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Development of a cost-effective and highly selective bioanalytical method for the analysis of Montelukast in plasma using LC-MS/MS

Prasanth Joseph¹; Chidella Kartheek Srinivas¹; Arun Kumar P¹; Saikat Banerjee¹; Samir Vyas²

¹Agilent Technologies, BENGALURU, India;

²Agilent Technologies, Mumbai, India

Introduction

Montelukast is a prescription drug that belongs to the class of leukotriene receptor antagonists. This is a combination drug available in tablet form or as granules. Montelukast oral tablets are used to treat symptoms of asthma and is also effective for allergies and exercise-induced bronchoconstriction.

A cost-effective highly selective and reproducible method is developed for the low-level quantification of Montelukast in plasma using Montelukast-D6 as an internal standard. An electrospray ionization (ESI) based multiple reaction monitoring method was developed on a 6470 triple quadrupole LC/MS (LC/TQ) system. A simple liquid-liquid extraction-based sample preparation is adopted for the extraction of drug from plasma.



Figure 1. 1290 Infinity II UHPLC coupled to the 6470 LC/TQ.

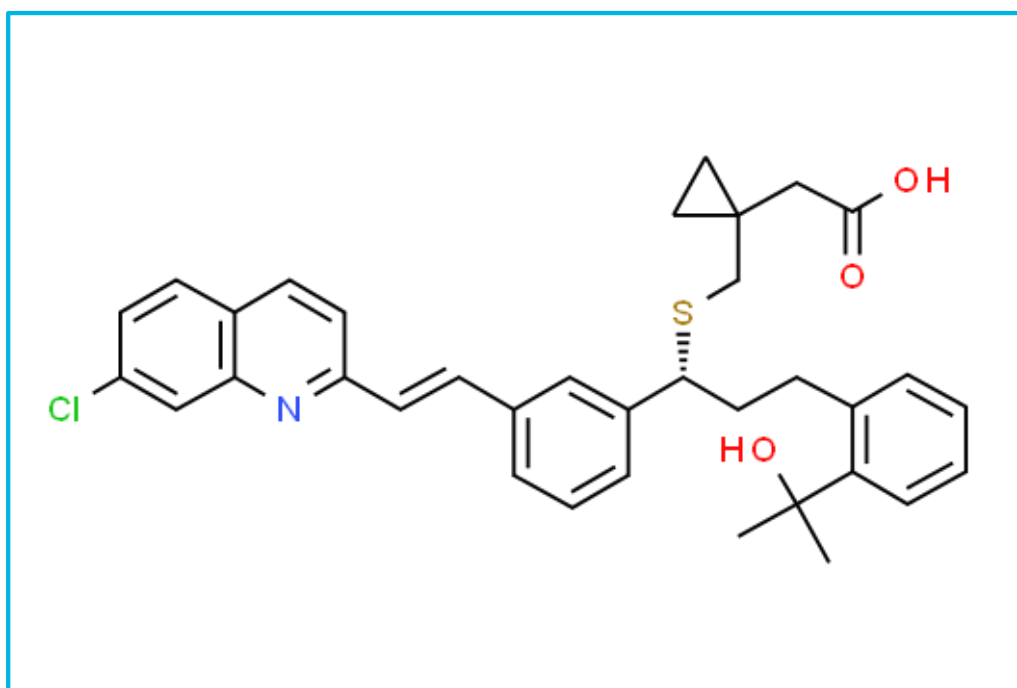


Figure 2. Chemical structure. of Montelukast

Experimental

Sample Preparation

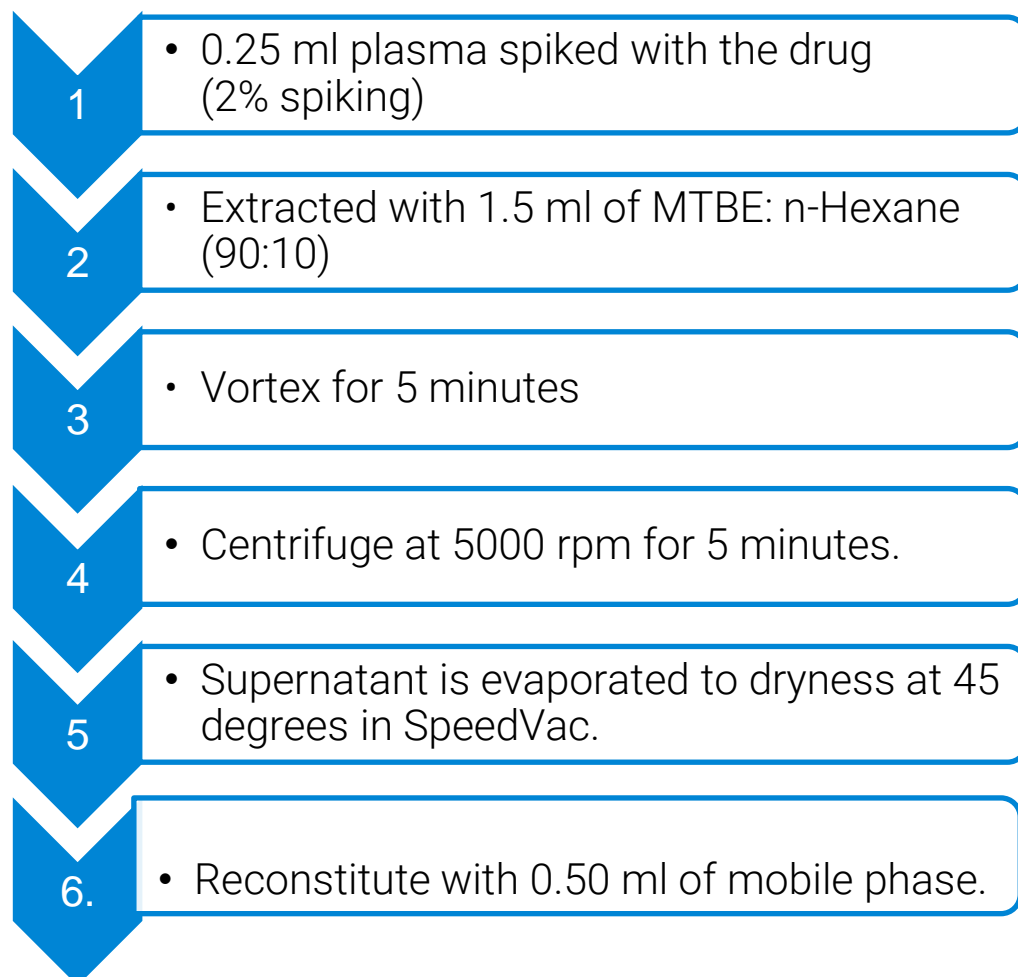


Figure 3. Liquid-liquid extraction protocol for the sample preparation of Montelukast

Chromatographic conditions

| | |
|---------------------|-----------------------------|
| Analytical column | XDB C18 (100 X3.0, 3.5um) |
| Flow rate | 0.5 ml/min |
| Mobile phase A | 0.1% Formic acid in water |
| Mobile phase B | Acetonitrile |
| Injection volume | 1 ul |
| Elution | Isocratic |
| Mobile phase ratio | 10:90 |
| Needle wash solvent | Acetonitrile: Water (60:40) |

Source parameters

| | |
|----------------------------|--------------------------|
| Ionization: ESI | Polarity: Positive |
| Sheath gas temp: 300°C | Sheath gas flow: 10l/min |
| Drying gas temp: 250°C | Drying gas flow: 8l/min |
| Cap Voltage: 3500V | Nozzle voltage: 0 |
| Nebulizer pressure: 40 psi | |

Method development

Montelukast method was developed using a 6470 LC/TQ installed with an Electrospray ionization source. Both Montelukast and the internal standard Montelukast-D6 were detected in positive ionization mode.

