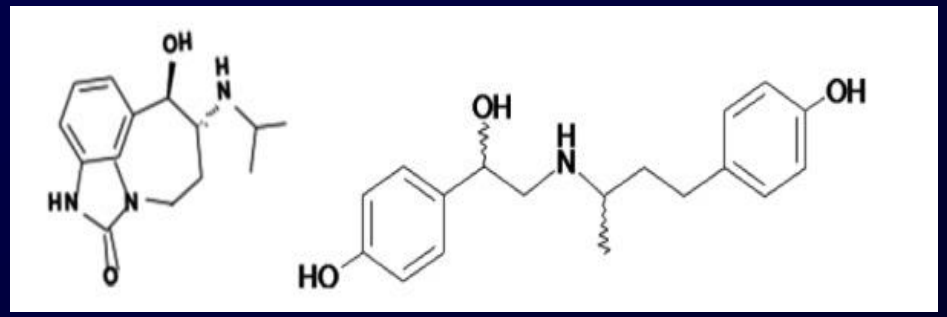
Military Laboratories For Quality Control
MLQC

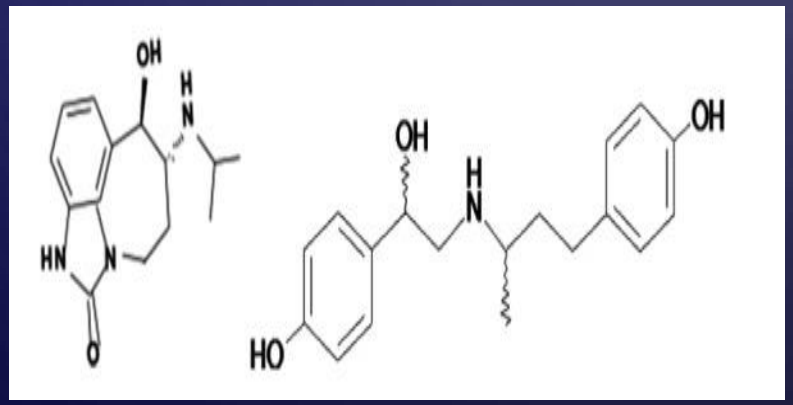
INTRODUCTION

IN THE PURSUIT OF OPTIMIZING LIVESTOCK PRODUCTION, ZILPATEROL AND RACTOPAMINE HAVE EMERGED AS POWERFUL BETA-AGONIST DRUGS WIDELY USED AS GROWTH PROMOTERS IN CATTLE. THESE COMPOUNDS ENHANCE FEED EFFICIENCY, INCREASE LEAN MUSCLE MASS, AND REDUCE FAT DEPOSITION, MAKING THEM VALUABLE TOOLS IN THE MEAT PRODUCTION INDUSTRY. HOWEVER, THEIR USE IS NOT WITHOUT CONTROVERSY. WHILE APPROVED IN THE UNITED STATES AND CANADA, THESE DRUGS ARE STRICTLY BANNED IN THE EUROPEAN UNION AND MANY OTHER COUNTRIES DUE TO CONCERNS OVER POTENTIAL HEALTH RISKS TO CONSUMERS AND ANIMAL WELFARE ISSUES.

THE PRESENCE OF RESIDUES FROM THESE DRUGS IN ANIMAL TISSUES, SUCH AS MUSCLE, LIVER, AND KIDNEY, POSES A SIGNIFICANT CHALLENGE TO FOOD SAFETY. TO PROTECT PUBLIC HEALTH, IT IS ESSENTIAL TO MONITOR AND CONTROL THE LEVELS OF THESE RESIDUES, ENSURING THEY REMAIN WITHIN PERMISSIBLE REGULATORY LIMITS. THIS IS WHERE ADVANCED ANALYTICAL TECHNIQUES, SUCH AS LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY (LC-MS/MS), PLAY A CRITICAL ROLE. LC-MS/MS IS A HIGHLY SENSITIVE AND PRECISE METHOD FOR DETECTING AND QUANTIFYING TRACE AMOUNTS OF ZILPATEROL AND RACTOPAMINE IN COMPLEX MATRICES LIKE ANIMAL TISSUES.



WHAT ARE ZILPATEROL AND RACTOPAMINE?



- **ZILPATEROL:**
  - A BETA-AGONIST DRUG USED TO PROMOTE LEAN MUSCLE GROWTH IN CATTLE.
  - APPROVED IN THE USA AND CANADA WITH SPECIFIC MAXIMUM RESIDUE LIMITS (MRLS).
- **RACTOPAMINE:**
  - ANOTHER BETA-AGONIST USED TO IMPROVE FEED EFFICIENCY AND INCREASE LEAN MEAT PRODUCTION.
  - ALSO APPROVED IN THE USA AND CANADA WITH DEFINED MRLS.
- **HEALTH CONCERNS:**
  - POTENTIAL RISKS TO HUMAN HEALTH IF RESIDUES EXCEED PERMISSIBLE LIMITS.
  - NEED FOR RELIABLE ANALYTICAL METHODS TO MONITOR RESIDUES IN FOOD PRODUCTS.

