cient of variation (*CV*) 1.2%]. Manipulation of samples does not lead to the loss of material, since absorptivity measurements of a directly prepared 5.1-μg/ml solution of I in ethanol afforded an absorptivity of 35.6.

The similar absorptivity values obtained between solutions of I prepared directly in ethanol and those prepared through an ether stage indicate that any residue from ether that could be present does not appear to make a significant absorbance contribution at 312 nm. To confirm this finding, 25-ml portions of ether were evaporated to dryness with a stream of dry nitrogen, any residue present was dissolved in 50.0 ml of ethanol, and the absorbance was measured. Values of 0.01 or lower were obtained.

Results from TLC examination of chlordiazepoxide formulations carried out over an extended period showed that all formulations contained well under 0.1% 2-amino-5-chlorobenzophenone (II). Thus, normally encountered levels of II in chlordiazepoxide formulations, while present in column eluates, do not interfere significantly with the absorbance of I at 312 nm. The absorbance maximums of a solution of I in ethanol (equivalent to 3% I using procedures described under *Experimental*) and a I–II mixture (equivalent to 3 and 1%, respectively) were nearly the same, 0.531 and 0.538, respectively. The level of II utilized was 10 times higher than pharmacopeial specifications (1–3) and was present in a much greater proportion relative to I than is ever encountered in the practical situation. Thus, it can be concluded that the absorbance contribution due to II, if present, will be well within experimental variation.

The accuracy of the proposed method for quantitating I in chlordiazepoxide was determined by analyzing aliquots of lactose triturate of I. The data obtained from this study (Table III) indicated a through-column recovery of 100.58% I (*CV* 3.9%). The precision of the proposed method was determined by analyzing replicate samples of a commerical capsule formulation, which indicated a coefficient of variation of 1.5% (Table III). This coefficient of variation of 1.5% compared very closely with coefficient of variation values obtained from ethereal solutions of I measured directly (1.6) and put through the trap column (1.5) (Table II) and with results obtained when mixtures of chlordiazepoxide and I were passed through the trap column (Table I, Samples 3–5, coefficient of variation of absorbance at 312 nm, 1.28%). In comparison, it would appear that the higher coefficient of variation values obtained from I-lactose triturate samples (Table III) reflect problems encountered in attempting to obtain a homogeneous triturate of a low level of I rather than the imprecision of the method.

Nine samples of chlordiazepoxide tablets and capsules, from a number of suppliers, were analyzed for I, employing the trap column method. The results from duplicate analyses were compared with values for I obtained from TLC and/or HPLC. Data obtained (Table IV) confirm the accuracy of the method of analysis and underline the suitability of the trap column procedure for the quantitation of decomposition product I in chlordiazepoxide.

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ACKNOWLEDGMENTS AND ADDRESSES

Received September 16, 1974, from the *Drug Research Laboratories, Health Protection Branch, Health and Welfare Canada, Ottawa, Canada K1A 0L2*

Accepted for publication January 21, 1975.

The authors acknowledge the assistance of technologists in the Analytical Chemistry Section in performing anâlyses and thank Mr. A. G. Butterfield and Mr. R. Sears for HPLC analyses.

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Spectrophotometric Determination of Acetaminophen and Dichloralantipyrine in Capsules

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Abstract □ A rapid method for the routine determination of acetaminophen and dichloralantipyrine in capsules is reported. The determination of acetaminophen is based on the ability of its hydrolytic product, *p*-aminophenol, to produce an intensive yellow color with vanillin. The determination of dichloralantipyrine is based on the fact that it, as well as its major metabolite chloral hydrate, produces a blue color with quinaldine ethiodide. No interferences were encountered, and good recovery and precision data were obtained.

Keyphrases □ Acetaminophen–dichloralantipyrine—spectrophotometric analysis of capsule formulation □ Dichloralantipyrine–acetaminophen—spectrophotometric analysis of capsule formulation □ UV spectrophotometry—analysis, acetaminophen and dichloralantipyrine in capsules

Several methods for the quantitative determination of acetaminophen in pharmaceutical preparations are available. Most of them are colorimetric and require the hydrolysis of acetaminophen to *p*-aminophenol (1–6). A significant contribution was provided by Vaughn (6), who capitalized on the fact that acetaminophen is readily hydrolyzed to *p*-aminophenol, which produces a stable yellow color with vanillin.

Archer and Haugar (7) found out that the addition compound, dichloralantipyrine, produces, upon the addition of quinaldine ethiodide, a blue color in proportion to its chloral hydrate content.

Table I—Recovery of Acetaminophen and Dichloralantipyrine Added

Acetaminophen, mg		Dichloralantipyrine, mg	
Added	Found	Added	Found
150	149.6	75.3	77
	151.9		75.5
	152.4		75.1
	148.1		75.1
	151.6		74.3
	149.7		74.9
Mean	150.5	Mean	75.3

Table II—Arithmetic Mean of Determination of an Accurately Weighed Capsule Mixture

Acetaminophen			Dichloralantipyrine		
Reported, mg	Found, mg	%	Reported, mg	Found, mg	%
120	120.0	100.0	60	58.8	99.7
	119.5	99.6		59.7	99.5
	118.8	99.0		60.3	100.5
	120.7	100.6		59.3	98.8
	119.6	99.7		59.0	98.3
	119.9	99.9		58.5	99.2
Mean	119.7	99.8		59.6	99.3

This report describes a quantitative UV spectrophotometric technique for the determination of acetaminophen and dichloralantipyrine in capsules containing 325 mg of acetaminophen, 100 mg of dichloralantipyrine, and 65 mg of isometheptene mucate.

