Composition/Concentration Monitor (CCM)

Introduction:

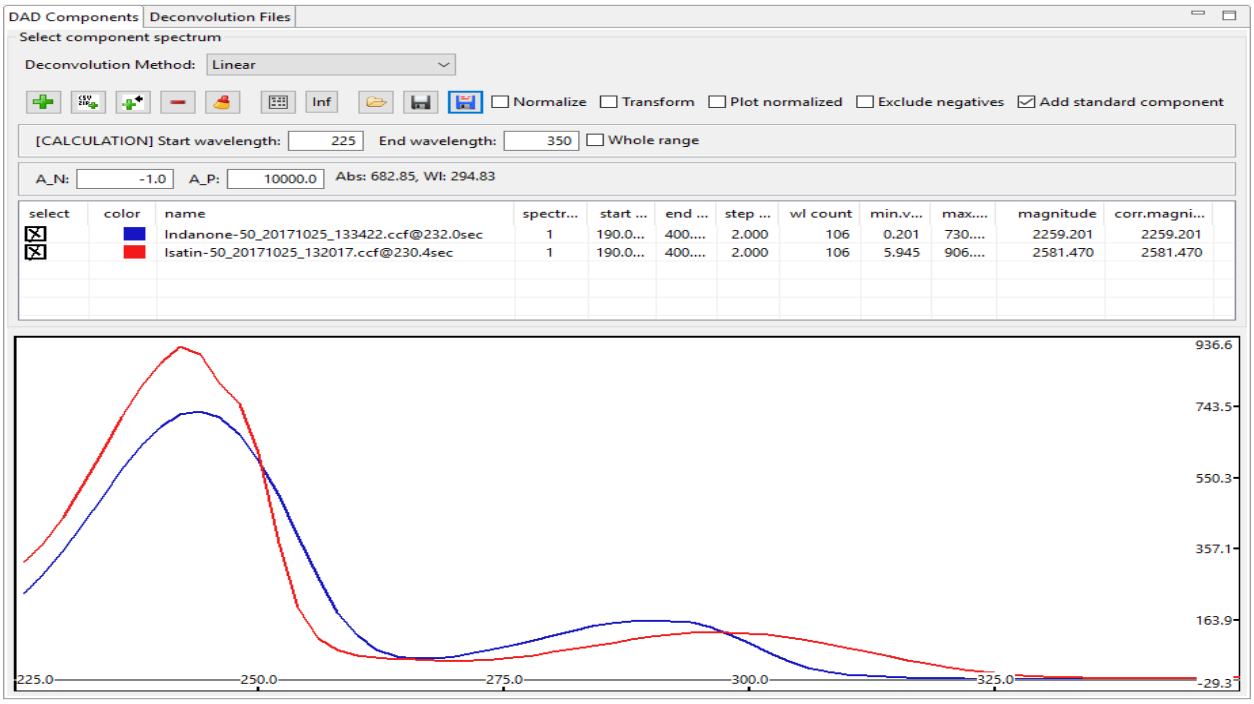
In 2013 we were encouraged by a Big-Pharma company to pursue the ability to simplify the purification of UV absorbing compounds on SMB ("simulated moving bed" liquid chromatography used for cost-effective large-scale purifications). Their candidate application was isolation of trace impurities in APIs ("active pharmaceutical candidates") and so they wanted to do relatively short runs on a fast-pace schedule using very little time to develop methods. A poster was presented at Prep 2015 and an oral presentation was given at Prep 2016 on this work. Our approach was to insert a flow cell before the recycling pump, connect flow cell to a spectrometer and light source using fiber optic cables, collect UV spectra, and mathematically deconvolve the UV spectra based on known spectra of components. At that time we assumed if spectra were sufficiently different, mathematical deconvolution would be possible in real-time. This effort was reasonably successful and we could watch deconvolved peaks in real-time. We observed movement of deconvolved peaks during the 6+ cycles after changing SMB parameters, as you would expect.

