INTRODUCTION

Trazodone is a tricyclic derivative used in the treatment of depression, panic attacks, and agoraphobia, as well as in treating anxiety and depressive behaviour and cocaine withdrawal. Studies have shown that in human lives, this compound is metabolised to m-chlorophenylpiperazine (mCPP) by the enzyme CYP3A4. mCPP is an intermediate in the production of three related substances nefazodone, trifluoperazine and meprobamate, which differ only in the substituent attached to the piperazinylpropyl moiety. For monitoring treatment compliance and therapeutic concentrations of trazodone, the use of specific, sensitive and reliable immunoassays enabling determination in biological fluids is advantageous. Moreover, there are reports of increasing seizures of mCPP as an MDMA-resembling compound and cocaine adulterant.

We report the development of two polyclonal antibodies, one enabling highly sensitive and specific determination of mCPP; the other exhibiting specificity for the active metabolite mCPP, nefazodone and the parent compound with good sensitivity. It is of value for the development of immunoassays for the screening of these compounds in therapeutic or toxicological applications.

Chemical structures:

![Chemical structures of trazodone, mCPP, nefazodone, and related compounds](image)

METHODLOGY

Trazodone and mCPP were coupled by way of a crosslinker to bovine thyroglobulin (BTV) as carrier. The resulting immunogen were administered subcutaneously to adult sheep on a monthly basis to provide target-specific polyclonal antibodies. IgG was extracted from the antisera and evaluated via competitive ELISA, to determine antibody sensitivity and specificity. The absorbance was measured at 450 nm and was inversely proportional to the concentration of the analytes.

Assay evaluation parameters:

- The calibration curves were generated with each of the analytes as standard 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.

### RESULTS

Results corresponding to the initial antibody evaluation are presented.

#### Specificity

Specificity, expressed as %cross-reactivity (%CR) was calculated as follows:

- For mCPP:
  \[ %CR = \left( \frac{IC50 \text{(mCPP)}}{IC50 \text{(cross-reactant)}} \right) \times 100 \]

- For trazodone:
  \[ %CR = \left( \frac{IC50 \text{(trazodone)}}{IC50 \text{(cross-reactant)}} \right) \times 100 \]

#### Analytical characteristics

- **Calibration range (ng/ml):**
  - mCPP: 0-20
  - Trazodone: Levels 1-8
  - Nefazodone: Levels 1-8

- **IC50:**
  - mCPP: Variable
  - Trazodone: Levels 1-8
  - Nefazodone: Levels 1-8

- **%CR:**
  - mCPP: <0.7
  - Trazodone: Levels 1-8
  - Nefazodone: Levels 1-8

- **%CV:**
  - Baseline: 1-2
  - Levels: 3-8

#### Calibration curves

![Calibration curves](image)

### CONCLUSION

This study reports the development of two highly sensitive polyclonal antibodies with different specificity profiles for trazodone, mCPP and nefazodone.

One antibody was specific for trazodone (%cross-reactivity 100%) without recognition of mCPP and nefazodone, this antibody presented a sensitivity value expressed as IC50 <0.15 ng/ml for the target molecule.

The other antibody exhibited a broader specificity profile with recognition of mCPP (%cross-reactivity 100%), nefazodone and trazodone (mCPP: 217.3 and 1593 respectively relative to mCPP) with sensitivity values IC50 <0.6 ng/ml for mCPP, <0.6 ng/ml for trazodone and <4.0 ng/ml for nefazodone.

The combined use of these antibodies will facilitate the drug administered. This is valuable for developing immunoassays for the determination of these compounds in different sample matrices for therapeutic and toxicological applications.

### REFERENCES