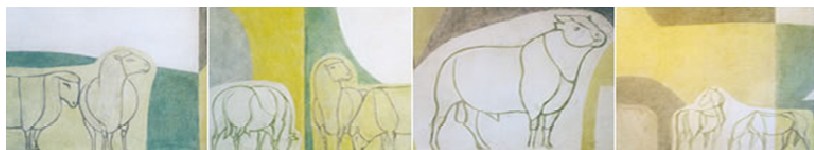




## VIAGGIO AL CENTRO DELLA DIREZIONE OPERATIVA CHIMICA

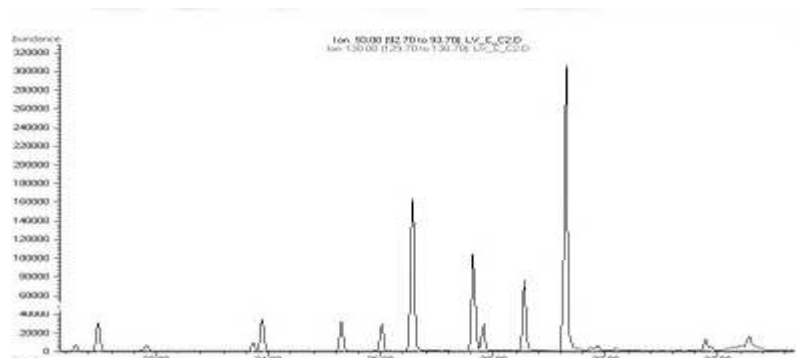
Francesco Necci Direzione Operativa Chimica

Roma 20 Novembre 2012

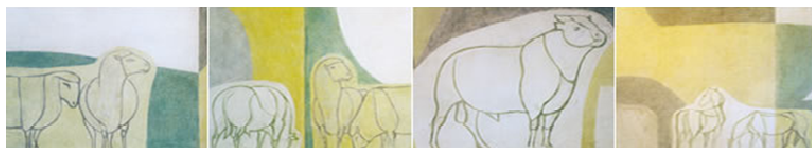


# INDICE

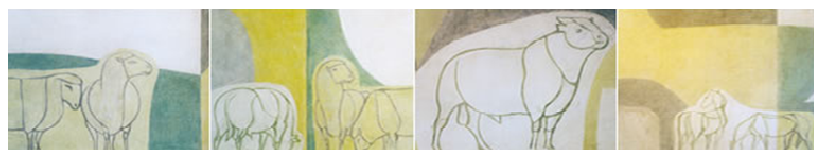
## Esempio di risultato positivo, metodo analitico di conferma



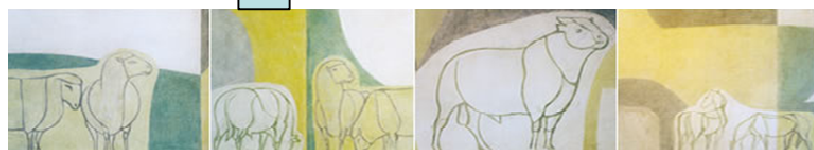
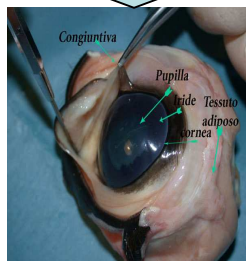
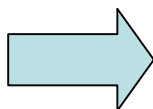
## Il palio di Siena: “Il Doping “



# Ma sarà veramente positivo???



# METODO ANALITICO DI CONFERMA SVILUPPATO VALIDATO E **ACCREDITATO** NELLA D.O.C



Istituto Zooprofilattico Sperimentale  
delle Regioni Lazio e Toscana

**BETA AGONISTI: CLENBUTEROLO,  
TERBUTALINA, SALBUTAMOLO,  
ISOSSISUPRINA, RACTOPAMINA,  
BROMBUTEROLO, BROMCLORBUTEROLO**

Redatto:  
L. GIANNETTI

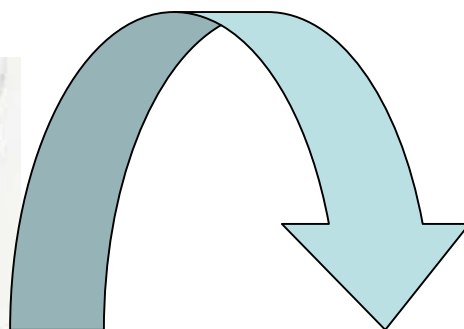
Verificato Responsabile Struttura Semplice:  
B. NERI

Verificato RQ:  
M. GUARDUCCI

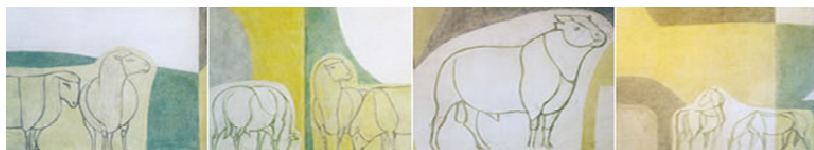
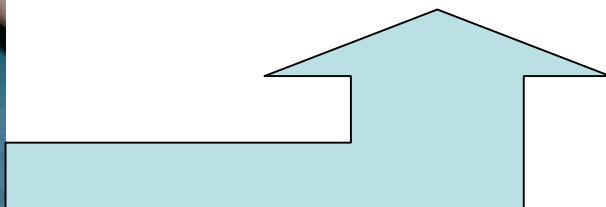
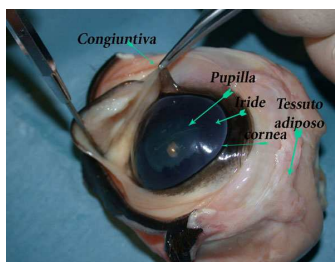
Approvato Responsabile di Struttura Complessa:  
B. NERI



# Pelo Bovino - Bulbo Oculare



**I  $\beta$ -Agonisti si legano alle cheratine del pelo e alla retina nel bulbo oculare portando quindi ad una maggiore probabilità di trovarli in un periodo di tempo più ampio rispetto ai liquidi di biologici**



# METODO ANALITICO DI CONFERMA



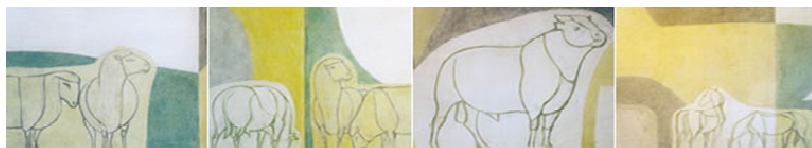
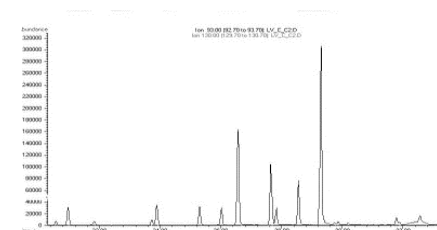
Prelevare 2.0mL di urina

- Aggiungere 3.0ml Tampone Sodio Acetato PH 5.2
- CH<sub>3</sub>COOH
- 50µL di  $\beta$  glucoronidasi/ sulfatasi
- Incubare una notte a 37°C



