

Letter to the editor:

ELLMAN'S METHOD IS STILL AN APPROPRIATE METHOD FOR MEASUREMENT OF CHOLINESTERASES ACTIVITIES

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<http://dx.doi.org/10.17179/excli2018-1536>

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Dear Editor,

It has been recently argued that Ellman's method has not sufficient accuracy for measurement of cholinesterases (ChEs) activities (Komersová et al., 2007; Sinko et al., 2007; Pohanka et al., 2011; Dingova et al., 2014). This method was first introduced and described by Ellman and his colleagues and is based on thiocholine-derivative (acetyl- or butyryl) hydrolysis by ChEs (true or pseudo) and reaction of resulting thiocholine with thiol reagent 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) and formation of 5-thio-2-nitrobenzoic acid (TNB) anion. Measurement of TNB ion absorption at 410 nm for pseudo-ChE and 440 nm for reticulocytic acetyl-ChE indirectly determines ChE activity (Ellman et al., 1961). Some authors proposed that indoxylacetate is better substrate than acetylthiocholine (ATCh) as it does not react with oxime antidotes and thiol used for Ellman's method (Pohanka et al., 2011). Moreover, DTNB which is unstable, interacts with free sulfhydryl groups in the sample, and may affect cholinesterase activity (Dingova et al., 2014). Other researchers showed that oximes react with ATCh (oximolysis), producing thiocholine and consequently TNB ion (Sinko et al., 2007). Some authors also mentioned that when the concentration of DTNB is far higher than the concentration of ATCh, hydrolysis rate of ATCh is decreased resulting in a lower measured ChE activity (Komersová et al., 2007).

Although above-mentioned problems have been stated about Ellman's method, each of them has its own logical solution. First, by simple appropriate dilution of sample, the thiol- and oxime-containing compounds in the sample are also diluted and hence the extent of reaction of DTNB with these substances is considerably reduced (Mohammadi et al., 2017). Second, molar attenuation coefficients of indoxylacetate and TNB are $3900 \text{ M}^{-1} \cdot \text{cm}^{-1}$ (Pohanka et al., 2011) and $13600 \text{ M}^{-1} \cdot \text{cm}^{-1}$ (Ellman et al., 1961) respectively. Thus, the detection limit for measurement of ChE activity by DTNB is about 3.5 times lower than that by indoxylacetate. Third, for DTNB concentrations of 0.2-0.598 mM, the best ratio of DTNB/ATCh concentrations is 1.25-3.74 which results in optimum rate of ATCh hydrolysis by ChE (Komersová et al., 2007).

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