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Research TDD

Analysis of Anti-psychotic Drug Penfluridol in Human Plasma by HPLC and MS/MS

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Abstract

*Penfluridol is a new long-acting neuroleptic drug known to be used widely for treating psychotic conditions. An internal standard was employed, which was penfluridol D7 (IS). The analyte and the IS were separated on a Phenomenex Kinetex C18, 5µm 100A, 100*4.6mm column using acetonitrile (40:60, v/v) as the mobile phase and 5 mM ammonium formate as the stationary phase. The retention time of Penfluridol and Penfluridol d7 was set at 2.94 and 2.75 mins respectively. Penfluridol and Penfluridol-d7 were detected and measured using the precursor to product ion transitions of m/z 524.300/203.100 m/z and 531.400/204.100 m/z, respectively. The investigation made use of an API-6500+ LC-MS/MS system running in multiple reactions monitoring (MRM) mode and outfitted with a turbo ion spray (TIS) source.We developed a highly sensitive, selective, and precise method for quantification of Penfluridol in human plasma.*

Keywords: Penfluridol, LC-MS/MS, Antipsychotic, Depot Drug, K_2EDTA

INTRODUCTION

Penfluridol is a new long-acting neuroleptic drug, first shown by Janssen Pharmaceuticals [2]. A number of studies relating to the pharmacology and clinical properties of the drug have been published so far [1, 4–12]. In contrast with this data, some attempts have been made so far to measure Penfluridol plasma levels in patients [5]. In recent studies some analysis on the drug has been published and researched which involved GPLC (Gas-Liquid Chromatography) [3], but no publication or method has been developed on the LC-MS/MS(Liquid Chromatography and Mass spectrometry) and here in our research laboratory we`ve discovered the Bio-analytical Estimation of the Penfluridol Drug on the LC-MS/MS. This method is sensitive enough for measuring concentrations corresponding to those in plasma after the usual therapeutic dosages. LC-MS/MS (Liquid Chromatography and Mass spectrometry) a highly sensitive and selective mass analysis capability of triple quadrupole mass spectrometry.

To the best of our knowledge hitherto there is no analytical method reported for simultaneous determination of drug Penfluridol. Therefore, an attempt has been made to develop a simple, accurate, rapid and reproducible LC-MS/MS method for simultaneous determination of Penfluridol in human plasma, in accordance with international guidelines [13, 14]. In this study, we have developed for the first time a rapid, accurate and precise High-performance Liquid-

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Chromatography and Mass Spectrometry method for measurement of penfluridol in the plasma of healthy volunteers.

MATERIALS AND METHODS

Penfluridol drug used for the study was procured from SimSon Pharma Limited, Mumbai, Maharashtra, India, which had the molecular formula-$C_{28}H_{27}ClF_5NO$ and IUPAC name 1-[4,4-bis(4-fluorophenyl)butyl]-4-[4-chloro-3-(trifluoromethyl)phenyl] piperidin-4-ol to analyse the drug efficiency in Human plasma a similar drug was used for comparing the analyte(penfluridol) in LC-MS / MS(Liquid Chromatography – Mass spectrometry) named Penfluridol D7 from the brand Alsa Chim(A Shimadzu group company) , having molecular formula $C_{28}H_{20}$ D_7ClF_5NO and IUPAC name 1-[4,4-bis(4-fluorophenyl)butyl]-4-[4-chloro-3-(trifluoromethyl)phenyl]-4-piperidinol, both molecules shared similar molecular weight, Exact mass, Mono isotopic mass, Physical properties and chemical properties. Penfluridol Pharmaco therapeutic group was found to be Pscycho-therapeutic which is then used to treat psychotic diseases.

Chromatographic System and Conditions

The LC-MS/MS system consisted of a Sciex Triple Quad 6500+ instrument with a binary pump, Autosampler injector with 10ul injection volume and MS detector. The software used was Analyst®1.7.1. Separation was achieved on a reverse phase Kinetix C_{18} 5µm 100*4.6mm column. The mobile phase used was a binary method in which Pump A consisted of – Acetonitrile (60%) and Pump B- 5mM Ammonium Formate Solution (40%) with flow rate of 1.000 ml/ minute. The retention of penfluridol observed was at Retention time 2.94 and penfluridol D7 at Retention time 2.75 sec. All the experiments were performed at ambient temperature.

Standard Solutions and Calibrator Preparation for Chromatographic Measurement

Stock standard solutions were prepared by dissolving about 2 mg of Penfluridol in 200 µl of Methanol and made up the volume up to the mark with methanol to produce a solution of about 1 mg/ml of concentration of Penfluridol. Prepared the Calibrators by using stock solutions at following concentration level 0.103ng/ml, 0.206ng/ml, 2.580ng/ml, 10.321ng/ml, 20.642/ng/ml, 30.808ng/ml, 41.078ng/ml and 51.347ng/ml and quality control samples at 0.104ng/ml, 0.302ng/ml,6.290ng/ml, 20.965ng/ml, and 41.930ng/ml Figure 9.

