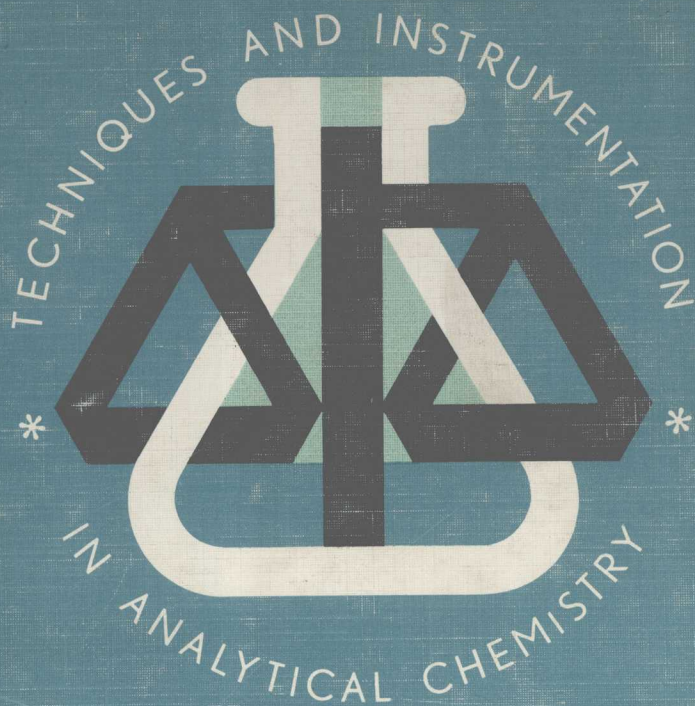


1



EVALUATION AND OPTIMIZATION OF LABORATORY METHODS AND ANALYTICAL PROCEDURES

A Survey of Statistical and Mathematical Techniques

D.L. Massart, A. Dijkstra and L. Kaufman

with contributions by S. Wold, B. Vandeginste and Y. Michotte

ELSEVIER SCIENTIFIC PUBLISHING COMPANY

TECHNIQUES AND INSTRUMENTATION IN ANALYTICAL CHEMISTRY — VOLUME 1

Evaluation and Optimization of Laboratory Methods and Analytical Procedures

A Survey of Statistical and Mathematical Techniques

Desiré L. Massart

Farmaceutisch Instituut, Vrije Universiteit Brussel

Auke Dijkstra

Laboratorium voor Analytische Chemie, Rijksuniversiteit Utrecht

Leonard Kaufman

Centrum voor Statistiek en Operationeel Onderzoek, Vrije Universiteit Brussel

with contributions by **Svante Wold**, **Bernard Vandeginste** and **Yvette Michotte**



ELSEVIER SCIENTIFIC PUBLISHING COMPANY
Amsterdam — Oxford — New York 1978

ELSEVIER SCIENTIFIC PUBLISHING COMPANY
335 Jan van Galenstraat
P.O. Box 211, 1000 AE Amsterdam, The Netherlands

Distributors for the United States and Canada:

ELSEVIER/NORTH-HOLLAND INC.
52, Vanderbilt Avenue
New York, N.Y. 10017

First edition 1978
Second impression 1980

Library of Congress Cataloging in Publication Data

Massart, D L
Evaluation and optimization of laboratory
methods and analytical procedures.

(Techniques and instrumentation in analytical
chemistry ; v. 1)

Bibliography: p.

Includes index.

1. Chemistry, Analytic--Statistical methods.

I. Dijkstra, Auke, joint author. II. Kaufman,
Leonard, joint author. III. Title. IV. Series.
QD75.4.S8M37 543 78-12667

ISBN 0-444-41743-5

ISBN 0-444-41743-5 (Vol. 1)
ISBN 0-444-41744-3 (Series)

© Elsevier Scientific Publishing Company, 1978

All rights reserved. No part of this publication may be reproduced, stored in a retrieval system or transmitted in any form or by any means, electronic, mechanical, photocopying, recording or otherwise, without the prior written permission of the publisher, Elsevier Scientific Publishing Company, P.O. Box 330, 1000 AH Amsterdam, The Netherlands

Printed in The Netherlands

Errata

Page 99, 2nd equation, 3426^2 should read 3436^2

Page 536, 2nd line, $2\pi\omega_0$ should read $\omega_0/2\pi$

3rd line, $\sum_{n=1}^{\omega_m/\omega_0}$ should read $\sum_{n=-\omega_m/\omega_0}^{\omega_m/\omega_0}$

