Individual and Simultaneous Spectrophotometric Determination of Dapsone and Metoclopramide HCl in Pharmaceutical Dosage Forms and Synthetic Binary Mixtures

Ahmed Ahmed OMRAN[1)]

Department of Chemistry, Faculty of Science, Al-Azhar University; Assiut, 71524, Egypt.
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A rapid, sensitive and selective spectrophotometric method has been developed for the quantitative determination of dapsone (DAP) and metoclopramide hydrochloride (MCP) in both pure and dosage forms. Individual and simultaneous methods are based on the diazo coupling reaction of these drugs with benzoylacetone (BAC) in alkaline medium. The resulting azo dyes exhibit maximum absorption at 437 and 411 nm with a molar absorptivity of 4.14×10^4 and 2.97×10^4 l mol^{-1} cm^{-1} for DAP and MCP, respectively. Simultaneous determination of DAP and MCP was developed utilizing first-order digital derivative spectrophotometry. All variables have been optimized. No interferences were observed from drug excipients and the validity of the methods was tested against reference methods.

Key words dapsone; metoclopramide; simultaneous determination; synthetic mixture; dosage form

Dapsone (DAP), 4,4′-diaminodiphenyl sulfone, is used to treat leprosy and other skin conditions.[2)] It may also be used to prevent malaria, for certain types of arthritis or other inflammatory conditions and pneumocystis carinii pneumonia (PCP). Metoclopramide hydrochloride (MCP), chemically, 4-amino-5-chloro-[2-(diethylamino)ethyl]-2-methoxybenzamide HCl is used to relieve certain stomach and esophagus problems such as diabetic gastroparesis and gastroesophageal reflux disorder (GERD).[3)] Oral MCP may also be used to prevent nausea and vomiting caused by other medications. Moreover, MCP may be used in the fields of surgery, gynecology, cardiology and radiology.

Due to their wide administration, considerable work has been done for determination of DAP and MCP include HPLC,[4—7)] electron capture gas chromatography[8—10)] and GC-MS[11)] but involve an expensive experimental setup. Official methods for the determination of these drugs are usually based on extraction of the drug as a free base and its subsequent determination by UV-spectrophotometry.[12,13)] Thus these methods suffer from strongly interferences of drug excipients and diluents. Spectrophotometric determination of these drugs has been proposed based on diazotization,[14—17)] ion pair complexes,[18)] charge transfer complexes,[19,20)] and the formation of coloured Schiffs base upon interaction with dimethylaminocinnamaldehyde[21)] and use of 9-chloroacridine[22)] and sodium 1,2-naphthoquinone-4-sulfonic[23)] as chromogenic reagents. These methods involve a time-consuming extraction procedures or heating and require strictly controlled reaction conditions. Many of these methods are less sensitive.

For routine quality control, development of a simple, rapid and sensitive spectrophotometric method is highly desirable. In this paper, the employment of benzoylacetone as a new coupling agent for simple, rapid, accurate, precise and an inexpensive method for determination of DAP and MCP in bulk and pharmaceutical preparations is described. Furthermore, a simple first derivative spectrophotometric procedure has been applied to the simultaneous determination of DAP and MCP in synthetic binary mixture and pharmaceutical preparations without prior separation.

Experimental

Apparatus A double-beam UV/Vis spectrophotometer (Perkin Elmer, lambda 3B) interfaced to IBM compatible computer with the capability of derivative mode operation was used. The spectra were recorded in 1-cm matched quartz cells. Suitable settings for recording the first-derivative spectra were: scan speed, 120 nm/min; response time, 2 s; spectral slit width, 2 nm; $\Delta\lambda$, 1 nm; the ordinate minimum and maximum were ±0.2 for the determination of DAP and MCP. The derivative spectra were obtained by numerical calculation with the aid of PECSS program using Savitzky–Golay algorithm.[24)] Measurement of pH was carried out with a Janco electronics LTD digital pH-meter with a combined glass Calomel electrode.

Reagents and Chemicals All chemicals were of analytical-reagent grade. Benzoylacetone (BAC, 2×10^{-3} M): Prepared by dissolving 32.4 mg of BAC in 100 ml of methanol. Sodium nitrite (1% m/v): Prepared by dissolving 1 g of sodium nitrite in 100 ml of distilled water. A 2% ammonium sulfamate was prepared by dissolving 2 g of ammonium sulfamate in 100 ml distilled water. Hydroxide–chloride buffer of pH=13 was prepared by mixing 25 ml 0.2 M KCl (14.9 g/l) and 66 ml of 0.2 M NaOH (8.0 g/l) and diluted to 100 ml with distilled water.[25)] Hydrochloric acid, 1 M solution was used.

