Validation in extraction method for determination of morphine in Plasma with NaY Zeolite

R. Zendehdel
Pharmacology Department, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
M. Zendehdel*
Chemistry Department, Arak University, Arak, Iran
M. H. Pirali
Medicinal Chemistry Department, School of Pharmacy, Tehran, Iran
M. Gh. Khansari
Pharmacology Department, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
M. Amini
Medicinal Chemistry Department, School of Pharmacy, Tehran, Iran
M. Darabi
Medicinal Chemistry Department, School of Pharmacy, Tehran, Iran

Archive of SID

Abstract

A method describe for cleaning up morphine from plasma using a liquid-liquid extraction with THF followed up solid-phase extraction with NaY zeolite. Samples were extracted using zeolite NaY column followed by reversed phase HPLC with fluorescence detection. Morphine has shown to have a retention time of 9.45 min using a mobile phase of water, methanol and acetonitrile at E_x= 235nm, E_m=349nm wavelengths. The total recovery of extraction was 107±12.7%. This method was based on an ex-calibration procedure and was linear between 20- 200 ng/ml of morphine. The lowest amount of morphine in plasma, which can be determined by this method, was 10ng/ml.

Keywords: Morphine, Plasma, Zeolite, HPLC.

1. Introduction

Zeolites are crystalline aluminosilicate with channel and pores that adsorbed cation, organic molecule or biological material. Literature review show that in some of biological separation such as amino acid extraction zeolites was used [(1)]. Many work show that NaY zeolite has been used for catalyses effects and its molecular absorbing properties [(2, 3)]. S.J Fisher et al studied size-exclusion chromatography with use ZSM-5 zeolite for trace analysis of polyaromatic hydrocarbons organocholrine pesticides[(4)].Hence; several works on biomedical application of natural zeolite(NZ) have been published during the last five years

* Corresponding author

www.SID.ir

[5,6].For example, recent paper reported a novel use of natural zeolite as a potential adjuvant in anti cancer treatment [7].Rivera et al are reported that the possible interaction between NZ and aspirin, and the results suggested that both products could be simultaneously administered[8]. In all of work zeolites were used as size exclusion while not affecting the target analyze, which are excluded from the zeolite pores, removes most of the interfering material .

Determination of morphine in biological sample has become a routine assay in many clinical and forensic toxicology laboratories, in which urine sample is the biological fluid that almost always is preferred. Since the sensitivity of morphine concentration in urine is 300ng/ml, other biological samples such as plasma have been interested [9]. Previously reported methods of measuring morphine concentration in plasma have used radioimmunoassay (RIA) [10] gas chromatography (GC) with electron-capture detection [11] or mass spectrometry [12] or high-performance liquid chromatography (HPLC) with electrochemical detection (ED)[13]or ultraviolet UV [14] and fluorescence detection [15]. A major problem associated with HPLC is the need of sophisticated extraction procedure to separate the compound of interest from a biological matrix prior to analysis. Such extraction procedures were introduced previously made use of either liquid-liquid extraction [16] or solid-phase extraction methods for example with use of C18-column [17, 18].Our group, in another work determined of morphine in the plasma of addicts with using of NaY zeolite that extract following high-performance liquid chromatography[19].

In this work, we describe a method for validation of cleaning up morphine from plasma using a liquid-liquid extraction with THF followed up solid-phase extraction with NaY zeolite.Hence, reproducibility within-day and between-day was studied.

2. Experimental

2.1. Component and Standards

NaY zeolite prepared from previous work in my laboratory [20]. Hence, solvents were HPLC-grade and obtained from Merck Co. (Germany). All other chemicals were from commercial sources and of analytical grade.

2.2. Chromatographic System

The HPLC system consisted of a model 6000A solvent delivery pump (Waters Assoc., Milford, MA, U.S.A.); a Model 7125 injector equipped with a 100-µl loop (Rheodyne, Cotati, CA, U.S.A) a µBondapak C_{18} Column (300 mm × 4mm I.D., 10µm), a model 474 fluorescence detector with an excitation wavelength of 235 nm and a 349 nm emission. Chromatography was performed at ambient temperature using a mobile phase consisting of 5% methanol, 3% acetonitrile, 0.5 mM sodium edetate, 0.012M potassium dihydrogen orthophosphate and 0.148 mM phosphoric acid in distilled water. The flow

Rate was 1.2mL/min.In this mobile phase we haven't interference that investigated in before work [19].

