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Development of a simple, selective and sensitive bioanalytical method for the analysis of Donepezil in plasma using LC-ESI-MS/MS

Chidella Kartheek Srinivas[1] ;Prasanth Joseph[1];Arun Kumar P[1]; Saikat Banerjee[1]; Samir Vyas[2]

[1]Agilent Technologies, BENGALURU, India;
[2]Agilent Technologies, Mumbai, India

Introduction

Donepezil is an FDA approved drug used to treat dementia in Alzheimer's patients. It is available in generic form. It belongs to a class of cholinesterase inhibitors and comes as a tablet that dissolves quickly in the mouth.

In this work, we used a triple quadrupole LC-MS/MS equipped with an electrospray ionization source operated in positive mode to quantify Donepezil in human plasma samples. The developed method consisting of a simple liquid-liquid extraction protocol and multiple reaction monitoring-based quantification was selective and highly reproducible. Assay performance was within current pharmaceutical and regulatory guidelines.

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

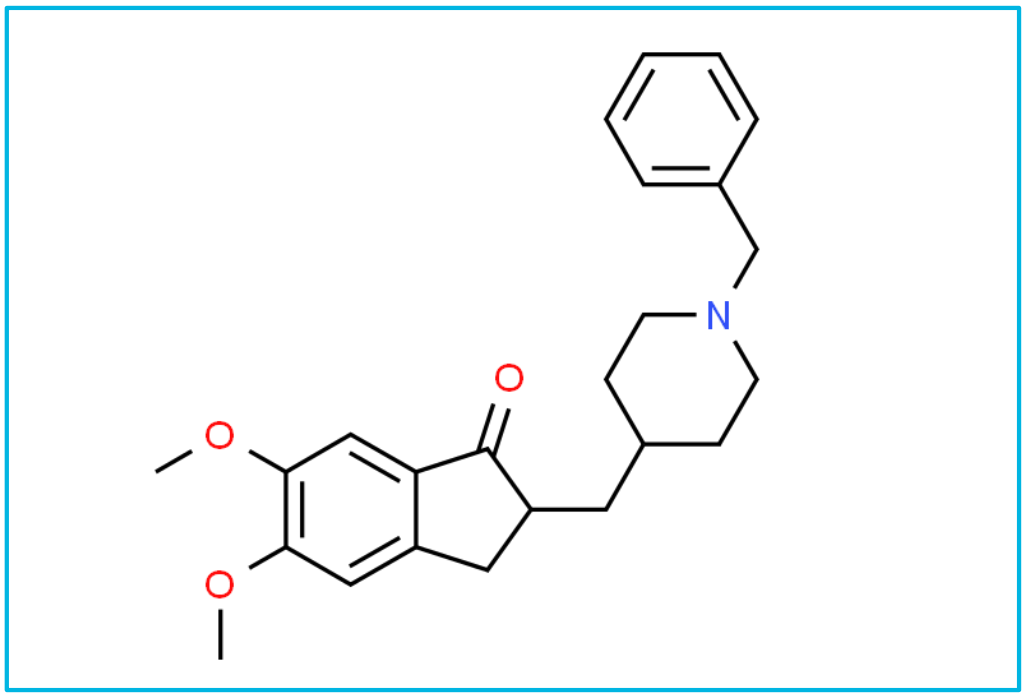

Figure 2. Chemical structure of Donepezil

Experimental

Sample Preparation

1. 0.25 ml plasma spiked with the drug (2% spiking)
2. Extracted with 1.5 ml of Ethyl Acetate: n-hexane (90:10)
3. Vortex for 5 minutes
4. Centrifuge at 5000 rpm for 5 minutes.
5. Supernatant is evaporated to dryness at 45 degrees in SpeedVac.
6. Reconstitute with 0.25 ml of mobile phase.

