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RESEARCH PAPER

Spectrophotometric Determination of Some Chemotherapeutic Agents Using Acetyl Acetone

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ABSTRACT

Acetyl acetone is introduced as a new coupling agent for the spectrophotometric determination of some chemotherapeutic agents, such as metoclopramide, dapsone, p-*aminobenzoic acid, and cisapride in both pure and dosage forms. The method is based on the diazo-coupling reaction of these chemotherapeutic agents with a new coupling agent, acetyl acetone, in an alkaline medium. The optimum reaction conditions and other analytical parameters are evaluated. The influence of the substrates commonly employed as excipients with these chemotherapeutic agents has been studied. The method is simple, rapid, and sensitive. The results obtained compare favorably with those obtained with other reference methods.*

Key Words: *Acetyl acetone; Chemotherapeutic agents; Spectrophotometry*

INTRODUCTION

Metoclopramide hydrochloride (MCP) is a potent antiemetic and antispasmodic agent. It has been found to be remarkably useful in the treatment of drug-induced nausea and vomiting, including cancer chemotherapy. Dapsone (DAP) is an antileprotic drug. Cisapride (CPD) is a substituted piperidinyl benxamide, chemically related to MCP, it has been used to stimulate gastrointenstinal motility and also used as an alternative to MCP in the management of diabetic gastroparesis. *p*-Aminobenzoic acid (PABA) is a skin-protective agent. In view of their pharmacological importance, considerable work has been done for their detection and quantification. Various analytical techniques have been

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developed for the determination of these drugs in both pure and dosage forms, including high-performance thin-layer chromatography (1), HPTLC (2), and electron-capture gas–liquid chromatography (3), but they involve expensive experimental setup. Official methods used for the determination of these drugs in pharmaceutical preparations are usually based on non-aqueous titrimetry or ultraviolet spectrophotometry (4–6).

The determination of these chemotherapeutic agents by spectrophotometric methods has been proposed based on diazotization (7–10), ion-pair complexes (11–13), charge-transfer complexes (14), and the formation of colored dye by the coupling reaction between the oxidation product of 3-methyl benzothiazolin-2-one hydrazone (MBTH) (15,16) and catechol (17). Other chromogenic reactions use sodium vanadate (18), ammonium meta vanadate (19), nitrous acid-cresyl fast violet acetate (CFVA) (20), and 9-chloroacridine (21). These methods involve a time-consuming extraction step or heating, and require strictly controlled reaction conditions. Many of these methods are not sensitive. In addition, fluorimetric (22) and polarographic (23) methods have been reported for the assay of cisapride. The widespread use of these chemotherapeutic agents has necessitated the development of a rapid, facile, and sensitive spectrophotometric method for their quality control.

In the present paper, a study of the spectrophotometric determination of chemotherapeutic agents is described. The proposed method is a simple, accurate, sensitive, and inexpensive method for the assay of MCP, DAP, PABA, and CPD in bulk and in pharmaceutical formulations, employing acetyl acetone as a new coupling agent.

MATERIALS AND METHODS

Apparatus

Jasco model UVIDEC-610 and Elico model CL-27 spectrophotometers were used for all absorbance measurements.

Reagents

All chemicals used were of analytical reagent grade.

- 5% Acetyl acetone (AA): prepared by diluting 5.1 mL of AA in 100 mL of methanol.
- Sulfamic acid (3%): freshly prepared by dissolving 3 g of sulfamic acid in 100 mL of distilled water.
- Sodium nitrite (0.1%): prepared by dissolving 1 g of sodium nitrite in 1000 mL of water.
- Sodium hydroxide (4 M): prepared by dissolving 16 g of sodium hydroxide pellets in 100 mL of water.
- Hydrochloric acid: 1 M solution was used.

Standard Solutions of Chemotherapeutic Agents

Separate aqueous solutions of MCP and PABA and methanolic solutions of DAP and CPD were prepared. The concentrations of these solutions are equivalent to 1000 $\mu g\,mL^{-1}$. Solutions of lower concentration were prepared by diluting the standard solutions.

