

“True” spectrum of the chromatographic peak

Yuri Kalambet,

Ampersand International Inc., Beachwood, OH

Sergey Maltsev

Ampersand Ltd., Moscow;

Yuri Kozmin

Shemyakin Institute of Bioorganic Chemistry, Moscow

kalambet@ampersand-intl.com
www.chromandspec.com

Introduction

While analyzing chromatograms, obtained with the help of a multi-channel (e.g. Photodiode Array or Mass-Spectral) detector [1], one of the first and most important goals is to find the peak spectrum for the purpose of spectrum identification or to define a database spectrum.

There are several ways to calculate the spectrum associated with the peak, some of the well-known ones are: peak apex, upslope, downslope, average spectra, and spectrum by factor analysis [2]. In most software packages some amount of expert work has to be done to select the spectrum that can be used for peak identification from all possible spectra. We offer a simple procedure to select best spectrum that matches additional criterion to be “purest”.

Inter-spectra angle definition

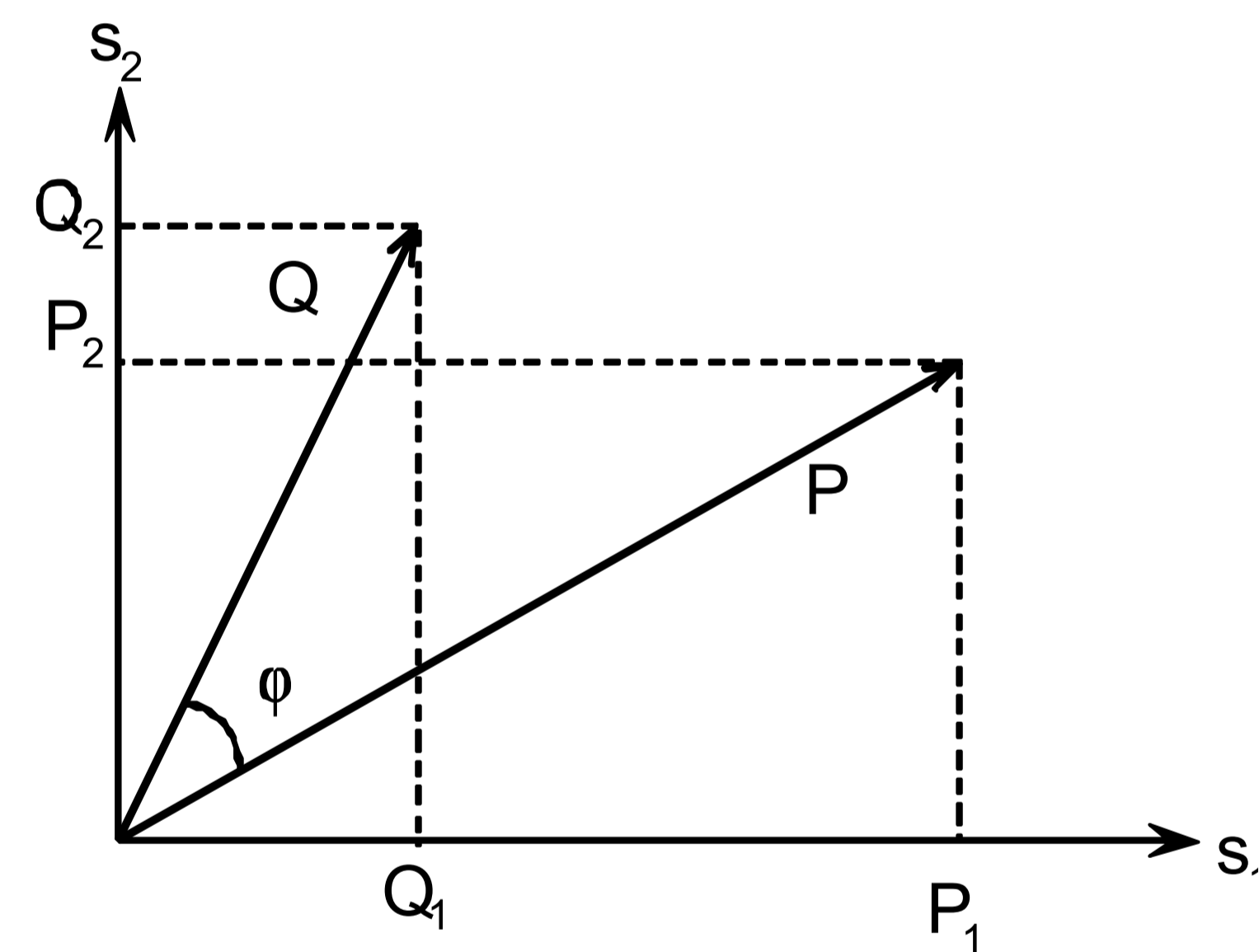


Figure 1. Definition of inter-spectra angle for the case of two channels (wavelengths), S_1 - response at channel 1, S_2 - response at channel 2, P and Q – spectra of different components. ϕ is inter-spectra angle.

