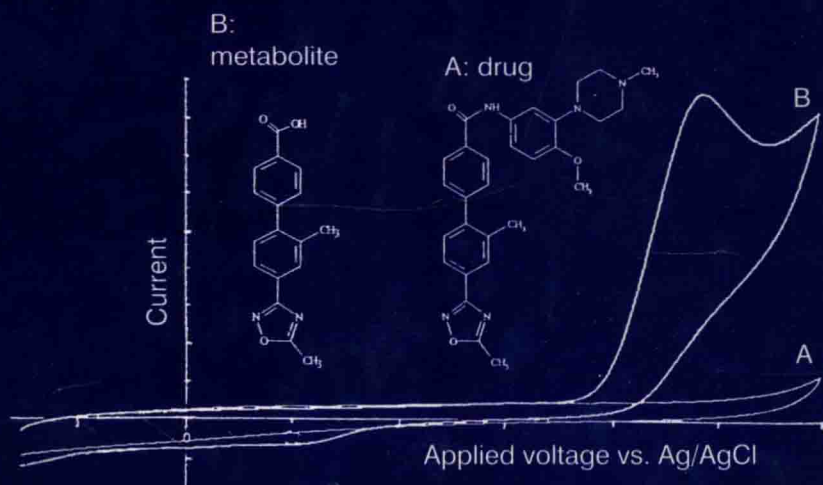


BIOFLUID ASSAY FOR PEPTIDE- RELATED AND OTHER DRUGS



Methodological Surveys in Bioanalysis of Drugs, Volume 24

Biofluid Assay for Peptide-related and Other Drugs

Edited by

Eric Reid

Guildford Academic Associates, Guildford, United Kingdom

Howard M. Hill

Corning Hazleton (Europe), Harrogate, United Kingdom

Ian D. Wilson

Zeneca Pharmaceuticals, Macclesfield, United Kingdom



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Biofluid Assay for Peptide-related and Other 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. Acknowledgements 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 - excitation] & 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	CE, capillary electrophoresis
immunosorbent assay	C.V., coefficient of variation
IA, immunoassay (RIA, radio-)	i.v., intravenous; s.c., sub-
i.s., internal standard	cutaneous; i.m., intramuscular
LLOQ/LOD, limit of quantifi-	QC/QA, quality control/assurance
cation (lower)/detection	SPE, solid-phase extraction

In some arts.: MeCN, acetonitrile; PCA/TCA, perchloric/trichloro-acetic 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* sub-series. #Former series title (affects shelving or procurement; info. wanted?): *Methodological Surveys in Biochemistry and Analysis.*

List of Authors with p. nos.*Primary author*

D.J. Anderson: (i) 78-81,
(ii) 183-193
Cleveland State Univ., OH
C.J. Bailey: 40-47
Zeneca Pharm'ls, Alderley Pk.
M. Bayliss: 141
Astra Charnwood, Loughborough
M. Bentley: 327-328
AS FOR Hill
C.D. Bevan: 299, & see Tiller
Glaxo Wellcome, Stevenage
K. Borner: 147-150
Klin. Chem./Biochem. Inst., Berlin
S. Braggio: 158
Glaxo Res., Verona, Italy
D. Browne: 254-256, & see
Robotham; AS FOR Hill
J.E.C. Burnett: 333-335, &
see Hill (i); AS FOR Hill
G.C. DiDonato: 57-58
Bristol-Myers Squibb Res.
Inst., Princeton, NJ
T.A. Enos: 126-127, & see
James, Simmonds; AS FOR James
J.D. Gilbert: 65-77
Merck Res., West Point, PA
K. Grob: 246-253
Kantonales Labor, Zürich
D. Gygax: 11-19
Ciba-Geigy, Basel
P. Heizmann: 304-311
Hoffmann-La Roche, Basel
H.M. Hill: (i) 48-56,
(ii) 223-224, (iii) 316-326,
& see Burnett, Thomas
Corning Hazleton, Harrogate
S-H. Hsu: 35-39
Rhone-Poulenc Rorer Central
Res., Collegeville, PA
A.J. Hutt: 154-157
Kings Coll., Chelsea, London
C.A. James: 82-88, & see Enos
Pharmacia & Upjohn, Crawley

