1-Dimethylamino-2-(2-hydroxy-1-propyloxy)propane (VI)—1-Dimethylamino-2-propanol (103 g., 1 mole) and 65 g. (1.1 mole) of propylene oxide were refluxed in 800 ml. of methanol for 18 hr. Methanol was evaporated under reduced pressure and the residue was fractionated. The fraction with a boiling point of 65° (0.2 mm. Hg) was the desired product; the yield was 24 g. (15%).

Anal.—Calc. for $C_8H_{19}NO_2$: C, 59.63; H, 11.79. Found: C, 59.86; H, 11.89.

1-Dimethylamino-2-(2-hydroxy-1-propyloxy)propane Methiodide (VII)—Compound VI (1 g., 0.006 mole) and 3 g. (0.021 mole) of methyl iodide in 25 ml. of ether containing a few drops of methanol were kept at 0° for 2 days. The resulting crystals were recrystallized from acetone–ether and then from acetone to afford 0.93 g. (55%) of material, m.p. 147–148°.

Anal.—Calc. for $C_9H_{22}INO_2$: C, 35.64; H, 7.26; I, 41.91; N, 4.60. Found: C, 35.79; H, 7.21; I, 41.72; N, 4.52.

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Colorimetric Method for Determination of Guanazole in Blood and Urine

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Abstract □ A colorimetric method, based on diazotization and coupling, for the determination of guanazole in aqueous solution, blood, and urine is described. The average relative standard deviation of the method is about 0.9%. Determination in blood involves precipitation of proteins with trichloroacetic acid and diazotization and coupling of guanazole in the filtrate. Determination in urine involves separation of guanazole from the diazotizable, naturally occurring urinary aromatic amines using ion-exchange chromatography.

Keyphrases □ Guanazole (3,5-diamino-1,2,4-triazole)—colorimetric analysis in aqueous solution, blood, and urine □ 3,5-Diamino-1,2,4-triazole (guanazole)—colorimetric analysis in aqueous solution, blood, and urine □ Colorimetry—analysis, guanazole in aqueous solution, blood, and urine

Guanazole (3,5-diamino-1,2,4-triazole)[1] is an experimental drug beneficial in the treatment of adult acute leukemia (1–6). No methods for its determination are published in the literature. This paper describes a method for the determination of guanazole in aqueous solution, blood, and urine. The method is based on diazotization and coupling (7, 8).

EXPERIMENTAL

Apparatus—Spectra and absorbance measurements were made with a recording spectrophotometer[2]. Matched cells with a 1-cm. optical path were used.

Materials—Dowex-50W (H^+) (12% cross-linkage, 200–400 mesh) was prepared as previously described (9). Dowex-1 (Cl^-) (10% cross-linkage, 200–400 mesh) was sedimented to remove the very fine and very coarse particles. Dowex-1 (Cl^-) was converted to Dowex-1 formate by passage of sodium formate and formic acid over the resin followed by appropriate water washes.

Guanazole was obtained in bulk[3].

The following reagent grade chemicals were used: concentrated hydrochloric acid, sodium nitrite, ammonium sulfamate, *N*-(1-naphthyl)ethylenediamine dihydrochloride, dimethyl sulfoxide, and trichloroacetic acid.

Procedure for Aqueous Solution—Two milliliters of 5.0 *N* HCl and 2 ml. of the guanazole aqueous solution were added to 2 ml. of dimethyl sulfoxide. Then 0.2 ml. of 0.5% $NaNO_2$ was added to this mixture. Immediately 0.2 ml. of 5.0% ammonium sulfamate was added and mixed thoroughly, followed by 0.2 ml. of 0.5% *N*-(1-naphthyl)ethylenediamine dihydrochloride[4]. After 10 min. the absorbance of the sample was compared with a blank at 504 nm. in the spectrophotometer. Quantitative comparisons were made with a guanazole standard curve prepared in a similar fashion.

Procedure for Blood—Eight milliliters of a 15% solution of trichloroacetic acid was added to 2 ml. of blood. This was then filtered through a prewashed filter paper, which was then washed with a few milliliters of distilled water. The filtrate was made up to 25 ml. in a volumetric flask. Two milliliters of the filtrate was added to 2 ml. of 5.0 *N* HCl, diazotized and coupled as described for the aqueous solution, and compared with a blank made up with water and the other reagents.

