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# Chromatographic Separation of Various Pharmacological Classes of Therapeutic Agents on Impregnated Thin Layer Silica Gel Plates

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## DESCRIPTION

Thin Layer Chromatography (TLC) is a useful liquid chromatographic technique use to separate and identify chemical substances in mixtures. Analytically, the technique has been used to identify and quantify therapeutic agents in pharmaceutical drug products and biological fluids. Surfactants are organic compounds with at least one hydrophilic group and one hydrophobic group in the molecule. Buffer solutions are solutions of constant PH values. To investigate the degree of separation of various pharmacological classes of therapeutic agents which are co-administered (based on prescriptions) on surfactant and PH control-impregnated thin layer chromatographic plates. Thin Layer Chromatography (TLC) is the separation of chemical substances between two phases (stationary solid phase and mobile liquid solvent phase) based on differences in solubility of chemical substances in the two phases [1]. Several studies have reported the chromatographic separations of chemical substances on silica gel-impregnated thin layer chromatography with the primary aim of improving separation of various classes of chemical substances. Studies [2,3] have reported the separation of aliphatic amines, sulfa drugs and phenolic compounds on metal salts or soap-impregnated silica gel plates. Furthermore, aromatic amines have been separated on surfactant-impregnated silica gel plates [4].

### *Experimental*

**Chemicals and reagents:** Acyclovir and pioglitazone, amlodipine and atenolol, enalapril, glibenclamide, hydrochlorothiazide and valsartan, lamivudine, sitagliptin. Cetylmethyl ammonium chloride, polysorbate 80 (tween 80) and sodium lauryl sulphate. Ammonia, acetic acid, sodium acetate, citric acid, sodium citrate, sodium dihydrogen phosphate and disodium hydrogen phosphate and iodine crystals were of analytical grade. Silica gel G6 was from BDH, Mumbai, India. Methanol, chloroform, ethyl acetate, toluene were of HPLC grade. The water was purified using a Milli-Q system.

**Method**: Thin layer chromatographic plates (10 cm × 20 cm) were prepared by mixing silica gel G6 with purified water in 1:3 volume ratios for 5 min to obtain homogeneous slurry. The resultant slurry was coated on the thin layer plates using a TLC applicator to give a 0.25-mm layer thickness. Silica gel impregnated plates were similarly prepared using surfactants and buffers of known PH values as impregnating agents. The plates were activated at a temperature of 105°C for 30 minutes after being air dried and then used in the analysis. The chromatographic separation of the drugs was carried out in a saturated development chamber. The developing solvent system composing of methanol: chloroform: ethyl acetate: ammonia (1.5:3:5:0.5) was about 1.5 cm in height in the development chamber. The solutions (5 µ g/ml) of studied drugs in methanol were applied about 2 cm above the bottom edge of the plate using micropipette. After development, the developed chromatograms were dried and the spots were visualized with short UV light and iodine vapor respectively. The Retardation ($R_F$) Factor values were calculated.

The results showed that out of ten therapeutic agents studied, enalpril, glibenclamide and sitagliptin were neither separated on plain silica gel plate nor on impregnated plates using the optimized developing solvent system. The remaining seven therapeutic agents exhibited some significant separations on surfactant and PH control-impregnated silica gel plates when compared to the plain silica gel plate (control). With the surfactants investigated, tween 80 gave the best separation for the ten therapeutic agents evidenced by Retardation Factor ($R_F$) values. The obtained retardation factor values are amlodipine ($R_F$ 0.81 ± 0.02) atenolol ($R_F$ 0.68 ± 0.04), hydrochlorothiazide ($R_F$ 0.74 ± 0.02), lamuvidine ($R_F$ 0.56 ± 0.03), pioglitozone ($R_F$0.65 ± 0.02) and valsartan ($R_F$0.37 ± 0.04) respectively. The retardation factor values obtained on plain silica gel plates (control) are amlodipine (0.75 ± 0.03), atenolol (0.61 ± 0.02), hydrochlorothiazide (0.65 ± 0.02), lamuvidine (0.48 ± 0.02), pioglitozone (0.59 ± 0.03) and valsartan (0.31 ± 0.02) respectively. With the ionic surfactants, sodium lauryl sulfate had a better separation than tween 80 on atenolol ($R_F$ 0.73 ± 0.03) and valsartan ($R_F$ 0.41 ± 0.03) respectively while cetrimide gave better separation on acyclovir ($R_F$ 0.68 ± 0.02) than tween 80. The results also indicated that pH control-impregnated silica gel plates had only significant effect on the $R_F$ values of hydrochlorothiazide at different PH values (2.5, 4.5, 6.8 respectively) when compared to the control (plain silica gel plate). For example, the $R_F$ values are 0.48 ± 0.02, 0.53 ± 0.03, 0.57 ± 0-03 for PH values 2.5, 4.5, and 6.8 respectively while the control $R_F$ value was 0.65 ± 0.02.

The Retardation Factor ($R_F$) value is used to quantify the movement of the chemical substances along the thin layer chromatographic plates. We define retardation factor as equal to the distance moved by the substance divided by the distance moved by the solvent and the value is always between zero and one. The developing solvent system composition of methanol: chloroform: ethyl acetate: ammonia (1.5:3:5:0.5 v/v) out of 18 solvent systems tried gave the best separation of the therapeutic agents studied probably due to their mixed polarity properties. With the PH control-impregnated silica gel plates, the acidity or basicity of active agents might have governed their separation. For instance at PH 2.5, acyclovir barely left the base line while valsartan gave almost the retardation factor value it had on plain silica gel plate probably because of their acidic properties.

## CONCLUSION

Six out of ten therapeutic agents investigated were very significantly separated on polysorbate 80 (tween 80) impregnated silica gel plates using the optimized developing solvent system. The implication is that when two or more of these six active agents are co-administered, their identifications in biological fluids are feasible using the optimized developing solvent system. Furthermore, their biological fluids concentration levels could be accurately quantified with the application of high performance thin layer chromatographic method.

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