- Portare a Ph 10-12 con NaOH 10N
- Estrarre con Etyl Acetato
- Purificare con colonnine XC(100mg)
- Eluire con CH<sub>3</sub>OH/NH<sub>4</sub>OH 95:5
- Portare a secco in corrente di azoto
- Riprendere il residuo con 0,5 mL di CH<sub>3</sub>OH al 50%

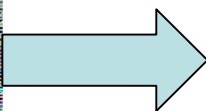
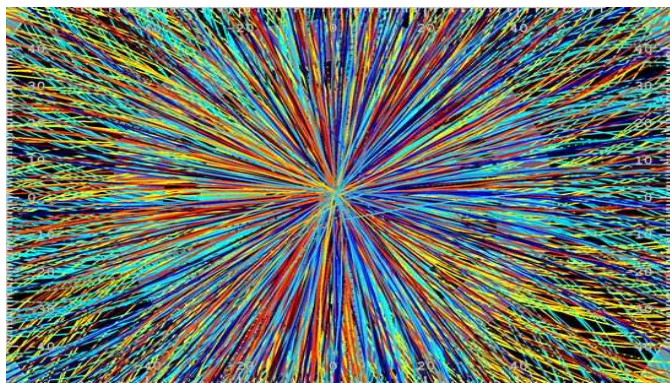
Analizzare in LC-MS-MS



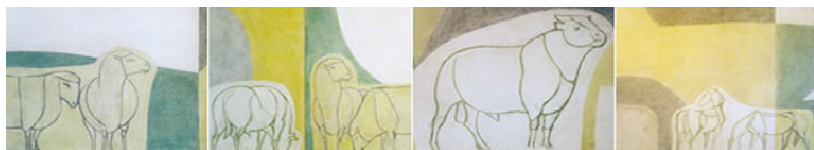
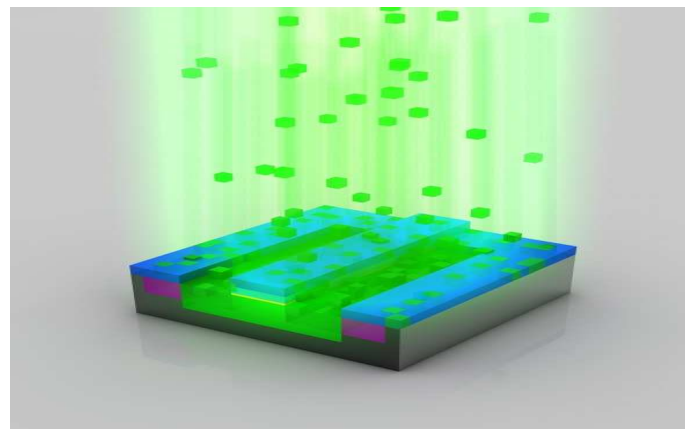
# PRINCIPIO LC-MS-MS

- Tale tecnica permette di identificare da una miscela complessa una o più sostanze in essa presenti
- Tecnica basata sulla frammentazione in “ioni” ( $m/z$ ) , caratteristici e specifici di ciascuna sostanza

Miscela complessa



Ioni di interesse  $m/z$



# IMPOSTAZIONI PARAMETRI STRUMENTALI

## Ioni di interesse rapporto m/z

Analita	Ione precursore (m/z)	Ione figlio(m/z)	Energia di collisione (eV)	Dwell Time ms
Clenbuterolo	277	203	20	100
		259	15	
Ractopamina	302	164	22	100
		284	20	
Brombuterolo	367	293	25	100
		349	15	
Bromclorbuterolo	323	249	20	100
		168	25	
Isossisuprina	302	284	20	100
		150	30	
Salbutamolo	240	148	20	100
		222	15	
Terbutalina	226	152	20	100
		125	30	

Tempo (min)	% Fase mobile A	% Fase mobile B
0	90	10
3	90	10
10	50	50
15	10	90
22	90	10
25	90	10

## Condizioni Spettrometro di massa :

**Ionizzazione:** ESI positivi

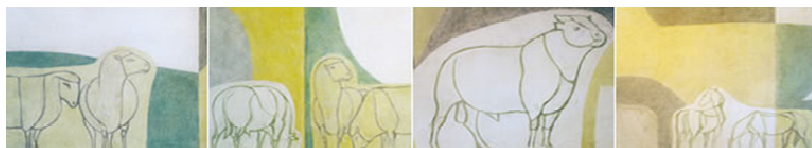
**Voltaggio capillare:** 5500 V

**Orifizio:** 60V

**Temperatura TIS:** 300 °C

**Flusso N<sub>2</sub>:** 60

**Flusso Aria:** 60





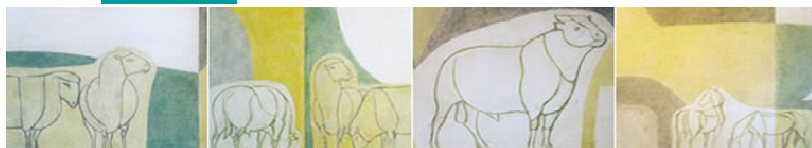
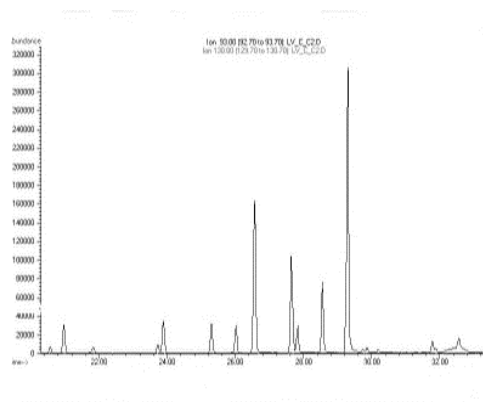
# LC-MS-MS



