Critical Evaluation of Extraction Procedures in Determination of Atropine and Scopolamine from *Datura* Powder

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Abstract □ The simultaneous quantitative extraction and determination of atropine and scopolamine from *Datura* powder has been performed. This combination procedure afforded relative precisions of 5.11 and 3.85% for atropine and scopolamine with relative accuracies of −3.02 and −1.47%, respectively. Duplicate determinations of duplicate extractions over a 2-month period indicated that for atropine, 23.92% of the variation was attributable to the determination procedure and 76.08% was attributable to the extraction method. For scopolamine, 29.09% of the variation was due to the determination procedure and 70.91% to the extraction method. Comparisons of the USP percolation, continuous extraction procedure, and differential solvent extraction procedures are given. A theoretical discussion of total variation in quantitative methods in relation to extraction and determination is developed. Attempts to identify an unknown peak related to scopolamine are reported.

Keyphrases □ Atropine, scopolamine, extraction from *Datura* powder—comparison, evaluation of various procedures □ Scopolamine, atropine, extraction from *Datura* powder—comparison, evaluation of various procedures □ *Datura* powder—extraction of atropine, scopolamine, comparison, evaluation of methods

The work of Brochmann-Hanssen and Svendsen (1) led Solomon *et al.* (2) to attempt the GLC determination of atropine and scopolamine from solutions and plant powders. This method provided good accuracy and precision, although dehydration products of atropine and scopolamine were formed. A minor GLC peak attributable to scopolamine was not determined. Wu Chu and Mika (3) are presently studying several substances as internal standards for this partition chromatographic method. Recently, Zimmerer and Grady (4, 5) reported a GLC and spectrophotometric assay for hyoscyamine sulfate, atropine sulfate, scopolamine bromide, and phenobarbital tablets and elixir. This method utilized homatropine as an internal standard.

Wu Chu *et al.* (6) reported an extraction procedure for atropine and scopolamine, utilizing a photodensitometric determination method adapted to the quantitation of plant powders. No attempt was made, however, to combine the extraction method with the GLC method of Solomon *et al.* (2).

Some initial considerations of the problems of combining an extraction and determination procedure indicated that a good quantitative method must not only have good accuracy and precision, but the extraction procedure must have a variation within the range of that of the determination method. Upon closer examination, if: s_q^2 = sampling variation in the total quantitation method, s_d^2 = sampling variation in the determination method, s_e^2 = sampling variation in the extraction method, and

$$s^2 = \sum \frac{(\bar{x} - x)^2}{N} = \sum \frac{(\text{expectancy of } x - \text{value of } x)^2}{\text{number of samples studied}} \quad \text{(Eq. 1)}$$

Table I—Overall Precision and Accuracy of Combined Extraction and Determination of a Standard Powder

Statistic	Alkaloid	
	Atropine	Scopolamine
$\bar{x}$, mg. %	21.87	6.81
95% $CL_{\bar{x}}$, mg. %[a]	21.60–22.14	6.78–6.87
$RSD \times 100\%$[b]	5.12	3.85
Absolute accuracy,[c] mg.	−0.66	−0.10
Relative accuracy[d]	−3.02	−1.47
N	64	64

[a] The mean will occur within these limits in 19 of 20 determinations. [b] Relative standard deviation = $SD/\bar{x} \times 100\%$. [c] Calculated as deviations from the median. [d] Relative accuracy = *absolute accuracy* $\times 100\%/\bar{x}$.

where $\bar{x}_q$ = expectancy of the total quantitation method, $\bar{x}_d$ = expectancy of the determination method, and $\bar{x}_e$ = expectancy of the extraction method, it can be shown that

$$s_q^2 = s_d^2 + s_e^2 \quad \text{(Eq. 2)}$$

Thus,

$$s_e^2 = s_q^2 - s_d^2 \quad \text{(Eq. 3)}$$

For n samples, where n is small enough to be corrected for the small sample bias, after expansion and subsequent simplification, Eq. 3 can be written as

$$\sum_{n=2}^{n=n} \frac{(\bar{x}_q + \bar{x}_d - 2x_n)(\bar{x}_q - \bar{x}_d)}{n-1} = \sum_{n=2}^{n=n} \frac{(\bar{x}_e - x_n)^2}{n-1} \quad \text{(Eq. 4)}$$

If the determination method is vastly improved without improving the extraction procedure,

$$\lim_{n \to N} (\bar{x}_d - x_n) = 0, \qquad \text{where } N \gg n \quad \text{(Eq. 5)}$$

By simplifying Eq. 4,

$$\sum_{2}^{N} \frac{(\bar{x}_q - x_n)^2}{N} = \sum_{2}^{N} \frac{(\bar{x}_e - x_n)^2}{N} \quad \text{(Eq. 6)}$$

Thus, an excellent determination procedure coupled with a relatively coarse extraction procedure will produce a quantitative method in which the variation of the extraction procedure is measured with excellent precision and accuracy. Consideration of this problem along with a study of the variation in extraction of *Datura* alkaloids in relation to an established determination method is the purpose of this paper.

