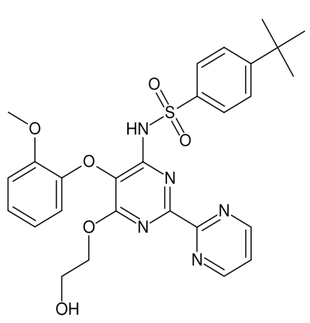
Fully Automated LC-MS/MS-based Determination of Bosentan and its Metabolites in Dried Blood Spots using the SCAP™ DBS System

Mirko Glinski[1], Norbert Ganz[1], Jasper Dingemanse[2], Laurent Nicolas[2], Werner Döbelin[3]

[1]Inovalab AG, CH-4153 Reinach, Switzerland
[2]Actelion Pharmaceuticals Ltd., CH-4123 Allschwil, Switzerland
[3]Prolab GmbH, CH-4153 Reinach, Switzerland

Contact: mirko.glinski@inovalab.ch
Contact: jasper.dingemanse@actelion.com
Contact: info@prolab.ch

Introduction



Bosentan (Ro 47-0203) is a dual endothelin receptor antagonist used for treatment of pulmonary arterial hypertension.

There are three major metabolites of Bosentan:
Hydroxy Bosentan (Ro 48-5033), the major metabolite in plasma, is the result of hydroxylation at the *t*-butyl group.

Desmethyl Bosentan (Ro 47-8634) is the product of *O*-demethylation of the phenolic methyl ether.

Hydroxy Desmethyl Bosentan (Ro 64-1056) is formed by both demethylation and hydroxylation.

Dried Blood Spot (DBS) analysis is a very promising alternative to conventional whole blood and plasma analysis used in drug discovery and development or later in therapeutic drug monitoring.

Advantages of DBS are
- Only low amount (25 µL) of test material needed
- Minimal sample processing needed
- Sample storage and shipment at ambient temperature possible

Automation of the DBS workflow (→DBS extraction) is needed to enable a throughput that can compete with conventional plasma analysis.

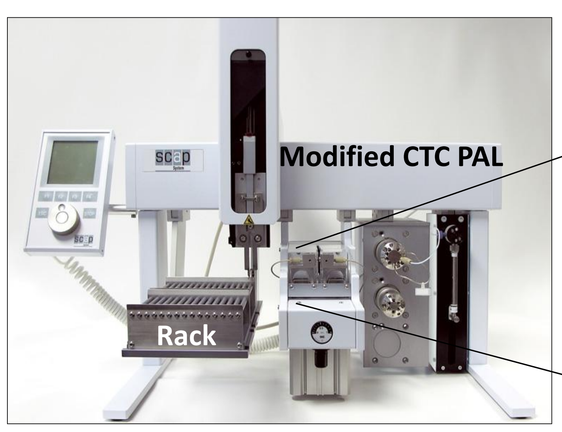
Summary

SCAP™ DBS System
- SCAP™ DBS System [2] allows the fully-automated DBS analysis via LC-MS/MS without need of time-consuming spot punching and manual extraction
- This makes high-throughput DBS analysis available urgently needed for the investigation of, e.g., large sets of clinical patient samples
- Simplified method development by online DBS extraction.
- No limitation regarding DBS card material (→Whatman DMPK-A, -B, -C, Ahlstrom 226)
- Variable adapters (2-5 mm) available for clamp module →variable extraction areas

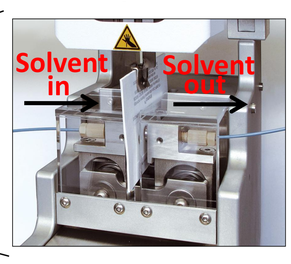
Analytical Method
- LLOQ for Bosentan, Hydroxy Bosentan and Hydroxy Desmethyl Bosentan is 2.0 ng/mL and 5.0 ng/mL for Desmethyl Bosentan
- ULOQ is 1500 ng/mL for all compounds
- r^2 values for all analytes are >0.99 showing good linearity of the method
- Intra-assay precision is ≤15.1% (LLOQ), intra-assay accuracy is between 91.6% and 119.7%
- Total run time is 8 min
- Carry-over is ≤0.033%
- Between 20 and 50 µl spotted blood no significant influence on metabolites' responses, for Bosentan the range is between 20 to 30 µL.

