

# DEVELOPMENT OF A HIGH SENSITIVITY ANTIBODY FOR THE DETECTION OF SALVINORIN A.

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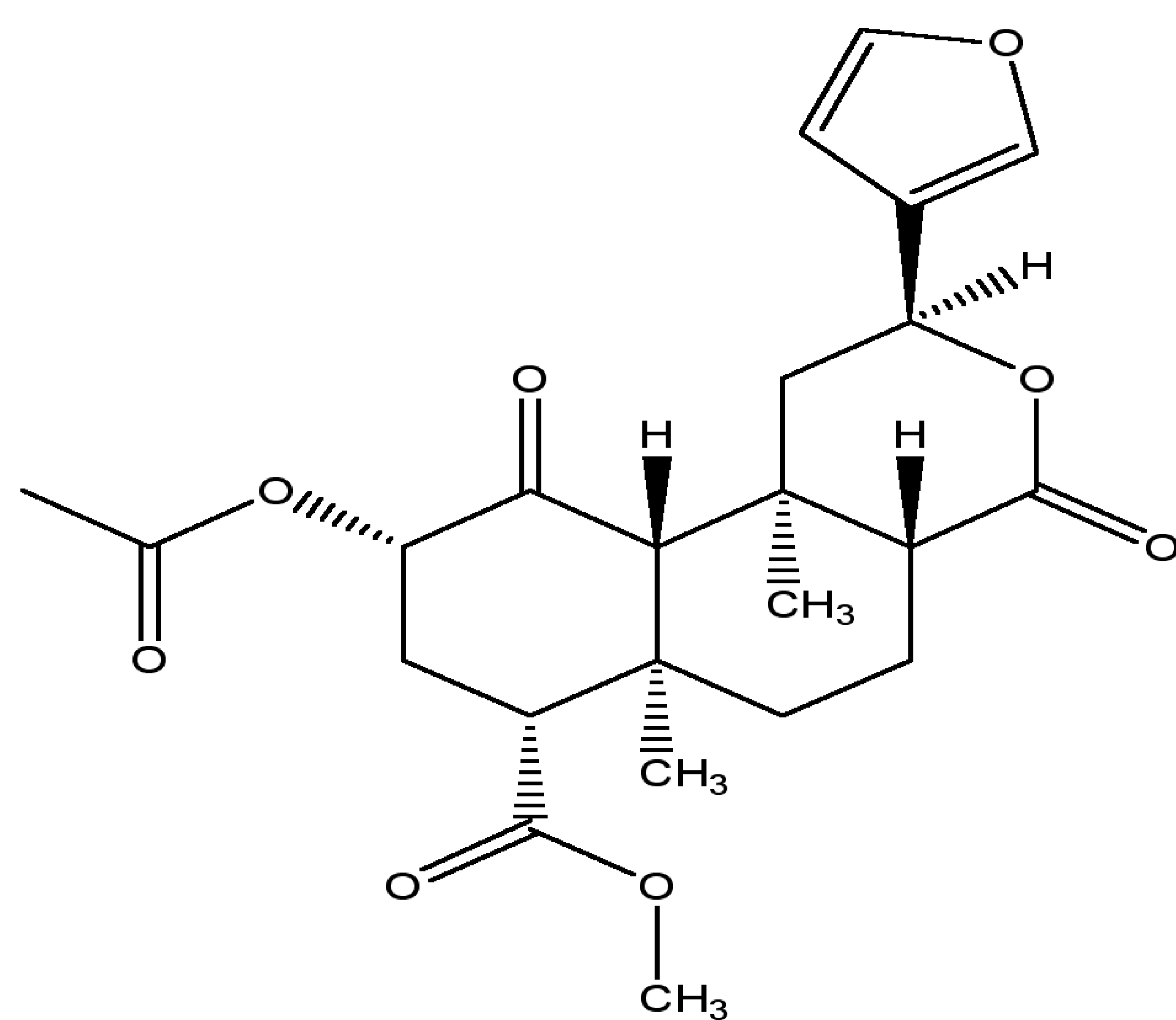
## INTRODUCTION

Salvinorin A is a potent psychoactive component of the indigenous Mexican plant *Salvia divinorum*<sup>1</sup> with strong affinity for the kappa-opioid receptor (KOR).<sup>2</sup> Its central nervous system effects and hallucinogenic properties have been compared to LSD, leading to its illegal status in many countries. Its use as a recreational drug is increasing due to its ready availability and potent activity.

