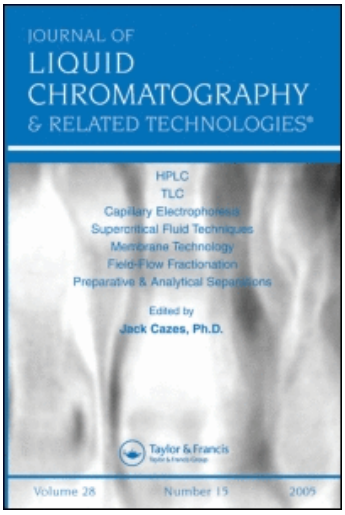

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A Simplified TLC System for Qualitative and Semi-Quantitative Analysis Of Pharmaceuticals

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A SIMPLIFIED TLC SYSTEM FOR QUALITATIVE AND SEMI-QUANTITATIVE ANALYSIS OF PHARMACEUTICALS

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ABSTRACT

There is a need at pharmacies, ports of entry, etc. for inexpensive rapid screening methods which could be used to determine if the right drug is present in the right amounts. These areas usually do not have ready access to full laboratory services for compendial testing. Such a method has been developed using thin-layer chromatography (TLC) making it possible to analyze drugs in areas without laboratory services. Solutions of the drug sample are spotted along with reference materials and the intensities visually compared. Since most drugs are colorless in white light, it is necessary to treat the developed chromatogram with some visualizing agent. Several different categories of pharmaceuticals have been made visible in white light by dipping the plate into a solution of iodine-KI. The stained spots make it possible to determine the drug content qualitatively and quantitatively within the range of specifications. The iodine staining method has been found to be a general method.

INTRODUCTION

One of the greatest health risks faced by many of the world's people is the lack of appropriate medicines for treating common as well as life-threatening illnesses. The second situation which is potentially equally hazardous is inadvertently treating patients with either the wrong medication, or the right medication at the wrong dose. The first problem can only be resolved by the establishment and maintenance of appropriate national and international human health services; the second requires the availability of a useful method for accurately validating the authenticity and dosage level of available medications. These concerns are naturally magnified when validations need to be carried out in non-laboratory, field environments. Indeed, in remote geographical locations a reliable but simple analysis method could be used to ensure that the available medications provide the necessary therapeutic index.

Drug monitoring by official methods require well-equipped laboratories and well-trained personnel which are generally not amenable to routine use in the field or in remote areas. This means that people in such areas will continue to run the real risk of receiving improper medication in the absence of a simple, rapid, inexpensive method.

To be useful, such a method must not only satisfy the basic requirements for qualitative and quantitative analysis, but be capable of being used by personnel with a minimum of technical training under nonideal environment conditions (e.g., lack of electricity, ambient temperatures).

One technique that offers the best possibility to satisfy these requirements is thin-layer chromatography (TLC). However, as normally practiced, TLC cannot be carried out without laboratory space or electricity. Another major problem with conventional TLC is that the spots of most drugs are colorless and cannot be directly visualized. Hence, even using the TLC technique, a method is needed that would readily allow the TLC

spots for the chromatographed drugs to be visible in white light (daylight).

This paper describes an inexpensive portable TLC method for simultaneously detecting and estimating as many as ten commonly used drugs by manually dipping the developed TLC plate into an iodine solution using a specially designed containment system. This plate-dipping approach has not been a general practice in TLC operations. As will be shown, this method - which can be carried out anywhere and is applicable in the absence of electricity- allows for routine semi-quantitative analysis of drugs in a rapid manner.

