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Research Article

A Novel Bioanalytical LC/MS/MS Assay for the Determination of Procyclidine in Human Plasma and its Pharmacokinetics Applications

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Abstract

Background: Parkinson's disease (PD) is a prevalent chronic degenerative nervous system disorder. Procyclidine is a medication used improve to motor functions.

Aim: Development of a bio-analytical procedure for rapid quantitative estimation of procyclidine in human plasma and its application in pharmacokinetics, bioavailability studies, and therapeutic drug monitoring to aid in the achievement of effective clinical results in Parkinson's disease management.

Methods: Sample clean-up was performed to extract procyclidine from plasma samples using liquid-lquid extraction technique. Chromatography was performed using 4mM Ammonium Acetate : Methanol 25 : 75 V/V. The pump flow rate was setted at 0.7 ml/min. ESI was setted at positive mode, and m/z 288.2 → 84, 302.2 → 98.1 for procyclidine and trihexyphenidyl as internal standard respectively. A comparative bioavailability study of procyclidine 5mg tablets generic product versus reference product was done in a crossover design with 24 subjects as an application of the validated bioanalytical methodology. The pharmacokinetic parameters used to evaluate the two products bioequivalence were C_{max}, AUC_{0-t}, AUC_{0-inf}, and $T_{max.}$

Results: The average procyclidine recovery was 91.118%. The quantitation limit was 0.1 ng/ml, and the correlation coefficient (r^2) was 0.9998. Furthermore, statistical analysis of the results obtained revealed no significant difference between the two products.

Conclusion: The established bioanalytical LC/MS/MS method was successfully applied for procyclidine quantification in human plasma. Furthermore, it is appropriate for use in pharmacokinetic studies and therapeutic drug monitoring in parkinson's disease management. This can ensure clinically effective drug levels in human plasma and avoid possible unwanted adverse outcomes.

Keywords: Procyclidine; Parkinson's Disease; LC/MS/MS; Validation; Liquid-liquid Extraction

Introduction

Procyclidine is a synthesized antispasmodic compound with a relative low toxicity. It has been demonstrated to be effective in treating the symptoms of parkinsonism and extrapyramidal dysfunction produced by tranquillizer medication [1].

The central cholinergic projections are very diffuse. The major routes originate in the mid and hindbrain from cholinergic neurons such as Meynert's nucleus basalis magnocellularis and extend to cortical neurons [2].

Procyclidine principally inhibits muscarinic (M) receptors M2, M1, and M4, the latter two of which are widely distributed

throughout the brain; M2 is the heart isoform of the receptor and is not commonly seen in other organs [3].

Plasma procyclidine profile were represented by one compartment oral model. After administration of 10mg procyclidine tablet, the average apparent volume of distribution was 1.01 L/kg. Elimination half life was 12.6 ± 4.8 hrs. The average maximum plasma concentration (C_{max}) was 116 ± 39.2 ng/ml. And the mean value of time to maximum plasma concentration (T_{max}) was 1.1 ± 0.36 hrs [4].

The only existing method for quantitative determination of procyclidine in either plasma or urine is besed on a gas-liquid chromatographic (GLC) procedure which includes an inconvenient initial isolation step and lacks adequate sensitivity for detailed pharmacokinetic studies [5].

An analytical method for the simultaneous determination of enantiomers of trihexyphenidyl, procyclidine and biperiden in human serum by liquid chromatography–electrospray ionization mass spectrometry (LC–ESI-MS) with on-line sample extraction. The method employs a column-switching technique [6-9].

The goal of this study was to develop a reliable bioanalytical technique to estimate procyclidine in biological fluids and to use it in pharmacokinetic and bioavailability studies, and therapeutic drug monitoring in patients to ensure efficacy and safety of Parkinson's disease (PD), and bipolar disorders treatment.

A comparative bioavailability study of procyclidine generic tablets versus reference tablets was performed according to international guidelines to examine the reliability of the established bioanalytical procedure for the quantitative determination of procyclidine in biological fluids. The protocol called for 24 healthy subjects with a seven days washout period [10].

