FHAL-P19 PROCJENA STABILNOSTI TIMOKINONA U SISTEMIMA ĆELIJSKIH KULTURA: UTJECAJ TEMPERATURE, IZLOŽENOSTI SVJETLOSTI I MEDIJUMA ZA ĆELIJSKU KULTURU

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Timokinon (TQ) posjeduje značajan *in vitro* terapeutski potencijal, ali njegova hemijska nestabilnost ograničava reproduktivnost eksperimentalnih rezultata. Ova studija istražuje kinetiku termičke i fotolitičke degradacije TQ-a u biološkim matricama radi uspostavljanja pouzdanih protokola rukovanja i skladištenja.

Rastvori TQ-a (10, 50 i 100 μM) analizirani su validiranom HPLC-UV metodom na sistemu Agilent 1260 Infinity (Agilent Technologies, SAD) i koloni Zorbax C18 (4,6x150 mm, 5 μm). Fotostabilnost je ispitivana u komori Suntest CPS+ (Atlas, SAD) prema ICH Q1B smjernicama (1.2×10^6 lux·h na 35°C tokom 9 h). Termička stabilnost praćena je 168 h na 5°C, sobnoj temperaturi, 25°C/60% RH i 40°C/75% RH u serum-slobodnim i kompletnim medijumima (10% fetalnog goveđeg seruma, Elabscience, Kina) RPMI 1640 i DMEM.

Fotolitički stres uzrokovao je brzu degradaciju, uz svega 0,01% preostalog TQ-a nakon 9 h. Termička degradacija pratila je koncentracijski zavisnu kinetiku pseudo-prvog reda. Na ambijentalnim uslovima potpuna degradacija nastupa za 24–96 h. Skladištenje na 5°C usporava proces, ali postotak ostatka nakon 7 dana pada ispod 42%. Sastav matrice značajno utječe na stabilnost; DMEM i prisustvo seruma pružaju djelimičnu zaštitu, dok RPMI daje manje konzistentne profile.

TQ pokazuje visoku nestabilnost u medijumima za kulturu ćelija. Svjetlost, propisana ICH smjernicama, izaziva brzu, gotovo potpunu degradaciju, zahtijevajući trenutnu zaštitu od svjetlosti od same pripreme uzoraka. Iako čuvanje na 5°C poboljšava kratkoročnu stabilnost, dugoročno dolazi do velikog gubitka analita. Stabilnost zavisi od matrice, uz veću postojanost u DMEM-u i medijumima sa serumom. Zaštita od svjetlosti i minimalno vrijeme skladištenja obavezni su za *in vitro* reproduktivnost.

Zahvalnica

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FHAL-P19 STABILITY ASSESSMENT OF THYMOQUINONE IN CELL CULTURE SYSTEMS: EFFECTS OF TEMPERATURE, LIGHT EXPOSURE, AND CELL GROWTH MEDIA

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Thymoquinone (TQ) has significant *in vitro* therapeutic potential, but its chemical instability limits experimental reproducibility. This study investigates TQ thermal and photolytic degradation kinetics in various biological matrices to establish robust handling protocols.

TQ solutions (10, 50, and 100 μM) were analyzed using a validated HPLC-UV method employing an Agilent 1260 Infinity HPLC system (Agilent Technologies, USA) with a Zorbax C18 column (4.6x150mm, 5 um). Photostability was assessed in a Suntest CPS+ chamber (Atlas, USA) per ICH Q1B guidelines (1.2×10^6 lux·h at 35°C for 9 h). Thermal stability was tracked for 168 h at 5°C, ambient, 25°C /60% RH, and 40°C /75% RH in serum-free and complete (10% fetal bovine serum, Elabscience, China) RPMI 1640 and DMEM.

Photolytic stress caused extensive degradation, with 0.01% TQ remaining after 9h. Thermal degradation showed concentration-dependent, pseudo-first-order kinetics. Ambient exposure led to complete degradation within 24-96 h. Refrigerated storage (5°C) reduced degradation but recovery fell below 42% after 7 days. Matrix composition significantly influenced stability; DMEM and serum presence provided a partial protective effect, while RPMI formulations yielded less consistent recovery profiles.

TQ exhibits high thermal and photolytic instability in cell media. ICH-compliant light triggers near-complete degradation, demanding immediate light protection during sample preparation. Although storage at 5°C improves short-term stability, substantial loss occurs over time. TQ stability is highly matrix-dependent, showing enhanced persistence in DMEM and serum-supplemented media. Strict light protection and minimized storage times are mandatory for reliable *in vitro* screening.

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