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A Fully Validated LC–MS/MS Method For Simultaneous Determination Of Oxybutynin And Its Metabolite N-desethyloxybutynin In Human Serum

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PURPOSE

Overactive bladder (OAB), with symptoms of urgency, frequency, and urge incontinence, substantially alter the health-related quality of life in millions of people. The most widely prescribed medicine for this condition is oxybutynin. Following oral administration, oxybutynin is rapidly absorbed and undergoes extensive upper gastrointestinal as well as first-pass hepatic metabolism via the cytochrome P450 system (i.e., CYP3A4) into multiple metabolites. Studies of transdermal and intravesical administration of oxybutynin, which completely avoids hepatic metabolism and pre-systemic gastrointestinal absorption, have shown a decrease in systemic adverse events.

In this study, a liquid chromatography (LC) tandem mass spectrometry (MS/MS) method was developed and validated for the simultaneous determination of oxybutynin and its major metabolite, N-desethyloxybutynin (N-DEO), in human serum.

METHODS

Chromatographic analysis of analytes was achieved on Waters® Alliance e2695 HPLC system under reverse-phase conditions (Waters Corporation; Milford, MA USA). Mass spectrometric detection was carried out on a triple quadruple mass spectrometer Waters® Acquity (Waters Corporation; Milford, MA USA) operating in positive ESI and multiple reaction monitoring (MRM).

Table 1. LC parameters

Parameters	Value
Analytical column	Phenomenex Luna® HILIC column (150 mmx 3.0 mm, 5 µm)
Guard column	Phenomenex Luna® security guard column (4.0 mm × 2.0 mm)
Column temperature	60 °C
Mobile phase A	Acetonitrile (98%)
Mobile phase B	10 mM ammonium formate buffer (2%)
Flow rate	0.6 mL/min
Running time	4.5 min
Injection volume	20 µL

Table 2. MS/MS parameters

Analyte	Q1 (m/z)	Q3 (m/z)	CV (V)	CE (eV)	Retention time (min)
Oxybutynin	358.33	142.17	45	26	1.7
N-DEO	369.40	142.10	60	24	1.7
Oxybutynin-D11	330.27	312.18	50	14	2.2
N-desethyl oxybutynin-D5	335.33	317.24	30	14	2.3

Q1 = Precursor ion, Q3 = Product ion, CV = Cone voltage, CE = Collision energy

Sample preparation

200 µL serum sample was fortified with 50 µL internal standard working solution and vortexed for 10 s. Samples were extracted with 1.5 mL of ethyl acetate on a high speed shaker for 20 min. This was followed by centrifugation at 20,800 × g for 30 min at 4°C. The supernatant was separated and evaporated under a gentle stream of nitrogen. After drying, the residue was reconstituted in 50 µL of mobile phase and centrifuged at 20,800 × g for 10 min at 4°C. 20 µL samples were injected into the LC-MS/MS system.