2.3. Sample Preparation

Six different concentrations from morphine were prepared for calibration curve (20, 50, 75, 100,150, and 200ng/mL). Stock solution was prepared by dissolving morphine in water followed by an appropriate dilution in plasma. Samples were kept at room temperature during analysis.

First step:

To 0.5mL of human plasma were added 0.25mL of zinc sulphate 0.7M, 2.5mL of bicarbonate buffer (pH=10.5), 6mL of THF. After we add some drop from morphine to this

Archive of SID

www.SID.ir

mixture. The mixture was mixed on a vortex mixer for 2 min, centrifuged for 3min (3000g) and the upper organic layer was separated.

Second step:

0.4g from NaY zeolite was dry packed into a column. The column has 30mm length and 5mm diameter that had been plugged with a wad of cotton at two ends. This column was kept in vacuum (0.075 bars) for 1 min. Organic layer was passed to this column in atmosphere pressure.

2.4. Validation of Methods

The organic layer (THF) was loaded to the column. The packing of zeolite Y should be stand for 5 min with THF period at room temperature under atmospheric pressure. The extract solution was dried under N_2 flow and again dissolved in a total volume of 200µl in HPLC mobile phase (chromatographic system) and 100µl of that were injected to the HPLC system.

3. Results and discussion

3.1. Method Modification

Calibration curves showed good linearity between peak-height and concentration from 0.2 to 2µ g/mL (y =0.026X+0.46; r^2 =0.993). We choose two concentrations 500 and 1000 ng/mL for this work. Because concentrations in plasma from subjects on morphine therapy are in the range 80-800ng/ml and in the tolerance development could be considered as low as500 ng/mL[21].In the first step we use from the plasma sample that concentration of morphine is 1000ng/mL. Fig. 1 shows typical chromatogram of plasma sample via extraction of morphine by THF described by others [16] that morphine peak can be seen at 4.75 min. There is an unknown peak at 5.63 min that interferes with morphine at interest concentrations. When we use from the sample that interest concentration of morphine is 500ng/mL unknown peaks completely cover morphine peak (see Fig. 2). To overcome this interference, several stationary and mobile phases were investigated to establish the optimum separation of morphine but an efficient separation was not achieved. Therefore, in second step we used a column loaded with the zeolite NaY to achieve an improve separation of morphine in plasma. Figure 3 shows chromatogram of plasma sample with 500 ng/mL morphine that passed from NaY zeolite column .It seems this zeolite absorbed inconvenience material and eliminate unknown peak and can be seen morphine peak at 5.03min. When more morphine added to all samples height of 5.03 min peak increases. Therefore this peaks related to morphine. In last section, after passing the extract solution (THF phase), the zeolite column was washed with 10ml methanol to prove that no morphine remained in the column. Figure 4 shows chromatogram of methanol extraction from zeolite column. It can be clearly seen the zeolite NaY absorbed the unwanted interferes unknown compounds. It seems that there is no any specific interaction between morphine and zeolite Y; therefore, this could be attributing to the other morphine analogues.

In order to achieve a better chromatographic separation, we examined a new chromatography condition, which was described in material and method sections. Fig. 5 shows a typical chromatogram from an injection of a plasma containing morphine at a concentration of 500ng/ml. Compared to sample of fig. 3 the separation was greatly improved while retention time increased to 9.7min.

The limit of detection was 10ng/ml in plasma and recovery was determined by comparing the peak heights of extracted plasma samples with the peak heights of standards of the same concentrations. The recovery (n = 4) at the concentration of 100ng/mL was 107.1±12.7%.

Archive of SID

www.SID.ir

3.2. Reproducibility Studies

The within run relative standard deviations (R.S.D. %) calculated from the peak height of morphine at analyst concentrations of 20, 50, 75, 100, 150 and 200ng/mL (four samples assayed for each concentration) and between run (R.S.D. %) obtained from independently prepared standards dilutions during 4 days (see Table 1). These data demonstrate adequate reproducibility for routine laboratory

4. Conclusion

We have introduced a method for validation by using THF and Y zeolite to clean up morphine in plasma. In this work we use from liquid-liquid extraction with THF followed up solid-phase extraction with NaY zeolite. Zeolite Y retained the unwanted interferes unknown compounds. Moreover our method is sensitive, reliable and reproducible for even 0.5mL of plasma, which may be useful for routine monitoring of plasma morphine concentration in future. Reproducibility within-day and between-day was studied. The recovery (n = 4) at the concentration of 100ng/mL was 107.1±12.7%.