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

Chromatographic conditions

Analytical column	SB C18 (100 X3.0, 1.8um)
Flow rate	0.4 ml/min
Mobile phase A	5mM ammonium formate with 0.1% formic acid
Mobile phase B	Acetonitrile
Injection volume	2 µl
Elution	Isocratic
Mobile phase ratio	20:80
Needle wash solvent	Acetonitrile: Water (60:40)

Source parameters

Ionisation: ESI	Polarity: Positive
Sheath gas temp: 275°C	Sheath gas flow: 8l/min
Drying gas temp: 200°C	Drying gas flow: 8l/min
Cap Voltage: 3500V	Nozzle voltage: 0
Nebuliser pressure: 40 psi	

Method development

The method was developed on an Agilent G6470 QQQ LC-MS/MS equipped with an Electrospray ionization source. Both Donepezil and the internal standard Donepezil-D7 were ionized in positive ionization mode.

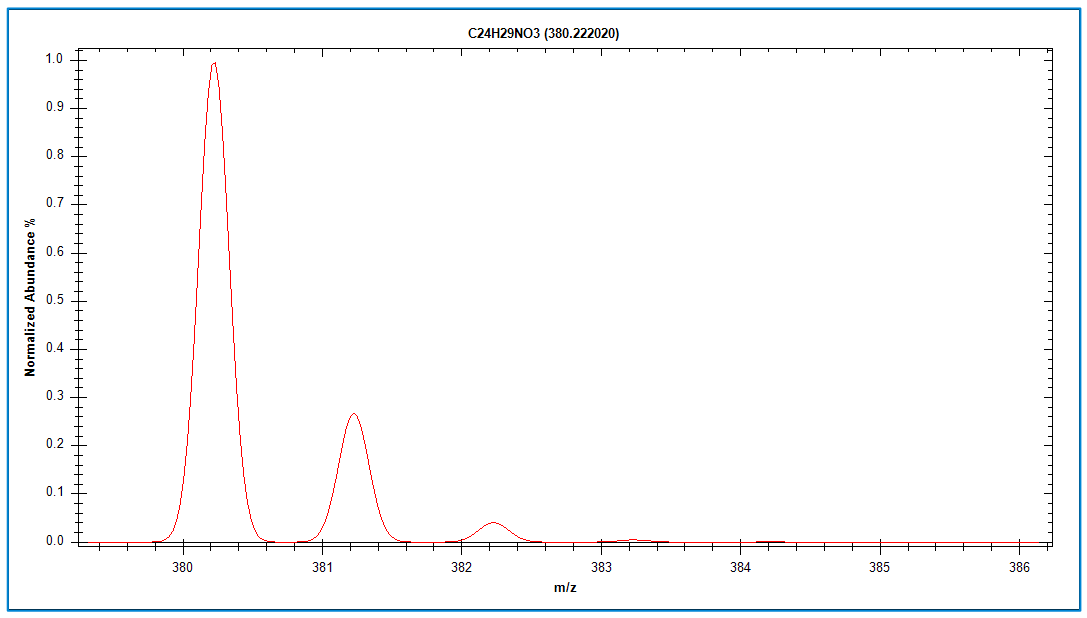

Figure 4. Isotopic pattern of Donepezil in Positive mode

Compound ID	Precursor ion m/z	Product ion m/z	Collision energy
Donepezil	380.2	91	40
Donepezil-D7	387.3	98	40

Table 1. MRM parameters for Donepezil in ESI positive mode

The calibration curve in the range of 0.1 ng/ml to 100 ng/ml was linear with weighing factor = $1/X^2$. The regression coefficient for relative response versus relative concentration of the analyte to the internal standard was 0.9993. The accuracy of the calibration standards in the linearity curve was between 96 and 106%.

As a part of the precision and accuracy batch, triplicate injections of LLOQ, LQC, MQC and HQC were performed to calculate recovery. Average recovery at LLOQ of 0.1 ppb was 107%. Average recovery at 0.5ppb (LQC), 40ppb (MQC) and 80 ppb (HQC) were 101%, 98% and 102% respectively.

