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Next-Level Sensitivity: Polyamine Profiling in Serum Using LC/MS/MS

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Introduction

Polyamines play an important role in cell growth and proliferation. They are widely studied as biomarkers and mechanistic indicators in oncology research and drug development[1]. In cancer biology, dysregulated polyamine metabolism is directly linked to tumor progression, immune modulation, and therapeutic response, making it an increasingly important pathway in precision oncology. Polyamine concentrations in biological samples can vary substantially across cell types and physiological states[2]. As a result, sensitive and reliable polyamine quantification is critical for pharmacodynamic biomarker development and for monitoring target engagement in emerging oncology therapeutics.

The polycationic nature of polyamines has posed several analytical challenges, such as nonspecific binding and poor chromatographic resolution. Derivatization converts the polar polyamines into less polar form and improves peak shapes. Isobutyl chloroformate is a common derivatization reagent. The reaction scheme is illustrated below in Figure 1. Such improvements are particularly valuable in high-throughput bioanalysis settings, where assay robustness and reproducibility are essential for decision-making in drug discovery pipelines.

In this study, a solid-phase extraction cleanup method was explored using Bond Elut HLB sorbent. This chemistry enables efficient extraction of analytes across a broad polarity range, delivering the high recovery, reproducibility, and robustness for the quantitative workflows[3]. This aligns well with the needs of translational research and regulated bioanalysis, where consistent sample preparation is key to generating actionable insights across large study cohorts.

This study introduces a new method of sample preparation, together with LC/MS analysis using the Agilent 6495D LC/TQ to improve the sensitivity of polyamine quantification in serum. The enhanced sensitivity supports detection of low-abundance polyamines, enabling earlier insights into disease biology and improving the utility of these measurements in oncology drug discovery and translational research.

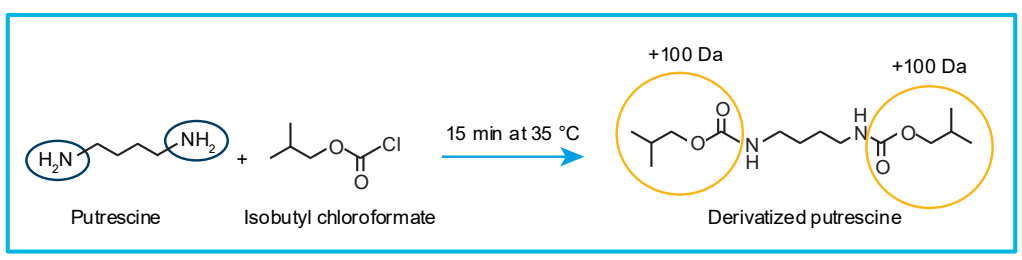

Figure 1. Carbamoylation of putrescine with isobutyl chloroformate.

Experimental

Sample preparation

The polyamine standards were spiked into serum at the concentration of 0.05 to 250 ng/mL. Three levels of QC spikes were prepared at 1.25, 12.5 and 125 ng/mL. 1,6-diaminohexane served as internal standard. Protein precipitation and carbamoylation steps were shown in Figure 2.

