Comparative study on release of two drugs in fixed dose combination using zeroorder and first derivative spectrophotometry

AnisYohana Chaerunisaa[1]*, Muhaimin[2]

**[1]Faculty of Pharmacy, Padjadjaran University, Jatinangor, 45363, Indonesia
[2]Department of Chemistry, Faculty of Education, University of Jambi, Jambi 36361, Indonesia**

Abstract : Fixed dose combination (FDC) is a formulation of two or more active ingredients combined in a single dosage form. Development and validation of an analytical UV using derivative has been increasingly used. This method offer sensitive, simple and robust method for analyzing active ingredient in pharmaceutical dosage forms, especially when it contains more than one drug. Drug assays can also be calculated by using mathematical equation from zero derivative or absorbance spectrophotometry. This current study aimed to compare release of drugs from fixed dose combination dosage forms (pellets and microparticles) calculated by first derivative with that by zero order spectrophotometry method. As drug models, propranolol HCl and carbamazepine were used as sample of highly and poorly soluble drugs mixed in a single dosage form. Propranolol HCl and carbamazepine were mixed and loaded into sugar cores, and the drug beads were coated with 10% w/w coating level of ethylcellulose containing 20-40% w/w HPC. As microparticles, propranolol HCl was used first primary emulsion while the second primary oil phase was carbamazepine. Other type of microparticles used the opposite system. Drug release either of propranolol HCl or carbamazepine measured by zero order spectrophotometry were smoother compared to those measured by first derivative method. The validation parameter including linearity, and accuracy, have been validated statistically and recovery studies confirmed the accuracy of the proposed method. In conclusion, drug release calculated by mathematical equation from zero order spctrophomotetry offered simple, cheap and robust butaccurate, sensitive, and precise method for the determination of drugs in fixed dose combination.
Keywords : Zero order, first derivative, spectrophotometry, drug release, fixed dose combination.

Introduction

Fixed-combination medicinal products have been increasingly used either to improve compliance or to benefit from the added effects of the two medicinal products given together. A fixed dose combination (FDC) is a formulation of two or more active ingredients combined in a single dosage form available in certain fixed doses. Fixed dose combination drug products may improve medication compliance of patients[1,2]. If the determination of the two drugs in the formulation containing two or more drugs, is required, the derivative spectrophotometric technique is suitable, particularly whenthe spectra of the two drugs overlap[3,4,5]. Derivative spectrophotometry has been used dueto its capacity to separate shoulders and weak signals[6,7,8],improving the

resolution of two or more analyticalmasked bands, and also, because is a selective analyseof drugs after simple dissolution of samples withoutcomplicated extraction[9,10,11,12].

Rapid developments in electronic and particularly in microcomputer technologies in the last ten years have led to a parallel growth of interest in the use of UV-Visible derivative spectrophotometry as an analytical tool for:

- The enhancement of the resolution of overlapping peaks, and
- The elimination or reduction of background or matrix absorption.

Today, the first and second derivative modes are a standard feature of most microprocessor UV-Visible spectrophotometers, and some instruments offer the third, fourth, and even up to the seventh order derivative modes[13].

Quantification of drugs as mixture could be conducted either as bulk[14, 15,16,18] or as pharmaceutical dosage forms [19,20,21,22,23,24]. Regarding the drug assay during the dissolution test of fixed dose combination, quatification of drug can be performed by a visible spectrophotometric method after dissolved in the release medium[20,21,22,24]. In order to obtain a simple, cheaper, faster, less environmental toxic method for the quantitative assay of drugs in tablets (drug assay and dissolution test), the drug assay can be calculated either by the "zero-crossing" technique to first-derivative spectrophotometry method or by the calculation of each drug in the sample mixture's absorbance and the absorbances of the standard solutions are measured at several wavelengths[13]. Furthermore, some visible spectrophotometric methods often require time-consuming pre-separation techniques to remove possible interferences from colored materials such as catechin, One of the simplest methods to overcome this limitation is derivatization of spectra. [13, 23,24,25].Recoveries do not differ significantly from 100% which showthere was no interference from the excipient used in formulation [20,21,22,24, 25].

This study aimed to comparethe application of a spectrophotometric method using first derivative spectrophotometry with zero order or absorbance spectrophotometry method.The validation procedure was carried out according to the ICH guideline and the parameters evaluated were specificity, linearity, range, precision, accuracy, and robustness.The method can be very useful tools for determining drug release from fixed dose combination, without tedious and time consuming separation procedures.

Experimentals

Materials

Spectrophotometric measurement has been conducted using propranolol HCl and Carbamazepin as model drug of highly and poorly soluble drugs in fixed dose combination (FDC). The FCD were developed as pellet and microparticles dosage forms. Materials Propranolol HCl, carbamazepine (BASF AG; Ludwigshafen, Germany), ethylcellulose (EC, Ethocel® standard 10 cP FP premium gradeColorcon, Kent, UK) ,hydroxypropylcellulose (HPC, Klucel®JF Pharm andKlucel®LF Pharm, Ashland Aqualon, Wilmington, USA), Non-pareilscore (Suglets® sugar spheres NF; 710-850µm, NP Pharma S.A., Bazainville, France)were used as received.

