



Research Article

# Stability Indicating Method to Analyze Benidipine and Chlorthalidone Using HPLC Technique: Establishment, Validation and Application to Tablets

Kadali Jagadeesh<sup>1,2</sup>, Nowduri Annapurna<sup>2\*</sup>

<sup>1</sup>Basic Science Department, Shri Vishnu Engineering College for Women, Bhimavaram, Andhra Pradesh, India- 534201.

<sup>2</sup>Department of Engineering Chemistry, AU College of Engineering (A), Andhra University, Visakhapatnam, Andhra Pradesh, India -530003.

## Article Info

### Article History:

Received: 9 August 2019  
Accepted: 21 September 2019  
ePublished: 10 March 2020

### Keywords:

-Benidipine  
-Chlorthalidone  
-Degradation  
-Tablets  
-HPLC  
-Analysis

## Abstract

**Background:** The combination of chlorthalidone and benidipine was used to manage hypertension. The mixture of chlorthalidone and benidipine in tablet dosage form has not been previously determined by any method. A stability indicating HPLC method was developed for the simultaneous determination of benidipine and chlorthalidone in bulk and tablets.

**Methods:** Chromatographic separation was accomplished in a reverse phase system using an isocratic elution with a mobile phase composed of methanol-0.1M dipotassium hydrogen phosphate buffer (40:60, v/v), at 1 ml/min flow rate. The photodiode array (PDA) detector set at 260 nm was used to detect and quantify benidipine and chlorthalidone. Benidipine and chlorthalidone tablet samples were subjected to degradation under acid, neutral, alkali, thermal, photo and oxidative. The proposed method was effectively adapted to quantify benidipine and chlorthalidone in the combined tablet formulation.

**Results:** The elution times for benidipine and chlorthalidone were approximately 4.573 min and 6.422 min, respectively. The method was validated within a concentration range of 2 - 6 µg/ml ( $R^2 = 0.9997$ ) for benidipine and 6.25 - 18.75 µg/ml ( $R^2 = 0.9998$ ) for chlorthalidone. Adequate results were obtained for precision (RSD% = 0.106% for benidipine and RSD% = 0.031% for chlorthalidone) and accuracy (99.95 - 100.25 % mean recovery for benidipine and 99.60 - 99.63% mean recovery for chlorthalidone). Robustness has also been found to be acceptable. During the degradation study, interference was not noticed in the analysis of studied drugs.

**Conclusion:** The findings demonstrated that the method could be useful for determination of the selected drug combination in routine analysis.

## Introduction

Benidipine is a  $Ca^{2+}$  channel blocker. It is a derivative of synthetic dihydropyridine with IUPAC name (3R)-1-Benzyl-3-piperidinyl methyl (4R)-2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydro-3,5-pyridinedicarboxylate.<sup>1-4</sup> Benidipine has anti-anginal and anti-hypertensive activities. Chlorthalidone is a thiazide-like diuretic agent. It is a sulfonamide with IUPAC name (RS)-2-Chloro-5-(1-hydroxy-3-oxo-2,3-dihydro-1H-isoindol-1-yl)benzene-1-sulfonamide.<sup>5-8</sup> It has anti hypertensive and diuretic activities. The chemical structures of benidipine and chlorthalidone are given in Figure 1.

