DEVELOPMENT OF ANTIBODIES FOR THE MEASUREMENT OF TRAZODONE AND RELATED COMPOUNDS.

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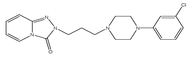
INTRODUCTION

Trazodone is a triazolopyridine derivative used in the treatment of depression, panic attacks, and agoraphobia, as well as insomnia, aggressive behaviour and cocaine withdrawal.[1-4] Studies have shown that in human liver, this compound is metabolised to m-chlorophenylpiperazine (mCPP) by the enzyme CYP3A4.[5] mCPP is also an intermediate in the production of three related substances nefazodone, etoperidone and mepiprazole, 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 rapid immunoassays enabling determination in biological fluids is advantageous. Moreover, there are reports of increasing seizures of mCPP as an MDMA-mimicking compound and cocaine adulterant.[6,7]

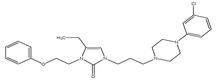
We report the development of two polyclonal antibodies, one enabling highly sensitive and specific determination of trazodone; the other exhibiting specificity for the active metabolite mCPP, nefadozone and the parent compound with good sensitivity. This is of value for the development of immunoassays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

Chemical structures:



TRAZODONE

mCPP



NEFAZODONE

METHODOLOGY

Trazodone and mCPP were coupled by way of a crosslinker to bovine thyroglobulin (BTG) as carrier. The resulting immunogens were administered separately to adult sheep on a monthly basis to provide target-specific polyclonal antisera. 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 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:

  Polyclonal antibody specific for trazodone
  %CR=[IC50(trazodone)/IC50 (cross-reactant)]x100

  Polyclonal antibody with broader specificity profile:
  %CR=[IC50(mCPP)/IC50 (cross-reactant)]x100

- 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

Results corresponding to the initial antibody evaluation are presented.

IC50 and specificity

Analyte	Polyclonal antibody specific for trazodone		
	Calibration range (ng/ml)	IC50 (ng/ml)	Specificity (% cross-reactivity)
Trazodone	0-10	0.14	100
mCPP	0-20	-	<0.7
Nefazodone	0-20	-	<1.4

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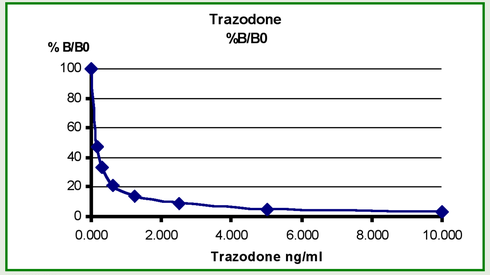
Calibration curve



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Intra-assay precision

Intra-assay precision (n=8x3)	
Trazodone	%CV
Level 1	0.8
Level 2	4.8
Level 3	2.2
Level 4	1.1
Level 5	1.7
Level 6	6.7
Level 7	9.0
Level 8	2.0

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IC50 and specificity

Analyte	Polyclonal antibody with broader specificity profile		
	Calibration range (ng/ml)	IC50 (ng/ml)	Specificity (% cross-reactivity)
Trazodone	0-10	0.5	1593
mCPP	0-20	8.5	100
Nefazodone	0-20	3.9	217.3

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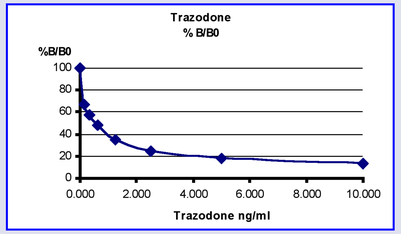
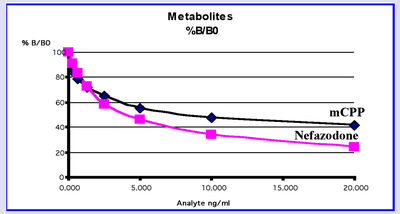
Calibration curves





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Intra-assay precision

Intra-assay precision (n=8x3)					
Trazodone	%CV	mCPP	%CV	Nefazodone	%CV
Level 1	1.8	Level 1	2.0	Level 1	2.9
Level 2	2.7	Level 2	1.4	Level 2	2.3
Level 3	10.8	Level 3	1.5	Level 3	1.1
Level 4	3.5	Level 4	1.7	Level 4	0.9
Level 5	0.35	Level 5	2.2	Level 5	1.9
Level 6	1.2	Level 6	3.8	Level 6	1.9
Level 7	1.3	Level 7	1.9	Level 7	2.1
Level 8	4.4	Level 8	1.5	Level 8	0.9

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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 of <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 (%cross-reactivity 217.3 and 1593 respectively relative to mCPP) with sensitivity values IC50 <8.5 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 determination of the drug administered. This is valuable for use in developing immunoassays for the determination of these compounds in different sample matrices for therapeutic and toxicological applications.

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