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Research Article

Simple and rapid LC-MS/MS method for determination of Piribedil in human plasma

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Abstract

A sensitive, simple, and fast LC-MS/MS method of analysis was developed and validated for the determination of piribedil in human plasma. Piribedil was extracted by protein precipitation using acetonitrile and separated on C18 Phenomenex Gemini column (150 × 4.6mm, 5 µm) using isocratic elution of 75% of ammonium acetate buffer (10 mM) and 25% acetonitrile at a flow rate of 1 ml.min-1 over 5 min run time. Piribedil and d8-Piribedil, as internal standard, were detected and quantified in positive ion mode via MRM at m/z 299/135 and 307/135 for piribedil and d8–piribedil, respectively. The suggested method for piribedil was validated according to FDA and EMA guidelines. The standard calibration curve was linear over the concentration range of 3.4–5952 pg.ml^{-1}. The intra-day precision was 2.45–9.94% and accuracy 92.78–99.97%. The inter-day precision was 2.14–5.47% and accuracy 95.73–101.99%. The recovery of analyte and IS was 96.94% and 111.18%, respectively. piribedil in plasma was stable at benchtop (short term) for 24 h, in autosampler tray for 48 h, in instrumentation room for 24 h (post-preparative), after 5 freeze-thaw cycles (–70 °C), and 11 days in the freezer (–70 °C). The validated method was successfully applied to a bioequivalence study of piribedil formulations involving 15 healthy Jordanian volunteers.

Keywords

LC–MS/MS, Method Validation, Piribedil, Human plasma, Bioanalytical, Pharmacokinetic study, Bioequivalence

Introduction

Piribedil is a derivative of alkoxybenzyl-4-(2-pyrimidinyl) piperazine (Fig. 1). A non-ergot dopamine receptor agonist with interesting pharmacology in that it is a D2 and D3 receptor agonist in addition to a 2-adrenoceptor agonist (Regnier et al. 1968; Gobert et al. 2003). Several effects on dopamine-related cerebral functioning have been observed (cognitive functions, motor regulation, behavioral disorders, visual perception and auditive) (Schück et al. 2002). Piribedil is an effective treatment for Parkinson's disease patients, in the control of tremors (Jenner 1992; Perez-Lloret and Rascol 2016). It's one of the most social and affordable medicine for Parkinson's disease patients (Kotvitska and Prokopenko 2020), it's also suggested for people with memory impairment (Wang et al. 2018), depression (Post 1978), intermittent claudication (Çelik et al. 2017), and restless legs syndrome (Louis et al. 2001).

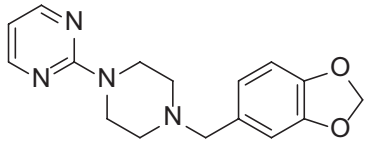

Figure 1. Chemical structure of Piribedil.

Only a few methods for piribedil in human plasma were published (Fanelli and Frigerio 1974; Sarati et al. 1991; Sarati and Caccia 1992; Altiokka et al. 2008; Uppuluri et al. 2018; Sultana et al. 2020). Fanelli and Frigerio (Fanelli and Frigerio 1974) described a method to detect piribedil in brain tissue and plasma by gas chromatography with flame ionization detector and liquid chromatography with mass fragmentographic technique. In addition, using a selective HPLC technology, Sarati et al. (Sarati et al. 1991) established a method for piribedil and of its basic metabolites determination in plasma using HPLC-UV. Moreover, a solid-phase extraction method has been developed to detect and quantify piribedil and its three principal basic metabolites before and after enzymatic hydrolysis in plasma and urine of rats (Sarati and Caccia 1992). A reversed-phase liquid chromatographic method for determining piribedil in human serum was introduced (Altiokka et al. 2008), with a LOQ and LOD of 96.6 and 290.4 pg.mL^{-1}. Determination of piribedil in human plasma has been developed by direct precipitation using HPLC–MS/MS method (Sultana et al. 2020). Piribedil was detected in plasma at concentrations from 0.02 to 5.00 ng. mL^{-1}. Piribedil was also extracted from rat plasma and brain samples using HPLC with fluorescence and photodiode array detectors (Uppuluri et al. 2018). In fluorescence and photodiode array detectors, linear response was achieved in the concentration ranges of (15–900), (450–9000) ng. g^{-1} in brain tissue and (5–300), (150–3000) ng. mL^{-1} in plasma (Venkateshwarlu and Patel 2022).

