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APPLICATION OF QUANTITATIVE THIN-LAYER CHROMATOGRAPHY IN DRUG ASSAY AND STABILITY TESTING

DETERMINATION OF CODEINE PHOSPHATE, NOSCAPINE, DIPHENHYDRAMINE HYDROCHLORIDE, PHENYLEPHRINE HYDROCHLORIDE, CAFFEINE, ETOFYLLIN, PHENOBARBITAL AND THIAMINE HYDROCHLORIDE BY *IN SITU* REFLECTANCE SPECTROSCOPY

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SUMMARY

The application of quantitative thin-layer chromatography involving scanning of remission in the assay of drugs in pharmaceutical preparations is described. The technique seems to be particularly useful in instances in which the usual analytical methods either cannot be applied or can be applied only with difficulty. Accuracy, reproducibility and the time required are discussed on the basis of the results of routine quality control tests. Possible applications of quantitative thin-layer chromatography in stability testing are also discussed.

INTRODUCTION

In the first Symposium held in 1971, we reported on the assay of drug mixtures by the scanning of remission[1]. We also discussed the separation and assay of drug mixtures that either could not be determined or could be determined only with difficulty by the usual analytical methods. Because our experiences with quantitative thin-layer chromatography (TLC) were very satisfactory, we used this method in other analytical determinations, especially in quality control. In the present paper we do not describe the technical details of the method, but rather, on the basis of examples, show why quantitative TLC determinations seem to be particularly useful in many instances.

The simplest and most rapid method for the assay of an active substance in a drug is to make measurements at the absorption maximum in the ultraviolet (UV) range. However, this method has the disadvantage that many substances absorb in this range and may cause interference. This also applies to the use of spectrophotometric analysis of multi-component mixtures and subsequent calculations, where overlapping bands may occur. Photometric measurements on a stained product by utilizing a reaction between the active substance and the reagent may be influenced by other active substances and/or excipients. When analytical difficulties of this

nature arise, quantitative TLC[1–8] is a practical method of solving these problems rapidly and satisfactorily. The substance to be analyzed is separated from interfering active substances and excipients by means of TLC and can be subsequently assayed. In our studies, we used the Zeiss chromatogram spectrophotometer as well as the Camag Z-scanner combined with the Zeiss PMQ II spectrophotometer, with comparable results.

SIMULTANEOUS ASSAYS

Simultaneous assay of codeine phosphate, noscapine and diphenhydramine hydrochloride in a drug mixture

Direct spectrophotometric measurements at the absorption maxima of the three substances in the UV range were not possible because of reciprocal interference. Owing to the unfavourable proportions of the active substances and the interfering excipients, the values obtained by spectrophotometric analysis of multi-component mixtures and subsequent calculations were not reproducible. The usual colour reactions for codeine, noscapine and diphenhydramine are based on a reaction with the nitrogen, which is, however, present in each of the three substances, so that this analytical possibility is also excluded. For these reasons, scanning of the three active substances after their separation on the chromatographic plate appeared to be useful. Cyclohexane–chloroform–diethylamine (90:18:12) was used as the solvent. Fig. 1 shows the good separation of the active substances, which is the most important prerequisite in quantitative *in situ* evaluations. The upper spots represent diphenhydramine, the centre spots noscapine and the bottom spots codeine.

The remission of the spots was scanned at the absorption maxima: codeine 285 nm, noscapine 313 nm and diphenhydramine 258 nm. The spots were evaluated horizontally in relation to the solvent flow. This technique permits the determination of three double samples and two standards for one substance within a shorter time than in a determination in the direction of solvent flow, because the slit, which is longer than the diameter of the spots, passes the substances without interruption. A

