ORIGINAL RESEARCH

The evaluation of Two Zwitterionic Hydrophilic Interaction Liquid Chromatography Materials for the Rapid Separation of Methamphetamine and Muscimol Pharmaceuticals

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ABSTRACT

The investigation aimed to use two Zwitterionic Hydrophilic Interaction Liquid Chromatography (ZIC-HILIC) columns to examine the retention behavior of methamphetamine and muscimol. The various spacer lengths between zwitterionic molecules in the stationary phases have been utilized to investigate methamphetamine and muscimol retention behavior. In hydrophilic interaction liquid chromatography (HILIC), the retention characteristic of these two drugs was examined using a mixture of sodium acetate buffer/acetonitrile as an eluent equipped with a UV detector. It has been established that hydrophilic and hydrophobic mechanisms primarily govern the separation of methamphetamine and muscimol medicines on two ZIC-HILIC columns. As previously stated, the ZIC-HILIC-1 column demonstrated a difference when compared to the ZIC-HILIC-5 columns. The combination of positively and negatively charged locations in a single molecule bonded to the surface of an adsorbent allows for distinct variations in methamphetamine and muscimol selectivity separation.

Keywords: Drug, Methamphetamine, Muscimol, Retention characteristic, Zwitterionic Hydrophilic Interaction Liquid Chromatography.

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INTRODUCTION

To study the analysis of polar and ionic molecules, hydrophilic interaction liquid chromatography (HILIC) has become a better option than both normal-phase chromatography and reverse-phase chromatography. It may form difficult to retain highly polar materials in reversed-phase mode, making analysis difficult.[1] In recent years, the interest in the HILIC approach has been driven by rising demand for the study of polar compounds, metabolites, and physiologically relevant chemicals in proteomics, glycomics, and clinical analysis.[2, 3] Some research suggests a multimodal separation process between the analyte and water attached to the stationary phase's surface or between the analyte and stationary phase. HILIC retention is caused by partitioning the injected analytes between the mobile phase and a water-rich layer in the hydrophilic HILIC stationary phase, according to the HILIC hypothesis that is the most widely accepted.[4] HILIC selectivity and retention are primarily influenced by altering the eluent's type and percentage of organic solvent, buffer type and concentration, and pH value residue often rises with an increase in organic solvent percentage. The pH impacts retention because it alters the ionization of the column material and the analytes being studied.[5] The stationary phases of HILIC may be classified into three groups: neutral, charged, and zwitterionic. Zwitterionic ion exchangers offer a novel approach to developing stationary phases for several modes of high-performance liquid chromatography.[6] The mix of positively and negatively charged sites in a single particle or within the functional groups of a single molecule bonded to an adsorbent's surface offers unique options to modify separation selectivity because hydrophilic interaction chromatography (HILIC) is one of the important techniques. Numerous studies and applications have been performed using ZIC-HILIC columns by Rasheed *et al.*[7-29] The study's initial goal was to investigate how methamphetamine and muscimol were retained utilizing two novel columns (ZIC-HILIC-1 and ZIC-HILIC-5). This research is unique because it first analyses the drug retention behavior using the ZIC-HILIC columns. This study discussed how zwitterionic ion-exchangers are classified based on their structure, distribution of oppositely charged groups. As a result, this was the second

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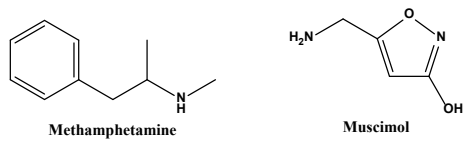

Figure 1: The chemical structures of methamphetamine and muscimol

of our research. The third goal of this study was to present a new and rapid method for determining methamphetamine and muscimol. (Figure 1). Methamphetamine is a stimulant in the amphetamine and phenethylamine class of psychoactive medicines. Teens can benefit from methamphetamine by increasing their alertness, attention, and energy. Desoxyn is a brand name for methamphetamine.[30] Methamphetamine is a central nervous system stimulant that is highly addictive. It has been shown that methamphetamine increases the amount of the naturally occurring chemical dopamine in the brain, where dopamine is implicated in physical movement, motivation, and rewarding behaviours. The ability of methamphetamine to rapidly release vast quantities of dopamine into the brain's reward areas considerably promotes drug-abusing behaviour.[31] Muscimol, also known as pantherine or agarin, is one of the significant hallucinogenic components of amanita muscaria and related species of mushrooms.[32] Muscimol is a strong and selective GABA A receptor agonist with a sedative, hypnotic, antidepressant, and hallucinogenic properties. According to studies, psychotropic chemicals produced from fungus are a viable tool for treating various mental and physical health issues. Distillation and purification of an alternative psychotropic chemical. It has been found to reduce tension and anxiety and ease muscular discomfort and promote restorative sleep. There have been some previous reports that have been utilized to analyse the medicines used in this investigation, such as *S. pneumoniae* liquid chromatography with tandem mass spectrometry (LC-MS/MS),[33] Gas chromatography mass spectrometry (GC/MS),[34] High Performance Liquid Chromatography Mass Spectrometry (HPLC/MS)[35] and High-performance liquid chromatography ultra-violet (HPLC–UV).[36]

Experimental

Chemicals

Methamphetamine and muscimol were purchased from Sigma-Aldrich. Acetonitrile (HPLC-grade) was obtained from Fisher Scientific. Acetic acid and sodium acetate were purchased from Merck. A milli-Q system (Millipore, USA) was utilized to purify the water, then used in all the experiments.