Solvent Solutions Preparation

For preparation of 7 reagents and solvents used in LC-MS/MS method detailed protocol was used as mentioned below:

1. *5mM Ammonium formate Solution (Buffer-A):* Weigh about 0.3150 g of Ammonium Formate and transfer into a 1000 ml of volumetric flask. Add 500 ml of Ultra-Pure water and mix well to dissolve and make up to 1000 ml with Ultra-Pure Water. Transfer it into reagent bottle mix well and degas in sonicator and keep at room temperature.
2. *Mobile Phase (Acetonitrile: 5mM Ammonium formate Solution: 60:40 v/v):* Pump A: Acetonitrile (60%), Pump B: 5mM Ammonium formate Solution (40%)
3. *Elution Solution (Acetonitrile: 5mM Ammonium formate Solution: 80:20 v/v):* Take 800 ml of Acetonitrile and 200 ml of 5mM Ammonium Formate by using measuring cylinder, transfer into reagent bottle, mix well and degas in sonicator and keep at room temperature.
4. *Diluent Solution (Methanol: Water: 50:50 v/v):* Preparation-Take 250 ml of methanol and 250 ml of ultra-pure water by using measuring cylinder, transfer into reagent bottle, mix well and degas in sonicator and keep at room temperature.
5. *Rinsing Solution (Methanol: Water: 70:30 v/v):* Preparation- Take 700 ml of Methanol add 300 ml of ultra-pure water by using measuring cylinder and transfer into 1000 ml of reagent bottle, mix well and degas in sonicator and keep at room temperature.
6. *Washing Solution (Methanol: Water: 10:90 v/v):* Preparation- Take 100 ml of Methanol add 900 ml of ultra-pure water by using measuring cylinder and transfer into 1000 ml of reagent bottle, mix well and degas in sonicator and keep at room temperature.

7. *0.1% Formic acid solution in Water (Buffer-B):* Take 500 ml of ultra-pure water, transfer into 1000 ml of volumetric flask, add 1 ml of Formic Acid into it and volume make up to the mark with ultra-pure water. Transfer it into reagent bottle mix well and degas in sonicator and keep at room temperature.

METHOD VALIDATION

The developed method was validated according to the USFDA and EMEA guidelines [13, 14]. The system suitability was evaluated by six replicate analytes of penfluridol with prepared stock concentration 542.890ng/ml and Matrix spiked concentration 21.716ng/ml.

Standard calibration curves were prepared in the mobile phase with six concentrations ranging from 0.103 ng/mL to 51.347ng/mL into the LC-MS/MS system keeping the injection volume constant. The peak areas were plotted against the corresponding concentrations to obtain the calibration graphs. To study the reliability and suitability of the developed method, recovery experiments were carried out at 65.615, 71.180 and 68.904 %.

Extraction Procedure

1. Aliquot 192 µl Blank Plasma add 8 µl of each analyte spiking solution of CC/QC except Blank and STD 0, add 8 µl diluent in Blank and STD 0, vortex for complete mixing.
2. Add 50 µl internal standard spiking solution (Penfluridol D7~108.770 ng/ml) to each pre labelled Ria vial except blank sample add 50 µl of diluent solution in blank sample and vortex for few seconds
3. Add 0.200 ml of 0.1% formic acid solution into all samples and Vortex for few second
4. Use SPE Method, Orochem Panthera Deluxe 30 mg, 1 ml Cartridges.

Solid Phase Extraction (SPE) method:

For the Solid phase extraction (SPE) method 200 µl of Plasma samples was used, using solid phase extraction technique, samples were loaded on the SPE Cartridges (Orochem, Panthera 30mg, 1ml) on the solid Phase extractor made by Athena technology (Mumbai, Maharashtra 400601, India).

Conditioning of the cartridges is done with 1 ml methanol and thus equilibrated with 1 ml water. Loaded the sample and washed with water followed by 10% Methanol. Eluted the analyte from cartridges with 500 µl of Elution solution and injected on LC-MS/MS (Sciex).

Chromatographic method

Penfluridol Quantification is done in Human K_2EDTA (K_2EDTA is the anticoagulant used for the study) plasma by using Sciex LC-MS/MS 6500+. Separation of penfluridol was done by using the column Kinetix C_{18} 5µm 100*4.6mm and mobile phase used as a binary method in which Pump A–Acetonitrile (60%) and Pump B-5mM Ammonium Formate Solution (40%) with flow rate of 1.000 ml/minute. The retention of penfluridol observed at Retention time 2.94 and penfluridol D7 at Retention time 2.75 s.

RESULT`S AND DISCUSSION

The method was validated for linearity in the range of 0.103 to 51.347 ng/ml. Chromatographic separation was achieved on LC-MS/MS method with Phenomenex Kinetexs $C_{18,}$ 5μm 100*4.6 mm column using 5 millimolar ammonium formate and acetonitrile (40:60, v/v) as mobile phase. The retention time of Penfluridol and Penfluridol D7 was found to be 2.94 and 2.75 mins respectively. MS parameters of Penfluridol analyte and Penfluridol D7 used as the internal standard in the experiment, results are presented in Table 1.