**Separa sostanze semplici  
da miscele complesse**

-Tipo largamente utilizzato C18

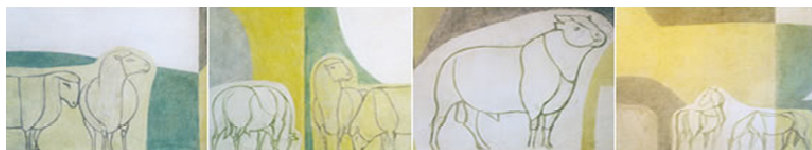
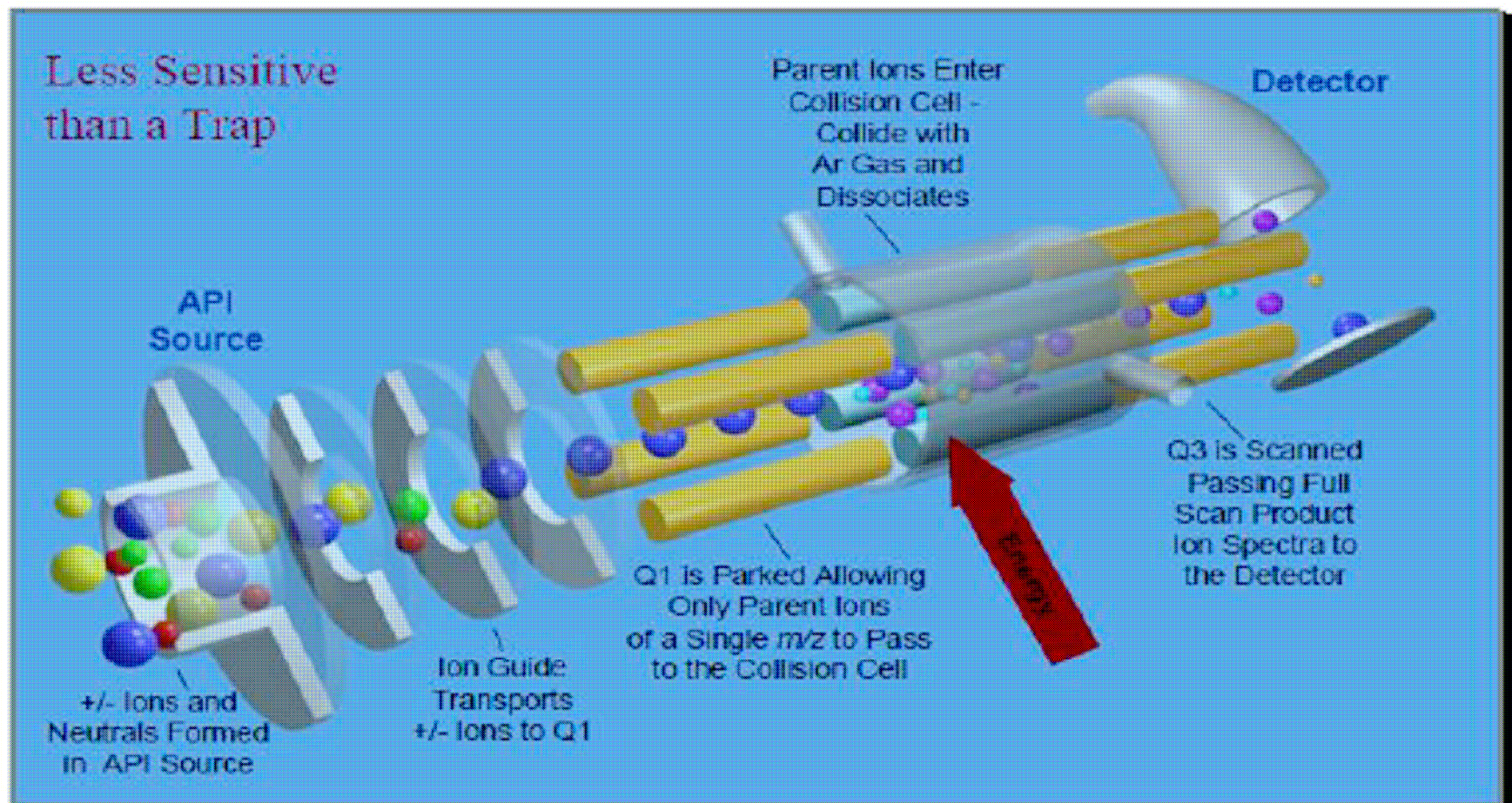
Il cui nome deriva dalla natura  
dell'impaccamento usato, una  
molecola costituita da 18 atomi  
di carbonio.





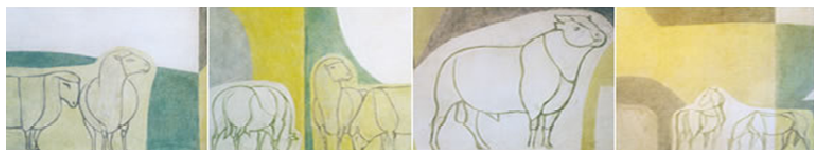
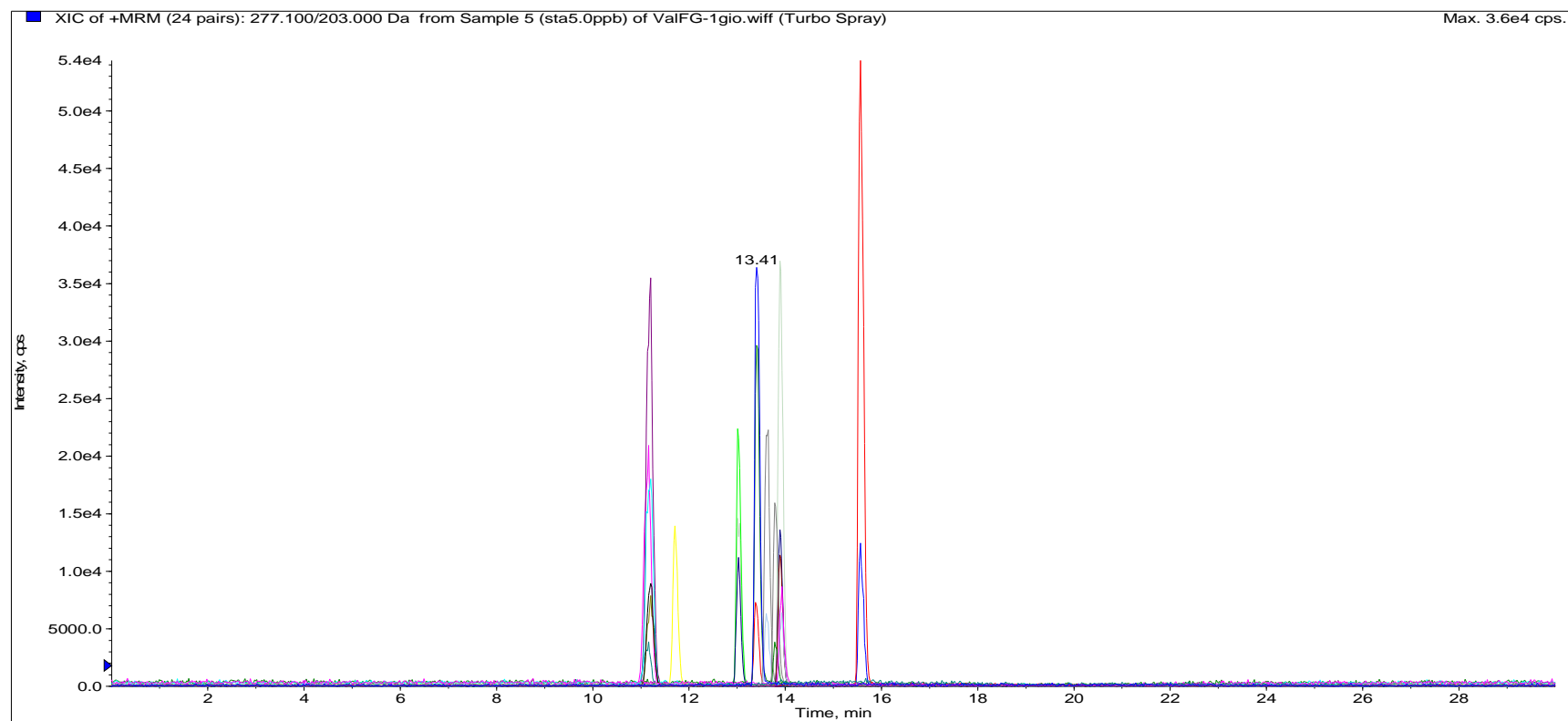
# QUADRUPOLO LC-MS-MS