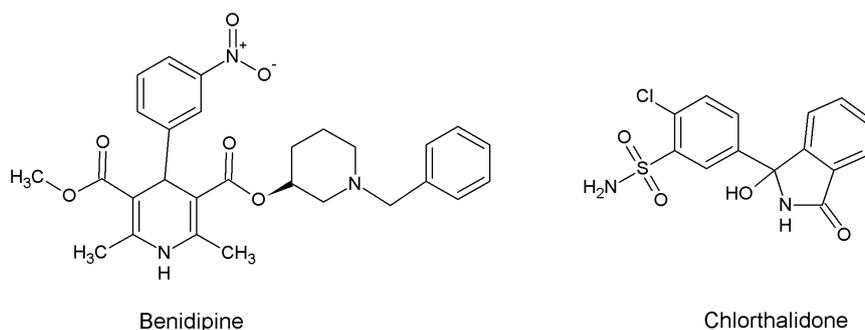
The combination of chlorthalidone and benidipine was used to manage increased blood pressure (hypertension).<sup>9</sup> This combination is available as tablet dosage form with brand names: Benitowa-CH (Akumentis Healthcare Ltd), Beniduce CH (Macleods Pharmaceuticals Pvt Ltd), Benidin CH (Lloyd Healthcare Pvt Ltd), Benina C (UTH Healthcare) and Benitowa-CH (Akumentis Healthcare Ltd).

In all brands, the strength of each tablet is 4 mg benidipine and 12.5 mg chlorthalidone. In this combination, benidipine relaxes the blood vessels and makes the heart very effective in pumping blood all through the body and chlorthalidone removes excess water and some electrolytes from the body by increasing the volume of urine generated. Literature study revealed few methods for the quantification of chlorthalidone and benidipine as a single component in bulk, formulation and human plasma. These methods are: spectrophotometric,<sup>10-17</sup> fluorimetric<sup>18</sup>, high-performance liquid chromatography (HPLC),<sup>19-21</sup> LC-MS,<sup>22,23</sup> GC-MS<sup>24</sup> and voltametric.<sup>25</sup>

To the best of our thorough literature survey, the mixture of chlorthalidone and benidipine in tablet dosage form was not previously determined by any method. The purpose of this investigation was to develop an effective and simple stability indicating method for the simultaneous quantification of chlorthalidone and benidipine using

\*Corresponding Author: Nowduri Annapurna, E-mail: drannapurna.nowduri@gmail.com

©2020 The Author(s). This is an open access article and applies the Creative Commons Attribution License (<http://creativecommons.org/licenses/by-nc/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, as long as the original authors and source are cited.



**Figure 1.** Chemical structures of benidipine and chlorthalidone.

reversed-phase HPLC. The developed method was validated and then adopted to analyze chlorthalidone and benidipine in tablets.

## Materials and Methods

### Materials

Standards of benidipine and chlorthalidone were supplied by Prayosha Healthcare Pvt, Ltd (Gujarat, India) and Pronter Pharma drugs Pvt. Ltd (Hyderabad, India). Benitowa-CH Tablets (Akumentis Healthcare Ltd, Gujarat, India) labeled to have 4 mg of benidipine and 12.5 mg of chlorthalidone was procured and used for analysis. Methanol (HPLC grade) was obtained from Merck specialties Ltd (Telangana, India). Dipotassium hydrogen phosphate, sodium hydroxide, phosphoric acid, hydrochloric acid and hydrogen peroxide were of analytical grade and procured from Sd Fine-Chem Limited (Tamilnadu, India). The water of HPLC grade (Milli Q, Millipore, USA) was utilized.

### Chromatographic conditions

The Waters Alliance 2695 model HPLC system composed of quaternary pump, autosampler and photodiode array detector was utilized. A C18 Kromasil column (5  $\mu$ m particles, 250 mm  $\times$  4.6 mm i.d) was used for separation and analysis of benidipine and chlorthalidone. The operating temperature was set at 25°C. The mobile phase utilized consisted of a combination of methanol and 0.1 M dipotassium hydrogen phosphate buffer (adjusted at pH 4.5 with orthophosphoric) in the ratio of 40:60 (v/v) in an isocratic mode flow rate at 1.0 ml/min. The injection volume was 10  $\mu$ l. The detector has been fixed at 260 nm for the simultaneous determination of benidipine and chlorthalidone. The chromatographic information was gathered and processed using a computer system with Water Alliance empower 2 software.

