Journal of Medical Sciences

ISSN 1682-4474

science alert

ANSInet
an open access publisher
http://ansinet.com

Research Paper

J. Med. Sci., 15 (5): 221-228
1st July, 2015
DOI: 10.3923/jms.2015.221.228

Simultaneous Determination of Paracetamol, Guaiphenesin and Chlorpheniramine Maleate Using Ultraviolet Spectroscopy in Combination with Multivariate Calibration

[1,2]Abdul Rohman, [1,3]Yani Ardiyanti, [1]Sudjadi and [1]Sugeng Riyanto

The method of choice for analysis of drugs in multi-component preparations is chromatographic based technique such as High Performance Liquid Chromatography (HPLC). However, chromatographic method is time consuming and requiring much effort. As a consequence, some simple methods such as UV spectrophotometry are continuously developed, especially in combination with the chemometrics software. The UV-vis spectrophotometry coupled with multivariate calibration of Partial Least Square (PLS) has been developed for quantitative analysis of paracetamol, guaiphenesin and chlopheniramine maleate in the presence of phenylpropanolamine without separation step. The calibration model is prepared by developing a series of sample mixture comprising these drugs in certain proportion. The evaluation of calibration model was based on coefficient of determination (R^2) and Root Mean Square Error of Calibration (RMSEC). The result showed that UV spectrophotometry combined with PLS can be used for quantitative analysis of drugs. The coefficient of determination (R^2) for the relationship between actual values and predicted values was higher than 0.99 indicating good accuracy. The RMSEC values obtained were relatively low indicating good precision. The accuracy of developed method was compared to that of HPLC. The developed method was successfully used for analysis of paracetamol, guaiphenesin and chlopheniramine maleate in tablet dosage form.

Key words: UV spectrophotometry, principal component regression, SMLR, HPLC, PLS, phenylpropanolamine, RMSEC

JMS (ISSN 1682-4474) is an International, peer-reviewed scientific journal that publishes original article in experimental & clinical medicine and related disciplines such as molecular biology, biochemistry, genetics, biophysics, bio-and medical technology. JMS is issued eight times per year on paper and in electronic format.

For further information about this article or if you need reprints, please contact:

Abdul Rohman
Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta, 55281, Indonesia

[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta, 55281, Indonesia
[2]Research Center of Halal Products, Universitas Gadjah Mada, Yogyakarta, 55281, Indonesia
[3]The National Agency of Drug and Food Control, District of Palangkaraya, Central Kalimantan, Indonesia

ANSInet
Asian Network for Scientific Information

INTRODUCTION

Paracetamol (PCT), Guaiphenesin (GG) and Chlopheniramine maleate (CTM) are active pharmaceutical ingredients frequently combined and widely used in several pharmaceutical preparations, especially in cold and cough formulation. The PCT is a popular antipyretic and analgesic agent (Hardman *et al*., 1996). The chemical structures of PCT, GG and CTM are shown in Fig. 1. In some countries, it is one of the most used medicines, as an alternative to acetylsalicylic acid (aspirin). The GG is is an expectorant used for treatment of productive cough, while CTM is a powerful first-generation alkyl amine antihistamine, H1-receptor antagonist, widely used for symptomatic relief of common cold and allergic rhinitis, with weak sedative properties (Redasani *et al*., 2013).

Some analytical methods have been used for determination of PCT, GG and CTM, either alone or in combination with other medicines, in pharmaceutical products, mostly based on chromatographic and electrophoretic techniques. Such methods are high performance liquid chromatography (Akhtar *et al*., 1994; Deconinck *et al*., 2011), gas chromatography (Harsono *et al*., 2005), FTIR spectrophotometry (Mallah *et al*., 2012), cyclic voltametry (Teixeira *et al*., 2009) and derivative spectrophotometry (Deshpande *et al*., 2012). Some of these methods are time consuming and requiring sophisticated instrument (Rohman *et al*., 2015), therefore, UV-vis spectrophotometry is continuously developed to overcome these difficulties.

