

# **The Future of Bioanalytical Technologies - Lessons from the Past**

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**Howard M Hill ([hillh@ukorg.huntingdon.com](mailto:hillh@ukorg.huntingdon.com))**

**Director**

**Pharmaceutical Analytical Services**

**Huntingdon Life Sciences**

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**Huntingdon**  
Life Sciences

# Overview

- **Scope**
- **Drivers for change**
- **Life cycle of a “typical analytical techniques”**
- **Rise and fall**
- **Developing technologies**
- **The future - replacing LC-MS(MS)!?**

# Scope

- **Regulatory Bioanalysis in support of PK/TK in animals and man**
- **High through put screening technologies may be the source of new bioanalytical technologies**
- **Only “small” molecules**
- **Big molecule approaches might have positive spin out**

# Why (How) Do New Technologies Arise?

## Basic Research

**“Relationships between “Research” and “Performance” depend on attitude.**

**Companies that rely on chemistry are more positive and farsighted.”**

**Science Policy Research Unit, Sussex University**

# Necessity is the Mother of Invention

- **Sensitivity**
- **Speed**
- **Ease of use**
- **Price (cost effectiveness)**
- **BUT**
- **Beware Fashion - Analysts want to use the latest technologies**

# Fashion or Necessity

- **Fashion is something barbarous, for it produces innovation without reason and imitation without benefit**
- **Critical Mass and Market**
- **Champions**
  - ◆ **not just the inventor**
  - ◆ **academic**
  - ◆ **marketing**

# Diverging Therapeutic Areas

- **Small molecules NCE**
- **Large molecules**
  - ◆ **Protein/Peptide - Ligand Assays**
  - ◆ **Oligonucleotides -**
  - ◆ **Gene Therapy - PCR**
  - ◆ **Vaccines - Ligand Assays**
- **Biomarkers**
  - ◆ **How important are pharmacokinetics**
- **Result**
  - ◆ **Less new drugs are coming to the market**
  - ◆ **Small molecules, smaller % of a decreasing market**
  - ◆ **Greater range of analytical technologies e.g. PCR; Immunoassays**

# Bioanalytical Techniques I have known

- Paper chromatography
- Thin layer chromatography
- Gas Chromatography
  - ◆ ECD “Lovelock”
  - ◆ NPD
  - ◆ Capillary columns “Grob”
- HPLC
  - ◆ UV
  - ◆ Fluorescence
  - ◆ Electrochemical Detectors
- HPLC (MS)
  - ◆ API interface (Sciex Biomolecular Analyser)
  - ◆ Ion Spray “Hennion”
  - ◆ Heated Beulizers
- Immunoassays
  - ◆ Where to for small molecules
  - ◆ Remember Zantac
  - ◆ Future is very good

# HPLC - Evolution → Enhancement

- **Pumps; pressurised; syringe; reciprocating pulse free!**
- **UV detectors; cells, noise, sensitivity**
- **Fluorescence; Electrochemical**
- **Sample Preparation**
  - ◆ **Liquid:Liquid**
  - ◆ **Solid Phase**
  - ◆ **Automation**
- **Column Technology; packing size**
- **Mobile Phases - Gradients**
- **Derivatization (post / pre column)**
- **Sample Focussing**
- **Column Switching - online concentration heart cutting**

# HPLC-UV; Fluorescence

**Is it dead or dying?**

- **In bioanalysis it will be a niche technology like GC has become**
- **Pharmaceutical Analysis it has remained the same for the last 30 years - there are “currently” no drivers for change - it will be there in a further 30 years! Maybe matchbox size with own in-built data system**

# Solid Phase Extraction

- **Analytichem (late 70's)**
  - ◆ **small range**
  - ◆ **high variability**
  - ◆ **quality of packing**
  - ◆ **packing size**
  - ◆ **channeling**
  - ◆ **impurities from the cartridge**
- **LC-MS(MS)**
  - ◆ **New lease of life for SPE**
  - ◆ **Greater range of “packings”**
  - ◆ **Improved quality of packing**
  - ◆ **Small packing volumes**

# The Cupboard - Why they failed

- Super critical fluid chromatography
- TLC plate scanners (Quantitative)
- Polarographs
- AASP
- ASTED
- Agilent / HP MSD
- etc etc
- Column switching
- Green fingers and persistence

# Remember the AASP

- **Analytichem Automate Sample Process**  
10 well cartridge
- **Failed because reliability**
- **Method Development (expensive in cartridges)**
- **96 well plates took over**