PERMISSIBLE LIMITS IN THE USA, CANADA AND CODEX STANDARD:

USA:

RACTOPAMINE:

MUSCLE: 30 MG/G (PPB)

ZILPATEROL:

MUSCLE: 12 MG/G (PPB)

CANADA:

RACTOPAMINE:

MUSCLE: 40 MG/G (PPB)

ZILPATEROL:

MUSCLE: 5 MG/G (PPB)

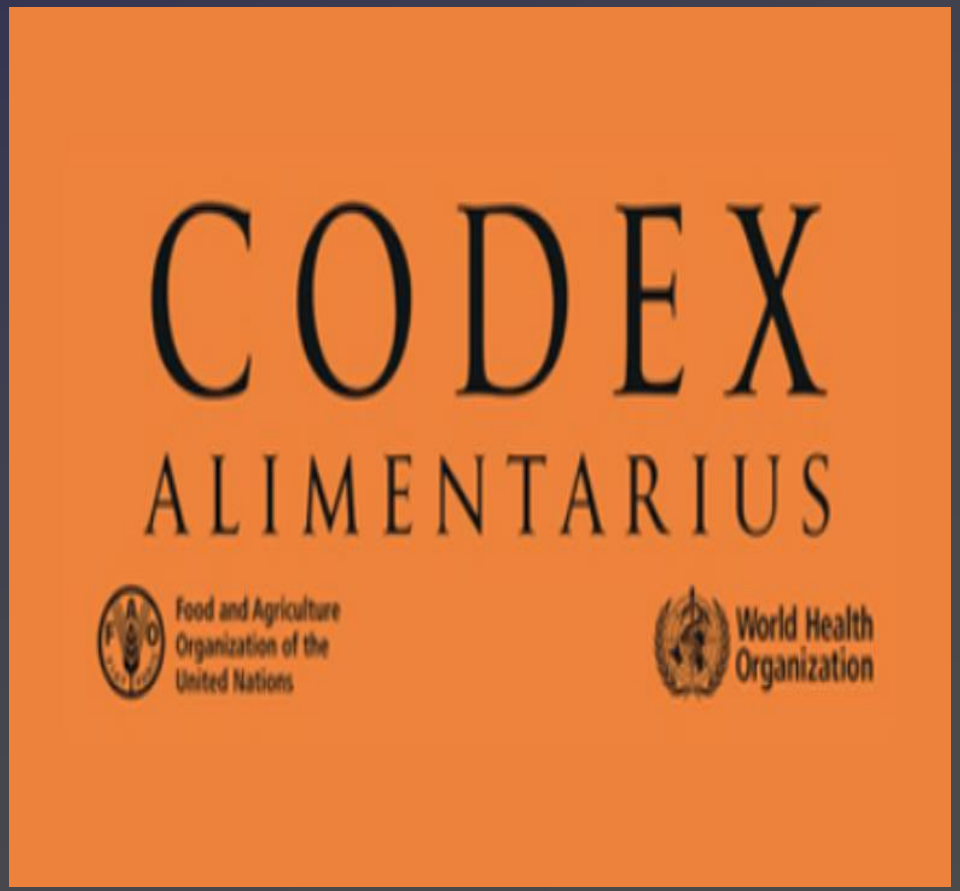
MAXIMUM PERMISSIBLE LIMIT IN CODEX:

RACTOPAMINE:

MUSCLE: 10 MG/G (PPB)

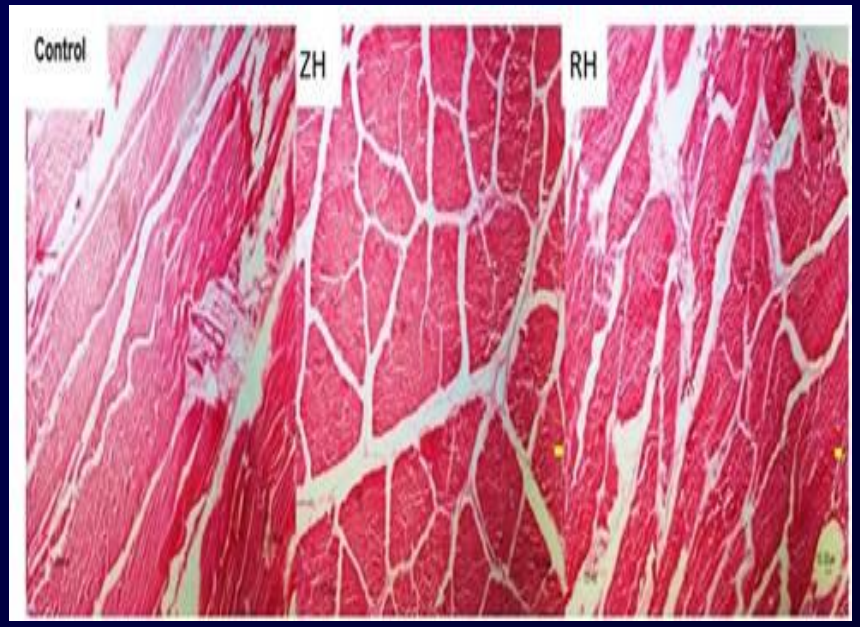
ZILPATEROL:

