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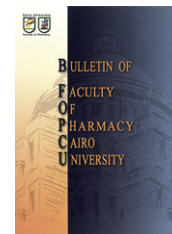
## ORIGINAL ARTICLE

# Stability indicating HPLC and spectrophotometric methods for the determination of bupropion hydrochloride in the presence of its alkaline degradates and related impurity

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**Abstract**    Four sensitive and selective stability-indicating methods for the determination of bupropion hydrochloride in the presence of its alkaline degradates, related impurity, 3-chlorobenzoic acid, and in its pharmaceutical formulation were developed. Method A is an isocratic reversed phase HPLC, good separation between bupropion hydrochloride, its alkaline degradates and related impurity was achieved using a mobile phase of 1.2% w/v ammonium dihydrogen phosphate pH 4.5 and acetonitrile (80:20, v/v) and detection at 210 nm. Method B is based on the first derivative ($D^1$) measurement of the drug at 259 nm, zero contribution point of its alkaline degradates and related impurity. Method C is based on the resolution of the drug, its alkaline degradates and related impurity by first derivative ratio spectra ($DD^1$). Method D is based on the determination of bupropion hydrochloride and its impurity by the $Q$ value method at 248 nm, 227 nm and at isoabsorptive point 237 nm. These methods are successfully applied for the determination of bupropion hydrochloride in bulk powder, pharmaceutical formulation and in the presence of its alkaline degradates and related impurity. The results obtained are statistically analyzed and there are no

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significant differences between the four methods and the official one with respect to accuracy and precision.

## 1. Introduction

Bupropion hydrochloride (Bup-HCl) is described chemically as shown in Fig. 1 as (±)-2-(tert-butyl amino)-1-(3-chlorophenyl) propan-1-one.[1] The drug is an antidepressant that acts as norepinephrine, dopamine reuptake inhibitor and nicotine antagonist. Bupropion hydrochloride is used also for depression, smoking cessation, obesity and attention deficit hyperactivity disorder.[2] Various methods were reported for the determination of Bup-HCl including spectrophotometry,[3–5] potentiometry,[3] conductometry,[3] titrimetry,[5] and polarography.[6] HPLC methods were used for the determination of Bup-HCl.[7–9] HPLC tandem mass spectrometry also was used for the identification and determination of the drug in human plasma, whole blood and urinary metabolites.[10–18] Electro kinetic chromatography was used for separation of bupropion enantiomers[19] and their enantiomeric determination in human plasma using coupled achiral-chiral liquid chromatography.[20] A validated thin-layer and gas chromatography were used for the determination of Bup-HCl in its pharmaceutical formulation.[21,22]

A literature survey reveals that there is no stability indicating HPLC or spectrophotometric methods were published for the drug. The novelty of this work is to establish stability indicating HPLC and spectrophotometric methods for the determination of Bup-HCl in the presence of both its alkaline degradates and its related impurity, 3-chlorobenzoic acid with identification of the degradates.

3-Chlorobenzoic acid is one of the Bup-HCl metabolites and is physiologically inactive[23,24] and is stated officially as related impurity in the raw material and pharmaceutical formulation.[25] The proposed methods are simple, rapid, accurate, reproducible and economical besides the HPLC method is less time consuming than the official USP one.[25]

## 2. Experimental

### 2.1. Instrumentation

1. HPLC Agilent 1200 series was equipped with a model series ED62962752 LC quaternary pump, Rheodyne injector Lot ID 7725 with a 20 μL loop and UV detector DE71360313. Separation and quantitation were made on column eclipse XDB-C18, 4.6 × 150 mm, 5 μm particle size serial number: USK H036684. The detector was set at $\lambda = 210$ nm (Germany).

2. A double beam UV–visible spectrophotometer (Shimadzu, Japan) model UV-1601 PC with quartz cell of 1 cm path length, was connected to IBM compatible computer and a HP 600 inject printer was used. The spectral band width was 2 nm and the wavelength scanning speed was 2800 nm min$^{-1}$. The absorbance spectra of the test and reference solution were recorded over the range of 200–

320 nm. The first derivative and derivative ratio were obtained using the accompanying software with $\Delta\lambda = 4$ and scaling factor = 10.

3. Mass spectrometer GCMS-QP 1000 EX working by electronic ionization mode. Shimadzu (Japan).

4. PH meter HANAA 8417 (Portugal).

5. Photodegradator UV light source LPS-220 PTI model A1010 (Italy).

6. TLC plates pre-coated with silica Gel GF, 0.25 mm thickness, fluorescent at 254 nm (E. Merck, Darmstadt, Germany).

7. Sonicator, Bandelin-Sonorex TK (Germany).

8. Centrifuge Hettich (Germany).

### 2.2. Materials

#### 2.2.1. Working standard

Bupropion hydrochloride was kindly supplied by Glaxo Smith Kline (GSK), Cairo Egypt; its purity was 99.90% ±0.59 according to the official HPLC method.[25]

#### 2.2.2. Pharmaceutical formulation

Wellbutrin® SR tablets are labeled to contain 150 mg of bupropion hydrochloride per tablet and manufactured by Glaxo Smith Kline, Cairo, Egypt. Batch No. 090665A.

#### 2.2.3. Degraded sample

2.2.3.1. Preparation of alkaline degradates. An accurately weighed 100 mg of Bup-HCl was dissolved in 10 ml methanol and transferred into 250 mL stopper conical flask, 100 mL 5 M aqueous sodium hydroxide solution was added and refluxed for 12 h. The solution was cooled at room temperature then neutralized with 5 M hydrochloric acid, filtered and evaporated to residue, weighed and diluted to 50 mL with methanol and filtered if necessary. The solution was tested for complete degradation by TLC using isopropanol–toluene–dichloromethane–acetic acid (5:5:5:0.5, by volume) as a developing system, no spot was observed at $R_f$ 0.25 corresponding for intact Bup-HCl. Only 2 spots were observed, one at $R_f$ 0.85 corresponding to 3-chlorobenzoic acid and the other remain at the starting line. The stock solution of the degradates was prepared in methanol 1 mg mL$^{-1}$. The structure of the degradates was elucidated using mass spectroscopy.

