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Short Communication

Determination of MDL 73,005 in human plasma by solid-phase extraction and high-performance liquid chromatography*

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Introduction

MDL 73,005EF, 8-azaspiro[4,5]decane-7,9-dione-8-[2-[[(2,3-dihydro-1,4-benzodioxin-2-yl-)-methyl]amino]ethyl]monomethanesulphonate, is a novel class of compound classified as a 5-HT$_{1A}$ partial agonist by its pharmacological activities in animal models [1–3]. This agent is being evaluated for the treatment of anxiety in man. In order to facilitate the studies of bioavailability and pharmacokinetics of this compound, a sensitive and reliable analytical procedure was needed to measure plasma concentration of MDL 73,005. This communication describes a reversed-phase high-performance liquid chromatography (HPLC) method developed for this purpose and the application of the assay to a bioequivalence study in human volunteers.

Experimental

Materials

Reagent grade chemicals were used throughout this study. HPLC grade solvents were used to make all solutions. Methanol and acetonitrile were obtained from Burdick and Jackson Laboratories (Muskegon, MI). Drug-free EDTA human plasma was supplied by Carolina Biological Supply (Burlington, NC). The SPE columns (cyano, end-capped, 100 mg 1.0 ml^{-1}) were purchased from Analytichem International (Harbor City, CA). Authentic compounds of MDL 73,005EF and internal standard (MDL 74,348) (Fig. 1) were obtained from Marion Merrell Dow Research Institute (Cincinnati, OH). The free base form of the drug was quantified.

Plasma standards

Standards of MDL 73,005 were prepared in drug-free EDTA plasma. An initial stock solution of 1.0 mg ml^{-1} (free base) was prepared in acetonitrile. Spiking solutions of 1 and 10 μg ml^{-1} were then prepared by further dilution of the stock solution with acetonitrile. Various volumes of these spiking solutions were added to a clean test tube and the organic solvent evaporated prior to the addition of human plasma to give plasma standards of 0, 25, 50, 100, 250, 500 and 1000 ng ml^{-1}. Plasma standards were prepared freshly for every run. The stock solutions in acetonitrile could be stored in a refrigerator and shown to be stable for up to 2 months.

Treatment of clinical human plasma samples

Previous studies in the authors' laboratory had shown that MDL 73,005 is unstable in aqueous and basic solutions. The most stable pH for the compound was shown to be at approximately 2. Hence, special precautions were taken to ensure that human plasma collected for the analysis of MDL 73,005

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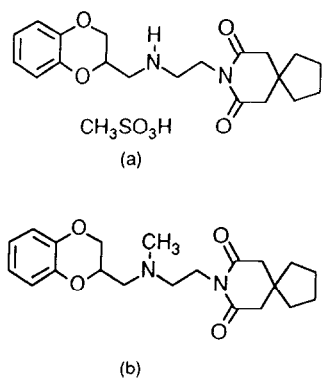

Figure 1
Chemical structures of (a) MDL 73,005 EF and (b) the internal standard (MDL 74,348).

should be acidified to pH ~2 immediately before storage in freezer. This was accomplished by adding 100 μl of a 2 M HCl solution per 1 ml of human plasma before freezing the sample.

Validation study

To test the accuracy and precision of the analytical method, 35 randomly coded plasma samples were analysed in duplicate over five days. A seven point (blank, 25, 50, 100, 250, 500 and 1000 ng ml^{-1}) calibration curve was analysed at the beginning and the end of each day's run. To determine the within-day precision of the method, six aliquots of three different concentrations were analysed on one day. Six different lots of blank plasma samples were assayed to check for endogenous interferences.

Application of the method

To show the feasibility of the assay, a comparative bioequivalence study was carried out where either a 10 mg capsule or tablet was randomly administered to eight human volunteers. Periodic plasma samples taken from each subject were randomly coded and analysed by this procedure. The analysts were unaware of the dose or sample time and the code was not broken until all of the samples were analysed.