### Preparations

#### Stock solution

Stock solution of benidipine (40  $\mu$ g/ml) and chlorthalidone (125  $\mu$ g/ml) was prepared by dissolving benidipine (4 mg) and chlorthalidone (12.5 mg) standards in diluent (methanol and 0.1 M dipotassium hydrogen phosphate buffer, ratio 40:60, v/v).

### Solutions for calibration graph

Stock solution (benidipine - 40  $\mu$ g/ml and chlorthalidone - 125  $\mu$ g/ml) was accurately diluted with diluent to obtain the following concentrations:

Solution 1: benidipine - 2  $\mu$ g/ml and chlorthalidone - 6.25  $\mu$ g/ml

Solution 2: benidipine - 3  $\mu$ g/ml and chlorthalidone - 9.375  $\mu$ g/ml

Solution 3: benidipine - 4  $\mu$ g/ml and chlorthalidone - 12.5  $\mu$ g/ml

Solution 4: benidipine - 5  $\mu$ g/ml and chlorthalidone - 15.625  $\mu$ g/ml

Solution 5: benidipine - 6  $\mu$ g/ml and chlorthalidone - 18.75  $\mu$ g/ml

### Solutions for validation

Stock solution (benidipine - 40  $\mu$ g/ml and chlorthalidone - 125  $\mu$ g/ml) was accurately diluted with diluent to obtain a solution with a concentration of 4  $\mu$ g/ml benidipine and 12.5  $\mu$ g/ml chlorthalidone.

### Tablet sample solution

Twenty tablets were finely powdered and weighed. A powder amount equal to 12.5 mg of chlorthalidone and 4 mg of benidipine was transferred to a 100 ml flask and sonicated for 20 min with 60 ml of diluent. The solution was filtered and the volume was diluted to 100 ml mark with the diluent (Concentration: benidipine - 40  $\mu$ g/ml and chlorthalidone - 125  $\mu$ g/ml). From this solution, 1 ml aliquot was transferred to 10 ml flask and the volume was diluted to 10 ml mark with the diluent to get a solution with a concentration of 4  $\mu$ g/ml benidipine and 12.5  $\mu$ g/ml chlorthalidone.

### Degradation of benidipine and chlorthalidone

- Hydrolysis with acid: 10 ml of tablet sample solution (benidipine - 40  $\mu$ g/ml and chlorthalidone - 125  $\mu$ g/ml) was mixed with 10 ml of 0.1N HCl and sonicated at ambient temperature for 30 min.
- Hydrolysis with alkali: 10 ml of tablet sample solution (benidipine - 40  $\mu$ g/ml and chlorthalidone - 125  $\mu$ g/ml) was mixed with 10 ml of 0.1N NaOH and sonicated at ambient temperature for 30 min.
- Wet degradation with water: 10 ml of tablet sample solution (benidipine - 40  $\mu$ g/ml and chlorthalidone -

125 µg/ml) was mixed with 10 ml of distilled water and sonicated at ambient temperature for 30 min.

- Oxidative degradation with hydrogen peroxide: 10 ml of tablet sample solution (benidipine - 40 µg/ml and chlorthalidone - 125 µg/ml) was mixed with 10 ml of 30% hydrogen peroxide and sonicated at ambient temperature for 30 min.
- Degradation with dry heat: A powder amount equal to 12.5 mg of chlorthalidone and 4 mg of benidipine was spread in a petri plate and placed in an oven set at 105°C temperature for 30 min.
- Degradation with sunlight: A powder amount equal to 12.5 mg of chlorthalidone and 4 mg of benidipine was spread in a petri plate and placed in direct sunlight for 24 hr.

Following degradation, each sample collected under each condition of degradation was diluted properly with diluent to get a solution with a concentration of 4 µg/ml benidipine and 12.5 µg/ml chlorthalidone.

