Automated Procedure for Determination of Free Aromatic Amine in Water-Soluble Radiopaques

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Abstract □ The determination of free aromatic amine in water-soluble radiopaques by diazotization in 2-methoxyethanol–water, coupling with naphthylethylenediamine, and colorimetry of the azo dye has been automated. Sensitivity, precision, and accuracy are satisfactory for purity testing and stability studies.

Keyphrases □ Radiopaques (water soluble)—automated determination of free aromatic amine impurities □ Amine impurities in water-soluble radiopaques—automated determination

Many water-soluble radiopaques currently used in urography, angiography, and intravenous cholecystography are derivatives of 2,4,6-triiodobenzoic acid with at least one acylated aromatic amino group in position 3 or 5. Acylation of the amino group is normally the last step of the synthesis. Therefore, testing of nonreacted amine as a potential impurity becomes mandatory. In fact, a quantitative determination of free aromatic amine is specified in several monographs for radiopaques listed in USP XVIII and BP 1968.

The purpose of the present investigation was to develop an automated technique for the determination of free aromatic amine in radiopaques as bulk products or as injectable solutions. The application of the procedure is for routine purity testing and for extensive stability studies of the injectable radiopaque solutions, where free aromatic amine may be formed by hydrolysis of the acylamino group.

Among the methods for the determination of aromatic amine reported in the literature, the Bratton–Marshall (1) reaction was considered most suitable for automation. This reaction is used in the USP XVIII monographs for meglumine diatrizoate injection (2), sodium diatrizoate injection (3), meglumine iodipamide injection (4), sodium iodipamide injection (5), meglumine iothalamate injection (6), and sodium iothalamate injection (7); it has already been automated for assay of ethopabate (8), sulfonamides, and other drugs (9, 10).

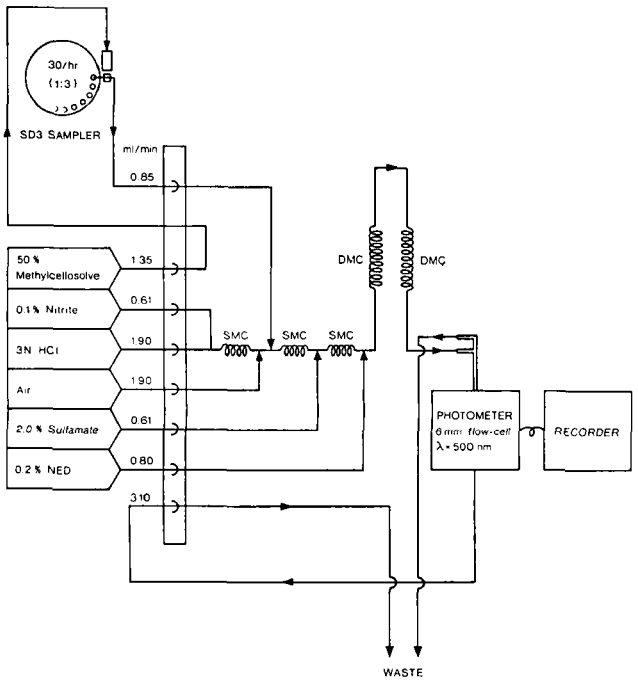

Figure 1—*Automated flow diagram (NED = naphthylethylenediamine).*

EXPERIMENTAL

Apparatus—The analytical train included the following modules: a SD3 sampler distributor[1] programmed at 30 samples/hr and set for a sample–wash ratio of 1:3, a proportioning pump[2], a flow photometer[3] equipped with a 6-mm continuous flow cell and a 500-nm interferential filter, and a recorder[4] with a chart speed of 152.4 cm/hr (60 in./hr).

Reagents[5] and Solutions—The following were used: 2-methoxyethanol[6]–distilled water (1:1, v/v), 3 *N* hydrochloric acid in 2-methoxyethanol–distilled water (1:1, v/v), 0.1% sodium nitrite in distilled water, 2% ammonium sulfamate in 2-methoxyethanol–distilled water (1:1, v/v), and 0.2% *N*-(1-naphthyl)ethylenediamine in 2 *N* hydrochloric acid–2-methoxyethanol (1:1, v/v).

Standard Solutions—The following standard solutions were prepared: 3-amino-2,4,6-triiodobenzoic acid, 3-amino-5-acetamido-2,4,6-triiodobenzoic acid, 3-amino-5-methylacetamido-2,4,6-triiodobenzoic acid, and 5-amino-2,4,6-triiodo-*N*-acetylisophthalamic acid.

Stock solutions were prepared by neutralizing 50 mg of each standard substance with 0.1 *N* NaOH and diluting to 100 ml in a volumetric flask with distilled water. These stock solutions were further diluted separately to obtain final concentrations in 2-methoxyethanol–water (1:1, v/v) ranging from 2.5 to 150 μg/ml.