2.2.3.2. Preparation of acid degradates. Acid degradates were prepared as mentioned above using 5 M hydrochloric acid and neutralized with 5 M sodium hydroxide. The material was tested for complete degradation as mentioned above.

2.2.3.3. Preparation of oxidative degradates. Using 10 mg Bup-HCl and 1 mL hydrogen peroxide 30%, the solution was diluted with 25 mL water and refluxed for 1 h. The material was tested for complete degradation as above and confirmed

by comparing it with 3-chlorobenzoic acid using HPLC method.

### 2.2.3.4. Preparation of photodegradates.
An amount of 100 mg of Bup-HCl was dissolved in 100 mL methanol and subjected to UV light source for 6 h.

The proposed alkaline, acidic, oxidative and photolytic degradates were tested for complete degradation using HPLC method.

### 2.2.4. Chemicals and reagents
All reagents and chemicals used were of analytical grade, and the solvents were of HPLC and spectroscopic grade.

1. 3-Chlorobenzoic acid 99% Aldrich, related impurity of Bup-HCl in USP.[25]
2. Acetonitrile and methanol of HPLC grade (SDFCL).
3. Dichloromethane, isopropanol (Fisher Scientific), hydrogen peroxide 30% (Merck), orthophosphoric acid, toluene, glacial acetic acid, sodium hydroxide, ammonium acetate (Adwic), hydrochloric acid (Riedel–de Häen) and ammonium dihydrogen phosphate (WINLAB).
4. The water for HPLC was prepared by double glass distillation and filtration through a 0.45 µm membrane filter.

### 2.3. Chromatographic conditions

Analysis was carried out on Agilent 1200 series instrument. However, for convenience, the elution was monitored at 210 nm. Separation was achieved by using ODS column (Eclipse XDB 150 mm × 4.6 mm, 5 µm) with mobile phase of (80:20, v/v) mixture of 1.2% w/v ammonium dihydrogen phosphate pH 4.5 adjusted with orthophosphoric acid if necessary and acetonitrile. The mobile phase was filtered using 0.45 µm disposable filter (Millipore, Milford, MA) and degassed by ultrasonic vibrations prior to use. The flow rate of the mobile phase was 1.5 mL min$^{-1}$. Twenty microliters of sample solution is injected each time.

### 2.4. Standard solutions

- Stock standard solutions of Bup-HCl, its alkaline degradates and related impurity, 3-chlorobenzoic acid, 1 mg mL$^{-1}$ prepared in methanol (for HPLC, $D^1$, $DD^1$ and $Q$ value).
- Working standard solutions of Bup-HCl, its alkaline degradates and related impurity 200 µg mL$^{-1}$ prepared in methanol (for HPLC) and 50 µg mL$^{-1}$ in methanol (for $D^1$, $DD^1$, and $Q$ value).

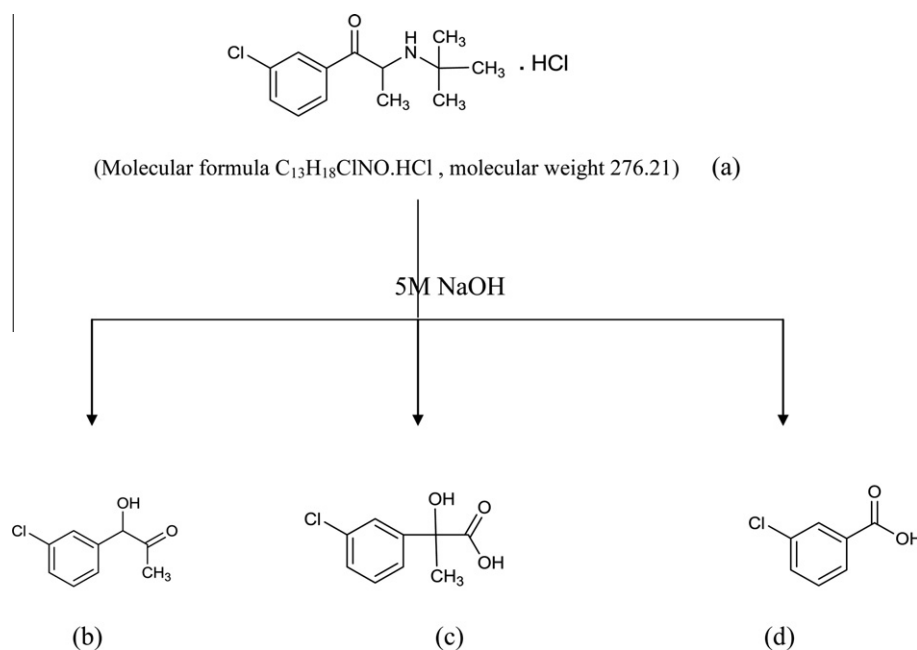### 2.5. Laboratory prepared mixtures

Aliquots of Bup-HCl were accurately transferred into a series of 10-mL volumetric flasks, to these flasks separate volumes of the solution of alkaline degradates or related impurity are added to prepare different mixtures containing 10–90% of the alkaline degradates and related impurity and the volume of each flask was completed with the same solvent used in the proposed methods.