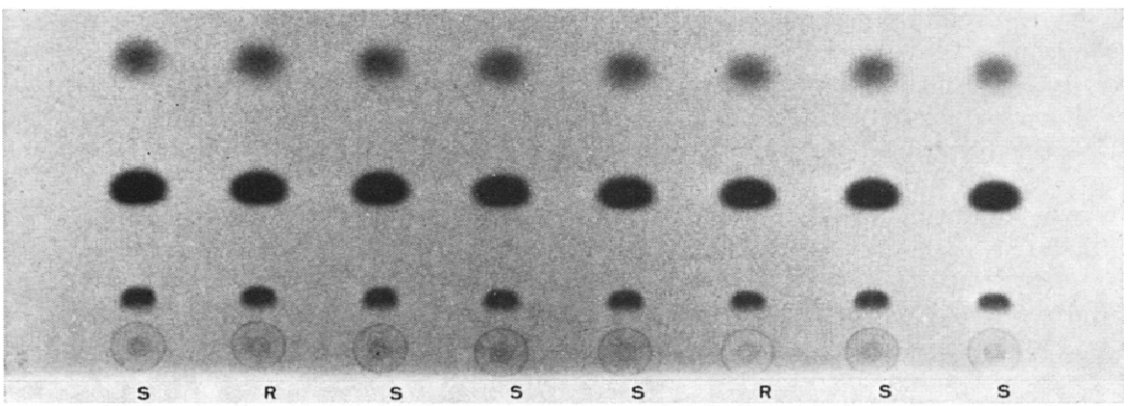

Fig. 1. TLC of a mixture of codeine phosphate, noscapine and diphenhydramine hydrochloride (S) on a thin layer of Silica Gel F_{254} (Merck) with cyclohexane–chloroform–diethylamine (90:18:12) as solvent. Chamber saturation; length of run 15 cm; pipetting volume 10 μl. Reference (R): codeine phosphate 20 μg + noscapine 50 μg + diphenhydramine hydrochloride 50 μg.

uniform length of run for all spots is a prerequisite for this rapid evaluation technique. In order to check this uniformity, we use silica gel plates with F_{254} fluorescence indicator. Prior to scanning, the plates are checked by means of a short-wave UV lamp, which renders visible all substances by fluorescence quenching.

Quantitative evaluations of the recorded peaks can be carried out according to the Kubelka–Munk equation or according to the simplified Tausch method[5], in which the amount of substance is plotted against the square of the remission peak area. The Vitatron recorder used permits the direct integration of the areas of the logarithmically scanned peaks[7]. This time-saving method of quantitative evaluation gives linear results in the range of amounts spotted. For more than a year, this method of spot evaluation, which we have also used in all subsequently described studies, has been applied to quality control in the assay of codeine phosphate, noscapine base and diphenhydramine hydrochloride in hard capsules. The method consists of shaking the capsules with 0.1 *N* HCl in order to dissolve the capsules and to convert the noscapine base into the soluble noscapine hydrochloride. Subsequently, the drugs are dissolved by the addition of methanol (70%); insoluble excipients are removed by filtration and 10 μl is spotted. For the statistical control of the method, 10 assays were carried out in one batch. The results are presented in Table I.

TABLE I

RESULTS IN ONE BATCH OF A MIXTURE OF CODEINE PHOSPHATE, NOSCAPINE AND DIPHENHYDRAMINE HYDROCHLORIDE (10 ASSAYS)

Time required: 1 h for 3 samples (1 double assay each); 2 h for 10 samples (1 double assay each).

Substance	*Label value (mg/capsule)*	*Found (mg/capsule)*	*Relative standard deviation (%)*
Codeine phosphate	20.0	20.6	3.7
Noscapine	50.0	51.8	2.9
Diphenhydramine hydrochloride	50.0	51.1	1.8

