DRUG DEVELOPMENT COMMUNICATIONS, 1(6), 517-537 (1974-1975)

A GENERALIZED APPROACH TO CONTENT UNIFORMITY ASSAYS

S. A. Fusari, D. Dittmar, and C. H. Perizzo

Parke Davis and Company, Detroit, MI 48232

ABSTRACT

In anticipation of the Content Uniformity requirements for USP XVIII and NF XIII, we developed a general procedure for the assay of intact capsules containing a variety of drugs. Using this procedure, whole capsules are dissolved in an appropriate aqueous medium and the drug dialyzed into a suitable recipient stream. The dialyzate is diluted if necessary, and then pumped to a spectrophotometer for final reading and assay. The use of this technique completely eliminates the need for opening capsules and making quantitative transfers of the drug contents.

This technique was so successful that it was extended to tablets containing both water-soluble and water-insoluble drugs. Water-insoluble drugs such as steroids are handled by

conducting the dialysis from aqueous alcoholic solutions. Excellent results have been obtained using this technique on chemically dissimilar compounds; examples of these assays will be given.

METHODS

We wish to discuss the application of a very old technique to the solution of a very modern problem. The technique is dialysis and the problem is Content Uniformity assays. Before proceeding further with the details of the work we did, we would like to relate some of the background work which ultimately led to the use of dialysis as a general type solution to the problem of Content Uniformity assays.

In anticipation of the Content Uniformity requirements for certain drug dosage forms in USP XVIII and NF XIII, we, like many others, started working on automated methods to ease the burden of the large number of assays which would be generated by the new test requirements. Our first thought was to try to adapt whatever assays we already had on these drugs to Content Uniformity assays. This was practical only in those cases where tablets were involved. It proved wholly impractical for the assay of capsules because it became apparent that we had to make quantitative transfers of the capsule contents

prior to starting the assays. Aside from being time consuming, making quantitative transfers proved impractical because most of our capsules are Kapseals, that is, the body and the cap are sealed together by a gelatin band. In these cases, the only way to make a quantitative transfer involved cutting the Kapseal and very carefully transferring the entire contents to a suitable vessel. At best, this procedure is fraught with difficulty, particularly where the capsule shell tends to become brittle. Many others have probably gone through the experience of trying to cut a capsule and having the capsule shatter, while at the same time sending puffs of powder into the air. At best, this is a very trying experience and considerable care has to be given to make it quantitative. In the case of capsules which pull apart easily, a lot of time still needs to be consumed in making quantitative transfers. The whole idea of opening capsules was rejected immediately because of the time and trouble involved in these manipulations.

Our first approach was to dissolve the intact capsule in a suitable aqueous medium and then extract the desired drug with an immiscible solvent using the Auto Analyzer. Since this procedure had always worked well on certain tablets and/or granu-

lations, we hoped that it would work equally well on a dilute solution containing dissolved gelatin as well as our drug.

We hoped that because of the gentle extraction conditions prevailing in the Auto Analyzer that we would avoid the formation of emulsions which so often plague manual extractions of gelatin-containing solutions.

We tried this approach and while it showed some promise, the idea was soon abandoned because gelatin would precipitate slowly when it came in contact with the immiscible solvent and it eventually clogged our extraction coils.

It thus became apparent that what we needed was not a piecemeal or individual drug assay approach to Content Uniformity assays, but instead, a general solution to the entire problem. Since our primary concern at the time was the assay of capsule dosage forms, we decided to use a method which would separate the gelatin from the drug we wished to assay. Dialysis seemed to be the most obvious and logical answer. Dialysis would effectively separate the gelatin from the drug we wished to assay; the dialyzate containing the desired drug could be treated as a "purified" solution of drug which could then be manipulated in whatever way seemed necessary for the eventual assay of the drug. This approach was tried and proved highly successful. It has been applied to many different drugs; it

proved so successful in the case of capsules that its used was automatically extended to tablets with the same degree of success. Due to the simple nature of this process, the use of dialysis enabled us to progress very rapidly with Content Uniformity assays. One basic manifold was employed with only minor variations to adjust dilutions. The technique we developed involves dissolving the intact capsule or tablet in an appropriate aqueous medium and then dialyzing the drug to be assayed into a suitable recipient stream. The dialyzate is diluted, if necessary, and then pumped to a spectrophotometer for final readout and assay. The use of this technique completely eliminates the need for opening capsules and making quantitative transfers of the drug contents.