We were inspired by these accomplishments and decided to continue development for more general applications. Since operating our own large-scale SMB during development was not realistic, we adapted our Java Software (JSW) to deconvolve spectra from Agilent DAD ("diode array detector" commonly used in analytical liquid chromatography applications). Our JSW already controlled Agilent HPLC (high performance liquid chromatography systems) in automated method development (AutoMDS) and automated prep purification (AutoPrep) applications. With the benefit of convenient in-house testing using standard Agilent HPLC with DAD, our mathematical department worked exclusively on this effort. We made significant progress and improved our mathematics algorithms so that we can deconvolve in real-time component spectra that are not significantly different. We also solved non-linearity problems associated with high concentrations in purification applications. We can even extract spectra of unexpected components.

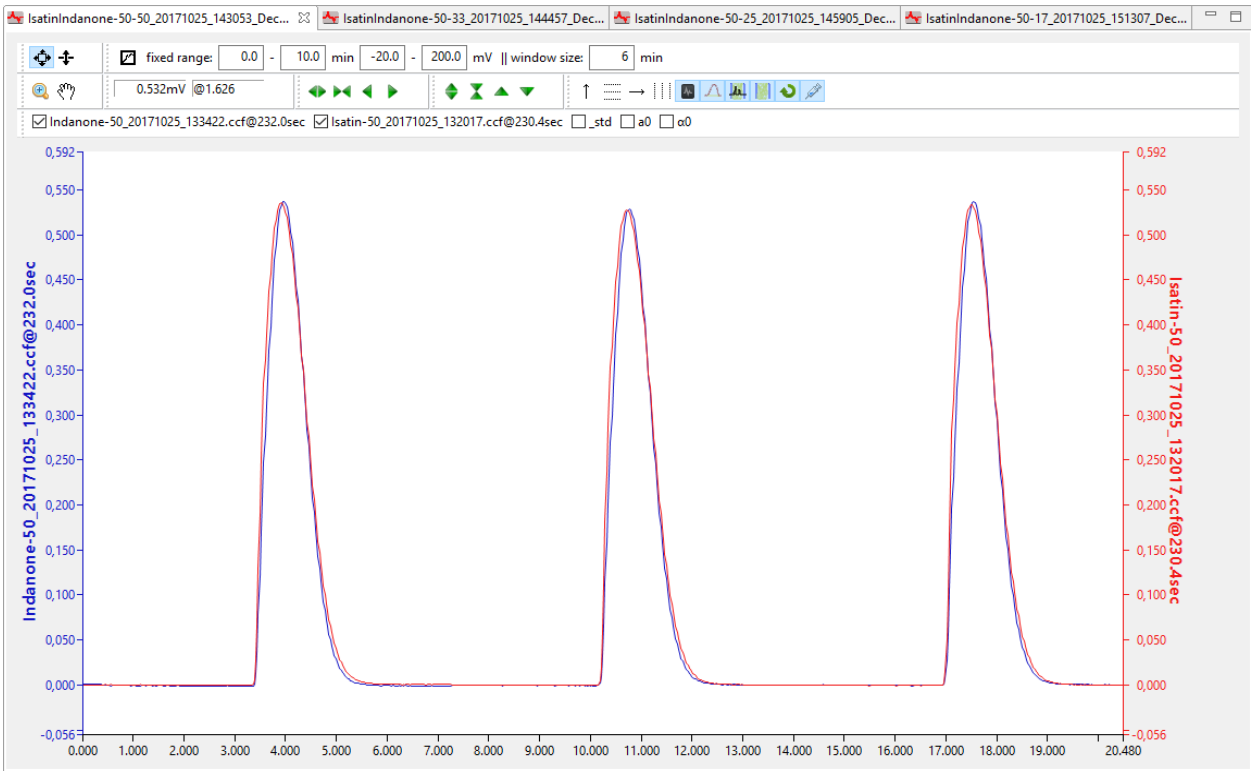
Examples presented below show real-time deconvolutions of triplicate FIA ("flow injection analysis", no column) injections on Agilent HPLC with DAD using Indanone and Isatin analytes. Indanone and Isatin solutions were added to autosampler vials using a syringe for measurement to create approximate mixtures of 50:50, 50:33, 50:25, 50:17, 50:0, 33:50, 25:50, and 17:50. Deconvolutions were made in real time using the WL range of 220-350 nm.