EXPERIMENTAL

All drugs were dissolved (extracted) in the required solvent, and 3 µL aliquot of the solution was spotted on a glass plate or plastic sheet coated with E. Merck Silica Gel 60-F254. TLC plates of size 5 X 10 cms were used as a matter of convenience. The sample drug and the standard solutions were spotted alternatively across the plate. The spots were developed in standard TLC containers. The spotted plate was developed using a suitable developing solution until the solvent front reached 1 cm from the top of the plate. After drying the plate, the spots were examined in the UV region using a Shimadzu CS-930 Dual Wavelength TLC Scanner at 254 nm. The plate was then dipped into a solution of iodine and potassium iodide, dried and measured again by the densitometer and a wavelength of 420 nm. The sample was spotted at a concentration representing 100% of the declared drug content. The actual concentration was determined by visually comparing the sample spot with those represented by known concentrations of the respective standards. The densitometer measurements were performed to verify that a semi-quantitative analysis could be attained by eye scanning.

Senanayake and Wijesekera (1) described the preparation of an iodine solution using weighed amounts of the chemicals and then sprayed the plates with the solution to visualize the spots. Their method of preparing the iodine and KI solution was altered for this work by using common kitchen measures to eliminate the need for weighing the chemicals.

To prepare the iodine solution, a half teaspoon (2.5 mL) of potassium iodide was dissolved in 200 mL of 95% ethanol. One level teaspoon (5 mL) full of iodine crystals was then dissolved in this solution (Solution A). A total of 25 mL of concentrated hydrochloric acid was carefully added to 75 mL of distilled water, followed by addition of 100 mL of 95% ethanol (Solution B). Solutions A and B were then mixed and stored in a sealed bottle.

RESULTS AND DISCUSSION

Numerous methods for visualization of colorless compounds in TLC have been described (e.g., ref. 2). Such methods involving the use of iodine have included spraying with an iodine solution or exposing the plate to vapors of iodine. Plate dipping methods have been used to enhance fluorescence (3) or to aid in the identification of specific compounds (4). Flinn, Juhl and Layloff (5) described the dipping of a plate having spots of theophylline into a special solution of iodine to make the theophylline spots visible, and explored the possibility of quantification.

The World Health Organization has identified a number of drugs as being essential throughout the world. Some of these drugs and other types of drugs were tested by the dipping method. Table 1 shows the results of dippings from different drug classes to test the overall applicability. The listed concentrations were determined as the best level for quantitative evaluation of the spots resulting from the iodine staining solution.

Initially, chromatograms from drugs spotted at a concentration of 0.5 mg/mL were stained with iodine. If the spots

TABLE 1
Regression Analysis of Pharmaceuticals

Drug	Concentration required for analysis(mg/mL)	Corr. Coeff. Iodine stain	UV	Percent Pound
Ampicillin	0.5	0.992	not visible	106
Benzylpenicillin	5.0	0.996	not visible	-
Chloramphenicol	10.0	0.993	too dark	120
Chloroquine diphosphate	0.5	0.999	0.988	110
Estradiol cypionate	5.0	0.993	0.926	-
Paracetamol	10.0	0.927	too dark	110
Praziquantel	5.0	0.982	0.976	100
Sulfamethoxazole	5.0	0.995	too dark	92
Theophylline	0.5	0.994	0.994	100
Trifluoperazine HCl	0.5	0.998	0.999	110

were not visible, the concentration was increased until acceptable spot intensities were obtained. Further, drug levels in actual formulated medications may vary; hence, it is important to spot a standard solution for each drug at levels that bracket the drug level listed in the medication. From these considerations, the concentrations of the drugs listed in Table 1 represent the good conditions for visualization.

Spraying the plates is the most common method for making the colorless compounds visible in TLC. Typically, each drug or drug functionality class requires a special spray which is specific for the chemical structure. The list of sprays is long and requires a number of chemicals and technical experience in chemistry to select the correct spray. In addition, many of the spray systems require several steps for their effective use. Iodine vapor and spraying with an iodine solution has been used for visualization of many drugs as a general detection method. Sulfuric acid sprays which produce a char of the organic compounds representing the TLC spots also have been extensively used to visualize TLC spots. These methods require a sprayer, propellant and a suitable hood facility due to the toxicity and corrosiveness of the spray. Further, a sulfuric acid spray must be performed in an efficient

hood followed by careful handling and disposal of the TLC plate after the spraying. The plate must be heated in an oven or on a hot plate to char the spots. Such requirements make spraying inappropriate for drug analysis in remote geographical areas lacking electricity and skilled laboratory personnel. Other concerns associated with the use of sprays in field environments include environmental damage, the need for availability of the propellant and sprayer, and operation of the spray equipment.

