Open Access Maced J Med Sci **electronic publication ahead of print,**
published on August 30, 2019 as https://doi.org/10.3889/oamjms.2019.750

ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences.
https://doi.org/10.3889/oamjms.2019.750
eISSN: 1857-9655
Public Health

Quantitative Estimation of Anti Hypertension Combination by Ratio Subtraction Spectrophotometry Method

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Citation: Bachri M, Masfria M, Syahputra S, Hafid H. Quantitative Estimation of Anti Hypertension Combination by Ratio Subtraction Spectrophotometry Method. Open Access Maced J Med Sci. https://doi.org/10.3889/oamjms.2019.750

Keywords: Irbesartan; Hydrochlorothiazide; Spectrophotometry ultraviolet; Ratio subtraction

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Received: 09-Jun-2019; **Revised:** 28-Jul-2019; **Accepted:** 29-Jul-2019; **Online first:** 30-Aug-2019

Funding: The study was financially supported by the DRPM Directorate of Higher Education Ministry of Research Technology and High Education, Indonesia through the "Thesis Research Grant" Research Grant 2019

Competing Interests: The authors have declared that no competing interests exist

Abstract

BACKGROUND: Irbesartan and hydrochlorothiazide are a group of anti-hypertensive drugs that are very effective and safe to use to reduce blood pressure and oedema. The combination has a small active ingredient content so that if the treatment didn't meet the requirements for therapeutic doses, it not achieved to the maximum therapy.

AIM: The research aims to the simultaneous determination of irbesartan and hydrochlorothiazide in tablets by Ratio subtraction spectrophotometry method.

METHODS: The absorption spectra and sample measurement in the Ratio subtraction method performed on Irbesartan at a wavelength of 247.6 nm and 273.6 nm for the Hydrochlorothiazide (HCT) using 0.1 N NaOH as a solution. This method is validated with linearity, accuracy, and precision in intraday and interday, LOD and LOQ and applied in the determination of a mixture of irbesartan and hydrochlorothiazide in the dosage tablet.

RESULTS: The validation test for IRB is 101.03 for accuracy, with a precision of 0.57; with precision testing at intraday 0.34 and interday 1.34, and LOD is 0.70 and LOQ is 2.12. Meanwhile, validation for HCT that the accuracy 100.34%; precision 0.89 and precision on intraday 1.20 and interday 1.18, and LOD 0.78 and LOQ 2.37 with IRB levels are 101.03 ± 0.63% and HCT is 100.59 ± 0.91%.

CONCLUSION: The ultraviolet spectrophotometric method in subtraction ratio method was validated a method of linearity, accuracy, precision in intraday and interday, LOD, and LOQ and according to ICH guidelines and successfully applied for the determination simultaneous of irbesartan and hydrochlorothiazide in the tablet's dosage form.

Introduction

Clinically the problem-related side effects may be solved by introducing low-dose combination drugs for first-line antihypertensive therapy. The combination of Irbesartan and Hydrochlorothiazide is one of the right combinations for antihypertensive and effective to reduce blood pressure and oedema in hypertensive patients who are usually accompanied by complications and minimal side effects, namely hyperkalemia [1]. The combined use of angiotensin receptor (AR) blockers and diuretics is better tolerated, but more costly, than generic ACE inhibitors and diuretics, mostly because of the absence of cough and much lower incidence of angioedema [2].

Irbesartan (IRB), with chemically names 2-Butyl-3 - [[20- (1H-tetrazole-5-yr) [1, 10-biphenyl] -4-yl] methyl] 1, 3-diazaspiro [4.4] non-1 -en-4-one is angiotensin II receptor (ARB) inhibitors are used to treat hypertension and diabetic nephropathy. Because blood vessels can narrow due to the influence of angiotensin II and serves to inhibit these effects, thus dilating blood vessels and reducing pressure on blood vessels [3]. Irbesartan is an angiotensin II antagonist (AT1 receptor subtype) which is the primary vasoactive hormone involved in RAAS and a strong vasoconstrictor, and is formed from angiotensin I through a reaction catalysed by angiotensin-converting enzyme (ACE, kininase II). Irbesartan works against angiotensin II by blocking the effects of vasoconstrictor and aldosterone secretion from angiotensin II by binding selectively to the angiotensin II AT1 receptor [4].

