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VALIDATION OF THE CONTENT UNIFORMITY TESTING OF USP XXI (1985) FOR TABLETS CONTAINING POTENT DRUGS.

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ABSTRACT

The content uniformity of tablets containing high potency, low dosage drugs can only be successfully maintained by application of GMP at all stages of the total manufacturing process including both formulation and quality control. This requires both understanding of the pharmaceutical technology involved and appropriate designing of content uniformity test.

The USP XXI (1985) has made the content uniformity test more stringent by applying both tests by attributes and variables. In this study the test has been challenged by using 27 batches of ethinyloestradiol 10 µg tablets having different degrees of homogeneity in powder mixes and tablets.

The results indicate that the test has a weak potential in determining the content uniformity of batches prepared from cohesive drug powders and characterised by skewed distribution

of drug content in tablets . It is the same drawback of the USP XX (1980) content uniformity test. This defect is due to the small sample size of 10 unit doses in the first step which is not sufficient to detect the presence of unit dosages containing high drug content in a batch of tablets having skewed distribution. Statistical analysis of the results using coefficient of skewness and nonparametric Lilliefors test has shown that some batches which have passed the USP XXI (1985) content uniformity test are pharmaceutically unacceptable. This indicates the importance of incorporating within the official specifications a test which includes an examination of the type of distribution and hence, increasing sample size is required.

INTRODUCTION

For unit doses particularly those dosage forms containing potent drugs where the dose is extremely small , it is not sufficient to comply with the drug content requirements based on 20 tablets . The content uniformity testing of such dosage forms is an essential part of their quality control. The reason being that, the amount of drug the patient receives in a unit dose should be a reflection of the labelled strength. It is also to ensure the maintenance of good manufacturing standards particularly where mixing problems are expected (1,2) .

Recognising these requirements the USP XVII (1965) and USNF (1965), included for the first time a content uniformity test for tablets containing 5 mg or less of drug content per tablet.

In the consecutive versions (USP 1970, 1975 and 1980) a content uniformity test for tablets containing 50 mg or less of drug per tablet is included. The inspection plan of all these was dependent on two stage test by attributes. The relative weakness of inspection plan by attributes have been extensively discussed (3 - 18). The main drawbacks which have been outlined are the small sample size in the first step of the test and the lack of determining the variation among the units and absence of examination of the type of distribution of dosage units in batches. Consequently, the inspection plan by variables has been suggested. Furthermore, it has been pointed out that unless the content uniformity test examines the type of distribution of drug content in the tablets, pharmaceutically unacceptable batches may pass the test (1,2 and 18). Examination of the type of distribution of drug content in unit doses requires increasing the sample size up to 50 units.

The new edition of the USP , i.e. USP XXI (1985) includes a content uniformity test based on an inspection plan by two stage test using both sampling by attributes and variables (19). However, the sample size of the first step is kept small, 10 unit doses. Although it becomes more stringent by applying both tests by attributes and variables, it still doesnot safeguard against unit doses containing high drug content for the same reasons outlined before.

A recent report (21) has proposed a control chart for the mean constructed from duplicate 20 tablet drug content assays

and 10 unit doses assays. The report suggests this test along with the USP XXI (1985) content uniformity to be used for validation and control of solid dosage form manufacturing process.

The current study will assess the USP XXI (1985) content uniformity test in order to evaluate its validity for controlling solid dosage form containing potent drugs. The type of distribution of drug content in unit doses is examined by using coefficient of skewness and nonparametric Lilliefors test.

METHODS

The present study has used data of different batches of ethinyloestradiol (EE) 10 µg tablets. Twenty seven batches were prepared using different particle sizes of EE ranging from very fine cohesive powder to a free flowing coarse powder (22). The weight of each tablet was around 55 mg, similar to commercial EE 10 µg tablets. Fifty tablets from each batch were withdrawn randomly and assayed individually (23). The results from each sample of 50 tablets have been subjected to USP XX (1980) and USP XXI (1985) C.U.tests and to other statistical tests.

