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Acetylacetone–Formaldehyde Reagent for the Spectrophotometric Determination of Some Sulfa Drugs in Pure and Dosage Forms

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Abstract. A new simple and sensitive spectrophotometric procedure for the determination of sulfacetamide sodium (I), sulfadiazine (II), sulfadimidine (III) and sulfathiazole (IV) is based on the reaction of the drug with acetylacetone–formaldehyde reagent to give a yellow product having λ_{max} at 400 nm. Optimization of the reaction conditions has been investigated. A linear correlation was obtained between absorbance at λ_{max} and the concentration. The Beer's law limits of I, II, III and IV are 4–80, 4–72, 4–60 and 4–80 μg/ml, respectively. For more accurate results, Ringbom optimum concentration ranges were evaluated to be 6–76, 8–66, 6–56 and 8–75 μg/ml for I, II, III and IV, respectively. The molar absorptivities and Sandell sensitivities for all sulfa drugs under consideration were evaluated. Relative standard deviations of 0.98, 1.07, 0.86 and 0.79% were obtained for I, II, III and IV, respectively. The method has been compared to the official method and found to be simple, accurate (*t*-test) and reproducible (*F*-test). The developed procedures were applied for bulk sulfa drugs and some of their dosage forms without interferences from additive and common prescribed drugs.

Key words: spectrophotometric analysis, sulfa drug determination, acetylacetone, formaldehyde.

Sulfonamides are widely used in the treatment of urinary tract infections, burns, conjunctivitis, and chloroquine-resistant malaria. They are also the drugs of choice for the treatment of nocardiosis, toxoplasmosis and several travellers diarrhoea, and meningococcal infections [1]. Sulfacetamide sodium is the monohydrate of the sodium salt of N^1-acetylsulfanilamide [6209–17–2], sulfadizaine is N^1-(pyrimidin-2-yl)sulfanilamide [68–35–9]. Sulfadimidine is N^1-(4,6-dimethylpyrimidin-2-yl)-sulfanilamide and sulfathiazole is N^1-thiazol-2-yl sulfanilamide [72–14–0]. Various spectrophotometric [2–8], fluorimetric [9, 10], flow injection analysis [11, 12],

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Raman spectrometric [13], atomic absorption spectrometric [14], differential scanning calorimetric [15], polarographic [16], titrimetric [17–19], microbiological [20, 21] and high-performance liquid chromatographic [22–24] methods for the determination of sulfonamides have been described. Titration with sodium nitrite solution to determine the aromatic amine function is the most widely used assay procedure for sulfonamides and their dosage forms in the official methods [25, 26].

Acetylacetone–formaldehyde reagent has been found to be valuable for the determination of primary amines in the UV region [27–29]. The objective of this investigation is to describe a modification of the acetylacetone–formaldehyde reagent method for colorimetric estimation of some sulfa drugs, (due to the availability of such drugs and their formulation in local market in Egypt). The change in the optimum conditions of reaction and preparation of acetylacetone–formaldehyde reagent solution was made to develop a new spectrophotometric method for determination of some sulfa drugs which is quick, simple, sensitive and selective. The procedure has been successfully applied to a variety of pharmaceutical dosage forms.

Experimental

Apparatus

A Perkin-Elmer Lambda 3B spectrophotometer with a 10-mm quartz cell was used for all spectrophotometric measuremets and an Orion Research Model 601A/ Digital Ionalyzer was used for checking the pH of acetate buffer solutions.

Reagents

All chemicals and reagents were of analytical or pharmaceutical grade. All solutions were prepared in doubly distilled water.

The acetylacetone–formaldehyde reagent solution was prepared by mixing freshly distilled acetylacetone (7.8 ml) and formaldehyde (15 ml; 36% w/w) with sodium acetate (16.0 ml; 0.2 *M*) and acetic acid (34.0 ml; 0.2 *M*) solutions. After keeping the solution in a boiling water bath for 5 min, it was cooled to room temperature, the pH was adjusted to 4.3 and the solution was diluted with water to 100 ml. The reagent solution was prepared fresh each day.

Standard sulfonamides were obtained from E1-Nasr Pharmaceutical Chemical Company (Cairo, Egypt). 100 mg of sulfa drug [sulfacetamide sodium (I), sulfadiazine (II), sulphadimidine (III) and sulfathiazole (IV)] were dissolved in 100 ml of 0.3 *M* HCl to obtain stock working solutions.

