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Short communication

Solid-phase extraction of methadone enantiomers and benzodiazepines in biological fluids by two polymeric cartridges for liquid chromatographic analysis

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Abstract

The aim of this work was to present the advantages of two polymeric cartridges (Oasis HLB from Waters and Abselut Nexus from Varian) for the solid-phase extraction of methadone enantiomers and its major metabolite, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) and of some benzodiazepines (diazepam, flunitrazepam, nitrazepam, oxazepam) in serum and urine in comparison with classical C18-bonded-silica cartridges or liquid extraction. After addition of serum or urine samples, these two cartridges were washed with a water–methanol mixture (95:5, v/v) and eluted with diethylether. After rapid evaporation, the residue was regenerated with mobile phase and injected either in a chiral column (Cyclobond I-2000 RSP) for methadone enantiomers and its metabolite or in a reversed-phase column (Symmetry Shield RP8) for benzodiazepines. The results showed that the chromatograms of blank serum and urine were cleaner than those obtained from classical solid-phase extraction or liquid extraction. The recoveries from these two polymeric cartridges were higher (95–102%) than those obtained by the two previous classical methods and the total time for extraction and solvent evaporation was also shorter (about 6–7 min). For methadone and benzodiazepine extraction, the use of acidic or alkaline buffer was not necessary.

Keywords: Polymeric extraction cartridges; Methadone; EDDP; Diazepam; Flunitrazepam; Nitrazepam; Oxazepam; Enantiomer separation

1. Introduction

The extraction of drugs and toxics in biological matrices for physical chemical analysis (HPLC, GC, MS), a key step of bioanalysis, is used to clean the samples by removing proteins and other interfering biological compounds before analysis. However, classical extraction techniques, such as liquid extraction and solid-phase extraction using reversed-phase silica sorbents have many problems when there are the differences in chemical nature (polarity, affinity, pH, etc.,) between extracted compounds and extraction solvents or solid-phase extraction sorbents. The classical commonly used sorbents are porous silica particles surface-bonded with C18 or other hydrophobic alkyl groups. The limitations of today's sorbents require the analyst to watch carefully and control closely the extraction procedure. Therefore, it is difficult and time-consuming to achieve high, reproducible recoveries for analysis of numerous drugs, especially a mixture of apolar compounds and polar ones, such as drugs and their polar metabolites. Recently, some polymeric extraction cartridges are commercialized and can be used at pH from 1 to 14

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and with many different polar and apolar organic solvents (methanol, chloroform, diethylether, etc.) contrary to classical reversed-phase silica extraction columns. Therefore, it is easier to find an appropriate extraction condition for a specific compound and especially for a mixture of analytes with different chemical properties, such as polarity, pH, affinity, etc. [1–4].

The aim of this work was to present the advantages of two polymeric cartridges (Oasis HLB from Waters and Abselut Nexus from Varian) for the solid-phase extraction of methadone and its major metabolite, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) and of some benzodiazepines (diazepam, flunitrazepam, nitrazepam, oxazepam) in serum and urine in comparison with liquid extraction for methadone or with C18-bonded-silica cartridge for benzodiazepines. These extracted compounds will be analyzed either by chiral HPLC for methadone enantiomers and its metabolite or by reversed-phase mode for benzodiazepines. These methods were applied to the determination of methadone enantiomers and its metabolite in some treated patient serum and also of oxazepam in some treated rabbit serum.

2. Materials and methods

2.1. Chemicals and reagents

All drugs, chemicals and solvents were of analytical purity and purchased from Sigma (St. Louis, MO, USA). Deionized water was purified by Milli Q.UV Plus system (Millipore, Milford, MA, USA).

2.2. Materials

The liquid chromatographic system consisted of a Waters pump Model 600E (Milford, MA, USA), a Waters UV–vis photodiode array detector Model 994, a Waters printer plotter Model 5200, a Shimadzu integrator Model CR-6A (Kyoto, Japan) and a Rheodyne injector Model 7125, fitted with a 50 μl loop. A column chiller system Model 7955 with a temperature range between 0 and 50 °C ± 0.2 °C was purchased from Jones Chromatography Inc. (Lakewood, Colorado, USA). An extraction vacuum apparatus for solid-phase extraction was purchased from Supelco (Bellefonte, PA, USA).

Two kinds of polymeric sorbents were used: 1 ml Oasis HLB extraction cartridge [poly(divinylbenzene-co-*N*-vinylpyrrolidone)] from Waters (Milford, MA, USA) and 1 ml Abselut Nexus extraction cartridge (chemical structure unknown) from Varian (Harbor, CA, USA), both for the extraction of these previous drugs in spiked serum and urine.

Two kinds of HPLC analytical columns (250 mm × 4.6 mm i.d., 5 μm) were used: a chiral column Cyclobond I-2000 RSP (β-cyclodextrin derivatized with R,S-hydroxypropyl ether-bonded phase, 5-μm) (Astex, Whippany, NJ, USA) for the analysis of methadone enantiomers and its major metabolite (EDDP) and a reversed-phase column SymmetryShield RP-8 (Waters) for the separation of four benzodiazepines. Each analytical column was connected with a guard column (10 mm × 3.2 mm i.d.) packed with the same bonded phase.

2.3. Extraction procedures

After addition of serum or urine samples (100–200 μl), these two polymeric cartridges were washed with 1 ml of water–methanol mixture (95:5, v/v) and eluted with diethylether (2 × 1 ml). About 200 mg anhydrous Na_2SO_4 were added in this extracted solvent by slightly shaking the collected glass tube (5 ml). After transferring the supernatant extracted solvent to a clean glass tube and rapid evaporation under nitrogen stream at about 40 °C, the residue was regenerated with 100 μl of mobile phase, then 50 μl aliquot was injected either into a chiral column for methadone enantiomers and its metabolite (EDDP) analysis or into a reversed-phase column for the separation of four benzodiazepines. For Oasis HLB extraction cartridge, the column was conditioned with 1 ml methanol then 1 ml deionized water before the addition of biological samples. This precondition was not necessary for Abselut Nexus extraction cartridge. For the analysis of methadone enantiomers and EDDP in spiked serum and urine, 100 μl of papaverine hydrochoride (2 μg/ml in water) used as an internal standard were added in the same time with the sample into the polymeric cartridges.

For the solid-phase extraction of four benzodiazepines using classical reversed-phase-bonded-silica technique, a Bond Elut C18 extraction column (1 ml) (Varian) was chosen for this study of comparison. This column must be preconditioned with a column volume of methanol and then with water before adding serum or urine samples (100–200 μl) mixed with the same volume of 0.1 M sodium borate buffer (pH 9.5). After drawing the column by vacuum, the matrix was washed twice with water, followed by 40 μl of methanol, then eluted with 2 × 1 ml methanol. The total eluent was dried under a stream of nitrogen and the residue was reconstituted in 100 μl of mobile phase. A 50 μl aliquot of this extract solution was injected into the analytical column.

A liquid extraction technique previously described [5] with slight modification was also chosen for the comparison study of methadone enantiomers and its metabolite EDDP. Briefly, samples were added with 100 μl of internal standard (papaverine hydrochloride), 300 μl of 10% anhydrous sodium carbonate in water and 4 ml of hexane. After vortexing then centrifuging, the upper layer was transferred, then dried and regenerated with 100 μl of mobile phase.

The recovery was calculated by the areas of peaks after extraction compared to those of peaks obtained from direct injection of standard solutions in mobile phase at the same concentrations.