In contrast, the dipping procedure and TLC system employed in this study was found to offer the following advantages:

1. Problems associated with spraying are eliminated by dipping the chromatogram in a solution of iodine and potassium iodide.
2. The solution used for dipping is the same for all drugs that have been tested, indicating that iodine offered the possibility of serving as a general staining reagent for a wide number of drugs.
3. The organic material comprising the binding in the plate coating (thin-layer) is only slightly stained by the iodine, providing a faint yellow background which allows the analyst to spot visualization for quantitative evaluation.
4. The toxicity and corrosive effects from the use of sprays are eliminated.
5. The plate iodine-dipping method is the best process required for TLC spot visualization in locations lacking laboratory facilities or electricity.

Some reluctance to the use of iodine dipping in the past has been due to fear of disturbing (via distortion or dissolving) the spot by the iodine solvent. However, no spot distortion occurs when the whole dipping process (immersing the spot and its withdrawal) is done quickly and without hesitation. Even if some distortion or dissolution occurred (which has not been observed), the sample and the reference spots should be similarly affected, which should still allow useful visual quantitative comparisons between the sample and standard spots.

Prior to employing the dipping method, spraying with a solution of iodine in methanol was attempted, but without success. The iodine evaporated so rapidly that the spots were not visible long enough to obtain a reliable comparison between any of the spots. Some drugs, such as the penicillins, were not visible in the UV; however, they could be visualized by the iodine dipping procedure.

The amount of drug applied to the plate varied with the ability of the iodine to stain that drug. Quantitative measurements could be made on some drugs when only 1.5 nanograms of the drug were spotted, while others required 30 nanograms to provide a sufficient contrast in the differences in drug quantity.

Clearly, the intensity of the spots for a given drug must vary detectably and linearly with concentration if the method is to be used as a quantitative measure of drug content of a medication. The USP generally requires individual dosage forms of drugs to be within 85 to 115% of the declared content. Hence, a method employed for screening purposes must be sensitive enough to determine drug levels within these limits. In practice, the intensity of the spots must visually show sufficient differences over the concentration range of standards for visual determinations under normal white light.

As mentioned above, each drug was tested using standards to determine the quantity of drug necessary to give a visible spot by the dipping process. After determining the concentration, spots were applied from enough different concentrations to cover the range specified by the USP methods.

The intensity of the spots was measured as a function of concentration by a densitometer in the UV (254 nm) for those drugs that could be successfully measured in this manner. After the UV measurement, the plates were dipped in the iodine-KI solution, and the density of the spots were measured in the visible at 420 nm. Those UV-absorbing drugs requiring relatively large quantities for visibility with iodine could not be measured in the UV since the high quantities provided comparable detection signals and therefore no change in contrast was observed.

The penicillin drugs were not visible in the UV, but could be detected after dipping in the iodine solution. Each drug was carefully examined visually under white light to determine if the eye could detect differences similar to those obtained using the densitometer.

Plots of intensity versus concentration were prepared for each drug using the densitometer measurements. Detection linearity was evaluated from regression analysis of the data over the range of concentrations used, including the use of the origin as a data point (zero concentration). The correlation coefficient for the drugs varied from 92 to 99+% which indicated that the intensities vary linearly with concentration over a broad concentration range. The background from the iodine stain may not be uniform, consequently the base line from the densitometer might vary. Peak height as determined by the densitometer was found to be the best way to measure the spot intensity.

Visually, it was found that the eye could detect differences of 10% in many cases. If a 15% spread in drug quantity was applied, then all drugs evaluated could be visually analyzed. Even when the change in intensity was not linear over the concentration range, visual inspection was found to be an effective means of estimating drug quantity.