Extraction procedure

Samples and standards were prepared by solid-phase extraction (SPE) on cyano columns. To each 1.0-ml aliquot of sample (sample or standard), 100 μl of an internal standard (MDL 74,348) working solution (6 μg ml^{-1}) was added. Each plasma standard sample was acidified by the addition of a 100 μl of a 2 M HCl solution. Clean SPE columns were preconditioned with two 1.0-ml volumes of methanol and two 1.0-ml volumes of water. Plasma samples were then added into the SPE columns. After the aqueous portion was withdrawn, the SPE column was rinsed with 1.0 ml of water and then 1.0 ml of methanol–water (80:20, v/v). The column was then dried for 5 min with the aid of a strong vacuum (*ca* 25 in. Hg). The compounds of interest were eluted from the column with 3 × 100 μl of 0.1% perchloric acid in acetonitrile. The sample was then transferred for HPLC analysis and 75 μl injected into the HPLC system.

Instrumentation

A component HPLC system (Waters, Milford, MA) consisted of a 600E system controller, a WISP Model 715 Auto-injector with a refrigerator unit operated at 5°C and a Model 480 UV absorption detector operated at 274 nm. A pre-packed 25 cm × 4.6 mm i.d. Spherisorb 3 ODS 2 (3 μm particle size) HPLC column (Phase Separation, Norwalk, CT) was operated with a mobile phase consisting of acetonitrile–0.02 M monobasic potassium phosphate buffer (pH 2.5)–triethylamine (50:50:0.05, v/v/v) delivered at a flow rate of 1.0 ml min^{-1}. Column temperature was maintained at 30°C with a column heater. Detector output was recorded and chromatograms analysed by a Beckman CALS laboratory data system (Beckman Instruments, Allendale, NJ).

Calculation

The peak heights of MDL 73,005 and internal standard (MDL 74,348) were determined using the laboratory data system described above. The peak height ratios of MDL 73,005 versus internal standard were calculated and plotted against the standard concentrations. The parameters found by a power fit linear regression [4] of the standards were used to calculate the concentrations of MDL 73,005 in the unknown samples. Since patient samples stored in freezer were diluted with the addition of 2 M hydrochloric acid, the appropriate correction factor was applied for the final calculation of MDL 73,005 plasma concentration.

Results and Discussion

HPLC conditions

The Spherisorb ODS column provided ideal separation for MDL 73,005 and the internal standard. Using the HPLC conditions described above, the retention time for MDL 73,005 was about 8.9 min and about 11.4 min for the internal standard. Figure 2 shows several typical LC chromatograms from extracted plasma standards.

Extraction efficiency and specificity

Using the SPE procedure described above, the extraction efficiency of MDL 73,005 from plasma at 50 ng ml^{-1} was 89.1%. Analyses of six different lots of human EDTA plasma from commercial source did not show any significant peaks that would interfere with the assay.

Validation study

The between-day accuracy and precision of the method were determined by assaying 35 randomly coded unknowns on five different days. The within-day accuracy and precision was determined by analysing six aliquots of three different concentrations on the same day. These data are shown in Tables 1 and 2. For between-day accuracy, the results ranged from 96.4 to 102.9%. The between-day assay precision of the method, as measured by the relative standard deviation (% RSD) ranged from 1.7 to 5.0%. Similar results were obtained for the within-day accuracy (98–103.3%) and precision (<3.5%).

