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Short Communication

An analytical method to determine small quantities of phenytoin in the presence of excess amounts of one of its prodrugs, the monomethane sulphonate salt of 3-(*N,N*-dimethylglycyloxymethyl)-5,5-diphenyl-hydantoin

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Introduction

The purpose of this work was to develop a method for the detection and determination of small quantities of phenytoin, an effective anticonvulsant drug [1], in the presence of large amounts of a water soluble cationic prodrug (monomethane sulphonate salt of 3-(*N,N*-dimethylglycyloxymethyl)-5,5-diphenylhydantoin) of phenytoin [2]. An earlier study by Varia *et al.* [3] revealed that the precipitation of phenytoin from concentrated solutions of this prodrug as a result of prodrug hydrolysis to phenytoin could be correlated with neither the hydrolysis rate of the prodrug, determined in dilute aqueous solution [3], nor the known aqueous solubility of phenytoin in water.

It was postulated that the lack of correlation was due to either a decreased rate of hydrolysis of the prodrug in concentrated solution or the solubilization of phenytoin by the prodrug as a result of either complex formation between the prodrug and phenytoin or the fact that the prodrug may form micellar solutions at higher concentrations. However, before these various hypotheses could be tested, an analytical technique was required to detect phenytoin in the presence of the prodrug that did not have an adverse effect on the performance and lifespan of the HPLC columns. Preliminary studies showed

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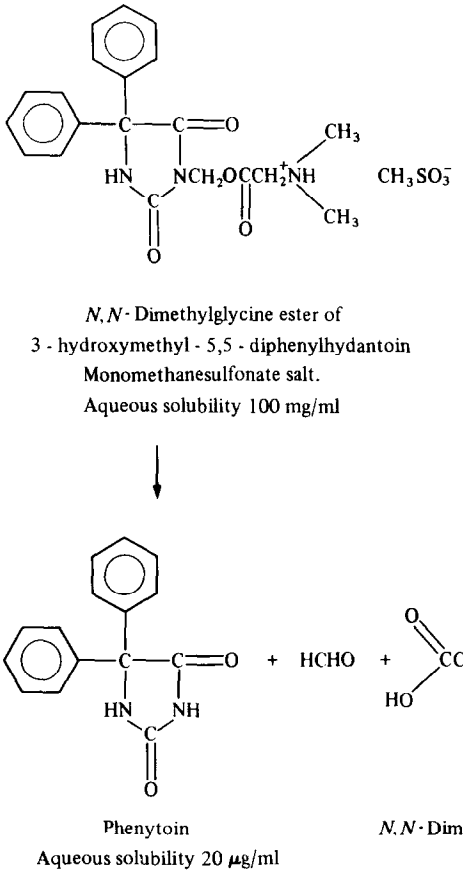

Scheme 1
The hydrolysis reaction of the monomethane sulphonate salt of 3-(*N,N*-dimethylglycyloxymethyl)-5,5-diphenylhydantoin, forming Phenytoin, *N,N*-dimethyl glycine and formaldehyde.

that the injection of high concentrations (up to 90 mg/cm^3) of the prodrug resulted in rapid and irreversible column deterioration.

Various procedures for phenytoin analysis have appeared in the literature [4–19], however, most were not directly applicable to the current problem because of the presence of the prodrug. A suitable method, that makes use of small disposable cation exchange columns to separate the prodrug from phenytoin prior to HPLC analysis of phenytoin, has been developed and is now described.

Experimental

Materials

Unless otherwise mentioned all reagents and solvents used were analytical grade. Phenytoin (Sigma Chemical Co., St. Louis, Mo.) as well as the internal standard, 5-(4-methylphenyl)-5-phenylhydantoin (Aldrich Chemical Co., Milwakee, Wis.), were obtained from commercial sources. The prodrug was synthesized according to the method reported by Varia *et al.* [2], recrystallized from ethanol–acetone–ether and dried

in a vacuum oven. The purity was determined by means of a differential scanning calorimeter (DSC 4, Perkin–Elmer, Norwalk, Connecticut). The melting point recorded (173–175°C) was the same as reported by Varia *et al.* [2].

Separation method

A suspension of the cation exchange resin: AG 50W-X4 100–200 Mesh, Hydrogen form (Bio-Rad Laboratories), was prepared by adding 750 cm^3 of distilled water to 500 g of resin. This suspension was washed with water containing increasing amounts (25, 50, 75 and 100%) of methanol. The concentration of methanol was gradually increased to prevent damage to the resin structure due to the addition of the organic solvent. After washing with pure methanol the ratio of methanol:water was gradually decreased, with the same ratios as before, to render the final purified resin as a suspension in water.