Iodine-stained spots fade on standing, so the spots should be carefully watched after the TLC sheet dries to determine the best time to view their relative differences (i.e.,time when the greatest contrast between spots and the thin-layer occurs). However, some spot fading does not decrease the ability to see relative differences in spot intensity, since the spots fade at the same rate. Sometimes when a glass thin-layer support is used the spot differences may be determined more easily from the backside of this support than from the front side. This is true with faded spots. The spots cannot be viewed from the back if the plastic TLC sheet is used. Spots resulting from alcoholic iodine solution alone tend to fade rapidly. Addition of the acidified potassium iodide decreases the iodine loss rate to permit a longer

time for observation. The KI solution tends to make the stain more intense with greater contrast between spots, and comparisons can be made after a reasonable elapsed time.

Analysis of formulations. The assay of a drug is determined by extracting (dissolving) the finished form of the medication in an appropriate solvent, additional solvent is added to produce the necessary concentration for spotting. Most drug forms contain excipients which can vary in quantity. For example, some of the low dosage forms (e.g., trifluoroperazine HCl, containing only 2 mg per tablet) has as much as 99% of its weight in excipients. A portion of some of the excipients may dissolve in the extracting solvent causing multiple spots to appear. Fortunately, most excipients are very polar (e.g., starch) or inorganic and will remain near or where spotted (origin), so they do not interfere in the assay. Drugs in capsule form usually have less excipients which also do not interfere especially if they remain at the origin. Most excipients are not highly soluble in the required solvent system. All insoluble material must be removed from the solution before spotting. This can be done by allowing the solid particles to settle and decant, filtration, or centrifuging (preferred).

Quantitative assay of the medications was accomplished by spotting an aliquot of the drug sample solution between two previously spotted levels of the drug standard. Hence, the two quantities of drug standard were selected to bracket a range of the drug therapeutic index which extends above and below the declared drug content in the medication. The drug content was estimated following plate development and iodine dipping by comparing the area and intensities of the sample spot with those of the two standards. This comparison can then be used to determine if the level of the drug in the medication is appropriate.

This rapid TLC iodine-dip screening method is not intended to replace existing drug monitoring methods, but rather to provide an alternate reliable drug monitoring capability for use in remote

geographical areas. Nonetheless, if this method is used as a general rapid screening method in well equipped laboratories, drugs that fall outside the limits of acceptance can be rechecked or reanalyzed using more sophisticated methods (e.g., HPLC). In general, however, for the drugs evaluated in this paper, employing the dipping and rapid screening method should, in most cases, eliminate the need for other analyses.

Dipping the developed plate into the iodine solution can be accomplished only after the TLC plate is completely dry. An appropriate container must be used for the iodine solution. The main requirements are that the plate must be dipped past the maximum point of spot migration and that the plate must be removed without hesitation.

This work has been directed towards several objectives, including the refinement and simplification of the overall procedure and the use of small volumes of solvent. The use of a small plastic bag to contain the iodine solution and the 5 X 10 cm TLC plates (or films) was found to be a useful way to minimize the solvent required, and make the complete system more portable. Larger containers and plates can be used, but this makes the operation more cumbersome, difficult and expensive.

The dipping system. The dipping system design was developed to effectively use a minimum volume of iodine solution employed a plastic bag approximately 8 cms wide by 10 cms long (Figure 1). The bag can be hung or taped on the wall or any vertical surface. A rigid flat object is placed across the bottom of the bag. The flat object may be plastic, wood, or glass with a thickness of approximately 1 or 2 mm and dimensions about 10 X 5 cms. The bottom of this object is attached slightly below the bottom of the dipping bag using a cellophane tape which acts as a hinge. About 10 mL of the iodine dipping solution is then poured into the bag.