Dialysis has also been extended to the assay of tablets containing water-insoluble drugs such as steroids. In this case, the dialysis is conducted from hydroalcoholic solutions into aqueous alcoholic recipient streams. Excellent results have been obtained. It is admitted that in the case of tablets containing either water-soluble or water-insoluble drugs, we could have also accomplished the assay by using the continuous filter instead of the dialyzer. However, we have found that the use of the dialyzer eliminates at least one dilution and allows for better wash between samples because less "lag" is encoun-

tered through the entire system. Furthermore, the absorbance given by individual samples is better than 95% of the steady state absorbance when using the dialyzer as opposed to only about 85% when using the continuous filter.

RESULTS AND DISCUSSION

Since our drug is contained in a tablet or capsule along with many other compounds, we have found it convenient to make up our standards in a placebo containing all the other ingredients of the tablet or capsule. This is to compensate for volume changes in the preparation of solutions and osmotic pressure differences for dialysis. Since our assays have all been concentration plot, only one standard concentration point is necessary. However, we purposely intersperse a standard before and after every 10 samples being assayed. The A values for the standard have varied hardly at all during the day's run. The ultraviolet curves obtained on dosage form dialyzates have exactly matched the ultraviolet curves of the pure drug.

The assay procedures have all been found to be extremely reproducible and highly accurate. The values obtained have always compared very closely to values obtained using our

present manual methods. Laboratory prepared samples containing known quantities of drug added to the dosage form placebo were used to study the confidence limits of the assay. These studies have indicated that 3 values for these assays have all been from 1-2%. Ten replicate assays of the same sample have shown the precision of these assays to be excellent; 3 values are usually about 1%.

Before proceeding further with additional details, we wish to cite some advantages of this procedure.

1. Intact capsules may be assayed regardless of whether they are natural, colored or opaque capsules.

2. The procedure is extremely versatile. The same basic manifold may be used to assay many chemically dissimilar compounds. Only very minor variations in the basic manifold have been found necessary for the assay of such widely dissimilar compounds as phenobarbital, diphenhydramine, sodium saccharin and prednisone.

3. Extraction of the drug has been entirely eliminated for all dosage forms we have worked with up to now. This has been very beneficial because aside from simplifying the manifold, it has lessened the occurrence of tubing failure during

analysis. As you all know, tubing failure is one of the major nuisances associated with automated analysis.

4. All assays have shown excellent accuracy and precision at minimum rates of 20 assays/hour.

5. The procedure is simple and virtually trouble free.

To digress for a moment, in talking to many people concerning our approach to Content Uniformity assays, it became apparent that very few people had ever used the dialyzer module in routine analysis. In fact, some people do not even own this module. We think the reason for this is that originally the dialyzer was used primarily as a filtering device; then, when the continuous filter became available, it largely replaced the dialyzer in spite of the fact that the dialyzer was capable of providing a service over and above mere filtration. This is an example of how a step forward in one direction occasioned a step backward in another. At any rate, we are very happy that a dialyzer module was available in our laboratories as part of an older set-up that had been used to run analyses for glucose, glycerol, ammonia etc. on fermentation broth samples. It was partially this latter use which suggested the use of dialysis for running Content Uniformity assays.

For the remainder of the paper, we would like to direct your attention to the figures, since they illustrate all of the points I have discussed.

Figure 1 shows the basic manifold which has been used in these assays. This manifold is used for substances in which the prepared samples are dialyzed directly and the dialyzate run directly to the spectrophotometer. This can be done for samples where the external dilution in sample preparation and the a (1%, 1 cm.) value of the substance given a dialyzate which does not require further dilution. Drugs assayed using this manifold include saccharin, oxymetholone, norethindrone, norethindrone acetate, methyltestosterone and prednisone.

Figure 2 shows a modification of the manifold used in Figure 1. The only change occurs after dialysis; the dialyzate is resampled and diluted prior to being read in the spectrophotometer. Using this manifold, certain substances such as Diphenylhydantoin, which is dialyzed as the sodium salt, can be converted to the acid form by diluting the resampled dialyzate with an acid alcohol solution before it is read in the spectrophotometer. Drugs assayed using this manifold are Diphenylhydantoin, Sodium Diphenylhydantoin, Diphenhydramine,