*Co-authors, with relevant name to
be consulted in left column*

M. Abermann - Nemes	M. Hail - DiDonato
J.A. Allanson -	E.L. Hand - Gilbert
Pleasance	G. Harrison - Wring
A. Arroyo - DiDonato	H. Hartwig - Borner
D. Ayres - Wring	M.J. Hemsley -
S.J. Bacon - Bailey	Noctor (i)
R.J. Barnaby -	E. Houghton
Braggio	- Seymour
G. Barnard - Venn	E. Jochem -
K. Bijmholdt-Sierat	van Amsterdam
- van Amsterdam	A.E. Jones
E. Bojti - Nemes	- Pleasance
J. Borlak - Bentley	I. Klebovich
E. Borner	- Nemes
- K. Borner	J. Körner - Heizmann
L. Botta - Gygax	P. Kwasowski
S.C. Bridges	- Stevenson
- Westwood	
N.K. Burton	S.F. Lane - Tiller
- James	R. Laplanche - Nuez
J.L. Byard - Seymour	B. Law - Lough
	J. Lee - Hsu
M. Churchill	M. Lee - DiDonato
- Mason, Moore	G.Ph.D. Leeflang -
S.D. Clarke -	van Amsterdam
Hill (i), Thomas	G. Lefèvre - Gygax
C.S. Creaser	M. Lemaire - Nuez
- Seymour	Y. Liu - Anderson (i)
S. Dayal - Wring	H. Lode - Borner
V. de Biasi - Hutt	J.M. Long - Enos
J.J. Dervan - Enos	
M. Ehrat - Gygax	P.V. Macrae - Venn
J.D. Ellis - Gilbert	J. McGowan - Moore
S. Feely - Seymour	D.A. McLoughlin
C. Fernández-Metzler	- Gilbert
- Gilbert	S. Maier - Heizmann
B. Ferraiolo	J. Maltas - Lough
- Mohler	A.J.G. Mank -
I.J. Fraser	Lingeman
- Pleasance	H. Marsh - Seymour
J.L. Furness - Bayliss	P. Martin - Wilson
	P. Maude - Robotham
W.A. Galloway -	C.M. Middlekoop -
Mason, Moore	van Amsterdam
C. Gooijer - Lingeman	K.G. Miller - Poole
R. Gora - Heizmann	M.J. Mills - Lough
P. Graf - Gygax	W. Morden - Wilson
S-J. Groves - Lough	I.M. Mutton -
P. Guntz - Nuez	Bevan, Tiller

Primary author

H. Lingeman: 275-287
Free Univ., Amsterdam

W.J. Lough: 142-146
Univ. of Sunderland

C. Mason: 137-140, &
see Moore - AS FOR Moore

M.A. Mohler: 3-10

R.W. Johnson Pharm. Res.
Inst., Raritan, NJ

J. Moore: 89-93, & see Mason
British Biotech, Oxford

K.B. Nemes: 103-104
Egis Pharm'ls, Budapest

T.A.G. Noctor: (i) 151-153,
(ii) 213-214, (iii) 257-261,
& see Hill (i) & (ii), Thomas
Corning Hazleton, Harrogate