Standard Procedure

An aliquot of the sample solution containing 50–400 μg of MCP, 2.5–40 μg of DAP, 25–200 μg of PABA, or 40–350 μg of CPD was transferred into a series of 25-mL standard flasks. To this solution was added 2 mL of 0.1% sodium nitrite, and the acidity was adjusted with 1 mL of 1 M hydrochloric acid. After 3 min, 1 mL of 3% sulfamic acid solution was added to each flask. Then volumes of 4 mL each of the 5% AA and 4 M sodium hydroxide solution were added, and the contents diluted to the mark with methanol and mixed well. After 10 min, the absorbance of the colored azo dye was measured at 460 nm for DAP, at 445 nm for CPD, and at 430 nm for both MCP and PABA against the corresponding reagent blank. A calibration graph was drawn and the regression equation calculated.

Pharmaceutical Formulations

An accurately weighed amount of powdered tablets (MCP, DAP, or CPD) equivalent to 25 mg was transferred to a 100-mL standard flask, the volume made up with distilled water (the sample was shaken thoroughly for about 30–40 min. Methanol was used for CPD and DAP), and then filtered. Appropriate aliquots of the drug solution were taken and the standard procedure was followed for analysis of drug content.

For the analysis of injection solution, the requisite volume was transferred to a 100-mL standard flask and diluted to the mark with distilled water. The drug content in the diluted solution was determined as described above. The same drug samples were also estimated by the reference methods (15,16) for comparison, and the results of the analysis are given in Table 1.

RESULTS AND DISCUSSION

The method involves the diazo-coupling reaction of these chemotherapeutic agents with acetyl acetone in an alkaline medium to give an orange-colored azo dye with maximum absorption at 430–460 nm. The absorption spectra of the above dyes are presented in Fig. 1. Under the reaction conditions, these chemotherapeutic agents are treated with nitrite solution in acidic medium, which undergoes diazotization to give the diazonium chloride. The diazonium chloride couples with the active methylene group of acetyl acetone to form an azo dye in an alkaline condition according to Sch. 1. Here MCP is used as the model compound, since the other chemotherapeutics behaved similarly to it (Sch. 1).

The factors affecting the color development, reproducibility, sensitivity, and adherence to Beer's law were investigated and are reported below.

Effect of Acetyl Acetone

The effect of the concentration of acetyl acetone was studied by measuring the absorbance at specified wavelengths in the standard procedure for solutions containing a fixed concentration of drugs and varying amounts (1–8 mL) of acetyl acetone. A volume of 4 mL of 5% AA solution in a total

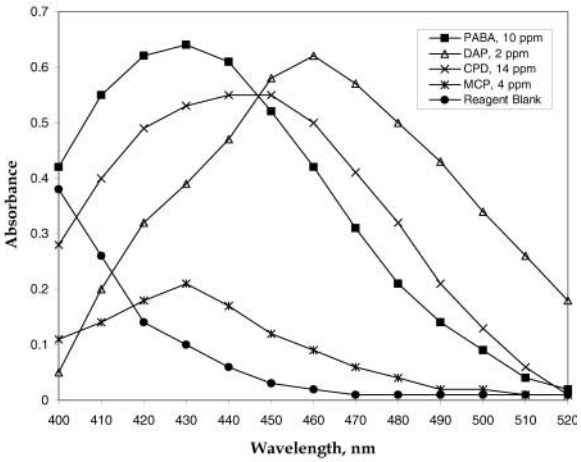


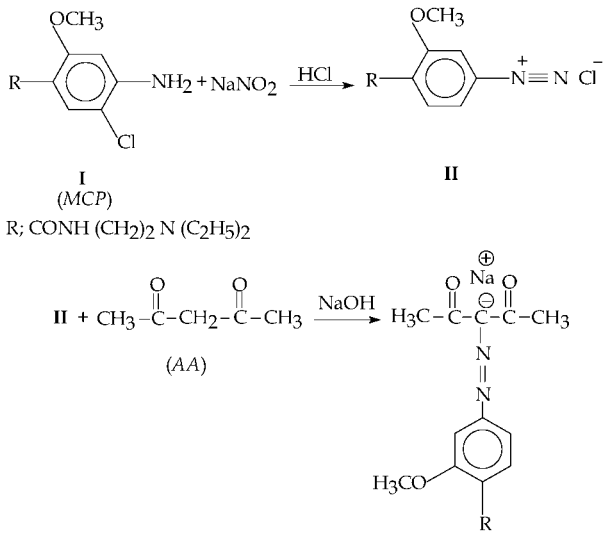
Figure 1. Absorption spectra of the reaction products of AA with MCP, DAP, PABA, and CPD.

