but not entirely evaporated, then cooled and titrated immediately." Thus the U. S. P. X method effects a partial elimination of the volatile bases and avoids any possible loss through overheating but subjects the alkaloids to possible hydrolyzation through prolonged contact with the moisture of the air.

Thus it is evident that the higher results returned by the Watkins and Palkin method are occasioned, in part, at least, through the inclusion by their method of the larger part of the volatile base content of the alkaloidal residue. Avoidance of loss by overheating and hydrolyzation of the alkaloids also may have contributed to the higher results returned by their method. Whether or not the Automatic Extractor described by Watkins and Palkin actually extracts more alkaloid from a given hyoscyamus than can be extracted by simple maceration or percolation is a question to be decided. Several different lots of hyoscyamus examined in this Laboratory returned essentially the same proportion of alkaloids by both the automatic extractor and simple percolation method, provided every other detail of the assay process was identical. While this number of comparisons is not conclusive it

may be indicative that the Automatic Extractor is not actually more efficient in extracting alkaloids from hyoscyamus than is the simple percolator.

In the assay of mydriatic drugs the U. S. P. X directs that the final chloroformic solution of the alkaloids be evaporated spontaneously. This is an extremely tedious operation and exposes the alkaloids to prolonged contact with the moisture of the air with possible resultant hydrolysis of some of the alkaloids. The dried-air-desiccator treatment of preparing mydriatic alkaloids for titration described above protects the alkaloids against hydrolysis and overheating and essentially eliminates any interfering volatile bases which may be present. Consequently, this treatment disposes of these disturbing factors which contribute to the disagreements often arising between analysts of mydriatic drugs and seems worthy of consideration for inclusion in the U. S. P. description of the assay process for mydriatic drugs.

SUMMARY.

As ordinarily segregated from vegetable drugs and their preparations mydriatic alkaloidal residues may contain large proportions of volatile bases which greatly increase the apparent alkaloidal content of the drugs or preparations. Effectual means of eliminating these volatile bases and so allowing a truer estimate of the actual alkaloidal content are described.

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RESEARCH LABORATORIES,
TAILBY-NASON COMPANY,
BOSTON, MASS.

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