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1.Introduction

Bupropion hydrochloride (BUP) (±)-2-(*tert*-Butylamino)-1-(3-chloropropiophenyl) propan-1-one hydrochloride [1] is approved as an atypical antidepressant that acts as weak dopamine and norepinephrine reuptake inhibitor to alleviate symptoms of depression. BUP has other therapeutic indications including sexual dysfunction, obesity, attention deficit hyperactivity disorder, and seasonal affective disorder [2]. It has recently been shown to have anti- inflammatory properties [2]. It also assists to decrease the craving and attenuating withdrawal symptoms for nicotine (NIC) in tobacco users trying to quit smoking and approved as an effective nicotine replacement therapy (NRT) [3]. In Australia and the United Kingdom smoking cessation is the only licensed indication of BUP. Treatment with oral sustained-release BUP combined with a NIC patch resulted in a higher rate of long-term smoking cessation compared with a placebo, and also with a single treatment of either NIC or BUP [4] .This synergistic effect has evoked trials for the construction of transdermal patches containing both drugs [5].

Literature survey revealed that BUP is official in United States Pharmacopeia (USP) [6] and that several methods were reported for the analysis of BUP in bulk powder, pharmaceutical preparations, with co- administered drugs, and in biological fluids including : titration [7,8] , polarography [9], conductometry [10], potentiometry [10,11], spectrophotometry [11-15] , spectroflourimetry [16] , Gas chromatography (GC) [17] , Thin layer chromatography (TLC) [18] , HPLC [19-30] and Ultra performance liquid chromatography) UPLC [30,32] . Enantiomeric separation of BUP enantiomers was also performed by electrokinetic chromatography for the quantitative analysis in pharmaceutical formulations [33]. However, BUP was never determined in the presence of a mixture of its degradation products and co- administered drug NIC (**Fig. 1a-e**), also there is no single stability

indicating RRLC method reported for the determination of BUP in pharmaceutical dosage forms or in active pharmaceutical ingredient (API) with its acid, alkaline, and oxidative degradation products. Therefore the objective of this manuscript is to establish and validate chromatographic methods with stability indicating nature to determine BUP in its bulk powder and its commercial tablets as well as in the presence of its different degradation products and co- administered drug NIC in laboratory prepared mixtures as well as to use one of the chromatographic methods to investigate the kinetics of alkaline and oxidative degradation processes of BUP. It also aims to reveal the spurious advantage of RRLC approach in achieving high throughout and improved sensitivity without affecting selectivity using short sub- 2 µm particle size columns.

2. Experimental

2.1. Instruments

The HPLC system (Agilent, USA) 1200 series, consisted of quaternary pump (G1311A) equipped with auto sampler (G1329A) and a Multiple wave detector (G1365B). Separation and quantification were performed on Intersil ODS3 (250 mm x 4.6 mm i.d, 5 µm particle size) analytical column (Japan).

The RRLC system (Agilent, Germany) 1200 SL, consisted of Binary pump (G1312B) equipped with auto sampler (G1329B) and a Diode array detector (G1315C). The stationary phase was Agilent XDB C18 (50 mm x 4.6 mm i.d, 1.8 µ particle size) RRLC column (USA).

Mobile phases were filtered through 0.45 µm miillipore membrane filters (Sartorius, Germany) and degassed before use. Dual UV detection was conducted at 250 and 224 nm. Samples were filtered through 0.45 µm millipore PTFE syringe filters prior to injection and all measurements were held at room temperature (25 ^{o}C ± 2).

Chemstation module for both instruments was used in data processing and acquisition. Additional instruments were used including: Metler Toledo analytical balance (AB265-S, Switzerland)

thermostatic water bath (Memmert, Germany), rotavapour (Buchi, Switzerland), precoated TLC plates, silica gel F_{254} (20 x 20 cm^2, 0.2 mm) aluminum plates (Macherey Nagel, Germany), Hamilton syringe 50 µL capacity, UV with short wavelength 254 nm lamp (Desaga, Germany), sonicator (Crest, New York) digital pH meter (HANNA pH 211, Romania).

2.2. Samples

2.2.1. Pure standard

BUP (99.66 %) was kindly supplied by GlaxoSmithKline (Cairo, Egypt) its purity was assessed using official HPLC method [6]. NIC was kindly supplied by Novartis Company; its purity was certified to be 100.10 %.

2.2.2. Pharmaceutical dosage form

Wellbutrin ® 150 mg SR tablets were kindly supplied by GlaxoSmithKline (Cairo, Egypt). Each tablet was labeled to contain 150 mg BUP batch number 0ZM9982.

2.3. Chemicals and solvents

All chemicals used in preparation of mobile phases were of HPLC grade while all solvents used throughout this work were of analytical grade. These included hydrochloric acid 36 % , triethylamine (Fischer scientific, UK), sodium hydroxide (Qualikens, India), hydrogen peroxide 30 % (Panerac, Spain), acetonitrile and methanol (Scharlau, Spain), o-phosphoric acid , glacial acetic acid, and ammonium acetate (Adwic Co., Egypt), octane sodium sulphonate, heptane sodium sulphonate, and dichloromethane (Fine chemical, Mumbai). Purified water was obtained by double distillation and filtration through 0.45 µm membrane filters was used throughout the whole work and indicated by "water".

2.4. Solutions

Stock standard solutions of BUP and NIC (1 mg mL^{-1}) were prepared by accurately weighing 100 mg ± 0.10 of each in separate 100 mL volumetric flasks and dissolving in water.

0.075 M ammonium acetate solution was prepared by dissolving 5.79 g in 1000 mL water.

Working standard solutions of BUP and NIC (100 µg mL^{-1}) for HPLC and BUP (20 µg mL^{-1}) for RRLC ,were separately prepared by convenient dilution of their stock standard solutions with the corresponding mobile phases for each method as described below.

3. Methods

3.1. Chromatographic conditions

HPLC was performed on Intersil ODS3 analytical column (250 mm x 4.6 mm i.d, 5 µm particle size). The mobile phase consisted of 0.075 M ammonium acetate buffer solution-methanol-acetonitrile-triethylamine (44:44:15:0.15, v/v), 2.5 g of sodium octane sulphonate added to 1 L of the mobile phase and pH adjusted to 6 ± 0.2 with o-phosphoric acid.