Page 545, Fig. 27.1, e^{t/τ_x} (ordinate) should read e^{-t/τ_x}

Page 549, 4th line, 3% should read 1%

INTRODUCTION

One of the major problems with which the analytical chemist is confronted is how to make the best possible use of the large amount of information on analytical principles, methods and procedures that is available in the analytical literature. As stated by Laitinen and Harris (1975), an analytical chemist can be judged in part by his skill in the critical selection of methods. Almost daily the analytical chemist is confronted with problems of optimizing analytical procedures or related problems such as the selection of the best procedure for solving a given problem. This situation is represented by the most recent definitions of analytical chemistry, stating that analytical chemists have to produce qualified, relevant information on materials and processes in an optimal way (Gottschalk, 1972 ; Kaiser, 1974). It is therefore surprising to note that analytical chemists in general do not seem to have taken pains to develop strategies for optimization. Until recently it was common for the choice of the best procedure for a given analytical problem to be made largely intuitively and based upon experience.

Recently, a number of papers have appeared that express the concern of an increasing number of analytical chemists with this situation. It has been our intention to discuss in this book the formal methods that are available at present for the optimization and selection of analytical methods.

Before it is possible to make a selection or to carry out an optimization, one must have criteria according to which this may be done. Consequently, the performance of analytical procedures has to be evaluated by determining one or more performance characteristics of the procedure that is to be optimized or of the procedures from which the best one is to be selected. The set of criteria has to be defined for each problem and will include quantities such as precision, accuracy, limits of detection and interferences. Up to now, most of these criteria have been used in quantitative analysis, and it is probable that another set of characteristics will be required for qualitative analysis. Measures of information may become important in this respect and, therefore, a large section is devoted to information theory. Performance characteristics are discussed

in Part I.

The next two parts of the book are devoted to the optimization of procedures. One can discern different levels of optimization (Beveridge and Schechter, 1970), and for the purpose of this book we consider three such levels :

(1) Selection of one existing procedure from several alternatives. This is the simplest possible stage. There are several alternatives ; each of these is evaluated and the one which corresponds best to the exigencies of the application is selected. The main problem here is the evaluation of the procedures. This is described in Part I.

(2) Optimization of a procedure for which the outline is given. For example, given that the determination will be carried out colorimetrically with dithizone, select the optimal wavelength, the best concentration of dithizone, the pH, etc. This kind of problem usually consists in the selection of the optimal value of one or more continuously adjustable parameters. Occasionally, one may also have to include discrete parameters such as the kind of detector to be used. These problems are discussed in Part II.

(3) Optimization of combinations of analytical procedures or attributes of methods. There are many instances in which analytical procedures are combined, for instance

the combination of tests in a clinical laboratory to yield the optimal diagnostic or discriminatory power ;

the combination of GLC stationary phases to form a preferred set ;

the combination of elementary steps in a separation procedure to yield an optimal multicomponent separation scheme.

Such combinatorial problems are discussed in Part III. The analytical laboratory as a whole may be considered as a combination of methods, apparatus, etc. Some optimization problems concerning the functioning of an analytical laboratory are therefore also included in Part III.

Laitinen (1973), in an Editorial in *Analytical Chemistry*, stated that an analytical method is a means to an end, and not an end in itself. Analytical chemists tend to overlook this and sometimes develop methods that are more precise, faster, etc., while forgetting the intended applications. In such

instances, one can hardly speak of optimization. The analytical procedure must be chosen in relation to the goal and questions such as "how useful is it to increase the precision of a procedure used for a particular purpose" then arise. Some problems of this kind are discussed in Part IV.

Problems of optimization in analytical chemistry are often related to other optimization problems. However, such analogies will only be recognized if problems are formulated in a more or less formal and generalized way. The analytical procedure and the analytical laboratory should be considered from the point of view of systems theory. Some aspects of such a generalizing approach are discussed in Part V. In fact, one observes that analytical chemists concerned with optimization problems intuitively follow a systems approach. It would therefore have been more logical to start this book with a discussion of systems theory, but as yet it is not possible to construct a complete systems theoretical picture of the analytical procedure and the analytical laboratory, and to some extent the topic is of academical interest. Therefore, we have started the discussion with those points which clearly are of direct value in analytical practice.