The spectrophotometric techniques result the most appealing approach to be adopted in pharmaceutical analysis, due to its simplicity, in spite of their low selectivity and sensitivity. Nevertheless, the assay of multicomponents in pharmaceutical preparation using spectrophotometric techniques results useless in many cases, due to a high number of components and the extensive spectral overlapping (Ragno *et al*., 2004), therefore, the chemometrics technique is applied to resolve this problem (Fisher and Jones, 1987).

Currently, the application of chemometric techniques, especially multivariate calibrations are playing a very important role in the multicomponent analysis of pharmaceutical mixtures (De Luca *et al*., 2009; Rohman *et al*., 2015). Some multivariate calibrations such as Principal Component Regression (PCR), Stepwise Multiple Linear Regression (SMLR) and Partial Least Squares (PLS) are the most adopted multivariate methods in pharmaceutical analysis and are frequently used for instrumental methods withouut separation techniques like ultraviolet and infrared spectroscopies (Rohman, 2012; El-Gindy *et al*., 2006). The objective of this study was to determine paracetamol (PCT), guaiphenesin (GG) and chlopheniramine maleate (CTM) in synthetic mixture and in tablet formulation using UV-spectrophotometry in combination with multivariate calibration.

MATERIALS AND METHODS

The standards of Paracetamol (PCT), Guaiphenesin (GG) and Chlorpheniramin maleate (CTM) were of Reference standard of Indonesian Pharmacopeia and were obtained from the National Agency of Drug and Food Control, Republic of Indonesia. The chemicals and reagents used were of pro analytical grade. The solvents used for HPLC were of liquid chromatography grade. The tablet dosage form was obtained from pharmacy in Yogyakarta.

Preparation of standard solution: The standard solutions were prepared freshly in methanol: hydrochloric acid 0.1 M (3:1) and used for preparing calibration (20 samples) and validation samples (10 samples). The composition of calibration and validation samples are shown in Table 1 and 2, respectively. Each solution mixture was scanned using UV-vis spectrophotometer (Shimadzu) at 200-400 nm. Each 2 nm, their absorbance were recorded and used for the optimization the calibration models.

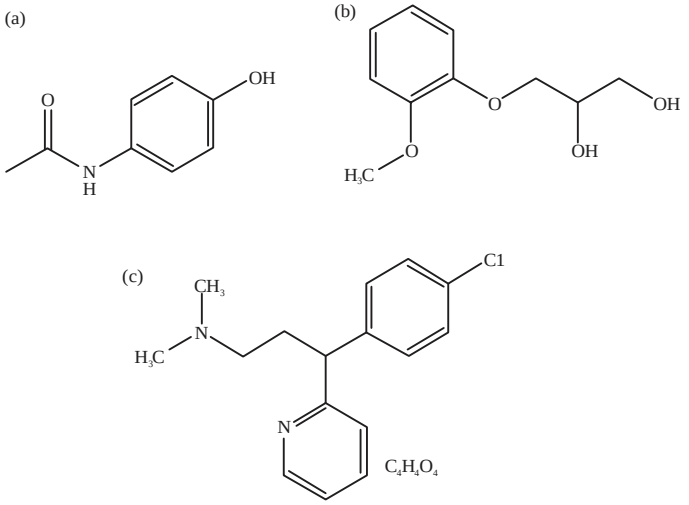

Fig. 1(a-c): Chemical structure of (a) Paracetamol, (b) Guaiphenesin and (c) Chlopheniramine maleate

Table 1: Composition of synthetic mixture consisting of PCT, GG, CTM and PPA-HCl used in calibration samples

	Synthetic mixture (μg mL^{-1})			
No. of samples	PCT	GG	CTM	PPA-HCl
1	10	16	3	5
2	11	11	8	8
3	4	5	7	19
4	4	19	6	8
5	8	6	15	4
6	3	16	7	8
7	10	15	10	10
8	2	17	16	10
9	9	7	2	6
10	15	12	2	11
11	15	17	8	10
12	8	17	10	17
13	2	6	15	15
14	6	8	8	9
15	8	5	6	18
16	12	11	13	9
17	13	8	14	6
18	6	7	20	9
19	13	11	14	4
20	5	3	9	4

PCT: Paracetamol, GG: Guaiphenesin, CTM: Chlopheniramine maleate, PPA-HCI: Phenylpropanolamine

