Retention Behavior of Basic Amino acids on Various Adsorbents layers in DMSO-1MHCl Mobile Phase

S.D. SHARMA¹, H. SHARMA² AND S.C. SHARMA³

¹Dean of Sciences, IFTM University, Moradabad (U.P)
²Associate Professor, Chitkara University, Punjab
³Professor, Chitkara University, Punjab

Received: November 11, 2015 | Revised: November 21, 2015 | Accepted: December 7, 2015
Published online: March 14, 2016
The Author(s) 2016. This article is published with open access at www.chitkara.edu.in/publications

Abstract: The four basic amino acids were chromatographed on various adsorbents layer and also on admixture layers. It is found that in DMSO – 1MHCl mobile phases, the adsorbent titanium (iv) tungstate and its admixture with silica gel-G were found more effective than other adsorbent. However, various basics amino acids are separated on these layers. Which have some real life importance.

Keywords: Basic amino acids, DMSO and Absorbent

1. INTRODUCTION

The planar chromatography of amino acids on silica gel-G [1-6] ion exchanger [7-9] and unconventional supports [10-12] has been studied earlier. However, TLC of amino acids has been successfully carried out on impregnated as well as on reversed phase [4,13]. Some work on the use of silica gel-G, admixture and impregnated silica gel-G layers has also been reported [12,13]. Much less attention has, however been devoted, to the use of mixed DMSO mobile phase on various adsorbent layers simultaneously.

We have therefore decided to chromatographic studies of basic amino acids on various adsorbent layer with mixed mobile phase containing DMSO. The retention behavior of amino acids on silica gel-G, titanium (iv) tungstate, talc, starch and alumina and their admixture was also studied. The advantage of this work lies in the separation of some of the amino acids present in the various drug samples such as Santevini (Plus), Astymin (Liquid) contain L-Lysine HCl and Hepacor contain L-Ornithine.

Inorganic ion exchanger materials have been used earlier in TLC of amino acids [9]. The selectivity and dual phenomenon of adsorption and ion exchange in addition to the effect of the mobile phase led to some useful separations of these acids. Titanium (iv) tungstate possess high thermal and chemical stability and reasonably higher exchange capacity among the zirconium phosphate type
ion exchanger. So to explore the separation potential of basic amino acids, it is used in admixture form with conventional supports.

Talc and other unconventional adsorbents has been used for TLC separation of amino acids and organic compounds [10-12]. Because of the utility of these inexpensive materials it has been decided to use with other conventional and unconventional adsorbents.

Although, the advantage of using dimethyl sulfoxide (DMSO) as mobile phase in paper and thin layer chromatographic separation of amino acids [9,12]. A literature survey reveals that DMSO has rarely been used as mobile phase for the separation of amino acids [4,9,12]. To explore the separating potential of this solvent, We decided to use a mixed mobile phase containing DMSO for TLC separation of basic amino acids.

2. EXPERIMENTAL

Amino acids from LOBA (India) and Silica gel-G (E. Merck, India), talc, Starch (Qualigens, Fine Chemicals) and other chemicals were of analytical reagent grade DMSO was used as a mobile phase.

Preparation of test solution and detector standard solution of individual amino acids and mixtures were prepared having concentration of 500ng/µl of each compound in water. 0.2% ninhydrin in acetone was used as detector.

The basic amino acids used were B₁-Ornithine (Orn), B₂-Lysine HCl, B₃ – Histidine (His) and B₄ – Arginine (Arg). The synthesis of ion exchanger, admixture of adsorbents, preparation of chromatoplates and procedure are reported earlier (9).

3. RESULTS AND DISCUSSION

The Planar chromatography of basic amino acids gave the interesting results on various adsorbent layers and its admixture. The admixture of, silica gel-G with talc, alumina and titanium (iv) tungstate layer were found to be excellent supports with numerous possibilities for amino acids separations in DMSO-1MHCl media. On the basis of Rf data, several binary and ternary separations of basic amino acids were observed (Table-1).

The Plots of Rf versus basic amino acids (with increasing molar mass) for different adsorbent layers reveal that on silica gel-G, all the basic amino acid shows lower Rf. This is probably due to the presence of extra –NH₂ group with its lone pair of electron in these amino acids. For all basic amino acids Rf is probably due to the lower solubility of these acids in DMSO-1MHCl mobile phase.

The all basic amino acids chromatographed on titanium (iv) tungstate layer (Fig.1)
Retention Behavior of Basic Amino acids on Various Adsorbents layers in DMSO-1MHC1 Mobile Phase

The amino acids Lys and His show appreciable migration due to lower adsorption. However Orn and Arg are strongly adsorbed on these layers causing lower $R_f$. The lower $R_f$ of Arg is probably due to its high molar mass and also the presence of guanidine group in it. Arg has the complex structure and possess the higher value of $pI$ ($pI = 10.8$), so it is strongly adsorbed on this layer. However, Lys show an appreciable migration on titanium (iv) tungstate layer. The unexpected behavior of Lys may be due to its less adsorption on that layers. It is evident from Fig-1 that on talc layer, all the amino acids show higher $R_f$. The higher RF values of all the basic amino acids might be because talc is non-polar and thus unable to retain the polar amino acids, resulting in high $R_f$.

