Stability Assessment of Phenoxybenzamine in Solution and in Matrix, and Its Quantitation in Human Plasma
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Purpose
Phenoxybenzamine is highly unstable in its free base form. Phenoxybenzamine is not stable in solution if prepared in the wrong solvent, shows light and oxygen sensitivity, and exhibits limited stability in matrix. Stabilization is mandatory at every step of the method and it makes developing an assay to quantitate such molecule particularly challenging. Post-extraction derivatization was found to be effective in converting unstable Phenoxybenzamine to a stable derivative.

Methods
Extraction on solid phase cartridges (Waters OASIS MCX) under acidic conditions followed by derivatization of the eluate yielded a stable derivative which could be easily chromatographed and quantitated. Reversed phase chromatography in isocratic mode was performed on an ACE 3 C18 30 x 4.6mm analytical column, and detection was performed on a SCIEX API 4000 LC-MS/MS with a turbo ion spray source operated in positive mode. Transitions monitored were 341.5 → 100.2 and 348.5 → 100.2 for Phenoxybenzamine derivative and Phenoxybenzamine-d7 derivative respectively. The method was validated over a range of 25 to 25000 pg/mL.

Results
Phenoxybenzamine stability in solution was first assessed. It was found that acidic conditions are crucial in order to maintain stability, since severe degradation occurred in half of the tested solvents. 0.1N HCL was found to be the most effective solvent, and stock, intermediate, working and reference solutions were prepared in 0.1N HCl.

Stability in matrix was also assessed. Degradation as high as 25% was observed in plasma within 5 hours at room temperature. Stability could be obtained (degradation less than 5%) within this time frame when cooling plasma down to 4°C, but increased degradation (12-13%) was observed when stability time was extended up to 20h. Plasma stabilization was needed, and acidification with o-phosphoric acid 85% was chosen to be the most effective. Stability in acidified plasma at room temperature showed variation of 6-7% over 5h and up to 25% over 20h. When cooled to 4°C, stability showed variation of 1% over 5h and less than 5% over 20h.

Extraction was performed on a MCX solid phase cartridge. Elution was performed using a solution of 5% diethylamine in methanol. The eluate was then incubated at 50°C for 60 minutes to complete the phenoxybenzamine’s chlorine atom displacement by the diethylamino group. Derivatization converted the unstable phenoxbezamine to a stable derivative. Evaporation to dryness and reconstitution with mobile phase yielded samples ready for injection.

Recovery was higher than 77% for the analyte and the internal standard. Precision and accuracy was below 5.8% of bias and 7.1% of coefficient of variation. The matrix effect was evaluated and found to have no impact on quantitation, including hemolyzed and lipemic plasmas. Freeze and thaw, short-term and long-term stability in matrix were evaluated and met acceptance criteria. The method was successfully validated over a range of 25 to 25000 pg/mL.

Conclusion
Through careful investigations, stability of Phenoxybenzamine in solution and in matrix could be assessed and controlled. Stabilization conditions were developed and quantitation of a stable derivative in human plasma was possible. The method was applied to a clinical study and has demonstrated excellent reproducibility.