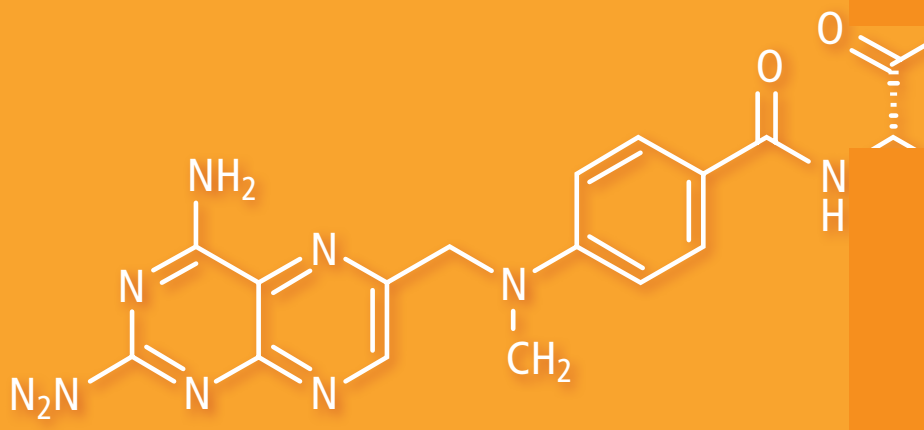
APPLICATION NOTE



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High Sensitivity Methotrexate Quantitation in Human Serum Utilizing PerkinElmer QSight® 220 Triple Quadrupole Mass Spectrometer

Introduction

This note outlines a rapid LC-MS/MS research method utilizing the QSight® 220 triple quadrupole mass spectrometer. The developed method provides exceptional results for the quantitation of methotrexate in serum especially in terms of LLOQs and linearity.

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2. Method

Methotrexate was purchased from Toronto Research Chemicals (Toronto, ON). The stock solution 25 mg/mL of Methotrexate in methanol was prepared and stored at -15°C. Human serum was purchased from Sigma -Aldrich (Milwaukee, WI) and cleaned up by protein precipitation by mixing one volume of human serum, one volume of 0.1% formic acid and two volumes of acetonitrile. The mixture was vortexed for 1 min followed by centrifugation for 15 min. The supernatant was transferred to clean container and used as a matrix for methotrexate quantitation. A series of methotrexate standards from 0.03 to 125ng/mL were prepared by spiking the stock solution into the clean protein precipitated human serum. All the solvents used in this application are HPLC grade.

2.1. Mass Spectrometry Conditions

The QSight® 220 equipped with an ESI source was used to perform the analysis. Table 1 highlights the instrument settings used during the method.

Table 1: Settings used on the QSight® 220 during the method

ESI Voltage (V)	5000
HSID Temp (°C)	300
Nebulizer Gas Setting	400
Drying Gas Setting	120
Heating Gas	350
Source Temp. (oC)	250
Dwell Time (ms)	100
Pause Time (ms)	5

Table 2: Selected MRM operating conditions for methotrexate

MRM	CE	CCL2	CCL4	EV
455.1/308.0	28	-100	-90	30
455.1/134.0	48	-100	-90	30

Quick Facts:

- Method for high sensitivity quantitation of methotrexate using the QSight® 220 triple quadrupole mass spectrometer.
- LLOQ achieved is 0.03 ng/mL with 5uL injection
- Liquid Chromatography: Shimadzu® Prominence UFLC®
- ESI Source
- Calibration Curve R^2 = 0.99999

2.2. LC Conditions

The separation was performed on a Shimadzu® Prominence UFLC® system which includes binary pumps, autosampler, degasser, column oven. A sample volume of 5 µL is injected into a Imtakt C8 column (2.0 x 75 mm, 3 µm) at room temperature. The LC time program is illustrated as Table 3. The total LC cycle time is 3 min and liquid flow rate is 0.4 mL/min.

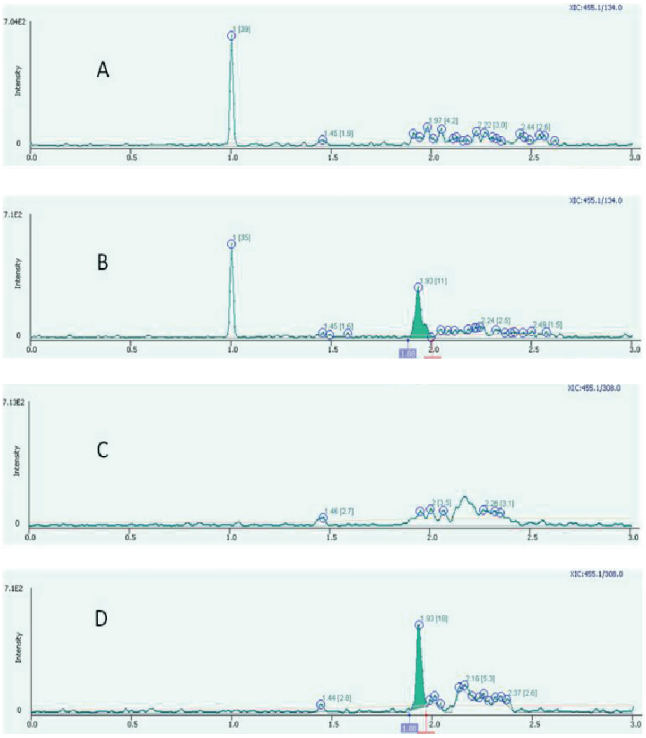
Table 3: LC Gradient Conditions; Solvent A is water with 0.1% formic acid, Solvent B is methanol with 0.1% formic acid