Coefficient of Skewness $\sqrt{b}_1$:

The degree of skewness is assessed by calculating the statistic $\sqrt{b}_1$, the coefficient of skewness:

$$\sqrt{b}_1 = \frac{m_3}{m_2^{3/2}} \qquad \text{where} \quad m_r = \sum_{i=1}^{n} \frac{(x_i - \bar{x})^r}{n}$$

n is number degrees of freedom and r = 2 for m_2 and r = 3 for m_3. For a symmetrical distribution, if all of the observations are considered, $\sqrt{b_1} = 0$; for a positively skewed distribution $\sqrt{b_1} > 0$. However, the value of $\sqrt{b_1}$ calculated for a sample observations from symmetrical distribution will not be exactly zero. The limiting values for a normal distribution for different sample sizes are given in the following Table (24):

Sample Size	Coefficient of Skewness	
	P = 0.05	P = 0.01
30	0.661	0.962
50	0.533	0.787
100	0.389	0.567

Fisher (25) suggested that this test to be an excellent test for the significance departure from normality.

Nonparametric Lilliefors test for normality :

Another test to check the normality of the distribution of drug content in unit doses is a nonparametric test for normality, Lilliefors test (26). It compares the empirical distribution function of the standardised sample values (observations within a sample) against the standard normal distribution function.

$$Z_i = (X_i - \bar{X}) / S .$$

where Z_i is the standardized sample observation of X_i, $\bar{X}$ is the sample mean and S is the standard deviation of sample observations. The empirical distribution function of the standardized sample values is plotted in Lilliefors graph paper.

The null hypotheses tested are :

H_o : the random sample has a normal distribution with unspecified mean and variance.

versus,

H_1 : the distribution function of the contents of EE in the tablets (X_i) is non - normal.

The test requires rejection of the null hypothesis at P = 0.05 if the test statistic T_2 (the largest vertical distance between the empirical distribution function and the standard normal distribution) exceeds the 0.95 quantile . Values of the 0.95 quantile according to the sample size are given in the following Table (27):

Sample Size	0.95 Quantile
30	0.162
50	0.125
100	0.089

In addition, if the cumulative relative frequency curve of the sample observations deviate outside the bounds of specific sample size, then it should be considered non-normal (27).

Combination of content uniformity and drug content assays ($\bar{X}_w$):

Bolton (21) has suggested control charts for the mean potency from duplicate drug content assays (20 tablets each) and 10 content uniformity assays . In case of negligible assay variation then:

$$\bar{X}_w = (2 \bar{X} \text{ composite} + \bar{X} \text{ CU}) / 3 .$$

where :

$\bar{X}_w$ = Weighted average .

$\bar{X}$ composite = Average of duplicate drug content assays (20 tablets each).

$\bar{X}$ CU = Average of content of 10 unit doses.

In case of coefficient of variation (CV) of drug content equals to 5% the control - chart limits of $\bar{X}_w = 100 \pm 2.74$, while in case of (CV) = 7% the control - chart limits of $\bar{X}_w = 100 \pm 3.83$. The value 100 is the target mean which is equal to the label claim, it gives more discriminating power to the proposal.

DISCUSSIONS

Results are summarized in Tables 1 and 2. The advantage of USP XXI (1985) C.U. Test over the previous one is obvious by having batch number 13 as an example. Batch number 13 passes the USP XX (1980) C.U.Test because the first 10 tablets are within the limits of $\pm$ 15% of the specified potency limits in the monograph, i.e. 102.5% multiplied by $\pm$ 15 %. However, it does not pass the USP XXI (1985) C.U.Test because of the following:

1 - The first 10 tablets have CV equal to 9.7% and one tablet lies outside the $\pm$ 15% limits, where the potency limits for this batch is 100 % of the label claim.

2 - The second step produces CV equal to 12.6% and one tablet has drug content equal to 15.1 µg.

Although the USP XXI (1985) C.U.Test becomes more stringent, it doesnot add much more discriminating power to the test because the sample size of the first step is still 10 unit doses. Both

TABLE (1).

SUMMARY OF RESULTS AFTER APPLICATION OF USP XX (1980) AND USP XXI (1985) CONTENT UNIFORMITY TESTS ON 27 BATCHES OF ETHINYLOESTRADIOL 10 µG TABLETS.