Methods

Preparation of pellets and microparticles

1. Drug combination as single layer pellets

Propranolol HCl and carbamazepine as model drugs with different solubility were mixed as drug layering suspension (10% w/w solid content) in isopropanol:water 70:30 w/w containing 20% w/w Methocel®E5(20% w/w based on propranolol HCl weight) as the binder to achieve 15% weight gain of each drug (based on initial weight of cores) in a fluidized bed coater (AeromaticStrea-I, Binzen, Germany).

Drug loaded cores were coated with 10% w/w coating level ethylcellulose containing 20-40% w/w HPC solution in isopropanol: water 88:12 w/w (7% solid content) in a fluidized bed coater Mini Glatt® (Glatt, GmbH, Binzen, Germany).

2. Microparticle blends containing propranolol HCl and carbamazepine

The first primary emulsion containing propranolol HCl (W/O/W) and second primary oil phase containing carbamazepine (O/W).For the W/O/W method, 43 mg propranolol HCl were dissolved in 0.25 g purified deionized water. Propranolol HCl aqueous solution was first emulsified by probe sonication for 30 s under ice-cooling into 3 ml dichloromethane containing 300 mg of ethyl cellulose. This gave the first primary emulsion containing propranolol HCl. For the O/W method, 300 mg of ethyl cellulose were dissolved in 3 ml dichloromethane. 43 mg carbamazepine were then dissolved in this organic phase. This process produced the second primary oil phase containing carbamazepine. Following, the first primary emulsion containing propranolol HCl and the second primary oil phase containing carbamazepine were dispersed in an external aqueous phase (800 ml aqueous PVA solution [0.25% w/v] containing 0.5 M NaCland NaOH at pH 12), with dispersion time intervals (DTI) of 0 and 60 min, and stirred for 4 h at 500 rpm with a propeller stirrer to allow microparticle hardening.

3. In vitro drug release studies

As pellet dosage forms, drug release were studied in a paddle apparatus (VK 7010, Agilent Technologies Deutschland GmbH, Böblingen, Germany), (100 rpm, 37°C, 900 ml, phosphate buffer pH 6.8, n = 3). Drug releases were measured by UV spectrophotometer with diode arrays (HP 8453, Agilent Technologies Deutschland GmbH, Waldbronn, Germany) at wavelengths 227 and 285(n = 3).

As microparticles, 10 mg microparticles (particle size: < 70 μm) were placed in 10 ml pH 7.4 phosphate buffer (USP XXIV) and shaken at 37 °C in a horizontal shaker (GFL 3033, Gesellschaftfür Labortechnik GmbH, Burgwedel, Germany) at 75 rpm. At predetermined time points, 1 ml samples were withdrawn and replaced with 1 ml fresh medium each 7 days, filtered and analyzed.

4. Development of the method

To determine concentration of propranolol HCl and carbamazepin in the sample solution, the sample's absorbance and the absorbances of the standard solutions are measured at several wavelengths. The zero-crossing technique consists in measuring the derivative value at a wavelength (wavenumber), at which the derivative of the interfering component accepts value zero - crosses the zero line.
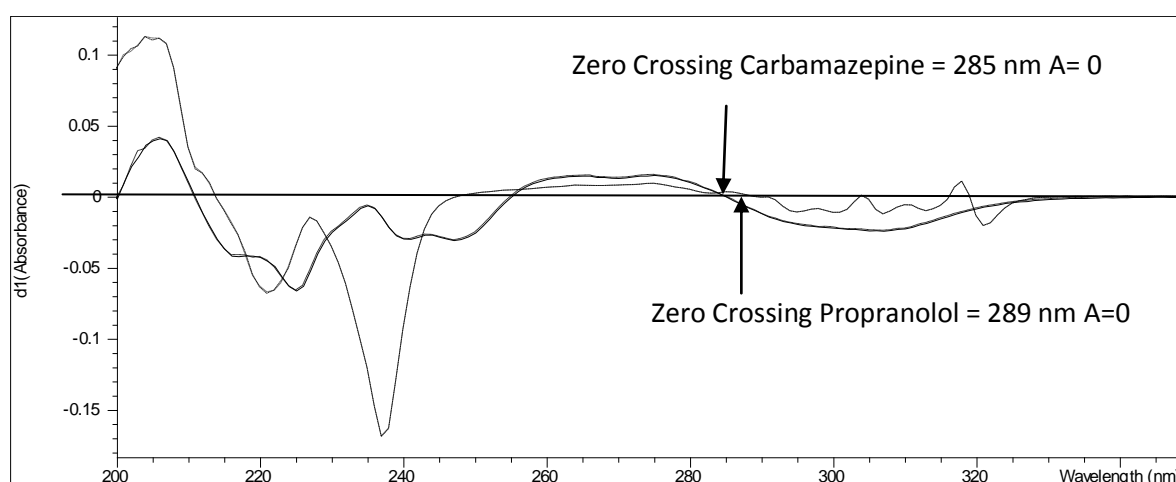
Figure 1. First order derivative spectra for propranolol HCl and carbamazepine