Table 2: Composition of synthetic mixture consisting of PCT, GG, CTM and PPA-HCl used in validation samples

	Synthetic mixture (μg mL^{-1})			
No. of samples	PCT	GG	CTM	PPA-HCl
1	14	18	18	7
2	5	17	9	4
3	9	4	11	7
4	14	9	7	14
5	12	7	16	11
6	8	2	6	17
7	2	15	16	18
8	6	10	19	5
9	18	11	11	6
10	7	19	3	9

PCT: Paracetamol, GG: Guaiphenesin, CTM: Chlopheniramine maleate, PPA-HCI: Phenylpropanolamine

Analysis of paracetamol, guaiphenesin and chlopheniramine maleate in tablet dosage forms using UV spectrophotometry: Twenty tablets were taken and subjected to mass homogeneity test. The tablets are crushed and an amount of powder equivalent to 1 tablet is taken and added with methanol: hydrochloric acid 0.1 M (3:1 v/v) until 100 mL. The solution is shaken vigorously for 30 min. The solution is filtered using Whatman paper and supernatant is taken and diluted with solvent and subjected to spectrophotometric measurement as described above. Spesific preparation using addition standard applied for chlorpheniramine maleatee analysis. The concentration of paracetamol, guaiphenesin and chlorpheniramine maleatee in tablet dosage forms is calculated based on the optimized calibration model.

Analysis of paracetamol, guaephenesin and chlorpheniramin maleate in tablet dosage forms using high performance liquid chromatography: Three separate methods were used for chromatographic analysis of three kinds of components using US pharmacopoeia as a standard method (USP., 2012). Twenty tablets were taken and subjected to mass homogenity test. The tablets are crushed and an amount of powder equivalent to 1 tablet was prepared in accordance with the respective procedures as shown in Table 3.

Data analysis: The level of paracetamol, guaiphenesin and chlorpheniramine maleate in tablet dosage is calculated with the aid of multivariate calibration of Partial Least Square (PLS). The PLS analysis is carried out using Minitab software version 16 (Minitab Corp., USA). The correlation between actual values of these drugs determined by HPLC and calculated values as determined by UV spectrophotometry in combination with PLS is performed using Excel.

RESULTS AND DISCUSSION

The overlay of absorption UV spectra of pure paracetamol (PCT), guaiphenesin (GG), chlopheniramine maleate (CTM) and phenylpropanolamine (PPA) in methanol: hydrochloric acid 0.1 M (3:1) at wavelength 200-400 nm are shown in Fig. 2, which exhibited excessive overlapping of drugs making the difficulty of analysis of these

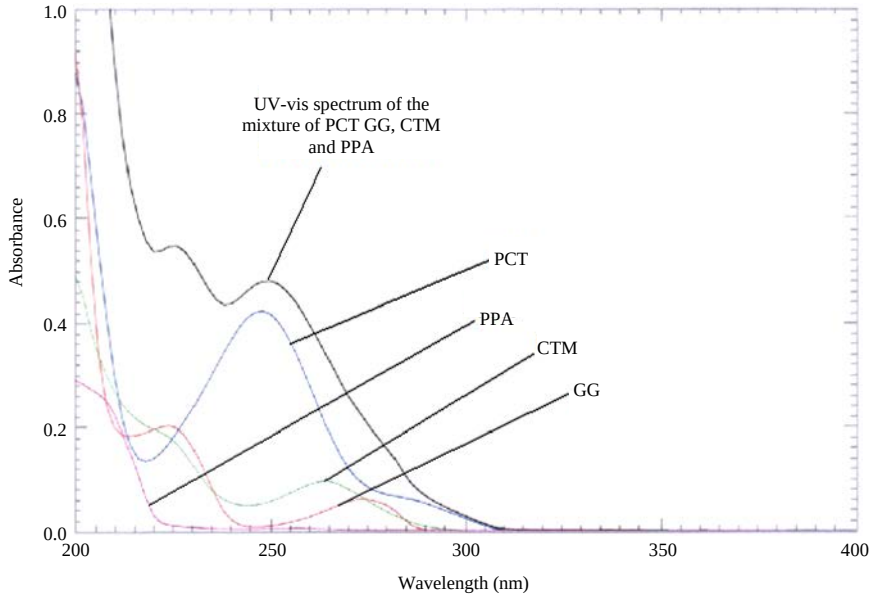

Fig. 2: Overlay of UV spectra of paracetamol, guaiphenesin and chlopheniramine maleate and phenylpropanolamine HCl

