

DEVELOPMENT OF A HIGHLY SENSITIVE ANTIBODY FOR THE DETECTION OF ZOLPIDEM AND ITS MAJOR METABOLITE

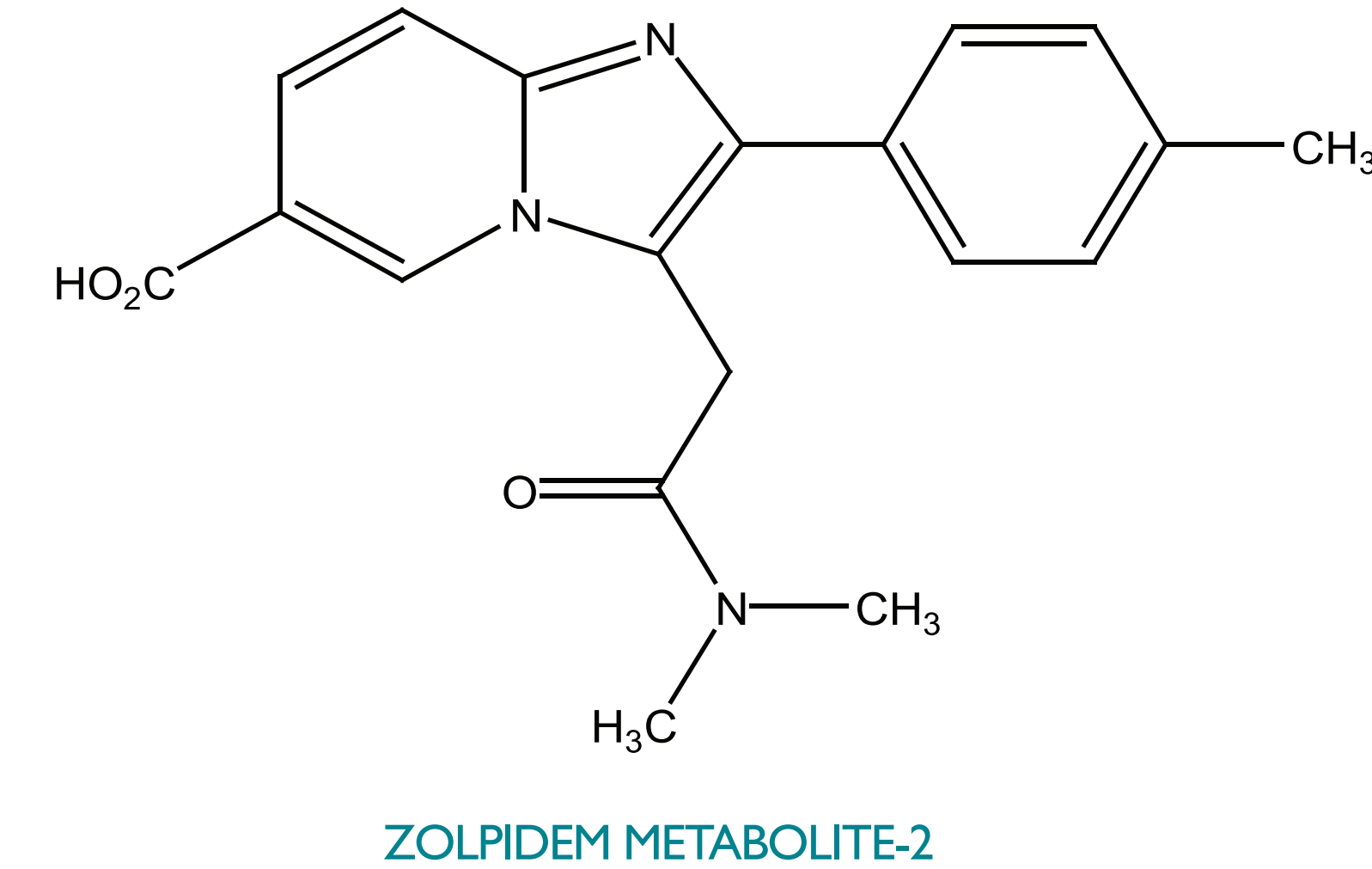
