Quality Control & Analytical Development
In The Pharmaceutical Industry
December 2011
Outline

• Quality Control & Analytical Development in the Drug Development Process
• Pharmacopeia and Compendia Methods
• USP General Chapters and Testing Methods
• ICH Guidelines and Good Manufacturing Practices
• Specifications
• Pending USP Monograph: Tenofovir Disoproxil Fumarate
• Determination and Control of Impurities in Pharmaceuticals
• Metal Analysis in the Pharmaceutical Industry
• Wrap Up
Quality Control and Analytical Development in the Drug Development Process
## Quality Control vs. Analytical Development

<table>
<thead>
<tr>
<th>Quality Control</th>
<th>Analytical Development</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Release testing</td>
<td>• Develop new analytical methods</td>
</tr>
<tr>
<td>• Stability testing</td>
<td>• Compose specifications for drug substance and drug product</td>
</tr>
<tr>
<td>• Cleaning Verification</td>
<td>• Compose analytical sections of regulatory filings</td>
</tr>
<tr>
<td>• Sampling</td>
<td>• Support process development and formulation development</td>
</tr>
</tbody>
</table>
Quality Control in the Drug Development Process

Quality Control

- Toxicology
- Pharmacokinetics
- Process Support
- Formulation Support
- Reference Materials
- Stability Testing
- Regulatory Filing Support
- Release Testing
- Clinical Trial Support

GMP/GLP Regulations
Analytical Development in the Drug Development Process

- Toxicology
- Pharmacokinetics
- Drug Substance Methods
- Drug Product Methods
- Specifications
- Physical Characterization
- Development Reports
- Clinical Trial Support
- Regulatory Filing Support
Analytical Requirements in Drug Development Process – HPLC Assay Method Example

**Pre-Clinical**
- Initial HPLC Method
  - Determination of Salt Form
  - Bioavailability/Toxicology Studies
  - Solution Stability

**Phase 1**
- Early HPLC Method
  - Batch Release
  - Early Stability
  - Clinical Support
  - Regulatory Filings (IND)
  - Scale Up
  - Bioavailability/Reproductive Toxicology
  - Formulation Development

**Phase 2**
- Late HPLC Method
  - Batch Release
  - Formal Stability of Drug Substance
  - ID Major Impurities
  - Formulation Support

**Phase 3**
- Batch Release
  - Formal Stability of Drug Substance and Product
  - Development of Specification
  - Finalization of Impurities and Degradants
  - Method Validation
  - Process Validation

**Phase 4**
- Batch Release
  - Regulatory Filings (IND)
  - Scale Up
  - Bioavailability/Toxicology Studies

**GMP**
**GLP**
Pharmacopeia and Compendia

Methods
What is a Pharmacoepia?

- In simple terms, the word *pharmacoepia* is a compound, created from two Greek words: *pharmakon* (medicine or charm) and *poiein* (to make).
- Defined as “a book containing a compilation of pharmaceutical products with their formulas and methods of preparation,” the word pharmacopeia has a rich, historic meaning with roots tracing back to 15th century Florence, Italy. At that time, a physician named Lodvice dal Pozzo Toshchanelli did a smart, yet simple thing—he produced a “little book of drug formulas” in response to a request from the local guild of pharmacists seeking information about quality standards in drug therapy. Little did he know that he would be setting standards for the future of worldwide public health.
- Centuries later across the Atlantic, America’s founding forefathers revived Toshchanelli’s idea and further defined the term “pharmacopeia.” It was used indiscriminately to label a variety of “drug books” regardless of whether they represented legal or authoritative standards.
US Pharmacopeia (USP)

- In 1820, the United States Pharmacopeia (USP) was published containing formulae for the preparation of 217 drugs considered to be the “most fully established and best understood” at the time. In 1888, the American Pharmaceutical Association created the National Formulary (NF), which included formulations and unofficial preparations for widely sold products.
- Yet it wasn’t until the passage of the Federal Food and Drugs Act in 1906 that standards in the USP were recognized as official standards for the United States. The Federal Food, Drug, and Cosmetic Act of 1938 further solidified USP’s role in U.S. law, designating USP, as well as NF and Homeopathic Pharmacopeia standards, as enforceable by the U.S. Food and Drug Administration (FDA). Finally in 1975, USP purchased the NF, combining the two publications under one cover to create the United States Pharmacopeia–National Formulary.
The Modern Pharmacopeia

- In modern times, the multi-billion dollar pharmaceutical industry produces thousands of drugs annually, although not much has changed with regard to the initial intent for a pharmacopeia.
- The *USP–NF* contains more than 4,000 monographs for prescription and over-the-counter products, dietary supplements, medical devices, and other health care products. In its present form, somewhat different than Toshchanelli’s original black book, the *USP–NF* is published annually and is available as a CD-ROM and online as well as in hardcover. USP
- also produces an Asian edition and a Spanish edition of the *USP–NF*. 
Monographs and General Chapters

- The USP–NF is a combination of two official compendia, the United States Pharmacopeia (USP) and the National Formulary (NF). Monographs for drug substances and preparations are featured in the USP. Monographs for dietary supplements and ingredients appear in a separate section of the USP. Excipient monographs are in the NF.

- A monograph includes the name of the ingredient or preparation; the definition; packaging, storage, and labeling requirements; and the specification. The specification consists of a series of tests, procedures for the tests, and acceptance criteria. These tests and procedures require the use of official USP Reference Standards. Medicinal ingredients and products will have the stipulated strength, quality, and purity if they conform to the requirements of the monograph and relevant general chapters.

- Tests and procedures referred to in multiple monographs are described in detail in the USP–NF general chapters.
Official Recognition

• The U.S. Federal Food, Drug, and Cosmetics Act designates the USP–NF as the official compendia for drugs marketed in the United States. A drug product in the U.S. market must conform to the standards in USP–NF to avoid possible charges of adulteration and misbranding. The USP–NF is also widely used by manufacturers wishing to market therapeutic products worldwide. Meeting USP–NF standards is accepted globally as assurance of high quality.
Other Pharmacopeias

- In addition to USP, there are three other large pharmacopeias in the world, which include
  - the European Pharmacopeia (EP) (www.pheur.org), the British Pharmacopeia (BP)
  - (www.pharmacopeia.org), and the Japanese Pharmacopeia (JP)
  - (http://jpdb.nihs.go.jp/jp14e/index.html), all of which share the goal of publishing and producing standards for pharmaceuticals.
- While its global counterparts are part of the ministries of health in their countries, USP has remained a practitioner-based, nongovernmental standards-setting organization. All pharmacopeias, however, seek to advance public health by ensuring the quality and consistency of medicines and promoting the safe and proper use of medications.
USP General Chapters and Test Methods
The USP

• The USP Contains

  – ~4100 Monographs

  – More than 200 tests, assays, and general chapters
Monographs

- Molecular Structure
  - Description
  - Identity
  - Assay
  - Impurities
General Chapters

- Frequently cited procedures
- Sometimes with acceptance criteria
- Provide a single source for information present many monographs
Assays

• Standard Preparation

• Sample Preparation

• Assay Procedure

〈351〉 ASSAY FOR STEROIDS

The following procedure is applicable for determination of those Pharmacopeial steroids that possess reducing functional groups such as α-ketols.

