ELSEVIER

Journal of Chromatography A, 740 (1996) 284–288

JOURNAL OF CHROMATOGRAPHY A

Short communication

High-performance liquid chromatographic determination of cyclohexylhydantoin and cyclohexylidenehydantoin formed during the synthesis of phenylhydantoin from hydantoin and cyclohexanone[1]

Amruta S. Tambe

Division of Organic Chemistry: Technology, National Chemical Laboratory, Pune 411008, India

Received 22 January 1996; accepted 26 February 1996

Abstract

DL-Phenylhydantoin can be converted into D-phenylglycine by an enzymatic process. D-phenylglycine is an important starting material in the production of β-lactams such as semisynthetic penicillins and cephalosporins. In our laboratory, the synthesis of phenylhydantoin was achieved from hydantoin and cyclohexanone in the presence of a base. An efficient and fast isocratic reversed-phase high-performance liquid chromatography method was developed for the determination of phenylhydantoin, cyclohexylhydantoin and cyclohexylidenehydantoin. Quantitative analysis was carried out by an external standard method.

Keywords: Cyclohexylhydantoin; Cyclohexylidenehydantoin; Hydantoins; Phenylhydantoin; Cyclohexanone

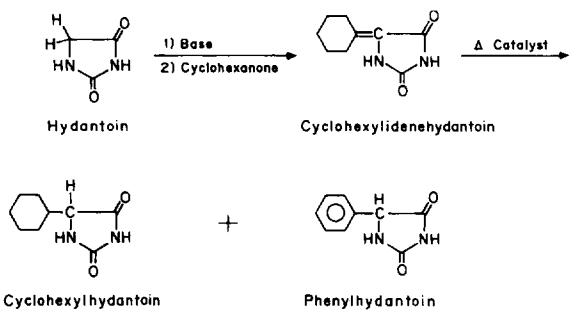
1. Introduction

DL-Phenylhydantoin can be converted into D-phenylglycine by an enzymatic process [1]. This is possible because of the spontaneous chemical racemization of five-substituted hydantoins even under mild basic catalysis conditions. D-Phenylglycine is an important starting material in the production of β-lactams such as semisynthetic penicillins and cephalosporins. DL-Phenylhydantoin can be prepared by reaction of urea and glyoxalic acid in benzene-containing solvents in the presence of acids [2] or by heating allantoic acid methyl ester with benzene and concentrated sulfuric acid [3,4]. A few Japanese patents report the preparation of phenylhydantoin by the Bucherer Bergs reaction [5,6] and from aldehydes or ketones by emulsifying with a surfactant [7]. The use of cation-exchange resin has also been reported [8]. In our laboratory, the synthesis of phenylhydantoin was achieved from hydantoin and cyclohexanone in the presence of a base (Scheme 1) and a patent has been filed recently [9]. In order to optimize the reaction conditions to get the maximum yield of phenylhydantoin, it was essential to develop an analytical method for the determination of phenylhydantoin, cyclohexylhydantoin and cyclohexylidenehydantoin in the final product.

In the literature, a HPLC method that uses a convex gradient was reported for the separation of phenylglycine and phenylhydantoin [12]. There are few references reporting enantioseparation of five-

[1] NCL Communication Number: 6225.

0021-9673/96/$15.00
PII S0021-9673(96)00171-9



Scheme 1. Synthesis of phenylhydantoin from hydantoin and cyclohexanone.

substituted hydantoins by GC [13] as well as by HPLC [14–18]. Huang and Ough [19] have recently reported the determination of amino acid hydantoins by HPLC with diode array detection. To our knowledge, simultaneous separation of the various substituted hydantoins has not been reported in the literature. In the present work, an isocratic reversed-phase HPLC method is described for the determination of three substituted hydantoins.

2. Experimental

2.1. Instrumentation

A Waters HPLC system, equipped with model 510 dual head reciprocating solvent delivery pumps controlled by a model 680 automated gradient controller, a Waters Lambda-Max model 481 LC spectrophotometer and a model U6K loop injector (vol= 2 μl) was used. A Waters data module (model 730) was used for recording the data. A Waters Novapak C_{18} Radial-Pak cartridge (10 cm×5 mm, 5 μm) fitted in RCM 100 Radial Compression Separation System was used as a reversed-phase column.