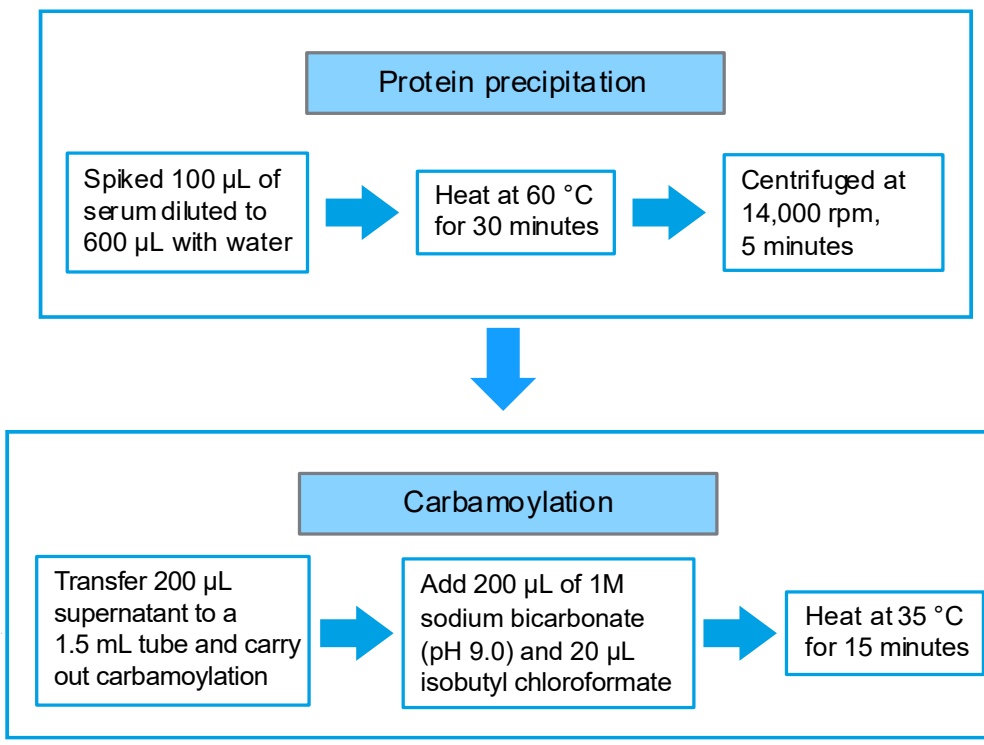

Figure 2. Protein precipitation and carbamoylation of polyamines in serum samples.

The carbamoylated samples were loaded onto Bond Elut HLB cartridges and elution was performed under 100% methanol at the speed of 1 drop/3 seconds on positive pressure manifold. The eluents were dried at 60 °C and reconstituted with 50:50 (v:v) mobile phase A:B.

LC/MS Analysis

The polyamines were separated using InfinityLab Poroshell 120 Aq-C18 column. Mobile phase A was 0.1% formic acid and mobile phase B 99% acetonitrile in water with 0.1% formic acid. The column temperature was set at 50 °C. MRM transition details are listed in Table 1.

Table 1. MRM transition parameters.

Compound	Precursor	Product (Quantifier)	CE
Agmatine	231.4	**116**, 158,140	20, 15, 20
Acetylputrescine	231.2	**157.1**,115.1,72	5, 14, 27
Acetylcadaverine	245.2	**171.1**,129.1,86	5, 18, 27
Acetylspermine	545.4	**255.2**, 471.3, 155.1	32, 14, 36
1,3-diaminopropane	275.2	201.1, **101**, 58	5, 13, 36
Putrescine	289.2	**215.1**, 115.1, 98	5, 13, 18
Cadaverine	303.3	229.1, **129.1**, 86.1	5, 13, 27
Spermidine	446.3	298.1, 372.2, **198.1**	13, 9, 27
Spermine	603.5	**529.3**, 155.1, 255.1	13, 36, 35
1,6-diaminohexane	317.2	243.1, **143.1**, 100.1	5, 14, 23

Chromatographic separation

Thirteen polyamines were well retained and separated with sharp peak shape. Nine polyamines (Table 1) were confidently quantified in spiked serum samples with the method. Ornithine, GABA and arginine calibration ranges need to be further optimized.

