Journal of Chromatography A, 1218 (2011) 2732–2736

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Review

TLC for pharmaceutical analysis in resource limited countries

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ARTICLE INFO

Article history:
Available online 13 December 2010

Keywords:
Thin layer chromatography (TLC)
High performance thin layer chromatography (HPTLC)
Pharmaceutical
Drugs

ABSTRACT

This review article discusses the sustainability and robust advantages of planar chromatography that are critical to the successful performance of product quality assessments in resource limited areas including field applications. Because of the robustness and ease of use, the training required for successful performance of the high performance thin layer chromatography (HPTLC) assessments is much lower than that of other technologies with comparable reproducibility such as high performance liquid chromatography (HPLC). Some of the successful applications of planar chromatography in resource limited countries are presented. It should be noted that these planar chromatographic technologies have much lower plate counts and therefore separation power than column technologies such as HPLC and gas liquid chromatography (GLC). However in finished pharmaceutical products there are generally few active ingredients which are assessed making the HPTLC adequate for these analyses. In addition at this time there is a much wider array of detection technologies available for HPLC and GLC.

Contents

1. Introduction

Chromatography has become the mainstay of organic analysis because it greatly simplifies both qualitative and quantitative assessments; in general, it is much simpler and more accurate to perform assessments on the individual components of a mixture when separated than directly on the mixture. The basics of chromatography were defined by Tswett [1] over 100 years ago and the great expansion in its use came about with technology improvements required to support the chemical industry which includes pharmaceutical, biotechnology, clinical and many other products. The primary technology changes include higher pressure liquid systems, more uniform and smaller particle stationary phases, modified stationary media, more robust and repeatable sample introduction systems, and a wide array of assessment detectors much more sophisticated than the visual detection which Tswett employed. However as these innovations evolved the chromatography systems required significant technology infrastructure to sustain them including spare part inventories and skilled technicians to perform repairs, corrective and preventive maintenance, and system updates. These more sophisticated systems in many instances have introduced significant sustainability issues. In this context sustainability is a measure of the probability of a failure of system and the availability of resources required to restore it to an operational state; the lower the probability of failure and/or the greater the availability of restoration resources the more sustainable.

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doi:10.1016/j.chroma.2010.12.022

For example, bicycles are low failure probability systems and readily restored to operational status which makes them very sustainable anywhere in the world. The Toyota Rav 4 vehicle is relatively sustainable in Tanzania because it has a modest probability of failure and, because Toyota has a large presence there, large amounts of resources are available to restore them. On the other hand Ford products which have a similar probability of failure are much less sustainable there because there are few Ford vehicles in use to justify maintenance of spare parts inventories and repair services. Interestingly solid state technology systems such as cell phones, digital cameras and computers have such a low probability of failure that the extreme difficulty of restoring them is not significant to their sustainability.

These sustainability issues are well known in HPLC where pumps, valves, columns and media are subject to wear and abuse and require significant infrastructure and resources to restore them. Since the TLC mobile phase development is driven by capillary action the only replacement component is the TLC plate which is approximately US$2. As in the case of the Toyota and Ford products, the sustainability of HPLC systems depends heavily on the amount of similar equipment to economically justify having available maintenance infrastructure. On the other hand the diode array detectors (DAD) have an extremely low probability of failure so these types of detection systems are sustainable in any market.