In this work, an LC-MS/MS method has been developed and validated to determine piribedil in human K_2EDTA plasma. It was demonstrated that this analytical procedure is simple, rapid, sensitive, and robust for piribedil plasma pharmacokinetic studies in humans.

Experimental

Chemicals and reagents

Piribedil and internal standard (IS) [$^2H_8$]–piribedil were obtained from ALSACHIM (Illkirch-Graffenstaden, France). LCMS grade Methanol was obtained from Sigma-Aldrich (Missouri, United States). LCMS grade Acetonitrile, ammonium acetate and water were obtained from Merck (Darmstadt, Germany). Acetic acid was obtained from ISOLAB (Eschau, Germany). Human K_2EDTA plasma was obtained from Pharmaceutical Research Unite (PRU) clinical site (Amman, Jordan).

Instrumentation and chromatographic conditions

The chromatographic method was accomplished by a Gemini C18 column (150 × 4.6mm, 5 µm) manufactured by Phenomenex (California, LA, USA) using a Shimadzu HPLC-LCMS 8060 system (Kyoto, Japan). The auto-sampler temperature was 4 °C. The analyte and IS were separated with an isocratic elution of (75: 25) ammonium acetate buffer (10 mM): acetonitrile, in a flow rate of 1 ml.min^{-1}. The mass spectrometric data were collected on a Shimadzu LCMS 8060 (Kyoto, Japan) with a triple quadrupole mass analyzer. A positive mode of ESI interface and multiple reactions monitoring (MRM) mode were intended for piribedil (m/z 299/135) and [$^2H_8$]–piribedil (m/z 307/135). The desolvation of spray analyte droplets was accomplished by adjusting the ESI parameters such as nitrogen gas at a flow rate of 3 L.min^{-1}. The analysis data were obtained by Lab solution software, version 5.9.1 from Shimadzu (Kyoto, Japan).

Preparation of standards and quality control samples

Standard solutions were prepared from stock solutions of Piribedil and IS of piribedil (0.2 µg.ml^{-1}), IS (104 µg.ml^{-1}) in methanol, and stored at -20 °C. Calibration standards and quality control (QC) samples were prepared by spiking blank plasma with piribedil at concentrations of 3.42, 6.85, 27.38, 136.90, 595.20, 2380.80, 5356.80, and 5952.00 pg.ml^{-1}. The final concentration of the QC samples was 3.42 pg. ml^{-1} (lower limit of quantification, LLOQ), 6.85 pg.ml^{-1} (low quality control-1, QCL-1), 27.38 pg.ml^{-1} (low quality control-2, QCL-2), 1785.60 pg.ml^{-1} (middle quality control, QCM), and 4464.00 pg.ml^{-1} (high quality control, QCH).

Sample preparations

To extract piribedil and IS from human plasma, protein precipitation by acetonitrile was used. An aliquot of 500 µL spiked human plasma sample was transferred to an Eppendorf micro tube (2 ml), and vortex-mixed with 50 µL IS (26.02 ng.ml^{-1}) for 30 s. 1 mL of cold acetonitrile was added to the sample and vortexed for 1 min then centrifuged (5 min at 13000 rpm, 2–8 °C). Finally,180 µL was injected into the LC-MS/MS unit (Reeder et al. 2022).

