

DEVELOPMENT OF SENSITIVE POLYCLONAL ANTIBODIES FOR THE DETECTION OF ZALEPLON AND ZOLPIDEM

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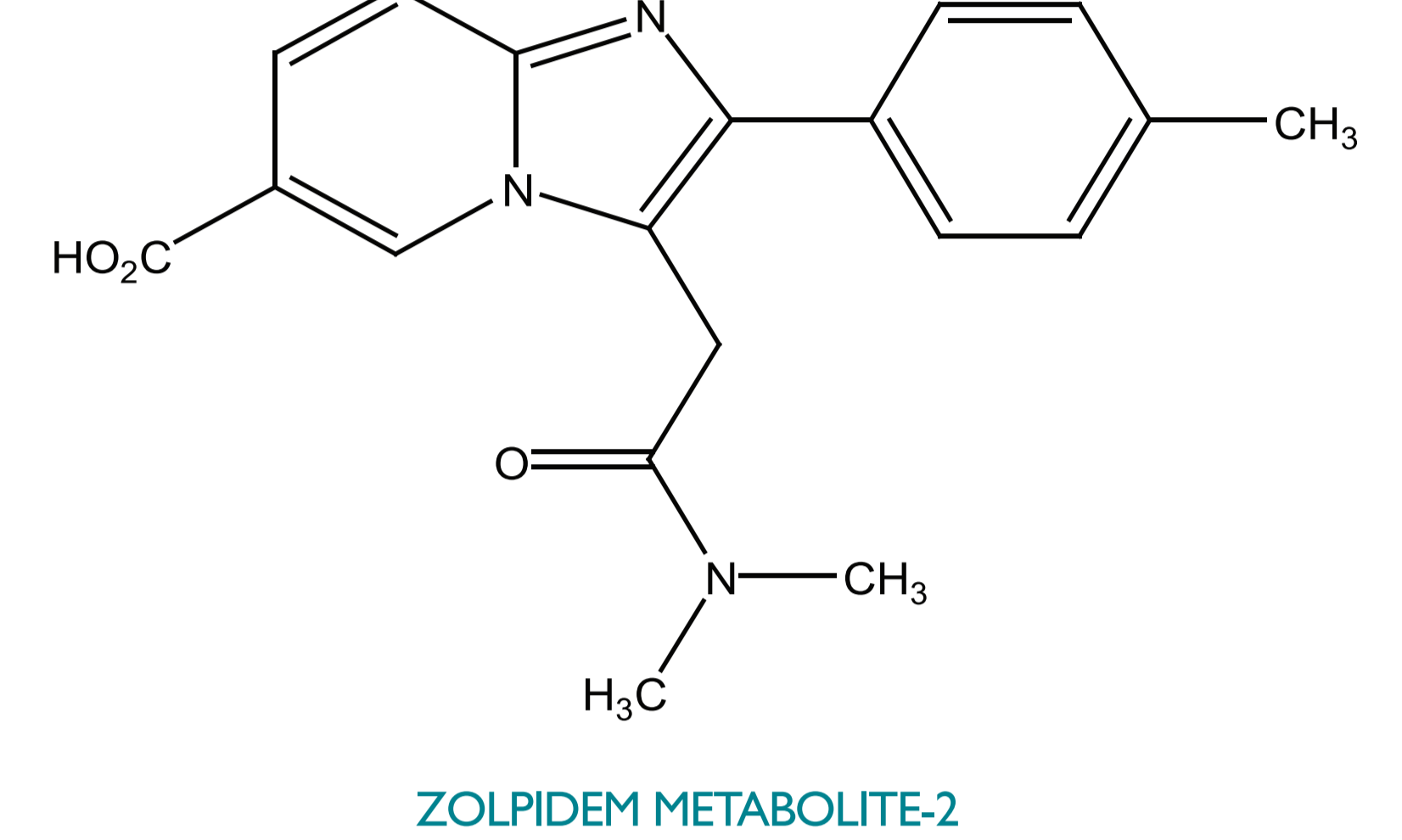
