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# The development and application of coupled HPLC–TLC for pharmaceutical analysis

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**Abstract**: Successful coupling of high-performance liquid chromatography (HPLC) and thin-layer chromatography (TLC) facilitates correlation of the retention factor ($R_f$) of a component in a TLC separation with a peak observed in HPLC, thus eliminating the need for isolation of the component. Experiments were designed to optimize flow and spotting rates for typical HPLC mobile phases, evaluating such parameters as band broadening and on-plate loading. Also investigated were the application of flow splitting and semi-microbore HPLC columns. The problems encountered with the development of TLC plates upon which the HPLC eluent has been applied as a linear streak were studied, as were detection methods for the resulting spots. This paper describes the successful development of the technique, using model compounds of pharmaceutical interest.

**Keywords**: *TLC; HPLC; coupled HPLC and TLC; interfaced HPLC and TLC.*

## Introduction

Both TLC and HPLC are extensively employed in the analysis of pharmaceuticals. This paper focuses on the identification and quantification of minor components which may be present at low levels, in drug substances and their formulations. The minor components include known and unknown process related impurities, degradation products and excipients. However, potential problems of quantification and identification may arise when specific correlation between the HPLC peaks and TLC spots is not established.

Currently, correlation of HPLC and TLC results requires isolation of observed components using one separation methodology, and subsequent chromatography using the complementary method. A typical approach would involve removing a spot from the TLC plate, extracting any components from the support, and finally analysing the extracted materials by HPLC [1, 2]. This method can have limited application where the compound may be irreversibly bound to the TLC support, or where on-plate degradation is evident or where isolation may cause chemical transformation.

An alternative approach involves collection of HPLC eluent, into discrete fractions, followed by application onto a TLC plate and then development of the plate. This technique has been used extensively, with both narrow and normal bore columns [3–10]. Work described in this paper exploits this approach, but eliminates the need for fraction collection and instead streaks the complete run *directly* onto a TLC plate.

Work has been carried out previously in the field of coupled HPLC–TLC, primarily to separate further a poorly resolved mixture of components [11–13]. The technique has also been developed utilizing narrow bore (0.7 mm) HPLC columns [14–16], however sample size is limited with this type of column. A further application of coupled HPLC–TLC is the application of a complete run onto a TLC plate, and using the plate as a permanent record of the HPLC trace, upon which further detection methods may be used [17]. A device is currently available, from Camag, that couples HPLC and TLC by applying fractions of the HPLC eluent onto a TLC plate, however problems arise with close eluting peaks.

The aim of the work detailed in this paper was to correlate results obtained from a fully optimized *conventional* HPLC separation with those obtained with a fully optimized TLC separation. This was achieved by applying eluent from a *complete* HPLC run onto a TLC plate, for subsequent development, thus ensuring that all components were detected and complete assignment and correlation of results obtained.

## Experimental

The technique was evaluated using two mixtures, a set of three anti-oxidants and a drug mixture. The anti-oxidants used were butylated hydroxytoluene, BHT (Sigma, Poole, Dorset, UK), 2-butylated hydroxyanisole, 2-BHA (Promochem, St Albans, Herts, UK) and 3-butylated hydroxyanisole, 3-BHA (Promochem). The drug substance used was 2-(2,4-difluorophenyl)-1,3-bis(1H-1,2,4-triazol-1-yl)-2-propanol, fluconazole (Fig. 1), which was present in a mixture with two potential impurities (designated X and Y), all components present at similar levels.

The work was carried out using an HPLC with diode-array detection (HP1090 Chemstation, Hewlett–Packard) connected to a TLC streaker (Camag Linomat III, BDH) by means of an adjustable needle valve (SGE, MCVT-1-50). The needle valve, which required calibration prior to use, was connected directly after the flow cell on the HP1090 (Fig. 2) and was employed to reduce or stop the eluent flowing on to the TLC plate. In this work, 30% of the eluent flow was directed on to the TLC plate, but this value may be varied depending on the application. The valve was connected to a fixed needle, which applied eluent to the plate, via a short piece of narrow bore PTFE tubing. The delay time of the system (i.e. time taken from injection to application on-plate, with no HPLC column) was determined for different flow rates. For example at 0.2 ml $min^{-1}$ the delay time was found to be 0.5 min. The injection volume used was 20 μl.