**Standard Preparation**—Dissolve in alcohol a suitable quantity of the USP Reference Standard specified in the individual monograph, previously dried under the conditions specified in the individual monograph and accurately weighed, and dilute quantitatively and stepwise with alcohol to obtain a solution having a concentration of about 10 µg per mL. Pipet 20 mL of this solution into a glass-stoppered, 50-mL conical flask.

**Assay Preparation**—Prepare as directed in the individual monograph.

**Procedure**—To each of the two flasks containing the Assay Preparation and the Standard Preparation, respectively, and to a similar flask containing 20.0 mL of alcohol to serve as the blank, add 2.0 mL of a solution prepared by dissolving 50 mg of blue tetrazolium in 10 mL of methanol, and mix. Then to each flask add 2.0 mL of a mixture of alcohol and tetramethylammonium hydroxide TS (9 : 1), mix, and allow to stand in the dark for 90 minutes. Without delay, concomitantly determine the absorbances of the solutions from the Assay Preparation and the Standard Preparation at about 525 nm, with a suitable spectrophotometer, against the blank. Calculate the result by the formula given in the individual monograph, in which C is the concentration, in µg per mL, of the Reference Standard in the Standard Preparation; and A₀ and Aₛ are the absorbances of the solutions from the Assay Preparation and the Standard Preparation, respectively.
General USP methods

- Liquid Chromatography <621>
- Gas Chromatography <621>
- Ion Chromatography <621>
- Mass Spectrometry <736>
- Particle Size <429>
- UV <197>
- IR <851>
- NMR <761>
- Titration <541>
- Many More
Liquid Chromatography

Waters Acquity UPLC

Waters Acquity UPLC – H Class

Agilent 1200

Waters Alliance

Agilent 1100
LC Detectors

• UV (Ultraviolet)
  – Most common LC detector
  – High sensitivity required for routine UV-based applications to low-level impurity identification and quantitative analysis.

• PDA (Photodiode Array) –
  – Similar to UV but can scan through the entire wavelength spectrum
  – Gives simultaneous data at all wavelengths.
  – Powerful development tool

• ELS (Evaporative Light Scattering) –
  – Provides solutions for the analysis of a wide variety of compounds that lack UV/Vis chromophores.
  – Specialized detector for trace analysis
LC Detectors (Cont.)

- **CAD (Corona Aerosol Detector)**
  - The Corona CAD detector detects any nonvolatile or semivolatile analyte, with or without a chromophore.
  - Specialized detector for trace analysis

- **RI (Refractive Index)**
  - Provides sensitivity, stability, and reproducibility for the analysis of components with limited or no UV absorption
Gas Chromatography

Agilent 7890

Agilent 6890
Gas Chromatography Detectors

• FID (Flame Ionization Detector)
  – Most common GC detector
  – The flame ionization detector (FID) is the most sensitive gas chromatographic detector for hydrocarbons.

• TCD (Thermal Conductivity Detector)
  – Senses changes in the thermal conductivity of the column effluent and compares it to a reference flow of carrier gas.
  – When an analyte elutes from the column the effluent thermal conductivity is reduced, and a detectable signal is produced.

• ECD (Electron Capture Detector)
  – Used for detecting electron-absorbing components (high electronegativity)
  – Used for halogenal compounds.
Gas Chromatography Detectors (Cont.)

- **NPD (Nitrogen Phosphorus Detector)**
  - Uses thermal energy to ionize an analyte.
  - Nitrogen and phosphorus can be selectively detected with a sensitivity that is $10^4$ times greater than that for carbon.

- **FPD (Flame Photometric Detector)**
  - Similar to FID in that the sample exits the column into a hydrogen diffusion flame.
  - Where the FID measures ions produced by organic compounds during combustion, the FPD analyzes the spectrum of light emitted by the compounds as they luminesce in the flame.
Mass Spectrometry

Thermo LCQ LC-MS

Micromass Quattro Micro LC-MS

Agilent 5975C GC-MS

Agilent 7500 ICP-MS
LC-MS

- **LCQ Ion Trap**
  - Used for structural elucidation of low level impurities
  - Has both an ESI and APCI Ion Source

- **Quattro Micro**
  - Triple Quad MS used for quantitation of low level impurities
  - Has an ESI source
GC-MS

- Agilent 5975C
  - Electron Ionization (EI) Source
  - Powerful development tour for identification of volatile impurities
ICP-MS

- Agilent 7500CE
  - Premier technique for trace metal analysis
  - He collision cell for removal of unknown matrix interferences
  - Organic Kit to allow use in organic media
Other Tools

Malvern 2000 Particle Size Analyzer

Varian 400 MHz NMR (x2)

Agilent 8453 UV

ThermoFisher Q20 DSC

Autopol V Polarimeter

Nicolet Avatar IR
Still More Tools

Metrohm KF (VKF, CKF)  Metrohm Autotitrators  Dionex ICS 3000 IC (x2)  Shimadzu TOC-V
ICH Guidelines and Good Manufacturing Practices
ICH Guidelines

- **Q** – Quality Guidelines
  - the conduct of stability studies, defining relevant thresholds for impurities testing and a more flexible approach to pharmaceutical quality based on Good Manufacturing Practice (GMP) risk management

- **E** – Efficacy Guidelines
  - the design, conduct, safety and reporting of clinical trials. It also covers novel types of medicines derived from biotechnological processes and the use of pharmacogenetics/genomics techniques to produce better targeted medicines

- **S** – Safety Guidelines
  - uncover potential risks like carcinogenicity, genotoxicity and reprotoxicity.

- **M** – Multidisciplinary Guidelines
  - Those are the cross-cutting topics which do not fit uniquely into one of the Quality, Safety and Efficacy categories.
ICH Quality Guidelines

- Q1  – Stability
- Q2  – Analytical Validation
- Q3  – Impurities
- Q4  – Pharmacopeias
- Q5  – Quality of Biotechnological Products
- Q6  – Specifications
- Q7  – Good Manufacturing Practices
- Q8  – Pharmaceutical Development
- Q9  – Quality Risk Management
- Q10 – Pharmaceutical Quality System
- Q11 – Development and Manufacture of Drug Substances
ICH Q7 Good Manufacturing Practices

• GMPs are a set of regulations, codes, and guidelines for the manufacture of
  – Drug Substance and Drug Products
  – Medical Devices
  – In vivo and in vitro diagnostic products
  – Food

• The Rules
  – The Federal Food, Drug, and Cosmetic Act
  – Code of Federal Regulations (CFR)
  – International Conference on Harmonization (ICH) Guidelines
  – FDA/EMEA Guidance Documents

• Basic GMP Requirements
  – Management Systems
  – Operating Procedures
  – Personnel Training
  – Data Accountability
  – Method Validation
  – Equipment Qualification and Calibration
  – Facilities
  – Certification Documentation
ICH Q7 Good Manufacturing Practices
Management Systems

• Well Defined Organizational Structure

• Well Defined Responsibility for Each Organizational Unit

• Change Management/ Configuration Management System

• Deviations and Investigations

• Corrective Actions Preventative Actions (CAPA)