RRLC was conducted on XDB C18 RRLC column (50 mm x 4.6 mm i.d, 1.8 µm particle size). The mobile phase was 0.075 M ammonium acetate buffer solution-methanol-acetonitrile-triethylamine (44:44:10:0.15 v/v), 2.5 g of sodium heptane sulphonate added to 1 L of the mobile phase and pH adjusted to 5 ± 0.2 with o-phosphoric acid. The flow rates were isocratic adjusted at 1.5 mL min^{-1} for HPLC and 0.5 mL min^{-1} for RRLC. Dual UV detection was conducted at 250 and 224 nm for both methods. Auto sampler injectors adjusted to deliver 20 and 2 µL for HPLC & RRLC methods, respectively. All measurements were held at ambient temperature (25^{o}C ± 2) for both methods. To reach good equilibrium, the analysis was usually performed after conditioning of the columns for about 1 hour.

3.2. Linearity and Construction of calibration curves

Aliquots of BUP were transferred from its working standard solutions into separate series of 10 mL volumetric flasks to obtain concentration ranges of 5 – 100 and 2 – 20 µg mL^{-1} for HPLC and RRLC, respectively in the corresponding mobile phases. Triplicate 20 µL or 2 µL injections were performed for each concentration and chromatographed using HPLC and RRLC systems, respectively.

Calibration curves relating the average integrated peak areas to the corresponding concentrations were constructed and the regression equations were then computed.

3.3. Stability study of Bupropion hydrochloride

BUP was stressed for acid, alkaline and oxidative degradation. Solutions were prepared by dissolving separately, about 10 mg of pure BUP in 10 mL of 5 M HCl, 10 mL of 1 M NaOH and 20 mL of 3 % methanolic hydrogen peroxide. The solutions were tightly closed away from light and placed in thermostatically controlled water bath set at 90 ^{o} C for 24 hours for acid degradation and kept aside at room temperature (25 ^{o} C ± 2) for 48 hours for alkaline and oxidative degradation or heated in a thermostatically controlled water bath at 70 ^{o} C for 2 hours and 80 ^{o} C for 3 ½ hours for alkaline and oxidative degradation, respectively.

Acid and alkaline degradation products solutions were then neutralized by 5 M sodium hydroxide or 1 M HCl to pH 7 and evaporated till dryness in rotavapour water bath at 70 ^{o} C while oxidative degradation product solution was evaporated to dryness at 40 ^{o} C in rotavapour water bath to stop the reaction. Residues of hydrolytic degradation products were separately dissolved in 50 mL methanol and filtered to get rid of the formed sodium chloride formed by neutralization process. The solution was further re- evaporated in rotavapour water bath at 40 ^{o} C to dryness. The residues of hydrolytic and oxidative degradation products were separately dissolved in 100 mL water to obtain stock solution of 100 µg $mL^{-1\ for}$ each.

The degradation processes were monitored by TLC plates using acetonitrile, dichloromethane, 20 % glacial acetic acid (5:5:0.25, v/v/v) as the developing system and the developed plates were visualized under UV lamp at 254 nm.

3.4. Analysis of laboratory prepared mixtures

For HPLC laboratory prepared mixtures of BUP, its degradation products (hydrolytic and oxidative) and co-administered drug NIC were prepared in the range of 10 – 50 % w/w. Mixtures of the same

range were prepared for RRLC without NIC then procedures were undertaken as described under each method where the concentration of the intact drug in each mixture was calculated from the corresponding regression equations.

3.5. Assay of pharmaceutical formulation (Wellbutrin SR tablets)

Ten tablets of Wellbutrin ®150 mg SR (150 mg BUP /tablet) were weighed and ground. An accurately weighed amount of the ground powder equivalent to 50 mg of BUP was transferred to 100 mL volumetric flask, 50 mL methanol was added and the solution was sonicated for 45 min and left to cool to room temperature. The volume was completed with water to obtain a concentration of 500 µg mL^{-1}. The solution was diluted with the mobile phase to reach concentrations of 10 µg mL^{-1}; for HPLC and 2 µg mL^{-1}; for RRLC. The recommended procedures were undertaken as described under each method.

The accuracy of the developed chromatographic methods was evaluated by spiking the pre-analyzed BUP solutions with extra 10, 50, and 70 µg using HPLC and 2, 10 and 14 µg using RRLC of pure BUP, and then the mean recovery % of the pure drug was calculated.

3.6. Kinetic investigation of the alkaline and oxidative degradation of BUP by HPLC

Accurately weighed amount of about 50 mg of BUP was dissolved in 50 mL distilled water or 50 mL methanol for alkaline and oxidative degradation, respectively (1mg mL^{-1}). One mL of each solution was transferred to separate series of test tubes followed by 1 mL of 1 M NaOH solution or 2 mL of 3% methanolic hydrogen peroxide solution. The test tubes were stoppered and placed in a thermostatic water bath at different temperatures, 50, 60, 70 ^{o}C & 60, 70, 80 ^{o}C for alkaline and oxidative degradation, respectively. Every 15 min starting from zero time to 1.25 hours, the content of each tube was neutralized to pH 7 using 1 mL of 1 M HCl or adding 1 mL of cold water. The content of each tube was immediately transferred to 10 mL volumetric flasks, filtered and chromatographed as described under linearity after completing the volume with the mobile phase.

The concentrations of the remaining BUP were calculated at each time interval and temperature. Logarithm of the percentage of the remaining BUP concentration was plotted against the corresponding time interval in hours for each temperature, and the regression equations were computed.

4. Results and discussion

4.1. Method development and optimization

BUP was subjected to alkali, acid hydrolysis, and oxidation in an attempt to develop stability indicating chromatographic methods; as according to ICH guidelines: "an ideal stability indicating method is one that quantifies the standard drug alone and also resolves its degradation products".

BUP was left with 1 M sodium hydroxide at room temperature (25 ± 2 ^{o} C) for 48 hours. Complete degradation giving two degradation products analyzed and separated by HPLC at retention times 2.4, 3.9 min of 1-(3-chlorophenyl)-1-hydroxy-2-propanone (Deg1) and 3- chlorobenzoic acid (Deg2), respectively with the disappearance of the peak of intact drug at retention time 6.9 min. The same degradation profile was also obtained when heating the drug with 1M sodium hydroxide at 70 ^{o} C for 2 hours (**Fig. 2A&B**).

Complete oxidation of BUP to 3- chlorobenzoic acid using 3% hydrogen peroxide in methanol at room temperature (25 ± 2 ^{o} C) for 48 hours or for 3 ½ hr in thermostatically controlled water bath set at 80 ^{o} C was emphasized by HPLC, where the peak of intact BUP at 6.9 min was gradually replaced with time with the peak of 3- chlorobenzoic acid at 3.9 **(Fig. 2C)**.

