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SPECTROPHOTOMETRIC DETERMINATION OF DRUGS HAVING PRIMARY AMINE GROUP WITH p-DIMETHYLAMINOCINNAMALDEHYDE

Özlen GÜZEL*, Aydın SALMAN

İstanbul University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, 34116 Beyazıt- İstanbul, TURKEY

Abstract

A new spectrophotometric method for the determination of each dapsone and metoclopramide HCl alone in two different tablet preparations is described. The reactions were completed at room temperature within 9 and 20 minutes, respectively. Maximum absorbances for the reaction products were observed at 548 nm (dapsone) and 553 nm (metoclopramide HCl). Beer's law was obeyed over the concentration range of 4-12 µg/mL for dapson and 4-24 µg/mL for metoclopramide HCl. The molar absorptivity, Sandell sensitivity detection and quantification limits were also determined. The results of the methods were in good agreement with those obtained by reference methods cited in the literature. It was observed that the proposed method became suitable for quality control and routine analyses of tablets containing dapsone or metoclopramide HCl.

Key words: *Dapsone, Metoclopramide HCl, p-Dimethylaminocinnamaldehyde, Spectrophotometric determination.*

Primer amin grubu içeren ilaçların p-DAC ile spektrofotometrik tayini

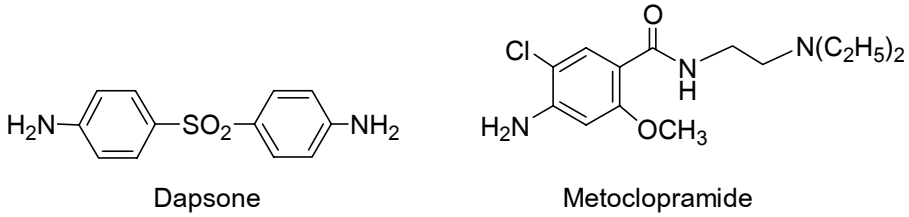
İki farklı tablet preparatındaki dapson ve metoklopramid HCl'in tayini için yeni bir metod verilmektedir. Reaksiyonlar oda sıcaklığında sırasıyla 9 ve 20 dakikada tamamlanmaktadır. Reaksiyon ürünlerinin maksimum absorbansları 548 nm (dapson) ve 553 nm (metoklopramid HCl) de gözlenmiştir. Dapson için 4-12 µg/mL ve metoklopramid HCl için 4-24 µg/mL konsantrasyon aralıklarında Beer kanunu geçerlidir. Molar absorptivite, Sandell duyarlılık tayini ve miktar tayini sınırları saptanmıştır. Bu metodların sonuçları, literatürde bildirilen metodların sonuçları ile uygunluk göstermektedir. Bildirilen metodun dapson veya metoklopramid HCl içeren tabletlerin kalite kontrol ve rutin analizleri için uygun olduğu görülmüştür.

Anahtar Kelimeler: *Dapson, Metoklopramid HCl, p-Dimetilaminosinnamaldehid, Spektrofotometrik miktar tayini.*

* **Correspondence:** Tel.: +90 212 455 57 00/15526; Fax: +90 212 440 02 52;
E-mail: ozlen_guzel@yahoo.com

INTRODUCTION

Dapsone (DAP), chemically 4,4'-diaminodiphenyl sulfone, has been known as an important antileprotic drug in addition to its antimalarial properties. Metoclopramide HCl (MET), chemically 4-amino-5-chloro-N-[(2-diethylamino)ethyl]-2-methoxybenzamide, is an antiemetic in treatment of gastrointestinal disordes. Chemical structures of DAP and MET are given in Scheme 1.



Dapsone Metoclopramide

Scheme 1. Structural formulas of DAP and MET.

In view of their pharmacological importance, considerable work has been done for their detection and quantification. In the literature, DAP has been quantitatively determined by colorimetry using diazo reaction (1-5), 9-chloroacridine (6), 4-chloro-5,7-dinitrofurazon (7) sodium 1,2-naphthoquinone-4-sulfonic (8) and by UV spectrophotometric method (9). MET is determined by colorimetry using chloranil (10), 1,2-naphthoquinone (11), diazo reaction (12-19) and by UV spectrophotometric method (20). p-Dimethylaminocinnamaldehyde (p-DAC) is used for the determination of primary and secondary amines (21,22).

In the present work a new spectrofotometric method was developed for the determination of the subjected drugs in pure and dosage forms. This spectrofotometric determination of DAP and MET in single-component commercial tablet preparations (Servidapson® tablet and Metpamid® tablet, respectively) is based on the reaction between drug's primary amine group and p-DAC in acidic medium. The result obtained from the developed spectrophotometric method was compared with those obtained by literature method and a good agreement was observed from this comparision.

