An Investigation on Liquid Chromatographic Technique for Simultaneous Measurement of Alprazolam and Mebeverine in Pharmaceutical Dose Form

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Abstract – A delicate, quick, exact and reproducible Isocratic, RP-LC strategy was created for assurance of Alprazolam and Mebeverine in pharmaceutical measurement frame. A Sunfire C18, 5 pm segment with portable stage containing methanol: cradle (0.02M KH2P04) (70:30). The stream rate was 1.0 ml/min and effluents were observed at 225 nm with help of PDA identifier. The maintenance time was 6.04 min. for ALP and 3.53 min. for MEB. The linearity was in the scope of 0.05-40 gg/ml for ALP and 0.2-40 pg/ml for MEB. The proposed strategy was approved regarding linearity, exactness, accuracy, specificity and strength. Because of straightforwardness, rate and exactness of technique, trust that the strategy will be helpful for routine quality control examination.

Keywords: Alprazolam; Mebeverine; RP-HPLC; Validation

INTRODUCTION

Alprazolam [ALP] is a white powder and undefined in nature. Synthetically it is 8-chloro-l-methyl-6-phenyl-4H-[1, (Sharma & Sharma, 2007), (Sharma & Sharma, 2007)] triazolo [4,3-a]-l, (Sharma & Sharma, 2007)] benzodiazepine[l] (Figure 1A). It is tie nonspecifically to benzodiazepine receptors BNZ-1, which intervenes rest, and BNZ-2, which influences muscle unwinding, anticonvulsant movement, engine coordination, and memory (Sharma & Sharma, 2007). Mebeverine (MEB) is white powder. Synthetically, it is 3, 4-Dimethoxybenzoic corrosive 4-[ethyl][2-(4-methoxyphenyl)- l-methylthethyl] amino]-butylester and both the medications are exceptionally dissolvable in water and liquor [3] (Figure IB). Mebeverine follow up on the gut muscles at cell level to unwind them. This mitigates excruciating muscles fits of gut without influencing its ordinary motility. It is additionally an inhibitor of calcium terminal renewal (Sharma & Sharma, 2007). Along these lines it has double activity which standardizes the little motility. The blend of Alprazolam with Mebeverine was helpful and safe in fractious gut disorder.

Writing overview uncovered that alprazolam can be assessed by spectrophotometry (Indian pharmacopeia, 2007) and by fluid chromatographic strategies (Hanysova, et. al., 2005, Perez-Lozano, et. al., 2004, Sharma & Sharma, 2011, Chaudhary, et. al., 2012, Wagh, et. al., 2014, Indian pharmacopeia, 2007) separately or in blend with different medications, and mebeverine can be evaluated by fluid chromatographic techniques independently or in mix with different medications (Radwan, et. al., 2006, Arayne, et. al., 2005, Kothapelly, et. al., 2006, Reddy, et. al., 2014, Makwana & Patel, 2013). The detailed techniques are profoundly modern, expensive, and tedious and require unique example preparation. In contrast with LC and different techniques, HPLC strategy is thought to be a decent option, and it ought to be generally investigated as a vital instrument in routine medication examination. UV Spectroscopy technique has been accounted for synchronous estimation of both alprazolam and mebeverine in consolidated dose shape (ICH-Rules, 2005).

As far as anyone is concerned, no article identified with the HPLC assurance of ALP and MEB in pharmaceutical dose frames has showed up in the writing. Show consider includes advancement of an elite thin layer fluid chromatographic strategy for the assurance of ALP and MEB in blend measurements frame. A noteworthy favorable position of HPLC is its capacity to dissect a few examples all the while utilizing a little amount of portable stage. This lessens the time and cost of examination, limits presentation dangers, and altogether decreases transfer issues of lethal natural solvents, in this way.
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diminishing the potential outcomes of condition contamination.

The point of the present work was to build up an exact, repeatable, and particular HPLC strategy for the assurance of ALP and MEB both as a mass medication and in formulation. The proposed strategy was approved by ICH rules [18] and its refreshed universal tradition.

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Figure 1: (A) Chemical Structure of Alprazolam (B) Chemical Structure of Mebeverine.