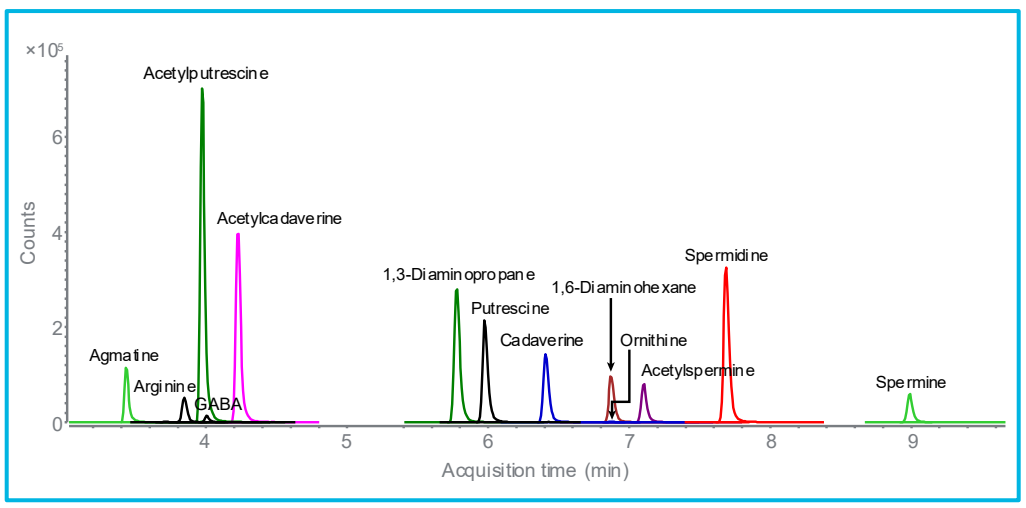

Figure 3. Chromatographic separation of polyamines in serum at a concentration of 5 ng/mL.

Method performance

The workflow performance was assessed based on method sensitivity, linearity, accuracy, precision, recovery, repeatability, and reproducibility. Workflow performance was evaluated based on two batches of data acquired on two different days.

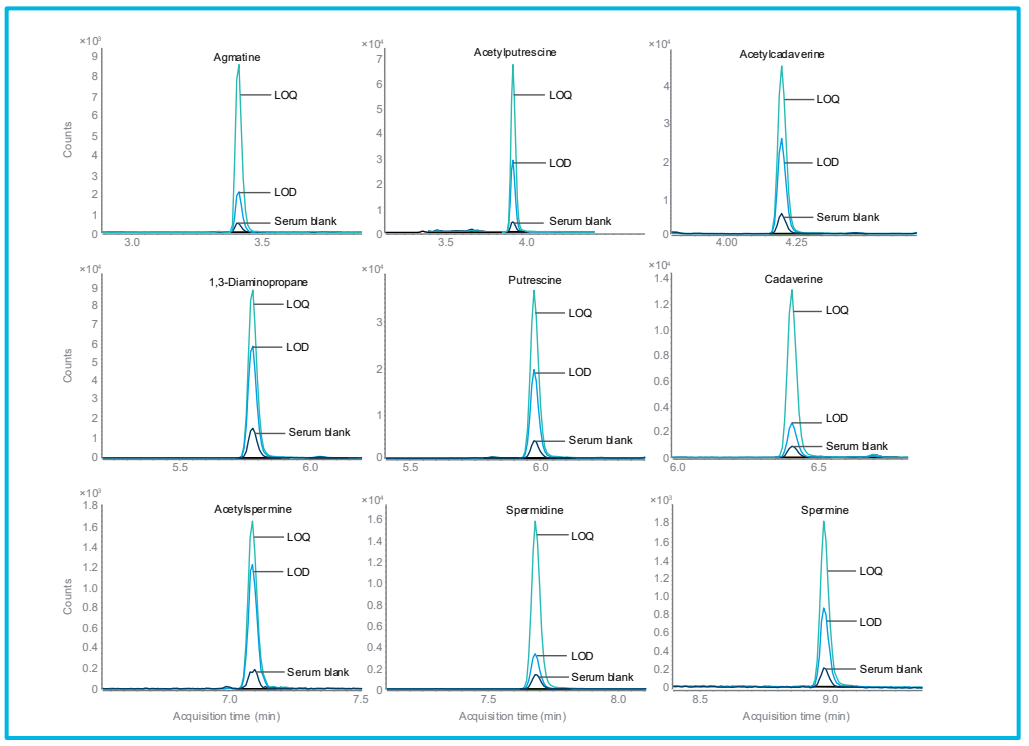

Figure 4. Overlaid MRM chromatograms of serum blank, LOD, and LOQ samples for the 9 polyamine targets.