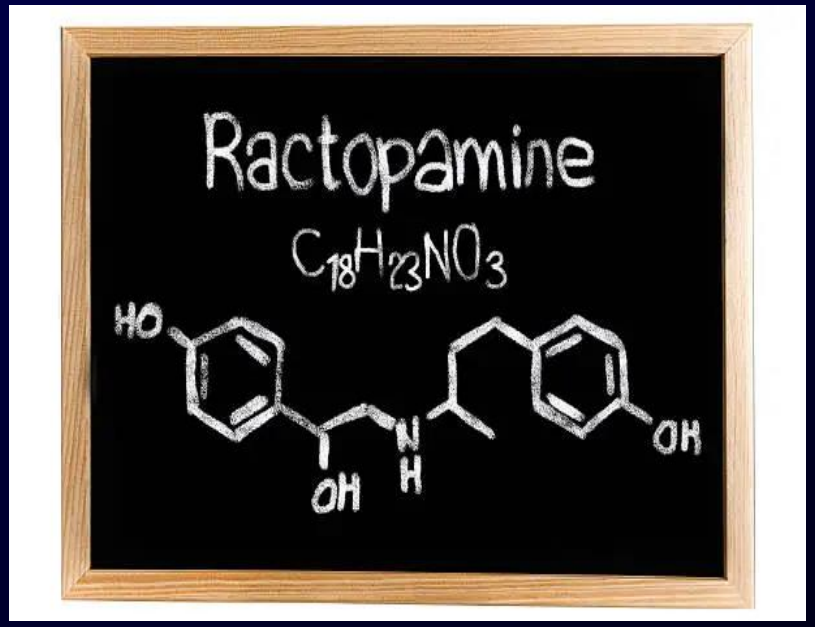
MUSCLE: 0.5 MG/G (PPB)



ANALYSIS OF RACTOPAMINE AND ZILPATEROL BY USING UPLC-MS/MS

WE DETECT AND QUANTIFY RACTOPAMINE AND ZILPATEROL IN KIDNEY, MUSCLE, AND LIVER TISSUES TO ENSURE COMPLIANCE WITH REGULATORY LIMITS, USING UPLC-MS/MS AND ACCORDING TO OASIS PRIME MCX METHOD .





Sample Collection and Preparation:

* Collect **5 g of the sample** (from liver, muscle, or kidney tissues).
* Ensure proper **homogenization** of the sample for consistency.

Extraction:
Perform **extraction** to isolate the target analytes (ractopamine and zilpaterol) from the sample matrix by methanol.

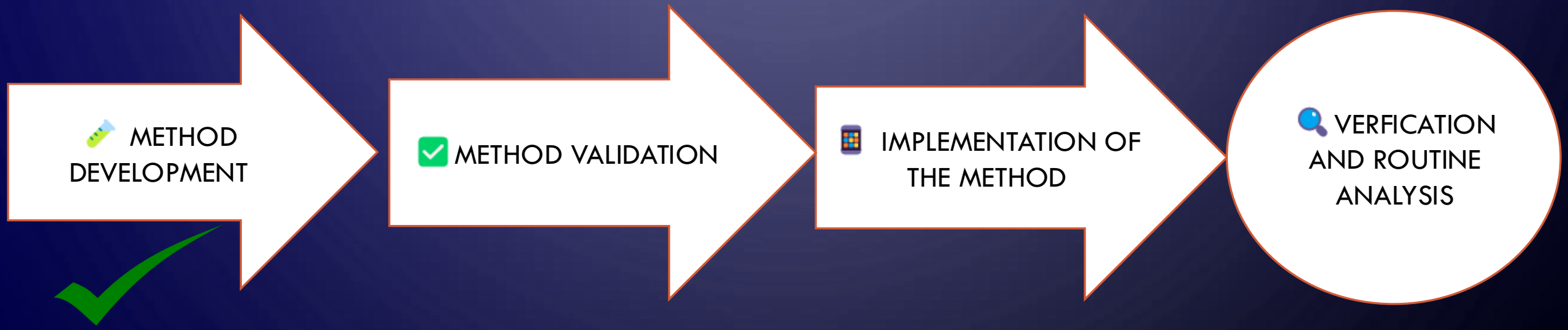
Solid-Phase Extraction (SPE):
Purify the extracted sample using the Oasis PRIME MCX SPE method to remove impurities and concentrate the analytes

Sample Injection:
Inject the purified sample into the UPLC-MS/MS system for analysis.

