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

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ARTICLE INFO

Article history:
Received 2 November 2017
Accepted 17 December 2017
Available online 18 December 2017

Keywords:
Green chemistry
Human urine
Micellar chromatography
Naltrexone hydrochloride (NTX)
Bupropion hydrochloride (BUP)

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 (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-Damony and Hassan, 2012; Khanmohammadi et al., 2009; Pulgarín 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...
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-deHaen (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 utilized 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...
reference method (Haritha et al., 2015). Also, intra and inter day precision were evaluated by analyzing different samples either in the same day or in different successive days.

2.6.3. Selectivity
It was assessed by determining the studied drugs in their laboratory prepared mixtures. These mixtures were prepared by transferring definite aliquots of the cited drugs into a series of 10 mL volumetric flasks, keeping the ratio NTX: BUP as 8:90. The procedure under linearity was followed and the concentrations of the studied drugs were calculated from the corresponding regression equation.

2.6.4. Limits of detection and quantification (LOD & LOQ)
They are the lowest detected or quantified amounts of the studied drugs with accepted reliability.

2.6.5. Robustness
Robustness can be checked by evaluating the effect of minute changes on the proposed methods, like the composition of the mobile phase, concentration of organic modifier and concentration of surfactant.

2.7. Analysis of NTX and BUP in their co-formulated tablet
Ten Contrave® extended release tablets were weighed to get the average weight of a tablet then crushed, finely powdered and mixed well. Tablet powder equivalent to 8 mg NTX/90 mg BUP was transferred to a 100.0 mL volumetric flask where 30.0 mL water was added. Sonication of the flask contents were done for 25 min, then the volume was completed to the mark using water as a solvent. Filtration through syringe filter was done. The solution was then diluted using the developing system to get the working solution for analysis using the same procedure under linearity, and then the concentrations of the studied drugs were computed from the corresponding regression equation.

2.8. Assay of BUP in spiked human urine
Portions of BUP stock solution were added in a series of 10.0 mL volumetric flasks to reach a concentration range of 1.2–2.4 μg/mL. The content of each flask was diluted to about 7.0 mL with the mobile phase, then one mL of human urine was added to each flask, and the volume of each flask was completed to the mark using the mobile phase as a diluting liquid and the content was mixed well. Chromatographing of the prepared solution was performed using the optimized conditions. A blank experiment was done at the same time. A calibration graph was plotted between peak area and concentration of the drug in μg/mL.

3. Results and discussion

3.1. Chromatographic performance optimization and system suitability
Excellent separation pattern and well-defined symmetrical peaks were acquired after optimization of the chromatographic conditions (Fig. 2).

3.1.1. Column selection
Two different columns were tried, LiChrosorb® RP C-18 column (150 mm × 4.6 mm i.d., 5-μm particle size) and Promosil ODS100A column (250 mm × 4.6 mm i.d., 5 μm particle size), Agela Technologies, USA. Optimum separation with excellent peak characters was acquired on using the first column.

3.1.2. Wavelength selection
Examination of the UV absorption spectra of the studied drugs in mobile phase (Fig. 3a and b) revealed that, NTX and BUP exhibit peak absorbance at 210 nm, so the ultraviolet detector was adjusted at 210 nm to determine both drugs in acceptable sensitivity.
3.1.3. Temperature effect

The effect of variation in column temperature was evaluated by changing it in the range of 25–65 °C. On increasing column temperature, the number of theoretical plates increased. Temperature increase did not affect resolution, peak shape or symmetry. Therefore, room temperature was taken as the working temperature to avoid any harmful effect on the column, Table 1.

3.1.4. Composition of the mobile phase

Many mobile phases with different composition were tried to reach optimum separation pattern and peak characters. The mobile phase modifications comprise the variation in pH, the type and concentration of organic modifier, the concentration of SDS, and the flow rate. The obtained results are shown in Table 1.

### A. Mobile phase pH

The mobile phase composition was changed in the range of 2.3–7.0. It was noticed that, on increasing the pH, the retention time and number of theoretical plates increased but the BUP peak broadened to a certain extent. The pH 3.5 was optimum with respect to resolution and retention time, Table 1.