The required level of the iodine solution is achieved by gently pressing the bulging bag containing the dip solution with the flat object described above. This is done so that the iodine solution can cover the bottom 9 cms of the developed plate using

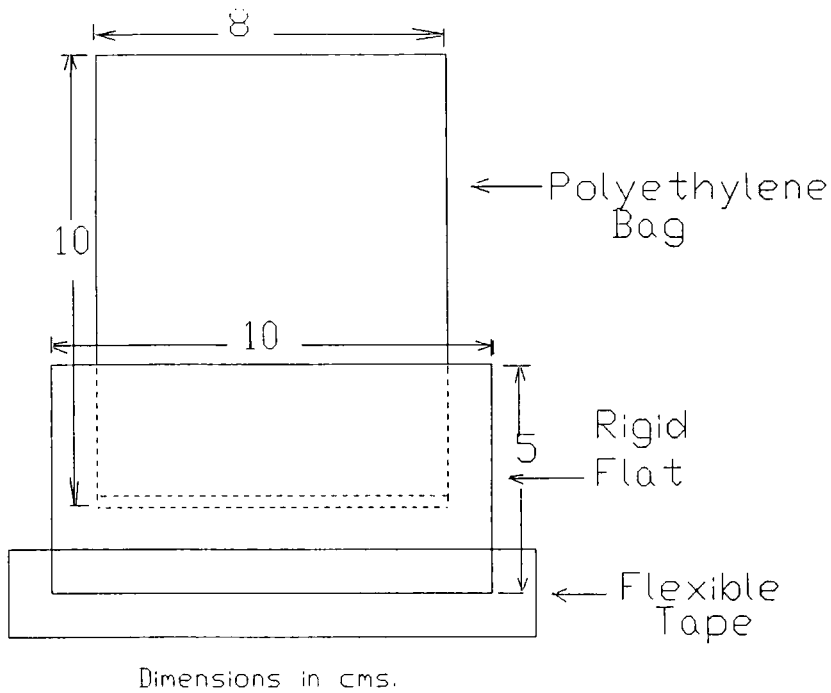

FIGURE 1 Polyethylene Staining Bag (Iodine).

a single, smooth, steady in and out motion to provide a rapid dip requiring not more than two seconds.

After several dippings the iodine solution forms a crust at the surface of the liquid. This crust resulting from solvent evaporation and smearing of the residue will disturb both the thin-layer and spots. The dipping solution should be replaced when this happens. The crusty plastic bag should be replaced or thoroughly washed and dried to remove the solid residue. The crust formation and bag clean-up or replacement can be delayed by resealing the dipping bag immediately after each use. The same dipping solution should not be used to develop more than 5 plates. Any remaining solution can be returned to a sealed container. Do not allow the solution to stand in the bag overnight. The plastic bag may be reused the next day if the bag has been cleaned and dried on the previous day.

