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Note

High-performance liquid chromatographic determination of metrizamide in plasma

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Metrizamide, 2-{[3-(acetylamino)-5-(acetylmethylamino)-2,4,6-triiodobenzoyl] amino}-2-deoxy-D-glucose, Amipaque®, is a non-ionic, water-soluble radiographic contrast medium intended for lumbar, thoracic and cervical myelography, ventriculography and cisternography. The absorption of metrizamide from cerebrospinal fluid to blood has been reported[1] using a technique based on the analysis of the specimen for its iodine content by a non-specific ashing technique[2].

This paper describes the development of a specific high-performance liquid chromatographic (HPLC) method for metrizamide in plasma. This assay technique has been used to measure quantitatively metrizamide in the plasma of human subjects undergoing radiographic evaluation in subarachnoid diagnostic applications.

EXPERIMENTAL

Materials

Metrizamide is a product of Winthrop Laboratories (New York, N.Y., U.S.A.). The internal standard for the assay, 3-dimethylaminomethyleneimino-2,4,6-triiodobenzoic acid, was synthesized at the Sterling-Winthrop Research Institute. The column was purchased from Whatman (Clifton, N.J., U.S.A.). Other chemicals were obtained commercially (reagent grade) and used without further purification.

Preparation of samples

Plasma standards were prepared by supplementing 1.0 ml of control human plasma with aliquots of an aqueous stock solution of metrizamide (1 mg/ml). Duplicate standards at concentrations of 0, 2, 4, 8, 12, 16 and 24 μg/ml of metrizamide were prepared.

Two sets of randomized and coded plasma samples, to be analyzed under single blind conditions, were prepared as described above. Each set contained quadruplicate plasma samples at final concentrations of 0, 2.5, 5.5, 9, 15 and 19 μg/ml in control human plasma. One set of samples was analyzed immediately and the other set was analyzed following a 5-day storage period in a −4° freezer. Freshly prepared

plasma standards were extracted and analyzed concommittantly with each set of un-
known samples.

Protein precipitation and analysis

To 1.0 ml of human plasma, containing the disodium salt of N,N'-1,2-ethane-
diylbis-[N-(carboxymethyl)glycine] (EDTA), as the anticoagulant, was added 50 μl
internal standard solution (1.5 mg/ml) and 0.2 ml of zinc sulfate solution (20%, w/v
in water). The sample was mixed and 0.2 ml of a saturated aqueous solution of barium
hydroxide was added. The tube was again mixed and a marble was placed over the
opening of the test tube which was heated on a steam bath for 2 min. The tube was
cooled and centrifuged.

A 25-μl aliquot of the clear supernatant solution was analyzed under the follow-
ing HPLC conditions. Column: Partisil 10 ODS (250 mm $\times$ 4.6 mm I.D.) with a
Corasil C_{18} precolumn (60 mm $\times$ 4 mm I.D.). Detector: Schoeffel Model SF 770
variable wavelength UV detector set at 244 nm. Mobile phase: distilled water–
methyl alcohol–glacial acetic acid (94.5:5:0.5). Flow-rate: 2.0 ml/min. Retention
time: metrizamide, 3.9 min; internal standard, 11.2 min. Pump: Milton Roy Model 709,
1000 p.s.i. Temperature: 20°, ambient.

Clinical study

Eight patients received a single injection of metrizamide in the subarachnoid
space as part of a routine neuroradiological procedure. Blood specimens were ob-
tained at 0, 2, 4, 6, 8, 24 and 48 h after administration. These were collected in vacuum
tubes containing EDTA as the anticoagulant. The samples were centrifuged and the
plasmas were separated and frozen until the analysis for metrizamide was performed.

RESULTS AND DISCUSSION

Recorder tracings from the HPLC analysis of both a control plasma sample
and a sample supplemented with 16 μg/ml of metrizamide are shown in Fig. 1.

A linear regression model was used to describe the relationship between the
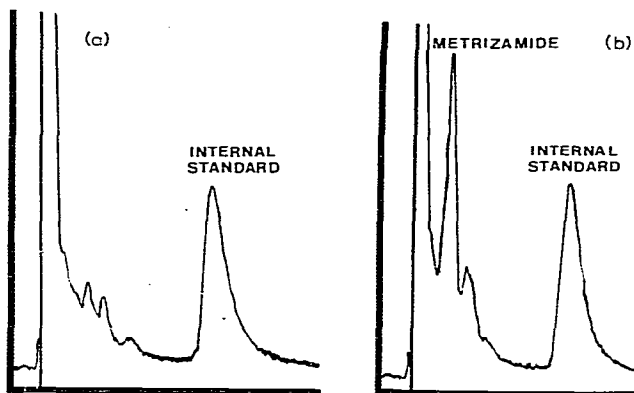peak-height ratio of metrizamide to the internal standard and the concentration of

Fig. 1. Chromatogram of control human plasma containing the internal standard (a) and the same
sample containing 16 μg/ml of metrizamide (b).

metrizamide in the plasma standards. To estimate assay sensitivity, accuracy, preci-
sion, and to determine whether metrizamide concentration changes upon storage of
frozen plasma samples, identical sets of plasma samples were assayed immediately
and after storage; the plasma parameters were in good agreement. The minimum
quantifiable level was determined by inverse prediction[3] as that concentration whose
lower 80% confidence limit just encompasses zero[4]. The overall mean sensitivity for
metrizamide in plasma was 0.72 μg/ml. A representative plot of the relationship
between relative peak height and the concentration of metrizamide added to plasma,
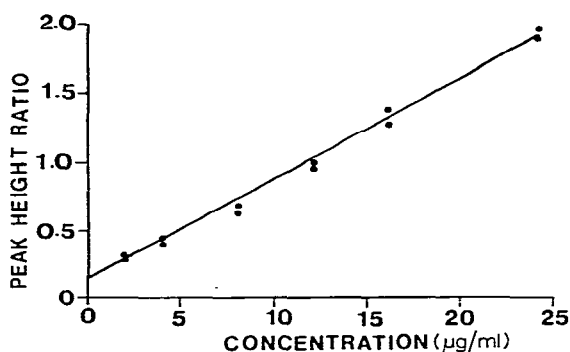is presented in Fig. 2.

