Content Uniformity in Rectal Suppositories

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Abstract □ The content variability in five types of suppositories was evaluated in terms of coefficient of variation. The observed content variability had a coefficient of variation in the range of 1.2–4.5; the method variability had a coefficient of variation in the range of 0.3–4.3. Method variability interferes with the assessment of the actual content variability and, according to the official content uniformity specifications, may lead to the rejection of complying samples or to the acceptance of noncomplying samples. The rationale and the structure of the official content uniformity specifications were studied. The performance of official content uniformity specifications declines as method variability increases. An alternative approach for the evaluation and the restriction of content variability, based on the coefficient of variation, may be advantageous in some instances.

Keyphrases □ Suppositories, rectal—content uniformity □ Content uniformity, suppositories—official specifications □ Variability, drug content—suppositories

In discrete dosage forms, the content accuracy and the content unit-to-unit uniformity of the active ingredient are basic and obvious requirements for assuring reliable and constant therapeutic effects. Several investigators (1–5) have studied the manufacturing, analytical, and statistical problems involved in the control of content uniformity of tablets and capsules. In fact, content uniformity specifications are already given for some tablets in the USP XVII (6) and in the NF XII (7).

But content nonuniformity may affect other dosage forms besides tablets and capsules. For instance, sedimentation, heterogeneous repartition during the melting-casting process, or weight variations (8) may result in an excessive unit-to-unit content variation in rectal suppositories, which are dosage forms designed either to develop a local therapeutic effect or to serve as the vehicle for a drug with a general action. Especially in the second instance, the content uniformity of the active ingredient is as important as for oral dosage forms. Nevertheless, little attention has been given to this subject, apart from Elste *et al.* (9) who investigated some assay methods of content in individual suppositories.

The present investigation was undertaken to study the analytical and statistical aspects of the content uniformity control in rectal suppositories and to give a further contribution to this subject.

EXPERIMENTAL

Materials—Five types of suppositories, containing from 0.19 to 30% active principle, were investigated:

1. DIN, *i.e.*, 2.3-g. suppositories containing 15 mg. of dehydroepiandrosterone sodium sulfate in a water-soluble base of polyethylene glycol 1450 and 6000,[1] glyceryl monostearate, propylene glycol, and succinonitrile.
2. VAL, *i.e.*, 2.0-g. suppositories containing 100 mg. dimenhydrinate in a water-insoluble base of theobroma oil.
3. GAM, *i.e.*, 2.7-g. suppositories containing 800 mg. phenprobamate in a water-insoluble base of triglycerides of natural fatty acids.[2]
4. MAL, *i.e.*, 2.1-g. suppositories containing 4 mg. dimefline hydrochloride (3-methyl-7-methoxy-8-dimethylaminomethylflavone hydrochloride) and 500 mg. aminopyrine in a water-insoluble base of triglycerides of natural fatty acids.[3]
5. TEF, *i.e.*, 2.1-g. suppositories containing 276 mg. of theophylline and 46 mg. of ethylenediamine (to form aminophylline) in a water-insoluble base of theobroma oil.

The suppositories were manufactured by the melting-casting process previously outlined (8). Before casting, the active ingredients were in suspension in the melted base and the homogeneity was maintained by continuous mechanical stirring.

Methods—Ten replicates of the following assays were done: (*a*) bulk drug used for the preparation of suppositories; (*b*) drug content in samples equivalent in weight to one suppository (samples were taken from a mass obtained by homogenizing, without melting, 30 suppositories of each type) and (*c*) drug content in individual suppositories after weighing each suppository to within a precision of 0.1 mg.

The drug of replicates (*a*) and the samples of replicates (*b*) and (*c*) belonged to the same production batch.

The assays were done manually by the following methods.

Bulk Drugs—Dehydroepiandrosterone Sodium Sulfate—The bromometric method for Δ^5-steroids, according to Gorac (10), was used.

Dimenhydrinate—Assay was done with perchloric acid in glacial acetic acid as proposed by Meulenhoff and Van Sonsbeek (11).

Phenprobamate—Kjeldahl's method of nitrogen determination, using the accelerator "selenium mixture Merck" proposed by Wieninger (12), was used.

Dimefline Hydrochloride—This method involved titration in glacial acetic acid after adding mercuric chloride solution, using perchloric acid and methyl violet as indicator. One milliliter of 0.1 *N* perchloric acid is equivalent to 35.98 mg. of dimefline hydrochloride.

Aminopyrine—Perchloric acid method in anhydrous medium (13) was used.