## Triple Quadrupole: Full Scan MS/MS



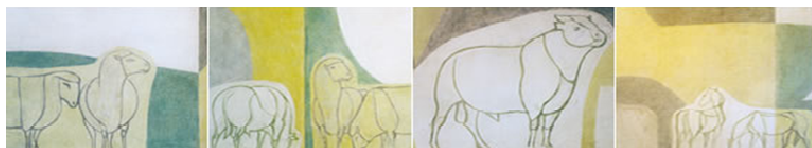
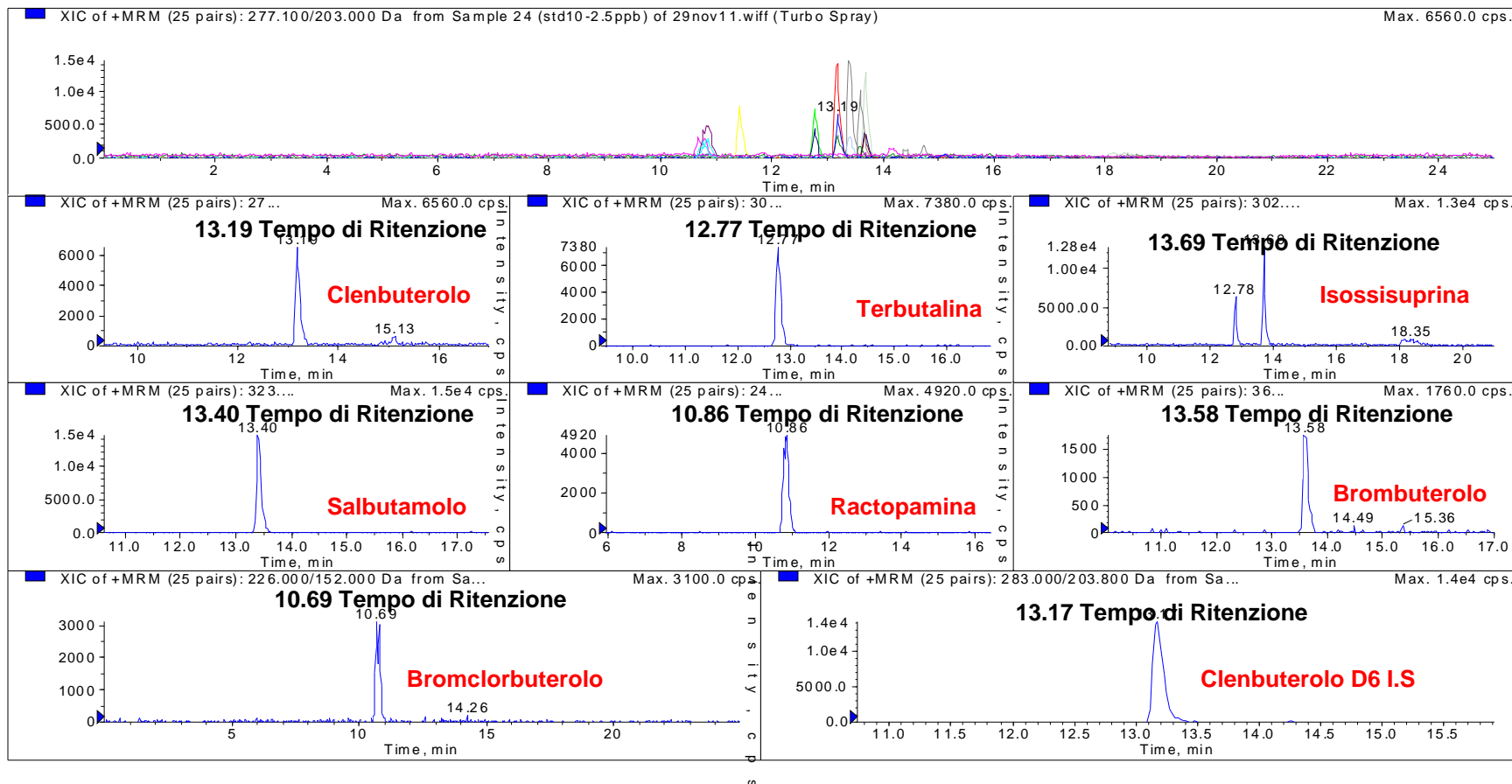


# Cromatogramma TIC (Total Ion Chromatogram)



# Esempio di Cromatogramma (XIC)

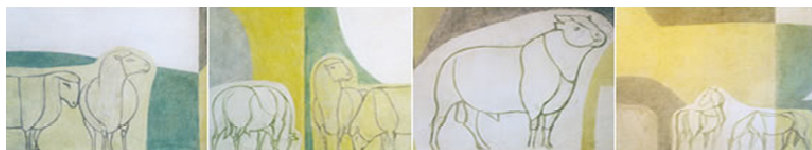
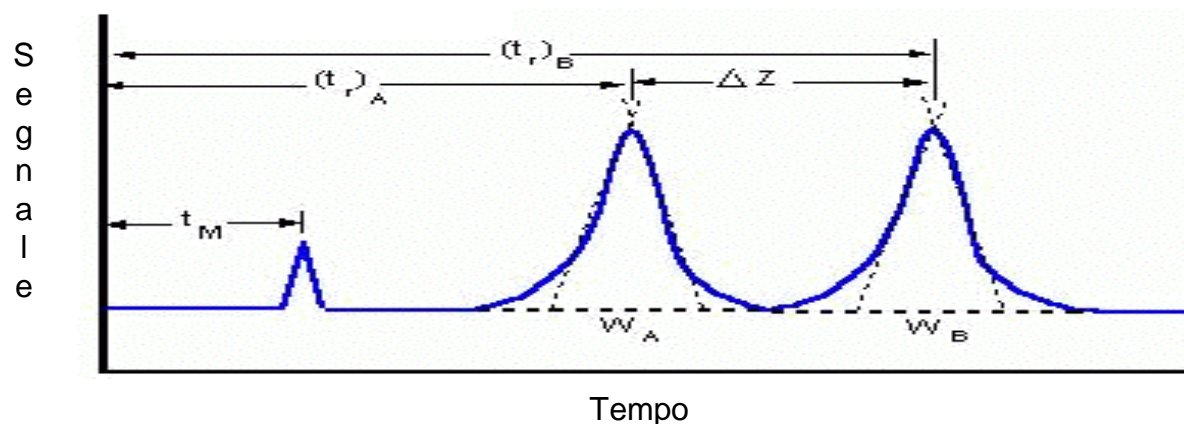
(campione addizionato 0.5 µg/L)



