Ion pair HPLC assay of metformin hydrochloride in human plasma

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ABSTRACT: OBJECTIVE A HPLC method was established for the assaying of metformin in human plasma. **METHODS** The mobile phase was composed of methanol-0.015mol• L^{-1} potassium dihydrogen phosphate (containing 0.03mmol• mL^{-1} sodium heptanesulfonate and adjusted to pH 4.7 by phosphoric acid) (40∶60), the flow rate was 1.0mL/min, the detection was carried out with a UV detection at 232nm, colum temperature was at 37℃. **RESULTS** The calibration curve of metformin was lined between 0.25μg• mL^{-1} ~4.0μg• mL^{-1}, (r=0.9993, n=6). Absolute recovery was more than 80%, within-day and between-day RSD were both less than 10%. **CONCLUSION** The method is simple, rapid, and accurate, and it is suitable for the determination of metformin in the human plasma.

KEY WORDS: ion pair HPLC; metformin; assay

离子对-高效液相色谱法测定人血中二甲双胍含量

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摘要:目的 建立离子对-HPLC法测定人血中二甲双胍含量的方法。方法 采用 ODS C_{18}色谱柱(4.6mm×250mm, 5μm);以甲醇-磷酸二氢钾溶液(含 0.03mol• mL^{-1}庚烷磺酸钠, pH4.7)(40∶60)为流动相,流速:1.0mL• min^{-1},紫外检测波长 232nm,柱温 37℃。结果 离子对-HPLC法测定人血中二甲双胍的线性范围 0.25~4μg• mL^{-1},相关系数 0.9993。日内差异、日间差异均小于 10%,绝对回收率大于 80%。结论 本法适用于二甲双胍血药浓度测定。

关键词:离子对-HPLC;二甲双胍;含量测定
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Metformin hydrochloride is one of the antidiabetic drugs. Because metformin hydrochloride is high polar, so it has a very short retention time when separated using reversed phase chromatography. Acetonitrile and perchloric acid were used as precipitating agent in recent years. According to the reference[1~3], in this report, we present a HPLC method to determine metformin concentration in human plasma. The sample preparation involves protein precipitation with trichloroacetic acid. Sodium heptanesulfonate was used as the ion-pair reagent, and it can solve the problem of short retention time of metformin, also without taking off the bubble machine online.

1 Materials and methods

1.1 Chemicals and reagents: The standard reference of metformin hydrochloride was provided by Guizhou Tianan Pharmaceutical Co. Ltd. The content was 99.95%, Sodium heptanesulfonate (the chemical reagent factory of Shandong king Yu industry) was HPLC-grade. Trichloroacetic acid, potassium dihydrogen phosphate and phosphoric acid were analytical grade. Water was redistilled. Other chemicals were analytical-grade reagents.

1.2 Instruments: HPLC system consists of Waters 717 pump, Waters 2487 UV detector. Data were collected with Waters chromatographic data station. WH-861 Swirl mixing device. The high-speed centrifuge provided by the company of the Abbott laboratories.

1.3 Chromatography condition: The analytical column was ODS C_{18} particle side 5μm column. 4.6mm × 250mm ID. The mobile phase was composed of methanol-0.015mol·L^{-1} potassium dihydrogen phosphate (containing 0.03mmol·mL^{-1} sodium heptanesulfonate and adjusted to pH 4.7 by phosphoric acid) (40∶60); The flow rate was 1.0mL·min^{-1}. The column temperature was maintained at 37℃. The detective wavelength was 232nm and peak area were measured. The count of the column plate of the theory is more than 1500.

1.4 Standard solution preparation: 50mg of the metformin standard was dissolved with redistilled water to 2mg·mL^{-1} in 250mL volumetric flask. The solution was stored at 4℃, it was diluted to the suitable concentrations of metformin when it is necessary.

1.5 sample preparation: To 0.5mL of human plasma samples in 2mL conical extraction tube, 50μl of trichloroacetic (70%) was added. The mixture was swirled for 1 min using a vortex agitator, and then centrifuged at 13 000r·min^{-1} for 6 min. The aqueous layer was shifted to the autosampler vial and 50 μl were injected into the column.

2 Results and Conclusions

2.1 Chromatography The chromatography of blank plasma and metformin were showed in Fig 1. The average retention time of metformin is 6.5 minutes and the endogenous components did not give any interfere peaks.

