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ORIGINAL ARTICLE / ÖZGÜN MAKALE

VALIDATION STUDY OF A SPECTROPHOTOMETRIC BINARY MIXTURE ANALYSIS

BİR SPEKTROFOTOMETRİK İKİLİ KARIŞIM ANALİZİNİN DOĞRULAMA ÇALIŞMASI

Özgür ÜSTÜNDAĞ[1*], Erdal DİNÇ[1]

[1] Ankara University, Faculty of Pharmacy, Department of Analytical Chemistry, 06560, Ankara, Turkey

ABSTRACT

Objective: *A rapid spectrophotometric analysis by applying a signal processing method was validated by intra-day and inter-day experiments, standard addition technique, recovery study and analysis of variance (ANOVA). For this purpose, a binary pharmaceutical formulation was evaluated consisting of candesartan cilexetil (CC) and hydrochlorothiazide (HT).*

Material and Method: *The absorption spectra of mixtures and tablet solutions in the spectral range 200-305 nm were measured by a Shimadzu UV-1601 dual-beam UV-VIS spectrophotometer with a constant gap width (2 nm).*

Result and Discussion: *Intra-day and inter-day experiments, standard addition technique, recovery study and analysis of variance (ANOVA) studies were carried out by using artificial mixtures were prepared for this purpose. These studies have shown that the validity and applicability of the method is good.*

Keywords: *Binary mixture, quantitative determination, signal processing, validation*

ÖZ

Amaç: *Bir sinyal işleme yöntemi uygulanarak hızlı bir spektrofotometrik analiz, gün içi ve günler arası deneyler, standart ekleme tekniği, geri kazanım çalışması ve varyans analizi (ANOVA) ile doğrulanmıştır. Bu amaçla, kandesartan sileksetil (CC) ve hidroklorotiyazidden (HT) oluşan ikili bir farmasötik formülasyon değerlendirildi.*

Gereç ve Yöntem: *200-305 nm spektral aralığındaki karışımların ve tablet çözeltilerinin absorpsiyon spektrumları, sabit bir boşluk genişliğine (2 nm) sahip bir Shimadzu UV-1601 çift ışınlı UV-VIS spektrofotometresi ile ölçülmüştür.*

Sonuç ve Tartışma: *Gün içi ve günler arası deneyler, standart ekleme tekniği, geri kazanım çalışması ve varyans analizi (ANOVA) çalışmaları yapılmış ve bu amaçla yapay karışımlar hazırlanmıştır. Bu çalışmalar yöntemin geçerliliğinin ve uygulanabilirliğinin iyi olduğunu göstermiştir.*

Anahtar kelimeler: *İkili karışım, kantitatif tayin, sinyal işleme, validasyon*

[*] **Corresponding Author / Sorumlu Yazar:** Özgür Üstündağ
e-mail / e-posta: ustundag@pharmacy.ankara.edu.tr, **Phone / Tel.:** +903122033171

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INTRODUCTION

Modern analytical methods are important for in clinical, biomedical, pharmaceutical analysis, industrial manufacturing quality control, environmental monitoring and forensic medicine. To this end, analytical chemists seek to meet the needs of better chemical measurements or to evolve more efficient procedures and increase the reliability of existing analytical methods to achieve the desired analytical results in many disciplines and the aforementioned fields. [1-3].

Analytical methods such as spectrophotometry [4], mass-spectrometry [5], chromatography [6] and electrophoresis [7], electrochemistry [8] and their joint devices have been used for analytical purposes. Mainly, LC and CE methods have been used in conjunction with different spectroscopic systems (separate techniques, namely LC-MS and CE-MS) to obtain more chemical data and reduce the complication of multicomponent substance analysis. Furthermore, these combined unit methods involve high costs and time for analysis [9-11]. Herewith the disadvantages of the mentioned separation techniques or combination analyzers, analytical chemists opt to use spectroscopic methods (rather than separation techniques) to enable rapid analysis at low cost when success is possible. In particular, derived spectrophotometry and its modified versions have been widely used for rapid quantitative separation of multicomponent mixtures without separation steps. In any case, these spectral methods cannot lead to good analysis because of strongly overlapping composite spectra, noise peaks at the main peaks, fundamental problems, reduced signal intensity and deterioration of the ratio of signal/noise (S/N) for higher orders [12]. Current progresses in signal processing methods have given us more opportunities to find prefable solutions for complex analytical problems [13, 14]. One of the latest introductions is the derived spectrophotometry (DS) application for spectral assay of components in preparations [15]. Several analytical methods for the analysis of CC and HT have been reported in pharmaceutical and biological studies, including derived spectrophotometric methods [16-19] and high performance liquid chromatography methods [20-22].