• Periodic Reviews

• Audits
ICH Q7 Good Manufacturing Practices
Operating Procedures

- Defines Processes and Activities Regularly Performed
- Standardizes the Operations to Complete Defined Processes and Activities
- Step-by-Step Instructions
- Controlled Documents

GMP-ONLINE-CONSULTANCY.COM

Handling of OOS Results

2. In those instances where an investigation has revealed a cause, and the suspect result is invalidated, the result will not be used to evaluate the quality of the batch or lot.
3. Invalidation of a discrete test result may be done only upon the conservation and documentation of a test event that can reasonably be determined to have caused the OOS result.
4. In those cases where the investigation indicates an OOS result is caused by a factor affecting the batch quality (i.e., an OOS result is confirmed), the result will be used in evaluating the quality of the batch or lot.
5. Statistical treatments of data will normally not be used to invalidate a discrete chemical test result. In very rare occasions and only after a full investigation has failed to reveal the cause of the OOS result, a statistical analysis may be valuable as one assessment of the probability of the OOS result as discordant, and for providing perspective on the result in the overall evaluation of the quality of the batch. Records are kept of complete data derived from all tests performed to ensure compliance with established specifications and standards.

5.2.2 Reporting and Documentation
5.2.2.1 Reporting
1. Copies of the final OOS investigation Report are distributed to the appropriate Quality management.
2. Preliminary assessment should be done within 2 working days (refer to attachment 1 Section II).
3. Complete investigation should be closed within 30 calendar days (refer to attachment 1 Section II to III).
4. For stability samples the timing for FDA Field Alert Reporting begins with the discovering the OOS result (these must be reported to the FDA within 3 working days after discovery of the result). For those products that are the subject of applications, regulations require submitting within three working days a field alert report (FAR) of information concerning any failure of a distributed batch to meet any of the specifications established in an application. For more details refer to SOP NDA Field alerts.
5. In the event of a confirmed failure (OOS result), notify all other appropriate parties via deviation procedure SOPs form sheet. For more details refer to SOP Deviations.

5.2.2.2 Documentation
1. All results including rejected data will be retained in laboratory records. A complete description of the reason for rejection will be included in
ICH Q7 Good Manufacturing Practices
Operating Procedures – Test Methods

- Defines Processes and Activities Required to Perform An Analysis
- Standardizes the Operations to Complete Analysis
- Step-by-Step Instructions
- Controlled Documents
ICH Q7 Good Manufacturing Practices
Personnel Training

• Training Requirements Detailed in a Training Syllabus

• Each Employee is Assigned a Training Syllabus

• The Training Syllabus includes Operating Procedures and Test Methods

• Training Record Must Record the Successful Completion of the Training Syllabus

• Should be Periodically Reviewed by Employee
ICH Q7 Good Manufacturing Practices

Data Accountability

- Sample Chain of Custody
  - Logbooks
  - Sample Labels

- Traceability of Sample Preparation

- Traceability of Reagents Used

- Traceability of Equipment Used

- Traceability of Raw Data
ICH Q7 Good Manufacturing Practices
Method Validation

• Demonstration that an Analytical Method is Capable of Performing the Prescribed Analysis
  – Accuracy
  – Precision
  – Specificity
  – Limit of Detection (LOD)
  – Limit of Quantitation (LOQ)
  – Linearity
  – Range
  – Robustness
  – Solution Stability
ICH Q7 Good Manufacturing Practices
Equipment Qualification and Calibration

• Instrument Qualification
  – Design Specification
  – Installation Qualification
  – Operation Qualification
  – Performance Qualification

• Instrument Calibration
  – Periodic Maintenance
  – Performance Verification
  – Daily System Suitability
ICH Q7 Good Manufacturing Practices

Facilities

• Complete Set of Facility Drawings
  – Design Specification
  – Blue Prints
  – Facility Qualification
  – Change Control

• Environmental Monitoring
Specifications
Specifications

• Defines Key Attributes of Drug Substance or Drug Product to be Tested

• Defines Acceptable Attribute Limits (Test Limits)

• Defines the Retest Period

• Defines the Test Methods to be Used

---

TRAMADOL HYDROCHLORIDE

STRUCTURAL FORMULA

<table>
<thead>
<tr>
<th>ITEMS</th>
<th>STANDARD</th>
</tr>
</thead>
<tbody>
<tr>
<td>APPEARANCE</td>
<td>White to off white crystalline powder</td>
</tr>
<tr>
<td>IDENTIFICATION</td>
<td>IR Spectrum</td>
</tr>
<tr>
<td>SPECIFIC OPTICAL ROTATION</td>
<td>C=1, ETHANOL</td>
</tr>
<tr>
<td>ASSAY (ON DRY BASIS) HPLC</td>
<td>98% to 100%</td>
</tr>
<tr>
<td>LOSS ON DRYING</td>
<td>NMT 0.5%</td>
</tr>
<tr>
<td>MELTING POINT</td>
<td>179 °C - 184 °C</td>
</tr>
<tr>
<td>SOLUBILITY IN WATER</td>
<td>Freely soluble in water</td>
</tr>
<tr>
<td>pH (1% w/v SOLUTION IN WATER)</td>
<td>4.5 - 6.0</td>
</tr>
<tr>
<td>PURITY (RELATED SUBSTANCES)</td>
<td></td>
</tr>
<tr>
<td>a) 2-Dimethylamino methyl cyclohexane</td>
<td>NMT 0.2%</td>
</tr>
<tr>
<td>b) Cis-isomer (HPLC)</td>
<td>NMT 0.3%</td>
</tr>
</tbody>
</table>
Specifications - Requirements

- Universal Tests
  - Description
  - Identity
  - Assay
  - Impurities
    - Organic
    - Inorganic
    - Residual Solvents

- Specific Tests
  - Water Content
  - Enantiomeric Purity
  - Particle Size
  - Polymorphism
Impurities

- Specific, stability indicating procedure to determine content of impurities in API and DP.
  - Calculation should be made by comparison with qualified Reference Standards
  - Alternatively, if a qualified Reference Standard of an impurity is unavailable or in short supply, calculation may be made by comparison with the active. In this case every attempt should be made to determine the Response Factor for the impurities. Retention time markers of key impurities should also be included in the system suitability samples

- Impurities in the DS should not be reported in results for the DP, unless they are also degradation products.

- Impurities below the Reporting Limit do NOT have to be reported to Regulatory Authorities, but should be tracked internally to establish trends or changes due to process changes or stability changes.
Pending USP Monograph: Tenofovir Disoproxil Fumarate
Pending Monograph

Tenofovir Disoproxil Fumarate

v.1 Authorized September 1, 2011

C₁₉H₂₅N₅O₁₀P · C₄H₄O₄

(50.635.51)

(R)-5-[[2-(6-Amino-9H-purin-9-yl)-1-methylthoxy]methyl]-
2,4,6,8-tetraoxa-5-phosphonanedioic acid, bis(1-
methylthyl) ester, 5-oxide, (E)-2-butenedioate (1:1);
Bis(hydroxymethyl) [[[(R)-2-(6-amino-9H-purin-9-yl)-1-
methylthoxy]methyl]phosphonate, bis(isopropyl carbonate)
(ester), fumarate (1:1);
9-[(R)-2-[Bis[[isopropoxycarbonyloxy]methoxy-
phosphinyl]methoxy]propyl]adenine fumarate (1:1)
[202138-50-9].