Method validation

The developed method was validated according to FDA (Food and Drug Administration 2018) and EMEA (Committee for Medicinal Products for Human Use 2011) guidelines for bioanalytical method validation. Selectivity, precision, sensitivity, accuracy, matrix effect, linearity, recovery, stability, and dilution integrity were all evaluated. The selectivity was evaluated by injection of eight different lots of hemolyzed and hyperlipidemic blank plasma. Caffeine, paracetamol, diclofenac, ascorbic acid, nicotine, aspirin, and ibuprofen were all examined as potential conco-

mitant medication interference. The linearity was assessed using calibration curves with eight standard levels. Sensitivity was tested by analyzing six triplicates of LLOQ against a calibration curve. The matrix effect was studied at two levels (QCL-1 and QCH) using eight different lots of blank plasma including hemolyzed, and hyperlipidemic. Inter- and intra- day precision and accuracy were assessed using six determination levels at LLOQ, QCL-1, QCL-2, QCM, and QCH were extracted and assessed against the calibration curve. The peak area for both the extracted analyte standard and the non-extracted standard was compared to establish piribedil and IS recoveries. The bioanalytical method's recovery was calculated for piribedil at three concentration levels (QCL-1, QCM, and QCH), and for IS at the QCH concentration. Dilution integrity at a concentration of five times the concentration of QCH was tested. Six replicates of each concentration were tested. Stability tests were performed under various conditions for the stock solutions and plasma samples to assess analyte stability. The stability of the stock solution was tested at room temperature and -20 °C by comparing the area of piribedil in stability sample to the area in the freshly prepared solution. Six duplicates at QCL-1 and QCH levels were used to examine bench top stability (24 h), long-term stability (11 days), freeze-thaw (five cycles), and autosampler stability (24 h).

Pharmacokinetic study

The pharmacokinetic parameters of piribedil were measured in fifteen healthy Jordanian volunteers in a pharmacokinetic study intended to measure the rate and extent of piribedil absorption from piribedil oral tablets. This study was approved by the Institutional Review Board/Independent Ethics Committee (IRB/IEC). This study was directed according to the Declaration of Helsinki for biomedical research, which specifies that all subjects must take an informed consent containing all information needed about the purpose of the study, the procedures, and the risks that could be happening. The volunteers have been stopped eating up to 10 h before dosing, but the water was available. The volunteers received a single 50 mg oral dose of piribedil with 250 mL water. 6 mL of blood samples were collected from a forearm vein in labelled K_2-EDTA blood tubes 9.0 mL at (pre-dose) and at 1, 2, 3, 4, 5, 6, 8, 10, 11, 12, 13, 14, 15, 16, 20, 24, 28, 32, 40, 48 and 72 hours postdosing. Samples were centrifuged at 4000 rpm for 5 min at 4 °C. Supernatant plasma was transferred to polypropylene tubes and stored in a freezer at -70 °C. pharmacokinetic parameters were assessed using WinNonlin (version 8.3) software (Scientific Consulting Inc., NC, USA).

Results and discussion

Method development

Both negative and positive ionization modes mass parameters were investigated for piribedil. The positive mode response was more suitable than the negative mode. Chromatographic parameters were optimized to achieve high resolution and improved piribedil signal intensity yet while maintaining a short run time. Piribedil detection was improved by the addition of acetic acid in the mobile phase. Different ratios of mobile phase were evaluated, and the optimum ratio was 25:75 acetonitrile: 10 mM ammonium acetate buffer solution. Many C18 column brands were tested and Phenomenex Gemini C18 (150 × 4.6mm, 5 m) was chosen as it gives rise to optimum peak shape. Piribedil and IS retention time was were 3.98 ± 0.40 and 3.62 ± 0.36 min, respectively, which facilitate a small run time (5 min) using a 1 $mL.min^{-1}$ flow rate. A one-step protein precipitation extraction approach was used for sample preparation as it is a simple and fast extraction method and the resulted drug recovery was suitable.