Our criterion is based on the local purity [3] parameter, attributed to every spectrum of the peak, that equals to the maximum of two angles between the spectrum being evaluated and its two left and right time-neighbors. The lower is this parameter, the “purer” is the spectrum. For homogeneous peaks that are far from detector overflow, all spectra should be proportional to each other according to Beer’s law, so angle between neighbors should equal zero, and any spectrum can be selected as “purest”. This theoretical ideal picture is

spoiled by noise influence, and signal-to-noise ratio is minimized somewhere near peak apex. Really, for peaks without detector overflow purest spectrum is near the peak top. As soon as concentration is high enough for some channels to be out of linear range, position of the purest spectrum shifts to up- or downslope portion of the peak. In the case of peak overlap “purity” profile becomes significantly disturbed, so spectra from contaminated parts of the peak become “impure”.

This “purest” spectrum can be used for all purposes of spectrum identification procedures and thus many evaluation procedures can be automated [4]. Surely, this spectrum is not “true”, but can be considered as a good estimate of the real one.

Results

All results were obtained using fast scanning detector of “Milichrom A02” chromatograph (Econova, Novosibirsk, Russia), but are easily applicable to any Diode-Array or Mass-spectral detector.

Conclusion

Best purity spectrum is a very simple concept that significantly improves performance of peak identification routines at a very low cost.

References

- 1.L.HUBER. Application of diode-array detection in high performance liquid chromatography 1989, Hewlett-Pachard Co.
2. E.R.MALINOWSKY. Factor analysis in chemistry. Wiley, NewYork,1991.
- 3.Yu. A. KALAMBET, Yu. P. KOZMIN, and M. P. PERELROYSEN. Computer spectrochromatography. Principles and practice of multi-channel chromatographic data processing, *J. Chromatogr.*, **1991**, 5421, 247-261.
4. Ю.А. Каламбет, Ю.П. Козьмин, С.А. Мальцев. Как получить правильный спектр хроматографического пика; «Партнеры и конкуренты», 2005, № 4, 27-30 // Yu.A.Kalambet, Yu.P.Kozmin, S.A.Maltsev. How to get correct spectrum of the chromatographic peak; “Partners and Competitors”, 2005, № 4, 27-30 (Russian)