The verified LC/MS/MS technique developed was in accordance with international requirements for the bioanalysis of biological samples [11], and the pharmacokinetic calculations were performed with WinNonLin software. Furthermore, statistical analysis (ANOVA) was performed using SAS software, and the 90% C.I. limits for the ratio of generic to reference products results were calculated to investigate the compliance with the confidence limits acceptance range of 80 to 125 percent [12].

Methods

Liquid chromatography tandem mass spectrometry bioanalytical method

Chromatographic conditions and Mass spectrometry parameters

The following method was developed in-house: the content of mobile phase is 4mM Ammonium Acetate : Methyl alcohol 25 : 75 V/V, the pump flow rate was 0.7 ml/min. The injection volume was 1ul. The Mass detector was set to positive ESI mode, m/z (mass to charge ratio) was 288.2 → 84, 302.2 → 98.1 for procyclidine and trihexyphenidyl as internal standard respectively. The fragmentor energy was set at 130 for both procyclidine and trihexyphenidyl, and collision energy was set at 18 for procyclidine, 20 for trihexyphenidyl.

Solutions preparation

Procyclidine Standard Solution

In a 100 ml volumetric flask, an accurately weighed 11.35mg of procyclidine hydrochloride working standard was transferred (equivalent to 10mg of procyclidine base). After that, 80 ml of methanol was added. The flask was sonicated for 10 minutes, then the volume was completed methanol, to obtain a 100 ug/ml procyclidine (Solution A). From (solution A) 1 ml was transfered to a 100 ml volumetric flask and completed to volume with methanol to obtain 1000 ng/ml procyclidine (Solution B).

Working solutions

Standard Solution used	mls taken	Final Conc. obtained (ng/ml)	Final volume (ml)
"Solution B"	0.01 ml	1	10
"Solution B"	0.05 ml	5	10
"Solution B"	0.1 ml	10	10
"Solution B"	0.5 ml	50	10
"Solution B"	1 ml	100	10
"Solution B"	2.5 ml	250	10
"Solution B"	5 ml	500	10
"Solution B"	10 ml	1000	10

Table a

The above dilutions are all prepared with methanol.

Trihexyphenidyl standard solution

In a 100 ml volumetric flask, an accurately weighed 10mg of trihexyphenidyl hydrochloride standard was transferred. After that, 80 ml of methanol was added. The flask was sonicated for 10 minutes, then volume was completed with methanol to obtain solution of 100 ug/ml trihexyphenidyl hydrochloride. From this solution, 0.4 ml was transferred to 100 ml volumetric flask and the volume was completed with methanol to obtain a solution of 400 ng/ml trihexyphenidyl hydrochloride.

Serial dilution preparation of standard procyclidine in human plasma

Standard concenetrations of procyclidine in plasma were prepared by transferring 25 ul aliquot of procyclidine standard solutions (in the range from 1 to 1000 ng/ml) to a 250 ul of blank plasma.

Preparation of plasma samples

Subject plasma samples (250 ul) were placed into centrifuge tubes, and 25 ul of trihexyphenidyl working solution 400 ng/ml was added, then vortex-mixed for half minute before adding 3 ml of diethylether: dichloromethane 70:30 v/v and vortex-mixing for about 60 to 120 seconds. The samples were then centrifuged at 3500rpm for five min at 4°C, and the transparent organic supernatant layer was transferred to a clean test tube and evaporated until drying. Then, 200 ul mobile phase was used to reconstitute the dry residue, which was then transferred to an insert vial for drug quantification on LC/MS/MS.

Quantitation

The unknown sample concentration was determined using a formula: Y = aX + b. Where Y: peak response ratio, X: unkown drug concentration in samples, a: slope, b: Y-Intercept.