The HPLC columns used were Spherisorb S3W, 3 μm packing size and 100 × 2 mm i.d. (Phase Sep, Jones Chromatography) for the analysis of BHT, 2-BHA and 3-BHA and a Hypersil ODS, 5 μm packing size and 100 × 2 mm i.d. (Hewlett–Packard) for the analysis of the drug mixture. Normal-phase HPLC analysis of the three anti-oxidants was carried out at 285 nm, using an eluent of 2% methanol in dichloromethane–hexane (45:55, v/v) (HPLC grade, Rathburn, UK), with a flow rate of 0.2 ml $min^{-1}$. Reversed-phase HPLC analysis was employed with the drug mixture at 261 nm, using an eluent of water–methanol–

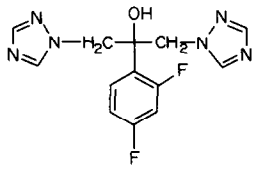


**Figure 1**
The structure of fluconazole.

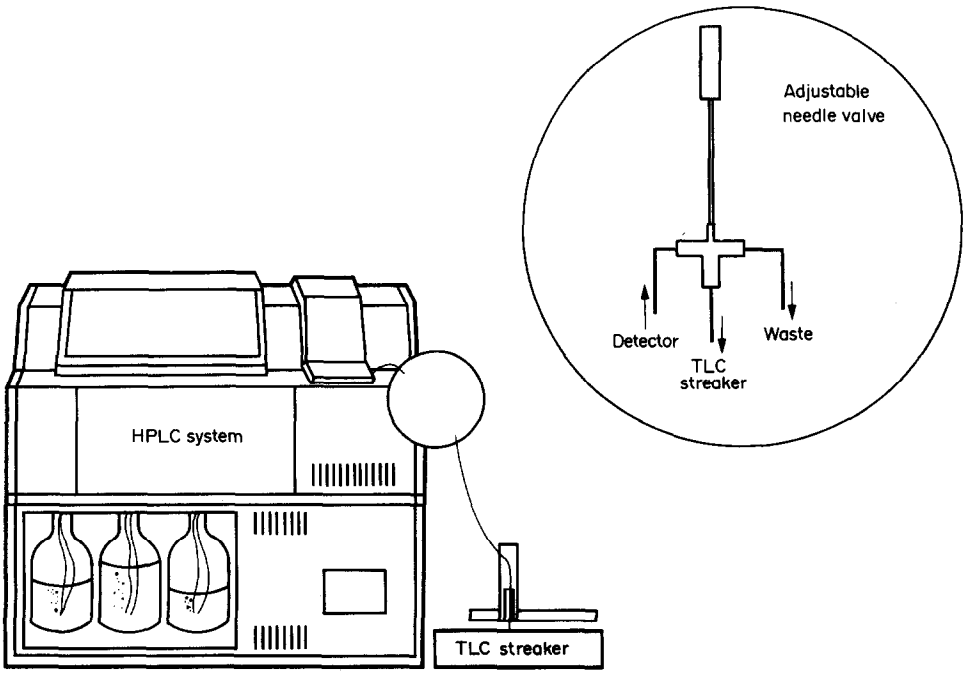


**Figure 2**
Schematic of coupled HPLC–TLC system.

acetonitrile (70:20:10, v/v/v) (HPLC grade, Rathburn) at a flow rate of 0.2 ml $min^{-1}$.

The TLC plates used were silica gel 60 $F_{254}$ (20 cm × 20 cm) pre-coated to a thickness of 0.025 cm with no pre-concentration zone (Merck, D.C. Fertigplatten Kieselgel $F_{254}$).

Linear development of the TLC plates, after streaking, was carried out using ascending, one-dimensional mode with chamber saturation (room temperature, 1 h). With the anti-oxidant mixture, the solvent system was dichloroethane–hexane (75:25, v/v) (GPR grade, BDH), and for the synthetic drug mixture, ethylacetate–methanol–ammonia (72:28:12, v/v/v) (GPR grade, BDH). The plate was removed from the solvent system when the solvent front had reached 4 cm from the top of the TLC plate.

The resulting spots on the TLC plate were detected using a scanning densitometer (Camag TLC Scanner II, BDH), with a band width of 10 nm, slit width of 0.6 mm and slit length of 12 mm. Detection, utilizing the absorbance mode, was at 285 nm for the anti-oxidant mixture and 261 nm for the drug mixture. The plate was scanned in tracks at intervals along the line of application of the eluent. A 1 cm interval was used at this stage as the work was of an exploratory nature and a smaller interval would have provided more information than was necessary at this stage. Each track started at just above the point of application of eluent and finished just before the solvent front. The densitometer was also used to acquire UV spectra directly from the TLC plate for the drug mixture.

