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ANALYSIS

DEVELOPMENT, VALIDATION AND APPLICATION OF THE HPLC METHOD FOR DETERMINATION OF MIANSERIN IN HUMAN SERUM

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Abstract: A high performance liquid chromatography method for the determination of mianserin in human serum was developed and validated. Doxepin was used as an internal standard. Mianserin was extracted from human serum using a liquid-liquid extraction with hexane:isoamyl alcohol (99:1, v/v). The sample was then dissolved in 0.05 M phosphoric acid (pH= 3.0), and after separation on a Hichrom RPB (250 x 4.6 mm, 5 mm) column, the analytes were measured by ultraviolet detection at 214 nm. The recovery ranged from 86.1 to 94.5% for mianserin. The method was specific and linear over the concentration range of 2.0 – 128.0 ng/mL. The limit of quantification (LOQ) was established at 2.0 ng/mL (CV=13.8%). The accuracy range was from 92.5 to 107.5%. The method was used to measure mianserin in human serum samples obtained from healthy volunteers who had received a single oral dose of 30 mg mianserin. Pharmacokinetic parameters obtained for the mianserin were in agreement with the existing data.

Keywords: mianserin, human serum, HPLC

Mianserin is a tetracyclic antidepresant drug widely used in clinical practice. Therefore there is a need for monitoring its concentrations in blood serum for diagnostic, as well as for bioequivalence purpose. Several methods using HPLC (1-4) and HPLC-MS (5) were reported to detected mianserin in blood serum. HPLC methods are specific and linear within various concentration ranges such as 1-60 ng/mL (5) or 10-200 ng/mL (4). LOQ's of these methods were from 1 ng/mL (5) to 10 ng/mL (4), with recovery ranging from 40-50% (5) to 97.5% (4).

After analysis of their performance, we found that none of these methods fulfills our expectations in respect of basic parameters such as range and sensitivity, or general efficiency of sample preparation. Therefore, our aim was to develop the HPLC method for the determination of mianserin in blood serum, with such features as simple and efficient extraction, wide useful range of mianserin concentrations, and suitability for validation procedure.

EXPERIMENTAL

Chemistry

Mianserin hydrochloride was obtained from Polfa, Pabianice, Poland. Doxepine hydrochloride (LOTH) was used as an internal standard. Acetonitrile, methanol, hexane, triethylamine, phosphoric acid (85%) and potassium dihydrogen orthophosphate purchased from J.T. Baker (Deventer, Holland) were HPLC and analytical-grade reagents. Sodium hydroxide (BDH, England) and isoamyl alcohol (3-methylpentan-1-ol) were obtained from Schuchardt, Munchen (Germany). Water (Aqua pro injectione) was obtained from Polfa, Lublin (Poland).

Preparation of solutions

The standard solution of mianserin and the internal standard of doxepin were prepared in methanol and stored at 4°C. The 0.05 M phosphoric acid solution was filtered through a Millipore membrane filter (0.45 um) from Nihon, Millipore (Yonezawa, Japan). Standard lyophilized serum (Serostandard N) for validation and preparation of calibration curve of the method was obtained from Biomed, Krakow.

Stock solution was prepared by dissolving 10 mg of mianserin in 10 mL of methanol. This solution was used to prepare different working solutions of mianserin in serum. Stock solution of the internal standard was prepared by dissolving 9.48 mg of doxepine-HCl in 10 mL of methanol. Working solution (2,37 μg/mL) was prepared by diluting 250 μL of stock solution to 100 mL in methanol.

Methanol solutions after dividing into aliquots were stored at –20°C. Serum aliquot, containing mianserin and internal standard, solutions were used immediately after preparation or stored overnight in the refrigerator.

HPLC system

The chromatographic separation was carried out on a Shimadzu liquid chromatograph consisting of LC-10 AS pump and SPD-10 AV detector in combination with the integrator (Chromax-2001, Poland). A Hichrom RPB (250 × 4.6 mm, 5 mm) column and Hichrom 12 × 4,6 mm Guard Cartridge were used.

Assay conditions

The mobile phase was prepared by mixing 34 parts (vol.) of 1% triethylamine aqueous solution with 16 parts off acetonitrile. For degassing and removing off possible solid particles, the mobile phase was filtered through a 0.45 µm Millipore membrane filter under vacuum. Then, pH was adjusted to 3.5 by the addition of phosphoric acid. The flow rate was 1/mL/min and the UV detector set at 214 nm.

Sample processing

To extract 0.5 mL of serum, 50 mL of the internal standard containing 118 ng of doxepine in methanol, 0.5 mL of 2 M sodium hydroxide and 5 mL of hexane-isoamyl alcohol (99:1) were added and then shaken for 45 min and centrifuged at 2000 × g for 10 min. After separation of layers and freezing at -30°C, the organic layer was decanted into a tube containing 250 µL of 0.05 M phosphoric acid, shaken for 30 min and centrifuged at 2000 × g for 10 min. An aliquot of the aqueous solution (100 mL) was injected onto the HPLC system.