Detector overload

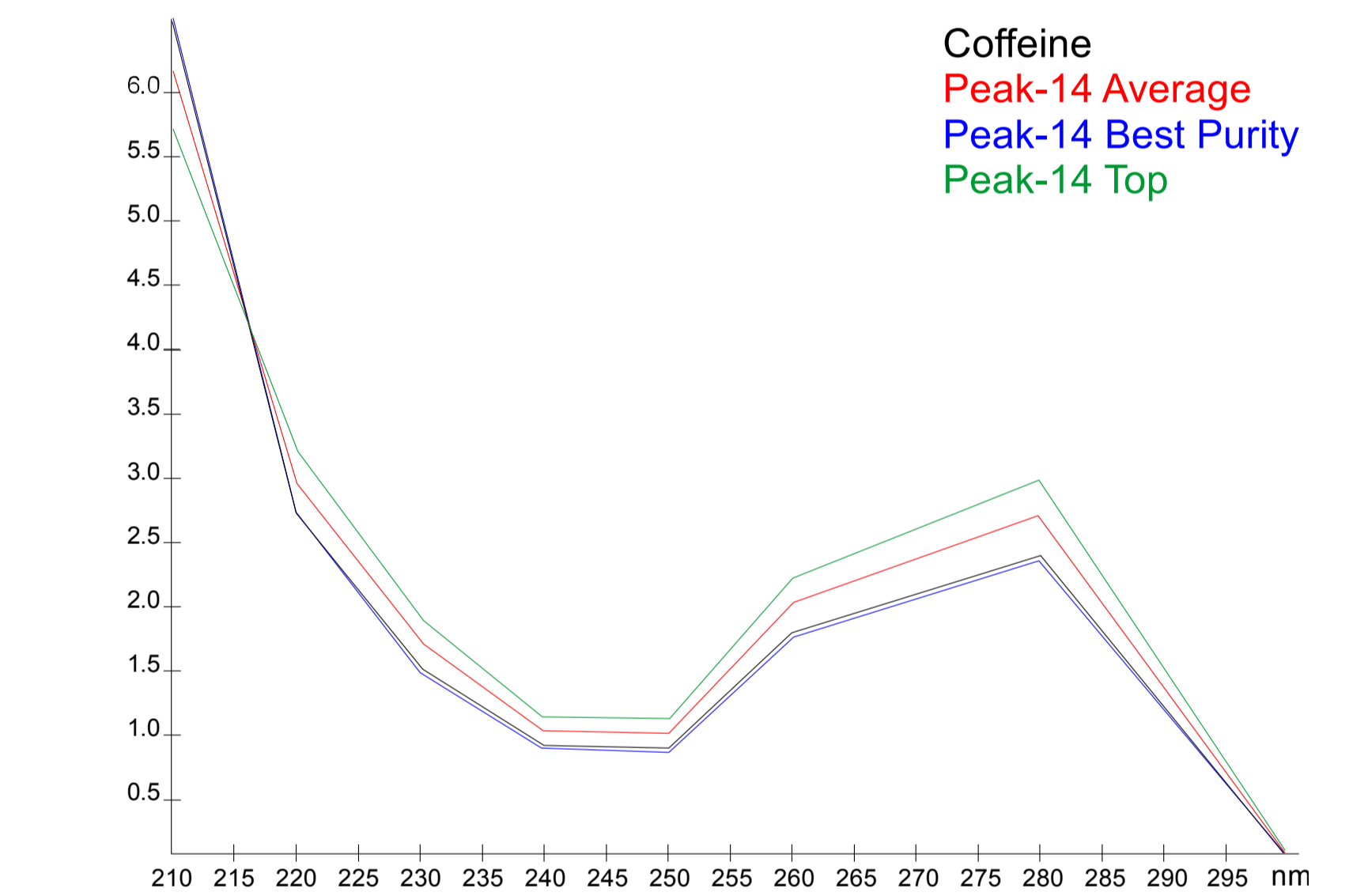
