10

Basic Principles of Optical Quantification in TLC

Mirko Prošek and Irena Vovk

National Institute of Chemistry, Ljubljana, Slovenia

I. INTRODUCTION

Quantitative thin-layer chromatography (QTLC) measured by direct photometric scanning has been performed for nearly 50 years. Despite its long history, this procedure has not achieved the reputation of being a very reliable quantitative analytical technique. Relatively large standard deviation has often been mentioned as one reason that QTLC was not acceptable as a reliable quantitative technique. The most important drawbacks were problems in sample application, development, scanning, and data processing. The opposition was unjustified (1). TLC is an open-bed technique with many not precisely controllable parameters, which on the one hand contributes to a large dispersion of measurements but on the other hand eliminates systematic errors. High accuracy can easily be obtained by using a large number of applications of the same sample and statistical methods. In addition, certain steps in the procedure can be strictly controlled and even automated. Major improvements in reproducibility, simplicity, and speed are obtained with automatic sample applicators, controlled development and drying conditions, and sophisticated computer-controlled scanning modes with the use of image processing.

The possibility of simultaneous development of up to 74 samples on one HPTLC plate makes planar chromatography one of the most informative microanalytical techniques. Many more expensive and sophisticated combination techniques such as gas chromatography/mass spectometry (GC/MS), high-performance liquid chromatography/MS (HPLC/MS), HPLC/inductively coupled plasma-MS (HPLC/ICP-MS), and capillary electrophoresis/MS (CE/MS), can generate even more data per second than planar chromatography and can collect data from different detectors at the same time but from only a single sample. Different samples can be compared only by use of software that enables collection and post-run parallel presentation of results. Samples can be evaluated together, but data are collected at different times and only in the case of very robust measurement conditions can data be compared.

Among the users of chromatography around the world today, it is possible to see renewed and increasing interest in TLC. Analysts have seen that sophisticated and specifically oriented techniques cannot be properly used if they are not planned according to the results obtained by prescreening using cheaper, less sensitive, but more informative techniques such as TLC.

The production of uniform TLC plates with different types of layers, instrumentalized programmable applicators and development systems, and the use of sophisticated, inexpensive computers, scanning devices, charge-coupled device (CCD) cameras, and printers open up new possibilities for reliable quantitative TLC.

Various modes of quantification in TLC are indicated in Fig. 1. In the simplest mode, substance is eluted from the plate and quantified with a spectrophotometer. Today elution is not often used for quantitative measurement, but it is very convenient for identification of compounds in separated spots on TLC plates with mass spectrometry (2,3). Direct in situ modes of quantification, using a densitometer, CCD camera, or flatbed scanner, are used for routine work. Most often,