## Procedures

### Calibration graphs

For this, five calibration solutions in the range of 2-6 µg/ml (benidipine) and 6.25-18.75 µg/ml (chlorthalidone) were prepared. These solutions were injected into the system and analyzed by the suggested conditions. Benidipine and chlorthalidone calibration curves were assessed by plotting the drug peak area versus drug concentration. Regression equations for benidipine and chlorthalidone were also determined.

### Determination of benidipine and chlorthalidone in tablets

The prepared tablet sample solution was subjected to analysis by the suggested conditions in triplicate. The benidipine and chlorthalidone peak areas were referred to corresponding regression equations to obtain the sample concentration and nominal percent of the labeled claim.

### Degradation study

Forced degradation experiments were conducted on benidipine and chlorthalidone combination tablets under different conditions as described in Q1A (R2) ICH guideline.<sup>26</sup> The conditions include: acid, alkali, wet, dry heat, oxidative and photolytic. The degraded solutions were injected in the system and analyzed with the suggested conditions. The obtained chromatograms were explored for

peak areas and additional peak appearance. The decrease in the drug peak area was considered as degradation. The occurrence of additional peaks and its resolution from the peaks of benidipine and chlorthalidone were considered as an indication of specificity and stability indicating nature of the method.

## Results and discussion

### Method optimization

The optimum conditions required for the quantitative analysis of benidipine and chlorthalidone with maximum sensitivity were determined by selecting the analytical column, varying the mobile phase composition, ratio and pH followed by observing its effects on peak width, peak symmetry, retention time and resolution. The chromatographic separation was executed on different HPLC columns including Kromasil C18, Waters C18 and Inertsil C18. Finally, Kromasil C18 column with ambient temperature was selected as it provided good resolution between peaks of benidipine and chlorthalidone with good peak shape.

Solvent systems like 0.1% orthophosphoric acid buffer with methanol, 0.1M sodium dihydrogen phosphate buffer with methanol and 0.1M dipotassium hydrogen phosphate with methanol were investigated with different ratios, pH and flow rate. Good peak shapes, good resolution with high sensitivity and reasonable retention time were obtained with the following conditions: solvent system as mobile phase - 0.1M dipotassium hydrogen phosphate and methanol in the ratio of 60:40, v/v; flow rate - 1.0 ml/min; pH - 4.5. The wavelength of 260 nm was found to be suitable for the quantification of benidipine and chlorthalidone. Using recommended conditions, benidipine and chlorthalidone were eluted at 4.573 min and 6.422 min (Figure 2), respectively.

### Method validation

The procedure was validated in accordance with the ICH Q2 (R1) regulations for pharmaceutical sample assessment.<sup>27</sup>

### System suitability

This was tested by injecting the standard solution (4 µg/ml benidipine and 12.5 µg/ml chlorthalidone) in five replicates. The relative standard deviation values for peak areas and retention times of selected drugs were determined. Plate count, peak asymmetry and resolution factor were also determined for both drugs. The determined values were

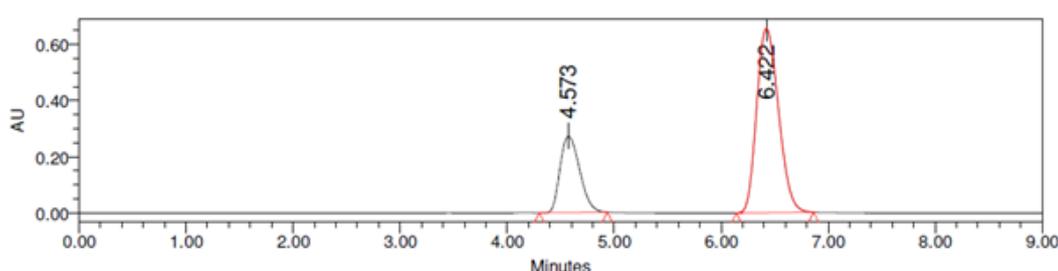


Figure 2. Elution of benidipine and chlorthalidone using recommended conditions.

compared with the recommended limits of ICH. All the values are within the acceptance limits, so the system is appropriate for the simultaneous analysis of benidipine and chlorthalidone. The results are shown in Table 1.

