

# IMMUNO-AFFINITY COLUMNS

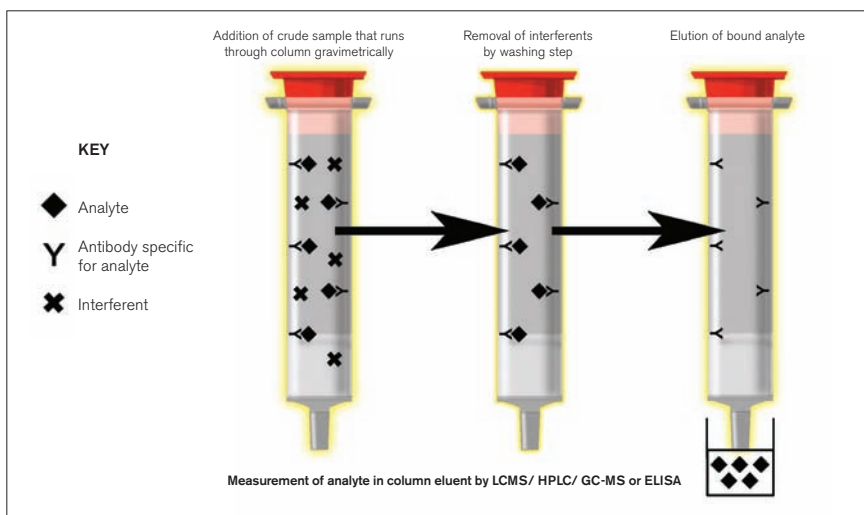
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Chapter 9

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## THE PRINCIPLE OF SAMPLE PURIFICATION BY IMMUNO-AFFINITY COLUMNS



### SINGLE ANALYTE IAC'S

Product Description	Cat. No.	Binding Capacity
<b>β – Agonists / Clenbuterol</b>	CB2184	
• Clenbuterol		50ng
• Salbutamol		50ng
• Mabuterol		50ng
• Mapenterol		50ng
• Terbutaline		50ng
• Cimaterol		30ng
• Pirbuterol		50ng
• Carbuterol		50ng
• Brombuterol		50ng
• Methylclenbuterol		50ng
<b>Corticosteroids</b>	DM2185	
• Dexamethasone		250ng
• βmethasone		250ng
• Flumethasone		250ng
<b>Stilbenes</b>	SJ2154	
• Diethylstilbestrol		50ng
• Hexestrol		50ng
• Dienestrol		50ng
<b>Trenbolone &amp; Nortestosterone</b>	TB2186	100ng
<b>Zeranol</b>	ZR2420	100ng

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**MULTI-ANALYTE IAC'S**


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Product Description	Cat. No.	Binding Capacity
<b>Growth Promoters</b>	GP1821	25ng
• Boldenone		25ng
• Zeranol		25ng
• Ractopamine		25ng
• Corticosteroids (Dexamethasone)		25ng
• 19 – Nortestosterone		25ng
• Stilbenes (Hexestrol)		25ng
• Trenzalone		25ng
• Stanozolol		25ng
• $\beta$ -Agonists (Clenbuterol)		25ng

The Multi-Analyte Immuno-affinity Column has been uniquely designed to include 9 different antibodies that can extract 9 different analyte types. Therefore with one sample being added to the column there is the potential to pull out 9 different growth promoters and their major cross reactants. This is a unique selling feature within the market.

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**KEY FEATURES OF KITS**


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- **Highly Specific** - lower signal to noise ratio, therefore limits of detection will be excellent
- **Recoveries are high with IAC's** (typically 80-90%)
- **Fast** (a full gravitational run through will take approximately 1 hour)
- **Safe** (only 1 solvent elution step)
- **Reusable** (can be used 10 times)
- **Supplied in packs of 5 columns** (allowing 50 sample preparations)
- **24 month expiry from date of manufacture** (long shelf life)
- **Size: 6ml** (can be automated in order to control flow rates)

Radox IAC's are available in pack sizes of 5 columns per kit

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## EXAMPLE OF A SINGLE ANALYTE IMMUNO-AFFINITY COLUMN

<b>Catalogue No.</b>	TB2186	
<b>Kit Format</b>	5 Columns	
	Column wash buffer (conc.)	2x100ml
	Column storage buffer (conc.)	2x100ml

## PRINCIPLE

Each Immuno-affinity column contains 1ml of gel having immobilized antibodies that are specific for trenbolone ( $\alpha$  and  $\beta$ ) and 19-nortestosterone. The employment of the Immuno-Affinity Columns (IACs) greatly reduces the time required for the analysis of trenbolone/19-nortestosterone and provides a very effective clean-up enabling analysis by ELISA [Trenbolone (Cat. No. TB2106) and 19-nortestosterone (Cat. No. NT2105)], high performance liquid chromatography (HPLC) and Gas chromatography mass spectrometry (GC-MS).

