8

Detection, Identification, and Documentation

Gerda Morlock

Scientific Consultant, Stuttgart, Germany

Karl-Arthur Kovar

University of Tübingen, Tübingen, Germany

I. INTRODUCTION

For detection and identification of chromatogram zones, in situ techniques are generally employed. As in an analytical disk (1), the information stored in the chromatogram can be used for various detection and identification methods, even successively, because the processes of chromatographic development and detection or identification are independent in both time and space. Detection in HPTLC takes place in the absence of the mobile phase and therefore offers much greater choices than any other chromatographic technique. This means that

1. Multiple subsequent detection of the same chromatogram is possible. In addition to recording, e.g., an absorbance or fluorescence scan using visible or ultraviolet (UV) light, a Fourier transform infrared (FTIR) or Raman spectrum can be recorded, and these methods can be followed by a suitable microchemical reaction or mass spectrometry (MS) to provide additional information.
2. Detection can be repeated with different parameters, e.g., portions of the chromatogram can be selectively evaluated.
3. Postchromatographic derivatization can easily be performed on the plate. A great variety of selective or specific reagents can be used to ease detection and identification.

Absorbance or fluorescence spectrometry and microchemical detection are commonly employed in TLC (Fig. 1). Bioactivity-based reactions, i.e., microbiological and biochemical detection methods, have gained interest for toxicologically relevant substances. In situ FTIR spectroscopy has become a practical method for detection and identification, and Raman spectroscopy has gained importance with the introduction of lasers as the light source. Furthermore, the combination of TLC with in situ MS (see Chap. 9) can be employed. Detection and identification of radioactively labeled substances by autoradiographic, fluorographic, spark chamber, or scanning techniques are discussed in a special chapter of this Handbook (see Chap. 12). The combination of TLC with flame ionization detection (Chap. 13) is used only for special purposes (2).

With all of these methods, accurate documentation is necessary to provide reliable and reproducible results. Factors of influence must be documented in detail. Nowadays, protocols and image documentation of the plate involve the use of computers, but drawing, sketching, tracing, photocopying, or photographing can also be used to obtain images of the plate.

II. DETECTION

For detection, physical, microchemical, microbiological, and biochemical methods are available in HPTLC. Physical detection methods mainly include either absorbance or fluorescence mea-