Chromatographic System

Analysis was performed using an HPLC system (Merck Hitachi), an HPLC device coupled with a UV detector (type L-4200) was used, and a Merck Hitachi L-6200 Pump, in-line degasser with a 20 µL injection loop. The N2000 workstation was used for processing the chromatographic data. The selection of a stationary phase is critical for good analysis. In this investigation, two columns (ZIC-HILIC-1 and ZIC-HILIC-5, 100 mm×4.6 mm I.D.) with varying chain lengths were chosen.[37] These columns were characterized as having a variety of functional groupings, resulting in a variety of HILIC mechanisms. An ultrasonic bath and a pH 740 (WTW) were employed.

Chromatographic Conditions

Acetonitrile and buffer acetate mixture using a mobile phase to analyze methamphetamine and muscimol. The injection volume was 20 µL, and elution was carried out at a 0.75 mL/minute flow rate at 25°C. The ultraviolet area at 350 nm was used to analyze methamphetamine and muscimol.

Preparation of standard solutions

Standard solutions of methamphetamine and muscimol were prepared daily. The solutions were prepared by dissolving an accurately weighed amount of methamphetamine and muscimol (10 mg) in 100 mL of eluent, a stock solution of methamphetamine and muscimol were prepared (100 µg/ mL). It had to be kept in the dark and in cool ambient (6°C).

The Results and discussions

During the experiment, two distinct stationary phase packing materials (ZIC-HILIC-1 and ZIC-HILIC-5) were tested. A comparative examination and the selection of the optimal analytical circumstances were the goals of this strategy. Various mobile phase compositions of ACN as the organic phase and ammonium acetate buffer as the aqueous phase

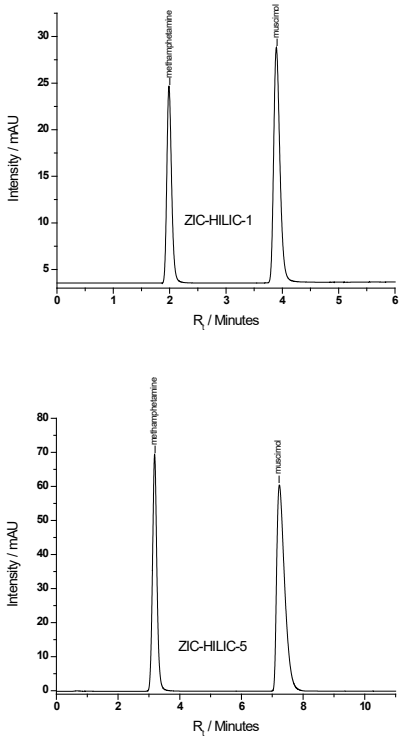

Figure 2: Chromatograms of the methamphetamine and muscimol using ZIC-HILIC-1 and ZIC-HILIC-5 columns.

Table 1: The partition coefficient ($LogP_{oct/wat}$) and acid dissociation constant (pKa) of methamphetamine and muscimol.[38]

Drug	*$LogP_{oct/wat}$*	*pKa*
Methamphetamine	2.07	9.87
Muscimol	-1.6	5.86 and 8.97

were utilized to investigate the separation mechanism. The influence of organic modifier content, pH, and ionic strength on the retention behavior of methamphetamine and muscimol in the mobile phases under inquiry were assessed. The chromatograms for methamphetamine and muscimol are shown in (Figure 2). Acetate buffer (pH 3.5) with 90% ACN and a buffer concentration of 30 mM (pH 3.5).

Study influence of the organic composition

Choosing the appropriate solvent is critical in separating and estimating drugs since acetonitrile is usually selected over methanol because of its low viscosity, which provides greater flow rates for the mobile phase. Because acetonitrile is a non-proteotic solvent, hydrogen bonds with the stationary phase functional group or the analytes are unlikely to form. The influence of the mobile phase (acetonitrile) contents on the analytical separation of methamphetamine and muscimol were investigated. When acetonitrile content was raised, muscimol retention time increased (Figure 3) in buffer acetate (pH 3.5, 35 mM). Otherwise, the retention time of methamphetamine decreased when the ACN content increased (Figure 3). This impact made it evident that methamphetamine and muscimol behaved differently. This difference in behavior is due to hydrophobicity values ($LogP_{ow}$), as indicated in Table 1.

