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Simultaneous determination of naltrexone and bupropion in their co-formulated tablet utilizing green chromatographic approach with application to human urine

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ABSTRACT

A rapid, simple and accurate micellar HPLC-method was adopted and validated for concurrent quantification of naltrexone hydrochloride (NTX) and bupropion hydrochloride (BUP). The proposed method was conducted on RP-18 LiChrosorb® column (150 mm × 4.6 mm i.d. 5-μm particle size) at 25 °C, as a stationary phase and a mixture of 0.175 M sodium dodecyl sulphate (SDS), 0.3% triethanolamine (TEA) and 12% *n*-propanol in 0.02 M *ortho (o)*-phosphoric acid of pH 3.5 as a developing system. It was pumped at a flow rate of 1.2 mL/min, with ultraviolet detection at 210 nm. The linearity ranges were 0.5–15.0 μg/mL and 1.2–18.0 μg/mL, with detection limits of 0.10 and 0.31 μg/mL and quantification limits of 0.30 and 0.93 μg/mL for NTX and BUP, respectively. The studied drugs were successfully quantified by applying the proposed method in their co-formulated tablet. The cited method was also applied for *in-vitro* quantification of BUP in spiked human urine without prior extraction.

1. Introduction

Naltrexone hydrochloride (NTX) is (5α)-17-(Cyclopropylmethyl)-4,5-epoxy-3,14-dihydroxymorphinan-6-one hydrochloride (Fig. 1a) (Moffat et al., 2011; The Merck Index, 2001). NTX is a nonselective opioid receptor antagonist; congener of naloxone. Bupropion hydrochloride (BUP) (Fig. 1b); 1-(3-Chlorophenyl)-2-[(1,1-dimethylethyl)amino]-1-propanone, a unicyclic aminoketone with noradrenergic and dopaminergic activity (Moffat et al., 2011; The Merck Index, 2001).

NTX is a monograph subject in United States Pharmacopeia (USP, 2011) and the British Pharmacopoeia (BP, 2015).

It was determined by several analytical methods in biological specimens and pharmaceutical formulations, including those based on chromatographic techniques either liquid chromatography (Zuccaro, et al., 1991; Clavijo et al., 2008; Iyer et al., 2007) or gas chromatography (Huang et al., 1997; Toennes et al., 2004; Mehrdad et al., 2009), electrochemical techniques (Ghorbani-Bidkorbeh et al., 2010; Norouzi et al., 2007; Fernandez-Abedul et al., 1997; Ganjali et al., 2009; Ghorbani-Bidkorbeh et al., 2011) and Spectroscopic techniques (El-Didamony and Hassan, 2012; Khanmohammadi et al., 2009; Pulgarın et al., 2003; Kossoski et al., 2013).

Regarding BUP, it is only official in United States Pharmacopeia (USP, 2011). Several techniques were used for the quantification of BUP including spectrophotometry (Misiuk and Zalewska, 2011; Meiling et al., 2002) and chromatography either in dosage form (Al-khamis, 1989; Ulu and Tuncel, 2012) or in biological fluids (Ma et al., 2015; Cooper et al., 1984; Borges et al., 2004). Some techniques were used to determine BUP together with its metabolites (Yeniceli et al., 2011; Yeniceli and Dogrukol-Ak, 2009; Wang et al., 2012; Hu et al., 2011).

In 2014, Food and Drug Administration (FDA) has approved a new combination for the treatment of obesity and controlling body weight containing BUP and NTX. This combination can be used efficiently in management of obesity by targeting the Central Nervous System (CNS) pathways that affect food intake. In this combination, BUP can lead to loss of appetite and increased energy output

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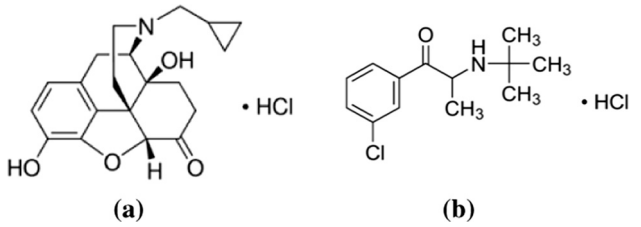

Fig. 1. The structural formulae of the studied drugs. (a) Naltrexone hydrochloride. (b) Bupropion hydrochloride.

by stimulating the pro-opiomelanocortin (POMC) neurons in the hypothalamus. NTX is used to suppress POMC inhibition and so leads to greater effect on POMC activation. BUP and NTX also affect the reward pathway that result in reduction of food needs (Wadden et al., 2011).

Few methods were adopted for the simultaneous quantification of both drugs including spectrophotometry and HPLC (Haritha et al., 2015; Phani et al., 2015).

