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N. V. Horlachuk, S. Yu. Cholach
I. HORBACHEVSKY TERNOPIL NATIONAL MEDICAL UNIVERSITY

DETERMINATION OF MIANSERIN IN BIOLOGICAL MATERIAL BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Introduction. *Mianserin is a derivative of tetracyclic antidepressants, belongs to the piperazine-azepine derivatives. Chemically, it is (±)-2-methyl-1,2,3,4,10,14b-hexahydrodibenzo[c,f]pyrazino[1,2-a]azepine. Mianserin exhibits anti-stress activity, which is very important in the treatment of patients with depression that is combined with anxiety.*

The aim of the study *– development of an effective technique for isolating mianserin from biological tissues and selecting optimal conditions for determination by HPLC in the presence of metabolites.*

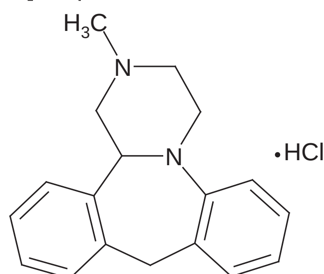
Research Methods. *Identification and quantification of mianserin isolated from biological material was performed by HPLC. The research was carried out on an Agilent 1200 liquid chromatograph, an Eclips C18 column, 150,0 mm long, 4,6 mm in diameter, and the sorbent particle size was 5 μm. The sample was injected into the chromatograph in the isocratic mode, the volume of the injected sample was 20 μl, the column temperature was 25 °C. A comparative evaluation of the efficiency of isolation of mianserin was performed from model liver samples acidified with 30 % acetic acid.*

Results and Discussion. *During the study of rat liver extracts simultaneously with the peak of mianserin (retention time of 3.37 min), the peak of the metabolite of mianserin, identified by us as demethylmianserin – M-7 (retention time of 2.52 min.) is recorded.*

Conclusions. *The efficiency of isolation of mianserin from model liver samples by two methods was compared. It was established that 28.9–33.6 % of mianserin is isolated with water acidified with oxalic acid. 56.5–59.8 % of the studied drug can be isolated with an acidified 30 % solution of acetic acid. To prepare the sample for analysis by HPLC, the conditions for their purification were worked out, the degree of extraction of mianserin from the studied sample is 99.8–100.0 %. Developed conditions for identification and quantification of mianserin by HPLC on an Eclips C18 column and detection at a wavelength of 214 nm. The limit of quantitative determination of mianserin in solutions is 0.5 μg/ml.*

KEY WORDS: **mianserin; isolation methods; high performance liquid chromatography (HPLC).**

INTRODUCTION. Mianserin is a derivative of tetracyclic antidepressants, belongs to the piperazine-azepine derivatives. Chemically, it is (±)-2-methyl-1,2,3,4,10,14b-hexahydrodibenzo[c,f]pyrazino[1,2-a]azepine:



Mianserin exhibits anti-stress activity, which is very important in the treatment of patients with depression that is combined with anxiety [1]. This drug is used for endogenous, psychogenic and somatogenic depressions. Recently, domestic and foreign sources have published proofs of the using of mianserin (lerivon) as an analgesic agent for headaches and fibromyalgia [2, 3]. Symptoms of intoxication are observed in cases of taking the drug in a dose higher than 1200.0 mg per day. Fatal cases of poisoning occur as a result of taking mianserin with MAO inhibitors [4, 5].

Determining the level of mianserin concentration in plasma, urine, and medical products are described by methods of high-performance liquid chromatography with mass spectrometric detection and gas-liquid chromatography [6, 7]. However, there are no methods for isolating this drug from internal organs submitted to the forensic medical examination office for suspected poisoning [8, 9].

The aim of the study – development of an effective technique for isolating mianserin from biological tissues and selecting optimal conditions for determination by HPLC in the presence of metabolites.

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MATERIALS AND METHODS. Identification and quantification of mianserin isolated from biological material was performed by HPLC. The research was carried out on an Agilent 1200 liquid chromatograph, an Eclips C18 column, 150.0 mm long, 4.6 mm in diameter, and the sorbent particle size was 5.0 mg. The sample was injected into the chromatograph in the isocratic mode, the volume of the injected sample was 20 µl, the column temperature was 25 °C.