Method application on comparative bioavailability study

Ethics

The bioequivalence study was carried out in compliance with the ICH and GCP principles set by (EMEA) and after the approval of Ethics Committee on the Procyclidine Hydrochloride 5mg tablet study protocol (Code: PRO-CLAP-BES-0519/0266). According to Drug Research Center internal systems, all paperwork and records were archived.

A documented informed consent was signed by the participant, clinical investigator, and other relevant parties. All research details were explained with participants prior to the commencement of the screening stage. Subjects were under no obligation to complete the bioequivalence research if they did not want to.

All study activities were overseen by the principal investigator and clinical investigator. Licensed doctors were in charge of subject physical examination and follow-up for vital sign measurements such as body temperature, blood pressure, pulse rate, breathing rate, and the occurrence of any side or adverse reactions through the study. Registered nurses collected blood samples.

Inclusion criteria

Subjects ranged in age from 18 to 55 years old, with calculated body mass index (BMI) within normal permissible range. Normal physiological examination and laboratory results were obtained. Subjects should not be alcoholics or drug addicts, and they should have no known history of either, as well as no clinical history for near study contribution. Nonsmokers were favored over smokers, and if must, the limit should not be more than 8 cigarettes per day.

Exclusion criteria

Any procyclidine hypersensitivity, gastrointestinal tract problems, hematological abnormalities, renal disease, auto-immune diseases, cardiovascular diseases, diabetics, liver disease, respiratory diseases, history of alcohol and drug abuse, positive human immunodeficiency virus, abnormal lab results, subject treated with any medication less than 2 weeks of the starting date of the bioequivalence study, blood donation or participation in clinical studies that requires more than half liter of blood loss within month and half prior the bioequivalence study starting date.

Subjects

The bioequivalence study included twenty-four healthy adult individuals who were submitted to a general physical examination, a neurological examination, and clinical lab analysis. The individuals were chosen because they are fulfilling all inclusion/exclusion criteria. Concurrent medication was not permitted during the research's duration, and no meals, drinks, coffee, or tea were

permitted for four hours following study dosage administration. They were served a standard breakfast at 11:00 a.m., followed by a lunch meal at 3:00 p.m.

Study design

The design of this study was a two-way crossover, single-dose, single-center, open-label randomized design to compare the bioavailability of generic againest reference procyclidine hydrochloride 5mg tablet in 24 healthy adult subjects under fasting conditions with a 7 days washout period.

Collection of sample

The number and timing of the blood sample collection as well as the wash out interval were planned in accordance with procyclidine pharmacokinetic properties.

In each study phase, 17 blood samples were obtained, with 5 ml collected at the following intervals: 0 (directly prior to dosing), 20 min, 40 min, 1, 1.33, 1.67, 2, 2.5, 3, 4, 6, 8, 10, 12, 24, 48 and 72 hours after procyclidine administration in tubes containing EDTA disodium as anticoagulant. The collected blood samples were centrifuged at 4000 r.p.m. for 10 minutes and plasma samples were separated in a plastic wassermann tube. The obtained samples were kept at -80°C until they were analysed. All sample/study related data was recorded on each sample tube. The total volume of blood collected for the entire study did not surpass 170 ml.

Analysis of samples

The qualtitative determination of procyclidine in subject plasma samples was conducted by LC-MS/MS using the established bioanalytical technique, which was validated according to international standards.

Calculation of the pharmacokinetics parameters

The following pharmacokinetic parameters were evaluated;

- Maximum plasma concentration (C_{max}).
- Time point of maximum plasma concentration (t_{max}).
- Elimination Half-life ($t_{1/2e}$).
- Elimination rate (K_{el}).
- Area under plasma concentration-time curve from zero to time t (AUC_{0-t}).
- Area under plasma concentration-time curve from zero to infinity (AUC_{0-inf}).

Statistical analysis

Statistical computerized program SAS software was used for analysis of variance (ANOVA). Procyclidine bioequivalence was proven within the recommended 90 percent confidence level of 80 to 125 percent on Ln-transformed data for C_{max}, AUC_{0-t}, and AUC_{0-inf} using the parametric method.