Detection and Quantification

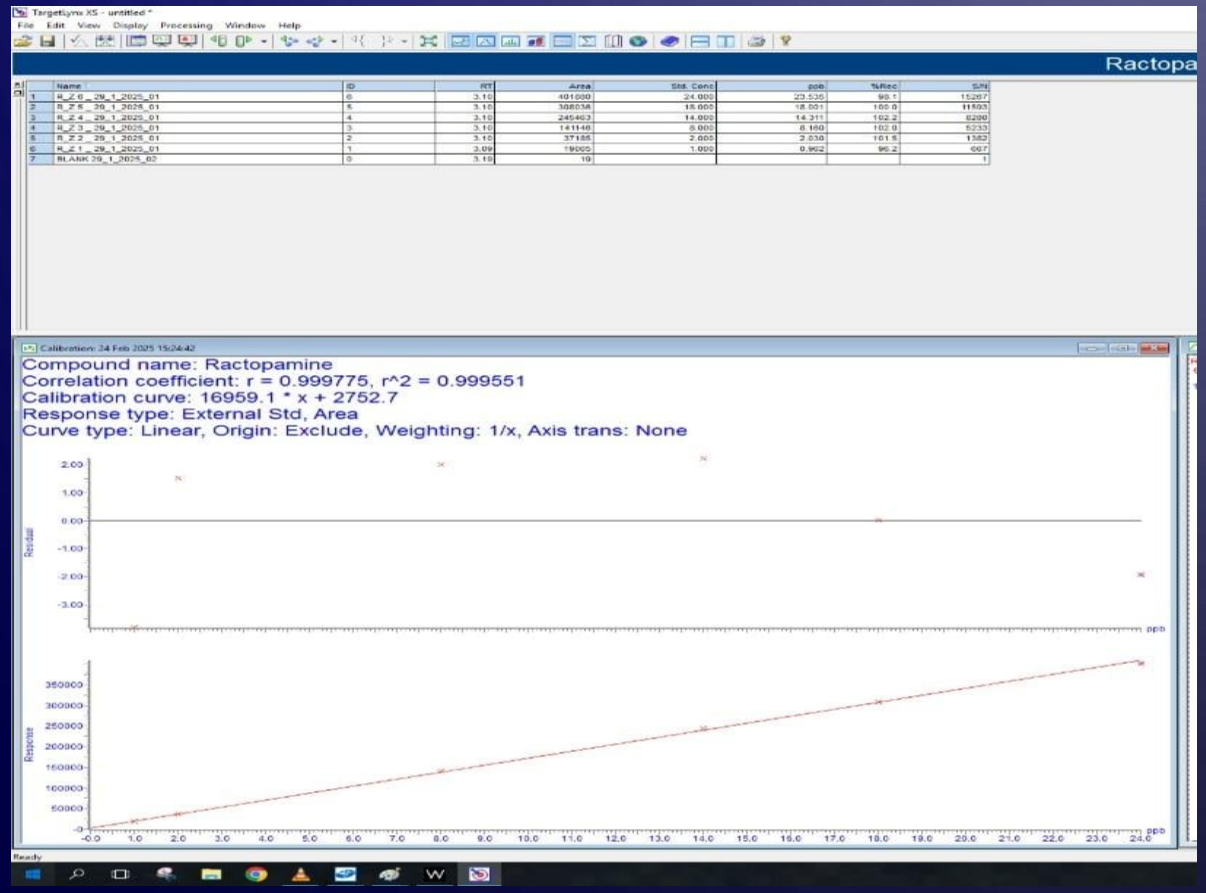
WHAT WE HAVE DONE AND WHAT WILL WE DO?

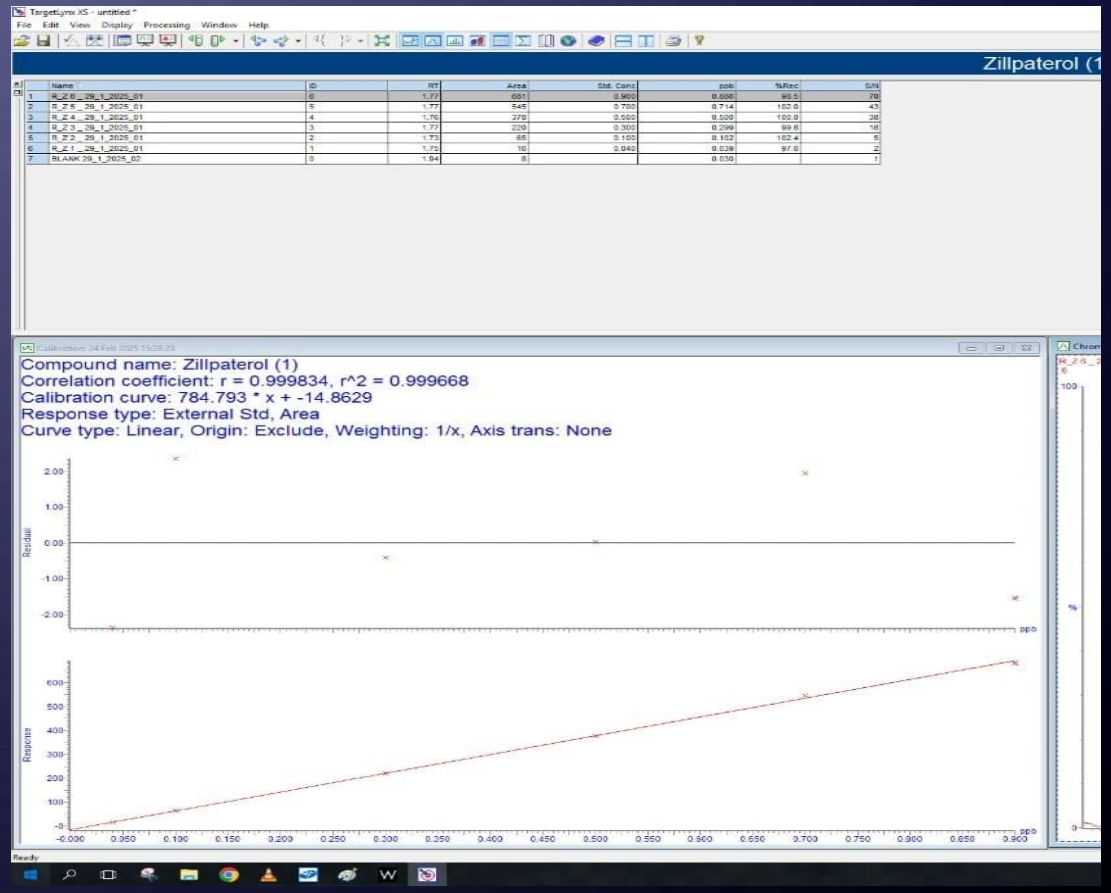
Analysis of Ractopamine and Zilpaterol in Cattle Tissues: From Method Development to Routine Application in Military Laboratories For Quality Control (MLQC)



WHAT WE HAVE ACHIEVED IN MILITARY LABORATORIES FOR QUALITY CONTROL AND WHAT WAS THE RESULT ?