Hydrochlorothiazide (HCT), with chemical names 6-Chloro-3, 4-dihydro-2H-1, 2, 4-

benzothiadiazine-7-sulfonamide 1, 1 dioxide is a group of diuretics and first-line drug therapy for the treatment of hypertension. Thiazide diuretics class of highly effective in preventing stroke and heart failure in patients with hypertension [3]. Hydrochlorothiazide is a treatment for mild to moderate hypertension. Often in more severe cases combined with other drugs to strengthen the effect, especially beta-blockers. Combination of ACE inhibitors, reducing resistance to activated renin-angiotensin-aldosterone system (RAAS) [2].

The combination of IRB with HCT significantly reduces BP in patients not controlled by IRB or HCT alone. The addition of IRB had positive effects on HCT induced biochemical abnormalities. In matrix studies, IRB appeared to blunt hypokalemia associated with HCT, and uric acid and total cholesterol levels were lower with the combination than with HCT monotherapy. IRB did not increase the HCT associated increases in serum triglycerides. Finally, IRB-HCT combination brings positive effects of IRB on undesired effects of diuretic, provides advantages of AR blockade, decreases resistance because of activated RAAS, simplifies treatment regimen, besides providing better BP control. Moreover, HCT may increase protective organ benefits of the AR antagonist by providing better BP control in this combination [1], [2], [4], [5], [6], [7].

The Irbesartan level was determined by ultraviolet spectrophotometry with acid solvents at wavelengths 224 and 246 nm, and hydrochlorothiazide assayed by ultraviolet spectrophotometry in an alkaline solvent at a wavelength of 274 nm and high-performance liquid chromatography method with buffer-acetonitrile mobile phase P (6: 4) and a mixture of monobasic sodium phosphate P 0.1 P M-acetonitrile (9: 1)) [3], [8]. Several articles have been published regarding the determination of the mixture of irbesartan and hydrochlorothiazide levels, among others, by using high-performance liquid chromatography, by capillary electrophoresis [8], [9], the zero-crossing method with spectrophotometry [10].

Spectrophotometry is a simple, fast and relatively easier method compared to other methods but the main problem with a mixture of binary or ternary spectrophotometry analysis is the determination of compounds simultaneously in the same mixture of drugs without prior separation [3.7]. Several studies of simultaneous spectrophotometric determination of the combinations of IRBs and HCTs have been published, among others, methods the zero-crossing method with spectrophotometry, Simultaneous equation and absorbance ratio, ratio subtraction coupled with constant multiplication, ratio difference and constant centre [9], [10], [11].

Ratio subtraction method (RSM) is one of the spectrophotometric methods that can be used to analyse two or more mixtures of drugs simultaneously without having to do a separation, easily applied to the routine analysis and without the need for derivatisation first. Several articles on the establishment of a mixture by using RSM has published, among others, the determination of omeprazole, tinidazole, and clarithromycin with ethanol, Benazepril and amlodipine with methanol and Timolol and dorzolamide [12], [13], [14], [15]. The ratio subtraction method shows that this method is very simple, accurate, and does not require complicated mathematical calculations using only constants in the spectrum of ratios that can be applied to the IRB and HCT levels. [16], [17], [18].

The purpose of this study is to prove the subtraction ratio method can be used to determine IRB and HCT levels in tablet dosage forms.

Material and Methods

Material

Pharmaceutical grades of IRB were from the National Agency of Drug and Food Control of the Republic of Indonesia, HCT was from PT Kimia Farma. Tablet C (PT. Sanofi) contained 300 mg IRB and 12.5 mg HCT, NaOH, Methanol.

Apparatus and conditions

UV-Visible Spectrophotometer (Shimadzu 1800) with a computer equipped with UV probe 2.43 software (UV-1800 Shimadzu), the absorption was recorded at a wavelength of 200-400 nm using UV-probe software, Analytical balance (Sartorius), sonicator (Branson 1510).

Preparation of standard stock solution

Carefully weighed of 50 mg IRB and HCT, then transferred to a 50 mL volumetric flask dissolved it in 0.1 N NaOH by adding it to the line. Stock solution concentration was 1000 µg/ml. Pipetted 5 mL of stock solution transferred to a 50 mL volumetric flask diluted it using 0,1 N NaOH by adding it to the line, and the concentration would be 100 µg/mL that is working solution.

Validation test

The solution standard for IRB and HCT for absorption spectrum was made by the selected wavelength points 247.6 nm for IRB, while the HCT used wavelengths of 273.4 nm. Determined zero orders from series C are used after manipulate to get a regression equation for each component [19], [20].

