

DEVELOPMENT OF A HIGH SENSITIVE POLYCLONAL ANTIBODY FOR THE DETECTION OF **RITODRINE**

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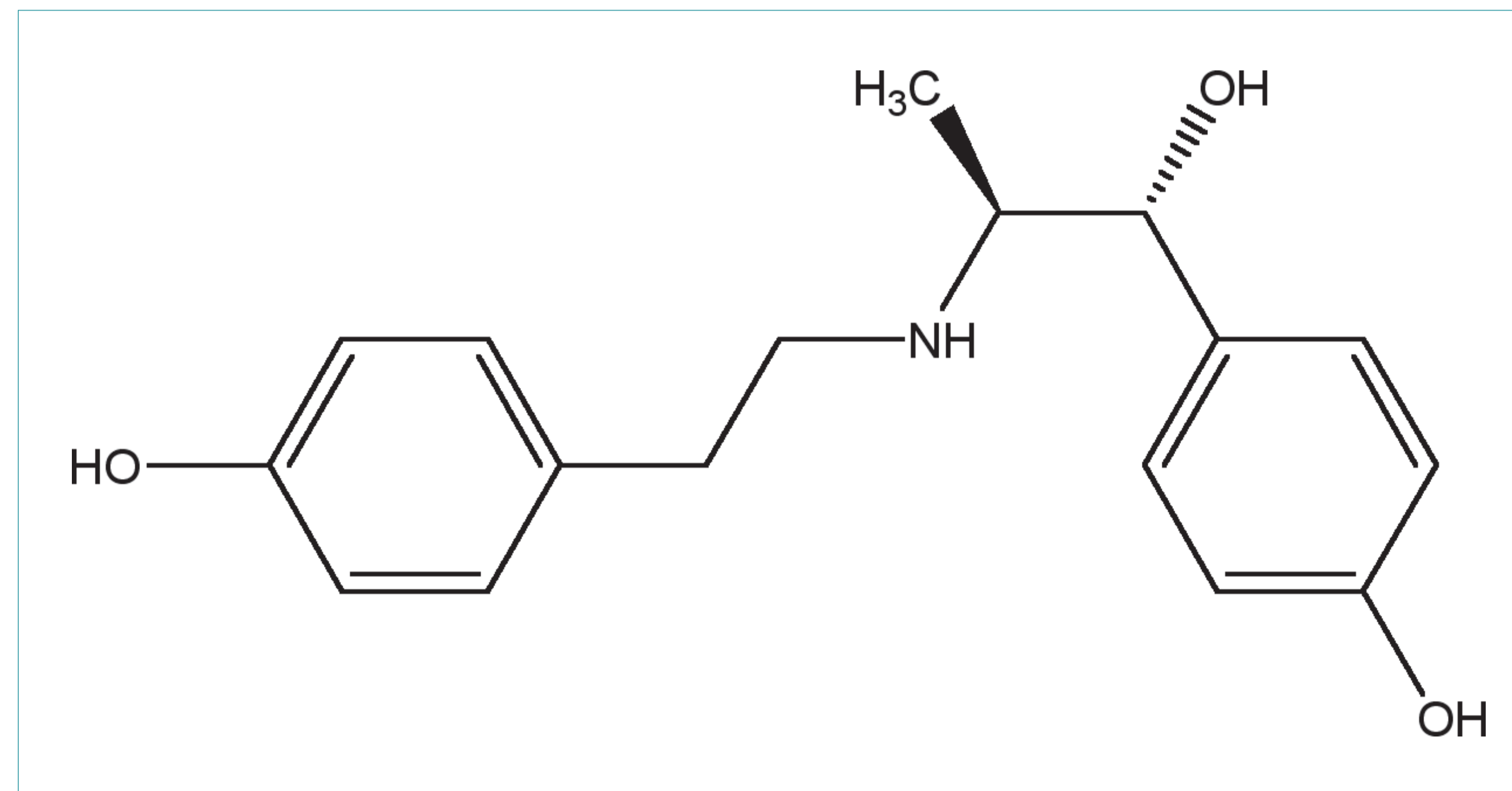
Introduction

Beta-Agonists have been clinically used in the treatment of cardiovascular and respiratory disorders in veterinary and human medicine. They have also been used illegally due to their anabolic effects. The US Food and Drug Administration and the EU have banned the use of the

beta-agonist ritodrine for humans and livestock, but its illegal use still occurs. The development of sensitive analytical methods for the detection of this compound is of interest for regulatory purposes.

This study reports the development of a highly sensitive polyclonal antibody to ritodrine, which is valuable for the development of sensitive immunoassays for the determination of this compound in test samples.

Chemical Structure



Ritodrine

Methodology

The ritodrine hapten was prepared via multi-step organic synthesis and conjugated to the carrier protein bovine thyroglobulin (BTG). The resulting immunogen 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 analytes 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:
 $\%CR = [IC50(\text{ritodrine}) / IC50(\text{cross-reactant})] \times 100$

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.