Thin layer chromatography (TLC) and the closely related high performance thin layer chromatography (HPTLC) are members of a class planar chromatography where the separation is effected by solvent capillary action causing the mobile phase to move across the chromatographic plate. Some of the technical advantages of planar chromatography have been previously discussed [2]. In contrast to forced flow, capillary flow chromatography requires no pumps, valves, pressure controls, etc., so the chromatography systems are much more sustainable in any world market. In addition, the stationary phases in these experiments are single use devices so the medium is replaced with each experiment; a general use HPTLC chromatography plate costs less US$15 and can be used to perform up to 18 assessment channels in the vertical mode and twice that number in the horizontal mode where the solvent wicks from two sides of the plate developing towards the middle. Generally each experiment is conducted by the development of the 18 assessment channels using the same development solvents. The detection may be visual such as Minilab® which makes the process very similar to the original Tswett experiments that are not only very sustainable but also limited to detection of compounds which absorb in the visible region and quantitation estimate is limited by the visual acuity of the observer. In addition there are no detection problems in the case of non-elution, thermal instability and masking by solvent, since all of the applied substances remain on the plate available for detection. Since the development is conducted on a single use medium, immobile substances cannot interfere with subsequent experiments; in HPLC such substances could become interfering substances should the polarity of the mobile phase be changed.

The use of densitometers, which is similar to the solid state detectors in terms of sustainability, provides more precise and accurate measures both within and among laboratories. Unlike the UV–vis and fluorescent detector systems in conventional HPLC which require the use of flow cells exposed to components in the mobile phase components, the densitometer in the TLC application does not require a cell which lessens the routine maintenance requirements.

The implementation of these sustainable technologies in the developing countries will greatly assist them in identifying counterfeit and substandard pharmaceutical products in their market places. The WHO has identified this illegal distribution as a major problem in the developing countries [3,4].

2. Planar chromatography applications in Africa

A recent literature search in PubMED for articles with key word subject headings “TLC, HPTLC” and “Africa” restricted to subheadings “pharmaceutical” quality (“standards” or “analysis”): pharmaceutical analysis, drug testing and quality control was conducted. This search strategy yielded 262 TLC hits; 8 of these were review articles while over 236 citations were for screening and profiling of natural products and 18 papers concerned analytical applications in pharmaceutical testing with the remaining concerning bioanalytical work and clinical applications.

Interestingly, the search yielded few publications on HPTLC application in analytical method development and successful testing of drugs. The distribution by country source was: Egypt (15), Tanzania (3), and Algeria (2). The larger number for Egypt is likely due to the fact that is has a well established pharmaceutical industry with supporting academic and regulatory programs. These publications included:

1. procedures, materials, and instrumentation for the different steps in the HPTLC assessments;
2. validation of results;
3. applications to bulk drugs, formulations, stability studies, biological samples (e.g., urine and plasma); and
4. hydrophobicity studies.

We are now presenting the summary of these original investigations under three categories viz Minilab applications in drug quality assurance programs particularly in resource constrained settings of Africa, HPTLC methods and applications and special application of planar chromatography in clinical sciences. The applications in the natural product screening are outside the scope of this review manuscript.

2.1. Use of TLC in pharmaceutical drug product testing

The low acquisition, operational and maintenance costs needed to successfully perform the TLC analytical technique are very important because it can provide product quality assessment capability in areas where laboratory facilities for pharmaceutical quality analysis are minimal or do not exist. Countries which have no product quality assessment resources are most at risk for having counterfeit/substandard medicines on their markets [3,4]. The majority of the poor quality products in the WHO reports had no drug at all or the wrong drug; these product quality failures are easily detected by TLC.

Kenyon et al. demonstrated that TLC can be used to provide a semi-quantitative yet versatile and robust testing of pharmaceuticals in a resource limited environment [5]. The techniques were used in Swaziland to analyze nineteen different marketed drugs all on the WHO Essential Medicines List. The former German Pharma Health Fund (GPHF), now the Global Pharma Health Fund, improved on the Kenyon approach by developing a TLC based testing kit called the Minilab®. The Minilab® kit is packaged in a sturdy suitcase which contains all necessary reagents and supplies to perform more than 1,000 TLC based pharmaceutical analyses [6]. The Minilab® kit can be purchased for about US$4000 and can be easily transported and deployed in a non laboratory environment. The implementation of the Minilab® based TLC testing requires minimal training of operators and a work bench with access to running water. Furthermore, the solvents used in a Minilab TLC testing (e.g., acetone, ammonia solution, ethyl acetate glacial acetic acid, Hydrochloric acid, methanol, sulphuric acid, toluene) are of low toxicity and are used in relatively small quantities thus require no extensive safety measures; the toxicity of the solvents is similar to those used in household products such as paints. Further to low acquisition costs,

the replacement cost for supplies used for TLC testing in a Minilab® is relatively inexpensive. A TLC plate costs about US$2 and the solvents used are the commonly used analytical grade reagents.

