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Discussion

High-performance liquid chromatographic determination of morphine, morphine-3-glucuronide, morphine-6-glucuronide and codeine in biological samples using multi-wavelength forward optical detection: a reply

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While studying the contents of Volume 571 (1991) of *Journal of Chromatography Biomedical Applications* my attention was raised by a paper by Gopal Chari, Anil Gulati, Rama Bhat and Ian R. Tebbett (pp. 263–270).

In the abstract of the paper, a method for the determination of morphine with a detection limit of 500 pg/ml in biological samples, with the use of UV detection, is promised to the reader. Because our laboratory is a very enthousiastic user of the same detector that is mentioned by the authors, I studied the paper more extensively. The detection limit of 500 pg/ml is very low. Attaining this with a sample volume of 0.4 ml and an injection of 1/3 (50 μl out of 150 μl) of the total extract this leads to an absolute limit of detection of 67 pg. We, until now, considered this beyond the potential of even this detector, especially for a compound with a relative low specific absorbance such as morphine at about 280 nm.

However I discovered some aspects of the presentation of the results that made me hesitate to try to reproduce the method immediately. Please let me comment on these:

(1) No wavelength was given with the chromatograms presented. In the text it was stated that "The best separation and detection was obtained at 280 nm wavelength". As Fig. 1 can hardly be considered informative, the reader could have been informed better, *e.g.*, by presenting the respective spectra of the compounds of interest. Further, it was stated that "The data for each chromatogram were saved ... were used to distinguish between peaks of M ..." (p. 265). In our experience, spectral data become uninterpretably noisy even when a chromatogram, recorded at the monitoring wavelength, shows peaks well above the (absolute) limit of detection [1]. It would have been informative to know the concentration level down to which spectral data are useful for identification, especially because retention times vary significantly, as can be concluded by comparing the chromatograms in Fig. 4 and comparing Fig. 4 with Fig. 2. This effect is recognized by the authors (p. 266).

(2) Fig. 3. (chromatogram of extracted blank

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plasma) shows that of the peaks in the relevant part of the chromatogram (between 3 and 6 min), the absorbance of the highest peak is about 0.01. Fig. 2 shows that the absorbances of the peaks representing the spiked plasma at the 20 ng/ml level (40 times the stated limit of detection) are only around 0.0025. This can only lead to the conclusion that one of them must be a misrepresentation.

(3) In Fig. 4, the height of the morphine peak is about 0.01 absorbance and the chemical "noise" (background = signal from endogenous peaks) is about 0.005 absorbance. This means that, although integration of the peak area could be correct, the spectral data for morphine and metabolites must be distorted significantly [see also comment (1)]. This indicates, in my opinion, that the stated "detection limit" [limit of detection (LOD) is the preferred term] of 500 pg/ml is not calculated from these chromatograms but extrapolated from the "absolute limit of detection" (A-LOD). The A-LOD is determined by measuring/injecting standard solutions [2,3] and is expressed in absolute mass units (*e.g.*, pg) and not concentration units (*e.g.*, pg/ml). When one is determining the LOD (or, in this case, LOQ = limit of quantification, would be more appropriate [2,3]) the background of a blank sample and not the instrument noise should be taken into account, otherwise over-optimism is gained in determining the LOD or LOQ.

(4) The authors describe the determination of the reproducibility under "*Precision*" (p. 268). This paragraph is very confusing. At first sight it appears as if the reproducibility of the retention times is tested. For the "Accuracy" [4,5] the reproducibility of the extraction–recovery is of primary importance. Details on reproducibility of the recovery are mentioned only briefly on p. 266 without giving further information on reproducibility; it is not made clear how intra- and inter-assay precision are determined, etc. Further, testing linearity within a range of 10–50 ng/ml is not very extensive.

(5) No explanation is given in the paper of why a re-equilibrium time of 5 min is needed after monitoring the column effluent for 15 min. One would not consider this necessary in an isocratic run. In this way a question is raised instead of being answered by the authors. In my opinion, this should not be the case in a scientific publication.

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