## FLOW RATES

The IAC gel has very good flow characteristics and is stored in a plastic column of internal diameter, which further assists flow rate. Generally the columns are run without the need for vacuum manifold or peristaltic pump, although these may still be used for equilibration and storage stages if desired. As a guide an entire run takes approximately 50 minutes if all stages are carried out by gravity. The IAC contains two frits to prevent disruption of the gel bed.

### Binding capacity

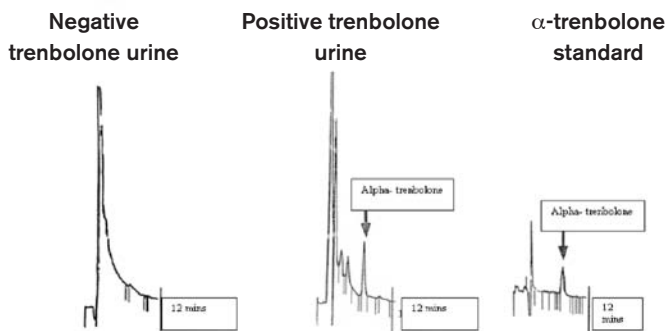
The IACs will bind 100ng of trenbolone and/or 19-nortestosterone

### Samples

Tissue and urine

## HPLC CHROMATOGRAPHS

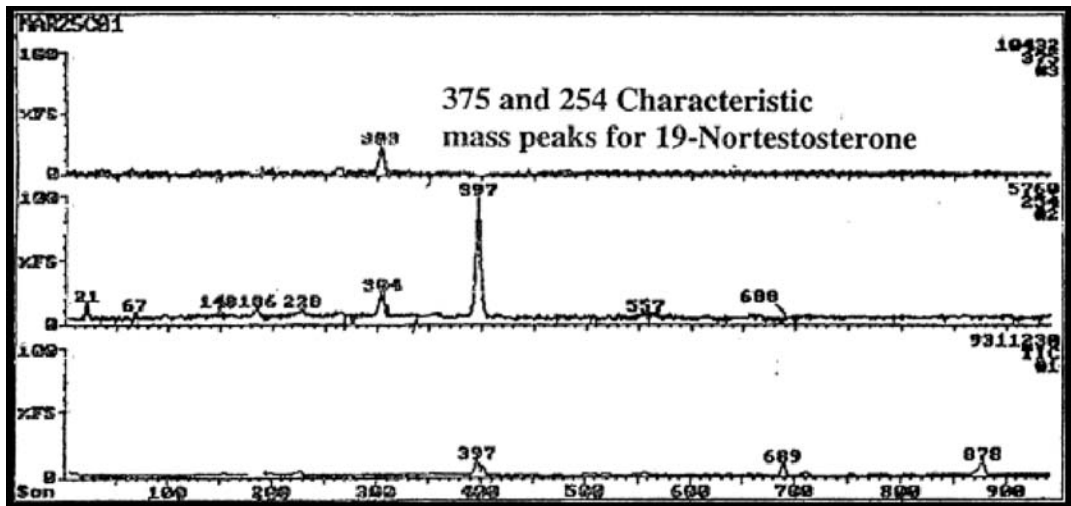
With IAC extracts, the quality of chromatographs is very good with only the peak(s) of interest being present. The bovine urine samples have been concentrated 9x by IAC i.e. 9ml urine was loaded onto an IAC and the evaporated eluate resuspended in 1ml HPLC mobile phase (70/30 MeOH/H<sub>2</sub>O), effectively concentrating the initial urine 9 fold. Chromatographs are shown for negative urine, trenbolone positive urine and an  $\alpha$  trenbolone standard.