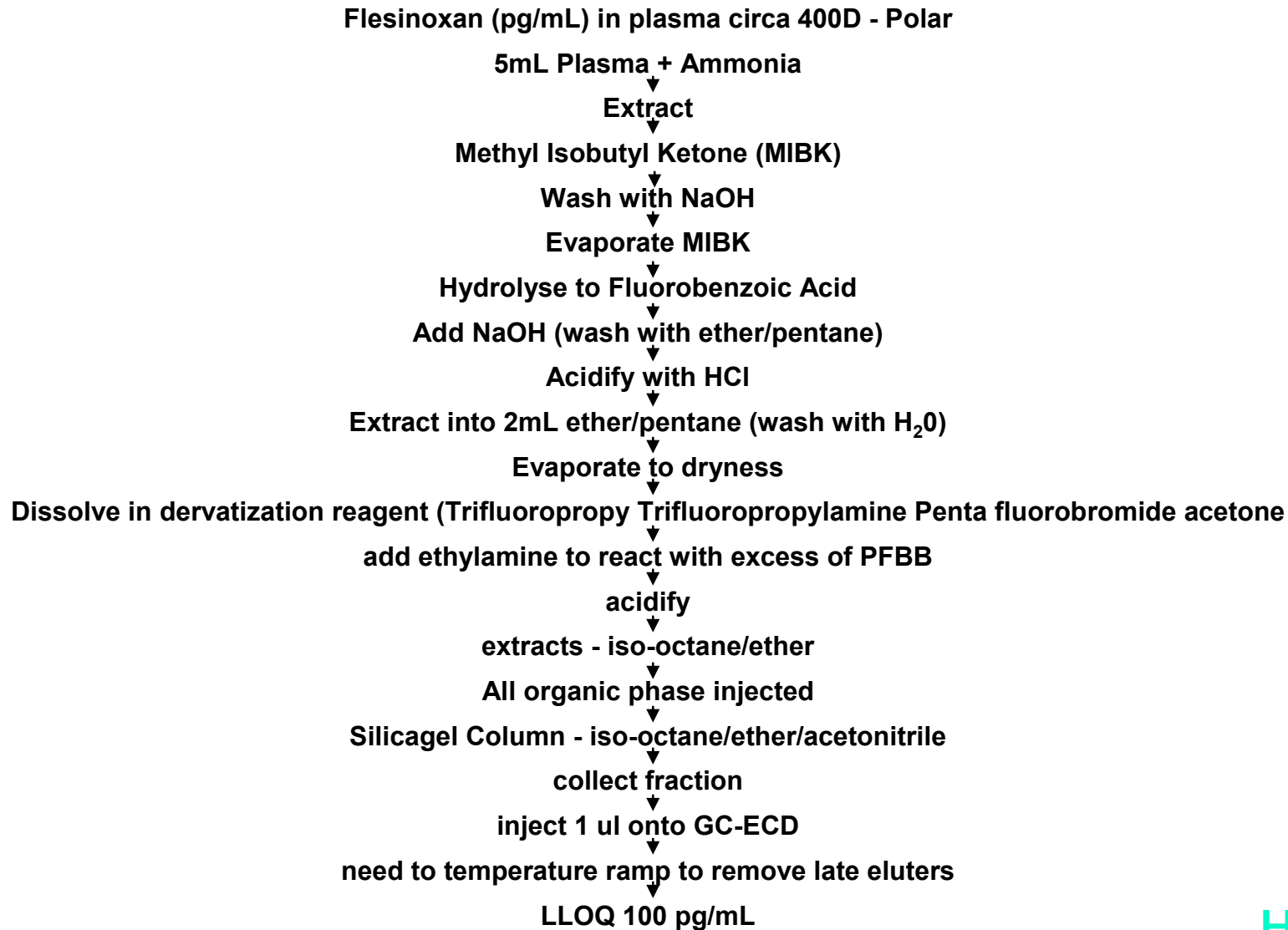
# Capillary Electrophoresis

- Archie Martin circa 1977/78 that CZE would replace hplc
- Reliability of “columns”
- Sample size
- Detection
- Expense LKB system only manufacturer
- CE - Enhancements
- Non-aqueous capillary electrophoresis (NACE)
- Micellar Electrokinetic chromatography (MEKC)
- MS - interface / Chips

# Niche Application

- **Chiral separations**
- **Biotechnology products - impurities**
- **Small molecules - stability indicating**
- **Green finger technique**

# Enhancement Techniques 1985?



AND you had to redistill and dry (where necessary) the solvents

# Potent Drugs Analytes (1987)

- HPLC and **current** fluorescence detectors are insufficiently sensitive for pg/mL levels
- Hence the Renaissance of GC
  - ◆ Bonded Phase fused-silica columns
  - ◆ Sensitive NPD and ECD
  - ◆ On column injectors
  - ◆ Cryogenic focussing
  - ◆ Need **clean** samples
- However, API cut short the renaissance