A system includes a DAD from multi-vendors for small scale applications or a FiberDAD from PDR for large scale applications. FiberDAD includes an inline flow cell coupled to a spectrometer and light source via fiber optic cables and can be operated in explosive environments.

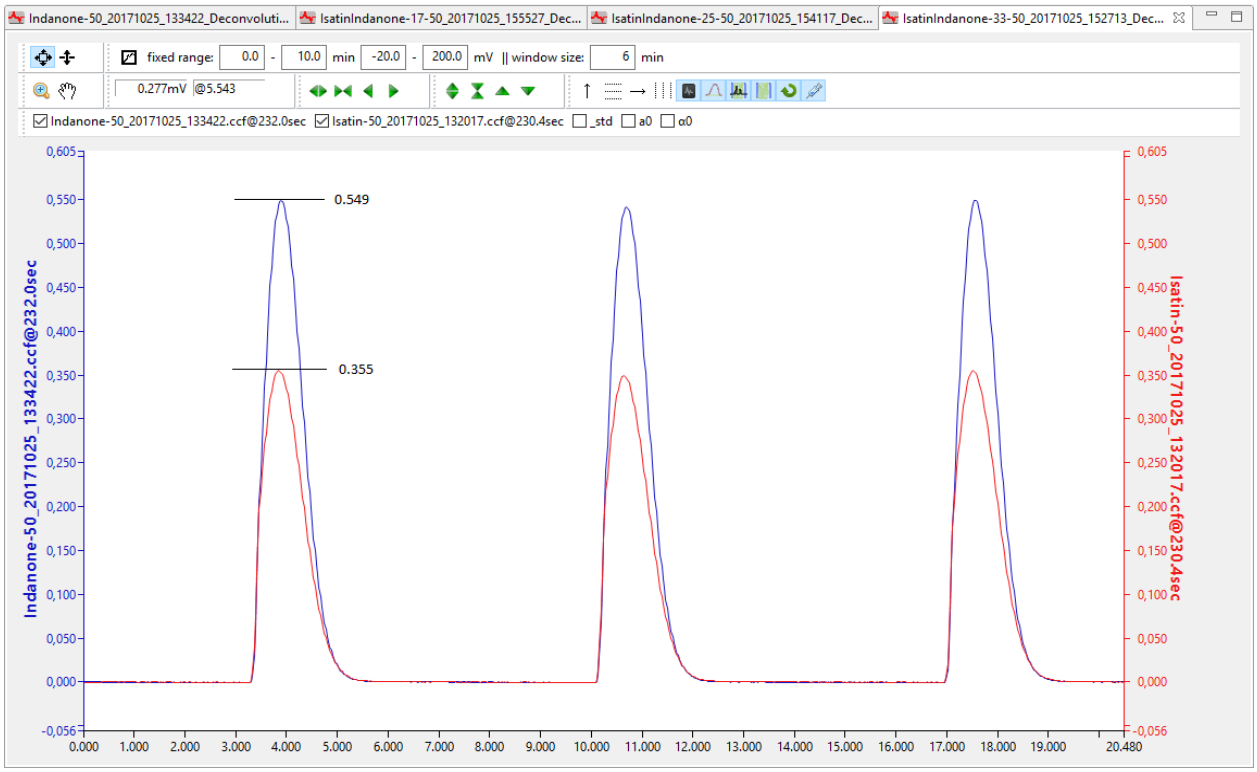
Indanone/Isatin Spectra:



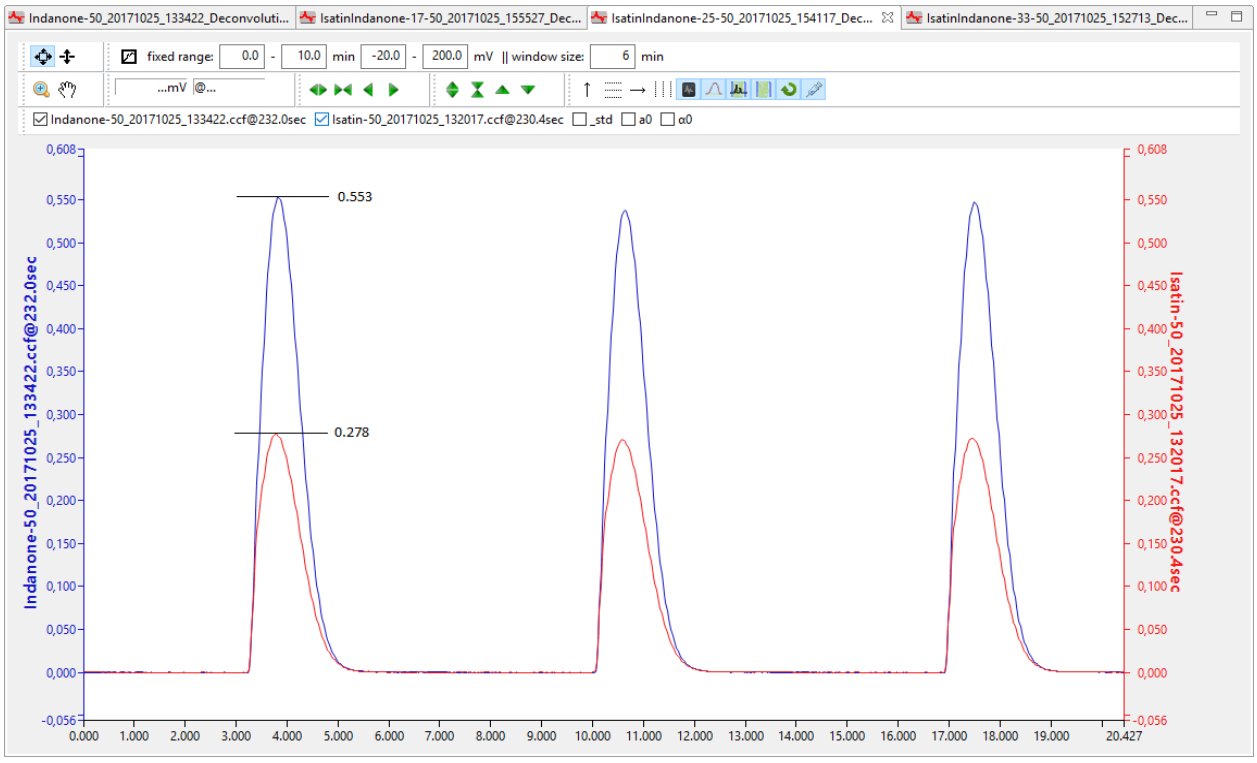
Indanone 50, Isatin 50, Isatin/Indanone = 1 (Deconvolution result: 0.538/0.536 = 1.0003):



