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Absolute Configuration Determination Report

GENERAL INFORMATION	
Customer	BioTools, Inc.
Sales Order Number	
Sample code (Our ref.)	**Penicillin-G**
Sample description (Your ref.)	**Penicillin-G**
VCD-spectrometer	ChiralIR w/ Dual*PEM*
Report prepared by	Jordan Nafie
Report validated and signed by	Rina K Dukor
Date	September 9, 2021
RESULTS	
Absolute Configuration of Penicillin G is (3S,5R,6R)	Confidence Level: **99%**
MEASUREMENT PARAMETERS	
Concentration	8mg / 125uL
Solvent	d6-DMSO
Resolution	4 cm^{-1}
PEM setting	1400 cm^{-1}
Number of scans/Measurement time	8 hours
Sample cell	BaF_2
Path length	100 µm
CALCULATION DETAILS	
Gaussian version	Gaussian 09
Total low-energy conformers used for Boltzmann sum	5
Methodology and basis set for DFT calculations	B3LYP / 6311Gdp w/ CPCM (DMSO)
Enantiomer used for calculation	3S,5R,6R
Total calculated conformers	9
Number of low-energy conformations shown in report	3

COMMENTS

The confidence level is a measure of the degree of congruence between a calculated and measured spectrum. If identical spectra are being compared the confidence level is 100%. The confidence level (CL) is not the likelihood that the assignment is correct. Rather it's a measure of quality or degree of agreement between calculated and measured spectra. With a CL of 99% for this molecule, the visual agreement between measured and calculated spectra is very good – this is a high confidence assignment. Since the sample obtained was the potassium salt, this was measured in d6-DMSO, and calculated as the salt form.

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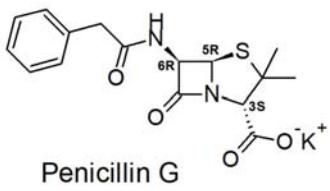
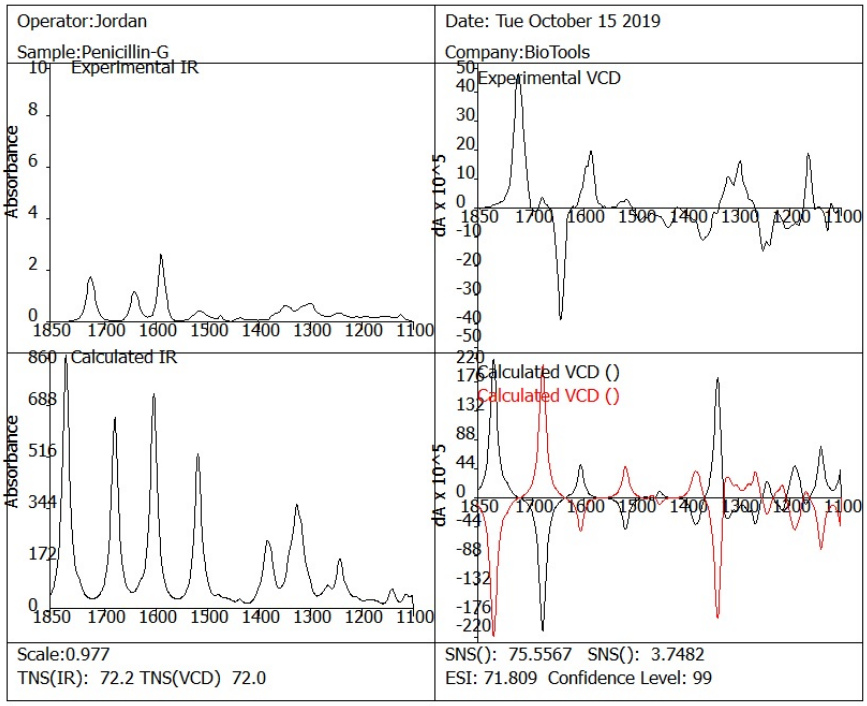
Structure of Penicillin G:



Penicillin G



CompareVOA Results.
Please note: In this plot the frequency scaling factor is not applied.

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Table 1. Numerical comparison describing the similarity in the range of 1100 - 1825 cm^{-1} between the calculated IR and VCD spectra for the **(3S,5R,6R)** enantiomer at the B3LYP / 6311Gdp w/ CPCM (DMSO) level and the observed IR and VCD spectra for **Penicillin G**.