## 3. Procedure

### 3.1. Construction of calibration curve

#### 3.1.1. HPLC method
Aliquots of Bup-HCl working standard solution (200 µg mL$^{-1}$ in methanol) equivalent to 100–1000 µg were accurately transferred into a series of 10-mL volumetric flasks, the volume was



(Molecular formula $C_{13}H_{18}ClNO.HCl$, molecular weight 276.21)   (a)

5M NaOH

(b)   (c)   (d)

**Figure 1** The alkaline degradates of bupropion hydrochloride (a) showing 1-(3-chlorophenyl)-1-hydroxy-2-propanone (b), 2-hydroxy-2-methyl-2-(3-chlorophenyl) acetic acid (c) and 3-chlorobenzoic acid (d).
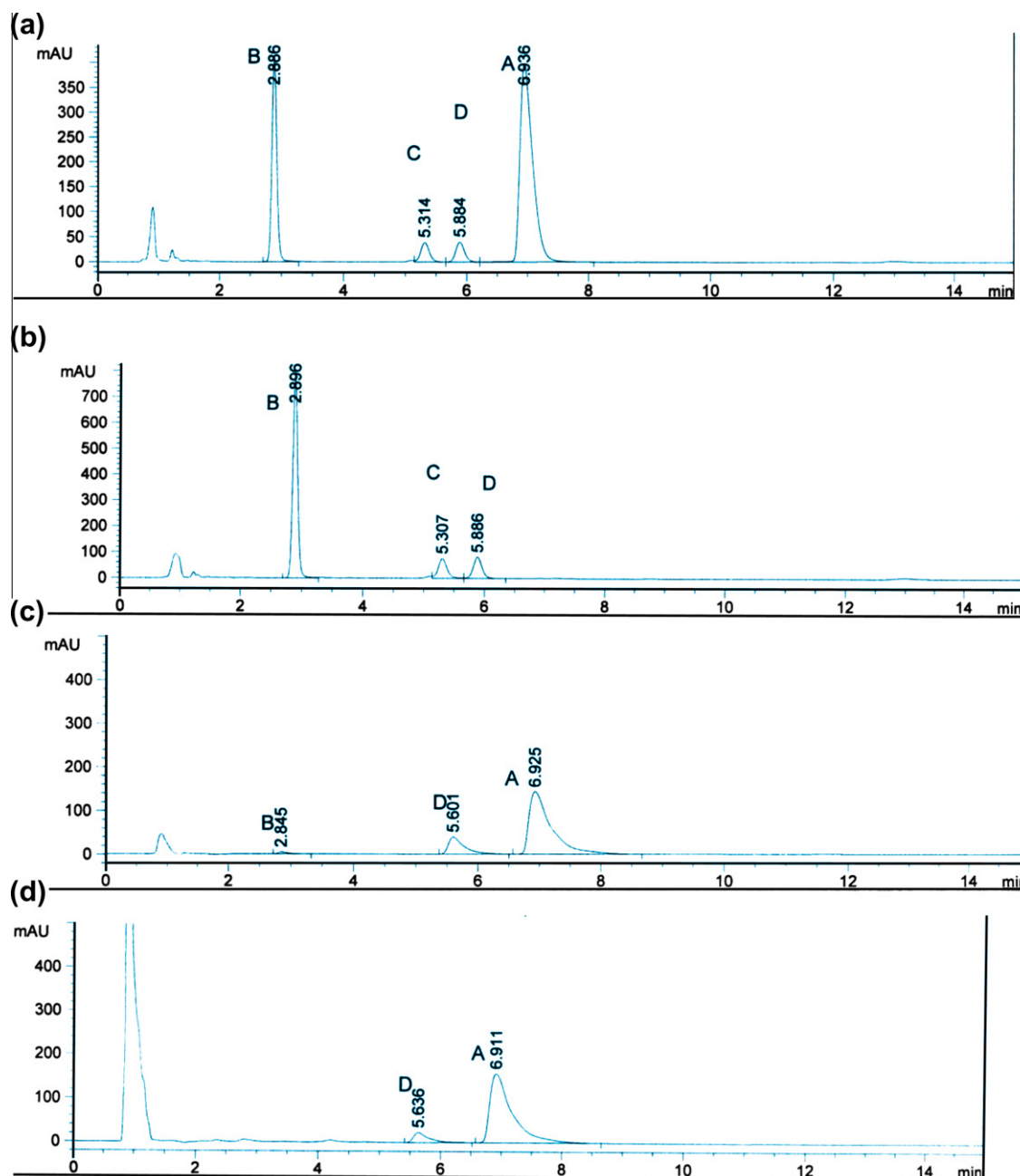
**Figure 2** (a) Chromatogram of mixture of bupropion hydrochloride 90 μg mL$^{-1}$ and its alkaline degradates 90 μg mL$^{-1}$. Showing: A – bupropion hydrochloride; B – 1-(3-chlorophenyl)-1-hydroxy-2-propanone; C – 2-hydroxy-2-methyl-2-(3-chlorophenyl) acetic acid; D – 3-chlorobenzoic acid. (b) Chromatogram of alkaline degradates 90 μg mL$^{-1}$ showing complete degradation. (c) Chromatogram of acid degradates 90 μg mL$^{-1}$ showing incomplete degradation. (d) Chromatogram of oxidative degradates 90 μg mL$^{-1}$ showing incomplete degradation.

completed to the mark with methanol. Triplicate 20 μL injections were made for each concentration and chromatographed under the condition described above. The calibration curve was constructed and the regression equation was computed.

### 3.1.2. $D^1$ method
Aliquots of Bup-HCl working standard solution (50 μg mL$^{-1}$ in methanol) equivalent to 20–250 μg were accurately transferred into a series of 10-mL volumetric flasks, the volume was completed to the mark with methanol. The values of $D^1$

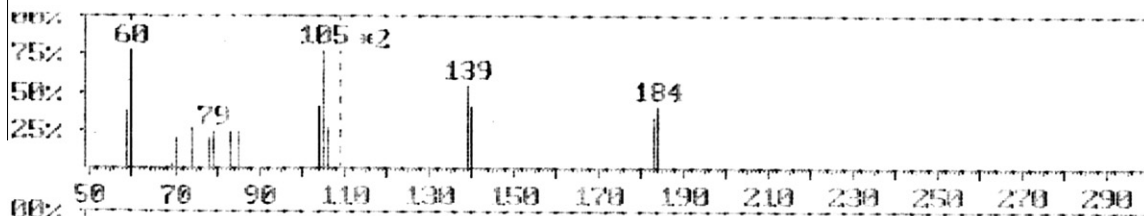amplitude using methanol as a blank were measured at 259 nm. The calibration curve was constructed and the regression equation was computed.