Calibration curve

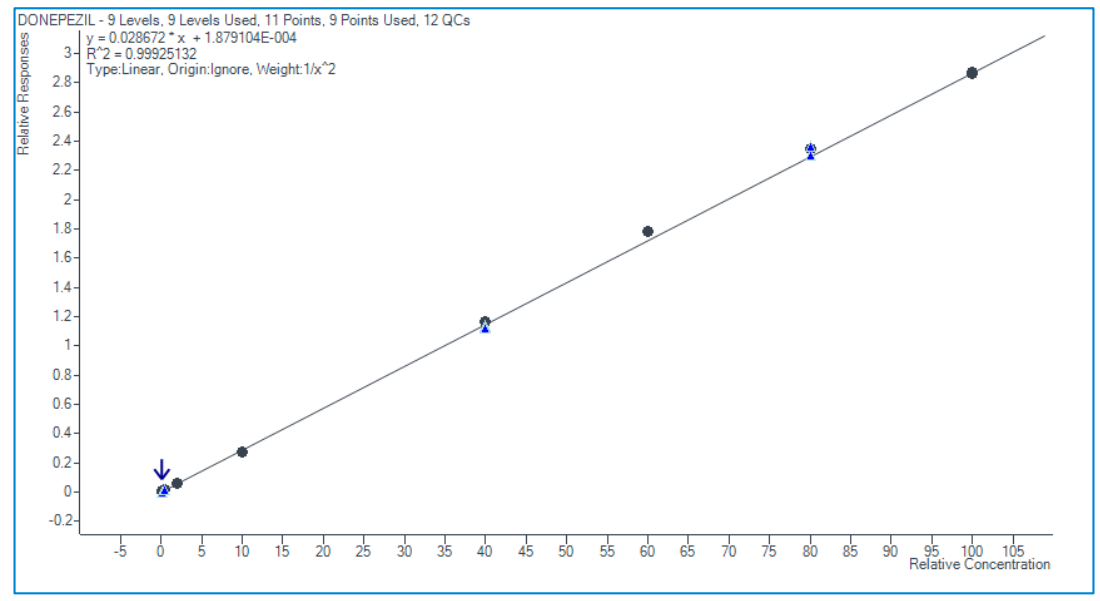

Figure 5. Calibration curve of Donepezil

Sample ID	Type	RT, min	Response	Calculated concentration	Accuracy (%)
Blank	BLANK	1.747	93		
Blank+IS	BLANK	1.747	241	0.0085	
0.1 ppb	CAL	1.747	1710	0.1058	105.8
0.2 ppb	CAL	1.747	3090	0.1993	99.7
0.5 ppb	CAL	1.747	8164	0.4928	98.6
2.0 ppb	CAL	1.747	31324	1.9417	97.1
10 ppb	CAL	1.747	156466	9.6055	96.1
40 ppb	CAL	1.747	661169	40.7788	101.9
60 ppb	CAL	1.747	1013018	62.1801	103.6
80 ppb	CAL	1.747	1333669	81.9101	102.4
100 ppb	CAL	1.747	1634651	100.0444	100
LLOQ1	QC	1.75	1959	0.1106	110.6
LLOQ2	QC	1.75	1765	0.0995	99.5
LLOQ3	QC	1.75	1960	0.1118	111.8
LQC 1	QC	1.75	9292	0.5727	114.5
LQC 2	QC	1.75	7994	0.4814	96.3
LQC 3	QC	1.75	8164	0.4731	94.6
MQC 1	QC	1.75	666329	39.5861	99
MQC 2	QC	1.75	666599	39.8187	99.5
MQC 3	QC	1.75	655234	39.138	97.8
HQC 1	QC	1.75	1335138	82.5444	103.2
HQC 2	QC	1.75	1347247	80.5089	100.6
HQC 3	QC	1.75	1319752	82.6568	103.3

Figure 6. Calibration table of Donepezil

25 injections of plasma sample prepared at the LLOQ level were carried out to evaluate the reproducibility of the response. % CV of area ratio for 25 injections was 2.5%.

