

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

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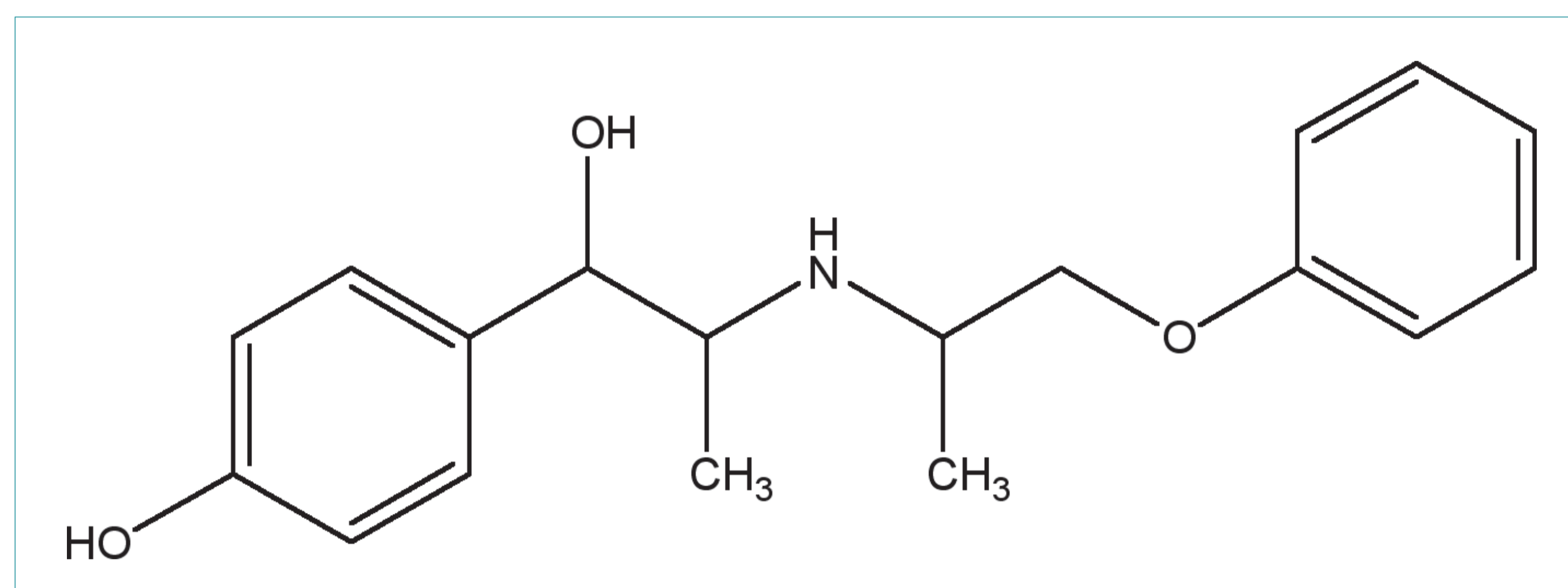
Introduction

Isoxsuprine is a di-phenolic beta(2)-adrenoceptor agonist employed in both veterinary and human medicine. When administered to meat producing cattle, it can act as a repartitioning drug, resulting in increased body weight, a leaner carcass and enhanced feed conversion. However, due to the general ban on beta-agonists within the EC, the use of isoxsuprine as a growth promoting agent is illegal.

The number of analytical methods for beta(2)-agonists that also include isoxsuprine is limited. Haasnoot *et al* (1994) developed an ELISA based method for the fenoterol type beta(2)-agonists, which exhibited low cross-reactivity for isoxsuprine. Brambilla *et al* (1992) described an EIA screening and mass spectrometric confirmation in bovine plasma and urine. Additional mass spectrometric (Dumasia and Houghton,

1991) and HPLC (Hashem and Lubczyk, 1991) methods have been described for equine applications. In view of its general availability and the absence of the analyte in the routine surveillance scheme, a highly sensitive and specific antibody for the detection of isoxsuprine has been developed. This is of value for the generation of rapid screening methods for this analyte.

Chemical Structure



Isoxsuprine

Materials and Methods

Isoxsuprine was modified with a cross-linker and conjugated directly to 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:
 $\%CR = [IC_{50}(\text{isoxsuprine}) / IC_{50}(\text{cross-reactant})] \times 100$

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.