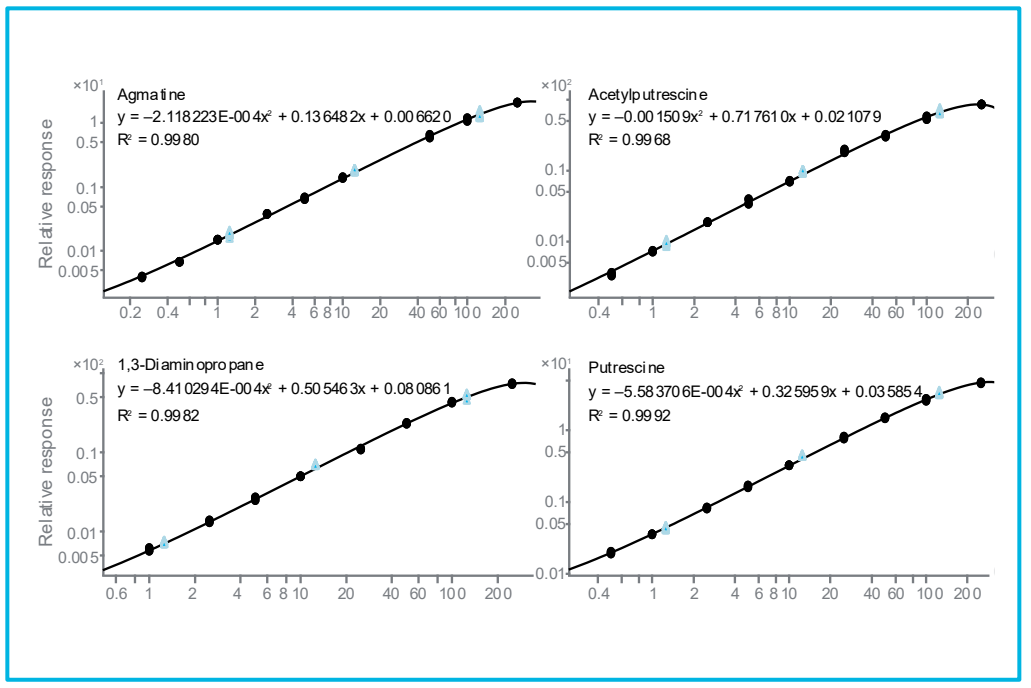

Figure 5. Example calibration curves of 4 polyamines.

Table 2. Analytical method performance.

No.	Compound Name	RT	R^2	Cal Range (ng/mL)	Acurracy (%)	LOD (ng/mL)	LOQ (ng/mL)	Recovery (%)	Recovery Repeatability (%)	Recovery Reproducibility (%)
1	Acetylputrescine	3.95	0.997	0.5 to 250	86.7-117.9	0.1	0.5	112.2	3.9	12.5
2	Acetylcadaverine	4.21	0.992	0.5 to 250	82.2-114.6	0.25	0.5	109.0	3.5	8.1
3	Acetylspermine	7.09	0.991	0.1 to 250	88.5-119.5	0.05	0.1	112.4	3.1	7.2
4	1,3-diaminopropane	5.78	0.998	1 to 250	88.8-108.0	0.5	1	114.4	1.4	5.0
5	Putrescine	5.98	0.999	0.5 to 250	95.3-107.6	0.25	0.5	113.3	1.5	6.5
6	Cadaverine	6.41	0.999	0.25 to 250	95.4-106.5	0.1	0.25	110.0	1.6	6.9
7	Spermidine	7.68	0.994	0.25 to 250	81.6-111.8	0.1	0.25	103.9	3.2	4.6
8	Spermine	8.98	0.990	0.2 to 500	81.8-115.1	0.1	0.2	104.9	8.0	8.0
9	Agmatine	3.41	0.998	0.25 to 250	88.4-110.8	0.05	0.25	110.9	5.1	11.5