Procedure

A. *Bulk samples*

Aliquots (0.1–2.0 ml) of sulfonamide solution were pipetted into a 25-ml measuring flask. After addition of the reagent solution (4.0 ml), the reaction mixture was kept in a water bath at 40 ± 1°C for 25 min. The solution was then diluted to the mark with water and the absorbance measured at 400 nm against a reagent blank prepared in a similar manner. The drug concentration was read from a calibration graph prepared under identical conditions.

B. *Pharmaceutical dosage forms*

Tablets: Weigh and thoroughly grind 20 tablets. Extract an accurately weighed portion of the obtained powder, equivalent to 100 mg of the sulfonamide, with 50 ml of 0.3 *M* HCl, with occasional shaking for

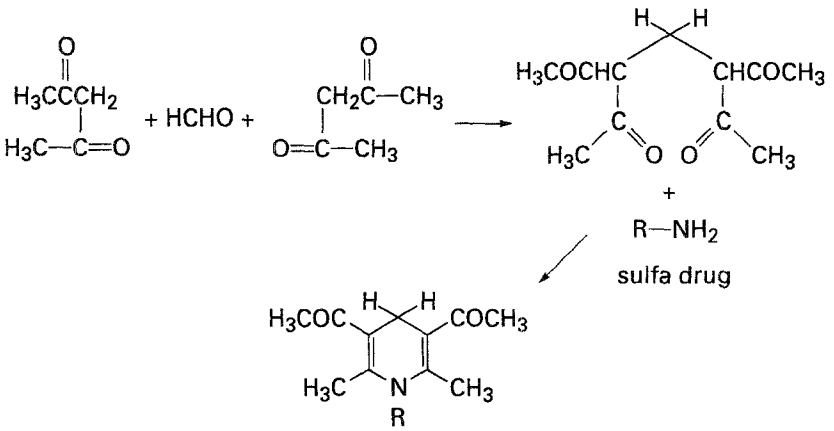
15 min. Filter the mixture into a 100-ml measuring flask, wash the residue several times with 0.3 *M* HCl and dilute to the mark. Use 4.0 ml of the reagent for color development and analyse as above.

Ophthalmic solutions: Dilute a suitable volume of the ophthalmic solution with 0.3 *M* HCl to obtain a solution containing 1 mg/ml of sulfacetamide sodium. This solution is then analysed as above.

Results and Discussion

Absorption Spectra

The absorption spectra of the reaction products from sulfonamides containing a primary aromatic amino group and acetylacetone–formaldehyde reagent show (Fig. 1) characteristic maxima at 340 nm (UV) and 400 nm (visible region). All the analytical measurements were made at 400 nm. Based on the literature [27–29] and our own findings, there is no possibility for the reaction of acetylacetone–formaldehyde with the secondary amino group attached to SO_2. The scheme for the chromogen formation due to the reaction of acetylacetone and formaldehyde with the primary aromatic amino group of a sulfa drug (RNH_2) can be outlined as follows (Scheme 1):



The reaction conditions were established by variation of one parameter at a time. For this reaction, various buffer media were used, viz, borate, acetate and universal buffers of different pH values. Only acetate buffer solution of pH 4.2–4.8 gave a stable color that was suitable for spectrophotometric measurements. The optimum pH values for each drug (I–IV) are summarized in Table 1, since the results are highly concordant at these pH values. This compares with the pH range of 1.5–2.0 used by Csiba [27] for the UV method. The reaction time was established by increasing it in increments of 5 min at a constant temperature of 40 ± 1°C and it was found that 25 min is sufficient to yield maximum absorbance. 4 ml of the reagent solution was enough to develop the colour to its full intensity. The final temperature after dilution was not critical.

Calibration

Typical calibration data for the four sulfonamides investigated obtained from linear regression analysis of absorbance readings vs. concentration of each drug (μg/ml)