MATERIAL AND METHOD

The absorption spectra of mixtures and tablet solutions in the spectral range 200-305 nm were measured by a Shimadzu UV-1601 dual-beam UV-VIS spectrophotometer with a constant gap width (2 nm). When applying the methods, Microsoft EXCEL and Wavelet Toolbox in Matlab 7.0 software were applied for calculations.

Commercial Tablet Product

A pharmaceutical tablet (ATACAND PLUS® Tablet, AstraZeneca Pharm. Ind., Istanbul, Turkey) including 16 mg CC and 12.5 mg HT per tablet was gathered from the Turkish market. CC and HT pure compounds were given away by National Pharm. Ind. Company, Turkey.

Standard Solutions

Standard CC and HT stock solutions were arranged respectively by dissolving 25 mg of each drug in 100 mL of methanol. A calibration range between 2.0-20.0 μg ml^{-1} for CC and HT in the above solvent was prepared for spectrum analysis from standard stock solutions for each active ingredient. For the standard addition method, sample solutions were arranged by adding the stock solution of each component to the tablets with three different collection rates for the six replicas to appraise the effect of the excipients in the analysis. Moreover, six synthetic sample solutions were prepared in three different sampling stages for analysis during the day and between days.

Sample Solutions Preparation

For testing tablets; twenty tablets of CC and HT were weighed and crushed to a fine powder. Transfer an equal amount of powder to a 100 ml volumetric flask and solved with methanol. The contents of the flask were mechanically stirred for 30 minutes. After filtration, the supernatant was diluted with methanol to an ultimate concentration. This sample preparation was repeated ten times.

RESULT AND DISCUSSION

The purpose of this work is to validate a rapid derivative spectrophotometric method (DS) for the simultaneous assay of CC and HT in mixtures and preparations. The CC and HT standards and the UV spectra of the tablet solution were measured between 200 and 305 nm as can be seen in Figure 1.

First-Derivative Spectrophotometry (DS)

In the application of this method, the first derivative spectra were obtained by using a $\Delta\lambda$=10 nm interval to calculate the derivative data of the original spectra of samples (Figure 2). The calibration graph, which was obtained by measuring the $dA/d\lambda$ values at 263.4 nm for CC and 249.6 nm for HT were used for the analysis. Regression equation, correlation coefficient and their statistical data were shown in Table 1. The calibration equation of the DS method was validated by using the quantitative assay of artificial mixtures. Recovery results and with relative standard deviation were shown in Table 2. The DS was applied to strongly overlapping spectra in the wavelength range 200.0 to 305.0 nm and the resulting spectrum is also shown in Figure 2.

The outcome of the regression analysis obtained are shown in Table 1. The amounts of CC and HT in the samples were computed using the calibration equations obtained from the linear regression analysis in Table 1.