Results and Discussion

Sensitivity

The presence of endogenous polyamines in serum was prominent, as shown in Figure 4. Hence, LOD was defined as the three-fold peak area of the blank, and LOQ was defined as the five-fold peak area of the blank. Using this method, the LODs achieved were between 0.05 to 0.5 ng/mL and LOQs were 0.2 to 1 ng/mL (Table 2). These values are superior to the reported LOQ values (>1 ng/mL) from literature[4].

Linearity and accuracy

The calibration range for each polyamine is from the LOQ to 250 ng/mL except for spermine, which was up to 500 ng/mL. All the calibration curves fit to a quadratic equation with $R^2 > 0.99$.

The accuracy for all targets across calibration range was well within the range of 81.6% to 117.9%.

Precision, recovery and robustness

Precision of retention time and area was determined using QC replicate injections (n=8). Excellent precision was obtained, with an area % RSD of <8.1 and RT % RSD of <0.05.

Recovery was found to be between 103.9% and 114.4%, indicating the SPE sample cleanup method was reliable in retaining and extracting targeted polyamines from serum.

Recovery repeatability was calculated as intra-day recovery %RSD. It was found to be <8.0%. Recovery reproducibility was based on inter-day recovery %RSD. It was found to be <12.5%. The repeatability, and reproducibility results confirmed the robustness of this workflow.

Agilent 6495D LC/TQ with Infinity II LC

Conclusions

A sensitive and robust end-to-end workflow was developed for serum polyamine quantitation using LC/MS/MS. The workflow features

- SPE extraction and cleanup supporting more reliable quantitation in complex biological matrices
- Improved chromatographic performance enabling clearer differentiation of closely related polyamines
- Accurate and precise quantitation across a wide dynamic range supporting dose-response studies and biomarker validation efforts in pharmaceutical development
- High sensitivity down to ppt level enabling detection of subtle metabolic changes
- Excellent recovery and robustness ensures scalability to large cohort high-throughput studies and reproducible assays in translational cancer research.

The workflow provides an integrated solution covering sample preparation, chromatography, MS detection, and consumables. Such integration is particularly valuable in pharmaceutical environments, where standardized, reproducible workflows are required to support regulated bioanalysis and translational research.

References

1. Byun, J. A.; Lee, S. H.; Jung, B. H.; Choi, M. H.; Moon, M. H.; Chung, B. C. Analysis of Polyamines as Carbamoyl Derivatives in Urine and Serum by Liquid Chromatography–Tandem Mass Spectrometry. BMC 2008, 22(1), pp.73–80.

2. Dai, Z.; Wu, Z.; Wang, J.; Wang, X.; Jia, S.; Bazer, F. W.; Wu, G. Analysis of Polyamines in Biological Samples by HPLC Involving Pre-Column Derivatization with o-Phthalaldehyde and N-Acetyl-L-Cysteine. Amino acids, 2014, 46(6), pp.1557–1564.

3. Excellent Retention and Reproducibility for Reliable Results. Agilent Bond Elut HLB. Agilent Technologies flyer, publication number 5994-6429EN, 2023.

4. Isaguirre, C.; Gendjar, M.; Nauta, K. M.; Burton, N. O.; Sheldon, R. D. Polyamine Quantitation by LC-MS Using Isobutyl Chloroformate Derivatives. Methods in Enzymology, Vol. 715; Academic Press, 2025; pp 437–458.

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