The standard substances were checked for purity by carbon and iodine microanalysis, acidimetric determination of the equivalent weight, and TLC.

Sample Solution—For preparation of sample solutions from bulk substance, 5 g was neutralized with 2 *N* NaOH, diluted to 25 ml with distilled water, and then diluted to 50 ml with 2-methoxyethanol. From injectable solutions, a sample of 1–5 ml was withdrawn and diluted accurately with distilled water and then with 2-methoxyethanol to obtain a 10% solution of the radiopaque in 2-methoxyethanol–water (1:1).

Automated Procedure—The schematic diagram of Fig. 1 indi-

[1] Colorimetric laboratory analyzer model 1500, C. Erba, Milan, Italy.
[2] C. Erba, Milan, Italy.
[3] Model FF1, C. Erba, Milan, Italy.
[4] Speedomax W, Leeds & Northrup.
[5] All chemicals were E. Merck reagent grade.
[6] Methylcellosolve.

Table I—Statistical Analysis of Regression Lines[a] ($y = bx + a$)

Parameter	Standardization Number									
	1	3	5	7	9	14	15	16	19	23
b	163.6	163.2	163.0	164.1	163.5	162.1	160.1	162.6	164.4	161.7
a	−0.16	0.09	−0.27	0.00	−0.15	0.70	0.46	0.31	0.14	0.33
r	0.999986	0.999993	0.999981	0.999979	0.999988	0.999948	0.999960	0.999960	0.999967	0.999977
n	7	7	7	7	7	7	7	7	7	7
s_b	0.39	0.27	0.44	0.47	0.35	0.93	0.49	1.02	0.93	0.66
s_a	0.49	0.49	0.49	0.49	0.49	0.51	0.51	0.81	0.81	0.64
s_{xy}	±0.40	0.28	0.47	0.49	0.37	0.75	0.40	0.42	0.38	0.31

[a] y = concentration (micrograms per milliliter), x = absorbance, b = regression coefficient (slope), a = shift of regression line, s_{xy} = standard deviation of scatter around regression line, s_b = standard deviation of b, s_a = standard deviation of a, and r = correlation coefficient.

Table II—Precision and Accuracy

$R\text{-}NH_2$ Given x_i, μg/ml	$R\text{-}NH_2$ Found x_i, μg/ml										Mean Value $\bar{x}_i$, μg/ml	Precision $\left(\frac{\Sigma(x_i - \bar{x}_i)}{n-1}\right)^{1/2}$	Accuracy $x_i - \bar{x}_i$, μg/ml
	1	3	5	7	9	14	15	16	19	23			
2.41	2.29	2.54	2.17	3.11	2.47	2.97	2.72	2.70	2.12	2.57	2.57	±0.32	+0.16
4.82	4.75	4.99	4.78	5.09	4.92	5.40	5.09	5.10	4.74	4.99	4.98	0.20	+0.16
9.65	9.82	9.88	9.51	9.35	9.99	9.78	9.82	9.75	9.82	9.69	9.74	0.19	+0.09
19.30	18.98	19.18	18.96	19.20	19.14	19.02	18.97	19.03	19.48	19.08	19.10	0.16	−0.20
48.28	48.26	48.07	48.96	47.92	48.41	47.06	47.85	47.85	48.15	48.06	48.06	0.49	−0.22
96.50	97.34	96.21	97.20	96.16	95.99	96.01	95.98	96.04	97.12	96.63	96.47	0.55	−0.03
144.78	144.61	145.04	144.60	145.16	145.13	145.45	145.21	145.19	144.30	144.71	144.94	0.36	+0.16