Table 1

Analysis of Chemotherapeutics by the Proposed and Reference Methods

Preparation	Analyte	Label Claim (mg)	% Recovery[a]±SD		*t*-Value[b]	*F*-Value[c]
			Reference Methods (15,16)	Proposed Method		
Tablets						
Perinorm	MCP	10	99.1 ± 0.6	99.8 ± 0.6	1.71	1.00
Reglan	MCP	10	99.8 ± 0.7	101.4 ± 0.5	2.62	1.96
Emenil	MCP	10	97.9 ± 0.5	99.8 ± 0.8	1.76	2.56
Cisawal	CPD	10	98.9 ± 0.9	99.8 ± 0.5	1.58	3.24
Ciza	CPD	10	100.1 ± 0.4	99.7 ± 0.4	1.69	1.00
Unipride	CPD	10	99.9 ± 0.3	99.1 ± 0.7	1.83	5.44
Dapsone	DAP	25	98.9 ± 0.5	99.8 ± 0.4	2.52	1.56
		100	99.9 ± 0.6	99.1 ± 0.7	2.10	1.36
Injections						
Perinorm	MCP	5 mg/5 mL	98.8 ± 0.5	98.9 ± 0.6	0.36	1.44
Reglan	MCP	5 mg/5 mL	99.8 ± 0.7	99.6 ± 0.5	0.69	1.96

[a]Average of five determinations.
[b]Tabulated value 2.78.
[c]Tabulated value 6.39.



Scheme 1. Proposed diazo-coupling reaction between MCP and AA.

volume of 25 mL was found to be sufficient. Higher reagent concentrations gave colors of higher intensity, but these faded very quickly with time.

Effect of Sodium Nitrite Concentration

The constant absorbance readings were obtained in the range 1–4 mL of 0.1% sodium nitrite for all the drugs. Hence, a volume of 2 mL of 0.1% sodium nitrite solution was used in a final volume of 25 mL of the reaction mixture. The excess of nitrite could be removed by the addition of 1 mL of 3% sulfamic acid.

Effect of Acid Concentration

Diazotization was carried out at room temperature and cooling to 0–5°C was not necessary. The constant absorbance readings were obtained in the range 0.5–2 mL of 1 M hydrochloric acid. A volume of 1 mL of 1 M hydrochloric acid was found to be sufficient, and thus used in the further experimental studies. Higher concentrations of acid gave lower absorbance values.

Effect of Sodium Hydroxide

To develop a quantitative method based on this reaction, a study was conducted to determine the most effective alkalis and the optimum alkali concentration to be used. Sodium hydroxide was found to be the most effective base compared to sodium carbonate or ammonia. The orange-colored azo dyes are unstable in ammonia and do not give maximum color intensity in sodium carbonate medium. The constant absorbance readings were obtained in the range 2–5 mL of 4 M sodium hydroxide solution for all the chemotherapeutics. Hence, a volume of 4 mL of 4 M sodium hydroxide was used in further studies.

Effect of Reaction Time

The colored azo dyes developed rapidly after addition of the reagents and attained maximum intensity after about 10 min at room temperature. The color was stable for a period of more than 5 hr for the above chemotherapeutics. The results are presented in Fig. 2.

Analytical Data

Beer's law range, molar absorptivity, slope, intercept, correlation coefficient, detection limit, and quantitation limits of the method are presented in Table 2.

Effect of Concomitant Substances

To test the accuracy of the method, recovery experiments were performed on synthetic mixtures prepared in the laboratory. The usual tablet diluents and excipients were found not to interfere with the analysis by the proposed method (Table 3).