RESULTS

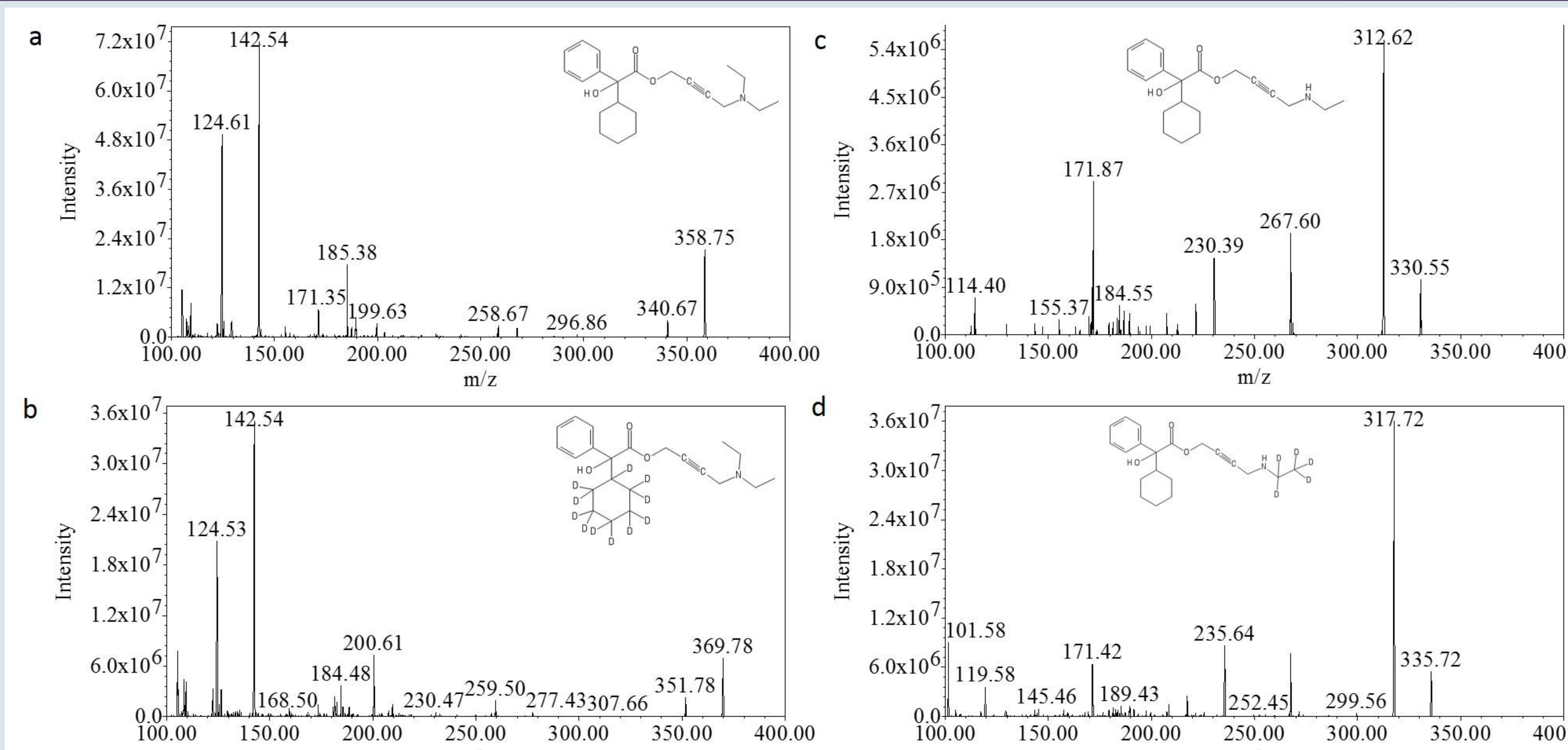

Figure 1. Full product ion scan of (a) oxybutynin and (b) oxybutynin-D11 (IS), (c) N-DEO and (d) N-desethyl oxybutynin-D5 (IS).