The more prominent BUP degradation pathway involved hydroxide ion catalysis of the free base form; where degradation involved the loss of *t*- butyl amino group[2]. At pH greater than 6 BUP (pka 7.9) is available as free base and is consequently liable to hydroxide ion catalysis by alkali or peroxide. Heating BUP with 5 M HCl lead to the protonation of the N-H of the *t*- butyl amino group and avoided the formation of the free base thus protecting the drug against the hydroxide ion

attack. Accordingly incomplete acid degradation of BUP to 3- chlorobenzoic acid and 1-(3-chlorophenyl)-1-hydroxy-2-propanone was obtained (**Fig.2D**).

Mobile phase/stationary phase matching trials were performed to achieve the best system that provided the best separation of BUP from its degradation products and NIC and optimum system suitability parameters according to USP [6]. For HPLC The results of these trials showed best results using 0.075 M ammonium acetate buffer solution-methanol-acetonitrile-triethylamine (44:44:15:0.15 , v/v), containing 0.25 % w/v sodium octane sulphonate with pH adjusted to 6 as shown in **Fig. 3**. The same composition was used for RRLC method in the ratio of 44:44:10:0.15, v/v containing 0.25 % (w/v) sodium heptane sulphonate and pH 5. The pH of mobile phases were adjusted with o-phosphoric acid. The flow rates were isocratic adjusted at 1.5 mL/min for HPLC and 0.5 mL/min for RRLC. The retention time of BUP was about 6.9 min using HPLC and was reduced by nearly 60 % to about 3.0 min using RRLC (**Fig. 4A&B).**

Quantification of the drug in both methods was performed using dual wave lengths detection at λ_{max} of the drug 250 nm and at 224nm for BUP degradation products; that showed poor absorbtivity at 250 nm. Consequently, dual wave lengths were used to preserve both the sensitivity of the method; by quantifying the drug at its $\lambda_{max;}$ and the stability indicating power of the method by detecting the drug degradation products at their optimum wavelength.

The effect of pH on some system suitability parameters; including retention time, and resolution, was carefully studied using the HPLC system as shown in **Fig. 5A&B**.

The retention time of the basic drug BUP (pKa 7.9) [34] decreased with decreasing pH until it became nearly constant at pH 6 probably due to the effect of ion pairing with the ionized drug, while the retention times of its degradation products increased with low pH due to the suppressed ionization of the carboxylic and hydroxyl groups in these compounds resulting in increased retention on the non polar ODS column. Separation of BUP from its degradation products was thereby based on the

use of a combination of ion pairing and ion suppression chromatography to obtain the optimum resolution between the studied drug and its degradation products .

4.2. System suitability tests

System suitability testing parameters were calculated according to USP [6] to ensure that the chromatographic systems were working correctly during the analysis after optimization of the chromatographic conditions using different mobile phases, pH, and flow rates. Capacity factor (K), selectivity factor (α), resolution (R), column efficiency, tailing factor (T) and relative standard deviation peak area of five replicate injections were parameters to be checked during the analysis as represented in **Table 1**. Validation of the two chromatographic methods was performed in accordance to ICH guidelines [35].

4.2. Method validation

4.2.1. Linearity and range

Calibration curves were constructed representing the relationship between integrated peak areas and the corresponding concentrations in the range of 10 – 100 µg mL^{-1} for the HPLC method and 2 – 20 µg mL^{-1} for the RRLC method.

The characteristic parameters for the regression equations were computed as illustrated in **Table 2**.

4.2.2. Precision

Repeatability and intermediate precisions of the proposed methods were evaluated using three different concentrations assayed three times in the same day and in triplicate on three successive days using the developed methods. The results in **Table 2** indicate satisfactory precision of the proposed methods.

4.2.3. Selectivity

The selectivity of the proposed methods was emphasized when analyzing laboratory prepared mixtures containing different percentages of the studied drug and its different degradation

products By applying the proposed HPLC and RRLC methods BUP could be determined without any interference from up to 50 % w/w of its degradation products and co- administered drug NIC (**Table 3).**

4.2.4. Peak purity

The selectivity of the proposed methods was further demonstrated by testing peak purity of the cited drug in pharmaceutical preparation matrix spiked with its different degradation products using DAD as illustrated in **Fig. 6A&B.**

The main feature of DAD is the possible collection of spectra across a range of wavelengths at each data point collected across a peak, and through software manipulations each spectra can be compared to determine peak purity [36]. Comparing peak spectra is probably the most popular method to discover an impurity. If a peak is pure all UV-visible spectra acquired during the peak's elution or migration should be very close to a perfect 100 % match [37]. Similarity factor and similarity / threshold ratio are used to express this match.

The similarity factors of BUP peak were 999.791 & 999.721, for HPLC and RRLC methods, respectively indicating that the spectra are very similar (similarity factor > 995) and were within the calculated thresholds of and > 999.841 for HPLC and > 996.651 for RRLC where similarity / threshold ratios were less than 1 (0.41 for HPLC and 0.37 for RRLC) indicating that the peaks of the investigated drug had passed the peak purity test [37].

The suggested methods were successfully applied for the analysis of pharmaceutical product ***Wellbutrin SR*** of the drug under study without interference from the excipients present. Also the standard addition technique was applied to evaluate the matrix effect on the recovery, and satisfactory results were obtained as presented in **Table 4**.

The results obtained by applying the proposed methods for the analysis of the studied compound in bulk powder and pharmaceutical preparations were statistically compared to the official USP methods [6].

The values of the calculated *t* and F were less than the tabulated ones which revealed that there was no significant difference with respect to accuracy and precision [38] as illustrated in **Table 3**.

4.3. Kinetic Study

The kinetics of alkaline and oxidative degradation of BUP was investigated using 1 M NaOH and 3% methanolic hydrogen peroxide, since the decomposition rates were convenient to obtain reliable kinetic data. When degradation processes were induced and monitored using the proposed HPLC method, regular decrease in the concentration of the drug with increasing time intervals was observed. The influence of temperatures on the degradation processes are shown in **Fig. 7.** Logarithm the percentage of the remaining concentration for the drug in every degradation process was plotted against the corresponding time interval in hours for each temperature where straight lines were obtained, and the regression equations were computed. Accordingly at the selected temperatures (50, 60, 70 ^{o} C) for BUP alkaline degradation and (60, 70, 80 ^{o} C) for BUP oxidative degradation and since sodium hydroxide (1 N) and hydrogen peroxide (3%) were found in excess ,the alkaline and oxidative degradation of BUP followed pseudo first order kinetics where the degradation rates depended on the remaining concentration of BUP and the temperature at which the degradation was conducted .

