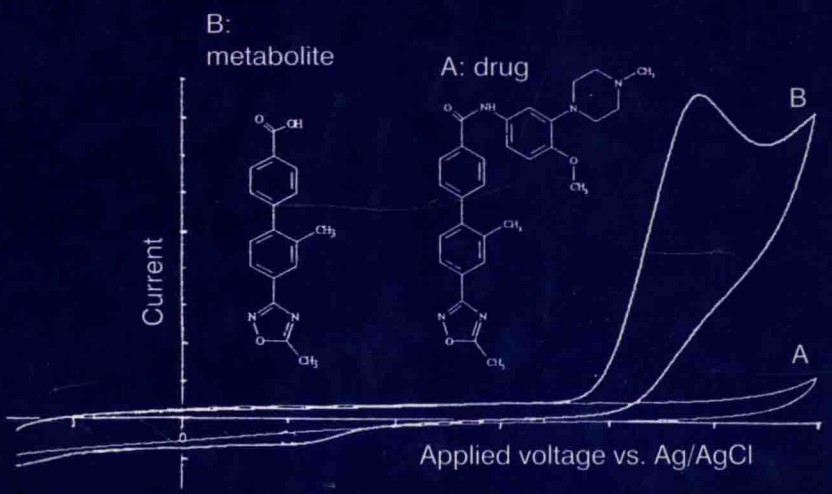
METHODOLOGICAL SURVEYS IN BIOANALYSIS OF DRUGS
Series Editor: Eric Reid ■ Volume 24

BIOFLUID ASSAY FOR PEPTIDE-RELATED AND OTHER DRUGS



Edited by Eric Reid, Howard M. Hill, and Ian D. Wilson

Methodological Surveys in Bioanalysis of Drugs, Volume 24

Biofluid Assay for Peptide-related and Other Drugs

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Biofluid Assay for Peptide-related and Other Drugs

METHODOLOGICAL SURVEYS IN BIOANALYSIS OF DRUGS

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Senior Editor's Preface

with some abbreviations and argued 'MS shorthand' policies

This volume reflects the continuing tradition of strong editing to achieve clear yet compact presentations that give adequate detail which, however, does not devour page space.- Validation evidence, whilst important, may not need multiple Tables such as prevail in other publications which, moreover, may have Figs. with unrectified miniscule lettering. Putting instrument-generated diagrams into legible and uncluttered form with elimination of 'junk', and curing faintness, is a growing burden now that fewer diagrams are hand-drawn.- Authors please note! Faintness marred some material in the previous volume (printers may use a camera without 'intelligent' adjustments). The present book may fulfil more consistently the aim of good appearance with fair homogeneity that embraces abbreviations.

The Bioanalytical Forum series, 21 years on.- The 1975 Forum was a pioneer venture to encourage problem-discussing amongst bioanalysts (mainly company-based), whose timidity made the Forum hard to set up. Its success has since been emulated by other meetings, and it is now common for company staff to 'go public' with their methods; but the series still flourishes as evidenced by the 1995 Forum (11th, at the usual University of Surrey venue) on which this book (not mere 'Proceedings') is based. For a decade the series was under 'Guildford Academic Associates' auspices. Now, with the same team and aided by an endowment, a 'Forum Syndicate' of the Chromatographic Society has responsibility. The 1997 Forum dates are 2-5 September.

Some features of the book.- The inclusion of Discussion remarks seems to be commendable rather than idiosyncratic, as hopefully applies also to insertions by this Editor. These include an Assay Compendium, based on therapeutic classes but now with diminished coverage of therapeutic and toxicological monitoring (for which our publisher now produces Abstracts); the emphasis is on more sensitive assays and on automation. Good indexing remains paramount, with the usual Analyte Index based on some chemical features which analytes not listed may also possess. In the past this Editor's Preface has alluded to some 'dislikes'; disfavoured vague terms include '2-D'/'multi-D' and 'hyphenated'. A traditional book feature - 'Special topic' - here comprises peptide-type analytes (as in Vol. 16), too diverse for comprehensive coverage.

Acknowledgements.- Dr D. Stephenson was Co-organizer for the 1995 Forum, to which SmithKline Beecham made an appreciated donation. Drs D. Thomas and M.V. Doig helped settle editorial policies for mass-spectrometry (MS) 'shorthand' [see overleaf]. For the benefit of fellow-bioanalysts, busy authors 'made time' to produce publication texts. Ackowledgements for allowing use of items already published elsewhere appear within the text.

Alertings to readers and other analysts and library staff

Abbreviations, especially for MS.- Settling policies for the book was prompted by diversity amongst Forum Abstracts, e.g.:

LC/MS/MS LC/MS-MS LC-MS/MS LC-MS-MS HPLC-MS/MS HPLC-MS-MS

as reflected in Journals, wherein guidance to authors is scant.

