Journal of Pharmaceutical and Biomedical Analysis 113 (2015) 239–264

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Review

Analytical methodologies for the determination of benzodiazepines in biological samples

Karolina Persona [a], Katarzyna Madej [a,*], Paweł Knihnicki [a], Wojciech Piekoszewski [a,b]

[a] Department of Analytical Chemistry, Faculty of Chemistry, Jagiellonian University, 30-060 Krakow, Poland
[b] Laboratory of High Resolution Mass Spectrometry, Regional Laboratory of Physicochemical Analysis and Structural Research, Faculty of Chemistry, Jagiellonian University, 30-060 Krakow, Poland

ARTICLE INFO

Article history:
Received 22 October 2014
Received in revised form 5 February 2015
Accepted 9 February 2015
Available online 17 February 2015

Keywords:
Benzodiazepines
Body fluids
Alternative biological materials
Sample preparation techniques
Analytical methods

ABSTRACT

Benzodiazepine drugs belong to important and most widely used medicaments. They demonstrate such therapeutic properties as anxiolytic, sedative, somnifacient, anticonvulsant, diastolic and muscle relaxant effects. However, despite the fact that benzodiazepines possess high therapeutic index and are considered to be relatively safe, their use can be dangerous when: (1) co-administered with alcohol, (2) co-administered with other medicaments like sedatives, antidepressants, neuroleptics or morphine like substances, (3) driving under their influence, (4) using benzodiazepines non-therapeutically as drugs of abuse or in drug-facilitated crimes. For these reasons benzodiazepines are still studied and determined in a variety of biological materials. In this article, sample preparation techniques which have been applied in analysis of benzodiazepine drugs in biological samples have been reviewed and presented. The next part of the article is focused on a review of analytical methods which have been employed for pharmacological, toxicological or forensic study of this group of drugs in the biological matrices. The review was preceded by a description of the physicochemical properties of the selected benzodiazepines and two, very often coexisting in the same analyzed samples, sedative-hypnotic drugs.

Contents

1. Introduction 240
2. Sample preparation techniques 240
   2.1. Sample preparation of conventional body fluids 242
   2.2. Sample preparation of alternative biological materials 243

Abbreviations: 2D UTLC, two-dimensional ultra-thin-layer chromatography; ACN, acetonitrile; AP, atmospheric pressure; APCI, atmospheric pressure chemical ionization; AP-MALDI-MS(MS), atmospheric pressure matrix-assisted laser desorption/ionization mass spectrometry; AuNPs, gold nanoparticles; BSA, bovine serum albumin; BSTFA, *N,O*-bis-(trimethylsilyl)trifluoroacetamide; BZD, benzodiazepines; C_{16}MIMBr, 1-cetyl-3-methylimidazolium bromide; C_{16}MPYB, *N*-cetyl-*N*-methylpyrrolidinium bromide; CE, capillary electrophoresis; CEC, capillary electrochromatography; CEC-MS(TOF), capillary electrochromatography-time of flight mass spectrometry; CI, chemical ionization; CPE, cloud-point extraction; CS, column-switching; CV, coefficient of variation; DAD, diode array detector; DFSA, drug facilitated sexual assault; DLLME, dispersive liquid–liquid microextraction; DPCAdSV, differential pulse cathodic adsorptive stripping voltammetry; DPV, differential pulse voltammetry; DUID, driving under the influence of drugs; ECD, electron capture detector; EDDP, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine; EI, electron ionization; EMIT, enzyme-multiplied immunoassay technique; ESI, electrospray ionization; GC, gas chromatography; HPLC, high performance liquid chromatography; HS-SPME, headspace solid-phase microextraction; LC, liquid chromatography; LC–HRMS, liquid chromatography–high resolution mass spectrometry; LLE, liquid–liquid extraction; LOD, limit of detection; LOQ, limit of quantification; MAE, microwave-assisted extraction; MALDI-MS, matrix-assisted laser desorption/ionization-mass spectrometry; MDA, 3,4-methylenedioxyamphetamine; MDEA, 3,4-methylenedioxy-*N*-ethylamphetamine; MDMA, 3,4-methylenedioxy-*N*-methylamphetamine; MECC, micellar electrokinetic chromatography; MISPE, molecularly imprinted solid-phase extraction; MRM, multiple reaction monitoring; MS, mass spectrometry; MSTFA, *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide; MTBSTFA, *N*-methyl-*N*-(tert-butyldimethylsilyl)trifluoroacetamide; NICI, negative-ion chemical ionization; NPD, nitrogen–phosphorus detector; PARAFAC, parallel factor analysis; PDA, photodiode array detector; PFSPE, packed-fibre solid-phase extraction; PLS, partial least squares; PSA, primary–secondary amine sorbent; QuEChERS, Quick Easy Cheap Effective Rugged Safe; RAM, restricted access material; SRMM–MECC, stacking and reverse migration micelles–micellar electrokinetic chromatography; SDS, sodium dodecyl sulfate; SERS, surface enhanced Raman spectroscopy; SPE, solid-phase extraction; SPME, solid-phase microextraction; RSD, relative standard deviation; TBDMS, tert-butyldimethylsilyl; THF, tetrahydrofuran; TMCS, trimethylchlorosilane; TOF, time-of-flight; UA-DLLME, ultrasound-assisted dispersive liquid–liquid microextraction; UPLC, ultra performance liquid chromatography; UV, ultraviolet detection.