At its Zero Crossing,Propranolol was measured at 285 nm, because Carbamazepine gave 0 absorbance (gave no interfere), while Carbamazepine was measured at 289 nm because Propranolol gave 0 absorbance.In this way there is no effect of one component on the other one(Fig. 1).The first derivative measurement using zero-crossing technique permits to eliminate the influence of the componentinterfering with the component

determined. A disadvantage of this measuring techniqueis a not too great precision of measurements. To overcome the unprecise measurement for drug release measurement, in this current study the result of drug assay during dissolution study were also calculated using mathematical equation from zero derivative or absorbance spectrophotometry[6,13]. Mathematical techniques to determine drug release in a mixture can becalculated from this expressions (Fig. 2).This method involves simple linear interpolation between adjacent wavelengths.
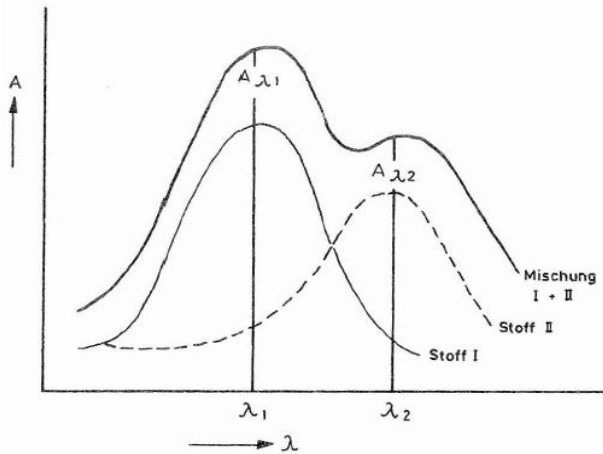
Figure 2. Zero order absorbance of a mixture

The absorbance for this calculation were measured in two different wavelengths. The wavelength were picked based on the maximum wavelength of carbamazepine which has highest absorbance difference value with propranolol (285 nm) and the maximum wavelength of propranolol which has highest absorbance difference value with carbamazepine (227 nm) (Fig. 3).
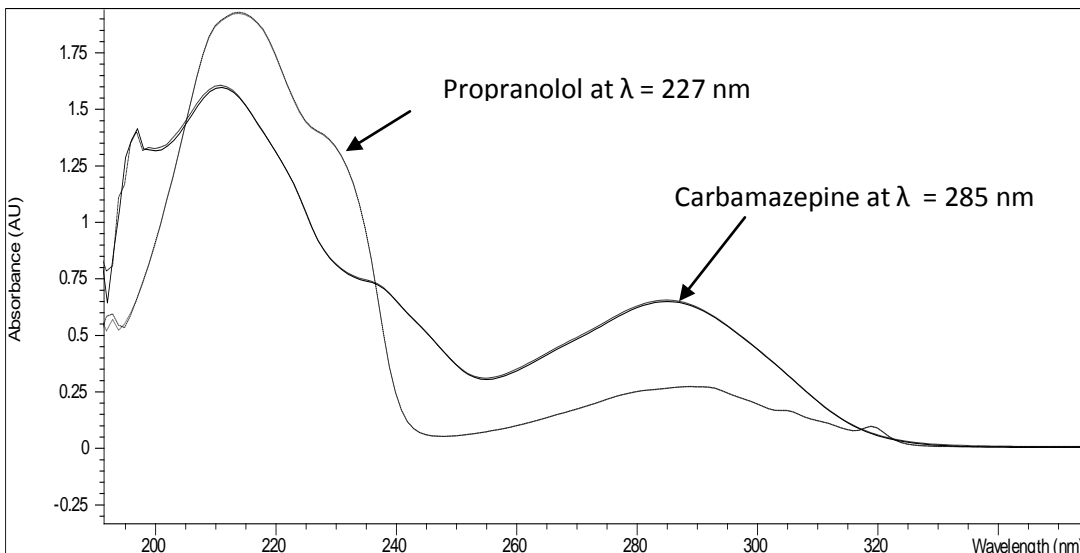
Figure 3. Absorbance of propranolol HCl and carbamazepine at 25mg/ml

From Fig. 2, mathematical equation to calculate concentration of each drug shown as follows:

$$A_{\lambda 1} = {}^I_{\lambda 1}A^{1\%}_{1\,cm} \cdot c_I + {}^{II}_{\lambda 1}A^{1\%}_{1\,cm} \cdot c_{II}$$

Eq. 1

$$A_{\lambda 2} = {}^{I}_{\lambda 2}A^{1\%}_{1\,cm} \cdot c_{I} + {}^{II}_{\lambda 2}A^{1\%}_{1\,cm} \cdot c_{II}$$

Eq. 2

$$c_{I} = \frac{A_{\lambda 1} \cdot {}^{II}_{\lambda 2}A^{1\%}_{1\,cm} - A_{\lambda 2} \cdot {}^{II}_{\lambda 1}A^{1\%}_{1\,cm}}{{}^{I}_{\lambda 1}A^{1\%}_{1\,cm} \cdot {}^{II}_{\lambda 2}A^{1\%}_{1\,cm} - {}^{I}_{\lambda 2}A^{1\%}_{1\,cm} \cdot {}^{II}_{\lambda 1}A^{1\%}_{1\,cm}}$$

$$c_{II} = \frac{A_{\lambda 2} \cdot {}^{I}_{\lambda 1}A^{1\%}_{1\,cm} - A_{\lambda 1} \cdot {}^{I}_{\lambda 2}A^{1\%}_{1\,cm}}{{}^{I}_{\lambda 1}A^{1\%}_{1\,cm} \cdot {}^{II}_{\lambda 2}A^{1\%}_{1\,cm} - {}^{I}_{\lambda 2}A^{1\%}_{1\,cm} \cdot {}^{II}_{\lambda 1}A^{1\%}_{1\,cm}}$$