Precision

Reparability of the methods was studied by repeating the methods six times. To study intra-day precision, a method was repeated three times in a day. Similarly, the method was repeated on three different days to determine inter-day precision.

The determination of precision is based on the relative standard deviation (RSD) value 2% [19], [20].

Intraday and interday precision

Intraday and interday are precision measurements with simultaneous samples. Intraday is a repetition that is done every day in one day, during every day at certain hours in a few days. Intraday and repetition at each concentration. Intraday is three repetitions on the same day (morning, afternoon, evening) and three repetitions with 3 different days (days 1, 2, 3). Determination of interday and intraday precision was seen from its relative standard deviation < 2.5% [19].

Accuracy

Accuracy test was calculated by measured recovery percentage in three specific points which were: 80%, 100%, and 120%. In each of the specific points, the test used 70% from the sample and 30% from the pure active substances (standard addition method) [19], [20].

Construction of Absorption Maximum Spectrum and Ratio Absorption Spectrum

The working solution was pipetted each containing 10 µg/mL IRB and 8 µg/mL HCT and a mixed solution of 10 µg/mL IRB and 8 µg/mL HCT then each transferred in a 25 mL volumetric flask Diluted using 0.1 N NaOH, and measured the absorption spectrum. Furthermore, are used 5-15 µg/mL IRB solution and 4-12 µg/mL for HCT and a mixture of the two drugs in the same range and prepared for made an absorption spectrum of RSM then scanned in the range of 200-400 nm and overlapped the third spectrum. Useful for the ratio subtraction method (RSM) extended ratio subtraction method (EXRSM) method, where the mixture of IRB and HCT shows overlapped spectra, IRB represents unextended spectrum and HCT representing an extended-spectrum [3], [9], [10].

Determination IRB and HCT mixture by RSM

The IRB and HCT can determined by using the ratio subtraction method by a standard spectrum of 8 µg/mL HCT´ as a divisor producing a new curve that represents IRB/HCT´ + HCT/HCT´ (constant) and 10 µg/mL IRB as a divisor producing a new curve that represents HCT/IRB´ + IRB /IRB´ (constant). Then the curve is subtracted with constant values (HCT/HCT´) and (IRB /IRB´), then multiplication curve by the standard spectrum of 8 µg/mL HCT´ for IRB and 10 µg/mL IRB´ for HCT which is the same divisor used, therefore the obtained spectrum is zero-order absorption spectrum of IRB and HC. Then can be summarised as the following [8], [9]:

$$\frac{X+Y}{Y^o} = \frac{X}{Y^o} + \frac{Y}{Y^o} + Constant \dots\dots\dots\dots\dots\dots\dots\dots .1$$

$$\frac{X}{Y^o} + Constant - Constant = \frac{X}{Y^o} \dots\dots\dots\dots\dots\dots\dots\dots .2$$

$$\frac{X}{Y^o} \; x \; Y = X \dots\dots\dots\dots\dots\dots\dots\dots\dots .3$$

$$Y = \frac{X+Y}{X^o} + \frac{X}{X^o} - \frac{Y}{X^o} \dots\dots\dots\dots\dots\dots .4$$

Determination of the constants directly derived from the curve with a straight line parallel to the X-axis is the wavelength in the region where y expanded

Note: X = IRB; Y = HCT; Y° = HCT is Divisor; and X° = IRB as Divisor.

The concentrations of IRB and HCT are calculated using the linear relationship between the absorbance at its λ max versus the corresponding concentration [8], [9].

Preparation of sample solution

Twenty tablets are weighed and crushed homogeneous. Furthermore, weighed the amount of powder equivalent to 300 mg of IRB and 12.5 mg of HCT and the equality of TRI contained in there is calculated. It should be weighed up to six repetitions. Subsequently incorporated into the flask 50 mL and diluted with 0.1N NaOH (with sonicator for 15 minutes), and then paid back with 0.1 N NaOH until the line mark, shaken until homogeneous. The solution is then filtered, approximately 10 mL of the first filtrate discarded. Taken 0.5 mL, put in a 25 mL flask and paid back with diluted with 0.1 N NaOH until the line marks to obtained solution in which there are IRB and HCT concentration of 10 µg/mL and 8 µg/mL, respectively. Measured absorption was at a wavelength of 200-400 nm.

Results

Spectrum overlapping studies were obtained from the maximal absorption spectrum of IRB and HCT as well as a mixture of IRB and HCT. In the maximal absorption spectrum of IRB the concentration

of 10 µg/ml was used and for HCT concentrations of 8 µg/ml, a ratio of 5: 4 was obtained for the IRB and HCT respectively and can be seen at Figure 1.