### 3.1.3. $DD^1$ method
Aliquots of Bup-HCl working standard solution (50 μg mL$^{-1}$ in methanol) equivalent to 20–250 μg were accurately transferred into a series of 10-mL volumetric flasks, the volume was completed to the mark with methanol. Spectrum of working standard solution of Bup-HCl was divided by either the
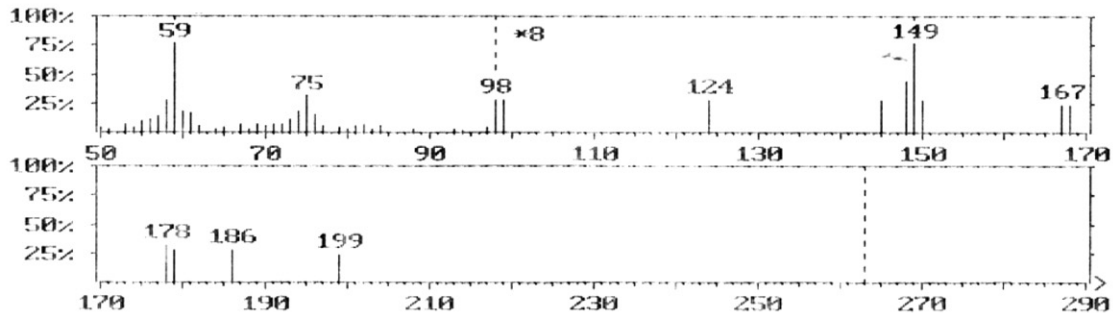
## (a) Bup-HCl
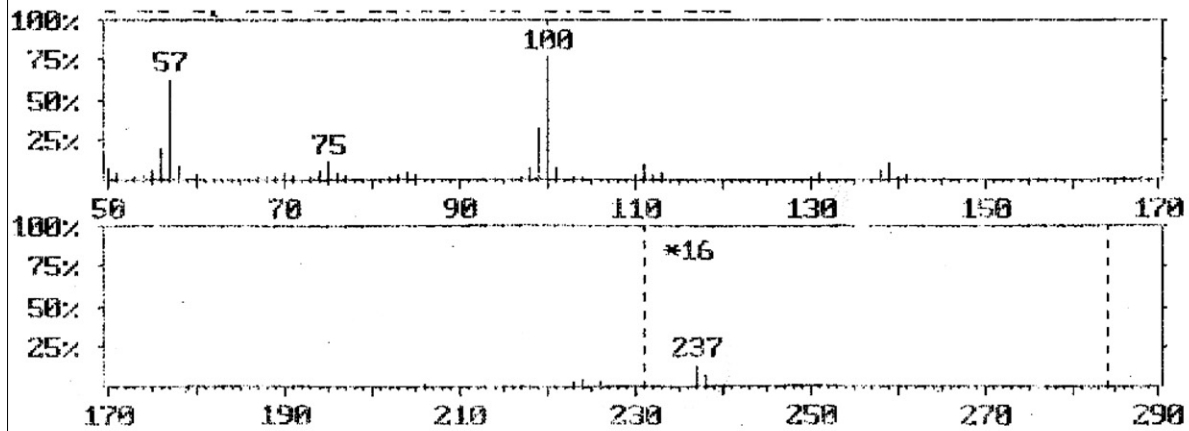


## (b) 1-(3-chlorophenyl)-1-hydroxy-2-propanone



## (c) 2-hydroxy-2-methyl-2-(3-chlorophenyl) acetic acid



## (d) 3-chlorobenzoic acid
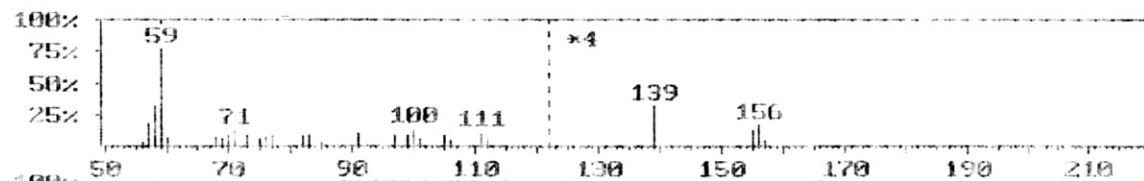


**Figure 3**    Mass spectra of Bup-HCl (a) and its alkaline degradates (b–d).

spectrum of 10 μg mL$^{-1}$ alkaline degradates or 5 μg mL$^{-1}$ in methanol related impurity. The DD[1] values were calculated for the obtained spectra with $\Delta\lambda = 4$ and a scaling factor = 10. The amplitudes at 240 and 244.8 nm were measured for the drug in the presence of alkaline degradates and related impurity, respectively. The calibration curve was constructed and the regression equation was computed.

### 3.1.4. Q value method

Aliquots of Bup-HCl and 3-chlorobenzoic acid working standard solutions (50 μg mL$^{-1}$ in methanol) equivalent to 40–160 μg were accurately transferred into a series of 10-mL volumetric flasks, the volume was completed to the mark with methanol. This method is based on measuring the absorbance at 237 nm of isoabsorptive point, at 248 and 227 nm

**Table 1** System suitability parameters of the proposed HPLC method.

| Product | Symmetry | Resolution | Theoretical plates | Retention time (min) |
|---|---|---|---|---|
| Bupropion hydrochloride | 0.48 | | 5714 | 6.9 ± 0.1 |
| | | 3.35 | | |
| 3-Chlorobenzoic acid | 0.83 | | 8207 | 5.8 ± 0.05 |
| | | 2.25 | | |
| 2-Hydroxy-2-methyl-2-(3-chlorophenyl) acetic acid | 0.86 | | 7380 | 5.3 ± 0.02 |
| | | 12.44 | | |
| 1-(3-Chlorophenyl)-1-hydroxy-2-propanone | 0.91 | | 6581 | 2.9 ± 0.07 |