**Table 1.** Benidipine and chlorthalidone system suitability results.

Statistical evaluation	ET	PR	PC	PA	Rs
<b>Benidipine</b>					
Mean*	4.602	3398873	6926	1.188	-
SD	0.0281	2372.76	13.221	0.004	-
% RSD	0.612	0.070	0.191	0.376	-
<b>Chlorthalidone</b>					
Mean*	6.436	8632165	8662	1.118	5.086
SD	0.0141	4749.61	26.025	0.004	0.065
% RSD	0.219	0.055	0.300	0.400	1.286
<b>Recommended limits</b>					
ICH limits	RSD: ≤2%	RSD: ≤2%	> 2000	≤ 2	≥ 2

\* mean of five values; ET - elution time; PR - Peak area response; PC - plate count; PA - peak asymmetry; Rs - resolution.

### Selectivity

Standard solution (4 µg/ml of benidipine and 12.5 µg/ml of chlorthalidone), tablet sample solution (4 µg/ml of benidipine and 12.5 µg/ml of chlorthalidone) and mobile phase blank solution were injected separately into the system and chromatograms obtained were compared. No interfering peak was observed near the retention times of benidipine and chlorthalidone in the chromatograms of tablet and mobile phase blank. This demonstrated the method's selectivity.

### Linearity

The linearity was determined using five point calibration curve that represented the relationship between the values of peak area and analyte (benidipine and chlorthalidone) concentration. Table 2 shows the calibration data. Linearity was confirmed through values of correlation coefficient (> 0.999).

### LOD and LOQ

LOD was defined as  $3.3 \times$  standard deviation of regression line's intercept/Slope of calibration graph. LOQ was defined as  $10 \times$  standard deviation of regression line's intercept/Slope of calibration graph. The determined values are shown in Table 2. The low values of obtained LOD and LOQ indicated the good sensitivity of the method.

**Table 2.** Calibration and sensitivity data of benidipine and chlorthalidone.

Parameter	Benidipine	Chlorthalidone
Linearity (µg/ml)	2-6	6.25-18.75
Slope (m)	84925	69123
Intercept (I)	2130	9755
Correlation coefficient (R <sup>2</sup> )	0.9997	0.9998
LOD (µg/ml)	0.004	0.005
LOQ (µg/ml)	0.013	0.016

### Precision

Intra-day and inter-day precision were performed with working standard solution at a concentration of 4 µg/ml benidipine and 12.5 µg/ml chlorthalidone. Precision results were expressed as relative standard deviation of peak area values. Acceptance criteria for precision stated that the percent relative standard deviation should be <2%. The results (Table 3) demonstrated the precision of the method.

**Table 3.** Precision data of benidipine and chlorthalidone.

Injection no.	Benidipine	Chlorthalidone
<b>Intra-day</b>		
Peak area <sup>§</sup>	3394260	8636063
SD*	3598.601	2683.512
RSD**	0.106	0.031
<b>Inter-day</b>		
Peak area <sup>§§</sup>	3392321	8635564
SD*	2577.360	3227.561
RSD**	0.076	0.037

\*SD - standard deviation; \*\*RSD - percent relative standard deviation; § - mean of six determinations; §§ - mean of three determinations

### Ruggedness

Ruggedness was performed using working standard solution (4 µg/ml of benidipine and 12.5 µg/ml of chlorthalidone) with two different analysts and two different HPLC systems (instrument 1 - Waters Alliance HPLC system; instrument 2 - Shimadzu HPLC system). Ruggedness results were expressed as relative standard deviation of peak area values. Acceptance criteria for ruggedness stated that the percent relative standard deviation should be <2%. From the results (Table S1 in supplementary data), the proposed method was demonstrated as rugged.