DEFINITION

Tenofovir Disoproxil Fumarate contains NLT 98.0% and NMT 102.0% of C₁₉H₂₅N₅O₁₀P · C₄H₄O₄, calculated on the anhydrous basis.

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

- Residue on Ignition (281): NMT 0.2%
- Heavy Metals, Method II (231): NMT 20 ppm
  [NOTE—Use Organic Impurities, Procedure 7 when the
  impurity profile includes adenine or tenofovir disoproxil
  monoester. Use Organic Impurities, Procedure 2 when the
  impurity profile includes desmethyl tenofovir disoproxil.]

ORGANIC IMPURITIES, PROCEDURE 1

Buffer: 0.01 M of dibasic sodium phosphate in water. 
Adjust with phosphoric acid to a pH of 5.5.
Solution A: Methanol, tertiary butyl alcohol, and Buffer
(11:1:28)
Solution B: Methanol, tertiary butyl alcohol, and Buffer
(27:1:12)
Mobile phase: See Table 1.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Solution A (%)</th>
<th>Solution B (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>30</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>45</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>50</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>60</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 1

This monograph has been developed under USP’s Pending Monographs Guideline and is not a USP–NF monograph.

http://www.usp.org

©2011 The United States Pharmacopeia. All Rights Reserved.
The Pending Monograph Approach

- The USP Pending Monographs approach enables Web-based publication of monographs for articles based on information in FDA applications and filings.

- The purpose of Pending Monographs is to have an official USP or NF monograph ready as soon as possible after FDA grants final product approval.

- Pending Monographs approach lets monograph development begin before FDA's approval process is complete, resulting in an official USP or NF monograph more rapidly than would be possible if monograph development started only after final FDA approval.
USP Non-US Monographs

• The USP approach for monographs for articles legally marketed outside the U.S. (Non-US Monographs) allows the creation of documentary standards (monographs) for drug products and their ingredients that have been approved in and are legally marketed in countries other than the U.S. and are intended to treat neglected diseases.

• Where appropriate, USP will provide reference materials (official USP Reference Standards) to support testing of an article against a Non-US Monograph.

• In order for a Non-US Monograph for a drug product and/or its drug substance to be developed, the Sponsor must:
  – Not have obtained final approval from the Food and Drug Administration (FDA) of the drug product in the U.S.
  – Have obtained approval for the drug product in a country with a stringent regulatory authority 1
  – Follow USP's Guideline for Submitting Requests for Revision to the USP-NF and label the Request "Non-US Monograph."
  – Inform USP if an application for approval of the drug product is filed in U.S. or if the approval status of the drug product or drug substance elsewhere changes.
Overview of the TDF Process

Adenine $\rightarrow$ HPA

\[
\begin{align*}
\text{TsO} & \quad \text{PO} \quad \text{Et} \\
\text{CH}_3 & \quad \text{OEt} \quad \text{OEt}
\end{align*}
\]

Diethyl PMPA

\[
\begin{align*}
\text{HO}_2\text{C} & \quad \text{CO}_2\text{H} \\
\text{CH}_3 & \quad \text{O} \quad \text{O} \quad \text{OEt} \quad \text{OEt} \quad \text{OH}
\end{align*}
\]

Tenofovir DF (Bis-POC PMPA Fumarate)

TDF USP Monograph

The USP Monograph for TDF contains ten distinct sections which are listed below:

- Definition
- Identification
- Assay
- Impurities
- Chloromethyl Isopropyl Carbonate
- Enantiomeric Purity
- Tenofovir Disoproxil Related Compound B
- Fumaric Acid Content
- Specific Tests
- Additional Requirements
Definition

• For the current USP Monograph TDF contains NLT 98.0% and NMT 102.0% of $C_{19}H_{30}N_5O_{10}P.C_4H_4O_4$ calculated on an anhydrous basis

• Anhydrous Basis: Assay calculated taking into account water and OVI content
Identification

- Two distinct tests
  - Infrared Absorption <197> (The IR spectrum of the sample must match the IR spectrum of a similarly measured reference material)
  - Enantiomeric HPLC RT (The retention time of the major peak from the sample solution corresponds to that of the standard solution)
Identification (Cont.)

- IR Testing
  - 3-4 mg of sample prepared in 300-500 mg of KBr
  - Sample spectrum is compared to a reference spectrum

- HPLC RT
  - Retention time of the main peak of reference standard compared to retention time of the main peak of the sample
  - The retention of both peaks should be within 2.0% of one another
Assay

• An assay measures the amount of a substance in a sample.

• In order to carry out an assay you need a reference standard.

• A reference standard is a standardized substance which is used as a measurement base for similar substances.

• USP's official Reference Standards are highly characterized specimens of drug substances, excipients, impurities, degradation products, dietary supplements, compendial reagents and performance calibrators. They are required for use in conducting official USP–NF tests and assays.
Assay (Cont.)

• To perform a HPLC assay an accurate amount of reference standard with a known potency is weighed out and made up to a known concentration (Typical between 0.1 to 0.5 mg/mL).

• The sample is then prepared in the exact same manner.

• After the samples have been run on the HPLC, the area counts per concentration of the sample are divided into the area counts of the standard to give the assay value

• Low assay numbers typically indicate high inorganic content, high water content or high OVI content.
Assay (Cont.)

• USP developed an isocratic HPLC method for assay of TDF

  – Buffer: 1 mL/L of TEA in water pH = 6.0
  – Mobile Phase: 9:11 ACN:Buffer
  – Diluent: 1:19 ACN:Water
  – Standard Solution: 0.05 mg/mL of USP TDF RS in diluent
  – Sample Solution: 0.05 mg/mL of sample in diluent
  – Detector: UV at 260 nm
  – Column: YMC Pack ODS-AQ 4.6x250 mm
  – Column Temp: 30°C
  – Flow Rate: 1 mL/min
  – Injection Volume: 10 µL
  – System Suitability: Tailing NMT 2.0, RSD NMT 2.0%
  – **Acceptance:** NLT 98.0% and NMT 102.0% of TDF calculated on an anhydrous basis
Impurities

- Residue on Ignition <281>: NMT 0.2%
- Heavy Metals <231>: NMT 20 ppm
- Organic Impurities:
  - Two HPLC Gradient Methods Developed by USP to give Total Impurity Content
The USP details eight impurities that must be quantitate for the release of the API. The allowable levels of these impurities differs depending on toxicology data.
Organic Impurities: Procedure 1

