Development and Validation of UPLC-MS/MS Method for Simultaneous Determination and Pharmacokinetics Studies of Doxorubicin and Sorafenib in Rat Plasma

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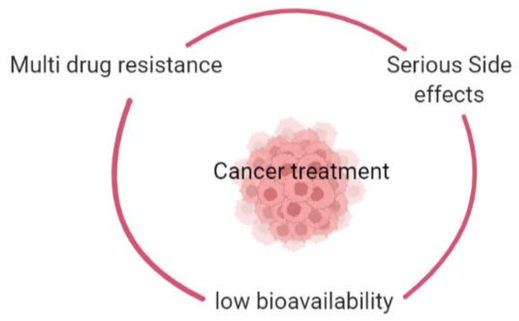
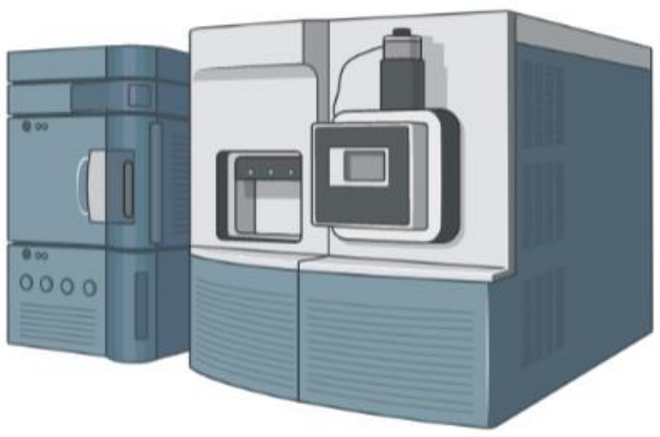
INTRODUCTION

- To overcome cancer treatment drawbacks is to use two or more agents as a combination therapy.
- **Doxorubicin (DOX)** and **Sorafenib (SOR)** could be used together as a co-therapy in the treatment of some resistant tumors when using a convenient drug delivery system (DDS).
- DOX is well known for multi-drug-resistance (MDR) and SOR is an agent that targets Raf which has an effect on multi-drug-resistance-1 (mdr-1) gene and thus influencing the sensitivity of cells to DOX.
- When these two agents are used in combination a reliable method for simultaneously detecting both drugs is needed.



OBJECTIVES

To develop a robust high thru-put ultra-performance liquid chromatography tandem mass spectrometry (UPLC MS/MS) method for the simultaneous determination of DOX and SOR in rat plasma.



METHODS

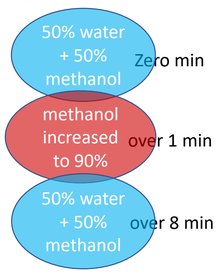
Stock and standard solutions:

- A series of standard solutions of suitable concentrations (7-2000 ng/mL) for the construction of calibration curve.
- 20 μL of ERL (IS) to each sample.
- Quality control (QC) samples were prepared at four different concentrations of DOX and SOR (20, 70, 600, and 800).

Instrumentation and UPLC-MS/MS Conditions:

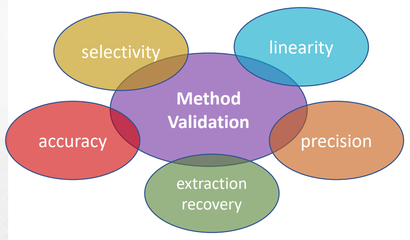
- UPLC-MS/MS ultraperformance LC system (Waters, Singapore)
- The MS/MS system was operated using MRM mode for quantitation using ESI in positive ionization mode.
- MRM was used to monitor the transitions from protonated precursor ions [M+H]+ to certain product ions of *m/z* 544 > 397.005 (DOX), *m/z* 465.05> 252.03 (SOR) and *m/z* 394 > 278 (ERL).

Chromatographic conditions:



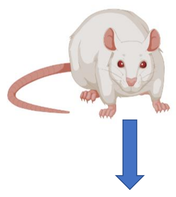
Method Validation:

- According to the U.S. Food and Drug Administration (US FDA) guidelines for the bioanalytical method.