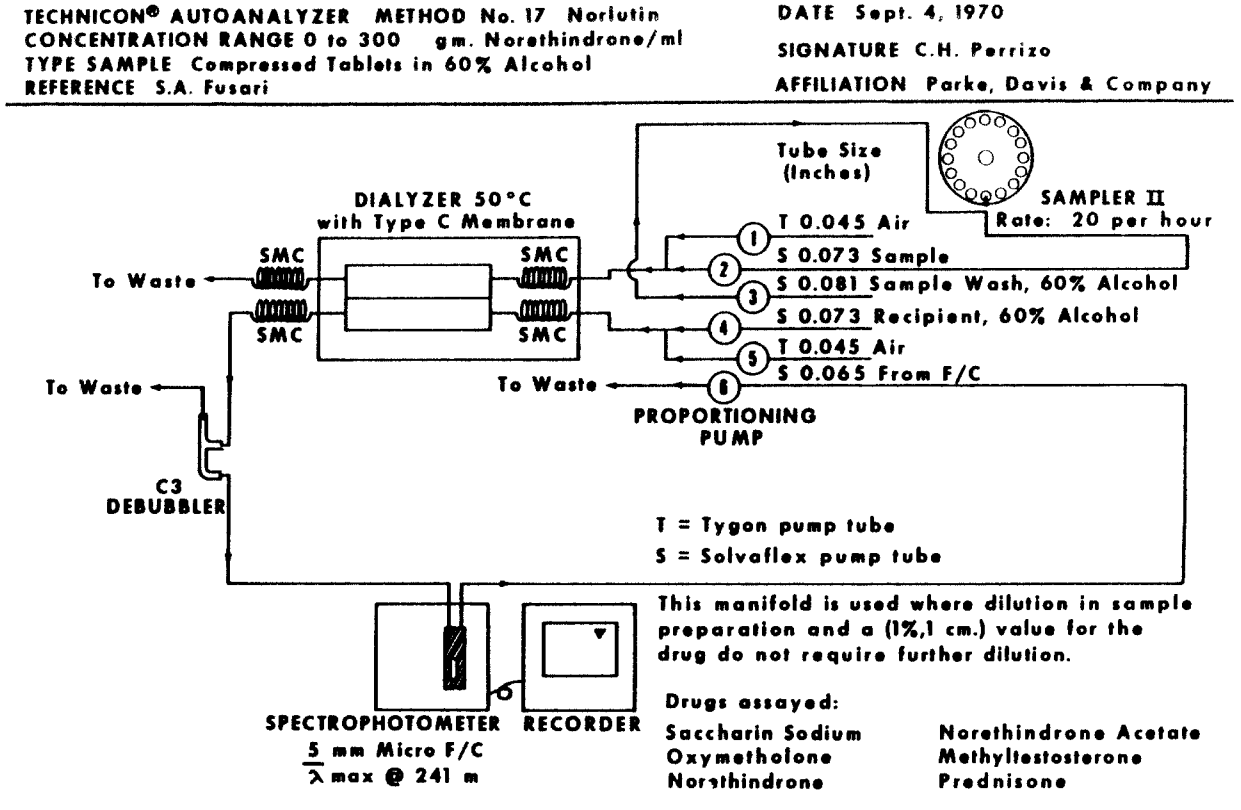


FIGURE 1
Basic Manifold

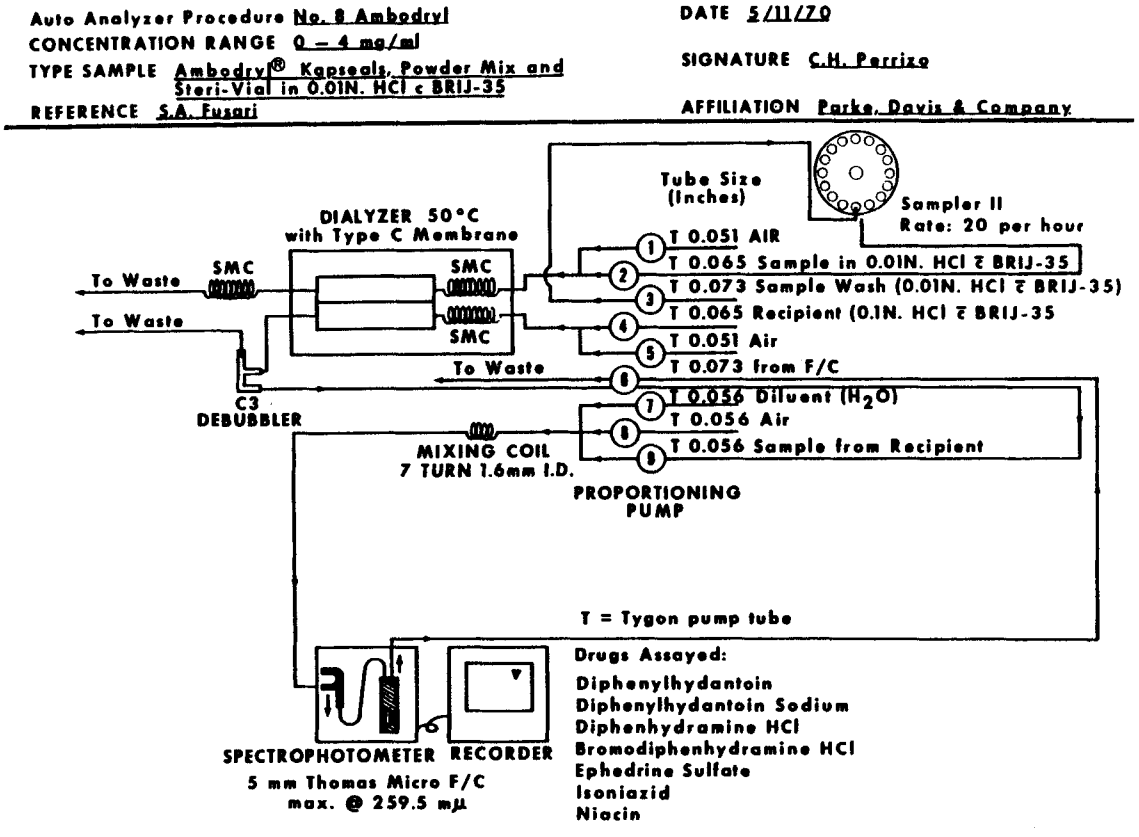


FIGURE 2
Modified Manifold

Bromodiphenhydramine Hydrochloride, Ephedrin Sulfate, Isoniazid and Niacin.

Figure 3 shows a modification of the manifold shown in Figure 2. The only change in the manifold is the addition of a sample diluent line prior to dialysis. This manifold was used for substances with high a(1%, 1 cm.) values where the sample dilution, during preparation, was kept at a minimum. Drugs assayed using this manifold include Phenobarbital, Propylthiouracil and Caffeine.

Table I is a recapitulation of the drugs assayed so far, using the dialysis technique. Notice the wide variety of substances assayed.

Figure 4 shows a comparison of the ultraviolet curves of the pure compound and the drug found in the dialyzate stream. In every case so far, it has been found that the ultraviolet curve of the dialyzate exactly matches the ultraviolet curve of the pure drug itself, or of the ultraviolet curve of the drug which has been dialyzed from a drug-placebo mixture. In most instances, the match is with the pure drug itself and no compensation is required by the placebo constituents.

Table II shows the results obtained when we ran a Confidence Limits study on samples. Known amounts of drug ranging