In addition to TLC testing the Minilab® includes a simple disintegration test and a physical examination protocol. The Minilab® system can be easily deployed in the field to carry out basic pharmaceutical product quality assessments in the supply chain. Using the system one can reliably detect products containing no or wrong drug, product with potentially poor dissolution or grossly substandard products. The Minilab® currently has testing methods for more than fifty drugs on WHO Essential Medicines List.

The ease of deployment with low operational costs of the TLC based analytical techniques has been a key to the vast increase in its use to detect counterfeit/substandard medicines in markets particularly in resource constrained settings [7,8].

The South East Asia region has been a focus for TLC implementation for pharmaceutical drug quality assessments. To help combat the marketing of counterfeit artemisinin-based antimalaria drugs [9,10] the United States Pharmacopeia (USP) Drug Quality Information (DQI) program in 2003 deployed the Minilab® at seventeen field test centers in three countries which did not have laboratory testing facilities [9]. The USP-DQI program also supported the deployment of Minilabs to assess the quality of antimalaria drugs in some African countries with no laboratory facilities [11,12]. Following this USP initiative, other organizations also have deployed TLC systems in other countries in this region to screen the quality of medicines [13]. The TLC systems have been recommended as an effective quality screening method particularly in those countries where there is a high prevalence of counterfeit products [14].

The WHO also has supported TLC-based Minilab® testing in countries with limited pharmaceutical product testing capacities. Between 2005 and 2008 the WHO supported the medicine regulatory authorities in Tanzania, Zambia and Uganda to acquire and deploy Minilabs as part of their drug quality assurance system. In Tanzania the Minilab® was deployed as part of an elaborate risk-based, tiered quality assurance system where basic testing was linked to a fully functional laboratory. Such an approach, provided opportunity for the regulatory authority to more than double the number of samples tested and to monitor key ports of entry where Minilab® also was deployed [15]. In Zambia, at the time of its deployment, the Minilab® was the only pharmaceutical product testing capability in the country [16].

The efforts by WHO and other international organizations to support the acquisition of Minilab® in countries with limited or no testing capacity along with research demonstrating its usefulness as a tool to detect counterfeit and substandard drugs have led to its widespread use. The GPHF currently estimates that there are more than 350 kits in use in more than 70 countries worldwide [17]. The robustness of Minilab® and low probability of failure have led to its use as a first line defense against counterfeit and substandard drugs in the supply chain of many countries.

2.2. Use of planar chromatography in therapeutic drug monitoring

The TLC technology has been applied as an inexpensive method for monitoring therapeutic drugs in resource constrained settings. They have been used for the semi-quantitative determination of Nevirapine in biological fluids: saliva, umbilical cord blood and plasma [18–20]. The procedure was shown to be sensitive, specific, robust, and able to detect sub-therapeutic concentrations of the drug, and the results compare well with HPLC at a lower cost. These findings indicate further the potential of TLC as an effective analytical tool for countries that do not have sophisticated laboratory services. HPTLC has also been used in clinical science in Africa where a method for the determination of tinidazole in human serum was developed and successfully applied at a University in Algeria in 1999, with results that favourably compared to those obtained with an HPLC based method [21].

2.3. Shortcomings of TLC as an analytical tool and current advances

Despite the advantages of the Minilab® TLC use in pharmaceutical analysis, it suffers from key two key shortcomings: the reliance on operators' visual acuity for visual estimation of drug content, and operators skill and technique for manually applying sample solution spots on the chromatographic plate with adequate precision. Because of these shortcomings, the methods developed by GPHF include use of two levels of standard solutions 100% and 80% whose spot sizes are compared to those of the sample which forms the basis of pass/fail decision. It has also been shown that improvement on reliability of Minilab® based TLC testing results can be improved by carrying out regular on the job training and proficiency testing for the operators [22]. Despite these inherent shortfalls the TLC techniques largely as a screening technology but the results are not suitable for compliance actions except those which are unequivocal such as no drug or wrong drug present in the sample.