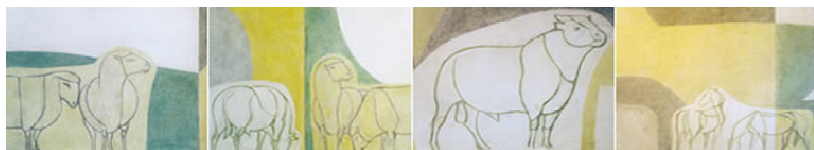
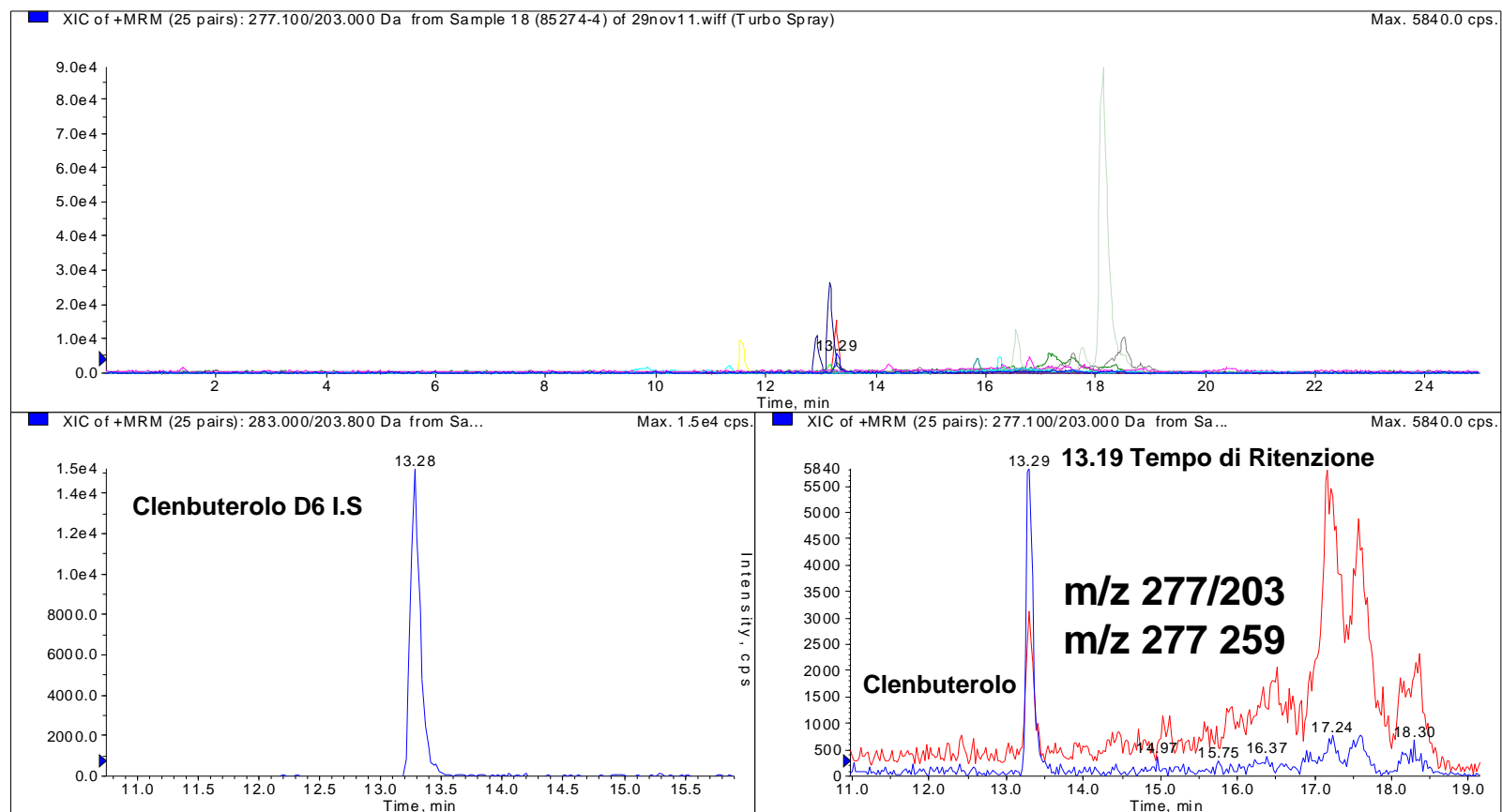


# TEMPO DI RITENZIONE

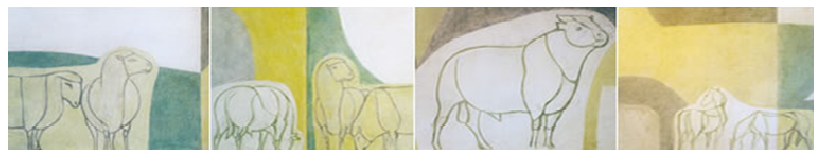
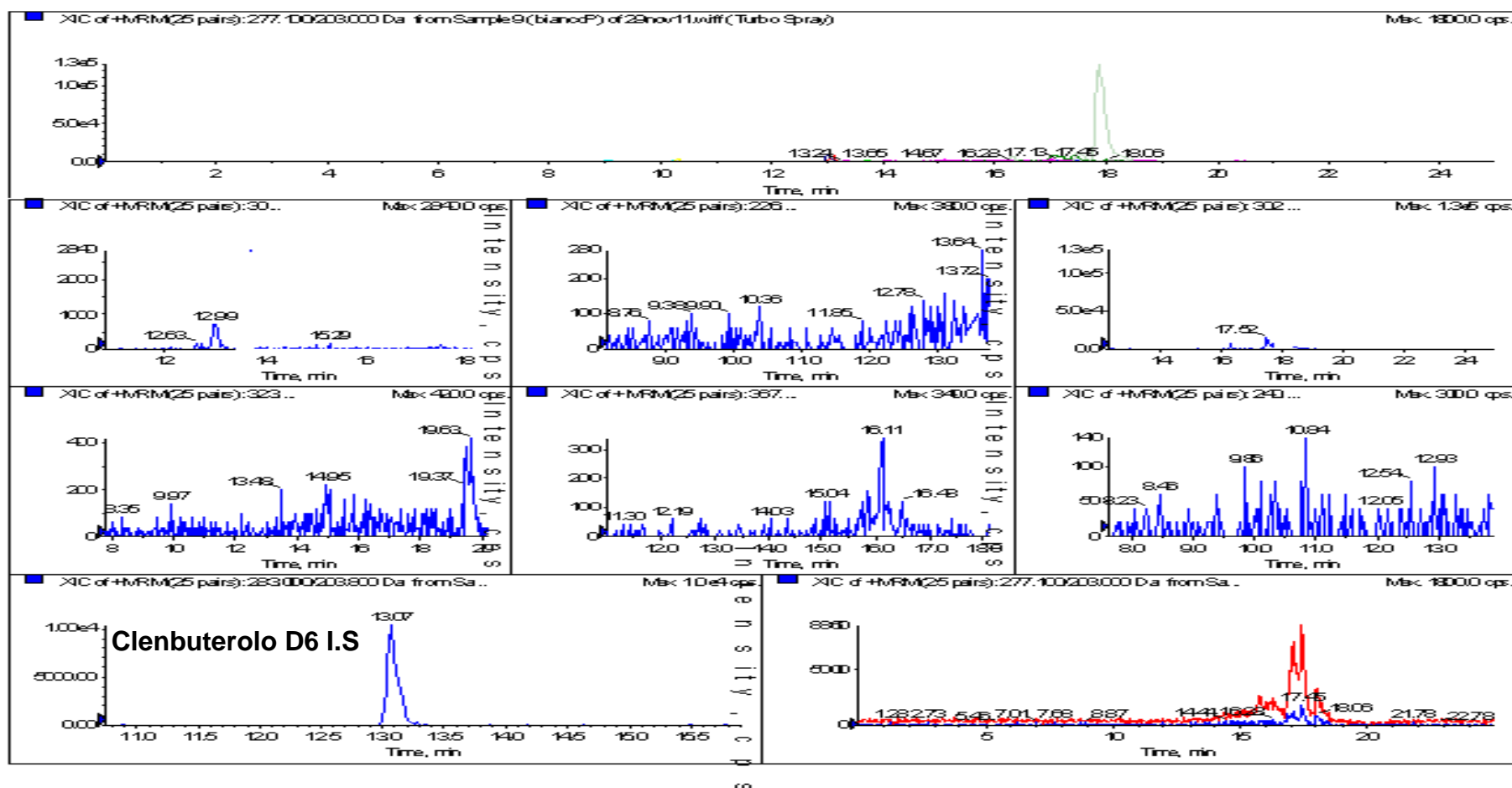
- Il tempo di ritenzione di un picco ( $t_r$ ) è il tempo che intercorre dall'introduzione del campione al momento in cui l'apice del picco raggiunge il rivelatore.