Method validation

Selectivity

Selectivity is the degree of interference with piribedil and IS by endogenous plasma components was evaluated by examining chromatograms generated from processed blank plasma samples. As illustrated in Fig. 2, endogenous components in drug-free plasma did not interfere with the analyte peak. Also, commonly used medications (caffeine, paracetamol, diclofenac, ascorbic acid, nicotine, aspirin, and ibuprofen) showed no interference.

Sensitivity

The method sensitivity was determined by signal to noise ratio in the LLOQ sample method to ensure less than five times response in the blank as compared to LLOQ. The value of 3.42 $pg.ml^{-1}$ was chosen as the LLOQ for piribedil. At LLOQ concentrations, piribedil's precision and accuracy were determined to be 9.78% and 99.97%, respectively, which indicate good sensitivity of the method.

Matrix effect

The quantitation of the matrix effect was evaluated by comparing the peak area of analyte and IS from aqueous samples (representing 100% recovery at QCL-1 and QCH levels) to the extracted post-spiked blank with aqueous samples QCL-1 and aqueous samples QCH, respectively. The precision for piribedil was found to be 1.90%, and 4.95% at QCL-1 and QCH concentration, indicating no significant matrix effect was detected in the method.

Linearity

The linearity was assessed over the concentration range of 3.42–5952.00 $pg.ml^{-1}$. The method was linear over tested range. A regression equation with a weighting factor ($1/x^2$) of piribedil to the IS concentration provided the best match for the concentration–detector response relationship for piribedil in human plasma. The weighted calibration curves created during validation have a mean correlation coefficient of 0.999 (Venkateshwarlu and Patel 2022)

