

Supplementary materials

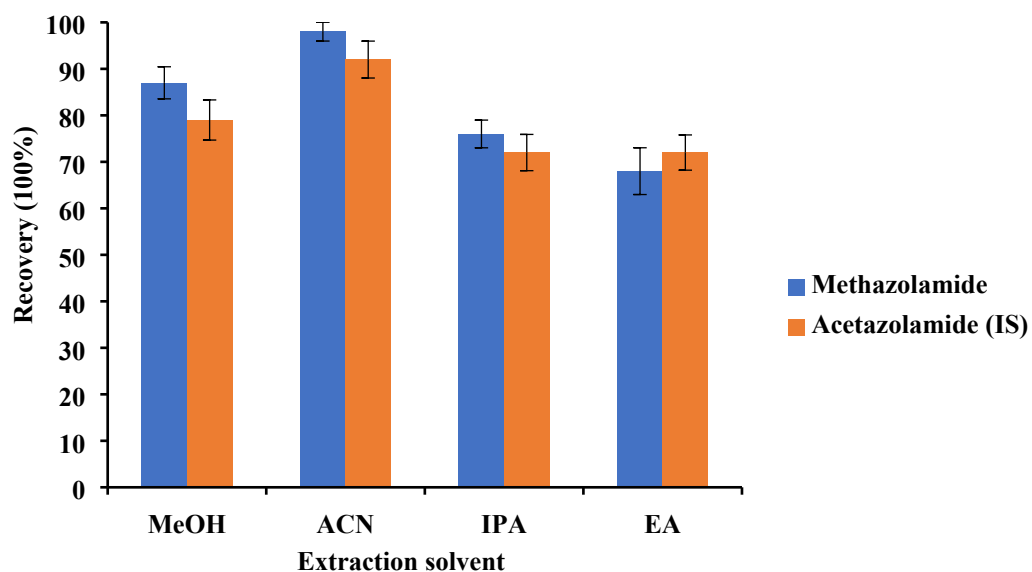


Figure S1 Optimization of extraction solvent

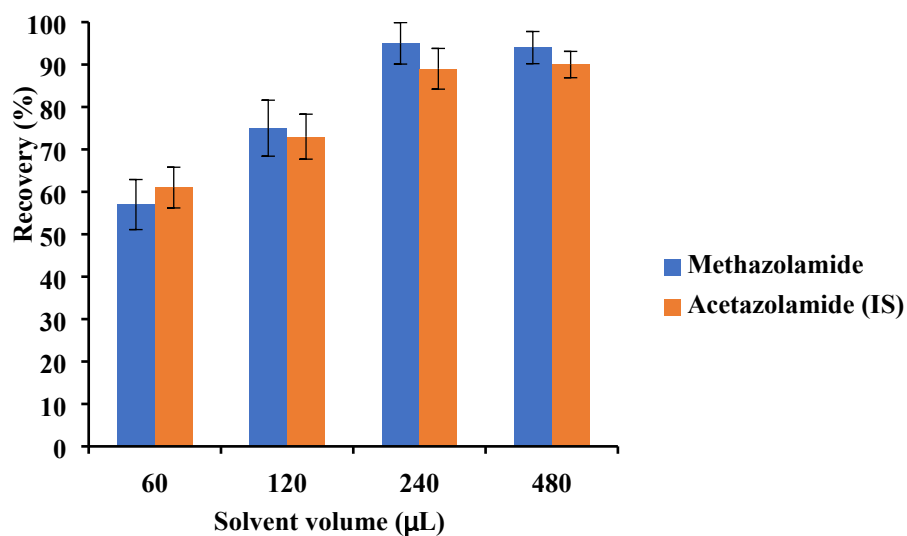


Figure S2 Optimization of solvent volume.