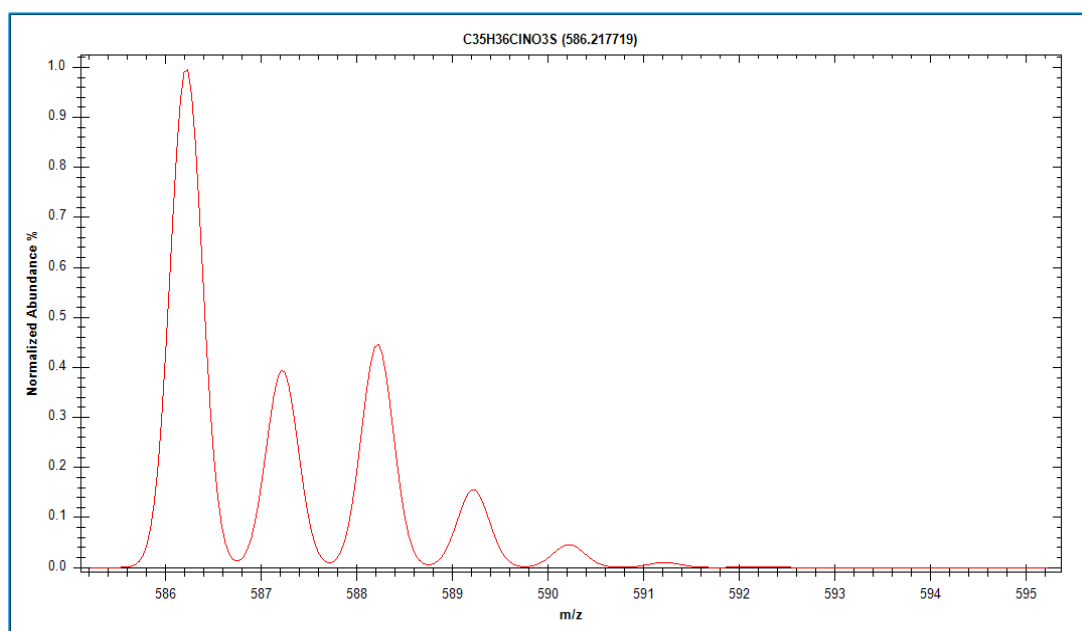


Figure 4. Isotopic pattern of Montelukast

| Compound ID | Precursor ion | Product ion | Collision energy |
|----------------|---------------|-------------|------------------|
| Montelukast | 586.2 | 568.1 | 16 |
| Montelukast | 586.2 | 422.1 | 28 |
| Montelukast D6 | 592.3 | 574.1 | 16 |
| Montelukast D6 | 592.3 | 427.1 | 28 |

Table 1. MRM parameters for Montelukast

Precision and accuracy of the batch was determined to verify the method performance in plasma samples. 3-orders of calibration curve concentrations were generated within the concentration range of 1 ng/ml to 1000 ng/ml and found to be linear. The regression coefficient obtained is 0.9993 when linearity plotted using "area ratio" against "concentration ratio" of analyte to internal standard with a weighing factor of $1/X^2$. The accuracy of each calibration standards measured from the linearity curve was between 96-104%.

