

A Rapid Screening and Quantitative LC-MS/MS Method of Etomidate and Modafinil in Blood and Urine using SCIEX Triple Quad 5500+ system

Zhang Chong, Sun Xiaojie, Liu Bingjie, Guo Lihai

SCIEX

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Introduction

Narcotic drugs and psychotropic substances (collectively referred to as narcotic drugs) are essential medications for maintaining human health. However, these substances have dual characteristics. When misused and exhibiting uncontrollable addictive properties, they can pose serious risks to individuals and society.

On September 6, 2023, China National Medical Products Administration, the Ministry of Public Security, and the National Health Commission jointly issued an announcement to include etomidate, modafinil, etc. in the second category of psychotropic drugs. The announcement will be officially implemented on October 1.

This experiment used the SCIEX Triple Quad 5500+ liquid chromatography-mass spectrometry system to establish a rapid screening and quantitative method for etomidate and modafinil. It has the advantages of simplicity, rapidity, and high sensitivity.

Testing methods

1. Sample preparation

Take 500 μ L of blood (or urine) in a centrifuge tube, add 1 mL of acetonitrile/methanol mixed solution (volume ratio, 1:1) for extraction, vortex for 30 s, centrifuge for 10 min (10000 r/min), take the supernatant, filter it with a 0.22 μ m organic membrane, and perform LC-MS/MS analysis.

2. LC condition

Column: Phenomenex Kinetex Biphenyl (2.1 \times 100mm, 2.6 μ m)

Flow rate: 0.3mL/min

Column temperature: 40 $^{\circ}$ C

Injection volume: 1 μ L

Gradient elution:

Phase A: Water (0.02% formic acid + 2mM ammonium formate)

Phase B: Acetonitrile: Water = 95:5 (0.02% Formic Acid + 2mM ammonium formate)

Time	A %	B %
0	95	5
0.5	95	5
4	0	100
6	0	100
6.1	95	5
8	95	5

3. Mass spectrometry conditions

SCIEX Triple Quad 5500+ system

Ion source: ESI source, positive mode

Ion source parameters:

IS voltage: 5500V

Curtain gas: 30psi

Nebulizer gas GS1: 50psi

Auxiliary gas GS2: 55psi

Source temperature TEM: 450 $^{\circ}$ C

Collision gas CAD: Medium

Table 1. Mass spectrometry parameters of etomidate and modafinil

Precursor ion	Product ions	Retention time (RT, min)	ID	Addition method	Declustering voltage (DP, V)	Collision Energy (CE, eV)
245.1*	141.0	4.09	etomidate -1	[M+H] ⁺	25	14
245.1	95.0	4.09	etomidate -2	[M+H] ⁺	25	32
167.0*	152.1	3.56	modafinil -1	[M-C ₂ H ₆ O ₂ NS+H] ⁺	40	32
296.1	129.1	3.56	modafinil -2	[M+Na] ⁺	68	17

* Quantitative ion pair

Table 2. Linear range and equation of etomidate and modafinil

Name	Linear range (ng/mL)	Linear equations	Correlation coefficient
Etomidate	0.02-200	Y=1.4396X+2246.05	0.9976
Modafinil	0.5-2000	Y=8.4713X+615.1036	0.9997

Results

1. Typical chromatograms of etomidate and modafinil (see Figure 1)

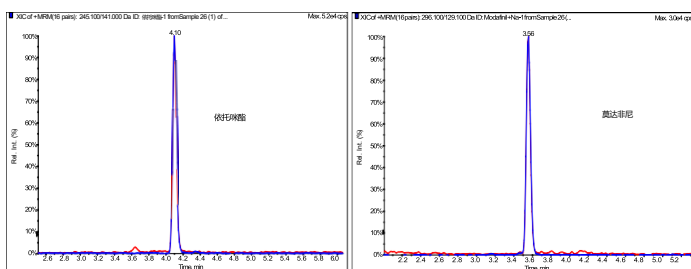


Figure 1. Typical chromatograms of etomidate (1ng/ml) and modafinil (20ng/ml)

2. Linear range of etomidate and modafinil (see Table 2)

Discussion

Selection of Modafinil Ion Pairs

When optimizing compound-dependent parameters using syringe infusion of the pure analytical standards, we found that the parent ions of modafinil [M+Na]⁺ (m/z 296.1), [M+H]⁺ (m/z 274), and [M+NH₄]⁺ (m/z 291.1) appeared in the first-stage full scan. In addition, the ion [M-C₂H₆O₂NS+H]⁺ (m/z 167.1) was also seen. Therefore, we searched and optimized the ion pairs for several types of ions with different adducting modes.

When selecting and optimizing the LC-MS method, additives such as formic acid and ammonium formate were added to the mobile phase to provide more protons and ammonium ions, but the ion pair responses of [M+H]⁺ (m/z 274) and [M+NH₄]⁺ (m/z 291.1) did not improve significantly. Through optimization, the selection of quantitative ion pairs is generally based on high response, less interference, and good linear relationship of the calibration curve. Finally, the ion pairs shown in Table 1 were selected in this experiment, with 167.1 > 152.1 as the quantitative ion.

Conclusion

In this paper, a qualitative and quantitative detection method for etomidate and modafinil was established on the SCIEX Triple Quad 5500+ system. This method has simple pretreatment and short analysis time, and is suitable for the detection and identification of etomidate and modafinil in blood and urine.

References

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