2.2. Reagents

HPLC-grade water was obtained from a Milli-Q system (Millipore, Bedford, MA, USA). Methanol was purified to chromatographic quality in our laboratory. Reagent grade orthophosphoric acid and triethylamine were used to prepare a stock buffer solution of triethylammoniumphosphate (0.7 *M*, pH=2.46). Solvents were filtered through a Millipore filter (0.45 μm) using a Millipore all-glass filter apparatus.

2.3. Chromatographic conditions

The mobile phase used was 35% methanol in phosphate buffer at a flow-rate of 1.0 ml/min. Detection was carried out at 214 nm. Chromatography was performed at room temperature.

2.4. Preparation of standard solutions

A standard mixture containing 30.5 mg of hydantoin, 37.0 mg of phenylhydantoin, 28.3 mg of cyclohexylhydantoin and 21.2 mg of cyclohexylidenehydantoin was prepared in 35% methanol in water in a 50-ml volumetric flask. Aldrich-grade hydantoin was used to synthesize substituted hydantoins in our laboratory and their purities were checked by HPLC as well as by NMR spectroscopy and microanalysis. The concentrations of the individual components of the standard solutions were as follows: cyclohexylidenehydantoin, 10.6 mg in 10 ml of methanol; cyclohexylhydantoin, 5.1 mg in 10 ml of methanol and phenylhydantoin, 27.6 mg and 70.2 mg in 50 ml of methanol–water (35:65, v/v). A series of calibration solutions were prepared by diluting 1 to 8 ml of stock standard solution to 10 ml in 35% methanol in water. The injection volume was 20 μl. An external standard calibration graph was plotted to find out the linearity of the UV detector (214 nm) response for various hydantoins.

2.5. NMR and other data

Cyclohexylhydantoin IR (Nujol): 3300, 1720 and 1680. ^{1}H NMR (200 MHz, [$^2H_6$]dimethyl sulfoxide): δ 1.15 (m, 5H, -CH_2 axial), 1.4 (m, 1H, cyclohexyl-C*H*), 1.65 (m, 5H, -CH_2 equatorial), 3.85 (d, 1H, CO-C*H*-NH), 7.3 (d, 1H, CH-N*H*-CO), 7.85 (s, 1H, CO-N*H*-CO). ^{13}C NMR (200 MHz, [$^2H_6$]dimethyl sulfoxide): 25.84, 26.20, 26.42, 39.77, 62.95, 158.34 and 175.90. Mass: 183 $(M+1)^+$, 100 (B). Analysis: calculated for $C_9H_{14}O_2N_2$: C, 59.34; H, 7.69; N, 15.38. Found: C, 58.82; H, 7.40; N, 14.89. m.p.: 220°C, literature value, 226°C [10].

Cyclohexylidenehydantoin IR (Nujol): 3300, 1720

and 1660. ^{1}H NMR (200 MHz, [$^{2}H_6$]dimethyl sulfoxide): δ 1.5 (s, 6H, -CH_2), 2.20 (s, 2H, =C-CH_2), 2.75 (s, 2H, NH-C=C-CH2), 9.66 (s, 1H, =C-NH-CO), 10.85 (s, 1H, CO-NH-CO). ^{13}C NMR (200 MHz, [$^{2}H_6$]dimethyl sulfoxide): 26.33, 27.31, 27.64, 28.17, 30.21, 122.95, 132.80, 154.58 and 165.85. Mass: 180 M^+, 113 (B). Analysis calculated for $C_9H_{12}O_2N_2$: C, 60.0; H, 6.66; N, 15.55. Found: C, 59.65; H, 6.96; N, 15.81. m.p.: 255°C, literature value, not reported.

Melting point of cyclohexylidenethiohydantoin: 263–265°C [11].

3. Results and discussion

A chromatogram showing the separation of hydantoin, phenylhydantoin, cyclohexylhydantoin and cyclohexylidenehydantoin is presented in Fig. 1. The analysis time was less than 10 min and the peaks were well resolved, as can be clearly seen from the retention time and capacity factor values presented in Table 1. Since the retention for hydantoin is poor, capacity factors were calculated considering the retention time of hydantoin as the dead volume of the column. Quantitative analysis of hydantoin was not carried out. The standard solutions were quite stable and gave fairly constant retention times over a period of twelve months.

Statistical data obtained for retention time and area counts for standard mixtures of individual components are given in Table 2. High repeatability was obtained during the analysis carried out over the course of twelve months. In the case of area counts, the first three digits were taken for the calculation of statistical data.

