DEVELOPMENT OF A HIGH SENSITIVITY ANTIBODY FOR THE DETECTION OF ZOLPIDEM

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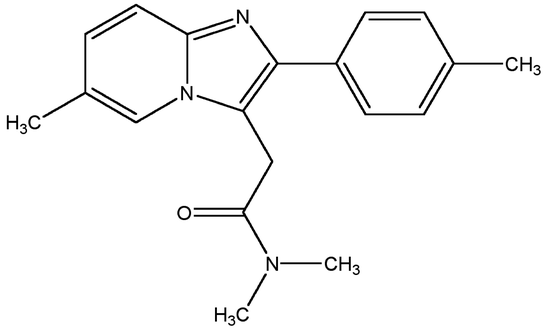
INTRODUCTION

Zolpidem is a short-action non-benzodiazepine hypnotic drug. Zolpidem has been documented as being responsible for some behavioural effects consistent with sedative properties such as suppression of locomotor activity and procumbent posture in animal models. Moreover, reports dealing with "drug-facilitated crimes" (robbery, mugging, sexual assault) related to benzodiazepines and benzodiazepine-like hypnotics are tending to increase because of their short half-life, their rapid excretion and/or relatively long delay between intake and urinary sampling.[1]

Immunoassays for the sensitive and specific determination of zolpidem are of interest for therapeutic and toxicological applications. This study reports the development of a **highly sensitive polyclonal antibody for zolpidem**, which is valuable for the development of effective immunoassays for the determination of this compound for therapeutic and toxicological applications.

Chemical Structure



Zolpidem

METHODOLOGY

The immugen comprising zolpidem hapten coupled to bovine thyroglobulin (BTG) as carrier was administered to adult sheep on a monthly basis and polyclonal antiserum was generated. IgG was extracted from the antiserum and evaluated via competitive chemiluminescent immunoassay on biochip to determine antibody performance. The light signal generated was inversely proportional to the concentration of the analyte. The assay was applied to the semi-automated bench top analyser Evidence Investigator.™

Assay evaluation parameters:

- The calibration curves were generated with each of the analytes as standards in the competitive assay. B/B0 values were calculated where B is the relative light unit (RLU) for x ng/ml of the analyte and B0 is the RLU in the absence of analyte.
- The IC50 for each analyte was calculated by taking 50% of the RLU from the zero calibrator and reading this RLU value from the x-axis (concentration in ng/ml) of the respective calibration curve. This concentration corresponded to the inhibitory concentration that produced 50% inhibition.
- Specificity: the specificity, expressed as %cross-reactivity (%CR) was calculated as follows: %CR=[IC50(zolpidem)/IC50 (cross-reactant)]x100
- Intra-assay precision was determined from the results of 2 replicates at different concentration levels within the same run. Results were expressed as %CV.
- Initial assessment of positive whole blood samples: 19 confirmed positive samples by GC/MS were analysed using the biochip immunoassay and results were compared.

RESULTS

Results corresponding to the initial antibody evaluation are presented.

IC50

Analyte	Calibration range (ng/ml)	IC50(ng/ml)
Zolpidem	0-125	1.4

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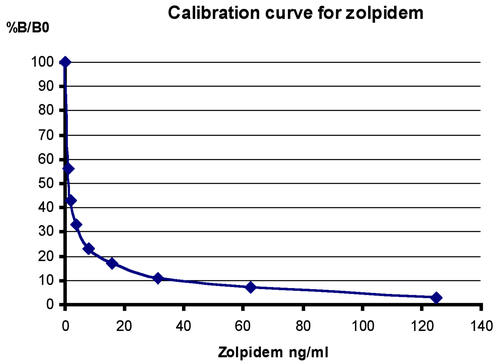

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Specificity

Analyte	%Cross-reactvity
Zolpidem	100
Alpidem	3.7
Zolpidem carboxylic acid	<1
Flumazenil	<1

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Precision

Intra-assay precision (n=2x8)	
Zolpidem	Whole blood
	%CV
Level 1	4
Level 2	7
Level 3	3
Level 4	1
Level 5	5
Level 6	8
Level 7	8
Level 8	1

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Intitial evaluation of positive whole blood samples

Sample	Zolpidem screening on biochip	GC/MS Zolpidem ng/ml
1	+	74
2	+	100
3	+	152
4	+	1500
5	+	198
6	+	39
7	+	64
8	+	850
9	+	74
10	+	246
11	+	1100
12	+	840
13	+	197
14	+	2500
15	+	200
16	+	1450
17	+	170
18	+	48
19	+	2500

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CONCLUSIONS

This study reports the development of a high sensitivity **polyclonal antibody for zolpidem**.

Initial evaluation of the antibody shows specificity for zolpidem (%cross-reactivity 100) with sensitivity value, expressed as IC50, of 1.4 ng/ml. The intra-assay precision for different concentration levels (n= 2x8) expressed as %C.V. was typically < 8%. Initial analysis of 19 positive whole blood samples indicated detection of the compound in this matrix with agreement with a confirmatory method.

The development of this highly sensitive polyclonal antibody to zolpidem provides a valuable means for the generation of more effective immunoassays for use in the determination of this compound in therapeutic and toxicological applications.

REFERENCES

1. Klupsch F., *et al.* Major metabolites of zolpidem: expeditious synthesis and mass spectra. *Chem. Pharm. Bull.* 2006, **54(9)**: 1318-1321.

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