DEVELOPMENT OF AN ANTIBODY FOR SPECIFIC HIGH SENSITIVITY DETECTION OF CARISOPRODOL.

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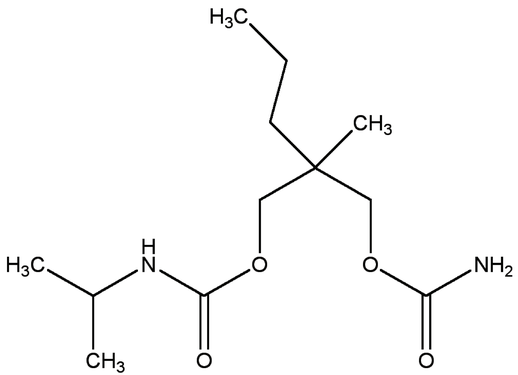
INTRODUCTION

Carisoprodol is a centrally acting skeletal muscle relaxant[1] and it is useful against various types of pain such as low back pain.[2, 3] The abuse potential of carisoprodol has been reported[4]. Carisoprodol is metabolized to meprobamate, which is a barbiturate-like drug that is abused and has associated toxicity and withdrawal.[4,5] Patients for whom carisoprodol is prescribed are at risk of meprobamate dependence.[6]

The use of immunoassays enabling specific and sensitive detection of carisoprodol are useful for clinical and toxicological applications. We report the development of a **high sensitivity polyclonal antibody for the specific detection of carisoprodol**, which is of value for the development of effective immunoassays for its determination and application to toxicology, therapeutic and clinical settings.

Chemical structure:



Carisoprodol

METHODOLOGY

The immunogen comprising carisoprodol 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 ELISA. The absorbance was read at 450 nm and was inversely proportional to the concentration of the analyte.

Assay evaluation parameters:

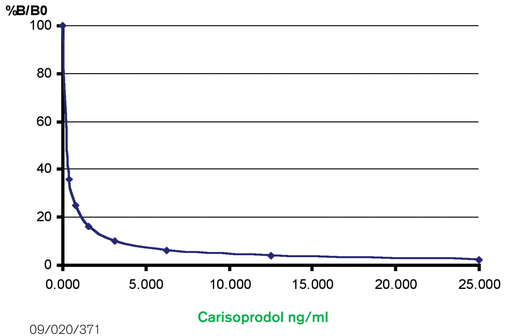
- The IC50 for each analyte was calculated by taking 50% of the optical density (OD) from the zero calibrator and reading this OD value from the x-axis (concentration in ng/ml) of the respective calibration curve. This concentration corresponded to the inhibitory concentration that produced 50% inhibition.
- Specificity: the specificity, expressed as %cross-reactivity (%CR) was calculated as follows: %CR=[IC50(carisoprodol)/ IC50(cross-reactant)]x100
- 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.
- Intra-assay precision was determined from the results of 3 replicates at different concentration levels within the same run. Results were expressed as %CV.

RESULTS

IC50 and specificity

Analyte	Calibration range (ng/ml)	IC50 (ng/ml)	Specificity (% cross-reactivity)
Carisoprodol	0-25	0.198	100
Meprobamate	0-25	-	<0.1

Calibration curve for carisoprodol



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Precision

Intra-assay precision (n=3x8)	
Carisoprodol	%CV
Level 1	1.3
Level 2	1.6
Level 3	0.4
Level 4	1.8
Level 5	2.1
Level 6	4.7
Level 7	1.7
Level 8	2.7

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CONCLUSION

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

Initial evaluation of the antibody shows specificity for carisoprodol (%cross-reactivity 100) with sensitivity value, expressed as IC50, of 0.198 ng/ml. No cross-reactivity with meprobamate (% cross-reactivity <0.1) was found. The intra-assay precision for different concentration levels (n= 3x8) expressed as %C.V. was typically < 5%.

The development of this highly sensitive polyclonal antibody to carisoprodol provides a valuable means for the generation of more effective immunoassays to be used in toxicology, therapeutic and clinical settings.

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