Calibration curve

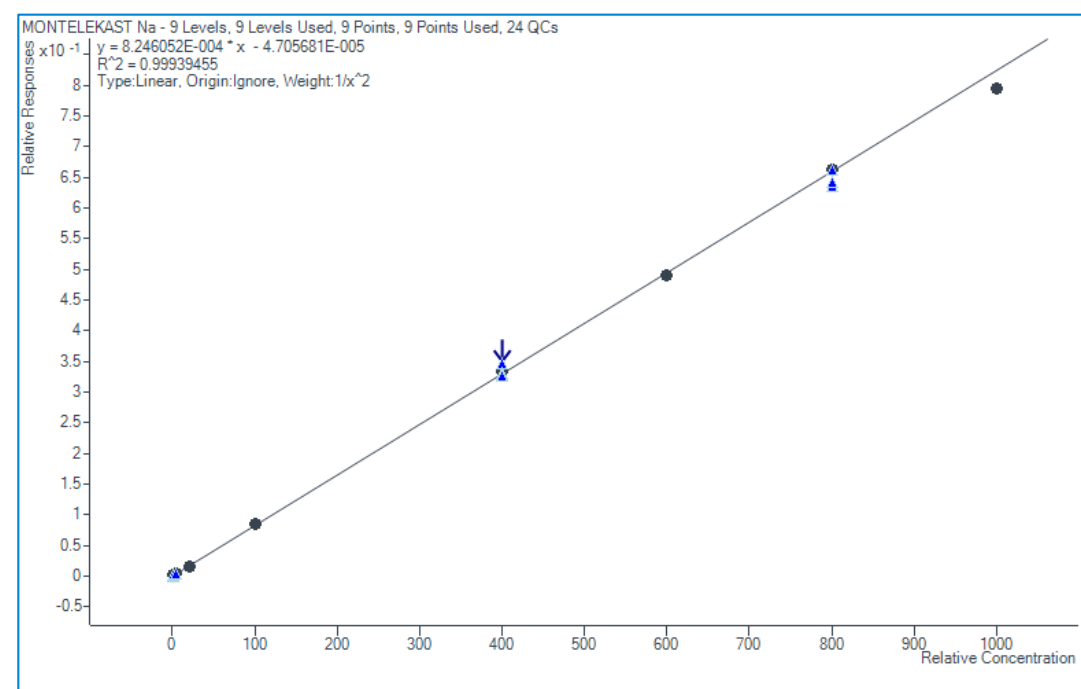


Figure 5. Calibration curve of Montelukast

Following the linearity studies, triplicate injections of LLOQ, LQC, MQC, and HQC were also submitted. Recovery for these QC samples at their respective concentration of 1, 5, 400 and 800 ppb were between 93-110%. The average area response at the LLOQ level was found to be 786 counts.

