Original article

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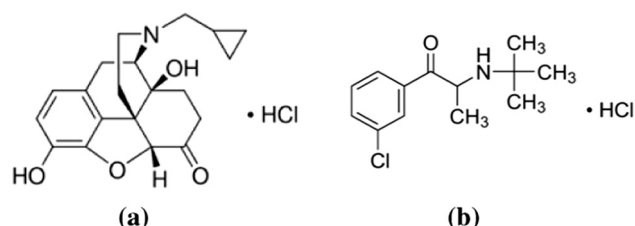
Fig. 1. The structural formulae of the studied drugs. (a) Naltrexone hydrochloride. (b) Bupropion hydrochloride.

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

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.
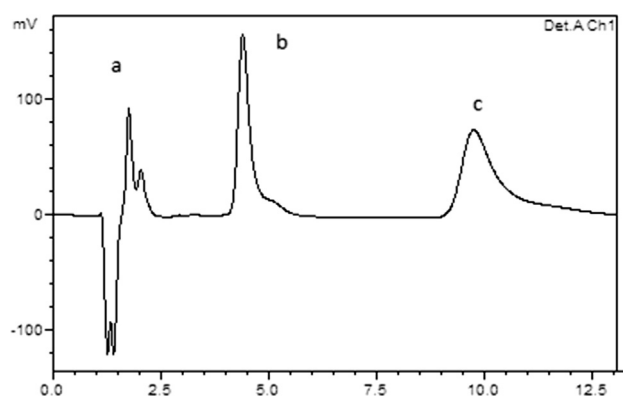
Fig. 2. Typical chromatograms of laboratory prepared mixture of the studied drugs under the described chromatographic conditions: (a): Solvent front. (b): NTX. HCl (12.0 μg/mL). (c): BUP. HCl (15.0 μg/mL).

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.

(a)

(b)

Fig. 3. Absorption spectra of the studied drugs in mobile phase: (a): NTX. HCl (5.0 μg/mL). (b): BUP. HCl (12.0 μg/mL).

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 reso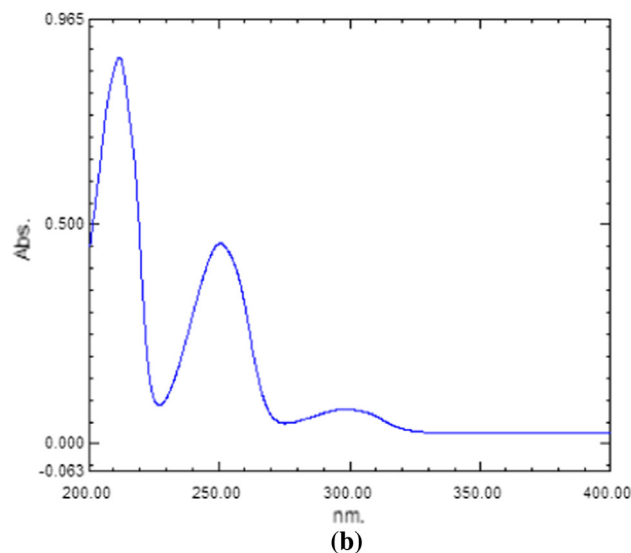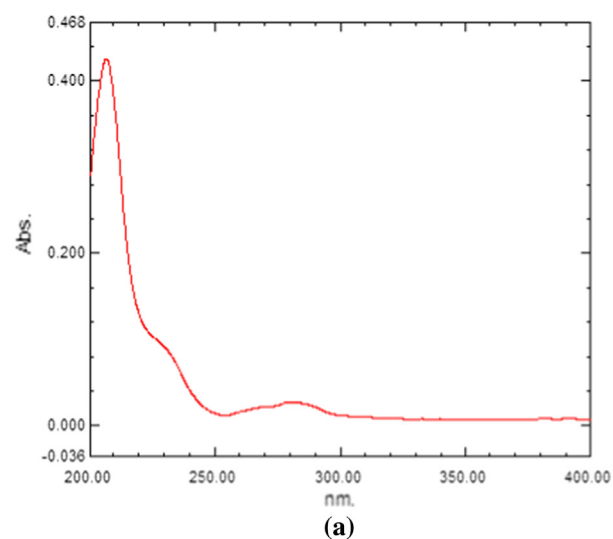lution, peak shape or symmetry. Therefore, room temperature was taken as the working temperature to avoid any harmful effect on the column, Table 1.