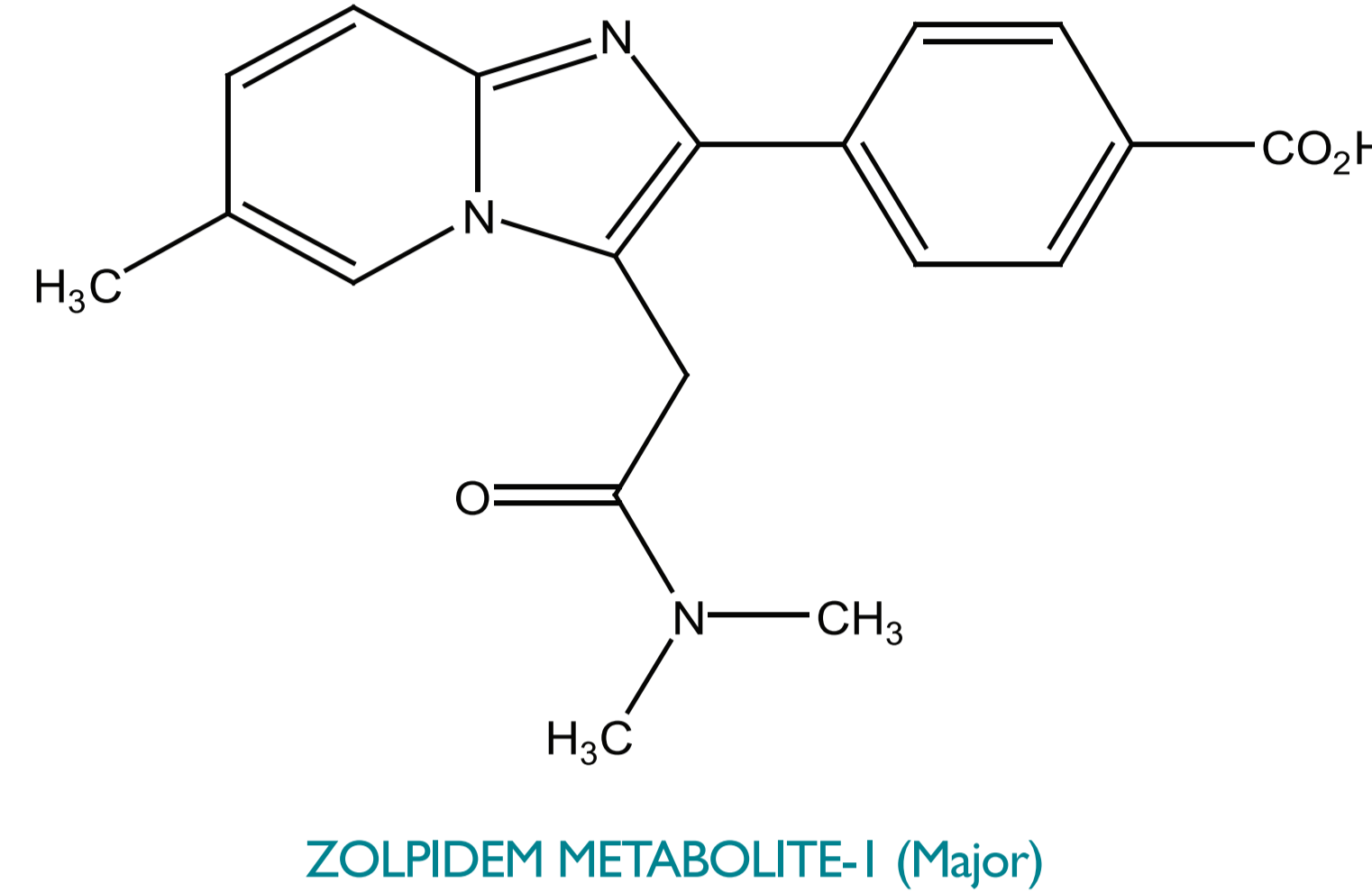
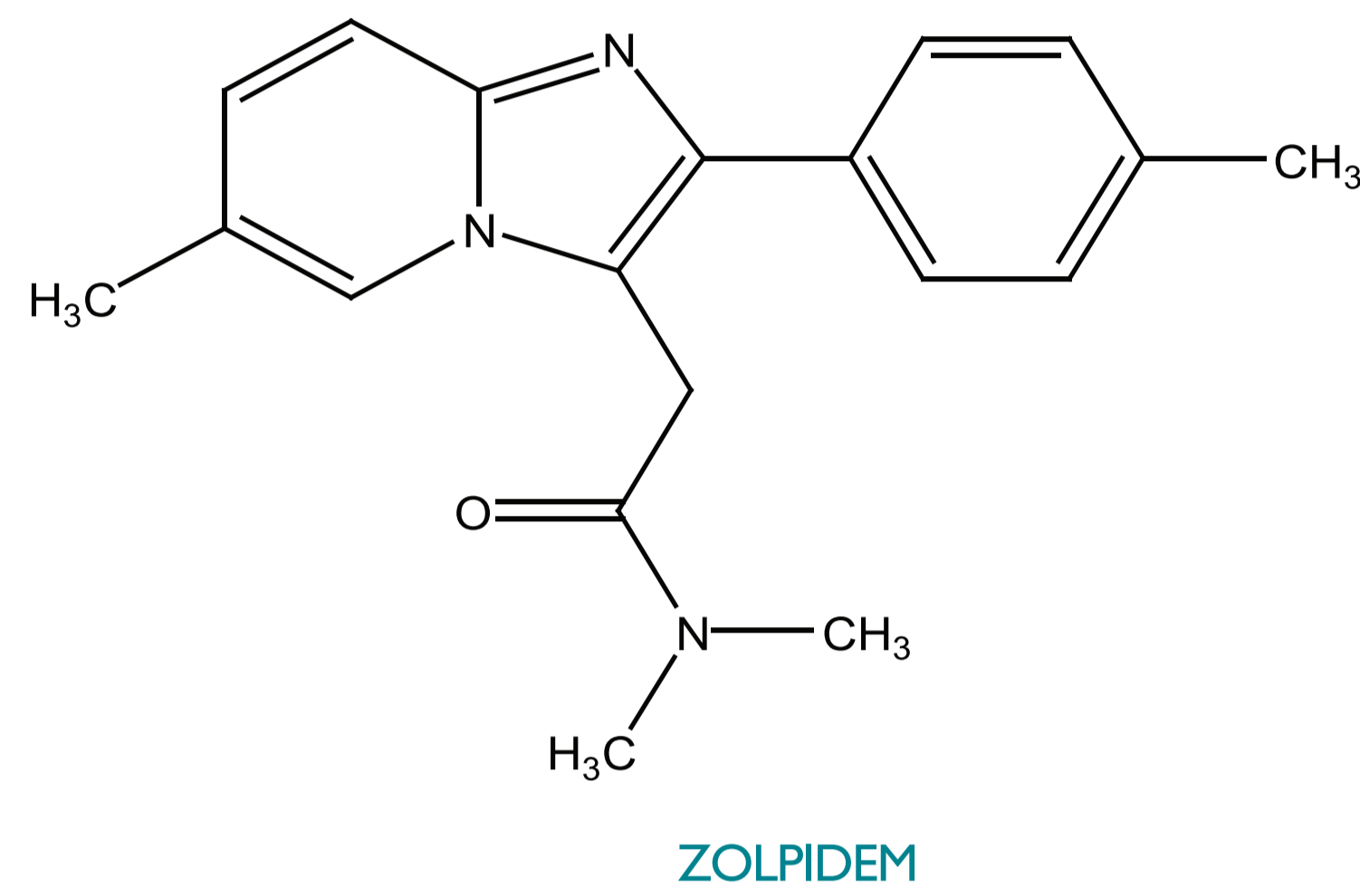