### Accuracy

Accuracy was evaluated by adding pure standards of benidipine and chlorthalidone to the pre-analyzed tablet sample solution at three levels of concentration (50%, 100%, and 150%). Accuracy results were expressed as percent recovery of added benidipine and chlorthalidone. Acceptance criteria for accuracy stated that the percent recovery should be within 98-102%. The percent recoveries of benidipine and chlorthalidone determined (Table 4) suggested excellent accuracy of the method.

### Robustness

The robustness was evaluated by investigating the change of flow rate, temperature, methanol ratio in the mobile phase, pH of mobile phase and detection wavelength on the system suitability parameters. Under studied conditions, no substantial changes were noticed in the system suitability parameters. The results demonstrated the robustness of the method (Table S2 in supplementary data).

**Table 4.** Accuracy data of benidipine and chlorthalidone.

Spiked level (%)	Concentration added ( $\mu\text{g/ml}$ )	Concentration found*( $\mu\text{g/ml}$ )	SD	Recovered(%)	RSD (%)
<b>Benidipine</b>					
50	2	1.999	0.0044	99.95	0.226
100	4	4.008	0.0023	100.19	0.055
150	6	6.015	0.0017	100.25	0.032
<b>Chlorthalidone</b>					
50	6.25	6.225	0.0036	99.60	0.063
100	12.50	12.452	0.0046	99.62	0.041
150	18.75	18.680	0.0137	99.63	0.077

\*mean of three values; SD – standard deviation; RSD – percent relative standard deviation.

**Table 5.** Degradation data of benidipine and chlorthalidone.

Condition	Ben peak area	Ben recovered %	Ben degraded %	Chl peak area	Chl recovered %	Chl degraded %
Undegraded	3398873	100.00	-	8632164	100.00	-
Acid	3345851	98.44	1.56	8069347	93.48	6.52
Base	3217033	94.65	5.35	8298099	96.13	3.87
Oxidation	3067483	90.25	9.75	7947633	92.07	7.93
Thermal	3323078	97.77	2.23	8516493	98.66	1.34
Photo	3325117	97.83	2.17	8506998	98.55	1.45
Water	3378820	99.41	0.59	8550158	99.05	0.95

Ben – benidipine; Chl – chlorthalidone.

**Table 6.** Benidipine and chlorthalidone content in Benitowa-CH tablet.

Labeled claim (mg)	Determined value (mg)	Recovered (%)	Mean recovery (%)	SD	RSD
<b>Benidipine</b>					
4	3.998	99.95	100.13	0.1587	0.159
4	4.008	100.19			
4	4.010	100.25			
<b>Chlorthalidone</b>					
12.5	12.450	99.6	99.62	0.0153	0.015
12.5	12.453	99.62			
12.5	12.454	99.63			

SD – standard deviation; RSD – percent relative standard deviation

### Specificity

The specificity was evaluated by analyzing tablet sample solution (4  $\mu\text{g/ml}$  of benidipine and 12.5  $\mu\text{g/ml}$  of chlorthalidone) that was exposed to different degradation conditions (acid, alkali, wet, dry heat, oxidative and photolytic). The degradation study was done to show the method's capability to quantify benidipine and chlorthalidone simultaneously in the presence of possible degradants. The results are summarized in Table 5. The chromatograms of degradation study are given in Figure S1 in supplementary data. From the chromatograms it was noticed that there was a clear separation of benidipine and chlorthalidone peaks from degradants. This indicated the good specificity and stability-indicating ability of the method.

### Benidipine and chlorthalidone content in marketed tablet product

Benidipine and chlorthalidone content of marketed tablet product, Benitowa-CH, was determined by the proposed method. The outcomes (Table 6) were in good agreement with the labeled claim.