C. Nuez: 96-102
Sandoz Pharma, Basel

S. Pleasance: 118-125
Glaxo-Wellcome, Beckenham

C.F. Poole: 194-208
Imperial Coll., London SW7

L. Robotham: 312-315
- AS FOR Noctor

M.A. Seymour: 215-218
- AS FOR Westwood

R.J. Simmonds: 128-136, &
see Enos, James
Pharmacia & Upjohn, Crawley

D. Stevenson: 219-222
Univ. of Surrey, Guildford

M.R. Taylor: 288-298, & see
Westwood; AS FOR Westwood

D. Thomas: 230-238, & see
Hill (i) - AS FOR Noctor

P.R. Tiller: 329-332
- AS FOR Bevan

P.H. van Amsterdam: 105-117
Solvay Duphar, Weesp, Belgium

R.F. Venn: 20-34
Pfizer Central Res., Sandwich

S.A. Westwood: 209-212, & see
Taylor
Horseracing Forensic Lab.,
Newmarket

*Co-authors, with relevant name to
be consulted in left column*

T.V. Olah - Gilbert	W. Speed - Enos, Simmonds
K. O'Neill - Bayliss	N. Stevens - Hutt
P. Oroszlan - Gygax	J. Stubbs - Mohler
G. Padbury - James	J. Taberner - Wilson
D. Perrett - Taylor	S.C. Tan - Hutt
C. Pfister - Gygax	S. Taylor - DiDonato
S.K. Poole - C.F. Poole	P. Teale - Seymour
S. Pratt - Hill (ii)	K. Tennant - Hill (i), (ii) & (iii), Bentley, Browne
J.R. Preston - Bayliss	T. Thompson - Burnett
B. Rashid - Stevenson	
S.A. Rees - James	
K. Reeve - Seymour	
L. Rich - Hsu	
S. Richards - Moore	D.J.K. van der Stel - van Amsterdam
C.J. Roberts - Seymour	H.J.C. van Schie - van Amsterdam
J.N. Robson - Hill (i), Noctor (ii), Thomas	T. Wangsa - Hsu
R.J. Ruane - Bailey	B. Warrack - DiDonato
P. Sadra - Pleasance	S.A. White - Hill (iii)
M. Saeed - Lough	D. Wilkinson - Bayliss
K.C. Saunders - Venn	V.J.C. Willson - Bayliss
F.C. Schwende - James	S.A. Wood - James
E. Sharp - Noctor (i)	K. Woodward - Taylor, Westwood
M. Shepherd - Browne	V. Wroblewski - Mohler
D.S. Siebert - Poole	A.S. Yuan - Gilbert
P. Solanki - Mason	

continued from left-hand column

I.D. Wilson: 239-245
Zeneca Pharm'ls, Alderley Pk.
S.A. Wring: 262-274
Glaxo-Wellcome, Triangle Pk., NC

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'.

Senior Editor's Preface, with some abbreviations and argued 'MS shorthand' policies v

List of Authors xi

#A PEPTIDE-TYPE AGENTS [Some background literature listed] .. 1

#A-1 Unique issues and analytical methods for pharmacokinetic and metabolism studies with protein/peptide pharmaceuticals - MARJORIE A. MOHLER, V. WROBLEWSKI, J. STUBBS & BOBBE FERRAILO 3

~~#A-2~~ Immuno and other 'biological' approaches in pharmacokinetics - D. GYGAX, L. BOTTA, M. EHRAT, P. GRAF, G. LEFÈVRE, P. OROSZLAN & C. PFISTER 11

#A-3 Analytical strategies for a new peptide drug - R.F. VENN, G. BARNARD, P.V. MACRAE & K.C. SAUNDERS 20

~~#A-4~~ A sensitive sandwich chemiluminescent enzyme immunoassay for the determination of administered salmon calcitonin in human plasma - S-H. HSU, JULIE WANGSA, JAMES LEE & LISA RICH 35

#A-5 Analytical procedures for monitoring high mol. wt. fragments derived from the rRicin immunotoxin ZD 0490 in human plasma - S.J. BACON, C.J. BAILEY & R.J. RUANE 40

#A-6 Sample preparation and subsequent steps: strategies for peptides and peptidomimetics - H.M. HILL, J.N. ROBSON, T.A.G. NOCTOR, K. TENNANT, D. THOMAS, S.D. CLARKE & J.E.C. BURNETT 48

#A-7n LC-MS profiling of peptidomimetics and peptides from biological sources - G.C. DiDONATO, ANNE ARROYO, M. HAIL, SUSAN TAYLOR, BETHANNE WARRACK & M. LEE 57

COMMENTS and annotations bearing on Sect. #A articles 59

#B AGENTS WITH PEPTIDE-TYPE TARGETS [Comment: 'proteases'] 63

#B-1 Development and cross-validation of RIA and LC-MS-MS methods for determining the fibrinogen-receptor antagonist L-734,217 in plasma - J.D. GILBERT, E.L. HAND, D.A. McLOUGHLIN, J.D. ELLIS, T.V. OLAH, A.S. YUAN & C. FERNÁNDEZ-METZLER 65

#B-2n Retention of fibrinogen in ion-exchange HPLC: evidence for a domain binding model - Y. LIU & D.J. ANDERSON 78

