

# Liquid chromatographic determinations of meta-chlorobenzoic acid in bupropion hydrochloride

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## Abstract

A simple, sensitive method for the determination of meta-chlorobenzoic acid in bupropion hydrochloride is described. Chromatographic separation of m-chlorobenzoic acid is achieved using a mobile phase consisting of n-hexane and ethanol (1000:50, v/v) at a flow rate of 1.0 ml/min on a Chiralpak ADH (250 × 4.6 mm). Absorbance is monitored at 235 nm. The method is linear for m-chlorobenzoic acid over concentration range of LOQ, 5.0 µg/ml to 15.0 µg/ml for m-chlorobenzoic acid with correlation coefficient greater than 0.99. This method is more selective and accurate than United States Pharmacopoeia method for the determination of m-chlorobenzoic acid in bupropion hydrochloride.

**Key words:** 1-(3-Chlorophenyl)-1,2-propanedione, meta-chlorobenzoic acid, bupropion Hcl, HPLC method, method validation

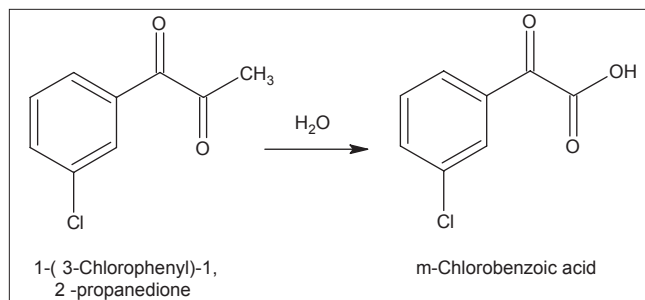
## INTRODUCTION

Bupropion hydrochloride (Bup-HCl) is described chemically as shown in Figure 1 as ( $\pm$ )-2-(tert-butyl amino)-1-(3-chlorophenyl) propan-1-one.<sup>[1]</sup> The drug is an anti-depressant 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.<sup>[2]</sup> It is a water-soluble salt of an aminoketone,<sup>[3]</sup> with a pKa of 7.9,<sup>[4]</sup> and it is also known with the generic name of amfebutamone hydrochloride. Bupropion is structurally related to phenylethylamines, cathinone (a CNS stimulant from leaves of *Catha edulis*), and to the anorectic drug diethylpropion.<sup>[5,6]</sup> Various methods were reported for the determination of Bup-HCl including

spectrophotometry,<sup>[7-9]</sup> potentiometry,<sup>[7]</sup> conductometry,<sup>[7]</sup> titrimetry,<sup>[9]</sup> and polarography.<sup>[10]</sup> HPLC methods were used for the determination of Bup-HCl.<sup>[11-13]</sup>

Bupropion monograph is official in USP.<sup>[14]</sup> Under organic impurities (Test 1) by HPLC, meta-chlorobenzoic acid is controlled at a limit of NMT 0.2%. The verification of bupropion hydrochloride is performed as per organic impurities in Bup-HCl API following USP<sup>[14]</sup> verification of compendial procedures. During verification of organic impurities (Test 1) By HPLC as per USP,<sup>[14]</sup> it is found that impurity at relative retention time (RRT) 0.58 i.e., 1-(3-Chlorophenyl)-1,2-propanedione of organic

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**Figure 1:** Reaction Scheme for the degradation of 1-(3-Chlorophenyl)-1,2-propanedione to m-chlorobenzoic acid

impurities of Test 2 appeared at the same retention time (RT) as that of meta-chlorobenzoic acid peak. Therefore, it is need to develop an accurate method for the determination of meta-chlorobenzoic acid in bupropion hydrochloride. Thus, the paper reports a normal phase method for the determinations of meta-chlorobenzoic acid in bupropion hydrochloride.

## EXPERIMENTAL

### Reagents and chemicals

Standard m-chloro benzoic acid and bupropion are obtained from Glenmark Generics Limited, Navi Mumbai, India. n - hexane, ethanol, and acetonitrile used were of HPLC grade and purchased from RFCL Limited, New Delhi, India.

### Analytical mode high-performance liquid chromatography

A waters (USA) 2695 series HPLC system equipped with 2695 series quaternary gradient pump, auto sampler with cooler and PDA system, and a Shimadzu LC 2010 CHT (Japan) HPLC module equipped with quaternary gradient pump, column oven, auto sampler, and DAD system were used for the analysis and validation of the proposed method. The data were recording using Empower 2 and LC solution software for Waters and Shimadzu system.

