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*Short Communication*

# Reversed-phase TLC on a bonded silica: an alternative to normal phase TLC for stability testing of drugs

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## Introduction

Thin layer chromatography (TLC) is a rapid, inexpensive and technically undemanding technique, which has been used extensively for drug stability studies. However, one of the limitations of TLC on silica is that the silica itself may give rise to artefactual decomposition of the drug. This obviously limits any interpretation of stability.

The recent commercial availability of chemically bonded reversed-phase (RP) TLC plates prompted an investigation of these as an alternative to TLC on silica for drug stability studies. The use of RP-TLC has been investigated for stability studies on Cas 434, *N*-(3-(4-*n*-butoxy-phenoxy)-2-hydroxy-*n*-propyl)-*N'*-(1,3-dimethyl-2,4 (1H, 3H) dioxo-6-pyrimidinyl)-ethylene diamine (Fig. 1), a cardioselective β-blocker, which was found to decompose on TLC using silica gel.

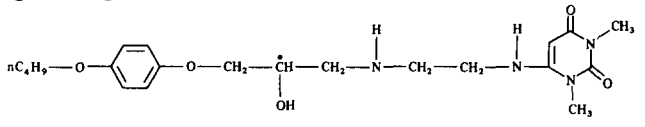

**Figure 1**
Cas 434, *N*-(3-(4-*n*-butoxy-phenoxy)-2-hydroxy-*n*-propyl)-*N'*-(1,3-dimethyl-2,4 (1H, 3H) dioxo-6-pyrimidinyl)-ethylene diamine; * denotes position of $^{14}C$-label.

Early bonded RP-TLC plates were hydrophobic, making it very difficult to apply aqueous samples such as biological fluids. Recent advances [1, 2] have resulted in a second generation of non-hydrophobic, fully wettable RP-TLC plates, to which water and biological fluids such as bile, deproteinized plasma and urine may be directly applied. $C_{12}$ bonded, fully wettable RP-TLC plates have therefore been used in these studies.

## Experimental

Radioactive $^{14}$C-Cas 434 was synthesized in these laboratories to a specific activity of 19.6 μCi mg$^{-1}$ and 97.5% radiochemical purity. Solvents were of technical grade. Silica gel TLC plates (20 × 20 cm, plastic backed, 0.25 mm thick, manufactured by Macherey-Nagel) were purchased from Camlab Ltd., Cambridge, UK. $C_{12}$ Bonded reversed-phase TLC plates (20 × 20 cm, glass backed, 0.25 mm thick, manufactured by Antec AG) were obtained from Fluorochem Ltd., Glossop, UK. All TLC plates were used without further pretreatment. The drug and its breakdown products were detected using autoradiography with Kodirex® X-ray film, Kodak Ltd., Hemel Hempstead, UK. The X-ray film was developed in DX 80® developer, and fixed in FX 40® fixer, Ilford Ltd., Basildon, UK. The extent of decomposition was determined by segmenting the TLC plate and determining the radioactivity in each segment by scintillation counting. Scintillator (NE 260) was purchased from Nuclear Enterprises Ltd., Edinburgh, Scotland, UK. Radioactivity was measured in a Packard model 2450 or 3255 liquid scintillation counter. All samples were analysed in triplicate.

The stability of the drug was assessed in aqueous solutions, buffered to pH 2, 4, 7, 10 and 12, at room temperature, and in urine, plasma and bile (from rat and dog), at −20°C, 4°C, ambient temperature and 37°C. Sample preparation was limited to the precipitation of plasma proteins using an equal volume of acetonitrile. Samples were applied to the TLC plate using a syringe. Chromatography was performed in ~25 × 25 × 10 cm glass TLC tanks (Shandon Southern, UK) pre-equilibrated with the solvent used for chromatography.

Solvent systems used for chromatography on silica were butanol–acetic acid–water (7:2:1 v/v), ethanol–chloroform–ammonia (10:9:1 v/v) and acetonitrile–ethanol–ammonia (3:1:1 v/v). The solvent system adopted for reversed-phase chromatography was isopropanol-water-ammonia (40:40:20 v/v). Ammonia solutions had a specific gravity of 0.88.