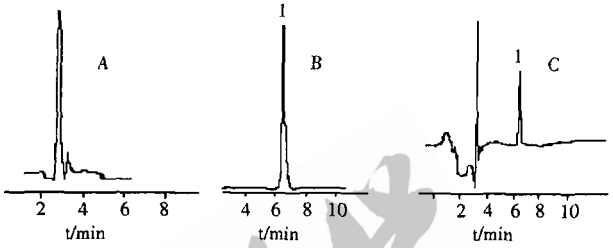

Fig 1 chromatograms of metformin

图 1 二甲双胍的 HPLC色谱图

A: blank plasma; B: solution of metformin; C: blank plasma spiked with metformin (1, metformin)

A:空白血浆;B:二甲双胍溶液;C:二甲双胍血浆混合液(1,二甲双胍色谱峰)

2.2 Standard curve

A standard concentration curve was obtained by adding metformin standard solution at concentration of 0.25, 0.5, 1.0, 2.0, 4.0μg·mL^{-1} to 0.5mL blank human plasma under the same experimental condition. Operation and determine according to the above-mentioned plasma sample. It was regressed between the peak area (A) and the concentration(C, μg·mL^{-1}), Calibration curve was linear over the range of 0.25 ~ 4.0μg·mL^{-1}. A linear equation was: $A = 1358548 \cdot C + 161784.3$ ($r = 0.9993$, $n = 6$). The limit of quantification of plasma metformin was 0.25 μg·mL^{-1}.

2.3 Reproducibility and recovery

To test the within-day and between-day reproducibility, 5 aliquots of each sample were assayed within 1d and 5d at drug levels of 0.25, 1.0, 4.0μg·mL^{-1}. The results were showed in the Tab 1. RSD was in 10.0%. It is reliable to determine the method, accord with the biological sample and analysis of the demand.

5 aliquots of each sample were assayed at drug levels of 0.25, 1.0, 4.0μg·mL^{-1} to test the recovery, write down peak area (As). Substituting plasma and trichloroacetic acid with water, determine according to the terms of above-mentioned chromatograms, write down the peak area (Ar). It is As/Ar·100% to calculate the absolute recovery, the results were showed in the Tab 1.

3 Discussion

3.1 Optimization of chromatographic condition

Tab 1 Recovery and precision for the determination of metformin hydrochloride in plasma ($n=6$)

表 1 人血中二甲双胍的回收率和精密度试验 ($n=6$)

Added (μg/mL)	Measurement (μg/mL)	Recovery(%)	Precision(RSD/%)	
			Between-day	Within-day
0.25	0.21	84±1.72	8.0	9.4
1	1.05	105±1.46	2.1	3.1
4	3.96	99±1.34	2.4	5.6

We operated experimentation according to the report[4], but the retention time of metformin was 2.5 min. Then we used sodium dodecyl sulfate as the ion-pair reagent according to the reference[2], however we could not take off the bubble as the mobile phase was being on the move, because a large number of bubble was made by the unstable chromatogram. Trying to use the sodium heptanesulfonate as the ion-pair reagent, we found that a number of bubble obviously reduced in the course. The bubble was being reduced when the temperature was being raised. We tested the column temperature from 20℃ to 37℃, the course of the chromatogram is steady at 37℃.

To study how did concentration of the sodium heptanesulfonate impact on retention time, we tested the concentration 0.01 ~0.1mmol·mL^{-1}. With the increase of its concentration, retention time also increased, the most suitable concentration was 0.03mol/L. The retention time of metformin is 6.5 min.

We tested pH of the mobile phase from 3.0 to 6.0. At the different of pH, there was no obvious change on retention time, but endogenous materia in plasma interfered obviously. Test confirmed that the pH 4.7 is suitable.

Extraction condition choose metformin is difficult to dissolve to the organic solvent, but the combining rate of albumen is low, It can be extracted from the plasma sample with method of precipitating albumen with trichloroacetic acid, acetonitrile, perchloric acid. The experiment showed that the peak had the best shape when it was precipitated by trichloroacetic acid. There were 100 l trichloroacetic acid as the concentration of 30% in 0.5mL plasma, then 70% trichloroacetic acid was 50 l. Through comparing, we adopted the sediment consumption of 50 l, in order to reduced the dilution effect to the plasma. If clear liquid have fat-soluble interference material exist, available dichloromethane extraction after precipitating, because metformin polar is high, the loss of metformin can be neglected in the course.

Reference

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