CONFIRMATION OF β-AGONISTS RESIDUES IN BOVINE RETINA AND LIVER USING HPLC-MS/MS AND EVALUATION OF MATRIX-DEPENDENT PROBLEMS

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INTRODUCTION

- **β-agonists or β-adrenergic agonists** are synthetic compounds commonly used to **treat diseases** (as bronchodilators and tocolytic agents) in veterinary medicine.
- Sometimes they are used as **growth promoters** in a fraudulent manner for increasing the weight of animals, in favour of a higher percentages of lean mass. The most frequently used are **clenbuterol**, **salbutamol** and **ractopamine.**
- Several cases of **human intoxication** have been reported such as food poisoning, cardiovascular and central nervous problems, due to the consumption of **meat containing residues** .
- The inappropiate use as growth promoters has led to their prohibition, as manifested in **Council Directive 96/22/EC**.
- In the **Commission Regulation (EU) Nº 37/2010**, maximum residue limits **(MRLs)** have been fixed for **clenbuterol** for specific animal species and different tissues.
- Minimum required performance limits **(MRPLs)** have been established according to the **Commission Decision 2002/657/EC** in the case of substances without MRLs.
- In the case of animal samples, it is important to consider the possible existence of **matrix effect**. This is an alteration or disturbance in the ionization efficiency caused by the presence in the sample of substances that co-elute with the analytes of interest. These alterations can cause problems in accuracy and sensitivity.
- **Retina and liver** can be very variable between different animals. Thus, when analyzing β-agonists in this type of samples it is necessary to evaluate the matrix effect in order to achieve accurate and sensible methods.

EXPERIMENTAL

Bovine Retina/Liver → Sample preparation → Purification procedure → Method validation → Matrix effect experiments

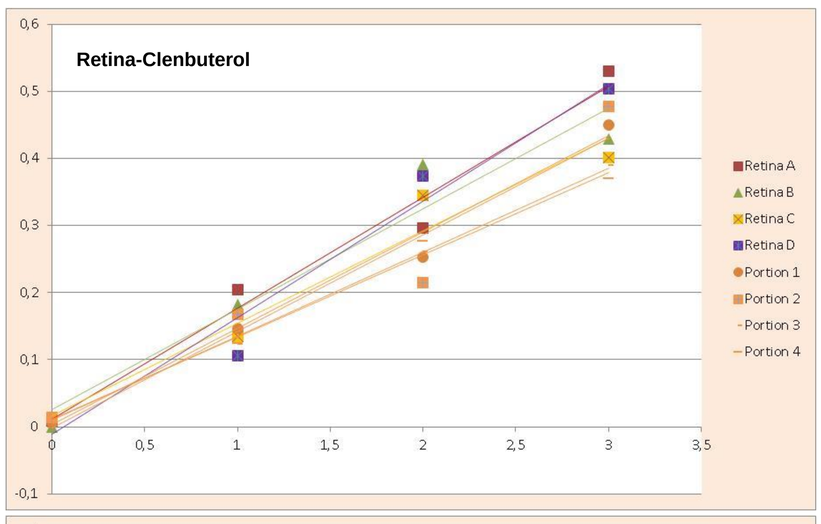
- Various sets of matrix-matched calibration curves were prepared to evaluate the presence of matrix effect in liver and retina samples.
- Samples from different animals were used, to assess inter-individual variations. Also, a pull of samples collected from different animals was prepared for each matrix. In the case of liver samples, different amounts of matrix were tested as well. Post-extraction experiments were performed.
- The analytical method was validated according to Commission Decision 2002/657/EC.

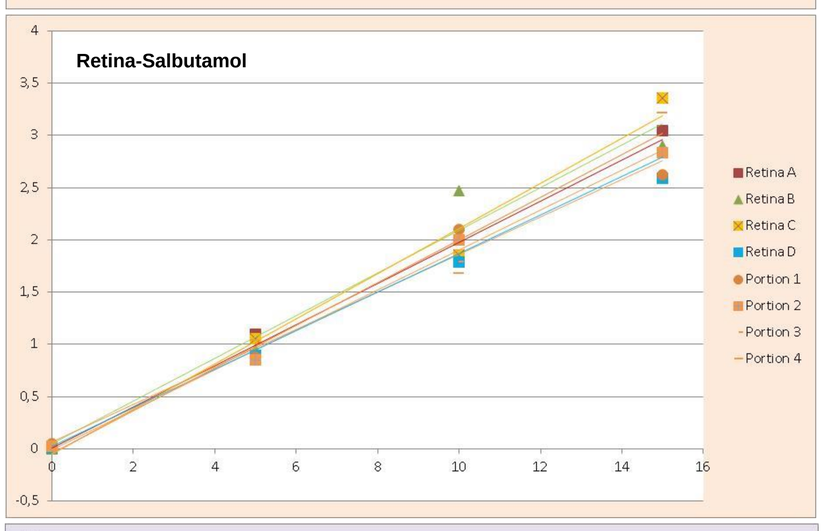
Table 1. Validation levels.

