

# Analysis of $\beta$ -agonists in liver and urine using molecular imprinted polymers and LC-MS/MS

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## INTRODUCTION

$\beta$ -Agonists act as growth promoters and also influence the lipolysis, transferring fat to muscle. These effects have made them misused in breeding of bovine and other farm animals.

Administration of  $\beta$ -agonists as growth promoters was banned in EU by directive 96/22/EC.

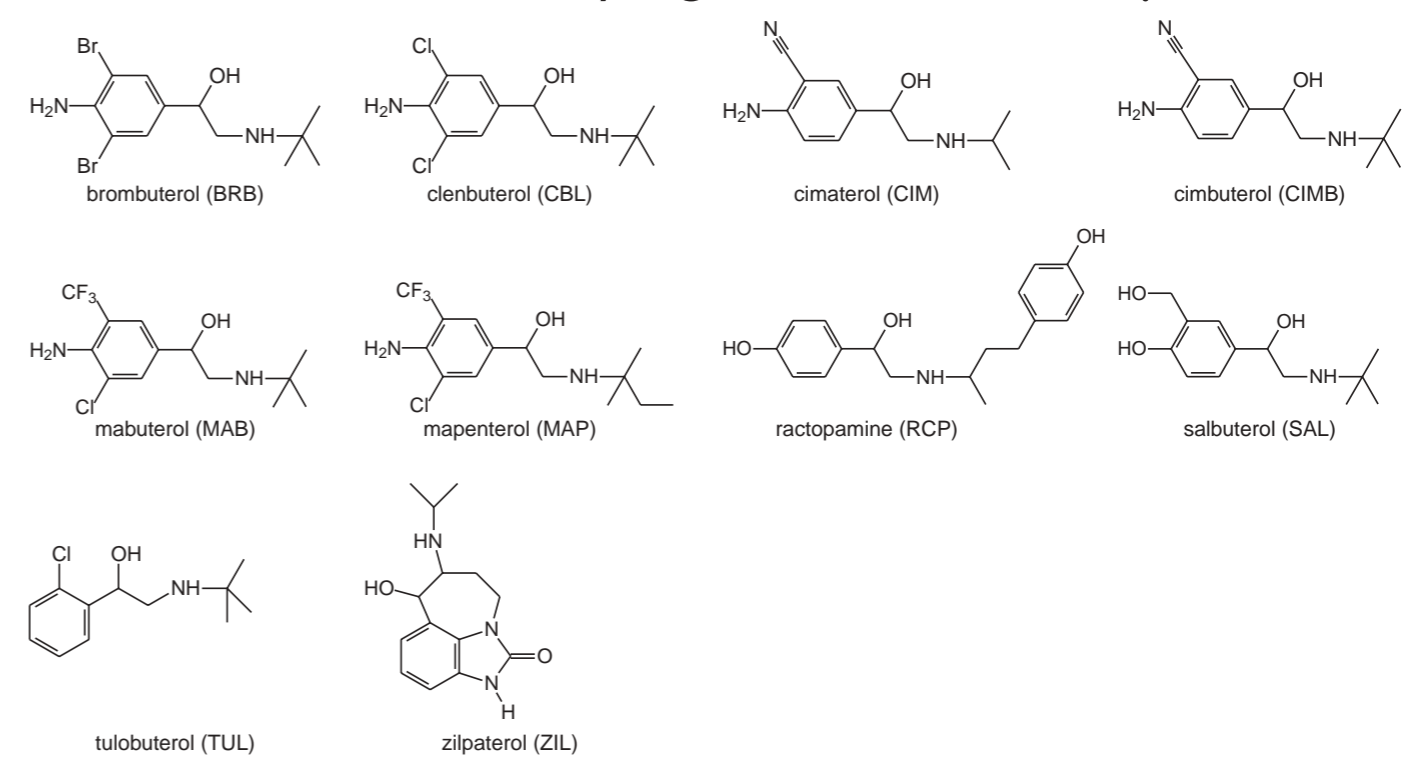
To control the implementation of this directive it is necessary to analyse these compounds in both live and slaughtered animals.