The SCAP™ (Sample Card And Prep) DBS Technology*

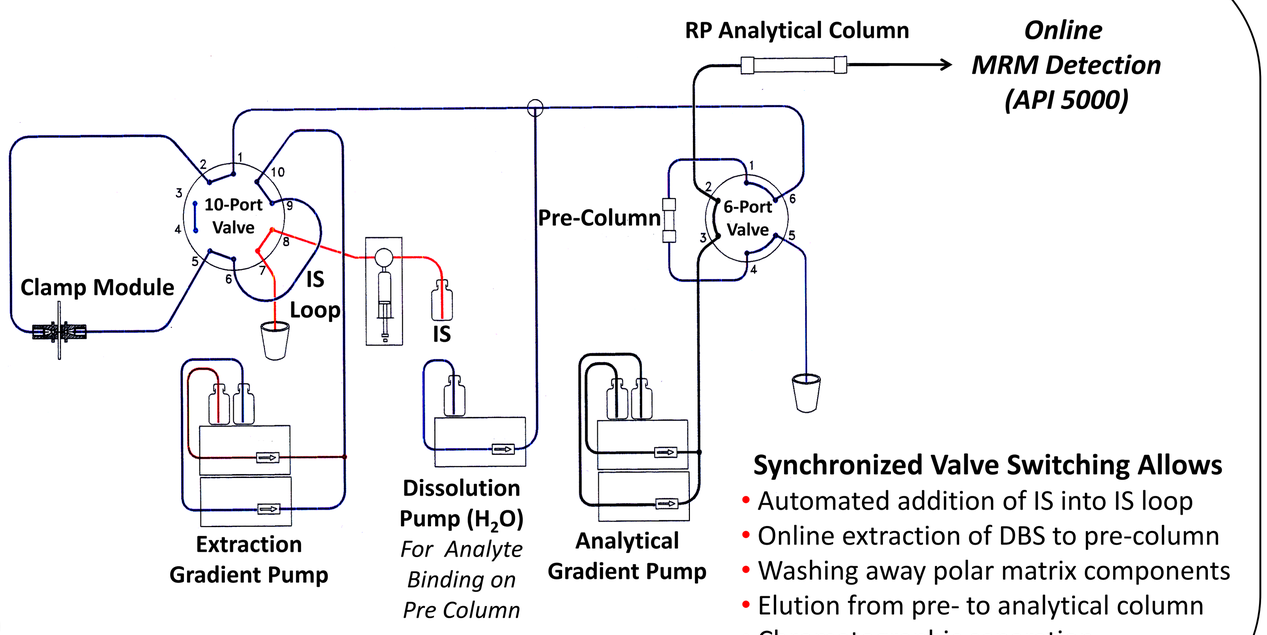
SCAP™ DBS System



Clamp Module



Cycle Composer Software from CTC Analytics for overall control
- Application of 25 µl blood on DBS card, 2 h drying at RT
- DBS cards are sequentially picked up from the autosampler rack by robotic gripper
- Transfer of DBS card to clamp module, integration into the HPLC flow path (limit: 200 bar)



Synchronized Valve Switching Allows
- Automated addition of IS into IS loop
- Online extraction of DBS to pre-column
- Washing away polar matrix components
- Elution from pre- to analytical column
- Chromatographic separation
- MS/MS detection

* based on the SCAP™ PLS (Pipette Liquid Sampling) System for fully automated online analysis of biofluids [1]