	USP XX (1980)	USP XXI (1985)
NUMBER OF BATCHES PASS.	20	19
NUMBER OF BATCHES FAIL.	8	9

N.B. 15 BATCHES ARE CONSIDERED PHARMACEUTICALLY UNACCEPTABLE, SEE TABLE (2).
BATCH NUMBER 9 HAS BEEN SAMPLED TWICE.

tests have rejected those batches characterised by gross inhomogeneity of drug content in unit doses such as batches nimber 1,2,3, 4 and 10. Batch number 11 has been rejected because the mean drug content is much below the specified limits. As shown in Table (1) , both tests are not significantly different within the range of batches used for thie study. Batches number 5,8, 9(a) and 9(b), 12,14 and 15 are pharmaceutically unacceptable batches as shown by examination of the range, mean and coefficient of variation (CV) of 50 tablets (Table 2). All these batches have

TABLE (2)

SUMMARY OF RESULTS OF 15 PHARMACEUTICALLY UNACCEPTABLE BATCHES OF ETHINYLOESTRADIOL 10 µG TABLETS (50 TABLETS EACH).

BATCH NUMBER	DRUG CONTENT RANGE	MEAN	µG/TABLET CV	$\sqrt{b_1}$	USP XX 1980	USP XXI 1985
1	4.6 - 48.5	11.9	57.8	3.224	FAIL	FAIL
2	9.2 - 16.1	10.2	10.3	3.751	FAIL	FAIL
3	0.1 - 29.0	11.2	63.2	0.600	FAIL	FAIL
4	4.3 - 18.6	11.9	25.4	0.257	FAIL	FAIL
5	9.2 - 12.9	10.9	7.6	0.343	PASS	PASS
6	9.5 - 13.9	11.5	8.2	0.471	FAIL	FAIL
7	9.4 - 12.2	11.0	5.0	0.017	FAIL	FAIL
8	8.1 - 11.2	9.7	7.1	0.088	PASS	PASS
9 (a)	9.1 - 16.8	10.2	11.5	3.761	PASS	PASS
9 (b)	9.0 - 11.3	9.8	5.0	1.134	PASS	PASS
9 (a+b)	9.0 - 16.8	10.0	9.2	4.427	PASS	PASS
10	8.9 - 53.5	10.8	57.2	6.737	FAIL	FAIL
11	6.9 - 9.6	8.0	6.9	0.419	FAIL	FAIL
12	8.5 - 13.4	10.0	8.3	1.217	PASS	PASS
13	8.7 - 15.1	9.8	12.0	2.708	PASS	FAIL
14	8.5 - 29.0	10.3	29.4	4.892	PASS	PASS
15	8.9 - 25.3	10.2	23.1	5.500	PASS	PASS

BATCH NUMBER 9(a) AND 9(b) ARE SAMPLES WITHDRAWN FROM THE SAME BATCH, EACH OF 50 TABLETS.

CV , COEFFICIENT OF VARIATION. $\sqrt{b_1}$, COEFFICIENT OF SKEWNESS. FOR LIMITS OF $\sqrt{b_1}$ SEE TEXT.

passed both tests, i.e. the USP XX (1980) and USP XXI (1985) C.U. Tests. The reasons could be summarized as follows :

1 - The tablets which are having drug content outside the specified limits are not among the first 10 units required by the test.

2 - The coefficient of variation of the first 10 tablets is lower than the specified one, 6%.

Another argument which can be used to critisize the C.U.Tests is illustrated by the following example. Assuming the normal distribution of drug content in tablets, the probability of having tablet with drug content outside ± 25% of USP XXI (1985) C.U. Test limits is zero (28). Checking the coefficient of skewness of batch number 5 and the Lilliefors graph (Fig. 1), the distribution of this batch is considered a reasonable approximation of a normal distribution. The batch sample of 50 tablets include one tablet outside the limits of ± 25% and 5 tablets outside the limits of the specified potency mean (102.5%) according to the USP XXI (1985) C.U.Test. These 6 tablets are number 28, 32, 36, 43, 46 and 48 in the 50 tablets sample. The first 10 tablets of batch number 5 have drug content within the specified requirements, consequently the batch has been accepted by the USP XXI (1985) C.U.Test.

Applying the proposal of Bolton (21), by calculating the weighted average $\bar{X}_w$: taking the first 10 tablets to calculate $\bar{X}$ CU and the rest 40 tablets to calculate $\bar{X}$ composite, then the $\bar{X}_w$ will be equal to 10.88 µg. Thus, batch number 5 will be not accepted because the mean itself is far from the target value recommended by the proposal, 100% of the label claim inspite of the CV of the first 10 tablets being equal to 5.4%.