We have chosen liver and urine as suitable matrixes, molecular imprinted polymers for the isolation of the  $\beta$ -agonists from the matrixes and LC-MS/MS for the quantification and confirmation. In our method we have included ten  $\beta$ -agonists: clenbuterol, brombuterol, cimaterol, cimbuterol, mabuterol, mapenterol, ractopamine, salbutamol, tulobuterol and zilpaterol. Deuterated analogs are used as internal standards for seven of the compounds.

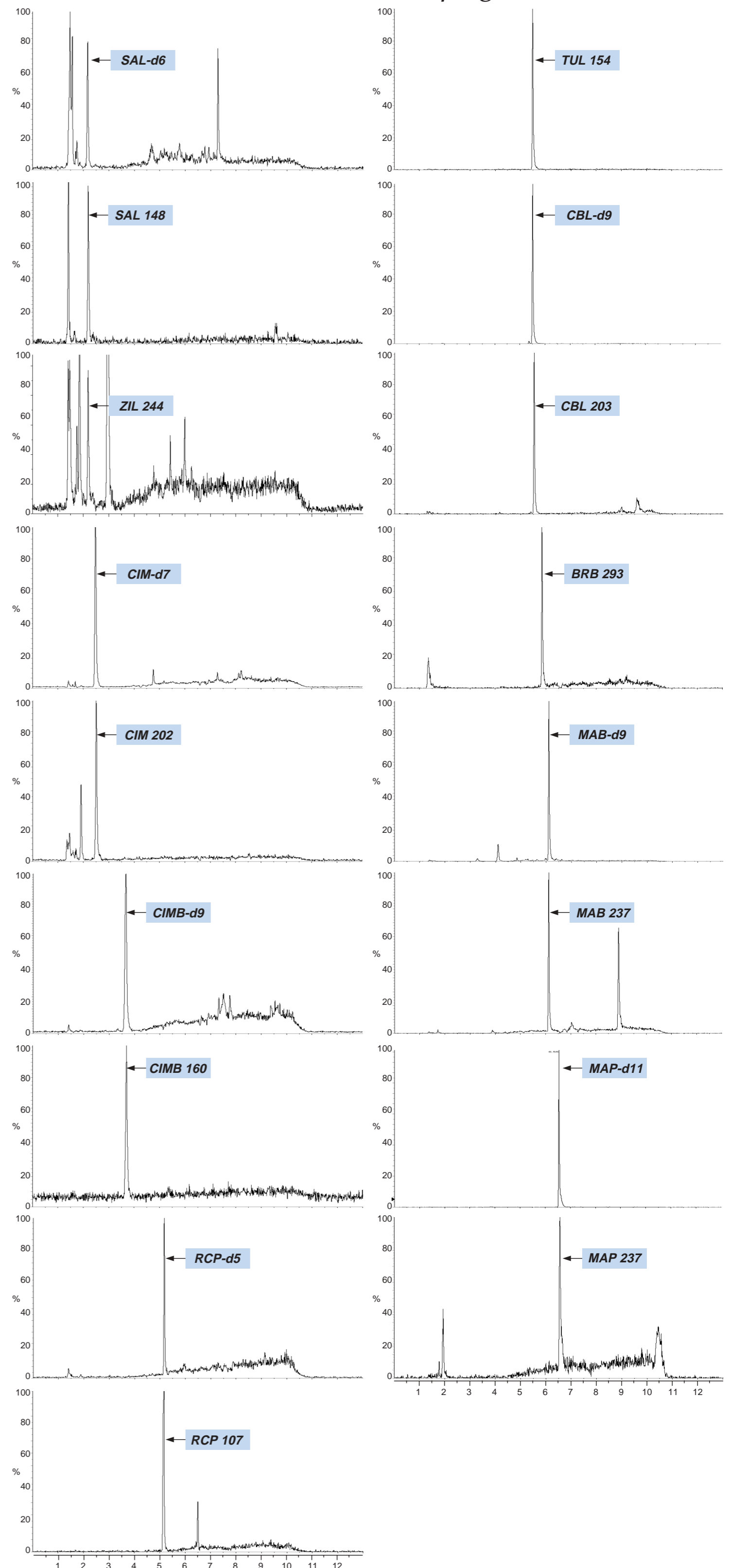
## Aim of the study

The aim of this study was to develop a method suitable for both screening and confirmation of the most common  $\beta$ -agonists at the proposed MRPL levels or lower and for clenbuterol at the MRL level, 0.5  $\mu\text{g}/\text{kg}$ . The method should be applicable for