Fully Automated Direct Application of Dried Blood Spots Combined with HPLC Coupled to Tandem Mass Spectrometry for Simultaneous Quantification of Bosentan and its Metabolites

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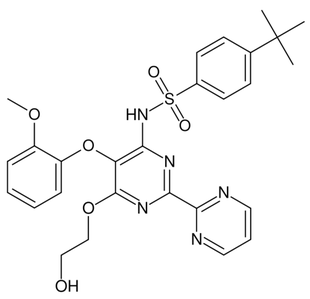
Introduction



Bosentan is a dual endothelin receptor antagonist used for treatment of pulmonary hypertension.

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

Desmethyl Bosentan is the product of *O*-demethylation of the phenolic methyl ester.

Hydroxy Desmethyl Bosentan 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 of test material needed
- No sample processing needed
- Sample storage and shipment at ambient temperature possible

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

Summary

SCAP™ DBS System

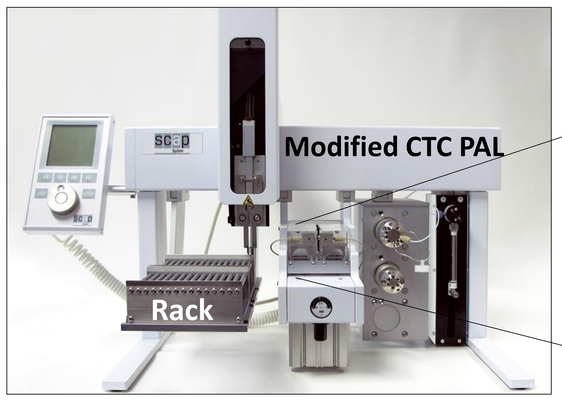
- SCAP™ DBS System [2] allows the fully-automated DBS analysis via LC-MS/MS without need of time-consuming spot punching and manual extraction.
- This makes high-throughput DBS analysis available urgently needed for the investigation of e.g. large sets of clinical patient samples.
- Simplified method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

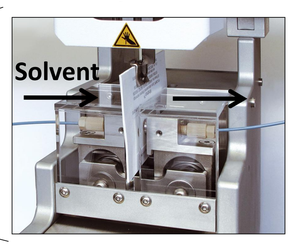
Analytical Method

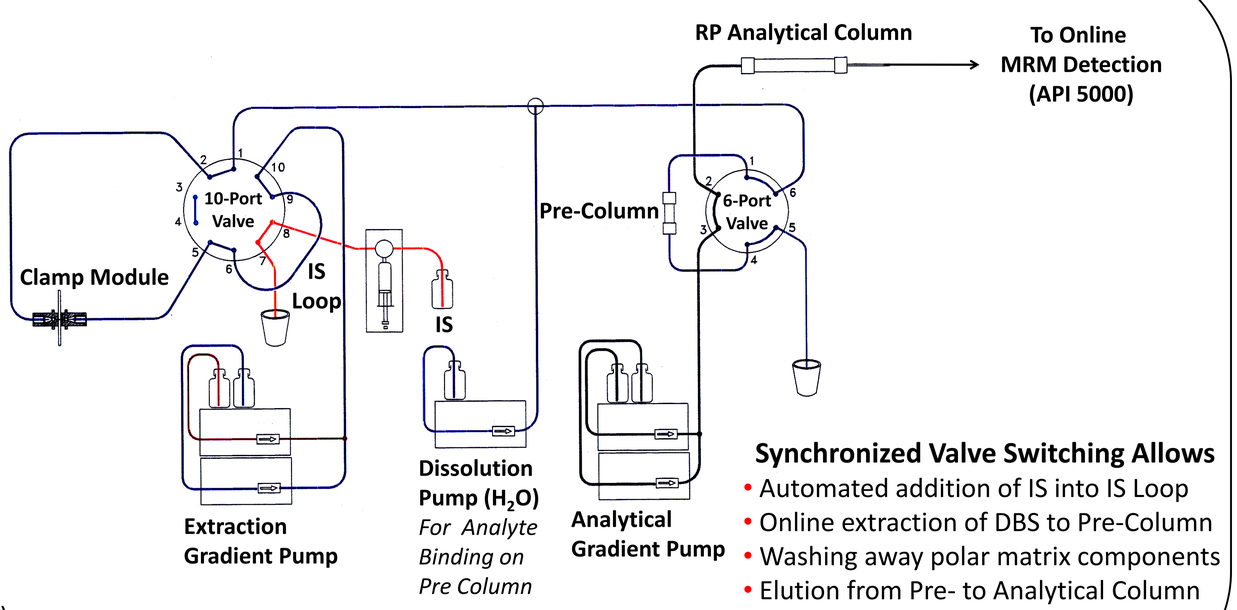
- LLOQ for Bosentan, Hydroxy Bosentan and Hydroxy Demethyl Bosentan is 2 ng/mL and 5 ng/mL for Desmethyl Bosentan.
- ULOQ is 1500 ng/mL for all compounds.
- r^2 values for all analytes are >0.99 showing good linearity of the method.
- Intra-assay precision is ≤15.1%, intra-assay accuracy is between 91.6% and 119.8%.
- Total run time is 8 min.
- Carry-over is ≤0.033%.
- Between 20 and 50 µl spotted blood no significant influence on analytes' responses.