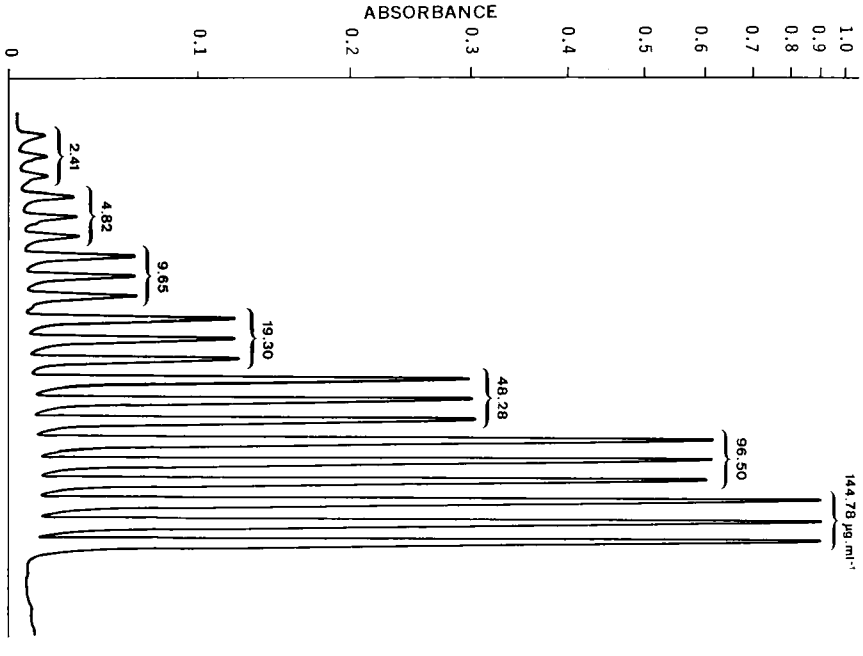

Figure 2—*Typical recording curve of 3-amino-2,4,6-triiodobenzoic acid standard solutions.*

cates the five phases of the automated procedure: (*a*) aspiration of the sample (30 sec) and washing (90 sec), (*b*) segmentation with air and combination with diazotizing reagent (reaction time of 40 sec at room temperature), (*c*) elimination of excess nitrous acid with ammonium sulfamate (40 sec), (*d*) coupling of the diazotized aromatic amine with naphthylethylenediamine (160 sec), and (*e*) continuous-flow photometry of the azo dye at 500 nm.

The flow rate of the reagents was set for obtaining the following molar ratio:

sample (200 μg/ml)	1
sodium nitrite	27
hydrochloric acid	17,000
ammonium sulfamate	325
N-(1-naphthyl)ethylenediamine	17

Prior to performing the analysis, the automated system was calibrated by triplicate assay of a series of analytical reference standards in the 2.5–150-μg/ml range. The linear regression was calculated from the recorded absorbance values (11).

Triplicate sets of five "standard" samples with a reference standard between each set were analyzed. The free amine concentration was determined from the mean absorbance measurement with the aid of the standard calibration curve[7].

RESULTS AND DISCUSSION

Linearity and Sensitivity—Figure 2 is a reproduction of a recording curve obtained by analyzing standards of sodium acetrizoate injectable solution with a free amine content in the 2.5–150-μg/ml range. The uniformity and linearity of triplicate analyses at

[7] If the mean error of the determination of one of the inserted standard samples was greater than two times the standard deviation of the calibration curves, the system was calibrated again and the new regression values were used for the following analyses. In practice, the deviation of the inserted standard sample was always ≤1.5 *SD* within the same working day.

Table III—Comparison of Automated and Manual Method

Injectable Radiopaque	Content			Free Amine Found, mg/100 ml		
	Salt, g/100 ml	Composition, %	Iodine, mg/ml	Automated Method	Manual Method	Differences
Sodium–meglumine diatrizoate	60	Meglumine 87 Sodium 13	296	3.9	3.7	+0.2
Sodium–meglumine diatrizoate	60	Meglumine 87 Sodium 13	296	4.2	3.95	$+0.2_5$
Sodium–meglumine diatrizoate	75	Meglumine 87 Sodium 13	370	5.6	5.8	−0.2
Sodium–meglumine diatrizoate	75	Meglumine 87 Sodium 13	370	6.0	6.1	−0.1
Sodium iothalamate	60	Sodium 100	282	14.4_5	15.0	-0.5_5
Sodium iothalamate	60	Sodium 100	282	12.3	12.2	+0.1
Sodium iothalamate	67	Sodium 100	400	8.1	7.9	+0.2
Sodium iothalamate	80	Sodium 100	479	23.1	23.3	−0.2
Sodium iothalamate	80	Sodium 100	479	8.6	8.6	±0.0
Meglumine iodamide	65	Meglumine 100	300	5.0	5.0	±0.0
Sodium–meglumine iodamide	62	Meglumine 77 Sodium 23	340	3.4	3.2	+0.2
Sodium–meglumine iodamide	80	Meglumine 85 Sodium 15	380	17.0	16.8	+0.2
Sodium–meglumine iodamide	80	Meglumine 45 Sodium 55	420	7.7	7.5	+0.2

the various concentrations and the return to the baseline between samples, indicating no overlap, can be noted.

Precision and Accuracy—Reproducibility of the automated technique was demonstrated by statistical analysis of 10 concentration *versus* absorbance regression lines randomly selected out of 36 calibrations performed with the same reference standards during 2 months (Tables I and II). The accuracy of the proposed procedure was evaluated by comparison of the results obtained by the automated technique with that of the USP XVIII (2, 3, 6, 7) procedure. Table III shows the excellent agreement between the two procedures for sodium and meglumine salts of diatrizoic acid and iothalamic acid.

The values for the manual method reported in Table III for iodamide salts were obtained by a manual method developed in these laboratories corresponding to the USP XVIII method for diatrizoate injectable solutions.

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