Standard Solution DAP and MCP hydrochloride were purchased from Sigma chemical Co. (St. Louis, MO, U.S.A.) and used without further purification and purity was confirmed by ^{1}H-NMR, Thin layer chromatography and by melting point measurements. Methanolic solution of DAP and aqueous solution of MCP were prepared daily by dissolving 100 mg of the sample in 100 ml of methanol or water, respectively (1000 μg/ml). Working solutions were prepared as required by dilution with methanol and distilled water for DAP and MCP, respectively.

Standard Procedure. Determination of DAP and MCP Utilizing BAC An aliquot of standard solution containing 0.3—5.0 ml (75 μg ml^{-1}) of DAP or 0.2—3.3 ml (100 μg ml^{-1}) MCP was transferred quantitatively into a series of 25 ml standard flasks. To this solution 1 ml of 1% sodium nitrite was added and the acidity was adjusted with 1 ml of 1 M hydrochloric acid. The solutions were shaken thoroughly for 2 min to allow the diazotization reaction to go to completion. To the resultant solution, 1 ml of 2% ammonium sulfamate solution was added to each flask with swirling for another 2 min to expel N_2. Added 2 ml of 2×10^{-3} M BAC solution and complete to the mark with hydroxide–chloride buffer and mixed well. After 5 min, the absorbance of the coloured azo dye was measured at 437 and 411 nm for DAP and MCP, respectively. The reference solutions were similarly made without adding the drug solution.

Procedure for the Determination of DAP and MCP in Dosage Forms Twenty tablets were accurately weighed and pulverized. A portion of the fine and homogenized powder equivalent to 100 mg of drug was accurately weighed and transferred into a 50 ml volumetric flask containing approx. 25 ml of methanol for DAP (distilled water for MCP). The mixtures were thoroughly shaken for 30 min, sonicated for 5 min, cooled and then the volume was brought to 50 ml with methanol and distilled water for DAP and MCP, respectively. The solutions obtained were filtered and the first 10 ml of

e-mail: ahmed_omran@yahoo.com

Table 1. Analysis of DAP and MCP in Various Dosage Forms

Sample	Proposed method[a]				Reference method[12]	*t*-test[b]	*F*-test[c]
	Amount taken (μg ml^{-1})	Amount found (μg ml^{-1})	%Rec±S.D.	CV (%)	%Rec±S.D.		
DAP							
Dapsone tab (25 mg)	2	1.99	99.5±0.6	0.39	99.8±0.8	1.94	1.56
	6	5.98	99.7±0.5	0.35	100.9±0.5	1.87	1.49
	10	10.03	100.3±0.5	0.24	99.9±0.4	1.83	1.17
Dapsone tab (100 mg)	4	4.02	100.5±0.4	0.28	99.7±0.5	1.78	1.11
	8	7.99	99.9±0.2	0.31	100.5±0.4	1.57	1.33
	10	9.98	99.8±0.6	0.42	100.2±0.5	1.72	1.29
MCP							
Primperan tab. (10 mg)	4	4.01	100.3±0.7	0.53	99.9±0.6	1.47	1.21
	6	6.0	100.0±0.3	0.77	99.5±0.4	1.14	1.45
	8	7.97	99.6±0.4	0.66	98.8±0.7	1.31	1.84
Vomistop tab. (7.5 mg)	4	3.99	99.8±0.5	0.39	100.3±0.6	0.98	1.10
	8	7.92	99.0±0.7	0.53	99.6±0.6	0.77	0.97
	10	9.97	99.7±0.6	0.28	100.6±0.5	1.13	1.08
Primperan inj. (10 mg/2 ml)	2	2.01	100.5±0.3	0.68	100.3±0.4	0.79	0.86
	3	2.97	99.0±0.5	0.72	100.1±0.2	0.37	0.58
	5	4.94	98.8±0.7	0.81	99.8±0.6	1.25	1.40
Meclopram tab. (10 mg)	4	3.98	99.5±0.6	0.71	99.8±0.5	1.31	1.23
	6	6.01	100.2±0.3	0.54	100±0.4	0.92	0.84
	8	7.97	99.6±0.7	0.45	96.8±0.7	1.11	1.05

a) Average of five determinations. *b*) Tabulated value 2.78. *c*) Tabulated value 6.39.

filtrate were discarded. Requisite amount (200 μg/25 ml) of the drug solution was taken and the above standard procedure was followed for the assay of drug content in tablets.