The mobile phase consisted of a 1 % aqueous solution of triethylamine and acetonitrile, taken in a volume ratio (34:16), the pH of the mobile phase was adjusted to 3.5 with a dilute solution of phosphoric acid. A solution of triethylamine (1 %) and phosphoric acid was prepared using deionized water. The liquid mobile phase was filtered by any convenient method. The speed of the mobile phase was 1 ml/min. Detection was carried out at a wavelength of 214 nm (matrix-diode detector).

Quantification was carried out by the method of absolute calibration, where the equations of the calibration curve were calculated using the software of the Emprower Pro device. A standard aqueous solution of mianserin was prepared in bidistilled water with a concentration of 100.0 µg/ml to construct a graduation graph. By diluting this solution, a series of standard mianserin solutions with concentrations of 0.5 was prepared; 1,0; 2,0; 5,0; 10,0; and 20.0 µg/ml. For this, 0.125; 0.25; 0.50; 1.25; 2.50 and 5.00 ml of a standard aqueous solution of mianserin (100.0 µg/ml) was infused into measuring flasks with a capacity of 25.0 ml and diluted with the mobile phase to the mark.

To develop a technique for isolating mianserin, the livers of people who died from injuries were used. 100.0; 200.0; 300.0; 400.0 and 500.0 µg of mianserin in the form of an aqueous solution were added to homogenized samples of biological material weighing 20.0 g. The mixtures were left at room temperature (20°C) for 24 hours, with occasional stirring. After the specified time, isolation was carried out.

Isolation of mianserin from model samples of biological material was carried out with water acidified with oxalic acid (O. O. Vasiliev's method), as well as with a 30 % solution of acetic acid. Water acidified with oxalic acid is most often used in the laboratories of the forensic medical examination bureau to isolate basic and acidic substances from organs.

The effectiveness of the isolation technique was tested on the organs of animals (rats). For this, a series of animals weighing 200.0–220.0 g were used during 18 hours. They received mianserin at a dose of 10.0 mg/kg three times. Animals were injected with an aqueous solution of the required amount of the drug through a probe into the stomach. After a day, the animals were decapitated under an anesthesia and the liver was used for research.

Methods of isolating mianserin from the liver. Samples of biological material were poured with a 30 % solution of acetic acid until the solid particles were completely covered. The mixture was stirred and left for 1 hour. The pH was monitored by the universal indicator papers. If necessary, a 50 % acetic acid solution was added for maintenance pH 2–3. Acid extracts were drained, and the biological material was infused two more times with new portions of 30 % acetic acid solution for 1 hour with periodic stirring and pH control. Extracts from each portion of biological material were combined and introduced in small portions of 30 % sodium hydroxide solution to pH 8–9 and mianserin was extracted twice with chloroform. The chloroform extracts were combined and the organic solvent was evaporated in a rotary evaporator at 40°C. After that, the dry residues were dissolved in 10.0 ml of chloroform.

Extraction purification by chromatography in a thin layer of sorbent. Before carrying out these studies, additional purification of chloroform extractions was carried out by using the method of chromatography in a thin layer of silica gel. “Silpearl” brand of silica gel (manufactured in the Czech Republic) was used to manufacture the fixed sorbent layer. A sorbent layer was applied to a 12×24 cm glass plate. The necessary amount of silica gel and medical plaster was ground in a porcelain mortar, 10.0–15.0 ml of distilled water was added to this mixture, and the resulting suspension was evenly applied to the surface of the plates. The plates were dried and activated for 1 hour in a drying cabinet at 105 °C. Before applying the extracts to the plates, they were eluted once in a solvent system, after which the plates were air-dried and reactivated in a thermostat for 30 min at 105 °C. At a distance of 2 cm from the lower edge of the plate, the start line was marked, the path of the solvent system was 20 cm.