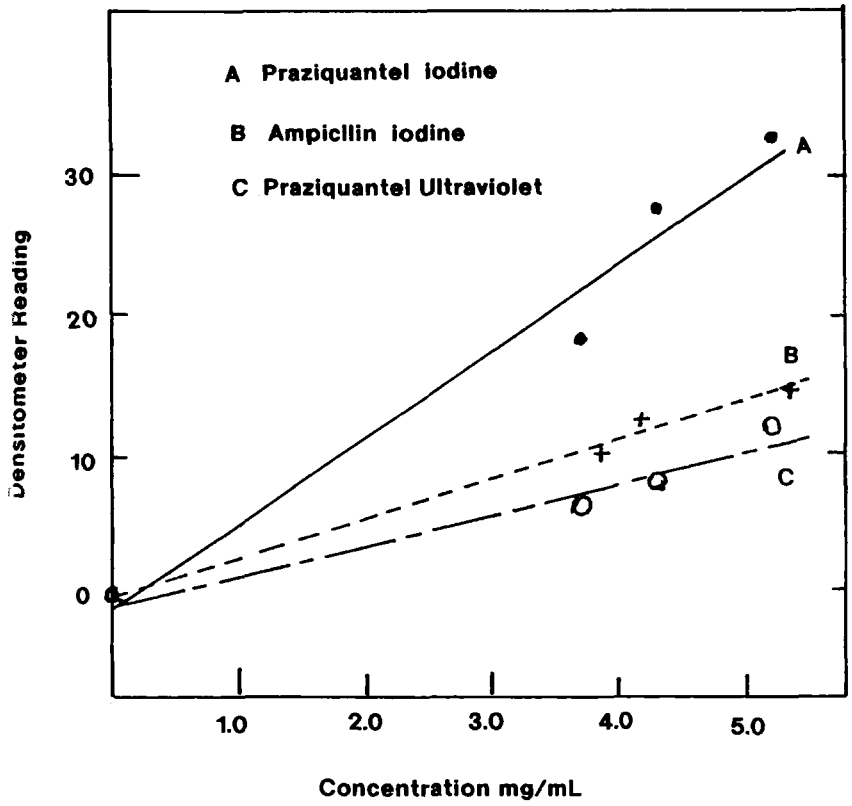

FIGURE 2 Change in Spot Density with Concentration (UV and Iodine Stain).

Data treatment. Plots of representative data of the corrected intensities are shown in Figure 2, with the densitometer density plotted versus concentration. The densitometer readings vary significantly when comparing results from the UV (without iodine staining) and visible (from iodine staining) detection are compared.

Regression analysis of the UV and visible densitometer data for praziquantel vs. concentration show different slopes; however, these differences in intensity can be visually observed as well.

The change of intensity of ampicillin is shown for the iodine stain only as this drug is not visible in the ultraviolet.

As long as the difference in spot intensity can be observed or detected as a function of drug quantity spotted, this method can be used to give a reliable estimate the amount of the drug present. The location of the spots relative to authentic standards of the drug can also be noted to identify the drug.

Figure 3 shows a densitometer trace in the ultraviolet for theophylline as a representative example to illustrate the change in densitometer signal intensity with drug quantity. The actual contrast for the different spots is similar, making it impossible to show the developed plate using UV or iodine staining for detection. Nonetheless, the eye could distinguish the slight differences in contrast which were obtained when the sample solution was spotted between spots from different concentrations of reference solutions of the same drug which representing 90 and 110% of the sample solution. This arrangement permitted a direct comparison to determine whether the drug was within USP specifications. There is a possibility that the spot density for some drugs may not show sufficient contrast between different concentrations to make judgment; however, in such cases the spot area may be used. It is recommended that one should evaluate both the spot area and its density in making such judgments. The spot contrast densities for theophylline is low, but the eye can detect these small differences.

In addition to the utility of this dipping method for rapid screening and analysis of pharmaceuticals in areas without electricity. Despite the utility of this method under these extreme operating conditions, it can also be used as an effective screening method in well-equipped laboratories by highly trained personnel for low-cost rapid screening of drugs. It also should be noted that iodine staining is not limited to pharmaceuticals: other organic compounds can be visualized by this method making quantitative screening possible for these compounds. In conclusion, the iodine dipping procedure and system described in

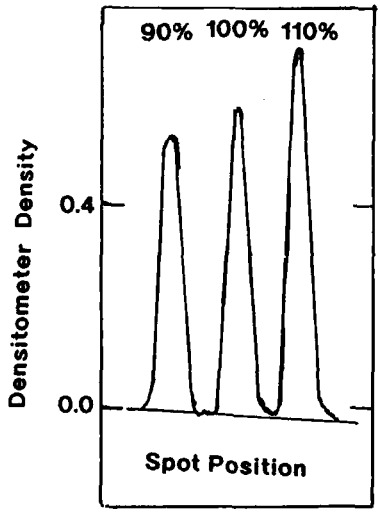

FIGURE 3 Densitometer Response at Three Concentrations of Theophylline. Concentrations Represent Specification Limits.

this paper has been demonstrated to be a reliable, simple, generalized method for drug analysis under raw field conditions which does not require the use of electricity, developing tanks, extensive chemicals, sprays, or costly detection systems. A manual detailing the equipment and methodology of this system is under preparation for publication as a special report of the World Health Organization (6).

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