For the analysis of ampoules, the requisite volume was transferred quantitatively into a 50 ml standard flask and diluted to the volume with methanol (H_2O for MCP). The drug content in the diluted solutions was assayed as mentioned above. The results of the analysis are given in Table 1.

Simultaneous Determination of DAP and MCP Using BAC To a 25 ml calibrated flask containing 22.0—380.0 μg of DAP and 20.0—332.5 μg MCP were added 1 ml of 1% sodium nitrite and 1 ml HCl (1.0 M). The solution was allowed to stand 2 min and 1 ml of 2% ammonium sulfamate was added. Add 2 ml of BAC solution (2×10^{-3} M) and complete to the mark with hydroxide–chloride buffer. The absorption spectra were recorded, against a blank solution similarly prepared, and the first derivative values of the absorption spectra were measured at 411 and 437 nm where the concentrations of DAP and MCP could be determined, respectively.

Results and Discussion

Optimization of Variables. Effect of Benzoylacetone The influence of BAC concentration was studied by measuring the absorbance at the specified wavelengths in standard procedure for solutions containing the same drug concentration at varying amounts of BAC. A volume of 2 ml BAC 2×10^{-3} M in a total volume of 25 ml was found to be sufficient for full color development.

Effect of Sodium Nitrite The optimum concentration of sodium nitrite solution was found to be 1 ml of 1% (w/v) solution in a total volume of 25 ml of the reaction mixture. The excess nitrite could be removed by the addition of 1 ml of 2% (w/v) ammonium sulfamate.

Effect of Acidity on Diazotization For diazotization reaction, the use of hydrochloric acid as reaction medium was found to give more satisfactory results than sulphuric acid. A suitable acidity, as evident from the full color development and the stability of the coupled products was found to be 1 ml of 1 M HCl per 25 ml of the final volume. Fortunately, diazotization was carried out at room temperature (25±2 °C) and there was no need for cooling to 0—5 °C.

Effect of Alkalinity Completing the mixture solutions to the mark with hydroxide–chloride buffer fulfilled the optimum alkalinity. No partial decolorization observed due to the excess volume of buffer quantity.

Effect of Reaction Time Full coloured azo dyes were developed rapidly after the sequence addition of the reagents and the maximum absorbance was attained after 5 min at room temperature (*ca.* 25 °C). The colour was stable for a period more than 3 h for both drugs and their mixture.

Effect of Foreign Species on Synthetic Mixtures A systematic study of the foreign potentially interfering species effect was performed on the simultaneous determination of DAP and MCP in laboratory prepared synthetic mixtures following the basic procedure for 10 sample systems. Tolerance was defined as the excipient or interfering concentration that produced an error not exceeding 3% in the determination of analyte. The tolerance limits (interferent/analyte; w/w molar ratio) for dextrose, glucose, sucrose, talc, starch, sodium algenate, magnesium stearate and gum acacia are >400, 400, 400, 300, 150, 40, 25, 20, respectively. The results clarify that the proposed first derivative method has a good tolerance level for the tested species.

Absorption Spectra and First-Derivative Mode The zero- and first-order derivative spectra of the coloured azo dyes of DAP (curve 1) and MCP (curve 2) and a mixture of both (curve 3) are shown in Figs. 1a and b, respectively. The coloured product shows maximum absorption at 437 nm for DAP and at 411 nm for MCP. The close overlap of the absorption spectra of DAP and MCP coloured products prevents the correct use of zero-order absorption measurements for their simultaneous determination in binary mixtures. To overcome such difficulty, derivative spectrophotometric method was applied. The derivatization of zero-order spectra leads to poor resolution of the first-derivative spectra of the mixture (*i.e.* absence of separated peaks). Thus a zero-crossing measurement technique was utilized. In Fig. 1b, zero-