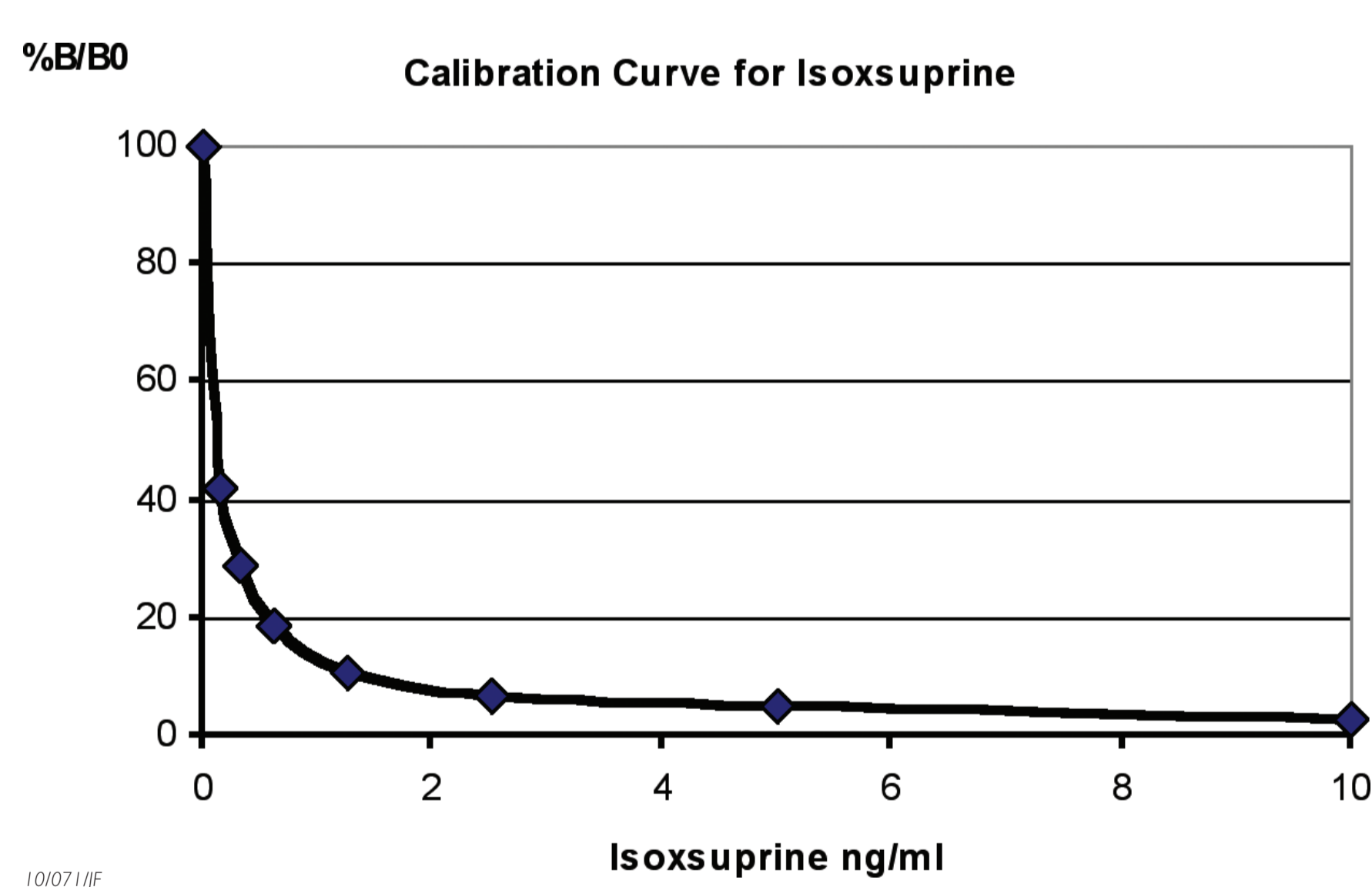
Results

Results corresponding to the initial antibody evaluation are presented:

Sensitivity

Analyte	Calibration Range (ng/ml)	IC ₅₀ (ng/ml)
Isoxsuprine	0-10	0.107

Typical calibration curve



Precision

Analyte	Intra-assay precision (n=8x2)							
	Level 1	Level 2	Level 3	Level 4	Level 5	Level 6	Level 7	Level 8
	%CV	%CV	%CV	%CV	%CV	%CV	%CV	%CV
Isoxsuprine	3.8	3.6	1.2	0.2	3.2	4.4	0.9	11.1

Specificity/Cross-reactivity (CR)

Analyte	% CR
Isoxsuprine	100
Ritodrine	<0.107
Fenoterol	<0.107
Ractopamine	<0.107
Salmeterol	<0.107
Clenbuterol	<0.107
Cimaterol	<0.107
Zilpaterol	<0.107
Mapenterol	<0.107
Clenpenterol	<0.107
Salbutamol	<0.107
Terbutaline	<0.107
Cimbuterol	<0.107
Clenproperol	<0.107
Brombuterol	<0.107
Bromchlorbuterol	<0.107
Mabuterol	<0.107

Conclusion

- Data indicate that the developed polyclonal antibody is sensitive and highly specific for isoxsuprine.
- This polyclonal antibody presented a sensitivity value expressed as IC₅₀ of 0.107 ng/ml for the target molecule. The intra-assay precision expressed as %CV was typically ≤10%
- The polyclonal antibody developed can be used in the development of effective immunoassays for the detection of low levels of this compound in test samples.

References:

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
2. Haasnoot W. *et al*. Determination of fenoterol and ractopamine in urine by enzyme immunoassay. *Analyst*, 1994, **119**: 2675-80.
3. Brambilla G. *et al*. Illegal use of isoxsuprine in animal productions: an ELISA screening and MS-MS confirmation online beef plasma and urine. Proceeding 3TD World Congress Foodborne Infections and Toxicant, Berlin, 1992, 1282.
4. Dumasia M.C. and Houghton E. Screening and confirmatory analysis of beta-agonists, beta-antagonists and their metabolites in horse urine by capillary gas chromatography-mass spectrometry. *J. Chromatogr.* 1991, **564**: 503-13.
5. Hashem A. and Lubczyk B. Determination of isoxsuprine in equine plasma by high-performance liquid chromatography with electrochemical detection. *J. Chromatogr.* 1991, **563**: 216-23.