Altasciences, 575 Armand-Frappier, Laval, Québec, H7V 4B3, Canada
Altasciences.com | contact@altasciences.com

Overcoming Recovery Challenges in Hemolyzed Samples for the Determination of Propafenone and 5-Hydroxy Propafenone by LC-MS/MS

Vinicio Vasquez, Milton Furtado, Mingluan Chen and Anahita Keyhani
Altasciences, Laval, Québec, Canada

OVERVIEW

PURPOSE

Formulation of optimal protein precipitation conditions required to circumvent the loss of 5-Hydroxy Propafenone in hemolyzed plasma.

METHOD

Different solvents were investigated to increase the recovery of 5-Hydroxy Propafenone.

RESULTS

Acidified methanol and acetonitrile as precipitation solvents showed a significant improvement in the extraction recovery of 5-Hydroxy Propafenone in hemolyzed plasma.

INTRODUCTION

Propafenone and its primary active metabolite 5-Hydroxy Propafenone (Figure 1) are antiarrhythmic drugs used in patients with atrial and ventricular fibrillation.

The development of an LC-MS/MS assay for 5-Hydroxy Propafenone was challenged by poor recovery in hemolyzed plasma when using a methanolic protein precipitation extraction, whilst non-hemolyzed plasma samples remained unaffected. Judicious selection of an appropriate precipitating solvent and solvent:plasma ratio demonstrated significant recovery improvement of 5-Hydroxy Propafenone in hemolyzed plasma, yielding results comparable to non-hemolyzed plasma.

METHOD

SAMPLE PREPARATION

Propafenone and 5-OH-PON were fortified in non-hemolyzed and hemolyzed human plasma (K_2EDTA) and extracted by protein precipitation with organic solvent(s) following the addition of deuterated internal standards. The supernatant was diluted before injecting.

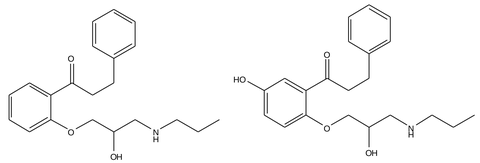
CHROMATOGRAPHY

Chromatographic separation was achieved using a C18 column with mobile phase composed of heptafluorobutyric acid (*aq*) and acetonitrile:methanol.

DETECTION

Sciex API5000 Triple Quadrupole, MRM acquisition in positive ion ESI :

- *m/z* 342 > m/z 116 (Propafenone)
- *m/z* 358 > m/z 116 (5-Hydroxy Propafenone)



PROPAFENONE
Chemical Formula: $C_{21}H_{27}NO_3$
Exact Mass: 341.20
Molecular Weight: 341.44

5-HYDROXYPROPAFENONE
Chemical Formula: $C_{21}H_{27}NO_4$
Exact Mass: 357.19
Molecular Weight: 357.44

Figure 1. Chemical Structure of Propafenone and 5-Hydroxy Propafenone

RESULTS

Propafenone and 5-Hydroxy Propafenone were initially extracted by protein precipitation from both non-hemolyzed and hemolyzed plasma with a MeOH:plasma ratio of 2:1. The recovery efficiency was ca. 90% for each analyte and corresponding deuterated internal standard. The method was subsequently modified to increase the ULQ, thereby allowing an increased MeOH:plasma ratio of 8:1. This ratio resulted in a relative recovery for 5-Hydroxy Propafenone and its corresponding deuterated internal standard of only 9% in hemolyzed samples (Table 1) in comparison to non-hemolyzed plasma; while an extraction recovery of 90% for Propafenone was maintained.

Table 1. Relative Recovery 5-Hydroxy Propafenone in hemolyzed samples using MeOH for Protein Precipitation with a proportion 8:1 solvent:plasma.

Plasma precipitation with MeOH	Low QC (6.00 ng/mL)		High QC (750.00 ng/mL)	
	Peak Area Drug	Ratio	Peak Area Drug	Ratio
Mean	64463.1506	0.1357	7526728.0272	15.7252
S.D.	915.0283	0.0014	342841.8083	0.4111
n	6	6	6	6
% C.V.	1.4	1.0	4.6	2.6
5% Hemolyzed Plasma precipitation with MeOH (Lots #1-4)	**Low QC (6.00 ng/mL)**		**High QC (750.00 ng/mL)**	
	Peak Area Drug	**Ratio**	**Peak Area Drug**	**Ratio**
Mean	5563.0923	0.1305	690397.8916	15.4251
S.D.	532.9457	0.0067	101921.6234	0.7125
n	12	12	12	12
% C.V.	9.6	5.1	14.8	4.6
Relative Recovery to Plasma (%)	**8.6**	**96.2**	**9.2**	**98.1**

RESULTS (CONTINUED)

A post-column infusion of hemolyzed blank plasma (Figures 2 and 3) ruled out the possibility of ionization suppression, suggesting a potential degradation, transformation or blood component binding promulgated by the increased quantity of methanol.