MATERIALS AND METHODS

Standard Powder—A standard powder was prepared from the leaf laminae (petiole removed) of 144 DS 2 (7) field-grown plants of

Table II—Contribution of Extraction and Determination to Total Variation[a] ($s_q^2 = s_d^2 + s_e^2$)

Source of Variation	DF	Atropine[b] F	Atropine[b] P	Atropine[b] % Contribution to Total MS[d]	Scopolamine[c] F	Scopolamine[c] P	Scopolamine[c] % Contribution to Total MS
Extraction							
Between months	1	1.6630	n.s.	48.52	0.4249	n.s.	12.36
Between weeks within months	2	0.2450	n.s.	7.15	1.6617	n.s.	48.34
Between days within weeks	4	0.1272	n.s.	3.71	0.2382	n.s.	6.93
Between extractions within days	8	0.5720	n.s.	16.69	0.1130	n.s.	3.29
Determination							
Within	16	Error term	—	23.92	Error term	—	29.09
Total	31	—	—	99.99	—	—	100.01

[a] Modified analysis of variance table. [b] Calculated as $y = (0.1784x + 1.8600)/w \times 100\%$, where y = milligrams percent of atropine, x = integrator counts adjusted to 200 atten., and w = weight (grams) of powder extracted. [c] Calculated as $y = (0.2472x + 0.1200)/w \times 100\%$, where y = milligrams percent of scopolamine, x = integrator counts adjusted to 200 atten., and w = weight (grams) of powder extracted. [d] Mean square of variable/sum of all mean squares × 100%.

Datura stramonium, forced-air dried at 50°, and ground to a 40-mesh powder.

Extraction Method—Ten grams of the standard powder was extracted by the method of Wu Chu *et al.* (6), scaled up from 4 g., and finally washed to volume in a 5-ml. rather than 10-ml. volumetric flask. Unless otherwise stated, this extraction method was referred to as the "differential solvent extraction method." The USP XVII (8) methods of percolation and continuous extraction procedure were utilized as stated, except for the addition of washed sand as a bulking agent. All continuous extraction procedures were run for 8 hr. to ensure exhaustive extraction.

Determination Procedures—Two-microliter samples of the final extract, brought to volume in a 5-ml. volumetric flask, were quantitated according to the GLC procedure of Solomon *et al.* (2).

Materials—All chemicals used for the chromatographic portion of this paper were analytical reagent grade. Atropine and scopolamine[1] and scopoline[2] produced a single spot on TLC. Silane-treated glass wool[3] was used (9).

Aposcopolamine Synthesis—Aposcopolamine was unavailable commercially, so it was synthesized according to the method of Willstätter and Hug (10). The resulting product was recovered as long needles (m.p. 96.5–97.0°), and the identity was confirmed by NMR and mass spectrometry.

Experimental Designs—The precision and accuracy study (Table I) was run on 64 independent extractions. The study of variation with time (Table II) represents the effect of heirarchal or nested time levels on the variation of the total quantitative method. The USP XVII percolation and continuous extraction procedures, as stated in the Belladonna Leaf Monograph, were compared (Table III), and these procedures were then compared to the differential solvent extraction method in a 3 × 3 Latin square design (11). All precautions for randomization were taken.

RESULTS AND DISCUSSION

The use of silane-treated glass wool to hold column contents in place decreased the formation of dehydration products but did not eliminate them entirely.

Attempts to identify the minor peak associated with scopolamine occurring at retention time 14.5 min. (236°), as previously reported (2), were not successful. Injection of pure scopoline, the compound considered as the most likely degradation product, afforded a single peak with a retention time 3 min. 24 sec. (170°). Mass spectral analysis of a collection of the effluent at 14.5 min. (236°) retention time for a combined running time of 20 hr. was inconclusive, because only two peaks were obtained (*m/e* 263 and 240). The placement of the scopoline GLC peak and the fact that fragments of at least *m/e* 263 could be obtained from the unknown compound did indicate, however, that the compound was probably not scopoline.

Since collection of the compound was not possible with the system available, it was hypothesized that dehydration of scopolamine was occurring similar to the formation of apoatropine from atropine. Synthetic apoatropine was prepared (parent ion *m/e* 285 representing 72% of the base peak of *m/e* 94 in the mass spectrum), but upon injection it afforded a single peak at retention time 16 min. 54 sec. The mass spectral studies of aposcopolamine indicated no fragments between *m/e* 285 (parent ion) and *m/e* 154. Thus, the partial spectrum of the unknown compound and the spectrum of the aposcopolamine definitely appeared dissimilar. The partition pattern of scopolamine and its dehydration product aposcopolamine was parallel to the partition pattern of atropine and apoatropine. Both dehydration products were detected about 1 min. before the parent compound on the methylsilicone gum on a silanized diatomite system. The unknown compound, however, had a retention time of 3.5 min. less than the parent scopolamine.