- **RESULTS OF METHOD DEVELOPMENT THAT APPLIED IN MLQC**
- **LINEARITY:**
  - EXCELLENT LINEARITY WAS OBSERVED FOR BOTH RACTOPAMINE AND ZILPATEROL.
  - **CORRELATION COEFFICIENT (R^2):** > 0.999 FOR BOTH COMPOUNDS.





- **SELECTIVITY:**
  - HIGH SELECTIVITY WAS ACHIEVED WITH WELL-RESOLVED PEAKS FOR RACTOPAMINE AND ZILPATEROL.
  - NO INTERFERENCE FROM MATRIX COMPONENTS WAS OBSERVED.
- **CHROMATOGRAPHIC PEAKS:**
  - **RACTOPAMINE:** RETENTION TIME = 3.15 MIN.
  - **ZILPATEROL:** RETENTION TIME = 1.8 MIN.
  - PEAKS WERE SHARP AND SYMMETRICAL, INDICATING GOOD SEPARATION.

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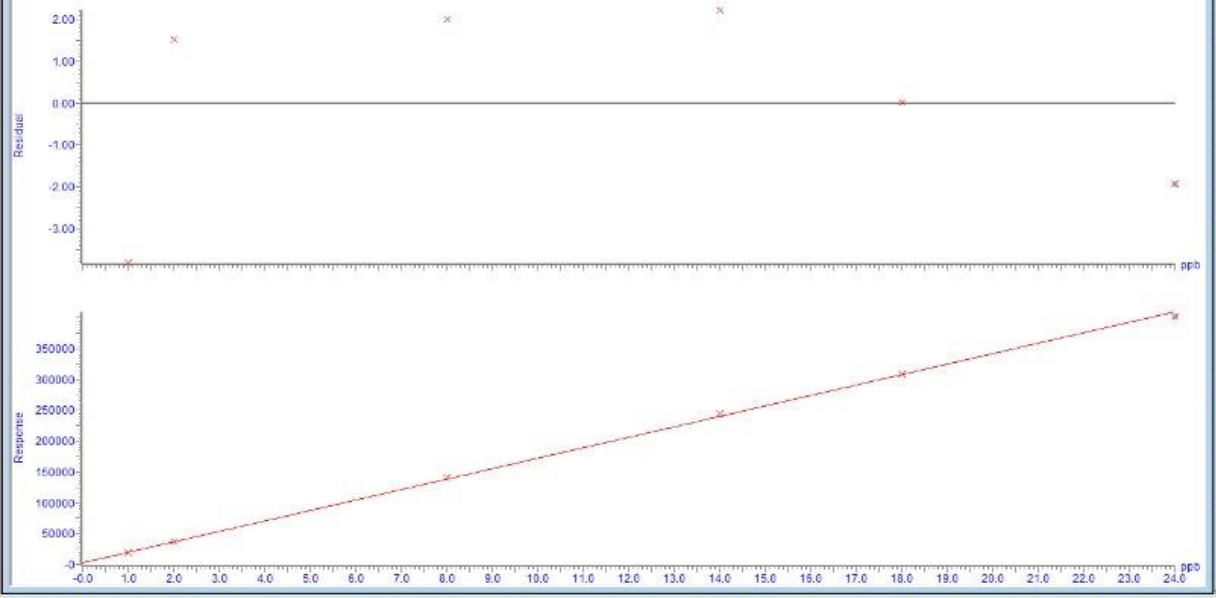
Ractopamine

