Journal of Chromatography, 377 (1986) 460—465
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 3052

Note

Liquid chromatographic determination of meglumine diatrizoate in human plasma and urine

URSULA TURPEINEN* and ULLA-MAJ POMOELL

Helsinki University Central Hospital, Meilahti Hospital Laboratory, Haartmaninkatu 4, 00290 Helsinki (Finland)

(First received October 9th, 1985; revised manuscript received November 15th, 1985)

Meglumine diatrizoate (Angiografin) is an ionic radiographic contrast agent consisting of 3,5-diacetamido-2,4,6-triiodobenzoic acid and 1-deoxy-1-(methylamino)-D-sorbitol (Fig. 1). Like other substances of this class of highly ionized contrast agents, its molecular composition precludes significant protein binding and it is generally excreted unchanged, principally by glomerular filtration [1].

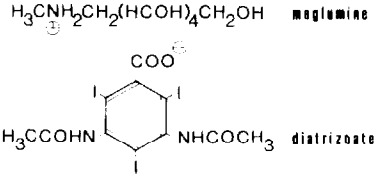

Fig. 1. Chemical structure of meglumine diatrizoate.

Previously described methods for the determination of contrast media in plasma and urine have relied on their iodine content, either by direct chemical analysis [2] or as radioactive iodine [3]. A spectrophotometric [4] and a polarographic [5] method have also been described for the determination of iodinated contrast agents. Most of the methods are, however, either cumbersome, involving the administration and measurement of radiolabelled compounds, or are not sufficiently specific.

High-performance liquid chromatography (HPLC) has been previously applied for the determination of contrast media such as iohexol [6, 7] and iothalamate [8, 9] in biological fluids.

0378-4347/86/$03.50

We describe here a selective HPLC method for measuring meglumine diatrizoate in plasma and urine. Proteins are removed by precipitation and the supernatant is diluted and applied on an amino column, using *p*-aminobenzoic acid as internal standard.

EXPERIMENTAL

Apparatus

The chromatographic system consisted of a Varian 5000 or 5500 liquid chromatograph combined with a Vista 402 chromatographic data system (Varian Assoc., Walnut Creek, CA, U.S.A.) and a Perkin-Elmer recorder 056-1002 (Hitachi, Tokyo, Japan). We used a 250 × 4.6 mm I.D. amino column, 5 μm particle size (Spherisorb S5 NH2, Phase Separations, Queensferry, U.K.). For the determination of the absorption spectrum of meglumine diatrizoate, a Cary 118 spectrophotometer (Varian) was used with the following settings: 0.5 A f.s., scanning speed 1 nm/s from 200 to 340 nm in 1-cm cuvettes.

Reagents

Angiografin solution (650 mg/ml) was from Leiras (Turku, Finland). The internal standard, *p*-aminobenzoic acid, was from Merck (Darmstadt, F.R.G.) and a 1.5 m*M* stock solution in water was stored at − 20°C. HPLC-grade acetonitrile was from Rathburn Chemicals (Walkerburn, U.K.). Potassium phosphate buffer (10 m*M*, pH 4.3) was filtered through a 0.45-μm (average pore size) filter before use. All other chemicals were of analytical-reagent grade.

Sample treatment

To 500 μl of human plasma spiked with meglumine diatrizoate, 500 μl of acetonitrile were added. After mixing and centrifugation (2 min at 7200 *g*), an aliquot of the clear supernatant was diluted 1:250—1:1000 with water, depending upon the expected amount of diatrizoate. To 100 μl of the dilution, 5 μl of 1.5 m*M* *p*-aminobenzoic acid were added as internal standard. The plasma samples from patients who had been given meglumine diatrizoate (1.5 ml/kg body weight) were treated as above.

The urine samples were diluted up to 1:1000 with water and 5 μl of internal standard stock solution were added to 50 μl of the diluted sample.

Chromatographic conditions

Aliquots of 2.5—20 μl of the sample dilution were injected into the chromatographic system. The mobile phase consisted of 10 m*M* potassium phosphate buffer (pH 4.3) and acetonitrile (25:75 or 13:87) at a flow-rate of 1.5 ml/min at room temperature. The detection wavelength was 238 nm, the detector range 0.01 A f.s. and the recorder chart speed 5 mm/min. With the Vista 402 data system we used, the attenuator setting was 4, corresponding to 0.008 A f.s.

Analytical variables

The calibration curve was prepared by the standard addition method.

Various amounts of meglumine diatrizoate were added to the urine and plasma samples, which were then processed as described above. The calibration curves were obtained by plotting the peak height versus the amount injected.

The accuracy was evaluated by comparing the absolute peak height of meglumine diatrizoate in spiked samples to the calibration curve.

RESULTS

UV absorption

The maximum absorption for meglumine diatrizoate was obtained at wavelength 238 nm. The calculated molar absorptivity of the contrast medium at 238 nm was $2.96 \cdot 10^4\ M^{-1}\ cm^{-1}$.

