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Uncertainty contribution of the baseline noise effect during an HPLC-UV elution of four vitamer compounds

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The sensitivity of the HPLC instrument is strongly required to track the inadequacy of many of biological compounds in their native medium. However, this sensitivity is influenced at this level, by the chromatographic parameters variability, where the integration errors arising from the baseline noise constitute an important effect. In fact, noise blurs the base of a peak making it difficult to locate where it starts and ends and therefore where to measure the area. This effect was inspected, using experimental data, obtained on a simultaneous HPLC-UV elution of four vitamers compounds. Considering that the detection of the integration points in the elution zone of the compounds of interest, is randomly oriented by the shape of the baseline noise, it is thus possible to limit the surface, of the base of the corresponding Gaussian peak, to a simple geometrical form. This approximation allows the elaboration of a model for calculating the peak area dispersion, relating to this effect. Assuming in one hand, a noise magnitude comparable to the constructor given threshold (50 µV) and on the other hand, performing this calculation for peak area quantities, corresponding to the commonly existing natural content of the analytes of interest. Consequently, the uncertainty estimation during an isocratic elution, in two separated zones of the same chromatogram, shows that for low-content samples, the contribution of integration can reach (2.5% to 5%) and (2% to 10%) of the overall uncertainty budget, respectively, for the first and the last eluted compounds.

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