EXPERIMENTAL

Apparatus:
UV-Vis spectrophotometer : A Shimadzu UV-1601 UV-visible Spectrophotometer with 1 cm matched glass cells was used for absorbance measurements.

Materials and Reagents:
Servidapson® tablet contains 100 mg dapson/tablet (Ciba-Geigy/Swiss).
Metpamid® tablet contains 10 mg metoclopramide HCl/tablet (Sifar-Istanbul/Turkey).
p-Dimethylaminocinnamaldehyde *(p-DAC)* (Merck-Schuchardt).
DAP and MET were kindly obtained from Ciba-Geigy/Swiss and Sifar-Istanbul/Turkey, respectively.

Standard Solutions:

Stock DAP solution (**I**) was prepared by dissolving 4 mg of DAP in 100 mL of methanol.

Stock MET solution (**II**) was prepared by dissolving 8 mg of MET in 100 mL of methanol.

Reagent Solutions:

p-DAC_1 solutions was prepared by 11.25 mg of p-DAC in methanol in 100 mL calibrated flask.
p-DAC_2 solution was prepared by dissolving 920 mg of p-DAC in methanol in 100 mL calibrated flask.

Buffer solution :

KCl-HCl buffered solution (pH=1.0) was prepared by mixing 25 mL 0.2 N KCl and 48.5 mL 0.2N HCl in 100 mL calibrated flask.
Other chemicals and solvents used in this study were of analytical reagent grade.

General Procedure

Preparation of calibration graphs

For preparation of DAP calibration graph, 1-3 mL of **I** was reacted with 1 mL of p-DAC_1, and 0.1 mL of KCl-HCl buffered solutions (pH=1.0), adjusted to 10 mL with methanol, kept at room temperature for 9 minutes (n=6), and the absorbances were measured at 548 nm against a blank solution.

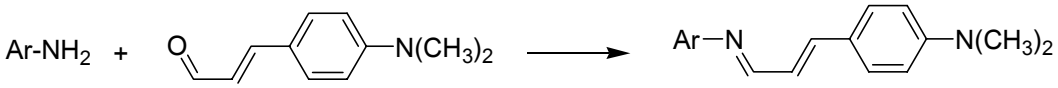
For preparation of MET calibration graph, 0.5–3 mL of **II** was reacted with 1 mL of 6 N HCl and 4 mL p-DAC_2 and adjusted to 10 mL with methanol, kept for 20 minutes at room temperature (n=6) and the absorbances measured at 553 nm against a blank solution.

Assay procedure for tablets

Accurately weighed quantities of powdered tablets equivalent to about 8 mg of the active substance were extracted with 3x15 ml of methanol, on a water bath, filtered and then diluted to 100 mL with methanol. The extracts were checked by TLC. Assays were performed as described under general procedure.

RESULTS AND DISCUSSION

In the present study, we developed visible spectrophotometric methods for determination of each drug alone in two different tablet preparations containing DAP and MET. The complex reactions of DAP and MET with p-DAC are based on the interaction between drug's primary amine group (Ar-NH_2) and p-DAC in acidic medium as shown in Scheme 2.



Scheme 2. Reaction between p-DAC and primary amines of drugs.

These reactions yielded red colored complex with maximum absorptions at 548 nm for DAP and at 553 nm for MET; the absorption spectra for these complex products were presented in Figure 1. The formation of these colored products were used in the development of the spectrophotometric method for the determination of each DAP and MET drugs alone in two different commercial tablet formulations.

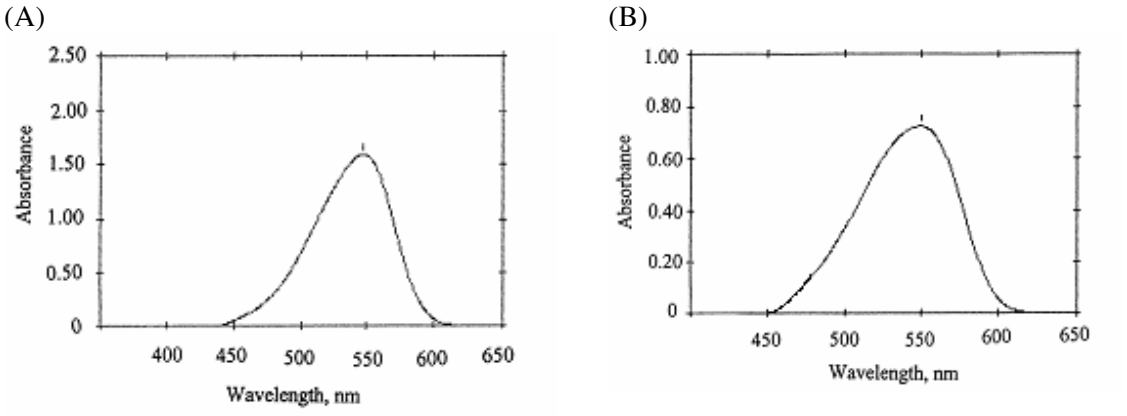


Figure 1. Absorption spectra of the reaction products of DAP (A) and MET (B) with p-DAC.