Shorthand now adopted, acceptable in some MS circles *(including* IUPAC *?)*, employs **hyphens**, not 'slashes' // unless 'options' are signified (e.g. FTICR/MS). A further policy [some individuals like it] saves multiple hyphens: **parenthetical sub-descriptions** (), e.g. for thermospray, or atmospheric pressure ionization, chemical (c) or electrospray (ESP/ESI; variant: ionspray, ISP). #Thus: LC-MS(APcI) or merely LC-MS(API) [*not* LC-API-MS], maybe amplifying the 'c' approach: n (*not* N)/p = negative/positive ion as in *examples:* LC-MS-MS(APncI) LC-MS-MS(TSP) and similarly electron impact, GC-MS(EI); fast atom bombardment, TLC-MS(FAB) Also: SIM = selected ion monitoring, or MRM if multiple reaction.

Other abbreviations.- HPLC prefixed NP/RP, normal/reverse(d) phase [ISRP, internal surface]. Detection: fluor = fluorimetric - ex[citation] & em[ission] nm maybe stated; EC = electrochemical [*any support for* ECh*?*]; **not** ECD, = electron-capture detection as in GC-ECD. FID = flame ionization; NPD = nitrogen-phosphorus.

Ab, antibody (mAb, monoclonal)	BSA, bovine serum albumin
ELISA, Enzyme-linked immunosorbent assay	CE, capillary electrophoresis
IA, immunoassay (RIA, radio-)	C.V., coefficient of variation
i.s., internal standard	i.v., intravenous; s.c., subcutanous; i.m., intramuscular
LLOQ/LOD, limit of quantification (lower)/detection	QC/QA, quality control/assurance
	SPE, solid-phase extraction

In some arts.: MeCN, acetonitrile; PCA/TCA, perchloric/trichloroacetic acid; TEA, triethylamine; PK/PD, pharmaco-kinetics/dynamics. Temperatures ° are Celsius. 'Multi-D' *disfavoured (unclear term).*

Bibliographic points affecting Libraries besides readers.-

- Now ***A*** and ***B*** distinction (specify! vol. nos. shared): *J. Chromatog.*
- Dual-title muddle (synonyms): *HRC* or *J. High Resolu. Chromatog.*
- Sloppy variants: *Pharmac./Pharmacol. Endocr./Endocrin./Endocrinol.*
- Vol. 23 of present series: erroneous 'Cumulative Index' phrase on spine (was in Vol. 22!).- *Mask over! (by tape?)*
- Earlier vols.- #*Analysis* subseries: 5, 7, 10 & even nos. up to 22 - all focused on drugs, as were many arts. in *Biochemistry* subseries. #Former series title (affects shelving or procurement; info. wanted*?*): *Methodological Surveys in Biochemistry and Analysis.*

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CONTENTS

Besides main contributions there are 'NOTES' (short articles) and 'COMMENTS'. The suffix '**n**' as in #A-7n distinguishes NOTES from main articles. COMMENTS (put at end of Section) comprise Forum discussion remarks and Editor's 'annotations'.

'n' as in #C-5n signifies a Note

'n' as in #E-4n signifies a Note

Section #A

PEPTIDE-TYPE AGENTS

The suffix '**n**' as in #A-7n distinguishes a 'NOTE' from main articles. COMMENTS (starting on p. 59) comprise Forum discussion remarks and Editor's 'annotations'.

Overleaf: some general literature (not bioanalytical)

Some general literature [see also ref. list in art. #A-1]

'Amino Acids, Peptides and Proteins', in the RSC Specialist Periodical Reports series; Vol. 26 (1995) includes surveys of peptide hormones and cyclic/modified/conjugated peptides.

In **Adv. Drug Res.**- Dutta, A.S. (1991) **21**, 147-286: 'Design and Therapeutic Potential of Peptides'. McMartin, C. (1992) **23**, 41-106: 'Pharmacokinetics of Peptides and Proteins...'. Fauchère, J-L. & Thurieau, C. (1992) **23**, 128-159: 'Evaluation of the Stability of Peptides and Proteins as a Tool in Peptide Drug Design'.

Entries in Sect. #ABC (p. 161) reflect the wide **therapeutic span** (prefix ° denotes a peptide-type drug).

Therapeutic usefulness may blossom for some gene-coded agents:- 'Antimicrobial Peptides' [Ciba Foundation Symp. #186 (1994); ed. J. Marsh & J.A. Goode; Wiley, Chichester, 283 pp.]. They are of diverse occurrence and chemical type, e.g. toad-skin magainins.

For hirudin (cf. art. #A-2), a review on its clinical potential gives good chemical and 'bio' background: Johnson, P.H. (1994) Annu. Rev. Med. **45**, 145-177.

'The Comparative Physiology of Regulatory Peptides' (1989; ed. S. Holmgren), Chapman & Hall, London, 392 pp.