Ethylenediamine—The method of De Lorenzi (14), based on the addition of formaldehyde to neutralized solutions of ethylenediamine hydrochloride and titration of the developed acidity with 0.1 *N* sodium hydroxide, was followed.

Theophylline—About 250 mg., exactly weighed, was dissolved in 30 ml. of anhydrous pyridine previously neutralized to thymolphthalein. The solution was then titrated with 0.1 *N* alcoholic potassium hydroxide to a deep-blue color. One milliliter of 0.1 *N* potassium hydroxide is equivalent to 18.02 mg. of anhydrous theophylline.

Drugs Vehicled in the Suppository Bases—DIN—The procedure described by Clark and Thompson (15) for the pure ingredient was adapted to suppositories. One suppository was dissolved in 100

[1] Carbowax 1450 and 6000, Union Carbide Corp., New York, N. Y.
[2] Imhausen H, Imhausen Werke, Witten-Ruhr, Germany.
[3] Imhausen W, Imhausen Werke, Witten-Ruhr, Germany.

Table I—Analytical Statistics Obtained on Bulk Drug, on Homogenized Suppositories, and on Individual Suppositories

Suppositories and Entries[a]	Content of Active Ingredient: Bulk Drug, %	Homogenized Suppository, mg./g.	Individual Suppository, mg./Suppository	Suppository Weight, mg.
DIN				
NC	98.27[b]		15	
ACF	98.35	6.06	14.7	2308
CV	0.29	4.33	2.44	1.62
R%	−0.6–0.3	−5.1–6.7	−5.1–2.4	−1.9–3.1
NR			−7.2–0.0	
FLM_{95}	98.15–98.55	5.87–6.25	14.4–14.9	2281–2335
VAL				
NC	100		100	
ACF	100.15	51.44	102.1	1977
CV	0.14	0.57	3.45	1.57
R%	−0.1–0.3	−1.1–0.9	−4.2–7.4	−2.3–2.5
NR			−2.1–10.3	
FLM_{95}	100.05–100.25	51.23–51.65	99.6–104.6	1956–1998
GAM				
NC	100		800	
ACF	99.86	300	784	2675
CV	0.36	1.13	1.84	1.48
R%	−0.6–0.7	−1.4–1.7	−2.0–2.8	−1.7–2.7
NR			−4.0–0.6	
FLM_{95}	99.60–100.12	297–302	773–795	2647–2703
MAL-DIM				
NC	100		4.00	
ACF	100.04	1.93	3.97	2060
CV	0.14	0.66	1.20	1.06
R%	−0.2–0.3	−0.6–1.1	−2.3–2.0	−1.4–2.2
NR			−3.0–1.3	
FLM_{95}	99.93–100.15	1.92–1.94	3.94–4.00	2046–2074
MAL-AMI				
NC	100		500	
ACF	100.03	242.5	499	
CV	0.15	0.30	1.74	
R%	−0.2–0.2	−0.5–0.4	−2.0–3.3	
NR			−2.2–3.2	
FLM_{95}	99.92–100.14	242.0–243.0	493–505	
TEF-ETHYL				
NC	100		46	
ACF	99.57	20.7	43.5	2088
CV	0.15	3.56	3.45	1.27
R%	−0.2–0.1	−6.2–3.2	−4.8–6.2	−2.0–2.0
NR			−10.0–0.4	
FLM_{95}	99.46–99.67	20.1–21.2	42.4–44.6	2069–2107
TEF-THEO				
NC	90.85[c]		276	
ACF	90.42	134	280	
CV	0.26	3.19	4.47	
R%	−0.5–0.4	−2.5–6.1	−6.6–5.4	
NR			−5.1–7.0	
FLM_{95}	90.25–90.59	131–137	271–289	

[a] NC = nominal content; ACF = average content found; *CV* = coefficient of variation; R% = range found, calculated in percentage of the ACF; NR = range in percentage of the NC; FLM_{95} = 0.05 *p* fiducial limits of the ACF. [b] Water content 1.73%. [c] Water content 9.15%.

ml. of methanol by refluxing for 15 min. and, after cooling, was diluted to 200.0 ml. with methanol. The mixture was left to rest, and 2.0 ml. of the decanted limpid liquid was evaporated to dryness in a test tube. One milliliter of antimony trichloride reagent (150 g. $SbCl_3$ in 50 ml. of acetic anhydride) was added, and the test tube was kept for 5 min. in a water bath at 50 ± 2°. After cooling, 2.0 ml. of acetic anhydride and 3.0 ml. of acetic acid were added. After 40 min., the developed color was measured and compared with a standard solution of dehydroepiandrosterone sodium sulfate submitted to the same procedure.