## Results and Discussion

Initial work on the stability of the drug concentrated on the development of traditional silica TLC systems. It was quickly noted that such TLC systems resulted in the breakdown of the drug. In the example shown in Fig. 2 two components are visible in the

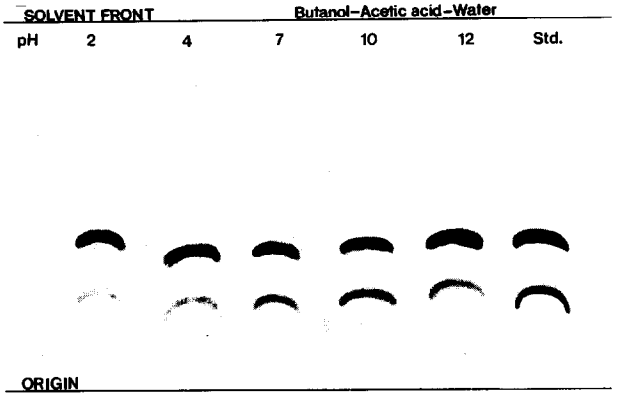


**Figure 2**
TLC autoradiogram of Cas 434 pH stability study on silica gel TLC plates. The standard gives 2 bands containing 66.7 (upper) and 30.2 (lower) % of the radioactivity on the plate.

autoradiogram. Decomposition of the freshly prepared standard is equal to or greater than that observed in the test samples, in this case simple aqueous solutions at different pH values (2, 4, 7, 10 and 12). Similar effects were seen with the biological samples. Two-dimensional TLC, using the same solvent system for both dimensions, demonstrated that breakdown occurred on the plate. The excellent shape of the bands and the lack of streaking suggests that decomposition occurred when the plate was dry, and not during chromatography.

When reversed-phase TLC on the $C_{12}$ bonded silica was attempted, no artefactual decomposition of the standard was seen. When samples of the drug in biological fluids, incubated at a variety of temperatures for up to 40 days, were chromatographed under the same conditions a number of decomposition products were readily observed (Fig. 3). Furthermore the extent of breakdown can be seen to be temperature dependent, with little decomposition at low temperature and extensive degradation at 37°C. With plasma samples it was necessary to remove protein before applying the sample to the plate to avoid pronounced streaking. Neither the artefacts produced by TLC on silica gel nor the genuine degradation products revealed by RP-TLC were investigated further but the fate of Cas 434 on TLC using silica gel provides a useful illustration of the problems which can arise using this method, whilst RP-TLC provides a practical solution. The ability to apply the sample directly to these RP-TLC plates represents a great saving in time compared with other, hydrophobic, RP-TLC plates. Because the hydrophobic RP-TLC plates will not accept samples in aqueous solutions the sample must either be freeze dried and dissolved in a suitable solvent, or the drug must be isolated by solvent extraction. The ability to apply aqueous samples has further advantages due to the low eluotropic properties of water which result in very small spots (or streaks) at the origin, important for good chromatography.

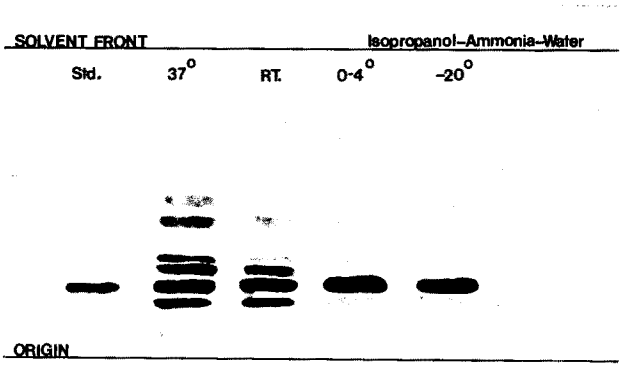


**Figure 3**
TLC autoradiogram of urine samples containing Cas 434. Chromatography on $C_{12}$ bonded silica after storage of samples for 41 days at −20°C, 4°C, ambient temperature and 37°C.

Reversed-phase TLC with bonded phases represents a useful alternative to normal phase TLC on silica gel, where this results in the artefactual decomposition of the drug. The development of fully wettable, non-hydrophobic RP-TLC plates is a significant advance, and makes the technique much more suitable for samples such as bile, urine and plasma.

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