Simultaneous Analysis of Carbapenems in Human Bodily Liquids Using HFIP as Buffer Additive in LC-ESI-MS/MS

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Introduction

Carbapenems are β-lactam antibiotics that have a broad spectrum of activity. Carbapenems are stable against a variety of β-lactamases. Due to the carbapenems' antibacterial activity against a wide range of gram-positive and gram-negative bacteria, the use of these drugs is extensive. LC-MS can be used for quantitative analysis of carbapenems in bodily fluids.

Reversed phase (RP) separation of basic analytes is often more efficient under basic conditions. Eluent components for LC-MS analysis have to be volatile and must not suppress ionization. Novel solvent - 1,1,1,3,3,3-hexafluoroisopropanol (HFIP, pKa=9.3 [1, 2]) was used as a component for basic buffer for chromatographic separation of carbapenems' in human bodily fluids (blood plasma, urine and cerebrospinal fluid) by UHPLC-ESI-MS/MS to provide alternative selectivity comparing with fluorinated stationary phases.

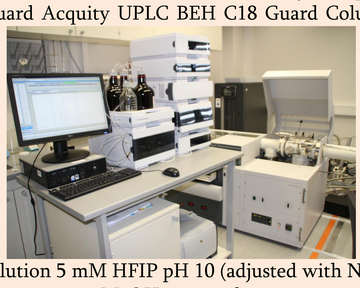
Materials and methods

Blood plasma sample preparation

500 µL of sample → Protein precipitation/ centrifugation/ evaporation to dryness under N_2 → Filtration/ injection

Chromatographic separation was performed using a Waters Acquity UPLC BEH C18 column (2.1 x 100 mm, 1.7 µm) equipped with Waters VanGuard Acquity UPLC BEH C18 Guard Column (2.1 x 5 mm, 1.7 µm).

Agilent 1290 Infinity UHPLC system



Varian 1320-MS Triple Quadrupole LC/MS

Gradient elution 5 mM HFIP pH 10 (adjusted with NH_4OH) with MeOH was used.

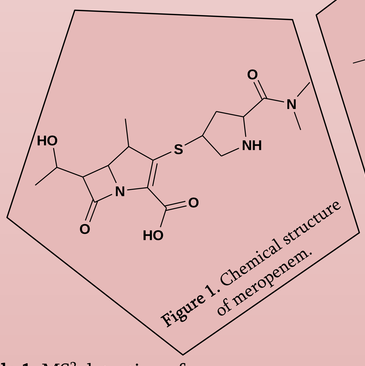


Figure 1. Chemical structure of meropenem.

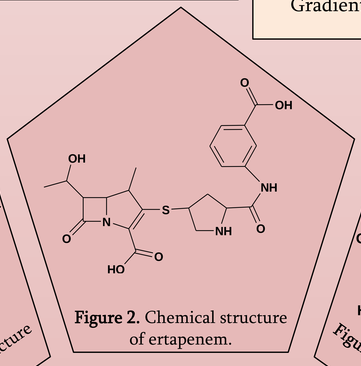


Figure 2. Chemical structure of ertapenem.

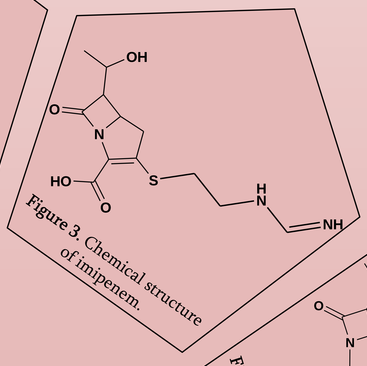


Figure 3. Chemical structure of imipenem.

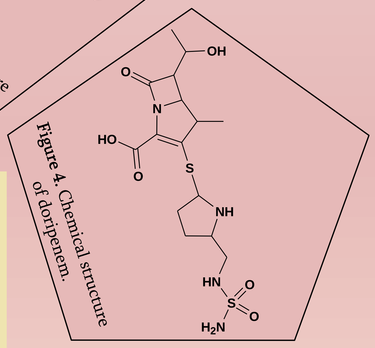


Figure 4. Chemical structure of doripenem.

Table 1. MS^2 detection of cabrapenems

Analyte	Parent ion $[M+H]^+$	Fragments, (m/z)
Imipenem	300	256
		103
Meropenem	384	340
		254
		200
		141
Doripenem	421	342
		318
		298
		274
Ertapenem	476	432
		346
Cilastatin[1]	359	342
		263
		219
		202

[1] Cilastatin is co-administered with imipenem.

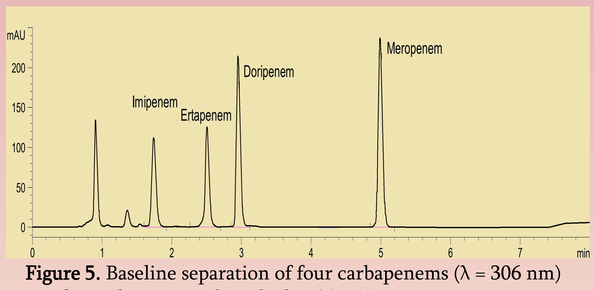


Figure 5. Baseline separation of four carbapenems (λ = 306 nm) from plasma sample spiked at 80 µg/mL concentration.

HFIP is mostly deprotonated (e.g. more hydrophilic) at pH 10. HFIP interacts strongly with protonated analytes at pH 10. Carbapenems' peak shapes are enhanced using HFIP buffer and retention of hydrophilic compounds is increased due to the change in buffer pH and strong interaction between HFIP and stationary phase surface.

Analytes' ionozation efficiency increased over 100 times using HFIP buffer. LOQ values (estimated from standard deviations from five replicate analysis) ranged from 0.05 to 0.1 µg/mL for all analytes.

In the side of ion-pairing ability with analytes, HFIP competes also over the stationary phase surface, composing a hydrophilic fluorous layer over the C18 statsionary phase. HFIP provides alternative selectivity in RP separation (comparing with C18, ion pairing agents or fluorinated stationary phases [4]).

Protein precipitation is quick and „dirty", but carbapenems tend to be unstable in sample solutions [3]. Matrix-matched calibration was employed with recoveries 100% (SD 8%). Change of eluent component to HFIP decreased matrix components' retention. Reduction of possible matrix effects was achieved.

Method was validated according to the European Medicines Agency guidelines. Calibration graphs were linear in concentration range 0.1-100 µg/mL with r^2>0.9998. The method within-day accuracy and precision values ranged from 100 ± 0.3% to 100 ± 9% and from 1.5% to 8.4%, respectively, for all analytes.

Conclusion

- ✓HFIP enhances the chromatographic separation from blood plasma peaks.
- ✓HFIP competes with deprotonated analyte over the stationary phase surface. The „fluorous phase" on stationary phase allows alternative retention of analytes' [1, 2, 4].
- ✓Using HFIP increases the retention of carbapenems and decreases the influence of matrix peaks on the chromatogram.
- ✓Alternative retention and higher analytes' peaks occured when HFIP was in use.

References

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Acknowledgments

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