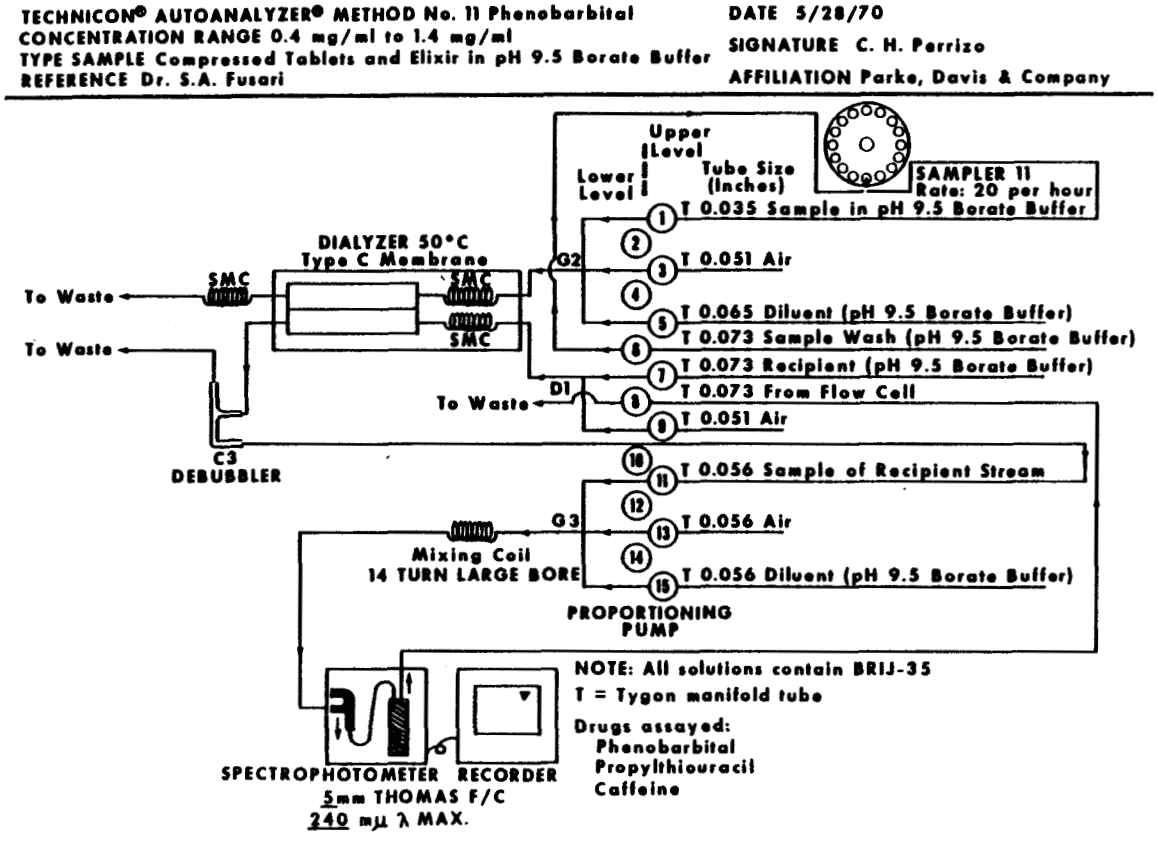


FIGURE 3
Modified Manifold

TABLE I

DRUGS ASSAYED

Capsules

Diphenhydramine (Benadryl)®
Bromodiphenhydramine Hydrochloride (Ambodryl)®
Diphenylhydantoin
Diphenylhydantoin Sodium (Dilantin)®
Ephedrine Sulfate

Compressed Tablets

Isoniazid (Niconyl)®
Niacin (Nicotinic Acid)
Saccharin Sodium
Oxymetholone (Adroyd)®
Norethindrone (Norlutin)®
Norethindrone Acetate (Norlutate)®
Methyltestosterone
Prednisone (Paracort)®
Phenobarbital
Propylthiouracil
Caffeine (Aspirin Compound)

from 75 to 125% of Label Claim and differing by about 2% in most instances, were added to a placebo in order to stimulate dosage forms containing varying amounts of active ingredients. As can be seen from the figure, we have no difficulty at all distinguishing between the various samples. The average recovery is 100.2% with a range from 99.1 - 101.4% and 3 limits for the recovery are 1.75%. This is typical for the assays we have been running.