Recent advancements in technology have contributed to a marked improvement of repeatability and reliability of TLC based testing. Automating the TLC sample application step has markedly improved repeatability of the sample application process, and thereby the overall test procedure. In addition the detection technology has been developed to measure the intensity of a spot of interest on the plate by which comparisons to standards can be related to drug content. With the aid of software, the complex mathematics needed to calculate the drug content from the reflected light can be easily performed. These two key developments have made TLC-Densitometry a reliable method for pharmaceutical drug analysis. The separation media also have been improved by reducing the particle size and uniformity which has evolved into HPTLC. The HPTLC offers all of the advantages of TLC but with improved separation capacity by marked improvement in plate numbers which approach those afforded by the conventional HPLC columns.

The above developments have increased the acquisition costs for HPTLC plates but the new systems have brought improved versatility, throughput and robustness to the TLC technique while retaining the low running and maintenance costs. In addition, where the Minilab® have been in use before, the existing basic chromatography skills can be adapted with minimal efforts for HPTLC-densitometer analysis. This has made (HP)TLC-densitometry testing a more useful technology for analysis in resource constrained settings since the costs generally are less than other analytical systems such as HPLC.

2.4. HPTLC-densitometry applications for drug testing in Africa

In order to be able to exploit the cost and convenience advantages of (HP)TLC-densitometry there is a need to improve and/or adapt existing methods, and develop new methods of analysis. There are currently a large number of HPTLC-densitometry papers originating from the India sub-continent which reflects the wide acceptance of this technology. These additional methods increase the testing inventory and upon qualification will be useful to further expand the use of the technology.

The International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) has published guidance (Q2R1) for the validation of pharmaceutical analytical methods [23]. Any new method or alteration/improvement of existing methods should be validated in

accordance with this guidance to demonstrate the reliability of the obtained results. As in all methods including HPLC, the following parameters are integral part of the validation requirements: accuracy, precision [repeatability and intermediate precision in terms of relative standard deviation (RSD)], specificity, linearity, range, limit-of-detection, limit-of-quantitation, and robustness.

Method validation is generally categorized into three different levels: single laboratory validation (SLV), peer verification (PVM), and full collaborative study. The SLV applies to a specific laboratory, technician and equipment. Staff members and students in our laboratories have developed and extensively validated in accordance with the ICH analytical method validation guidelines for SLV HPTLC-densitometry methods for the assessments of: Metronidazole [24], Quinine [25], Nevirapine [26], a fixed dose combination of Lamivudine and Zidovudine [27], Sulfamethoxazole and Trimethoprim [28], and an improved method for a triple ARV combination Lamivudine, Stavudine and Nevirapine [29]. These methods will be reported elsewhere.

The PVM applies for a limited number of labs (2–7) and is intended to provide information on how a method is interpreted outside of the original lab. This stage would involve the originator lab performing extensive SLV on new, existing or improved analytical method. This is followed by preparation of method protocols, composite samples and standard materials which are sent to secondary labs to perform the methods. We recently published an extensive successful two-laboratory PVM investigation on the use of HPTLC-densitometry to perform assays of Lamivudine–Zidovudine, Mebendazole, Nevirapine, and Quinine composite samples [30]. For the four samples excellent reproducibility was obtained. In addition the method used less toxic organic solvents thus moving towards a greening of chromatography.

The highest level of validation is the full collaborative study which requires eight or more labs providing acceptable data using the same method protocols, composite samples and standard materials. The different levels of validation range in their degree of ruggedness; the most rigorous form of method validation is the full collaborative study. A successful collaborative study provides a high level of confidence that the method is reproducible.