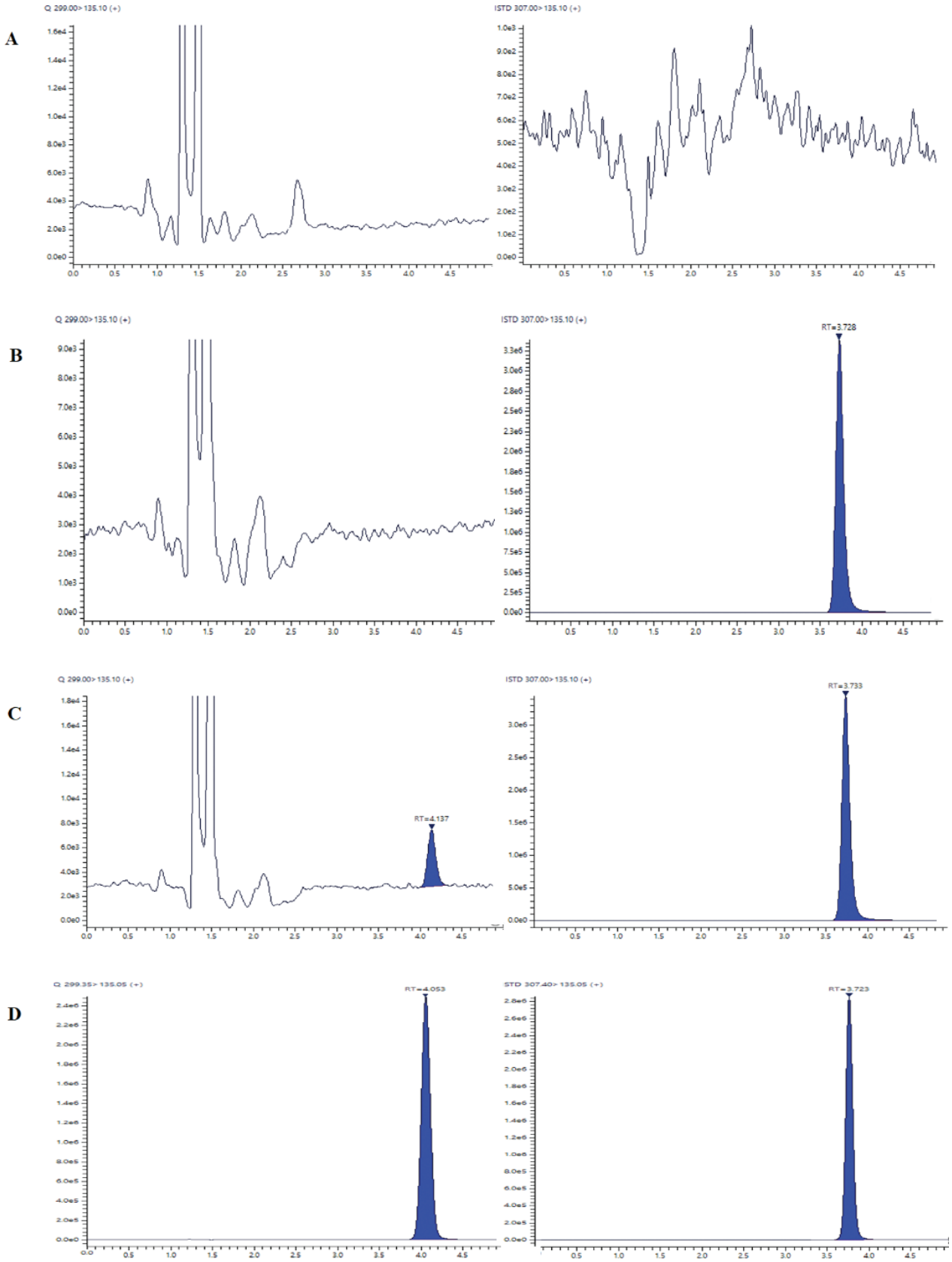

Figure 2. MRM ion-chromatogram of Piribedil (m/z 299.35 > 135.05) and d8-Piribedil IS (m/z 307.40 > 135.05) in (**A**) blank plasma (without analyte and IS), (**B**) blank plasma with IS, (**C**) Piribedil at LLOQ with IS and (**D**) real subject sample at Cmax after administration of 50 mg dose of Piribedil.

Table 1. Intra-day and inter-day precision and accuracy for piribedil.

QC Level	Intra-day (n=18)				Inter-day (n=6)			
	Measured concentration (pg/mL)	Mean concentration found (pg/mL)	Accuracy (%)	CV (%)	Measured concentration (pg/mL)	Mean concentration found (pg/mL)	Accuracy (%)	CV (%)
LLOQ	3.42	3.42	99.97	9.94	3.42	3.49	101.99	5.47
QCL-1	10.27	9.742	94.89	3.30	10.27	9.83	95.73	3.42
QCL-2	102.67	100.45	97.83	4.49	102.67	104.01	101.30	5.34
QCM	1785.6	1776.4	99.48	2.87	1785.6	1778.8	99.62	2.58
QCH	4464.0	4141.8	92.78	2.45	4464.0	4230.5	94.77	2.14

Table 2. Stability data for piribedil in human plasma samples (n = 6).

Stability test	Spiked concentration, (pg / mL)	Mean ± SD (pg / mL)	Accuracy / stability (%)	CV (%)
Autosampler stability (at 25°C for 24 h)	10.27	10.57 ± 0.62	102.96	5.84
	4464.0	4453.51 ± 53.07	92.12	3.64
Bench top stability (24 h at room temperature)	10.27	9.73 ± 0.58	94.75	5.95
	4464.0	3853.64 ± 130.44	86.33	3.38
Freeze-thaw stability (five cycles)	10.27	10.59 ± 1.29	103.16	12.15
	4464.0	3984.78 ± 82.20	89.26	2.06
Reinjection stability (68 h)	10.27	10.28 ± 0.33	100.14	3.24
	4464.0	4409.20 ±76.51	98.77	1.74
Long-term stability (at -70 °C for 11 days)	10.27	8.99 ± 0.44	87.63	4.89
	4464.0	4059.42 ± 95.61	90.94	2.36

Precision and accuracy

The developed method accuracy was assessed in terms of % recovery. % Recovery was found to be from 92.78 to 99.97% for intra-day study, and within 94.77 to 101.99% for inter-day accuracy. Inter- and intra- day precision were evaluated using six and eighteen replicates respectively. The CV% for both precisions was in the range of 2.41 to 9.94%. All results are summarized in Table 1.

