Altasciences CLINICAL RESEARCH ALGORITHME PHARMA

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Effects of Temperature and Anticoagulant on the Stability Evaluation of Tetracyclines in Whole Blood: Drug-Matrix Equilibrium

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OVERVIEW

PURPOSE

An alternative and effective handling procedure for sample collection is presented to ensure drug-matrix stability. The procedure originated from an investigation of the effect of temperature on the whole blood stability of the tetracyclines minocycline and doxycycline.

METHOD

Human whole blood containing K_2EDTA, sodium heparin or sodium fluoride/potassium oxalate (NaF/KO) was incubated at 37ºC and fortified with either minocycline or doxycycline, then maintained at 4ºC or 22ºC. Plasma was harvested at different timepoints from 0 to 120 min, samples were extracted by protein precipitation and tetracyclines analyzed by LC-MS/MS.

RESULTS

The responses of minocycline and doxycycline in whole blood (K_2EDTA) at 4ºC demonstrated substantial loss within 15 min, then achieved a steady-state. In contrast, response was essentially similar at 22ºC, suggesting a slow equilibration process between red blood cells (RBCs) and plasma as the root cause for initial response loss at 4ºC. Furthermore, this equilibrium appears affected by choice of anticoagulant.

INTRODUCTION

The integrity of analyte in whole blood is critical as the sample processing needs to demonstrate analyte stability from blood collection through to plasma generation and final storage. As standard procedure, blood samples are kept at *ca.* 4ºC following collection in order to maintain drug integrity prior to harvesting plasma. In the current investigation, the tetracyclines minocycline and doxycycline (Figure 1) were used to determine the impact of temperature, incubation time, and anticoagulant on whole blood drug stability. Blood stability determined at different temperatures and with different anticoagulants indicated a potential disequilibrium within matrix components shortly after sample collection. This kinetic imbalance could influence accurate drug quantitation.

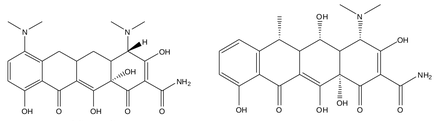

Figure 1. Structures of Minocycline and Doxycycline

METHODS

SAMPLE PROCESSING

- Whole blood samples were fortified at 37ºC with minocycline (4500 ng/mL) and doxycycline (1125 ng/mL) then maintained at either 4ºC or 22ºC. Plasma was harvested at 0, 15, 30, 60 and 120 min and the tetracyclines extracted by methanolic protein precipitation containing deuterated internal standard. Each experiment was conducted in whole blood containing either K_2EDTA, NaF/KO or sodium heparin. For each timepoint, three aliquots were prepared.

CHROMATOGRAPHY

- Agilent Technologies Series 1100 binary pump and autosampler
- Reversed-phase chromatography with gradient elution

DETECTION

- SCIEX API 5000, (+) ESI
  - Minocycline MRM: *m/z* 458.2 > 441.2
- SCIEX API 3000, (+) ESI
  - Doxycycline MRM: *m/z* 445.2 > 428.1

RESULTS

EFFECT OF TEMPERATURE ON MINOCYCLINE AND DOXYCYCLINE WHOLE BLOOD STABILITY

Within the first 15 min of the whole blood stability incubation at 4ºC in K_2EDTA, average losses of 18% and 25% were observed for minocycline and doxycycline, respectively (Tables 1 and 2). Thereafter, the response remained stable for the balance of the incubation period (i.e. 2 hr). The trend of rapid response loss followed by steady-state kinetics suggests degradation/instability was an unlikely cause, but would be rather explained by a drug equilibration process between RBCs and plasma. Further support for this hypothesis is derived from the fact that no loss of signal was observed at 22ºC for both tetracycline compounds, consistent with equilibration being established more rapidly at higher temperature, thus allowing more efficient partitioning during plasma generation.

Table 1. Whole Blood Stability Evaluation of Minocycline in Human Plasma K_2EDTA at 4ºC and 22ºC

Kinetic Times (min)	4 °C		22 °C	
	Average minocycline peak area	% Deviation	Average minocycline peak area	% Deviation
0	62031	N/AP	64357	N/AP
15	51668	-16.7	70097	8.9
30	50883	-18.0	66454	3.3
60	50947	-17.9	66004	2.6
120	49313	-20.5	62628	-2.7

Table 2. Whole Blood Stability Evaluation of Doxycycline in Human Plasma K_2EDTA at 4ºC and 22ºC

Kinetic Times (min)	4 °C		22 °C	
	Average doxycycline peak area	% Deviation	Average doxycycline peak area	% Deviation
0	326625	N/AP	296323	N/AP
15	265679	-18.7	327063	10.4
30	243708	-25.4	311255	5.0
60	237469	-27.3	297000	0.2
120	225696	-30.9	265729	-10.3

The impact of temperature on whole blood stability for the two tetracyclines was tested with various anticoagulants at 4ºC and 22ºC (Tables 3 to 6). For blood containing sodium heparin or NaF/KO, a steady-state response was observed regardless of temperature, such that all timepoints were within 10% of time zero, thus meeting acceptance criteria. Therefore, the choice of both anticoagulant and temperature impacts equilibration between RBCs and plasma.

Table 3. Whole Blood Stability Evaluation of Minocycline in Human Plasma Sodium Heparin at 4ºC and 22ºC

Kinetic Times (min)	4 °C		22 °C	
	Average minocycline peak area	% Deviation	Average minocycline peak area	% Deviation
0	115893	N/AP	126522	N/AP
15	112582	-2.9	120036	-5.1
30	114281	-1.4	121057	-4.3
60	119495	3.1	118909	-6.0
120	121363	4.7	119916	-5.2

Table 4. Whole Blood Stability Evaluation of Minocycline in Human Plasma NaF/KO at 4ºC and 22ºC

Kinetic Times (min)	4 °C		22 °C	
	Average minocycline peak area	% Deviation	Average minocycline peak area	% Deviation
0	100294	N/AP	111709	N/AP
15	104758	4.5	110971	-0.7
30	103276	3.0	111383	-0.3
60	104071	3.8	112156	0.4
120	106676	6.4	115792	3.7

Table 5. Whole Blood Stability Evaluation of Doxycycline in Human Plasma Sodium Heparin at 4ºC and 22ºC

Kinetic Times (min)	4 °C		22 °C	
	Average doxycycline peak area	% Deviation	Average doxycycline peak area	% Deviation
0	687443	N/AP	704737	N/AP
15	674423	-1.9	667446	-5.3
30	648126	-5.7	689404	-2.2
60	673296	-2.1	650189	-7.7
120	651815	-5.2	679688	-3.6

Table 6. Whole Blood Stability Evaluation of Doxycycline in Human Plasma NaF/KO at 4ºC and 22ºC

Kinetic Times (min)	4 °C		22 °C	
	Average doxycycline peak area	% Deviation	Average doxycycline peak area	% Deviation
0	690513	N/AP	692479	N/AP
15	709417	2.7	694436	0.3
30	698200	1.1	692126	-0.1
60	674880	-2.3	697187	0.7
120	663820	-3.9	702829	1.5

CONCLUSION

We have demonstrated that analyte equilibration in whole blood is both temperature and anticoagulant-dependent, and that sample handling prior to plasma generation can impact drug availability, thus biasing accuracy for plasma-based assays. Sample processing at 22ºC offers an alternative and effective procedure for compounds demonstrating variability during blood stability determinations.

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