

# A novel protocol for the analysis of diltiazem to aid interpretation

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**AIMS:** The calcium-channel blocker diltiazem is a highly unstable drug in blood and is rapidly converted by chemical and enzymatic processes to desacetyldiltiazem. Therefore, the measurement of diltiazem alone may not be a true reflection of the concentration immediately after death or sample collection. This protocol describes a means of determining the concentration of diltiazem and desacetyldiltiazem in blood to evaluate the possible degree of in vitro instability with resultant implications for the interpretation.

**METHODS:** Diltiazem plasma calibration (0.3125, 0.625, 1.25, 2.5, 5 and 10 mg/L) and quality control standards (1 mg/L and 5 mg/L) are produced from pure reference material and refrigerated for 1 day to allow initial production of desacetyldiltiazem. The standards are then frozen at -20°C. Continuous instability and conversion of the diltiazem (even at -20°C) enables the determination of desacetyldiltiazem. A 10 mg/L diltiazem plasma standard is prepared freshly and rapidly and is serially diluted to the concentrations of the initial diltiazem frozen standards. This is used for the measurement of diltiazem in the quality control standards, frozen standards and the case sample(s) and determines the current diltiazem concentration. The desacetyldiltiazem concentration is calculated by subtracting the current diltiazem concentration (e.g. 8.2 mg/L) of the frozen standards from the original concentration (e.g. 10 mg/L); in this example producing a desacetyldiltiazem concentration of 1.8 mg/L. The resultant desacetyldiltiazem concentrations of all original frozen standards can be used as calibrants to measure the desacetyldiltiazem concentration in the quality control standards and the case sample(s). The methodology is controlled by the analysis of the quality control standards. For example, addition of the current diltiazem concentration (e.g. 3.7 mg/L) and the desacetyldiltiazem concentration (e.g. 1.3 mg/L) based on the separate calibration curves should be equivalent to the starting concentration (in this example, 5 mg/L).

The protocol can be applied to any method capable of identifying diltiazem and desacetyldiltiazem. For the purpose of this paper and based on existing in-house methodology, HPLC with diode array UV detection following liquid-liquid extraction was used and found to be a rapid and easy method of analysis.

**RESULTS:** The literature indicates therapeutic diltiazem concentrations are typically <0.3 mg/L in plasma, with levels usually >1 mg/L and >6 mg/L associated with overdose and death, respectively. Diltiazem concentrations between 0.54 mg/L and 7.60 mg/L and desacetyldiltiazem concentrations between 0.60 mg/L and 7.14 mg/L have been found in the analysis of 7 cases. The desacetyldiltiazem concentration was higher than the corresponding diltiazem concentration in 4 of the cases analysed. This indicated a possible high degree of degradation prior to analysis and measurement of only diltiazem may have resulted in a concentration that could have been considered to be only within the therapeutic range. For example in one case, a diltiazem concentration of 0.54 mg/L was measured with a corresponding desacetyldiltiazem concentration of 7.14 mg/L – which could be due to overdose followed by degradation.

However, as desacetyldiltiazem is also a metabolite of diltiazem *in vivo*, the possibility of chronic use/metabolism must also be considered. Conversely, if the combined diltiazem and desacetyldiltiazem concentration is lower than that found in fatalities, the results could rule out ingestion of an overdose, having taken into account any instability.

**CONCLUSIONS:** The protocol has enabled the measurement of diltiazem and desacetyldiltiazem in case samples. Although diltiazem overdosage is uncommon it has been a useful aid in evaluating the toxicological significance if suspected. It is strongly recommended that laboratories consider the concentrations of both diltiazem and desacetyldiltiazem when investigating such cases.

**KEYWORDS:** *Diltiazem, Instability, Interpretation*

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