Study influence of the pH buffer

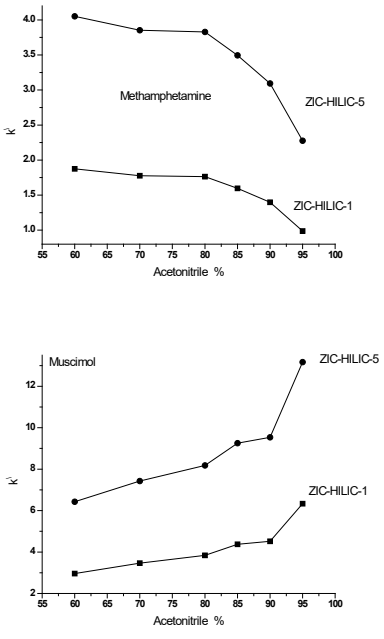

Figure 3: The influence of ACN % variation on methamphetamine and muscimol behaviour using ZIC1-HILIC-1 exchanger and ZIC-HILIC-5 exchangers.

The pH of the buffer is a major chromatographic factor as it can influence both the stationary and polar solutes charging status. The buffer pH value impact was studied in the mobile phase using different pH values from (3–5.5) of the acetate buffer at constant ACN 90% and buffer concentration 35 mM. The results shown in Figure 4 showed that the retention time of muscimol decreased by raising the value of the buffer pH, the pKa (5.86 and 8.97) and the isoelectric point (6.02) values for muscimol. Thus, muscimol is a cation form in pH 3 to 5.5. Contrastingly is the behavior of the methamphetamine when changing the pH of the beaver from 3 to 5. It shows a slight

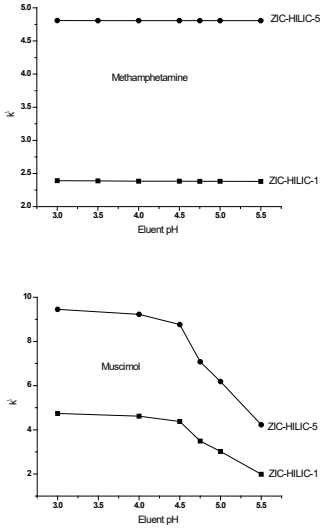

Figure 4: Effect of eluent pH of the buffer on methamphetamine and muscimol.

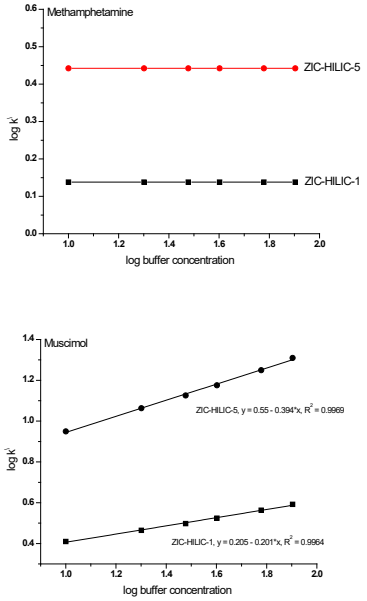

Figure 5: Effect of eluent concentration on methamphetamine and muscimol.

decrease in the pH reaction thanks to the unchanged charge of methamphetamine.

Study Influence of the ionic strength of the buffer

Adjusting the buffer solution concentration is essential for developing a HILIC technique because the HILIC mechanism's intricacy of ionic strength considerably influences chromatographic analysis. The effect of the ionic strength of the buffer on the retention behavior of methamphetamine and muscimol was studied at concentrations ranging from 10–80 mM (pH 3.5) at 90% ACN in the eluent. The results are shown in (Figure 5). It is noticeable that increasing the concentration of buffer in the eluent increases the retention factor of muscimol for both (ZIC-HILIC-1 and ZIC-HILIC-5) columns. This is due to the hydrophilicity of the muscimol ($LogP_{oct/wat}$ = -1.6). As for methamphetamine, the picture is different. Notably, the retention factor for methamphetamine remains nearly constant as buffer concentration increases. Hence, methamphetamine's uncharged molecule can be attributed. To better understand methamphetamine's retention behavior, it is necessary to consider the drug's physical-chemical features, the pKa value of methamphetamine (9.87). As a result, methamphetamine should be an uncharged molecule.

CONCLUSION

This article demonstrates the interaction between two medications and a two-zwitterionic stationary phase. The ZIC-HILIC-5 column has the greatest retention of methamphetamine and muscimol medicines than the ZIC-HILIC-1 column. The explanation could be related to a geometrical arrangement of zwitterion molecules in the ZIC-HILIC-5 column, which have the highest retention of medicines. For muscimol separation, the hydrophilic interaction is the primary mechanism. In comparison, methamphetamine studies indicate that the hydrophobic interaction is the retention mechanism.

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