The trend towards a more formal approach of the selection of analytical methods is not really new, but it has definitely grown stronger in the last few years. It is one of the principal concerns of the very recent field of chemometrics (Kowalski, 1975). It is not only felt among those who make this their research field in general analytical chemistry, but also by analytical chemists who are concerned more directly with analytical practice, such as clinical chemists and by those who need the results of analytical determinations, such as physicians using laboratory tests for medical diagnosis. At about the same time that concepts such as information were introduced into general analytical chemistry, clinical chemists began to use multivariate data analysis techniques to investigate which analytical methods yield the most diagnostic information. If one looks at the literature cited by the "generalists" and the clinical chemists, one finds that they cite different literature and that,

in general, there seems to be very little communication between the two groups.

In some applications, formal methods for the investigation of the performance of analytical methods were introduced many years ago. This is the case, for example, with official analytical chemists, who have developed methods for the evaluation of errors likely to occur in analytical procedures. Many analytical chemists from other specialities, but who are also concerned with the evaluation of analytical methods, seem, however, to ignore the existence of such methods.

We have tried to combine the knowledge stored in these (and other) different specialities in the hope of stimulating a more systematic application of formal selection methods in analytical chemistry. Our first idea was to limit this book to newer methods or concepts, such as information theory and operational research; but it was soon clear that it would be meaningless to try and make a synthesis and not include classical statistical concepts. Therefore, a number of chapters on classical statistical methods were added. Because, on the other hand, we did not want to duplicate the material already available in several books on statistics in chemical analysis, we have tried to eliminate statistical methods designed for the evaluation of results rather than of procedures, and we have not tried to discuss the subject exhaustively. This is also true for all other chapters. More specialized knowledge should be sought in the original literature or specialized books and chapters to which we refer. Since this book was written to introduce a number of formal optimization techniques to analytical chemists and not for specialists, we have not tried to cover the existing literature exhaustively. Instead, we usually have given some references to books, review articles and a few illustrative articles.

In writing this book, we have started from the belief that some of the newer mathematical methods or theories, such as pattern recognition, information theory, operational research, etc., are relevant to some of the basic aims of analytical chemistry, such as the evaluation, optimization, selection, classification, combination and assignment of procedures or sub-procedures - in short all those processes that intervene in determining exactly which analytical procedure or

programme should be used. Unfortunately, most chemists are daunted by the task of learning how these mathematical methods function and this is not helped by the difficulty of establishing a link between the formal mathematics found in most books on this subject and analytical problems.

We have tried to treat the mathematical topics as lucidly as possible and to illustrate the text with examples, in some instances abandoning a rigorous mathematical treatment. However, we have tried to compensate for this by including a series of mathematical sections. The level of the mathematics is higher in Chapters such as 2, 3 and 4, where the subject treated is not completely unfamiliar to most analytical chemists. In those chapters where the subject matter is probably new to most analytical chemists, only the most elementary explanations are given, often in words, because we think it more important to emphasize the underlying philosophy than to explain the mathematics. In doing so, we hope we have removed the barriers of applying formal methods to optimization problems in analytical chemistry. One major difficulty encountered when writing this book was the mathematical symbolism. We have tried to present a coherent set of symbols throughout the book but, because of the diversity of the methods described, we have not been entirely succesful in this respect. Nevertheless, we think that the symbols used should be sufficiently clear.

Parts of the draft of this book were read by E. Defrise-Gussenhoven (Vrije Universiteit Brussel ; Chapters 2 and 3), H.C. Smit (University of Amsterdam ; Chapter 10), D. Coomans (Vrije Universiteit Brussel ; Chapter 20), G. Kateman (Catholic University of Nijmegen ; Chapters 26 and 27) and P.M.E.M. van der Grinten (DSM, Heerlen ; Chapters 26 and 27). Their suggestions were very valuable. The authors are also indebted to S. Peeters, who typed the final text, A. Langlet-De Schrijver and L. Maes, who prepared the figures, and C. Uytterhaegen-Hendrickx, G. De Boeck, A. Van Gend, F. Gheys and M. Segers-Geeroms who typed the manuscript. The research leading to the idea of writing this book was helped financially by organizations such as the Fonds voor Geneeskundig Wetenschappelijk Onderzoek and the Fonds voor Kollektief en Fundamenteel Onderzoek and was stimulated by collaboration with members from the Studiegroep voor

XVI

Laboratorium optimalisering and the Centrum voor Statistiek en Operationeel Onderzoek of the Vrije Universiteit Brussel, and with the following students, who obtained degrees based on research on subjects covered in this book : H. De Clercq, M. Detaevernier, J. Smeyers-Verbeke, J.H.W. Bruins Slot, P.F. Dupuis, G. van Marlen, P. Cley, T. Koppen and A. Eskes. The proofs were read by A. Kaufman. The authors express their thanks to all of these persons and organizations.

