# Drugs Identification in Urine, Bile and Gastric Contents using Thin Layer Chromatography in Multiple Screening Systems

A method of simultaneous identification of 25 molecules in human urine, bile and gastric contents using liquid-liquid extraction followed by thin layer chromatography (TLC) using multiple screening systems is described. The analytes were extracted at 25°C under isocratic conditions using chloroform after acidification with 1 to 2 drops of HCl 6 N for 10 mL of the biological sample, and dichloromethane after alkalization with 1 to 2 drops of NaOH 10 N for 10 mL of the biological sample. Employing LLE, the best conditions were achieved with double extraction of 10 mL of the biological sample, pH=9.5 for alkaline extraction and pH=2 for acid extraction. The organic extractums were filtered and dehydrated using anhydrous sodium thiosulfate powder and concentrated after evaporation of the organic solvents at 65°C. The extraction residues were solubilized in 500 µl of methanol and spotted with the molecules of reference onto four TLC plates (10 cm × 10 cm). The TLC plates were put into twin-through development chambers previously incubated 30 minutes for saturation namely TA (methanol:ammoniac 5% (50:0.750, v/v)), TD (chloroform:acetone (40:10, v/v)), TE (Ethyl acetate:methanol:ammoniac (42.3:5:2.5, v/v/v)), TB (cyclohexan:toluene:diethylamine (37.5:7.5:5, v/v/v)). The mobile phase migrates by capillarity through the stationary phase, driving at different speeds the molecules to be separated. The migration time (several minutes) depends on various parameters. When the solvent front has moved through a

distance considered as sufficient (a few centimetres), the TLC plates were removed and dried, then exposed to ultraviolet light, the retardation factors Rf of each visible spot was measured. Some chemical processes might also be used to reveal spots. The total number of substances present in the biological sample was determined by counting the number of spots found on each TLC plate, the biggest number among the four counted values is considered as the default number of the present substances. A mathematical formula was applied to guess all possible matches according to a data table of $R_f$ profiles of standards already calculated by the same method. The validation parameters obtained in LLE were linearly range of 50-1000 µg $mL^{-1}$ biological fluid ($r \geq 0.9815$). This method has shown its suitable applicability in order to rapidly identify a wide verity of substances of toxicological interest present in the biological samples. Moreover, it's inexpensive and could be suggested in various routine drug screening processes, especially for toxicological/forensic analysis.

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