VAL—One suppository was added to 100.0 ml. of water in a stoppered flask and heated to 45° until the base melted. The flask was shaken and cooled to room temperature. The content was filtered, and 5.0 ml. of the filtrate was diluted to 100.0 ml. with 0.01 *N* sodium hydroxide. The absorbance was determined at 276 mμ using 0.01 *N* sodium hydroxide as blank. As the standard, a solution of dimenhydrinate in 0.01 *N* sodium hydroxide was used. Dimenhydrinate was taken from the same batch used for manufacturing the suppositories.

GAM—One suppository was stirred in 50 ml. of petroleum ether (40–70°) until the base dissolved. The insoluble drug was quantitatively filtered on a tared sintered-glass funnel and washed with three 20-ml. portions of petroleum ether. The funnel was dried to constant weight in a vacuum over P_2O_5. The weight of the residue (melting at 100–105°) was taken as the phenprobamate in the sample.

MAL: Assay of Dimefline Hydrochloride (*MAL-DIM*)—One suppository was dissolved in 50.0 ml. of chloroform. A portion of 20.0 ml. of this solution was evaporated to dryness on a water bath. To the residue, 50 ml. of 0.1 *N* hydrochloric acid was added. The mixture was heated and stirred on a water bath at 45° for 5 min., cooled, diluted to 100.0 ml. with 0.1 *N* hydrochloric acid, and filtered; 25.0 ml. of the filtrate was diluted to 50.0 ml. with 0.1 *N* hydrochloric acid. On this solution the absorbance was determined at 309 mμ using 0.1 *N* hydrochloric acid as blank. The *a* for dimefline hydrochloride is 61.9.

MAL: Assay of Aminopyrine (*MAL-AMI*)—To another portion of 20.0 ml. of the chloroformic solution, prepared for the MAL-DIM assay, 20 ml. of glacial acetic acid was added. The titration was done with 0.1 *N* perchloric acid, using methyl violet as indicator. One milliliter of 0.1 *N* perchloric acid corresponds to 23.13 mg. of aminopyrine.

TEF: Assay of Ethylenediamine (*TEF-ETHYL*)—One suppository was stirred in 50 ml. ethyl ether until the base dissolved. After extraction with four 20-ml. portions of water, the aqueous extracts

were filtered through a filter paper and diluted to 100.0 ml. with water (Solution A). To this solution, 0.1 N hydrochloric acid was added until the solution was neutral to methyl orange indicator. Five milliliters of 40% aqueous formaldehyde, previously neutralized to the same indicator, was added; the developed acidity was titrated with 0.1 N sodium hydroxide. One milliliter of 0.1 N sodium hydroxide corresponds to 6.0 mg. of anhydrous ethylenediamine.

TEF: Assay of Theophylline (*TEF-THEO*)—Exactly 1 ml. of Solution A, prepared as already described, was diluted to 250.0 ml. with 0.01 N sodium hydroxide, and the absorbance was measured at the maximum (about 275 mμ). The amount of drug in the sample was calculated according to the British Pharmacopoeia (16) taking 65.0 as the value of a for anhydrous theophylline.

RESULTS

Compliance with the Official Content Uniformity Specifications (OCUS)—The content ranges of the active ingredient (Table I, Entry NR) in the investigated suppositories were within the limits prescribed in the first step of the OCUS given by USP XVII (6) for some oral dosage forms. In fact, in the 10 specimens investigated, the active ingredient found was always within the $M \pm 0.15\ M$ limits (M = nominal content). The OCUS may, therefore, be extended to rectal suppositories, where a heterogeneous distribution of discrete particles of the active ingredients between the individual dosage units is likely, as in oral dosage forms with a relatively small quantity of active ingredient compared to the inert ingredient (3, 4).

Assay Method for Content Uniformity Tests—The large amount of interfering vehicle and the small quantities of active ingredient present in the individual suppositories necessitated different assay methods for the bulk drug from those for the content of each suppository. Table I shows the performance of these assay methods in the different conditions, namely: (*a*) on the bulk drug, (*b*) on the homogenized suppository mass, and (*c*) on the individual suppositories. The analytical intrinsic variability, expressed by the coefficient of variation (CV), was usually smaller in the assays of the bulk drug than in the content assays on suppositories. In several instances, the difference between the CV of the two types of assay was significant to a $p < 0.05$ level. [The critical value for the ratio between the larger and the smaller CV is 1.56 (17).]