### Table 1

Optimization of the chromatographic conditions for separation of NTX and BUP by the proposed HPLC method.

| Parameter                  | NTX | BUP | NTX | BUP | Resolution (Rs) | Tailing Factor (T) | Selectivity (α) |
|----------------------------|-----|-----|-----|-----|-----------------|--------------------|-----------------|
| No. of theoretical plates (N) |     |     |     |     |                 |                    |                 |
| Column temperature ° C      |     |     |     |     |                 |                    |                 |
| Room temperature            | 130  | 827 | 2.306 | 6.540 | 5.984           | 1.830              | 2.302           | 2.836           |
| 40° C                       | 120  | 785 | 3.597 | 9.860 | 6.026           | 1.095              | 1.641           | 2.741           |
| 50° C                       | 805  | 4799| 3.411 | 9.691 | 5.977           | 1.024              | 1.446           | 2.841           |
| 65° C                       | 1492 | 810 | 3.167 | 9.467 | 6.625           | 1.017              | 1.231           | 2.989           |
| pH of mobile phase          |     |     |     |     |                 |                    |                 |
| 2.3                         | 124  | 763 | 4.457 | 11.373 | 5.741           | 1.898              | 2.274           | 2.552           |
| 3.5                         | 1300 | 827 | 2.306 | 6.540 | 5.984           | 1.830              | 2.302           | 2.836           |
| 5                           | 1219 | 749 | 3.609 | 9.503 | 5.715           | 1.639              | 2.345           | 2.633           |
| 6                           | 857  | 378 | 3.646 | 9.740 | 4.289           | 1.441              | 2.229           | 2.672           |
| 7                           | 8143 | 7512| 4.033 | 11.062 | 6.982           | 0.861              | 1.135           | 2.743           |
| Type of organic modifier of Conc 12% (v/v) |     |     |     |     |                 |                    |                 |
| Propanol                    | 1300 | 827 | 2.306 | 6.540 | 5.984           | 1.830              | 2.302           | 2.836           |
| Methanol                    | 1766 | 1059| 2.783 | 7.200 | 6.461           | 1.367              | 1.123           | 2.587           |
| Acetonitrile                | 1992 | 742 | 1.696 | 5.554 | 6.413           | 2.352              | 1.205           | 3.276           |
| Ethanol                     | 1791 | 131 | 1.460 | 4.928 | 3.012           | 2.178              | 2.814           | 3.376           |
| Conc of SDS (M)             |     |     |     |     |                 |                    |                 |
| 0.175                       | 941  | 267 | 3.336 | 8.608 | 3.616           | 1.695              | 2.177           | 2.580           |
| 0.15                        | 1300 | 827 | 2.306 | 6.540 | 5.984           | 1.830              | 2.302           | 2.836           |
| 0.08                        | 1483 | 697 | 7.405 | 19.608 | 6.109          | 1.995              | 2.137           | 2.648           |
| 0.05                        |     |     |     |     |                 |                    |                 |
| NTX retention time was 11 min, and BUP was not eluted till 25 min. |

Conc of the organic modifier (Propanol) |     |     |     |     |                 |                    |                 |

Conc of the organic modifier (Propanol) |     |     |     |     |                 |                    |                 |

Effect of flow rate (mL/min.) |     |     |     |     |                 |                    |                 |

| 1.0                         | 941  | 267 | 3.336 | 8.608 | 3.616           | 1.695              | 2.177           | 2.580           |
| 1.2                         | 1234 | 834 | 3.577 | 9.086 | 5.746           | 2.223              | 2.651           | 2.540           |

Number of theoretical plates (N) = 5.54 * \( \left( \frac{t_R}{W_h} \right)^2 \).
Resolution (Rs) = \( \frac{d}{2t_{R1} + t_{R2}} \).
Tailing factor (T) = \( \frac{W_t}{2t_{R2}} \).
Selectivity factor (Relative retention) (α) = \( \frac{t_{R2}}{t_{R1}} \).
B. Type of organic modifier

Different organic solvents of concentration 12% v/v were tested as modifiers including: methanol, acetonitrile, ethanol and n-propanol. The optimum resolution and perfect performance was attained on using n-propanol (Table 1).