Small cation exchange columns were prepared by pipetting 1 cm^3 of the purified resin suspension into glass wool stoppered pasteur pipettes. The pH of each column was adjusted to pH 2.5 to ensure minimum hydrolysis of the prodrug during separation and stoppered with small rubber caps in order to prevent the columns from drying. A 20 μl aliquot of the internal standard solution [50.0 mg/cm^3 in methanol–water (70:30, v/v)] as well as 20 μl of the standard or sample solutions was added onto the columns, respectively and eluted with 3 cm^3 distilled methanol.

The eluents were collected in silannized (5% v/v dimethylmonochlorosilane (OVSC — Ohio Valley Speciality Chem. Co., RT. 6, Brant) in hexane) glass tubes. The contents of each tube was evaporated under a gentle stream of nitrogen at 70°C. Prior to analysis the analytes were reconstituted with 100 μl of methanol–water (70:30, v/v).

Analytical method

Samples were analysed by means of a modular liquid chromatograph consisting of an Altex Model 110A pump, Rheodyne injector with 20 μl loop, a Waters Associates Model 450 variable wavelength detector and a Columbia Scientific Industries Model CS 138 integrator. Separations were performed on 5 μm Hypersil ODS (250 mm × 4.6 mm i.d.) columns with methanol–aqueous 0.1 M acetate buffer pH 6.0 (60:40, v/v) isocratic elution.

Reproducibility

In order to determine the reproducibility of the method a regression curve was constructed using phenytoin solutions containing 10.35, 20.74, 41.23, 80.22 and 164.94 μg per cm^3, respectively.

Five runs of each concentration of phenytoin were performed using the described method. Both peak heights and integration values (AUC) were measured in order to test the reproducibility of the method.

Results and Discussion

Table 1 shows the ratio of peak heights as well as the ratio of the AUC of the sample to internal standard for 5 samples of each concentration. Table 2 shows the mean values and standard deviations.

Little difference between the two methods of evaluation (peak heights versus AUC) was found. The method showed great reproducibility with a highest relative standard deviation of only 5.56% at the 41.23 μg/cm^3 concentration. The method gave a standard

Table 1
The ratio of peak heights of the sample:internal standard, as well as the ratio of the AUC of the sample:internal standard using 5 different concentrations of phenytoin ($N = 5$)

Concentration (mg/ml)	Sample no.	Ratio of sample:int. std. Peak heights	AUC
10.35	10a	0.115	0.094
	b	0.115	0.094
	c	0.118	0.094
	d	0.116	0.095
	e	0.113	0.095
24.74	24a	0.328	0.238
	b	0.330	0.244
	c	0.333	0.262
	d	0.316	0.235
	e	0.325	0.240
41.23	40a	0.558	0.411
	b	0.523	0.404
	c	0.589	0.446
	d	0.519	0.388
	e	0.530	0.393
80.22	80a	0.969	0.735
	b	0.955	0.710
	c	0.966	0.728
	d	0.970	0.742
	e	0.960	0.719
164.90	160a	1.893	1.397
	b	1.914	1.404
	c	1.941	1.431
	d	1.962	1.407
	e	2.028	1.492

Table 2
The mean and coefficient of variation for the five concentrations of phenytoin used

Peak heights Concentration (mg/ml)	Mean RSD
10.35	0.1155–1.73
24.74	0.3264–1.99
41.23	0.5438–5.42
80.22	0.9642–0.62
164.94	1.9403–3.09

AUC-integration Concentration (mg/ml)	Mean RSD
10.35	0.0945–1.90
24.74	0.2438–4.38
41.23	0.4085–5.56
80.22	0.7271–1.65
164.94	1.4250–3.15

curve linear over a wide concentration range from 1.0 to 160.0 $\mu g/cm^3$. Excellent regression ($r = 0.9995$ and $y = 0.0116x + 0.0302$) using peak heights as well as using AUC ($r = 0.9994$ and $y = 0.0082 + 0.0703$) was found.