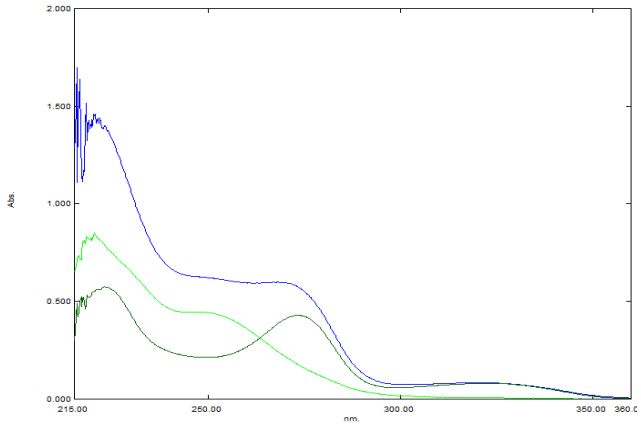

Figure 1: Overlain spectrum of IRB (10 µg/mL), HCT (8 µg/mL) and mixture (IRB 10 and HCT 8 µg/mL)

Based on Figure 1, the overlapping spectrum of IRB and HCT shows that HCT is more extended than IRB so that HCT is used as the initial divisor for the subtraction method ratio. According to Kamal et al. (2016), the determination of overlapping spectrum studies is the starting point for determining the levels using the ratio subtraction method. This analysis of the use of length used for analysis, which shows that the initial divisor is used for the ratio subtraction method, where the initial divisor is used the next step [8].

Method validation

The developed method is validated for linearity, precision, and accuracy. The validation results are shown in Table 1.

Table 1: Validation Methods of IRB and HCT in Ratio subtraction method

No.	Parameter	IRB	HCT
1	Analytical wavelengths	247.6 nm	273.6 nm
2	Concentration (µ/ml	5-15	4-12
3	Regression equation	Y_{irb} = 0.0482X - 0.0083	Y_{hct}= 0.0585X – 0.0068
4	Correlation coefficient	0.9994	0.9988
5	Accuracy (%)	101.03	100.59
6	Precision (RSD) (%)	0.57	0.89
7	Interday (% RSD)	1.34	1.18
8	Intraday (% RSD)	0.34	1.20
9	LOD (µg/mL)	0.70	0.78
10	LOQ (µg/mL)	2.12	2.37

Based on Table 1, It can be seen that the calibration curves of IRB and HCT were linear in the range of 5-15 µg/mL and 4-12 µg/mL, respectively.The regression equations of calibration curves were Y_{irb} = 0.0482X - 0.0083, r = 0.9994 for IRB and Y_{hct}= 0.0585X – 0.0068, r = 0.9988 for HCT. Relative standard deviations (% R.S.D.) for interday were found to be 1.34 and 1.18 for IRB and HCT, respectively. The intraday precision showed % R.S.D 0.34 and 1.20 for IRB and HCT, respectively. The LOD for IRB and HCT was found to be 0.7005 µg/mL and 0.7834 µg/mL respectively. The LOQ for IRB and HCT was found to be 2.1227 µg/mL and 2.3741 µg/mL respectively. Based on the table above shows all the interday and intraday results meet the requirements < 2% RSD, this means that the RSM method can be stated to have good precision because after testing on intra-day and intra-day it gives insignificant results in statistical calculations and meets ICH requirements 2015 [19].

Accuracy

The percentage recoveries of a drug from marketed formulation were determined by standard addition of pure drugs at three (80%, 100%, and 120%) known concentrations and excellent recoveries were obtained at each level. The accuracy studies are shown in Table 2

Table 2: Accuracy study

No.	Drug	Concentration (%)	Mean % recovery
1	IRB	80 %	100.97
2	IRB	100%	101.08
3	IRB	120%	101.03
4	HCT	80 %	99.80
5	HCT	100%	100.37
6	HCT	120%	100.37

The percentage of recovery for IRBs is three levels, 80%, 100%, and 120% respectively 100.97, 101.08 and 101.03, while for HCT is 99.80, 100.37 and 100.37, that is mean the validation of RSM is a requirement with ICH 2015 [19].