C. SDS concentration

The effect of variation of SDS concentration on chromatographic performance was studied in the range of 0.05–0.175 M. It was found that 0.175 M SDS is the most suitable concentration as the peak symmetry of BUP and NTX was increased with optimum resolution (Table 1).

D. Concentration of organic modifier

The influence of variation in n-propanol concentration was studied in the range of 5.0–12.0% V/V. The study revealed that, the optimum system suitability parameters were achieved when using 12.0% n-propanol (Table 1).

So, the mobile phase was well optimized to be a mixture of 0.175 M sodium dodecyl sulphate (SDS), 0.3% triethanolamine (TEA) and 12% n-propanol in 0.02 M o-phosphoric acid of pH 3.5.

3.1.5. Flow rate

The effect of flow rate variation was studied where different values of flow rate were tried. The optimum separation with reasonable retention times was attained at a flow rate of 1.2 mL/min, shown in Table 1.

3.2. Method validation

The proposed method was validated according to the ICH-guidelines.

3.2.1. Linearity

Under the optimized experimental conditions, a linear relationship was established by plotting the peak area against the drug concentration in µg/mL. The linearity ranges were 0.5–15.0 µg/mL and 1.2–18.0 µg/mL for NTX and BUP, respectively (Table 2). Linear regression analysis of the data gave the following equations:

\[ P = 21935.355 + 285115.858 C \quad (r = 0.9999) \] for NTX

\[ P = -126511.703 + 344823.744 C \quad (r = 0.9998) \] for BUP

where P is the peak area, C is the concentration of the drug in µg/mL and r is the correlation coefficient.

3.2.2. Accuracy and precision

It was proved by statistical comparison of the results obtained by the cited method and those obtained by reference method (Haritha et al., 2015). Statistical analysis (Miller and Miller, 2005) showed no significant difference between the compared methods with respect to accuracy and precision, respectively (Table 3).
Intra and inter-day precision were evaluated by replicate assay of three samples either on the same day or on three successive days (Table 4). Excellent repeatability and intermediate precision were proved by the small values of relative standard deviations.

### 3.2.3. Selectivity
Method selectivity was evaluated via interference observation due to the additives in the formulation. No interference was noticed due to these compounds with the studied drugs in the cited method. Also, no interference coming from the human urine matrix in case of BUP analysis, so there is no need for the previous extraction before performing the assay.

### 3.2.4. Detection and quantification limits (LOD and LOQ)
LOQ was quantified as the lowest measurable below which the standard curve is nonlinear. LOD was quantified as the lowest detectable concentration (ICH guidelines, 2005).

LOQ and LOD values for NTX and BUP were presented in Table 2.

### 3.2.5. Robustness
It was assessed by estimating the reflections of minor variations in the experimental parameters on the proposed method. The studied parameters were the mobile phase pH (3.5 ± 0.1), concentration of n-propanol (12 ± 0.5% (v/v)) and SDS concentration (0.175 M ± 0.01). These deliberate variations didn’t affect the method performance.

### 3.3. Applications
#### 3.3.1. Laboratory prepared mixture analysis
The suggested method was used for determination of NTX and BUP in laboratory prepared mixtures (Fig. 2) and the results were compared statistically with those of the comparison method (Haritha et al., 2015) (Table 5).

#### 3.3.2. Analysis of the dosage form
This procedure was successfully used for quantification of NTX and BUP in their co-formulated tablet (Fig. 4, Table 6).