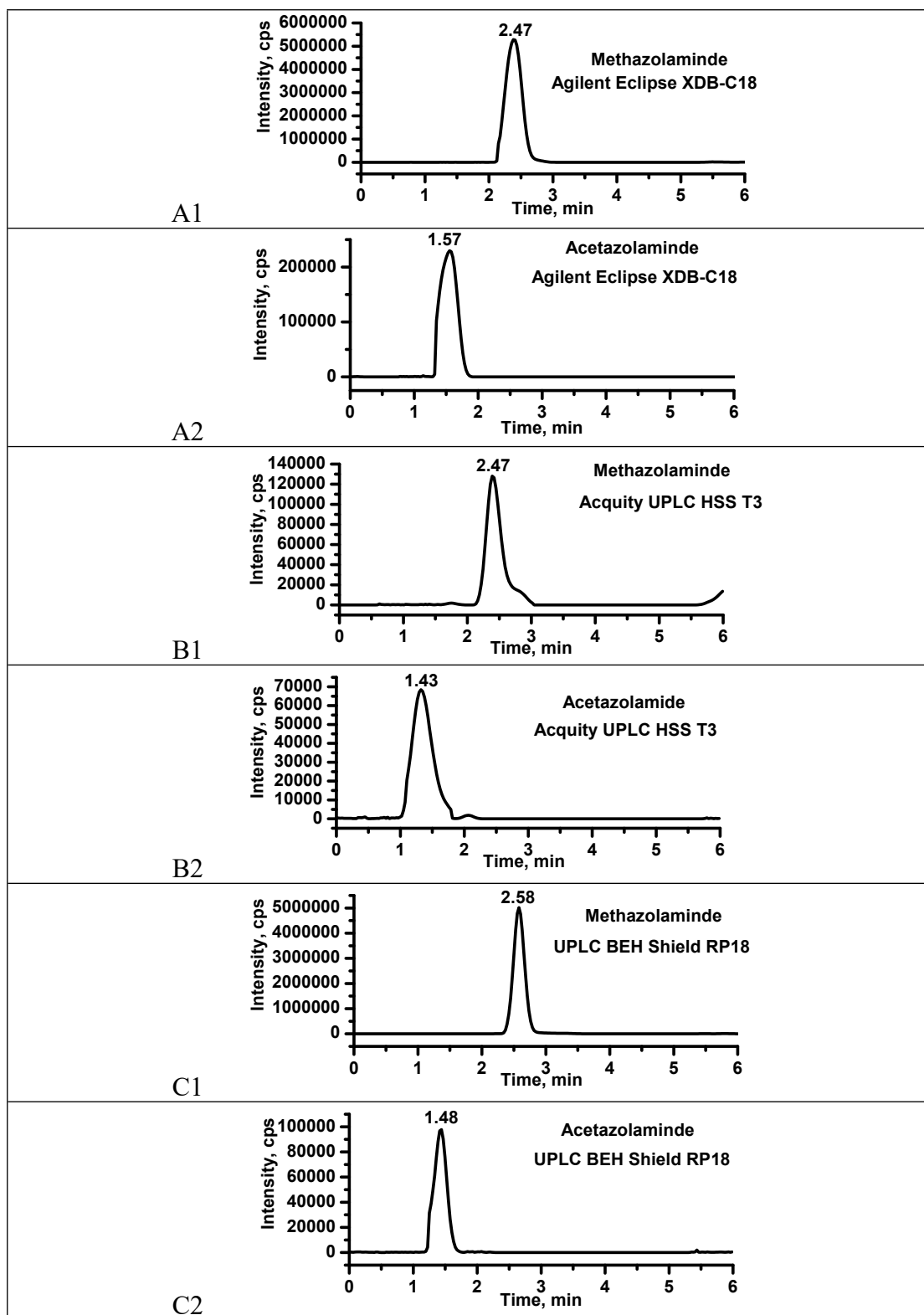


Figure S3 Comparison of Agilent Eclipse XDB-C₁₈ (A1-A2), Acquity UPLC HSS T₃ (B1-B2), UPLC BEH Shield RP₁₈ (C1-C2)