Eq. 4

To calculate the release of propranolol HCl and carbamazepine in dissolution sample,concentration of each drug was calculated using the equation:

$$A^{1\%}_{1\,cm} = \varepsilon^{1\%}_{1\,cm}$$

$$C_{propranolol} = \frac{A_{\lambda\,prop} \cdot \varepsilon^{CBZ}_{\lambda\,CBZ} - A_{\lambda\,CBZ} \cdot \varepsilon^{CBZ}_{\lambda prop}}{\varepsilon^{prop}_{\lambda prop} \cdot \varepsilon^{CBZ}_{\lambda cbz} - \varepsilon^{prop}_{\lambda CBZ} \cdot \varepsilon^{CBZ}_{\lambda prop}}$$

Eq. 5

$$C_{CBZ} = \frac{A_{\lambda\,CBZ} \cdot \varepsilon^{prop}_{\lambda prop} - A_{\lambda\,prop} \cdot \varepsilon^{prop}_{\lambda CBZ}}{\varepsilon^{prop}_{\lambda prop} \cdot \varepsilon^{CBZ}_{\lambda cbz} - \varepsilon^{prop}_{\lambda CBZ} \cdot \varepsilon^{CBZ}_{\lambda prop}}$$

Eq. 6

5. Validation of method parameters:

Linearity

Standard stock solutions were prepared by dissolving 50 mg carbamazepine in 500 ml volumetric flask and the volume was made up with Buffer phosphate pH 6.8 to get a concentration of 0.1 mg/ml (Carbamazepine solubility). 12.5 gram Propranolol HCl was dissolved in 50 ml volumetric flask and the volume was made up with Buffer phosphate pH 6.8 to get a concentration of 250 mg/ml (Propranolol HCl solubility).

Aliquots of a 100 μg/mL standard solution were transferred to 10 mL volumetricflasks to obtain the final concentrations of1.562; 3.125; 6.25; 12.5, and 25.0 μg/mL. Each solution wasprepared in triplicate. The linearity was evaluated by linear regression analysis, which was calculated bythe least square regression method. The absorbances were measured at 227 and 285nm.

Precisions

Standard solution of propranolol HCl and carbamazepine were transferred to a 100 mL volumetric flask and was properly diluted to 25 μg/mL. A portion of each of these solutions was transferred to the sample cell of the spectrophotometer and the absorbance were measured and recorded. For Intra-day precision: six different sample at the same concentration were measured in triplicate under the same experiment condition and on the same day.For Inter_day precision: the samples were measured on two different days.

Accuracy

The accuracy of method was determined in terms of % recovery standard. Recovery studies were carried out by addition of standard drug solution at the level of 80, 100 and 120% to the pre analyzed drug. The results should be within 100±10%, which indicating a good degree of sensitivity of method towards detection of analytes in samples.

Results and Discussion

Drug release comparison

In the present study, a new, reliable, reproducible, simple UV method for the determination of propranolol HCl and carbamazepine as drug model infixed dose combination as pellets and microparticles dosage forms were developed and evaluated. The method were developedby comparing drug release calculated by zero order absorbance and by first derivative spectrophotometry, using phosphate buffer as release media. In zero order spectrofotometry, the absorptivity of both propranolol HCl and carbamazepine in sample solution were measured at 227 and 285 nm and drug concentration for drug release calculation of both drugsfrom the dosage form were calculated using Eq. 5 and 6 respectively.When using first derivative method, the absorptivity ofsample solution were measured at 285 and 289 nm as zero crossing of both drugs. The drug concentrations were then calculated to drug release.   Release of drugs from pellets containing propranolol HCl and carbamazepine coated by Ethyl cellulose : HPC with different ratio (60:40, 70:30, 80:20 and 90:10) at different coating level calculated by zero order spectrophotometry shown in Fig. 4. Drug release calculated by first derivative spectrophotometry shown in Fig. 5.When calculated by zero order method, drug release were smoother compared to those calculated by first derivative.

(a)   (b)