Analytical variables

A linear calibration curve, $y = 0.508x + 7.35$; $r = 0.991$, for peak height (y, mm) versus quantity of meglumine diatrizoate (x, pmol) was obtained with plasma samples spiked with known amounts of the contrast medium from 20 to 156 pmol per injection. With urine samples spiked with known amounts of meglumine diatrizoate, a similar calibration curve was obtained, $y = 0.480x + 5.91$; $r = 0.992$. The linear range was from 20 to 118 pmol per injection. The minimum detectable amount of meglumine diatrizoate (signal-to-noise ratio > 10) in both cases was 20 pmol per 20-μl injection when the detector was set at 0.01 A f.s. corresponding to a 1 μM sample concentration of meglumine diatrizoate.

The accuracy, defined as 100 × amount found/amount added, was 88% at the 40-pmol level and 97% at the 79- and 155-pmol levels. The mean was 94%.

As shown in Table I, the within-assay coefficient of variation (C.V.) calculated from the values for three samples was 2.6–3.0% and the between-assay C.V. was 5.2—5.6%.

TABLE I

DATA ON PRECISION

Sample	Meglumine diatrizoate concentration (mean ± S.D.) (μM)	Coefficient of variation (%)
Within-assay (n = 10)		
A	6.5 ± 0.16	3.0
B	14 ± 0.32	2.9
C	30 ± 0.62	2.6
Between-assay (n = 5)		
D	6.1 ± 0.65	5.6
E	12 ± 1.1	5.6
F	27 ± 3.0	5.2

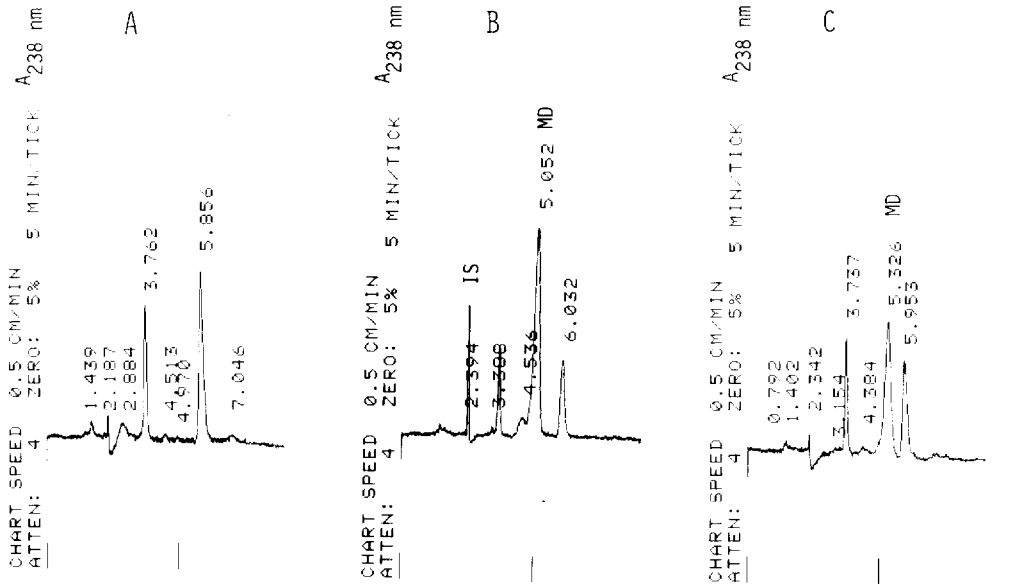

Fig. 2. Chromatograms of urine samples. (A) Blank urine; (B) urine sample with added meglumine diatrizoate (MD) and internal standard (IS); (C) a patient's urine containing 3.8 m*M* meglumine diatrizoate. The numbers indicate time of elution (min after sample injection) using 87% acetonitrile in the mobile phase.