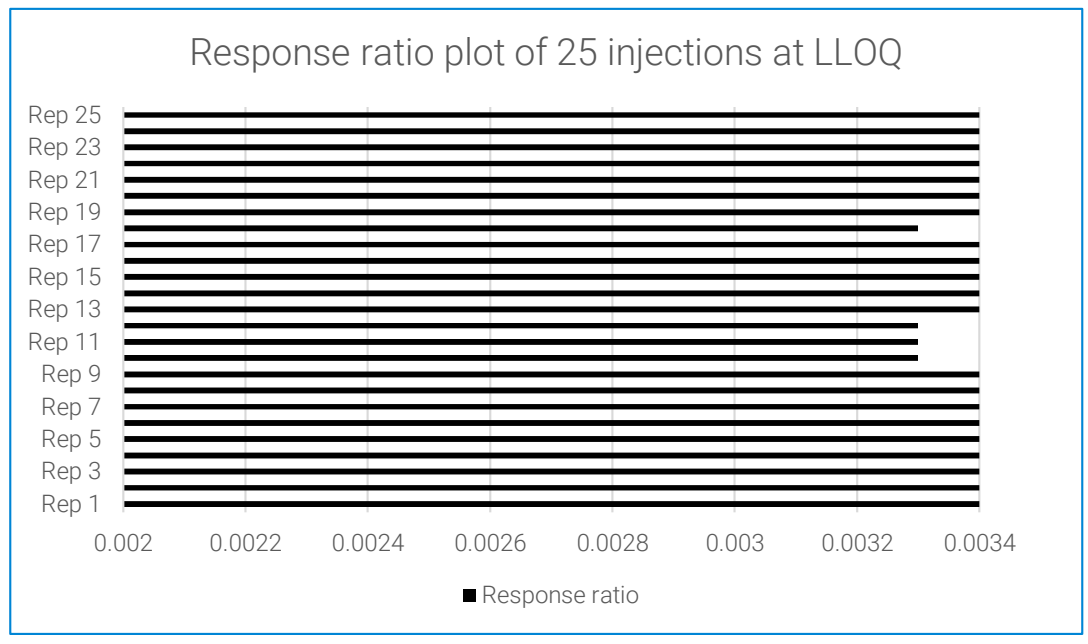

Figure 7. Reproducibility (area ratio plot) of 25 injections of Donepezil at LLOQ.

Results and Discussion

MRM chromatogram at LLOQ (0.1 ppb)

Signal to noise ratio calculated for the LLOQ level was more than 20:1, where the noise calculation was performed by the peak to peak algorithm.