REFERENCES

- G.S.G. Beveridge and R.S. Schechter, Optimization : Theory and Practice, McGraw-Hill, New York, 1970.
G. Gottschalk, Z. anal. Chem., 258 (1972) 1.
R. Kaiser, Z. anal. Chem., 272 (1974) 186.
B.R. Kowalski, J. Chem. Inf. Computer Sci., 15 (1975) 201.
H.A. Laitinen, Anal. Chem., 45 (1973) 1585.
H.A. Laitinen and W.E. Harris, Chemical Analysis, McGraw-Hill, New York, 1975.

CONTENTS

Introduction	XI
Part I Evaluation of the performance of analytical procedures	
Chapter 1. Performance characteristics of analytical procedures	3
Chapter 2. Precision and accuracy	7
2.1. General discussion of concepts	7
2.1.1. Introduction	7
2.1.2. Categories of errors in analytical chemistry	7
2.1.3. Precision and accuracy as criteria	10
2.1.4. Definition and measurement of precision (repeatability, reproducibility)	11
2.1.5. Definitions of bias and accuracy	14
2.1.6. A demonstration of the importance of laboratory bias	16
2.1.7. Some studies on precision	20
2.1.8. The total error	21
2.2. Mathematical	22
2.2.1. Frequency distributions	22
2.2.2. The mean of a frequency distribution	23
2.2.3. The variance and standard deviation of a frequency distribution	24
2.2.4. Discrete and continuous random variables	25
2.2.5. Parameters of a continuous random variable and their estimation	28
2.2.6. Some special distributions	32
Chapter 3. Evaluation of precision and accuracy-comparison of two procedures	39
3.1. General discussion of methods and concepts	39
3.1.1. Introduction	39
3.1.2. Evaluation of method bias using tests on the mean (t-test)	42
3.1.3. Non-parametric tests for the comparison of methods	47
3.1.4. Comparison of two methods by least squares fitting	49
3.1.5. Recovery experiments	55
3.1.6. Comparison of the precision of different methods (F-test)	57
3.2. Mathematical section	58
3.2.1. Theory of statistical tests ; statistical decisions	58
3.2.2. Parametric and non-parametric statistical tests	63
3.2.3. Tests for ordinal scales	63
3.2.4. Tests for interval or arithmetical scales	66
3.2.5. Two-dimensional frequency distributions	73
3.2.6. Two-dimensional random variables	76
3.2.7. Multi-dimensional random variables	79
3.2.8. The fitting of a straight line by the least squares method	81
Chapter 4. Evaluation of precision and accuracy. Analysis of variance	87
4.1. General discussion	87
4.1.1. Introduction ; definitions	87
4.1.2. A one-way layout	90
4.1.3. Fixed- and random-effects models	96
4.1.4. A two-way layout	97
4.1.5. Applications	101
4.1.6. Bartlett's test for the comparison of more than two variances	105
4.2. Mathematical section	107
4.2.1. The general linear model	107
4.2.2. The analysis of variance	112