(c)   (d)
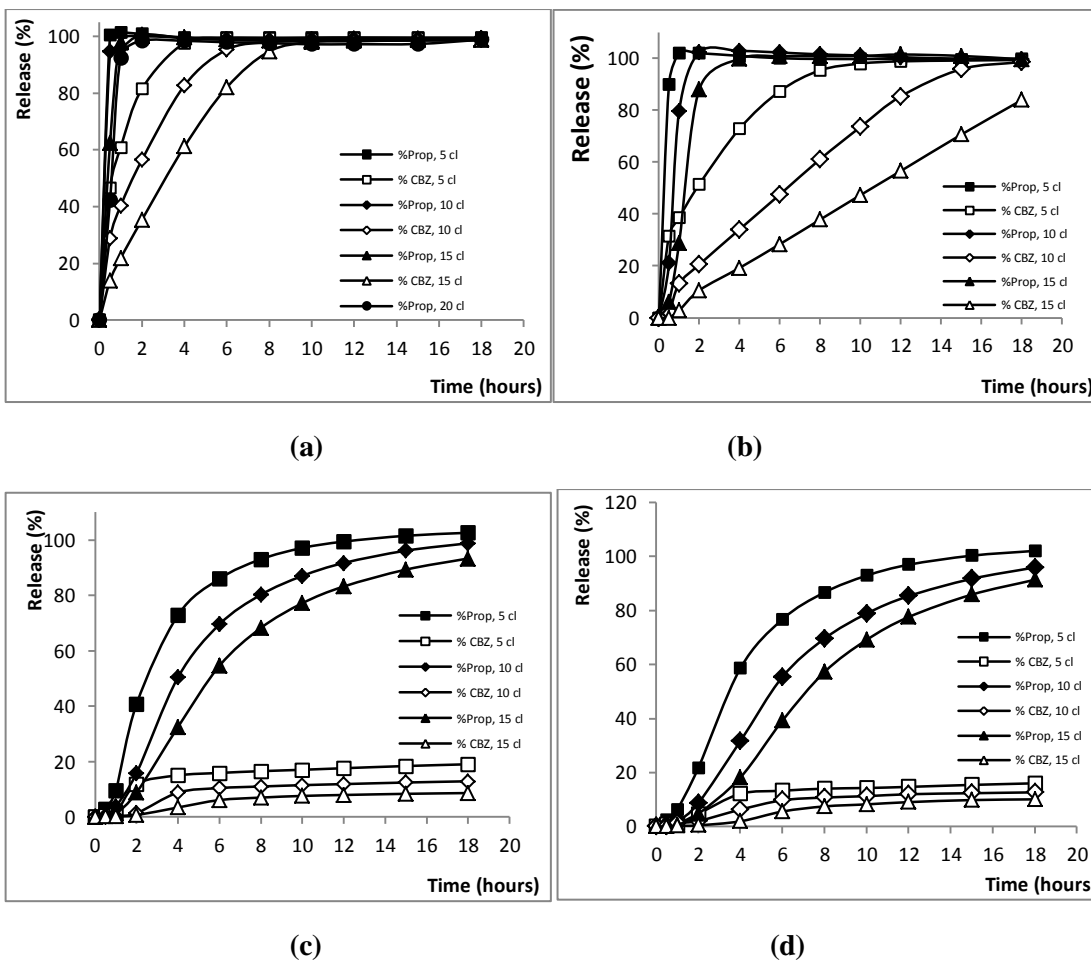
Figure 4. Release of 15% drug loading Propranolol HCl and carbamazepine from pellet coated by ethylcellulose 60:40 (a), 70:30 (b), 80:20 (C) and 90:10 (d) at different coating level calculated by zero order spectrophotometry