3.1. External standard quantitative analysis

Linearity of response for D-phenylhydantoin (1.0–10.0 μg) at 212 nm has already been reported [12]. We obtained a linear response for DL-phenylhydantoin at 214 nm up to 28.0 μg. This was required as the percentage of phenylhydantoin in the product was high. A linear relationship for cyclohexylidene and cyclohexylhydantoin was also obtained with UV detection at 214 nm up to 10 μg. Regression analysis

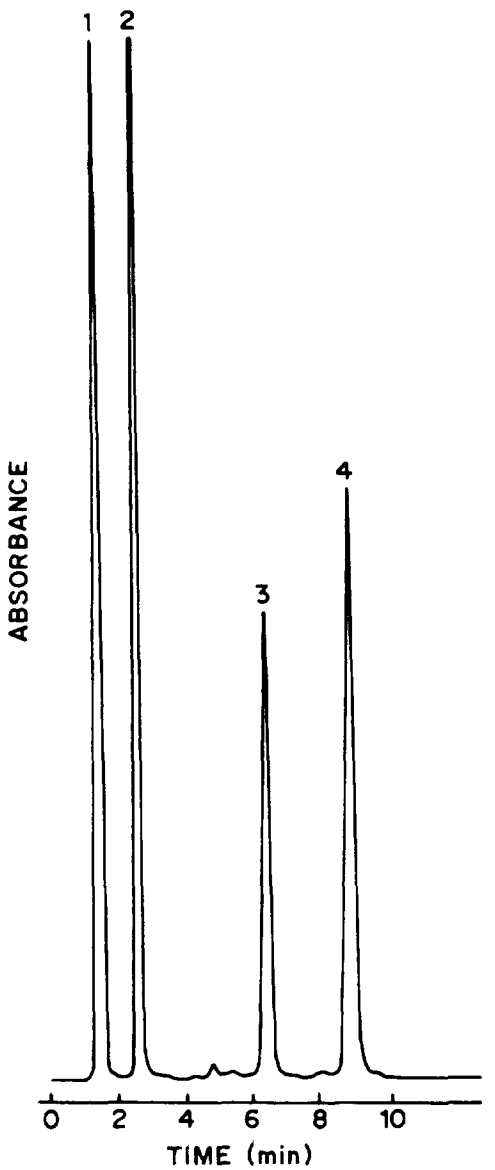


Fig. 1. Separation of hydantoin, phenylhydantoin, cyclohexylhydantoin and cyclohexylidenehydantoin by HPLC. Column, NovaPak C_{18} Radial Pak cartridge (10 cm×5 mm, 5μm); mobile phase, 35% methanol in phosphate buffer, pH 3; flow-rate, 1.0 ml/min; UV detection, 214 nm. Peaks: 1, hydantoin; 2, phenylhydantoin; 3, cyclohexylhydantoin and 4, cyclohexylidenehydantoin.

by the least squares method (Table 3) showed an excellent correlation between the peak areas and the amounts injected. Table 4 shows the results obtained for an unknown mixture.

Table 1
Retention times and relative retention values of various hydantoins

Number	Name of the compound	Retention time, t_R (min)	Capacity factor
1	Hydantoin	1.30	–
2	Phenylhydantoin	2.39	0.84
3	Cyclohexylhydantoin	6.34	3.88
4	Cyclohexylidenehydantoin	8.86	4.25

Table 2
Statistical data obtained for the analysis of various hydantoins

Number	Compound name		Parameter				
			S	R.S.D.	X	CL	n
1	Phenylhydantoin	t_R	0.071	2.97	2.39	±0.035	14
		A	6.36	1.58	403	±3.14	9
2	Cyclohexylidenehydantoin	t_R	0.19	2.13	8.93	±0.118	9
		A	5.56	2.36	235.4	±3.273	10
3	Cyclohexylhydantoin	t_R	0.064	1.03	6.24	±0.055	4
		A	5.57	1.63	342	±5.149	4

t_R=retention time; A=area counts; S=standard deviation; R.S.D.=relative standard deviation; X=average value, CL=confidence limits; n=number of observations.