Table 3: United State Pharmacopoeia 35 chromatographic system of analysis PCT, GG and CTM in tablet dosage form

Chromatography procedure	Paracetamol analysis	Guaiphenesin analysis	Chlopheniramine analysis
Final solution	0.25 mg mL^{-1}	0.02 mg mL^{-1}	0.0067 mg mL^{-1}
Solvent	Methanol, phosphoric acid solution 0.1%	Water, methanol	Methanol, phosphoric acid solution 0.1%
Mobile phase	Water-Methanol-acetic acid glacial (790:200:10)	Water-Methanol-acetic acid glacial (60:40:1.5)	Methanol and water (60:40) containing 0.34 g of monobasic potassium phosphate, 0.05 g of triethylamine hydrochloride, 0.25 g of sodium lauryl sulfate and 0.1 mL of phosphoric acid in each 100 mL
Column	OS	ODS	Phenyl
Injection volume	20 µL	20 µL	20 µL
Detector	UV 280 nm	UV 276 nm	UV 214 nm

PCT: Paracetamol, GG: Guaiphenesin, CTM: Chlopheniramine maleate

drugs using UV spectrophotometry without any treatment. Some efforts have been tried to solve this problems such as the use of H-point standard addition methods (Sabry and Khamis, 2000), treatment of UV spectra such as derivative spectra (Tomsu *et al*., 2004; Palabiyik *et al*., 2004) and the use of chemometrics of multivariate calibration (Rohman, 2012; Rohman *et al*., 2015). Multivariate calibration using principle component regression and partial least square are reported to overcome the spectral overlapping to facilitate calibration models (Marwada *et al*., 2014; Mohamed and Mikre, 2009). The wavelength range of 200-320 nm was throughout taken into account because the absence of absorbance after 320 nm for all the drugs. Quantitative analysis of PCT, GG and CTM using ordinary spectrophotometry was proved to be inaccurate because of the extensive overlapping of the UV spectral curves. In addition, quantitative analysis of PPA was not performed due to the lack of chromophore in PPA. However, during the optimization for quantitative analysis, PPA was added in the drug mixture.

In order to be succesfull during the modelling for quantitative analysis of PCT, GG and CTM, it is advisable that the mixture of studied drugs and the evaluated dosage form is similar in term of Uv spectra as shown in Fig. 3. Quantitative analysis of PCT, GG and CTM was performed with the aid of PLS calibration. Furthermore, some wavelengths have been optimized during PLS modelling. The wavelength capable of providing the best correlation between actual value of PCT, GG and CTM and its predicted values was selected. Finally the wavelength of 240-350 nm was prefereed for quantification of PCT, GG and CTM simultaneously due to its capability provide the highest values of coefficient of determination (R^2) and the lowest values of error expressed as RMSEC. The correlation between actual value and predicted values of PCT, GG and CTM as determined using UV spectrophotometry at 240-350 nm without any separation processes was shown in Fig. 4. The R^2 values obtained for such correlation is high, namely 0.999972, 0.999826, 0.999725 for PCT, GG and CTM, respectively. Meanwhile, the RMSEC values obtained is relatively low, i.e., 0.022019, 0.067889, 0.083875 for PCT, GG and CTM, respectively. The relative standard deviation for precision evaluation for each studied drugs are 0.27, 0.58, 0.85% for PCT, GG and CTM, respectively.