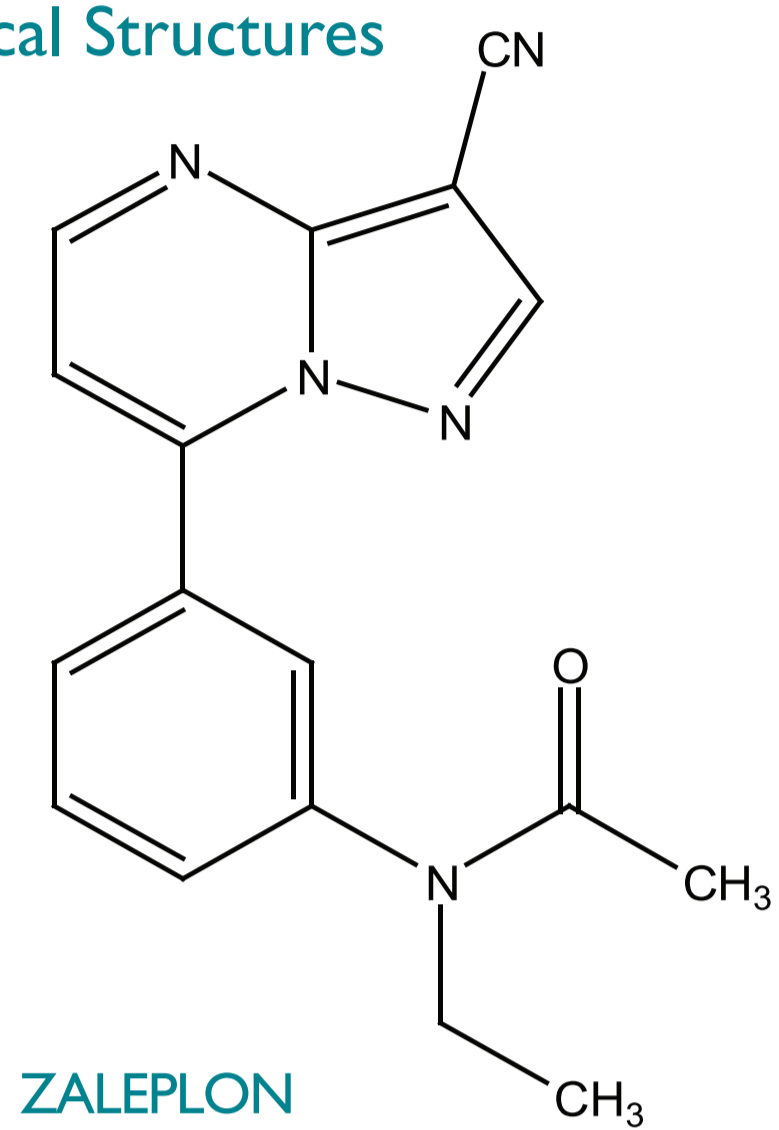
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Introduction