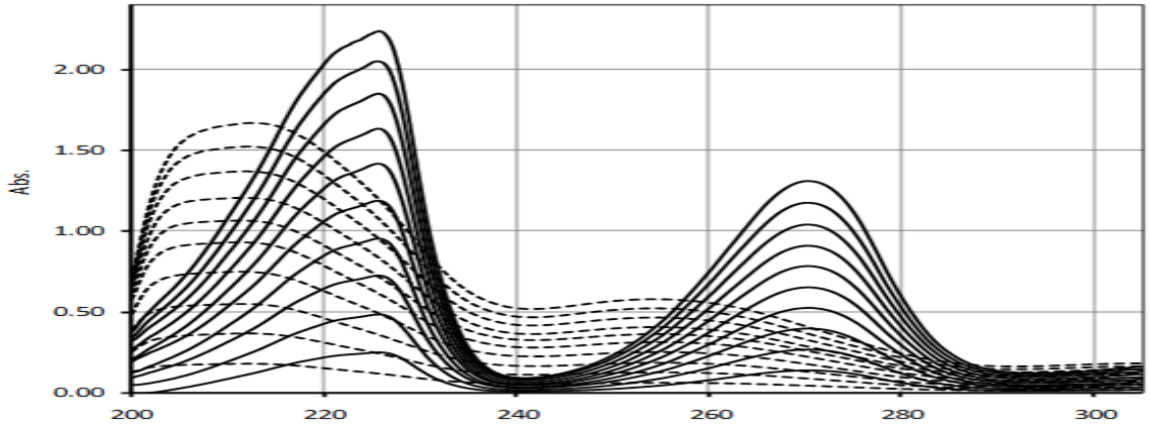

Figure 1. The UV-Absorption spectra of 2.0-20 µg mL^{-1} CC (---) and HT (—) in methanol

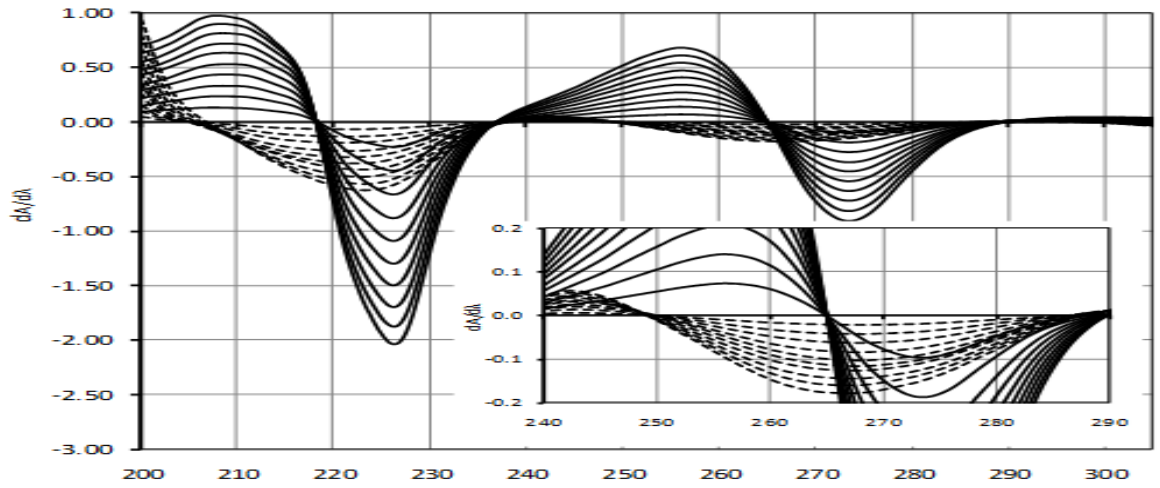

Figure 2. DS of CC (---) (2.0-20.0 µg mL^{-1}) and HT (—) (2.0-20.0 µg mL^{-1}) in methanol (Δλ=10 nm)

Table 1. Statistical outcome for the DS method

Method	**DS**	
Parameter	**CC**	**HT**
λ (nm)	264.3	249.6
m	-0.0081	0.0249
n	-0.0121	0.0024
r	0.9989	0.9999
SE(m)	1.56x10-4	1.22x10-3
SE(n)	2.93x10-4	9.82x10-5
SE(r)	2.83x10-3	1.78x10-3
LOD (µg ml^{-1})	0.34	0.46
LOQ (µg ml^{-1})	1.15	1.55

Validation of the Proposed Methods

A validation kit consisting of 16 artificial solutions of dissimilar concentrations in methanol in a working range of 2.0-20.0 µg ml^{-1} for CC and HT was produced. This validation set tested the accuracy

and precision of the DS method. The results gathered by performing the DS method to artificial mixtures prepared as a verification set are shown in Table 2.