Fig. 2. Extracted standard curve of control human plasma augmented with metrizamide; each con-
centration was prepared and chromatographed in duplicate.

For the range of concentrations used in the single blind study, results were
precise, accurate and reproducible. Table I summarizes the results for the analysis of
the "unknown" plasma concentrations. There was no statistically significant difference
among concentration groups with respect to mean percent differences. Over the range
of these samples the method has an estimated precision of 5.1% (standard deviation).
The estimated accuracy ranged from 3% low to 10% high over the concentration
range. A logarithmic transformation was applied to the plasma data prior to
statistical analysis to minimize the observed correlation between mean response and
variance of the quadruplicate sets[5].

The mean concentrations in the plasma of subjects at intervals after subarach-
noid administration of metrizamide is shown in Fig. 3. Although the dose varied from
2.0 to 3.2 g of total iodine (4.1 to 6.6 g metrizamide), when the mean plasma concen-
trations are plotted on a semi-logarithmic scale against time, a linear relationship
appears. This suggests first-order kinetics with an apparent half-life of about 11 h.
Golman[1], using mean values of his measurements, has reported that "the biological
half-life of metrizamide in the subarachnoid space was found to be 11 h". The mean
rate constant for excretion of metrizamide was reported as 0.09 ± 0.01 (ref. 1) which
corresponds to a half-life of 7.7 h; the range of reported values was from 0.23 (half-
life of 4 h) to 0.04 (half-life of 17.3 h). The agreement with our estimated half-life in
plasma suggests that the rate-limiting step in the excretion of metrizamide is the trans
fer from the subarachnoid space into the plasma.

TABLE I

CONCENTRATIONS FOUND IN PLASMA SAMPLES SUPPLEMENTED WITH METRIZ-AMIDE

Mean % difference = Mean of [antilog (log$_{10}$ assayed — log$_{10}$ seeded) — 1] × 100. MQL = minimum quantifiable level.

| Seeded levels (μg/ml)	Assayed level (μg/ml)	
	Day 1	Day 2
0	<MQL	<MQL
0	<MQL	<MQL
0	<MQL	<MQL
0	<MQL	<MQL
2.50	2.55	2.61
2.50	2.37	2.71
2.50	2.46	2.89
2.50	2.34	2.81
Geometric mean	2.43	2.75
% S.E.	1.96	2.24
Mean % difference	−2.9	+10.1
5.50	5.39	5.41
5.50	5.31	5.51
5.50	5.83	5.45
5.50	6.12	4.98
Geometric mean	5.65	5.33
% S.E.	3.40	2.34
Mean % difference	+2.8	−3.0
9.0	9.04	9.38
9.00	9.65	9.37
9.00	9.40	9.38
9.00	10.3	11.1
Geometric mean	9.59	9.78
% S.E.	2.80	4.33
Mean % difference	+6.5	+8.7
15.0	17.2	16.6
15.0	15.5	15.7
15.0	15.9	16.4
15.0	15.7	16.5
Geometric mean	16.1	16.3
% S.E.	2.37	1.27
Mean % difference	+7.1	+8.6
19.0	20.3	20.9
19.0	20.9	21.2
19.0	20.3	19.7
19.0	21.3	19.8
Geometric mean	20.7	20.4
% S.E.	1.19	1.88
Mean % difference	+8.9	+7.3
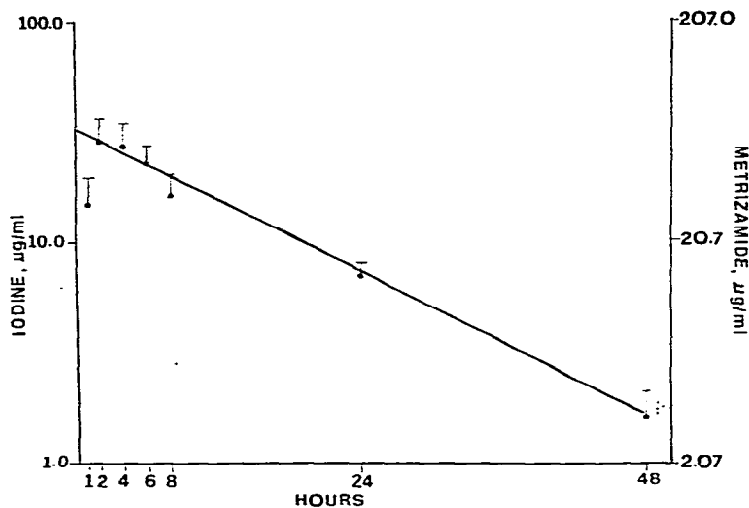
Fig. 3. Mean concentration of metrizamide in plasma of human subjects. Vertical lines denote 1 S.E. ($n = 8$).

In summary, an accurate, specific, reproducible and precise HPLC assay has been developed for the measurement of metrizamide concentration in human plasma. This method permitted estimation of the apparent first-order terminal elimination half-life in the plasma of human subjects that received metrizamide as a diagnostic agent.

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