APPLICATION NOTE

Chiral Separation of Ketamine and its Key Metabolites

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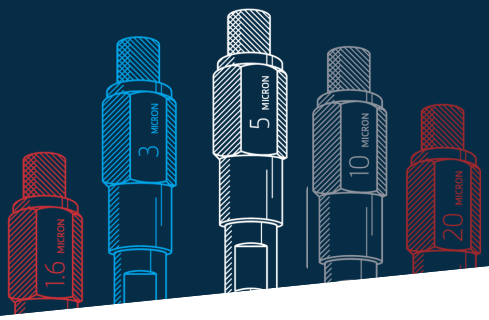

DAICEL Chiral Technologies

INTRODUCTION

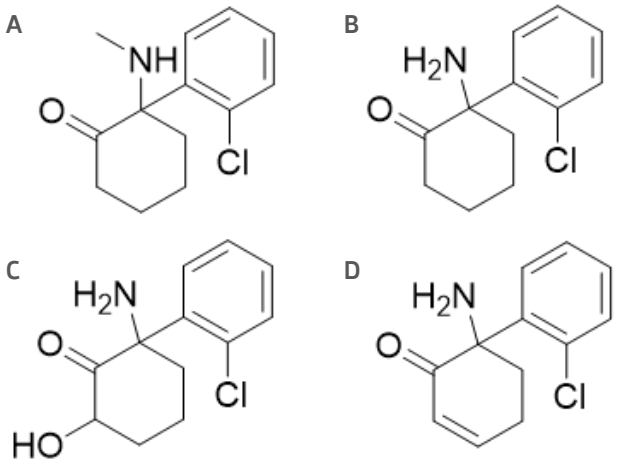

Figure 1: Ketamine (A), Norketamine (B), Hydroxynorketamine (C), and Dehydronorketamine (D)

Ketamine (Figure 1A) is a small-molecule therapeutic agent that exists as a pair of enantiomers. The S-enantiomer has been shown to have higher affinity for the N-methyl-D-asparatet (NMDA) receptor in the brain, and has been an approved FDA treatment of depression for decades[1]. The R enantiomer has gained attention in recent years for its potentially long-lasting antidepressant effects, with fewer dissociative side effects[2].

Following administration of ketamine, it undergoes rapid hepatic metabolism to produce major metabolites, including norketamine and hydroxynorketamine (Figure 1B and 1C respectively). These metabolites retain the original chiral center present in ketamine, and exhibit their own pharmacological activity[3]. For example, specific enantioemrs of 1C and 1D have been implicated in a sustaining antidepressive effects after the full clearance of ketamine from circulation[4]. The separation and quantification of ketamine, and critically the metabolites, is therefore critical to help support continued pharmacokinetic, pharmacodynamic, and mechanistic studies across preclinical and clinical applications.

This application note is focused on establishing a robust enantioselective separation for ketamine and its key metabolites using Daicel polysaccharide-based chiral stationary phases (CSPs). Method development was conducted in high-performance liquid chromatography (HPLC) mode with reversed phase (RP) solvents to afford mass spectrometry (MS)-compatible conditions. Further optimization was performed to fully separate the metabolite isomers, and single isomer injections (when available) were performed to assign elution order

EXPERIMENTAL

Chromatographic Conditions for Separation of Ketamine and its Metabolites		
Column	CHIRALPAK® ID-3 (150 mm x 4.6 mm i.d., 3 µm) Part #: 84524	CHIRALPAK® IN-3 (250 mm x 4.6 mm i.d., 3 µm) Part #: 93525
Mobile Phase	20 mM Ammonium Bicarbonate (aq.) pH = 9.0/Acetonitrile = 70-30	
Flow Rate	1.0 ml/min	
Detection	UV 220 nm ref. 450 nm	
Temperature	25°C	
Sample	10 µg/ml in water	
Injection Volume	10 µl	

Chidiebere Ogbu of the Heien Group at the University of Arizona kindly provided the racemic ketamine and racemic metabolites; the single isomers standards were purchased from Sigma Aldrich. The solvents used were all purchased from Scientific Equipment Company (SECO), were HPLC-grade, and were used as-is.

Screening and optimization for HPLC mode was performed on an Agilent 1200 configured with high-pressure mixing quaternary mobile phase delivery system, vacuum degasser, autosampler, temperature controlled column compartment, and photodiode array UV detector. The instrument was controlled by an Agilent ChemStation Version RevB.04.03.