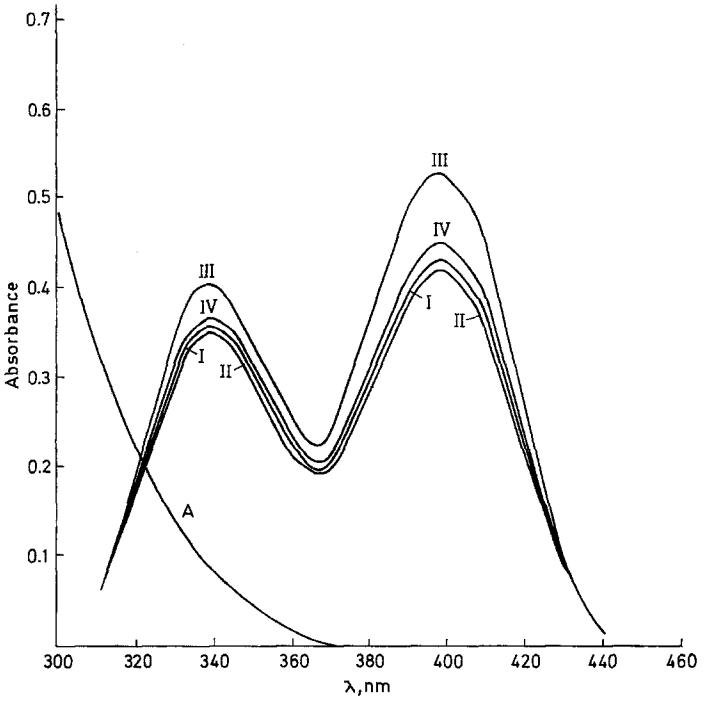


Fig. 1. Absorption spectra for (*A*) acetylacetone–formaldehyde reagent and its derivatization products with 20 μg/ml of *I*, *II*, *III* and *IV*

Table 1. Analytical and spectral characteristics of the colored products, precision and accuracy

Parameters	I	II	III	IV
pH	4.4	4.5	4.3	4.5
Beer's law limits, μg/ml	4–80	4–72	4–60	4–80
Molar absorptivity, 1/mol/cm	2.19×10^3	2.12×10^3	3.02×10^3	2.27×10^3
Sandell's sensitivity, μg cm^{-2}/0.001 A	0.116	0.118	0.092	0.112
Ringbom optimum concentration, μg/ml	6–76	8–66	6–56	8–75
Range of error, %	±1.2	±1.14	±1.67	±1.04
Relative standard deviation, %	0.98	1.07	0.86	0.79
Regression equation* Slope (*b*)	0.0086	0.0085	0.0108	0.0089
Intercept (*a*)	+0.023	−0.035	−0.027	+0.015
Correlation coefficient (*r*)	0.9985	0.9996	0.9988	0.9996
Calculated *t* value (2.310)**	1.316	0.978	0.769	1.136
Calculated *F* vlaue (2.450)**	1.217	1.053	0.971	1.088

* $A = a + bC$, where C is the concentration in μg/ml.

** Theoretical values for 95% confidence.

gave the slopes, intercepts, and correlation coefficients in Table 1. The Beer's law limits for drugs I, II, III and IV were 4–80, 4–72, 4–60 and 4–80 μg/ml, respectively. Moreover, Ringbom optimum concentration ranges can be calculated, which give more accurate results, i.e. 6–76, 8–66, 6–56 and 8–75 μg/ml for drugs, I–IV, respectively. The apparent molar absorptivities of the resulting colored products

range from 2.12×10^3 to 3.02×10^3 l/mol/cm whereas Sandell sensitivities range from 9.2 to 11.8 ng/cm^2. The performance of the proposed method was assessed by comparison with the official method [26]. Mean values obtained in Student's *t*- and *F*-tests [30] showed the absence of any systematic error in the method (Table 1).

Precision

Ten replicate determinations were made on the same solution containing 40 μg/ml of each of the investigated sulfonamides. The following coefficients of variation were obtained: 0.98, 1.07, 0.86 and 0.79% for I–IV, respectively.

Interferences

Sulfonamides are usually formulated in tablet form. Therefore, the effect of common tablet excipients and additives on the procedure was investigated. It was found that lactose, sucrose, fructose, glucose, starch, magnesium stearate, calcium phosphate, talc powder and acacia had no effect on the determinations (Table 2). An investigation was carried out to determine the effect of other drugs which are commonly prescribed with sulfa drugs, e.g., streptomycin sulfate, chloramphenicol, menadione and phthalylsulfathiazol, but it was found that none of these drugs interferred (max. error $\leqslant 2\%$).