From the slopes of the regression lines, it was possible to calculate the apparent first order degradation rate constant (K_{obs}) and the half-life at each temperature in **Table 5** according to the following equations:

$$\mathbf{Log\ (C_t / C_o) + 2 = - K_{obs}\ t, \quad t½ = 0.693 / K_{obs}}$$

Where C_t= concentration remaining at time t, C_o = initial concentration, K_{obs}= apparent rate constant, t½ is the half life.

According to the above equation half lives of BUP are constant at each temperature and don't depend on the drug's concentration.

By plotting Log K_{obs} values versus 1/T (inverse absolute temperature), the Arrhenius plots were obtained (**Fig.7),** which were found to be linear over the selected temperature ranges. The activation energies were calculated by applying the following equation:

Log K_{obs} = Log A- Ea/2.303RT

Where R is the universal gas constant and T is the absolute temperature in kelvin, Ea is the activation energy and A is the frequency factor, and found to be 17.36 kcal mole $^{-1}$ with frequency factor (A) = 1.377 X 10 ^{11} s $^{-1}$ and 19.23 kcal mole $^{-1}$ with frequency factor (A) = 2.786 X 10 ^{11} s^{-1}, for BUP alkaline and oxidative degradation, respectively. These values suggested intermediate instability of the drug towards oxidation and alkaline degradation.

5. Conclusion

The proposed methods are accurate, precise, and highly selective and could be used for purity testing, stability studies, quality control and routine analysis of bulk powders and pharmaceutical preparations.

The use of dual wavelength detection gave this work a great advantage of maintaining the sensitivity of the developed methods by quantification of the drug at its λ_{max} with simultaneous detection of degradation products ; of poor absorbtivity at the same wavelength; which is an important demand according to FDA definition for a stability indicating method. This work has revealed the great advantage of RRLC approach in achieving high throughout by reducing the retention time by about 60 % and improved sensitivity about 5 times without affecting selectivity. Another advantage of RRLC is reducing the cost by minimizing the amount of solvent and samples

used. Kinetic study of BUP alkaline and oxidative degradation has revealed that BUP is highly susceptible to alkaline and oxidative degradation where careful buffering, choice of solvents and protection against oxidation are strongly recommended during processing and formulation as well as during packaging and storage to obtain a BUP containing products of high stability.

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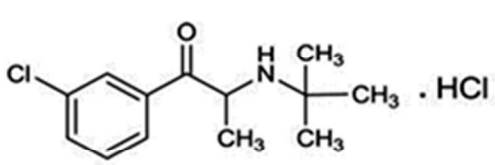
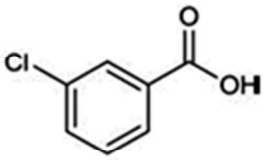
Figures citation

Figure	*Citation*
1	Fig. 1 Chemical structures of bupropion HCl , its degradation products and co-administered drug Nicotine (a) bupropion HCl, (b) m- chlorobenzoic acid , Deg 1 (c) 1-(3-chlorophenyl)-1-hydroxy-2 propanone, Deg 2 (d) nicotine
2	HPLC chromatograms of (A) Bupropion HCl, (B) complete alkaline degradation, (C) complete oxidative degradation, (D) incomplete acid degradation.
3	HPLC Chromatogram of laboratory prepared mixtures of Bupropion HCl and nicotine in the presence of Bupropion degradation products and co-administered drug nicotine at 250 nm (——) and 224 nm (- - - -).
4	Chromatograms of Bupropion HCl and its degradation products by (A) HPLC (B) RRLC methods using dual wavelength detection at 250 and 224 nm, showing the reduced through output of RRLC by 60% reduction in the time of analysis
5	Effect of pH on retention time (A) and resolution (B) of Bupropion HCl and its degradation

	products using the proposed HPLC method.
6	Peak purity of Bupropion HCl in drug product matrix spiked with its degradation products using (A) HPLC (B) RRLC systems. Symmetry factors were = 999.935 & 999.963 that were within the calculated thresholds > 999.841 & > 999.901 for HPLC & RRLC, respectively
7	Kinetic plots for Bupropion HCl degradation by (A) 1 M NaOH (B) 3% methanolic hydrogen peroxide at various temperatures and Arrhenius plots of (C) alkaline (D) oxidative degradation processes

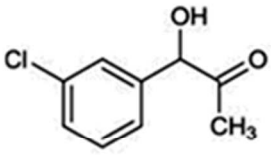


(a)



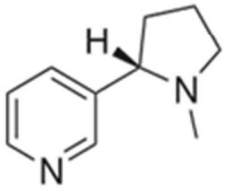
(b)

(Common hydrolytic and oxidative degradate)



(c)

(Hydrolytic degradate)



(d)

Fig. 1 Chemical structures of bupropion HCl , its degradation products and co-administered drug nicotine (a) bupropion HCl, (b) m- chlorobenzoic acid ,Deg 1 (c) 1-(3-chlorophenyl)-1-hydroxy-2 propanone, Deg 2 (d) nicotine.