MATERIALS AND TECHNIQUES

The fluid chromatographic framework comprise of Waters arrangement 2998 (Shelton, USA) outfitted with a PDA locator, arrangement 515 quaternary isocratic pump and manual injector rheodyne valve with 20 pL settled circle. The analytes were observed at 225 nm. Chromatographic investigation was performed on Sunfire C18 section having 250 mmx 4.6 mm i.d. furthermore, 5 pm molecule measure. Every one of the medications and synthetic substances were weighed on Mettler Toledo electronic adjust.

Dagnostically unadulterated ALP and MEB were acquired as blessing tests from Sun Pharmaceutical Pvt. Ltd., Baroda, India. Methanol (HPLC Review) SRL Private Ltd. Mumbai, India and Potassium Dihydrogen Ortho Phosphate Cradle - S. D. Fine Pvt. Ltd., Mumbai, India was acquired.

Chromatographic conditions

The Sunfire C18 section equilibrated with portable stage methanol: cradle (0.02M Potassium Dihydrogen Ortho Phosphate Support) (70:30) was utilized. The stream rate was kept up at 1 mL/min, eluent were observed with PDA identifier at 225 nm, and the infusion volume was 20 pL. Add up to run time was kept 8 min.

Preparation of standard stock solution

Alprazolam and Mebeverine were measured (10 mg) and exchanged to 10 ml volumetric flagon containing few ml of methanol. Volume was made up to the check with methanol to yield an answer containing 1000 pg/ml of alprazolam and mebeverine. Aliquot from the stock arrangement were suitably weakened with portable stage to acquire working standard of 100 pg/ml High mountain and MEB.

Strategy Validation

The strategy was approved for exactness, accuracy, linearity, detection limit, quantitation farthest point and robustness.

Linearity

Fitting aliquots of High mountain and MEB working standard arrangement was taken in various 10 mL volumetric carafes and weakened up to the check with portable stage to acquire last convergences of 0.05, 0.5, 5, 10, 20, 40 pg/mL of ALP and 0.2, 0.5, 5, 10, 20, 40 pg/mL of MEB. The solution were infused utilizing a 20 pL settled circle framework and chromatograms were recorded. Alignment bends were built by plotting normal pinnacle territory versus focuses and relapse conditions were figured (n=5).

Accuracy

The repeatability ponder was completed by assessing reaction six times and results are accounted for as far as relative standard deviation. The intra-day and between day exactness think about (moderate accuracy) was completed by evaluating the reactions 3 times around the same time and on 3 distinctive days for three unique centralizations of 0.05, 5, 20 of ALP and 0.2, 5, 20 of MEB and the outcomes are accounted for as far as relative standard deviation.

Precision

The precision of the method was controlled by ascertaining recuperation of High mountain and MEB by technique for standard expansion. Known measure of ALP (0, 0.05, 0.1, 0.15 pg/mL), MEB (0, 5, 10, 15 pg/mL) were added to a pre evaluated test arrangement, and the measure of High mountain and MEB were assessed by estimating the pinnacle zones and by fitting these qualities to the straight-line condition of adjustment bend.

Detection limit point of confinement and quantitation constrain

The breaking point of discovery (LOD) is characterized as the most minimal grouping of an analyte that can dependably be separated from foundation levels. Farthest point of measurement (LOQ) of an individual logical system is the most minimal measure of analyte that can be quantitatively decided with appropriate exactness and precision. LOD and LOQ were figured utilizing following condition according to ICH rules.

LOD = 3.3 xo/S; LOQ = 10 xo/S;
Where $o$ is the standard deviation of y-captures of relapse lines and S is the slant of the adjustment bend.

**Specificity**

The specificity was assessed by spiking regularly utilized excipients (starch, powder and magnesium stearate) into a pre measured amount of medication. The chromatogram is taken by proper weakenings. Created spot territory and maintenance time of ALP and MEB were resolved and impact of meddling compound was explored.

**Robustness**

Robustness of the strategy was examined by purposely changing the exploratory conditions like stream rate and level of natural stage.

**Arrangement security**

**Security of test solution were learned at 25 ± 2°C for 24 h. Framework reasonableness**

A framework reasonableness test was a vital piece of the strategy improvement to confirm that the framework is satisfactory for the examination of ALP and MEB to be performed. Framework reasonableness trial of the chromatography framework was performed before Validation run. Six reproduce infusions of a framework reasonableness standard and one infusion of a check standard were made. Region, maintenance time (Rt), limit factor, section productivity, symmetric factor, and flag to commotion proportion for the five appropriateness infusions were resolved.