'Cell Signalling: Experimental Strategies' [Vol. 21 (1991) of PRESENT SERIES, ed. E. Reid *et al.*] embraces IL's and other cytokines (p. 32), PA (p. 323), etc.; see p. vii and Index. Also it has a notable review (LeVine & Brown, with 85 refs.; not specially for peptide ligands) on receptor investigation as featured too, with guidance on binding assays, in Vol. 13 (1984; Plenum), 'Investigation of Membrane-located Receptors'. The calcitonin theme of the Vol. 13 art. by J.M. Moseley & co-authors is informative in respect of Hsu's art., #A-4 below.

#A-1

UNIQUE ISSUES AND ANALYTICAL METHODS FOR PHARMACOKINETIC AND METABOLISM STUDIES WITH PROTEIN/PEPTIDE PHARMACEUTICALS

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Analytical methods in disposition studies for biotechnology products range from the straightforward to the elaborate. Many protein-analysis methods do not positively identify the analyte. IA's and bioassays are often used. IA's are relatively specific and easy to perform, while in vitro bioassays are more difficult but illuminate biological effects. Radiolabelled proteins can be advantageous in respect of detection and analysis of the molecular form of the protein; but many limitations exist. Along with these methods, additional analysis by MS, chromatography or electrophoresis can help. MS can aid protein analyte identification, while HPLC in IE, RP and size exclusion modes may help in isolating protein products from complex biological matrices. PAGE is a notably powerful and much used technique for protein mixtures.*

PK parameters vary widely for proteins, maybe due to minor molecular variations; the profile of decline partly depends on assay sensitivity and sampling duration. Metabolism reflects proteolysis and possibly adding-on of groups. Degradation at extravascular injection sites may affect bioavailability, for which assay limitations may give artefactual results. Complexing to binding proteins may affect metabolism and/or clearance, and hence activity and toxicity. As the complexes are usually not covalent, their study may need non-denaturing analytical methods; disposition results may suffer if analysis does not distinguish bound and unbound forms. Studies by approaches now outlined show the usefulness for safety evaluation of the data obtainable.

This article aims to survey the unique issues and various analytical methods that are encountered in protein/peptide disposition studies (as more fully discussed elsewhere: [1, 2]).

†addressee for any correspondence

**Abbreviations* [others in later footnotes & Preface]: Ab, antibody (mAb, monoclonal); IA, immunoassay; ELISA, enzyme-linked immunosorbent assay; IE, ion-exchange; PAGE, polyacrylamide gel electrophoresis; PK, pharmacokinetic(s). 'Bioassay' may be enzymic.

The issues that need to be addressed in protein disposition studies* are similar to those for conventional small-molecule drugs. The primary aims are to find the fate of the active parent, to identify metabolites and binding proteins, and to determine the mechanisms of clearance. It is also important to identify the sites of catabolism and to correlate the drug's concentration with the pharmacological effect. In metabolism studies a major difference between conventional and protein drugs in biological matrices lies in the analytical methods used to isolate and measure the drug. With conventional drugs, endogenous proteins are removed by precipitation or extraction procedures so that methods such as HPLC or GC can be applied. With protein drugs such isolation approaches are precluded.

IA's and bioassays (sometimes enzymic) are often employed in protein PK and metabolism studies [3]. IA's are relatively specific and easy to perform, while *in vitro* and *in vivo* bioassays are more difficult but provide information regarding biological effects. Radiolabelled proteins can be useful in detecting and analyzing the drug molecular form. Complementary analyses can be of value.- MS can significantly aid identification of the protein analyte, while IE, RP or size-exclusion chromatography serves to separate out proteins from biofluids and electrophoresis to resolve and size-characterize the protein species of interest [4]. It is often useful to employ a combination of analytical techniques for the sake of reliable conclusions.

Antibodies (Ab's) in relation to analysis

As a much used IA approach, ELISA's are sensitive, do not employ radioactive reagents and can be completely automated†. The main drawback of IA's is lack of positive identification of the analyte, e.g. its exact biochemical form or sequence. There can also be interferences in IA's from diverse endogenous or exogenous materials including Ab's, binding proteins, metabolites and non-specific interferants from the biological matrix [5]. Ab's can also have a major impact on quantitation of the administered protein. In pre-clinical studies with recombinant human tissue factor, the presence of Ab's to the factor interfered with ELISA quantification of plasma levels. Conversely, recombinant tissue factor in plasma interfered in quantitation of tissue factor Ab's using a similar assay method.

A unique issue encountered in protein disposition studies is the production of Ab's. Their presence in an animal model with a human protein drug can be reckoned on; but Ab production has also been observed in clinical studies [6-9]. The immunogenicity of a protein is governed by its features including primary/secondary/tertiary structure. Stepping-up of dosing amounts, frequency or duration increases the cumulative dose,

*In contexts such as this, 'protein' embraces small polypeptides.
†For automated ELISA see S.A. Westwood's art., #D-3.