Zaleplon and zolpidem are nonbenzodiazepine hypnotic drugs used for the treatment of insomnia.⁽¹⁾ For monitoring their use or misuse, the availability of efficient immunoassays is relevant. Zaleplon is rapidly and almost completely absorbed following oral administration and peak concentrations are reached in approximately one hour. 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 two sensitive polyclonal antibodies, one developed for the detection of zaleplon, the other 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

Two immunogens were produced by conjugation of derivatised zaleplon (through the CN position) and derivatised zolpidem to bovine thyroglobulin (BTG) as carrier. The two immunogens were administered separately to adult sheep on a monthly basis for the generation of polyclonal antisera. IgG was extracted from the antisera and evaluated via competitive immunoassay.

The antibodies were immobilized on a biochip platform (9mm x 9mm), which is also the vessel for the immunoreactions. The semi-automated analyser Evidence Investigator (EV3602, Radox 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,** expressed as % cross-reactivity (%CR) was calculated as follows:

$$\%CR = [IC50(\text{analyte}) / IC50(\text{cross-reactant})] \times 100$$
- 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.

Results

Initial evaluation results are reported.

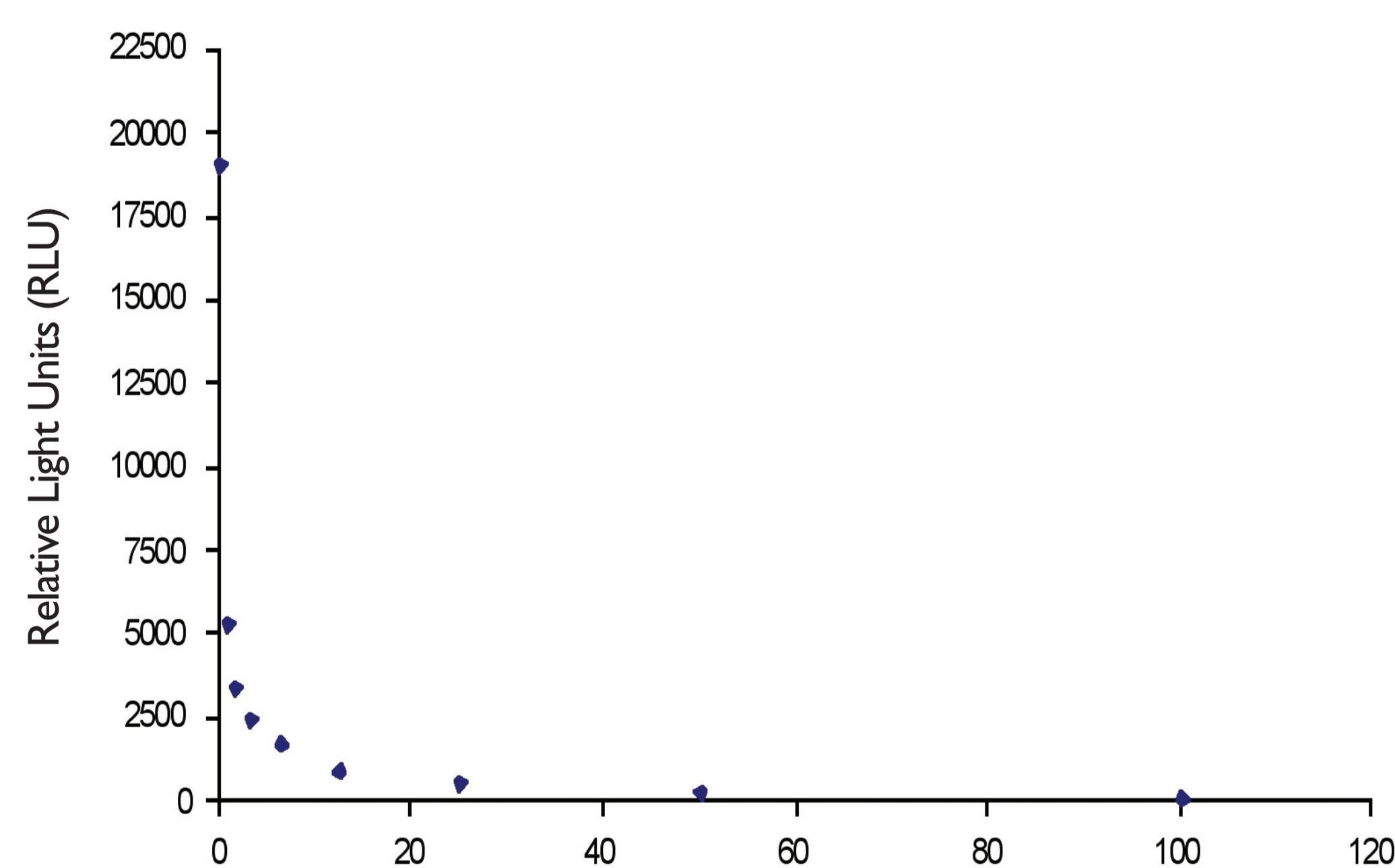
Polyclonal antibody for the detection of zaleplon

Sensitivity

| Analyte | Calibration range (ng/ml) | IC50 (ng/ml) |
|----------|---------------------------|--------------|
| Zaleplon | 0-100 | 0.232 |

10/09/0481RDS

Calibration curve



10/09/0481RDS

Precision

Intra-assay precision (n=6) %CV <7

10/09/0481RDS

Specificity

| Compound | Specificity (% cross-reactivity) |
|----------|----------------------------------|
| Zaleplon | 100 |