**Table 2** Characteristic parameters of bupropion hydrochloride by the proposed methods.

| Parameters | HPLC | Derivative spectrophotometry | Ratio derivative in presence of total degrades | Ratio derivative in presence of impurity | Q value for the drug | Q value for related impurity |
|---|---|---|---|---|---|---|
| Range ($\mu$g mL$^{-1}$) | 10–100 | 2–25 | 2–25 | 2–25 | 4–16 | 4–16 |
| Linearity | | | | | | |
|   Slope | 66.271 | 0.0309 | 2.6934 | 2.1243 | 1.2981 | 1.9173 |
|   Intercept | −29.935 | −0.0167 | 0.6116 | 0.579 | 0.255 | 0.4003 |
|   Correlation coefficient (r) | 0.9999 | 0.9999 | 0.9998 | 0.9998 | 0.998 | 0.997 |
| RSD% of slope | 0.23 | 0.19 | 0.04 | 0.16 | 1.59 | 1.92 |
| RSD% of intercept | 8.77 | 1.35 | 0.59 | 1.49 | 3.08 | 3.66 |
| Mean ± RSD% (accuracy) | 100.63 ± 1.12 | 99.97 ± 1.23 | 99.78 ± 0.91 | 99.35 ± 1.22 | 99.65 ± 1.38 | 100.40 ± 1.20 |
| Precision RSD% | | | | | | |
|   Repeatability[a] | 1.22 | 1.35 | 1.59 | 1.57 | 0.84 | 1.08 |
|   Intermediate precision[b] | 1.84 | 1.72 | 1.92 | 1.86 | 1.98 | 1.53 |
| Limit of detection ($\mu$g mL$^{-1}$) | 0.49 | 0.49 | 0.34 | 0.43 | 0.58 | 0.39 |
| Limit of quantitation ($\mu$g mL$^{-1}$) | 1.65 | 1.62 | 1.15 | 1.42 | 1.93 | 1.30 |

[a] The intraday ($n = 3$), average of three different concentrations repeated three times within day.
[b] The interday ($n = 3$), average of three different concentrations repeated three times in three successive days.

corresponding to the maxima for Bup-HCl and its related impurity. Their absorptivity values at the selected wavelengths were calculated.

### 3.2. Pharmaceutical formulation analysis

Ten tablets of Wellbutrin® SR tablets were accurately weighed and finely powdered. A portion of the powder equivalent to 100 mg of Bup-HCl was accurately weighed, dissolved in 70 mL of methanol with the aid of ultrasound for 30 min. A sufficient quantity of methanol was added to produce 100 mL. The solution was centrifuged for 1 h at 3500 rpm and then filtered; the final concentration was 1 mg mL$^{-1}$. Further dilutions of the sample solution were carried out with methanol to prepare the working standard solution. The general procedures for HPLC, D$^1$, DD$^1$ and Q value methods described under construction calibration were followed and the concentration of Bup-HCl was calculated.

## 4. Results and discussion

The International Conference of Harmonization (ICH) guideline entitled "stability testing of new drugs substances and products" requires the stress testing to be carried out to elucidate the inherent stability characteristics of the active substance.[26] An ideal stability indicating method is one that quantifies the standard drug alone and also resolves its degradation products and related impurity.

Bupropion hydrochloride was subjected to alkali, acid hydrolysis, oxidation and UV light. Refluxing Bup-HCl with 5 M sodium hydroxide, complete degradation results in formation of 3 degradates confirmed and separated by HPLC at retention time 2.9, 5.3 and 5.8 min. for 1-(3-chlorophenyl)-1-hydroxy-2-propanone, 1-(3-chlorophenyl)-1,2-propanedione which undergoes benzil-benzilic acid rearrangement in alkaline medium to form 2-hydroxy-2-methyl-2-(3-chlorophenyl) acetic acid and the third one was 3-chlorobenzoic acid, respectively, while no peak at retention time 6.9 min was observed corresponding of Bup-HCl (Figs. 1 and 2).

Upon refluxing Bup-HCl with 5 M HCl for 24 h, incomplete degradation results, and formation of 1-(3-chlorophenyl)-1-hydroxy-2-propanone and 3-chlorobenzoic acid. Reflux with hydrogen peroxide for 1 hour, incomplete degradation of Bup-HCl and formation of 3-chlorobenzoic acid. Exposure of the methanolic solution of Bup-HCl to photo degradation UV lamp for 6 hours, the drug remains stable.

This was confirmed by mass spectrometry (Fig. 3) showing mass ion peak m/z at 184, 199 and 156 for the degradates: 1-(3-chlorophenyl)-1-hydroxy-2-propanone, 2-hydroxy-2-methyl-2-(3-chlorophenyl) acetic acid and 3-chlorobenzoic acid. The latter is stated officially as related impurity was also found as one of the degradation products.
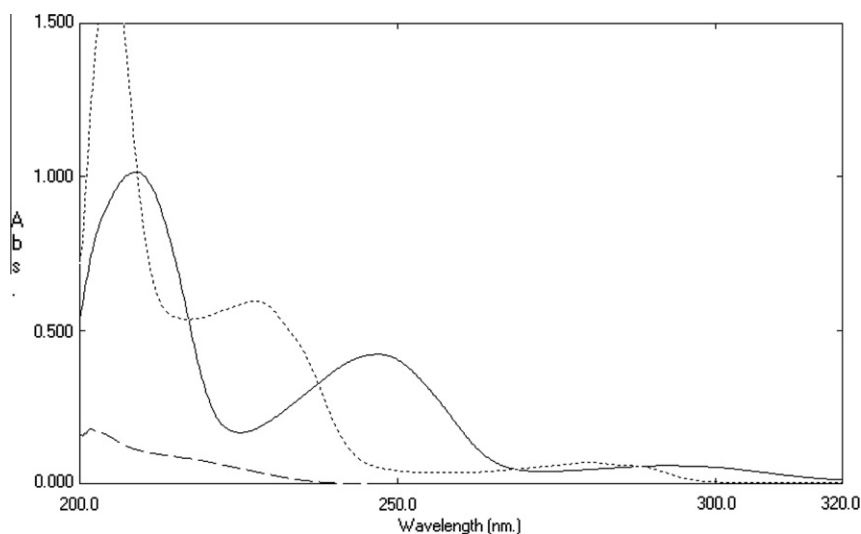
(a) HPLC method

Several trials were carried out to obtain a good resolution between the drug and its alkaline degradates or impurity.

