Abstract

Inadequately treated acute and chronic pain remains a major cause of suffering and dissatisfaction in pain therapy. Migraine prophylactic drugs and analgesics are concurrently administered to manage migraine pain. Poly pharmacy precipitates drug interactions and altered therapeutic efficiency. Hence there is a need to study the drug interactions between analgesics and drugs used in migraine therapy. Migraine prophylactic drugs such as propranolol hydrochloride, amitriptyline, flunarizine dihydrochloride, carbamazepine, levetiracetam and analgesics such as paracetamol and aceclofenac were selected for in vitro and in vivo interaction assessment. The first objective of the current study was to develop simple and sensitive analytical and bio-analytical methods for simultaneous estimation of the selected drugs used in migraine prophylaxis & analgesics which was followed by carrying out drug interactions between them. The second objective was to develop a simple bio-analytical method for the simultaneous estimation of drugs used in the treatment of diabetic neuropathy in human plasma.

1. Method development and in vitro & in vivo drug interaction assessment

Analytical and bio-analytical methods (in rat plasma) were developed for the simultaneous estimation of the selected drug combinations by Reverse Phase High Performance Liquid Chromatography. The elution was carried out on Phenomenex Luna C18 (100R, 250 x 4.60 mm, 5 µm) column using the optimized mobile phase system. The developed methods were validated as per ICH (analytical methods) and USFDA (bio-analytical methods) guidelines. Using the developed methods, in vitro & in vivo drug interaction studies were performed to assess the correlation between drug dissolution in vitro and drug plasma concentration in vivo at various time intervals. In vitro drug release studies were performed by using a USP dissolution rate apparatus (apparatus 2, 100 rpm, 37 ± 0.5 °C) at pH 1.2 (simulated gastric fluid). The test
samples were withdrawn at different time intervals and measured by RP-HPLC method. Based on the results of *in vitro* dissolution studies, the drug combinations exhibiting significant drug interaction were selected for drug interaction assessment *in vivo*. The plasma drug concentrations of migraine prophylactic drugs in the presence and absence of analgesics were determined. The changes in pharmacokinetics of one drug in the presence of other were assessed.

Synthetic mixture of paracetamol, aceclofenac, propranolol hydrochloride and levetiracetam were separated using acetonitrile and phosphate buffer of pH 7 (40:60 v/v) and with retention times 2.8, 2.9, 3.5 and 3.6 min respectively. Propranolol hydrochloride and paracetamol were found to be linear in the range of 10 to 70 µg/mL (R² value of PRL - 0.998, of PAR - 0.99), aceclofenac was found to be linear in the range of 10 to 80 µg/ml (R² value of 0.999) and levetiracetam was found to be linear in the range of 5 to 50 µg/ml (R² value of 0.995). In spiked rat plasma, propranolol hydrochloride, paracetamol, aceclofenac and levetiracetam were found to be linear in the concentration ranges of 150 to 900 ng/mL (R² value of 0.997), 100 to 700 ng/mL (R² value of – 0.99), 100 to 800 ng/mL (R² value of 0.998) and 50 to 300 ng/mL (R² value of 0.998) respectively.

Synthetic mixture of paracetamol, aceclofenac and amitriptyline hydrochloride and were separated using acetonitrile, methanol and phosphate buffer of pH 7 (55:5:40 v/v/v) and with retention times 1.8, 2.9 and 5 min respectively. Linearity range in analytical method for paracetamol, aceclofenac and amitriptyline hydrochloride were found to be 10 to 80 µg/mL, 20 to 80 µg/mL and 10 to 60 µg/mL (R² value of PAR- 0.995, of AFC - 0.996, and of AMP - 0.991). In spiked rat plasma, linearity range for amitriptyline hydrochloride was 25 to 175 ng/mL (R² value of
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0.998), for PAR was 100 to 600 ng/mL (R² value of - 0.997) and for aceclofenac was 200 to 800 ng/mL (R² value of - 0.997)