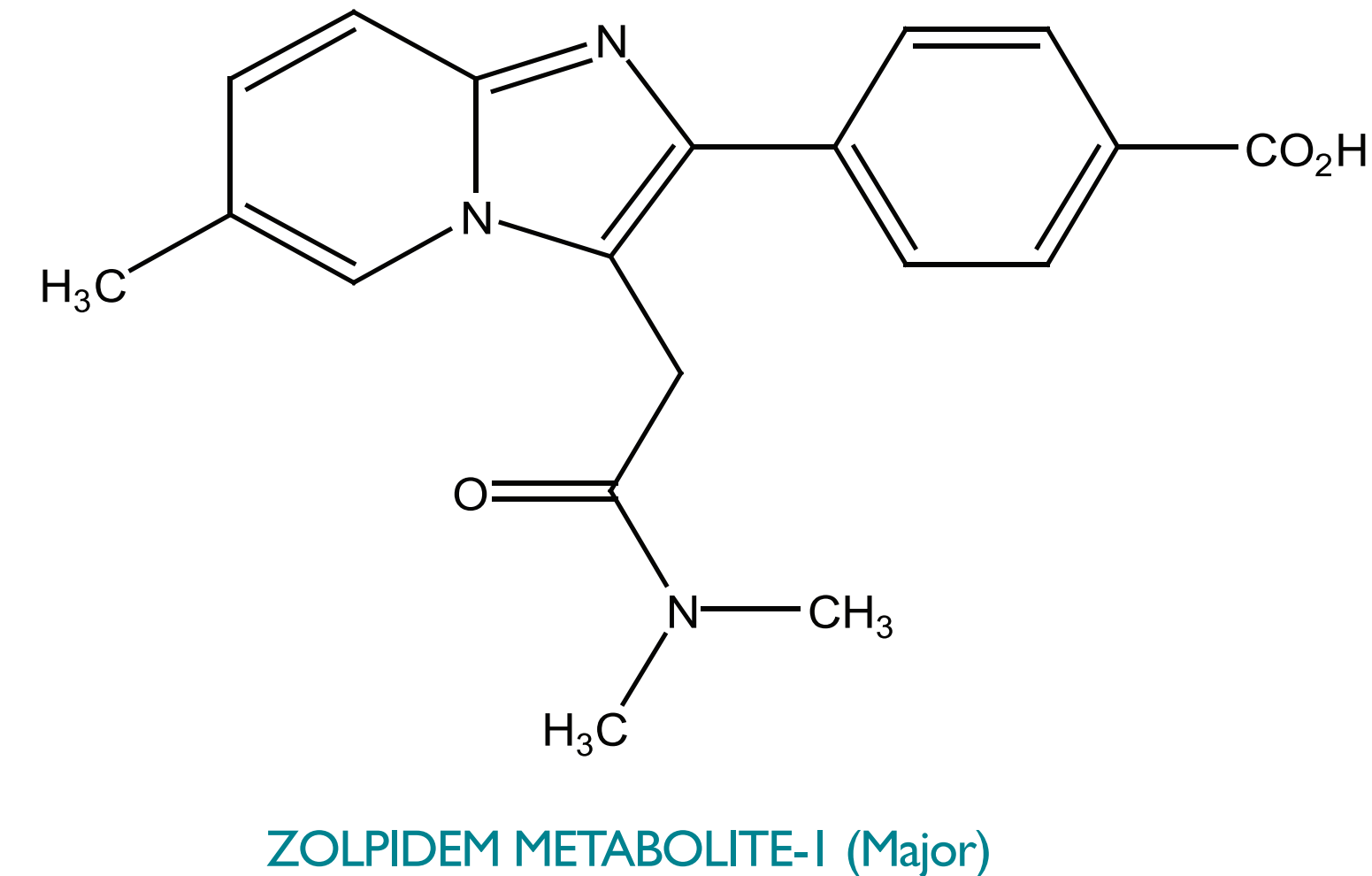
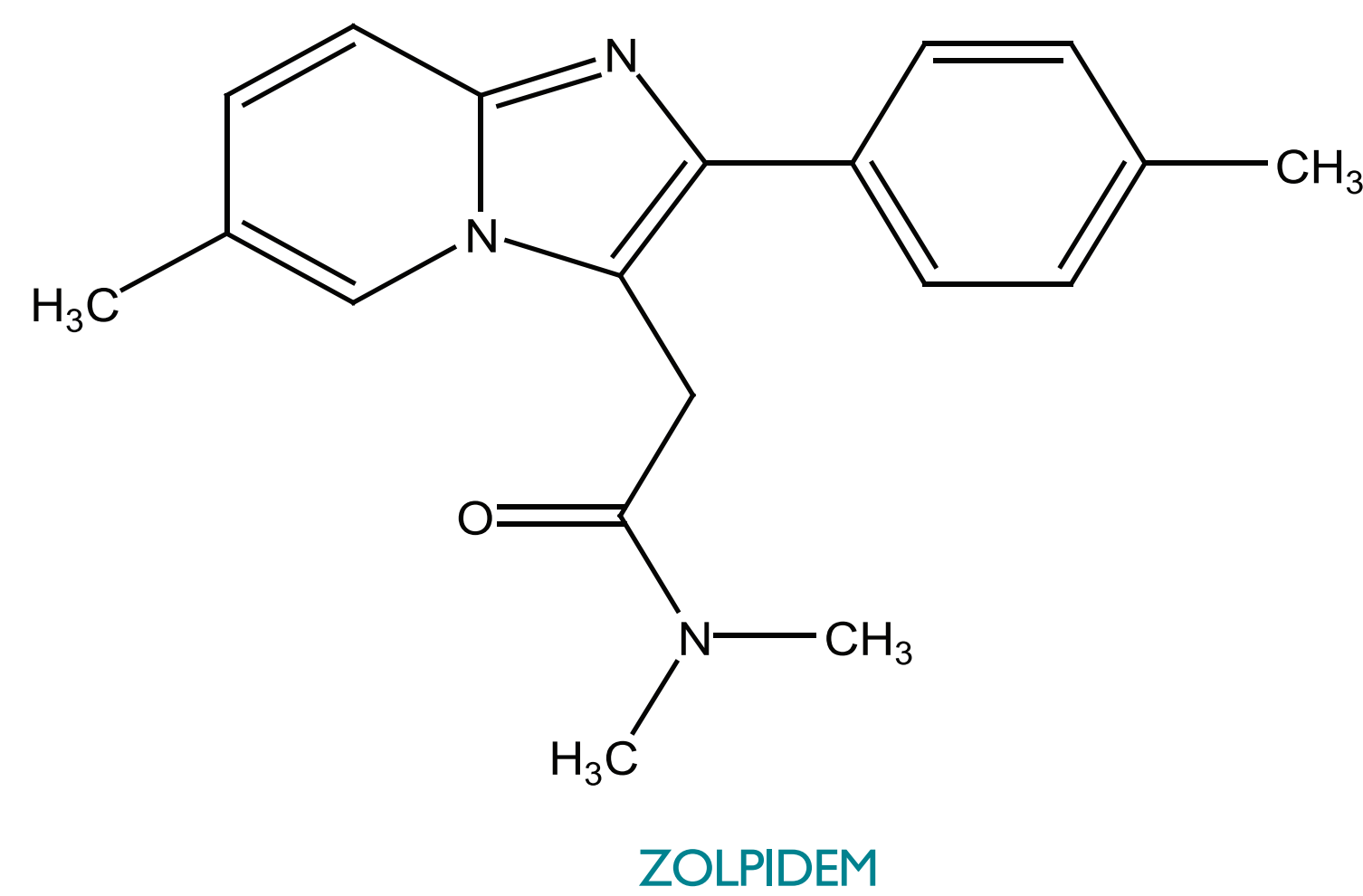
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Introduction