### Chromatographic conditions

The analysis was carried out on amylase-based chiralpak AD-H (250 mm × 4.6 mm, 5 μm, Daicel Chemical Industries Limited, Japan) using a mobile phase consisting of n-hexane/ethanol (1000/50, vol/vol) and diluents (Acetonitrile: Ethanol = 9:1) with UV detector at 235 nm at a flow rate of 1.0 ml/min. The column was maintained at 25°C throughout the analysis. A 20-μl sample of concentration was injected, and chromatogram was recorded for 60 minutes.

## METHODS

### Preparation of blank solution

Into a 50 ml separating funnel, transferred 20 ml diluents and added 20 ml n-hexane to it and shaken well for 5 minutes. Hold the separating funnel for 2 minutes to separate the 2 layers. Discard the bottom layer, and use the upper hexane layer for analysis.

### Preparation of test solution

About 200.0 mg of sample is accurately weighed into 20 ml volumetric flask. 10-15 ml of diluents is added and sonicated to dissolve. Volume was made up with diluent and mixed well. Into a 50 ml separating funnel transferred this solution and added 20 ml n-hexane to it and shaken well for 5 minutes. Hold the separating funnel for 2 minutes to

separate the 2 layers. Discard the bottom layer and use the upper layer for analysis.

### Preparation of reference solution

About 5.0 mg of m-chlorobenzoic acid standard is accurately weighed into 50 mL volumetric flask. 25-30 mL of diluents is added and sonicated to dissolve. Volume was made up with diluent and mixed well. Further dilute 2 ml of this solution to 20 ml diluents and mixed. Into a 50 ml separating funnel transferred this solution and added 20 ml n-hexane to it and shaken well for 5 minutes. Hold the separating funnel for 2 minutes to separate the 2 layers. Discard the bottom layer, and use the upper hexane layer for analysis.

### System suitability test

The relative standard deviation of 6 replicate injections of reference solution is not more than 5%, set as the system suitability criteria of the proposed method.

### Validation

#### Specificity and selectivity

The ability of the method to determine accurately and specifically the analyte of interests in the presence of other components in a sample matrix (that may be expected to be present in the sample matrix) under the stated conditions of the test (specificity = 100% selectivity).

#### Linearity

The ability of the method to obtain test results proportional to the concentration of analyte. The linearity was determined by dividing the response with the respective concentrations and to plot these 'relative responses' as a function of the concentration, on a log scale. The line obtained should be horizontal over the full linear range, with a positive deviation at low concentrations and a negative deviation at high concentrations.

#### Accuracy and precision

The accuracy of an analytical method is the extent to which test results generated by the method and the true value agree. The precision of a method is the closeness of agreement between independent test results obtained under stipulated conditions.

#### Limit of detection (LOD)

The limit of detection is the point at which a measured value is larger than the uncertainty associated with it. It is the lowest concentration of analyte in a sample that can be detected but not necessary quantified.

$$\text{LOD} = 3.3 \times S_o/b$$

Where  $S_o$  and  $b$  are standard deviation and slope of the calibration line, respectively.

### Limit of quantitation (LOQ)

The lowest concentration or amount of analyte that can be determined quantitatively with an acceptable level of repeatability precision and trueness.

$$\text{LOQ} = 10.0 \times S_o/b$$

### Ruggedness

The (intra – laboratory tested) behavior of an analytical process when small changes in the environmental and/or operating conditions are made.

### Robustness

A measure of the capacity of the analytical procedure to remain unaffected by small but deliberate variations in method – performance parameters, which provides an indication of its reliability during normal usage.

## RESULTS AND DISCUSSION

On studying the structural similarity of 1-(3-Chlorophenyl)-1,2-propanedione (Impurity at RRT 0.58) and m-Chlorobenzoic acid, it was observed that m-chlorobenzoic acid was the degradation product of 1-(3-Chlorophenyl)-1,2-propanedione. During analysis on contact with water, 1-(3-Chlorophenyl)-1,2-propanedione undergoes degradation to give m-chlorobenzoic acid. The reaction is shown in Figure 1.

### System suitability

The relative standard deviation of 6 replicate injections of reference solution was found to be 2.8%.

### Validation

#### Specificity/selectivity

Specificity of the method was evidenced by comparing blank, meta-chlorobenzoic acid, bupropion, impurity at different relative retention time (RRT) and spiking with meta-chlorobenzoic acid with these impurities. From the experimental data [Table 1], there are no interfering peaks at retention time of meta-chlorobenzoic acid from the chromatogram [Figures 2-4]. In the modified method, all the impurities of bupropion hydrochloride are well separated from m-chlorobenzoic, i.e., method is selective for determination of content of m-chlorobenzoic acid.