### Table 4
Precision data for the determination of the studied drugs by the proposed HPLC method.

| Parameters | NTX concentration (µg/mL) | BUP concentration (µg/mL) |
|------------|---------------------------|---------------------------|
|            | 3.0 | 6.0 | 9.0 | 12.0 | 15.0 | 18.0 |
| Intraday   | % Found | % Found | % Found | % Found | % Found | % Found |
| (% S.D.)   | 99.18 | 98.76 | 99.36 | 100.13 | 100.30 | 97.99 |
| % RSD      | ±0.70 | ±1.59 | ±1.47 | ±1.84 | ±1.02 | ±1.26 |
| % Error    | 0.71 | 1.59 | 1.48 | 0.83 | 1.04 | 1.07 |
| Interday   | % Found | % Found | % Found | % Found | % Found | % Found |
| (% S.D.)   | 99.14 | 99.13 | 99.18 | 100.36 | 100.30 | 99.79 |
| % RSD      | ±1.16 | ±1.26 | ±1.84 | ±1.84 | ±1.02 | ±1.26 |
| % Error    | 1.17 | 1.27 | 1.85 | 1.04 | 1.60 | 1.26 |

N. B. Each result is the average of three separate determinations.

### Table 5
Assay results for the determination of the studied drugs in laboratory prepared mixtures of their pharmaceutical ratio.

| Combination | Proposed method | Comparison method (Haritha et al., 2015) |
|-------------|-----------------|----------------------------------------|
|             | Amount taken (µg/mL) | Amount found (µg/mL) | % Found | % Found | % Found | % Found |
| NTX/BUP mixture 8/90 | NTX | BUP | NTX | BUP | NTX | BUP | NTX | BUP |
| 0.8         | 9 | 0.7946 | 8.9251 | 99.33 | 99.17 | 100.52 | 102.39 |
| 1.2         | 13.5 | 1.2107 | 13.6497 | 100.89 | 101.11 | 102.00 | 100.89 |
| 1.6         | 18 | 1.5946 | 17.9251 | 99.66 | 99.38 | 100.34 | 102.84 |
| Mean        | 99.96 | 99.95 | 101.22 | 101.41 |
| ±S.D.       | 0.82 | 1.02 | 0.91 | 1.52 |
| t-test      | 1.88(2.57) | 1.42 (2.57) |
| F-test      | 1.22(19.16) | 2.21(19.16) |

N. B. Each result is the average of three separate determinations.

* The figures between parentheses are the tabulated t and F values at P = .05 (Miller and Miller, 2005).
declares good agreement with the results obtained using the reference method (Haritha et al., 2015).

### 3.3.3. Application to biological fluids

BUP is well absorbed and undergoes hepatic metabolism via oxidation, hydroxylation and reduction. Peak plasma concentrations reached within 3 h for sustained-release preparations and 2 h for immediate release. Several metabolites are produced, which are pharmacologically active.

Greater than 60% of a dose is excreted in urine within 24 h, 87% over 96 h and 10% in the stool, with less than 1% being the parent drug (Moffat et al., 2011).

Urine peaks significantly interfered with the NTX peak at the specified chromatographic condition. So, BUP alone could be determined in urine, as shown in Fig. 5.

### Table 6

| Preparation          | Proposed method | Comparison methods (Haritha et al., 2015) |
|----------------------|-----------------|------------------------------------------|
|                      | Amount taken (µg/mL) | Amount found (µg/mL) | % Found | % Found |
| NTX                  |                  | NTX            | BUP       | NTX  | BUP  | NTX | BUP |
| Contrave* tablet     | 0.8              | 9              | 8.091     | 8.8878 | 101.14 | 98.75 | 100.52 | 102.39 |
|                      | 1.2              | 13.5           | 1.1817    | 13.7245 | 98.48  | 101.66 | 102.00 | 100.89 |
|                      | 1.6              | 18             | 1.6091    | 17.8878 | 100.57 | 99.38  | 100.34 | 102.84 |
| Mean ±S.D.           |                  | 100.06         | 99.93     | 1.40  | 1.53  | 904  | 52 |
| t-test               |                  | 1.33(2.57)*    | 1.27(2.57)* | 1.37(9.55)* | 1.01(9.55)* |
| F-test               |                  | 1.01(9.55)*    | 1.39(9.55)* |

* The figures between parentheses are the tabulated t and F values at P = .05 (Miller and Miller, 2005).