* Corresponding author. Tel.: +48 12 6635602; fax: +48 12 6635601.
E-mail address: madejk@chemia.uj.edu.pl (K. Madej).

http://dx.doi.org/10.1016/j.jpba.2015.02.017
0731-7085/

3. Analytical methods 243
3.1. Chromatographic techniques 246
3.1.1. Liquid chromatography 246
3.1.2. Gas chromatography 259
3.2. Chromatography-related techniques 260
3.3. Electrochemical methods 260
3.4. Miscellaneous methods 261
4. Conclusions 261
Acknowledgements 262
References 262

1. Introduction

Benzodiazepines (BZD) are a large group of drugs that were introduced to medical practice in the 1960s. Owing to their therapeutic effect (namely: anxiolytic, sedative, somnifacient, anticonvulsant, diastolic and muscle relaxant), BZD belong to the most often prescribed and applied pharmaceuticals [1]. Nowadays, there are over 50 BZD derivatives available worldwide, with the vast majority being under the international control of the Convention on Psychotropic Substances. According to the International Narcotics Control Board, in the last ten years the most commonly used BZD were: alprazolam, chlordiazepoxide, diazepam, flunitrazepam, lorazepam, lormetazepam, nitrazepam, temazepam, and triazolam [1]. It is estimated that every year about 10–20% of adults living in the developing countries take these drugs [2]. Furthermore, the effectiveness and safety in their use, as well as the low costs of these types of pharmaceuticals affect their spread.

Due to their properties, BZD belong to the most commonly prescribed medications worldwide and also, as a result, to the most commonly abused pharmaceuticals. Despite the fact that overdoses happen more often than in the case of any other drugs, BZD are believed to be relatively safe, mainly because of the ability of the human organism to adapt quite quickly to an increased level of the drug in the blood stream. However, the safety of using BZD decreases when co-administered with alcohol or other sedatives, antidepressants, neuroleptics and morphine-like substances, as it can cause a synergistic effect [1]. Taking BZD together with alcohol is especially dangerous, as this combination may cause significant lethargy, thus increasing the probability of traffic or home accidents [3,4]. Abuse of BZD action based on losing consciousness and memory, and sex crimes connected with them, stands for a particular problem. A victim under the influence of the drug has no recollection of the events and, therefore, BZD determination in biological materials becomes essential evidence in drug-facilitated crimes [5]. Another problem is connected with a number of cases of driving under the influence of drugs (DUID) [6,7]. The problem is now so significant that in England and Wales, in the period from February 2010 to March 2011, more than 100 cases (out of 376 controlled people) were reported as driving under the influence of BZD [7].

The structures of BZD are diversed, however a heterocyclic diazepine ring is most often condensed with a benzene ring. Derivatives of 1,4-benzodiazepine belong to the most common sub-group, with their prototype of chlordiazepoxide, which is a derivative of the amidine type. Introduction of a lactam structure in the place of the amidine group gave the most representative drugs in this group, which may be considered as derivatives of diazepam (Table 1). From the physico-chemical point of view BZD are lipophilic compounds with a relatively high octanol-water partition coefficient (e.g. for diazepam $\log P = 2.8$). They are quickly absorbed upon oral intake, and therefore the bioavailability depends mainly on their form and the way of administration (e.g. bioavailability of diazepam from a tablet is 100% but from suppository 50–60%). Some physicochemical and pharmacological properties of individual drugs from the BZD group are presented in Table 1.

The metabolism of BZD covers 3 pathways: hydroxylation, demethylation and glucuronidation. Biotransformation reaction, where 1,4-benzodiazepines like diazepam, nordazepam (also known as nordiazepam) and temazepam are mainly metabolized and excreted as oxazepam or oxazepam glucuronide, belongs to the best-known metabolic pathways. Similarly, clorazepate is metabolized to oxazepam through nordazepam. Oxazepam is the most often found metabolite of the majority of 1,4-benzodiazepines in urine [1,2,8]. Most of the products of metabolism can also be pharmacologically active and may be used in treatment.

The hazardous interactions of BZD with alcohol and other pharmaceuticals exhibiting similar pharmacodynamic action, and their use in drug-facilitated crimes, as well as driving under the influence of the drugs explains the legitimacy of BZD determination and the search for more effective methods for isolation of them from biological material.

Our review covers development of analytical methodologies, including sample preparation techniques and subsequently used analytical methods for the determination of BZD drugs, which were reported in majority of the important publications over the last ten years.

2. Sample preparation techniques

Generally, the choice of sample preparation technique depends on the following main factors: sample matrix, analyte, the aim of analysis, and the analytical technique used in connection with the last issue.

Liquid–liquid extraction (LLE) and solid-phase extraction (SPE) procedures are predominately used for the isolation of BZD from biomatrices. The use of these extraction techniques usually requires an appropriate pretreatment of a sample, like adjustment of the sample pH or protein precipitation. Some BZD and/or their phase I metabolites can be conjugated with glucuronic or sulfuric acid. Therefore, cleavage of conjugates is often performed; especially in urine. In the case of keratin matrices' (hair, nails) analysis, additional initial procedures of washing, cutting and pulverization before an extraction process are necessary.

In some cases of BZD determinations by the GC–MS method, the obtained extracts, especially from whole blood, as well as from oral fluid, were submitted to the derivatization step with such reagents as *N*-methyl-*N*-(tert-butyldimethylsilyl)trifluoroacetamide (MTBSTFA) [9,10], acetonitrile (ACN)/MTBSTFA (80:20, v/v) [11,12], tetramethylammonium hydroxide and propyliodide (first step) and triethylamine/propionic anhydride, 1:1, v/v (second step) [13], MTBSTFA/ACN/ethyl acetate (20:40:40, v/v/v) [14]. The extracts of urine samples, analyzed for oxazepam by the fluorescence method, were derivatized with cerium(IV), which is a catalyst, in concentrated ortho-phosphoric acid (85%) [15].