T4045

Analysis of Anamorelin Stereoisomers in Human Plasma Using Coupled Achiral/Chiral HPLC with Tandem Mass Spectrometric Detection

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PURPOSE

This study was to determine if anamorelin (R, R) isomeric form (Fig.1) undergoes *in vivo* conversion to its (R, S), (S, R) or (S, S) stereoisomers when administered to humans. In this presentation, we report LC-MS/MS based quantification methods using chiral LC and coupled achiral/chiral LC for the separation and determination of potentially low levels of (S, S), (R, S), and (S, R) stereoisomers in the presence of high levels of anamorelin (R, R) in clinical plasma samples.

METHOD(S)

The four anamorelin stereoisomers were extracted from plasma (50 uL) by using protein precipitation.

HPLC : Shimadzu Nexera X2 integrated system (LC-30AD pumps with SIL-30 AC autosampler)

Columns: 1) Betasil Phenyl, 5μ, 100x2.1 mm, Thermo Scientific
2) Luk Cellulose-1, 5μ, 250x4.6 mm, Phenomenex

Mobile Phase A : 5 mM Ammonia bicarbonate

Mobile Phase B : Acetonitrile

Mobile Phase C: 40/60 Acetonitrile/5 mM Ammonium bicarbonate

Mobile Phase D: 20/80/0.02 Acetonitrile/H_2O/Formic Acid

6-Port Valve Switch: VICI E60 6-port valve

Mass Spectrometer: AB Sciex API 5000 LC-MS/MS System

Polarity: Positive

Scan type: Multiple Reaction Monitoring (547→276)

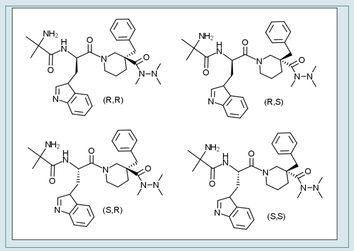

Figure 1. Anamorelin (R,R isomeric form) and its stereoisomers structures.

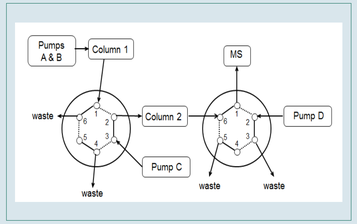

Figure 2. Columns and valve-switching setup

RESULT(S)

(S, S) isomeric form can be analyzed by chiral column alone (column 2 in Fig 2), but this column cannot separate (S,R) isomeric form from (R, R) anamorelin. In spiked standard and in dosed human clinical sample, high level of (R,R) anamorelin will cause, in case of *in vivo* interconversion, a merged peak for (R,S), (R, R) and (S, R) stereoisomers (Fig 3).

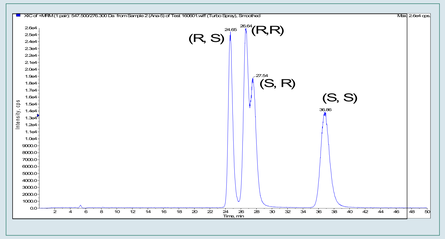

Figure 3. Chiral column alone resulted in incomplete separation.

Separation and analysis of each (R, S) and (S, R) isomeric form of anamorelin from the rest of the stereoisomers was achieved through the combination of one achiral reverse phase column and one chiral column connected with 6-port switching valve to the mass spectrometer (Fig 2).

The (R, S) and (S, R) isomeric forms resulted in one peak away from (S, S) and (R, R) isomeric form peak in the first achiral Betasil Phenyl column (Fig. 4, left). This (R, S) and (S, R) isomeric form peak was further resolved in the second chiral Luk Cellulose-1 column (Fig 4, right).

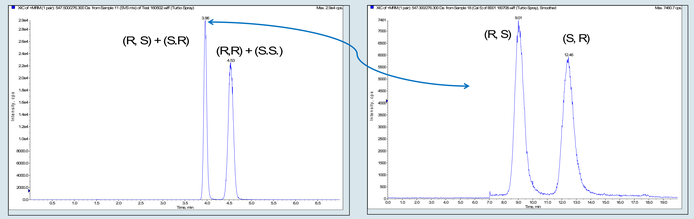

Figure 4. Four anamorelin stereoisomers are separated into two peaks in first achiral column (left). The combined (R,S) + (S,R) peak is further separated in second chiral column (right).

Calibration curves were constructed with eight different standard concentrations of each stereoisomer ranging from 0.5 to 50 ng/mL in human plasma, using least-squares linear regression with a weighting of $1/x^2$ factor. Typical anamorelin stereoisomer calibration curves observed in method qualification are shown in Fig. 5 to Fig. 7.

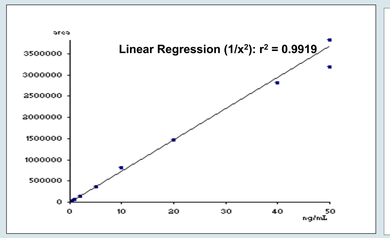

Figure 5. A typical calibration curve for anamorelin (R, S) stereoisomer.

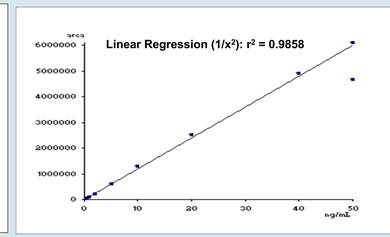

Figure 6. A typical calibration curve for anamorelin (S, R) stereoisomer.

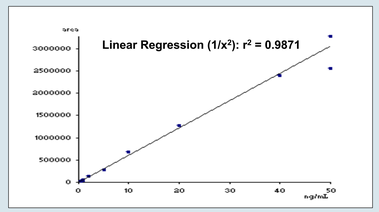

Figure 7. A typical calibration curve for anamorelin (S, S) stereoisomer.

	QCs	1.50	15.0	37.50
(R,S)	Mean	1.50	15.0	36.8
	% Bias	0.1	-0.1	-1.8
	% CV	7.5	4.6	7.9
	n	3	3	3
(S,R)	Mean	1.59	15.4	37.2
	% Bias	5.9	2.4	-0.9
	% CV	4.2	5.6	8.5
	n	3	3	3
(S,S)	Mean	1.53	15.3	36.4
	% Bias	2.0	2.0	-2.9
	% CV	8.5	8.0	12.9
	n	3	3	3

Table 1. Precision and accuracy results for three anamorelin stereoisomers quality control samples in method qualification.

CONCLUSION(S)

Four anamorelin stereoisomers separation and quantification was achieved through combined achiral/chiral columns and chiral column alone coupled with tandem mass spectrometric detection.

There were no detectable levels of the (R, S), (S, R) or (S, S) stereoisomers of anamorelin in clinical samples. These results indicate that anamorelin does not undergo *in vivo* chiral inversion following oral administration.

ACKNOWLEDGEMENT

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