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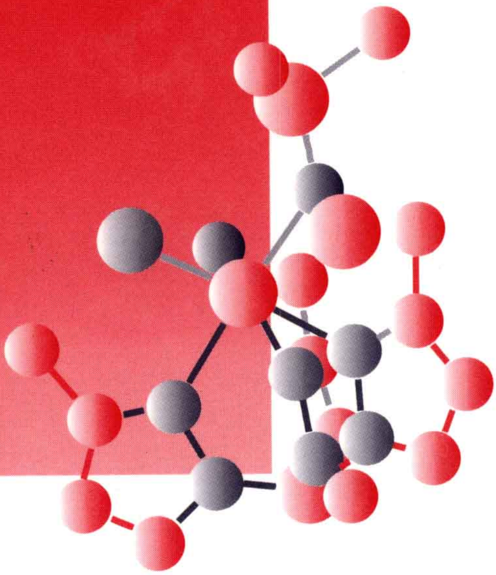

Electrochemical Detection in HPLC

Analysis of drugs and poisons

R.J. FLANAGAN, D. PERRETT and R. WHELPTON

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Analysis of Drugs and Poisons

Robert J. Flanagan
Medical Toxicology Unit, Guy's and St Thomas' NHS Foundation Trust, London, UK

David Perrett
William Harvey Research Institute, Barts & The London, Queen Mary's School of Medicine and Dentistry, London, UK

Robin Whelpton
Department of Chemistry, Queen Mary, University of London, London, UK

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ISBN 0-85404-532-5

A catalogue record for this book is available from the British Library

Published by The Royal Society of Chemistry,
Thomas Graham House, Science Park, Milton Road,
Cambridge CB4 0WF, UK

Registered Charity Number 207890

For further information see our web site at www.rsc.org

Typeset by Alden Bookset, Northampton, UK
Printed by Athenaeum Press Ltd, Gateshead, Tyne & Wear, UK

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Preface

The aim of this book is to give a balanced account of the application of HPLC with electrochemical detection (ED) in analytical toxicology and associated areas, including therapeutic drug monitoring, drug metabolism and pharmacokinetic studies. Many other reviews, book chapters and other sources of information on ED in HPLC tend to give an instrument manufacturer's view, not the view of the analyst, or are devoted to the analysis of easily oxidised species, such as catecholamines and related compounds. The emphasis in this monograph is on exogenous compounds, although catecholamines and other endogenous species are sometimes discussed to exemplify particular approaches or when such compounds have been used as drugs.

Although ED can give high sensitivity and selectivity and can be relatively inexpensive in operation, it is not an easy technique to use, the ever-present concerns being electrode deactivation or other more subtle factors that may act to influence response. The authors all have experience of using HPLC with both amperometric and coulometric detectors. This volume gives practical and useful information on the applications and limitations of the technique in the analysis of drugs and poisons in biological and related specimens, which tends to pose different problems to those encountered in the analysis of catecholamines and of pharmaceutical preparations.

Introductory chapters give information on basic electrochemistry and HPLC-ED, and on the specialised area of HPLC-ED of thiols. The major portion of the book is devoted to summary details of over 400 published HPLC-ED methods that are discussed in a standard format (column, eluent, internal standard, ED conditions, extraction procedure, limit of quantitation, *etc.*). These data are not always available *via* published abstracts and, wherever possible, sufficient information is given for the reader to decide whether a particular approach is worth pursuing. Chemical structures are given for most analytes and internal standards to ensure unambiguous identification and to illustrate possible electroactive moieties. Problems and pitfalls, and alternative techniques when appropriate, are emphasised throughout. Literature coverage is comprehensive up to the end of 2003.

Acknowledgements

We thank Steve Binder (Bio-Rad, Hercules, CA) and C.F.M. van Valkenberg (Antec, Leiden) for helpful comments on the manuscript, and the Series Editor, Prof Roger M. Smith, and the Royal Society of Chemistry for their help and encouragement.

