

DEVELOPMENT OF HIGHLY SENSITIVE ANTIBODIES FOR THE DETECTION OF TRICYCLIC ANTIDEPRESSANTS, TRAZODONE AND RELATED COMPOUNDS.

El Ouard Benchikh, Philip Lowry, R.Ivan McConnel, S. Peter Fitzgerald, Randox Laboratories Ltd., 55 Diamond Road, Crumlin, Co Antrim, BT29 4QY, UK.

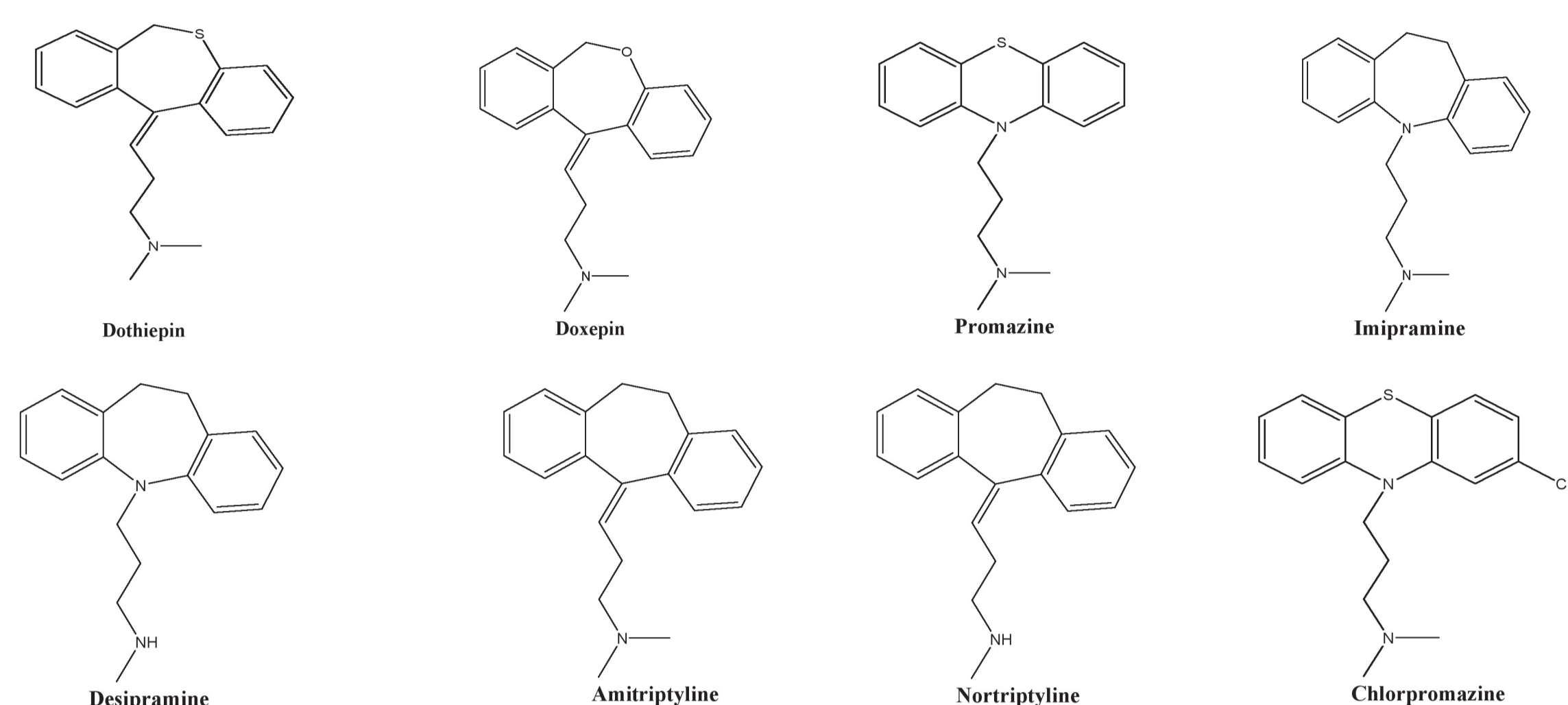
INTRODUCTION

Tricyclic antidepressants (TCAs) are indicated for the treatment of clinical depression, neuropathic pain, nocturnal enuresis and attention deficit hyperactivity disorder (ADHD). Whilst therapeutic drug monitoring (TDM) of TCAs is well established in the treatment of depression, TCA overdose remains the most common cause of death from prescription drugs.^(1,2)