The chiral columns used for screening included CHIRALPAK® IA-3, IB N-3, IC-3, ID-3, IE-3, IF-3, IG-3, IH-3, IJ-3, IK-3, IM-3, and IN-3, and were 4.6 mm inner diameter (i.d.) by 150 mm length and a 3 µm particle size.

DISCUSSION

Initial screening was performed with an 8 peak mixture of the 4 racemic compounds under gradient conditions. Starting at 90% aqueous/10% acetonitrile (ACN) and holding for 2 minutes, the gradient was increased to 90% ACN over 18 minutes, held for 6 minutes, then set back to 90% aqueous for 8 minutes, totaling 34 minutes per method.

Several partial separations (3-4 peaks) were observed IB N-3, IC-3, IE-3, and IH-3. Both ID-3 and IN-3 showed nearly complete separation (6 peaks on IN-3; 7 on ID-3), which suggested further optimization could achieve full resolution of all 8 expected peaks. To improve the separations, the gradient conditions were converted to isocratic methods, using 70-30 = Aqueous-ACN as a starting point. These conditions provided good retention for all compounds, and baseline separation on both columns for all racemic compounds, with the exception of ketamine (Figure 2 – ID; Figure 3 – IN). A slight partial separation of ketamine is observed on IN.

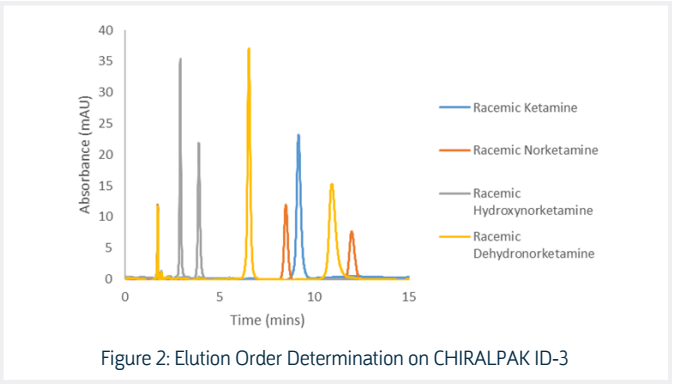

Figure 2: Elution Order Determination on CHIRALPAK ID-3

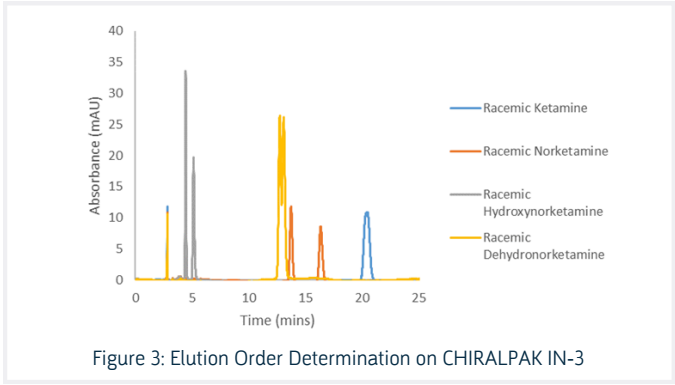

Figure 3: Elution Order Determination on CHIRALPAK IN-3

Single isomers were available for R ketamine, R norketamine, and 2R,6R hydroxynorketamine, so these were also run on each column to fully assign the elution order. On ID, R ketamine could not be determined due to the co-elution with S ketamine, R norketamine is 2nd eluting, as is 2R,6R hydroxynorketamine. The elution order is conserved on IN, with a note that the partial separation for racemic ketamine allows for the determination of R ketamine being the 2nd eluting peak.

CONCLUSIONS

Suitable conditions for the separation of ketamine and its metabolites were found on CHIRALPAK ID-3 and IN-3. Each column offers distinct advantages: ID-3 provides faster analysis times, while IN-3 affords partial resolution of ketamine. Depending on the specific application goal, one or both columns may be used in tandem to generate a more complete data set.

Importantly, both methods are fully MS-compatible, enabling direct analysis of ketamine metabolism from biological samples without extensive sample preparation. This ensures high quality, accurate analyses that will support ongoing research into the pharmacological effects of ketamine and its downstream metabolites.

As scientific understanding of ketamine metabolism continues to evolve, these robust chiral separation strategies may also facilitate the discovery of novel active metabolites with potential therapeutic value.

REFERENCES

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