Applications

The proposed procedure was applied to the determination of sulfonamides in dosage forms (Commercial products randomly collected from local pharmacies). Table 3 lists the results obtained by the proposed and official methods [26] based on

Table 2. Effect of excipients and other drugs on the recovery of sulfa drugs

Excipients and Drugs	Amount (mg/ml)	Recovery[a] ± SD (%)			
		I	II	III	IV
Lactose	1.0	99.1 ± 0.73	100.9 ± 0.89	98.5 ± 1.07	101.2 ± 1.04
Sucrose	1.5	98.8 ± 0.91	98.5 ± 1.05	101.0 ± 0.99	97.9 ± 1.33
Fructose	2.0	100.5 ± 0.62	99.3 ± 0.77	98.8 ± 0.93	98.2 ± 1.24
Glucose	1.5	98.5 ± 1.07	99.5 ± 0.61	98.4 ± 1.16	101.5 ± 1.18
Starch	1.0	100.8 ± 0.83	101.0 ± 0.78	99.3 ± 0.84	98.5 ± 1.16
Magnesium stearate	2.0	101.2 ± 1.11	99.0 ± 0.72	101.5 ± 1.14	98.1 ± 1.23
Calcium phosphate	1.5	98.7 ± 1.16	98.5 ± 1.08	98.4 ± 1.17	101.6 ± 1.09
Talc	2.0	98.3 ± 1.22	98.0 ± 1.21	102.0 ± 1.30	101.8 ± 1.15
Acacia	1.5	101.5 ± 1.05	98.8 ± 1.03	102.2 ± 1.33	97.8 ± 1.36
Streptomycin sulfate	1.0	98.3 ± 1.13	98.0 ± 1.22	98.5 ± 1.11	101.8 ± 1.18
Chloramphenicol	1.5	98.0 ± 1.26	101.5 ± 0.97	102.0 ± 1.28	98.4 ± 1.12
Menadione	2.0	98.7 ± 0.98	98.0 ± 1.20	98.8 ± 1.01	97.6 ± 1.40

[a] Average of six determinations at 0.04 mg/ml level.

Table 3. Assay of sulfa drugs in bulk dosage form by the proposed and official methods using standard addition procedure

Sample	Taken (μg/ml)	Drug added (μg/ml)	Proposed method		Official method	
			Found* (μg/ml)	Recovery ±SD** (%)	Found* (μg/ml)	Recovery ±SD** (%)
Sulfacetamide sodium	20	–	19.9	99.5±0.2	20.3	101.5±1.3
		20	40.3	100.75±0.3	39.5	98.75±1.2
		20	59.3	98.83±0.7	60.8	101.33±1.4
Sulfacetamide sodium (eye drops)	40	–	39.7	99.25±0.5	39.6	99.00±0.8
		16	56.5	100.84±0.6	57.0	101.79±1.5
		32	71.5	99.31±0.6	71.0	98.61±1.3
Sulfadiazine	32	–	32.2	100.63±0.4	31.7	99.06±0.6
		12	44.5	101.14±0.9	45.0	102.27±1.7
		24	55.5	99.11±0.8	55.2	98.57±1.1
Sulfadiazine tablets[a]	24	–	23.8	99.17±0.7	24.5	102.08±1.6
		16	39.9	99.75±0.3	40.5	101.25±1.2
		32	56.4	100.71±0.6	55.0	98.21±1.4
Sulfadimidine	36	–	36.2	100.56±0.5	35.6	98.89±1.1
		12	48.5	101.04±1.1	47.5	98.96±1.3
		24	60.6	101.00±1.0	59.0	98.33±1.5
Sulfadimidine tablets[b]	28	–	27.8	99.29±0.7	28.3	101.07±1.3
		20	48.3	100.63±0.6	47.5	98.96±1.1
		32	59.0	98.33±0.9	59.0	98.33±1.2
Sulfathiazole	16	–	16.1	100.63±0.7	15.8	98.75±1.0
		16	32.3	100.94±0.8	31.5	98.44±1.3
		32	48.5	101.04±1.1	47.2	98.33±1.2
		48	63.5	99.22±0.9	63.0	98.44±1.3

* Mean of six determinations.

** Recovery of added amount, assuming lable claims correct.

[a] The Nile Co. for Pharmaceutical and Chemical Industries, Cairo, Egypt.

[b] El-Nasr Pharmaceutical and Chemical Co., Cairo, Egypt.

electrometric titration with sodium nitrite solution to determine the aromatic amine function using the standard addition procedure. The results indicate good agreement with the official methods. The proposed colorimetric method can be recommended for routine analysis in the majority of drug quality control laboratories. Another favorable characteristic of the method is that the absorbances of the colored products formed are stable for at least 12 h.

On comparing the results obtained by the proposed method with those of the pharmacopoeial method [26] using the *t*-test for the accuracy and *F*-test for the precision assessment [30], the calculated values did not exceed the corresponding theoretical values, indicating insignificant differences between results. The proposed method is more accurate, with high recoveries amounting to 99.74 ± 1.4% compared with 100.24 ± 2.03% using the official method.

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