Table 2. Recovery outcome calculated by using artificial mixtures

DS			
Found (µg mL^{-1})		**Recovery (%)**	
CC	**HT**	**CC**	**HT**
1.91	6.25	95.3	104.1
3.87	6.26	96.7	104.3
5.68	6.35	94.7	105.8
7.45	6.26	93.1	104.3
9.84	6.20	98.4	103.3
12.10	6.15	100.8	102.4
13.99	6.19	100.0	103.2
15.84	6.06	99.0	101.0
17.70	5.99	98.3	99.8
19.64	6.04	98.2	100.7
7.75	2.00	96.9	100.1
8.39	4.14	104.9	103.5
7.62	6.19	95.3	103.1
7.73	8.40	96.6	105.1
7.55	10.40	94.4	104.0
7.64	12.45	95.4	103.8
	Mean	97.1	103.1
	SD	2.70	1.58
	RSD	2.78	1.53

To appraise the accuracy and precision of the DS method, precision and accuracy survey were applied daily at three dissimilar concentrations (2.0, 8.0 and 16.0 µg ml^{-1} for CC, HT) during the calibration of the DS method. Thus, 6 dissimilar solutions were used and the prepared solutions were used for intra-day and inter-day studies. The results can be seen in Table 3.

Before the DS method was implemented to the commercial tablet preparation, a standard addition technique was used to test the interference effects of tablet excipients on CC and HT. The results can be seen in Table 4.

Recovery and other calculations for CC and HT were performed by subtracting the quantity of CC and HT from the tablets. These surves were performed with five replicas at three dissimilar concentration grades.

Variance Analysis (ANOVA)

For comparison purposes, ANOVA test was performed for the results obtained from 6 different samples for each concentration in order to statistically evaluate the accuracy and precision of the analysis results obtained intra-day and inter-day with the DS method. It was determined that (with % 95 of confidential limit) there was no significant difference between the analysis results obtained (Table 5).

Table 3. Intra-day and inter-day outcome by the DS method

Intra-day Results						
	Added (µg mL^{-1})	**Found (µg mL^{-1})**	**SD**	**RSD**	**RE**	**Recovery (%)**
CC	2	1.97	0.05	2.60	-1.06	98.3
	8	7.80	0.19	2.48	-1.01	97.5
	16	15.87	0.13	0.81	-0.33	99.2
HT	2	2.04	0.04	1.77	0.72	101.9
	8	8.10	0.17	2.15	0.88	101.3
	16	16.02	0.14	0.85	0.35	100.1
Inter-day Results						
CC	2	1.98	0.04	1.87	-0.76	98.8
	8	7.76	0.17	2.18	-0.89	97.0
	16	15.85	0.19	1.22	-0.50	99.0
HT	2	2.04	0.06	2.89	1.18	101.9
	8	8.19	0.23	2.80	1.14	102.3
	16	16.03	0.16	0.97	0.40	100.2

Table 4. Standard addition outcome by the DS method

	CC			**HT**		
	Added (µg mL^{-1})					
	3	7	12	3	6	10
No.	**Found (µg mL^{-1})**					
1	2.95	7.07	12.38	3.10	6.01	10.22
2	2.93	6.83	12.34	2.99	6.24	10.20
3	2.89	7.33	12.40	2.88	6.00	9.87
4	2.99	7.08	12.36	3.00	6.20	10.46
5	2.89	6.92	12.31	2.98	5.89	10.20
	Recovery (%)					
No.	**CC**			**HT**		
1	98.4	101.1	103.1	103.4	100.2	102.2
2	97.8	97.6	102.8	99.7	103.9	102.0
3	96.4	104.8	103.3	96.0	100.0	98.7
4	99.8	101.1	103.0	100.1	103.3	104.6
5	96.5	98.9	102.6	99.3	98.1	102.0
Mean	97.8	100.7	103.0	99.7	101.1	101.9
SD	1.42	2.73	0.28	2.62	2.42	2.11
RSD	1.45	2.71	0.27	2.63	2.40	2.07
RE	-2.23	0.68	2.99	-0.29	1.12	1.91

Tablet Analysis

The outcomes gathered by applying the proposed technique to the CC-HT commercial preparation solutions are shown in Table 6. Accomplished results have been gathered for the quantitative determination of tablets containing CC and HT. In the determination of tablets, no interference with the tablet excipients in the determination of the concerned compounds was monitored when the DS method was applied to commercially available tablets.