Tolerability and safety

All incidences of suspected adverse responses to the study formulations were documented, as were the subjects' medical histories, physical examinations, and laboratory tests.

Measurement of blood pressure and heart rate

Tolerability assessments comprised blood pressure (cyctolic and diastolic) and pulse rate measures before drug administration (Zero time) and at regular intervals (at 2, 4, 6, and 10 hours) post administration. Normal blood pressure is 120/80 mmHg with a resting heart rate of 50 to 100 beats per minute.

Results

Bioanalytical method validation

Chromatograms of procyclidine

Figures (1), (2), and (3) demonstrate that procyclidine and trihexyphenidyl were well separated, with a retention time of 1.7 minute. Sharp and symmetrical peaks showed a good baseline with minimal tailing, allowing for reliable peak response measurement. The in house developed chromatographic conditions was developed to provide for literature [5-9] a valid bioanalytical method for accurate determination of procyclidine in plasma with a reasonable extraction procedure and chromatographic conditions.

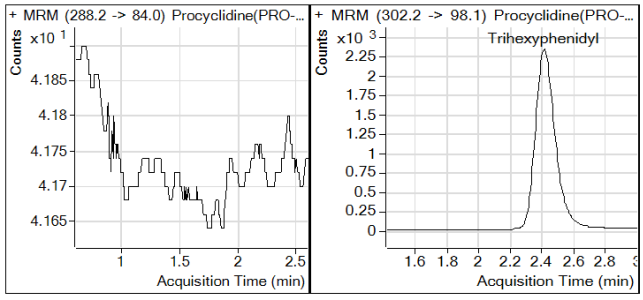

Figure 1: Chromatogram - an MRM Data of Blank Plasma Spiked with Internal Standard Trihexyphenidyl.

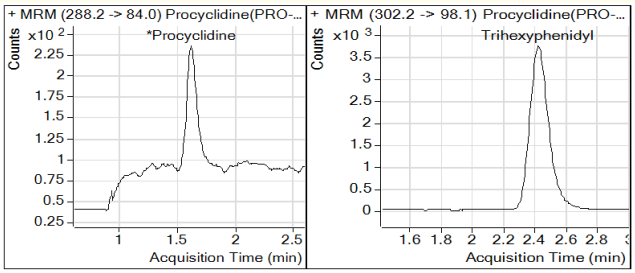

Figure 2: Chromatogram - an MRM Data of Blank Plasma Spiked with 0.1ng/ml Procyclidine and Internal Standard Trihexyphenidyl.

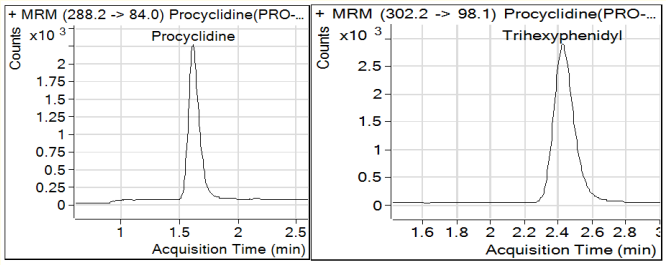

Figure 3: Chromatogram - an MRM Data of Blank Plasma Spiked with 25 ng/ml Procyclidine and Internal Standard Trihexyphenidyl.

Figure 4: Plasma Concentration (Mean ± S.D.) of Procyclidine following Single Dose Administration of Procyclidine 5 mg Tablets of Generic and Reference Products.

Linearity, accuracy, and precision

The peak area ratios of different standard concentrations of procyclidine in plasma ranging from 0.1 to 100 ng/ml was linear (r^2 0.9998). The C.V.% of the average results of inter-day variation was 1.410% according to FDA guidelines [11]. This increases the possibilities of its use in procyclidine pharmacokinetics and bioavailability studies.