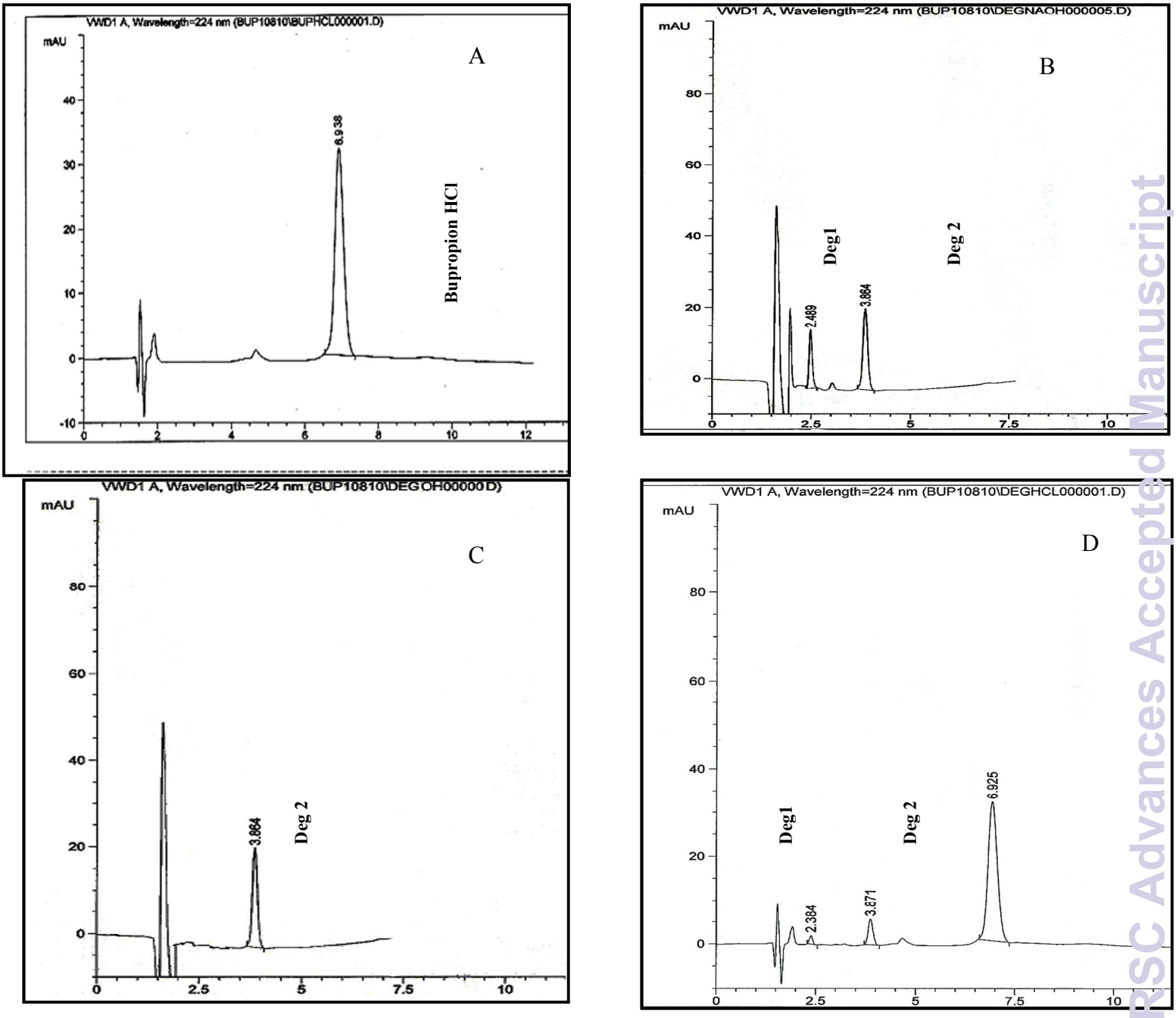


Fig. 2 HPLC chromatograms of (A) bupropion HCl, (B) complete alkaline degradation, (C) complete oxidative degradation, (D) incomplete acid degradation.

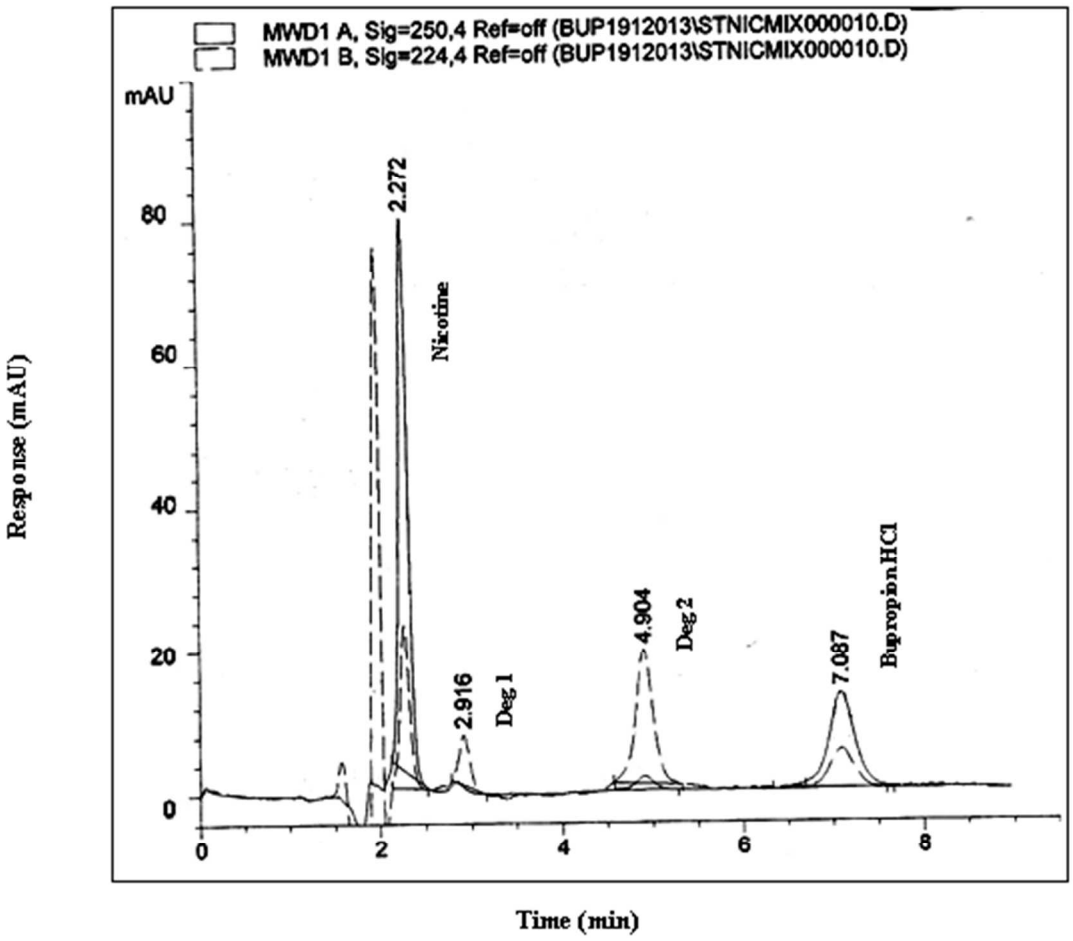


Fig. 3 HPLC Chromatogram of laboratory prepared mixtures of bupropion HCl and nicotine in the presence of bupropion degradation products and co-adminsterd drug nicotine at 250 nm (——) and 224 nm (- - - -).