Quantitative assay of phenylephrine hydrochloride and caffeine

The absorption maxima of both substances are located at 273 nm and therefore could not be measured in the UV range. Also, in this instance there was the possibility of separation on the TLC plate, followed by quantitative scanning of remission. The two active substances were readily separated with *n*-propanol–25% ammonia solution (88:12)[9], which is an alkaline solvent. However, while the phenylephrine spot was easily determined with reproducible results, the reproducibility of the caffeine results was not satisfactory[10]. Benzene–diethyl ether–glacial acetic acid–methanol (60:30:9:1) improved the reproducibility of the caffeine results significantly, but the phenylephrine spot could not be evaluated, so that we were not able to analyze the two substances simultaneously on one plate. The analysis of phenylephrine hydrochloride was satisfactory only in the alkaline solvent on silica gel plates, and that of caffeine only in the acidic solvent. Within the concentration ranges spotted, the calibration curves were linear. Table II presents the results of 10 assays of one batch.

TABLE II

RESULTS IN ONE BATCH OF A MIXTURE OF PHENYLEPHRINE HYDROCHLORIDE AND CAFFEINE (10 ASSAYS)

Time required: 2 h for 3 samples (1 double assay each); 4 h for 10 samples (1 double assay each).

Substance	*Label value (mg/tablet)*	*Found (mg/tablet)*	*Relative standard deviation (%)*
Phenylephrine hydrochloride	5.0	5.07	2.7
Caffeine	50.0	50.2	2.5

INTERACTIONS BETWEEN THE ACTIVE SUBSTANCE AND EXCIPIENTS

Quantitative assay of etofyllin in soft capsules

The application of quantitative TLC may also be advantageous in instances in which rapid measurement of the active substances in the drug is not possible owing to the presence of interfering excipients. One of our products contains etofyllin, 7-(β-hydroxyethyl)theophylline, which is suspended together with another active substance in an oil–wax mixture in soft capsules. To separate the etofyllin from the lipophilic excipients, the latter had to be removed by repeated shaking with *n*-hexane, followed by a spectrophotometric determination. This procedure necessitates a time-consuming preparation of the capsules. The solvent *n*-propanol–ethyl acetate–water–butanol (40:30:30:20) permitted a good separation of the lipophilic and hydrophilic substances.

The capsules were shaken with 1 *N* HCl, diluted with ethanol and filtered, and the appropriate amounts were spotted and scanned quantitatively in remission. Ten assays were carried out in one batch according to both methods, and recoveries, reproducibilities and the times required in the two methods were compared (Table III).

TABLE III

RESULTS IN ONE BATCH OF ETOFYLLIN (10 ASSAYS)

Label value 55 mg per capsule.

Method	*Found (mg/capsule)*	*Relative standard deviation (%)*	*Time required (h)*
TLC	55.2	1.4	2
Spectrophotometry	56.6	2.4	5

In this connection we should point out the application of quantitative TLC in the raw material control of etofyllin. TLC showed an additional spot in one of the etofyllin lots delivered and further studies revealed that this spot consisted of the 9-isomer of 7-(β-hydroxyethyl)theophylline. As this isomer could be separated from the 7-(β-hydroxyethyl)theophylline with the solvent *n*-propanol–ethyl acetate–water–butanol (40:30:30:20), we scanned the spot quantitatively at 270 nm. This 9-isomer was isolated from the substance. The relative standard deviation of eight samples of the 9-isomer on one plate was 1.8%. In comparison with the isolated 9-isomer as a standard, the content in the raw material was 5.4%.

Assay of phenobarbital in capsules with unknown excipients

Another possibility for the application of chromatogram spectrophotometers is the quantitative determination of an active substance when the composition of the excipients is unknown. We studied capsules with a phenobarbital content stated to be 15 mg per capsule and spectrophotometric analysis according to U.S.P. XVIII yielded a result of approximately three times this phenobarbital content. In order to avoid further time-consuming analytical processes for the separation of the interfering substances, we determined the content by TLC separation with subsequent scanning. The solvent chloroform–2-propanol–25% ammonia solution (45:45:10) permitted a good separation of phenobarbital from the remaining substances. For the statistical control of quantitative TLC of phenobarbital by scanning of remission, eight samples of the pure substance were spotted and scanned on one plate. The relative standard deviation was 2.3%. This method yielded a content of 15.8 mg per capsule in the product studied.