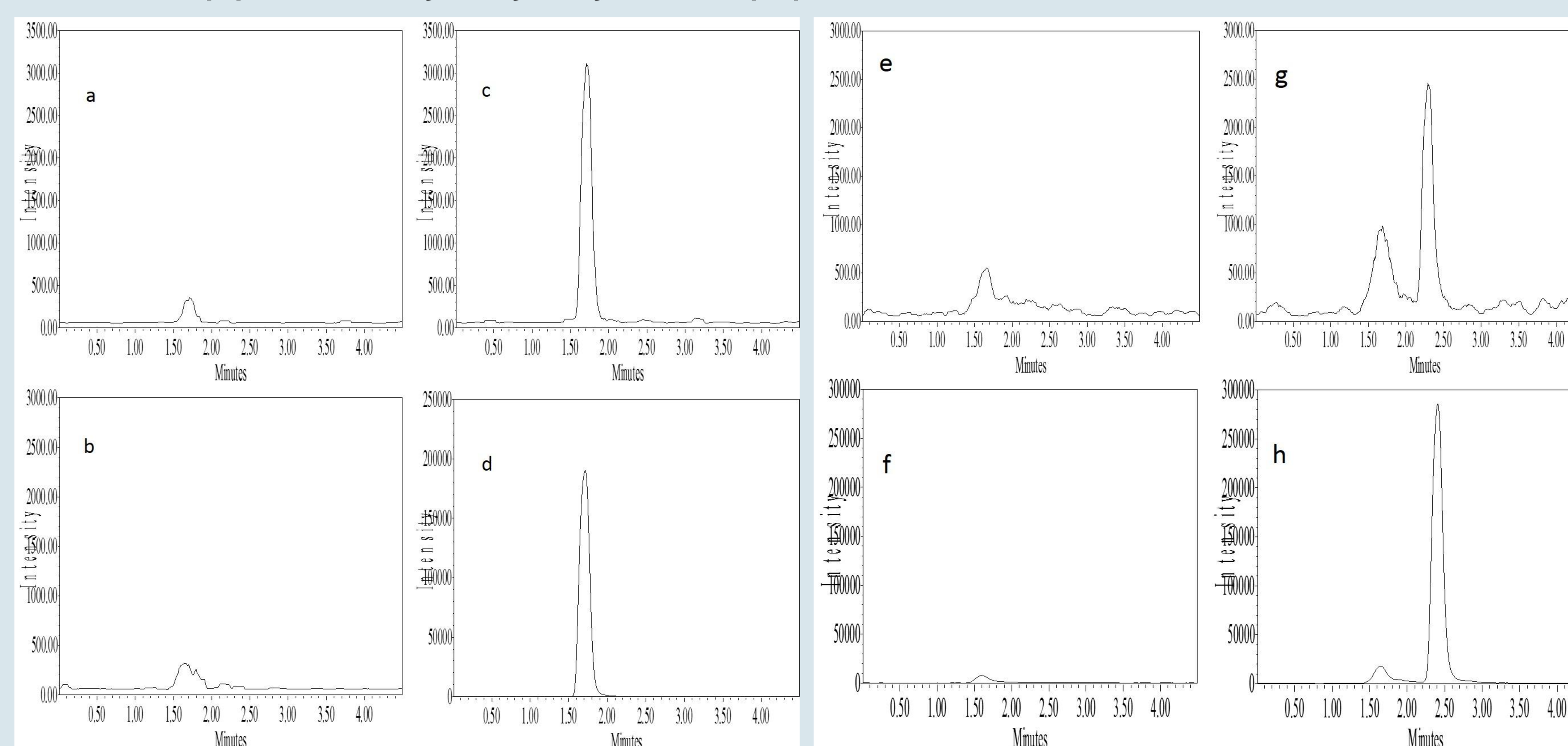

Figure 2: Representative MRM chromatograms (a) and (b) blank serum sample; (c) serum spiked with LLOQ of oxybutynin (50 pg/mL) and (d) oxybutynin-D11 (30 ng/mL); Representative MRM chromatograms (e) and (f) blank serum sample; (g) serum spiked with LLOQ of N-DEO (200 pg/mL) and (h) N-desethyl oxybutynin-D5 (30 ng/mL).

Table 3. Recovery and matrix effect

	Recovery (Mean% ± RSD, n=6)		Matrix effect (Mean% ± RSD, n=6)	
	Oxybutynin	N-DEO	Oxybutynin	N-DEO
LQC	97.7 ± 8.7	106 ± 12	91.9 ± 7.7	92.6 ± 12.6
MQC	98.0 ± 3.9	103 ± 4	94.3 ± 4.2	98.7 ± 5.6
HQC	98.3 ± 2.8	96.6 ± 5.4	95.5 ± 2.5	99.9 ± 2.7

Table 4. Intra-day and inter-day accuracy and precision

Nominal conc. (ng/mL)	Intra-day (n=6) Mean conc. (ng/mL)	Intra-day CV (%)	Intra-day Accuracy (%)	Inter-day (n=18) Mean conc. (ng/mL)	Inter-day CV (%)	Inter-day Accuracy (%)
Oxybutynin						
0.05	0.0511	6.00	102	0.0508	11.2	102
0.15	0.148	5.24	98.4	0.145	5.71	96.7
2.5	2.52	6.35	101	2.49	4.86	100
25	25.6	1.77	102	25.4	2.95	101
N-DEO						
0.2	0.215	8.84	108	0.205	10.7	103
0.6	0.574	6.25	95.7	0.565	6.57	94.1
10	10	4.06	100	9.59	5.09	95.9
100	102	2.92	102	102	5.91	102

Table 5. Summary of stability

Storage condition	level	Oxybutynin (n=6) Mean Conc (ng/mL) ± SD	Oxybutynin Bias (%)	N-DEO (n=6) Mean Conc (ng/mL) ± SD	N-DEO Bias (%)
Freeze–thaw stability	LQC	0.144 ± 0.009	-4.04	0.575 ± 0.059	-4.10
	HQC	25.6 ± 0.7	2.52	104 ± 2	4.02
Benchtop stability	LQC	0.152 ± 0.006	1.61	0.567 ± 0.033	-5.45
	HQC	25.1 ± 0.4	0.318	100 ± 2	0.034
Processed sample stability	LQC	0.15 ± 0.014	-0.158	0.615 ± 0.051	2.54
	HQC	25.5 ± 0.7	1.86	102 ± 1	2.32
Long-term stability (30 days)	LQC	0.146 ± 0.08	-2.71	0.561 ± 0.040	-6.41
	HQC	26.2 ± 5.1	4.62	108 ± 5	8.86
Stock solution stability	LQC	0.140 ± 0.005	-6.43	0.569 ± 0.041	-5.11
	HQC	27.3 ± 5.4	9.20	91.8 ± 6.8	-8.16

SD: standard deviation, %Bias = {(Mean stability samples/Nominal concentration×100) −100}

CONCLUSION

The developed LC-MS/MS method for simultaneous quantification of oxybutynin and N-DEO in extracted human serum was fully validated with similar or higher sensitivity for oxybutynin and N-DEO compared to previously published methods. Additionally, the method described here has the advantage of being simple, rapid and utilizing a much lower volume of human serum compared to previously reported methods. In conclusion, a reliable method was developed for quantification of oxybutynin and N-DEO following transdermal application; this serum sample analysis method will be employed for future clinical PK studies.

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