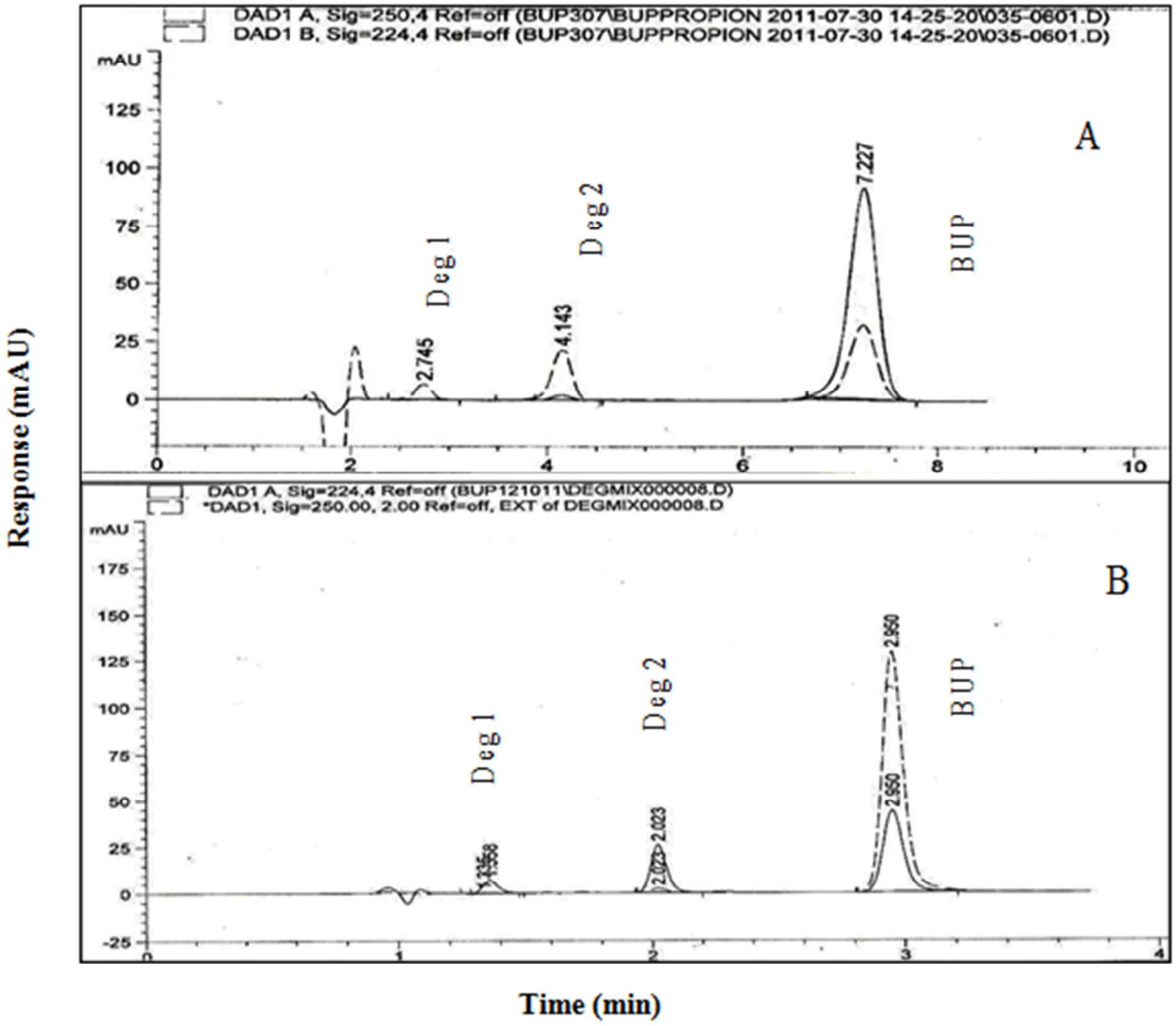


Fig.4 Chromatograms of bupropion HCl and its degradation products by (A) HPLC (B) RRLC methods using dual wavelength detection at 250 and 224 nm, showing the reduced through output of RRLC by 60% reduction in the time of analysis.

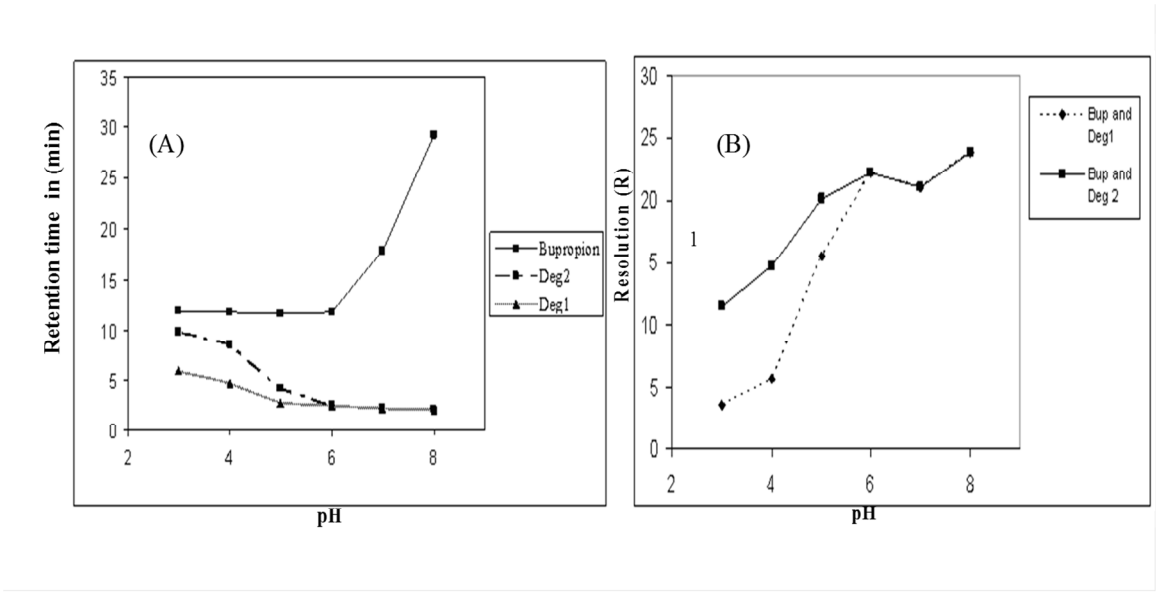


Fig. 5 Effect of pH on retention time (A) and resolution (B) of bupropion HCl and its degradation products using the proposed HPLC method.

