Supporting Information

SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF NOVEL ADENINE-HYDROGEN SULFIDE SLOW RELEASE HYBRIDS DESIGNED AS MULTI-TARGET CARDIOPROTECTIVE AGENTS

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Contents of Supporting Information: Supplementary Materials and Methods

Validation of the Analytical Method

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Specificity

Ten samples have been spiked at the MQC levels and processed as described along with ten blank samples. The acceptance criterion was the absence of any peak at the elution time of CMQT and IS higher than 20% of the LOQ. The results showed that no interference has been occurring at the corresponding wavelengths.

System suitability

A standard solution of MTQ and ISTD at the MQC level has been analyzed using the described methodology in order to assess the system suitability parameters. The results are tabulated in the following Table.

Peak	t _R (min)	USP Plate Count	USP Resolution	USP Tailing
MTQ	1.19	24417		0.95
ISTD	1.32	33603	4.34	0.96

Calibration model

The calibration model has been assessed for spiked plasma samples. Linear regression analysis has been employed with no weighting factors whereas the 0,0 point has neither been included nor the calibration curve forced through it. The internal standard methodology has been used and the analytical signal has been expressed as the ratio of MTQ to ISTD. The equation obtained is referred below

$$\text{Area}_{\text{MTQ}}/\text{Area}_{\text{ISTD}}=0.934(\pm 0.006)+0.310\pm(0.011)$$

The back calculated values showed %Er values lower than 10.29% rendering the calibration curve determined, valid. The plot of the residuals against the corresponding level showed no systematic trend proving that the calibration curve can be used for the determination of thiosulfates in plasma.

Recovery

The recovery has been measured using spiked samples at the LLOQ, LQC, MQC, HQC and ULOQ levels. Acceptance criteria were set as follows: %Er<20% at LLOQ and %Er<15% at all the other levels. The results are summarized to the following Table. All values lie between the acceptance criteria.

Level	Added (μmol L ⁻¹)	Found(μmol L ⁻¹)	%Er
LLOQ	0.25	0.26	-4.00
LQC	0.75	0.72	4.00
MQC	9	9.13	-1.44
HQC	15	14.72	1.87
ULOQ	20	18.91	5.45

Repeatability and intermediate precision

The repeatability and intermediate precision has been measured using spiked samples at the LLOQ, LQC, MQC, HQC and ULOQ levels. Acceptance criteria were set as follows: %RSD<20% at LLOQ and %RSD<15% at all the other levels. The results are summarized to the following Table. All values lie between the acceptance criteria.

Level	Added ($\mu\text{mol L}^{-1}$)	%RSD - repeatability	%RSD -intermediate precision
LLOQ	0.25	7.15	9.12
LQC	0.75	1.32	4.38
MQC	9	1.94	5.44
HQC	15	1.50	3.20
ULOQ	20	2.30	5.81

Detectability

The detectability has been assessed by the calibration curve data using the following equations as

$$LOD = 3.3 \times \frac{\text{Std dev of the response}}{\text{slope}}$$

$$LLOQ = 10 \times \frac{\text{Std dev of the response}}{\text{slope}}$$

The corresponding values were calculated as 0,077 $\mu\text{mol L}^{-1}$ and 0,233 $\mu\text{mol L}^{-1}$.

Carry-over effect

Five repeated injections of ULOQ followed by the blank concentration have been performed and the results have been evaluated as described before. It has been found that the instrumental response is lower than 20% than this attributed to the LLOQ level of the analyte and less than 5% for the ISTD.

Stability of the analytes

In order to assess the stability of the analytes under the analysis conditions. All procedures were attempted at the LQC and HQC levels. The assessment has been performed for a. three cycles of freeze – thaw using spiked samples that remained frozen for 24hrs and then thawed and processed at room temperature b. short term stability using samples remaining at room temperature for three hrs c. autosampler stability employing processed samples that remained in the autosampler for 9hrs and d. dried processed samples that have been reconstituted the next laboratory day in order to be injected to the UPLC system. The results are shown in the following Table. For the freeze-thaw samples it has been observed that their stability was marginally acceptable after three cycles so the stability cycle has been reduced to two. The last procedure afforded acceptable results.

Stability test	Level	%RSD - repeatability
Freeze-thaw 3 cycles	LQC	20.76
	HQC	18.37
Freeze thaw 2 cycles	LQC	12.35
	HQC	12.18
Short term	LQC	6.88
	HQC	14.82
Autosampler	LQC	8.1
	HQC	14.3
Dry-reconstitute	LQC	2.51
	HQC	3.01