Production of antisera to phenylbutazone and oxyphenylbutazone for use in immunochemical detection assays

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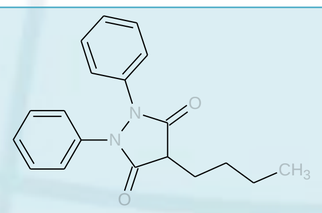
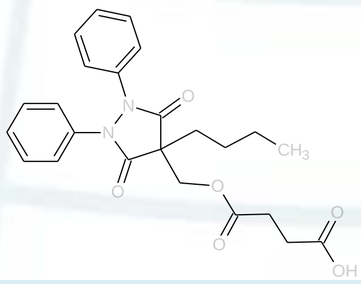
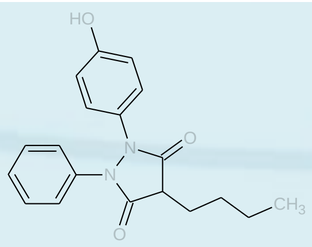
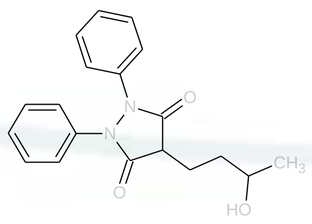
Background

- Phenylbutazone (PBZ) is a non-steroidal anti-inflammatory drug authorised to treat horses suffering from musculoskeletal disorders. Due to adverse effects in humans it is not permitted for use in food producing animals in the EU
- However some equine and bovine samples have been found to be non-compliant using physicochemical methods of analysis. There is a need for less expensive, high-throughput, rapid screening methods such as immunoassays
- PBZ is the predominant analyte in blood; its main metabolite, oxyphenylbutazone (OPBZ), and a second metabolite, γ–hydroxyphenylbutazone (HPBZ), appear in smaller quantities; suxibuzone (SBZ) is a prodrug of PBZ which is converted rapidly to PBZ and OPBZ after administration, hence its detection is not required
- This study set out to produce an antibody capable of detecting the target analytes (PBZ and OPBZ) below the 5 ppb (ng mL^{-1}) concentration as recommended by the Community Reference Laboratory

Antisera Production

- PBZ, OPBZ, HPBZ and SBZ were employed as haptens and rendered immunogenic via coupling to a carrier protein; corresponding hapten-horseradish peroxidase enzyme labels were prepared at the same time
- Two rabbits were immunised with each immunogen to produce polyclonal antisera; the antisera were assessed for sensitivity to PBZ, OPBZ, HPBZ and SBZ using the SBZ enzyme label (found to be the superior label in combination with all of the antisera)

Hapten	Structure	Coupling Reaction
PBZ		A carboxy derivative of PBZ was prepared by reaction with diazotised amino benzoic acid. The added carboxylic acid group was used to couple with the carrier protein via carbodiimide reaction
SBZ		The carboxylic acid on SBZ was activated with the cross-linker 1,1'carbonyldiimidazole before reaction with the carrier protein
OPBZ		Sulfhydryl groups were introduced to the carrier protein and used to react with the crosslinker, *p*-maleimidophenyl-isocyanate (PMPI). The isocyanate group was then employed to couple with OPBZ
HPBZ		The hydroxyl group on HPBZ was activated with the cross-linker N,N'-disuccinimidyl carbonate before reaction with the carrier protein

Results

	IC_{50}s (ng mL^{-1})				CR (%)			
Rabbit	**PBZ**	**OPBZ**	**HPBZ**	**SBZ**	**PBZ**	**OPBZ**	**HPBZ**	**SBZ**
PBZ-1	18.3	53.9	>100	94.6	100	34	ND	19
PBZ-2	5.5	57.2	>100	5.5	100	10	ND	100
SBZ-1	4.6	89.2	>100	9.1	198	10	ND	100
SBZ-2	3.6	>100	>100	1.4	39	ND	ND	100
OPBZ-1	5.8	5.6	>100	2.2	97	100	ND	255
OPBZ-2	>100	>100	>100	>100	ND	ND	ND	ND
HPBZ-1	7.7	>100	12.7	7.3	165	ND	100	174
HPBZ-2	0.9	9.3	3.9	3.7	433	42	100	105

The sensitivity (IC_{50}) and specificity (CR) of each antiserum in combination with SBZ-HRP in an ELISA. ND = Not determined

Conclusions

- SBZ and HPBZ haptens produced antisera with IC_{50}s of <5 ng mL^{-1} for PBZ; OPBZ hapten produced antiserum with an IC_{50} of 5.6 ng mL^{-1} for OPBZ
- The figures suggest that these antisera could deliver detection capabilities below the recommended 5 ppb (ng mL^{-1})

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