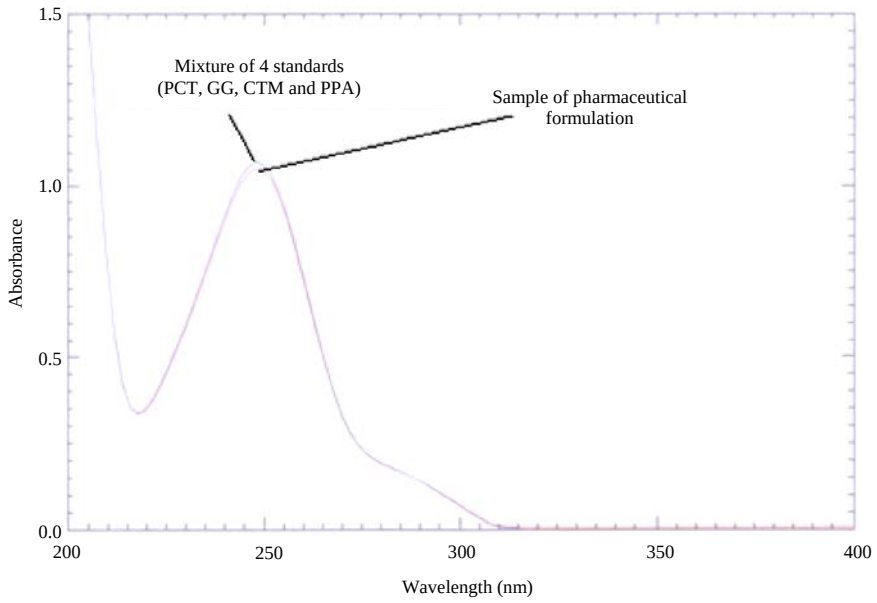

Fig. 3: UV spectra overlay of the drug mixture (paracetamol, guaiphenesin, chlopheniramine maleate and phenylpropanolamine HCl) and formulation containing these four drugs

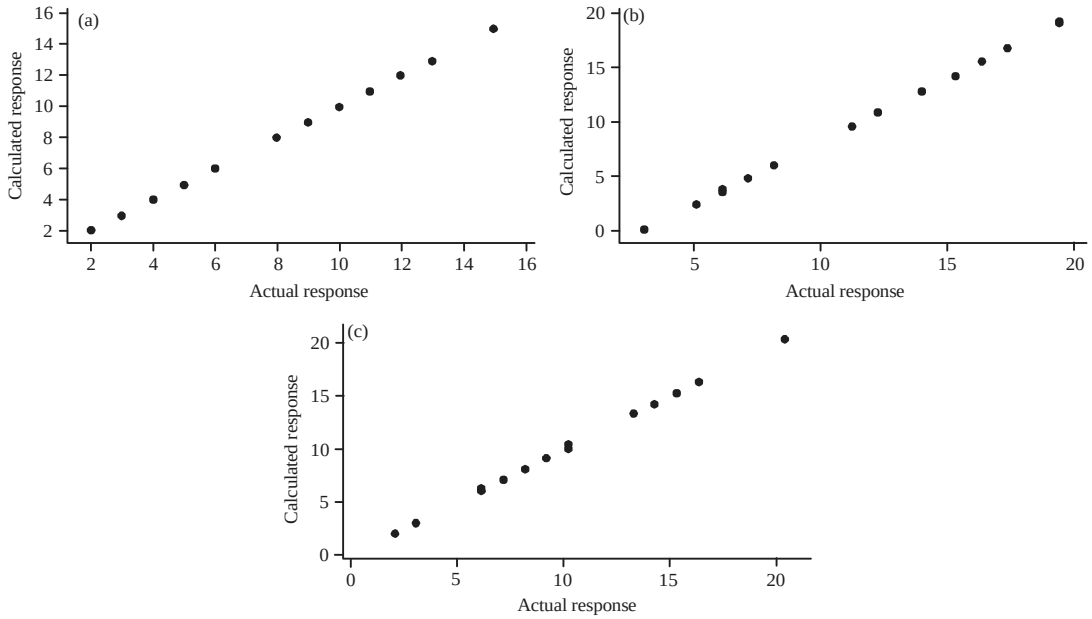

Fig. 4(a-c): Correlation between actual value and predicted values of PLS response plot (a) Paracetamol, (b) Guaiphenesin and (c) Chlopheniramine maleate as determined using UV spectrophotometry at 240-350 nm with the aid of partial least squares

