

DEVELOPMENT OF A HIGHLY SENSITIVE POLYCLONAL ANTIBODY FOR THE DETECTION OF ZILPATEROL

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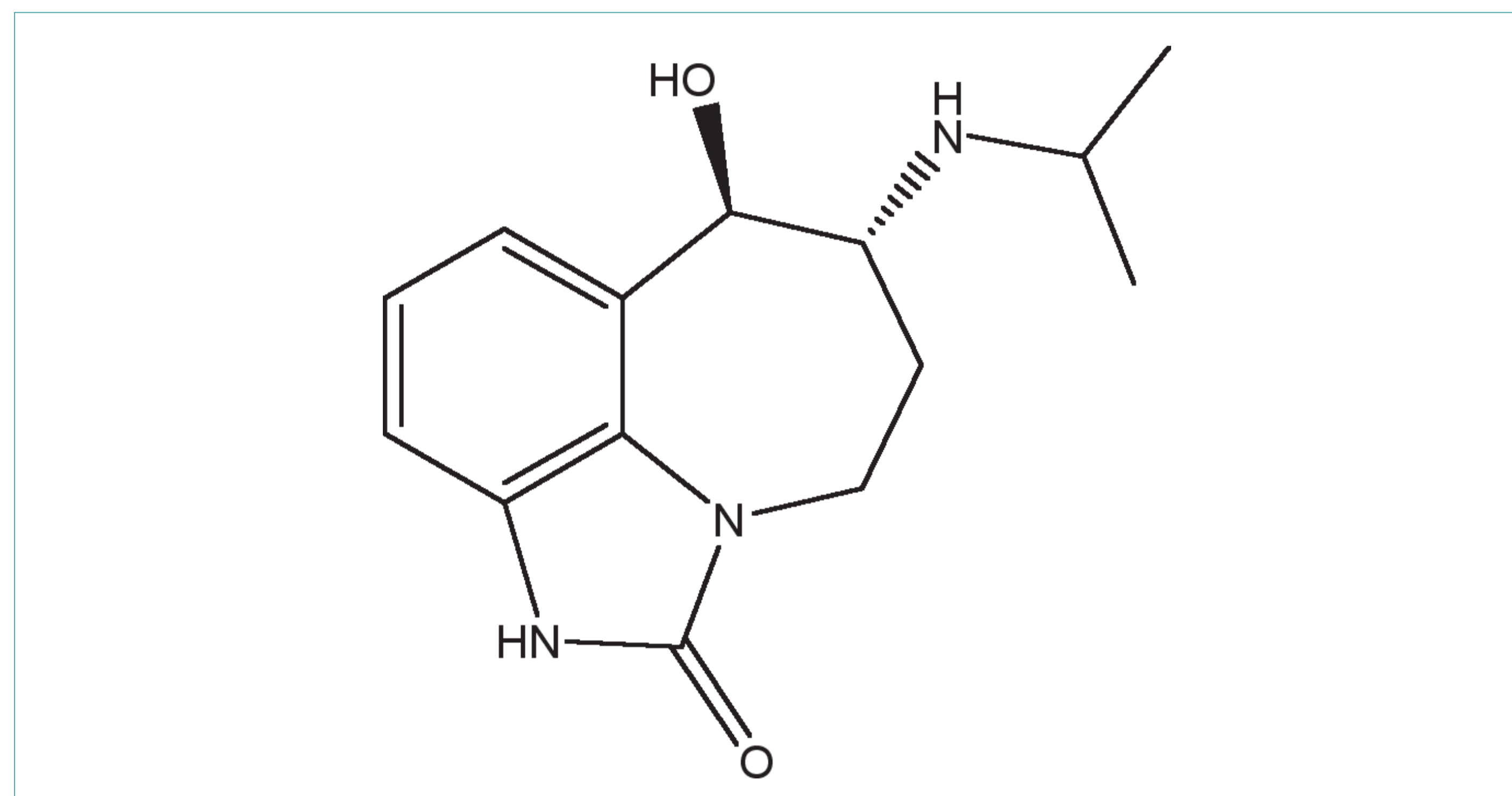
Introduction

Zilpaterol (Zilmax) is a β -adrenergic agonist. β -adrenergic agonists are used to increase carcass leanness, and promote animal growth. Illegal use of this compound has been reported in several countries. The

use of immunoassays enabling specific and sensitive determination of zilpaterol are useful for veterinary drug residue screening. We report the development of a highly specific polyclonal

antibody for the specific detection of zilpaterol, which is of value for the development of immunoassays and biosensors for its determination and application in Veterinary Drug Residue surveillance schemes.

Chemical Structure



Methodology

The immunogen, comprising zilpaterol hapten coupled to bovine thyroglobulin (BTG) as carrier was administered to adult sheep on a monthly basis to generate target-specific polyclonal antiserum. IgG was extracted from the antiserum and evaluated via competitive ELISA. The absorbance was read at 450 nm and was inversely proportional to the concentration of the analyte.

Assay evaluation parameters:

The calibration curves were generated with each of the analyte as standards in the competitive assay. B/B0 values were calculated where B is the absorbance measured at 450 nm for x ng/ml of the analyte and B0 is the absorbance measured at 450 nm in the absence of analyte.

The IC50 for each analyte was calculated by taking 50% of the optical density (OD) from the zero calibrator and reading this OD 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/Cross-reactivity

The specificity, expressed as %cross-reactivity (%CR) was calculated as follows:
%CR=[IC50(zilpaterol)/IC50 (cross-reactant)]x100

Precision

Intra-assay precision was determined from the results of 2 replicates at different concentration levels within the same run. Results were expressed as %CV.