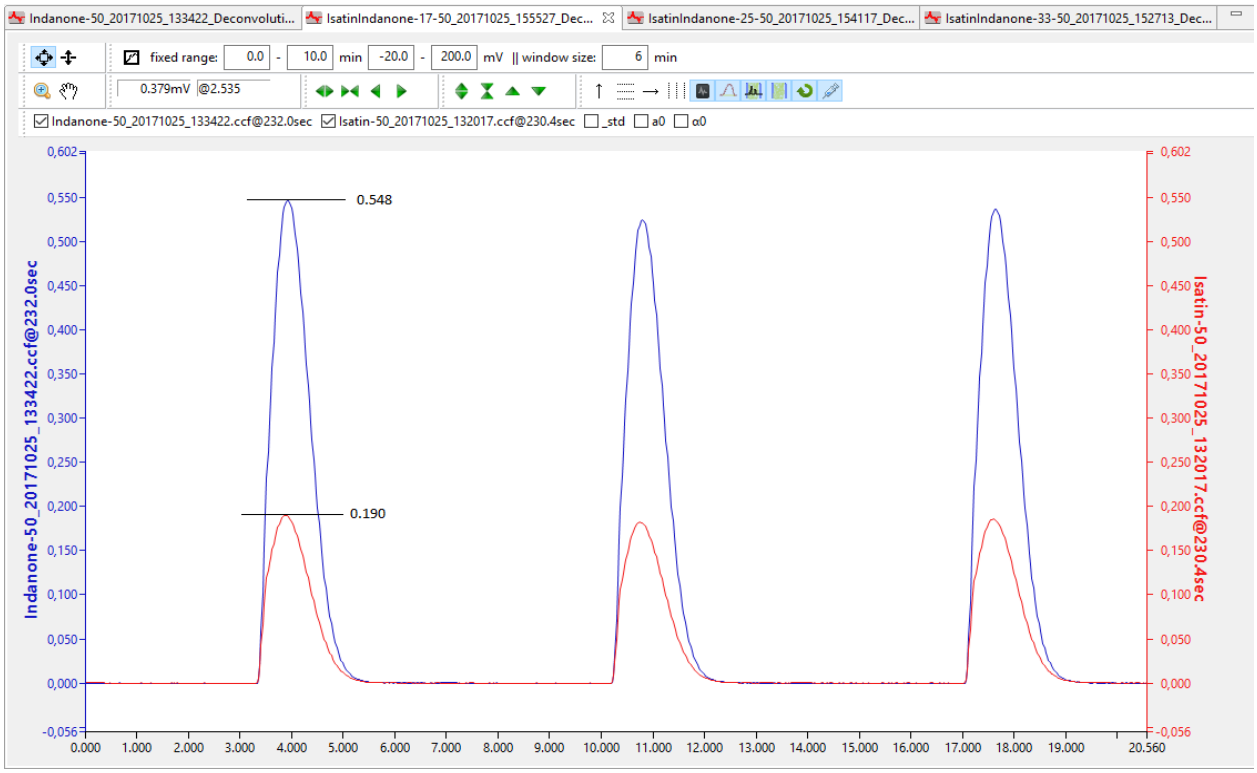
Indanone 50, Isatin 33, Isatin/Indanone = 0.66 (Deconvolution result: 0.355/0.549 = 0.646):



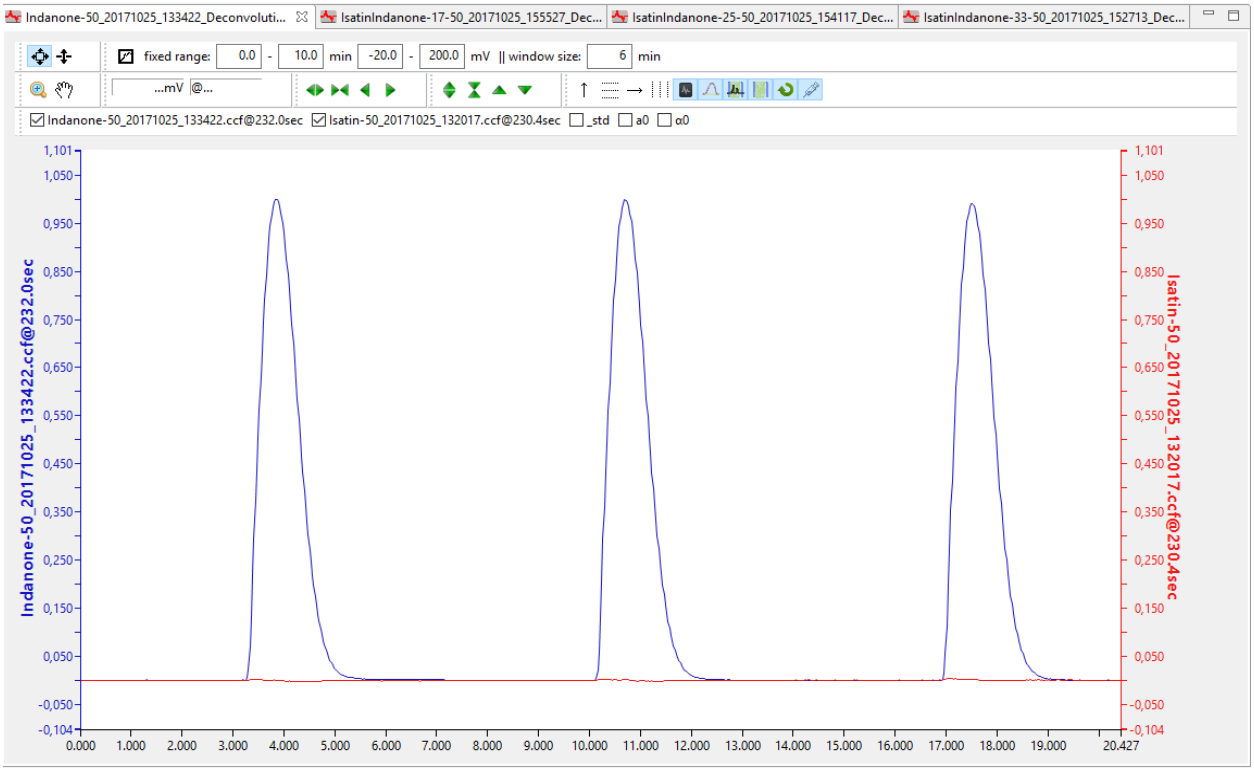
Indanone 50, Isatin 25, Isatin/Indanone = 0.5 (Deconvolution result: 0.278/0.553 = 0.502):