| Batch Table | | Sample | | MONTELEKAST Na | | MONTELEKAST D6 | | MONTELEKAST Na Results | | MONTELEKAST D6 Results | | Qualif. | | MONTELEKAST Na | | Qualif. | | | | |
|----------------|----------------|--------|-------|------------------|------------|----------------|--------|------------------------|-------------|------------------------|-------------|------------|--------|----------------|----|---------|---------|-------|-------|----|
| Name | Data File | Type | Level | Acq. Date-Time | Exp. Conc. | RT | Resp. | MI | Calc. Conc. | Accuracy | Final Conc. | ISTD Conc. | Ratio | Height | RR | Ratio | MI | Resp. | Ratio | MI |
| BLANK | Blank01-r001.d | Blank | | 01-06-2019 18:26 | | 1.896 | 11 | | 277.9678 | | 277.9678 | 278.0 | 4 | 0.2292 | | 1.645 | 48 | 72.9 | | |
| BLANK+IS | Blank+IS.d | Blank | | 01-06-2019 18:31 | | 1.230 | 9 | | 0.0680 | | 0.0680 | 0.0 | 1 | 0.0000 | | 1.651 | 996168 | 62.1 | | |
| MONTE 1 PPB | STD 1.d | Cal | 1 | 01-06-2019 18:36 | 1.0000 | 1.665 | 798 | | 0.9891 | 98.9 | 0.9891 | 1.0 | 217 | 0.0008 | | 1.651 | 1030278 | 61.8 | | |
| MONTE 2 PPB | STD 2.d | Cal | 2 | 01-06-2019 18:39 | 2.0000 | 1.665 | 1645 | | 2.0528 | 102.6 | 2.0528 | 2.1 | 446 | 0.0016 | | 1.651 | 999000 | 61.8 | | |
| MONTE 5 PPB | STD 3.d | Cal | 3 | 01-06-2019 18:43 | 5.0000 | 1.665 | 3927 | | 4.9464 | 98.9 | 4.9464 | 4.9 | 1065 | 0.0040 | | 1.651 | 974008 | 62.6 | | |
| MONTE 20 PPB | STD 4.d | Cal | 4 | 01-06-2019 18:48 | 20.0000 | 1.665 | 15384 | | 19.7857 | 98.9 | 19.7857 | 19.8 | 4132 | 0.0163 | | 1.651 | 965641 | 62.0 | | |
| MONTE 100 PPB | STD 5.d | Cal | 5 | 01-06-2019 18:52 | 100.0000 | 1.665 | 82298 | | 103.9122 | 103.9 | 103.9122 | 103.9 | 22277 | 0.0856 | | 1.651 | 960982 | 62.5 | | |
| MONTE 400 PPB | STD 6.d | Cal | 6 | 01-06-2019 18:56 | 400.0000 | 1.659 | 296078 | | 403.4498 | 100.9 | 403.4498 | 403.4 | 78382 | 0.3326 | | 1.651 | 890086 | 61.4 | | |
| MONTE 600 PPB | STD 7.d | Cal | 7 | 01-06-2019 19:01 | 600.0000 | 1.659 | 439762 | | 593.6190 | 98.9 | 593.6190 | 593.6 | 116183 | 0.4896 | | 1.651 | 898515 | 62.0 | | |
| MONTE 800 PPB | STD 8.d | Cal | 8 | 01-06-2019 19:05 | 800.0000 | 1.659 | 539221 | | 802.9347 | 100.4 | 802.9347 | 802.9 | 142084 | 0.6621 | | 1.645 | 814463 | 62.1 | | |
| MONTE 1000 PPB | STD 9.d | Cal | 9 | 01-06-2019 19:09 | 1000.0000 | 1.659 | 651275 | | 965.1465 | 96.5 | 965.1465 | 965.1 | 170901 | 0.7958 | | 1.645 | 818372 | 61.4 | | |
| MONTE 1 PPB | LLOQ-r001.d | QC | 1 | 01-06-2019 19:13 | 1.0000 | 1.659 | 794 | | 1.0488 | 104.9 | 1.0488 | 1.0 | 218 | 0.0008 | | 1.651 | 970266 | 61.9 | | |
| MONTE 1 PPB | LLOQ-r002.d | QC | 1 | 01-06-2019 19:18 | 1.0000 | 1.665 | 794 | | 1.0967 | 109.7 | 1.0967 | 1.1 | 211 | 0.0009 | | 1.651 | 926178 | 61.8 | | |
| MONTE 1 PPB | LLOQ-r003.d | QC | 1 | 01-06-2019 19:22 | 1.0000 | 1.665 | 769 | | 1.0143 | 101.4 | 1.0143 | 1.0 | 210 | 0.0008 | | 1.651 | 974253 | 62.6 | | |
| MONTE 5 PPB | LQC-r001.d | QC | 3 | 01-06-2019 19:26 | 5.0000 | 1.659 | 3610 | | 4.7084 | 94.2 | 4.7084 | 4.7 | 989 | 0.0038 | | 1.651 | 941206 | 62.7 | | |
| MONTE 5 PPB | LQC-r002.d | QC | 3 | 01-06-2019 19:31 | 5.0000 | 1.659 | 3646 | | 4.6643 | 93.3 | 4.6643 | 4.7 | 988 | 0.0038 | | 1.651 | 959679 | 61.4 | | |