## Results and Discussion

*Instrumentation development*

The adjustable needle valve used was necessary as the large flow rates employed with conventional HPLC would have required large volumes of eluent to be applied on to the TLC plate. Experience has shown that current TLC plate coatings are incompatible with such large volumes of solvents, as they cause the coating to lift off the plate.

A further benefit of the Linomat TLC streaker was the ability to apply a flow of nitrogen to the plate, however as this would have introduced another variable into the experiment it was not used in this exploratory work.

The TLC streaker required modification as the conventional time taken for a TLC plate to travel 20 cm is usually 1 min. This time was increased to about 4.5 min by altering the motor clock frequency. Despite this increase, the limited run time available for HPLC remains a drawback.

*Experimental design*

Initial experiments were set up to investigate the optimum flow rate for both polar and non-polar mobile phases. Leaving the splitter set to deliver 30% of flow onto a TLC plate, the flow rate of the HPLC system was systematically changed. The width of the solvent band on application to the TLC plate was measured at various flow rates [Fig. 3(a) and (b)]. The results indicated that low flow rates should be used, particularly with polar mobile phases (below 0.6 ml $min^{-1}$). With non-polar mobile phases, flow rates of up to 1 ml $min^{-1}$ could be used without excessive spreading of the eluent. The use of higher flow rates would be possible, but a lower fraction of the eluent flow should

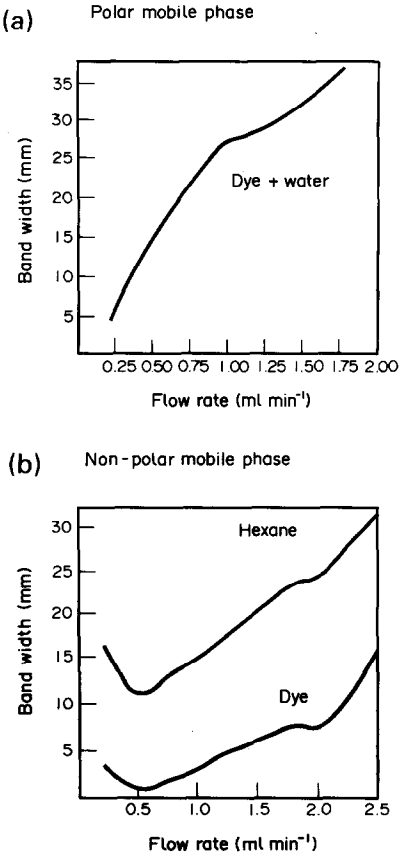


**Figure 3**
Solvent band spread versus flow rate, in both (a) polar and (b) non-polar mobile phases.

be applied to the TLC plate by adjustment of the splitter needle valve. With polar mobile phases, partitioning during application was noted (i.e. migration of the component to the outer edges of the solvent band). To give optimum results, a flow rate of 0.2 ml $min^{-1}$ was chosen for all further experimentation.

Intial TLC work with scanning densitometry using the anti-oxidant mixture illustrated a significant noise level, making detection of low