(a)   **(b)**

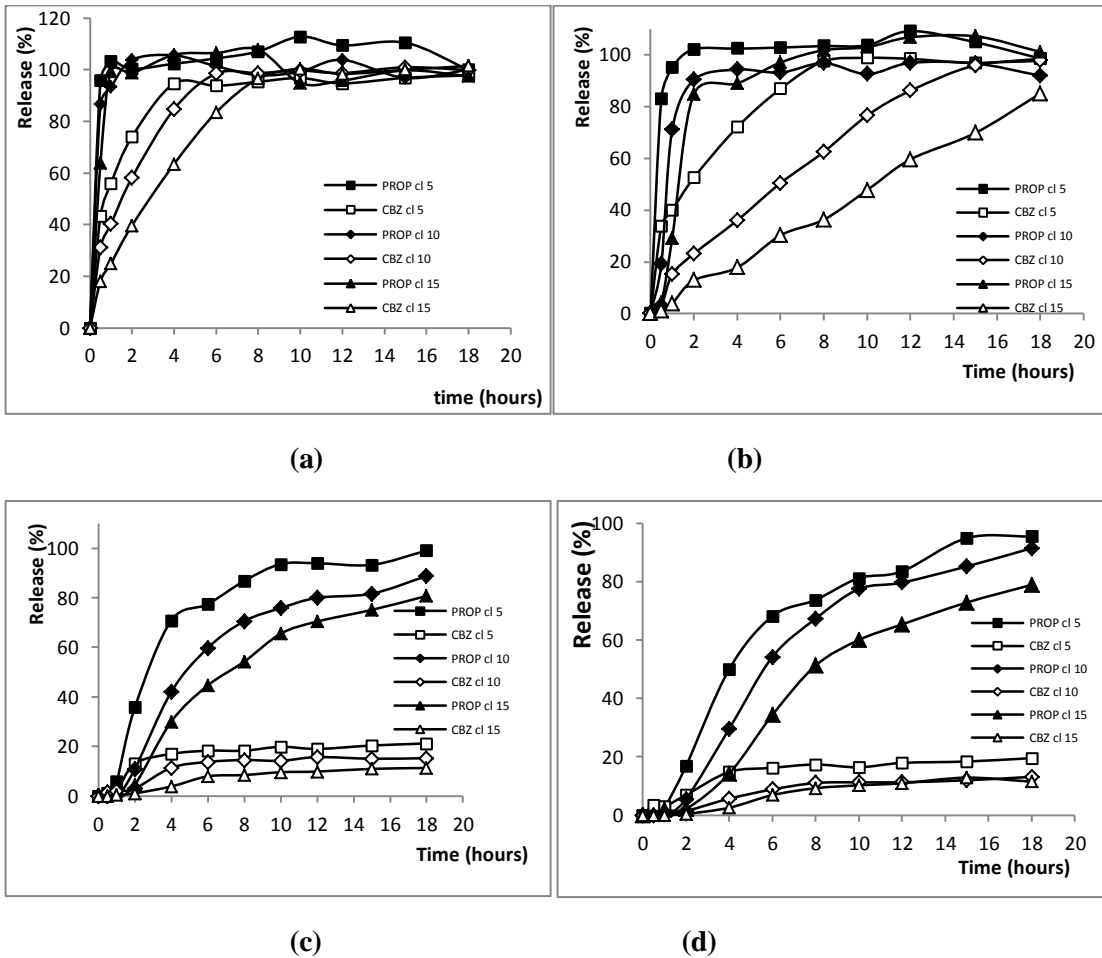**(c)**   **(d)**

Figure 5. Release of 15% drug loading Propranolol HCl and carbamazepine from pellet coated by ethylcellulose 60:40 (a), 70:30 (b), 80:20 (C) and 90:10 (d) at different coating level measured by first derivative spectrophotometry

Release study of drugs from different dosage form to which microparticles containing propranolol HCl and carbamazepine using Ethyl cellulose as polymer matrix were also studied. Drug release calculated by zero order spectrophotometry shown in Fig. 6.while that by first derivative spectrophotometry shown in Fig. 7. When calculated by zero order method, drug release were also smoother compared to those calculated by first derivative.
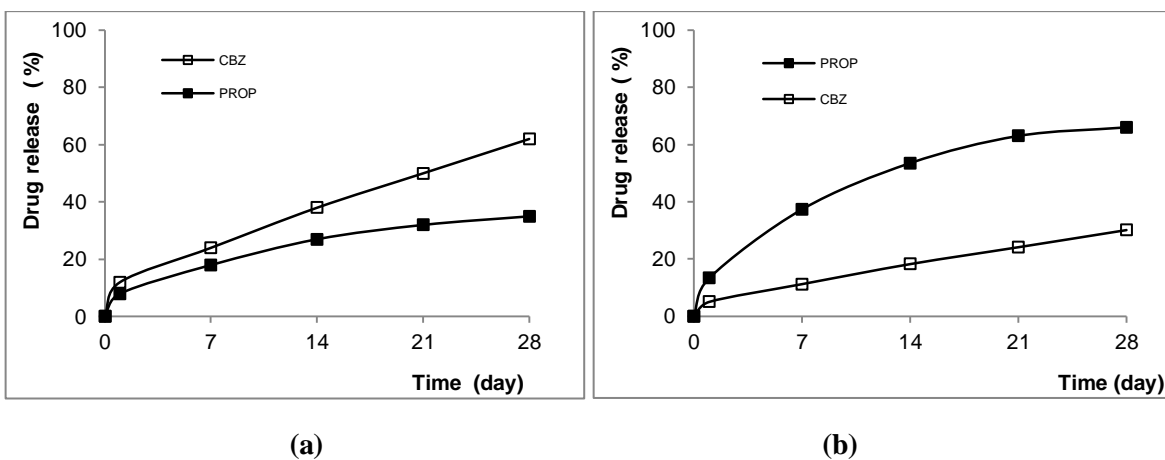

(a)   **(b)**

Figure 6. Release of Propranolol HCl and carbamazepine from microparticles blend with ethylcellulose as plymer matrix with Propranolol HCl asfirst primary emulsion, carbamazepine as second primary oil phase(a), and carbamazepine as first primary emulsion, propranolol HCl as second primary oil phase(b), at DTI 60 minutes calculated by zero order spectrophotometry
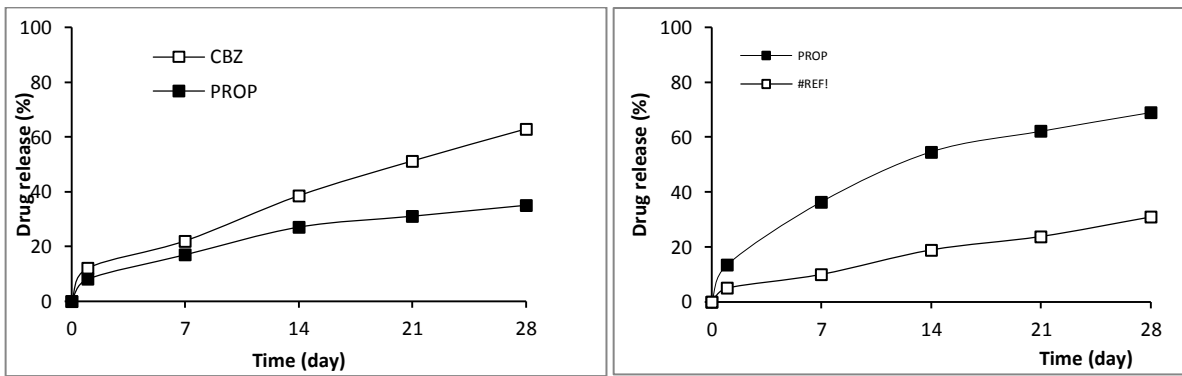
(a)   **(b)**