| MONTE 5 PPB | LQC-r003.d | QC | 3 | 01-06-2019 19:35 | 5.0000 | 1.659 | 3637 | | 5.0226 | 100.5 | 5.0226 | 5.0 | 972 | 0.0041 | | 1.651 | 888234 | 61.8 | | |
| MONTE 400PPB | MQC-r001.d | QC | 6 | 01-06-2019 19:39 | 400.0000 | 1.659 | 284913 | | 396.0306 | 99.0 | 396.0306 | 396.0 | 75465 | 0.3065 | | 1.651 | 872569 | 61.9 | | |
| MONTE 400PPB | MQC-r002.d | QC | 6 | 01-06-2019 19:43 | 400.0000 | 1.665 | 296199 | | 402.2789 | 100.6 | 402.2789 | 402.3 | 78817 | 0.3317 | | 1.651 | 893042 | 61.8 | | |
| MONTE 400PPB | MQC-r003.d | QC | 6 | 01-06-2019 19:48 | 400.0000 | 1.659 | 296759 | | 419.8603 | 105.0 | 419.8603 | 419.9 | 79401 | 0.3462 | | 1.651 | 857259 | 62.6 | | |
| MONTE 800PPB | HQC-r001.d | QC | 8 | 01-06-2019 19:52 | 800.0000 | 1.659 | 551106 | | 778.9340 | 97.4 | 778.9340 | 778.9 | 144700 | 0.6423 | | 1.645 | 858065 | 61.8 | | |
| MONTE 800PPB | HQC-r002.d | QC | 8 | 01-06-2019 19:56 | 800.0000 | 1.659 | 554871 | | 771.2006 | 96.4 | 771.2006 | 771.2 | 145912 | 0.6359 | | 1.645 | 872591 | 61.9 | | |
| MONTE 800PPB | HQC-r003.d | QC | 8 | 01-06-2019 20:01 | 800.0000 | 1.659 | 549067 | | 802.2796 | 100.3 | 802.2796 | 802.3 | 144506 | 0.6615 | | 1.651 | 830012 | 62.6 | | |
| MONTE 1 PPB | STD 1-r001.d | QC | 1 | 01-06-2019 20:05 | 1.0000 | 1.665 | 811 | | 1.0929 | 109.3 | 1.0929 | 1.1 | 221 | 0.0009 | | 1.651 | 949495 | 62.6 | | |
| MONTE 1 PPB | STD 1-r002.d | QC | 1 | 01-06-2019 20:09 | 1.0000 | 1.665 | 769 | | 1.0466 | 104.7 | 1.0466 | 1.0 | 205 | 0.0008 | | 1.651 | 930217 | 62.5 | | |
| MONTE 1 PPB | STD 1-r003.d | QC | 1 | 01-06-2019 20:13 | 1.0000 | 1.665 | 802 | | 1.0795 | 107.0 | 1.0795 | 1.1 | 217 | 0.0008 | | 1.651 | 951235 | 62.4 | | |
| MONTE 1 PPB | STD 1-r004.d | QC | 1 | 01-06-2019 20:18 | 1.0000 | 1.659 | 702 | | 0.9570 | 95.7 | 0.9570 | 1.0 | 193 | 0.0007 | | 1.651 | 945969 | 61.6 | | |
| MONTE 1 PPB | STD 1-r005.d | QC | 1 | 01-06-2019 20:22 | 1.0000 | 1.659 | 706 | | 0.9581 | 95.8 | 0.9581 | 1.0 | 193 | 0.0007 | | 1.651 | 950241 | 62.2 | | |
| MONTE 1 PPB | STD 1-r006.d | QC | 1 | 01-06-2019 20:26 | 1.0000 | 1.654 | 811 | | 1.0296 | 103.0 | 1.0296 | 1.0 | 224 | 0.0008 | | 1.645 | 1011321 | 62.1 | | |
| MONTE 400PPB | SST-r001.d | QC | 6 | 01-06-2019 20:30 | 400.0000 | 1.665 | 278497 | | 402.5705 | 100.6 | 402.5705 | 402.6 | 74352 | 0.3319 | | 1.651 | 839062 | 62.4 | | |
| MONTE 400PPB | SST-r002.d | QC | 6 | 01-06-2019 20:35 | 400.0000 | 1.659 | 262763 | | 400.2227 | 100.1 | 400.2227 | 400.2 | 70068 | 0.3300 | | 1.645 | 796303 | 61.7 | | |
| MONTE 400PPB | SST-r003.d | QC | 6 | 01-06-2019 20:39 | 400.0000 | 1.659 | 288142 | | 399.9099 | 100.0 | 399.9099 | 399.9 | 76726 | 0.3297 | | 1.645 | 873897 | 61.2 | | |
| MONTE 400PPB | SST-r004.d | QC | 6 | 01-06-2019 20:43 | 400.0000 | 1.659 | 282297 | | 400.9871 | 100.2 | 400.9871 | 401.0 | 77792 | 0.3306 | | 1.645 | 834161 | 62.1 | | |
| MONTE 400PPB | SST-r005.d | QC | 6 | 01-06-2019 20:48 | 400.0000 | 1.659 | 287539 | | 397.9799 | 99.5 | 397.9799 | 398.0 | 76496 | 0.3281 | | 1.651 | 876298 | 62.4 | | |
| MONTE 400PPB | SST-r006.d | QC | 6 | 01-06-2019 20:52 | 400.0000 | 1.659 | 282105 | | 397.4675 | 99.4 | 397.4675 | 397.5 | 74809 | 0.3277 | | 1.651 | 860846 | 62.5 | | |