Since the assay method on the bulk drug must usually be changed or modified for assaying the content in individual suppositories, its performance cannot be taken as a standard for content assay. The most representative standard is the assay with the elimination of the unit-to-unit content variability, *i.e.*, the assay on samples, of the size of one dosage unit, taken from homogenized suppositories.

Relationship between Content Variability and Observed Unit-to-Unit Variability—The unit-to-unit variability found in the content assays of the individual suppositories is composite and depends on the variability inherent in the analytical method as well as on the actual content variability.

It has been assumed that the different variables follow a Gaussian distribution (1, 18), so that they may be related by the following equation:

$$CV^2_{\text{obs.}} = CV^2_{\text{ana.}} + CV^2_{\text{con.}} \qquad \text{(Eq. 1)}$$

where $CV_{\text{obs.}}$ is the CV observed, $CV_{\text{ana.}}$ is the CV inherent in the analytical method, and $CV_{\text{con.}}$ is the CV depending on the actual content variability. Equation 1 shows that $CV_{\text{con.}}$ cannot be estimated directly from $CV_{\text{obs.}}$, unless $CV_{\text{ana.}}$ is negligible compared to $CV_{\text{obs.}}$. Some $CV_{\text{ana.}}$'s found, represented by the CV obtained on the homogenized suppository mass, were rather large, namely in the same range as those found by other authors in assays of the content in individual tablets or capsules with drug combinations or with small quantities of active ingredient compared to the inert ingredients (3, 18–21). The $CV_{\text{ana.}}$, therefore, interfered with the estimation of the $CV_{\text{con.}}$. As a matter of fact, the $CV_{\text{obs.}}/CV_{\text{ana.}}$ ratio was not significantly greater ($p < 0.05$) than 1 in the DIN, TEF–ETHYL, and TEF–THEO content assays (Table II). Thus, in these suppositories, there is no proof of an actual content variability, since this is concealed by the assay method variability. Conversely, the presence of an actual content variability is demonstrable at a $p < 0.05$ level in the VAL, GAM, MAL–DIM, and MAL–AMI suppositories (Table II).

Table II—Analysis of the Variabilities Observed in the Assays

Suppository Type	Active Ingredient, %	$\frac{CV_{\text{obs.}}{}^a}{CV_{\text{ana.}}}$	$\frac{CV_{\text{obs.}}{}^a}{\sqrt{CV^2_{\text{ana.}} + CV_W{}^2}}$
DIN	0.65	0.56	0.53
VAL	5.0	6.03	1.99
GAM	29.3	1.63	0.99
MAL–DIM	0.19	1.82	0.96
MAL–AMI	24.3	5.80	1.58
TEF–ETHYL	2.06	0.97	1.00
TEF–THEO	13.4	1.40	1.31

[a] The critical value for the ratio is 1.56 (α = 0.05, one-tailed).

The actual content variability may depend on a heterogeneous dispersion of the active ingredient in the suppository mass, or on the suppository-to-suppository weight variability, or on both. The relationship between the different coefficients of variation is

$$CV^2_{\text{con.}} = CV_W{}^2 + CV^2_{\text{het.}} \qquad \text{(Eq. 2)}$$

where CV_W represents the unit-to-unit weight variability, and $CV_{\text{het.}}$ is the coefficient of variation due to heterogeneous dispersion of the active ingredient in the vehicle. From Eqs. 1 and 2, a heterogenous distribution of the active ingredient is demonstrable when $CV_{\text{obs.}} > \sqrt{CV^2_{\text{ana.}} + CV_W{}^2}$. This seems to be the case of VAL and of MAL–AMI suppositories (Table II). Table II shows that it is not possible to relate heterogeneous distribution to the percentage of active ingredient in the dosage form, as is often the case in solid oral dosage forms (22).

Correlation between Content and Weight—The correlation between content and weight may be studied using the linear regression (23) of content (C) over the suppository weight (W) in the equation

$$C = a + bW \qquad \text{(Eq. 3)}$$

Comparison becomes easier by standardizing the regression, *i.e.*, by adopting as units average content and average weight. By this procedure, Eq. 3 shows a strict weight dependence of content when $a = 0$ and $b = 1$.

The regressions calculated by the least-squares method are given in Table III. Consistency with the hypothesis that $a = 0$ and $b = 1$ was tested with the Student's t test. Even though the regressions obtained apparently differ greatly from the theoretical one in which weight depends on content (expressed by $C = W$), the obtained results do not disprove such dependence. Data scatter, however, prevents any valid conclusion.

