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DEVELOPMENT OF A SENSITIVE POLYCLONAL ANTIBODY FOR

THE DETECTION OF DEXTROMETHORPHAN AND THE MAIN METABOLITE DEXTRORPHAN

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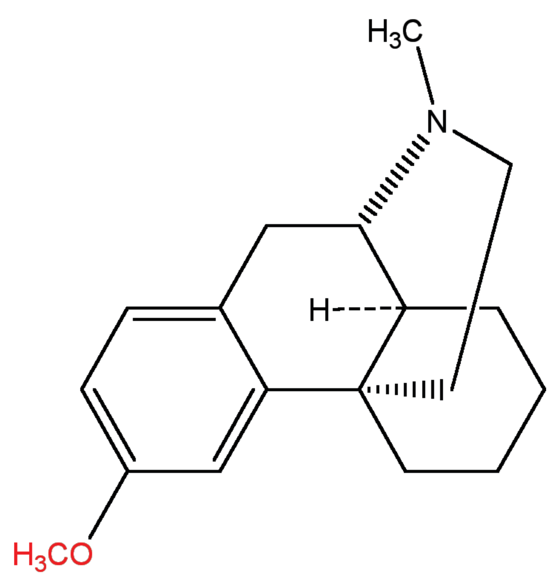
Introduction

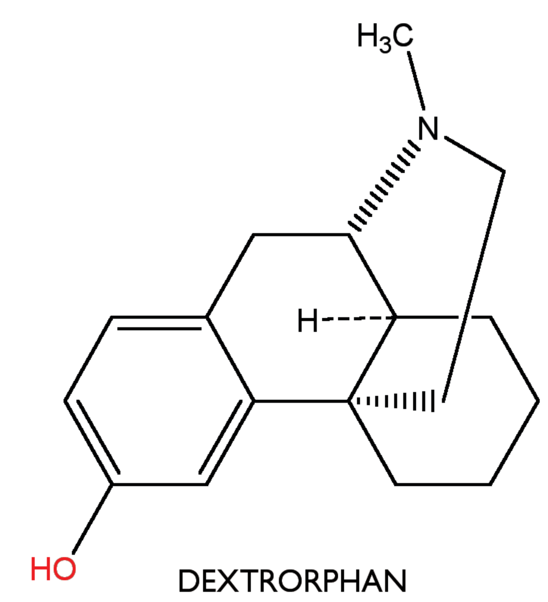
Dextromethorphan is a synthetic analog of codeine. It is used for the temporary relief of coughs caused by minor throat and bronchial irritation and is one of the active ingredients in many over-the-counter cold and cough medicines. At doses higher than medically recommended, this drug is classified as a dissociative psychedelic drug. After oral administration, dextromethorphan is well absorbed with peak plasma levels observed after 2.5 hours. [1,2] It is then widely distributed and rapidly and extensively metabolised by the liver. The cytochrome P450 2D6 isoenzyme is responsible for the conversion of dextromethorphan to its main metabolite dextrorphan. The development of efficient immunoassays enabling the detection of dextromethorphan and its main metabolite in biological fluids is of value for monitoring the use or misuse of this compound.

This study reports the development of a sensitive **polyclonal antibody** to **dextromethorphan/dextrorphan** for use in immunoassay development for forensic or toxicological applications.

Chemical Structures



DEXTROMETHORPHAN



DEXTRORPHAN

Methodology

Dextromethorphan was derivatised through the O-position and conjugated to bovine thyroglobulin (BTG) as carrier. The resulting immunogen was administered to adult sheep on a monthly basis to generate polyclonal antiserum. IgG was extracted from the antiserum and evaluated via competitive ELISA. Absorbances were read at 450nm.

Assay evaluation parameters:

- Sensitivity: the calibration curves were generated with each of the analytes as standards in the competitive ELISA. B/B0 values were calculated, where B is the absorbance measured at 450nm for x ng/ml of the analyte and B0 is the absorbance measured at 450nm in the absence of analyte. The IC50 for each analyte tested was calculated by taking 50% of the absorbance of the zero calibrator and reading this absorbance 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: specificity, expressed as % cross-reactivity (%CR), was calculated as follows: %CR = [IC50(dextromethorphan) / IC50(cross-reactant)] x 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 values.