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		No. of theoretical plates (N)		Capacity factor (K')		Resolution (Rs)	Tailing Factor (T$_f$)		Selectivity (α)
		NTX	BUP	NTX	BUP		T$_{f1}$	T$_{f2}$	
Column temperature ° C	Room temperature	1300	827	2.306	6.540	5.984	1.830	2.302	2.836
	40° C	1207	785	3.597	9.860	6.026	1.095	1.641	2.741
	50° C	8052	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	1245	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)	15%	767	564	2.899	7.557	4.649	1.355	2.024	2.607
	12%	941	267	3.336	8.608	3.616	1.695	2.177	2.580
	10%	1483	697	7.405	19.608	6.109	1.995	2.137	2.648
	5%	846	345	3.835	10.222	4.147	1.684	2.122	2.666
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) = $5.54\left(\frac{t_R}{Wh/2}\right)^2$.

Resolution (Rs) = $\frac{2\Delta t_R}{W_1+W_2}$.

Tailing factor (T) = $\frac{W_{0.5}}{2f}$.

Selectivity factor (Relative retention) (α) = $\frac{t_{R2}-t_m}{t_{R1}-t_m}$.

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:

$$P = 21935.355 + 285115.858\ C \quad (r = 0.9999)\ \text{for NTX}$$

$$P = -126511.703 + 344823.744\ C \quad (r = 0.9998)\ \text{for BUP}$$

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).

Table 2
Analytical performance data for the determination of the studied drugs by the proposed HPLC method.

Parameter	Value	
	NTX	BUP
Linearity and range (μg/mL)	0.5–15.0	1.2–18.0
Correlation coefficient (r)	0.9999	0.9998
Slope	285115.858	344823.744
Intercept	21935.355	−126511.703
$S_{y/x}$,S.D. of the residuals	15894.967	51032.813
S_a,S.D. of the intercept	8648.109	31893.779
S_b,S.D. of the slope	1095.553	3130.906
S.D.	1.470	1.190
%RSD[a]	1.469	1.192
%Error[b]	0.518	0.420
LOD[c]	0.100	0.305
LOQ[d]	0.303	0.925

[a] Percentage relative standard deviation.
[b] Percentage relative error.
[c] Limit of detection.
[d] Limit of quantitation.

Table 3
Application of the proposed method for the analysis of the studied drugs in their pure forms.

Studied drug	Proposed method	Comparison method (Haritha et al., 2015)		
NTX	Conc. taken (μg/mL)	Conc. Found (μg/mL)	% Found	% Found
	0.5	0.490	98.02	100.52
	1.0	0.979	97.90	102.00
	1.5	1.536	102.38	100.34
	3.0	3.011	100.37	102.00
	6.0	5.986	99.77	
	9.0	9.044	100.49	
	12.0	11.895	99.12	
	15.0	15.059	100.39	
$\bar{X} \pm SD$		99.81 ± 1.47	101.22 ± 0.91	
t-test		1.74 (2.23)[*]		
F-test		2.59 (8.88)[*]		
BUP	1.2	1.182	98.48	102.39
	2.4	2.361	98.36	100.89
	3.6	3.611	100.31	102.84
	6.0	6.024	100.41	99.50
	9.0	8.988	99.87	
	12.0	11.951	99.59	
	15.0	15.289	101.93	
	18.0	17.794	98.85	
$\bar{X} \pm SD$		99.73 ± 1.19	101.41 ± 1.52	
t-test		2.12 (2.23)[*]		
F-test		1.63(4.35)[*]		

- Each result is the average of three separate determinations.
[*] Figures between parentheses are the tabulated t and F values at p = .05 (Miller and Miller, 2005).

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 6
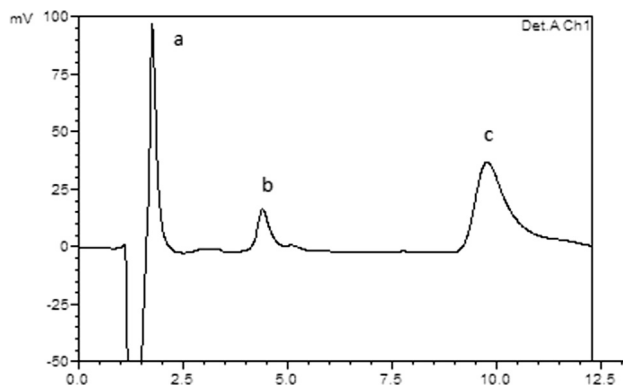

Fig. 4. Typical chromatograms of co-formulated tablets of the studied drugs under the described chromatographic conditions. (a): Solvent front. (b): NTX. HCl (0.8 μg/mL). (c): BUP. HCl (9.0 μg/mL).