# LC-MS-MS

- **MS “always” had possibilities: I.e. sensitivity, selectivity**
- **Mid-1970s Hammersmith Hospital - Harry Draffan “quoted” as saying no need for chromatography**

# LC-MS-MS (circa 1987)

## Bruins Overview

**Combination of LC-MS fundamental problem is the inability of MS vacuum to handle the gas load of the evaporated eluate.**

**Remedies include:**

- ◆ **Moving belt to remove eluent and introduce the sample into the ion source**
- ◆ **Direct introduction of “small” volume of eluent**
- ◆ **Miniaturisation of LC system**
- ◆ **Thermospray - additional pumping and heat to the column eluate**
- ◆ **Ionization at atmospheric pressure**

# LC-MS-MS (circa 1987)

## Bruins

### The Future:

- ◆ **Moving Belt continues to receive attention**
- ◆ **Thermospray is the best choice at present**
- ◆ **Continuous flow FAB is still in its infancy but will rapidly find application**
- ◆ **API with ion spray interface is potentially apt**

**HOWEVER, it is only available from Sciex and Hitachi and will only be successful when it becomes available from traditional manufacturers of analytical MS computer combinations**

# More Hyphenations - enhancements

- **Quadrupole Time of Flight**
  - ◆ **Q-TOF**
  - ◆ **Q-STAR**
  - ◆ **Good resolution**
  - ◆ **Compromised sensitivity**
- **Q Trap**
- **TSQ Quantum Triple Quadrupole**
  - ◆ **Good Resolution**
  - ◆ **High Stability**
  - ◆ **Good Sensitivity**
  - ◆ **Eliminate / Reduce Matrix Effects**

# LC - MS (MS) - enhancements

**Making the most of what we have**

- **More hyphenation; more refinements**
- **Photoionizer**
- **Cone voltage fragmentation**
- **Adjusting the variables**
  - ◆ **Mobile phase and pH (post column)**
  - ◆ **Collision cell energy**
  - ◆ **Matrix effect issues**
- **MUX**
- **Turbulent flow**

# Other Issues

- **These consume innovative energy resources**
  - ◆ **21 CFR 11**
  - ◆ **producing QA'd data and tabulations**
  - ◆ **Process of accepting data**
  - ◆ **Data systems**
- **Sample Preparation**
  - ◆ **Solid phase technologies**
  - ◆ **Automation systems - transferability**
- **Other Regulation issues**

# The Future

- **Chips (nano fluidics)**
- **Biosensors**
- **Microdialysis**
- **Molecular Imprinting  
(Antibodies)**

# Current Drugs

- Potent
- Multiple “polar” groups
- 400-700D
- 10-1,000 pg/mL
- Not GC friendly
- Not LC-UV
- LC-EC possibly but?
- Confirm LC-MS (MS) as the technology

# Biosensors

## ■ Range of Transducers

- ◆ Electrochemical
- ◆ Optical
- ◆ Photothermal
- ◆ Amperometric
- ◆ Acoustic

# Why Biosensors

- **Many high throughput screening systems use chip technologies for screening, e.g. enzymes, receptors, single or multiple cells**

# Screening - High throughput

- **Receptor - Activation**
- **Nanotechnologies (Microfluids) Chips**
- **Ligand based assays**
- **Enzyme Targets**
- **Cell - Cellular based assays**