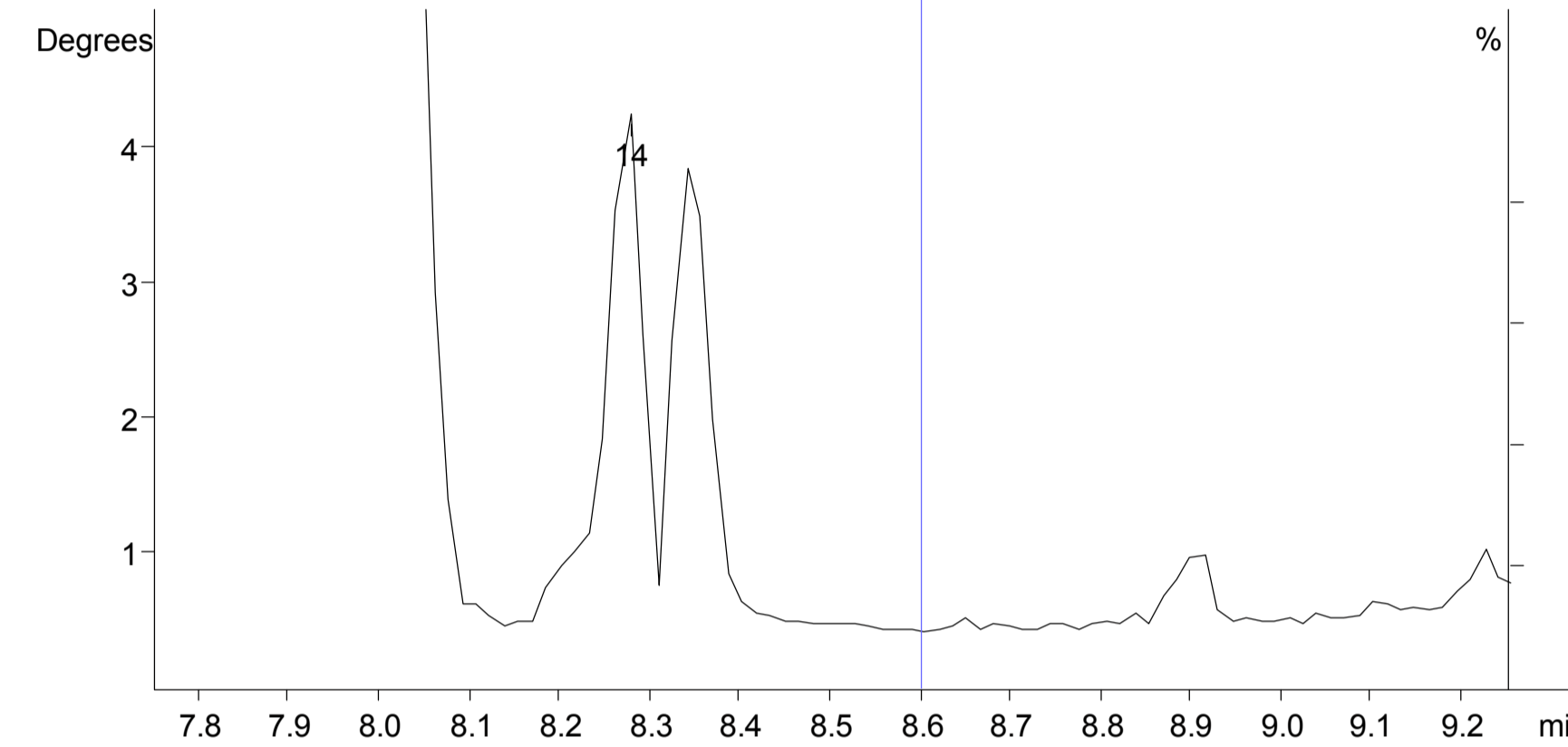
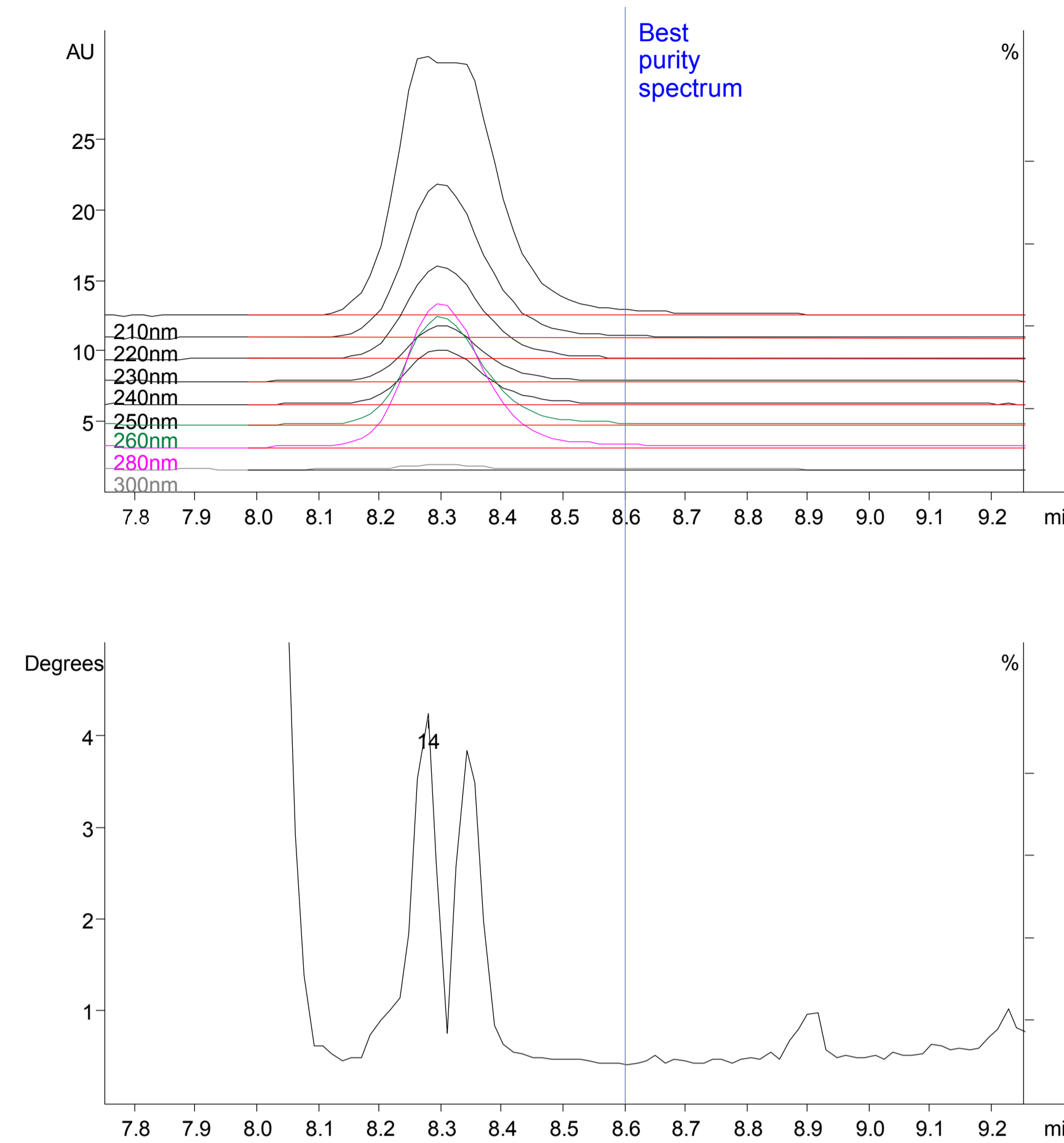