Intra-day and Inter-day precision for determination of penfluridol results are presented in Table 2 and 3. Penfluridol tested and found to be stable in human plasma at different conditions, results are presented

in Table 4. % Recovery of analyte and internal standard was found consistent at all three level; result is presented in Table 5.

Method was found selective at 0.103 ng/ml in 8 different plasma lot. Sensitivity was found reproducible at concentration level 0.103 ng/ml with % nominal 98.1216 and %CV 4.85 (Figures 1–7).

Table 1. MS Parameters of penfluridol.

Analyte / IS	Q1 Mass	Q3 Mass	Dwell (m/sec)
PEN (Analyte)	524.300	203.100	300.00
PEN-D7 (IS)	531.400	204.100	300.00

Table 2. Intra day precision for determination of penfluridol.

	LLOQC	LQC	M1QC	MQC	HQC
AVG.	0.0982	0.2882	5.8212	19.3677	36.9180
SD	0.00966	0.04283	0.10924	0.31603	0.91779
%CV	9.84	14.86	1.88	1.63	2.49
%NOMINAL	94.391	95.419	92.546	92.381	88.047

Table 3. Inter day precision for determination of penfluridol.

	LLOQC	LQC	M1QC	MQC	HQC
AVG.	0.0979	0.2911	6.1348	20.5363	38.9596
SD	0.00945	0.02687	0.33179	1.32213	3.03502
%CV	9.65	9.23	5.41	6.44	7.79
%NOMINAL	94.151	96.385	97.533	97.955	92.916

Table 4. Stability of penfluridol.

Name of Stability	% Stability	Condition
Bench top (27 Hours 37 Minutes)	QC % Change value -12.252% for LQC and -9.319% for HQC	Ambient temperature
Freeze-thaw (-70°C±10°C after 4th Cycles)	QC % Change value -2.550% for LQC and -11.458% for HQC	-70°C ±10°C and room temp.
Auto-sampler /Wet extracted (50 Hours 45 Minutes)	QC % Change value -13.245% for LQC and -9.477% for HQC	10°C
Whole blood (02 Hours 03 Minutes_	QC Mean % Peak Area Ratio is 102.0085% for LQC and 100.8206% for HQC	Ambient temperature
Stock solution (After 06 days)	Mean % Stability is 100.529% for LQC and 101.942% for HQC	2-8°C

Table 5. Percentage recovery.

	Analyte	Internal standard
Recovery	68%	58%

Mass Spectrums

We prepared the tuning solutions of penfluridol containing the concentrations of 10ng/mL solution and injected into the mass spectrometer (Sciex 6500+). Which showed the full mass range of the

targeted penfluridol analyte m/z Q1 524.2 Dalton and Q3 203.200 Dalton, as shown in Figure 1 and Figure 2.