One of the potential disadvantages when using multivariate calibrations is over-fitting of the regression model. It means that the model generates an optimistic model on the set of data used for calibration but the model would not perform well on other data sets with similar material (Miller and Miller, 2005). Cross-validation of calibration samples using “leave-one out” technique can be used to assess this problem. One of the calibration samples is left out from PLS model and the remaining samples are used to make PLS model. Furthermore, the removed sample is calculated using the new developed PLS model. This procedure was repeated; leaving each calibration sample out in turn. Then, the difference between the actual and predicted value for each specimen is calculated. The sum of the squares of these differences is called the Predicted Residual Error Sum of Squares (PRESS). The smaller the PRESS value, the better the

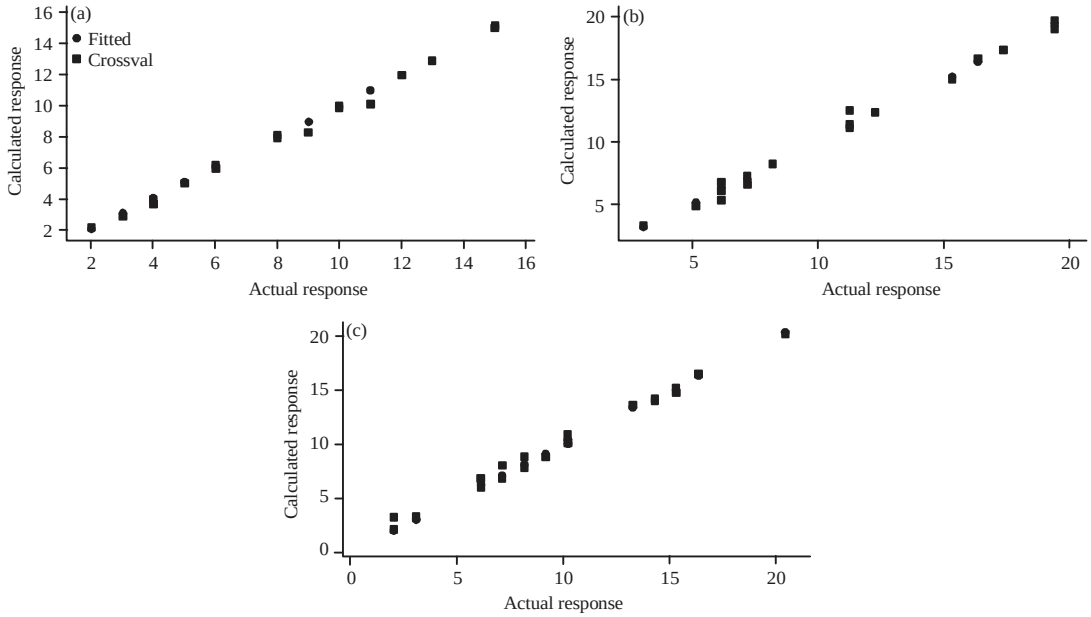

Fig. 5(a-c): Partial Least Squares (PLS) model for the correlation between actual value and predicted values of PLS response plot (a) Paracetamol, (b) Guaiphenesin and (c) Chlopheniramine maleate during cross validation

Table 4: Analytical results of PCT, GG and CTM in tablet dosage form obtained by UV spectrophotometry compared with those official methods (HPLC)

	PCT (%)		GG (%)		CTM (%)	
No.	UV Spectrophotometry	HPLC	UV Spectrophotometry	HPLC	UV Spectrophotometry	HPLC
1	97.73	98.44	101.65	102.18	98.42	107.35
2	97.32	99.46	103.99	101.75	101.19	106.45
3	99.10	101.10	100.34	101.02	101.85	107.85
4	99.44	100.78	102.58	100.25	99.64	104.46
5	98.15	100.07	104.34	101.05	95.23	104.09
6	98.45	98.42	102.77	100.17		105.86
AV (%)	98.37	99.71	102.61	101.07	99.27	106.01
RSD (%)	0.82%	1.15	1.45	0.79	2.64	1.43

AV: Average, HPLC: High performance liquid chromatography, PCT: Paracetamol, GG: Guaiphenesin, CTM: Chlopheniramine maleate

predictive power of the model (Rohman and Man, 2011). The PRESS values obtained are 1.693 (PCT), 3.445 (GG) and 4.915 (CTM). Regarding this result, the over-fitting does not happen in the developed PLS model, because the values of PRESS are relatively small. Figure 5 revealed the correlation between actual value and predicted values of PCT, GG and CTM during cross validation.