Figure 2. Elution profiles (a), purity angle derivative profile (b)

and spectra (c) of overloaded peak. Neither average, nor peak top spectra will provide correct component spectra. Best Purity spectrum provides correct answer.

Spectrum type	Angle with library spectrum
Average	4.67
Peak top	9.28
Best purity	0.46

Peak overlap

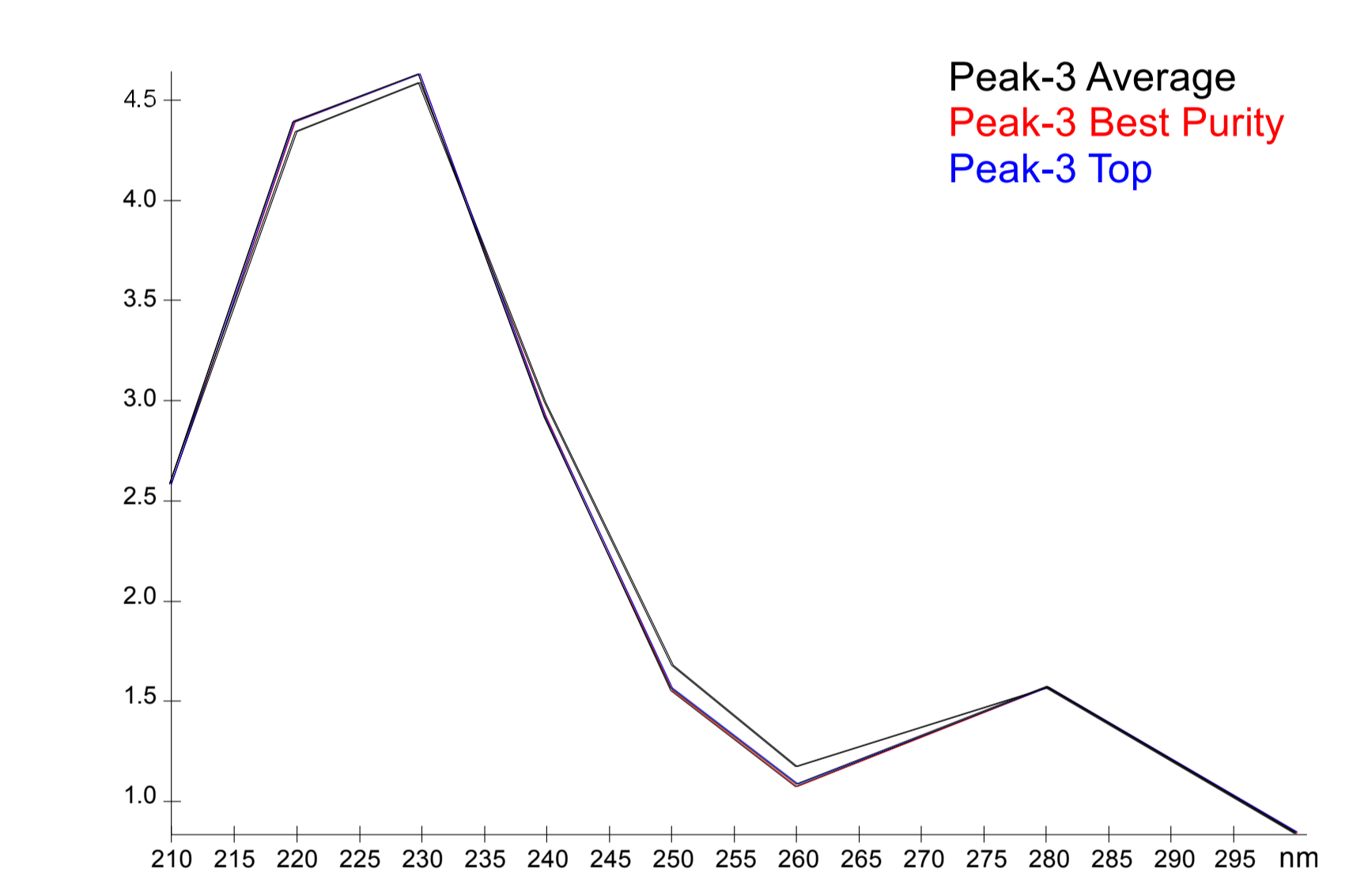
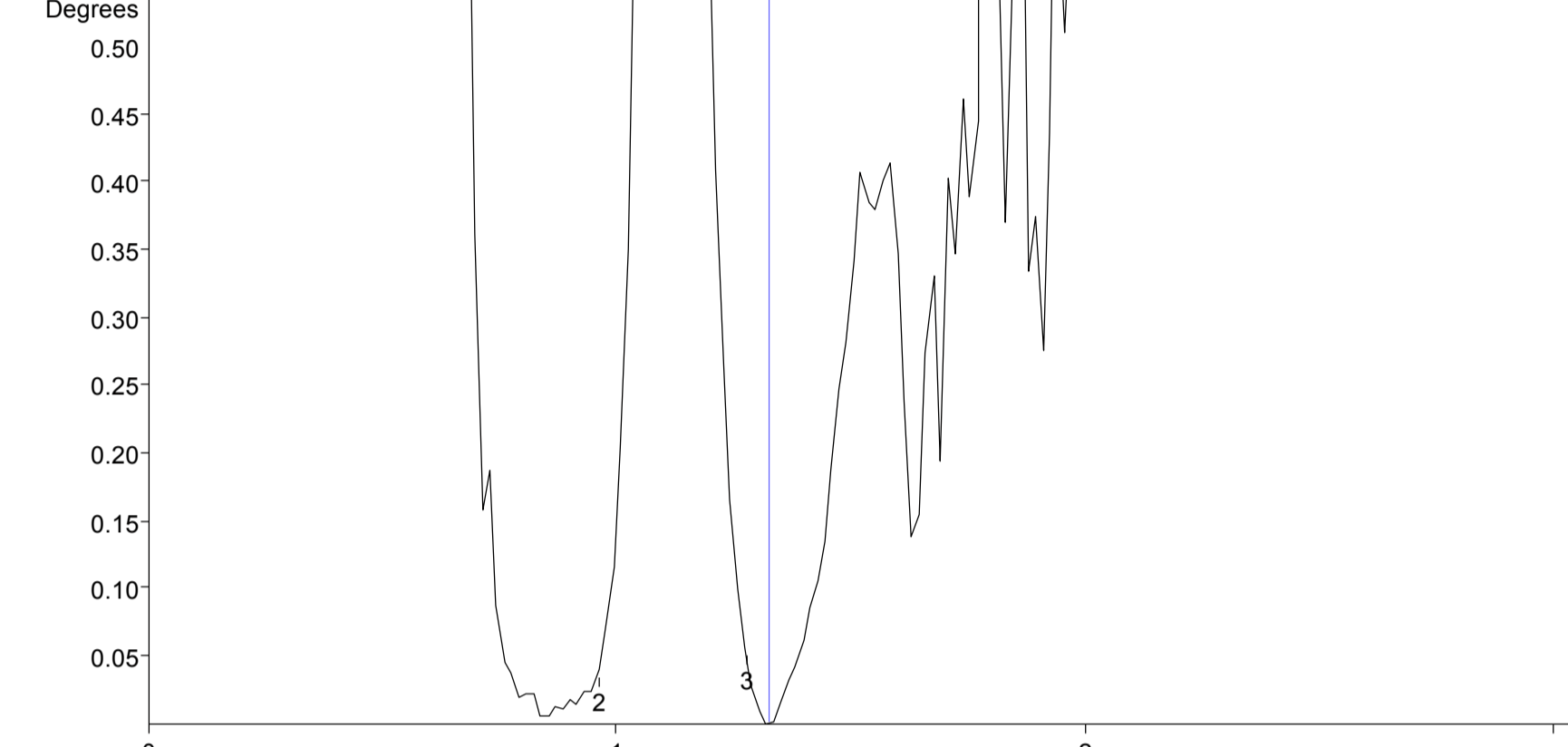
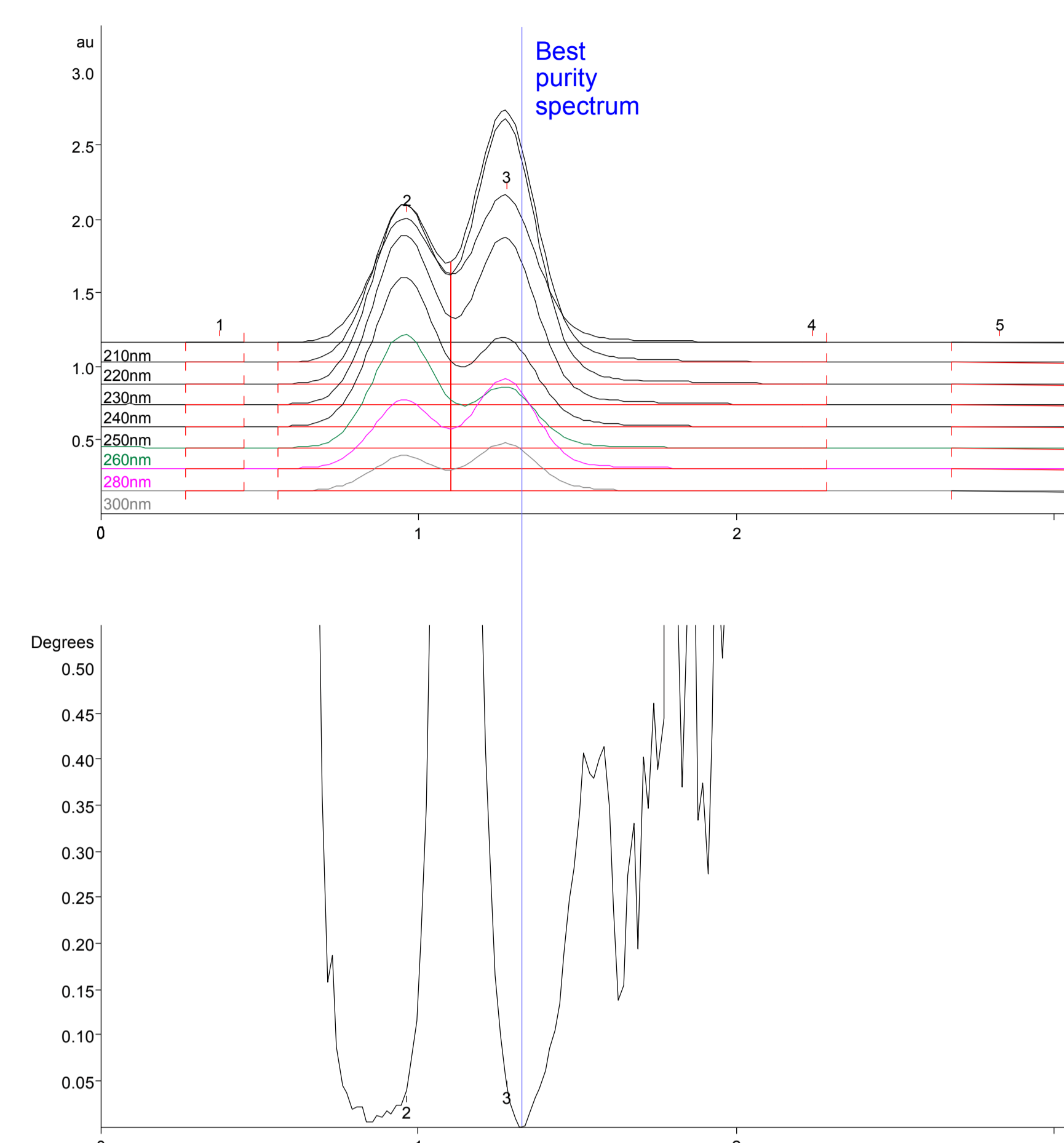