Cal. (1100-1825cm^{-1})	Numerical comparison	Observed **Penicillin G**
(3S,5R,6R)	scaling factor	0.977
	IR similarity (%)	72.2
	[a]∑ (%)	75.5567
	[b]Δ (%)	71.809
	Confidence Level (%)	99

[a]∑: single VCD similarity, gives the similarity between the calculated and observed VCD spectra.
[b]Δ: enatiomeric similarity index, gives the difference between the values of ∑ for both enantiomers of a given diastereoisomer.

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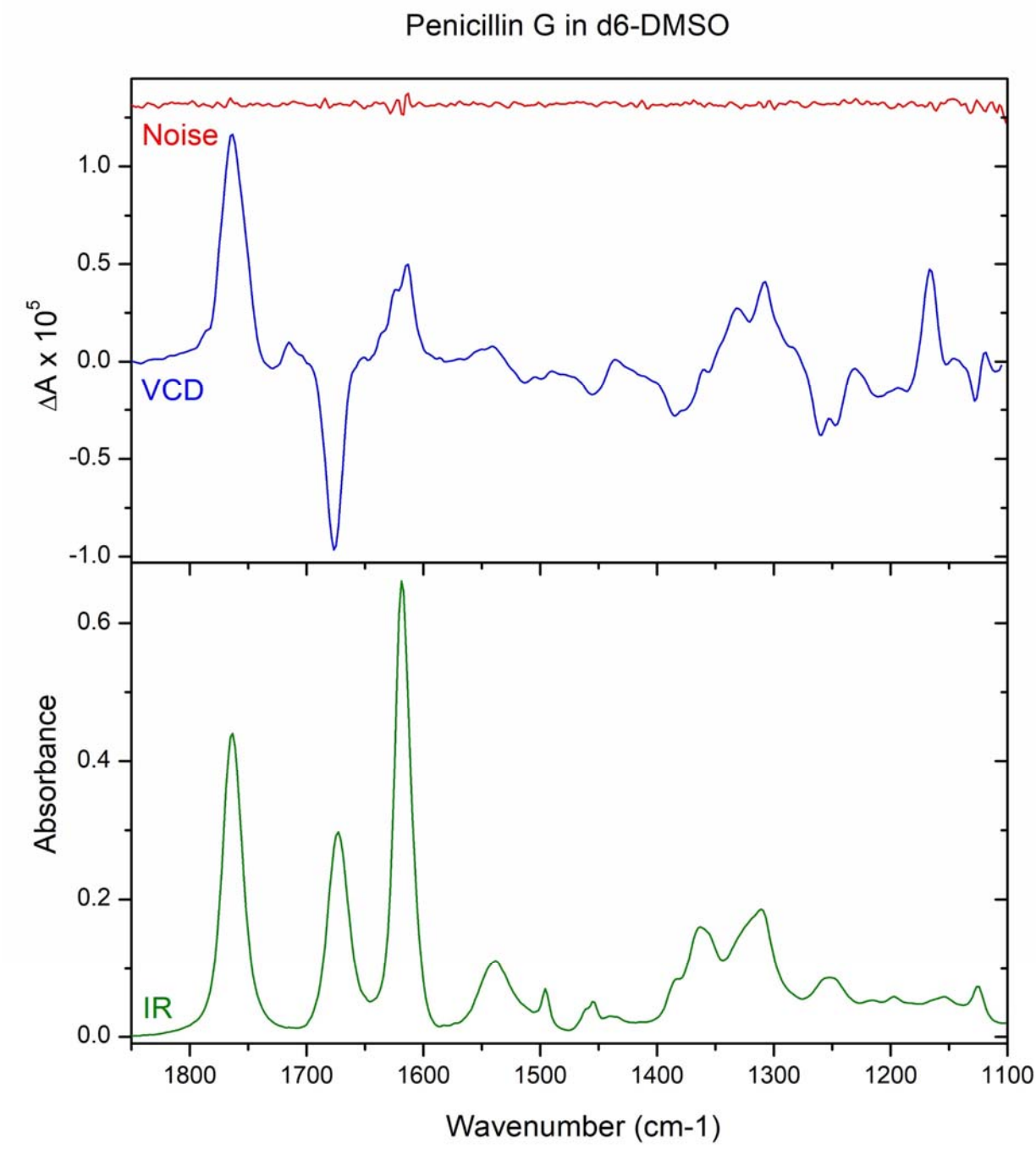

IR (lower frame) and VCD (upper frame) spectra of **Penicillin G** in d6-DMSO; 100um path-length cell with BaF_2 windows; 8 h collection for enantiomer and solvent; instrument optimized at 1400 cm^{-1}. Solvent subtracted IR and VCD spectra are shown. Uppermost trace is the VCD noise spectrum.

