**INTRODUCTION**

Pitofenone hydrochloride is an anti spasmodic drug which is chemically 2-[4-[2-(1-Piperidinyl) ethoxy] benzoyl] benzoic acid methyl ester. Pitofenone is an anti spasmodic compound and has a inhibitory effect on acetylcholinesterase. According to the literature survey it was found that few analytical methods such as HPLC and UV Spectrophotometric analysis were reported for the estimation of Pitofenone individually or with some other combination. In the present investigation, a new LC-MS/MS method has been developed for the estimation of Pitofenone in human plasma using Fenpivemirium as an internal standard.

**MATERIALS AND METHODS:**

**Experimental**

**Instrumentation:** To develop a LC/MS/MS method for quantitative estimation of Pitofenone HCl. The HPLC system was an LC Agilent 1100 series, consisted in a binary pump, an in-line degasser, an autosampler, a column thermostat and an in-line filter. The HPLC system was coupled to an MS mass spectrometer detector (Bruckner Daltonics Instruments). The ion transition monitored was m/z Pitofenone (Q Mass: 368.1;Q3 Mass 112.1), Internal standard (Q Mass: 338.3;Q Mass 239.1).

**Chromatographic separation was performed at 45°C on a ZORBAX Eclipse XDB – C 18, 4.6 × 150 mm, 5 um, column, protected by an in-line filter. The HPLC system was operated with an electrospray interface (ESI) operated in the positive ionization mode.

**Preparation of calibration curve solutions and samples:** The calibration curve standards spiked solutions were prepared by adding 200 µl of drug stock solution (1.0 mg/mL) with diluents (Methanol: Water, 50:50 v/v) in different vials and add 20 µl internal standard fenpivemiriumsolution (1.5 µg/mL) and vortex for 15 seconds. Prepare the calibration curve standards by spiking the respective calibration curve standards spiked solutions in screened blank plasma in different vials to obtain final concentration of 1, 2, 5, 25, 50, 125, 500, 800 and 1000 ng/mL of Pitofenone.

**Preparation of quality control solutions and samples:** The quality control spiking solutions were prepared by adding 20 µl of drug stock solution (1.0 mg/mL) with diluents (Methanol: Water, 50:50 v/v) in different vials and add 20 µl internal standard solution (1.5 µg/mL) and vortex for 15 seconds. Prepare the quality control solutions by spiking the respective quality control spiking solutions in screened blank plasma in different vials to obtain lower limit of quantification quality control (LLOQ QC), lower quality control (LQC), middle quality control (MQC), higher quality control (HQC) and upper limit of quantification quality control (ULOQ QC) samples of concentrations of 1, 3, 7, 50, 800 and 1000 ng/mL of Pitofenone.

**RESULTS AND DISCUSSION:**

The method validation of bio analysis was performed as stated in US-FDA guideline. Specificity, selectivity, system suitability, % recovery, linearity, limit of detection, limit of quantitation, accuracy and precision were analyzed.

**TABLE 1: OPTIMIZED HPLC CONDITIONS FOR THE ESTIMATION OF PITOFENONE**

<table>
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<tr>
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<td>Mobile phase</td>
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</tr>
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<td>Flow rate (ml/min)</td>
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| Injection | 45ºC on a ZORBAX Eclipse XDB – C 18, 4.6 × 150 mm, 5 um, column, protected by an in-line filter. The HPLC system was coupled to an MS mass spectrometer detector (Bruckner Daltonics Instruments). The ion transition monitored was m/z Pitofenone (Q Mass: 368.1;Q3 Mass 112.1), Internal standard (Q Mass: 338.3;Q Mass 239.1).

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**TABLE 2: SELECTIVITY DATA OF PITOFENONE**

| Precision and accuracy was evaluated by analysing three precision and accuracy batches. Each precision and accuracy batch consists of calibration curve and six replicates of LOQQC, LQC, MQC, HQC and ULOQ QC. Precision and accuracy was evaluated both inter and intra batches. The mean accuracy for each concentration level ranged from 91.2 to 102.8 and the mean precision for each concentration level ranged from 1.90 to 7.83.

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**TABLE 3: PRECISION STUDY OF THE METHOD (INTRA-CLASS)**

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**TABLE 4: PRECISION STUDY OF THE METHOD (INTER-CLASS)**

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**TABLE 5: RECOVERY STUDY OF THE METHOD (INTER-CLASS)**

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**TABLE 6: RECOVERY STUDY OF PITOFENONE**

**Stability studies:** Stability studies were performed to evaluate the stability of Pitofenone HCl both in aqueous solution and in plasma after exposing to various stress conditions. Pitofenone HCl and Fenpivemirium Bromide stock solutions (0.1 mg/ml) remained stable when stored at refrigerator conditions for 7 days including the storage at room temperature for 8 h. Pitofenone HCl was found to be stable for three freeze and thaw cycles. Pitofenone HCl was stable and did not show any degradation when stored in the freezer for 45 days.

**CONCLUSION:** From all results, it was concluded that the developed LC/MS/MS method is simple, sensitive, accurate, precise, and selective. Percentage recovery shows that the method is free from interference of matrix. The analytical method presented here has proved to be useful for investigation of the characteristics of Pitofenone in human plasma in pharmacokinetic and pharmacogenetic studies.

**ACKNOWLEDGMENTS:** The authors gratefully acknowledge Climegenetic International Ltd, Bangalore, India for providing necessary facilities for carrying out this study.