![Typical chromatograms of BUP in urine under the described chromatographic conditions. (a): Blank urine. (b): BUP in urine, where peak a corresponds to BUP HCl (1.8 µg/mL).](image)

### Table 7

| Parameter | Amount taken (µg/mL) | Amount found (µg/mL) | % Found |
|-----------|----------------------|----------------------|---------|
| 1.2       | 1.223                | 101.94               |
| 1.8       | 1.753                | 97.41                |
| 2.4       | 2.423                | 100.97               |
| Mean ±S.D.|                      | 100.11               | 2.39    |
| % RSD     |                      | 2.38                 |
| % Error   |                      | 1.38                 |

On application of the optimized chromatographic conditions, a linear relationship was established by plotting the peak area against BUP concentration in µg/mL:

\[
P = 234983.667 + 259.440 C \quad (r = 0.9977)
\]

where \( P \) is the peak area, \( C \) is the concentration of the drug in µg/mL and \( r \) is the correlation coefficient.

BUP samples were successfully analyzed using the proposed method (Table 7).

### 4. Conclusion

This work offers a micellar HPLC method for the concurrent quantification of NTX and BUP in their tablet form; also, this method can be applied for the determination of BUP in spiked human urine samples with excellent sensitivity and accuracy without prior extraction procedure. The studied drugs were separated with acceptable resolution in a time less than ten minutes.

This method has many advantages over the already published methods, that it is more sensitive with respect to LOD and LOQ. Also, the published work deals with the studied drugs in dosage form only without application in biological fluids. Over the mentioned merits of this method, it can be considered as a green analytical one so; it can be regarded as an environmentally friendly method of analysis.

Validation procedure according to ICH-guidelines was applied to the cited method to ensure its accuracy and precision.

### References

Al-khamis, K.I., 1989. Rapid determination of bupropion in human plasma by high performance liquid chromatography. J. Liq. Chromatogr. 12, 645–655.

Borges, V., Yang, E., Dunn, J., Henion, J., 2004. High-throughput liquid chromatography-tandem mass spectrometry determination of bupropion and its metabolites in human, mouse and rat plasma using a monolithic column. J. Chromatogr. B 804, 277–287.

BP, 2015. The British Pharmacopoeia, Her Majesty’s Stationary Office, London, vol. II, p. 475, 1195, vol. III, p. 1223.
Clavijo, C., Bendrick-Pearl, J., Zhang, Y.L., Johnson, G., Gasparic, A., Christians, U., 2008. An automated, highly sensitive LC-MS/MS assay for the quantification of the opiate antagonist naltrexone and its major metabolite beta-naltrexol in dog and human plasma. J. Chromatogr. B 874 (1–2), 33–41.

Cooper, T.B., Suckow, R.F., Glassman, A., 1984. Determination of bupropion and its major basic metabolites in plasma by liquid chromatography with dual-wavelength ultraviolet detection. J. Pharmaceutical Sci. 73, 1104–1107.

El-Dibamony, A.M., Hassan, W.S., 2012. Spectrophotometric and Fluorimetric methods for determination of naltrexone in urine, serum and tablets by oxidation with cerium(IV). J. Chem. Soc. 57, 1404–1408.

Fernandez-Abedul, M., Velazquez-Rodriguez, M., Barreira-Rodriguez, J., Costa-Garcia, A., 1997. Voltammetric determination of naltrexone in pharmaceuticals. Anal. Lett. 30, 1491–1502.

Ganjali, M.R., Alipour, A., Rahi, S., Norouzi, P., 2009. Design and construction of a naltrexone selective sensor based on computational study for application in pharmaceutical analysis. Int. J. Electrochem. Sci. 4, 1153–1166.

Ghorbani-Bidkorbeh, F., Shahrokhian, S., Mohammadi, A., Dinarvand, R., 2010. Electrochemical determination of naltrexone on the surface of glassy carbon electrode modified with Nafion-doped carbon nanoparticles: application to determinations in pharmaceutical and clinical preparations. J. Electroanal. Chem. 638, 212–217.