In a brief conclusion, a rapid approach, DS, was applied to the simultaneous spectral quantification of CC and HT in their artificial mixtures and tablets. It was performed with analytical validation parameters to indicate the validity and applicability of the method.

Table 5. Results of ANOVA test

Compound	Source of Variation	SS	df	MS	F-calculated	P-value	F-crit
CC	Between groups	36.86	5	7.37	1.10	0.38	2.53
	Within groups	200.45	30	6.68			
	Total	237.31	35				
HT	Between groups	26.48	5	5.30	1.20	0.33	2.53
	Within groups	132.71	30	4.42			
	Total	159.20	35				

Table 6. Tablet assay by the DS method

Method	DS	
	CC (mg)	HT (mg)
Mean	15.80	12.76
SD	0.13	0.22
RSD	0.82	1.71
SE	0.04	0.07
CL	0.08	0.14

16.0 mg of CC and 12.5mg of HT per tablet

AUTHOR CONTRIBUTIONS

Conception: *Ö.Ü., E.D.*; Design: *Ö.Ü.*; Supervision: *Ö.Ü.*; Resources: *Ö.Ü., E.D.*; Materials: *Ö.Ü., E.D.*; Data collection and/or processing: *Ö.Ü.*; Analysis and/or interpretation: *Ö.Ü., E.D.*; Literature search: *Ö.Ü.*; Writing manuscript: *Ö.Ü., E.D.;* Critical review: *Ö.Ü.*; Other: *Ö.Ü., E.D.*

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Siddiqui, M.R., Alothman, Z.A., Rahman, N. (2017). Analytical techniques in pharmaceutical analysis: A review. *Arabian Journal of Chemistry*, *10*, 1409-1421. [CrossRef]

2. Valagaleti, R., Burns, P.K., Gill, M. (2003). Analytical support for drug manufacturing in the United States—from active pharmaceutical ingredient synthesis to drug product shelf life. *Drug Information Journal, 37,* 407-438. [CrossRef]

3. Ju, H. (2013). Grand challenges in analytical chemistry: towards more bright eyes for scientific research, social events and human health. *Frontiers in Chemistry, 1*, 5. [CrossRef]

4. El-Didamony, A.M., Saad, M.Z., Saleem, N.O. (2015). Spectrophotometric determination of some analgesic drugs in pharmaceutical formulations using N-bromosuccinimide as an oxidant. *Journal of the Association of Arab Universities for Basic and Applied Sciences, 17*, 43-50. [CrossRef]

5. Baghel, U.S., Singh, A., Singh, D., Sinha, M. (2017). Application of mass spectroscopy in pharmaceutical and biomedical analysis. Spectroscopic Analyses-Developments and Applications, IntechOpen book series, London. [CrossRef]

6. Khateeb, L.A., Al-Hakami, W.E. (2020). Reliable chromatographic determination of non-steroidal anti-inflammatory drugs in real samples matrices. *International Journal of Environmental Analytical Chemistry*. [CrossRef]

7. Baciu, T., Botello, I., Borrull, F., Calull, M., Aguilar, C. (2015). Capillary electrophoresis and related techniques in the determination of drugs of abuse and their metabolites. *TrAC Trends in Analytical Chemistry, 74*, 89-108. [CrossRef]

8. Özdokur, K.V. (2020). Voltammetric determination of isoniazid drug in various matrix by using CuOx decorated mw-cnt modified glassy carbon electrode. *Electroanalysis, 32*, 489-495. [CrossRef]