Figure 6. Calibration table of Montelukast

Carryover was also evaluated by injecting the extracted blank sample after injection of the highest concentration standard. Area counts obtained for the blank after the injection of the highest concentration standard was less than 5% of the area of the LLOQ sample. Signal-to-noise ratio was calculated for LLOQ with the peak-to-peak algorithm and found to be more than S/N=30:1.

LLOQ chromatogram

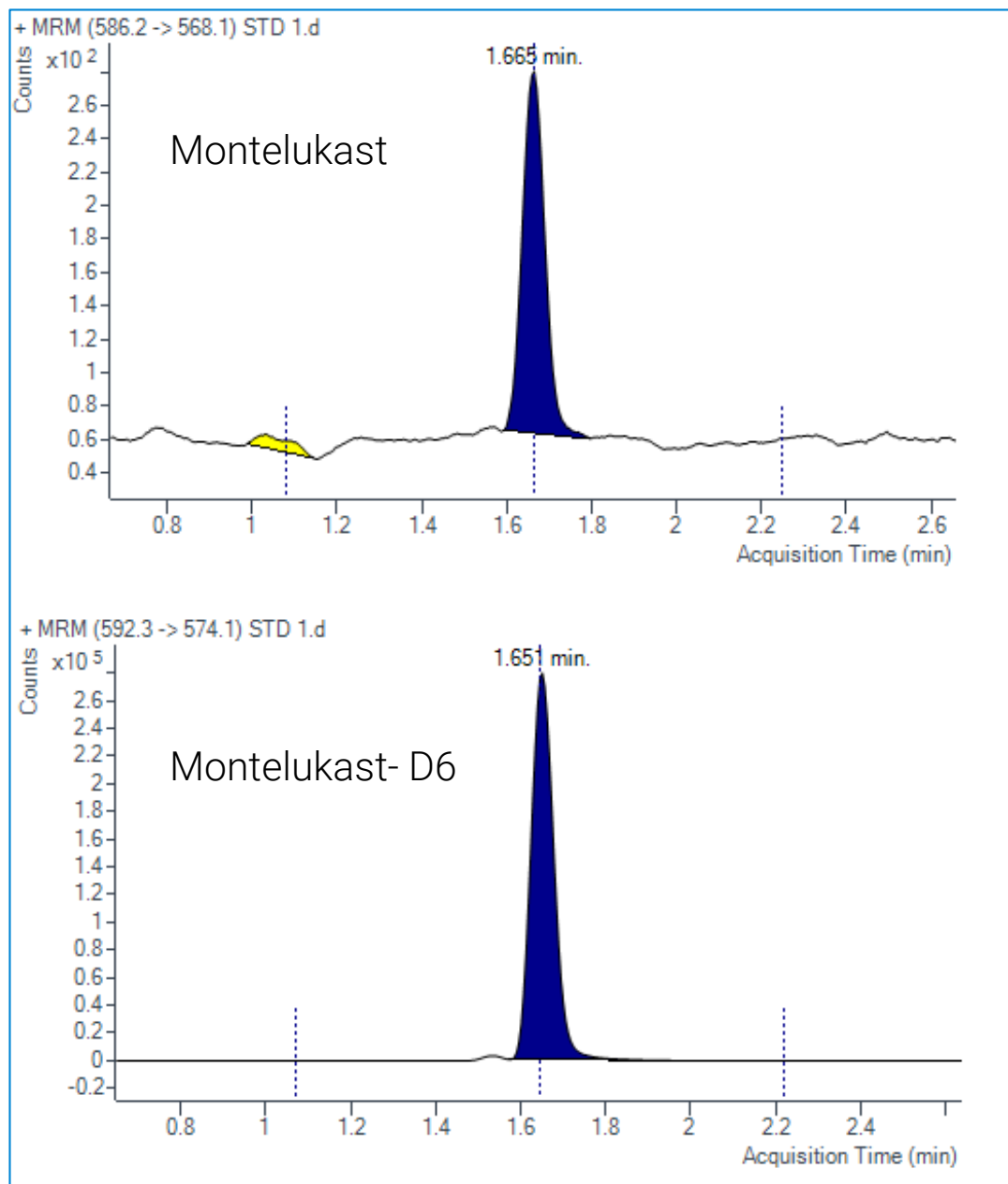


Figure 7. LLOQ chromatogram of Montelukast

The reproducibility of area ratio was measured by performing 300 injections of prepared plasma samples at the LLOQ level. % CV of area ratio for 300 injections was calculated as 6.4%.