Figure 7. Release of Propranolol HCl and carbamazepine from microparticles blend with ethylcellulose as plymer matrix with Propranolol HCl as first primary emulsion, carbamazepine as second primary oil phase(a), and carbamazepine as first primary emulsion, propranolol HCl as second primary oil phase(b), at DTI 60 minutes calculated by first derivative spectrophotometry

The propranolol HCl release from microparticle blends (with dispersion time interval, DTI 60 min) (first primary emulsion propranolol and second primaryemulsion carbamazepine) were slower than carbamazepine release(Fig. 6 and 7. a). Whereas, propranolol HCl release was faster than carbamazepine from EC microparticle blendswith first primary emulsion carbamazepineand second primary oil phase propranolol, DTI 60 min) (Fig. 6 and 7 b). From Fig. 6 and 7, it can be concluded that release calculated by zero order method, were smoother compared to those calculated by first derivative. The measuring technique of first derivative spectrophotometry has disadvantage of non great precision of measurements. It gave unstable measurement to give non smooth curve in release calculation.

Validation of method

Themethods were validated based on ICH; analytical methodvalidation guidelines. The values of R^2 for linearity,%RSD for precision and recovery study for accuracywere within the limits of acceptance criteria of ICH.It indicates repeatability, reproducibility and accuracyof the proposed method.The linearity of the method was established form zero order spectra by measurement of the absorbance ofstandard solutions containing various concentrations of propranolol HClin the presence of constantconcentration of carbamazepine or vice versa. The analytical results obtained aresummarized in Table 1. The linearity of the calibration curves and the adherence of the method to Beer's law arevalidated by the high value of the correlation coefficient. Data of precision showed a good accuracy which indicated that the proposed spectroscopic method is highly precise during one analysis and between different runs.

Table 1.Validation parameters of samples

Parameters		Experimental Value		STANDARD	COMMENTS
		Propranolol	**CBZ**		
1	Linearity (R^2)	0.999	0.995	>0.999	Good linearity for Prop (>0.999)
	Precision (%RSD)				Method provides good precision and reproducibility
	Inter day Precision	0.12	0.04 - 0.44		
2	Intra day Precision	0.28	0.11 - 0.39	< 2.0%	

The recovery studywere conducted and were calculated using zero order spectrophotometry using Eq. 5 and 6. The result shown by Table 2.

Table 2. Results of recovery study

sampel	Experiment		Teoritical		Recovery (%)	
	CBZ (µg/ml)	Prop (µg/ml)	CBZ (µg/ml)	Prop (µg/ml)	CBZ	Prop
1	5.0112	10.7289	5	10	100.22	107.28
2	4.1120	4.31090	4	4	102.80	107.77
3	8.2784	4.19439	8	4	103.48	104.85

The proposed calculation method using zero order spectrophotometry for simultaneous estimation of drugs in fixed dose combination which in this experiment were propranolol HCl and carbamazepine was found to be simple,accurate, precise, sensitive, and provide the smooth release curve. Themethod can be employed to perform the routine analysisconducted in quality control laboratories.

Conclusions

The zero order and first derivative spectrophotometry method has been developed for thesimultaneous estimation of drug release from fixed dose combination propranolol HCl and carbamazepine as pellets and microparticlesformulation. The zero order spectrophotometry and mathematical equations to calculate drug assay was found to give smoother drug release due to more precise calculation. The methods were validated based on ICH analyticalmethod validation guidelines. The values of validation parameters showed that this method offered simple, rapid but more precise calculations for drug release study from fixed dose combinations.

References

1. Chou T. C., 1998, *Drug combinations*: *From laboratory to practice*, J. Lab Clin.Med. ;131:6-8., Mosby Inc.
2. U.S. Department of Health and Human ServicesFood and Drug Administration Center for Drug Evaluation and Research (CDER), 2010,*Guidance for Industry Codevelopment of Two or More Unmarketed Investigational Drugs for Use in Combination* http://www.fda.gov/ Drugs/ Guidance ComplianceRegulatory Information/Guidances/default.htm
3. Dikran, S. B., Hussan, M J M, 2009, First- and Second-Order Derivative Spectrophotometryfor Individual and Simultaneous Determination ofamoxicillin and cephalexin, *National Journal of Chemistry,* Volume 34,260- 269
4. Pawar1, V, Pai, S., and Roa, G.K., 2008,Development and Validation of UV Spectrophotometric Method for Simultaneous Estimation of Montelukast Sodium and Bambuterol Hydrochloride in Bulk and Tablet Dosage Formulation, *Jordan Journal of Pharmaceutical Sciences*, Volume 1, No. 2,
5. Rajput, SJ, and Dobaria, HB.,2006, Dual wavelength spectrophotometric estimation of rabeprazole sodium and domperidone maleate in their combined pharmaceutical dosage form. *Indian Drugs*, 43, 905-908.
6. Simionescu S., Ion R M., 2015, Melatonin Dosage By First-Derivative Spectrophotometry, *The Scientific Bulletin of Valahia University – Materials And Mechanics* – Nr. 10 (Year 13) 2015
7. Dhole S M., Yeole M P.,2015, First derivative UV-spectrophotometric method for simultaneous determination of simvastatin and ezetimibe in tablet dosage form, *Der Pharmacia Lettre*, 2015, 7 (3):124-128
8. Roggia I, Ziegler S, Cruz I.B.M., Ourique1 A.F., Gomes P., 2016, A Derivative UV Spectrophotometric Method for The Determination of Methylxanthines and Tannins in Guarana Bulk (*Paullinia cupana*), *International Journal of Pharmaceutical Sciences and Research,*Vol. 7, Issue 6
9. Kamal, A H, El-Malla S F, Hammad S F., 2016, A Review on UV Spectrophotometric Methods for Simultaneous Multicomponent Analysis,*European Journal of Pharmaceutical and Medical Research,*2016,3(2), 348-360
10. El-Zinati A M., Abdel-Latif M A., 2015, Simultaneous Determination of Paracetamol and Tramadol in PharmaceuticalTablets by Derivative UV-Vis Absorption Spectrophotometry, *The Open Analytical Chemistry Journal*, 2015, 8, 1-6

