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**Innovative thin-layer chromatographic method combined with
fluorescence detection for specific determination of Febuxostat:
Application in biological fluids**

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ABSTRACT

A newly developed thin-layer chromatographic (TLC) method coupled with fluorescence detection for specific determination of Febuxostat (FEB) was designed. The proposed method adopts exposure of FEB on a developed TLC plate to hydrochloric acid vapors, resulting in an large enhancement of its weak fluorescence, permitting its specific and sensitive determination in real human plasma and urine after excitation at 345 nm on 60 F₂₅₄ silica gel plates using toluene–ethyl acetate–methanol–glacial acetic acid; (30:10:5:0.1,v/v/v/v) as mobile phase. The retention factor (R_f) value for FEB was 0.33 ± 0.03 with a correlation coefficient of 0.9974 in the concentration range of 2.5–50 ng/band. Upon using polynomial regression, the correlation coefficient was greatly improved (0.9999), with detection and quantification limits of 0.55 and 1.67 (ng/band), respectively. The proposed method was validated according to the International Conference of Harmonization and was successfully used for specific and selective determination of FEB in its commercial dosage form without excipient interference. Moreover, the proposed method was extended to efficient determination of the studied drug in real human plasma and urine samples in the presence of its metabolites without any interference, allowing clinical application of the proposed method for direct FEB determination in biological fluids as well as in pharmacokinetics studies and for quality control of the pharmaceutical dosage form without sample pretreatment or exhausting extraction steps.

Graphical abstract

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