Table 1

Within-day and between- day precision (n=4)

	20 ng/ml	50 ng/ml	70 ng/ml	100 ng/ml	150 ng/ml	200 ng/ml
Within-day (R.S.D. %)	15	14	10	8	9	7
Between-day (R.S.D. %)	15	13	12	12	8	9.8

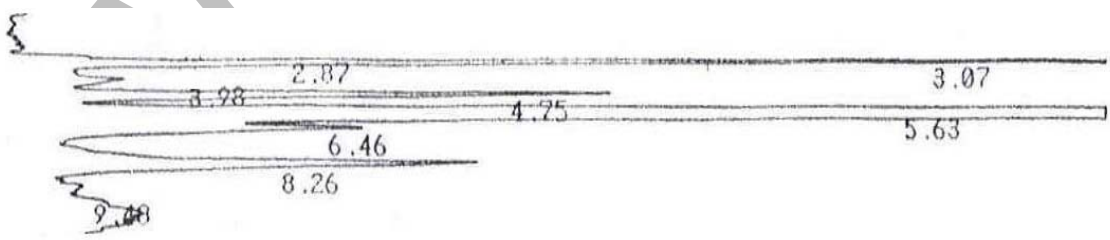

Fig 1. Chromatogram showing extracted plasma blank spiked with 1000ng/mL of morphine by THF

Archive of SID

www.SID.ir

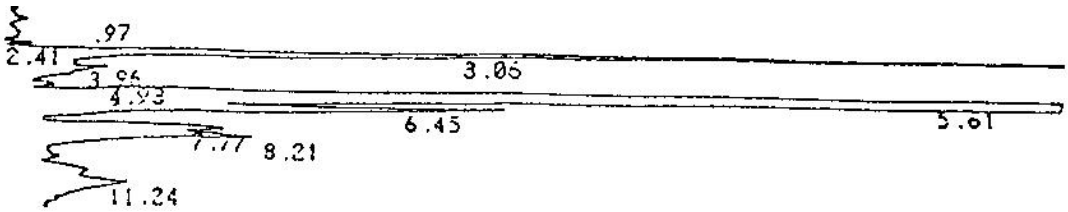

Fig 2. Chromatogram showing extracted plasma blank spiked with 500ng/mL of morphine by THF

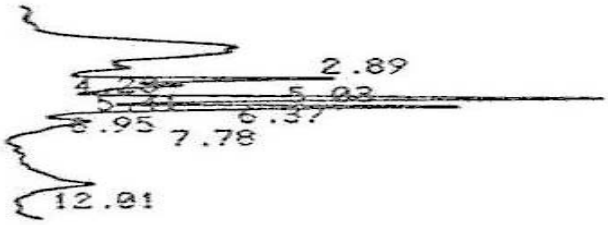

Fig 3. Chromatogram showing extracted plasma blank spiked with500ng/mL of morphine by THF

Followed by zeolite Y column.

