DEVELOPMENT OF A POLYCLONAL ANTIBODY FOR THE DETECTION OF **SALMETEROL**

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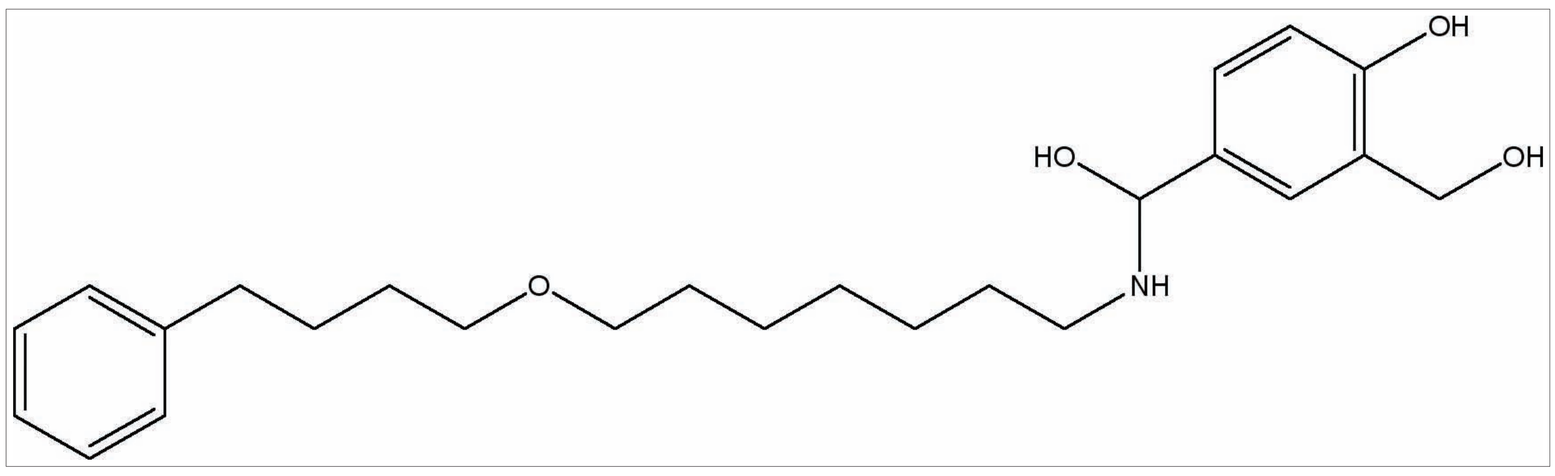
Introduction

Salmeterol (Serevent) is a potent and long-acting ß2-adrenoceptor agonist used as a bronchodilator for the prevention of bronchospasm in patients with asthma and chronic obstructive pulmonary disease (COPD). Anabolic effects of salmeterol are similar to those of clenbuterol and are dependent on the route of administration. The presence of drug residues in animal tissues is a food safety concern. Meat products obtained from beta agonist-fed animals may pose a potential risk to consumers' health. Individuals suffering from muscular tremors, vomiting, nervousness and cardiac palpitations are at particular risk.[1,2] Illegal use of this compound has been reported in several countries. Due to the potential health risks, the development of analytical methods for the detection of this analyte is relevant. A limited number of analytical methods (HPLC and LC-MS) have been reported in the literature for the quantification of the beta(2)-agonist salmeterol[3,4] and no immunological methods have been published to date.

We report the development of a highly sensitive polyclonal antibody for salmeterol, which is of value for the development of immunoassays for the determination of this compound in test samples.

Chemical Structure



Methodology

Salmeterol 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: the specificity, expressed as %cross-reactivity (%CR) was calculated as follows: %CR = [IC50 (salmeterol) / IC50 (cross-reactant)] x 100
- 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)
Salmeterol	0-40	0.95

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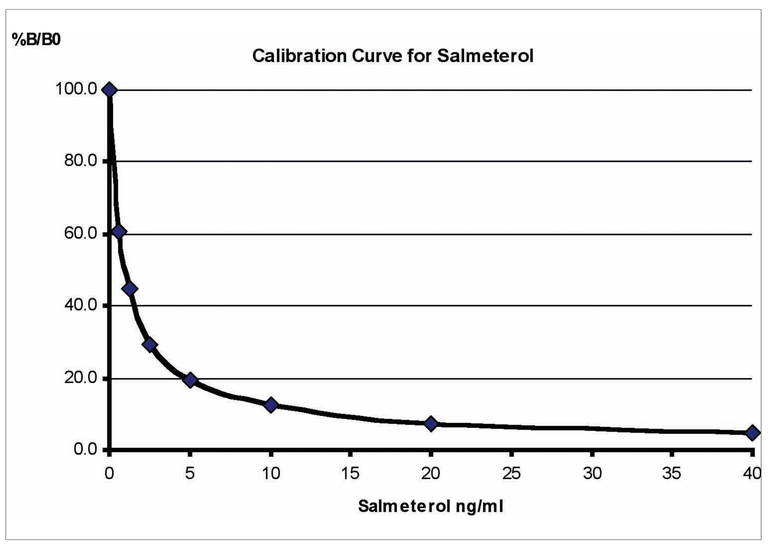
Specificity/Cross-reactivity (CR)

Analyte	%CR
Salmeterol	**100**
Cimaterol	0.25
Ritodrine	<0.19
Isoxsuprine	<0.19
Fenoterol	<0.19
Ractopamine	<0.19
Clenbuterol	<0.19
Zilpaterol	<0.19
Mapenterol	<0.19
Clenpenterol	<0.19
Salbutamol	<0.19
Terbutaline	<0.19
Cimbuterol	<0.19
Clenproperol	<0.19
Brombuterol	<0.19
Bromchlorbuterol	<0.19
Mabuterol	<0.19

Typical calibration curve



Precision

Intra-assay precision (n=8x2)	
Salmeterol	**%CV**
Level 1	0.7
Level 2	3.6
Level 3	1.8
Level 4	4.1
Level 5	0.9
Level 6	3.9
Level 7	8.9
Level 8	7.8

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Conclusion

- Initial data indicate that the developed polyclonal antibody is highly sensitive and specific for the detection of salmeterol.

- The antibody presented a sensitivity value expressed as IC50 of 0.95 ng/ml. The intra-assay precision expressed as %CV is typically ≤9.0%

- The antibody is of value for the development of sensitive, specific screening methods for the detection of salmeterol in test samples.

References:

1. Mitchell, G. A. and G. Dunnavan. Illegal use of β-adrenergic agonists in the United States. *J. Anim. Sci.*, 1998, **76:** 208-211
2. Martinez-Navarro, J. F. Food poisoning related to consumption of illicit β-agonist in liver. Lancet, 1990, **336(8726)**: 1311
3. James, G. Ryall *et al.* Systemic administration of β2-adrenoceptor agonists, formoterol and salmeterol, elicit skeletal muscle hypertrophy in rats at micromolar doses. *British Journal of Pharmacology*, 2006, **147**: 587-595.
4. P. Van Eenoo *et al.* Quantitative detection of salmeterol after inhalation in equine urine by liquid chromatography/tandem mass spectrometry. *Rapid Communication in Mass Spectrometry,* 2002, **16(18)**: 1755-1759

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