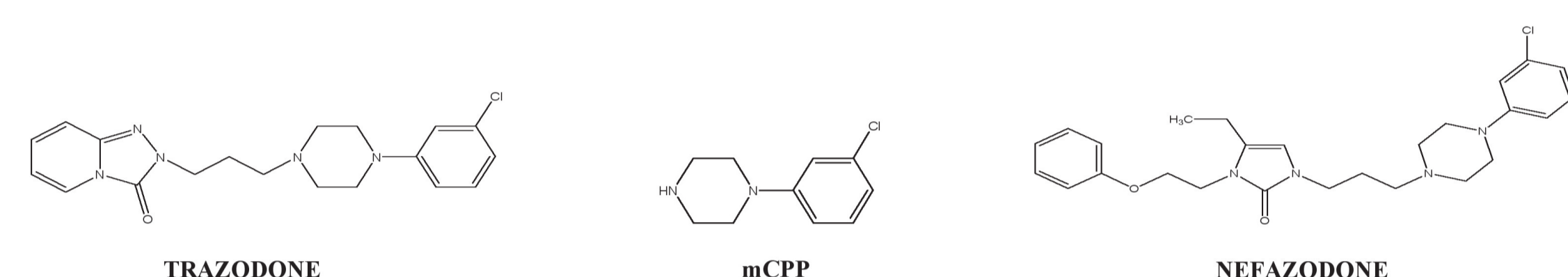
Trazodone is used in the treatment of depression, panic attacks, and agoraphobia, as well as insomnia, aggressive behaviour and cocaine withdrawal.^(3,6) Studies have shown that in human liver, this compound is metabolised to m-chlorophenylpiperazine (mCPP) by the enzyme CYP3A4.⁽⁷⁾ mCPP is also an intermediate in the production of nefazodone, etoperidone and mepiprazole.

For TDM and toxicological applications, the use of specific, sensitive and rapid immunoassays enabling determination of these compounds in biological fluids is advantageous. We report the development of a highly sensitive **monoclonal antibody presenting a broad specificity profile against TCAs** and **two highly sensitive polyclonal antibodies with different specificity profiles for trazodone, mCPP and nefazodone**. This is of value for the development of immunoassays for the screening of these compounds.

Chemical structure of some TCAs:



Chemical structures of trazodone and related compounds:



RESULTS

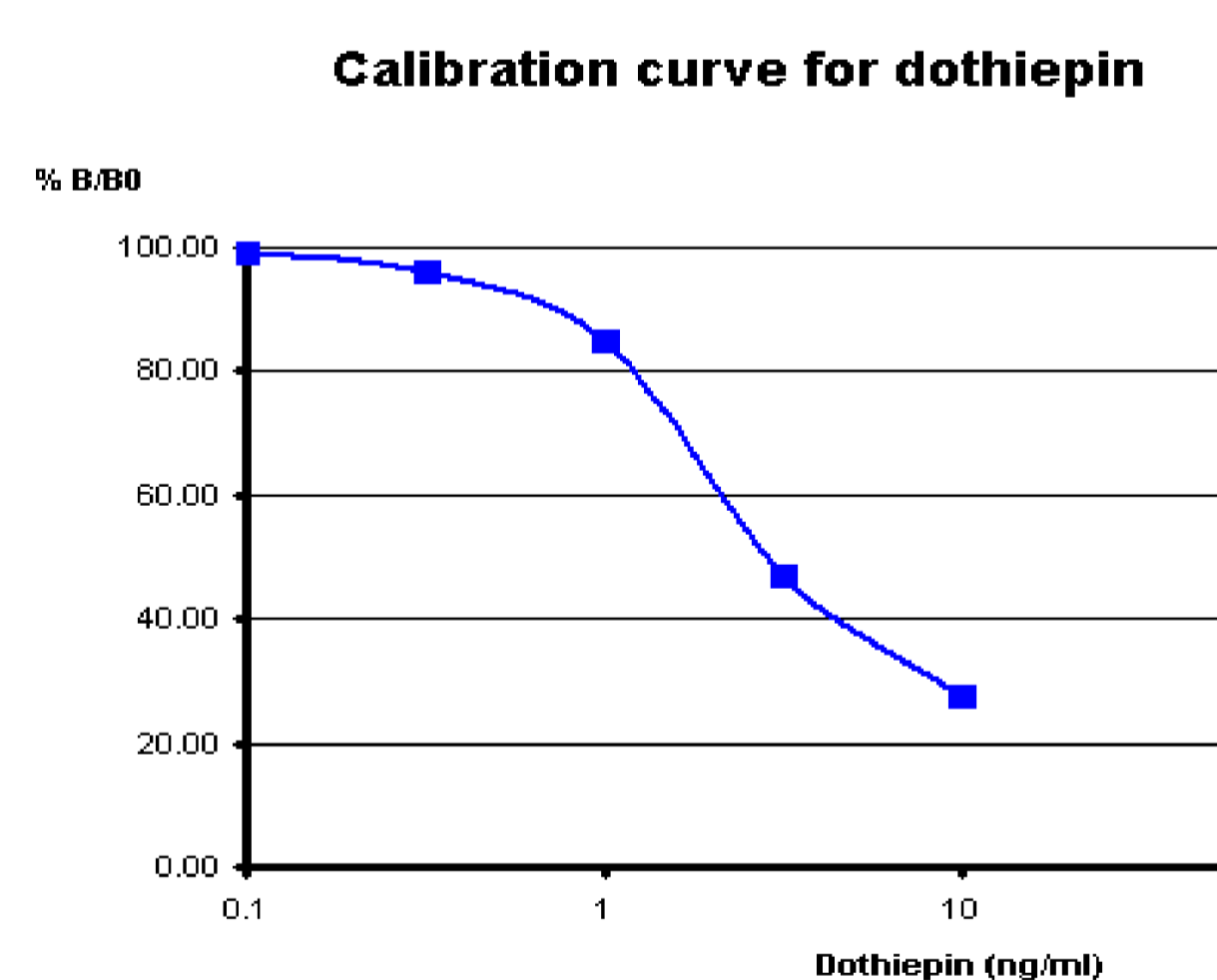
Evaluation of the purified generic monoclonal antibody against TCAs.

IC50 and specificity

TCA	IC50 (ng/ml)	Specificity (% cross-reactivity)
Dothiepin	2.93	100.00
Nortriptyline	2.3	127.39
Protriptyline	1.16	252.59
Lofepamine	1.96	149.49
Doxepin HCl	2.75	106.55
Cyclobenzaprine	1.31	223.66
Trimipramine	1.28	228.91
Imipramine HCl	1.59	184.28
Chlorpromazine HCl	6.51	45.01
Amitriptyline HCl	0.9	325.56
Desipramine	1.08	271.30
Nordoxepin HCl	6.82	42.96
Norclomipramine	4.04	72.52
Promazine	1.24	236.29
Norchlorpromazine	2.58	113.57

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Calibration curve



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Developed generic monoclonal antibody	Commercial system 1		Commercial system 5	
	Total TCAs tested	Specificity (%Cross-reactivity)	Total TCAs tested	Specificity (%Cross-reactivity)
15	12	>100	14	>100
	1	>70	7	>50
Sensitivity: ≤ 1.0 ng/ml (relative to dothiepin)	2	>40	3	10
	Commercial system 2		Commercial system 6	
≤ 0.35 ng/ml (relative to imipramine)	Total TCAs tested	Specificity (%Cross-reactivity)	Total TCAs tested	Specificity (%Cross-reactivity)
	11	>100	14	>100
Sensitivity	10ng/ml	>90	5	>40
	7	≤12	3	>15
Commercial system 3	Commercial system 7		Commercial system 8	
	Total TCAs tested	Specificity (%Cross-reactivity)	Total TCAs tested	Specificity (%Cross-reactivity)
14	>100	10	100	
Sensitivity NS	3	>60	1	50
	4	<30	6	<25
Commercial system 4	Commercial system 8		Commercial system 8	
	Total TCAs tested	Specificity (%Cross-reactivity)	Total TCAs tested	Specificity (%Cross-reactivity)
14	>100	11	100	
Sensitivity NS	8	>90	5	>50
	4	≤20	5	<45

* NS=not stated

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METHODOLOGY

Monoclonal antibody against TCAs:

Selection of fusion wells was carried out by ELISA binding assay using an HRP-labelled tracer blend. Selection of colonies and further antibody evaluation was performed by competitive ELISA immunoassays in 96-well microtiter plates. Competition between free analyte and horseradish peroxidase (HRP) labelled conjugate for antibody binding sites after incubation of 1 hour at 37°C was measured by reading the absorbance at 450 nm. The absorbance was inversely proportional to the concentration of the analyte.