# Urina bovina positiva per Clenbuterolo



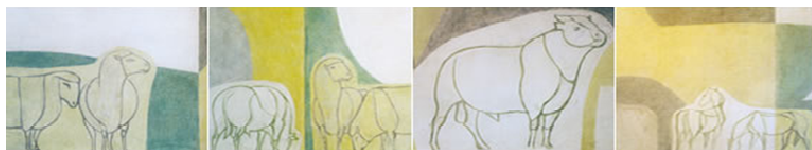
# Campione bianco urina bovina



## Parametri da verificare per la conferma

- Tempo di Ritenzione degli analiti
- Presenza di entrambi gli ioni (m/z)  
**(Clenbuterolo 277/203- 277/259)**
- Assenza di picchi cromatografici interferenti

**Campione confermato  
NON CONFORME**





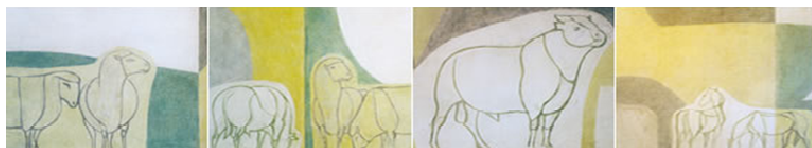
# GRAZIE PER L'ATTENZIONE!!!!



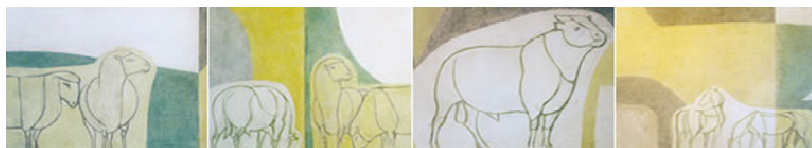
Francesco Necci

Roma 20 Novembre 2012

# DOMANDE .....

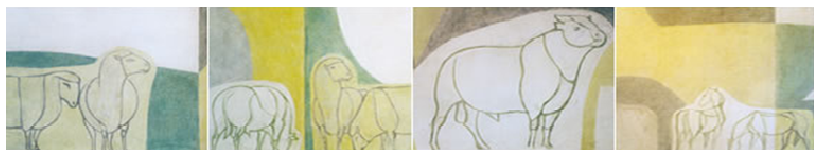


# IL Palio di Siena: IL “DOPING”



# Storia...

- Il **Palio di Siena** è una competizione fra le contrade di Siena nella forma di una giostra equestre di origine medievale.
- La "*Carriera*", come viene tradizionalmente chiamata la corsa, si svolge normalmente due volte l'anno: il 2 luglio si corre il *Palio di Provenzano* (in onore della Madonna di Provenzano) e il 16 agosto il *Palio dell'Assunta* (in onore della Madonna Assunta).

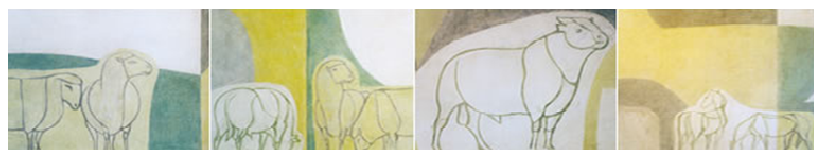




# DOPING COME E PERCHE'

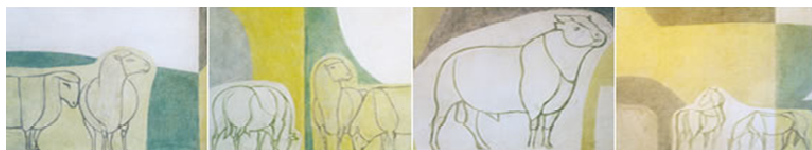
Doping è un termine inglese che tradotto in italiano significa grosso modo: *fare uso di droghe o sostanze stupefacenti*;

Doping nell' ambito sportivo significa: ***usare sostanze o procedimenti destinati ad aumentare artificialmente il rendimento in occasione di una gara sportiva***



# TIPI DI DOPING

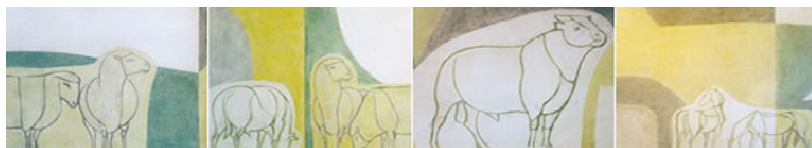
- Periodo pre-gara per tentare di aumentare le masse muscolari e la forza fisica.
- Durante la gara, per ridurre il senso di fatica, o per stimolare il S.N.C
- Dopo la gara, per riacquistare il più velocemente possibile le energie



# Principali classi e sostanze dopanti

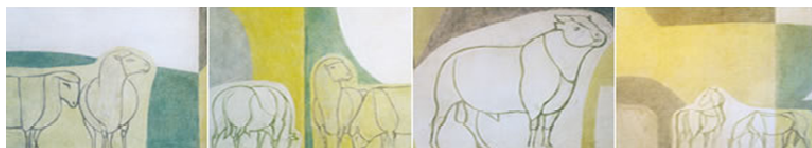
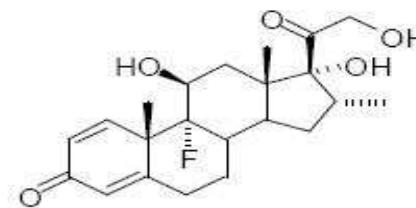
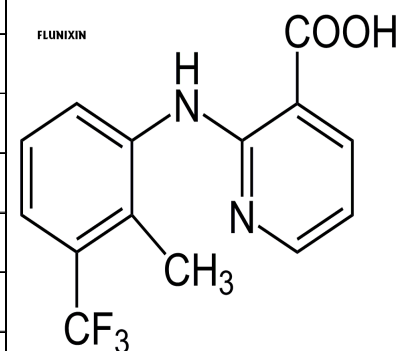
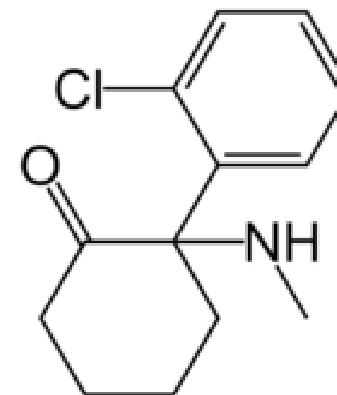


- **Stimolanti:** amfetamina caffeina cocaina
- ***Narcotici:*** buprenorfina pentazocina,
- ***Agenti anabolizzanti*** :stanozololo testosterone nandrolone
- ***Antinfiammatori:*** Flunixin ,Ibuprofene
- ***Cortisonici:*** Desametasone ,Flumetasone