Ghorbani-Bidkorbeh, F., Shahrokhian, S., Mohammadi, A., 2011. Preparation of a naltrexone HCl potentiometric sensor and its application to pharmaceutical analysis and drug determination in biological fluids. J. Food Drug Anal. 19, 445–451.

Haritha, A., Kumar, P.B.R., Priya, R.V., Sekhar, K.C., 2015. Analytical method development and validation for simultaneous estimation of naltrexone hydrochloride and bupropion hydrochloride in oral dosage form (tablets) by RP-HPLC techniques. J. Global Trends Pharmaceut. Sci. 6 (2), 2600–2606.

Huang, W., Moody, D.E., Foltz, R.L., Walsh, S.L., 1997. Determination of naltrexone and its major metabolite, hydroxybupropion in rat plasma by LC-MS and its application to pharmacokinetics. Die Pharmazie 66, 924–928.

Hu, L., Wang, Z., Xu, R., Ma, J., Wang, X., Zhang, X., 2011. Determination of bupropion and its main metabolite in rat plasma by LC-MS and its application to pharmacokinetics. Die Pharmazie 66, 924–928.

Huang, W., Moody, D.E., Foltz, R.L., Walsh, S.L., 1997. Determination of naltrexone and 6-β-naltrexol in plasma by solid-phase extraction and gas chromatography-negative ion chemical ionization-mass spectrometry. J. Anal. Toxicol. 21, 252–257.

International Conference on Harmonization (ICH), Harmonized Tripartite Guidelines. Validation of analytical procedures: Text and Methodology, Q2 (R1). Current Step 4 Version, Parent Guidelines on Methodology Dated November 6 1996, Incorporated in November 2005. Through (http://www.ich.org/LOB/media/MEDIA417.pdf) (accessed June 9, 2017).

Iyer, S.S., Kellogg, G.E., Karnes, H.T., 2007. A LC-electrospray tandem MS method for the analysis of naltrexone in canine plasma employing a molecular model to demonstrate the absence of internal standard deuterium isotope effects. J. Chromatogr. Sci. 45, 694–700.

Khanmohammadi, M., Mobedi, H., Mobedi, E., Kargarosha, K., Bagheri Garmarudi, A., Ghaseemi, K., 2009. Quantitative determination of naltrexone by attenuated total reflectance–FTIR spectrometry using partial least squares (PLS) wavelength selection. Spectroscopy 23, 113–121.

Kossoski, A.d.C., Pontarolo, R., Barreira, S.M., Germano, S., Maluf, D.F., 2013. Analytical validation of a quantification method by UV-vis spectrometry of naltrexone hydrochloride in capsules. Lat. Am. J. Pharm. 32, 616–620.

Ma, J., Wang, S., Zhang, M., Zhang, Q., Zhou, Y., Lin, C., Lin, C., Wang, X., 2015. Simultaneous determination of bupropion, metropolon, midazolam, phenaecitin, omeprazole and tolbutamide in rat plasma by UPLC-MS/MS and its application to cytochrome P450 activity study in rats. Biomed. Chromatogr. 29, 1203–1212.

Mehrdad, R., Khosrou, A., Rassoul, D., Sanaz, V.G., Mohsen, A., 2009. A simple and sensitive analytical method for determination of naltrexone level in plasma by GC-MS. Chromatographia 70, 1491–1494.

Meiling, Q., Peng, W., Yingshu, G., Junling, G., Ruonong, F., 2002. Development and validation of an HPLC method for the determination of bupropion hydrochloride in tablets. J. Chinese Pharmaceut. Sci. 11, 16–18.

Miller, J.N., Miller, J.C., 2005. Statistics and Chemometrics for Analytical Chemistry. Pearson Education Limited, Harlow.

Misiuk, W., Zalewska, M., 2011. Spectroscopic investigations on the inclusion interaction between hydroxypropyl-β-cyclodextrin and bupropion. J. Mol. Liq. 159, 220–225.