### Conclusion

A new stability indicating HPLC method was established for the simultaneous analysis of benidipine and chlorthalidone in tablets. All parameters of validation agreed with the acceptance criterion of ICH. The system suitability shows excellent analytical efficiency and reproducibility. This method allowed the accurate quantification of benidipine and chlorthalidone, so it can be used in quality control analysis. The degradation products formed under different degradation conditions were well resolved from benidipine and chlorthalidone, showing that the procedure was capable of indicating stability.

### Acknowledgements

Author, Kadali Jagadeesh, would like to thank Dr. G. Srinivasa Rao, Principal, Shri Vishnu Engineering College for Women, Bhimavaram & Dr. T. Sree Rama Murthy, Head, Department of Basic Sciences for providing necessary facilities to carry out the present research work.

### Conflicts of interest

Authors declare that no conflicts of interest present.

### Supplementary Data

Supplementary file contains Table S1–S2 and Figure S1 which is available on the journal's web site along with the published article.

### References

1. Yao K, Nagashima K, Miki H. Pharmacological, pharmacokinetic, and clinical properties of benidipine hydrochloride, a novel, long-acting calcium channel blocker. *J Pharmacol Sci.* 2006;100(4):243-61. doi:10.1254/jphs.DTJ05001X
2. Liao Y, Asakura M, Takashima S, Ogai A, Asano Y, Asanuma H, et al. Benidipine, a long-acting calcium channel blocker, inhibits cardiac remodeling in pressure-overloaded mice. *Cardiovasc Res.* 2005;65(4):879-88. doi:10.1016/j.cardiores.2004.11.006
3. Yamamoto E, Kataoka K, Dong YF, Nakamura T, Fukuda M, Nako H, et al. Benidipine, a dihydropyridine L-type/T-type calcium channel blocker, affords additive benefits for prevention of cardiorenal injury in hypertensive rats. *J Hypertens.* 2010;28(6):1321-9. doi:10.1097/HJH.0b013e3283388045
4. Tomino Y. Renoprotective effects of the L-/T-type calcium channel blocker benidipine in patients with hypertension. *Curr Hypertens Rev.* 2013;9(2):108-14. doi:10.2174/15734021113099990005
5. Roush GC, Buddharaju V, Ernst ME, Holford TR. Chlorthalidone: mechanisms of action and effect on cardiovascular events. *Curr Hypertens Rep.* 2013;15(5):514-21. doi:10.1007/s11906-013-0372-1
6. Mehta RT, Pareek A, Purkait I. Chlorthalidone, not hydrochlorothiazide, is the right diuretic for comparison. *Clin Hypertens.* 2018;24(1):4. doi:10.1186/s40885-018-0089-1
7. DiNicolantonio JJ, Bhutani J, Lavie CJ, O'Keefe JH. Evidence-based diuretics: focus on chlorthalidone and indapamide. *Future Cardiol.* 2015;11(2):203-17. doi:10.2217/fca.14.83
8. Cooney D, Milfred-LaForest S, Rahman M. Diuretics for hypertension: Hydrochlorothiazide or chlorthalidone?. *Cleve Clin J Med.* 2015;82(8):527-33. doi:10.3949/ccjm.82a.14091
9. Anuj S. Benidipine + Chlorthalidone. <https://www.1mg.com/generics/benidipine-chlorthalidone-405010>. Accessed on July 2019.
10. Kumar M, Shukla AK, Bishnoi RS, Jain CP. Development of UV spectrophotometric method for the determination of benidipine hydrochloride by using quality by design (QbD) approach. *Int J Appl Pharm.* 2018;10(4):92-7. doi:10.22159/ijap.2018v10i4.26623
11. Malek S, Prajapati L, Joshi A, Kharodiya M. Spectrophotometric method for the determination of benidipine hydrochloride in pharmaceutical formulation. *Trop J Pharm Life Sci.* 2018;5(1):1-7.