Polyclonal antibodies against trazodone and related compounds:

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:

$$\%CR = \left[\frac{IC50(\text{Dothiepin})}{IC50(\text{Cross-reactant})} \right] \times 100$$

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

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

Intra-assay precision was determined from the results of 3 replicates at different concentration levels within the same run. Results were expressed as %CV.

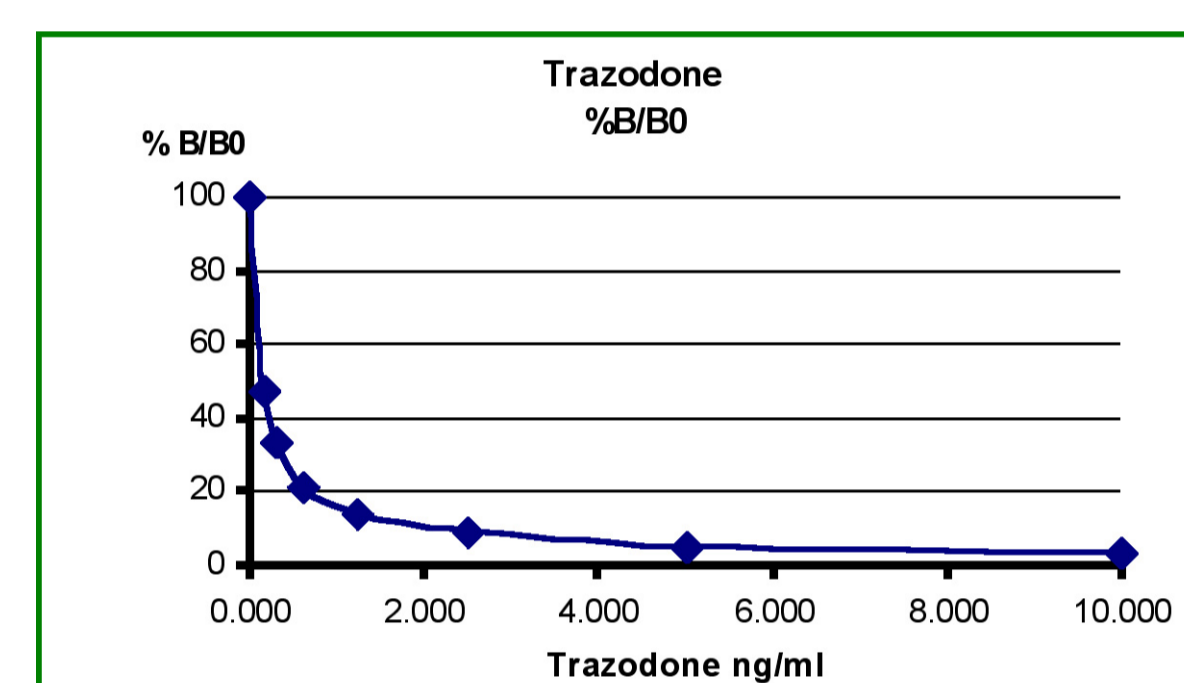
Evaluation of the polyclonal antibodies against trazodone and related compounds.