**Investigation of showcased formulation**

Twenty tablets were weighed precisely and finely powdered. Tablet powder proportional to 0.25 mg ALP and 135 mg of MEB were taken in 10 ml volumetric jar. Methanol (5 ml) was added to the above carafe and the jar was sonicated for 10 minutes. The arrangement was sifted utilizing Whatman channel paper No.41 and volume was made up to the stamp with the versatile stage.

Proper volume of the aliquot was exchanged to a 10 ml volumetric jar and the volume was made up to the stamp with the versatile stage to acquire an answer containing 0.1 pg/ml of High mountain and 54 pg/ml of MEB. The arrangement was sonicated for 10 min. It was infused according to the above chromatographic conditions and pinnacle region were recorded. The evaluation was done by keeping this incentive to the straight line condition of adjustment bend.

**RESULTS AND DISCUSSION**

**Enhancement of portable stage**

The target of the strategy advancement was to accomplish chromatographic crests for dynamic medication fixings with less hilter kilter factor. The portable stage methanol: buffer(0.02M Potassium Dihydrogen Ortho Phosphate Support) (70:30 %v/v) was observed to be palatable which gave symmetric pinnacle. Overlaid UV spectra of the medications demonstrated that ALP and MEB retained apparently at 225 nm (Figure 2), so discovery was completed at 225 nm. The maintenance time for ALP was 3.53 min. Furthermore, MEB was 6.04 min (Figure 3). The hilter kilter factor for High mountain and MEB was 0.45 and 0.57, separately. The portable stage stream rate was kept up at 1 ml/min. Overlain chromatograms of ALP and MEB were appeared in Figure 4.

![Figure 3: Chromatogram of Standard ALP (10 pg/ml) and MEB (10 pg/ml).](image-url)

**Figure 3: Chromatogram of Standard ALP (10 pg/ml) and MEB (10 pg/ml).**

The adjustment bend for High mountain and MEB was observed to be straight in the scope of 0.05-40 pg/ml and 0.2-40 pg/ml with a connection coefficient of 0.995 and 0.999. The synopsis of Validation and framework appropriateness parameters were appeared in Table 1.

The intra-day and between day exactness thinks about were done. The low RSD esteem demonstrate that the strategy is exact. The exactness of the strategy was dictated by figuring recuperation of ALP and MEB by technique for standard expansion. The rate recuperation was observed to be 98.29-101.39 for High mountain and 99.42 - 100.55 for MEB, separately. This qualities show that the strategy is exact (Table 2).

As far as possible for ALP and MEB was observed to be 0.080 pg/mL and 0.016 pg/mL, separately, while quantitation constrain was observed to be 0.243 pg/mL and 0.050 pg/mL, individually The above information demonstrates that a nano gram amount of the medication can be precisely and absolutely decided. Robustness examine was performed by purposely changing the trial conditions like stream
rate from 1 ml/min to 0.8 ml/min and 1.2 ml/min. The synthesis of versatile stage was changed fluctuating the extent of methanol by 2%. In both the conditions the recuperation of the medication was resolved and the RSD was observed to be under 2% (Table 3). Dependability of standard and test arrangement of ALP and MEB were assessed at room temperature for 24 hr. The medication was observed to be steady with a measure of medication discovered over 98%.

The proposed strategy was effectively connected to the assurance of ALP and MEB in its measurements shape. The % recuperation was observed to be 100.51 10.58 and 100.55 ± 1.08, individually which was equivalent with the marked sum (n=3) (Table 4). The chromatogram of showcased definition was appeared in Figure 5.