Batches number 9(a), 9(b), 12, 14 and 15 have been prepared from cohesive EE powder (mean particle size 7.9 µm). According to random mixing theory (29), this particle size distribution of drug powder, if well dispersed, will produce tablets with

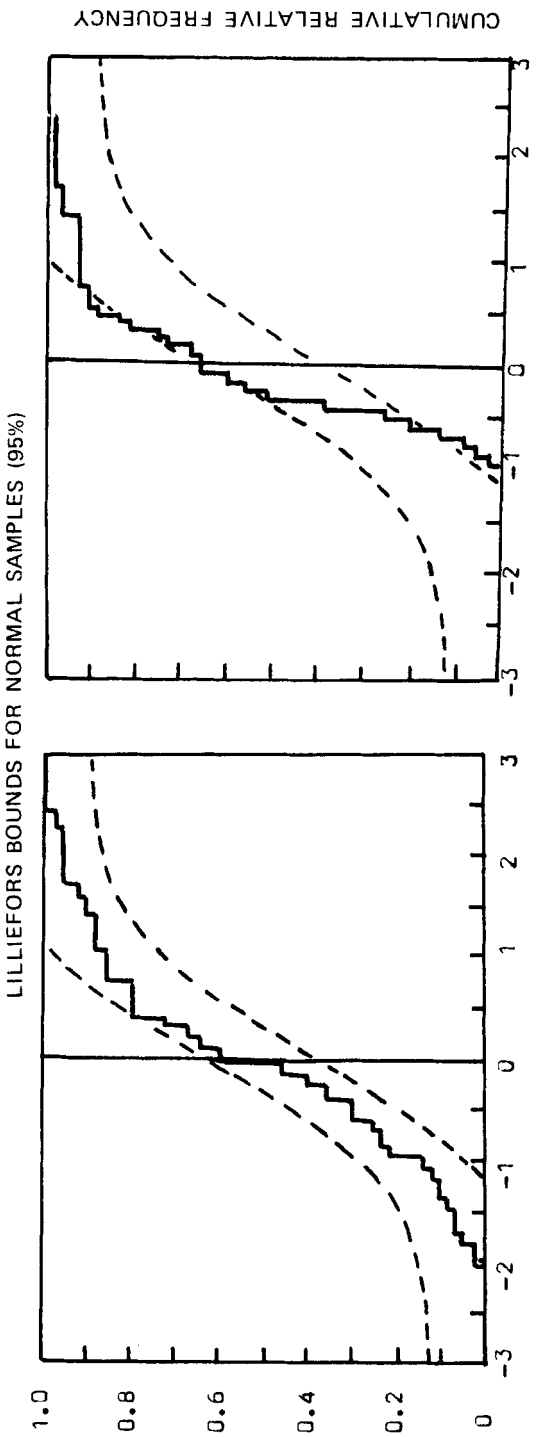

FIGURE (1)

LILLIEFORS GRAPH PLOT FOR BATCH NUMBER 5.
(SAMPLE SIZE, 50 UNITS).

FIGURE (2)

LILLIEFORS GRAPH PLOT FOR BATCH NUMBER 9a.
(SAMPLE SIZE, 50 UNITS).

pharmaceutically acceptable content uniformity (30). However , because of the cohesive nature of the drug powder, drug agglomerates have not been well dispersed. Consequently, positively skewed distributions have been produced. Positively skewed distributions are considered pharmaceutically unacceptable because tablets will be containing unit doses with high drug content (1).

Interpretation of data (Table 2) indicates that, there is a high probability of withdrawing samples showing low CV values particularly when the sample size is small, e.g. less than 50 units. This is true, because the number of drug agglomerates is very small relative to the number of tablets. Accordingly , evaluation of content uniformity of tablets containing potent drugs is very difficult and should be accompanied by examination of the type of distribution. The defect in the official C.U.Tests is not only due to the small sample size in the first step. The argument could be explained by assuming the C.U.Test is applied according to the following conditions :

1 - One stage inspection plan.

2 - Sample size of 30 units.

3 - Coefficient of variation of 6%.

4 - Other limits follow USP XXI (1985) C.U.Test.

The results have been found to be as follows :

1 - Batch number 9(a) fails, it shows CV equal to 13.3%.

2 - The second sample of batch number 9, i.e. 9(b) passes,it shows CV equal to 4.7% and fulfills the other requirements.

3 - Batch number 12 passes, it shows CV equal to 5.9% and fulfills the other requirements.

