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Dried blood spot techniques for the quantification of endothelin receptor antagonists and phosphodiesterase-5 inhibitors: Development, validation, and clinical application in patients

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Recent developments in the treatment of pulmonary arterial hypertension (PAH) have significantly improved the prognosis of this serious disease changing it from an acute to a chronic clinical condition. Still many patients have a high symptom burden due to the complex therapy, its side effects, and the disease itself. Therapeutic drug monitoring of the targeted PAH medication might improve the known dose-dependent beneficial outcomes by correlating the measured drug concentrations with clinical symptoms, adverse events, or using such determinations to detect nonadherence. The endothelin receptor antagonistst (ERAs) ambrisentan and bosentan and the phosphodiesterase 5 inhibitors (PDE-5Is) sildenafil and tadalafil are part of the oral targeted therapy for PAH and the most frequently prescribed medication in this disease.

The objectives of the present work were (1) to develop dried blood spots (DBS) quantification methods for ambrisentan, bosentan, sildenafil, and tadalafil and to validate the methods according to the pertinent guidelines of the US Food and Drug Administration (FDA) and European Medicines Agency (EMA), including DBS specific challenges; (2) to examine the plasma : DBS relationship ex vivo; (3) to perform a bridging study for clinical validation; (4) to investigate saliva as sampling matrix for therapeutic drug monitoring purposes; (5) to establish a DBS sampling kit; and (6) to acquire a national shipping certificate; and ultimately (7) to implement the DBS technique in the existing plasma drug monitoring.

Based on the pre-existing validated plasma quantification methods for ambrisentan, bosentan, and sildenafil the DBS methods from extraction to quantification were established. The methods were validated according to the respective guidelines of the FDA and EMA. Additionally, several DBS-specific parameters were included in the validation process such as the haematocrit effect, punch device carry-over, and the experiments concerning stability, matrix effect, and extraction recovery were extended. A clinical bridging study was conducted to generate simultaneously collected whole blood samples, DBS samples, and saliva samples. Correlation of the plasma and DBS samples was performed to establish correlation factors for the conversion of measured DBS samples into plasma concentrations. The clinical validation was further investigated by comparing the measured plasma concentrations versus the

corresponding calculated plasma concentrations. The relationship of the DBS and corresponding plasma concentrations was also investigated ex vivo, which confirmed the correlation factors. The saliva samples were analysed within the plasma quantification methods and also correlated to the respective plasma concentrations to examine the correlation and therefore, the matrix as a possible, non-invasive sample matrix for therapeutic drug monitoring. A DBS sampling kit was developed and applied in the clinical study thus confirming that it enables the patients to self-sample DBS at home. The kit includes an instruction sheet, an analysis request form, a data sheet, the sampling requirements (lancet, alcohol wipes, DBS cards), and the storage and shipping requirements (desiccant, Ziploc® bag, security envelope, self-addressed envelope). The acquired Certificate for the National "Kompaktbrief" simplified and cheapened the national shipping of the sampled DBS cards because the 3-fold packaging could be omitted and the size of the envelope could be reduced. Finally, the DBS methodology was implemented into clinical routine of a therapeutic drug monitoring previously exclusively based on plasma concentrations.

Within the present work, DBS methods for ambrisentan, bosentan, sildenafil, and tadalafil were developed from sampling to clinical implementation and validated according to the pertinent guidelines and expert recommendations.

The DBS methodology is still under development and the establishment of international guide-lines on sampling procedure, quantification analysis, and clinical validation should be pursued to further improve the applicability of this promising sampling technique.