several matrixes: liver, urine and muscle from different species. Preferably it should also take environmental consideration by using a minimum of organic solvents.

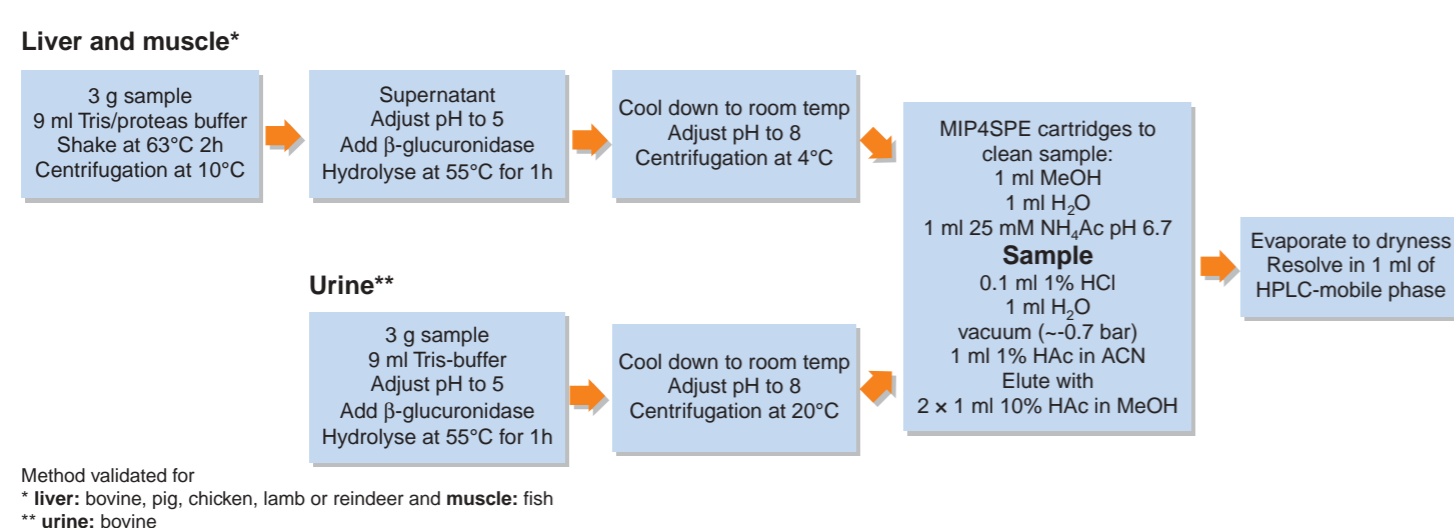
## The structure of the 10 $\beta$ -agonists in the study



## MRM chromatograms of bovine liver samples spiked at validation levels with the different $\beta$ -agonists



## Flow chart of the analytical method



Method validated for  
\* liver: bovine, pig, chicken, lamb or reindeer and muscle: fish  
\*\* urine: bovine

## LC-MS/MS parameters

### HPLC

**Column:** Chromolith Performance, RP-18e, 100 x 4.6 mm  
**Flow:** 1 ml/min  
**Solvent A:** 5 mM NH<sub>4</sub>COOH, pH 4  
**Solvent B:** 5% solvent A in ACN  
**Gradient:** from 90% A: 10% B to 25% A: 75% B in 8 min