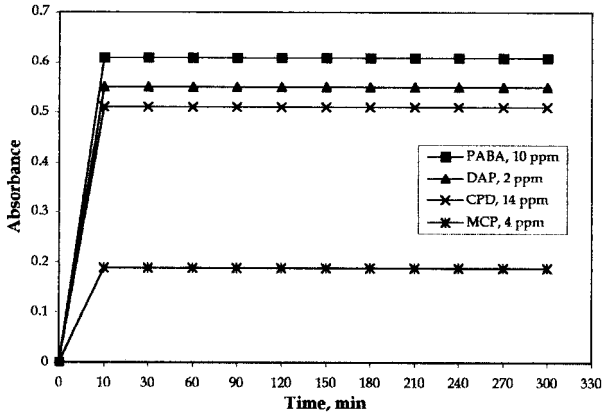


Figure 2. Effect of reaction time of azo dyes.

Table 2

Optical Characteristics and Analytical Data

Parameter	MCP	DAP	PABA	CPD
Beer's law limit ($\mu g\,mL^{-1}$)	2–16	0.1–1.6	1–8	1.6–14
Molar absorptivity ($L\,mol^{-1}\,cm^{-1}$)	2.20×10^4	1.68×10^5	1.28×10^4	1.54×10^4
Sandell's sensitivity ($\mu g\,cm^{-2}$ per 0.001 abs. unit)	0.0175	0.0014	0.0107	0.0301
Correlation coefficient (r)	0.999	1.000	0.9999	0.9999
Regression equation (Y[a])				
Slope (b)	0.0627	0.5600	0.1022	0.0340
Intercept (a)	−0.0279	0.0712	−0.0298	−0.0074
% RSD ($n=7$)	0.012	0.082	0.098	0.012
Detection limit, D_L ($\mu g\,mL^{-1}$)	0.0440	0.0032	0.0271	0.0437
Quantitation limit, Q_L ($\mu g\,mL^{-1}$)	0.1334	0.0098	0.0822	0.1324

[a] $Y = a + bx$, where x is the concentration ($\mu g\,mL^{-1}$). $D_L = 3.3\sigma/S$, $Q_L = 10\sigma/S$, where σ is standard deviation of blank, and S slope of calibration.

Table 3

Recovery of Some Chemotherapeutic Agents from Various Excipients by the Proposed Method

Analyte	Amount Present (mg)	Excipients (mg)						% Recovery[a] ± SD
		Talc	Dextrose	Starch	Sodium Algenate	Gelatin	Gum Acacia	
MCP	100	250	350	250	100	50	100	99.9 ± 0.9
	120	150	250	100	80	75	50	100.1 ± 0.8
DAP	100	300	200	250	100	50	80	100.6 ± 1.15
PABA	100	290	200	255	100	50	100	99.5 ± 0.45
CPD	100	250	300	200	75	75	80	100.4 ± 1.25

[a]Average recovery from five experiments.

Effect of Solvents

The choice of diluent for the reaction mixture was also studied. Water, methanol, ethanol, acetone, and isopropanol were tested as diluting solvents. Methanol was the best and thus selected for the experimental studies.

Application of the Method

The proposed method was applied to the quantitative determination of MCP, DAP, CPD, and PABA in bulk and in pharmaceutical formulations. The results of the analysis of Perinorm®, Reglan®, Emenil®, Cisawal®, Ciza®, Unipride®, and Dapsone® tablets and injection solutions presented in Table 1 compare favorably with other reference methods (15,16).

Precision

The precision of the proposed method was evaluated by replicate analysis of drug samples containing three different concentrations (low, medium, and high) (Table 4). The within-day precision showed a coefficient of variation (CV) of 1.65% or 0.27% at low concentration (2 or $4\,\mu g\,mL^{-1}$). The between-day precision evaluated over a period of 5 days showed a CV of 1.18% or 0.26% at low concentration. The low values of both the within- and between-day CVs at low concentration reflect the high precision of the proposed method.

Accuracy

The results of the drug samples in tablets and injections using the proposed and reference