In order to determine the optimal conditions for the assay procedures, some experimental parameters were investigated. These are explained below.

For example, various solvents were tested and methanol was found to be suitable for our experimental conditions. In case of methanol, p-DAC is very soluble while the excipients of tablets is not soluble. To find the optimal pH medium for the assay, various quantities of acidic buffer solutions were added; maximum absorbance was obtained when 0.1 mL of KCl-HCl buffered solution, pH=1 and 1 mL of 6 N HCl were used for DAP and MET, respectively. Similarly, the amounts of reagent which produced optimal absorbances, 4 fold reagent for DAP and 800 fold reagent for MET, were determined by gradually increasing the mole ratio of p-DAC to the substances. DAP solution was found to proceed quantitatively after 9 minutes and the MET solution after 20 minutes, at room temperature, as shown in Figure 2.

Under the optimum conditions described above, the calibration graphs for DAP and MET were obtained by using the relationship between concentration and its corrosponding absorbance.

The molar absorbtivity, Sandell sensitivity (S), concentration range, regression equation and correlation coefficient for each drug are shown in Table 1. A linear relationship was found between the absorbances at λ_{max} and the concentrations of MET and DAP in the range 4-24 μg/mL and 4-12 μg/mL, respectively. Regression analysis of the Beer's law plotted at λ_{max} reveals a good correlation (r = 0.9980-0.9986). The graphs showed a negligible intercept, which were calculated by the least-squares method's regression equation, A=a + bC (where A is the absorbance of 1 cm layer, a is the intercept, b is the slope, and C is the concentration of the measured solution in μg mL^{-1}). The high molar absorptivities ($3.12x10^3$ - $2.582x10^4$) of the

resulting colored complexes indicated high sensitivity of the methods. The method was found to be sensitive with the high ε values. Limit of detection (LOD) and limit of quantitation (LOQ) were determined using the formula: LOD or LOQ = κSD/b, where κ=3 for LOD and 10 for LOQ, SD and b for standard deviation of the intercept and slope, respectively. The results were as shown in Table 1.

The proposed method was compared with the UV method cited in USP XXII (9) for DAP and BP 1993 (20) for MET and the results obtained were statistically evaluated (Table 2).

For the proposed method, calculated *t* and *F* values are lower than theoretical values for both substances.

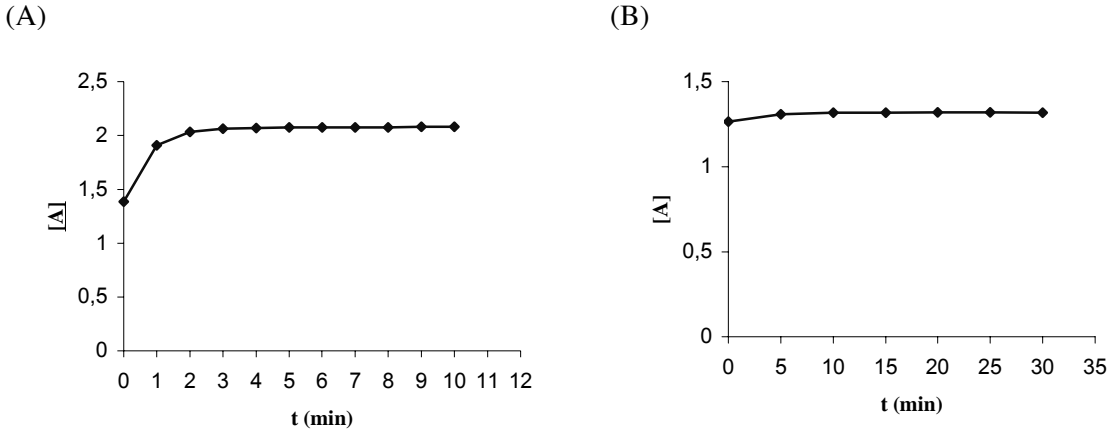


Figure 2. The effect of duration on the reaction of DAP (A) and MET (B) with p-DAC.

Table 1. Optimal characteristics and statistical data of the regression equations for the DAP and MET reactions with p-DAC.