### MS/MS

**Instrument:** Applied Biosystems 4000 Q TRAP™ LC/MS/MS System  
**Scan type:** MRM  
**Ionization:** ES+  
**Temperature:** 650°C  
**Ion spray voltage:** 5kV

### MRM transitions:

Substances	Parent ions (m/z)	Daughter ions (m/z)	Declustering Potential (V)	Collision Energy (V)	Collision Cell Exit Potential (V)
BRB	367	<b>293</b>	76	27	16
		349		19	10
CBL	277	<b>203</b>	46	25	14
		259		17	16
CIM	220	<b>202</b>	46	17	14
		160		27	12
CIMB	234	<b>160</b>	36	21	14
		216		13	14
MAB	311	<b>237</b>	61	25	16
		293		19	8
MAP	325	<b>237</b>	56	29	14
		217		37	18
RCP	302	<b>107</b>	46	47	6
		284		11	10
SAL	240	<b>148</b>	41	27	10
		222		17	14
TUL	228	154	51	23	14
		172		19	12
ZIL	262	<b>244</b>	46	19	14
		185		35	12
CBL-D9	286	<b>204</b>	46	25	14
CIM-D7	227	<b>209</b>	46	17	14
CIMB-D9	243	<b>225</b>	36	13	14
MAB-D9	320	<b>302</b>	61	19	8
MAP-D11	336	<b>238</b>	56	29	14
RCP-D5	307	<b>289</b>	46	11	10
SAL-D6	246	<b>228</b>	41	17	14

Daughter ions in bold type are used for screening and the others are for confirmation.

## Validation

Validation was performed according to Commission Decision 2002/657/EC at suggested MRPL levels (0.5-2.0  $\mu\text{g}/\text{kg}$ ).

Decision limit (CC $\alpha$ ) and Detection capability (CC $\beta$ ) calculated by calibration curve procedure.

Substances	Level ( $\mu\text{g}/\text{kg}$ )	Bovine liver		Pig liver		Chicken liver		Bovine urine		Fish muscle	
		CC $\alpha$	CC $\beta$	CC $\alpha$	CC $\beta$	CC $\alpha$	CC $\beta$	CC $\alpha$	CC $\beta$	CC $\alpha$	CC $\beta$
BRB	1.0	0.21	0.35	0.28	0.47	0.11	0.20	0.12	0.21	0.11	0.19
CBL	0.5	0.07	0.12	0.12	0.20	0.04	0.06	0.05	0.09	0.08	0.14
CIM	1.0	0.14	0.23	0.18	0.30	0.04	0.07	0.13	0.22	0.14	0.23
CIMB	1.0	0.17	0.29	0.38	0.64	0.06	0.10	0.18	0.30	0.14	0.23
MAB	1.0	0.12	0.21	0.24	0.40	0.04	0.07	0.10	0.18	0.10	0.17
MAP	1.0	0.12	0.21	0.18	0.31	0.04	0.07	0.12	0.21	0.12	0.21
RCP	2.0	0.35	0.60	0.68	1.15	0.21	0.35	0.30	0.50	0.22	0.37
SAL	1.0	0.14	0.25	0.18	0.30	-	-	0.24	0.42	0.11	0.19
	2.0	-	-	-	-	0.15	0.26	-	-	-	-
TUL	1.0	0.15	0.26	0.19	0.33	0.11	0.18	0.21	0.35	0.13	0.22
ZIL	1.0	0.19	0.32	0.27	0.46	0.15	0.25	0.18	0.30	0.15	0.26

## CONCLUSIONS

We have developed and validated a sensitive method for ten  $\beta$ -agonists in liver, urine and fish muscle. Validation of the compounds in bovine muscle is going on.

Confirmation according to Commission Decision 2002/657/EC was achieved for all compounds at the proposed MRPL levels. Salbutamol, that usually is difficult to detect because of low absolute recovery, could be included and confirmed at 1  $\mu\text{g}/\text{kg}$  (2  $\mu\text{g}/\text{kg}$  in chicken liver) because of the high sensitivity of the MS/MS instrument.