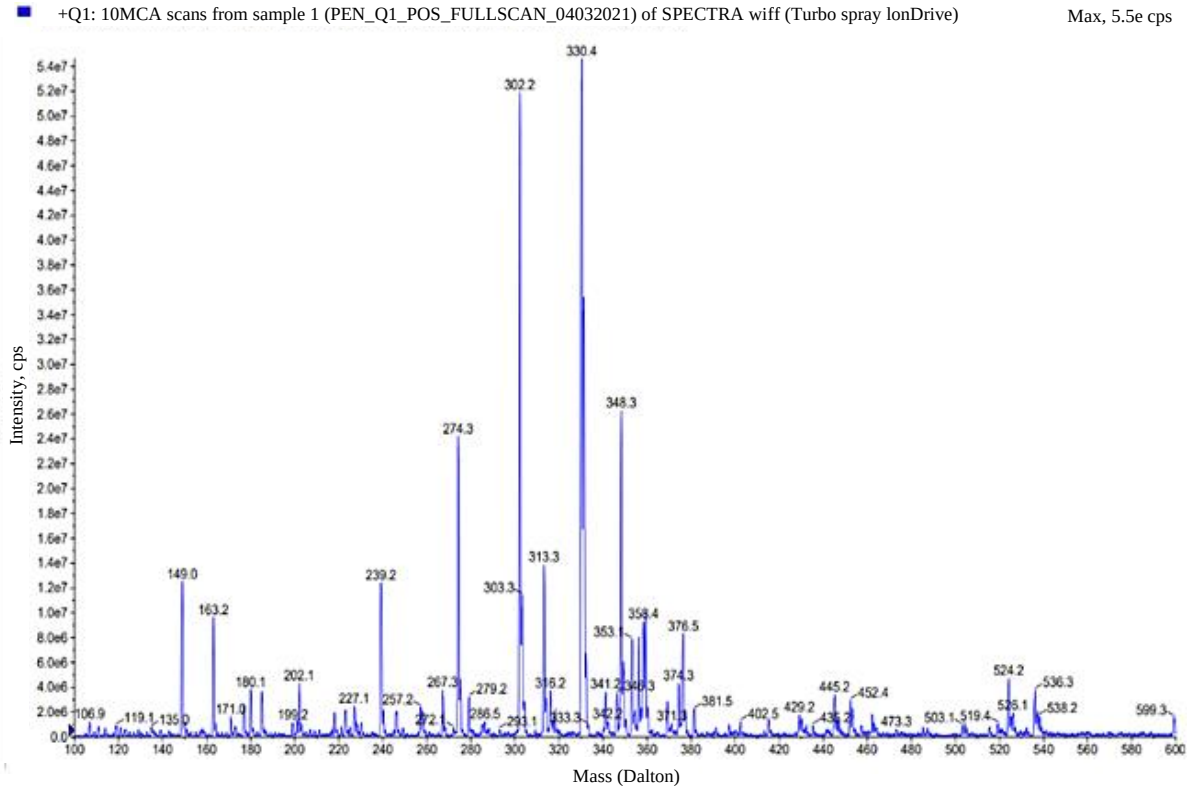

Figure 1. ms/ms spectrum of penfluridol (10ng/ml), q1 mass 524.300 dalton.

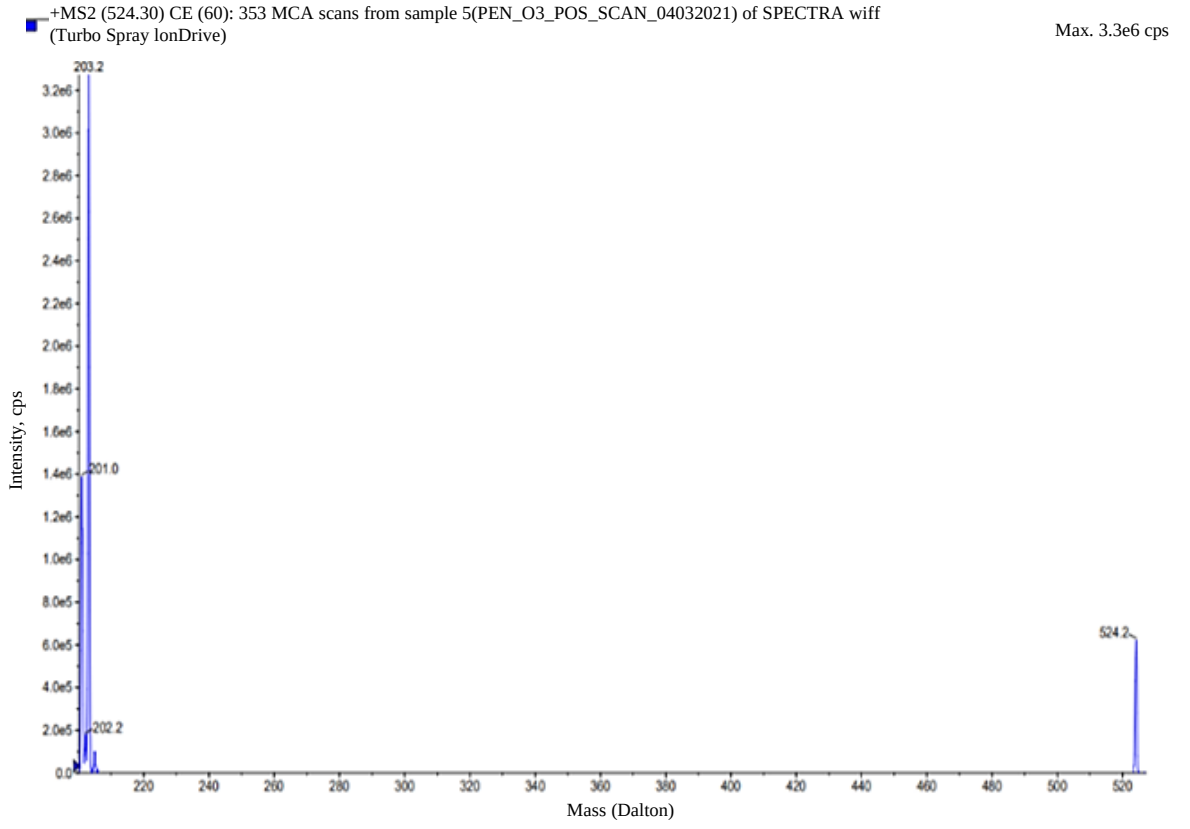

Figure 2. ms/ms spectrum of penfluridol (10ng/ml), q1 and q3 mass 524.300 dalton and 203.100 Dalton.