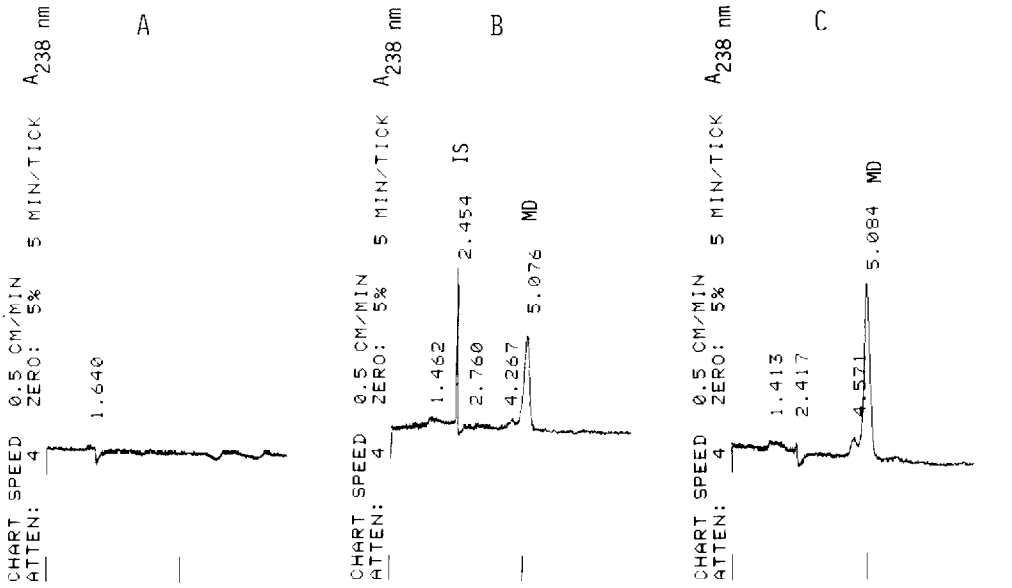

Fig. 3. Chromatograms of plasma samples. (A) Blank plasma; (B) plasma sample with added meglumine diatrizoate (MD) and internal standard (IS); (C) a patient's plasma containing 3.6 m*M* meglumine diatrizoate. Conditions as in Fig. 2.

Analysis of urine and plasma samples

Representative chromatograms are seen in Figs. 2 and 3 obtained with (A) blank urine and plasma, (B) urine and plasma containing added amounts of meglumine diatrizoate and the internal standard, and (C) urine and plasma from a patient given 1.5 ml of meglumine diatrizoate per kg body weight. A sharp and symmetrical peak of meglumine diatrizoate is seen at a retention time of 5.1–5.3 min. The internal standard elutes at 2.4–2.5 min. In all cases, meglumine diatrizoate was well separated from the other peaks. No interfering peaks were found in the various plasma blanks examined. In the urine samples, there are two endogenous compounds but they do not interfere with the present assay.

DISCUSSION

The purpose of the present study was to develop a rapid and practical method for the determination of the contrast agent, meglumine diatrizoate. The contrast agents in current use are generally safe, but have certain undesirable side-effects [10]. The clinical utilization of these compounds should involve the evaluation of the relation between administered dose and achieved concentration in plasma. This requires the availability of specific methods for the determination of contrast media in biological fluids. In contrast to most of the earlier methods for the determination of these compounds [2, 3, 11], our technique is based on the quantification of the whole organic molecule, rather than its iodine content, and is simple and rapid. Because of the high molar absorptivity of meglumine diatrizoate, we can dilute the samples up to 1:2000 and so reduce interference from endogenous compounds.

The percentage of acetonitrile in the mobile phase greatly affects the retention of meglumine diatrizoate. If the separation is not satisfactory, the acetonitrile content of the mobile phase can be increased up to 87% to achieve the best separation of meglumine diatrizoate.

The reasonable linearity and degree of precision (Table I) were obtained without an internal standard. However, *p*-aminobenzoic acid can be used as internal standard and it is well separated from the other peaks (Figs. 2B and 3B).

Our preliminary results show that the present method is also applicable to the quantification of other contrast media in plasma and urine. Metrizamide, methylglucamine iodamide and methylglucamine iothalamate are all eluted under these conditions. With slight modifications of the mobile phase composition, the separation can be further optimized.

In summary, our method is an accurate, selective and rapid HPLC determination of meglumine diatrizoate in human plasma and urine. The assay can be applied for the analysis of specimens obtained during clinical trials.

REFERENCES

1 W.R. Cattell, Invest. Radiol., 5 (1970) 473.
2 J.J. Moran, Anal. Chem., 24 (1952) 378.

3 M.D. Blaufox, D.R.S. Sanderson, W.N. Tauxe, K.G. Wakim, A.L. Orvis and C.A. Owen, Am. J. Physiol., 204 (1963) 536.
4 P. Purkiss, R.D. Lane, W.R. Cattell, I. Kelsey Fry and A.G. Spencer, Invest. Radiol., 3 (1968) 271.
5 M. Mastragostino, A. Testoni and P. Lanza, Anal. Lett., 17 (1984) 173.
6 J. Edelson, G. Palace and G. Park, J. Chromatogr., 274 (1983) 428.
7 E. Krutzén, S.-E. Bäck, I. Nilsson-Ehle and P. Nilsson-Ehle, J. Lab. Clin. Med., 104 (1984) 955.
8 S. Boschi and B. Marchesini, J. Chromatogr., 224 (1981) 139.
9 T. Prueksaritanont, M.-L. Chen and W.L. Chiou, J. Chromatogr., 306 (1984) 89.
10 M.A. Bettmann, Am. J. Roentgenol., 139 (1982) 787.
11 F. Medzihradsky, P.J. Dahlström, T.B. Hunter, J.R. Thornbury and T.M. Silver, Invest. Radiol., 10 (1975) 532.