Calibration curve

Calibration curve was prepared using serum containing mianserin in concentrations of: 2, 4, 8, 16, 32, 64 and 128 ng/mL. An aliquot of 50 mL of the internal standard solution in methanol (118 ng) was added to each sample prior to extraction. The samples were extracted as described above. The calibration curve was constructed by plotting the "F" coefficients (area of mianserin peak divided by the area of the internal standard peak) on the „y" axis, *versus* their respective concentrations of mianserin („x" axis). The regression line was obtained by least square analysis. Calibration curve data are given in Table 1.

Results and Discussion

Method validation

Specificity of the method

It was shown that the method was specific, as the chromatogram of blank serum without added mianserin or doxepin failed to show any peaks (Figure 1) where the relevant peaks were expected (Figure 2).

Limits of detection (LOD) and quantitation (LOQ)

LOD was estimated by comparison of the noise level and mianserin peak height. At noise/signal ratio (N/S) = 3, the LOD was 1 mg/mL. LOQ was accepted as the lowest mianserin concentration (2 ng/mL) used for the calibration curve (CV=13.8%) (6).

Recovery

The recovery of mianserin after sample preparation was measured by comparing the peak area found in plasma sample with the peak area obtained by direct injection of pure standard with equivalent amounts of mianserin. Three concentrations were investigated: 4.0; 16.0 and 64.0 ng/mL. The corresponding recoveries were: 94.5% (CV=7.3%; n=6); 92.5% (CV=4.2%; n=6); 86.1% (CV=5.7%; n=5).

Linearity and concentrations range

Calibration curve was prepared by adding mianserin methanol solutions with the internal standard to serum portions to obtain concentrations range from 2 ng/mL to 128 ng/mL of serum. These values were plotted on the "x" axis while the "y" axis was used to plot the values of the F factor (area of the mianserin peak/area of the internal standard peak) (Table 1). Within the range of mianserin concentrations from 2 ng/mL to 128 ng/mL the method was found to be linear. Regresion parameters of mianserine were: slope 0.00496, y-intercept 0.00309, r = 0.9925.

Table 1. Calibration curve for mianserin in blood serum (n=6).

Mianserin concentration (ng/mL of serum)	F Mean ± SD	CV %
2.0	0.0107 ± 0.0147	13.8
4.0	0.0148 ± 0.0011	7.2
8.0	0.0310 ± 0.0014	4.5
16.0	0.0580 ± 0.0026	4.4
32.0	0.1080 ± 0.0022	2.1
64.0	0.1920 ± 0.0107	5.6
128.0	0.4040 ± 0.0183	4.5

F = (area of the mianserin peak) / (area of the internal standard peak)
SD – standard deviation
CV – coefficient of variation
r = 0.9925
F = 0.00496 + 0.00309 × (concentration)

Table 2. Reproducibility and accuracy of the analytical method (within day)

	Nominal concentrations of mianserin in serum (ng/mL)		
	4	16	128
	Assayed mianserin concentrations		
Mean	3.7 (n=6)	17.2 (n=6)	122.7 (n=6)
SD	0.1	2.3	4.4
CV% (reproducibility)	7.7	2.3	4.4
Accuracy%	92.5	107.5	95.9

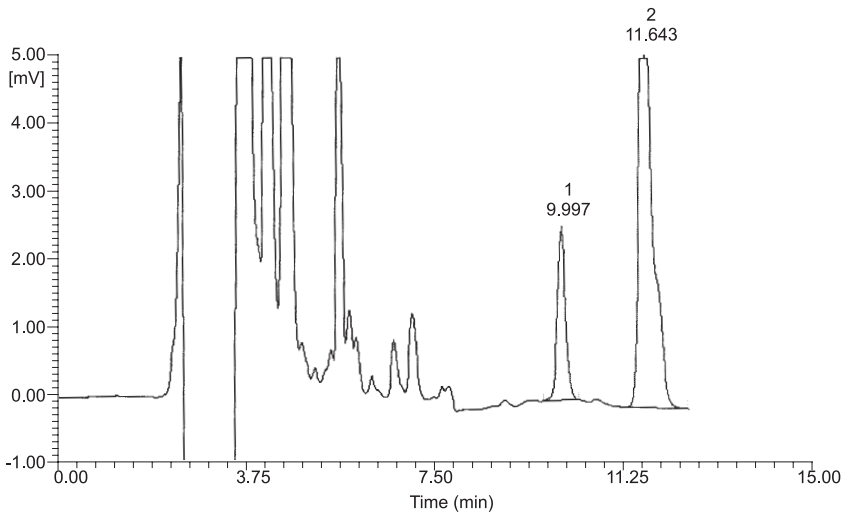

Figure 1. Chromatogram of blank serum of a healthy volunteer at time "0" before administration of mianserin.