11. S.R.Karajgi, Shahid Momin, R.B.Kotnal, N.V.Kalyane, 2016, Novel and validated first derivative UV Spectrophotometric method for the determination of Tolperisone hydrochloride in pharmaceutical preparations, *Journal of Chemical and Pharmaceutical Sciences*, Volume 9 Issue 4, October - December 2016

12. Saraan, S M D, Sinaga, S M, Muchlisyam, 2015, Development Method for Determination of Ternary Mixture of Paracetamol, Ibuprofen and Caffeine in Tablet Dosage Form Using Zero-crossing Derivative Spectrophotometric*, International Journal of PharmTech Research,*2014-2015, 7(2), pp 349-353.

13. Owen AJ., 1995, *Uses of Derivative Spectroscopy; Application Note,* Agilent Technologies

14. Pekamwar S S, Kalyankar T M, Tambe B V, Wadher S J, 2015, Validated UV-Visible Spectrophotometric method for simultaneous estimation of Cefixime and Moxifloxacin in Pharmaceutical DosageForms, *Journal of Applied Pharmaceutical Science,* Vol. 5 (01), pp. 037-041, January, 2015

15. Rekha K, Santosh J, Dilshadbee T, Ashpak T, 2015, Estimation of Ciprofloxacin Hydrochloride in Bulk and Formulation by Derivative UV-Spectrophotometric Methods, *International Journal of Advances in Scientific Research* 2015; 1(03): 137-144.

16. Rajanit, S., Paras, V., Hashumati R., 2015, Development and Validation of First Order Derivative Spectrophotometric method for simultaneous estimation of Nifedipine and Metoprolol Succinate in Synthetic Mixture, *International Journal of Pharma Sciences and Research (IJPSR),*Vol. 6 No.2

17. Sinaga, S M, Tarigan, T O, Muchlisyam, 2015, Determination of Mixture of Theophylline and Ephedrine Hidrochloride in Tablets by Derivative Spectrophotometric Method, *International Journal of PharmTech Research*, 2015,8(6),pp 273-283

18. Panditi, V R, Vinukonda, A, 2011, Validated First Order Derivative Spectroscopic Method for the determination of Stavudine in Bulk and Pharmaceutical Dosage Forms*, Int.J. ChemTech Res.*2011,3(1)

19. Pradhan N, Rajkhowa H, Giri H., Shrestha B., 2015, Simultaneous Spectrophotometric Estimation of Moxifloxacin Hydrochloride and Doxorubicin Hydrochloride, *International Journal of Pharmacy And Pharmaceutical Sciences*, Vol 7, Issue 11

20. Patel, BH, Patel, MM, Patel, JR, Suhagia. BN, 2007, HPLC analysis for simultaneous determination of rabeprazole and domperidone in pharmaceutical formulation. *Journal of Liquid Chromatography and Related Technologies*, 30, 439-445.

21. Patel, PG,Vaghela,NM,Rathi, SG,Rajgor, NB, Bhaskar.VH,2009, Derivative spectrometry method for simultaneous estimation of Rupatadine and Montelukast in their combined dosage form.,*J. of young Pharm.*1, 354-358.

22. Srinubabu, G., Sudharani, *B.,* Sridhar L., RaoJLA., 2006, Development and Validation of Liquid Chromatographic and UV Derivative Spectrophotometric Methods for the Determination of Famciclovir in Pharmaceutical Dosage Forms, *Chem. Pharm. Bull.* 54(6) 819—822 (2006)

23. Rangari, N T, Puranik, P K, Chaudhari, S R, Spectrophotometric Determination of Losatan Potassium inPure Form and in tablet dosage form., *International Journal of PharmTech Research,.* 2015,8(1),pp 142-145.

24. Dudhe P.B., Kamble M.C., Komerwar A., Sonawane A.M., Van S., 2016, Development and Validation of First Order Derivative Methodfor Metronidazole in Bulk and Tablet Using UV VisibleSpectroscopy, *International Journal of ChemTech Research*, 2016,9(4),pp 140-144.

25. Muchlisyam, Sinaga, S M.,Haro, G., 2016, Two Novel Ultraviolet Spectrophotometric Method for Determination Binary Mixture of Isoniazide and Rifampicin in Tablet Dosage Form, *International Journal of PharmTech Research*, 2016,9(8),pp 301-312.

✷✷✷✷✷