	DAP	MET
Parameter	Developed method	Developed method
λ_{max} (nm)	548	553
Beer's law limit [a] (μg mL^{-1})	4-12	4-24
Molar absorptivity (1 mol^{-1} cm^{-1})	$3.12x10^{3}$	$2.582x10^{4}$
Sandell's sensitivity (μg cm^{-2} per 0.001 absorbance unit)	0.0079	0.013
Regression equation [b]		
Slope±SD	$21.05x10^{-2}$±$45.6x10^{-3}$	$76.2x10^{-4}$±$14.7x10^{-3}$
Intercept±SD	$-48.64x10^{-2}$±$5.3x10^{-3}$	$-12.1x10^{-3}$±$9x10^{-4}$
Correlation coefficient, *r*±SD	$99.80x10^{-2}$±$2.9x10^{-3}$	$99.86x10^{-2}$±$1.1x10^{-3}$
LOD (μg mL^{-1})	0.039	1.12
LOQ (μg mL^{-1})	0.1330	3.740

[a] Average of six determination

[b] $A = a + bC$ (where C is the concentration of drug in μg mL^{-1}

Table 2. Analysis of DAP and MET in tablets by the proposed method and comparison UV method

Preparation	Label claim, mg / tablet	mg/tablet±Standard deviation, % (n=6) Developed method	UV method	*t*-test [a]	*F*-test [a]
Servidapson®	100	100.25 ± 0.88	101.80 ± 0.54	2.02	2.65
Metpamid®	10	9.5 ± 1.03	9.4 ± 0.97	1.86	1.13

[a]Theoretical values at 95% confidence limit; t=2.23 and F=5.05

Precision

The precision of the proposed method was investigated by intra-day and inter-day determinations of DAP and MET at three different concentrations of DAP (4, 8 and 12 μg/mL) and MET (4, 12 and 24 μg/mL). The intra-day studies were performed in one day (for each level n=5) and inter-day studies in five days over a period of two weeks. The intra and inter-day precisions expressed as relative standard deviation values (RSD %) for DAP were found to be within 0.87-1.99 % and 0.99-2.03 %, respectively. For MET these values were found within 0.67-1.78 and 0.89-1.89 (Table 3). The data proved good precision for the developed method.

Table 3. Results from intra-day and inter-day precision experiments.

	Actual concentration (μg/mL)	intra-day X_{ort}±SE	RSD (%)	% Bias	inter-day X_{ort}±SE	RSD (%)	% Bias
DAP	4	4.01 ± 20.26	1.99	-0.25	4.03 ± 20.56	2.03	-0.75
	8	8.03 ± 4.42	0.87	-0.37	8.06 ± 5.01	0.99	-0.75
	12	12.01 ± 3.46	1.02	-0.08	12.07 ± 5.58	1.65	-0.58
MET	4	3.99 ± 18.21	1.78	0.25	4.03 ± 19.14	1.89	-0.75
	12	12.06 ± 3.34	0.99	-0.50	12.26 ± 4.19	1.26	-2.16
	24	23.96 ± 1.13	0.67	0.16	24.02 ± 1.51	0.89	-0.08

SE: Standart error

Robustness and Ruggedness

The robustness of the proposed method was examined by evaluating the influence of small variations of the procedure variables such as time of the reaction, added reagent volume and pH of the medium, For the ruggedness of the method, the proposed method was carried out by two analysts and no considerable difference was observed (100.66 ± 0.65 % vs. 100.81 ± 0.71 %).

Table 4. Results from robustness experiments

		Average of found concentration	S.D.	R.S.D. (%)	Recovery (%)
DAP	Added reagent volume (1+0.05mL)	5.08	0.025	0.492	101.6
	Added reagent volume (1-0.05mL)	5.03	0.036	0.715	100.6
	Time of reaction (9+0.5 min.)	5.07	0.044	0.867	101.4
	Time of reaction (9-0.5 min.)	5.01	0.053	1.057	100.2
	KCl-HCl buffered Solutions (pH=1.0+0.5)	5.06	0.035	0.691	101.2
	KCl-HCl buffered Solutions (pH=1.0-0.5)	5.03	0.042	0.834	100.6
MET	Added reagent volume (4+0.05mL)	5.10	0.057	1.117	102.0
	Added reagent volume (4-0.05mL)	5.09	0.049	0.962	101.8
	Time of reaction (20+0.5 min.)	5.03	0.032	0.636	100.6
	Time of reaction (20-0.5 min.)	5.04	0.063	1.250	100.8

Table 5. Analysis of DAP and MET from various excipients by the proposed method.

Name of the compound	Amount present (mg)	Excipients (mg)						% Recovery ± SD[a]
		Talc	Dextrose	Starch	Sodium algenate	Gelatin	Gum acacia	
DAP	100	10	10	10	5	5	5	99.8±0.9
MET	10	20	30	20	20	10	20	99.5±1.0

[a] Average recovery from five experiments.

The obtained reproducible results (Table 4) showed that none of these variables and changes significantly affected the assay of drugs.

The developed method produced accurate and reproducible results and was applicable to the available dosage form, tablets.

Accuracy

In order to determine the accuracy of the proposed method, recovery measurements are performed on synthetic samples. The tablet excipients used were found not to interfere with the measurements. The results are shown in Table 5.

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