Chapter 5. Reliability and drift	127
5.1. The <i>a posteriori</i> approach ; quality control	127
5.1.1. Control chart methods for detection of drift	127
5.1.2. Operational research methods	129
5.1.3. Other statistical methods	135
5.2. The <i>a priori</i> approach ; ruggedness of a method	136
Chapter 6. Sensitivity and limit of detection	143
6.1. Introduction	143
6.2. Sensitivity and the analytical calibration function	145
6.3. Decision limit	147
6.4. Detection limit	150
6.5. Determination limit	151
6.6. Discussion	151
6.7. Gas chromatographic detectors	153
Chapter 7. Selectivity and specificity	157
7.1. Introduction	157
7.2. Quantification of selectivity and specificity	159
7.3. Some examples	162
Chapter 8. Information	165
8.1. Introduction	165
8.2. Information content	166
8.3. An application to thin-layer chromatography	170
8.4. An application to gas chromatography	176
8.5. Discussion	178
8.6. Tests of fit	181
Chapter 9. Practicability	185
9.1. Introduction	185
9.2. Cost	186
9.3. Time aspects	191
9.4. Some relationships between characteristics	192
Chapter 10. Characterization of continuous procedures	195
10.1. Continuous versus discontinuous procedures	195
10.2. Noise and drift	196
10.3. Response as a function of time	201
10.4. Discussion	205
10.5. Stochastic processes (mathematical)	207
 Part II Experimental optimization	
Chapter 11. Survey of experimental optimization methods	213
11.1. Introduction - the need for formal methods	213
11.2. Optimization strategies	216
Chapter 12. Factorial analysis	219
12.1. Description of the method	219
12.2. Mathematical section	226
12.3. Examples	231
12.4. Worked example	236
Chapter 13. Simultaneous experimental designs	243
13.1. Complete factorial designs	243
13.2. Incomplete factorial designs	244
13.2.1. Neglection of higher order terms	244
13.2.2. Partial factorials	245
13.2.3. Latin squares	245
13.2.4. Fractional factorials	247
13.3. Use for optimization purposes	254
Chapter 14. Sequential experimental designs	257
14.1. One-parameter methods	257
14.1.1. The use of Fibonacci numbers	258
14.1.2. The uniplex method	263
14.1.3. An application	265

14.2. Multiple parameter methods	266
14.2.1. The simplex method	267
14.2.2. The modified simplex method	270
14.2.3. An example	272
14.2.4. Other applications	273
14.2.5. Difficulties in the application of simplex optimization methods. Comparison with factorial designs	274
Chapter 15. Steepest ascent and regression methods	279
15.1. Introduction	279
15.2. Steepest ascent methods	279
15.3. Regression methods	284
15.3.1. Location of the optimum	284
15.3.2. Determination of the optimum from regression equations	294
15.4. A comparison of methods	295
15.5. Mathematical section : multiple regression	296
15.5.1. Surface fitting	296
15.5.2. Fitting of plane surfaces	297
15.5.3. Fitting of a quadratic surface	300
Part III Combinatorial problems	
Chapter 16. An introduction to combinatorial problems in analytical chemistry	305
16.1. Introduction	305
16.2. Some examples of combinatorial problems	306
16.3. Pattern recognition and related techniques	309
16.4. Multivariate statistical techniques	314
16.4.1. Introduction	314
16.4.2. Hotelling's T^2	315
16.4.3. Multivariate analysis of variance (MANOVA)	316
16.4.4. Measures of correlation	316
16.4.5. Analysis of covariance	317
16.4.6. Techniques for reducing a set of variables	318
16.5. Operational research	319
Chapter 17. Preferred sets - some selection procedures	325
17.1. Quantitative multicomponent analysis	325
17.2. Least squares solution	328
17.3. Choosing an optimal set of wavenumbers	332
17.4. The information content of combined procedures	337
17.5. Selection of an optimal set of stationary phases	339
17.6. Discussion	344
17.7. Matrix algebra	347
17.7.1. Introduction	347
17.7.2. Some definitions	348
17.7.3. Operations on matrices	354
17.7.4. Eigenvalues and eigenvectors	356
Chapter 18. Preferred sets - the classification approach	361
18.1. The classification problem	361
18.2. Classification techniques	362
18.3. Measures of resemblance	364
18.4. Clustering procedures	366
18.4.1. Types of clustering procedures	366
18.4.2. The average linkage algorithm	367
18.4.3. Operational research techniques	369
18.4.4. The selection of the preferred set from the classification	372
18.5. Information and classification	372
18.5.1. A comparison of the information theoretical and numerical taxonomic approaches	372
18.5.2. Classification using an information theoretical criterion	373
18.6. Applications	377