Table 4

Within-Day and Between-Day Studies on Some Chemotherapeutics

	Within-Day			Between-Day		
Preparation	Analyte Taken ($\mu g\,mL^{-1}$)	Analyte[a] Found ($\mu g\,mL^{-1}$) ±SD	CV%	Analyte Taken ($\mu g\,mL^{-1}$)	Analyte[a] Found ($\mu g\,mL^{-1}$) ±SD	CV%
MCP						
Perinorm tablet	4	3.98 ± 0.07	1.65	4	3.89 ± 0.05	1.18
	8	7.96 ± 0.03	0.36	8	7.94 ± 0.05	0.60
	12	12.05 ± 0.03	0.27	12	11.95 ± 0.03	0.26
Reglan tablet	4	3.99 ± 0.03	0.63	4	3.91 ± 0.05	1.18
	8	7.99 ± 0.03	0.31	8	7.98 ± 0.03	0.36
	12	11.96 ± 0.04	0.29	12	12.05 ± 0.03	0.30
Emenil tablet	4	4.01 ± 0.04	0.85	4	3.90 ± 0.04	1.18
	8	7.94 ± 0.05	0.60	8	7.99 ± 0.03	0.31
	12	11.92 ± 0.04	0.34	12	11.96 ± 0.04	0.29
CPD						
Cisawal tablet	4	3.89 ± 0.05	1.18	4	3.91 ± 0.05	1.18
	8	8.01 ± 0.02	0.52	8	7.96 ± 0.03	0.36
	12	11.96 ± 0.04	0.34	12	11.97 ± 0.03	0.21
Ciza tablet	4	3.90 ± 0.05	1.18	4	4.01 ± 0.04	0.85
	8	8.01 ± 0.02	0.29	8	7.99 ± 0.03	0.31
	12	11.96 ± 0.04	0.29	12	12.10 ± 0.02	0.15
Unipride tablet	4	3.98 ± 0.08	2.06	4	3.89 ± 0.05	1.18
	8	7.94 ± 0.05	0.60	8	7.96 ± 0.03	0.36
	12	11.97 ± 0.03	0.21	12	11.95 ± 0.03	0.26
DAP						
Dapsone (25 mg) tablet	0.4	0.39 ± 0.02	2.4	0.4	0.38 ± 0.02	3.9
	0.8	0.82 ± 0.02	1.9	0.8	0.84 ± 0.02	2.5
	1.2	1.19 ± 0.02	1.7	1.2	1.21 ± 0.04	3.1
Dapsone (100 mg) tablet	0.4	0.38 ± 0.02	2.9	0.4	0.41 ± 0.04	2.9
	0.8	0.79 ± 0.08	2.1	0.8	0.82 ± 0.02	1.9
	1.2	1.23 ± 0.04	1.2	1.2	1.19 ± 0.02	1.7
MCP						
Perinorm injection	4	3.99 ± 0.03	0.63	4	3.98 ± 0.03	0.68
	8	7.96 ± 0.03	0.36	8	7.99 ± 0.03	0.31
	12	12.10 ± 0.02	0.15	12	11.98 ± 0.04	0.32
Reglan injection	4	3.90 ± 0.05	1.18	4	3.99 ± 0.03	0.63
	8	7.94 ± 0.04	0.52	8	7.96 ± 0.03	0.36
	12	11.97 ± 0.03	0.26	12	11.92 ± 0.04	0.34

[a]Average of five determinations.

methods are shown in Table 1. A statistical analysis of the results by Student's *t*- and *F*-tests showed no significant difference in accuracy and precision between the proposed and reference methods (Table 1).

CONCLUSION

The introduction of acetyl acetone as a new coupling agent for the determination of some chemotherapeutics provides a method with good

Table 5

Comparison of the Proposed Method with Other Spectrophotometric Methods

Reagents	Range (ppm)	Remarks	Reference
Catechol	10–40	Less sensitive than the proposed method	17
MBTH	2–24	Less sensitive	15
Bromothymol blue	1–10	Extractive and time-consuming	11
9-Chloroacridine	20–200	Less sensitive	21
Resorcinol	1–100	Less sensitive and required heating	8
Chloranil or bromanil	40–160	Required heating at 80°C and less sensitive	14
$NaVO_3$	20–120	Less sensitive and required heating	18
Acetyl acetone	2–16 for MCP 0.1–1.6 for DAP 1–8 for PABA 1.6–14 for CPD	Most sensitive, rapid, and a facile work	Proposed method

sensitivity and wide applicability. The developed method is simple and rapid. It is unaffected by a slight variation in the experimental conditions. The proposed method does not involve any stringent reaction conditions, and is more sensitive than other spectrophotometric methods (Table 5). Determination does not require extraction or heating. The wide applicability of the new method for routine quality control is well established by the analysis of MCP, DAP, and CPD in commercially available samples.

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