Table 1
Between-day accuracy and precision for MDL 73,005 (n = 10)

Conc. added (ng ml^{-1})	Found (ng ml^{-1})	SD	RSD (%)	Accuracy (%)
0.0	0.0	—	—	—
27.5	28.3	1.4	5.0	102.9
45.0	44.8	1.5	3.4	99.6
110.0	112.9	5.6	5.0	102.6
200.0	192.7	7.0	3.6	96.4
550.0	544.5	9.5	1.7	99.0
840.0	863.1	30.3	3.5	102.8

Table 2
Within-day precision for MDL 73,005 (n = 6)

Conc. added (ng ml^{-1})	Found (ng ml^{-1})	SD	RSD (%)	Accuracy (%)
50.0	49.4	1.4	2.8	98.8
200.0	204.0	2.0	1.0	102.0
600.0	620.0	22.0	3.5	103.3

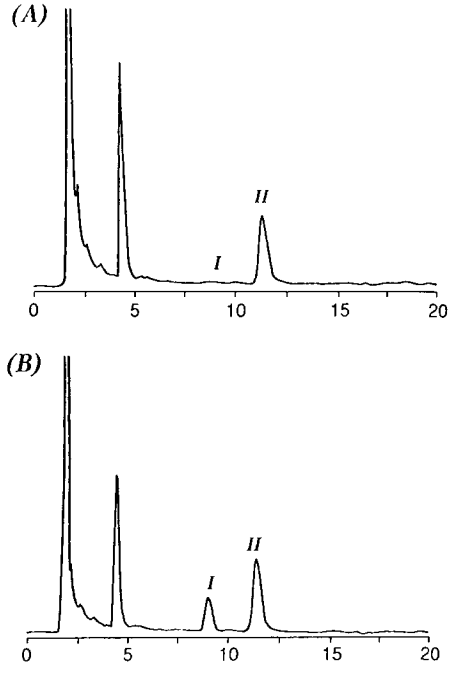

Figure 2
Chromatograms of extracted plasma standards: (A) blank; (B) 100 ng ml^{-1}. Peak I = MDL 73,005; II = internal standard.

Application of the method

This assay procedure has been applied successfully to the analysis of samples from the human bioequivalence study. Figure 3 shows a typical chromatogram from a human subject who was dosed with a 10 mg capsule of MDL 73,005EF. Figure 4 shows a complete plasma concentration–time profile from a human volunteer.

Stability

Acidified controls of MDL 73,005 prepared in plasma at 50, 200 and 600 ng ml^{-1} exhibited no significant degradation over a period of 3 months when stored at −20°C. A cycle of freeze–thaw of the above control samples also demonstrated no noticeable degradation of the compound.

Conclusions

The described method for the analysis of MDL 73,005 in human plasma is specific, sensitive and rugged. The use of SPE allows rapid and efficient sample clean-up and provides future potential for automated (robotic) sample preparation. The assay is sensitive

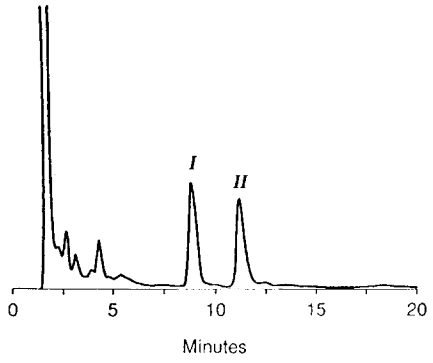

Figure 3
Chromatogram of an extracted plasma sample from a human volunteer receiving an oral capsule dose of 10 mg of MDL 73,005EF. Time point = 1.5 h post-dose.

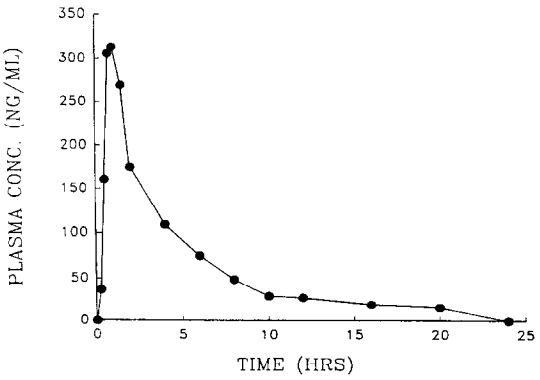

Figure 4
Representative plasma concentration–time profile of a subject after a single oral dose of 10 mg capsule of MDL 73,005.

enough to apply to bioavailability and pharmacokinetic studies in animals and humans.

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