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Simultaneous determination of 18 psychoactive agents and 6 metabolites in plasma using LC–MS/MS and application to actual plasma samples from conscription candidates

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ABSTRACT

In Korea, an increasing number of people attempt to evade military conscription by posing as mental health patients. To verify the authenticity of mental illness, there is a need to detect wide range of psychoactive agents in biological specimens of conscription candidates. In this study, we developed and validated a liquid chromatography–tandem mass spectrometry (LC–MS/MS) method for simultaneous determination of 18 psychoactive agents and 6 metabolites in human plasma. The method was characterized by the use of a simple, fast and cheap protein precipitation as sample preparation, a rapid run time (11 min) and a low volume of plasma sample (200 μL). The analytes were monitored under the scheduled multiple reaction monitoring (sMRM) positive and negative mode using electrospray ionization (ESI). The essential validation parameters including selectivity, linearity, accuracy, precision, matrix effect and recovery were satisfactory. The limit of detection ranged from 0.0005 to 0.001 μg/mL, and limit of quantitation ranged from 0.005 to 0.025 μg/mL. The developed method was successfully applied to 323 actual plasma samples submitted by Korea central physical examination center of military manpower administration in 2016, and is expected to contribute to the rapid and accurate disposition of military service.

1. Introduction

Draft evasion is an intentional decision not to comply with the national military conscription policies. In most countries where conscription is applied, refusing to submit a draft is considered a criminal offense [1]. In Korea, an increasing number of people seek to evade conscription. According to the military manpower administration (MMA), from April 2012 to July 2015, 123 suspects were prosecuted on suspicion of draft evasion, with 9 in 2012, 45 in 2013, 43 in 2014, and 26 in 2015 (year to July). The variety of ways to evade conscription by failing the physical examination include intentional tattoo, physical damage, eye disease, weight control, and fake mental illness. Of these, the latter accounted for the largest proportion (26.8%), followed by intentional tattoo (25.2%), eye disease (16.3%), and weight control (14.6%) [2].

Detection of psychoactive agents in biological specimens is necessary to check whether the conscription candidates suffer from mental illness. In Korea, the central physical examination center (CPEC) of MMA carries out the physical examinations. CPEC also check whether the individual has received drug treatment through domestic and overseas consignment inspections. This background check is time consuming and unnecessary budget expenditure. Therefore, CPEC signed a business agreement with the national forensic service (NFS) in August 2015 for rapid and cost-effective physical examination of the military service. CPEC has been submitting plasma samples for analysis to the NFS since August 2015. They have requested the analysis of 18 psychoactive agents classified as anticonvulsants, antipsychotics, antidepressants, central nervous system stimulants, antiparkinson agents, and anxiolytics. The agents are atomoxetine, aripiprazole, benztropine, buspirone, clobazam, clonazepam, duloxetine, escitalopram, gabapentin, lamotrigine, levetiracetam, modafinil, olanzapine, oxcarbazepine, paliperidone, topiramate, ziprasidone,

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and zonisamide. In addition to these parent drugs, we added some of their active or major metabolites, including 10-OH-carbazepine, 6-hydroxybuspirone, 7-aminoclonazepam, dehydroaripiprazole, desmethylclobazam, and lamotrigine-N2-oxide (Fig. 1).

Simultaneous pharmacotherapy with different classes of drugs is used in many psychiatric disorder treatments. Concurrent therapy is used to reduce the risk of adverse effects in high-dose or long-term use, and to treat various symptoms [3]. Antidepressants and anticonvulsants are widely used in the treatment of psychiatric disease such as depression, mood and anxiety disorders, and schizophrenia [4]. Anticonvulsants and antipsychotics are used to treat bipolar disorder [5]. Sempio et al. noted that combined therapy is being used to reduce the risk of sides effects, such as the increased risk of sudden cardiac death associated with antipsychotics and serotonin toxicity for antidepressants [3]. Therefore, the simultaneous analysis for a wide range of drugs is needed in developing an analytical method for psychiatric drugs. Currently, many analytical procedures using liquid chromatography–tandem mass spectrometry (LC–MS/MS) have been described for the determination of several groups of drugs that include anticonvulsants, antipsychotics, or antidepressants, and there are some multi-drug methods for the simultaneous determination of these groups [3,4,6–10]. The procedures published by Sempio et al. covered the 88 psychoactive drugs including benzodiazepines, antipsychotics, and antidepressants [3], and Steuer et al. described the quantitation methods for 40 antidepressants and neuroleptics [4]. However, there is no method that covers all our target psychoactive agents needed for verification the authenticity of mental illness, including acid and basic compounds with different polarities.