Zolpidem is a nonbenzodiazepine hypnotic drug used for the treatment of insomnia.⁽¹⁾ For monitoring its use or misuse, the availability of efficient immunoassays is relevant. Zolpidem is metabolised to 4-[3-(2-N,N-dimethylamino-2-oxoethyl)-6-yl]-6-methylimidazo[1,2-a]pyridin-2-yl]benzoic acid (80%) and to a lesser extent to 3-(2-N,N-dimethylamino-2-oxoethyl)-2-(4-methylphenyl)imidazo[1,2-a]pyridin-6-yl carboxylic acid.⁽²⁻⁵⁾ Due to the rapid and varied inter-individual metabolism of zolpidem,^(6,7) the development of screening tests which detect zolpidem and its main metabolite would enable the detection of the drug beyond approximately 8-24 hours.

We report the development of a sensitive **polyclonal antibody** developed for the detection of **zolpidem and its major metabolite**. This is of value for the development of effective immunoassays for application to toxicological, forensic and clinical settings.

Chemical Structures



Methodology

The immunogen was produced by conjugation of derivatised zolpidem to bovine thyroglobulin (BTG) as carrier. The immunogen was administered to adult sheep on a monthly basis for the generation of polyclonal antiserum. IgG was extracted from the antiserum and evaluated via competitive immunoassay.

The antibody was immobilized on a biochip platform (9mm x 9mm), which is also the vessel for the immunoreactions. The semi-automated analyser Evidence Investigator (EV3602, Randex Laboratories Ltd., Crumlin, Northern Ireland) was used.

Assay evaluation parameters:

- Sensitivity:** calibration curves were generated over 9 concentrations. B/B₀ values were calculated, where B is the signal measured for x ng/ml of the analyte and B₀ is the signal measured in the absence of analyte. The IC₅₀ was calculated by taking 50% of signal from zero calibrator and reading the value from the x-axis (concentration in ng/ml) in the calibration curve.
- Specificity:** specificity, expressed as % cross-reactivity (%CR), was calculated as follows:
%CR = [IC₅₀(zolpidem) / IC₅₀(cross-reactant)] × 100
- Precision:** intra-assay precision was determined from the results of 6 replicates at different concentration levels within the same run. Results were expressed as %CV values.