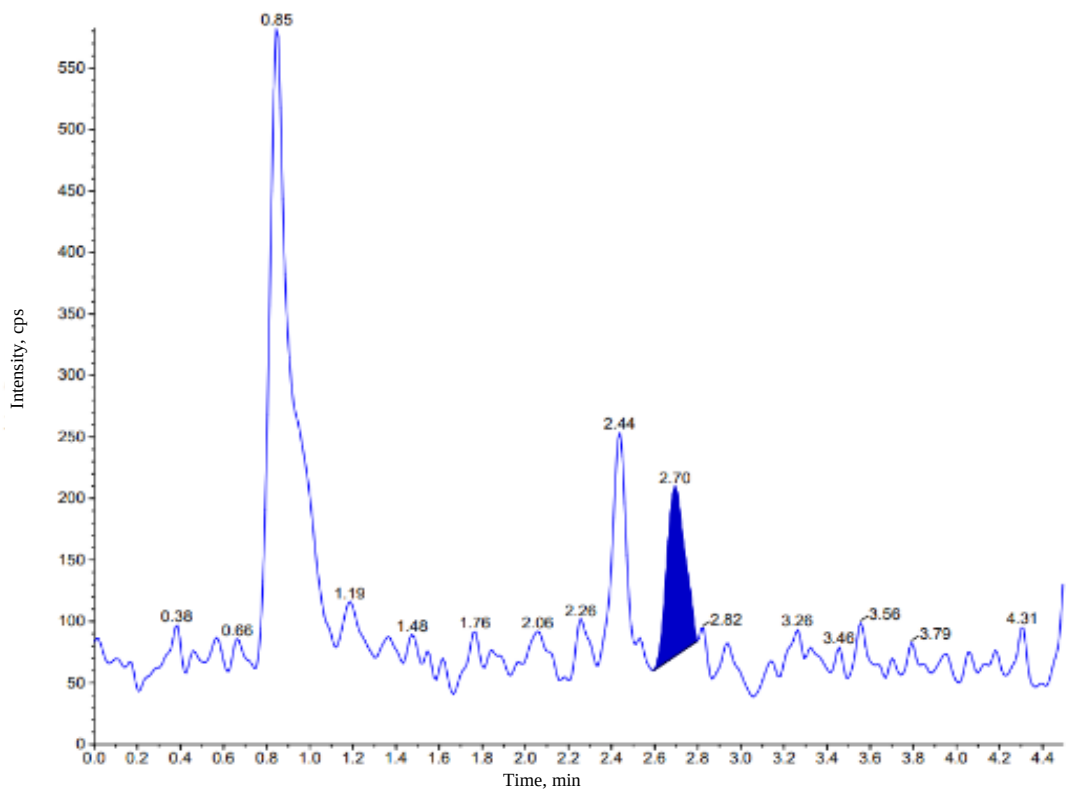

Figure 3. Penfluridol blank matrix chromatogram.

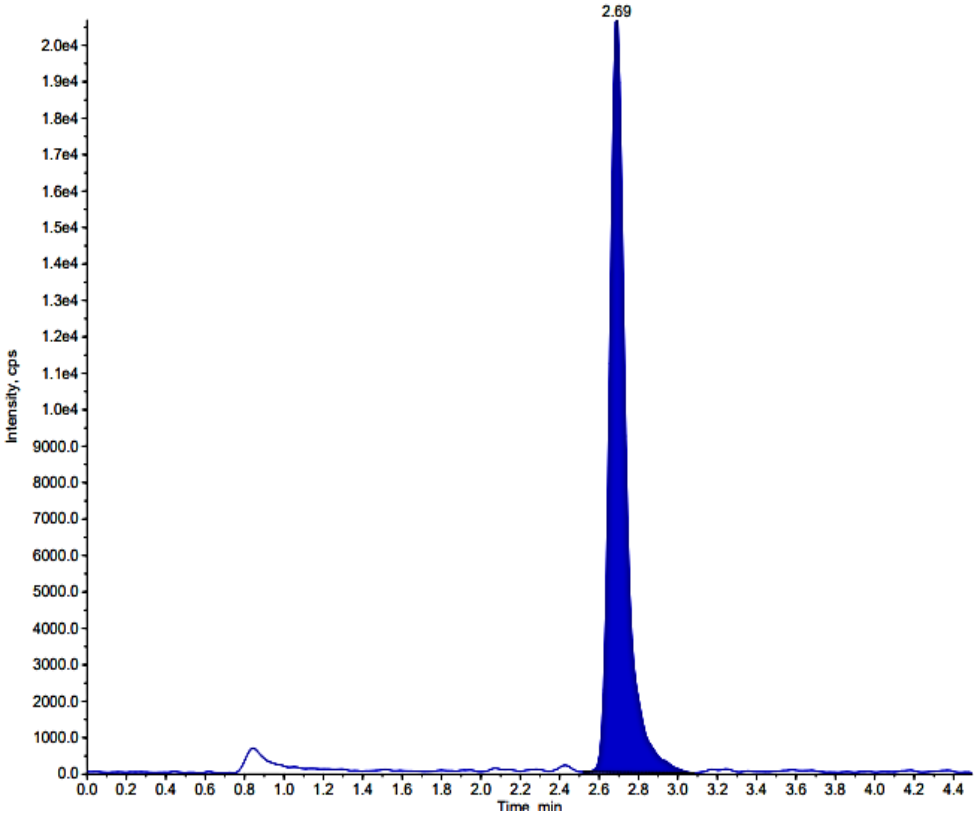

Figure 4. Penfluridol standard 1; LLOQC concentration is 0.102 ng/ml.