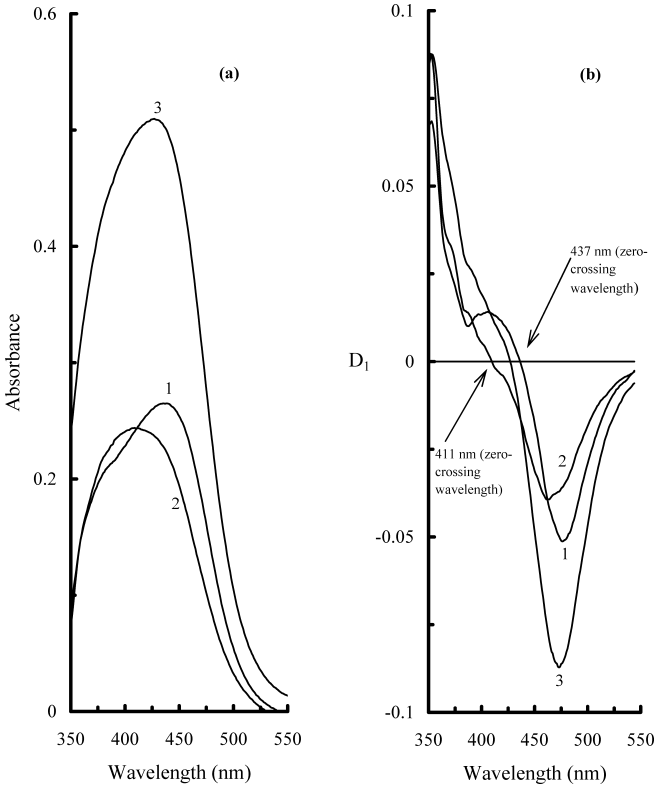

Fig. 1. (a) Zero- and (b) First-Derivative Spectra of Azo Products of DAP (Curve 1), MCP (Curve 2) and Mixture of DAP and MCP (Curve 3)
[DAP]=1.49 μg ml^{-1} and [MCP]=4.03 μg ml^{-1}.

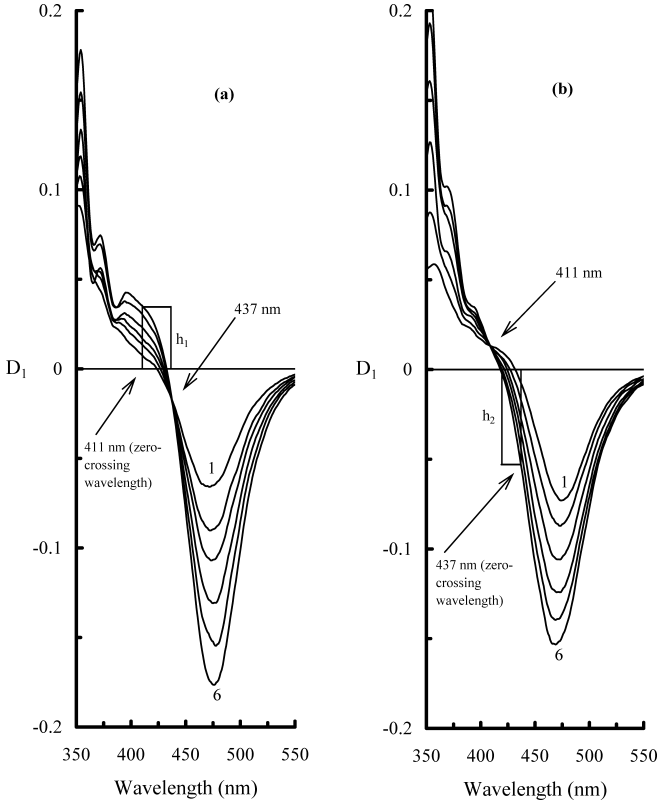

Fig. 2. First-Derivative Spectra of DAP and MCP Azo Products
(a) [MCP]=4.03 μg ml^{-1}; [DAP]=0.89, 1.79, 2.38, 2.98, 3.58 and 4.17 μg ml^{-1}. (b) [DAP]=1.49 μg ml^{-1}; [MCP]=2.02, 4.04, 6.05, 8.07, 10.89 and 12.01 μg ml^{-1}. These concentrations correspond lines 1, 2, 3, 4, 5 and 6, respectively. h_1 and (h_2) are the first derivative intensities of various concentrations of DAP (MCP) in the presence of constant concentration of MCP (DAP).

crossing wavelengths of DAP and MCP azo dyes were 437 and 411 nm, respectively.

To select the derivative order, a first-, second-, third- and fourth-order derivative spectra of DAP and MCP azo dyes were studied. The study revealed that the first-derivative spectra are simple and give results of highest accuracy and lowest detection limits.

Figure 2a shows a set of first-derivative spectra of mixtures containing 4.03 μg ml^{-1} of MCP plus increasing amounts of DAP (0.89—4.17 μg ml^{-1}). Figure 2b represents a further set performed by keeping the DAP concentration constant at 1.49 μg ml^{-1}, while MCP concentration was varied from 2.02—12.01 μg ml^{-1}. Figure 2 indicates that when the concentration of MCP (DAP) was kept constant and the DAP (MCP) concentration was varied, the peak amplitudes at 437 nm (411 nm) was unaltered. The amplitudes at 411 nm (h_1) and 437 nm (h_2) were proportional to DAP and MCP concentrations, respectively.