9. Chhonker, Y.S., Edi, C., Murry, D.J. (2018). LC–MS/MS method for simultaneous determination of diethylcarbamazine, albendazole and albendazole metabolites in human plasma: Application to a clinical pharmacokinetic study. *Journal of Pharmaceutical and Biomedical Analysis, 151*, 84–90. [CrossRef]

10. Sáiz, J., García-Ruiz, C., Gómara, B. (2017). Comparison of different GC-MS configurations for the determination of prevalent drugs and related metabolites. *Analytical Methods, 9,* 2897-2908. [CrossRef]

11. Maurer, H.H. (1995). Toxicological Analysis of Drugs: GC-MS Screening and Confirmation. In: P. Mangin and B. Ludes (Eds.), Acta Medicinæ Legalis, (pp 489-492). Springer, Berlin, Heidelberg. [CrossRef]

12. Daubechies, I. (1992). Ten Lectures on Wavelets, Society for Industrial and Applied Mathematics, Philadelphia, p.357. [CrossRef]

13. Dinç, E., Baleanu, D. (2007). Continuous wavelet transform applied to the overlapping absorption signals and their ratio signals for the quantitative resolution of mixture of oxfendazole and oxyclozanide in bolus. *Journal of Food and Drug Analysis, 15*(2), 109-117. [CrossRef]

14. Dinç, E., Baleanu, D., Üstündağ, Ö. (2003). An approach to quantitative two-component analysis of a mixture containing hydrochlorothiazide and spironolactone in tablets by one dimensional continuous daubechies and biorthogonal wavelet analysis of UV-spectra. *Spectroscopy Letters, 36,* 341-355. [CrossRef]

15. Redasani, V.K., Patel, P.R., Marathe, D.Y., Chaudhari, S.R., Shirkhedkar, A.A., Surana, S.J. (2018). A review on derivative UV-spectrophotometry analysis of drugs in pharmaceutical formulations and biological samples review. *Journal of the Chilean Chemical Society, 63*(3), 4126-4134. [CrossRef]

16. Charoo, N.A., Bashir, M., Abdalla, E., Ali, K.I.H. (2009). Determination of candesartan cilexetil in tablet dosage forms and dissolution testing samples by first derivative UV spectrophotometric methods. *Analytical Letters, 42*(14), 2232-2243. [CrossRef]

17. Belal, T.S., Daabeesb, H.G., Abdel-Khalekb, M.M., Mahrousb, M.S., Khamisb, M.M. (2013). New simple spectrophotometric method for determination of the binary mixtures (atorvastatin calcium and ezetimibe; candesartan cilexetil and hydrochlorothiazide) in tablets. *Journal of Pharmaceutical Analysis, 3*(2), 118-126. [CrossRef]

18. Gangola, R., Kaushik, S., Sharma, P. (2011). Spectrophotometric simultaneous determination of hydrochlorothiazide and telmisartan in combined dosage form. *Journal of Applied Pharmaceutical Science, 1*(1), 46-49. [CrossRef]

19. Erk, N. (2003). Application of first derivative UV-spectrophotometry and ratio derivative spectrophotometry for the simultaneous determination first derivative UV-spectrophotometry and ratio derivative spectrophotometry. *Pharmazie, 11,* 2580-2590. [CrossRef]

20. Khedr, A.E. (2008). Simultaneous determination of candesartan cilexetil and hydrochlorothiazide by high-performance liquid chromatography. *Journal of King Abdulaziz University, 15*(2), 3-13. [CrossRef]

21. Bonthu, M.G., Atmakuri, L.R., Jangala, V.R. (2018). Simultaneous determination of candesartan and hydrochlorothiazide in human plasma by LC-MS/MS. *Brazilian Journal of Pharmaceutical Sciences, 54*(1):17381. [CrossRef]

22. Annapurna, M.M., Venkatesh, B., Chaitanya, R.K. (2017). Quality by design approach (QbD) for the simultaneous determination of anti-hypertensive drugs (candesartan, irbesartan and hydrochlorothiazide) by RP-HPLC. *Journal of Analytical&Pharmaceutical Research, 4*(5), 00118. [CrossRef]