Figure 3. Elution profiles (a), purity angle derivative profile (b) and spectra (c) of overlapped peaks. Best Purity spectra provides the best answers.

Spectrum type	Angle with library spectrum
Average	2.12
Peak apex	1.04
Best purity	1.01

Peak with impurity

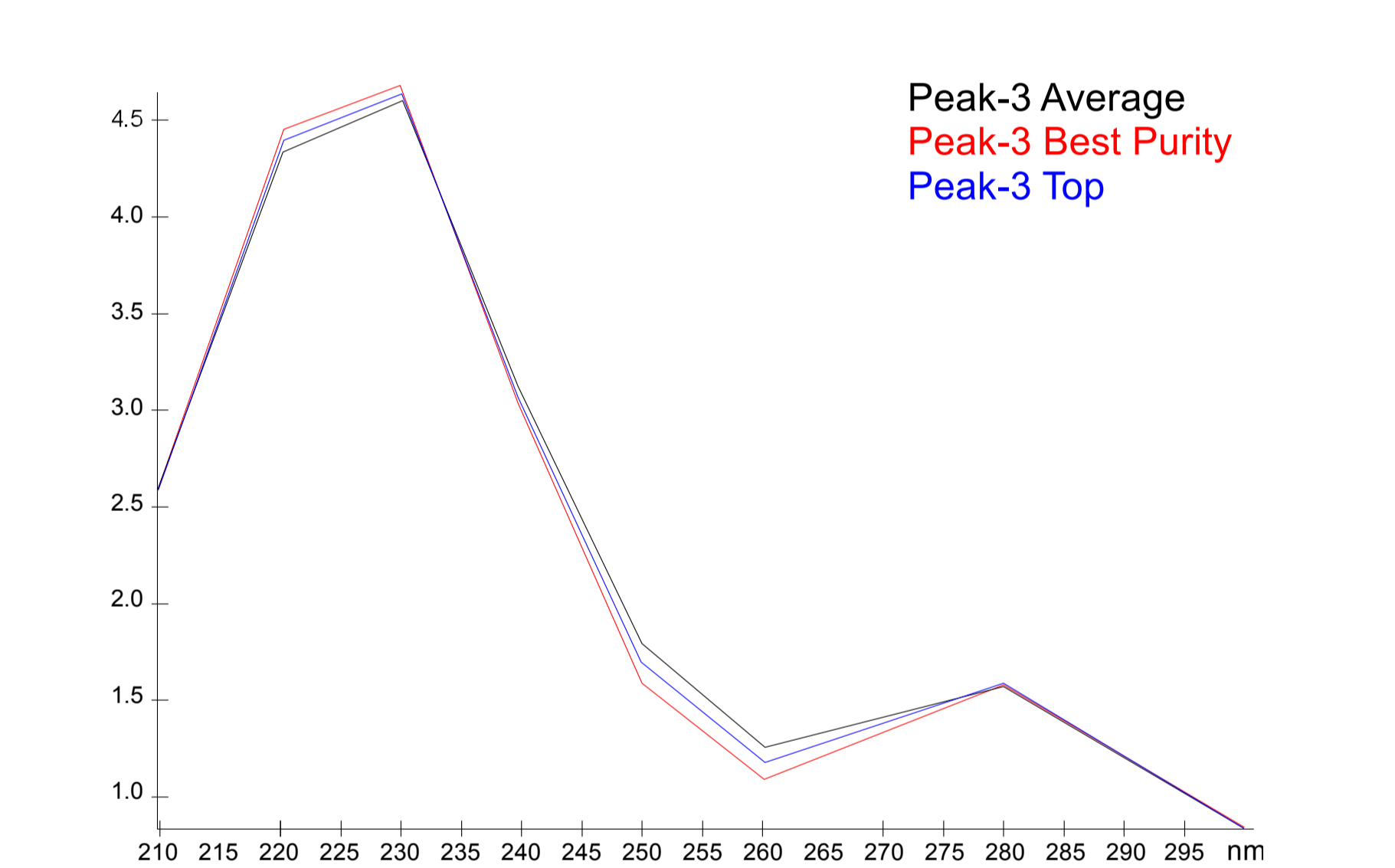