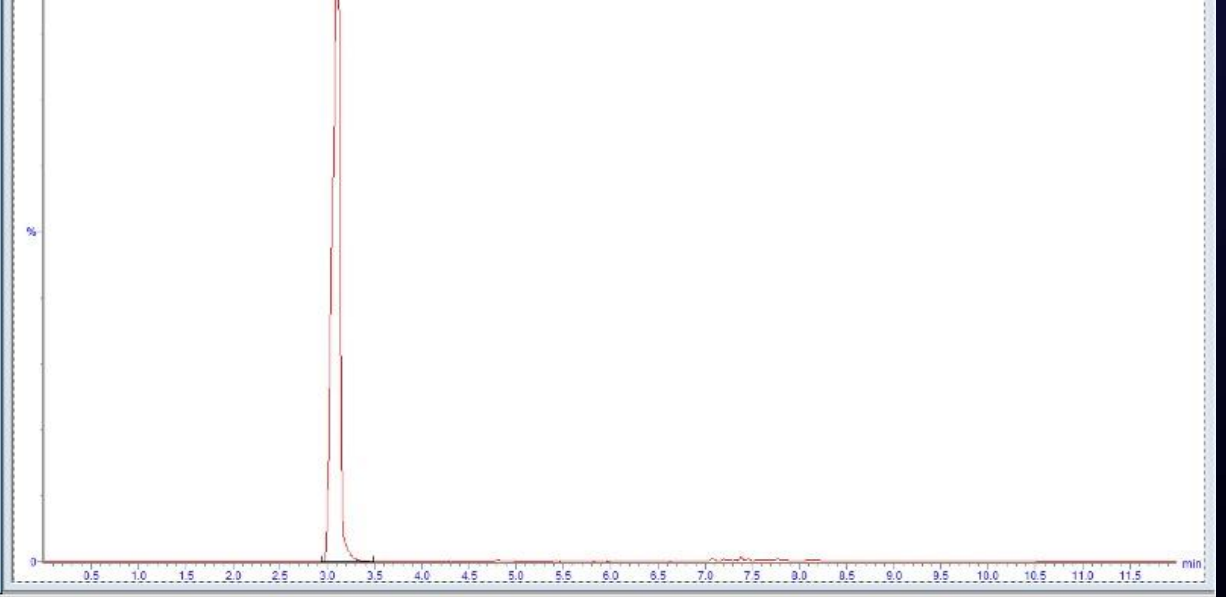
	Name	ID	RT	Area	Std. Conc	ppb	%Rec	S/N
1	R_Z 6 _ 29_1_2025_01	6	3.10	401880	24.000	23.535	98.1	15267
2	R_Z 5 _ 29_1_2025_01	5	3.10	308038	18.000	18.051	100.0	11503
3	R_Z 4 _ 29_1_2025_01	4	3.10	245463	14.000	14.311	102.2	8280
4	R_Z 3 _ 29_1_2025_01	3	3.10	141146	8.000	8.160	102.0	5233
5	R_Z 2 _ 29_1_2025_01	2	3.10	37185	2.000	2.030	101.5	1382
6	R_Z 1 _ 29_1_2025_01	1	3.09	19065	1.000	0.962	96.2	667
7	BLANK 29_1_2025_02	0	3.19	19				1

Calibration: 24 Feb 2025 15:24:42

Compound name: Ractopamine
Correlation coefficient: r = 0.999775, r^2 = 0.999551
Calibration curve: 16959.1 * x + 2752.7
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None



Chromatogram



Ready

Ractopamine

CAP NUM

3:24 PM
2/24/2025

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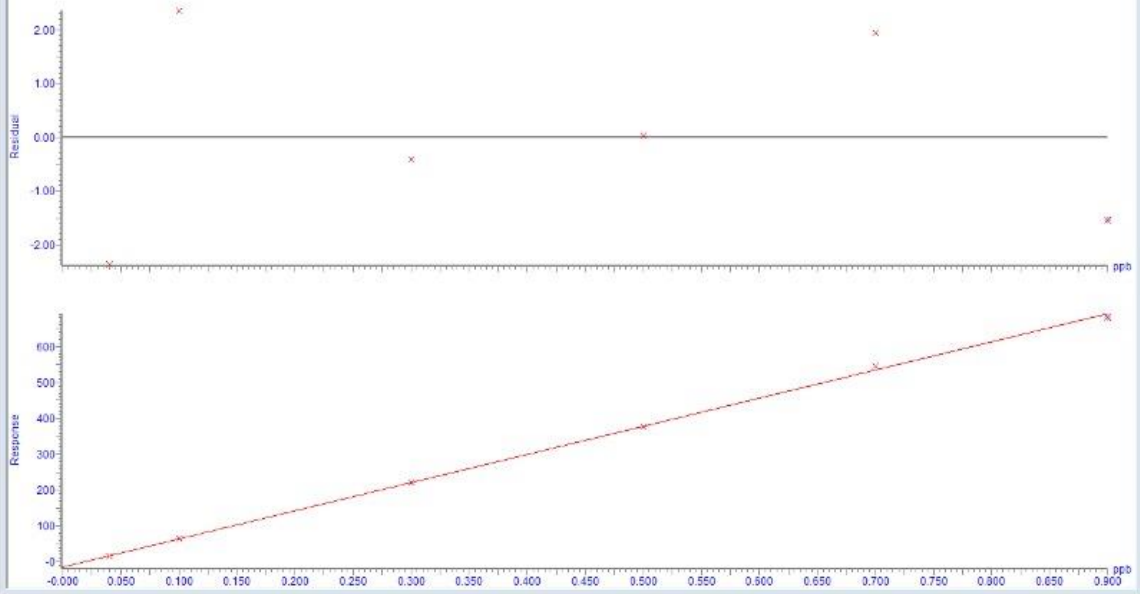
Zillpaterol (1)

	Name	ID	RT	Area	Std. Conc	ppb	%Rec	S/N
1	R_Z 6 _ 29_1_2025_01	6	1.77	681	0.900	0.886	98.5	70
2	R_Z 5 _ 29_1_2025_01	5	1.77	545	0.700	0.714	102.0	43
3	R_Z 4 _ 29_1_2025_01	4	1.76	378	0.500	0.500	100.0	38
4	R_Z 3 _ 29_1_2025_01	3	1.77	220	0.300	0.299	99.6	16
5	R_Z 2 _ 29_1_2025_01	2	1.73	65	0.100	0.102	102.4	5
6	R_Z 1 _ 29_1_2025_01	1	1.75	16	0.040	0.039	97.6	2
7	BLANK 29_1_2025_02	0	1.94	8		0.030		1