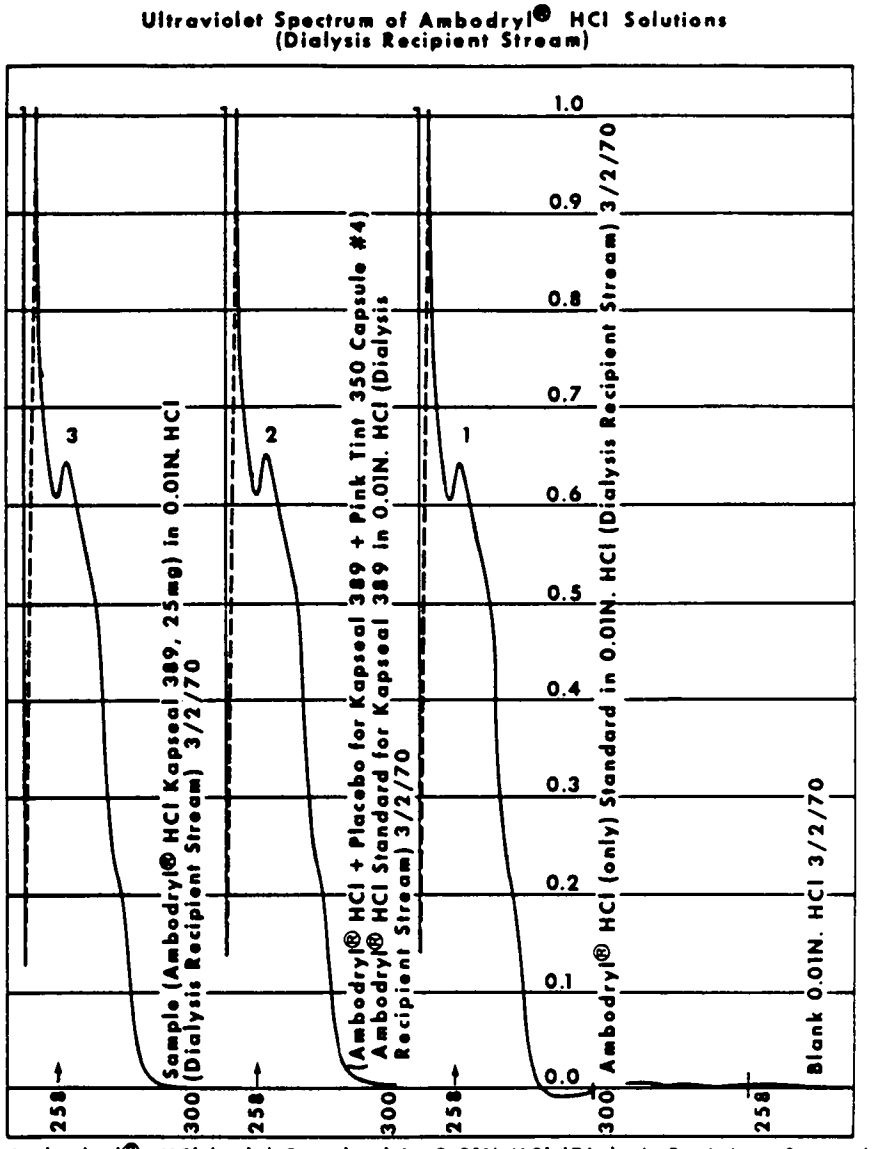


FIGURE 4

Ultraviolet curves of pure and dialyzed material

TABLE II

CONFIDENCE LIMITS STUDY

Auto Analyzer Assay for Ambodryl[R] HCl in Kapseal 389

A A Assay of Known Weighed Samples of Ambodryl[R] Hydrochloride
(to which Placebo for Kapseal 389 and empty gelatin capsules have been added)

Sample	mg Added	A		mg Recovered		% Recovery		Average
		1st	2nd	1st	2nd	1st	2nd	Recovery
1	75	0.502	0.502	75.5	75.8	100.7	101.1	100.9
2	80	.543	.533	80.3	80.5	100.4	100.6	100.5
3	85	.566	.567	85.1	85.6	100.1	100.7	100.4
4	87	.580	.580	87.2	87.6	100.2	100.7	100.5
5	89	.599	.592	90.1	89.4	101.2	100.4	100.8
6	91	.613	.603	92.2	91.1	101.3	100.1	100.7
7	93	.627	.615	94.3	92.9	101.4	99.9	100.7
8	95	.633	.628	95.2	94.9	100.2	99.9	100.1
9	97	.643	.642	96.7	97.0	99.7	100.0	99.9
10	99	.660	.653	99.2	98.6	100.2	99.6	99.9
11	101	.672	.666	101.0	100.6	100.0	99.6	99.8
12	103	.687	.685	103.3	103.5	100.3	100.5	100.4
13	105	.698	.693	105.0	104.7	100.0	99.7	99.9

14	107	.721	.710	108.4	107.3	101.3	100.3	100.8
15	109	.731	.720	109.9	108.8	100.8	99.8	100.3
16	111	.744	.735	111.9	111.0	100.8	100.0	100.4
17	113	.750	.743	112.8	112.2	99.8	99.3	99.6
18	115	.763	.755	114.7	114.0	99.7	99.1	99.4
19	120	.794	.791	119.4	119.5	99.5	99.6	99.6
20	125	.827	.822	124.4	124.2	99.5	99.4	99.5