Micellar chromatographic methods offers a lot of merits over traditional chromatographic ones, these include the lower cost, higher selectivity and possibility of analyzing both polar and non-polar analytes. On the other hand, the major advantage of micellar chromatographic analysis is the possibility of analyzing biological samples by direct injection to the column, without sample pretreatment; this is attributed to the solubility of the biological fluid proteins in the micelle in contrast to the organic solvent that causes their precipitation (Pramauro and Pelizzetti, 1988). Also, micellar chromatographic methods can be regarded as green analytical technique so; lower hazards for the environment and the operators were encountered on applying these methods (Saroj et al, 2017).

Regarding the literature, there are no reported micellar liquid chromatographic methods for determination of NTX and BUP simultaneously, in their combined tablet or in biological fluids. So, the main goal of this work is to develop simple, sensitive, accurate, economic and environmentally friendly analytical method for the quantification of BUP and NTX either in their bulk form or in their combined tablet and then extend the method to quantify BUP in spiked human urine.

2. Experimental

2.1. Apparatus

Shimadzu LC-20AD Prominence liquid chromatograph equipped with an SIL-20 AD auto sampler and a SPD-20A UV detector with CTO-20A column oven. Mobile phases were filtered using Whatman® Nylon membrane filters 0.2 μm, ø47 mm. The gases were removed by a Prominence degasser DGU-20A5R. The pH measurements were done using a Consort NV P-901 pH Meter (Belgium). Also, an ultrasonic bath, model Branson 2800 was employed. A&D GR300 analytical balance and Shimadzu UV-1800 Spectrophotometer were also used.

2.2. Materials and reagents

NTX and BUP bulk powder were purchased from Cayman chemical company, Ann Arbor, United States of America (USA); their purity was certified to be 99.9%. Acetonitrile, n-propanol and *o*-phosphoric acid 85–90%, w/v (HPLC grade) were purchased from Sigma-Aldrich (Eschenstrasse, Germany). Ethanol (HPLC grade) was obtained from Riedel-deHäen (Sleeze, Germany). Methanol (Supra-gradient HPLC grade), Scharlab, Spain. Triethanolamine (TEA), sodium dodecyl sulphate (SDS) and water for HPLC were obtained from Lobachemie, Mumbai, India. Urine samples completely free from drugs were obtained from healthy adult volunteers were utilied in this work.

2.3. Pharmaceutical formulations

Contrave® extended-release tablets (NDC 51267-890-99), each tablet contains 8 mg NTX and 90 mg BUP. It was manufactured by Orexigen Therapeutics Inc., La Jolla, California, USA.

2.4. Standard solutions

Preparation of standard solutions 50 μg/mL (NTX) and 120 μg/mL (BUP) was done using distilled water for HPLC. Suitable dilution of the stock solutions was carried out using the mobile phase.

2.5. Chromatographic performance optimization and system suitability

2.5.1. Column selection

Performance investigation was optimized by using two different columns. The first one was LiChrosorb® RP C-18 column (150 mm × 4.6 mm i.d., 5-μm particle size), Sigma-Aldrich, and the second one was PromosilODS100A column (250 mm × 4.6 mm i.d., 5 μm particle size), Agela Technologies, USA.

2.5.2. Wavelength selection

The UV-absorption spectra of NTX and BUP (dissolved in mobile phase) were plotted for appropriate selection of detection wavelength.

2.5.3. Temperature effect

It was studied by applying different temperature levels over the range of 25–65 °C.

2.5.4. Composition of the mobile phase

Many changes in the mobile phase were done to get the optimum chromatographic performance. These modifications were the pH of the mobile phase, the type and concentration of organic modifier and concentration of surfactant.

2.5.5. Flow rate

Different mobile phase flow rates were tried to get the optimum separation pattern and acceptable resolution.

2.6. Method validation

The developed analytical method was fully validated according to ICH-Q2B guidelines (ICH-guidelines, 2005).

2.6.1. Linearity

Aliquots of NTX and BUP stock solutions were accurately and separately transferred into two groups of 10-mL volumetric flasks and the volume of each was completed to the mark with the mobile phase and mixed well to obtain concentration ranges of 0.5–15 μg/mL for NTX and 1.2–18 μg/mL for BUP. Passing of 60–70 mL of the mobile phase was done to condition and pre-wash the stationary phase. Samples were then chromatographed using the suitable chromatographic parameters. The average peak area against concentration in μg/mL was plotted then the corresponding regression equations were computed.

2.6.2. Accuracy and precision

They were proved by statistical comparison of the results obtained from the proposed method by those obtained from a

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