Within-day and between-day precision and accuracy were examined at three levels of drug concentrations within the predicted range. Furthermore, the intra-day and inter-day accuracy results revealed an average recovery percentage of 96.494 percent and 98.642 percent, respectively. The outcomes of freeze-thaw, short-term, and long-term stability study revealed that the average procyclidine recovery was higher than 95%, indicating that both the targeted drug and the internal standard were stable under the conditions tested.

Comparative bioavailability study

Clinical observation

Most of the individuals tolerated the drug to some extent. There were no treatment-related adverse events or lab abnormalities detected. Throughout the study, blood samples were taken at the right time. There were no individuals who dropped out of the trial due to pharmacological adverse effects.

Pharmacokinetic data and assessment of bioequivelance

The results shown in tables (1) and (2) revealed that the mean values for C_{max} was 64.288 ± 18.076 ng/ml and 63.006 ± 18.694 ng/ml, t_{max} was 1.680 ± 0.768h and 1.618 ± 0.922h, $t_{1/2e}$ 12.154 ± 3.926 and 12.470 ± 4.697h, AUC_{0-t} 1066.631 ± 383.695 ng.h/ml and 1029.670 ± 341.410 ng.h/ml, for generic tablets and reference tablets respectively which were consistent with those described in the literature [4].

Statistical analysis

The study point estimate (%) results for C_{max}, AUC_{0-t}, and AUC_{0-inf} were 102.517, 102.985%, and102.957% respectively. The 90% confidence intervals of C_{max}, AUC_{0-t}, and AUC_{0-inf} were 96.592 to 108.804%, 94.425 to 112.320%, and 94.487 to 112.187% respectively (Table 3). As a result, 90 percent confidence intervals are provided within FDA acceptance criteria [12].

Subject	T_{max} (h)	C_{max}(ng/ml)	AUC_{0-t} (ng.h/ml)	AUC_{0-inf} (ng.h/ml)	K_{el} (h^{-1})	$T_{1/2}$ (h)
Mean	1.618	63.006	1029.670	1059.203	0.061	12.470
CV%	55.786	29.045	32.459	34.956	27.909	37.669
Range (Median)	0.667-4.000 (1.330)	37.434-90.982 (57.164)	579.781-1973.379 (924.613)	582.265-2220.673 (944.902)	0.025-0.092 (0.061)	7.542-27.387 (11.461)

Table 1: Pharmacokinetics Parameters of Procyclidine following administration of Reference Product.

Subject	T_{max} (h)	C_{max}(ng/ml)	AUC_{0-t} (ng.h/ml)	AUC_{0-inf} (ng.h/ml)	K_{el} (h^{-1})	$T_{1/2}$ (h)
Mean	1.680	64.288	1066.631	1099.230	0.062	12.154
CV%	45.705	28.117	35.973	39.439	26.048	32.303
Range (Median)	0.667-4.000 (1.500)	39.364-95.195 (62.254)	712.561-2042.376 (903.516)	715.993-2373.330 (907.210)	0.027-0.088 (0.065)	7.836-25.413 (10.657)

Table 2: Pharmacokinetics Parameters of Procyclidine following administration of Generic Product.

Pharmacokinetic Parameter	90% Confidence intervals of parametric means		
	Point estimate (%)	Lower limit (%)	Upper limit (%)
C_{max}	102.517	96.592	108.804
AUC_{0-t}	102.985	94.425	112.320
AUC_{0-inf}	102.957	94.487	112.187

Table 3: 90% C.I. for Generic and Reference Products.

Blood pressure and pulse rate

The reported blood pressure and pulse rate readings were all close to normal and within acceptable ranges (Figures 5 and 6).

Figure 5: Blood pressure systolic/diastolic (Mean ± SD) after single oral administration of Procyclidine generic and reference products.

Figure 6: Pulse rate (Mean ± SD) after single oral administration of Procyclidine generic and reference products.