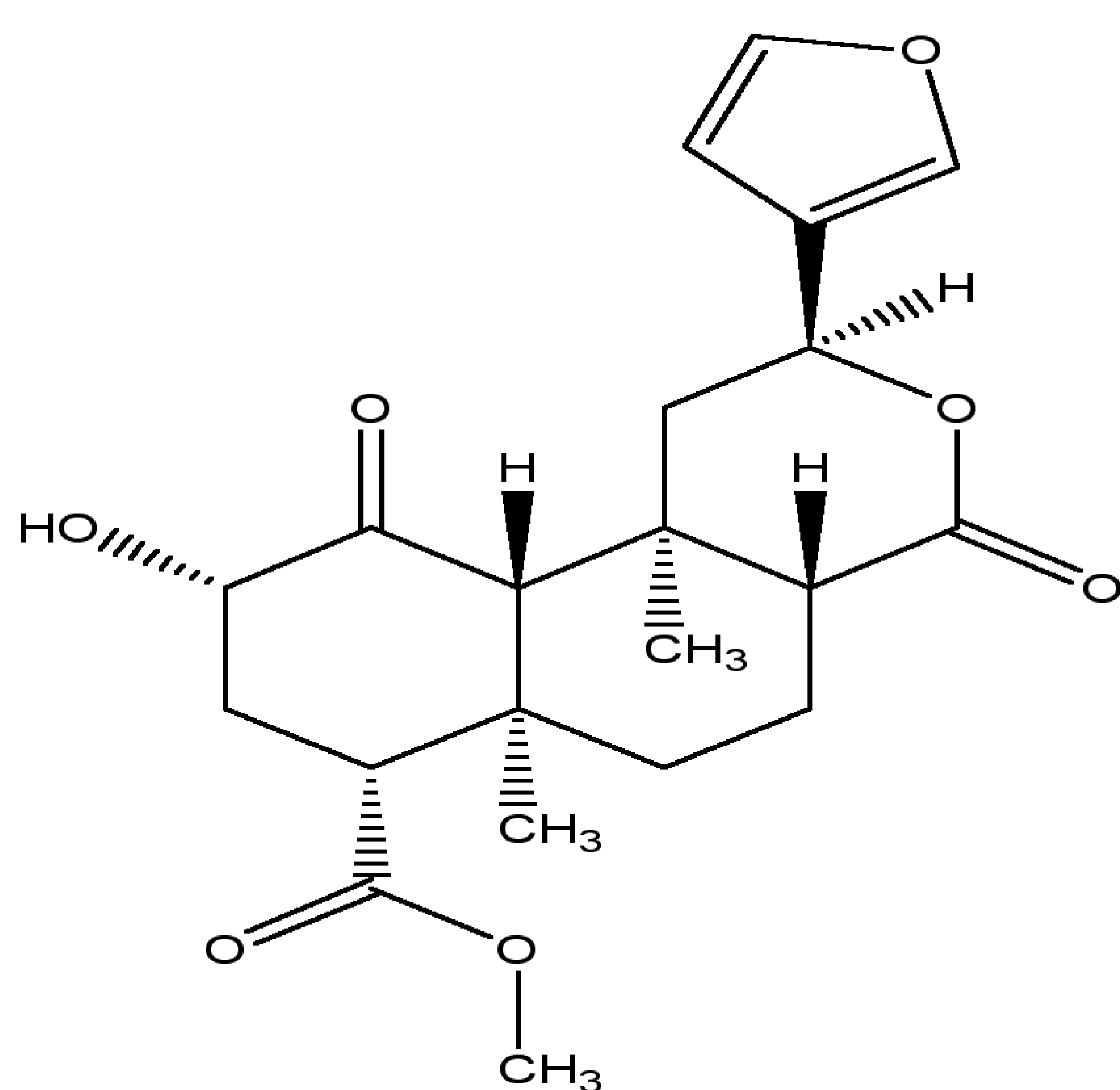
The use of immunoassays enabling specific and sensitive detection of this compound are useful for toxicological, therapeutic and clinical applications.