Table III—Regressions of Content over the Weight of Different Types of Suppositories

Suppository Type	Regression[a]	t[b] of Difference ($a = 0$; $b = 1$)
DIN	$C = (0.68 \pm 0.52) + (0.32 \pm 0.52)\ W$	1.31
VAL	$C = (0.64 \pm 0.77) + (0.36 \pm 0.77)\ W$	0.83
GAM	$C = (-0.09 \pm 0.21) + (1.09 \pm 0.21)\ W$	0.42
MAL–DIM	$C = (0.76 \pm 0.40) + (0.24 \pm 0.40)\ W$	1.92
MAL–AMI	$C = (-0.24 \pm 0.56) + (1.24 \pm 0.56)\ W$	0.42
TEF–ETHYL	$C = (-0.05 \pm 0.89) + (1.05 \pm 0.89)\ W$	0.06
TEF–THEO	$C = (-1.16 \pm 0.99) + (2.16 \pm 0.99)\ W$	1.18

[a] The regressions are standardized on average content and on average weight units. [b] t = 2.31 for α = 0.05.

Correlation between Drugs in Combination—The TEF suppositories contain a combination of ETHYL and of THEO; the MAL suppositories contain a combination of DIM and of AMI. Since in both suppository types, the two ingredients were assayed simulta-

Table IV—Linear Regressions between Active Ingredients in MAL and TEF Suppositories[a]

$$\text{DIM} = (0.52 \pm 0.18) + (0.48 \pm 0.18)\ \text{AMI}$$
$$t_a = 2.95;\ t_b = 2.70$$
$$\text{ETHYL} = (0.94 \pm 0.27) + (0.07 \pm 0.27)\ \text{THEO}$$
$$t_a = 3.48;\ t_b = 0.26$$

[a] The critical t value is 2.26 ($\alpha = 0.05$).

neously in each individual suppository, the possible correlation between the two ingredients may be investigated by the linear regression. The regressions found, standardized into units of average content, are given in Table IV.

No statistically significant correlation was found between ETHYL and THEO, and a very small one ($0.05 > p > 0.02$) was found between DIM and AMI. For these suppositories the content uniformity found on one of the two combined drugs is not transferable to the other one, since each active ingredient varies independently.

Content Accuracy—Content accuracy, *i.e.*, correspondence between the actual average content and the labeled content, may either be investigated on a composite sample or evaluated from the average of the contents of the individual dosage units. In the latter case, the coefficient of variation of the mean (*CVM*) indicates the precision by which content accuracy is evaluated. The $p = 0.05$ confidence limits are the product of *CVM* multiplied by the appropriate t value (2.26 for 9 degrees of freedom).

Table I shows that the $p = 0.05$ confidence limits of the average content covered or were very close to the labeled content in most instances. The largest difference between the actual average content and the labeled content was found for TEF–ETHYL suppositories (−5.4%). Nevertheless, the TEF–ETHYL suppositories may still conform to the specification that the content must be between 95 and 105% of the nominal content, since the upper confidence limits are only 3% lower than the nominal content. Therefore, there is no proof to a $p = 0.05$ level that the content found is lower than 97%.

It should be emphasized that the content accuracy found is not the true one, since it depends also on the accuracy of the assay method. The performance of the method in terms of accuracy cannot be investigated with statistical tools, as in the case of uniformity, but must be evaluated by comparing different methods, preparing samples with known quantities of the substance under investigation, *etc.* (24). While theoretically it is impossible to be sure that an assay method is 100% accurate, in practice some methods, especially when a comparison with a proper standard is involved, yield an acceptable accuracy.

In the case of TEF–ETHYL suppositories, it was found that the method used was inaccurate because of suppository base interference. The alternative accurate method, which is now under investigation, has a wide intrinsic variability and, therefore, is not suitable for evaluating content uniformity. The results of the study on content accuracy of TEF–ETHYL suppositories will be the subject of another paper.

DISCUSSION

The samples of the different types of rectal suppositories conformed to USP XVII OCUS (16) for tablets. It may be shown, however, that this does not imply that every sample taken from the investigated lots of suppositories complies with the OCUS or that the OCUS are appropriate for restricting content variability in rectal suppositories. In fact, the OCUS call for comment before evaluating their adaptability to more general conditions.

Structure of the OCUS—The OCUS are based on sampling plans devised for "attributes" and for the restriction of "defective" specimens in a lot. The content is a continuous variable, which is transformed into an attribute by confronting the content found to the $M \pm 0.15\ M$ limits (M is the labeled content), a transformation which implies by itself a loss of information. Nevertheless, the OCUS "attributes" plan was adopted by USP XVII after a multilaboratory study (2), mainly because it was shown that some variables, *e.g.*, the weights of sterile solids, occasionally may not follow a Gaussian distribution (25). In these cases, the "attributes" plan may be advantageous because it is more "robust," *i.e.*, less susceptible to the biasing effects of non-Gaussian distributions on the evaluation of variability.