5.0 ml of the chloroform extract of mianserin obtained after dissolving the dry residues was evaporated to a small volume (about 0.5 ml) and applied in a continuous strip to the start line using a microsyringe. The plates were dried in air and placed in a chamber saturated with vapors of solvent systems. For examining samples containing mianserin, the solvent system was used for separation: methanol-acetonitrile-25 % ammonia (30:10:5). After raising the solvent system 20 cm above the starting line, the plates were removed, dried in air, and the zones of the studied drug and its metabolites were detected using UV rays (λ=252 nm).

The layer of silica gel from the zones containing mianserin, as well as its metabolites, was removed with a scalpel, and each substance was eluted three times with chloroform, portions of 5.0 ml each. The chloroform solutions were combined, filtered through a glass filter and subjected to HPLC analysis. In parallel, mianserin was isolated from rat liver.

RESULTS AND DISCUSSION. During the study of rat liver extracts simultaneously with the peak of mianserin (retention time of 3.37 min), the peak of the metabolite of mianserin, identified by us as demethylmianserin – M-7 (retention time of 2.52 min.) is recorded (Fig.).

The conclusion about the presence of a metabolite was made after peaks with the same retention time during the examination of the liver of ten experimental animals.

The graduation graph for the quantitative determination of mianserin in the range of concentrations of 0.2–20.0 μg/ml is characterized by a linear dependence, which is expressed by the equation $Y=2.54 \cdot 10^3 – 4.13 \cdot 10^2$ (at r=0.9998), where Y is the area mianserin peak, X – mianserin concentration, μg/cm³, relative error of quantification 0.86 %.

It was found that during the using purification by chromatography in a thin layer of sorbent, the yield of mianserin from chloroform solutions of dry residues is 99.8–100 %. The degree of extraction of mianserin at pH 8–9, respectively, is 97.1–98.3 %.

Water acidified with oxalic acid from model liver samples isolates 28.9–33.6 % of mianserin. Using acetic acid, it is possible to isolate 56.5–59.8 % of mianserin (Table).

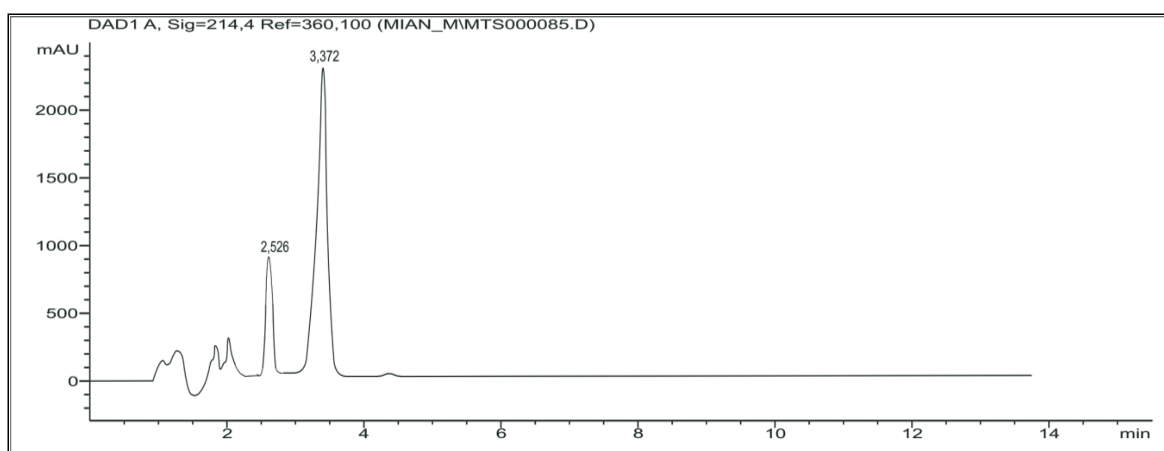

Fig. Chromatogram of mianserin with metabolite isolated from rat liver.

