A NOVEL PROTOCOL FOR THE ANALYSIS OF DILTIAZEM TO AID INTERPRETATION

Dr S. P. Elliott and Dr C. Seneviratne, Regional Laboratory for Toxicology, City Hospital, Birmingham, U.K.

Aim

The aim of this study was to aid the interpretation of cases involving diltiazem by determining the concentration of diltiazem and its metabolite/breakdown product deacetyldiltiazem (desacetyldiltiazem).

Introduction

The calcium-channel blocker diltiazem is a highly unstable drug in blood and is rapidly converted by chemical and enzymatic processes to deacetyldiltiazem (even in fluoride containers). 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 deacetyldiltiazem in blood to evaluate the possible degree of *in vitro* instability with resultant implications for the interpretation. Deacetyldiltiazem is also a pharmacologically active metabolite.

The literature indicates therapeutic diltiazem concentrations are typically <0.3 mg/L in *in life* plasma, with levels usually >1 mg/L and >6 mg/L associated with overdosage and death, respectively. However, in such cases there was not always measurement/estimation of the amount of deacetyldiltiazem present nor details of the provenance of deacetyl standards.

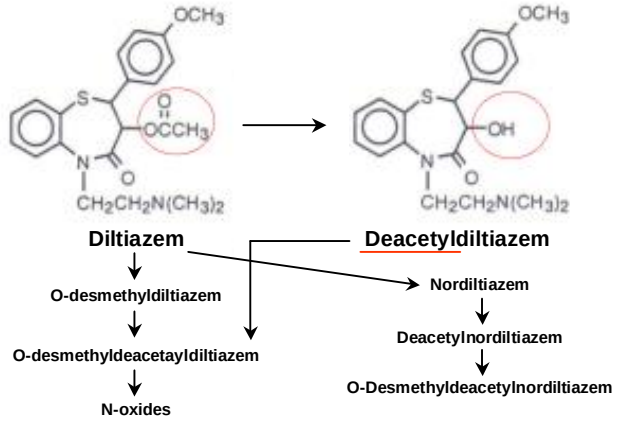

Figure 1. Diltiazem and metabolites. Deacetyldiltiazem is also a breakdown product.

Method (1)

Diltiazem and deacetyldiltiazem analysis performed using high performance liquid chromatography with diode-array UV detection (HPLC-DAD). 500 µL of internals standard (2 mg/L desipramine (in 0.2M Na_2CO_3) was added to 500 µL of sample and extracted with 5 mL of 1-chlorobutane and back extracted into 100 µL of 0.05M H_2SO_4. Chromatography was based on a Waters 5 micron OD/CN column using 30% acetonitrile-buffer mobile phase. 220 nm was used as the detection wavelength. Limit of detection was 0.03 mg/L. Identity of deacetyldiltiazem as sole breakdown product confirmed by LC-MS (M+H = 373)

Method (2)

1. Deacetyldiltiazem standards produced from fresh diltiazem plasma standards that are allowed to degrade. Standards and QCs are then frozen at –20ºC. Continuous instability and conversion of the diltiazem still occurs at –20ºC.

2. Fresh diltiazem plasma standard prepared and rapidly serially diluted to measure diltiazem content in the QCs, frozen (deacetyl) standards and the case sample(s). This determines the current diltiazem concentration.

3. Deacetyldiltiazem concentration 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 deacetyldiltiazem concentration of 0.8 mg/L.

4. Resultant deacetyldiltiazem concentrations of original frozen standards used as calibrants to measure the deacetyldiltiazem concentration in the QCs and the case sample(s).

5. Methodology controlled by the analysis of QCs (1 mg/L and 5 mg/L). For example, addition of the current diltiazem concentration (e.g. 3.7 mg/L) and the deacetyldiltiazem 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).

Results

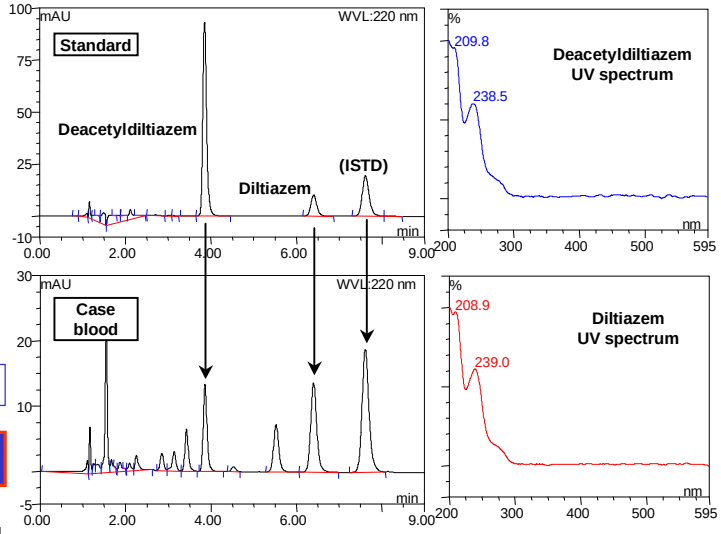

Figure 2. UV chromatograms of diltiazem and deacetyldiltiazem in frozen degraded 5mg/L plasma standard and Case 7 (Table) blood sample. Other peaks in case chromatogram are additional metabolites (based on UV spectra). UV spectra of metabolites are identical.

Case	Circumstances	Sample	Diltiazem (mg/L)	Deacetyldiltiazem (mg/L)	Diltiazem:Deacetyl ratio
1	Not known	Blood (plain)	1.28	6.89	0.19
		Blood (preserved)	0.54	7.14	0.08
2	Hanging	Blood (plain)	2.08	2.72	0.76
		Blood (preserved)	3.04	2.34	1.30
3	Not known	Blood (preserved)	1.15	2.65	0.43
4	Found in bed	Blood (plain)	1.76	0.87	2.02
5	Found at home	Blood (plain)	7.60	5.94	1.28
6	Found at home	Blood (plain)	1.25	0.60	2.08
7	Found in bed	Blood (plain)	0.84	2.80	0.30

Table. Results of analysis in fatalities where diltiazem ingestion was suspected. Based on the circumstances of death and the presence of other drugs, diltiazem was the likely cause of death in only Case 5 (also possibly Case 1 due to a delay in receipt).

• Diltiazem concentrations between 0.54 mg/L and 7.60 mg/L and deacetyldiltiazem concentrations between 0.60 mg/L and 7.14 mg/L.

• Deacetyldiltiazem concentration higher than the corresponding diltiazem concentration in 4 of the cases analysed. This would indicate 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 Case 1, a diltiazem concentration of 0.54 mg/L was measured with a corresponding deacetyldiltiazem concentration of 7.14 mg/L – which could be due to overdosage followed by degradation. However, as deacetyldiltiazem 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 - as in the majority of cases presented above.

Conclusions

The method has enabled the measurement of diltiazem and deacetyldiltiazem 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 deacetyldiltiazem when investigating such cases.

References

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