STABILITY TESTING

TLC is one of the most frequently used methods for the stability testing of a pharmaceutical substance. In TLC, the use of suitable solvents permits the separation of decomposition products from the unchanged substance. It is practical to evaluate the spot of the active substance quantitatively on the TLC plate and to determine the extent of the loss compared with the originally declared value.

Stability testing of thiamine hydrochloride

By means of quantitative TLC, we studied the stability of thiamine hydrochloride in a liquid product. Normally, cellulose plates are used in the TLC of thiamine. However, the product studied contains an additional substance which interacts by tailing and thereby renders quantitative scanning of the thiamine spots impossible. The solvent butanol–glacial acetic acid–water (20:10:70) on silica gel permitted the separation of the interfering substance and the removal of the thiamine spots from the starting point to the extent that quantitative scanning was possible.

Immediately after production of a batch, eight assays were carried out as a control of the method. The mean was 1.27 mg per millilitre of the liquid (theoretical value 1.3 mg/ml) and the relative standard deviation was 2.1%. The decrease in content was then determined by this method on samples of the liquid stored for 1,

TABLE IV

DECREASE OF THIAMINE HYDROCHLORIDE IN A LIQUID PRODUCT

Time passed (years)	*TLC method (%)*	*U.S.P. XVIII method (%)*
Initial analysis	101.2	104.0
1	91.1	90.8
2	81.7	81.5
3	76.1	77.7
4	66.3	68.5
5	58.2	57.7

2, 3, 4 and 5 years. For comparison, thiamine was determined in the samples by the common method of fluorescence measurement of the thiamine oxidation product, thiochrome. This method is also described in U.S.P. XVIII. Table IV shows the percentage of the decrease in both methods. The results correspond satisfactorily; quantitative TLC requires less time.

Compared with the claimed thiamine hydrochloride content, the product contained an additional 30%, so that after storage for three years the drug still contains the amount claimed on the label.

Stability testing of noscapine hydrochloride in aqueous solution

It is well known that light causes decomposition of noscapine hydrochloride, as shown by a brown discoloration. In order to obtain a method for future stability testing of a particular product, we exposed a 5% noscapine hydrochloride solution to UV light at 20° for several weeks. Samples taken at intervals were chromatographed with cyclohexane–chloroform–diethylamine (90:18:12) as solvent on silica gel; the noscapine spot was evaluated by scanning of remission at 313 nm and compared with the results of a noscapine hydrochloride standard. On this chromatogram, the short-wave UV lamp reveals an additional spot above the noscapine spot only indistinctly, while the fluorescence of this decomposition product is clearly visible under the long-wave UV lamp. The relative standard deviation of eight samples of an unchanged substance was 1.7%. The results of this accelerated stability test are presented in Table V. They show that quantitative determination of the decrease of noscapine hydrochloride in the stability testing of a formulation is possible.

TABLE V

STABILITY TESTING OF NOSCAPINE HYDROCHLORIDE IN AQUEOUS SOLUTION

Time passed (weeks)	*Noscapine hydrochloride content (%)*
Initial analysis	98.8
1	95.3
2	94.0
4	91.6
6	90.9

DISCUSSION

Quantitative TLC can be used successfully in the quality control of pharmaceutically active substances. The analysis of several active substances in one drug in routine checks could be carried out in a comparatively short time; the reproducibility and accuracy of the method are satisfactory. It should be emphasized that a number of other analytical methods could not be applied owing to the reciprocal interference of the active substances. In instances in which the presence of interfering excipients would necessitate the use of time-consuming separation techniques, quantitative TLC proved to be superior because of the accuracy of the results obtained and the short period of time required. This method is also suitable for testing the stability of substances in pharmaceutical products. The spot of the unchanged substance can be

scanned after separation from the decomposition products by TLC and the result obtained can be compared with the initial value.

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