Table – **Results of isolation of mianserin from model liver samples**

Added mianserin	Isolated with water acidified with oxalic acid		Metrological characteristics	Isolated with a 30 % solution of acetic acid		Metrological characteristics
mcg	mcg	%		mcg	%	
100	3.5	28.9	X = 31.22	59.2	56.5	X = 58.0
200	64.8	29.7	S = 1.93	113.6	56.8	S = 1.45
300	98.6	31.5	$S\overline{x}$ = 0.86	173.1	57.7	$S\overline{x}$ = 0.65
400	128.1	32.4	ΔX = 2.40	239.2	59.2	ΔX = 1.80
500	168.0	33.6	ε = 7.96 %	282.5	59.8	ε = 3.10 %

CONCLUSIONS. 1. The efficiency of isolation of mianserin from model liver samples by two methods was compared. It was established that 28.9–33.6 % of mianserin is isolated with water acidified with oxalic acid. 56.5–59.8 % of the studied drug can be isolated with an acidified 30 % solution of acetic acid.

2. To prepare the sample for analysis by HPLC, the conditions for their purification were worked out, the degree of extraction of mianserin from the studied sample is 99.8–100 %.

3. Developed conditions for identification and quantification of mianserin by HPLC on an Eclips C18 column and detection at a wavelength of 214 nm. The limit of quantitative determination of mianserin in solutions is 0.5 μg/ml.

LITERATURE

1. Frahnert C. Analisis of eighteen antidepressants, four atypical antipsychotics and active metabolites in serum by liquid chromatography, a simple tool for therapeutic drug monitoring / C. Frahnert, M. Rao, K. Grasmader // J. Chromatogr B. Analyt Technol. Biomed. Life Sci. – 2012. – **794**, No. 1. – P. 35–47.

2. Dulsire C. Analysis of side effects of mianserin / C. Dulsire // Clin. Pharmacology. – 2004. – **11**, No. 4. – P. 83–86.

3. Filatov E. G. Treatment of headache with Lerivone / E. G. Filatov, A. D. Solovyova // Journal of Neurology and Psychiatry. – 2000. – **9**, No. 3. – P. 58–62.

4. Schaper A. Suicide with mianserine / A. Schaper, E. Farber // Toxicol. Clin Toxicol. − 2002. − No. 40. − P. 343−345.

5. Koski A. Relation of postmortem blood alcohol and drug concentrations in fatal poisoning involving propoxyphene, mianserine and promazine / A. Koski, E. Vuori, I. Ojanpera // Hum. Exp. Toxicol. – 2005. – **119**, No. 6. – P. 344–348.

6. Analytical method development and validation of mianserin hydrochloride and its metabolite in human plasma by LC-MS / B. Chauhan, S Rani, S. Guttikar [et al.] // J. Chromatogr B. Analyt Technol Biomed Life Sci. – 2005. – **823**, No. 2. – P. 69–74.

7. Dodd S. Chiral determination of mianserine and its metabolite in human blood plasma by high-performance liquid chromatography / S. Dodd, G. Burravs, T. Norman // J. Chromatogr. B. Biomed. Sci. Appl. – 2000. – **748**, No. 2. – P. 439–444.

8. Ven K. Effects of the antidepressant mianserin in zebrafish / K. Ven, D. Keil, L. Moens // Chemosohere. – 2006. – **65**, No. 10. – P. 1836–1845.

9. Ridout F. Effects of mianserin on car driving skills / F. Ridout, I. Hindmarch // Psychopharmacology. – 2011. – **154**, 4. – P. 356–361.

REFERENCES

1. Frahnert, C., Rao, M., & Grasmader, K. (2012). Analysis of eighteen antidepressants, four atypical antipsychotics and active metabolites in serum by liquid chromatography, a simple tool for therapeutic drug monitoring. *J. Chromatogr B. Analyt. Technol. Biomed. Life Sci.,* 794 (1), 35-47.

2. Dulsire, C. (2004). Analysis of side effects of mianserin. *Clin. Pharmacology,* 11 (4), 83-86.

3. Filatov, E.G., & Solovyova, A.D. (2000). Treatment of headache with Lerivone. *Journal of Neurology and Psychiatry,* 9 (3), 58-62.

4. Schaper, A., & Farber, E. (2002). Suicide with mianserine. *Clin. Toxicol.,* 40 (2), 343-345.

5. Koski, A., Vuori, E., & Ojanpera, I. (2005). Relation of postmortem blood alcohol and drug concentrations in fatal poisoning involving propoxyphene, mianserine and promazine. *Hum. Exp. Toxicol.,* 119 (6), 344-348.