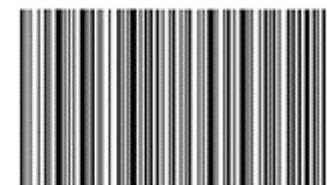
# MOLECOLE RICERCATE PER IL PALIO DI SIENA

<i>Molecole palio</i>	<i>Classe</i>	<i>Tecnica di analisi</i>
Butorfanolo	Narcotici	ELISA
Detomidina	Narcotici	ELISA
Ketamina	Narcotici	ELISA
Fentanyl	Narcotici	ELISA
Lidocaina	Narcotici	ELISA
Tramadol	Narcotici	ELISA
Mepivacaina	Narcotici	ELISA
Procaina	Narcotici	ELISA
Flunixin	AINS	ELISA
Ibuprofene	AINS	ELISA
Fenilbutazone	AINS	ELISA
Ketoprofene	AINS	ELISA
Desametasone	Cortisonici	ELISA
Metilprednisolone	Cortisonici	ELISA
Triamcinolone	Cortisonici	ELISA
Acetonide		





# KIT ANTIDOPING



**100523**  
**Identificativo Cavallo**

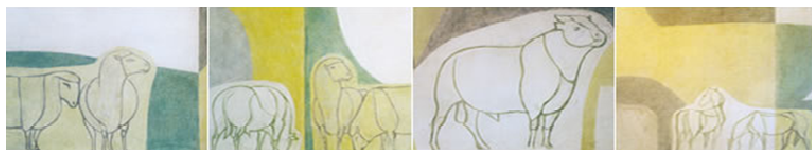


# Numero Campioni Analizzati



**74 campioni**  
**02/07/2012**

**77 campioni**  
**16/08/2012**



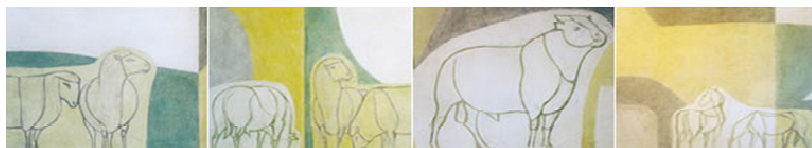
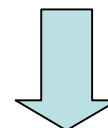
# N°KIT ELISA

**N° ANALITI DA RICERCARE :15**

**N° CAMPIONI DA ANALIZZARE:  
77 (depositati in doppio)**

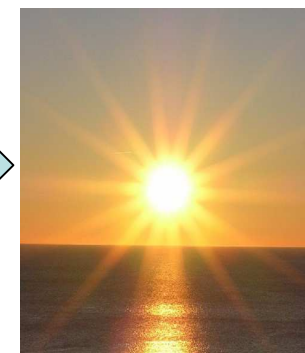
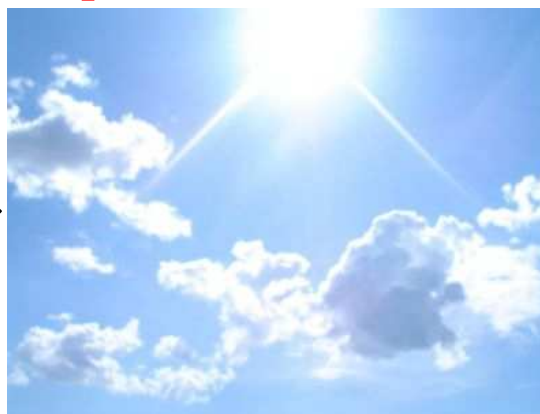
**N° POZZETTI E.L.I.S.A :96 per piastra**

**TOTALE: 2 Piastre per ogni analita**

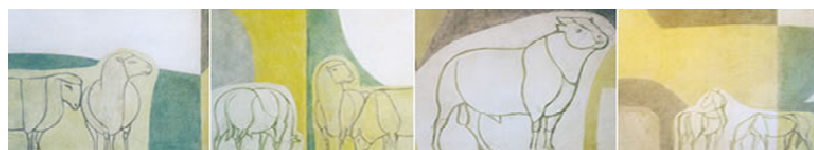
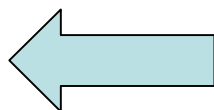




# Tempi di risposta 24h!!!!

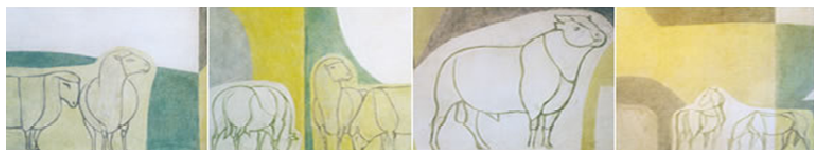


ORE 00.30...





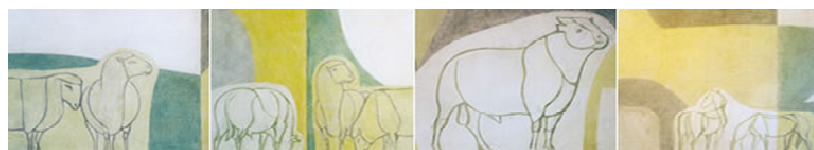
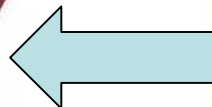
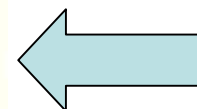
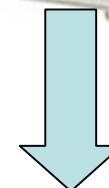
# CATENA DI MONTAGGIO D.O.C