**Figure 4** Zero order absorption spectra of bupropion hydrochloride 10 µg mL$^{-1}$ in methanol (____), its alkaline degradates 10 µg mL$^{-1}$ in methanol (_ _ _ _) and 3-chlorobenzoic acid 10 µg mL$^{-1}$ in methanol (..........).
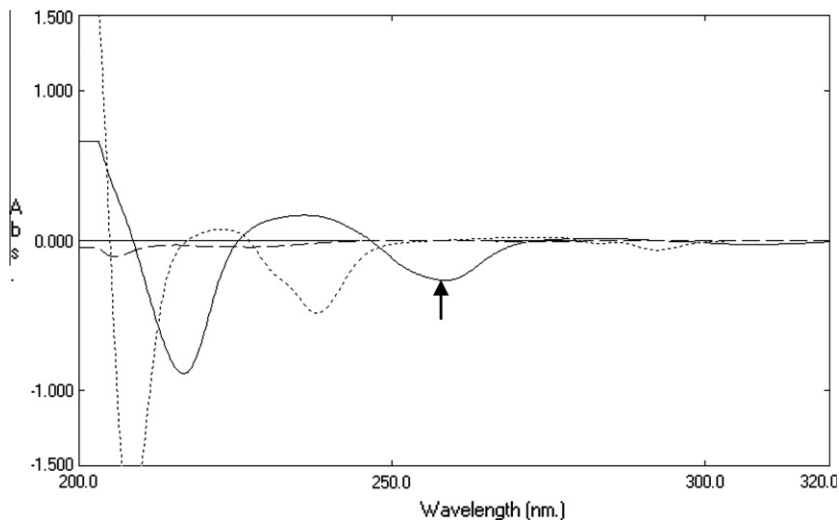


**Figure 5** First derivative absorbance of bupropion hydrochloride 10 µg mL$^{-1}$ in methanol showing the selected wavelength at 259 nm (____), its alkaline degradates 10 µg mL$^{-1}$ in methanol (_ _ _ _) and 3-chlorobenzoic acid 10 µg mL$^{-1}$ in methanol (..........).

These trials involved the use of different mobile phases with different ratios, different pH and flow rates.

The best resolution with sharp and symmetric peaks were obtained upon using 1.2% ammonium dihydrogen phosphate pH 4.5 and acetonitrile (80:20, v/v), and a flow rate of 1.5 mL min$^{-1}$. The retention time for Bup-HCl was found to be 6.9 ± 0.2 min. The same mobile phase with pH 6 delayed the retention time of the drug to 14 min.

System suitability of the procedure is shown in Table 1. Characteristic parameters for regression equation of HPLC method and correlation coefficient obtained by least squares treatment of the results were given in Table 2. The regression equation was computed and found to be:

$$Y = 66.271\,C - 29.935 \quad r = 0.9999$$

where $Y$ is the peak area, $C$ is the concentration of Bup-HCl in µg mL$^{-1}$ and $r$ is the correlation coefficient.

(b) Spectrophotometric methods

(1) D$^1$ method

The zero-order absorption spectra of Bup-HCl, alkaline degradates and 3-chlorobenzoic acid showed severe overlapping which interfere with the direct determination of Bup-HCl (Fig. 4). Derivative spectrophotometry is an analytical technique of great utility for extracting both qualitative and quantitative information from spectra composed of unresolved bands, and for eliminating the effect of baseline shift.[27] A rapid, simple, and low cost spectrophotometric method based on measuring the peak amplitude of D$^1$ spectrum of Bup-HCl at 259 nm (corresponding to zero-contribution of the degradates and related impurity) was developed with good selectivity without interference of alkaline degradates and related impurity. In order to optimize D$^1$ method, different solvents, smoothing and scaling factors were tested, methanol is the best solvent, a smoothing factor $\Delta\lambda = 4$ and a scaling
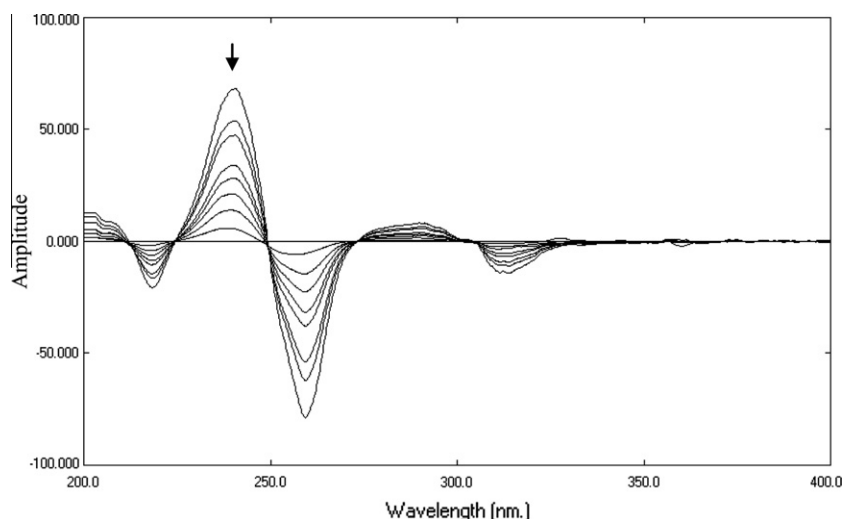
**Figure 6**   First derivative of ratio spectra of bupropion hydrochloride 2–25 µg mL$^{-1}$ with the divisor of alkaline degradates 10 µg mL$^{-1}$ in methanol showing the selected wavelength at 240 nm.
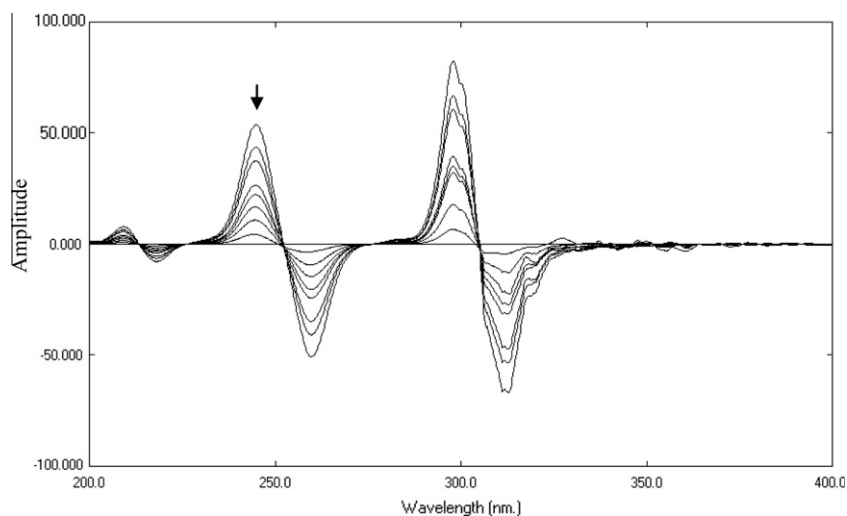