#### Solution stability

The study reveals that m-chlorobenzoic acid in bupropion hydrochloride is stable in the diluents for 48 hours when stored at room temperature under laboratory light conditions. There were no significant changes with initial data up to 48 h.

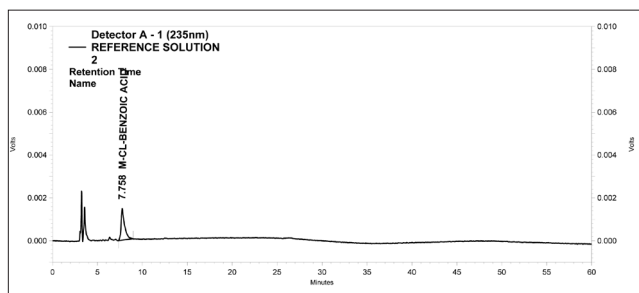
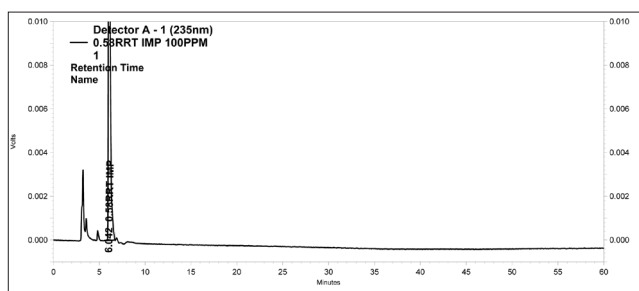
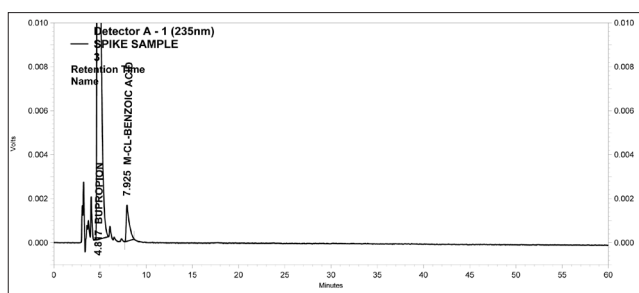
#### Linearity

The linearity regression studies demonstrated the acceptability of the method for the quantitative

**Table 1: Selectivity of the proposed method**

Selectivity	Retention time
Impurity at RRT 0.38	Not eluting
Impurity at RRT 0.58	6.042 minutes
Impurity at RRT 0.71	Not eluting
Impurity at RRT 0.78	5.617 minutes
Impurity at RRT 0.92	Not eluting
Impurity at RRT 1.14	4.992 minutes
Impurity at RRT 1.63	6.217 minutes
Impurity at RRT 1.63	6.867 minutes
Impurity at RRT 2.30	4.858 minutes
Impurity at RRT 2.74	3.725 minutes
m-chloro benzoic acid	7.925 minutes
Bupropion HCl	4.817 minutes

HCl = Hydrochloride, RRT = Relative retention time

**Figure 2: Standard Chromatogram of m-chlorobenzoic acid****Figure 3: Standard Chromatogram of 1-(3-Chlorophenyl)-1,2-propanedione****Figure 4: Standard Chromatogram of Bupropion and m-chlorobenzoic acid**

determination of m-chlorobenzoic acid. For linearity range determination, the data generated was analyzed by linear regression analysis to calculate the slope, intercept, and the correlation coefficient. The method follows a linear range over 5 µg/ml to 15 µg/ml for m-chlorobenzoic acid

(i.e., 50% to 150%) [Table 2] with a correlation coefficient greater than 0.99 [Figure 5].

### Accuracy

The accuracy of the method was determined by spiking the known amount of m-chlorobenzoic acid at LOQ, 50%, 100%, and 150% w/w of the specified limit. The accuracy in determination was checked at 3 different concentration levels, and it was observed that the recovery of the m-chlorobenzoic acid is within the prescribed range of 80-120%.