Extraction efficiency

One-step protein precipitation using acetonitrile proved to be simple, effective, and robust. Recovery was assessed by comparing the piribedil peak area ratio of "treated samples" with those "un-treated samples", as showed by the following formula:

$$\text{Recovery (\%)} = \frac{\text{Mean area ratio of drug for untreated samples}}{\text{Mean area ratio of drug for treated samples}} \times 100\,\%$$

The piribedil and the IS recoveries were reproducible and good. The overall mean recoveries of piribedil were 96.94% and the CV was less than 2.0%. The IS recovery was 111.18%, and the CV was less than 3.0%.

Dilution integrity

The dilution integrity was assessed for a sample at a concentration of 5 times ULOQ, which was named dilution QC. Dilution QC samples were further diluted with interference-free plasma dilutions for determining the dilution integrity of the samples. The method was accurate and precise up to 22320pg/ml^{-1}, with a dilution factor of 5.

Stability

Piribedil was stable in human plasma for 24 h at 25 °C. The extracted plasma samples results indicated that piribedil was stable in the auto-sampler for 24 h at 25 °C. Long-term stability was proven for frozen QC samples after 11 days at -70 °C. Samples were found to be stable after being subjected to five freeze and thaw cycles. As Table 2 shows, there was no significant loss of piribedil during sample storage nor repeated freezing and thawing conditions.

Application to a pharmacokinetic study

The suggested and validated LC-MS/MS method has been successfully implemented for measuring the pharmacokinetic parameters of piribedil in 15 healthy males after being administered one prolonged-release tablet containing 50 mg piribedil under fasting conditions. The results of the pharmacokinetic parameters illustrated that the average maximum plasma concentration (C_{max}) ± SD of piribedil for the twenty subjects was 350.91 ± 199.49 pg. mL^{-1} and reached the average time ± SD of 10.87 ± 10.95 h. The other parameters were the area under the curve ($AUC_{0\text{-}t}$ and $AUC_{0\text{-}\infty}$) for piribedil and those were found to be 4618.12 ± 3299.34 pg.h.ml^{-1} and 4080 ± 3028.12 pg.h.ml^{-1} for $AUC_{0\text{-}t}$ and $AUC_{0\text{-}\infty}$, respectively as illustrated in (Table 3).

Table 3. Pharmacokinetic parameters (Mean ± SD) of oral Piribedil (50 mg) prolonged-release tablet.

Parameters	Piribedil
C_{max} (ng.ml^{-1})	350.91 ± 199.49
T_{max} (h)	10.87 ± 10.95
$AUC_{0\text{-}t}$ (pg.h.ml^{-1})	4618.12 ± 3299.34
$AUC_{0\text{-}\infty}$ (pg.h.ml^{-1})	4080 ± 3028.12
K_{el} (h^{-1})	0.055 ± 0.026

Conclusion

A rapid, simple, and sensitive LC-MS/MS for piribedil quantification in human plasma was developed and fully validated according to FDA and EMA guidelines. The used protein precipitation extraction technique gave consistent and reproducible recoveries for piribedil. The method was accurate and precise for the determination of piribedil in human plasma throughout a concentration range of 3.42–5952 $pg.ml^{-1}$ and should be useful for regular monitoring of drug concentrations in pharmacokinetic investigations. The method was successfully applied to determine piribedil in healthy subjects and pharmacokinetic parameters were calculated.

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