Development and clinical validation of an LC-UV method to quantify dolutegravir in dried blood spots

Abdulafeez Akinloye[1], Babatunde Adeagbo[1], Oluseye Bolaji[1] and Adeniyi Olagunju[1,2]

[1]Department of Pharmaceutical Chemistry, Obafemi Awolowo University, Ile-Ife, Nigeria; [2]Department of Molecular and Clinical Pharmacology, University of Liverpool, Liverpool, UK

Correspondence to: aeolagunju@oauife.edu.ng

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INTRODUCTION

- Dolutegravir (DTG) is now the preferred component of first-line antiretroviral therapy (ART) in all population groups[1].
- Facilitating clinical pharmacology studies of dolutegravir in key populations (e.g. neonates and pregnant women), quantitative analysis methods compatible with micro-sampling and adaptable in resource-limited settings are desirable.
- Here, a method to quantify dolutegravir in dried blood spots (DBS) using liquid chromatography with ultraviolet detection (LC-UV) was developed, validated and applied in a pharmacokinetic (PK) study in HIV-positive women receiving DTG-containing ART.

METHODS

- Validation samples (over the concentration range of 400 - 10000 ng/mL) were prepared by spotting 50 µL of DTG-spiked whole blood on DBS cards.
- Extraction was by simple protein precipitation using methanol.
- Chromatographic separation was achieved with a gradient elution of acetonitrile/potassium phosphate monobasic buffer (pH 5) on a reverse-phase C18 column, at a flow rate of 1 mL/min using pioglitazone as the internal standard. Detection was by UV at a wavelength of 260 nm.
- Clinical validation was conducted by collecting DBS from participants (n = 10) at 8 time points (0.25-24 hours) after dose (paired plasma at 1 and 12 hours) from finger prick.
- DBS-derived plasma concentrations were obtained from DBS concentrations using (DBS/(1-Hct)) x 0.99 (DBS: measured DTG in DBS; Hct: mean haematocrit for female (0.40 L/L)[2]; 0.99 = plasma bound ratio of DTG).
- The method was used to quantify DTG, and PK parameters were estimated from concentration-time data using non-compartmental analysis.
- DBS-derived and measured plasma concentrations correlation was evaluated using linear regression and Bland-Altman plots.

RESULTS

- Accuracy ranged between 102.4 and 114.8% and precision ranged between 3.4 and 14.7% (Table 1).
- The mean recovery was 41.3% (%CV: 13.6).
- The method was specific and selective for dolutegravir with no interference at its retention time.
- Compared with plasma, DBS concentration was 37.5% (%CV: 6.1) lower.
- DBS-derived concentrations were used to characterise PK of DTG (Figure 1 and Table 2).
- A strong predictable correlation exists between DBS-derived and measured DTG plasma concentration (Figure 3 & 4).

Table 1: Accuracy and Precision for the quantification of dolutegravir in dried blood spot

QCs (ng/mL)	Inter-day (measured concentration)		Intra-day (measured concentration)	
	Accuracy (%)	Precision (%CV)	Accuracy (%)	Precision (%CV)
LQC (500)	112.3	12.5	107.1	14.7
MQC (4500)	114.8	8.42	109.9	3.4
HQC (8000)	107.5	11.6	102.4	3.8

Table 2: DBS-derived vs measured plasma dolutegravir PK parameters. Data are presented as mean (%CV)

PK parameters	DBS-derived values (n = 10)	Plasma (measured) (n = 10)[3]
C_{max} (µg/mL)	2.70 (24.7)	3.34 (16)
C_{24} (µg/mL)	1.34 (35.6)	0.83 (26)
AUC_{0-24} (µg.h/mL)	37.80 (23.2)	43.40 (20)

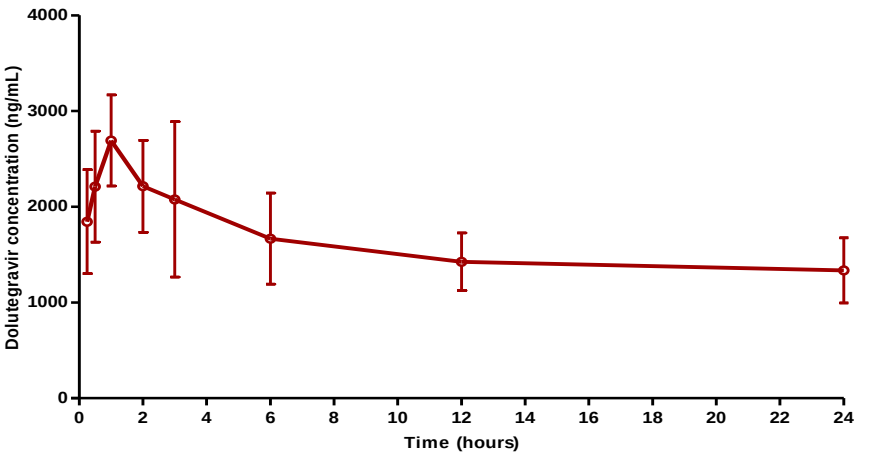

Figure 2: Concentration-time profile of dolutegravir (DBS-derived)

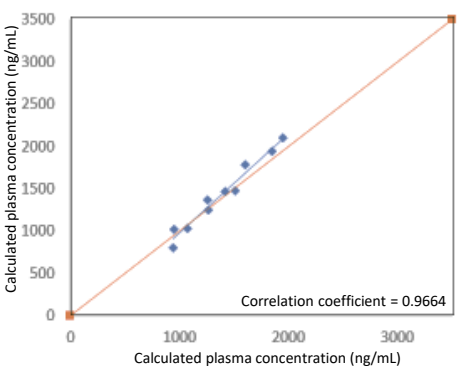

Figure 3: Theoretical vs measured plasma concentration for dolutegravir. Orange line = line of true identity

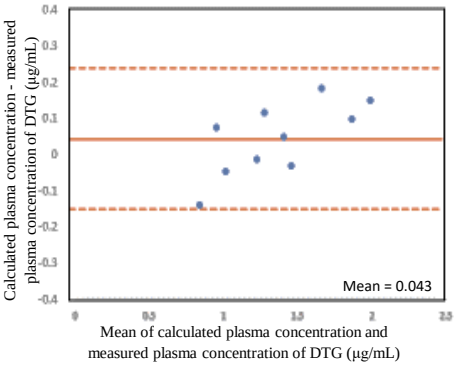

Figure 4: Bland-Altman plot for dolutegravir using mean haematocrit for female. The continuous line is the mean and the broken lines represents 95% CI (±0.1 SD)

CONCLUSION

- The developed method is simple, accurate and precise. Its application will expand opportunities to undertake clinical PK studies of DTG in key populations especially in limited-resource settings.
- PK of DTG was successfully characterised using DBS method.
- The reasons for lower PK parameters for DTG compared to previous studies using plasma samples warrant further investigation.

REFERENCES

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