4 - Batch number 14 fails, it shows CV equal to 15.4%.

5 - Batch number 15 passes, it shows CV equal to 5.6% and fulfills the other requirements.

Thus, increasing the sample size increases the efficiency of the test as already pointed out in many reports (17, 18). However, three pharmaceutically unacceptable batches have been accepted. The argument is not related to the sample size only, it is also concerned with the type of distribution as pointed out before.

Lilliefors test for normality and skewness test have been used to examine the type of distribution of batch number 9 . In order to impart more certain ty on the results the sample size is increased to 100 units. All samples exhibit positively skewed distributions (table 2) which have been supported by Lilliefors test (Fig 2, 3 and 4). Lilliefors graphs indicate evidences of deviating from normality, this is obvious for 9(a) in Fig 2 , T_2 = 0.188 and the cumulative relative frequency curve crosses bounds for normal samples. Sample 9(a + b) behaves similarly as shown in Fig 4 , T_2 = 0.25 and cumulative frequency curve crosses bounds for normal samples. However, Lilliefors graph for 9(b) in Fig 3 doesnot show the same extent of deviation from normality, T_2 = 0.150 and the cumulative relative frequency curve is within the bounds of normal sample. Taking into account the value of the coefficient of skewness for 9(b), 30 tablets ($\sqrt{b_1}$ = 1.333) and 50 tablets ($\sqrt{b_1}$ = 1.134) the distribution is significantly skewed and considered deviating from normality.

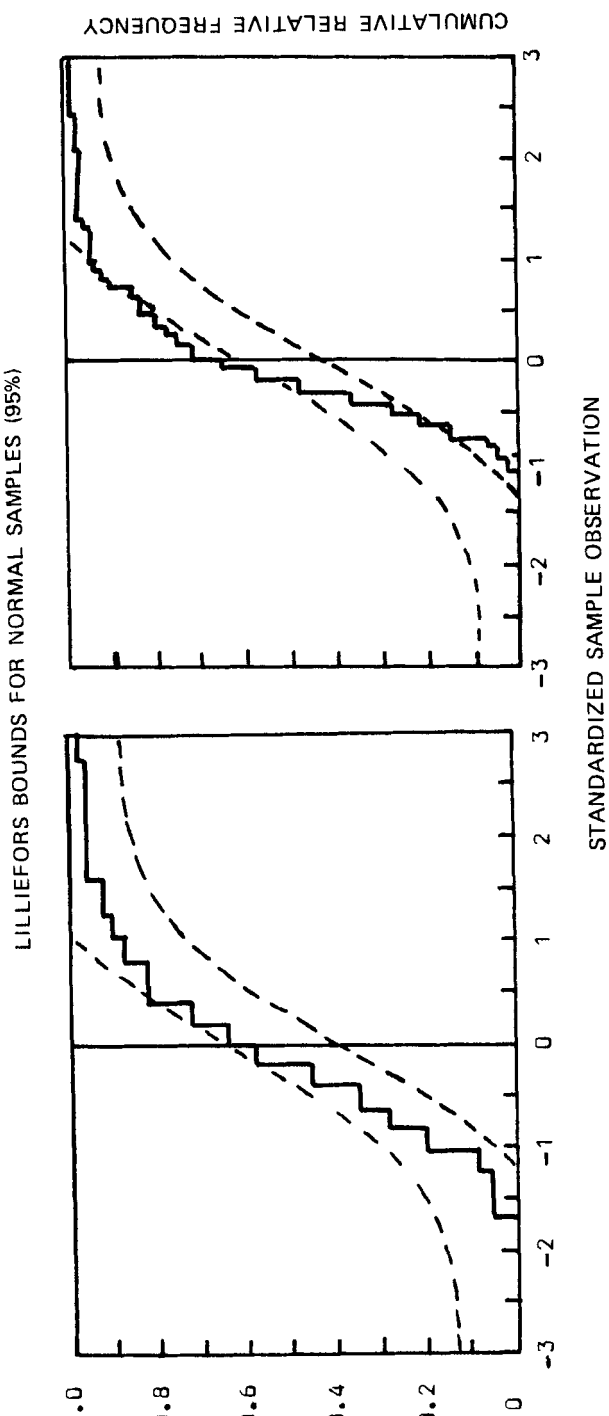

FIGURE (3).

LILLIEFORS GRAPH PLOT FOR BATCH NUMBER 9b.
(SAMPLE SIZE, 50 UNITS).