Quantification and Analytical Data The wavelengths (λ; nm), regression equations, linear ranges (μg ml^{-1}), correlation coefficients, detection limits (μg ml^{-1}) and quantitation limits (μg ml^{-1}) for determination of DAP by using zero-order and (first-order) derivative spectrophotometry are 437 (411), $A=0.167C+0.004$ ($D_1=0.0048C+1.1\times10^{-4}$), 0.9—15.0 (0.88—15.2), 0.9998 (0.9999), 0.0414 (0.0401) and 0.1254, (0.1215), respectively. The corresponding results for MCP determination are 411 (437), $A=0.088C+0.017$ ($D_1=0.0043C+8.3\times10^{-4}$), 0.8—13.2 (0.8—13.3), 0.9995 (0.9997), 0.0332 (0.0329) and 0.1006 (0.0996) by using zero- and (first-) order derivative spectrophotometric methods. A, absorbance; D_1, first derivative intensity; C, the concentration in μg ml^{-1} and number of specimens, $n=10$.

Reaction Sequence The method involves the diazo coupling reaction of DAP and/or MCP with BAC in an alkaline medium to give an orange coloured azo dyes with a maximum absorption of 437 and 411 nm for DAP and MCP, respectively. The absorption spectra of DAP, MCP and DAP-MCP mixture are shown as in Fig. 1. In the first step, the drug is treated with nitrite solution in acidic medium, undergoes diazotization to give the diazonium ion. In the second step, the diazonium ion is coupled with the active methylene group of BAC to form an azo dye in an alkaline medium. The reaction can be represented as shown in Chart 1. Here MCP is used as an example since DAP behaves in a similar manner.

Applications. Individual Determination of DAP and MCP in Pharmaceutical Formulations The reproducibility of the method was checked by ten replicate determinations at 5 μg ml^{-1} of DAP or MCP and the standard deviation (S.D.) was found to be 0.7—1.2% and 0.8—1.3% for DAP and MCP, respectively. The present method was successfully applied for a direct determination of DAP and MCP in various pharmaceutical formulations. The result of an assay of Dapsone, Vomistop, Meclopram and Primperan tablets and injection solutions are presented in Table 1 and compared favorably with those reported by the official method.[12] Table 1 clearly indicates that the proposed method to be highly sensitive and gives reproducible results.

Simultaneous Determination of DAP and MCP in Syn-

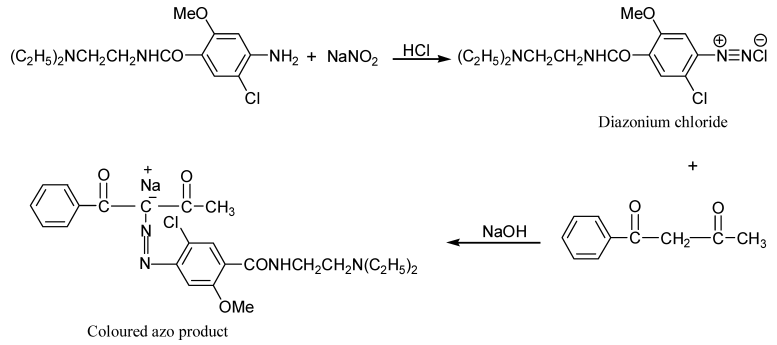

Chart 1. Proposed Reaction Mechanism of MCP with BAC

thetic Mixtures Simultaneous determination of DAP and MCP in synthetic mixtures was performed using the proposed procedure. Satisfactory results were found for different ratio of mixtures, from DAP : MCP = 1 : 9 to 9 : 1, and the mean recoveries were 97.7 and 101.3% for DAP and MCP, respectively.

In Pharmaceutical Preparations Due to the difficulty to find out both DAP and MCP in one pharmaceutical preparation, analytical samples were prepared by mixing two commercial products dapsone 25 mg and primperan 10 mg (Metoclopramide HCl preparation) produced by Memphis and El-Nile companies, respectively. 0.5 g of primperan and 1.25 g dapsone formulations were taken and the amounts found (mean ± standard deviation of five determinations) by the proposed derivative procedure were 0.46 ± 0.03 and 1.3 ± 0.04 for MCP and DAP at zero-crossing wavelengths of 437 and 411 nm, respectively. The results indicate a reasonable harmony with the composition quoted by supplier.

Conclusion

The results obtained in this work present a first time practical application of benzoylacetone as a new coupling agent for the determination of DAP and MCP in both pure and dosage forms. The proposed method is simple, rapid, and selective and offers the advantages of high sensitivity and a wide range of determination without the extraction or heating. Moreover, the combination of the proposed method with spectral differentiation allows the simultaneous determination of DAP and MCP without previous separation. This should be useful in routine quality control.

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