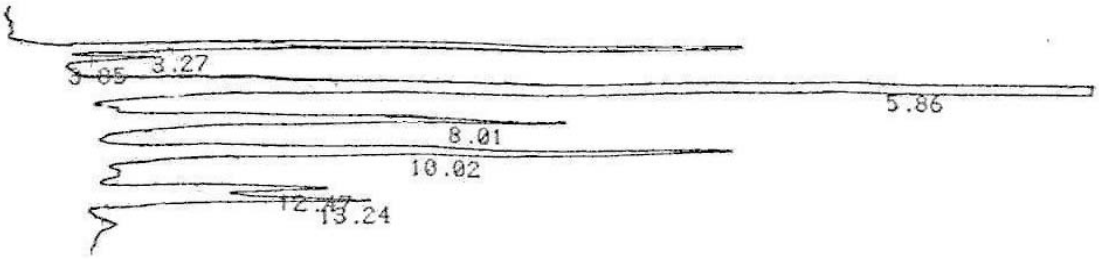

Fig 4. Chromatogram showing adsorbed molecules of plasma from zeolite Y

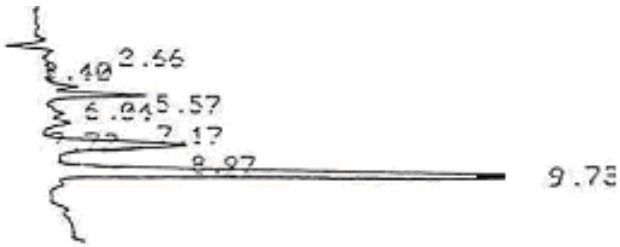

Fig.5.Chromatogram showing extracted plasma blank spiked with 500 ng/ml of morphine.

www.SID.ir

References

1- Yonset, S., Schaefer-Treffeufeldt, W., Kiss, A., Sextl, E. and Naujok, H., Ger. offen. DE 4, 217, 203 (cl.co7c227/40), 25 Nov 1993 Aool. 23May 1992:12pp

2- Kooti, M., Zendehdel, M. and Pouramini, M. M., *J. of Inclusion Phenomena and Macro cyclic Chemistry*, **42**, 265(2002).

3- Zendehdel, M., Fouroghi far, N. and Gaykani, Zm, *J. of Inclusion Phenomena and Macro cyclic Chemistry*, **53**, 42 (2005).

4- Fisher, S. J., Alexander, R. and Kagi, R. I., *J.of Chromatog*, **624**, 205(1993).

5- Park, M. and Komarneni, S., *Zeolites*, **18**, 171(1997).

6- Farias, T., Ruiz-Salvador, A. R. and Rivera, M., *Micropor and Mesopor Mat*, **61**, 117(2003).

7- Pavelic, K., Katic, M., Sverko, V., Marotti, T., Bosnjak, B., Balog, T., Stojkovic, R., Radacic, M., Colic, M. and Poljak-Blazi, M., *J. Cancer Res.Clin.Oncol*. **128**, 37 (2002).

8- Riveria, A., Rodriguez-Albelo, L. M., Rodriguez-Fuentes, G., Altshuler, E., in: Galarneau, A., Renzo, F. Di, and Fajula, F., *J. Vedrine (eds.)*, vol.135, Elsevier, Amesrterdam, p.**32**-P-07 (2001).

9- Levine, B. and Snialeh, J. E., *J.of Forensic science*, **43**, 388 (1998).

10- McQuay, H. J., Carroll, D., Faura, C. C., Gavaghan, D. J., Hand, C. W. and Moore, R. A., *Clin. Pharmacol. Ther*, **48**, 236(1990).

11- Gjerde, H., Fongen, U., Gundersen, H. and Christophersen, A. S., *Forensic Sci. Int*., **51**, 105 (1991).

12- Edlund, P. O., *J. Chromatogr*. **206**, 109 (1981)

13- Neill, G. P., Davies, N. W., and McLean, S., *J. Chromatogr*, **565**, 207 (1991).

14- Wasels, R. and Belleville, F., *J. Chromatogr. A*, **674**, 225(1994).

15- Posey, B. L. and Kimble, S. N., *J. Anal. Toxicol*, **7**, 241 (1983).

16- Schneider, J. J. and Ravenscroft, P. J., *J. Chromatogr*., **497**, 32 (1989).

17- Huwyler, J., Rufer, S., Kusters, E. and Drewe, J., *J. Chromatogr. B Biomed. Appl*, **674**, 57(1989).

18- Gerostamoulos, J. and Drummer, O. H., F*orensic Sci. Int. Forensic Science International*, **77**, 53 (1996).

19- Ghazi-Khansari, M., Zendehdel, R., Pirali-Hamedani, M. and Amini, M., 364 (1-2), 235 (2006)

20- Breck, D. W. and Tonawanda, N. Y., *Assigned to union carbide, pat*. No. **3130007** patented, (1964).

21- Tagliaro, F., Franchi, D., Dorrizzi, R. and Marigo, M., *Journal of Chromatogr*. **488**, 215 (1989).

Archive of SID

www.SID.ir