Moffat, A.C., Osellon, M.D., Widdop, B., Galichet, L.Y., 2011. Clark’s Analysis of Drugs and Poisons in Pharmaceuticals, Body Fluids and Postmortem Material, vol. 2, fourth ed. The Pharmaceutical Press, London.

Norouzi, P., Ganjali, M., Zare, M., Mohammadi, A., 2007. Nano level detection of naltrexone hydrochloride in its pharmaceutical preparation at Au microelectrode in flowing solutions by fast fourier transforms continuous cyclic voltammetry as a novel detector. J. Pharm. Sci. 96, 2009–2017.

Phani, R.C., Chaitanya, D., Prasanthi, B., 2015. RP-HPLC and spectrophotometric methods for the simultaneous estimation of bupropion HCl and naltrexone HCl. Int. J. Pharmaceut. Sci. Res. 6 (7), 1000–1009.

Pramauro, E., Pelizzetti, E., 1988. Micelles: a new dimension in analytical chemistry. Trends Anal. Chem. 7, 260–265.

Pulgarin, J.A.M., Bermejo, L.F.G., A., Lara, J.L.G., 2003. Spectrofluorimetric determination of naltrexone by a kinetic method using the stopped-flow technique. Anal. Chim. Acta 495, 249–259.

The Merck Index, 2001. An Encyclopedia of Chemicals, Drugs, and Biological. Royal Society of Chemistry, London.

Saroj, S.D., Shah, P., Jairaj, V., Rathod, R., 2017. Green analytical chemistry and quality by design: a combined approach towards robust and sustainable modern analysis. Curr. Anal. Chem. 13. https://doi.org/10.2174/1573411013666170615140836.

Toennes, S.W., Kauert, C.F., Grüsser, S.M., Jäkel, W., Partecke, G., 2004. Determination of naltrexone and 6-β-naltrexol in human plasma following implantation of naltrexone pellets using gas chromatography–mass spectrometry. J. Pharmaceut. Biomed. Anal. 35, 169–176.

Ulu, S.T., Tuncel, M., 2012. Determination of bupropion using liquid chromatography with fluorescence detection in pharmaceutical preparations, human plasma and human urine. J. Chromatogr. Sci. 50, 433–439.

USP, 2011. The United States Pharmacopoeia 34, the National Formulary 29, the US Pharmacopoeial Convention, Rockville, MD (2011). vol. III, vol. II, p. 2445, p. 3782, p. 4608.

Wadden, T.A., Foreyt, J.P., Foster, G.D., et al., 2011. Weight loss with naltrexone SR/bupropion SR combination therapy as an adjunct to behavior modification: the COR-BMOD Trial. Obesity (Silver Spring) 19 (1), 110–120.

Wang, X., Vernikovskaya, D.I., Abdelrahman, D.R., Hankins, C.D., Ahmed, M.S., Nanovskaya, T.N., 2012. Simultaneous quantitative determination of bupropion and its three major metabolites in human umbilical cord plasma and placental tissue using high-performance liquid chromatography–tandem mass spectrometry. J. Pharmaceut. Biomed. Anal. 70, 320–329.

Yeniceli, D., Dogrukol-Ak, D., 2009. A LC method for the determination of bupropion and its main metabolite, hydroxybupropion in human plasma. Chromatographia 70, 1703–1708.

Yeniceli, D., Senen, E., Korkmaz, O.T., Dogrukol-Ak, D., Tuncel, N., 2011. A simple and sensitive LC-ESI-MS (ion trap) method for the determination of bupropion and its major metabolite, hydroxybupropion in rat plasma and brain microdialysates. Talanta 84, 19–26.

Zuccaro, P., Altieri, L., Betteo, P., Pacifici, R., Ricciarello, G., Pin, L.A., Sternieri, E., Pichini, S., 1991. Determination of naltrexone and 6β-naltrexol in plasma by high-performance liquid chromatography with coulometric detection. J. Chromatogr. B 667, 485–490.