We report the development of a high sensitivity polyclonal antibody for the specific detection of **salvinorin A**, which is of value for the development of effective immunoassays for its determination and application to toxicology, forensic and clinical settings.

## Chemical Structures



**SALVINORIN A**



**SALVINORIN B**

## MATERIALS AND METHODS

### Polyclonal antibody against salvinorin A

The immunogen comprising salvinorin A coupled to bovine thyroglobulin (BTG) as carrier was administered to adult sheep on a monthly basis to provide target-specific polyclonal antiserum. IgG was extracted from the antiserum and evaluated via competitive ELISA. Competition between free analyte and horseradish peroxidase labelled conjugate for antibody binding sites after 1 hour incubation at 25°C was measured by reading the absorbance at 450nm. The absorbance was inversely proportional to the concentration of the analyte.

### Assay evaluation parameters:

- The IC<sub>50</sub> 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.
- The calibration curves were generated with each of the analytes as standards in the competitive assay. B/B<sub>0</sub> values were calculated where B is the absorbance measured at 450 nm for x ng/ml of the analyte and B<sub>0</sub> is the absorbance measured at 450 nm in the absence of analyte.
- Specificity: the specificity, expressed as %cross-reactivity (%CR) was calculated as follows:  
%CR = [IC<sub>50</sub> (salvinorin A)/IC<sub>50</sub> (cross-reactant)] x 100
- 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