The "attributes" plan of the OCUS, however, is not flexible; even a minor change in sample size, in acceptance number, or in critical limits radically changes the performance of the plan. Furthermore, the OCUS plan does not take intrinsic analytical variability into account and so sometimes leads to wrong conclusions.

The TEF–ETHYL suppositories, for instance, would not comply with the OCUS in approximately 1.7% of the samplings. More generally, an ideal lot with a perfect content uniformity, investigated for compliance with the OCUS with an assay procedure having an intrinsic variability expressed by a $CV_{\text{ana.}} = 5$, would not comply in approximately 1% of the samplings. Conversely, the same analytical variability might result in acceptance of a bad lot.

One reason for the choice of the "attributes" plan for the OCUS was the extensive experience already acquired with similar plans for the control and restriction of weight variability in different types of dosage forms. It should be remembered, however, that weight uniformity is investigated with a very precise and accurate method, namely the analytical balance, with an inherent $CV_{\text{ana.}}$ usually smaller than 0.1. When introduced into the equation

$$CV^2_{\text{obs.}} = CV^2_{\text{ana.}} + CV_W{}^2 \qquad \text{(Eq. 4)}$$

the $CV_{\text{ana.}}$ is negligible, so CV_W is directly evaluatable from the $CV_{\text{obs.}}$. Moreover, for measuring weight, there is only one well-known and well-defined analytical method independent of a particular drug or formulation.

On the other hand, content uniformity is tested by methods which may have large intrinsic variabilities, interfering with the evaluation of the true content variability. Further, each drug and often each formulation call for a particular assay method, with a different intrinsic variability and, therefore, also a different consequence on the evaluation of true content variability.

For these reasons, the experience acquired with the weight uniformity specifications cannot be transferred to the control of content uniformity, unless the assay method is extremely precise and accurate.

Another point which should be emphasized is that the results obtained with the OCUS depend on the precision *and* on the accuracy of content of active ingredient and of its assay method. As a matter of fact, the name "test for content uniformity" is misleading, since actually the allowed variability decreases with the increase of inaccuracy, as shown in Fig. 1. Since the inaccuracy actually found depends also on the random composition of the sample and is biased by the imprecision and the inaccuracy of the assay method, a single specimen may sometimes be classified as within the $M \pm 0.15\ M$ limits and sometimes as outside. The OCUS do not provide for a clear discrimination between good and defective specimens or for an explicit and fixed margin for content variability, so that among other drawbacks the OCUS cannot be transferred to the production control charts or to the inspection of portions of the lot which are larger than the sample sizes required by the OCUS.

When discussing weight variability, it was shown that the confidence in the results increases with the precision of the assay method. Since compliance with the OCUS depends both on precision and on accuracy, the assay method used for the OCUS must be accurate, a requirement which does not always go hand in hand with precision, economy, and sometimes even feasibility. This, for instance, is the case with biological assay methods, where the investigation of content uniformity can be performed only with inaccurate and unspecific methods (26). The need for different methods of testing accuracy and uniformity of content is becoming more and more obvious with the introduction of automated methods of analysis and is considered in the new editions of the USP and of the NF (21, 27, 28). But different methods, one for accuracy and the other for uniformity of content, are incompatible with the very structure of the OCUS.

In conclusion, the OCUS have several disadvantages. These are: (*a*) lack of flexibility so that it is very difficult to adapt the OCUS to different sample sizes, to the control of larger portions of a lot, or to control during production; (*b*) misleading results due to the neglect of the intrinsic analytical variability of the assay method; (*c*) dependence of content variability on content accuracy, which in

some instances prevents the use of precise methods for investigating content uniformity; (*d*) indefinite allowance for variability, impeding generalization, and the transfer of the OCUS to production control charts; (*e*) "yes or no" type of results, whereas often the knowledge of the degree of compliance or noncompliance of the sample is desirable to alert the producer or consumer to the presence of a borderline condition; (*f*) difficulty of gaining better knowledge of the OCUS through the experience acquired by content variability investigations, since most practical and theoretical studies evaluate the content as a continuous variable and express content variability in terms of standard deviation; and (*g*) loss of information due to the transformation of a continuous variable into an attribute.

These disadvantages are of secondary importance when the intrinsic variability of the assay method is small. But when the analytical variability is large enough to interfere with the evaluation of the actual content variability, or when a precise but inaccurate method is appropriate for checking content uniformity, an alternative approach to the control and the restriction of content variability seems desirable, *e.g.*, one based on a "variables" sampling plan, as already advanced by Breuning and King (29), or based on the *CV* since many published investigations evaluate content uniformity through the *CV* method.