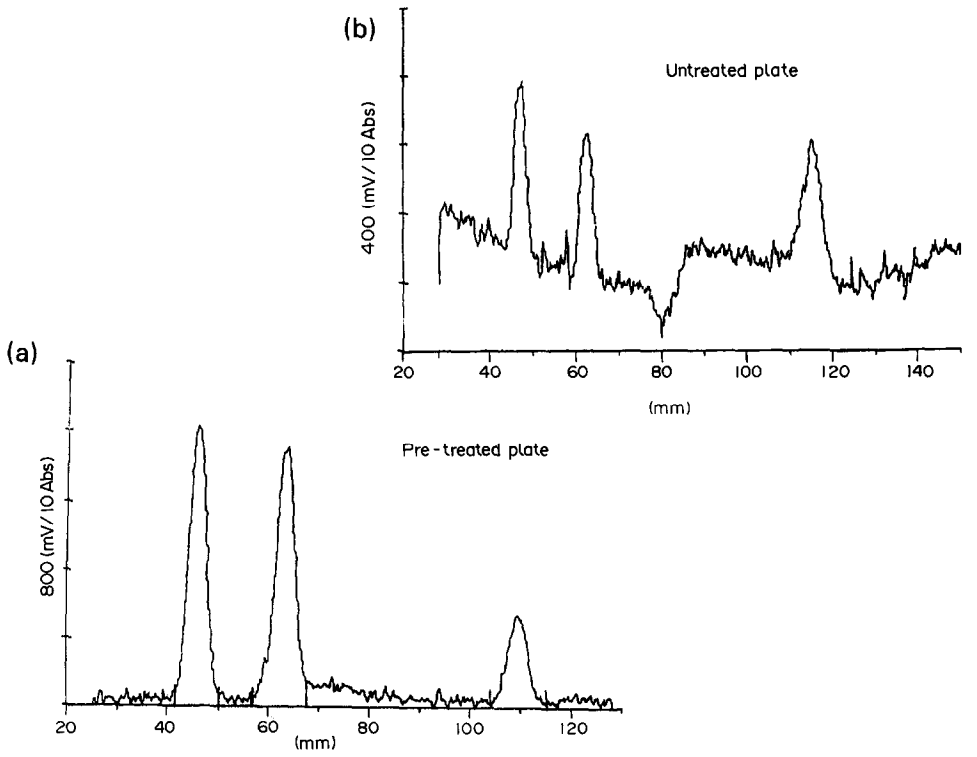


**Figure 4**
Comparison of signal-to-noise ratio obtained from scanning densitometry using both (a) pre-treated and (b) untreated TLC plates.

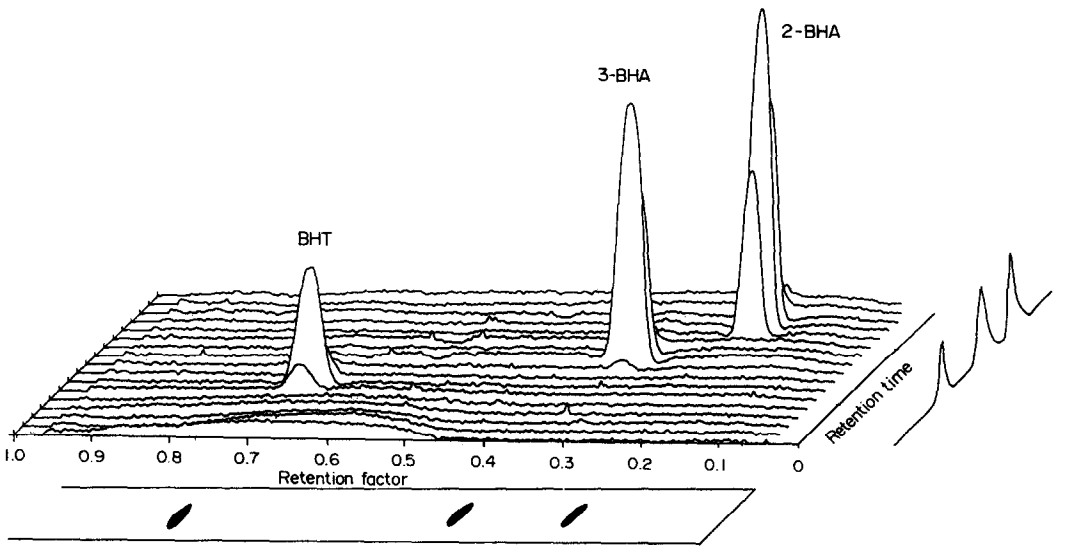


**Figure 5**
Correlation of HPLC retention times and TLC retention factors for BHT (butylated hydroxytoluene), 2-BHA and 3-BHA (butylated hydroxyanisole).

levels of compounds on-plate and their subsequent integration difficult [Fig. 4(a)]. To overcome this problem, TLC plates were prerun in methanol. Peak detection was consequently improved sufficiently to allow integration [Fig. 4(b)] and this measure is thus to be recommended.

Using the anti-oxidant mixture, with individual components present at 1 mg $ml^{-1}$, and the conditions detailed in the experimental section, a 3-D representation of the TLC plate was achieved as shown in Fig. 5. From this, HPLC retention times and TLC retention factors can be correlated directly.

The sensitivity of the scanning densitometer with the three anti-oxidants was investigated using solutions of 0.2 and 0.1 mg $ml^{-1}$, of the individual components and the coupled HPLC–TLC conditions previously described. At a concentration of 0.2 mg $ml^{-1}$, all components were visible in the 3-D representation (Fig. 6). In the 3-D representation of the 0.1 mg $ml^{-1}$ plate the BHT peak/spot was barely detected, but both of the peaks/spots due to isomers of BHA were visible. From this work the limits of detection for these compounds, was found to be approximately 3 μg for 3-BHA and 2-BHA, and approximately 6 μg for BHT.