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

SCAP™ DBS System







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 the 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]

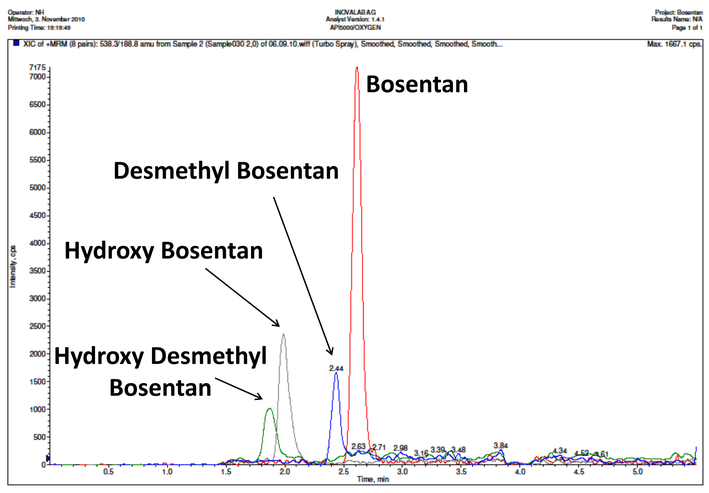
Results

Precision and Accuracy of Analytical Method

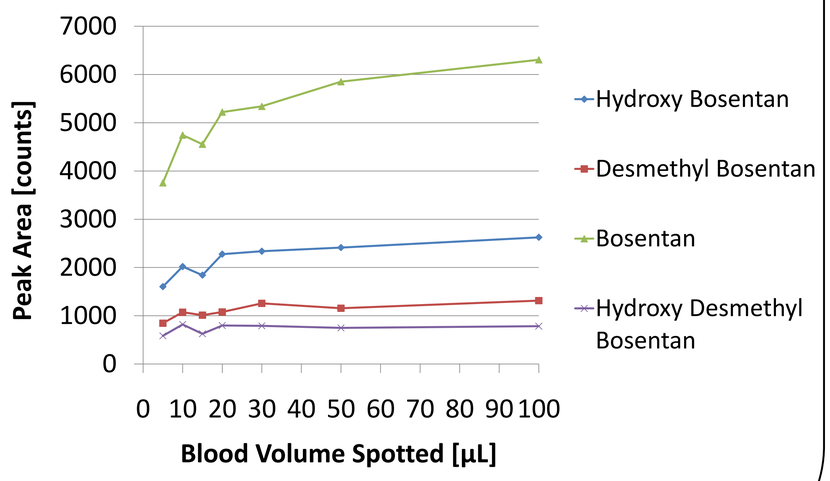
Intra Assay Precision [%]			
ng/mL	Bosentan	Hydroxy Bosentan	Hydroxy Desmethyl Bosentan
2 (LLOQ)	2.6	15.1	8.9
10	5.1	4.5	7.4

Intra Assay Accuracy [%]			
ng/mL	Bosentan	Hydroxy Bosentan	Hydroxy Desmethyl Bosentan
2 (LLOQ)	119.8	107.1	112.4
10	98.0	91.6	104.8

Sensitivity of Analytical Method, all Analytes at LLOQ



Dependency of Analytes' Responses on Spotted Blood Volume



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).

Acknowledgements: We gratefully acknowledge Jasper Dingemanse and Laurent Nicolas from Actelion Pharmaceuticals Ltd for their donation of the test items.