FIGURE (4).

LILLIEFORS GRAPH PLOT FOR BATCH NUMBER 9 a+b
(SAMPLE SIZE, 100 UNITS).

Batches number 12 and 13 have been prepared under similar conditions to batch number 9 except the time of wet mixing during granulation stage has been increased from 5 minutes (B9) to 15 minutes and 30 minutes for B12 and B13 respectively. Results as shown in Table 2 indicate that there is no significant difference between the CV values of the three batches. Furthermore , all these batches exhibit positively skewed distributions . Consequently, the three batches 9, 12 and 13 have similar properties with respect to the distribution of drug content within the batch. Then, if B12 is examined in more details using both Lilliefors test and skewness test, the following results have been obtained :

1 - When the sample size is 50 tablets : The Lilliefors test as shown in Fig 5 , indicates that the evidence is not sufficient to conclude that the distribution is non-normal because T_2=0.075 and the cumulative relative frequency curve doesnt cross the bounds of 50 units. Further examination by skewness test , the value of $\sqrt{b}_1$ is 1.217 which indicates positively skewed distribution, i.e. deviated from normality. This is due to the presence of one tablet containing 13.4 µg withdrawn with the last 10 tablets . It should not be considered as an outlier, because it is interpreted as an indication of the presence of drug agglomerates in powder mixes containing cohesive drug powders as pointed out in earlier reports (1,2).

2 - When the tablet containing 13.4 µg (maximum drug content) is removed : The sample size becomes 49 tablets instead of 50 tablets. The mean drug content is 9.97 µg, CV is equal to 6.8%

and $\sqrt{b_1}$ is equal to 0.053 which indicate that the distribution is approaching normality. The range of drug content in tablets 8.5 - 11.4 µg, consequently the batch passes C.U.Test.

As indicated above batches number 9, 12 and 13 have similar properties, now considering B13 sample as another sample of B12, then the examination of the distribution of B13 shows significant deviation from normality (Fig 6 and Table 2).

Collecting these data together for B9 (a) , B9 (b), B9(a+b), B12 and B13, it is confirmed that the problem of determining the content uniformity of such tablets is that there is a possibility of withdrawing samples of tablets tend to only show a distribution approaching normality with low values for coefficient of variation of drug content. Such type of batches will be having chances of passing easily USP XXI (1985) C.U.Test. The type of distribution should be carefully examined and assessed, otherwise misleading conclusions about the uniformity of tablets containing potent drugs could be obtained.

Applying this approach of data analysis on batch number 15, a batch which has been prepared using the same cohesive drug powder and experimental conditions employed for preparation of B9 except the mixer is different. The first 30 tablets of the sample gives mean content equal to 9.9 µg per tablet, CV equal to 5.6% and $\sqrt{b_1}$ equal to 1.254. Lilliefors test indicates deviation from normality as shown in Fig 7 (T_2 = 0.2, the cumulative frequency curve crosses the bounds for normal sample of 30 units). Increasing sample size to 50 units, the mean drug content is 10.2 µg, i.e. within the