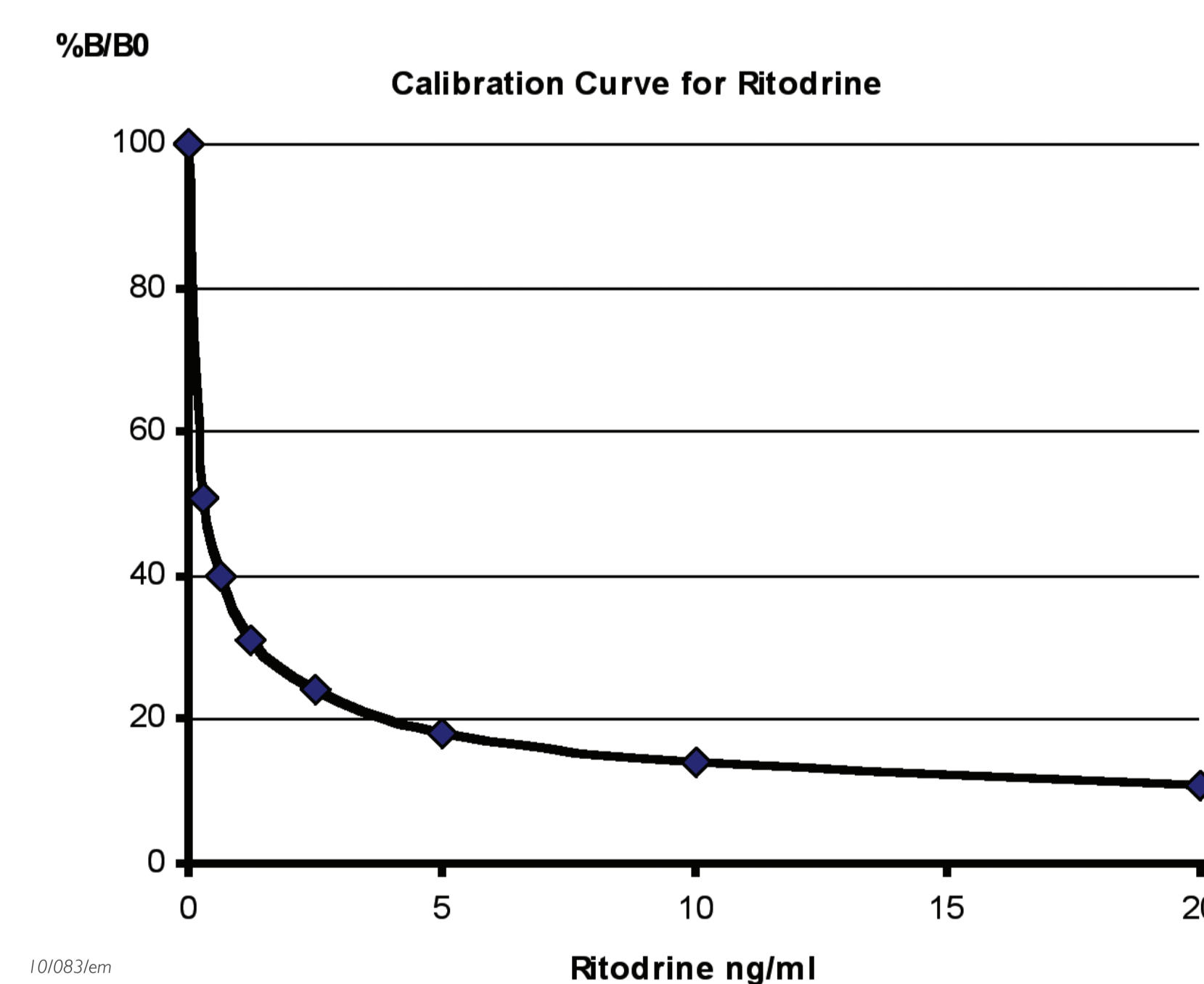
Results

Results corresponding to the initial antibody evaluation are presented:

Sensitivity

Analyte	Calibration Range (ng/ml)	IC50 (ng/ml)
Ritodrine	0-20	0.32
Isoxsuprine	0-200	9.37

Typical calibration curve



Precision

Analyte	Intra-assay precision (n=8x2)							
	Level 1 %CV	Level 2 %CV	Level 3 %CV	Level 4 %CV	Level 5 %CV	Level 6 %CV	Level 7 %CV	Level 8 %CV
Ritodrine	1.6	0.9	0.9	0.8	1.0	3.6	2.1	0.0

Specificity/Cross-reactivity (CR)

Analyte	% CR
Ritodrine	100
Isoxsuprine	3.46
Fenoterol	<0.16
Ractopamine	<0.16
Salmeterol	<0.16
Clenbuterol	<0.16
Cimaterol	<0.16
Zilpaterol	<0.16
Mapenterol	<0.16
Clenpenterol	<0.16
Salbutamol	<0.16
Terbutaline	<0.16
Cimbuterol	<0.16
Clenproperol	<0.16
Brombuterol	<0.16
Bromchlorbuterol	<0.16
Mabuterol	<0.16

Conclusion

- Initial data indicate that the developed polyclonal antibody is specific and sensitive for the detection of ritodrine, exhibiting 100% specificity for ritodrine, 3.46% cross-reactivity with isoxsuprine.

- The antibody presented a sensitivity value expressed as IC50 of 0.32 ng/ml for ritodrine. The intra-assay precision expressed as %CV is typically <4.0%.

- The antibody is of value for the development of sensitive, specific screening methods for the detection of ritodrine 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