- Gradient Method developed to quantitate seven separate impurities

<table>
<thead>
<tr>
<th>Name</th>
<th>Relative Retention Time</th>
<th>Relative Response Factor</th>
<th>Acceptance Criteria NMT (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fumaric Acid</td>
<td>0.12</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tenofovir</td>
<td>0.14</td>
<td>2.1</td>
<td>0.15</td>
</tr>
<tr>
<td>Adenine</td>
<td>0.16</td>
<td>4.4</td>
<td>0.15</td>
</tr>
<tr>
<td>Tenofovir isoproxil Monoester</td>
<td>0.24</td>
<td>1.5</td>
<td>1.0</td>
</tr>
<tr>
<td>Tenofovir disoproxil ethyl ester</td>
<td>0.80</td>
<td>0.72</td>
<td>0.15</td>
</tr>
<tr>
<td>Tenofovir isopropyl isoproxil</td>
<td>0.82</td>
<td>0.94</td>
<td>0.30</td>
</tr>
<tr>
<td>Tenofovir disoproxil</td>
<td>1.00</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tenofovir disoproxil carbamate</td>
<td>1.40</td>
<td>0.73</td>
<td>0.15</td>
</tr>
<tr>
<td>Tenofovir disoproxil dimer</td>
<td>1.76</td>
<td>1.0</td>
<td>0.15</td>
</tr>
<tr>
<td>Any individual unspecified impurity</td>
<td>-</td>
<td>1.0</td>
<td>0.10</td>
</tr>
<tr>
<td>Total impurities</td>
<td>-</td>
<td>-</td>
<td>2.0</td>
</tr>
</tbody>
</table>
### Organic Impurities: Procedure 2

- Gradient Method developed to quantitate five separate impurities

<table>
<thead>
<tr>
<th>Name</th>
<th>Relative Retention Time</th>
<th>Relative Response Factor</th>
<th>Acceptance Criteria NMT (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tenofovir</td>
<td>0.21</td>
<td>2.0</td>
<td>0.15</td>
</tr>
<tr>
<td>Tenofovir isoproxil Monoester</td>
<td>0.50</td>
<td>1.6</td>
<td>0.80</td>
</tr>
<tr>
<td>Tenofovir isopropyl isoproxil</td>
<td>0.79</td>
<td>1.0</td>
<td>0.15</td>
</tr>
<tr>
<td>Desmethyl tenofovir disoproxil</td>
<td>0.87</td>
<td>1.0</td>
<td>0.15</td>
</tr>
<tr>
<td>Tenofovir disoproxil</td>
<td>1.00</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Specified unidentified impurity</td>
<td>1.05</td>
<td>1.0</td>
<td>0.15</td>
</tr>
<tr>
<td>Any individual unspecified impurity</td>
<td>-</td>
<td>1.0</td>
<td>0.10</td>
</tr>
<tr>
<td>Total impurities</td>
<td>-</td>
<td></td>
<td>1.0</td>
</tr>
</tbody>
</table>
Another mandatory analytical test for an API is Organic Volatile Impurities (OVI).

OVI’s are tested by dissolving the sample up in a specified diluent and initiating GC analysis on the solution.

Typically, the OVI’s (e.g. Methanol, Ethanol, Acetone, ACN, IPA etc) tested for are from the solvents used in the API process.

Either a direct injection or headspace method can be used.

Limits are set based on ICH Q3.
Chloromethyl Isopropyl Carbonate (Cont.)

- USP developed a HS GC method for quantitation of CMIC (OVI)
  - Std Solution: 0.3 mg/mL of USP CMIC RS, 0.05 mg/mL of t-butyl alcohol RS and 0.05 mg/mL of DCM RS in NMP
  - Sample Solution: 200 mg/mL of TDF in NMP
  - Detector: Flame Ionization
  - Column: DB1 0.32mm x 30m
  - Temperature
    - Headspace Oven: 85° C
    - Loop: 110° C
    - Transfer Line: 120° C
    - Injector: 200° C
    - Detector 270° C
    - Ramp: Hold at 45° C for 5 mins then ramp at 10° C/min to 220° C. Hold for 10 min
  - Carrier Gas: Nitrogen at 0.5 mL/min
  - Split Ratio: 20:1
  - Vial Pressure: 14 psi
  - Vial Equilibration: 45 mins
  - Injection Size: 1 mL
  - Acceptance Criteria: NMT 0.15% CMIC
Enantiomeric Purity

- The majority of API’s produced have at least one chiral center.
- Common for a compound to have enantiomers and/or diastereomers.
- The efficacy and safety profile of different enantiomers and diastereomers is often very different to the parent compound.
- Therefore it is necessary to test the chiral purity of the API using specialized chiral columns.
- Traditional chiral tests are run using normal phase conditions but recent advances in column technology has seen the emergence of reverse phase chiral methods.
Enantiomeric Purity (Cont.)

- USP developed an isocratic HPLC method for the enantiomeric purity of TDF
  - Buffer: 0.1 M ammonium acetate in water, pH 6.8
  - Mobile Phase: 15:85 Methanol:Buffer
  - Standard Solution: 0.25 mg/mL of USP TDF RS in mobile phase
  - Sample Solution: 0.25 mg/mL of sample in mobile phase
  - Detector: UV at 260 nm
  - Column: Chromtech Chiral AGP 4.0x150 mm
  - Column Temp: 15° C
  - Flow Rate: 0.8 mL/min
  - Injection Volume: 10 µL
  - System Suitability: Resolution NLT than 1.5 between enantiomers
  - Acceptance Criteria: NMT 1.0% undesired enantiomer
TDF Related Compound B

• Tenofovir DF related compound B is a compound with some genotoxic concerns and has a very low acceptance limit (5 ppm).

• In order to analyze down to ppm range accurately, it is necessary to move beyond the realm of traditional detection (UV, PDA, FID) and use more ‘exotic’ detectors.

• For TDF related compound B, an LC-MS quantitation method was developed. The method uses Electrospray Ionization to generate the ions of interest.

• Other techniques for low level detection include GC-MS, GC-NPD, GC-FPD, GC-ECD LC-CAD, LC-ELS etc.

• These methods are often extremely challenging to develop.
TDF Related Compound B (Cont.)

- USP developed an LC-MS method for analysis of this impurity
  - **Buffer:** 0.1 M ammonium acetate in water,
  - **Mobile Phase:** 40:60 ACN:Buffer
  - **Standard Solution:** 0.01 µg/mL of USP TDF Related Compound B RS in methanol
  - **Sample Solution:** 2 mg/mL of sample in methanol
  - **Detector:** ESI MS (+ve ion mode)
  - **Column:** YMC-Pack ODS AQ 4.6x150 mm
  - **Column Temp:** 30° C
  - **Flow Rate:** 1 mL/min
  - **Injection Volume:** 10 µL
  - **System Suitability:** RSD NMT 10%
  - **Acceptance Criteria:** NMT 5 ppm of TDF Related Compound B
Fumaric Acid Analysis

- Titration is often a valuable tool in the pharmaceutical industry to perform basic analysis of acids and bases.
- For TDF, titration is used to analyze the fumaric acid content of the API.
- The majority of pharmaceutical companies use automatic titrators although burettes and pipettes are still in use.
Fumaric Acid Analysis (Cont.)

- USP developed an titration method for analysis of fumaric acid content of TDF
  - Sample Solution: 5 mg/mL of TDF in Water
  - Analysis: Titration 250 mL of sample solution with 0.1 N Sodium Hydroxide
  - Acceptance Criteria: 17.5% - 19.0% of fumaric acid on an anhydrous basis
Specific Tests

- Water Testing <921> NMT 1.0%

- To calculate water content the Karl Fisher method is used.