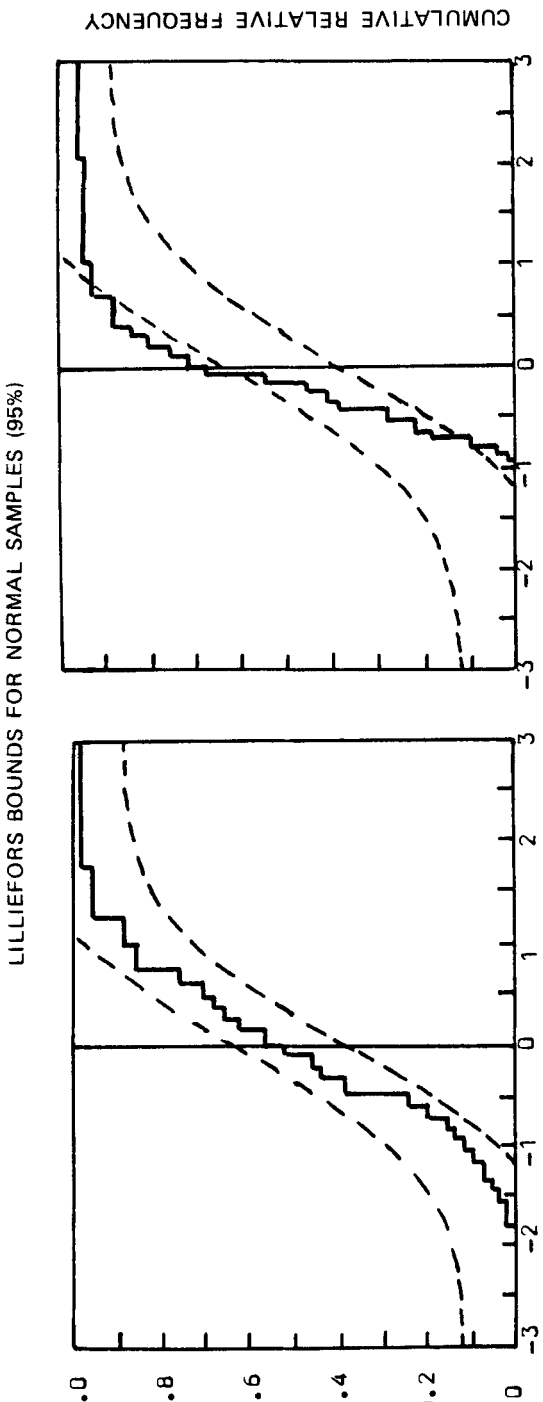

FIGURE (5)

LILLIEFORS GRAPH PLOT FOR BATCH NUMBER 12. (SAMPLE SIZE , 50 UNITS).

FIGURE (6)

LILLIEFORS GRAPH PLOT FOR BATCH NUMBER 13. (SAMPLE SIZE , 50 UNITS).

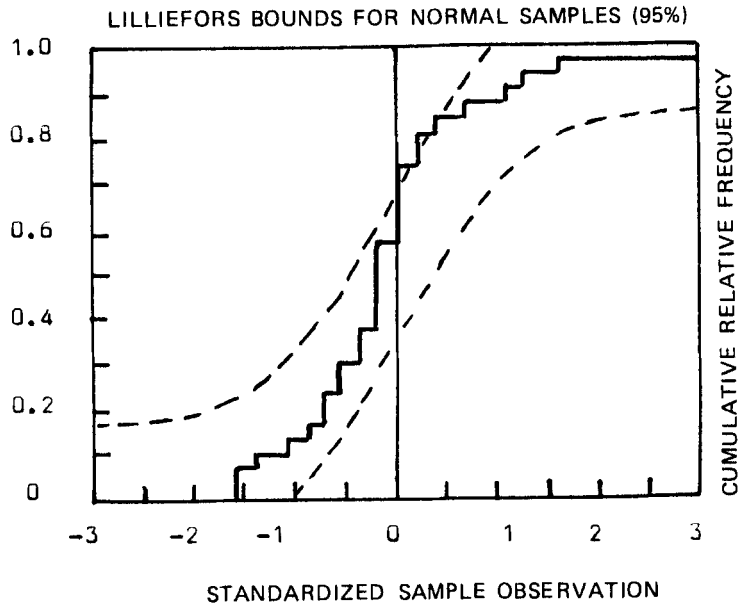

FIGURE (7)

LILLIEFORS GRAPH PLOT FOR BATCH NUMBER 15.
(SAMPLE SIZE , 30 UNITS).

limits, the CV increases significantly 23.1% and the $\sqrt{b_1}$ indicates significant positively skewed distribution (Table 2). This is due to the presence of unit doses with high drug content 15.1 and 25.3 μg per tablet. However, this batch has passed USP XXI (1985) C.U.Test.

Referring to Bolton's proposal (21), if the first 10 tablets are considered to calculate $\bar{X}$ CU and the rest of 50 tablets are considered to calculate $\bar{X}$ composite, the $\bar{X}_w$ of batches number 9 (a), 9(b), 12, 14 and 15 will be fallen within the specified limits of the proposal. Although these batches are

pharmaceutically unacceptable, all have been accepted by the proposal. This is because the $\bar{X}$ CU and $\bar{X}$ composite are close to the target mean (label claim) and the CV of the first 10 tablets is low, mostly close to 5% .

CONCLUSION

In conclusion, any test for content uniformity depends on small sample size and neglect examination of the type of distribution will not be efficient as a tool to validate the manufacturing process of solid dosage forms containing potent drugs. The same argument is true for any official tests which are used to control content uniformity of these drugs.

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