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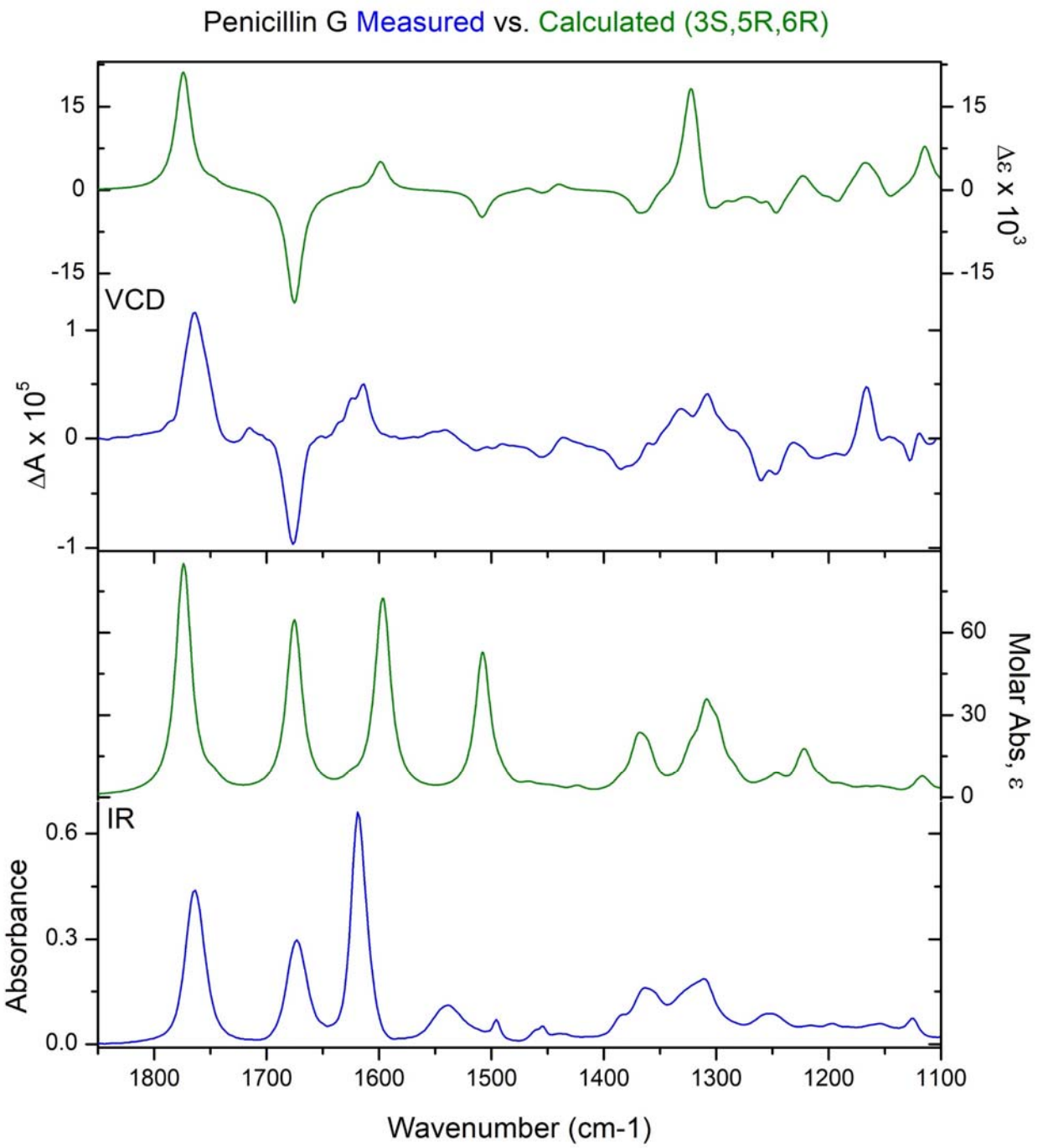

IR (lower frame) and VCD (upper frame) spectra observed for **Penicillin G** (left axes) compared with Boltzmann-averaged spectra of the calculated conformations for the **(3S,5R,6R)** configuration, (right axes).