6. Chauhan, B., Rani, S., Guttikar, I., Zope, A., & Padh, H. (2005). Analytical method development and validation of mianserin hydrochloride and its metabolite in human plasma by LC-MS. *J. Chromatogr. B. Analyt. Technol. Biomed. Life Sci.,* 823 (2), 69-74.

7. Dodd, S., Burravs, G., & Norman, T. (2000). Chiral determination of mianserine and its metabolite in human blood plasma by high-performance liquid chromatography *J. Chromatogr. B. Biomed. Sci. Appl.,* 748 (2), 439-444.

8. Ven, K., & Keil, L. (2006). Effects of the antidepressant mianserin in zebrafish. *Chemosohere,* 65 (10), 1836-1845.

9. Ridout, F., & Hindmarch, I. (2011). Effects of mianserin on car driving skills *Psychopharmacology,* 154 (4), 356-361.

Н. В. Горлачук, С. Ю. Чолач

ТЕРНОПІЛЬСЬКИЙ НАЦІОНАЛЬНИЙ МЕДИЧНИЙ УНІВЕРСИТЕТ ІМЕНІ І. Я. ГОРБАЧЕВСЬКОГО МОЗ УКРАЇНИ

ВИЗНАЧЕННЯ МІАНСЕРИНУ В БІОЛОГІЧНОМУ МАТЕРІАЛІ МЕТОДОМ ВИСОКОЕФЕКТИВНОЇ РІДИННОЇ ХРОМАТОГРАФІЇ

Резюме

Вступ. *Міансерин є похідним тетрациклічних антидепресантів, належить до похідних піперазину-азепіну. Хімічно це (±)-2-метил-1,2,3,4,10,14b-гексагідроксибензо-[c,f]піразино [1,2-a]азепін. Він проявляє антистресову активність, що дуже важливо при лікуванні пацієнтів з депресією, яка поєднується з тривогою.*

Мета дослідження *– розробити ефективну методику виділення міансерину з біологічних тканин та вибрати оптимальні умови для визначення методом високоефективної рідинної хроматографії за наявності метаболітів.*

Методи дослідження. *Ідентифікацію та кількісне визначення міансерину, виділеного з біологічного матеріалу, і його метаболітів проводили методом високоефективної рідинної хроматографії. Дослідження виконували на рідинному хроматографі Agilent 1200, колонці Eclips C18 довжиною 150,0 мм, діаметром 4,6 мм, частинки сорбенту були розміром 5 мкм. Пробу вводили у хроматограф, її об'єм становив 20 мкл, температура колонки – 25 °С. Здійснювали порівняльну оцінку ефективності виділення міансерину з модельних зразків печінки, підкислених 30 % оцтовою кислотою.*

Результати й обговорення. *Під час дослідження екстрактів печінки щурів одночасно з піком міансерину (час утримування – 3,37 хв) спостерігали пік його метаболіту, який ми ідентифікували як деметилміансерин – М-7 (час утримування – 2,52 хв).*

Висновки. *Ефективність виділення міансерину з модельних зразків печінки було порівняно двома методами. Встановлено, що з водою, підкисленою щавлевою кислотою, виділяється 28,9–33,6 % міансерину. Підкисленим 30 % розчином оцтової кислоти можна виділити 56,5–59,8 % досліджуваного препарату. З метою підготовки зразка для аналізу методом високоефективної рідинної хроматографії розроблено умови його очищення, ступінь вилучення міансерину з досліджуваного зразка становить 99,8–100,0 %. Розроблено також умови для ідентифікації та кількісного визначення препарату методом високоефективної рідинної хроматографії на колонці Eclips C18 і детектування при довжині хвилі 214 нм. Межа кількісного визначення міансерину в розчинах становить 0,5 мкг/мл.*

КЛЮЧОВІ СЛОВА: **міансерин; методи виділення; високоефективна рідинна хроматографія.**

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Address for correspondence: *N. V. Horlachuk, I. Horbachevsky Ternopil National Medical University, Maidan Voli, 1, Ternopil, 46001, Ukraine, e-mail: horlachuk@tdmu.edu.ua.*