### Precision

The % RSD for 6 replicate injections of standard solution of m-chlorobenzoic acid is found to be 1.31% for system

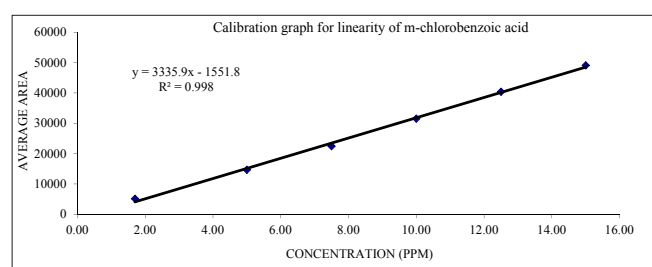


Figure 5: Linearity graph of proposed method

Table 2: Linearity of the proposed method

Conc. (%)	m-chlorobenzoic acid area	Average area
LOQ	5133	
50	14151	14709
	15267	
75	22409	22501
	22593	
100	31833	31606
	31378	
125	40851	40499
	40147	
150	49662	49196
	48730	
Slope	3335.58	
Intercept	-1551.7783	
Correlation Coefficient	0.999	

LOQ = Limit of quantification

precision. Method precision is performed by estimating the m-chlorobenzoic acid in control samples and 6 spiked samples on the same day. The % RSD for m-chlorobenzoic acid from 6 samples was calculated and was found to be well within the desired limits.

### Limit of detection

The limit of detection is determined from the linearity experiment wherein a lower concentration of m-chlorobenzoic acid is analyzed. The LOD concentration is found to be 0.6 ppm i.e., 0.01% of test concentration for m-chlorobenzoic acid. The RSD for 6 replicate injections of m-chlorobenzoic acid is found to be 13.24%. The signal to noise ratio is above 3. The results are reported in Table 3.

### Limit of quantitation

The limit of quantification is determined from the linearity experiment wherein a lower concentration of m-chlorobenzoic acid is analyzed. The LOQ concentration is found to be 1.7 ppm i.e., 0.02% of test concentration for m-chlorobenzoic acid. The RSD for 6 replicate injections of m-chlorobenzoic acid is found to be 6.40%. The signal to noise ratio is above 10. The results are reported in Table 3.

### Ruggedness

Ruggedness is performed by estimating the m-chlorobenzoic acid in 3 control samples and 6 spiked samples by 2 different analysts, using different HPLC columns on 2 different days. The % RSD for m-chlorobenzoic acid from these 12 samples is estimated and found to be well within the desired limits.

### Robustness

The robustness of the method was tested by making deliberate changes in the chromatographic conditions such as flow rate, temperature, and change in the ratio of mobile phase. It provides an indication of its reliability during normal usage. It was found that the results are comparable with that under normal condition, hence minimizing the errors arising due to these changes. The experiment is

Table 3: Data sheet for limit of detection and limit of quantitation

Chromatogram	Area of m-chlorobenzoic acid	Chromatogram	Area of m-chlorobenzoic acid
LOD	1712	LOQ	5208
LOD	1764	LOQ	5335
LOD	1265	LOQ	5484
LOD	1504	LOQ	5164
LOD	1325	LOQ	5079
LOD	1491	LOQ	4528
Average	1510	Average	5133
Std Deviation	199.983	Std Deviation	328.491
%RSD	13.24	%RSD	6.40
S/N	5.12	S/N	15.8

LOD = Limit of Detection, LOQ = Limit of quantitation

carried out at a temperature of 20°C the m-chlorobenzoic acid peak appears at 7.858 minute. When the experiment is carried out at a temperature of 30°C, the m-chlorobenzoic acid peak appears at 7.650 minute. When the experiment is carried out at a flow rate of 0.8 ml/min and 1.2 ml/min, the m-chlorobenzoic acid peak appears at about 9.508 minutes and 6.783 minutes, respectively. The effect of mobile phase composition n-hexane: Ethanol (1000: 45 and 995: 55), then m-chlorobenzoic acid peak appears at about 8.467 minutes and 6.942 minutes, respectively. The results of the study performed showed that there was no difference in retention time found, and separation was also not affected.

## CONCLUSIONS

M-chlorobenzoic acid is the degradation product of 1-(3-Chlorophenyl)-1,2-propanedione. During analysis on contact with water 1-(3-Chlorophenyl)-1, 2-propanedione undergoes degradation to give m-chlorobenzoic acid. In the present method, all the impurities of bupropion hydrochloride are well separated from m-chlorobenzoic, i.e., method is selective for determination of content of m-chlorobenzoic acid. In addition, the proposed HPLC method is a simple, precise, accurate, and rapid as evidenced from the validation data, and the method has wider linear dynamic range with good accuracy and precision. Therefore, the method can be used routinely for regular analysis of bupropion for the determination of m-chlorobenzoic acid.

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