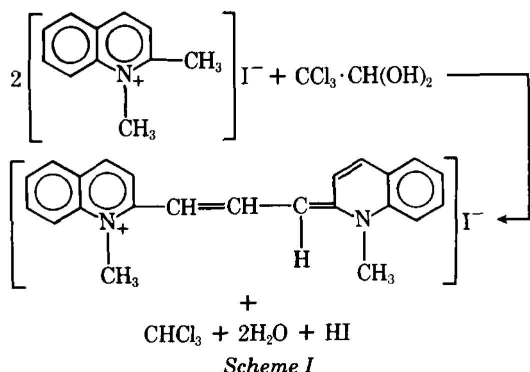
EXPERIMENTAL[1]

Materials and Reagents—The following were used (all of analytical grade): acetaminophen–dichloralantipyrine–isometheptene mucate capsules[2], 5% vanillin[3] in isopropanol, acetaminophen[4], dichloralantipyrine[5], 1.5% (w/v) quinaldine ethiodide[6] solution (dissolve 1.5 g of quinaldine ethiodide in 100 ml of water and filter if necessary), and 0.1 *N* ethanolamine[3] (dissolve 6.1 g of ethanolamine in water and dilute to 1 liter).

Acetaminophen Assay—The colorimetric curve is prepared as follows. Accurately weigh about 120 mg of acetaminophen in 100 ml of 4 *N* HCl. Transfer by pipet 0.5, 1.0, 1.5, 2.0, and 2.5 ml of the standard solution to five test tubes. Add 5 ml of 4 *N* HCl and place the tubes in a beaker of boiling water for 1 hr. Allow the tubes to cool and transfer the contents quantitatively to 50-ml volumetric flasks.

Add 10 ml of vanillin reagent to each flask and dilute to volume. Mix well and measure the absorption, after appropriate dilution, at 395 nm against a blank (6 ml of 4 *N* HCl, 10 ml of vanillin reagent, and enough water to make 50 ml). The absorbances obtained plot into a straight line.

Acetaminophen Sample Preparation and Assay—Weigh and finely powder the sample to correspond to 120 mg of acetaminophen based on label claim. Transfer it to a suitable container and add about 50 ml of 4 *N* HCl. Agitate with mechanical shaker for 20 min, filter through paper into a 100-ml volumetric flask, and dilute to volume with 4 *N* HCl. Mix well and transfer 1 ml to a test tube. Add 5 ml of 4 *N* HCl and heat for 1 hr in boiling water. Then proceed as directed under *Acetaminophen Assay* beginning with: "Allow the tubes to cool and . . . ." Calculate the amount of *p*-aminophenol from the calibration curve and multiply it by the factor 1.385 and the dilutions used.



Scheme I

Dichloralantipyrine Assay—The calibration curve is prepared as follows. Dissolve 250 mg of chloral hydrate in water and dilute to 500 ml. Dilute 10 ml of this solution to 100 ml with water, so that the standard chloral hydrate solution contains 50 μg/ml. Transfer by pipet 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, and 1.6 ml of the standard solution to seven test tubes and pipet 1 ml of water into an eighth test tube to serve as a blank. Add to each tube 1 ml of quinaldine ethiodide solution and 6 ml of isopropanol, mix well, add 0.5 ml of 0.1 *N* ethanolamine, dilute to 10 ml with water, and mix. Then place the tubes in a water bath at 60° for 1 hr. Remove the tubes from the water bath, cool, and measure the absorption at 605 nm after appropriate dilutions. The absorbances obtained plot into a straight line.

Dichloralantipyrine Sample Preparation and Assay—Weigh an aliquot from the previously finely powdered capsules to correspond to 75 mg of dichloralantipyrine based on label claim. Transfer it to a suitable container so that it can be diluted with water to contain 60 μg/ml. Transfer by pipet 1 ml to a test tube and proceed as described under *Dichloralantipyrine Assay*, beginning with: "Add to each tube 1 ml of quinaldine ethiodide solution . . . ." Calculate the amount of chloral hydrate from the calibration curve and multiply it by the factor 1.63 and the dilutions used.

RESULTS AND DISCUSSION

Vaughn (6) reported that complete hydrolysis of acetaminophen with 1 *N* HCl is not attained even after 45 min, so he chose 10 min in boiling water for convenience. The present assay uses 4 *N* HCl and 1 hr in boiling water to obtain as complete an hydrolysis as possible. However, studies in which the duration of time in boiling water was reduced to 20 min gave identical results. The hydrolytic product, *p*-aminophenol, produced a stable, intense yellow color upon the addition of vanillin reagent, with a maximum absorption peak at 395 nm. The amount of acetaminophen in each capsule was determined by taking into account that the ratio of acetaminophen to *p*-aminophenol is 1:1.385.