The differential extraction method was very satisfactory for combination with GLC analysis. The photodensitometric determination method (6) originally reported with this extraction procedure was not as accurate or precise as the GLC method. However, it had the advantage that when the alkaloid was in low concentration, more of the final chloroform extract could be applied to the TLC plate without too much danger of overloading. The GLC determination method was more accurate and precise but dilute solutions required injection of more than 5 μl., which produced tremendous tailing and even occasional extinction of the flame.

Examination of Table I indicates that the overall relative precision of the combined extraction and determination of the standard powder was 5.12 and 3.85% with relative accuracies of −3.02 and −1.47% for atropine and scopolamine, respectively. The 95% confidence limits of the mean (95% $CL_{\bar{x}}$) indicated that these data were repeatable.

The contribution of the combined extraction and determination components over a 2-month period was examined (Table II). The variation between months or between weeks within months represented the largest component of variation for both atropine and scopolamine, although the components of variation between months, between weeks within months, between extraction sets within weeks (between days within weeks), and between extractions within days were all nonsignificant for both alkaloids quantitated using the determination variance component as the error term. Further examination indicated that 23.92% of the variation for atropine and 29.09% of the variation for scopolamine were attributable to the determination method, while 76.08% of the variation for atropine and 70.91% of the variation for scopolamine were functions of the extraction method. These analyses indicated that the extraction and determination methods were comparable; even though the extraction method was more variable than the determination method, the difference was not so extensive that the

[1] Aldrich Chemical Co.
[2] K & K Laboratories.
[3] Applied Science Laboratories.

Table III—Comparison of Continuous Extraction, Percolation, and Differential Solvent Extraction Procedures[a]

Source of Variation	DF	Atropine[b] F	Atropine[b] P	Scopolamine[c] F	Scopolamine[c] P
Between treatments	2				
Continuous extraction *vs.* percolation	1	17.174	<0.001	30.96	<0.001
Continuous extraction and percolation *vs.* differential solvent	1	191.450	<0.001	39.85	<0.001
Between days	2	2.553	<0.01	<1	n.s.
Between replicates	2	1.256	n.s.	<1	n.s.
Treatment *vs.* days	4				
Continuous extraction *vs.* days	1	2.86	<0.05	<1	n.s.
Percolation *vs.* days	1	12.69	<0.001	4.57	<0.05
Differential solvent *vs.* days	1	<1	n.s.	3.12	n.s.
Continuous extraction and percolation *vs.* days	1	27.65	<0.001	2.38	n.s.
Pooled error	43	Error term	Error term	Error term	Error term
Total	53	—	—	—	—

[a] 3 × 3 Latin square design with one structural restriction (11). [b] Milligram percent per gram dry weight of powder. [c] Milligram percent per gram dry weight of powder.

total variation could be considered a total function of either procedure. A comparison, however, of the USP XVII extraction methods with the differential solvent method was indicated.

Statistical analysis of the secondary and tertiary interactions for atropine and scopolamine (Table III) indicated that only the differential solvent method was nonsignificant over the days tested in this experiment. The continuous extraction ($p < 0.05$), percolation ($p < 0.001$), and continuous extraction and percolation combination ($p < 0.001$) methods varied over the period studied (3 randomized days). However, only the percolation method had a significant contribution for days when scopolamine was analyzed ($p < 0.05$). All the methods did not vary significantly between replicates (three) within days as would be expected. These data were interpreted to indicate that although the USP extraction methods may be satisfactory for total alkaloidal quantitation, they may be too variable for the present determination needs. Therefore, this would be an experimental example of the conclusion reached in Eq. 6.

When the orthogonal comparison of the combination of continuous and percolation extraction methods *versus* the differential solvent extraction method was considered, the $F_{1,43}$ value was highly significant for both alkaloids studied ($p < 0.001$). When the continuous extraction and percolation methods were compared, they were highly significantly different ($p < 0.001$) in this study, although USP XVII lists these methods as alternate extraction procedures.

SUMMARY

The combination of the differential solvent extraction and GLC determination methods produced an analytical procedure which was both precise and accurate. Analyses over a 2-month period indicated that time did not produce a significant variation in results. Comparison with USP XVII extraction methods indicated that the differential solvent extraction method was less variable. Comparison of the continuous extraction and percolation methods, listed as alternate quantitative extraction procedures in the belladonna leaf assay, demonstrated that these methods were not as satisfactory extraction procedures in this study as the differential solvent method.

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