Content Uniformity Specifications Based on the Restriction of the Coefficient of Variation (CUS-*CV*)—According to Eq. 1 the basic acceptance condition of a CUS-*CV*, which takes into account the analytical variability, is

$$CV^2_{\text{con.}} = CV^2_{\text{obs.}} - CV^2_{\text{ana.}} \leqslant AQL^2 \qquad \text{(Eq. 5)}$$

where *AQL* is the acceptance quality level for content uniformity. Since the $CV_{\text{ana.}}$ depends on the assay method used for the content determination of the particular drug in the individual dosage form, the content uniformity specification must state for each drug and for each dosage form: (*a*) the official method for the content assay in the individual dosage units, and (*b*) the maximum method variability allowed for accepting the results (as for biological assay methods).

It has been shown (30) that the $CV_{\text{con.}}$ consistent with a 95% acceptance probability of USP XVII OCUS is 5.4 in the first sample of 10 and 6.2 in the composite sample of 30 dosage units. The *AQL*

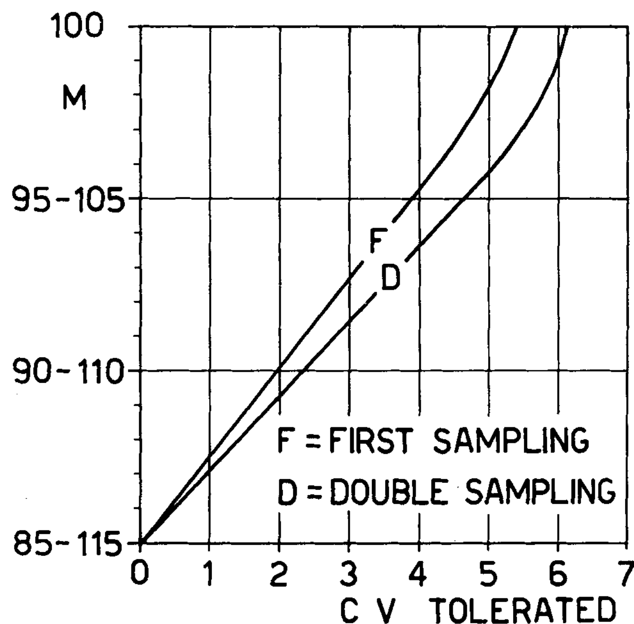

Figure 1—*Relationship between the content accuracy and the* CV *tolerated for the producer, according to the content uniformity specification of USP XVII. Ordinate: average content, as a percentage of nominal content; abscissa:* CV *tolerated for the producer. The tolerated* CV *is maximum for an average content equal to the nominal value and vanishes when the actual content approaches 85 or 115% of the labeled content. The "first" and the "double" sampling curves reflect the implications of the first and second sampling steps described in the USP specification.*

Table V—Limits for $CV_{\text{con.}}$ at Different Sample Sizes

Sample Size, units	$CV_{\text{con.}}$[a] *AQL'*	*UQL*
10	3.7	6.9
15	4.1	6.6
20	4.3	6.4
30	4.5	6.3
60	4.7	6.0
100	4.9	5.9

[a] Calculated according to Eq. 5.

for content uniformity may, therefore, be located between 5.4 and 6.2, *e.g.*, restrictively at 5.4.

The *AQL'* of a sample should give a 95% confidence that the *CV* in the lot is equal to or lower than 5.4. Therefore, the *AQL'*'s were calculated from the one-tailed 95% fiducial limits of Table H of Davies (17) for different sample sizes and are given in Table V. Also, the unacceptable quality level (*UQL*), *i.e.*, the quality found which gives 95% confidence that the $CV_{\text{con.}}$ in the lot is higher than 5.4, depends on the sample size and is given in Table V.

In an actual inspection for uniformity, one may, for instance, start with a sample of 10 units and then take any further decision, *i.e.*, upon acceptance, rejection, or expansion of the sample, on comparing the $CV_{\text{con.}}$ found with the values of Table V. In this way, the sample size is open and flexible.

As already pointed out, the OCUS link together two types of quality levels: one for uniformity and one for accuracy.