Standard for 1st run: A = 0.665 = 40 = 100.2%, range 99.1-101.4%

Standard for 2nd run: A = 0.662 = 0.584%

= 1.75%

Thus: 99% Confidence Limits = $\pm$ 1.75%

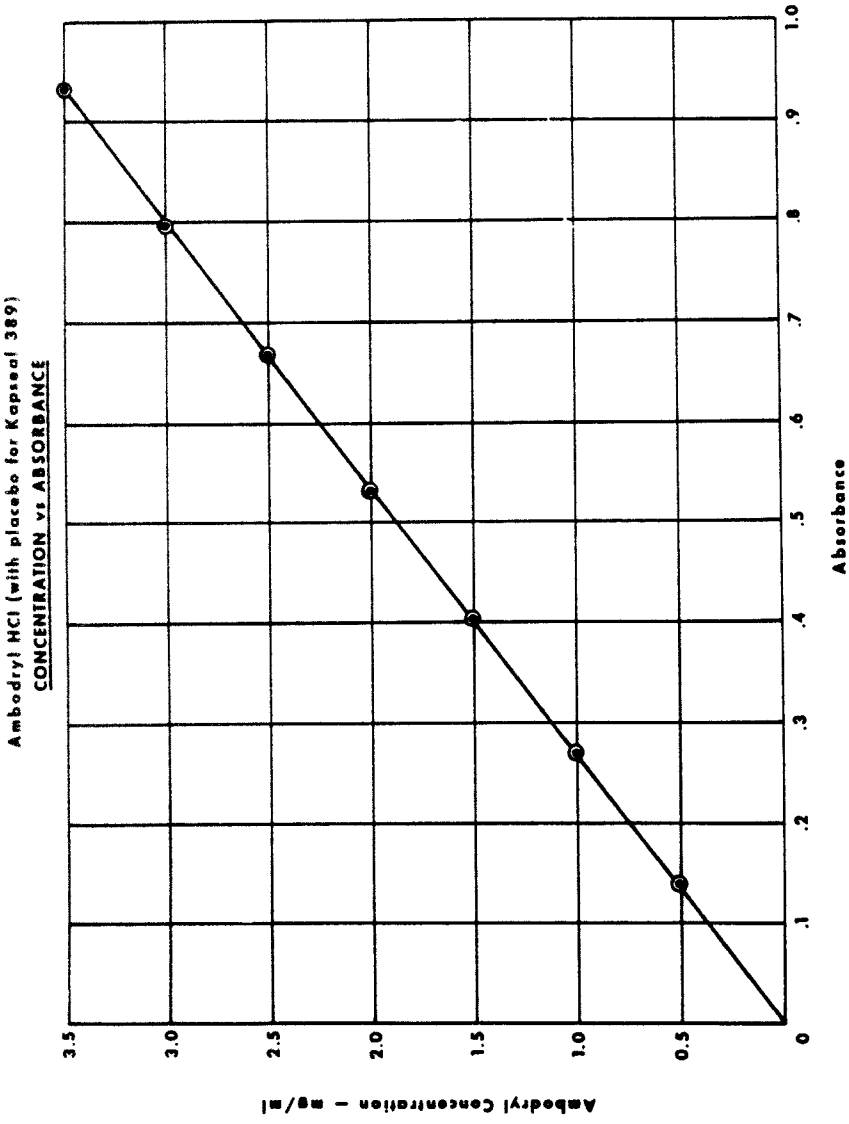
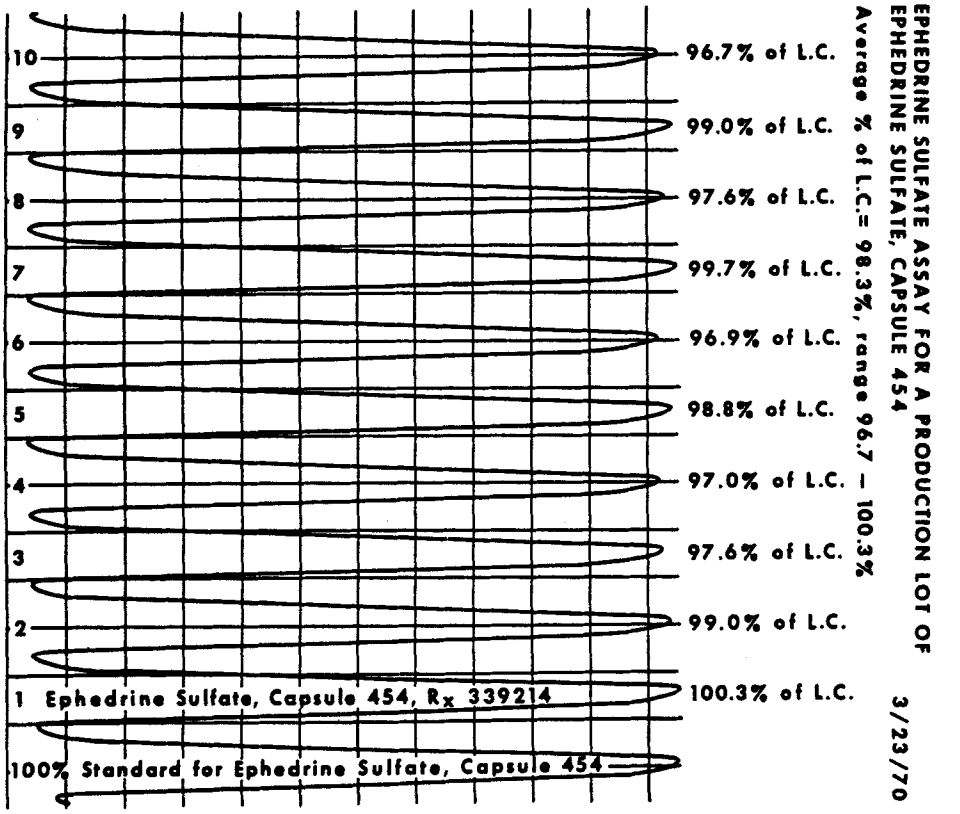