Results

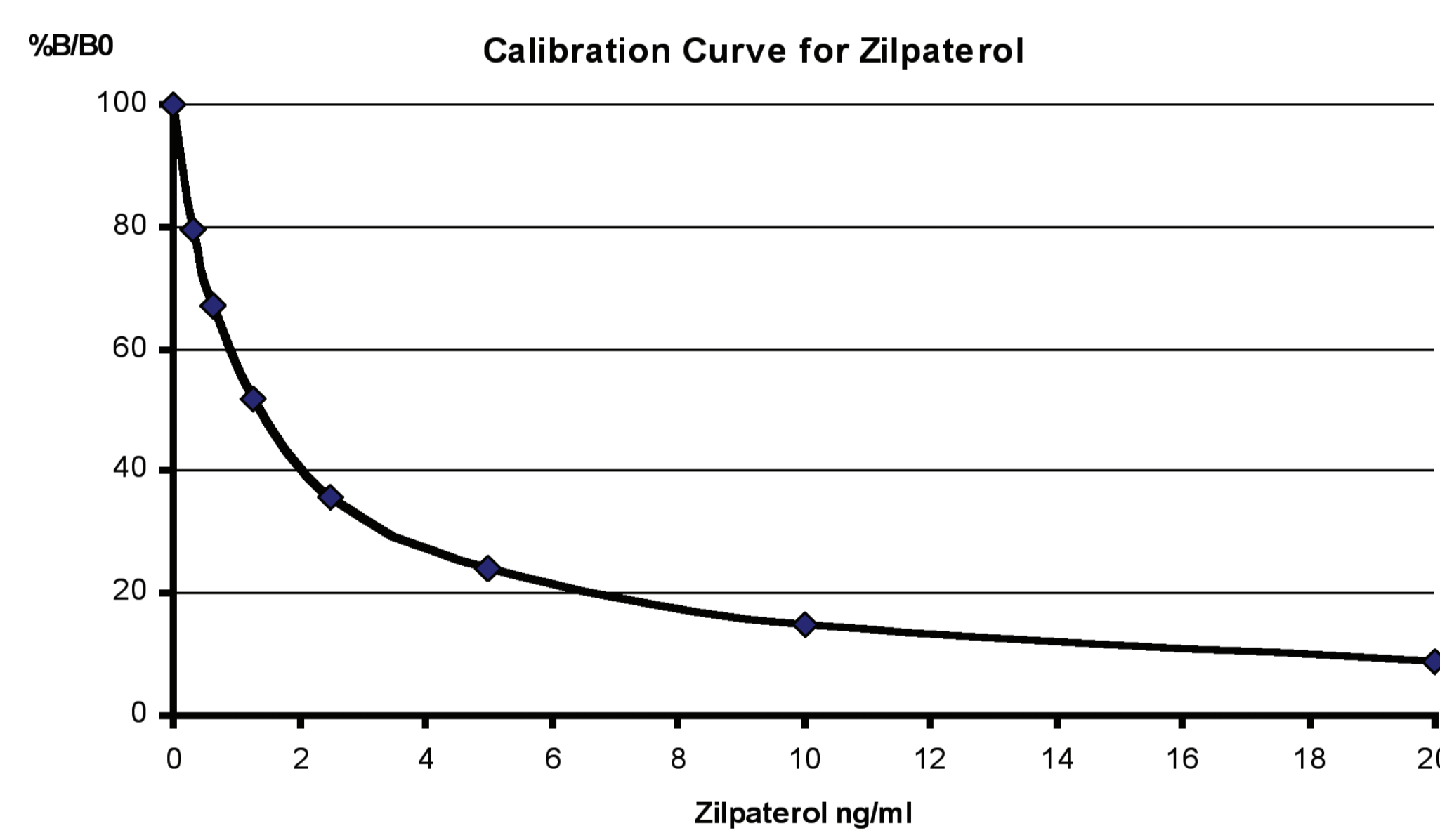
Results corresponding to the initial antibody evaluation are presented:

Sensitivity

Analyte	Calibration Range (ng/ml)	IC50 (ng/ml)
Zilpaterol	0-20	1.34

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Typical calibration curve



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Precision

Analyte	Intra-assay precision (n=8x2)							
	Level 1	Level 2	Level 3	Level 4	Level 5	Level 6	Level 7	Level 8
Zilpaterol	%CV 1.9	%CV 1.2	%CV 1.4	%CV 0.9	%CV 1.6	%CV 2.0	%CV 3.3	%CV 7.1

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Specificity/Cross-reactivity (CR)

Analyte	% CR
Zilpaterol	100
Ritodrine	<0.67
Isoxsuprine	<0.67
Fenoterol	<0.67
Ractopamine	<0.67
Salmeterol	<0.67
Clenbuterol	<0.67
Cimaterol	<0.67
Mapenterol	<0.67
Clenpenterol	<0.67
Salbutamol	<0.67
Terbutaline	<0.67
Cimbuterol	<0.67
Clenproperol	<0.67
Brombuterol	<0.67
Bromchlorbuterol	<0.67
Mabuterol	<0.67

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Conclusion

- Data indicate that the developed polyclonal antibody is highly specific and sensitive for the detection of zilpaterol.
- The antibody presented a sensitivity value expressed as IC50 of 1.34 ng/ml for zilpaterol. The intra-assay precision expressed as %CV is typically $\leq 7.5\%$.
- The antibody is of value for the development of sensitive, specific screening methods for the detection of zilpaterol in test samples.

Reference:

1. 96/22/EC, Council Directive 96/22/EC of 29 April 1996 concerning the prohibition on the use in stockfarming of certain substances having a hormonal or thyrostatic action and of beta-agonists, and repealing Directives 81/602/EEC, 88/146/EEC and 88/299/EEC. OJ., L196. L125. 23.5.1996