# Electroanalytical Biosensors

- **Enzyme based Biosensors**
- **Immuno Sensors**
- **Receptor based Sensors**

# Biosensors

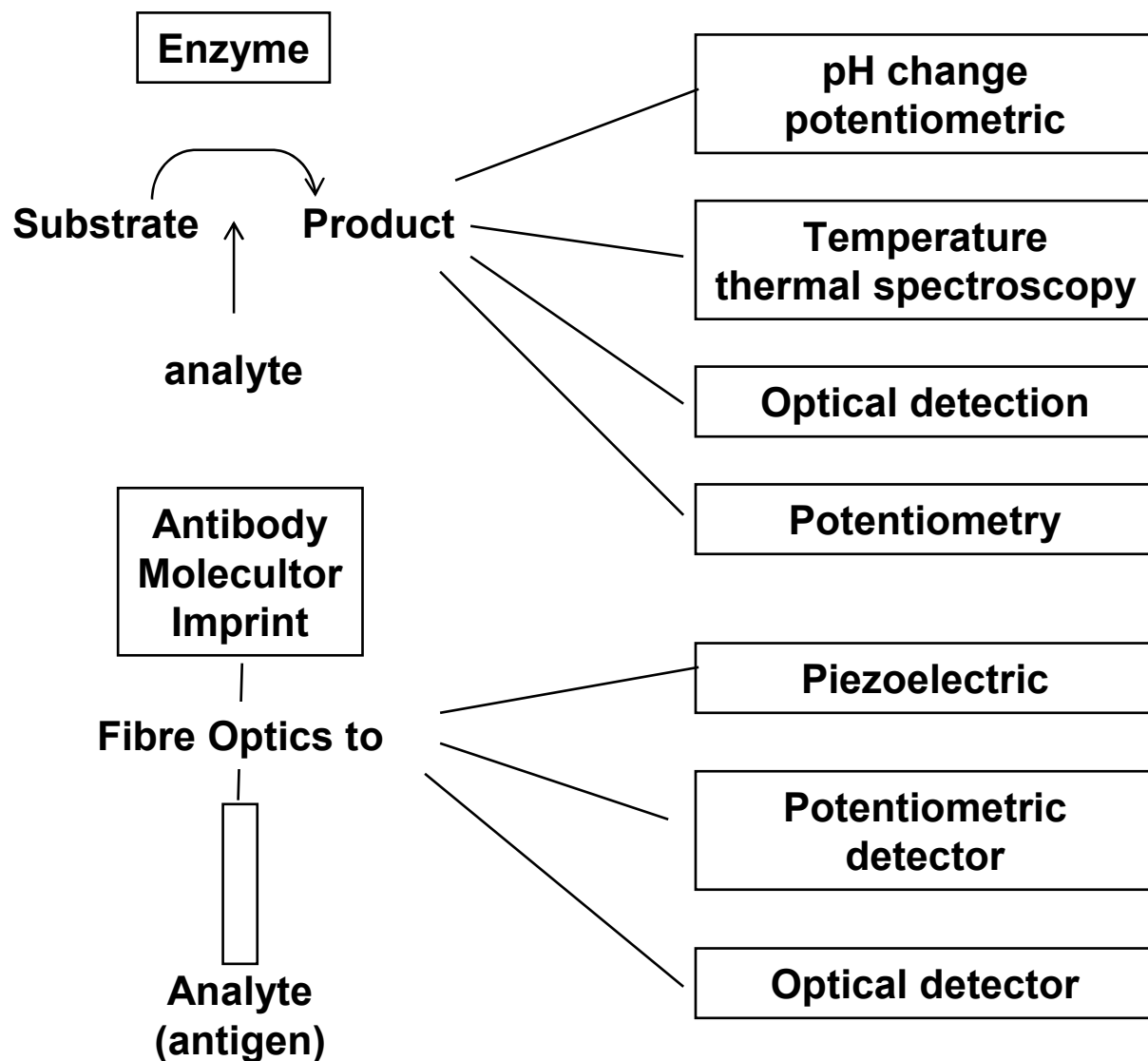
- Immobilized Bioreceptors e.g. enzymes, antibodies, single (multiple cells)
- Entrapped behind a membrane
- Entrapped within a polymer matrix
- Covalent bonding
- Bulk Modification (using a paste)
- Biological receptors within SAM or BLM
- Immunosensors
- Molecular sensors

**NB Entrapment techniques can stabilize biological materials**

- Nano technologies

SAM = self assembled monolayers  
BLM = bilayer lipid membrane

# Schematic Representations of a Biosensor



# Microdialysis / Sample Preparation

- **Driver - Glucose Monitor for Diabetics**
- **Glucose Oxidase - on micro chip (detector) online to subcutaneous microdialysis**
- **Caveat - Yes it may be an easy target like another assay for theophylline - but you have to start somewhere !!!**
- **Application for animal studies e.g. BAS Coulex, on-line sample collection**

# Automated Clinical Chemistry and Immunoassay Analysers

- Optimised reaction condition
- Highly reproducible
- “Calibration Curves” hard wired
- One point “correction/adjustment”
- Checks for the unusual
- Low skill level to operate
- BUT identifying problems and solving them is important
- Clinical Chemistry Chip

# LC-NMR

- Pumps
- RP18 Column
- Photodiode Array detector
- PEEK connection
- LC-NMR flow probe
- (Mobile Phase D<sub>2</sub>O; pD adjusted with pH meter)
- Bruker 600 MHz
- 4mm id - 120microL cell
- Spectra obtained in Stopped Flow Mode
- NOT (yet) a routine bioanalytical tool - a problem solving system - pattern “recognition” metabonomics

# Summary Conclusions

- **Is there sufficient “basic” research in bioanalysis in Industry in universities?**
- **Do the manufacturers want to or see a need to make the quantum leap?**
- **We still have not made the most “enhanced” LC-MS technology**
- **So API Triple Quadrupoles coupled to (HP LC) is here to stay ....**