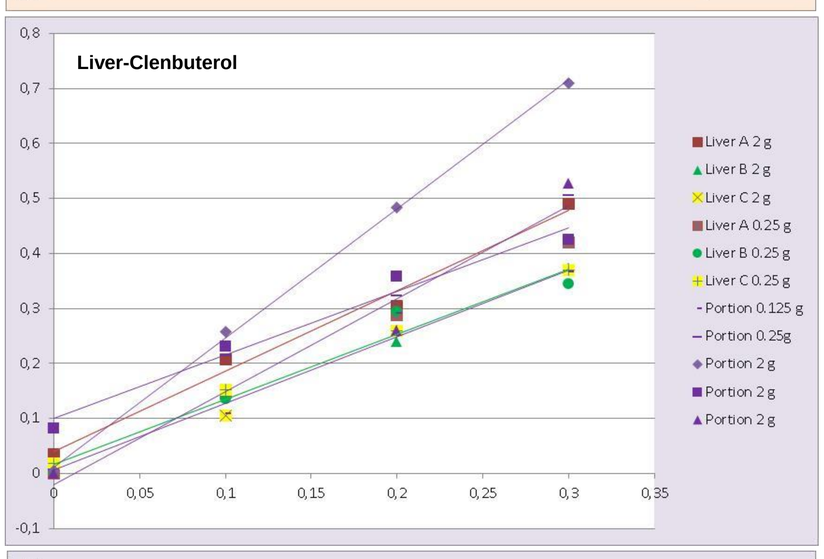
β-agonist	Liver (µg/Kg)	Retina (µg/Kg)
Clenbuterol	0.2	2
Mapenterol	0.2	2
Mabuterol	0.2	2
Clenproperol	0.5	5
Clenpenterol	0.5	5
Cimaterol	0.5	5
Terbutaline	1	10
Salbutamol	1	10
Ractopamine	1	10

RESULTS AND DISCUSSION

- Matrix effect is both matrix and compound dependent, and it can result in an increased signal or ionization or in a decrease (see different calibration lines in figures on the right corner).
- In retina, the existence of rotational effect is clear, and it may be originated in the extraction procedure, mainly in the SPE step. However, the intercept of the curve is hardly affected. The problem seems to disappear when using a pull of samples, but a pull is not representative of individual matrices.
- With regard to the liver, both problems appeared, and they could not be related with the amount of sample. Apparently, liver is a more difficult one, making it difficult to find a representative option to use as reference matrix.
- A confirmatory method based on SPE and HPLC-MS/MS has been developed for detecting 9 β-agonists in bovine retina and liver. The limits of the validated method were: CCα ≤ 1.5 and CCβ ≤ 2.6 µg kg^{-1} in retina, and CCα ≤ 0.18 and CCβ ≤ 0.30 µg kg^{-1} in liver.







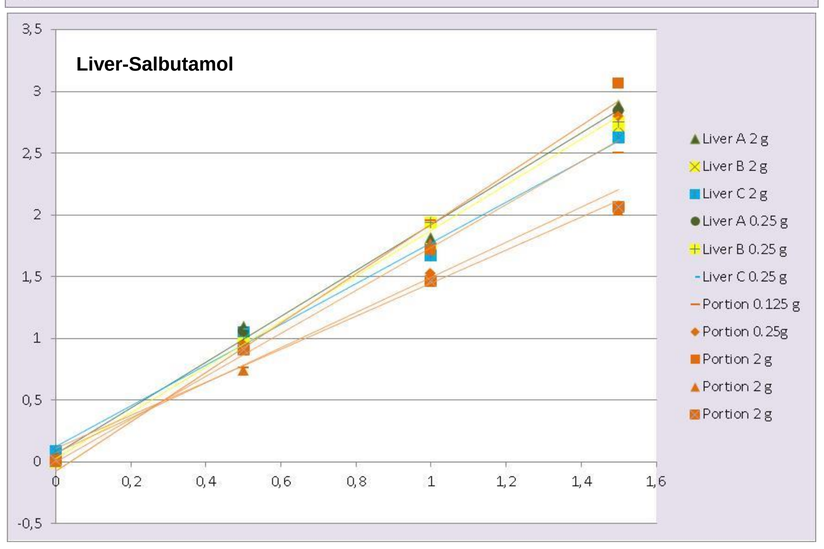


Table 2. Calibration curves prepared for studying matrix effect in β-agonists determination in bovine **retina** (m: slope; b: y-intercept; R^2>0.95).