The requirements implied in the OCUS must, therefore, be translated into terms of *AQL* for accuracy consistent with the *AQL* for uniformity. According to Breuning and King (29), this condition is satisfied by expressing accuracy as the *CVM*. In a sample of 10 and for a $CV_{\text{con.}} = 5.4$, the $CVM = 5.4/\sqrt{10} = 1.71$. This *CVM* means that about 99% of the averages of the assays on 10 units, or of the assays of composite samples of 10 units, should be within 95 and 105% of the nominal content, a condition which in fact is similar to the requirements of several monographs of USP XVII.

In sum, the CUS-*CV* may be outlined as the *AQL'* of the $CV_{\text{con.}}$ found on the basis of Eq. 5 should be within the values given in Table V for the different sample sizes. Since the OCUS implies also a specification for content accuracy, it must be added that the average content, assayed with a given accurate method on a composite sample of 10 homogenized dosage units, or calculated from 10 assays on individual dosage units, should be within 95 and 105% of the labeled content. The analytical variability must be considered in terms of $CV_{\text{ana.}}$ or of $CVM_{\text{ana.}}$ in order to rid the found average content of the bias due to analytical variability.

These specifications are both flexible and transferable to production control charts.

The main argument against a CUS-*CV* plan is that it is less "robust" than the "attributes" plan when the distribution of the investigated variable is non-Gaussian (25). A non-Gaussian distribution, however, seems an exception rather than the rule for the content of active ingredients (19). On the other hand, the advantages of the greater "robustness" of the OCUS are diminished by the greater room for uncertainty inherent in an "attributes" plan. In fact, according to Pietra and Setnikar (30), the *AQL* implied by the OCUS attribute plan for a *lot* is equal to 5.4 (or to 6.2 after the second sampling) and the *UQL* is equal to 12. This difference for lot *CV* is greater than the differences for sample *CV* listed in Table V. Although lot conditions are not comparable to sample conditions, the latter usually imply a larger margin of uncertainty but are, nevertheless, the actual conditions for inspecting uniformity.

Given the advantages and disadvantages both of the "attributes" plan and of the *CV* plan, reconsideration of the general approach to content uniformity control would seem to be indicated. The present investigation aims to be a contribution to this subject.

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USP Collaborative Study of the Assay of Atropine and Scopolamine Dosage Forms

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Abstract ☐ Several tablet and injection dosage forms of atropine and scopolamine are covered by USP XVIII monographs. Gas chromatographic unit dose assay and content uniformity procedures were developed for these monographs. Interlaboratory reliability was evaluated by means of a collaborative study. The methods afford substantial improvements in sensitivity, specificity, and speed over previous official methods.

Keyphrases ☐ Atropine, scopolamine dosage forms—analysis ☐ Scopolamine, atropine dosage forms, analysis—collaborative study ☐ Content uniformity method—atropine, scopolamine dosage forms ☐ GLC—analysis

Previous official methods of assaying dosage forms for belladonna alkaloids have relied largely on titrimetry. These methods lacked sensitivity and specificity. Unit doses could not be assayed and decomposition products were not excluded. Such problems aroused some criticism. A notable exception was an IR method (1) which, although failing in sensitivity, did offer specificity with some control over decomposition. Assay methods for USP XVIII were desired which would be accurate, reliable, and highly specific and yet be sufficiently sensitive, precise, and rapid to allow content uniformity determinations on unit doses.

Various other approaches to belladonna alkaloid analysis may be noted. A colorimetric method (2, 3) was applied to preparations containing phenobarbital along with the alkaloids, and a dye–complex method was applied to atropine tablets and elixir (4). Neither of these approaches distinguishes one belladonna alkaloid from another. A fluorometric method has been reported for atropine (5). Paper chromatography (6, 7), partition-column chromatography (2, 3, 8), countercurrent distribution (9), TLC (10), and TLC with densitometry (11) have all achieved separation of scopolamine from atropine–hyoscyamine.

Initial efforts in the gas chromatography of belladonna alkaloids were reported by Kazyak and Knoblock (12), Brochmann-Hanssen and Fontan (13), Jain and Kirk (14), and Solomon *et al.* (15). Penner (16) studied atropine assay by GLC, both as a silyl derivative and later untreated, using tetraphenylethylene as the internal standard. Alber (17) recently reported a broad study of the gas chromatography of drugs and alkaloids using the methylphenylpolysiloxane liquid phase which was used in this collaboration.

The procedures developed for this collaborative study are related to a method previously reported (18) for dose forms of belladonna alkaloids containing phenobarbital. Other official methods[1] were developed earlier for belladonna alkaloids using anthracene as an internal standard for control of injection volume alone.

MATERIALS

Methylene Chloride—Gas chromatography or 99 mole % grade was used.

[1] Hyoscyamine sulfate tablets NF and morphine and atropine sulfates tablets NF.