- The KF method is used to determine water content in API utilizing the quantitative reaction of water with iodine, sulfur dioxide an alcohol and a base.

- Three main types of KF
  - VFK
  - CFK
  - Oven CKF
Additional Requirements

- Packaging and Storage: Store in tight light resistance containers at 2 – 8°C
- Labeling: If a test for organic impurities other than Procedure 1 is used then the labeling states which test the article applies
Determination and Control of Impurities in Pharmaceuticals
ICH Guidelines: Q3 Impurities

• Scope
  – Impurities in Drug Substance (Q3A(R))
  – Impurities in Drug Product (Q3B(R))
  – Residual Solvents (Q3C)

• Identification of Impurities
• Qualification of Impurities
• Reporting of Impurities
• Specification of Impurities
ICH Q3A(R): Impurities in Drug Substance

- **Organic impurities**
  - Starting materials, by-products, intermediates, degradation products, reagents, ligands and catalysts

- **Inorganic impurities**
  - Reagents, ligands and catalysts, metals, inorganic salts, other materials (e.g. filter aids, charcoal)
  - Residual solvents (see Q3C)

- **Excluded**
  - Previously registered drugs
  - Clinical-trial materials
  - The following types of drug substance
    - Biological/biotechnological, peptide, oligonucleotide, radiopharmaceutical, fermentation product, semi-synthetic products derived there from, herbal products and crud products of animal or plant origin
  - Extraneous contaminants (controlled by GMPs)
  - Polymorphs
  - Enantiomers

- **Unusually Toxic Compounds**
  - *Lower threshold can be appropriate if the impurity is unusually toxic*
ICH Q3A(R): Threshold for Impurities in Drug Substances

• Identification Thresholds
  – The limit above which (the structure of) an impurity should be identified

• Qualification Thresholds
  – The limit above which an impurity would be considered qualified if adequately tested in safety studies and/or clinical studies

• Reporting Thresholds
  – The limit above which an impurity should be reported to regulatory authorities
### ICH Q3A(R): Threshold for Impurities in Drug Substances

<table>
<thead>
<tr>
<th>Maximum daily dose</th>
<th>Reporting Threshold</th>
<th>Identification Threshold</th>
<th>Qualification Threshold</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 2g/day</td>
<td>0.05%</td>
<td>0.10% or 1.0 mg per day intake (whichever is lower)</td>
<td>0.15% or 1.0 mg per day intake (whichever is lower)</td>
</tr>
<tr>
<td>&gt;2g/day</td>
<td>0.03%</td>
<td>0.05%</td>
<td>0.05%</td>
</tr>
</tbody>
</table>

1. The amount of drug substance administered per day
2. Higher reporting thresholds should be scientifically justified
3. **Lower threshold can be appropriate if the impurity is unusually toxic**
ICH Q3B(R): Impurities in Drug Product

- Degradation products
  - Drug substance
  - Reaction of drug substance and excipients and/or immediate container closure system

- Excluded
  - Clinical-trial materials
  - Previously registered drugs
  - Impurities in the drug substance
    - "Generally, impurities present in the new drug substance need not be monitored or specified in the new drug product unless they are also degradation products"
  - Impurities in the excipients
  - Leachables
  - The following types of drug substance
    - Biological/biotechnological, peptide, oligonucleotide, radiopharmaceutical, fermentation product, semi-synthetic products derived there from, herbal products and crude products of animal or plant origin
  - Extraneous contaminants (controlled by GMPs)
  - Polymorphs
  - Enantiomers

- Unusually Toxic Compounds
  - Lower threshold can be appropriate if the impurity is unusually toxic
**ICH Q3B(R): Threshold for Impurities in Drug Products**

<table>
<thead>
<tr>
<th>Maximum daily dose(^1)</th>
<th>Threshold(^2,3)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Reporting Thresholds</strong></td>
<td></td>
</tr>
<tr>
<td>≤ 1 g</td>
<td>0.1%</td>
</tr>
<tr>
<td>&gt;1 g</td>
<td>0.05%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Maximum daily dose(^1)</th>
<th>Threshold(^2,3)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Identification Thresholds</strong></td>
<td></td>
</tr>
<tr>
<td>&lt; 1 mg</td>
<td>1.0% or 5 µg TDI, whichever is lower</td>
</tr>
<tr>
<td>1 mg – 10 mg</td>
<td>0.5% or 20 µg TDI, whichever is lower</td>
</tr>
<tr>
<td>&gt;10 mg – 2 g</td>
<td>0.2% or 2 mg TDI, whichever is lower</td>
</tr>
<tr>
<td>&gt; 2 g</td>
<td>0.10%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Maximum daily dose(^1)</th>
<th>Threshold(^2,3)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Qualification Thresholds</strong></td>
<td></td>
</tr>
<tr>
<td>&lt; 10 mg</td>
<td>1.0% or 50 µg TDI, whichever is lower</td>
</tr>
<tr>
<td>10 mg – 100 mg</td>
<td>0.5% or 200 µg TDI, whichever is lower</td>
</tr>
<tr>
<td>&gt;100 mg – 2 g</td>
<td>0.2% or 3 mg TDI, whichever is lower</td>
</tr>
<tr>
<td>&gt; 2 g</td>
<td>0.10%</td>
</tr>
</tbody>
</table>

1. The amount of drug substance administered every day
2. Thresholds for degradation products are expressed either as a percentage of the drug substance or as a total daily intake (TDI) of the degradation product. **Lower thresholds can be appropriate if the degradation product is unusually toxic**
3. Higher thresholds should be scientifically justified
Purpose of Stability Studies

- To establish
  - Identity, potency, quality and purity (CFR 211.137) to assure the *safety* and efficacy product during its shelf life when stored under the intended, labeled conditions
  - How the product changes over time (potency, *impurities*, dissolution, microbiological) due to the influence of temperature, humidity, and light
  - Expiration dating for clinical supplies and commercial product
  - Retest dates for API
    - Expiry date may also be established after which API must be discarded
  - Marketed container closure system
  - Appropriate storage conditions
Metabolites and Impurities: Toxicity of Analgesics


Metabolites vs. Impurities

• “Impurities that are also significant metabolites present in animal and/or human studies are generally considered qualified” (Section 7. Qualification of Impurities, ICH Q3A R(2))

• Provided the impurity in the drug substance does not significantly increase the exposure levels of the metabolite. Considerations:
  – Major versus minor metabolite
  – Primary vs. secondary metabolite
  – Species differences (esp. human vs. animal)
  – Route of administration
  – Others
EMEA Guidance, January 2007

- Impurities classified as genotoxic where there are positive findings established in vitro and in vivo genotoxicity tests…that have a potential for direct DNA damage
- Impurities with sufficient evidence for threshold-related mechanism
  - Establish Permitted Daily Exposure (PDE) levels derived from NOEL using principles in Q3C
- Impurities without sufficient evidence for threshold-related mechanism
  - Control to level As Low As Reasonably Practical (ALARP)
  - Conduct Pharmaceutical Assessment
  - Conduct Toxicologic Assessment (AMES test). Results from test on API with low levels of impurity unacceptable
  - Establish Threshold of Toxicological Concern (TTC)
Examples of Genotoxic Moieties