Matrix/Analyte		Clenbuterol		Clenpenterol		Clenproperol		Mabuterol		Mapenterol		Ractopamine		Salbutamol		Cimaterol		Terbutaline	
		m	b	m	b	m	b	m	b	m	b	m	b	m	b	m	b	m	b
INDIVIDUAL	Retina A	0,166	0,011	0,159	-0,014	0,149	0,039	0,056	0,000	0,158	0,024	0,080	0,010	0,197	0,010	0,113	-0,032	0,180	0,012
	Retina B	0,150	0,026	0,206	-0,053	0,208	-0,069	0,227	-0,012	0,256	-0,028	0,124	-0,061	0,193	0,084	0,115	0,007	0,226	-0,038
	Retina C	0,138	0,017	0,163	0,019	0,162	-0,022	0,208	-0,021	0,179	-0,004	0,111	-0,028	0,217	-0,055	0,199	0,047	0,162	0,001
	Retina D	0,174	-0,012	0,142	-0,057	0,143	-0,059	0,181	-0,045	0,173	-0,015	0,097	0,027	0,173	0,021	0,091	-0,015	0,111	0,140
PULL	Portion 1	0,012	0,124	-0,065	0,169	-0,023	0,178	0,081	0,190	0,002	0,269	0,016	0,080	0,105	0,187	0,020	0,216	0,037	0,201
	Portion 2	0,003	0,144	-0,004	0,135	0,028	0,134	0,053	0,154	0,005	0,296	0,021	0,094	0,023	0,198	-0,002	0,186	0,136	0,161
	Portion 3	0,010	0,125	-0,018	0,151	-0,046	0,158	-0,037	0,179	-0,009	0,281	0,050	0,072	0,021	0,185	-0,034	0,201	0,028	0,187
	Portion 4	0,018	0,121	-0,018	0,155	0,033	0,156	0,056	0,156	0,000	0,296	-0,017	0,081	-0,046	0,204	-0,049	0,231	-0,029	0,186
STANDARD	1	0,014	0,146	-0,081	0,222	-0,049	0,128	0,007	0,029	-0,027	0,206	-0,023	0,083	0,037	0,189	0,008	0,089	0,070	0,151
	2	0,009	0,120	0,000	0,162	0,016	0,117	0,006	0,173	0,018	0,230	-0,007	0,044	0,028	0,160	0,043	0,171	0,058	0,132

Table 3. Calibration curves prepared for studying matrix effect in β-agonists determination in bovine **liver** (m: slope; b: y-intercept; R^2>0.95).

Matrix/Analyte		Clenbuterol		Clenpenterol		Clenproperol		Mabuterol		Mapenterol		Ractopamine		Salbutamol		Cimaterol		Terbutaline	
		m	b	m	b	m	b	m	b	m	b	m	b	m	b	m	b	m	b
INDIVIDUAL 2g	Liver A	1,461	0,040	1,372	0,011	1,666	0,038	1,838	0,043	2,073	0,051	0,707	0,050	1,854	0,066	2,487	-0,041	1,798	0,031
	Liver B	1,169	0,016	1,575	-0,039	1,737	0,040	2,268	0,015	2,459	0,037	0,817	0,030	1,797	-0,015	1,780	0,006	1,548	-0,009
	Liver C	1,208	0,007	1,392	0,013	1,741	0,026	1,859	0,040	2,176	0,024	0,629	0,100	1,647	0,125	1,337	0,208	1,483	0,124
INDIVIDUAL 0.25g	Liver A	1,398	0,004	1,796	-0,034	1,295	0,016	2,259	0,008	2,453	-0,006	0,730	-0,004	1,879	0,051	2,228	0,050	1,665	0,004
	Liver B	1,185	0,017	1,627	0,032	1,322	0,027	1,590	0,036	1,987	0,012	0,762	-0,012	1,848	0,028	2,487	0,020	1,794	0,018
	Liver C	1,166	0,025	1,531	0,047	1,352	0,024	2,003	0,014	2,180	0,025	0,616	0,018	1,691	0,098	2,201	0,047	1,473	0,122
PULL	0.125g	1,263	0,004	1,568	-0,002	1,266	-0,001	2,369	0,006	2,330	0,013	0,593	-0,025	1,737	0,003	2,274	0,027	1,702	0,024
	0.25g	1,604	0,021	1,896	-0,006	1,404	0,062	2,562	-0,015	3,002	0,014	0,624	0,008	1,789	-0,024	1,956	0,030	1,709	0,094
	2 g	2,345	0,012	2,630	-0,074	2,600	-0,027	3,846	-0,009	7,857	-0,021	0,534	0,054	1,997	-0,075	3,378	-0,077	2,352	-0,121
	2 g	1,158	0,100	3,555	-0,049	3,250	0,099	3,794	-0,033	6,096	0,136	0,448	0,028	1,422	0,072	3,310	-0,062	1,767	-0,039
	2 g	1,690	-0,020	3,033	0,018	2,753	0,002	3,388	0,018	6,430	0,069	0,379	0,039	1,342	0,107	1,820	0,076	1,578	0,005
STANDARD	1	1,252	0,002	1,789	-0,002	1,289	0,004	1,787	0,003	2,274	-0,013	0,702	-0,032	1,621	0,049	2,124	0,041	1,493	0,019
	2	1,304	0,008	1,470	0,017	1,039	0,034	1,750	-0,009	2,709	0,005	0,640	0,004	1,784	0,121	1,939	0,076	1,605	0,073

CONCLUSIONS

Matrix-matched calibration is commonly used for quantitation, but there are disadvantages associated with this approach. It is hard to collect blank matrix for a particular matrix, as interindividual variations (sample or animal-dependent) are frequent, especially for liver. Also method-dependent variations/effects are unavoidable.

REFERENCES

Can be supplied by authors under petition (Corresponding author's e-mail: patricia.regal@usc.es)

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