The second test case involved the synthetic drug mixture. With this mixture spectra were obtained, both from HPLC and scanning densitometry. Spectra alone however could not have permitted correlation of HPLC and TLC

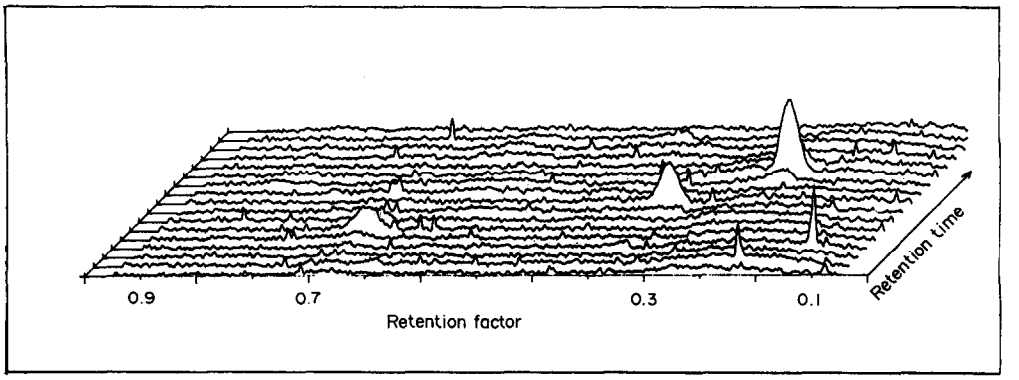


**Figure 6**
Limits of detection for BHT (butylated hydroxytoluene), 2-BHA and 3-BHA (butylated hydroxyanisole). (20 μl injection of 0.2 mg $ml^{-1}$ solutions.)

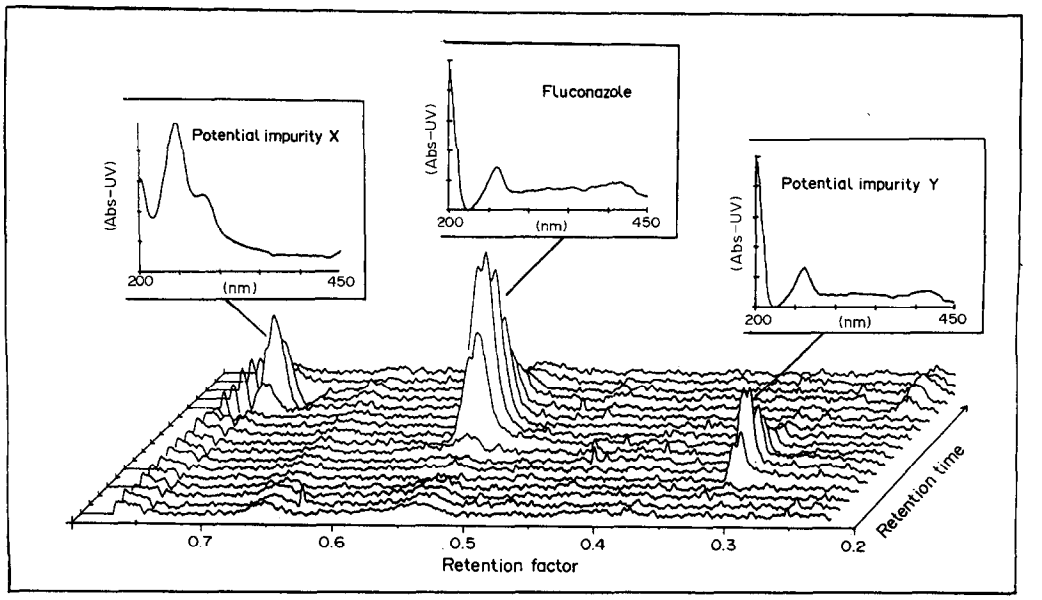


**Figure 7**
TLC of fluconazole and two potential impurities.

results as two peaks/spots gave identical spectra (Fig. 7). However it can be seen that the use of coupled HPLC–TLC overcomes this problem and facilitates the correlation of data concerning impurities potentially present in a drug mixture, without the need to isolate the components involved.

The peak splitting illustrated in Fig. 7 is due to the high aqueous content of the mobile phase and occurred despite the low eluent volume applied onto the TLC plate. The problem could have been overcome by the use of pre-concentration zone plates or wettable $C_{18}$ reversed-phase plates, however during the development stage these plates were not available.

## Conclusions

The work outlined in this paper has demonstrated that coupled HPLC–TLC has been successfully achieved and will permit the direct correlation of HPLC and TLC chromatograms, thereby allowing tracking of process related impurities and degradation products in both drug substances and their formulations. Rapid method development is possible for normal phase HPLC–TLC, however reversed-phase HPLC–TLC is more problematic due to the aqueous content of the mobile phase. The success of the technique is dependent on optimization of several parameters (e.g. flow rate and stream splitting) and is therefore seen primarily as a development tool and is not for routine methods.

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