Structural Alerts for Mutagenicity

Group 1: Aromatic Groups
- N-Hydroxaryls
- N-Acylated aminooxylyls
- Aza-aryl N-oxides
- Aminooxylyls and alkylated aminooxylyls

Purines or Pyrimidines, Intercalators, PNAs or PNAHs

Group 2: Alkyl and Aryl Groups
- Aldehydes
- N-Methylols
- N-Nitrosamines
- Nitro Compounds
- Carbamates (Urethanes)
- Epoxides
- Aziridines
- Propiolactones
- Propiosultones
- Aziridines
- Halogen
- Hydrazines and Azo Compounds

Group 3: Heteroatomic Groups
- Michael-reactive Acceptors
- Alkyl Esters of Phosphonates or Sulfonates
- Halo-alkenes
- Primary Halides (Alkyl and aryl-CH₃)

Legend: A = Alkyl, Aryl, or H
Halogen = F, Cl, Br, I
EWG = Electron withdrawing group (CN, C=O, ester, etc)
The Threshold of Toxicological Concern

- Applicable to compounds without sufficient evidence for a threshold-related mechanism
- Maximum Daily Intake = 1.5 μg/day
- Assumes life-time exposure of 70 years
- Produces additional cancer risk of 1 in 100,000
- Compares with actual life-time cancer risk of 1 in 4
- Applies to Marketing Applications
- Does not address investigational drugs or short-term exposure
Industry Position: Staged TTC Approach

Rationale for Determining, Testing and Controlling Specific Impurities in Pharmaceuticals that possess Potential for Genotoxicity

Prepared by the PhRMA Task Force on Genotoxic Impurities M. Andino, Pfizer; D. De Antonis, Pfizer; C. Beels, GlaxoSmithKline; J. DeGeorge, Merck; F. De Knaep, Johnson&Johnson; D. Ellison, Merck; J. Fagerland, Abbott; R. Frank, Noramco; B. Fritschel, Johnson&Johnson; E. Harpur, Sanofi-Aventis; C. Humfrey, Astra Zeneca; G. Mohan, Sanofi Aventis; A. Jacks, Noramco; N. Jagota, Wyeth; J. Mackinnon, GlaxoSmithKline; R. Mauthe, Pfizer; L. Mueller, Hoffman-La Roche; D. Ness, Eli Lilly; M. O’Donovan, Astra Zeneca; C. Riley, ALZA; M. Smith, GlaxoSmithKline; G. Vudathala, Sanofi-Aventis; L. Yotti, Bristol-Myers Squibb

What is a Staged TTC?

ICH Thresholds

- Qualification
- Identification
- Reporting

Maximum Daily Dose (mg)

Staged Threshold of Toxicological Concern

- ADI=120 ug
- ADI=40 ug
- ADI=20 ug
- ADI=1.5 ug

Drug Substance
PhRMA Decision Tree

Categorization, Qualification and Risk Assessment of Impurities

Class 1: Genotoxic Carcinogens
- Eliminate Impurity?
  - No
  - Risk Assessment
    - No or unknown
      - (Staged) TTC
        (see Table 1)

Class 2: Genotoxic, Care unknown
- Threshold Mechanism?
  - Yes
    - Impurity Genotoxic?¹
      - Yes
        - API Genotoxic²
          - No
            - Control as an ordinary impurity
      - No
        - PDE (e.g. ICH Q3 appendix 2 reference)
          - Not tested
            - Class 3: Alert – Unrelated to parent
          - Yes
            - Class 4: Alert – Related to parent

Class 3: Alert – Unrelated to parent
- Class 5: No Alerts

¹Either tested neat or spiked into API and tested up to 250 μg/plate
²If API is positive, risk benefit analysis required
³Quantitative risk assessment to determine ADI
# Allowable Daily Intake for Clinical Trials

Table 1: Allowable daily intake (μg/day) for genotoxic impurities during clinical development, a staged TTC approach

<table>
<thead>
<tr>
<th>Duration of Exposure</th>
<th>≤1 mo.</th>
<th>&gt;1-3 mo.</th>
<th>&gt;3-6 mo.</th>
<th>&gt;6-12 mo.</th>
<th>&gt; 12 mo.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allowable Daily Intake (μg/day) for all Phases of development</td>
<td>120&lt;sup&gt;a&lt;/sup&gt;</td>
<td>40&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>or</td>
<td>or</td>
<td>or</td>
<td>or</td>
<td>or</td>
<td></td>
</tr>
<tr>
<td>Alternative maximum level of allowable impurity based on percentage of impurity in API</td>
<td>0.5%</td>
<td>0.5%</td>
<td>0.5%</td>
<td>0.5%</td>
<td></td>
</tr>
</tbody>
</table>

a. Probability of not exceeding a $10^{-6}$ risk is 93%.
b. Probability of not exceeding a $10^{-5}$ risk is 93%, which considers a 70-year exposure.

**General Notes:**
1. Impurities are controlled to the lower value in the table (i.e., either the ADI or the percentage of impurity in API).
2. ADIs are based on linear extrapolation (Bos et al., 2004) of data from Munro et al. (1999) with the assumption that 50% of genotoxic compounds are carcinogenic.
3. Known carcinogens should have compound-specific risk calculated (see text and Figure 1). Consideration should be given to lowering the exposures for potentially sensitive populations (e.g., pediatric) by a factor of ten.
Case Study: The Viracept Issue

ROCHE 'ABANDONS' POOR AIDS PATIENTS
Loaded boxes are seen in the stockroom of Swiss pharmaceutical company Roche in Kaiseraugst, Switzerland, in this Sept. 22, 2006 file picture. Roche Holding AG, with revenues of $35 billion last year, increased its first half year profit 24 percent, the company reported on Thursday July 19, 2007. (AP Photo/Keystone, Martin Ruetschi)
**Counter Ions: Potential Generation of Alkylation Agents**

- **Alkyl- and Aryl Sulfonates and Halides**
  - Alkyl- and arylsulfonates (mesylate, esylate, besylate, tosylate, noyslates) and halides are common pharmaceutical salts, produced by the reaction of the free base of the drug with the corresponding acid*.
  - One potential side reaction is the reaction of (residual) free sulfonic acid or halide and lower alcohols (such as methanol, ethanol and iso-propanol) to produce the corresponding alkyl sulfonate ester, or alkyl halides.
  - Alkyl sulfonate esters and alkyl halides are powerful alkylating agents and known mutagens and carcinogens*.
  - Thus the use of alcohols should be used advisedly during the processing of sulfonate, and halide salts. Alternatively, the resultant sulfonate esters or alkyl halides should be controlled to levels below the TTC.