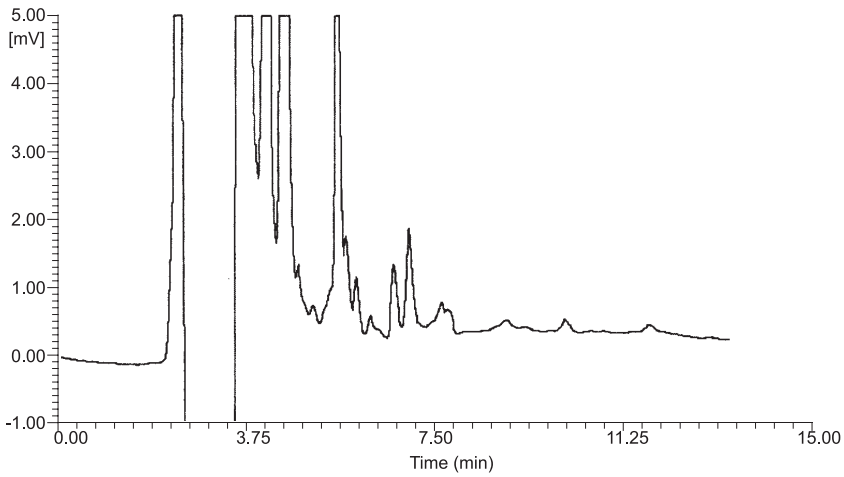

Figure 2. Typical chromatogram of serum after administration of mianserin, a single oral dose of 30 mg, after extraction, to a healthy volunteer, 1 – mianserin peak, 2 – doxepine peak (internal standard).

Reproducibility and accuracy

To determine these parameters on the "within day" basis three concentrations of mianserin in control serum were used: 4 ng/mL, 16 ng/mL and 128 ng/mL (Table 2). For "between days" basis 32 ng/mL and 64 ng/mL concentrations were used (Table 3). Reproducibility was defined as dispersion of results in form of a standard deviation (SD), expressed as coefficient of variation (CV%). Accuracy of the method was defined as an agree-

Table 3. Reproducibility and accuracy of the analysis method (between days)

	Nominal concentrations of mianserin in serum (ng/mL)	
	32	64
	Assayed mianserin concentrations	
Mean	28.5 (n=12)	58.4 (n=13)
SD	1.6	4.8
CV% (Reproducibility)	5.6	8.2
Accuracy%	89.1	91.2

Table 4. Pharmacokinetic parameters of mianserin after a single oral dose of 30 mg of mianserin.

Mianserin	Our method Concentration N=12	Dahl method (1) Concentration N=15
T_{max} (h)		
Mean ± SD	2.08 ± 0.7	2.0 ± 1.0
Range	1-3	1-3
C_{max} (ng/mL)		
Mean ± SD	39.42 ± 11.11	34.5 ± 22.2
Range	21.1-60.7	10.5-94.5
AUC_{0-8} (ng h/mL)		
Mean ± SD	299.92 ± 117.2	198.0 ± 107.7
Range	199-490	53.7-458.4

Table 5. Cross–validation

Validation parameters	Determination of mianserin		
	our HPLC method	HPLC method (4)	LC-MS method (5)
Linearity by linear regression with calibration/range (ng/mL)	2 – 128	10 – 200	1 – 60
LOD (ng/mL)	1	5	-
LOQ (ng/mL)	2	10	1
Inter- and intra-assay accuracy (%)	89.1 – 107.5	85 – 115	94.4 – 112.3
Recovery (%)	86.1 – 94.5	95.7	40 – 50

ment of the obtained assay result with the nominal concentration value.

Accuracy was calculated as follows: (mean assayed concentration) / (nominal concentration) × 100%.

Application

The method was used to assay mianserin in human serum samples obtained before and after administration of an oral dose of 30 mg of mianserin in twelve (N = 12) healthy volunteers. The results are given in Table 4.

The methods most commonly used for mianserin determination are HPLC (1-4) and HPLC-MC (5) techniques. Our method of mianserin determination is an extensive modification of the procedure described by Kurata et al. for rat plasma (2). We have further developed this method to be used for mianserin determination in the human blood serum; we have validated it and checked pharmacokinetic parameters of mianserin in human body.

A comparison of validation parameters of our method with other methods (4, 5) is presented in Table 5. Although method by Chauhan et al. (5) has LOQ of 1 ng/mL, its range is limited to 1-60 ng/mL and the recovery is low (only 40-50%). A recovery of method by Hefnawy and Aboul-Enein (4) is 97.5% with LOQ too high (10 ng/mL) for some applications. On the other hand, it has the widest range from 10 to 200 ng/mL. Our method combines the best parameters of the other two, with its LOQ equal to 2 ng/mL, acceptable range of 2-128 ng/mL and good recovery of 86.1–94.5%.

Our pharmacokinetic data compare well with parameters obtained by Dahl (Table 4).

The results obtained indicate, that our method offers an optimal combination of short extraction time, sensitivity and specificity, and the results of its validation show the proper choice of extraction and HPLC parameters. The developed method can find

an application when it is necessary to determine the levels of mianserin in the human blood serum for diagnostic purposes, monitoring of the drug's level, or for bioequivalence testing.

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