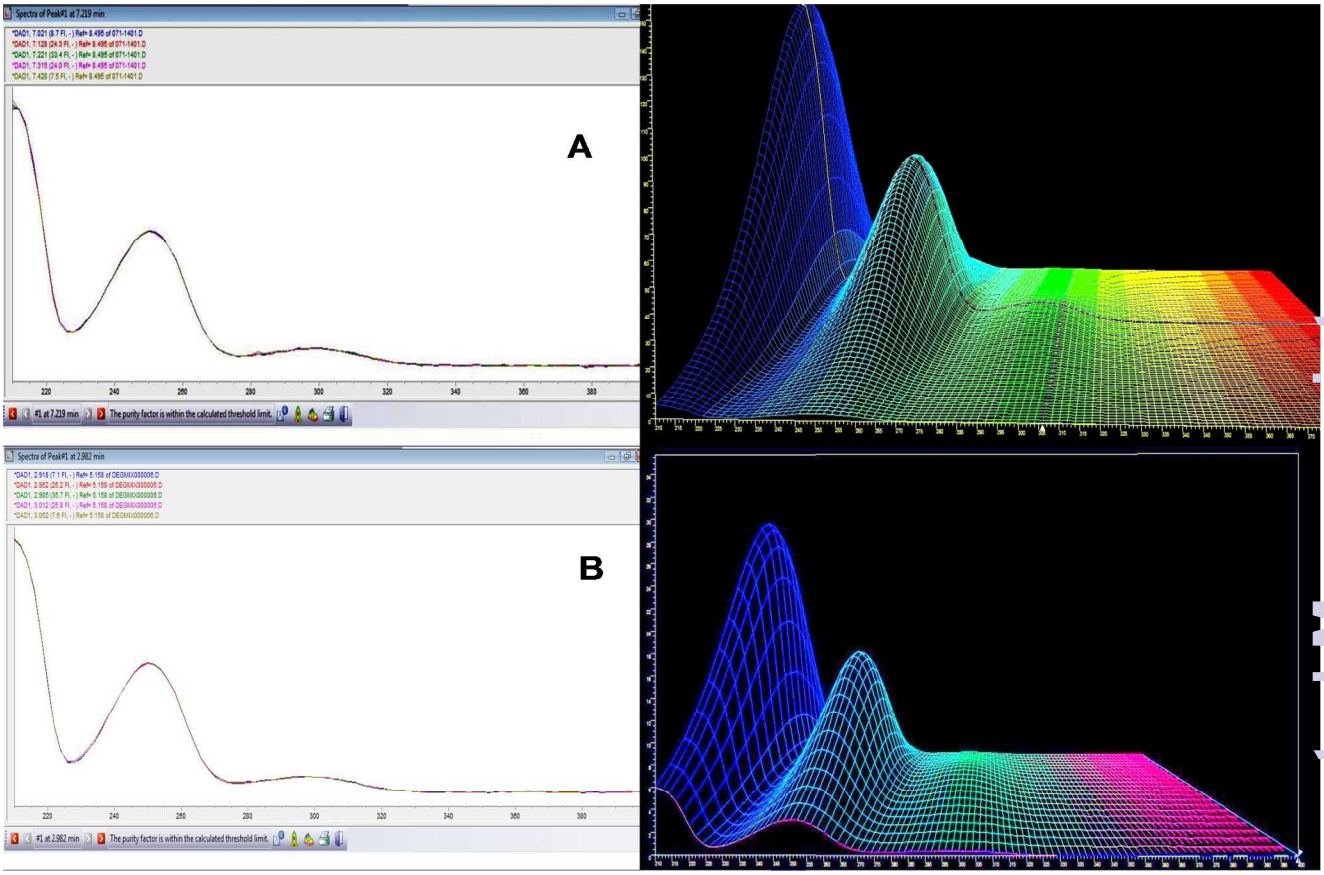


Fig.6 Peak purity of bupropion HCl in drug product matrix spiked with its degradation products using (A) HPLC (B) RRLC systems. Symmetry factors were = 999.935 & 999.963 that were within the calculated thresholds > 999.841 & > 999.901 for HPLC & RRLC, respectively.