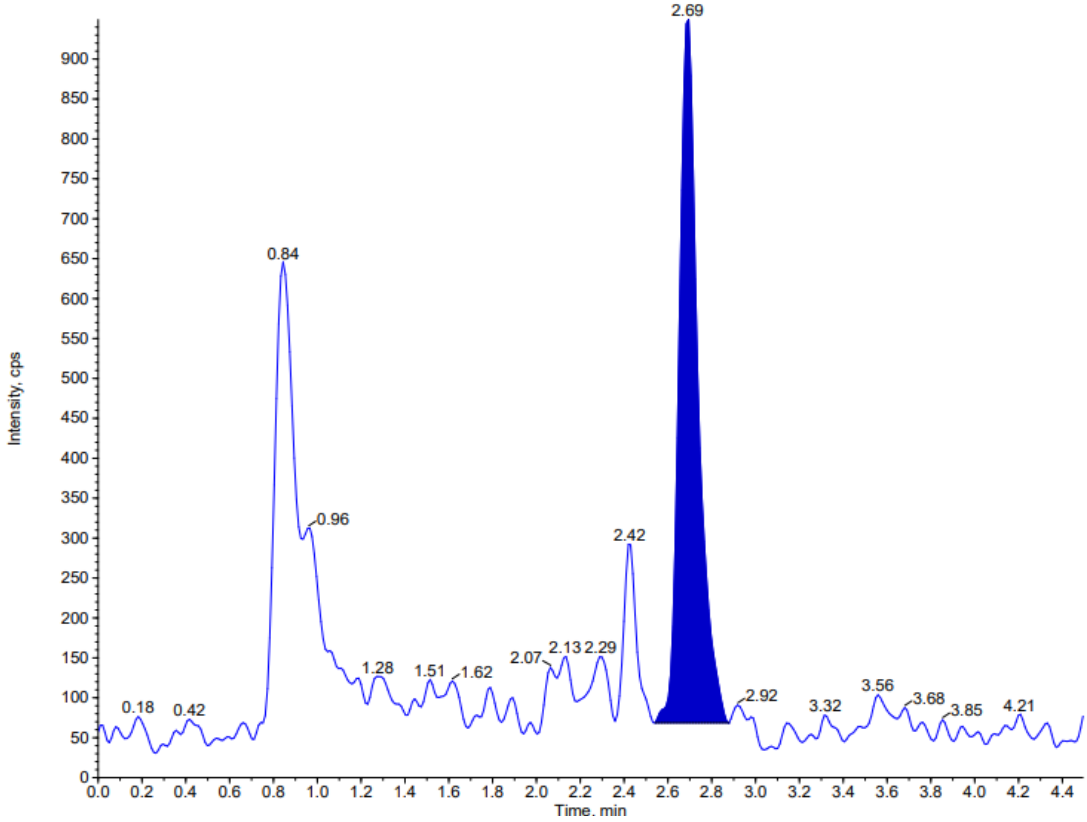

Figure 5. Penfluridol standard graph concentration is 30mg/ml.