Contamination of Nelfinavir (Viracept) by Ethylmethyl Sulfonate (EMS)

- The reaction of methane sulfonic acid with residual ethanol that was not completely removed after equipment cleaning
- Resulted in very high levels (~1000 ppm) of ethyl methanesulfonate ester in Viracept (nelfinavir mesylate), leading,
- To product recall (July, 2007).
Source of Ethyl Methane Sulfonate in Methane Sulfonic Acid
(Source: Roche: Viracept Recall – An Update, July 23, 2007)
Concentration of Ethyl Methane Sulfonate in Methane Sulfonic Acid Holding Tank (Source: Roche: Viracept Recall – An Update, July 23, 2007)

EMS content Oct 2006 - 2007
(root cause investigation)

API Batch Numbers
- Blue dot indicates first production after MSA tank cleaning
- Red dot indicates first production after tank sat idle for 77 days
- Green dot indicates topping off of the MSA tank
Viracept – Follow Up Actions

• Roche conducted in-depth studies to show threshold-level of toxicity for EMS of 25 mg/kg/day in mouse, corresponding to
  – AUC = 350 µM.h
  – C_{\text{max}} = 315 µM
• Translated to 2 mg/kg/day for EMS in humans
• Highest exposure predicted in humans 0.055 mg/kg/day, which translates to:
  – AUC = 13 µM.h
  – C_{\text{max}} = 0.85 µM
• Acceptance criteria for EMS in DP agreed with EMEA and FDA

Metal Analysis in the Pharmaceutical Industry
Heavy Metals

• Heavy metals
  – Several definitions proposed, based on:
    • density,
    • atomic number,
    • chemical properties,
    • toxicity
  – Include transition metals, some metalloids, lanthanides, and actinides

• Medical usage:
  – Include all toxic metals irrespective of their atomic weight as well as semimetals such as arsenic.
Limits for Residues of Metal Catalysts in Drugs

- Only draft guideline

- 5 Classes
  - Highly toxic/known toxic metals (Class 1A, 1B)
    e.g. Pt, Pd (1A), Ir, Rh, Ru, Os (1B)
  - Significantly toxic (Class 1C)
    e.g. Mo, Ni, Cr, V
  - Metals with low safety concern (Class 2)
    e.g. Cu, Mn
  - Metals with minimal safety concern (Class 3)
    e.g. Fe, Zn

# Limits for residues of metal catalysts in drugs

<table>
<thead>
<tr>
<th>Classification</th>
<th>Oral Exposure</th>
<th>Parenteral Exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Potential Daily Exposure (µg/day)</td>
<td>Conc. ppm</td>
</tr>
<tr>
<td><strong>Potential Daily Exposure</strong></td>
<td><strong>Conc. ppm</strong></td>
<td><strong>Potential Daily Exposure</strong></td>
</tr>
<tr>
<td><strong>Metal of significant safety concern:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Class – 1A</td>
<td>100</td>
<td>10</td>
</tr>
<tr>
<td>(Pt, Pd)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Class – 1B</td>
<td>100**</td>
<td>10**</td>
</tr>
<tr>
<td>(Ir, Rh, Ru, Os)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Class – 1C</td>
<td>300</td>
<td>30</td>
</tr>
<tr>
<td>(Mo, Ni, Ch, V)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Metals with low safety concern:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Class – 2</td>
<td>2500</td>
<td>250</td>
</tr>
<tr>
<td>(Cu, Mn)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Metals with minimal safety concern:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Class – 3</td>
<td>13000</td>
<td>1300</td>
</tr>
<tr>
<td>(Fe, Zn)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Specific limits set for inhalation exposure to Platinum; Chomium VI, Nickel
** Sub class limit: total amount of listed metals should not exceed indicated limit

Sources of Heavy Metals in Drugs

• API / Excipients
  – Raw Materials
  – Starting materials
  – Catalysts / metal reagents
  – Process Equipment
• Drug product
  – Ingredients
  – Process Equipment
  – Container
• Sabotage/lack of ethics
Detection Techniques

Heavy Metal <231>
Residue on Ignition <281>
Loss on ignition <733>
Plasma Spectrochemistry <730>
Color test – USP <231>

• Principle
  – Metallic impurities reacted with sulfide ions
  – Precipitation at pH 3.5 of dark-colored sulfides
  – Comparison with a standard

• Limitations:
  – Detects Pb, Hg, Bi, As, Sb, Sn, Cd, Ag, Cu, and Mo
  – Qualitative test (limit test)
  – Sample size: 2.0/(1000L), with L =limit in %
  – False positive or negative possible

• 3 methods
  – aqueous solution
  – ashing
  – wet digestion


USP listed metals, at 20ppm in method 1
**Alternative methods**

- Atomic absorption spectroscopy (AAS)
- Inductively coupled plasma optical emission spectrometry (ICP-OES)
- Inductively coupled plasma mass spectrometry (ICP-MS)
  - Most sensitive (0.01 – 1 ppb in solution)
  - Fast
  - Multi element
  - Multi isotope identification
Heavy Metals (USP)

• Heavy Metals / Toxic metals
  – Aluminium (Al)
  – Beryllium (Be)
  – Cadmium (Cd)
  – Chromium (Cr)
  – Copper (Cu)
  – Indium (In)
  – Iron (Fe)
  – Lead (Pb)
  – Lithium (Li)
  – Magnesium (Mg)
  – Manganese (Mn)
  – Mercury (Hg)
  – Molybdenum (Mo)
  – Nickel (Ni)
  – Osmium (Os)
  – Palladium (Pd)
  – Platinum (Pt)
  – Rhodium (Rh)
  – Rubidium (Rb)
  – Strontium (Sr)
  – Thallium (Tl)
  – Tin (Sn)
  – Tungsten (W)
  – Zinc (Zn)
  – Antimony (Sb)
  – Arsenic (As)
  – Boron (B)
  – Selenium (Se)

Metalloids / non metals

• Other Inorganic Impurities
  – Barium (Ba)
  – Bismuth (Bi)
  – Cobalt (Co)
  – Gold (Au)
  – Ruthenium (Ru)
  – Silver (Ag)
  – Thorium (Th)
  – Vanadium (V)
  – Uranium (U)
  – Gadolinium
  – Bromine (Br)
  – Germanium (Ge)
  – Iodine (I)
  – Sulfur (S)
  – Tellurium (Te)
  – Radionuclides

Needed by humans

ICP-MS
Inductively Coupled Plasma-Mass Spectrometry

- Plasma Operates at temperatures above 7000°K (Suns surface temp around 5800°K)
- Obliterates everything fed it into free atomic charged ions
- No need to worry about maintaining molecular structure for proper identification
What Can We Measure

Limits of detection in parts per billion (ng/ml)

Values reflect actual experimental data gathered over more than 1 year and are calculated from 3 sigma of an aqueous blank.
New vs Old
ICP-MS Heavy Metals

Fig. 2. Comparison of average (%) recoveries of elements: USP Heavy Metals test vs. ICP-MS Heavy Metals test.

ICP-MS Applications

- **Product Testing**
  - Heavy Metals
  - Catalyst removal
    - Ru Residue content
- **Manufacturing / Investigations**
  - Cleaning Validations i.e. can be based on the elimination of metallic catalyst from reactor rinse
  - ROI residue suspects
  - Visual Inspection failures i.e. leached out metal contaminates from reactor batches or containers
- **R&D Support**
  - Salts residue / removal verification
  - Catalyst Clean Up
Wrap Up
Other Topics

• Microbial for API
• Different types of water
• Instrument qualification
• Method qualification and validation
• Laboratory investigation
Q&A

• Thank You

• Questions?