FIGURE 5
Linearity plot for Bromodiphenhydramine

Figure 6 presents an actual run done on 10 Ephedrine Sulfate Capsules at 20 samples/hour. These capsules show an average assay of 98.3% with a range of 96.7% of L.C. to 100.3% of L.C. These capsules easily pass the Content Uniformity requirements.



FICURE 6

Ephedrine Sulfate Capsules

Figure 7 is an actual run done on 10 Norethindrone Acetate Tablets at 20 samples/hour. These tablets show an average assay of 99.1% of L.C. with a range of 95.7% of L.C. to 100.9% of L.C. They, too, easily pass the Content Uniformity requirements.

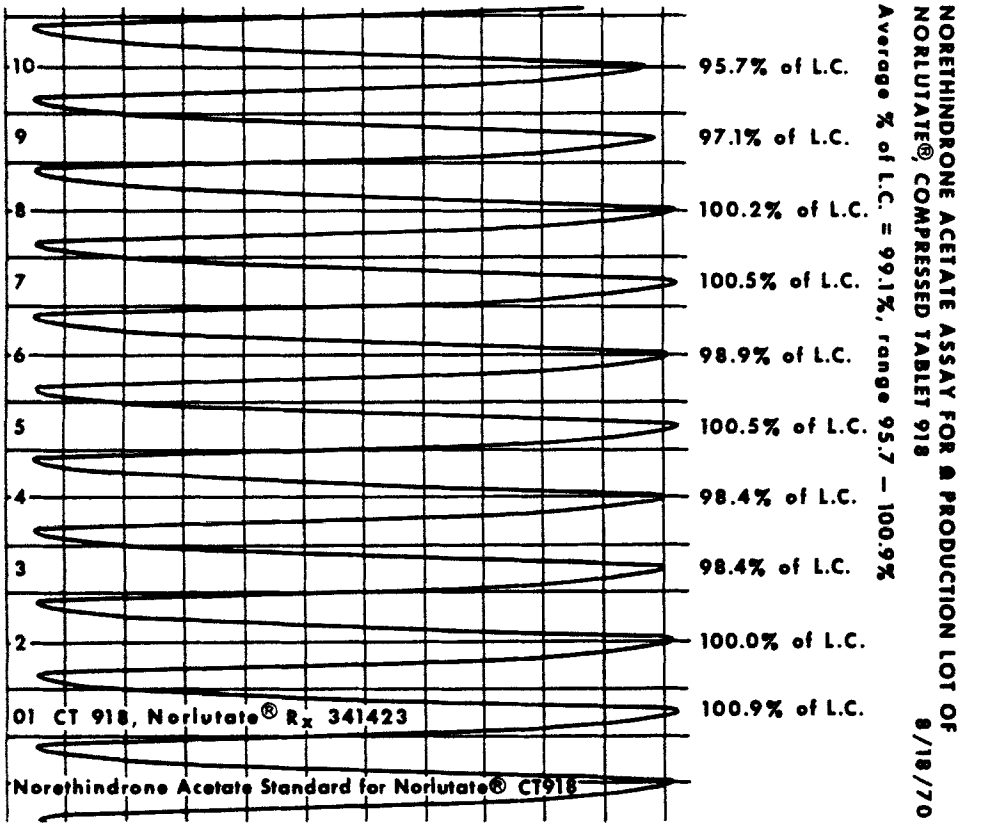


FIGURE 7

Norethindrone Acetate Tablets

We shall not bore you by presenting an endless array of figures with essentially the same data for different drug dosage forms. As can be seen from the figures, the method works extremely well and it is quite versatile. While we are sure that there are other ways of achieving the same end, particularly with tablets, this approach has been extremely simple, versatile and efficient. It has certainly helped us to progress rapidly on Content Uniformity assays.