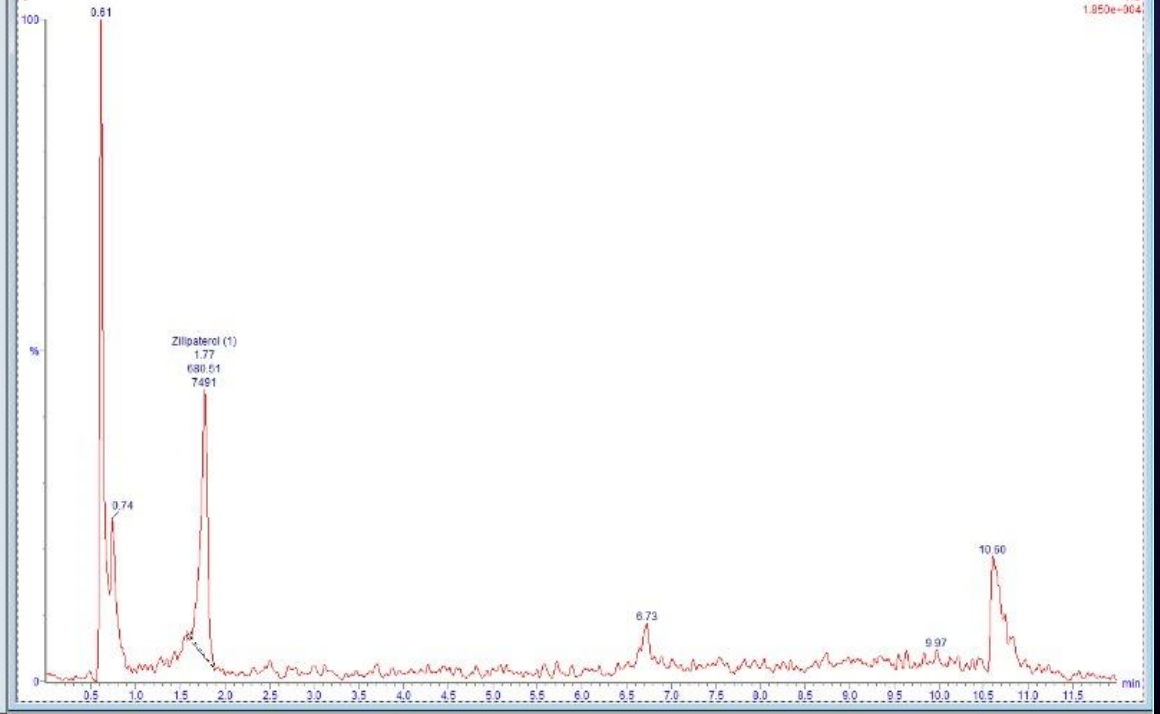
Calibration: 24 Feb 2025 15:28:23

Compound name: Zillpaterol (1)
Correlation coefficient: r = 0.999834, r^2 = 0.999668
Calibration curve: 784.793 * x + -14.8629
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None