List of Abbreviations

AASP	advanced automated sample processor
aq	aqueous
BAS	Bioanalytical Systems
BAL	British Anti-Lewisite (dimercaprol)
BALF	bronchoalveolar lavage fluid
CBA	carboxylic acid
CE	capillary electrophoresis
CoPC	cobalt phthalocyanine
CPE	carbon paste electrodes
CSF	cerebrospinal fluid
CV	cyclic voltammetry
CZE	capillary zone electrophotesis
DC	direct current
DEA	diethylamine
DHA	dihydroartemisinin
DHBA	dihydroxybenzylamine
DMAD	*N,N*-dimethylaminododecane
DOPA	3-(3,4-dihydroxyphenyl)alanine
DOPAC	3,4-dihydroxyphenylacetic acid
DTT	dithiothreitol
EC	electrochemical
ECD	electron capture detection
ED	electrochemical detection
EDTA	ethylenediaminetetra-acetic acid (or sodium salt)
ESA	Environmental Science Associates
FIA	flow-injection analysis
f.s.d.	full-scale deflection
GC	gas chromatography
GCE	glassy carbon (working) electrode

GSH	reduced glutathione
GSSG	oxidised glutathione
HFBA	heptafluorobutanoic acid
5-HIAA	5-hydroxyindoleactic acid
HIV	human immunodeficiency virus
HMDE	hanging mercury drop electrode
HPLC	high-performance liquid chromatography
HPLC-ED	HPLC with electrochemical detection
i.d.	internal diameter
i.v.	intravenous
LC	liquid chromatography
LoD	limit of detection
LoQ	limit of quantitation
LLE	liquid-liquid extraction
LLoQ	low limit of quantitation
MDA	methylenedioxyamphetamine (3,4-methylenedioxyamphetamine)
MDEA	methylenedioxyethylamphetamine
MDMA	methylenedioxymethylamphetamine
MS	mass spectrometry/spectrometric
MTBE	methyl *t*-butyl ether
NAC	*N*-acetyl-L-cysteine
NEM	*N*-ethylmaleimide
NAPM	*N*-(4-anilinophenyl)maleimide
ODS	octadecylsilyl
OPA	*o*-phthaldialdehyde
OSA	octanesulphonic acid
PAD	pulsed amperometric detection
PC	personal computer
PCA	perchloric acid
PFPA	pentafluoropropionic acid
PGE	porous graphite (working) electrode
PITC	phenylisothiocyanate
RSD	relative standard deviation
SAM	self-assembled monolayers
SCE	standard calomel electrode

SCX	strong cation-exchange
SDS	sodium dodecylsulphate
S/N	signal-to-noise
SPE	solid phase extraction
SPME	solid phase microextraction
TBA	tetrabutylammonium ion
TEA	triethylamine
THF	tetrahydrofuran
Tris	tris(hydroxymethyl)aminomethane
UV	ultraviolet

Contents

CHAPTER 1

General Introduction

1 Introduction

Electrochemical detection (ED) is used for the sensitive detection and measurement of electro-active analytes in many areas of analytical chemistry and biochemistry. These applications range from electrode sensor devices *via* flow injection analyses (FIA) to direct measurements of neurochemicals in brain tissue using *in vivo* cyclic voltammetry. In separation science, ED is used to detect and measure responsive analytes in flowing streams following analysis by high-performance liquid chromatography (HPLC) or capillary electrophoresis (CE). The use of ED with HPLC is by far and away the most important application of ED in flowing systems (Tables 1.1 and 1.2). The use of HPLC-ED grew by 500% between the 1980s and the 1990s. However, its popularity should be compared to some 20,000 papers that employed HPLC in combination with fluorescence detection and some 10,000 with MS detection (the most rapidly increasing combination at present). Most published HPLC methods use UV/visible detection, but the numbers are more difficult to quantify as this is not always made explicit in titles or abstracts.

Unlike UV or fluorescence detectors, ED does not exploit a physical property of an analyte, but an induced chemical change that results from an electrochemical reaction. ED must, therefore, be considered to be a type of post-column chemical reaction detector. ED differs, however, from other post-column reactors used in HPLC in that no reagents (other than electrons) or reaction devices are normally required to effect the chemical change in the analyte. In addition, the reaction kinetics are usually fast leading to minimal extra-column effects.

General aspects of electrochemistry have been covered in a number of books. In addition, the principles of ED when specifically applied to HPLC and/or CE have been reviewed.[1–7] A brief overview of this area is given in Chapter 3.