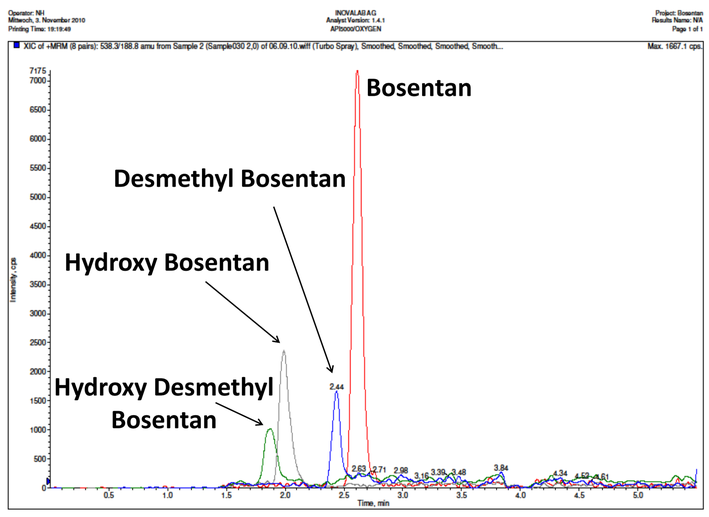
Results

Precision and Accuracy of Analytical Method

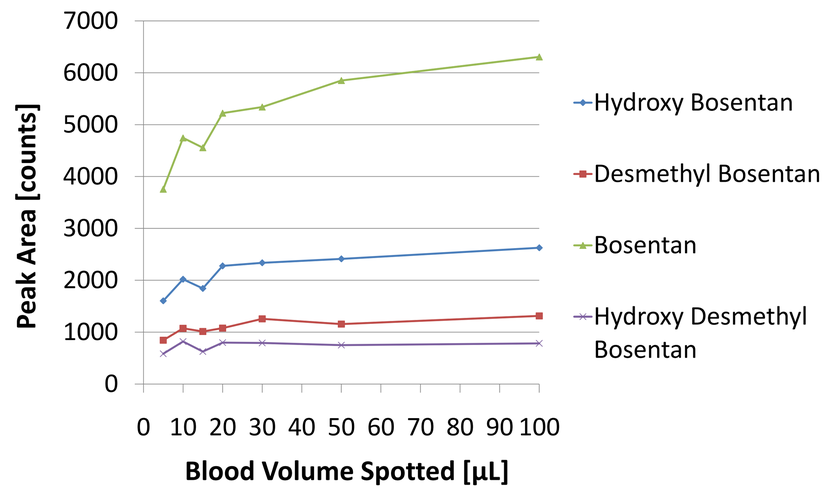
Intra Assay Precision [%]			
ng/mL	Bosentan	Hydroxy Bosentan	Hydroxy Desmethyl Bosentan
2 (LLOQ)	2.6	15.1	8.9
10	5.1	4.5	7.4
96	12.2	4.7	12.0
1500	7.0	7.3	5.6

Intra Assay Accuracy [%]			
ng/mL	Bosentan	Hydroxy Bosentan	Hydroxy Desmethyl Bosentan
2 (LLOQ)	119.7	107.1	112.4
10	98.0	91.6	104.8
96	100.4	97.6	109.2
1500	100.2	100.6	106.1

Sensitivity of Analytical Method, all Analytes at LLOQ



Dependency of Analytes'* Responses on Spotted Blood Volume



** The concentration of analytes was 20 ng/mL each.*

For the experiments FTA DMPK-A Cards (Whatman) were used. These chemically-treated cards lyse cells and denature proteins on contact.

References:

1. König S, Yildiz O, Hermann N, Steurer A, Singrasa M, Döbelin W. A Novel Concept for Sample Collection and Sample Preparation. Poster WP 427, ASMS, Salt Lake City (2010).
2. Heinig K, Wirz T, Bucheli F, Döbelin W. Determination of Tamiflu® and active metabolite in dried blood spots using the SCAP™ DBS system and column-switching LC-MS/MS. Poster No. 155, LC-MS Symposium, Montreux (2010).