**Figure 7**   First derivative of ratio spectra of bupropion hydrochloride 2–25 µg mL$^{-1}$ with the divisor of impurity 5 µg mL$^{-1}$ in methanol showing the selected wavelength at 244.8 nm.

factor = 10. Fig. 5 shows a suitable signal to noise ratio and the spectra show good resolution. A linear correlation was obtained between peak amplitude at 259 nm and the corresponding concentration in the range of 2–25 µg mL$^{-1}$, from which the linear regression equation was computed and found to be:

$$Y = 0.0309\,C - 0.0167 \quad r = 0.9999$$

where $Y$ is the peak amplitude at 259 nm, $C$ is the concentration of Bup-HCl in µg mL$^{-1}$ and $r$ is the correlation coefficient.

(2) DD$^1$ method

In order to improve the selectivity of the analysis of Bup-HCL in the presence of its alkaline degradates or related impurity, DD$^1$ spectrophotometric method was established. The main advantage of the method is that the whole spectrum of interfering substance is canceled. Accordingly, the choice of

the wavelength selected for calibration is not critical as in first derivative D$^1$ method. In order to optimize DD$^1$ method, several divisor concentrations of the alkaline degradates and related impurity were used and different smoothing and scaling factors were tested. The best result was obtained when using 10 and 5 µg mL$^{-1}$ as a divisor for alkaline degradates and related impurity at 240 and 244.8 nm, respectively, using $\Delta\lambda = 4$ and scaling factor = 10 as shown in Figs. 6 and 7. Beer's law was obeyed in concentration range 2–25 µg mL$^{-1}$, from which the linear regression equation was computed as shown in Table2.

$$Y_1 = 2.6934\,C + 0.6116 \quad r = 0.9998 \text{ in the presence of degradates}$$
$$Y_2 = 2.1243\,C + 0.5790 \quad r = 0.9998 \text{ in the presence of impurity}$$

where $Y_1$ and $Y_2$ are the peak amplitudes at 240 and 244.8 nm, $C$ is the concentration of Bup-HCl in µg mL$^{-1}$ and $r$ is the correlation coefficient.

**Table 3** Evaluation of the selectivity of the proposed methods for the determination of bupropion hydrochloride in the presence of its alkaline degradates and its related impurity.

| Sample number | % Added of alkaline degradates or impurity | Methods | | | | | | Q value |
|---|---|---|---|---|---|---|---|---|
| | | HPLC | | D[1] | | DD[1] | | |
| | | In presence of alkaline degradates | In presence of impurity | In presence of alkaline degradates | In presence of impurity | In presence of alkaline degradates | In presence of impurity | |
| 1 | 10 | 99.98 | 98.85 | 98.12 | 101.59 | 98.69 | 100.31 | |
| 2 | 20 | 99.00 | 99.50 | 101.60 | 101.60 | 98.36 | 98.36 | 101.72 |
| 3 | 50 | 101.35 | 101.53 | 98.98 | 100.77 | 101.9 | 100.85 | 101.84 |
| 4 | 70 | 101.85 | 101.96 | 102.62 | 102.62 | 98.53 | 98.53 | 98.12 |
| 5 | 80 | 100.99 | 98.65 | 102.05 | 102.05 | 98.25 | 98.25 | 99.58 |
| 6 | 90 | 98.35 | 99.56 | 101.69 | 98.14 | 98.25 | 98.88 | |
| Mean ± RSD% | | 100.25 ± 1.38 | 100.01 ± 1.40 | 100.84 ± 1.82 | 101.13 ± 1.57 | 99.00 ± 1.45 | 99.20 ± 1.12 | 100.32 ± 1.79 |

**Table 4** Determination of bupropion hydrochloride in pharmaceutical formulation by the proposed methods and application of standard addition technique.

| Parameter | HPLC | Derivative spectrophotometry | Derivative ratio in presence of total degradates | Derivative ratio in presence of impurity | Q value | USP[25,c] |
|---|---|---|---|---|---|---|
| Mean ± RSD% | 99.82 ± 0.86 | 99.42 ± 1.61 | 98.88 ± 0.85 | 99.12 ± 1.02 | 99.02 ± 1.29 | 99.96 ± 1.18 |
| Variance | 0.73 | 2.56 | 0.70 | 1.03 | 1.62 | 1.38 |
| $n$ | 5 | 5 | 5 | 5 | 5 | 5 |
| $t$-Test (2.306)[a] | 0.84 | 0.56 | 0.14 | 0.27 | 0.26 | |
| $F$-ratio (6.39)[a] | 1.89 | 1.85 | 1.97 | 1.35 | 1.17 | |
| Recovery[b] | 100.07 ± 1.83 | 99.59 ± 1.25 | 98.77 ± 1.60 | 98.86 ± 1.39 | 99.97 ± 1.76 | |

[a] Theoretical value for $t$ and $F$ for $P = 0.05$.
[b] For standard addition of different concentrations of bupropion hydrochloride.
[c] Official HPLC method using C18 (4.6 mm × 15 cm, 5 μm) column, mobile phase methanol and pH 7 phosphate buffer (65:35), flow rate 1.2 mL min$^{-1}$ and detection at 224 nm. The retention time of bupropion hydrochloride was at 12.5 min.