Collaborative studies are not always practical or possible for laboratories to manage because they require significant commitments of personnel, equipment, and support resources concurrently in multiple laboratories. It is important that laboratories employ a level of validation that is suitable for the method's intended use; it should ensure that the methodology is accurate, precise and rugged for the specified analyte and concentration range. Given this challenge and level of complexity in organizing the collaborative studies there is a need to identify technologies that would not seem to burden participating laboratories in terms of time and financial resources. In analytical testing HPTLC-densitometry is at the heart of this requirement putting down the cost and also time required to have results similar to those obtained with HPLC. No such a study has been reported involving labs from Africa. To our present knowledge only one such a study was reported for HPTLC of sucralose involving 14 labs from 5 different countries [31].

To date there have been several HPTLC validated methods published; the earliest dates back in 2001 where a method for the simultaneous determination of benazepril hydrochloride and hydrochlorothiazide was developed [32]. In this paper separation was carried out on Merck HPTLC aluminum sheets of silica gel 60 F (254), using ethyl acetate–methanol–chloroform (10:3:2, v/v/v) as mobile phase. The performance method was compared to HPLC. The same group developed a method for the separation of lisinopril and hydrochlorothiazide in binary mixtures in 2001 [33]. Since then the group has been very active and has published papers on: Nicergoline in the presence of its hydrolysis-induced degradation product in 2002 [34], rabeprazole in the presence of its degradation products in 2003 [35], zolpidem hemitartrate in 2003 [36], vincamine in the presence of its degradation products [37], alfuzosin hydrochloride [38], cilostazolin in 2007 [39] and sulpiride and mebeverine hydrochloride in 2010 [40] The later seven methods are stability indicating assays. They have sufficient resolution to allow the determination of the active substance of interest in the presence of the potential interfering substances from the formulation matrix and/or degradation products. The results of several of these assessments were compared to results obtained with HPLC and very good correlations were obtained.

These experiments represent an important milestone in the application of planar chromatography in pharmaceutical analysis because in these cases the use of the HPTLC plates made it possible to resolve the drug substances from impurities and achieve comparable separations to HPLC. Further in 2009 a comparison performance in the determination of oxybutynin hydrochloride and its degradation products using HPTLC-densitometry and HPLC were modeled using a chemometric approach [41]. The validation of all these methods has been based on extensive SLV studies.

3. Detectors in planar chromatography

Planar chromatography offers very versatile possibilities for the detection of the materials separated. Detection with eyes includes direct observation of colored materials, visualization by spray reagent, UV lamps, iodine vapor staining, and oxidation by concentrated acids. Advancement in technology has made it possible to use densitometer, allowing very high peed scanning of separated materials to acquire the entire spectrum in matter of few minutes. Densitometers with a video or digital camera to capture the chromatogram images on the layer placed inside a lighting module (e.g., CAMAG DigiStore 2) and software for image analysis (e.g., CAMAG VideoScan) have been used as an alternative to optical/mechanical slit scanning densitometers, e.g., for the determination of phospholipids in pharmaceutical products [42] and quetiapine in tablets [43]. HPTLC–MS hyphenation has been made possible for the analysis of several compounds, e.g., tetracycline antibiotics [44], caffeine quantification [45], and screening of combinatory libraries [46].

4. Conclusions

The widespread distribution of the GPHF Minilab® to perform rapid quality assessments in resource limited countries to detect markedly substandard and counterfeit pharmaceutical products has established a large cadre of people who can competently perform the TLC experiment. With some additional training we have shown that some of these individuals can be trained to successfully perform HPTLC. With the addition of densitometry and automatic sample application equipment along with some additional training these individuals can perform the TLC–HPTLC assessments with repeatability and reproducibility results comparable to those obtained with HPLC. We intend to move these technologies and procedures to other laboratories so they also can support due diligence efforts to help assure that pharmaceutical products purchased comply with the contractual procurement Terms and Conditions.

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