Conclusions

This study reports the development of a highly sensitive polyclonal antibody for the detection of zolpidem and its main metabolite, which consequently extends the detection window.

This antibody is of value for the development of efficient immunoassays to monitor use or abuse of this drug.

References:

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6. Reidy, L., et al. *J. Anal. Toxicol.* 2008, 32: 688-694.
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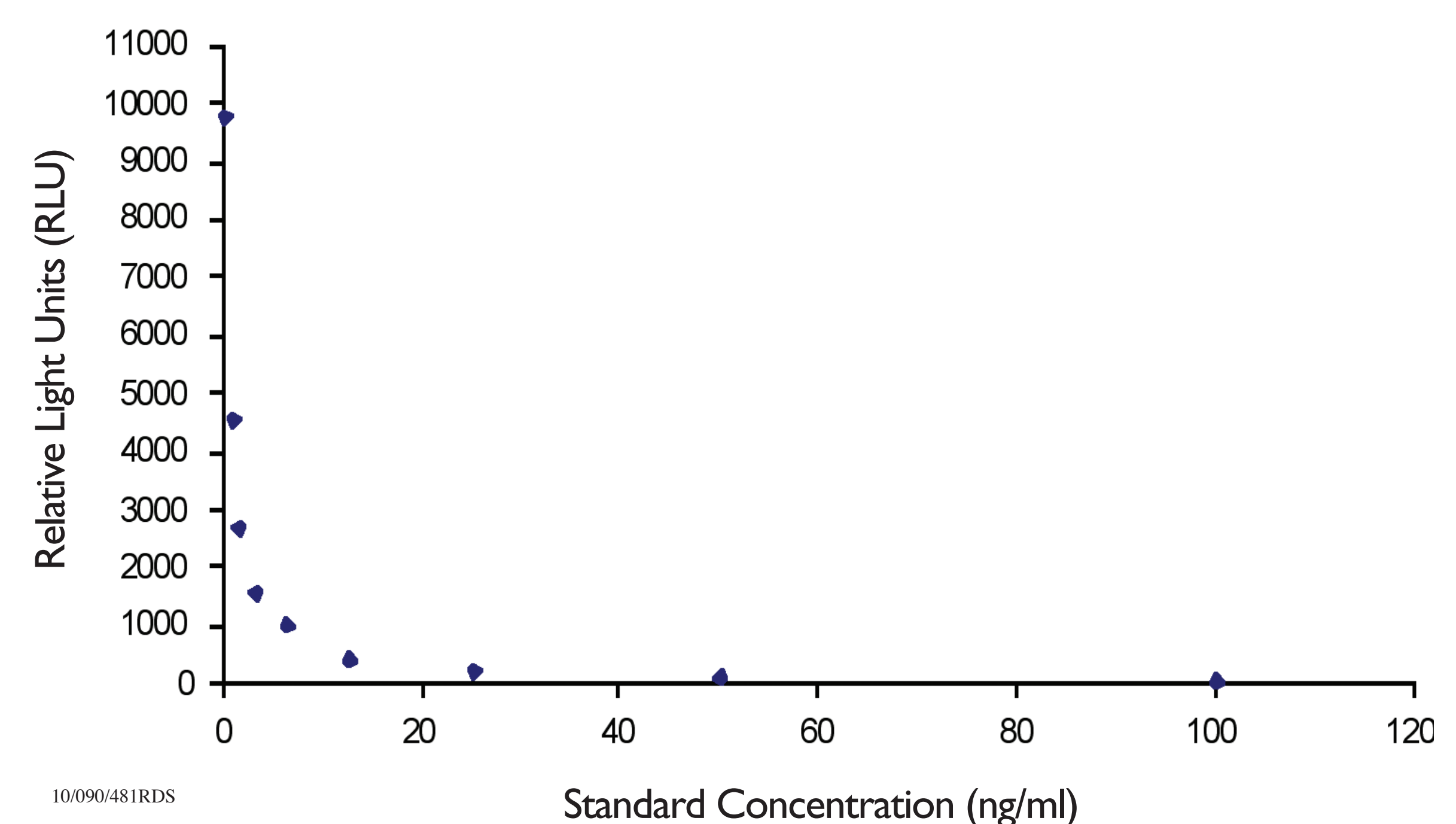
Results

Initial evaluation results are reported.

Polyclonal antibody for the detection of zolpidem Sensitivity

Analyte	Calibration range (ng/ml)	IC ₅₀ (ng/ml)
Zolpidem	0-100	0.693

Calibration curve



Precision

Intra-assay precision (n=6) %CV <10

Specificity

Analyte	Specificity (% cross-reactivity)
Zolpidem tartrate	100
Zolpidem phenyl-4-carboxylic acid	71
Zolpidem 6-carboxylic acid	<1
Alpidem	<1