The blood pressure results shown in figure 7 for the generic product show that all approaches normal values, with reported mean values of systolic blood pressure of 117, 116, 115, 116, 115 mmHg and diastolic blood pressure of 75, 75, 73, 74, 73 mmHg at zero (predose), 2, 4, 6, and 10 hours of drug administration, respectively.

In contrast, mean systolic blood pressure readings for the reference product were 115, 117, 111, 114, 115 mmHg and 73, 75, 71, 72, 73 mmHg for diastolic blood pressure at zero (predose), 2, 4, 6, and 10 hours of drug administration, respectively (Figure 8).

Figure 7: Blood pressure systolic/diastolic and Procyclidine plasma Conc (Mean ± SD) after single oral administration of reference product.

Figure 8: Blood pressure systolic/diastolic and Procyclidine plasma Conc (Mean ± SD) after single oral administration of generic product.

Discussion

The established bioanalytical technique demonstrated to be sensitive, specific, and fully validated, with linear (r^2 of 0.9998) range of 0.1 to 100 ng/ml, and therefore in accordance with FDA Guidelines [11]. And so, it might be used in bioavailability and clinical research, clinical trials, and therapy monitoring of procyclidine to ensure safety and efficacy in the management of Parkinson's disease (PD).

The chromatographic conditions developed in-house introduced a new bioanalytical approach for published literature data [5-9] with a reasonable extraction procedure and chromatographic conditions and using trihexyphenidyl as a structurally related internal standard. It is worth noting that the development of a precise and reliable bioanalytical assay was critical for assuring accurate and precise therapeutic monitoring and assessing the validity of generic drugs for commercial usage with targeted clinical response [13].

The significance of procyclidine therapeutic drug monitoring stems from its effectiveness in the treatment of Parkinson's disease [1,2]. In the current investigation, the pharmacokinetics parameters of procyclidine were measured using the established validated bioassay technique, which might be evidence for the effective implementation of this approach in clinical studies used to evaluate procyclidine outcomes in patients.

Furthermore, routine random medication samples should be selected from hospital and community pharmacies and subjected to quality checks to ensure that the dosage form retains its integrity and that the stated claim of active component meets the necessary standard [14].

Since medication errors (MEs) are a big issue in every healthcare system across the world, the developed method can provide accurate data for minimization of MEs resulting from incorrect maintenance dosing [15].

Chlorpromazine-procyclidine and imipramine have been shown in studies to be helpful in retarded depressive symptoms such as apathy and anorexia. In agitated depressive states, chlorpromazine-procyclidine may be more instantly efficacious, whereas imipramine may be more beneficial in depressed patients with phobic anxiety characteristics. According to previous findings, the inclusion of an anticholinergic drug to the treatment of depressive disorders with chlorpromazine may improve outcomes [16].

Anticholinergics such as trihexyphenidyl, procyclidine, benztropine, ethopropazine, and biperiden have also been shown in trials to be effective in the treatment of dystonia [17].

Further research revealed that procyclidine can modulate prepulse inhibition of the human acoustic startle response. Procyclidine reduced response amplitude and heart rate 1 to 2 hours after treatment compared to pulse-alone trials. This suggests

that the use of anticholinergics should be investigated in prepulse inhibition studies in schizophrenia [18].

The results of the comparative bioavailability study revealed that the 90 percent confidence limit for AUC_{0-t}, AUC_{0-inf}, and C_{max} was in the range of 80 to 125 percent, implying that both the generic and reference products of procyclidine were bioequivalent as per FDA acceptance limits [12].

Conclusion

The new bioanalytical technique for quantifying procyclidine in plasma has been completely validated and may be used in bioavailability research, clinical trials, and therapeutic drug monitoring. Furthermore, the comparative bioavailability study revealed that both the generic and reference medications were bioequivalent.

Procyclidine is an effective therapy for Parkinson's disease management, and therapeutic monitoring is an important approach for accomplishing therapeutic goals as a result of monitoring patients' drug levels to minimize subtherapeutic or toxic drug levels.

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