Ratio Subtraction Method

This method is operated using the UV probe 2.43 application by doing a manipulate from the data set spectrum, when the mixtures of IRB and HCT, where the spectrum of HCT is more extended, the determination of IRB in the mixture can be done by scanning the zero-order spectra of IRB and HCT, dividing them by a carefully chosen concentration of HCT´ standard (8 µg/mL) as a divisor. The choice of this divisor is very crucial because it can affect the results of the spectrum obtained. After dividing the new ratio, the spectrum is generated which represents IRB/HCT´ + constant as in Figure 2 (step 1).

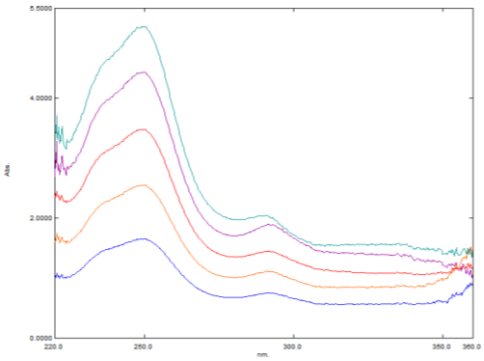

Figure 2: Spectra of mixtures IRB and HCT using 8 µg/mL of HCT´ as a divisor

The results of the spectrum are subtracted constant, namely HCT/HCT´ to produce the spectrum shown in Figure 3 (step 2).

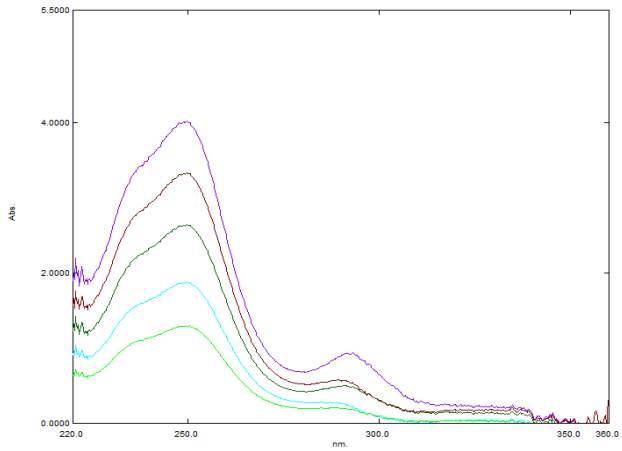

Figure 3: Spectra of mixtures IRB and HCT using 8 µg/mL of HCT´ as a divisor and after subtraction with constants (HCT/HCT´)

Then proceed with the multiplication of the obtained spectra by the divisor HCT´ (8 µg/mL) as shown in Figure 4.

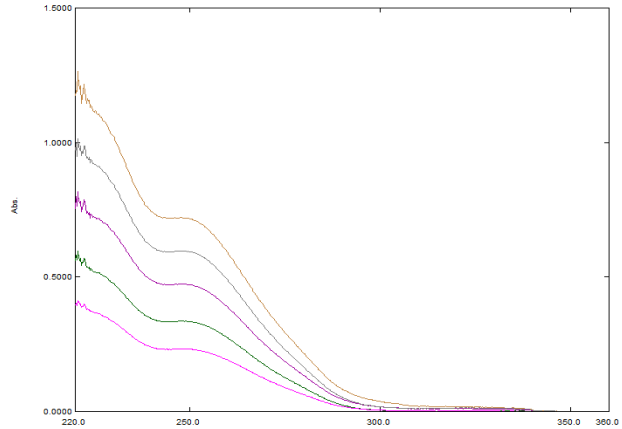

Figure 4: The zero-order absorption spectra of IRB by the proposed RSM after multiplication by the divisor

Finally, the original spectra of IRB can be obtained, Figure 4, which was used for direct estimation of IRB at 247.6 and calculation. The concentration of zero-order curves of IRB at 247.6 against the corresponding concentrations.

The determination of HCT can be obtained by the spectra of IRB by a carefully chosen concentration of the standard IRB´ (10 µg/mL) producing ratio spectra representing the constants IRB/IRB´. The scanned zero-order IRB and HCT were divided by the IRB´ (10 µg/mL) standard as a product of the new ratio spectra which represent HCT/IRB´ + constant as shown in Figure 5.

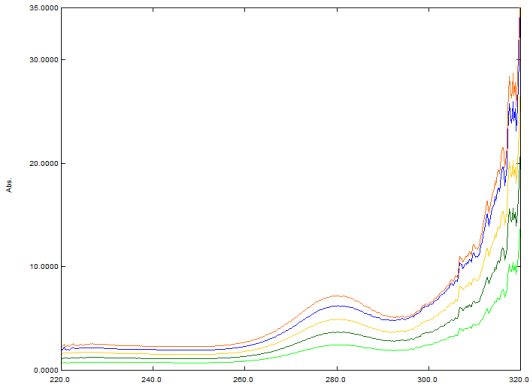

Figure 5: Spectra of Mixtures IRB and HCT using 10 µg/mL of IRB´ as a divisor

The spectra are subtracted by the constant so that the new ratio spectra, which represent HCT/IRB´ are shown in Figure 6.

