

# Quantitative Thin-Layer Chromatography



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# Quantitative Thin-Layer Chromatography

A Practical Survey



Springer

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# Preface

Thin-layer chromatography (TLC) is a rather ignored quantification technique. The method is widely used for education purposes and qualitative analysis. The analysis of herbs, in particular, is often performed by TLC due to the simultaneous separation of different samples facilitating their differentiation at first glance. The aesthetic results of individual coloured TLC zones are certainly appreciated by many people who enjoy looking at such results. Another critical aspect of TLC is the humidity dependence of adsorption chromatography. This is certainly the major reason why adsorption TLC was labelled “irreproducible” and “unreliable” and why industry prefers closed systems such as HPLC. Another reason for avoiding TLC is that analyses using highly automated HPLC were generally superior to TLC, which relies more on the skill of the analyst. Last, but not least, the odour of vanillin reagent reminded laboratory staff on a daily basis that chemical analysis is a part of chemistry and not computer science.

All these aspects that spoke against TLC are now just history. Modern equipment and working practices have overcome all these problems. TLC calibration curves are now linear over more than three orders of magnitude. Modern sample application, development chambers, and reagent spray or dipping devices provide the required degree of automation, reliability, and independence of the local environment (temperature, humidity, etc.) associated with robust analytical methods. Nevertheless, quantitative TLC, unlike HPLC, is still mostly done in resource-limited laboratories with incomplete instrument support.

This book is written as a self-study guide for professional scientists to refresh their understanding of modern TLC. It presents the complete theory of quantitative TLC analysis. It is also written for newcomers who want to use quantitative TLC but have limited access to older books which are often unavailable or difficult to obtain. The main concept was to collect in one place all the knowledge necessary to perform quantitative TLC. The chapters follow a modular style facilitating access to information relevant to the individual operations of a successful TLC analysis. The book starts with a chapter on history followed by a chapter on theory (including practical hints for fast and reliable method development). Chapter 3 introduces the different stationary phases and Chap. 4 the various mobile phases. Chapter 5

describes sample pre-treatment techniques and methods for sample application. Chapter 6 deals with the different development techniques. Chapter 7 adopts a dictionary-like style to introduce the many methods of general and specific staining reactions for visualization. Chapter 8 concentrates on analysis linked to bio-effective methods bridging the gap with biology. Chapter 9 describes all known measurement methods for evaluating TLC separations. Chapters 10 and 11 contain the theory for spectrometric methods linked to Chap. 12 that deals with chemometric methods of data analysis to maximize the information contained in the measured data. The book ends with two chapters (Chaps. 13 and 14) on basic statistics and planning and validation of TLC analyses.

Our hope is that this book will demonstrate that quantitative planar chromatography is a practical alternative in liquid/solid and liquid /liquid separations. We hope that the liquid/solid separation method, which is TLC's strength, will thus find new friends. In 1987 Friedrich Geiss wrote in the preface of his book *Fundamentals of Thin Layer Chromatography* "TLC is here to stay". We believe indeed that TLC will consolidate its position in analytical chemistry. Taking the biological measurement techniques of Chap. 8 into account, we hope that TLC will even extend its range and position among separation methods.

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# Acknowledgements

From my time in the industry, I personally remember the delighted expressions of laboratory staff looking at a nicely stained TLC plate. I remember in the mid-1980s that TLC was widely used for quantification purposes. I also remember that my technicians were always suspicious of TLC due to its non-linear calibration curves. It was difficult for them to accept that a doubling of the amount of substance did not result in a doubling of the measurement signal. All this was the reason for me to write a TLC book that helps to overcome this problem. I am indebted to the ladies of the Offenburg library who nearly instantly provided the desired papers for this book. I wish to thank Trisha Cornforth for her help in interpreting my German text into English, and I wish to thank my wife Marion and my daughter Johanna for their understanding and patience.

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October 2010

Bernd Spangenberg



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