Francesco Necci

Roma 20 Novembre 2012

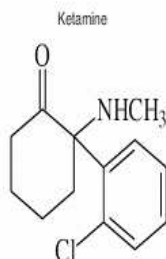
# SCHEMA DI LAVORO



# KIT ANTIDOPING

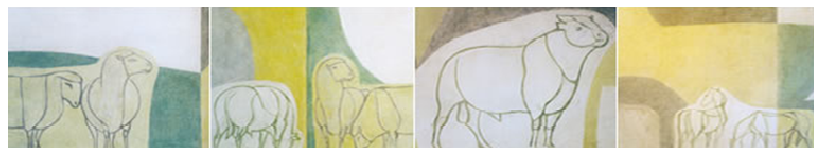
## KETAMINE

Product # 109410  
& 109415 (5 Ki

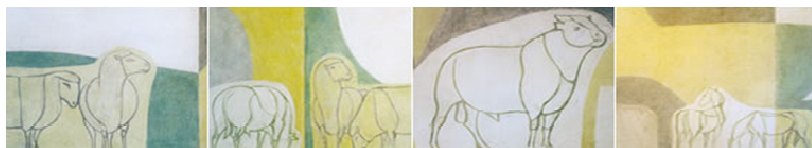
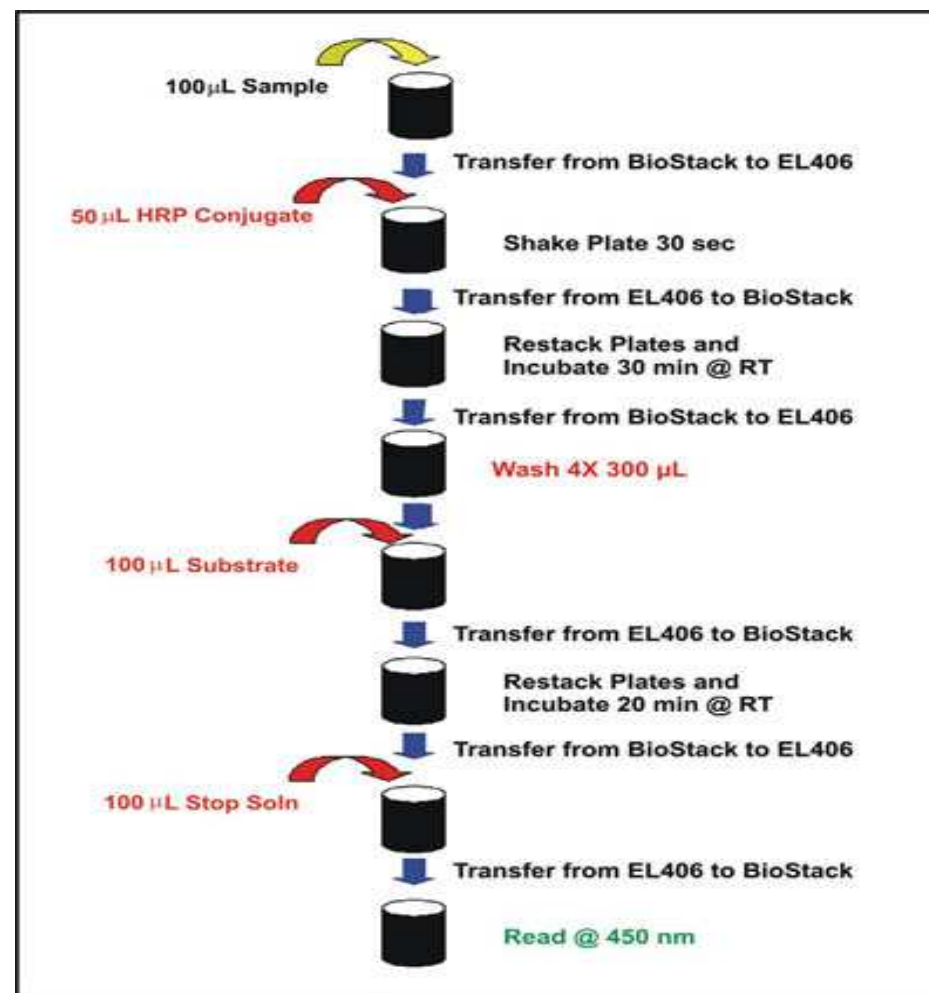
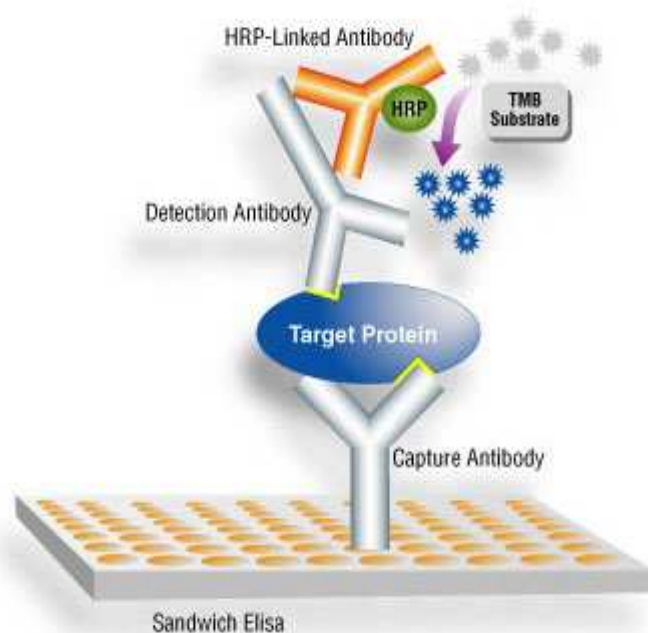


Note: "Typical" data is a representation. Variances in data will occur.

SENSITIVITY			
I-50 in EIA Buffer			
Ketamine	8ng/ml		
Norketamine	181ng/ml		
I-50 in Equine Urine (Diluted 1:4)		I-50 in Canine Urine (Diluted 1:4)	
Ketamine	96ng/ml	Ketamine	41 ng/ml
Norketamine	1232ng/ml	Norketamine	657 ng/ml
I-50 in Equine Plasma			
Ketamine	43ng/ml		
Norketamine	304ng/ml		



# PROCEDURA KIT



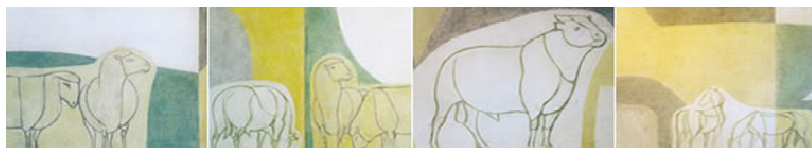


# RISULTATI



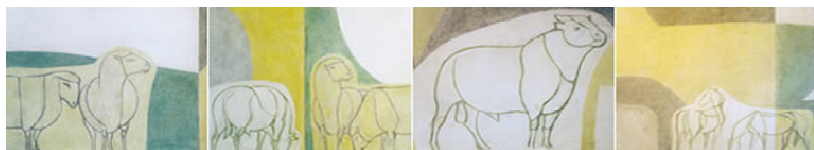
02/07/2012	LIDOCAINA
02/07/2012	METILPREDNISOLONE
02/07/2012	BUTORFANOLO, METILPREDNISOLONE PROCAINA
02/07/2012	BUTORFANOLO, DESAMETASONE
02/07/2012	PROCAINA
02/07/2012	BUTORFANOLO, DESAMETASONE,IBUPROFENE METILPREDNISOLONE
16/08/2012	IBUPROFENE
16/08/2012	IBUPROFENE
16/08/2012	IBUPROFENE, LIDOCAINA
16/08/2012	FENILBUTAZONE, DESAMETASONE,FLUNIXIN

**TOTALE: 6 CAMPIONI NON REGOLAMENTARI 02/07/2012**  
**4 CAMPIONI NON REGOLAMENTARI 16/08/2012**



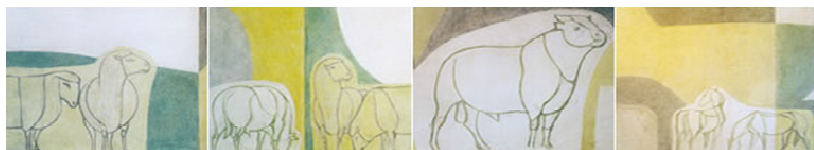
# VINCITORI PALIO DI SIENA 2012

Anno	Contrada	Fantino	Cavallo
02/07/2012	ONDA 	Luigi Boschelli detto Trecciolino	IVANOV 
16/08/2012	VALDIMONTE 	Jonatan Bartoletti Detto scompiglio	LO SPECIALISTA 



# VINCITORI MORALI PALIO DI SIENA 2012

- Dr. Bruno Neri
- Dr. Luigi Giannetti
- Elisa Gennuso
- Andrea Giorgi
- Luca Alessandrone
- Dario Lucchetti
- Tabita Mauti
- Daniela Barchi
- Francesco Necci



# MARIE CURIE (1867-1934)



- Uno scienziato nel suo laboratorio non è soltanto un tecnico, è anche un fanciullo posto di fronte a fenomeni naturali che lo impressionano come un racconto di fate

Grazie a tutti per l'attenzione