In this work, a LC–MSMS method for simultaneous determination of 18 psychoactive agents and 6 metabolites in human plasma was developed and validated. Actual plasma samples from conscription candidates in 2016 were analyzed to demonstrate the applicability of the developed method. Furthermore, type and frequency of drugs related to draft evasion were also investigated.

2. Material and methods

2.1. Chemicals

Aripiprazole (EP, 100%), atomoxetine (EP, 99.9%), benztropine (USP, 99.6%), buspirone (EP, 100%), duloxetine (EP, 99.9%), escitalopram (EP, 100%), gabapentin (EP, 99.9%), lamotrigine (EP, 100%), levetiracetam (EP, 99.9%), modafinil (EP, 100%), olanzapine (USP, 99.6%), oxcarbazepine (EP, 99.8%), paliperidone (USP, 99.6%), topiramate (USP, 99.6%), ziprasidone (EP, 95.6%), and desmethylclobazam (EP, 100%) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Zonisamide (98%) and lamotrigine-N2-oxide (98%) were obtained from Toronto Research Chemicals (Toronto, ON, Canada). 6-Hydroxybuspirone (99.9%) was purchased from TLC Pharmaceutical Standards (Aurora, ON, Canada). Dehydroaripiprazole (95%), and 10-OH-carbazepine (98%) were provided by Santa Cruz Biotechnology (Dallas, TX, USA). Clobazam (1.0 mg/mL in methanol), clonazepam (1.0 mg/mL in methanol), 7-aminoclonazepam (1.0 mg/mL in acetonitrile), trimipramine-d_3 (100 μg/mL in methanol), and topiramate-d_{12} (100 μg/mL in methanol) were obtained from Cerilliant (Round Rock, TX, USA). High performance liquid chromatography-grade acetonitrile and methanol were purchased from Fisher Scientific Co. (Fair Lawn, NJ, USA). Ammonium formate and formic acid were obtained from Fluka (St. Louis, MO, USA).

2.2. Sample preparation

For the LC–MS/MS analysis, plasma samples were prepared by protein precipitation with acetonitrile. Seven hundred microliters of acetonitrile and 50 μL of internal standard (IS) comprising 0.1 mg/L of trimipramine-d_3 and 0.5 mg/L of topiramate-d_{12} were

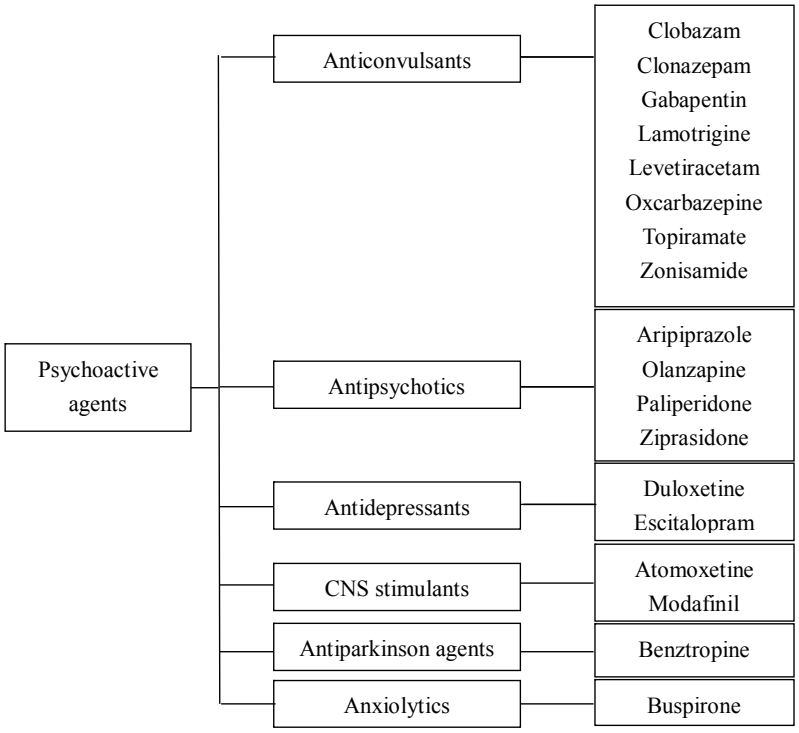

Fig. 1. List and classification of 18 psychoactive agents.