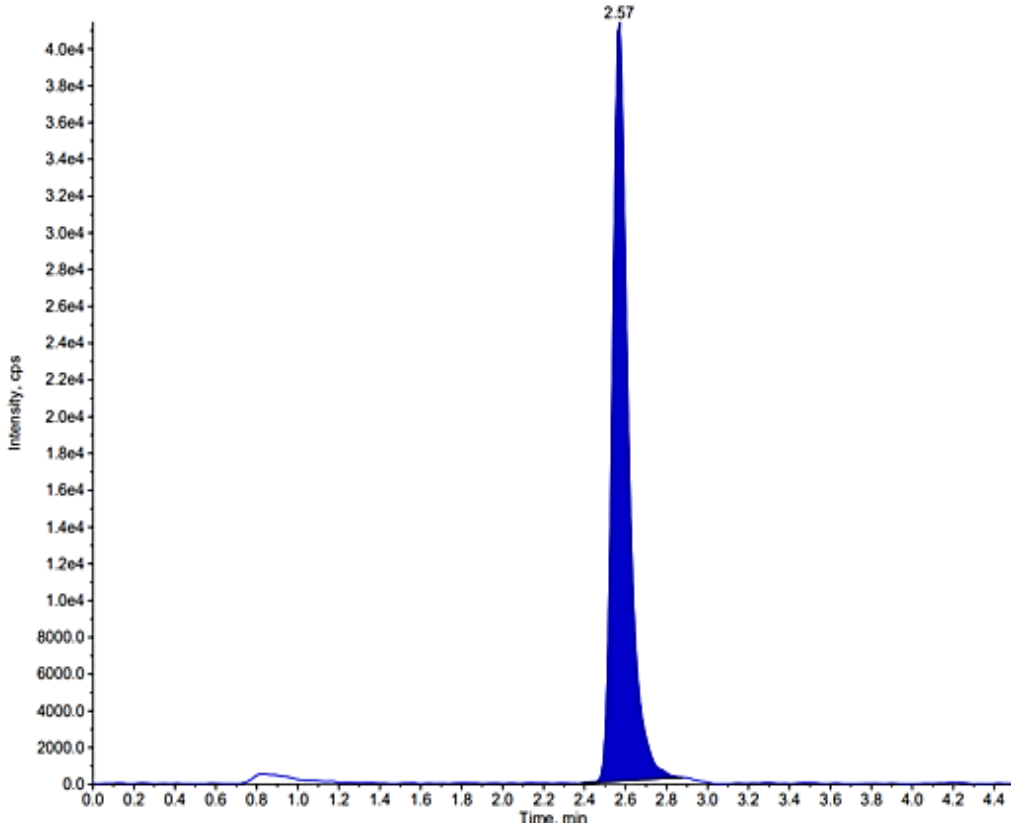

Figure 6. Penfluridol-d7 (internal standard) concentration 10µg/ml.

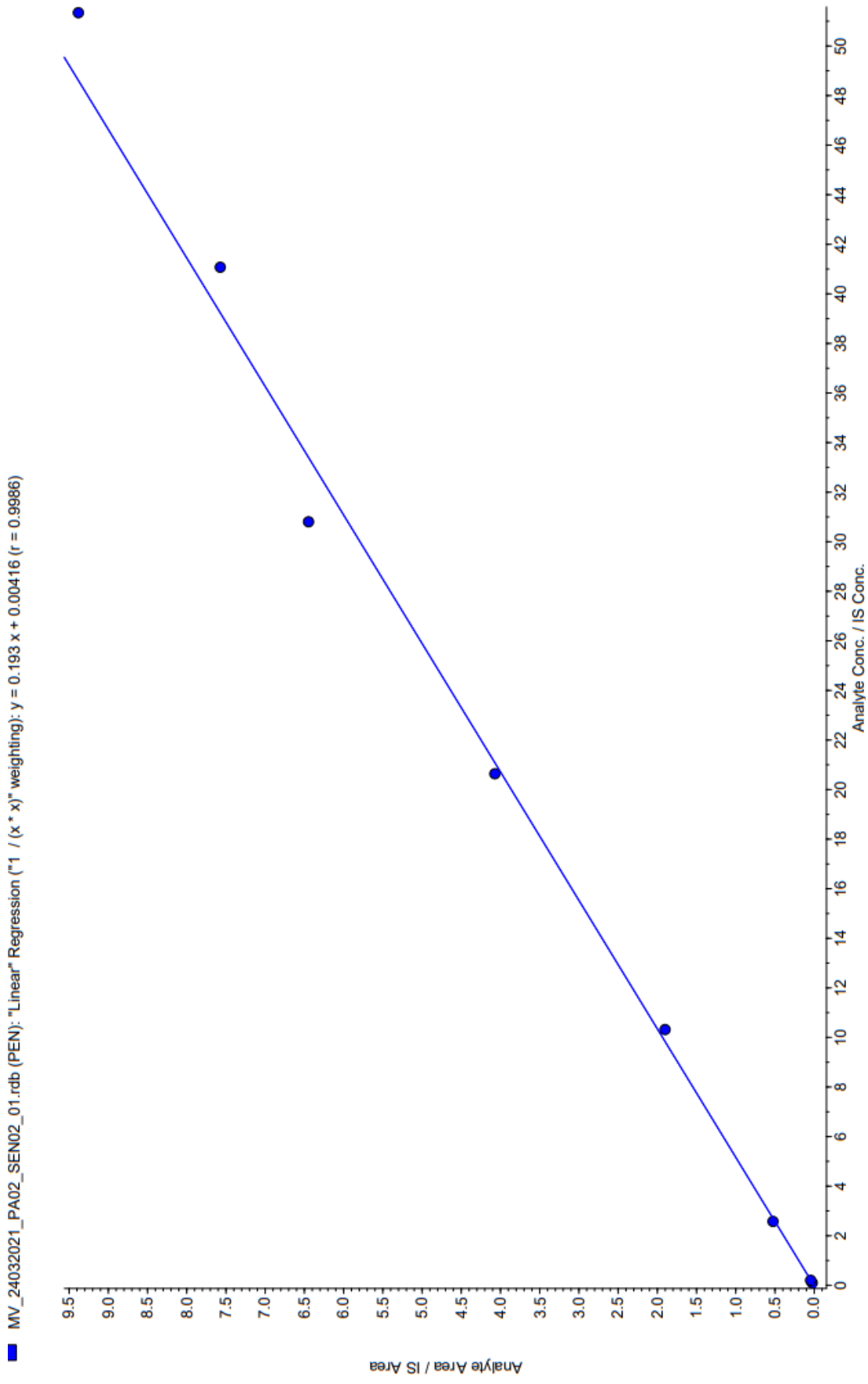

Figure 7. Calibration curve of penfluridol.

