Title: Innovative HPTLC Method with Fluorescence Detection for Assessment of Febuxostat-Montelukast Combination and Study of Their Protective Effects Against Gouty Arthritis

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The present study was designed to evaluate the potential protective effects of the cysteinyl leukotriene receptor blocker montelukast (MNK) and the xanthine oxidase inhibitor febuxostat (FBX) and their combination on a model of acute gouty arthritis induced by intraarticular injection of monourate sodium crystal (MUC) injection in rats. Additionally, we established an HPTLC method for the quantitative determination of both drugs simultaneously and applied this method to detect this combination in rat plasma. In this study, the treated male Wistar rats were grouped as follows: a negative control group injected with phosphate buffer saline (PBS) and a positive control group injected with MUC in their tibiotarsal joint. Four groups were treated orally with diclofenac (4 mg kg$^{-1}$), MNK (10 mg kg$^{-1}$), FBX (5 mg kg$^{-1}$) and MNK plus FBX before MUC injection
in their tibiotarsal joints. MUC injection in joints without pretreatment led to a progressive significant increase in joint diameter and heavy leucocytic infiltration compared to the PBS-injected joints. Treatment with diclofenac or a combination of MNK and FBX significantly decreased both joint diameters and leucocytic infiltration compared to the MUC group. MNK or FBX treatment attenuated leucocytic infiltration and significantly decreased joint diameters compared to the MUC group. Thus, MNK and FBX can prevent the development of MUC-induced acute gouty arthritis in rats. Also, MNK can be an alternative preventive treatment, particularly in elderly patients who cannot tolerate NSAIDs or corticosteroid. Furthermore, a new thin-layer chromatographic method combined with fluorescence detection was developed and validated for the simultaneous estimation of a mixture of FBX and MNK in spiked human plasma. In this method, we employed TLC aluminium plates precoated with silica gel G 60F 254 as the stationary phase and chloroform : methanol (9 : 1, v/v) as the mobile phase. The optimized mobile phase selected for development gives compact bands ($R_f$ values are 0.28 and 0.63 for FBX and MNK, respectively). The emission intensities were measured using a K400 optical filter after excitation at 322 nm. The linear regression data for the calibration plots showed excellent linear relationship ($r = 0.9990$ and 0.9996 for FBX and MNK, respectively), and the linearity range was 10.0–800.0 ng per band for both drugs. According to ICH-guidelines, this method was validated for precision, accuracy, robustness and selectivity. Also, the limits of detection and quantitation were calculated. In addition, stability studies on the studied mixture were performed. Statistical analysis proved that this method is reproducible and selective for the estimation of a mixture of FBX and MNK.