#B-3	Bioanalytical strategies to support the development of an HIV protease inhibitor, including GC-MS, HPLC and chiral analysis - C.A. JAMES, N.K. BURTON, T.A. ENOS, R.J. SIMMONDS, S.A. WOOD, S.A. REES, G. PADBURY & F.J. SCHWENDE	82
#B-4	Analysis of pseudopeptidyl matrix metalloproteinase inhibitors in plasma - J. MOORE, M. CHURCHILL, J. MCGOWAN, C. MASON, S. RICHARDS & W.A. GALLOWAY	89
	COMMENTS and an annotation bearing on Sect. #B articles ..	94
#C	VARIOUS DRUG ANALYTES: PROBLEMS AND STRATEGIES	95
#C-1	Brain distribution of a CNS-active drug studied by microdialysis coupled with MS-MS(TSP) in flow injection mode - C. NUEZ, R. LAPLANCHE, P. GUNTZ & M. LEMAIRE	96
#C-2n	A highly sensitive GC method for determining deramciclane and its N-des metabolite in rat and dog plasma - KATALIN B. NEMES, M. ABERMANN, E. BOJTI & I. KLEBOVICH	103
#C-3	A sensitive, specific and rugged determination of flesinoxan in plasma by HPLC-EC - D.J.K. VAN DER STEL, P.H. VAN AMSTERDAM, K. BLJMOLDT-SIERAT, E. JOCHEM, G.Ph.D. LEEFLANG, C.M. MIDDLEKOOP & H.J.C. VAN SCHIE ..	105
#C-4	Determination of the 5-HT receptor agonist 311C90 in human plasma by LC-MS-MS - S. PLEASANCE, I.J. FRASER, A.E. JONES, J.A. ALLANSON & P. SADRA	118
#C-5n	LC-MS-MS assay of fenticonazole in human plasma - T.A. ENOS, R.J. SIMMONDS, J.M. LONG, W. SPEED, J.J. DERVAN & C.A. JAMES	126
#C-6	Development and application of LC-MS-MS bioanalytical assays - Myths and realities! - R.J. SIMMONDS, T.A. ENOS & W. SPEED	128
#C-7	A strategy for the development of bioanalytical methods using LC-MS - C. MASON, J. MOORE, M. CHURCHILL, P. SOLANKI & W.A. GALLOWAY	137
#C-8n	Ultra-trace bioanalysis: three approaches for assay of a novel pharmaceutical at the pg/ml level - M. BAYLISS, V.J.C. WILLSON, J.R. PRESTON, K.E. O'NEILL, J.L. FURNESS & D. WILKINSON	141
#C-9n	Drug bioanalysis made easy? - W.J. LOUGH, S-J. GROVES, B. LAW, J. MALTAS, M.J. MILLS & M. SAEED	142
#C-10n	Fluorimetric detection of non-fluorescent quinolones after post-column photoreaction - K. BORNER, E. BORNER, H. HARTWIG & H. LODE	147