The PLS model was subsequently used to predict the level of independent samples in prediction/validation models. The prediction performance was assessed using R^2 and RMSEP values obtained; the small RMSEP and the high R^2 values indicated that the prediction model of new sample has less error. Using PLS model, the RMSEP values obtained are 0.22, 0.26 and 0.21% for the prediction model of PCT, GG and CTM, respectively.

The developed method was further used for determination of PCT, GG and CTM in tablet dosage form. The results obtained by UV spectrophotometry are compared with those analyzed using official methods (HPLC). HPLC is the most used method for analysis of pharmaceutiicals, especially in complex mixture (Mishra *et al*., 2013). The levels of PCT, GG and CTM obtained by UV spectrophotometry in combination with PLS and those by HPLC are shown in Table 4. It is konwn that results obtained by UV spectrophotometry are comparable to those determined by HPLC. It can be concluded that UV spectrophotometry can be an alternative technique for determination of PCT, GG and CTM di tablet dosage form.

CONCLUSION

This study established the feasibility of simultaneous determination of PCT, GG and CTM in synthetic and pharmaceutical preparations by a simple and rapid method without any separation step. A comparative study of the use of HPLC and UV spectrophotometry with the aid of multivariate calibration (PLS) methods for analysis PCT, GG and has been

accomplished. Although, the HPLC method is more specific than UV spectrophotometry, HPLC needs expensive equipment and materials. The UV spectrophotometry and PLS calibration has been successfully used for quantitative analysis of PCT, GG and CTM in synthetic and pharmaceutical preparations simultaneously without excessive sample treatment.

ACKNOWLEDGMENTS

The authors thank to Faculty of Pharmacy, Gadjah Mada University for its financial support during this study. The the National Agency of Drug and Food Control, district of Yogyakarta, Indonesia was acknowledged for providing HPLC instrument make this research possible.

REFERENCES

Akhtar, M.J., S. Khan and M. Hafiz, 1994. High-performance liquid chromatographic assay for the determination of paracetamol, pseudoephedrine hydrochloride and triprolidine hydrochloride. J. Pharm. Biomed. Anal., 12: 379-382.

De Luca, M., F. Oliverio, G. Ioele and G. Ragno, 2009. Multivariate calibration techniques applied to derivative spectroscopy data for the analysis of pharmaceutical mixtures. Chem. Intell. Lab. Syst., 96: 14-21.

Deconinck, E., P.Y. Sacre, S. Baudewyns, P. Courselle and J. De Beer, 2011. A fast ultra high pressure liquid chromatographic method for qualification and quantification of pharmaceutical combination preparations containing paracetamol, acetyl salicylic acid and/or antihistaminics. J. Pharmaceut. Biomed. Anal., 56: 200-209.

Deshpande, S.D., A.V. Deosarkar and S.G. Walode, 2012. Absorption ratio, derivative spectroscopy and RP-HPLC methods for estimation of guaifenesin and ambroxol hydrochloride in tablet. Der Chemica Sinica, 3: 759-765.

El-Gindy, A., S. Emara and A. Mostafa, 2006. Application and validation of chemometrics-assisted spectrophotometry and liquid chromatography for the simultaneous determination of six-component pharmaceuticals. J. Pharm. Biomed. Anal., 41: 421-430.

Fisher, B.V. and R. Jones, 1987. Chemometrics in pharmaceutical analysis. J. Pharmaceut. Biomed. Anal., 5: 455-467.

Hardman, J.G., L.E. Limbar, P.B. Molinoff, R.W. Ruddon and G.A. Gilman, 1996. Goodman and Gilman's the Pharmacological Basis of Therapeutics. 9th Edn., McGraw-Hill, New York, Pages: 631.

Harsono, T., M. Yuwono and G. Indrayanto, 2005. Simultaneous determination of some active ingredients in cough and cold preparations by gas chromatography and method validation. J. AOAC Int., 88: 1093-1098.