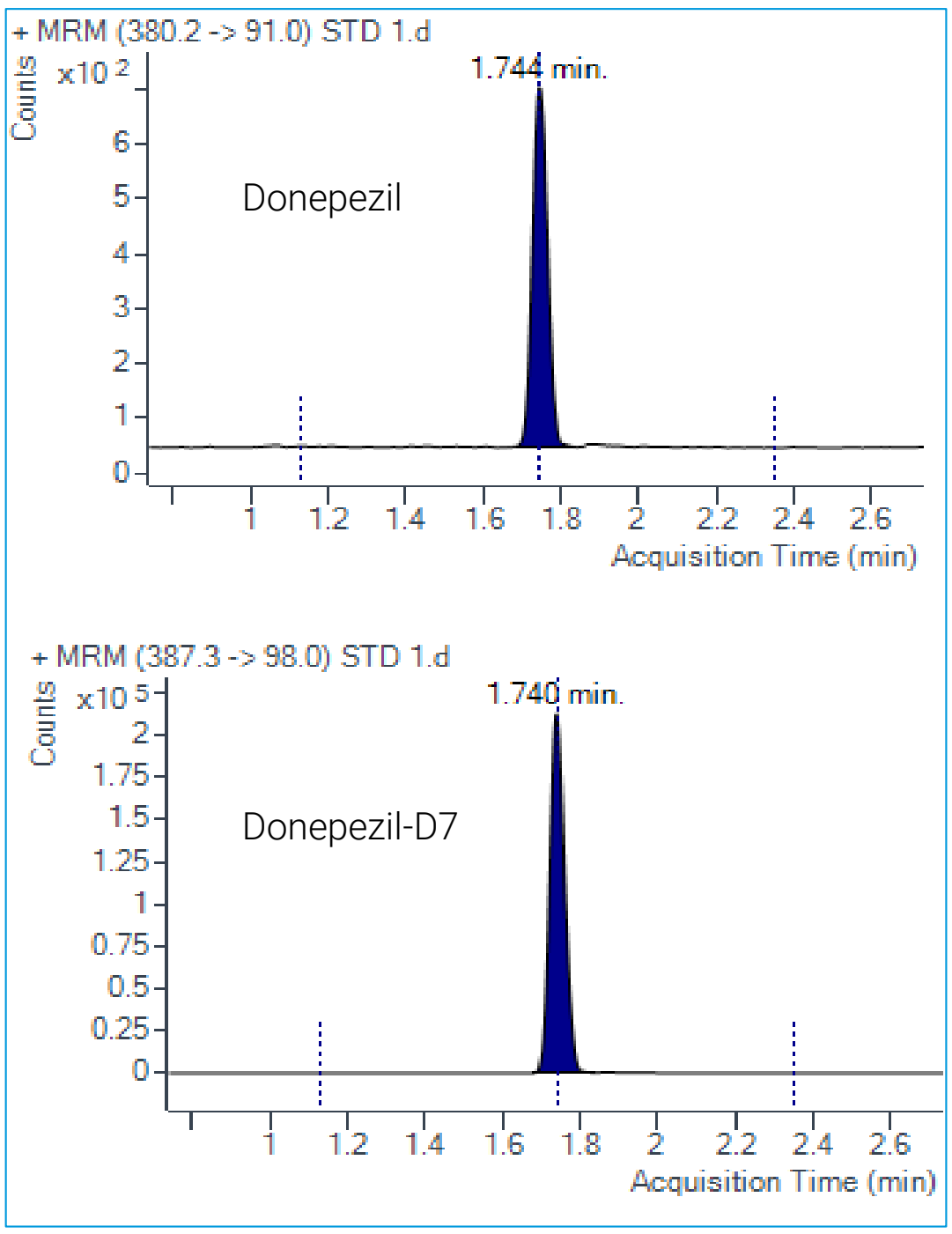

Figure 8. MRM chromtoagram of Donezepil at LLOQ

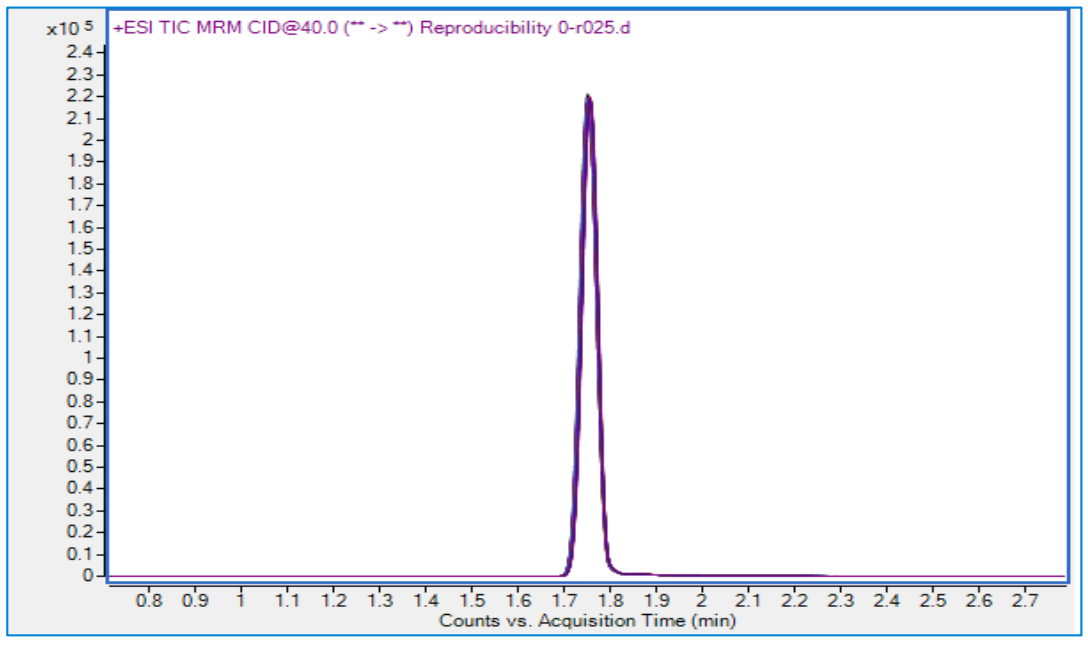

Figure 9. Overlay of 25 injections of system suitability standard of Donepezil

Recovery of QC samples

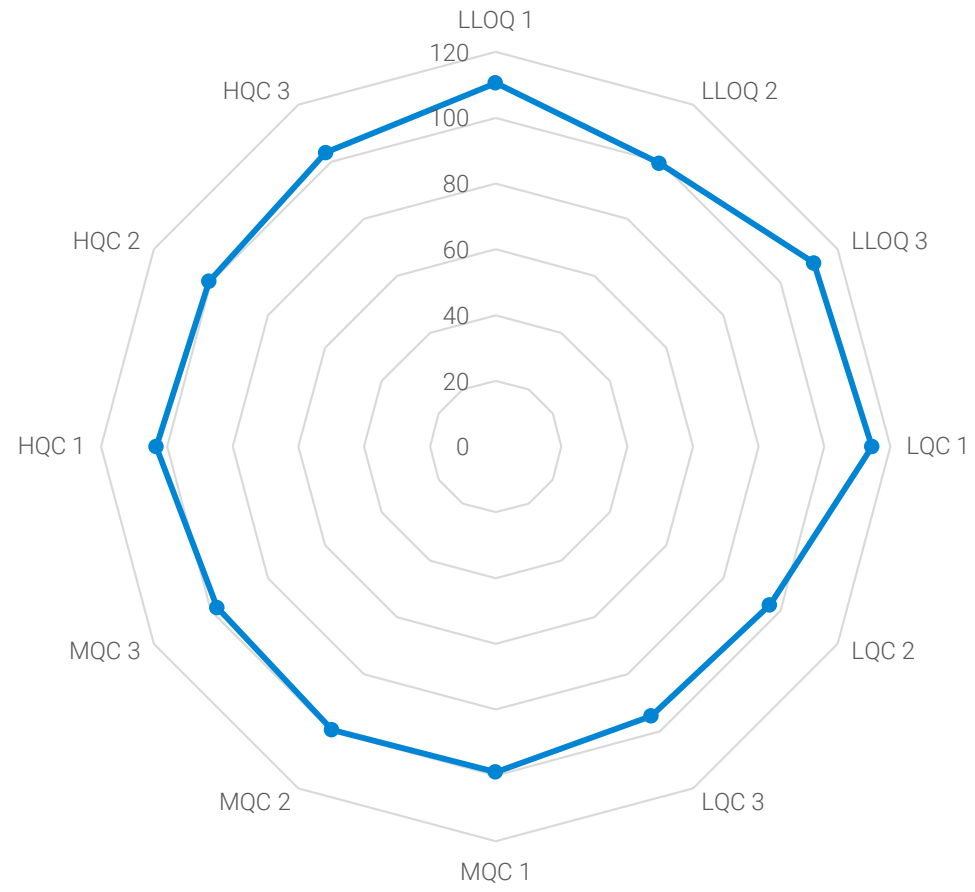

Figure 10. RADAR plot denoting recovery [%] of Donepezil in QC samples

Conclusions

- The developed MRM based method shows good sensitivity and is linear from 0.1 ng/ml to 100 ng/ml
- The developed bioanalytical method was based on a simple sample preparation that demonstrated selectivity and recovery.
- Developed method found to be highly reproducible over the precision and accuracy batch.

References

- Transl Clin Pharmacol2018;26(2):64-72; https://doi.org/10.12793/tcp.2018.26.2.64
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