Procedure for Urine—Fifty milliliters of the urine sample was passed through a Dowex-1 formate column (1.0 cm. o.d. × 10 cm.) under a pressure of 1.0–2 p.s.i. The column was washed with 50 ml. of deionized water, the effluent and wash were combined, and the pH was adjusted to 1–2. The acidified sample was passed through a Dowex-50W (H^+) column (1.0 × 10 cm.) by gravity. The eluate was discarded, and the column was then washed with 50 ml. of 0.1 *N* HCl. Guanazole was eluted from the column with 200 ml. of 2.5 *N* HCl. This chromatographic procedure separates guanazole from the diazotizable, naturally occurring urinary aromatic amines. An

[1] NSC 1895.
[2] Cary 14.
[3] From the National Cancer Institute, U. S. Public Health Service.
[4] This solution is to be kept in a dark bottle and should be prepared fresh every week.

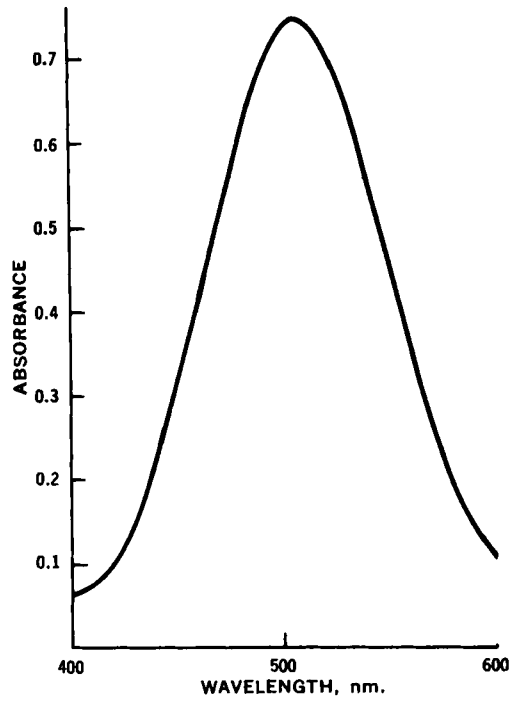

Figure 1—*Visible spectrum of the dye formed. Guanazole was added to water and then diazotized and coupled.*

aliquot of the 2.5 *N* HCl eluate was taken for diazotization and coupling as described for the aqueous solution and was compared with a blank urine sample treated as already described. If the eluate is too dilute to be assayed accurately, it can be concentrated by evaporating some of the water to a known volume.

RESULTS AND DISCUSSION

Figure 1 shows the visible spectrum of the dye formed; the maximum absorbance is at 504 nm. Absorbance is linear with concentration up to an absorbance value of 0.7. The molar absorptivity in the final solution is 17,500 l. M^{-1} cm.$^{-1}$. Table I shows the recovery of guanazole from samples of aqueous solution, human blood, and human urine. The recovery seems to be quantitative. The average relative standard deviation is 0.9%.

The absorbance value of the blank for human blood is very close to zero; the value for human urine in a 1-cm. cell is 0.12 ± 0.11 (standard error). Treatment of urine with ion-exchange chromatography, as described under the *Experimental* section, decreases the value of absorbance of the blank to 0.005 (0.05 in a 10-cm. cell).

Dimethyl sulfoxide is used to prevent the dye from precipitating. It is not needed if absorbance is read immediately after the addition of the coupling reagent or if the concentration of guanazole is low.

Table I—Recovery of Guanazole from Samples of Aqueous Solution, Blood, and Urine

	Guanazole Added, mcg./ml.	Recovery, %
Aqueous Solution		
	1.0	99.5
	3.0	99.2
	7.0	98.4
	9.9	100.5
	15.0	100.7
Average recovery ± *SD*		99.7 ± 1.0
Human Blood		
	12.0	98.4
	36.0	97.5
	84.0	99.2
	120.0	98.7
	180.0	97.0
Average recovery ± *SD*		98.2 ± 0.9
Human Urine		
	4.0	97.2
	10.0	99.2
	25.0	98.4
	40.0	97.5
	60.0	97.8
Average recovery ± *SD*		98.0 ± 0.8

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