![Figure 4: Overlay Chromatogram of ALP (0.05-40 ug/ml) and MEB (0.2-40 ug/ml).](image)

**Table 1: Summary of Validation and System Suitability Parameters**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>ALP</th>
<th>MEB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity (pg/ml)</td>
<td>0.05-40</td>
<td>0.2-40</td>
</tr>
<tr>
<td>Retention Time (min)</td>
<td>6</td>
<td>3.5</td>
</tr>
<tr>
<td>Detection limit (pg/ml)</td>
<td>0.08</td>
<td>0.01</td>
</tr>
<tr>
<td>Quantitation limit (pg/ml)</td>
<td>0.24</td>
<td>0.05</td>
</tr>
<tr>
<td>Accuracy (%)</td>
<td>99.42-100.55</td>
<td>98.29-101.39</td>
</tr>
<tr>
<td>Precision (%RSD)</td>
<td>Intra-day (n=3)</td>
<td>0.70-1.31</td>
</tr>
<tr>
<td>Inter-day (n=3)</td>
<td>0.82-1.81</td>
<td>1.14-1.82</td>
</tr>
<tr>
<td>Specificity</td>
<td>Specific</td>
<td>Specific</td>
</tr>
<tr>
<td>Robustness</td>
<td>Robust</td>
<td>Robust</td>
</tr>
<tr>
<td>Solvent suitability</td>
<td>Suitable for 24 hrs.</td>
<td>Suitable for 24 hrs.</td>
</tr>
<tr>
<td>Retention time (min)</td>
<td>6.04</td>
<td>3.53</td>
</tr>
<tr>
<td>Theoretical Plates</td>
<td>5053</td>
<td>6903</td>
</tr>
<tr>
<td>Asymmetric factor</td>
<td>0.45</td>
<td>0.57</td>
</tr>
<tr>
<td>Resolution</td>
<td>2.57</td>
<td></td>
</tr>
</tbody>
</table>

**Table 2: Accuracy Study of Alp and Meb by Proposed Rp-Lc Method.**

<table>
<thead>
<tr>
<th>Level</th>
<th>Amount Added (pg/ml)</th>
<th>Amount Recovered (pg/ml) (n=3)</th>
<th>% Recovered ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEB</td>
<td>ALP</td>
<td>MEB</td>
<td>ALP</td>
</tr>
<tr>
<td>0</td>
<td>10 + 0</td>
<td>0.1+0</td>
<td>0.1</td>
</tr>
<tr>
<td>50</td>
<td>10+5</td>
<td>0.1+0.01</td>
<td>0.14</td>
</tr>
<tr>
<td>100</td>
<td>10+10</td>
<td>0.1+0.1</td>
<td>0.20</td>
</tr>
<tr>
<td>150</td>
<td>10+15</td>
<td>0.1+0.15</td>
<td>0.24</td>
</tr>
</tbody>
</table>

**Table 3: Robustness Data for Alp and Meb by Proposed Rp-Lc Method.**

<table>
<thead>
<tr>
<th>Method Parameter</th>
<th>Normal Condition</th>
<th>Deliberate Change</th>
<th>%RSD of peak area (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow rate</td>
<td>1.0 ml/min</td>
<td>0.8 ml/min</td>
<td>0.49</td>
</tr>
<tr>
<td></td>
<td>1.2 ml/min</td>
<td>0.60</td>
<td>1.06</td>
</tr>
<tr>
<td>Mobile phase ratio</td>
<td>Methanol: Buffer (70:30)</td>
<td>72.28</td>
<td>1.01</td>
</tr>
<tr>
<td></td>
<td>68.32</td>
<td>1.12</td>
<td>1.81</td>
</tr>
<tr>
<td>pH of mobile phase ratio</td>
<td>5.0</td>
<td>5.2</td>
<td>0.58</td>
</tr>
<tr>
<td></td>
<td>4.8</td>
<td>0.69</td>
<td>1.02</td>
</tr>
</tbody>
</table>

**Table 4: Analysis of Marketed Formulation.**

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Amount drug taken (mg/mL)</th>
<th>Amount of drug found (mg/mL)</th>
<th>% Drug found Mean ± SD (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALP</td>
<td>MEB</td>
<td>ALP</td>
<td>MEB</td>
</tr>
<tr>
<td>0.1</td>
<td>10</td>
<td>0.105</td>
<td>10.05</td>
</tr>
</tbody>
</table>

![Figure 5: Chromatogram of Marketed Formulation ALP (0.1ug/ml) and MEB (10ug/ml).](image)

**CONCLUSION**

Proposed think about portrays RP-LC strategy for the concurrent measurement of ALP and MEB in its definition. The technique was approved and observed to be straightforward, delicate, exact and exact. Measurable investigation demonstrated that...
technique was repeatable and specific for the concurrent examination of ALP and MEB with no obstruction from the excipients. The method was effectively utilized for assurance of medication in their plan.

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