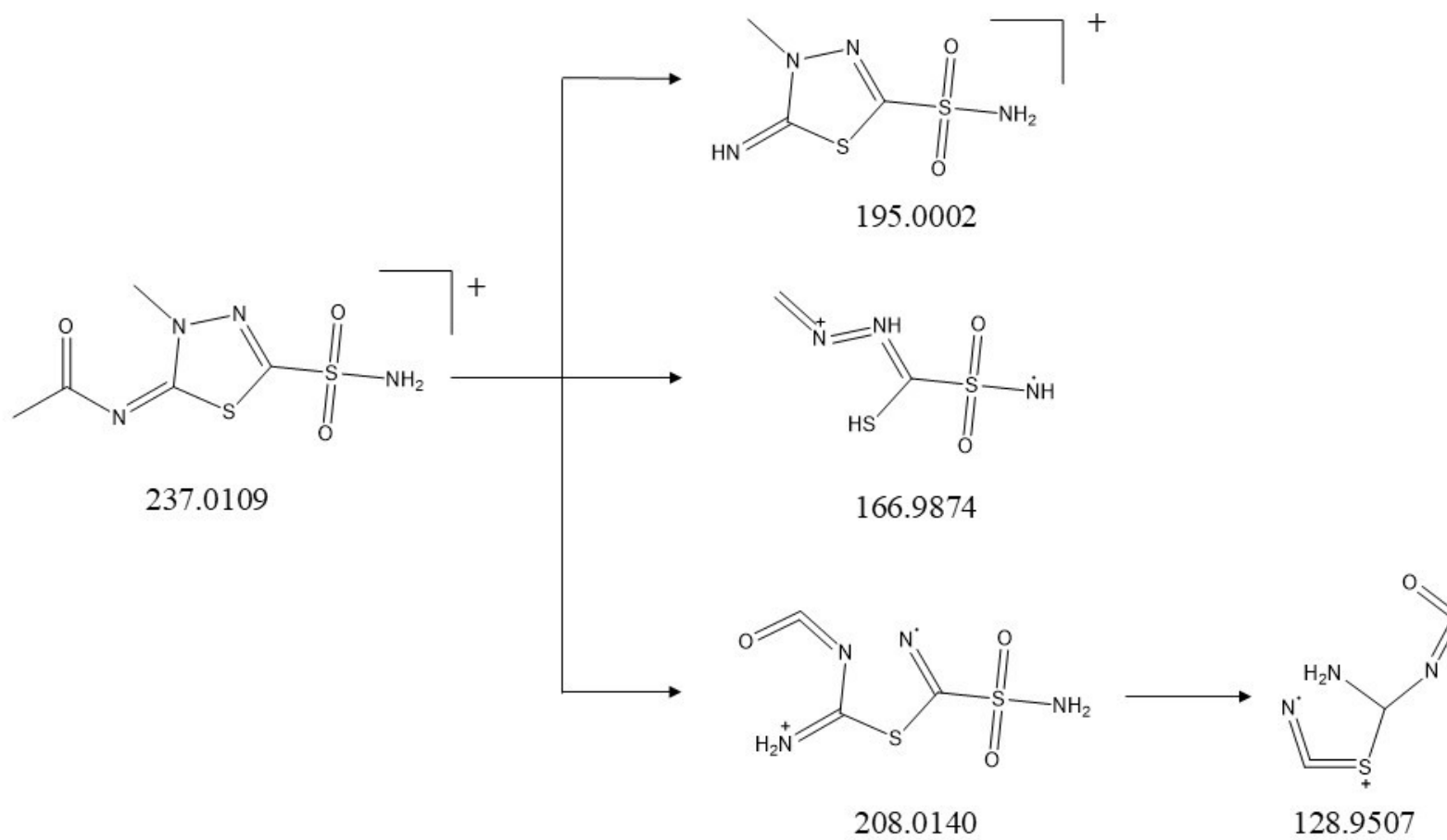


Figure S4 The fragmentation scheme of methazolamide

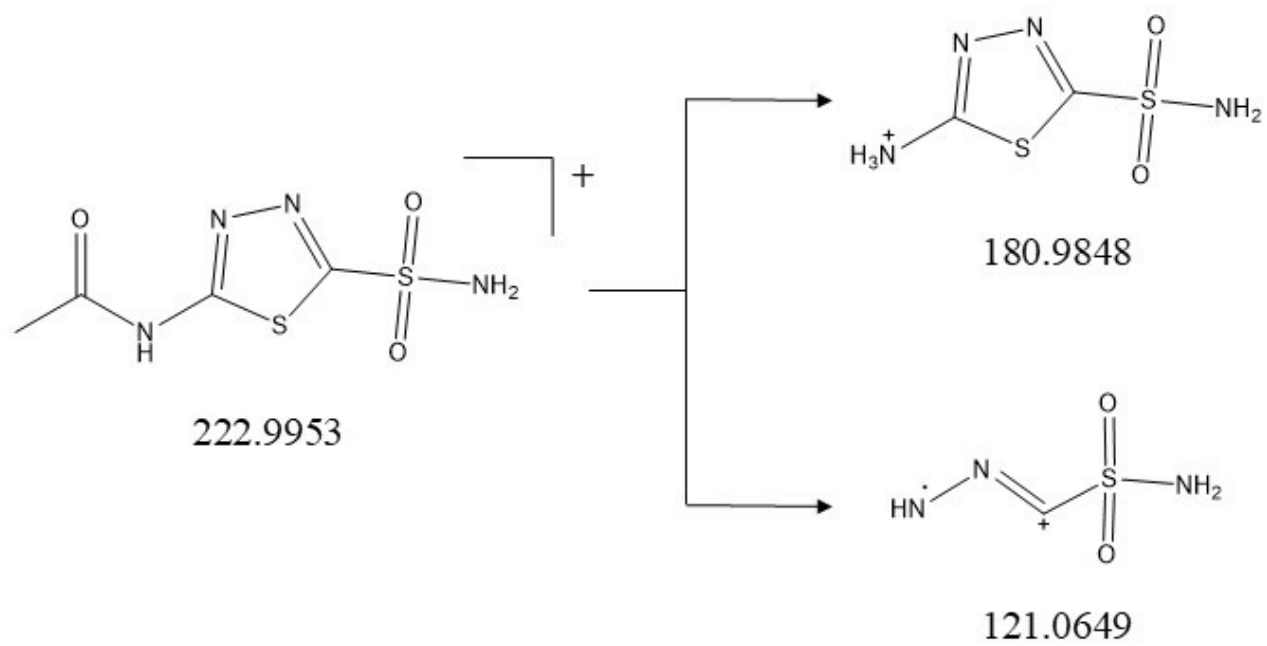


Figure S5 The fragmentation scheme of acetazolamide

Table S1. Comparative assessment of LC-based methods for the quantification of methazolamide and related substances

Sample matrix	Pretreatment	Methods	Run time (min)	Sensitivity (LOQ)	Ref.
Whole blood, plasma and urine	Liquid-liquid extracction	HPLC-UV	9	For Whole blood, plasma: 5.0 µg/mL; For Urine: 10 µg/mL	20
Tablets	Solvent extraction	HPLC-UV	15	10 µg/mL	21
Rabbit aqueous humor	Dilution and filtration	HPLC-UV	10	500 ng/mL	22
Rabbit aqueous humor	Protein precipitation	HPLC-UV	11	500 ng/mL	23
Crude methazolamide products and several batches of commercial methazolamide products	Dilution and filtration	HPLC-single Q MS	30	n.d.	10
Rat plasma	Protein precipitation	UHPLC-Q-Orbitrap MS	6	10 ng/mL	This work

n.d.: not found.