Chromatogram

R_Z 6 _ 29_1_2025_01 Smooth(Mn,2x2)
6
F1 MRM of 2 channels ES+
TIC
1.950e+004

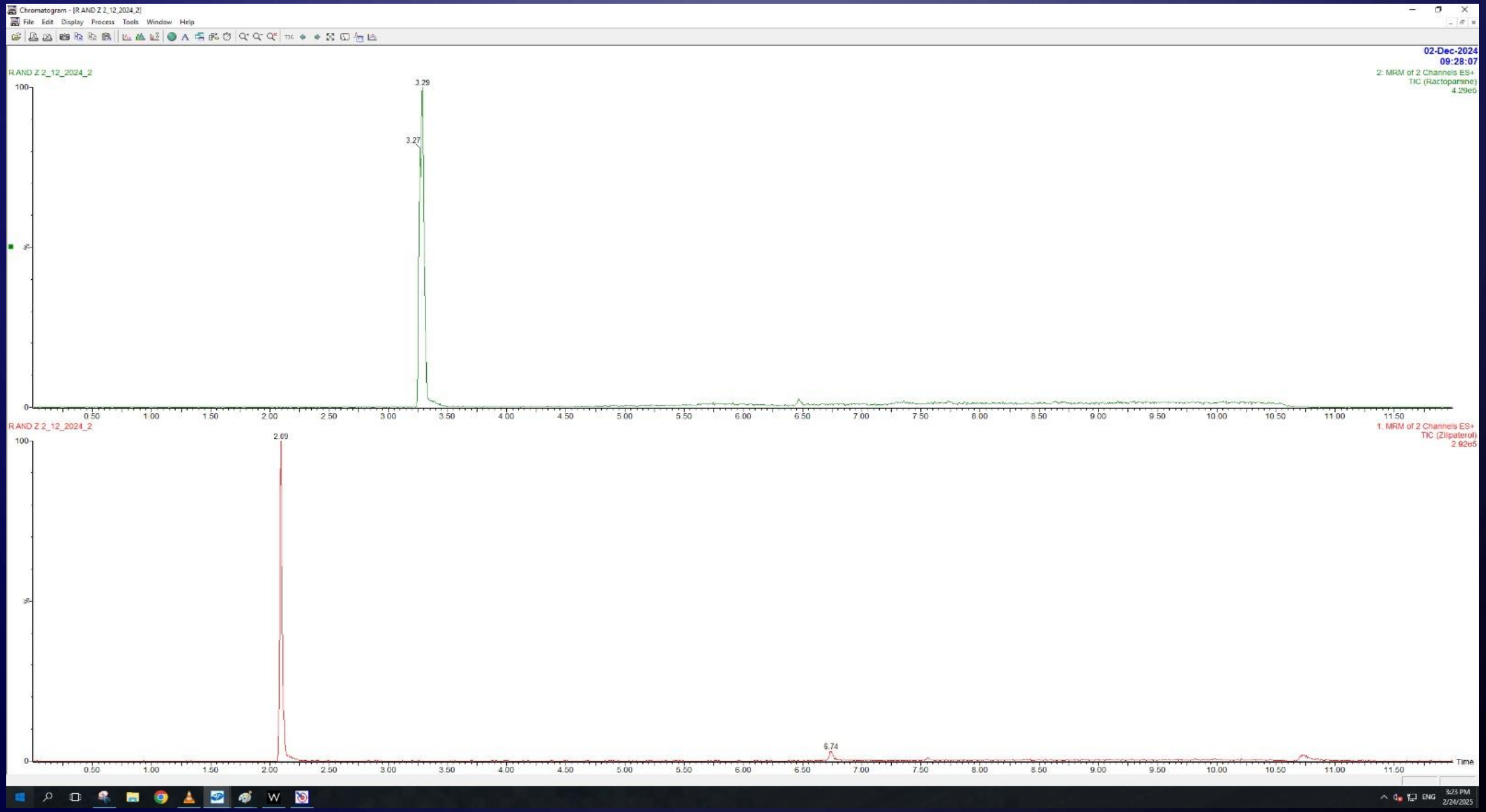


Ready

Zillpaterol (1)

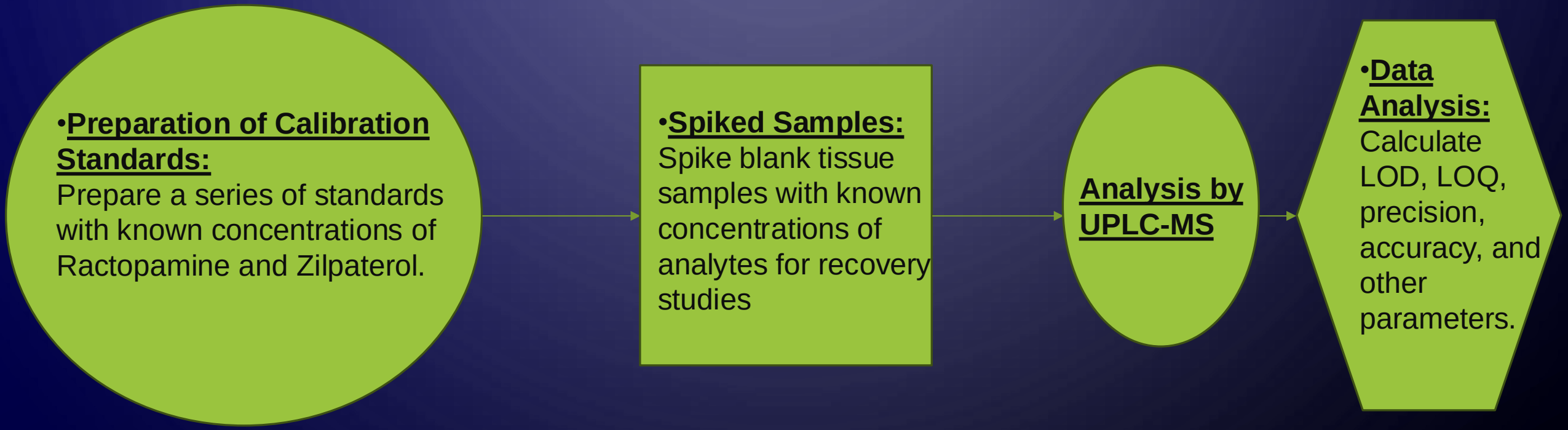
CAP NUM

ENG 3:28 PM 2/24/2025


Chromatogram - [R AND Z 2_12_2024_2]
File Edit Display Process Tools Window Help
02-Dec-2024
09:28:07
R AND Z 2_12_2024_2
2: MRM of 2 Channels ES+
TIC (Ractopamine)
4.29e6
3.29
3.27
100
%
0
0.50 1.00 1.50 2.00 2.50 3.00 3.50 4.00 4.50 5.00 5.50 6.00 6.50 7.00 7.50 8.00 8.50 9.00 9.50 10.00 10.50 11.00 11.50
R AND Z 2_12_2024_2
1: MRM of 2 Channels ES+
TIC (Zilpaterol)
2.92e5
2.09
6.74
100
%
0
Time
0.50 1.00 1.50 2.00 2.50 3.00 3.50 4.00 4.50 5.00 5.50 6.00 6.50 7.00 7.50 8.00 8.50 9.00 9.50 10.00 10.50 11.00 11.50
ENG
3:23 PM
2/24/2025

OUR NEXT STEP WITH UPLC-MS/MS

- WE WILL PERFORM **METHOD VALIDATION** (THE NEXT STEP) BY APPLYING THE FOLLOWING VALIDATION PROTOCOL



ASPIRATIONS

- TO BECOME A GLOBALLY RECOGNIZED LEADER IN THE ANALYSIS OF VETERINARY DRUG RESIDUES AND FOOD ADDITIVES.

- ENSURING FOOD QUALITY AND SAFETY BY PROVIDING ACCURATE AND RELIABLE ANALYTICAL SERVICES.

- RECEIVING SAMPLES.

- OBTAINING ISO 17025 ACCREDITATION

THANK YOU FOR LISTENING