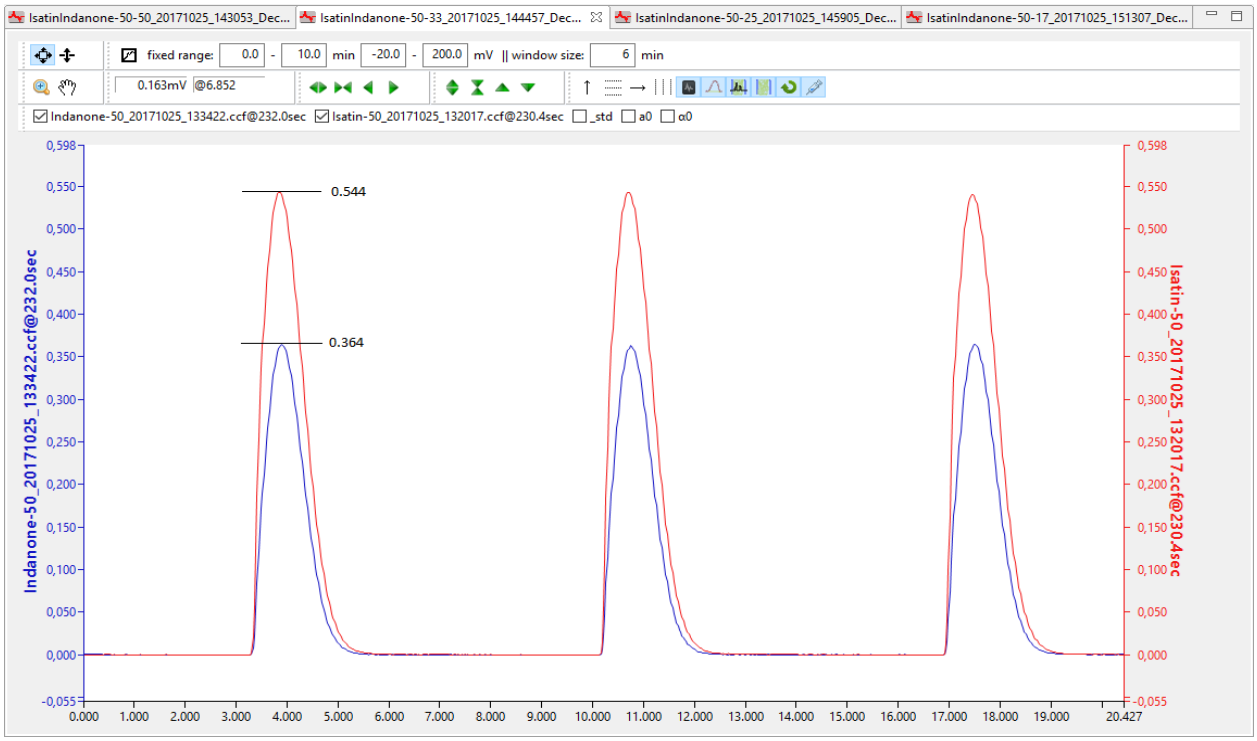
Indanone 50, Isatin 17, Isatin/Indanone = 0.33 (Deconvolution result: 0.190/0.548 = 0.348):