Time (min)	Procedure Solvent B composition (%)
0	5
0.1	5
0.6	95
1	95
1.1	5
3	5

3. Results

3.1. Extracted Ion Chromatogram (EIC)

Figure 1: (A) 455.1/134.0 Serum Blank, (B) 455.1/134.0 at a Concentration of 0.03 ng/mL of Methotrexate in Human Serum, (C) 455.1/308.1 Serum Blank, (D) 455.1/308.1 at a Concentration of 0.03 ng/mL of Methotrexate in Human Serum.



3.2. Linearity

The calibration curves generated for both MRM transitions using 1/x weighting are displayed in Figure 2 and Figure 3. Excellent linearity values, (R^2=0.9999) were obtained for concentrations ranging from 0.03 to 125 ng/mL for both of the monitored MRM transitions.

Table 4: Intra-day variability (%RSD)

MRM	QC1(low)	QC2(med)	QC3(high)
455.1/134.0	5.1	1.2	1.0
455.1/308.0	6.9	1.8	0.5

Table 5: Inter-day variability (%RSD)

MRM	QC1(low)	QC2(med)	QC3(high)
455.1/134.0	2.6	3.0	3.5
455.1/308.0	7.0	3.6	3.8

Figure 2: Calibration Curve for 455.1/134.0

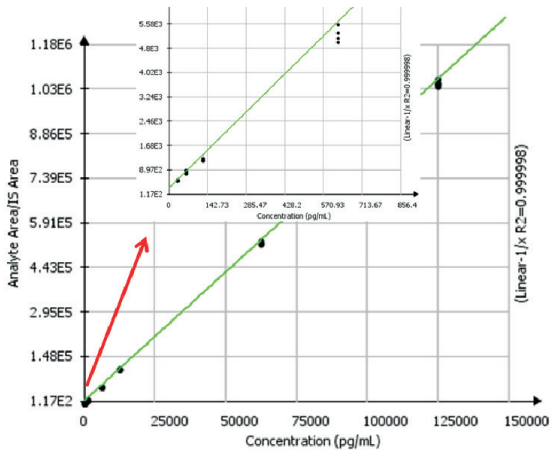
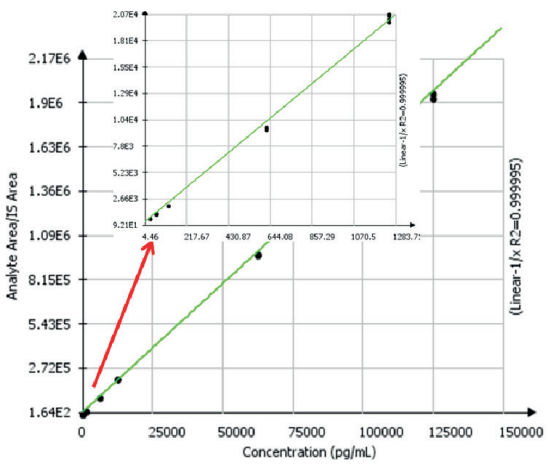


Figure 3: Calibration curve for 455.1/308.0



3.3. Quantitation Results

The inter and intra-day variability for this experiment were excellent as shown in Table 4 and 5. The intra-day variability is determined by processing 4 replicates of each QC sample and the inter-day variability is determined with 4 replicates in 3 batches.

4. Conclusion

A fast, sensitive, and accurate LC-MS/MS method was developed for methotrexate in serum using the PerkinElmer QSight® 220 triple quadrupole mass spectrometer. With an LLOQ of 0.03 ng/mL (using a 5 µL injection), the method falls well within the range necessary for methotrexate related clinical research applications. The linearity achieved by this method (R^2=0.9999) further illustrates the exceptional performance of the QSight® 220 for methotrexate quantitation.

5. Contact Information

To learn more about PerkinElmer Mass Spectrometry, our products or services please visit our website or contact us directly.

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