Title: Ultrasensitive Spectrofluorimetric Method for Rapid Determination of Daclatasvir and Ledipasvir in Human Plasma and Pharmaceutical Formulations

Authors: Mohammad Nabil Abo-Zeid, Noha N. Atia, Samia M. El-Gizawy, Salwa R. El-Shaboury

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Address: Department of Pharmaceutical Analytical Chemistry, Faculty of Pharmacy, Assiut University, Assiut 71526, Egypt

Direct-acting antivirals (DAAAs) represent a revolution in the treatment of chronic hepatitis C which have emerged at an extremely rapid pace over the past few years. DAAAs act directly on the hepatitis C virus at various points in the viral life cycle to inhibit viral production. Among these novel DAAAs, are daclatasvir (DCS) and ledipasvir (LDS). Herein, a novel, fast, simple, ultrasensitive and cost-effective spectrofluorimetric method was designed for determination of DCS and LDS in miscellaneous matrices. The method is based on investigation of the native fluorescence of the cited drugs. The relative fluorescence intensity (RFI) was measured at $\lambda_{ex}/\lambda_{em}$ equal to 315/381 nm for DCS and 332/387 nm for LDS. Under the optimum conditions, the linear ranges of calibration curves were 0.2-30 and 6-120 ng mL$^{-1}$ for DCS and LDS, respectively with correlation coefficients $\geq 0.9998$. The detection limits were 0.047 and 1.939 ng mL$^{-1}$ for DCS and LDS, respectively indicating ultrasensitivity of the proposed method. Consequently, this permits in vitro and in
vivo application of the proposed method in spiked and real human plasma with good percentage recovery (96.6–103.6%). The method was validated in compliance with ICH guidelines and US-FDA guidelines. Furthermore, the application was extended to analysis of DCS and LDS in its pharmaceutical formulations (either alone or in presence of other co-formulated drugs) and in synthetic mixture with sofosbuvir or ribavirin.

**Keywords:**

Direct-acting antivirals, Daclatasvir, Ledipasvir, Native fluorescence, Real human plasma, Pharmaceutical formulations