Study design:

- Four groups of wistar healthy male rats n=4.
- **Group I:** water for injection intraperitoneal (IP) and 0.9 normal saline orally.
- **Group II:** single intraperitoneal injection (IP) of DOX 5 mg/kg .
- **Group III:** a single dose of SOR 40 mg/kg orally.
- **Group IV:** DOX IP 5mg/kg and SOR 40 mg/kg orally



Pharmacokinetics analysis

Statistical analysis results between each testing group (II-IV) were expressed as mean ± SED, and $p < 0.05$ was considered significant.

Analysis of variance (ANOVA) and Tukey's multiple comparisons test were used.

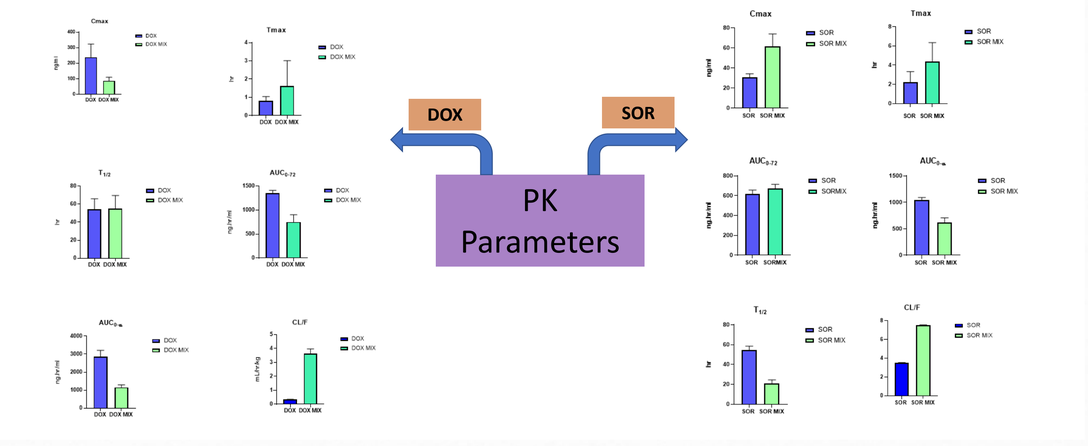
RESULTS

Evaluation of the intraday and interday accuracy and precision for the determination of DOX and SOR in rat plasma by the proposed UPLCMS/MS method.

Concentration ng/mL	Intraday				Interday			
	DOX		SOR		DOX		SOR	
	Mean recovery (%) ±RSD	E_r (%)	Mean recovery (%) ±RSD	E_r (%)	Mean recovery (%) ±RSD	E_r (%)	Mean recovery (%) ±RSD	E_r (%)
800	93.35 ± 3.19	-6.52	95.08 ± 2.55	-3.98	96.68	-3.19	90.65019	-8.38
600	92.98 ± 1.30	-6.87	91.81± 0.35	-6.68	97.16	-2.68	90.33589	-8.15
70	91.40 ± 4.46	-8.85	95.92 ± 2.66	13.95	95.63	-4.67	97.95673	15.97
20	94.61 ± 1.96	-2.74	92.11 ± 1.64	-7.03	94.77	-2.58	87.30114	-2.26

Evaluation of the extraction recovery and matrix effect for the determination of DOX and SOR in rat plasma by the proposed UPLC-MS/MS method.

Concentration ng/mL	Extraction recovery				Matrix effect			
	DOX		SOR		DOX		SOR	
	Mean recovery (%) ±RSD	E_r (%)	Mean recovery (%) ±RSD	E_r (%)	Mean recovery (%) ±RSD	E_r (%)	Mean recovery (%) ±RSD	E_r (%)
800	108.89 ± 3.15	7.79	108.81 ± 0.46	8.98	100.79	7.40	113.03	10.37
600	99.11 ± 14.64	-2.78	92.12 ± 1.42	-7.71	101.69	5.70	98.91	-0.68
70	111.86 ± 3.20	-11.52	103.54 ± 11.74	4.19	111.67	-0.42	121.33	1.92
20	120.50 ± 3.47	-8.06	104.806 ± 34.40	6.76	123.67	-1.44	106.94	0.22



DISCUSSION & CONCLUSION

- Our method showed good reliability, selectivity, accuracy, sensitivity and recovery for the simultaneous detection of DOX and SOR.
- The combination therapy showed a significant impact on some of the PK parameters such as *C*max, (*t*max), *t*1/2, AUC0-t, CL and *V*d.
- The combination showed a synergetic effect on SOR.

Futurework

- Analysis for the efficacy in cancer cells in vitro is on going.

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