DISCUSSION

Chromatograms of Penfluridol analysis in human Plasma was analysed using the computer-based Analyst software (version number 1.7.1) manufactured by Sciex. For the analysis of Penfluridol the concentration is calculated from the equation by using the regression analysis of the spiked plasma calibration standard with the reciprocal of the square of the drug concentration weighing factor (1/concentration* concentration i.e., $1/X^2$) for analyte.

$Y=mx+b$

Where x, m, Y and b are as follows:

x= concentration of analyte
m= slope of calibration curve
Y= peak area ratio of analyte to internal standard
b= y-axis intercept of the calibration curve

Study Sample Analysis

This method is found suitable for Quantification of Penfluridol in Human Plasma samples for 20mg Dose of Penfluridol, plasma concentration for area under curve is presented in Figure 8.

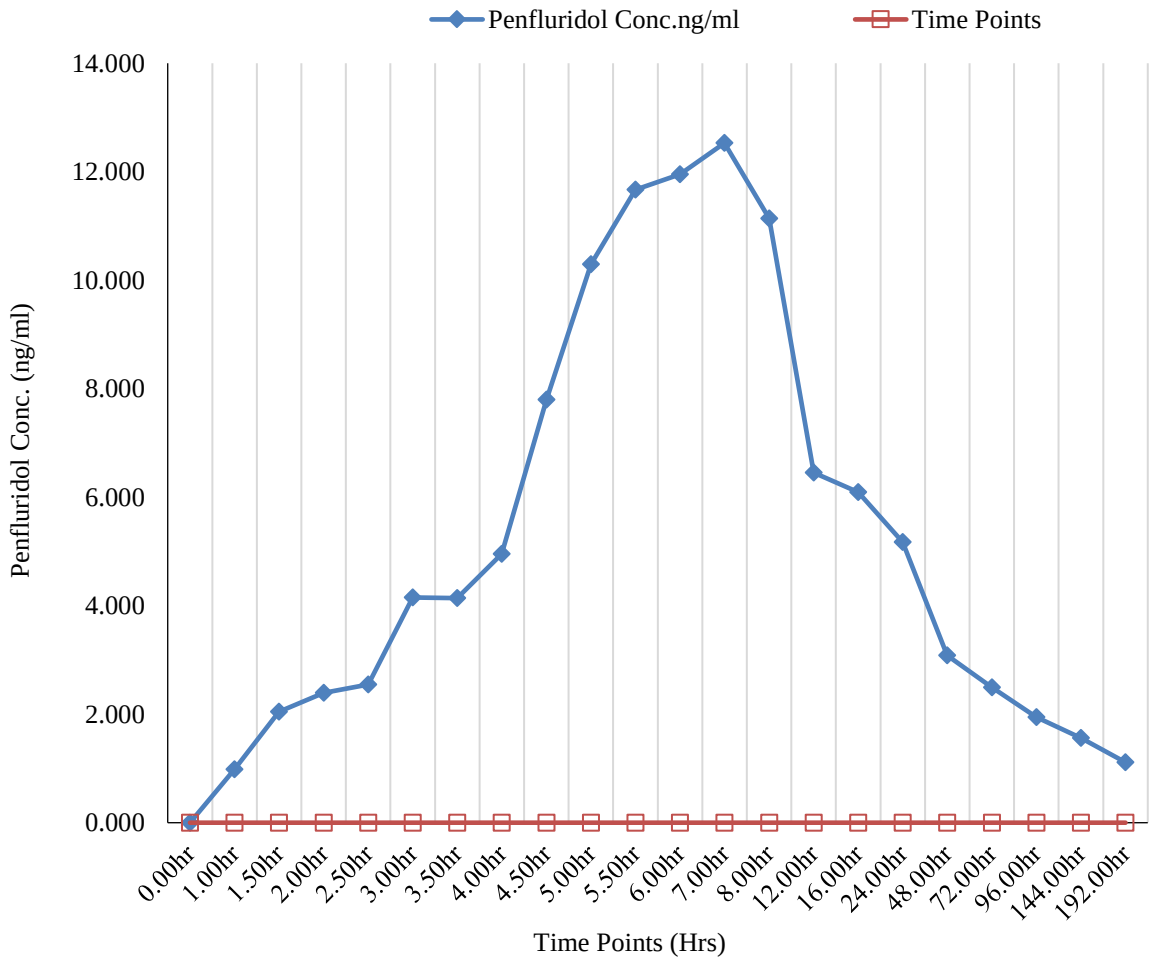

Figure 8. Plasma concentrations with respect to time points.

CONCLUSION

Pharmacokinetic research involving 24 healthy Indian volunteers was conducted using the approach. Evaluated prime pharmacokinetic parameters Cmax, tmax, AUC0t and AUC0-∞ for reference and test products. Study data was verified through the use of an expensive sample reanalysis (ISR). A

bioequivalence study of a 20 mg Penfluridol pill was successfully conducted using this newly designed method, which has a chromatographic run time of 4.50 min.

Acknowledgement

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