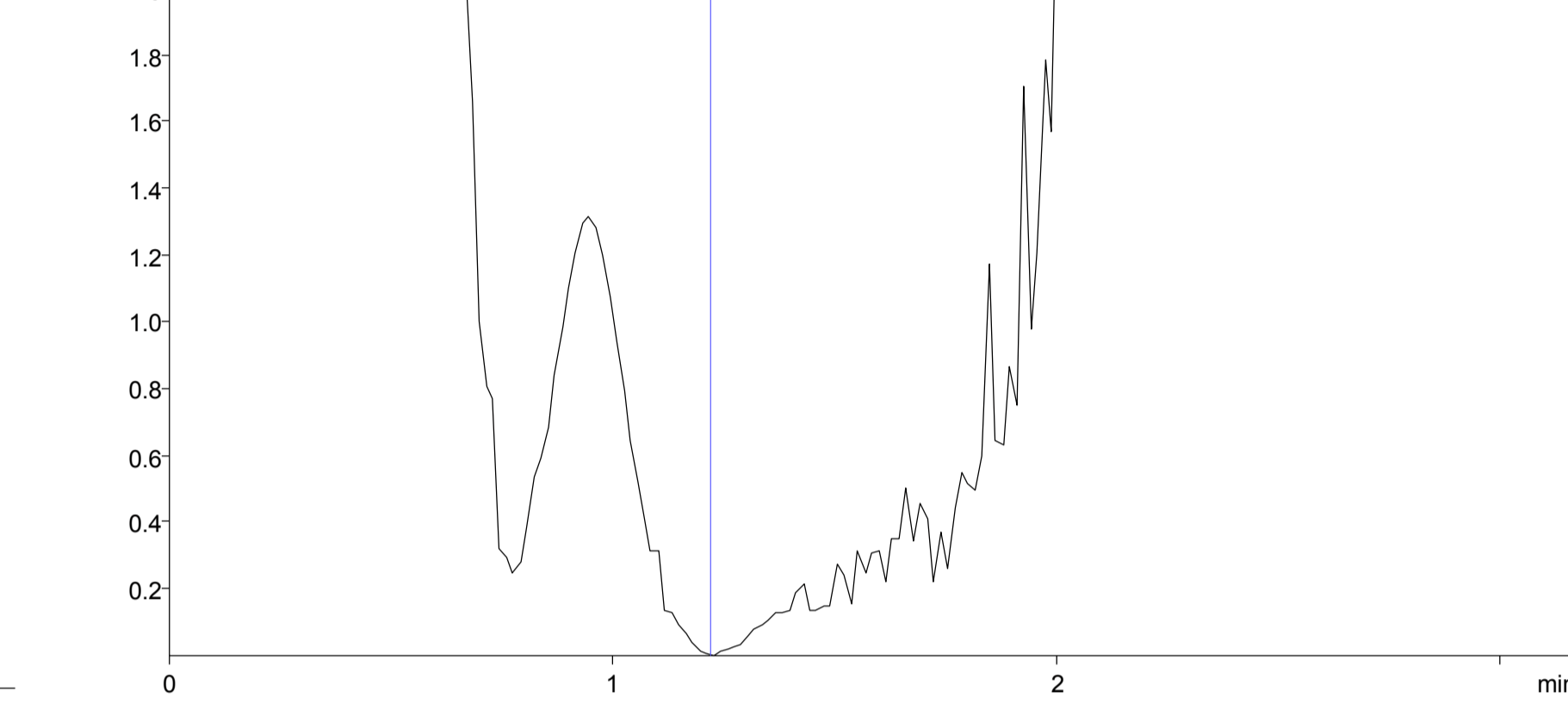
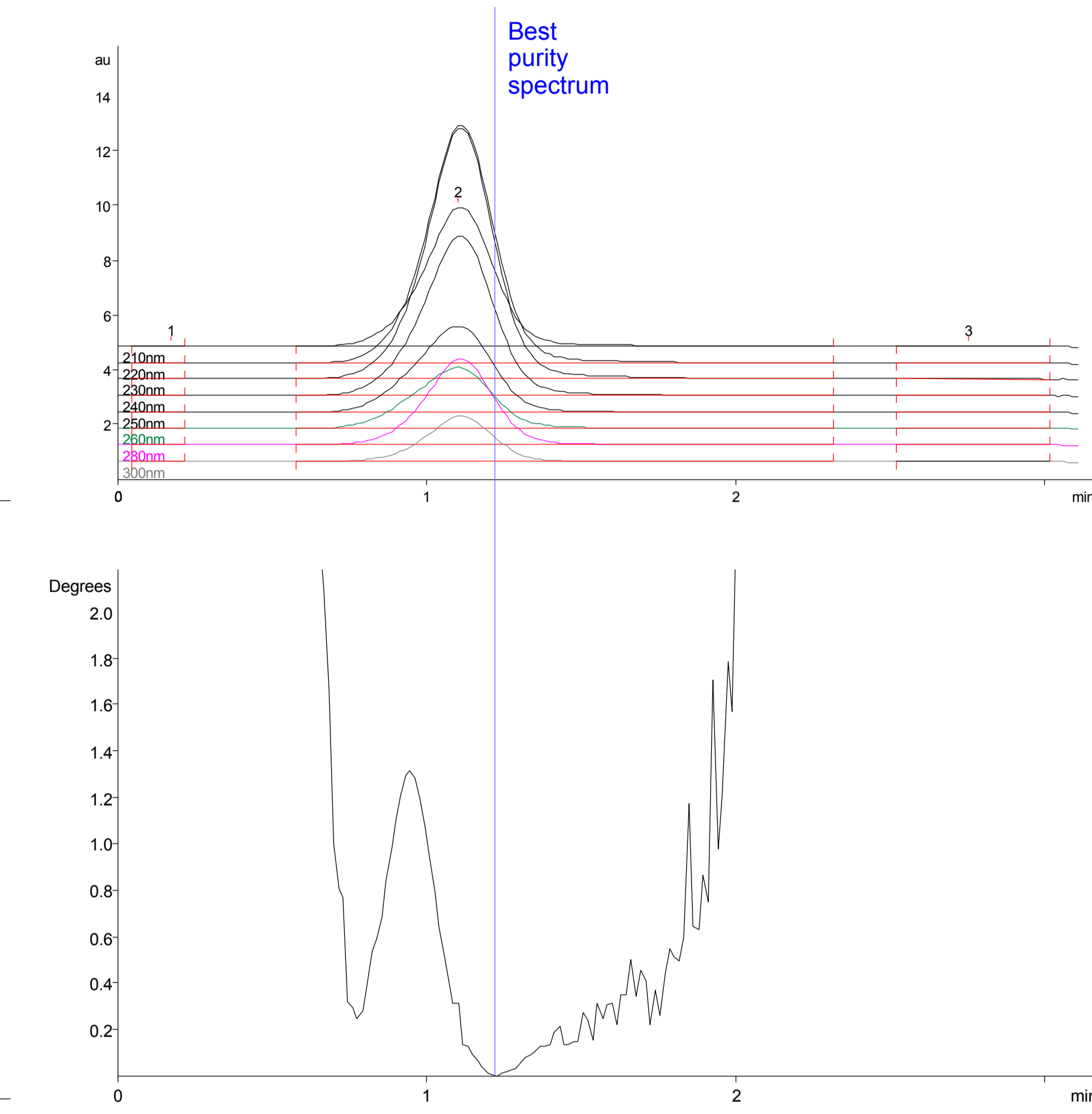


Figure 4. Elution profile (a), angle derivative profile (b) and spectra of the peak with impurity. Best purity spectrum provides the best answer.

Spectrum type	Angle with library spectrum
Average	3.23
Peak apex	2.24
Best purity	1.43