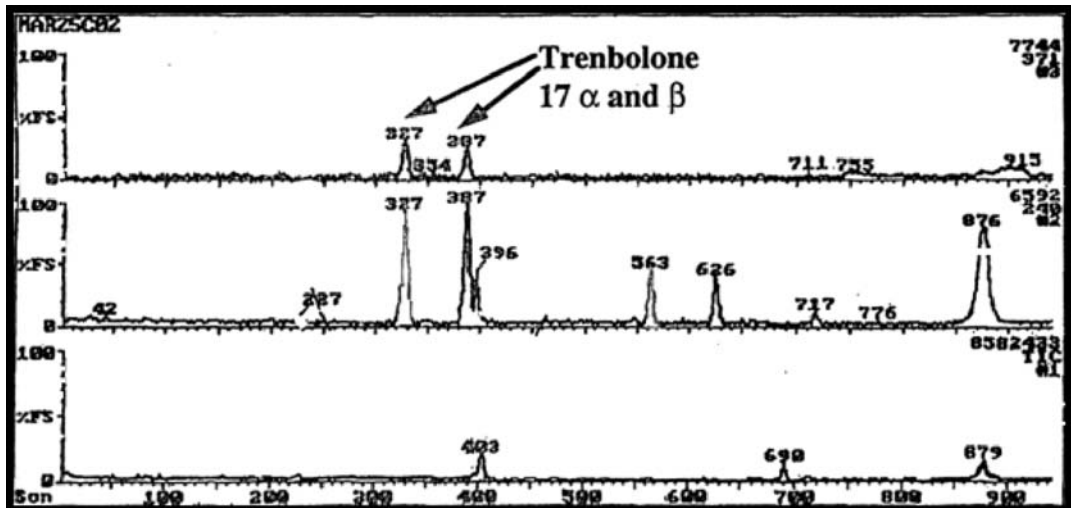
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\* All Randox recombinant proteins are expressed in *E.coli* with an amino terminal hexahistidine tag (unless otherwise stated)

## GC-MS DETECTION OF 19-NORTESTOSTERONE IN URINE AFTER IAC EXTRACTION



## GC-MS DETECTION OF TRENBOLONE IN EQUINE URINE AFTER IAC EXTRACTION



## ELISA LINEARITY &amp; RECOVERY AFTER IAC EXTRACTION

IAC provide linear recovery of analyte over a wide concentration range. Trenbolone/19-nortestosterone was loaded onto IACs over a wide concentration range (0 – 200ng/ml load). Trenbolone and 19-nortestosterone were loaded simultaneously and the recovery of each determined by trenbolone and 19-nortestosterone ELISA kits (TB2106 and NT 2105 respectively).

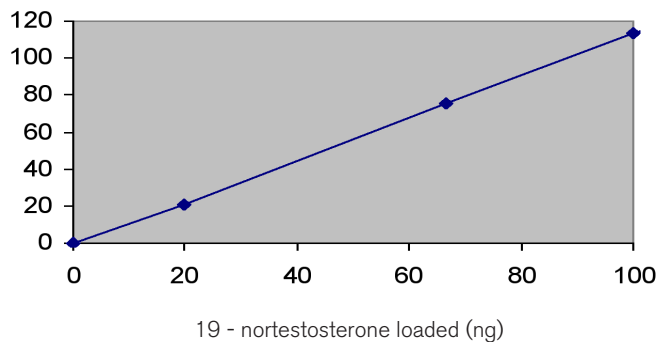
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## Immuno - Affinity Columns

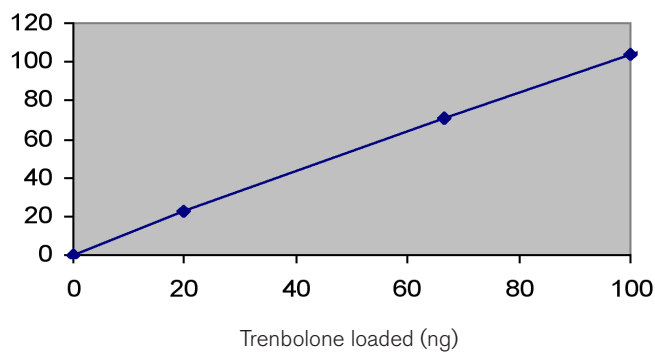
Total Load (ng)	Recovery (%)	
	19-Nortesterone	Trenbolone
Trenbolone and 19-Nortesterone		
20	105.0	114.0
66.7	113.3	106.7
100	113.4	104.4
200	100.8	99.8
Mean Recovery (%)	108.1	106.2

The following graphs show linearity for trenbolone and 19-nortesterone

19 - Nortestosterone recovery from immunoaffinity columns



Trenbolone recovery from immunoaffinity columns



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