Quantitative determination of glycopyrrolate in human plasma by liquid chromatography – electrospray ionization mass spectrometry

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Introduction

Glycopyrrolate (GLY), as a synthetic quaternary ammonium compound, has been used for decades as an antisialogogue, vagolytic and to reduce gastric volume [a]. Despite that the use of GLY has declined during the last twenty years, many anesthesiologists still routinely make use of GLY, particularly by painful and anxiety-provoking intramuscular injection [b]. The work presented here deals with the development of a quantitative LC-MS/MS tool for the determination of the quaternary ammonium anticholinergic GLY in human plasma samples using volatile ion-pairing reagents. During the bioanalytical method validation, matrix effect is assessed according to Matuszewski et al. [c].

Aim

Our aim is to achieve a liquid chromatography separation with MS detection of a permanently charged compound and to validate the method with focus on matrix effect. The assessment of matrix effect is a crucial step during the bioanalytical method validation. According to Matuszewski et al. [c], the degree of ion suppression for an analyte and an internal standard may be different in different lots of the same plasma. In that respect, matrix effect, absolute recovery and process efficiency were determined for our method using four different lots of plasma.

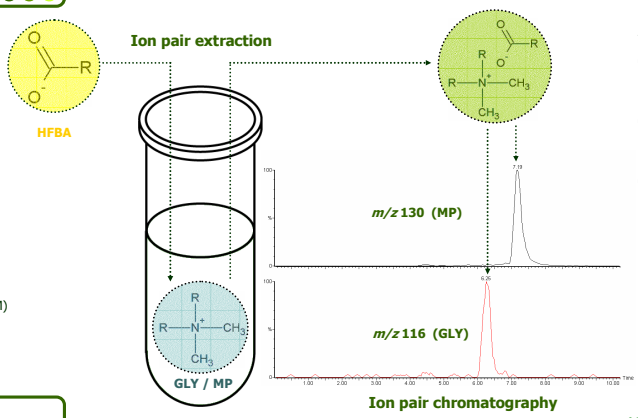
Materials

1. Extraction procedure:

- 1ml plasma + 1ml 0.2M ammonium formiate + 4ml CH_2Cl_2
- 10min rotary mixer; centrifugation (20min; 2254 x *g*; 25°C)
- 750µl supernatant removed
- + 1ml 0.1M HFBA + 4ml CH_2Cl_2
- 10min rotary mixer; centrifugation (20min; 2254 x *g*; 25°C)
- remove upper phase; lower CH_2Cl_2 phase evaporated to dryness
- redissolve in 200µl of eluent A
- mepenzolate (MP) was used as an internal standard

2. HPLC Conditions:

- Alliance 2790 (Waters®)
- Column:
  Atlantis™ dC18, 3µm, 150 x 2.1mm (Waters®)
- Mobile Phases:
  Eluent A: HFBA (15mM) – ammonium formiate buffer (20mM) (adjusted to pH 3.30 with formic acid)
  Eluent B: MeOH
- Flow Rate: 100µl/min; isocratic elution at 30% A and 70% B
- Injection Volume: 20µl



3. MS Conditions:

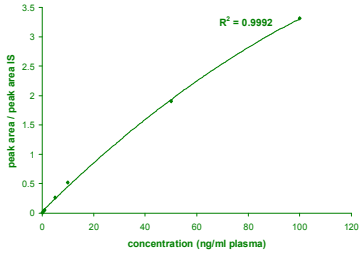
- Mass Spectrometer: Q-TOF (Waters®)
  Capillary Voltage: 3000V
  Cone Voltage: 35V
- MS/MS: collision energy 30 eV
  Glycopyrrolate *m/z* 318 -> *m/z* 116
  Mepenzolate (IS) *m/z* 340 -> *m/z* 130
- Software: Masslynx 4.0®, Quanlynx®

4. Quality control levels:

QC $level_1$: 0.253 ng/ml GLY
QC $level_2$: 2.525 ng/ml GLY
QC $level_3$: 25.25 ng/ml GLY

Results

- The quantification of GLY in plasma has been validated. A calibration curve has been set up covering the range from 0.1 to 100.9 ng/ml plasma (see figure). A quadratic calibration curve gave the best fit based on statistical regression analysis comparison, with a correlation coefficiënt of 0.9992.
- To complete this bioanalytical validation, also matrix issues have to be investigated. The procedures described by Matuszewski et al. [c] were adopted. Target compounds were spiked at QC levels into four different plasma samples and the matrix effects were evaluated using these samples. Intercomparison of the samples provides the relative matrix effect.



- Formulas used were:

$$\text{Absolute matrix effect} = \frac{\text{post}-\text{pure}}{\text{pure}} \cdot 100\%$$

$$\text{Absolute recovery} = \frac{\text{pre}}{\text{post}} \cdot 100\%$$

$$\text{Process efficiency} = \frac{\text{pre}}{\text{pure}} \cdot 100\%$$

	QC $level_1$	QC $level_2$	QC $level_3$
absolute matrix effect (n=3; %)	110.5 ± 20.13	144.0 ± 11.59	120.5 ± 6.842
relative matrix effect (n=4; %)	12.33 ± 5.472	12.19 ± 6.309	23.30 ± 4.144
absolute recovery (n=3; %)	83.54 ± 4.436	90.67 ± 11.11	84.15 ± 8.578
process efficiency (n=3; %)	90.34 ± 11.48	128.0 ± 9.113	98.42 ± 3.995

Conclusion

As can be seen in the table, ionization enhancement is present (matrix effect > 100%). As expected, relative matrix effect exceeds the precision of determination of QC levels in pure isocratic elution solvent (QC $level_1$ 6.44 ± 4.37%; QC $level_2$ 4.09 ± 2.16%; QC $level_3$ 16.88 ± 1.89%). Nevertheless, if we compare the RSD% of the drug-to-internal standard ratio for samples spiked postextraction to standards in pure isocratic elution solvent (10.29% versus 8.07%), it comfirms that the absolute and relative matrix effects for both compounds have practically no effect on quantification of GLY. As a result of this study, we conclude that this LC-MS/MS method is suitable for the absolute quantification of the drug glycopyrrolate in human plasma samples.

References

[a] C. A. Bernstein, J.H. Waters, M.C. Torjman, D. Ritter, *J. Clin. Anesth.* 1996, *8* : 515-518
[b] E. Kentala, M. Salonen, J. Kanto, *Acta Anaesthesiol. Scand.* 1990, *34* : 17-20
[c] B. K. Matuszewski, M. L. Constanzer, C. M. Chavez-Eng, *Anal. Chem.* 2003, *75* : 3019-3030