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

#C-11n	Compounds posing an endogenous blank problem: retinoids - T.A.G. NOCTOR, M.J. HEMSLEY & E. SHARP	151
#C-12n	Chiral-HPLC resolution of carboxyibuprofen, a major urinary metabolite of ibuprofen - S.C. TAN, N. STEVENS, V. DE BIASI & A.J. HUTT	154
#C-13n	An HPLC assay directly on plasma using an ISRP - S. BRAGGIO & R.J. BARNABY	158
	COMMENTS bearing on Sect. #C articles, and annotations including retinoids (cf. #C-11 & #D-1) [#C-3 comments are on p. 337; HPLC-MS-MS theme pursued in #E-1]	159
#ABC	ASSAY COMPENDIUM (<i>in therapeutic groups</i>) - E. REID ...	161
#D	SAMPLE TREATMENT INCLUDING PROTEIN REMOVAL AND SPE	181
	[Perspective: p. 182; also pertinent: #A-6, #C-6 etc.]	
#D-1	Direct-injection HPLC: chromatography of biofluids without protein removal - D.J. ANDERSON	183
#D-2	New approaches to solid-phase extraction - C.F. POOLE, SALWA K. POOLE, DONNA S. SIEBERT & K.G. MILLER	194
#D-3	Some experiences with SPE in bioanalysis - SELINA C. BRIDGES, M.R. TAYLOR, S.A. WESTWOOD & K. WOODWARD	209
#D-4n	Rapid sample preparation approaches, notably disc- based SPE, for HPLC-MS-MS(API) - T.A.G. NOCTOR, M. HEMSLEY & J.N. ROBSON	213
#D-5	Immunoaffinity as a sample clean-up step for detecting drugs at low levels in equine body fluids - M.A. SEYMOUR, J. BYARD, S. FEELY, H. MARSH, K. REEVE, C.J. ROBERTS, P. TEALE, C.S. CREASER & E. HOUGHTON	215
#D-6n	Drug extraction by selective SPE using immobilized antibodies: trials with morphine and clenbuterol - B. RASHID, P. KWASOWSKI & D. STEVENSON	219
#D-7n	Sample collection: issues, problems and solutions - H.M. HILL, K. TENNANT, T.A.G. NOCTOR & S. PRATT	223
	COMMENTS and annotations bearing on Sect. #D articles	225
#E	APPROACHES FOR ANALYTICAL SEPARATION AND DETECTION	229
#E-1	Impact of atmospheric pressure ionization on HPLC approaches - D. THOMAS, S.D. CLARKE, H.M. HILL, T.A.G. NOCTOR & J.N. ROBSON	230
#E-2	Advances in planar chromatography - P. MARTIN, J. TABERNER, I.D. WILSON & W. MORDEN	239

#E-3	Capillary-GC advances: large-volume injection and on-line LC-GC - K. GROB	246
#E-4n	A comparison of hot and temperature-programmed GC injection in relation to pseudoephedrine - D. BROWNE, K. TENNANT & M. SHEPHERD	254
#E-5	Bioanalytical applications of enantioselective HPLC - T.A.G. NOCTOR	257
#E-6	Cyclic voltammetry to optimize EC detection with HPLC, exemplified by drug-analysis case studies - S.A. WRING, D. AYRES, S. DAYAL & G. HARRISON	262
#E-7	Diode laser-induced fluorescence detection in HPLC and capillary electrophoresis (after pre-column derivatization) - H. LINGEMAN, A.J.G. MANK & C. GOOLJER	275
#E-8	Analysis of aspirin metabolites in urine and plasma by MECC with direct sample introduction - M.R. TAYLOR, S.A. WESTWOOD, K. WOODWARD & D. PERRETT ..	288
#E-9n	Freeze-thaw switching in electrophoretic capillaries - C.D. BEVAN & I.M. MUTTON	299
	COMMENTS and annotations bearing on Sect. #E articles....	300
#F	DATA ACQUISITION AND VALIDITY IN RELATION TO DRUG APPRAISAL	303
#F-1	Haemolytic plasma: unexpected problems with a validated assay in routine analysis - P. HEIZMANN, R. GORA, J. KÖRNER & S. MAIER	304
#F-2	A database for bioanalytical results in drug development - D. BROWNE, P. MAUDE & L. ROBOTHAM	312
#F-3	Current validation and GLP issues with respect to bio-analytical methods - H.M. HILL, K. TENNANT & S.A. WHITE ..	316
#F-4n	Post-validation problems with incurred samples - M. BENTLEY, J. BORLAK & K. TENNANT	327
#F-5n	High throughput determination of drug-protein binding using immobilized HSA and LC-MS - P.R. TILLER, I.M. MUTTON, S.F. LANE & C.D. BEVAN	329
#F-6n	Validation of endogenous analytes used as pharmacodynamic markers - J.E.C. BURNETT, T. THOMPSON & H.M. HILL ..	333
	COMMENTS and annotations on Sect. #F articles and #C-3	336
	ANALYTE INDEX	339
	GENERAL INDEX	345
	Corrections to past vols., particularly Vol. 23	350

'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

Marjorie A. Mohler[†], [¶]Victor Wroblewski, John Stubbs & Bobbe Ferraiolo

R.W. Johnson Pharmaceutical Research Institute,
P.O. Box 300, Route 202, Raritan, NJ 08669, U.S.A.

and [¶]Bayer Corporation, West Haven, CT 06516, U.S.A.

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.