18.7. Correlation and distance	378
18.7.1. Measures of distance between elements	379
18.7.2. Correlation and distance based on correlation	380
18.7.3. Distance between groups	382
Chapter 19. Factor and principal components analysis	385
19.1. Introduction	385
19.2. A short presentation of FPCA	387
19.3. Basis of FPCA	388
19.3.1. Relation to multiple regression (MR)	388
19.3.2. FPCA as a general model for a group of similar objects	389
19.3.3. Geometrical interpretation	390
19.3.4. Limitations of the method	391
19.4. Estimation of the parameters in the FPCA model	392
19.4.1. Scaling of data	392
19.4.2. The number of factors, r	393
19.4.3. Estimation of loadings and factors (components)	396
19.5. Information from the data analysis	399
19.6. Rotations and transformations of the parameter vectors	401
19.6.1. Transformations to simplify the parameter vectors	402
19.6.2. Transformations to find correlations with external variables	402
19.7. Additional data in analytical applications of FPCA	403
19.8. Other analytical chemical applications of FPCA	404
19.9. Conclusions	406
Chapter 20. Supervised learning methods	409
20.1. Introduction	409
20.2. Statistical linear discriminant analysis	412
20.2.1. Classification	412
20.2.2. Applications	415
20.3. Non parametric methods	418
20.3.1. The learning machine and related methods	418
20.3.2. The nearest neighbor method	422
20.3.3. SIMCA	423
20.4. Feature selection	428
20.4.1. General aspects	428
20.4.2. Methods conditioned on separation	430
20.4.3. Some applications	431
Chapter 21. Operational research : linear programming, queueing theory and some related methods	435
21.1. Linear programming	435
21.2. Game theory	438
21.2.1. Some examples	438
21.2.2. Mathematical	441
21.3. Queueing	446
21.3.1. Models and assumptions	446
21.3.2. The M/M/1 and M/M/n systems	448
21.3.3. Applications in analytical chemistry	451
21.4. Simulation techniques	456
21.4.1. Introduction	456
21.4.2. Applications in analytical chemistry	459
Chapter 22. Partial enumeration methods	463
22.1. The optimal configuration of apparatus in a (clinical) laboratory	463
22.2. Selection of representative patterns	466
22.2.1. The problem	466
22.2.2. The mathematical model	468
22.2.3. An application : the selection of GLC probes	473
Chapter 23. Graph theory and related techniques	475
23.1. Graph theory : a shortest path application	475
23.2. Graph theoretical classification methods	480
23.3. Dynamic programming	481
23.4. Sequencing and coordination methods	486

23.4.1. The PERT technique	486
23.4.2. A heuristic sequencing method	490
Chapter 24. Multicriteria analysis	493
24.1. Introduction	493
24.2. Utility functions	493
24.3. Outranking relations	495
24.4. Interactive methods	499
Part IV Requirements for analytical procedures	
Chapter 25. The diagnostic value of a test	503
25.1. Introduction	503
25.2. The rule of Bayes	509
25.3. Optimal dichotomous decisions	512
25.4. The ROC curve	514
25.4.1. Use in the selection of a cut-off point	514
25.4.2. Use in rating a test	517
25.5. Cost-benefit considerations	521
Chapter 26. Requirements for process monitoring	525
26.1. Introduction	525
26.2. Description of the fluctuations	527
26.3. Monitoring with continuous analysers	531
26.3.1. The distortion of a Gaussian peak	531
26.3.2. Measuring stochastic fluctuations	533
26.4. Shannon's sampling method - discrete sampling	535
26.5. Influence of errors	537
26.6. Determining average compositions	538
Chapter 27. Requirements for process control	543
27.1. Introduction	543
27.2. Measurability	544
27.2.1. Dead time	544
27.2.2. Sampling time	545
27.2.3. Precision	546
27.2.4. The total measurability	546
27.3. Some applications	548
Part V Systems approach in analytical chemistry	
Chapter 28. Analytical chemistry and systems theory	555
28.1. The scope of analytical chemistry	555
28.2. Systems theory	559
Chapter 29. The analytical procedure	563
29.1. The black box	563
29.2. Some input and output variables and their relations	566
29.3. The combination of black boxes	570
29.4. An example	572
29.5. Some other ways of describing analytical procedures	574
29.6. Quality control	575
29.7. The analytical procedure as a subsystem	576
Chapter 30. The analytical laboratory	579
30.1. Introduction	579
30.2. Some notes on the organization of the laboratory	580
30.3. Methods for simulating the analytical laboratory	582
Appendix	587
Subject index	593

PART I

**EVALUATION OF THE PERFORMANCE
OF ANALYTICAL PROCEDURES**