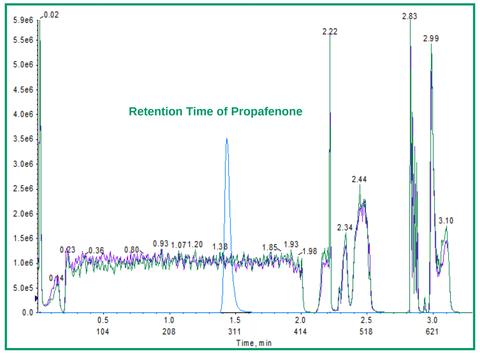


Figure 2. Representative chromatograms of Propafenone blank plasma post-column infusion with MeOH precipitation (green) and with ACN precipitation (purple).

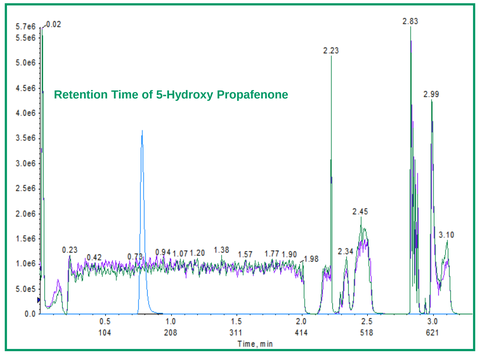


Figure 3. Representative chromatograms of 5-Hydroxy Propafenone blank plasma post-column infusion with MeOH precipitation (green) and with ACN precipitation (purple) .

RESULTS (CONTINUED)

Table 2. Relative Recovery 5-Hydroxy Propafenone in hemolyzed samples using ACN for Protein Precipitation with a proportion 8:1 solvent:plasma.

5% Hemolyzed Plasma Precipitation with ACN (Lots #1-4)	Low QC (6.00 ng/mL)		High QC (750.00 ng/mL)	
	Peak Area Drug	Ratio	Peak Area Drug	Ratio
Mean	69228.3635	0.1351	8032342.7442	15.8526
S.D.	2030.9720	0.0042	215613.3501	0.4895
n	12	12	12	12
% C.V.	2.9	3.1	2.7	3.1
Relative Recovery to Plasma (%)	**107.4**	**99.5**	**106.7**	**100.8**

Table 3. Relative Recovery 5-Hydroxy Propafenone in hemolyzed samples using MeOH and 0.2% HCOOH in MeOH for Protein Precipitation with a proportion 8:1 (solvent:plasma).

5% Hemolyzed Plasma precipitation with MeOH (Lots #5-6)	Low QC (6.00 ng/mL)		High QC (750.00 ng/mL)	
	Peak Area Drug	Ratio	Peak Area Drug	Ratio
Mean	4100.8844	0.1245	548259.3365	14.7416
S.D.	435.9226	0.0042	28995.7459	0.4298
n	6	6	6	6
% C.V.	10.6	3.4	5.3	2.9
Relative Recovery (%)	**13.6**	**92.9**	**16.2**	**94.7**
5% Hemolyzed Plasma precipitation with 0.2% HCOOH in MeOH (Lots #1-2)	**Low QC (6.00 ng/mL)**		**High QC (750.00 ng/mL)**	
	Peak Area Drug	**Ratio**	**Peak Area Drug**	**Ratio**
Mean	30455.8410	0.1372	3477347.8125	15.6145
S.D.	824.8024	0.0045	69578.4749	0.4843
n	5	5	6	6
% C.V.	2.7	3.2	2.0	3.1
Relative Recovery to Plasma (%)	**101.1**	**102.4**	**102.9**	**100.3**

RESULTS (CONTINUED)

Therefore, acetonitrile (Tables 2) and 0.2% formic acid in methanol were investigated (Tables 3) as alternative precipitating solvents, evaluated at an 8:1 solvent:plasma ratio. For each precipitating solvent, 100% relative recovery to plasma could be obtained for each analyte in hemolyzed plasma.

Further, given the success with acidified methanol as precipitating solvent, the supernatant of initial hemolyzed samples which were precipitated with methanol (Tables 3, Lots #5-6) were treated with formic acid (0.2% v/v final). The acidification of supernatant increased the relative recovery of 5-Hydroxy Propafenone from 15% to 99% (Table 4, Lots #5-6), suggesting an efficient release of analyte from a blood component rather than a degradation/transformation.

Table 4. Relative Recovery 5-Hydroxy Propafenone, hemolyzed samples acidified to 0.2% HCOOH post precipitation with MeOH. Protein Precipitation with a proportion 8:1 (solvent:plasma)

5% Hemolyzed Plasma, acidified to 0.2% HCOOH post precipitation with MeOH (Lots #5-6)	Low QC (6.00 ng/mL)		High QC (750.00 ng/mL)	
	Peak Area Drug	Ratio	Peak Area Drug	Ratio
Mean	29368.8396	0.1320	3337876.9375	15.3758
S.D.	1041.2573	0.0067	82284.0154	0.3991
n	6	6	6	6
% C.V.	3.5	5.1	2.5	2.6
Relative Recovery (%)	**97.5**	**98.5**	**98.8**	**98.7**

CONCLUSION

Acidified methanol and acetonitrile as precipitation solvents at a 8:1 ratio (solvent:plasma) showed a significant improvement in the extraction recovery of 5-Hydroxy Propafenone in hemolyzed plasma. Further tests would be necessary to determine the root cause for the low recovery using MeOH in high proportions to plasma and identify a possible chemical complex.

ACKNOWLEDGMENTS

The authors would like to thank Jeff Plomley for the technical editing of this poster's abstract.