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	99.18	100.45	97.85	99.59	101.93	98.85
		100.13	101.56	100.54	100.37	99.36	100.26
		98.76	98.43	100.22	101.25	99.54	101.34
	(x̄)	99.36	100.15	99.54	100.40	100.28	100.15
	±S.D.	±0.70	±1.59	±1.47	±0.83	±1.44	±1.25
	%RSD	0.71	1.59	1.48	0.83	1.43	1.25
	%Error	0.41	0.92	0.85	0.48	0.83	0.72
Inter-day	% Found	97.99	99.16	99.45	99.59	101.93	98.85
		99.13	97.84	97.65	98.78	98.74	97.65
		100.30	100.36	101.33	97.56	100.12	100.14
	(x̄)	99.14	99.12	99.48	98.64	100.26	98.88
	±S.D.	±1.16	±1.26	±1.84	±1.02	±1.60	±1.25
	%RSD	1.17	1.27	1.85	1.04	1.60	1.26
	%Error	0.67	0.73	1.07	0.60	0.92	0.73

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) % Found	
	Amount taken (μg/mL)		Amount found (μg/mL)		% Found			
	NTX	BUP	NTX	BUP	NTX	BUP	NTX	BUP
NTX/BUP mixture 8/90	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	99.58	100.34	102.84
							102.00	99.50
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).

Table 6
Assay results for the determination of the studied drugs in their co-formulated tablet.

Preparation	Proposed method						Comparison methods (Haritha et al., 2015)	
	Amount taken (µg/mL)		Amount found (µg/mL)		% Found		% Found	
	NTX	BUP	NTX	BUP	NTX	BUP	NTX	BUP
Contrave® tablet	0.8	9	0.8091	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
							102.00	99.50
Mean					100.06	99.93	101.22	101.41
±S.D.					1.40	1.53	0.91	1.52
t-test							1.33(2.57)[*]	−1.27(2.57)[*]
F-test							1.37(9.55)[*]	1.01(9.55)[*]

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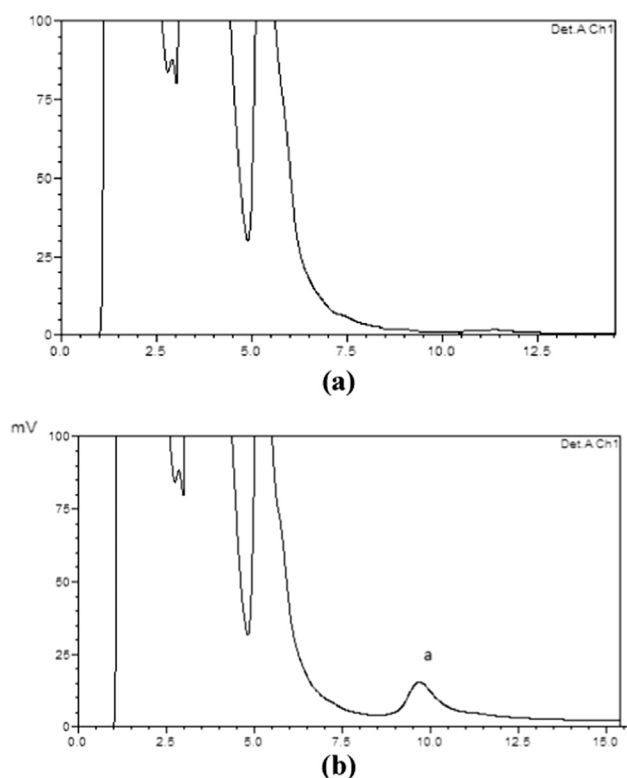

Fig. 5. Typical chromatograms of BUP in urine under the described chromatographic conditions. (a): Blank urine. (b): BUP in urine, where peak a corresponds to BUP HCl (1.8 µg/mL).

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 7
Assay results for the determination of BUP in spiked human urine using the proposed method.

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			100.11
±S.D.			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.

declares good agreement with the results obtained using the reference method (Haritha et al., 2015).

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