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

09/008/371

Calibration curve



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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

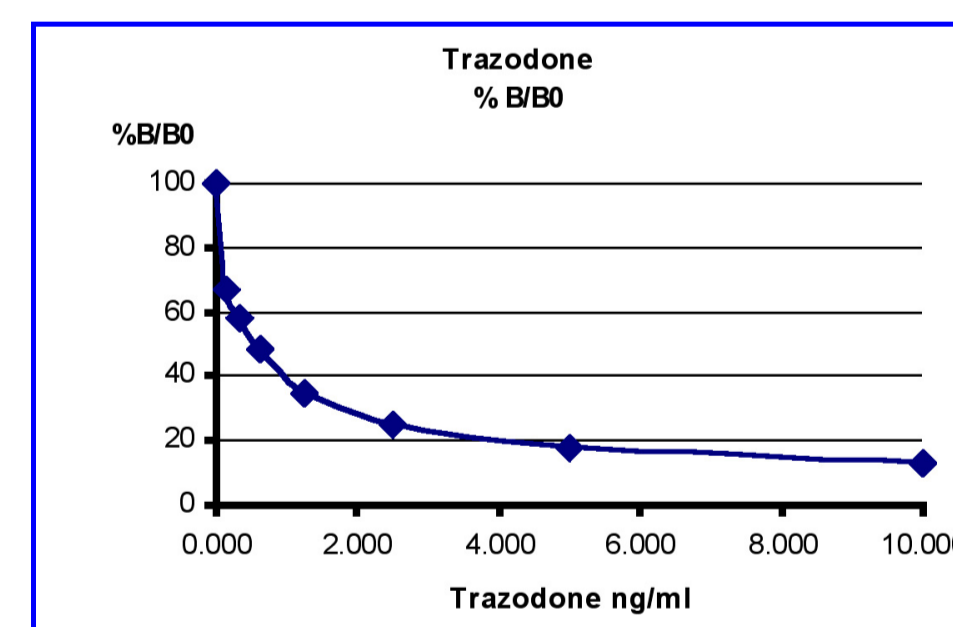
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IC50 and specificity

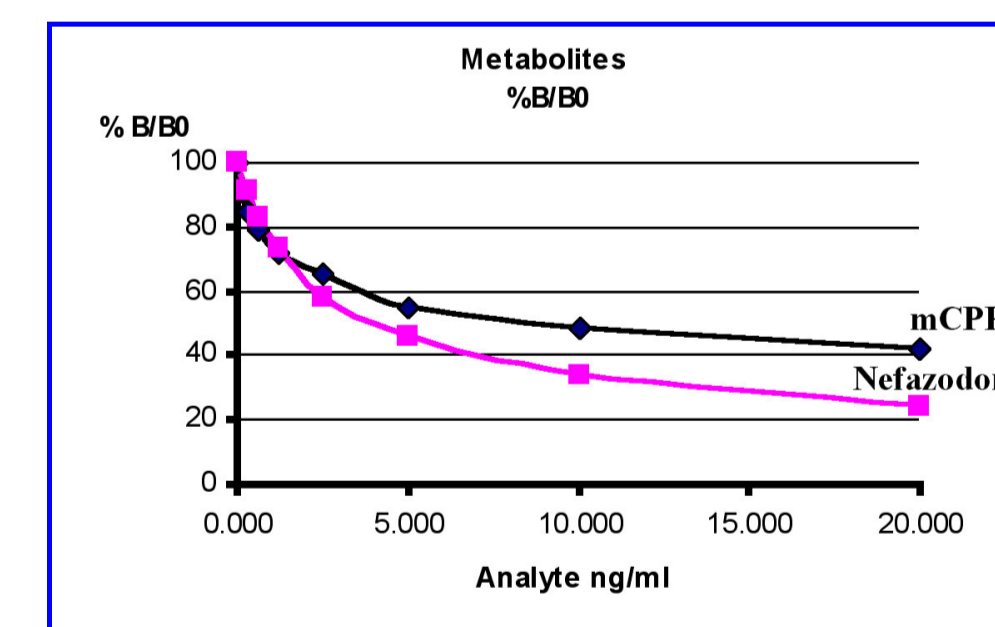
Analyte	Polyclonal antibody with broader specificity profile		
	Calibration range (ng/ml)	IC50 (ng/ml)	Specificity (% cross-reactivity)
Trazodone	0-10	0.5	1593
mCPP	0-20	8.5	100
Nefazodone	0-20	3.9	2173

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Calibration curves



09/008/371



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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CONCLUSIONS

Monoclonal antibody against TCAs.

- The developed generic monoclonal antibody exhibits **high sensitivity and specificity for a wide range of TCAs** and their metabolites.
- This represents a **significant improvement** for use in developing **more effective immunoassays** applicable to TDM and toxicology fields.

Polyclonal antibodies against trazodone and related compounds.

- 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 2173 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.