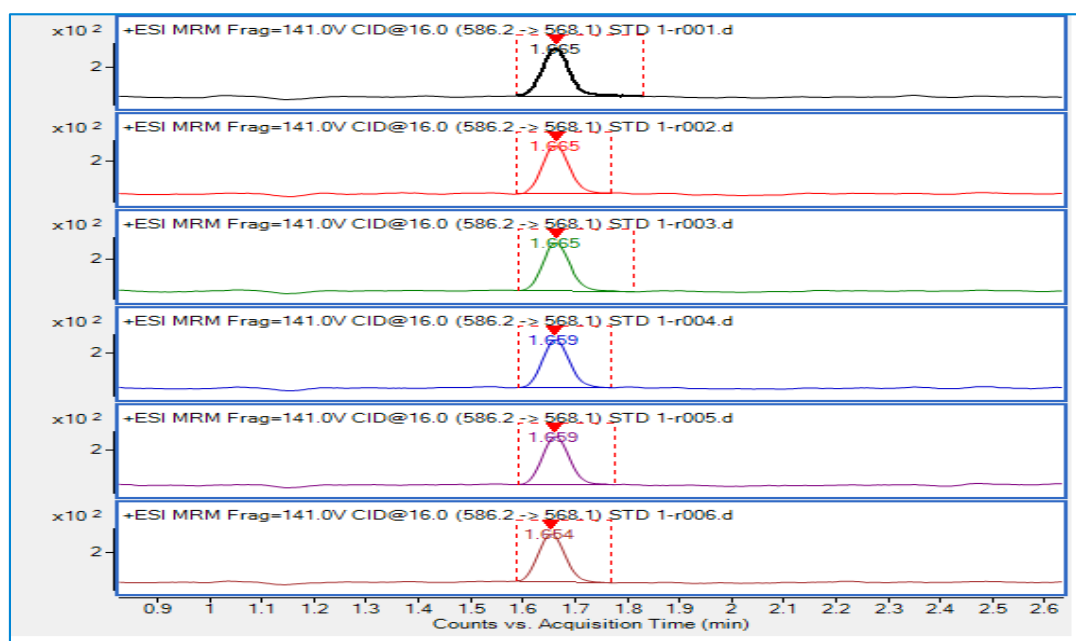


Figure 8. Reproducibility at LLOQ of Montelukast

Recovery of QC samples

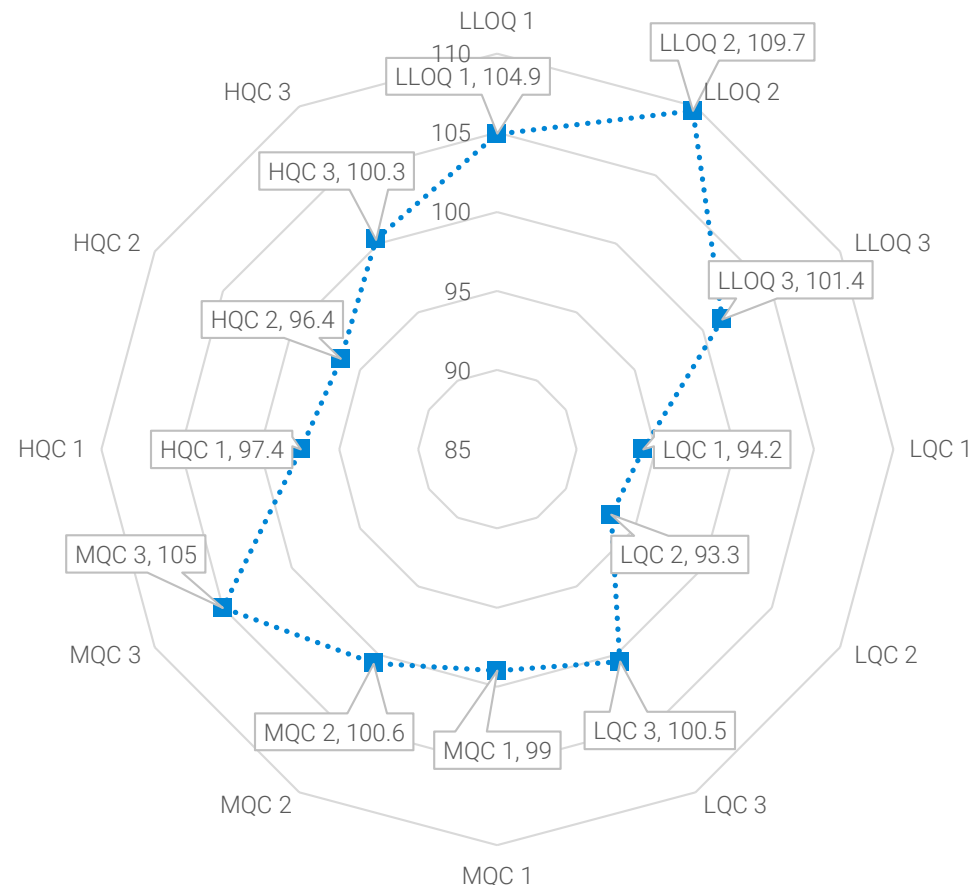


Figure 9. RADAR Plot of recovery of Montelukast in QC samples

Conclusions

- A MRM based Montelukast method was developed showing good sensitivity and linearity from 1 ng/ml to 1000 ng/ml
- The developed method is cost-effective, highly reproducible and shows good recovery from plasma matrix.

References

- Ezzeldin et al. Chemistry Central Journal 2014, 8:17 <http://journal.chemistrycentral.com/content/8/1/17>
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