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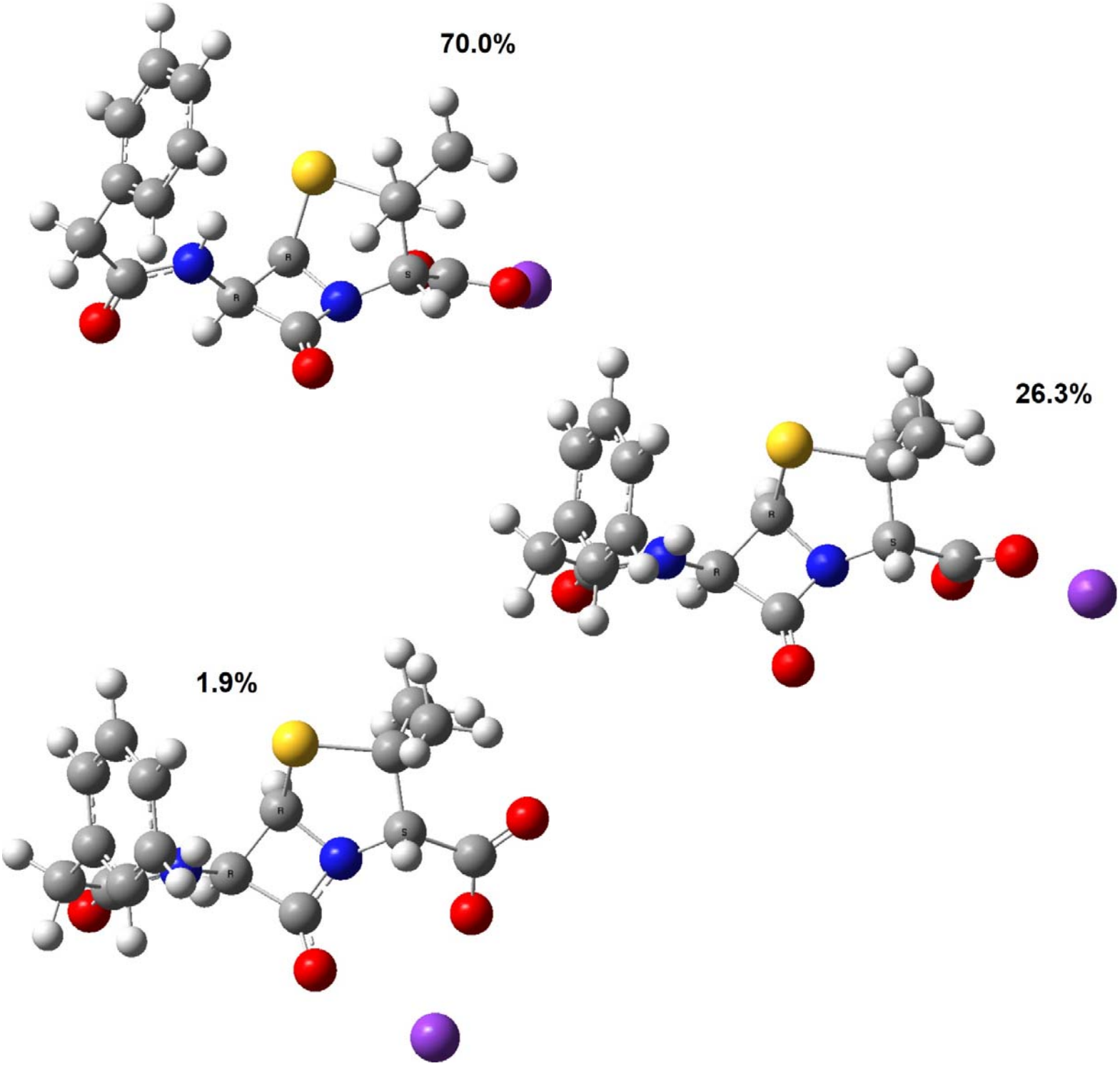
3 LOWEST ENERGY CONFORMERS - (3S,5R,6R) Configuration:



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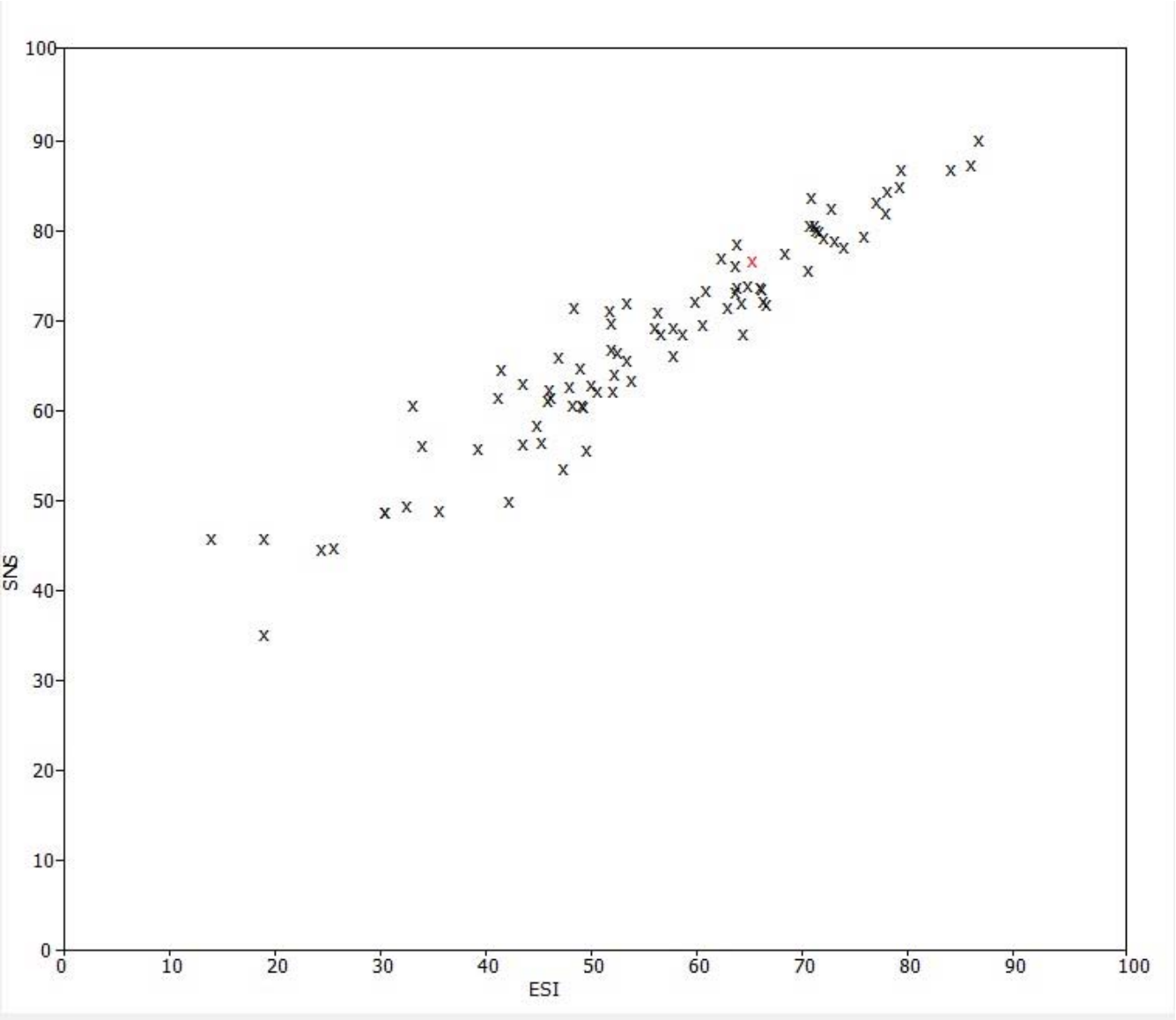

Plot of ESI (similarity of correct enantiomer minus incorrect enantiomer to calculated) vs SNS (overall similarity of correct enantiomer to calculated) for a library of correct assignments verified independently by X-Ray other method (Black X marks). Red X is Penicillin G.