The fairy-tale of a multi-analyte LC/MS/MS-assay for quantification of low pg/mL levels of active drug compounds and metabolites of a topical fixed dose combination product.
Agenda

• **Introduction:**
  – The product
  – FDA request

  – Serum analysis - PPT + Automated SPE

• **Assay generation 1 – CRO1: (2010 – today)**
  – Assay transfer #1 and 1.1
  – Plasma analysis - LLE

• **Assay generation 2 – CRO2 (2013 – today)**
  – Assay transfer #2
  – Rare matrices
**Introduction**

The product

- **Daivobet® Gel** – topical fixed dose combination product

- **VitD3 analogue** calcipotriol and corticosteroid **betamethasone Dipropionate** (BDP)

- First launch of Daivobet® Gel: treatment of **scalp** psoriasis.

- Same concentrations of active ingredients as Daivobet® Ointment for treatment of **body** psoriasis (already marketed product).

- Human PK-data was *indicated* at pre-IND (June 2004) *not* to be needed!
Introduction

FDA request at EoPh2 Dec 2004

“investigate *systemic concentrations* at Maximum Use conditions using the *most sensitive technique*”

Even though…:

• Animal data indicated *lower or equal systemic absorption* with the new gel formulation compared to the ointment.

• Absorption study with radiolabeled drug product had been conducted with original (ointment) formulation showing very low absorption (0-4% of dose) in psoriasis patients and healthy subjects.

• However, FDA noted at EoPh2 that very *sensitive methods* had been developed for *preclinical assays* for the parent compounds (LLoQ 20 – 40 pg/mL).
Assay generation 0
In-house regulated BioA group at LEO

Calcipotriol

MC1080 (main metabolite)

Betamethasone Dipropionate (and betamethasone 17-propionate = main metabolite)

Extraction and LC/MS/MS:

- PPT followed by Automated SPE (with both normal and reversed phase washing steps)

- Assays for TK: LC/MS/MS (SCIEX API3000 or API4000) – measurement as NH$_4$-adducts (great enhancement of ES$^+$ response)

Complicated!!
Assay generation 0

Major challenges

• Calcipotriol and MC1080 difficult to separate by LC (co-eluting) and by MS (isobaric)

• Massive and variable ion suppression – sometimes almost complete loss of sensitivity for calcipotriol and MC1080

• Noisy baseline including many peaks from endogenous compounds resembling the APIs

• Only stable-isotope labelled ISs for parent compounds available

• Timelines – risk of delay of clinical development and NDA-submission
Hypothesis: variable ion suppression caused by formation of “unbreakable” Na-adducts

- Possible solution: add Li-salt in reconstitution solution to compete with the Na-adduct formation

- Idea: why not quantify via Li-adducts instead of NH₄-adducts?

- Li-acetate added to both re-con solution and mobile phase

Bingo!!

- Baseline noise markedly reduced but analyte peak response retained

- Calcipotriol ion transition not present in MC1080 ion track → partly “separation” by choice of MS-transitions
Assay generation 0
Be careful: High risk of precipitation of Li-salts

- **Never** leave any tubing without flow when filled with Li-containing solvent – **blocking** of tubes, spray needle and autoinjector needle **will happen**!

- Frequent MS-interface cleaning and even venting of MS may be needed

**Measures that help:**

- **Orthogonal spray** as introduced with API4000 and newer models limits contamination of the MS-orifice and ion-path

- Column switching + use of external LC-pump providing Li-free solvents continuously limiting the amount of Li-solvents into MS-interface

- **Wash gradient** providing Li-free solvents for tubes at the end of **each** run

- **High curtain gas** setting (50 instrument units) – less contamination of MS while peak response is retained
Assay generation 0
Method validated and Clinical PK data obtained

- **Study design:** Daivobet® Gel used on the scalp and concomitant use of Daivobet® Ointment on the body

- In general, **very low plasma levels**

- **Only the metabolites** could be measured

- **NDA approval** of Daivobet® Gel obtained in **May 2008**

Need for further human PK – June 2010:

- Indication to be extended (in US) for use of Daivobet® Gel for psoriasis on the body

- New Max-Use Human PK-study needed

- Problem: In-house bioA-department at LEO had been closed down and all regulated BioA outsourced in 2008
Assay generation 1
Assay transfer # 1.0

- CRO (in EU) chosen and method transfer initiated June 2010

- Method set-up unsuccessful, but why?

- First of all: **Complicated assay**

**Modifications suggested by CRO:**

- Change from serum to plasma

- Use of LLE instead of SPE

- Different columns tested (Acquity HSS T3 column, Fortis C18, 1.7 um and more)
Method validation initiated (Sept. 2010), but…:

- Deterioration of column and loss of sensitivity → post-column addition of Li-acetate attempted, but resulted in variable RT

- Unclear extracts → spinning vials 5 min. at high speed (5000 g)

- Many instrument break downs

And in the middle of it all (Dec.: 2010):

- “We suggest to move activities to our US-facilities!”

- Gas supply insufficient at EU-site

- More API4000 instruments available at US-site
Assay generation 1

Happy end!

• Suggestion to move assay to US-site accepted and by April 2011 a successful validation had been completed.

• Later on, partial validations were conducted to introduce stable isotope labeled analogues as ISs for all analyte

This method has now to supported:

• Max-Use PK studies with Daivobet® Gel and Enstilar® (Spray formulation) in adults completed

• 2 similar ongoing studies in adolescents (phase IV commitment)

• PK Study with Daivobet® Ointment in Japanese subjects completed
Assay generation 1

LLoQ in human plasma

Betamethasone dipropionate (30 pg/mL)

Betamethasone 17-propionate (30 pg/mL)

Betamethasone-d10 dipropionate (IS)

Betamethasone 17-propionate-d5 (IS)
Assay generation 1
LLoQ in human plasma

Calcipotriol (50 pg/mL)
MC1080 (20 pg/mL)
Calcipotriol-d4 (IS)
MC1080-d4 (IS)
Analysis of rare matrices
Assay transfer #2

To support:

• **Dermatopharmacokinetic** clinical study in Japan:
  – Compare Daivobet® Ointment and Gel to support a jNDA for the Gel
  – Measurement of BDP and calcipotriol in **stratum corneum**
  – Stratum corneum sampled by **tape-stripping**

• New CRO chosen

• Method based on the same principles as the plasma method

• Very successful method transfer!
Analysis in rare matrices
Assay transfer #2

Many modified versions of the method:

• Animal PK/PD (Skin biopsies)

• Matrix from Ex vivo skin penetration studies:
  – Human and minipig Skin biopsies
  – Different types of receptor fluid
  – Analysis of both parent compounds and metabolites

Analysis of metabolites in metabolizing skin layers

Metabolite measurement as indicator for availability of BDP and calcipotriol in skin:

• Avoiding impact of contamination of samples by unabsorbed drug product from the skin surface
Metabolite concentrations in skin
Expressed as % of applied dose (ex vivo) of parent compounds

![Graph showing metabolite concentrations in skin expressed as % of applied dose (ex vivo) of parent compounds. The graph compares formulations A, B, and C with data points for two compounds, MC1080 and B-17-P.]
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