Table 3
Linear regression analysis by the least squares method for a plot of peak area versus the amount injected (μg) for substituted hydantoins

Number	Compound	n	A	B	r
1	Cyclohexylhydantoin	5	26 776	772 260	0.99959
2	Cyclohexylidenehydantoin	5	8053	11 897	0.9995
3	Phenylhydantoin	4	−3071	11 477	0.9967

A=y-axis intercept; B=slope; r=correlation coefficient; n=number of calibration points.

This very simple, fast, and efficient method allowed us to find out the exact percentage of the above-mentioned three components in the reaction product and therefore to optimize the reaction conditions; e.g. concentration of base, temperature of the reaction, etc.

4. Conclusion

A very good separation is obtained between hydantoin, phenylhydantoin, cyclohexylhydantoin and cyclohexylidenehydantoin by HPLC. The method described can be used to find out the amount of

Table 4
Results obtained from calibration graph for unknown sample

Number	Compound name	Amount (from graph)	Amount (Actual)	% Error	R.S.D. (Area)
1	Cyclohexylidenehydantoin	2.1465	2.12	1.25	1.63
2	Cyclohexylhydantoin	6.052	6.12	1.11	1.62

substituted hydantoins. The statistical analysis shows that the method is precise and reproducible. Regression analysis showed an excellent correlation between peak areas and the amounts injected.

Acknowledgments

The author gratefully acknowledges Dr. T. Ravindranathan, Head, OCT Division, National Chemical Laboratory, Pune, India, for providing the standards and samples for the HPLC analysis.

References

[1] R. Oliveri, E. Fassetti, L. Angeloni and L. Degene, Enzyme Microbiol. Technol., 1 (1979) 201.
[2] M. Yamada and S. Takahashi, Jpn. Kokai Tokkyo Kiho JP 05 339 244, December 21, 1993, Appl. 92/149 575, June 9, 1992.
[3] W. Hendei, E. Kloimstein, K. Fitzinger, A. Viehboeck and K. Haidinger, Eur. Pat. Appl. EP 599,198 June 1, 1994, At Appl. 9 212 355, November 27, 1992.
[4] W. Hendel, E. Kloimstein, K. Fitzinger, A. Viehboeck and K. Haidinger, Ger Offen DE 4 301 244, July 21, 1994, Appl. January 19, 1993.
[5] K. Takeuchi, H. Ogata, M. Senda, M. Nakamura, K. Odaka and Y. Sato, Jpn. Kokai Tokkyo Koho JP 02 169 577, June 29, 1990, Appl. 88/321 925, December 22, 1988.
[6] K. Takeuchi, H. Ogata, M. Senda, M. Nakamura, K. Odaka and Y. Sato, Jpn. Kokai Tokkyo Koho JP 02 169 578, June 29, 1990, Appl. 88/321 926, December 22, 1988.
[7] T. Yagawa, T. Tsuji and T. Nagai, Jpn. Kokai Tokkyo Koho JP 6 107 257 January 13, 1986, Appl. 84/128 832.
[8] K. Takeuchi, Y. Sato, M. Senda, T. Kono, Y. Shimokawa and T. Okamura, Jpn. Kokai Tokkyo Koho JP 60 222 465, November 7, 1985, Appl. 84/77 366.
[9] U.R. Kalkote, R.R. Joshi, R.A. Joshi and T. Ravindranathan, Appl. July 28, 1994, NE-82/94.
[10] J. Edelson, J.D. Fissekis, C.G. Skinner and W. Shive, J. Am. Chem. Soc., 80 (1958) 2698.
[11] H.L. Yale, J. Am. Chem. Soc., 75 (1953) 675.
[12] O.L. Guevara, R. Quintero and P. Padilla, J. Chromatogr., 329 (1985) 428.
[13] H. Lickefett, K. Krohn, W.A. Konig, B. Gehrcke and C. Syldatk, Tetrahedron: Asymmetry, 4 (1993) 1129.
[14] C. Yun and Y.P. Haur, J. Appl. Polym. Sci., 49 (1993) 851.
[15] W.D. Hooper, N.J. Oshea, J. Nerida and M.S. Quing, Chirality, 4 (1992) 142.
[16] S. Eto, H. Noda and A. Noda, J. Chromatogr., 579 (1992) 253.
[17] C. Rosini, C. Bertucci, D. Pini, P. Altemura and P. Savadori, Chromatographia, 24 (1987) 671.
[18] J.H. Maguire, J. Chromatogr., 387 (1987) 453.
[19] Z. Huang and C.S. Ough, J. Agric. Food Chem., 39 (1991) 2218.