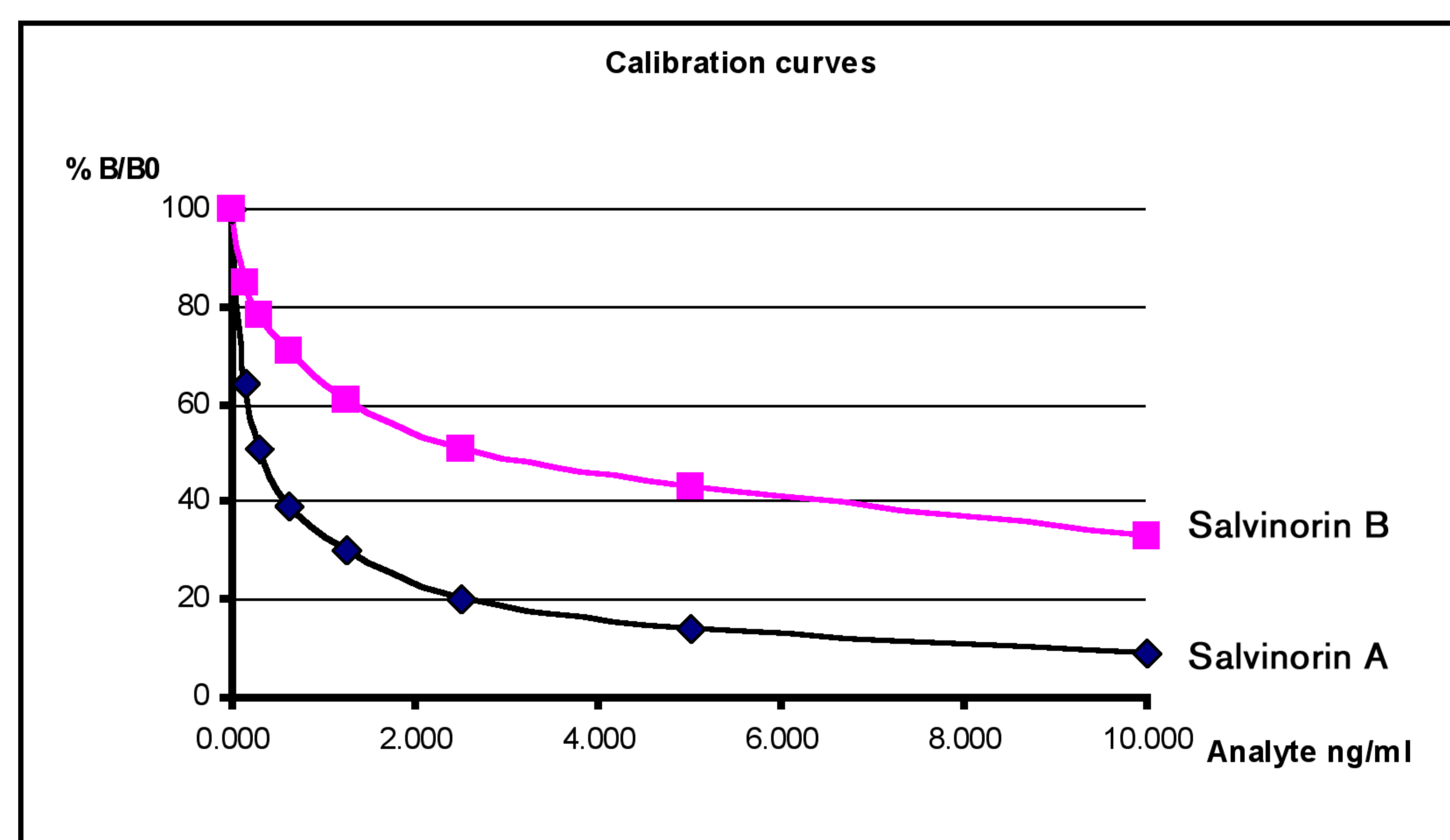
### Evaluation of the polyclonal antibody against salvinorin A

#### IC<sub>50</sub> and specificity

Analyte	Calibration Range ng/ml	IC <sub>50</sub> ng/ml	Specificity (% cross-reactivity)
Salvinorin A	0-10	0.33	100
Salvinorin B	0-10	2.8	12

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#### Calibration curves



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### INTRA-ASSAY PRECISION (n=8X3)

Salvinorin A	%CV
Level 1	2.2
Level 2	2.4
Level 3	4.8
Level 4	2.0
Level 5	4.3
Level 6	6.8
Level 7	5.5
Level 8	2.3

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## CONCLUSIONS

- This study reports the development of a **high sensitivity polyclonal antibody for the detection of salvinorin A**.
  - Initial evaluation of the antibody shows specificity for salvinorin A with cross-reactivity of 12% for salvinorin B.
  - For the calibration range of 0-10 ng/ml, the sensitivity value expressed as IC<sub>50</sub> is 0.33 ng/ml.
  - The intra-assay precision (n=3), expressed as %CV, is typically <7 for eight different concentration levels.
- The use of this antibody will facilitate the development of effective immunoassays for the determination of salvinorin A with applications in toxicology, forensic and clinical settings.

## REFERENCES

- Valdés, L.J., *et al.* Ethnopharmacology of ska María Pastora (*Salvia divinorum*, Epling and Játiva-M). *J. Ethnopharmacol.* 1983, **7(3)**: 287-312.
- Roth, B.L., *et al.* Salvinorin A: a potent naturally occurring nonnitrogenous kappa opioid selective agonist. *Proc. Natl. Acad. Sci.* 2002, **99(18)**: 11934-11939.