(3) *Q* value method

The absorbance ratio method is a modification of the simultaneous equations. *Q* analysis is based on the relationship between absorbance ratio value of binary mixture and relative concentrations of such a mixture.[28] This method was used for the determination of Bup-HCl and its related impurity in the presence of each other. From the overlain spectrum of Bup-HCl and its impurity, three wavelengths were selected, 248 nm, the maximum of the drug, 237 nm, the isoabsorptive point for both Bup-HCl and its impurity, and 227 nm corresponding to related impurity 3-chlorobenzoic acid (Fig. 4). The method employs *Q* values and the concentration of Bup-HCl was determined by using the following formula:

$$C_1 = \frac{Q_0 - Q_2}{Q_1 - Q_2} \times \frac{A}{a_1}$$

$$Q_0 = \frac{\text{Absorbance of sample at 248 nm}}{\text{Absorbance of sample at 237 nm}}$$

$$Q_1 = \frac{\text{Absorptivity of drug at its maximum 248 nm}}{\text{Absorptivity of drug at isoabsorptive point 237 nm}}$$

$$Q_2 = \frac{\text{Absorptivity of impurity at its maximum 248 nm}}{\text{Absorptivity of impurity at isoabsorptive point 237 nm}}$$

$A$ = Absorbance of mixture at isoabsorptive point
$a_1$ = Absorptivity of cited drug at isoabsorptive point

And the concentration of related impurity was determined by using the following formula:

$$C_2 = \frac{Q_0' - Q_4}{Q_3 - Q_4} \times \frac{B}{b_1}$$

$$Q_0' = \frac{\text{Absorbance of sample at 227 nm}}{\text{Absorbance of sample at 237 nm}}$$

$$Q_3 = \frac{\text{Absorptivity of impurity at its maximum 227 nm}}{\text{Absorptivity of impurity at isoabsorptive point 237 nm}}$$

$$Q_4 = \frac{\text{Absorptivity of drug at its maximum of impurity 227 nm}}{\text{Absorptivity of drug at isoabsorptive point 237 nm}}$$

$B$ = Absorbance of mixture at isoabsorptive point
$b_1$ = Absorptivity of impurity at isoabsorptive point

Linearity was obeyed in concentration range 4–16 μg mL$^{-1}$, from which the linear regression equation was computed as shown in Table 2.

$$Y_1 = 1.2981\,C_1 + 0.2255 \quad r = 0.9975$$
$$Y_2 = 1.9173\,C_2 + 0.4003 \quad r = 0.9970$$

where $Y_1$ and $Y_2$ are the $Q_0$ and $Q_0'$ values of Bup-HCl and its related impurity, respectively, $C_1$ and $C_2$ are the concentrations of Bup-HCl and its related impurity in μg mL$^{-1}$ and $r$ is the correlation coefficient.

**Table 5** Statistical comparison between the proposed methods and official USP method in bulk powder.

| Parameter | HPLC | Derivative spectrophotometry | Ratio derivative in presence of total degradates | Ratio derivative in presence of impurity | $Q$ value | USP[25,c] |
|---|---|---|---|---|---|---|
| Mean[a] ± RSD% | 100.63 ± 1.12 | 99.97 ± 1.23 | 99.78 ± 0.91 | 99.35 ± 1.22 | 99.65 ± 1.38 | 99.90 ± 0.59 |
| Variance | 1.27 | 1.52 | 0.82 | 1.46 | 1.89 | 0.35 |
| $n$ | 5 | 5 | 5 | 5 | 5 | 5 |
| $t$-Test (2.306)[b] | 0.25 | 0.91 | 0.81 | 0.39 | 0.72 | |
| $F$-ratio (6.39)[b] | 3.68 | 4.39 | 2.39 | 4.23 | 5.48 | |

[a] Average of five determinations.
[b] Values of theoretical $t$ and $F$ values at $P = 0.05$.
[c] Official HPLC method using C 8 (3.9 mm × 15 cm, 5 µm) column, mobile phase 0.025 M phosphate buffer, methanol, tetrahydrofuran (51:39:11 v/v), flow rate 1.1 mL min$^{-1}$ and detection at 250 nm. The retention time of bupropion hydrochloride was at 27.4 min.

The $Q$ method was highly selective towards the cited drug and its impurity and determines the cited drug in the presence of 20–80% of its impurity and vice versa.

The proposed HPLC, D$^1$ and DD$^1$ methods were checked by the analysis of laboratory prepared mixtures of Bup-HCl and its alkaline degradates or related impurity in different ratios as presented in Table 3. Bupropion hydrochloride could be determined in the presence of up to 90% of alkaline degradates or related impurity.

Method validation was performed according to ICH guidelines[26] for all the proposed methods. Table 2 shows results of accuracy, repeatability and intermediate precision of the methods.

The proposed methods were applied to the determination of Bup-HCl in commercial tablets. Five replicate determinations were made and satisfactory results were obtained in good agreement with the labeled claims (Table 4). Statistical comparison of the results was performed with regard to accuracy and precision using student's $t$-test and the $F$-ratio at 95% confidence level (Table 4). There is no significant difference between the proposed methods and the official one with regard to accuracy and precision.

The validity of the proposed methods was assessed by applying the standard addition technique, which showed accurate results and there is no interference from excipients as shown in Table 4.

Statistical comparison of the results of analysis of bulk powder obtained by the proposed methods and the official HPLC method[25] was also done using student's $t$-test and the $F$-ratio at 95% confidence level (Table 5), it is clear that there is no significant difference between the proposed methods with regard to accuracy and precision.

## 5. Conclusion

The proposed HPLC and spectrophotometric methods provide simple, accurate and reproducible procedure for the determination of Bup-HCl in pharmaceutical formulations, without any interference from the excipients and in the presence of its alkaline degradates and related impurity. The chromatographic method was found to be more specific and selective than the spectrophotometric methods, while the spectrophotometric methods had the advantage of low cost, rapid and environmental protecting. The proposed methods were completely validated and can be used for the routine analysis of Bup-HCl in quality control laboratories, where economy and time are essential.

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