Synthetic mixture of paracetamol, aceclofenac, carbamazepine and flunarizine dihydrochloride were separated using acetonitrile and phosphate buffer of pH 5 (90:10 v/v) and analytes were monitored at 210 nm with retention times 1.8, 1.83, 2.93 and 5 min respectively. In analytical method, linearity range of paracetamol, aceclofenac, carbamazepine and flunarizine dihydrochloride were found to be 10 to 100 μg/mL (R² value of - 0.998), 30 to 100 μg/mL (R² value of - 0.995), 10 to 80 μg/mL (R² value of - 0.997) and 10 to 70 μg/mL (R² value of - 0.995). In spiked rat plasma, linearity range of paracetamol, aceclofenac, carbamazepine and flunarizine dihydrochloride were found to be 100 to 800 ng/mL (R² value of 0.991), 300 to 800 ng/mL (R² value of 0.998), 200 to 1400 ng/mL (R² value of 0.997) and 50 to 300 ng/mL (R² value of 0.996) respectively. The developed methods were validated as per ICH and USFDA guidelines and the results were well within the limits.

In vitro and in vivo drug interaction studies: No significant changes were observed in the concentrations of the migraine prophylactic drugs when assessed in vitro and in vivo with aceclofenac. However, the combination of drugs such as propranolol hydrochloride, amitriptyline hydrochloride and carbamazepine with paracetamol exhibited drug interaction which was statistically significant. The drugs exhibiting significant interaction in vitro were chosen for in vivo interaction assessment. Dissolution in vitro and the achievement of the plasma Cmax of amitriptyline hydrochloride was faster in the presence of paracetamol. In vitro, paracetamol also dissolved quickly and reached its maximum Cmax and Tmax faster in the presence of amitriptyline hydrochloride. A significant delay in the in vitro dissolution of propranolol hydrochloride and carbamazepine was recorded in the
presence of paracetamol and the paracetamol dissolution was prolonged in the presence of carbamazepine. A shortened $t_{1/2}$, but no change in $C_{\text{max}}$ was observed when carbamazepine was administered along with paracetamol. Pharmacokinetic parameters of paracetamol when administered in the presence and absence of carbamazepine were more or less similar. Propranolol hydrochloride when administered with paracetamol exhibited prolonged $T_{\text{max}}$ and its $t_{1/2}$ was found to be extended. Paracetamol also exhibited a delay in $T_{\text{max}}$ and shortened $t_{1/2}$ when co-administered with propranolol hydrochloride.

The developed methods were simple, sensitive, precise, accurate, robust and stable in the solutions in which they were prepared. The developed methods were successfully employed in the in vitro and in vivo drug interaction assessment. A correlation between the results of in vitro percentage drug dissolution and in vivo plasma drug concentrations was observed for the selected drug combinations.

2. Development of bio-analytical method for simultaneous estimation of pregabalin, gabapentin and duloxetine hydrochloride in human plasma by GC-FID

Pregabalin, duloxetine hydrochloride and gabapentin were extracted after spiking to human plasma. The drugs were derivatized using ethyl chloroformate and analysed by GC method. The derivatized analytes were separated in Rtx-5 capillary column (cross bond of 5% diphenyl and 95% dimethyl polysiloxane) was used for the separation with dimensions of 30m × 0.25mm. Nitrogen was used as a carrier gas and a combination of zero air and hydrogen were used for the generation of flame. Various temperature programmings were attempted and a program of initial temperature $80^0 \text{C}$ for 5 min and a gradual rise in temperature to $160^0 \text{C}$ at the ramp rate of $100^0 \text{C} /\text{min}$ and $160^0 \text{C}$ was maintained for 5 min in a total run time of 10 min. Carrier gas pressure programming was performed by setting the initial pressure at
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83.7 Kpa for 3.5 min and pressure was gradually increased at the rate of 20 Kpa/min up to 120 Kpa.

The derivatized duloxetine hydrochloride, gabapentin and pregabalin were eluted at retention times of 2.38, 2.8 and 8.08 min respectively. The linearity range of duloxetine hydrochloride, gabapentin and pregabalin were found to be 5 - 50 µg/ mL (R² - 0.999), 10 - 35 µg/ mL (R² - 0.996), and 10-100 µg/ mL (R² - 0.997) respectively. The developed method was validated as per USFDA guidelines and all the validation parameters were well within the limits.

The developed GC method was simple, sensitive, precise and accurate. The method can be used for pharmacokinetics determination and drug interaction assessment.