On the Silica gel-G – Talc (1:1w/w) admixture layer the $R_f$ values are high, because of very low adsorption (Fig.2). So there is no significant effect on adding of talc on silica gel-G layers. The admixture layer of titanium (iv) tungstate and silica gel-G (1:1) show a variation in retention behavior of basic amino acids. The variation in the $R_f$ values of basic amino acids on this layer is due to the combined effect of ion exchange behavior of titanium tungstate and adsorption. It is interesting to note that the adding of ion exchanger in silica gel-G show a very significant behavior of basic amino acids. It is observed that the $R_f$ values of His are higher than those of other basic amino acids. It may probably be due to the presence of imidazole ring in the structure of His, which is not interact with silica gel-G –titanium(iv) tungstate admixture layer and consequently show higher $R_f$.

On talc-alumina layer, all the basic amino acids are strongly adsorbed and show lower $R_f$. On unconventional admixture with conventional adsorbent has no any significant effect on the retention behavior of amino acids.

<table>
<thead>
<tr>
<th>Adsorbents</th>
<th>Amino acids</th>
<th>Separated From</th>
</tr>
</thead>
<tbody>
<tr>
<td>Titanium(iv) tungstate</td>
<td>Orn (0.15)</td>
<td>Lys (0.60), His (0.35), Arg (0.10)</td>
</tr>
<tr>
<td></td>
<td>Arg (0.10)</td>
<td>Lys (0.60), His (0.35)</td>
</tr>
<tr>
<td>Silica gel-G -Titanium(iv) tungstate (1:1)</td>
<td>Arg (0.05)</td>
<td>Lys (0.42), Orn (0.15), His (0.80)</td>
</tr>
<tr>
<td>Silica gel-G -Talc (1:1)</td>
<td>Orn (0.10)</td>
<td>Lys (0.60), His (0.35)</td>
</tr>
<tr>
<td></td>
<td>His (0.35)</td>
<td>Lys (0.60)</td>
</tr>
</tbody>
</table>

Table 1: Important Separation achieved

The amino acids Lys and His show appreciable migration due to lower adsorption. However Orn and Arg are strongly adsorbed on these layers causing lower $R_f$. The lower $R_f$ of Arg is probably due to its high molar mass and also the presence of guanidine group in it. Arg has the complex structure and possess the higher value of $pI$ ($pI = 10.8$), so it is strongly adsorbed on this layer. However, Lys show an appreciable migration on titanium (iv) tungstate layer. The unexpected behavior of Lys may be due to its less adsorption on that layers. It is evident from Fig-1 that on talc layer, all the amino acids show higher $R_f$. The higher RF values of all the basic amino acids might be because talc is non-polar and thus unable to retain the polar amino acids, resulting in high $R_f$.

On the Silica gel-G – Talc (1:1w/w) admixture layer the $R_f$ values are high, because of very low adsorption (Fig.2). So there is no significant effect on adding of talc on silica gel-G layers. The admixture layer of titanium (iv) tungstate and silica gel-G (1:1) show a variation in retention behavior of basic amino acids. The variation in the $R_f$ values of basic amino acids on this layer is due to the combined effect of ion exchange behavior of titanium tungstate and adsorption. It is interesting to note that the adding of ion exchanger in silica gel-G show a very significant behavior of basic amino acids. It is observed that the $R_f$ values of His are higher than those of other basic amino acids. It may probably be due to the presence of imidazole ring in the structure of His, which is not interact with silica gel-G –titanium(iv) tungstate admixture layer and consequently show higher $R_f$.

On talc-alumina layer, all the basic amino acids are strongly adsorbed and show lower $R_f$. On unconventional admixture with conventional adsorbent has no any significant effect on the retention behavior of amino acids.
Plots of $R_p$ Vs amino acids Con various adsorbent layer in DMSO-1MHCl media
Retention Behavior of Basic Amino acids on Various Adsorbents layers in DMSO-1M HC1 Mobile Phase

Plots of $R_F$ Vs Amino acids on Various admixture layers in DMSO-1M HC1 media
4. CONCLUSION

From the above discussion it is clear that the basic amino acids are separated from each other on various adsorbents layer and its admixture. On the above discussion it is clear that titanium tungstate has a significant role for the separation of basic amino acids. On the other hand, the admixture of silica gel –G and titanium (iv) tungstate Gave the better results than other admixture layers.

REFERENCES