With UV detectors, selectivity is adjusted by varying the detection wavelength, lower wavelengths often giving enhanced sensitivity and a response from a wider range of analytes. A modest degree of selectivity is achieved by using UV detection in the aromatic region (240–270 nm) and traditionally 254 nm has proved popular. However, at lower wavelengths (200–210 nm), the absorption of the eluent, of other eluent constituents or of oxygen become limiting. Relatively few compounds show useful absorption at wavelengths higher than 340 nm (the lower limit of the

Table 1.1 *Publications on electrochemical detection used with different analytical modes*

Method	*No of publications*
ED + HPLC	9189
ED + CE	180
ED + FIA	111
ED + sensors	171
ED + nanotechnology	100

(Data based on a search of SCIRUS Database, December 2003 and a personal CE database (D. Perrett))

deuterium lamp emission). Generally, responses are usually independent of eluent conditions. In EC detection both sensitivity and selectivity are adjusted by varying the potential maintained between the working and reference electrodes, higher potentials, up to a local maximum, giving increased sensitivity. However, higher potentials usually induce a response from more compounds and therefore compromise selectivity. In oxidative mode, oxidation of eluent constituents becomes limiting at higher applied potentials, whilst in reductive mode, interference from dissolved oxygen can prove difficult to exclude. The response at the electrode is also very dependent on the eluent composition, especially its pH. Thus, as in all analytical methods, it is the signal-to-noise (S/N) ratio that is important and the detection conditions eventually adopted for a separation are a compromise between the electrochemical response of the analyte, the optimum eluent for both detection and elution, and interference from the sample matrix or from noise or drift from electronic or other sources.

Table 1.2 *Literature publications on applications of electrochemical detection, 1980–2003*

Applications	*No of publications*
Pharmaceuticals	3222
Clinical chemistry	1890
Neuroscience	1478
Biochemistry	1405
Chemical	2881
Environmental	1136
Industrial	829
Forensic	201

(Data based on a search of SCIRUS, December 2003)

In HPLC-ED the column eluate flows over the surface of an 'inert' electrode maintained at an appropriate positive or negative potential relative to a reference electrode. At the electrode surface analytes possessing electroactive functional groups undergo oxidation or reduction (oxidation being loss of electrons and *vice versa*). The electrons released (or donated) travel *via* the electrode and the change in current can be measured and related to the concentration of the analyte. Modern electronics allow the applied (working) potential to be held within very tight limits while at the same time measuring and amplifying the very small currents created. Hence these detectors can be very sensitive. A crude comparison of the sensitivity and applicability of the most common HPLC detectors towards favoured analytes under similar analytical conditions is given in Table 1.3. Both EC and fluorescence detectors can be at least 100 times more sensitive towards responsive compounds than a standard UV detector and are much more selective. Unfortunately, with time EC reaction products tend to accumulate at the electrode surface leading to loss of activity and hence loss of detector response – this is the major reason EC detection remains a relatively specialised field.

2 HPLC-ED in Analytical Toxicology

HPLC is widely used in analytical toxicology. UV/visible absorption (including diode-array and scanning instruments) and fluorescence detection remain of paramount importance, with pre- or post-column derivatisation sometimes being used to enhance sensitivity and/or selectivity. Modern UV detectors are in the main a considerable improvement on their predecessors. HPLC-MS and HPLC-MS-MS are being used increasingly in quantitative work, although the capital costs involved remain relatively high. Nevertheless, ED still finds a role in certain applications and there is a considerable body of literature associated with this topic. ED requires more care and thought in routine use than spectrophotometric detectors, principally because of the problems of electrode deactivation. On the other hand, running costs can be minimal and good sensitivity/selectivity can be attained with a number of analytes.

The aim of this volume is to give information to aid the use of HPLC-ED in the analysis of drugs and poisons in biological and related specimens. The available information (column, eluent, detection potential, extraction procedure, internal standard, sensitivity, *etc.*) is presented in a standard format in Chapters 6 and 7. These data are not always given in published abstracts and, wherever possible, sufficient information is given for the reader to decide whether a particular approach is worth pursuing. Chemical names or structural formulae are given to aid identification of electroactive moieties. The use of alternative techniques, including CE-ED, is emphasised as appropriate. Additional topics, such as analyte stability, are also discussed where relevant. Note that unambiguous details of the working and reference electrode combinations used in a particular application are not always given in published work – in such cases an informed guess as to the ED conditions actually used has had to be made.