Results

Results corresponding to the initial antibody evaluation are presented

Sensitivity and specificity

Analyte	Calibration range (ng/ml)	IC50 (ng/ml)	Specificity (% cross-reactivity)
Dextromethorphan	0-10	0.078	100
Dextrorphan	0-10	0.145	53.8
Desmethyl Dextromethorphan	0-10	0.285	27.4
3-Methoxy Morphinan	0-10	>10	<0.8
3-Hydroxy Morphinan	0-10	>10	<0.8

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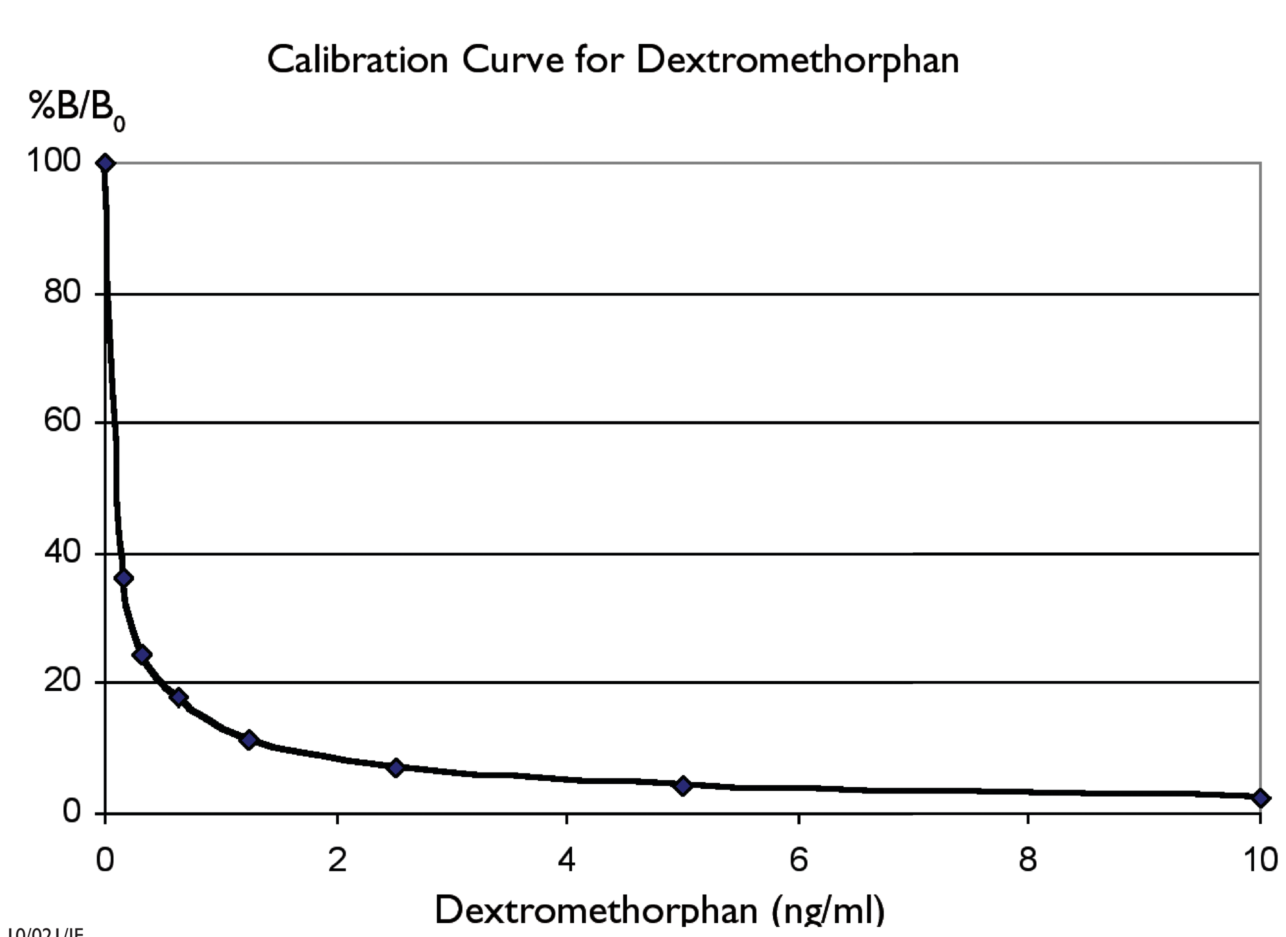
Calibration Curve



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Precision

Dextromethorphan	Intra-assay precision (n=2x8)							
	Level 1	Level 2	Level 3	Level 4	Level 5	Level 6	Level 7	Level 8
%CV	5.3	2.4	5.5	3.4	5.0	1.5	3.4	9.8

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Conclusions

Initial data indicate the development of a highly sensitive **polyclonal antibody** for the detection of dextromethorphan **(%cross-reactivity 100%)** and its main metabolite **dextrorphan (%cross-reactivity 53.8%)** with sensitivity values of 0.078 ng/ml and 0.145 ng/ml respectively (calibration range 0-10 ng/ml). %Cross-reactivity with desmethyl dextromethorphan was 27.4% whereas it was <0.8% with 3-methoxy morphinan and 3-hydroxy morphinan.

The intra-assay precision (n=2x8) expressed as %CV is typically <10%.

The antibody is of value for the development of sensitive immunoassays for the detection of dextromethorphan and dextrorphan in test samples.

References

1. International Programme on Chemical Safety, INCHEM, Poisons Information Monograph, Dextromethorphan.
2. US National Highway Traffic Safety Administration (NHTSA), Drugs and Human Performance Fact Sheets, Dextromethorphan.

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