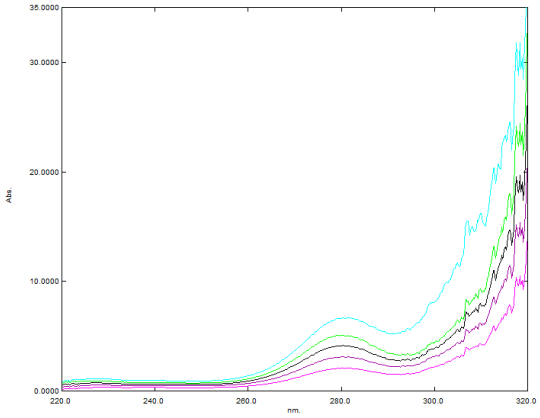

Figure 6: Spectra of Mixtures IRB and HCT using 10 µg/Ml Of IRB´ as a divisor after Subtraction with Constants (IRB/IRB´)

Then the spectra are multiplication by the IRB´ divisor (10 µg/mL) which is the same as before. Finally, the original spectra of HCT, Figure 7, which can be used for determination of HCT at 273.4 nm in the form of corresponding regression equation curves of HCT at 273.4 nm against the corresponding).

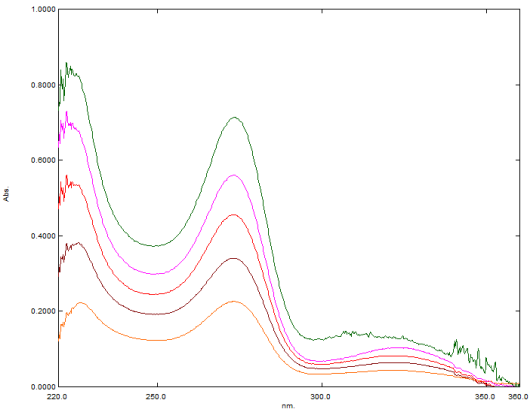

Figure 7: The Zero Order Absorption Spectra of HCT By The Proposed RSM

The methods are considered to be complementary to each other as the two components of interest in the mixture can be determined. The results obtained upon using the suggested methods for the analysis of IRB and HCT in marked tablets.

Table 3: Results of simultaneous estimation of IRB and HCT by RSM

Component	Claim on the label (mg)	The content (mg)
IRB	300	313.60 ± 1.88
HCT	12.5	12.66 ± 0.20

Based on Table 3 can be seen that the proposed ratio subtraction method gives accurate and precise have been developed and validated for irbesartan and hydrochlorothiazide in the marketed formulation (tablets) without prior separation and is easily applied for routine analysis. The most interesting feature of the ratio subtraction method is its simplicity and rapidity. The validation method has been demonstrated by a variety of tests for linearity, accuracy, and precision. The proposed methods were successfully applied to the determination of these drugs in commercial tablets.

Discussion

Based on the result above, it can be seen that there are irbesartan and hydrochlorothiazide mixture in the tablet dosage form is a requirement for Indonesian pharmacopoeia. This method means that pharmaceutical preparations containing the composition of the two substances can be used as a mixture of anti-hypertensive drugs. Its combination is primarily indicated for moderate-to-severe hypertension, but it could be useful for other irbesartan indications, as well. The combination significantly reduces BP in patients not controlled by IRB or HCT alone. Therefore, it is possible to evaluate the clinical efficacy under 2 main titles: BP-lowering efficacy, and the end-organ protection. The combination are fixed-dose combinations achieved BP targets in 77% of patients with systolic, 83% for diastolic and 69% for both BP levels [1], [2], [4], [5], [6], [7]

Acknowledgement

The authors wish to thank for Minister of Research, Technology and Higher Education of Republic Indonesia, Prof. Runtung., Rector of Universitas Sumatera Utara and Prof Erman Munir., Chairman of the USU Research Institutes in conducting appointment of research implementation of DRPM KEMENRISTEKDIKTI for Thesis Research Scheme no 202/UN5.2.3.1 /PPM/KP. DRPM/2019

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