12. Karasaka A. First order derivative spectrophotometric method for the determination of benidipine hydrochloride pharmaceutical preparations and forced degradation study. *Opt Spectrosc.* 2015;118(6):1002-6. doi:10.1134/S0030400X1506003X
13. Singhvi I, Chaturvedi SC. Spectrophotometric methods for estimation of benidipine hydrochloride from tablets. *Indian J Pharma Sci.* 1998;60(2):113-4.
14. Buridi K, Shantiswarup L, Raghubabu K. Validated visible spectrophotometric methods development for the determination of benidipine hydrochloride based on complex and internal salt formation reactions. *Int J Inst Pharma Life Sci.* 2012;2(1):25-33.
15. Narmeen SA, Medea AH, Rebwar OH. Spectrophotometric determination of chlorthalidone in pharmaceutical formulations using different order derivative methods. *Arab J Chem.* 2017;10(Suppl 2):S3426-33. doi:10.1016/j.arabjc.2014.02.002
16. Ingle SU, Patil PA, Kulkarni V, Patil SV, Salunke PA, Wagh RS. Development and validation of UV spectrophotometric method for chlorthalidone in bulk and pharmaceutical dosage forms. *World J Pharm Res.* 2014;3(9):958-63.
17. Padmalatha H, Anupama V, Parijatha B, Rama K. Spectrophotometric estimation of chlorthalidone in pharmaceutical formulation using MBTH & FC reagent. *World J Pharm Pharm Sci.* 2014;3(2):1433-40.
18. Ayad MM, Hanaa S, El-Adl SM, El-Kousy N, Alham AT. Quantitative determination of chlorthalidone by fluorescence labelling using 5-dimethylaminonaphthalene-1-sulphonyl chloride. *STP Pharma Sci.* 1996;6(3):221-4.
19. Sravani M. Estimation of benidipine by using HPLC. *Int J Pharma Chem Res.* 2017;3(4):775-806.
20. Singh B, Patel DK, Ghosh SK. A reversed-phase high performance liquid chromatographic method for determination of chlorthalidone in pharmaceutical formulation. *Int J Pharm Pharm Sci.* 2009;1(2):24-9.
21. Sonawane S, Jadhav S, Rahade P, Chhajed S, Kshirsagar S. Development and validation of stability-indicating method for estimation of chlorthalidone in bulk and tablets with the use of experimental design in forced degradation experiments. *Scientifica.* 2016;2016:1-9. doi:10.1155/2016/4286482
22. Kang W, Yun HY, Liu KH, Kwon KI, Shin JG. Determination of benidipine in human plasma using liquid chromatography-tandem mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci.* 2004;805(2):311-4. doi:10.1016/j.jchromb.2004.03.023
23. Kang W, Lee DJ, Liu KH, Sunwoo YE, Kwon KI, Cha IJ, et al. Analysis of benidipine enantiomers in human plasma by liquid chromatography-mass spectrometry using a macrocyclic antibiotic (Vancomycin) chiral stationary phase column. *J Chromatogr B Analyt Technol Biomed Life Sci.* 2005;814(1):75-81. doi:10.1016/j.jchromb.2004.10.006
24. Magara H, Kobayashi H, Kobayashi S. Determination of benidipine hydrochloride in human plasma by capillary column gas chromatography-negative ion chemical ionization mass spectrometry. *J*

- Chromatogra B Biomed Sci Appl. 1993;617(1):59-63. doi:10.1016/0378-4347(93)80421-y
25. Karadas N, Sanli S, Gumustas M, Ozkan SA. Voltammetric and RP-LC assay for determination of benidipine HCl. J Pharm Biomed Anal. 2012;66:116-25. doi:10.1016/j.jpba.2012.03.025
26. International Conference on Harmonization (ICH) of technical requirements for the registration of pharmaceutical for human use stability testing of new drugs substance and products Q1A (R2). Geneva, Switzerland; 2003.
27. International Conference on Harmonization; Validation of Analytical Procedures: Text and Methodology, Q2 (R1). IFPMA. Geneva, Switzerland; 2005.