Figure 1 shows a chromatogram (A) of a mixture of phenytoin (1.068 mg/cm^3) and prodrug (0.695 mg/cm^3) introduced directly into the column. In the same figure chromatogram B shows the absence of the prodrug peak (arrow) after a sample of the same solution was subjected to separation on the ion exchange column. This shows that no significant hydrolysis occurs on the column. Apart from the column deterioration problem the detection of very low concentrations of phenytoin in the presence of up to 92 mg/cm^3 prodrug precluded direct HPLC injections. Figure 2 shows a typical chromatogram of a sample containing phenytoin and the internal standard. No prodrug peaks have been found even at prodrug concentrations as high as 92.0 mg/cm^3, showing the cation exchange columns to have adequate capacity to handle such high concentrations.

Figure 3 shows the fairly good linear plots obtained during kinetic studies of prodrug hydrolysis to phenytoin at very low prodrug concentrations with expected small differences between consecutive samples. This showed the method to be reliable enough to distinguish between small differences in phenytoin concentrations as the reactions proceed. The various rates of hydrolysis of prodrug as a function of the initial prodrug concentration were determined at constant pH and ionic strength (0.5 M).

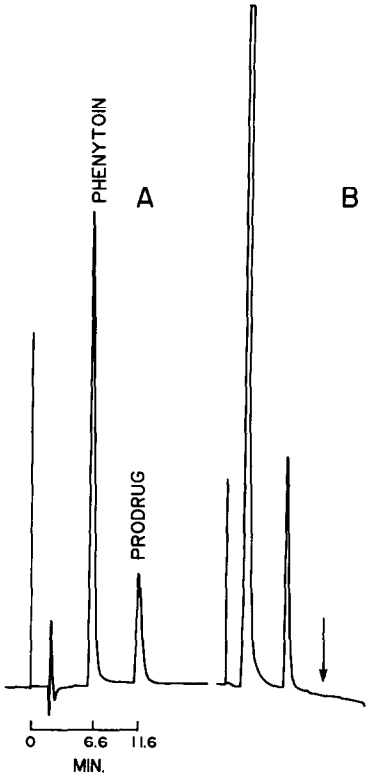

Figure 1
Chromatogram (A) represents a mixture of phenytoin (1.068 mg/cm^3) and prodrug (0.695 mg/cm^3) in methanol, injected directly onto the HPLC column while chromatogram B represents an analysis of a sample of the same solution that was subjected to ion exchange chromatography, showing the removal of prodrug by the column.

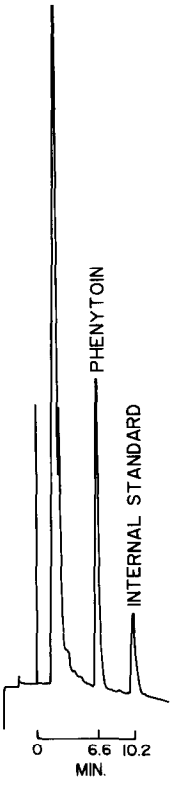

Figure 2
A typical chromatogram showing phenytoin and the internal standard peak as well as the retention times obtained.

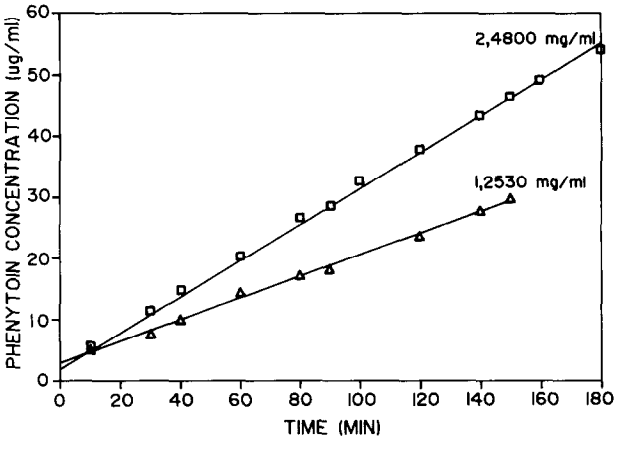

Figure 3
Curves showing the rate of hydrolysis of the prodrug as a function of various prodrug concentrations (1.253 and 2.480 mg/cm^3) in water. .

Conclusion

The developed analytical method is shown to exhibit excellent reproducibility and reliability enabling the determination of phenytoin concentrations from 1 up to 160 $\mu g/cm^3$ in the presence of prodrug concentrations up to 92.00 mg/cm^3. The cation exchange columns used also are shown to have sufficient capacity to separate phenytoin from large amounts of prodrug. Because of the instability of the prodrug in aqueous solutions the separation has to be rapid. Separation was obtained in less than 1 min with a subsequent step, the drying of the samples under nitrogen before analysis, being rate limiting.

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