Archer and Haugar (7) showed that quinaldine ethiodide reacts with the addition compound, dichloralantipyrine, to produce a blue color in proportion to its chloral hydrate content (Scheme I). This reaction is specific for dichloralantipyrine and chloral hydrate, and substances such as trichloroacetic acid, acetic acid, chloroform, bromoform, and glucose do not produce a blue color.

The quantity of dichloralantipyrine was based on the fact that each mole of dichloralantipyrine produces 2 moles of chloral hydrate, so the ratio of dichloralantipyrine to chloral hydrate is 1:1.63. Isometheptene mucate did not interfere with either assay, and the results of adding known amounts of acetaminophen and dichloralantipyrine are shown in Table I. The assay of the capsules is reported in Table II.

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ACKNOWLEDGMENTS AND ADDRESSES

Received November 6, 1974, from the *Department of Pharmaceutics, Brooklyn College of Pharmacy, Long Island University, Brooklyn, NY 11216*
Accepted for publication February 4, 1975.
Presented in part at the APhA Academy of Pharmaceutical Sciences national meeting, New Orleans, November 1974.
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High-Speed Liquid Chromatographic Analysis of Sulfasalazine (Salicylazosulfapyridine)

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Abstract □ A high-speed liquid chromatographic method for analysis of sulfasalazine (salicylazosulfapyridine) in bulk powder and tablet dosage form is presented. Analysis is accomplished with a reverse-phase partition column and 10% 2-propanol in pH 7.7 phosphate buffer as the mobile phase. The method of analysis utilizes a simple, one-step, solubilization procedure with dimethylformamide, addition of an internal standard, and chromatography. The method is specific for sulfasalazine in the presence of starting materials, degradation products, or by-products from its manufacture.

Keyphrases □ Sulfasalazine—high-speed liquid chromatographic analysis, bulk powder and tablet dosage form □ Salicylazosulfapyridine—high-speed liquid chromatographic analysis, bulk powder and tablet dosage form □ High-speed liquid chromatography—analysis, sulfasalazine bulk and tablet forms

Several analytical methods have been reported for the determination of sulfasalazine {salicylazosulfapyridine, 5-[*p*-(2-pyridylsulfamoyl)phenylazo]salicylic acid} (I). These methods include spectrophotometry (1), titration with titanium trichloride (1), polarography (2), and nonaqueous potentiometric titration (2). However, most of these methods fail to determine this compound selectively in the presence of its byproducts of synthesis or degradation products. Conversely, a chromatographic method would be expected to provide simple, rapid, and specific separation and quantitation of this compound in the presence of its impurities.

Although GC has not been investigated, its use for the underivatized I probably would be unsuccessful since this compound was thermally labile when subjected to differential thermal analysis[1]. TLC, although specific, has the disadvantage of potentially long analysis times and requires a number of manipulative steps which may adversely affect precision. For example, a relative standard deviation of 2–6% resulted just from spotting and chromatography (3). (Quantitation in these experiments was effected directly on the plate with a densitometer.) Consequently, even larger errors would be expected in the overall assay if the compound is removed from the TLC plate and eluted from the adsorbent prior to quantitation.

The present study was undertaken because high-speed liquid chromatography (HSLC) appears to offer several advantages (4) for the analysis of I that other methods do not possess. For example, it can be used for thermally labile compounds, the precision is good, and the method is specific and rapid. This technique has been successfully utilized to assay I in tablet dosage forms as well as the bulk chemical.

Table I—Column Packing Materials

Column	Packing	Column Dimensions
a	Neutral alumina[a], 100–200 mesh	1 m × 0.3 cm o.d.
b	Ether stationary phase chemically bonded on a controlled porous surface[b]	1 m × 0.3 cm o.d.
c	Strong anion-exchange resin coated on a controlled porous surface[c]	0.6 m × 0.3 cm o.d.
d	Spherical siliceous particles with a controlled porous surface[d]	0.6 m × 0.3 cm o.d.
e	Diphenyldichlorosilane stationary phase chemically bonded on a controlled porous surface[e]	0.6 m × 0.3 cm o.d.
f	Octadecyltrichlorosilane stationary phase chemically bonded on a controlled porous surface[f]	1.2 m × 0.3 cm o.d.

[a] AG-7, Bio Rad. [b] Permaphase ETH, duPont. [c] Zipax SAX, duPont. [d] Corasil II, Waters Associates. [e] Corasil/phenyl, Waters Associates. [f] Corasil/C_{18}, Waters Associates.

EXPERIMENTAL

Apparatus—A liquid chromatograph[2] equipped with a UV photometric detector (254-nm radiation using a low pressure mercury source) was used. The UV detector can operate at a sensitivity of 0.02 absorbance unit full scale (aufs).

Columns—The packing materials shown in Table I were used in precision-bore stainless steel columns. With the exception of Columns d, e, and f, which were purchased commercially, the columns

[1] Unpublished data.

[2] Model ALC 202, Waters Associates.