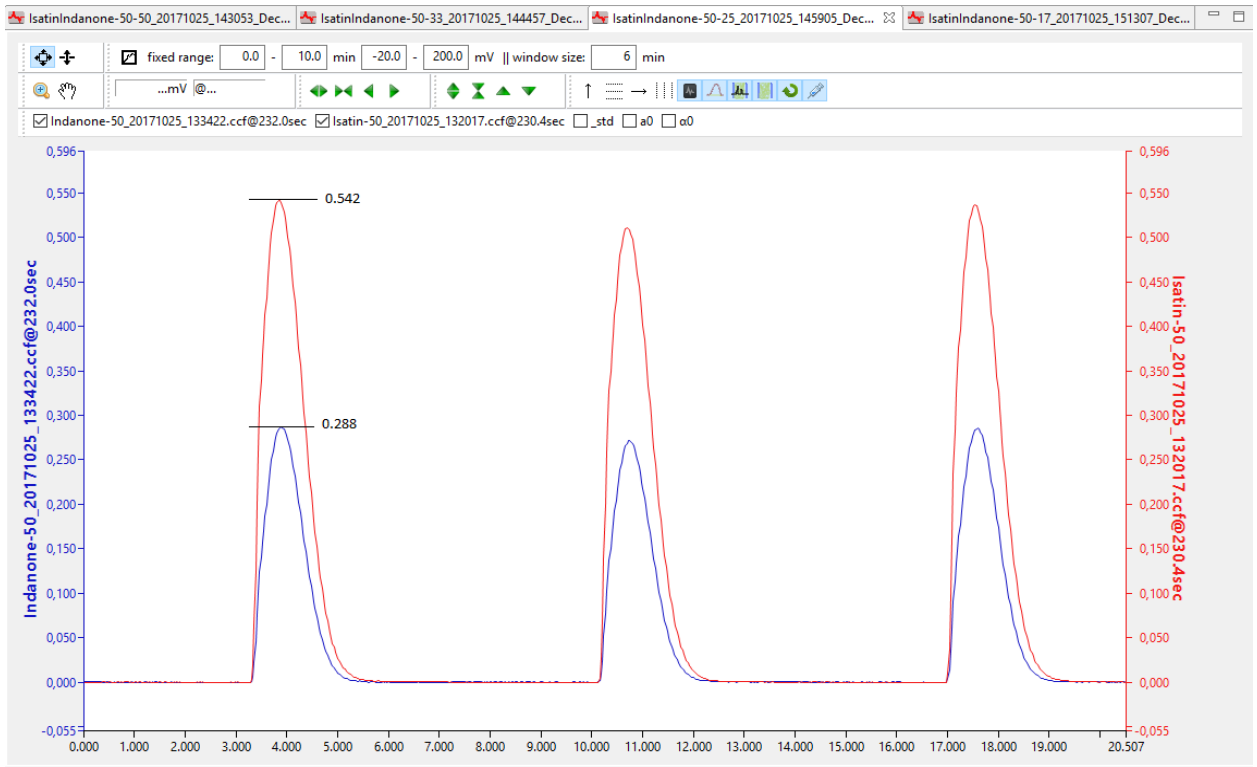
Indanone 50, Isatin 0:



Indanone 33, Isatin 50, Isatin/Indanone = 1.5 (Deconvolution result: 0.544/0.364 = 1.494):



Indanone 25, Isatin 50, Isatin/Indanone = 2 (Deconvolution result: 0.542/0.288 = 1.88):



Indanone 17, Isatin 50, Isatin/Indanone = 3 (Deconvolution result: 0.541/0.188 = 2.87):