Mallah, M.A., S.T.H. Sherazi, S.A. Mahesar and A.R. Khaskheli, 2012. Simultaneous quantification of ibuprofen and paracetamol in tablet formulations using transmission Fourier transform infrared spectroscopy. Am. J. Anal. Chem., 3: 503-511.

Marwada, K.R., J.B. Patel, N.S. Patel, B.D. Patel, D.V. Borkhatariya and A.J. Patel, 2014. Ultraviolet spectrophotometry (dual wavelength and chemometric) and high performance liquid chromatography for simultaneous estimation of meropenem and sulbactam sodium in pharmaceutical dosage form. Spectrochim. Acta Part A: Mol. Biomol. Spectrosc., 124: 292-299.

Miller, J.N. and J.C. Miller, 2005. Statistics and Chemometrics for Analytical Chemistry. 5th Edn., Pearson Education Ltd., England, ISBN-13: 978-0131291928, pp: 213-239.

Mishra, A., A.K. Mishra, O.P. Tiwari and S. Jha, 2013. HPLC analysis and standardization of Brahmi vati-An Ayurvedic poly-herbal formulation. J. Young Pharmacists, 5: 77-82.

Mohamed, A.E.I. and W. Mikre, 2009. Determination of lamivudine and stavudine in pharmaceutical preparations using chemometrics-assisted spectrophotometry. Saudi Pharm. J., 17: 275-281.

Palabiyik, I.M., E. Dinc and F. Onur, 2004. Simultaneous spectrophotometric determination of pseudoephedrine hydrochloride and ibuprofen in a pharmaceutical preparation using ratio spectra derivative spectrophotometry and multivariate calibration techniques. J. Pharm. Biomed. Anal., 34: 473-483.

Ragno, G., G. Ioele and A. Risoli, 2004. Multivariate calibration techniques applied to the spectrophotometric analysis of one-to-four component systems. Anal. Chim. Acta, 512: 173-180.

Redasani, V.K., A.P. Gorle, R.A. Badhan, P.S. Jain and S.J. Surana, 2013. Simultaneous determination of chlopheniramine maleate, phenylephrine hydrochloride, paracetamol and caffeine in pharmaceutical preparation by RP-HPLC. Chem. Ind. Chem. Eng. Quart., 19: 57-65.

Rohman, A. and Y.B.C. Man, 2011. Application of Fourier transform infrared (FT-IR) spectroscopy combined with chemometrics for authentication of cod-liver oil. Vibrat. Spectroscopy, 55: 141-145.

Rohman, A., 2012. Application of Fourier transform infrared spectroscopy for quality control of pharmaceutical products: A review. Indonesian J. Pharm., 23: 1-8.

Rohman, A., D. Silawati, Sudjadi and S. Riyanto, 2015. Simultaneous determination of sulfamethoxazole and trimethoprim using UV spectroscopy in combination with multivariate calibration. J. Med. Sci., 15: 178-184.

Sabry, S.M. and E.F. Khamis, 2000. Application of H-point standard additions method to spectrophotometric and spectrofluorimetric determinations of glafenine and glafenic acid in mixtures. Talanta, 51: 1219-1231.

Teixeira, M.F.S., L.H. Marcolino-Junior, O. Fatibello-Filho, F.C. Moraes and R.S. Nunes, 2009. Determination of analgesics (dipyrone and acetaminophen) in pharmaceutical preparations by cyclic voltammetry at a copper(II) hexacyanoferrate(III) Modified carbon paste electrode. Curr. Anal. Chem., 5: 303-310.

Tomsu, D., M.C. Icardo and J.M. Calatayud, 2004. Automated simultaneous triple dissolution profiles of two drugs, sulphamethoxazole-trimethoprim and hydrochlorothiazide-captopril in solid oral dosage forms by a multicommutation flow-assembly and derivative spectrophotometry. J. Pharm. Biomed. Anal., 36: 549-557.

USP., 2012. The united states pharmacopeia and the national formulary. U.S. Pharmacopeial Convention (USP), Rockville MD., pp: 2041-2042, 3382-3383.