10/07/1481RDS

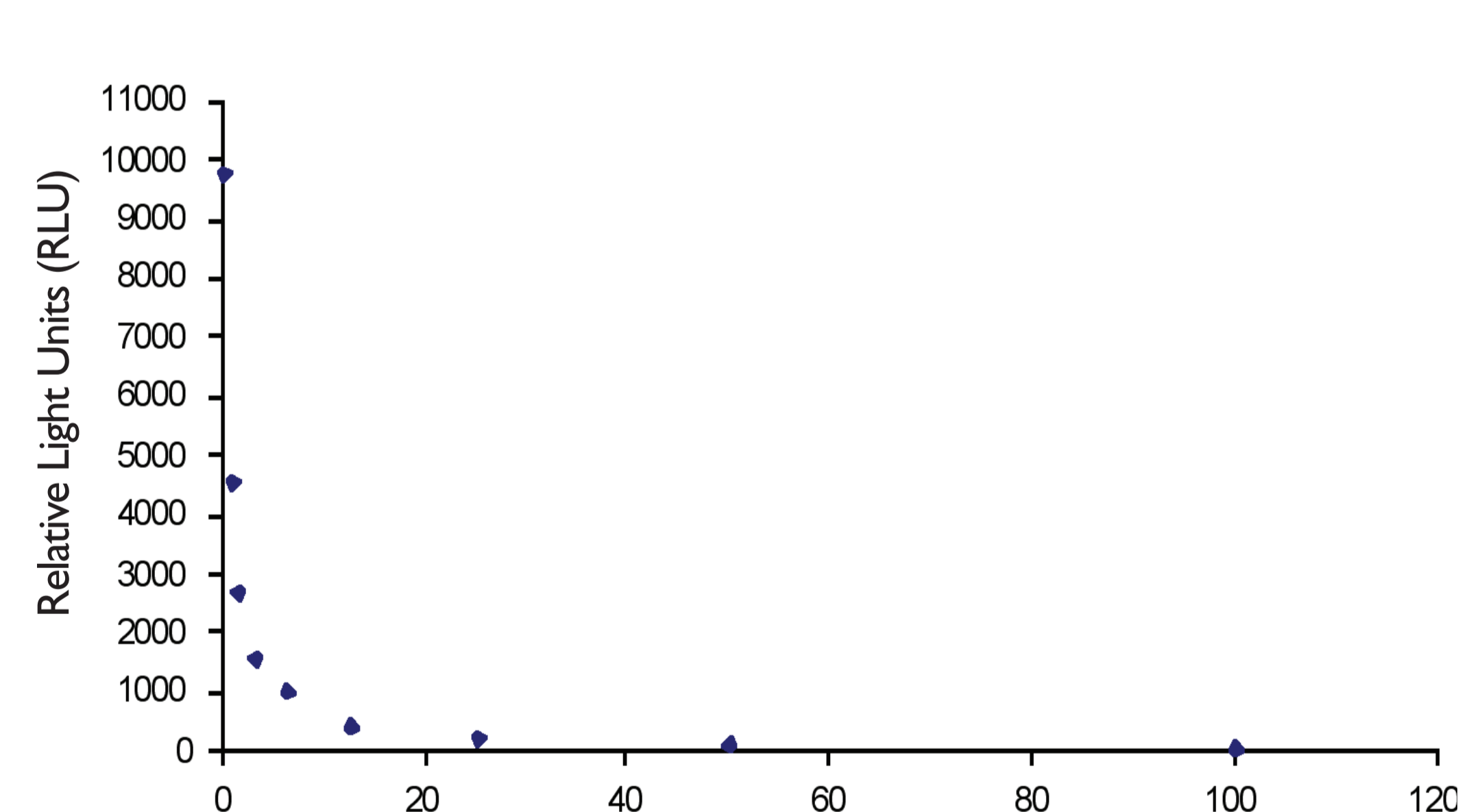
Polyclonal antibody for the detection of zolpidem

Sensitivity

| Analyte | Calibration range (ng/ml) | IC50 (ng/ml) |
|----------|---------------------------|--------------|
| Zolpidem | 0-100 | 0.693 |

10/09/0481RDS

Calibration curve



10/09/0481RDS

Precision

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

10/09/0481RDS

Specificity

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

10/06/1481RDS

Conclusions

This study reports the development of two highly sensitive polyclonal antibodies, one for the detection of zaleplon and the other for the detection of zolpidem. The latter also detects the main metabolite and consequently extends the detection window.

These two antibodies are of value for the development of efficient immunoassays to monitor use or abuse of these drugs.

References:

1. What's wrong with prescribing hypnotics? *Drug Ther. Bull.* 2004, 42(12): 89-93.
2. Ascalone, V., et al. *J. Chromatogr.* 1992, 581: 237-250.
3. Hempel, G. and Blaschke, G. *J. Chromatogr.* 1996, B675: 131-137.
4. von Moltke, L. et al. *Br. J. Clin. Pharmacol.* 1999, 48: 89-97.
5. Salva, P. and Costa, J. *Clin. Pharmacokin.* 1995, 29: 142-153.
6. Reidy, L., et al. *J. Anal. Toxicol.* 2008, 32: 688-694.
7. Villain, M., et al. *Forensic Sci. Int.* 2004, 143: 157-161.