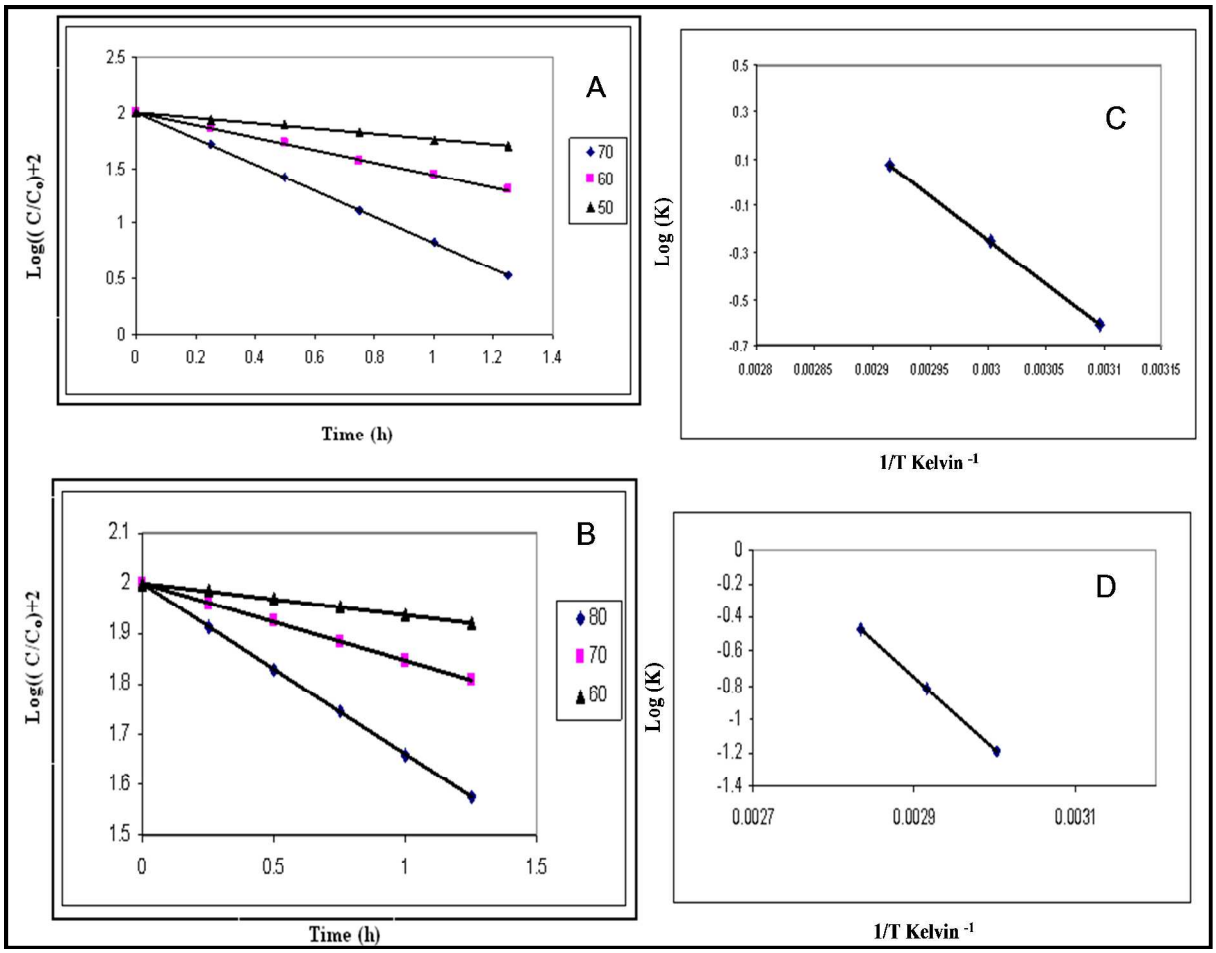


Fig.7 Kinetic plots for bupropion HCl degradation by (A) 1 M NaOH (B) 3% methanolic hydrogen peroxide at various temperatures and Arrhenius plots of (C) alkaline (D) oxidative degradation processes.

Table 1 System suitability test results of the proposed HPLC and RRLC methods for the determination of Bupropion HCl

Chromatographic parameters	HPLC method		RRLC method	Reference values
Retention time(min)	Bupropion HCl	7.22	2.95	_____
	Deg1	2.9	1.36	
	Deg2	4.9	2.02	
	Nicotine	2.4	___	
Capacity factor (K)	Bupropion HCl	3.8	2.27	K>2
	Deg1	0.9	0.51	
	Deg2	2.3	1.24	
	Nicotine	0.6	___	
Selectivity(α)	Bupropion HCl	____	___	α>1
	Deg1	4.2	4.45	
	Deg2	1.7	1.8	
	Nicotine	6.3	___	
Resolution(R)	Bupropion HCl	____	___	R>1.5
	Deg1	16.9	21.23	
	Deg2	8.5	12.04	
	Nicotine	22	___	
Tailing factor(T)	Bupropion HCl	1.03	1.18	T<_2
	Deg1	0.83	1.41	
	Deg2	0.94	1.16	
	Nicotine	1.1	___	
RSD% of peak areas	Bupropion HCl	0.014	0.70	<1,n=5
	Deg1	0.036	0.75	
	Deg2	0.084	0.29	
	Nicotine	0.052	____	
Theoretical plates(N)	Bupropion HCl	3699	6587	>2000
	Deg1	3971	4977	
	Deg2	3384	2482	
	Nicotine	2130	___	

Table 2 Characteristic parameters of assay validation of HPLC and RRLC methods for the determination of Bupropion HCl

Parameters	**HPLC**	**RRLC**
Linearity range, µg/mL	5 – 100	2-20
LOD, µg/mL	1.33	0.2
LOQ, µg/mL	4.02	0.62
Accuracy [a]	99.61 ± 0.51	99.30 ± 1.09
Precision RSD%		
Repeatability [b]	0.01- 0.04 %	0.15-1.52 %
Intermediate precision [b]	0.77-1.22 %	0.64-1.29 %
Regression equation	A= 30.18 C-6.164	A= 14.957 C+1.5438
Slope	30.18	14.957
SE of slope	0.125	0.063
Confidence limit of the slope	29.872-30.487	14.79643-15.11854
Intercept	-6.16	1.54
SE of intercept	7.28	0.71
Confidence limit of intercept	-23.9-11.73	-0.28130-3.3690
Correlation coefficient (r)	0.9999	0.9999
SE of estimation	12.13	0.93

[a] Mean of five determination ± SD

[b] n=9

Table 3 Selectivity of the proposed HPLC and RRLC methods for the determination of Bupropion HCl in laboratory prepared mixtures with its degradation products and co-administered drug nicotine

RRLC		HPLC			
Degradation products %(w/w)	**Recovery*%**	**Degradation products %(w/w)**	**Recovery*%**	**Nicotine and degradation products% (w/w)**	**Recovery*%**
5	100.96	5	98.89	10	101.29
10	100.23	10	99.67	20	98.95
20	100.14	20	100.59	30	100.51
30	99.06	30	99.08	40	99.89
40	99.58	40	99.01	50	99.06
50	100.14	50	99.01		
Mean ± RSD%	**100.02 ± 0.64**	**Mean ±RSD**	**99.38 ± 0.66**	**Mean ± RSD**	**99.94± 0.99**

* Mean of three determinations.

Table 4 Statistical comparisons between results obtained by applying the proposed HPLC, RRLC and the official USP methods for the determination of bupropion HCl in drug substances and drug products

Parameters	Drug substances			Pharmaceutical product (Wellbutrin® SR 150mg B.N. 0ZM9982)		
	HPLC	RRLC	USP method [a]	HPLC	RRLC	USP method [a]
Mean	99.61%	99.30%	99.66%	103.85%	103.13 %	104.46%
SD	0.512	1.087	0.888	1.537	1.910	1.047
n	5	5	5	5	5	5
Variance	0.262	1.182	0.789	2.362	3.648	1.096
SE	0.229	0.486	0.397	0.687	0.854	0.468

***t*- value(2.306)** [b]	0.109	0.574	0.734	1.366
F- value (6.400) [b]	3.011	1.498	2.155	3.328
Recovery [c] **± RSD %**			100.35 ± 0.846	100.25 ± 1.623

[a] Official HPLC method(USP).

[b] The values between parenthesis are the theoretical values of *t* and F at (P= 0.05).

[c] For standard addition of three different concentrations of bupropion hydrochloride.

Table 5 Degradation rate constant K_{obs} and half life ($t_{1/2}$) for Bupropion HCl in 1 M NaOH by the proposed HPLC method

Temperature(°C)	K_{obs} (h^{-1})	Half life($t_{1/2}$)
50	0.5595	1.23832
60	1.29	0.537151
70	2.7708	0.25579