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MEASUREMENT OF MEPHENYTOIN (3-METHYL-5-ETHYL-5-PHENYLHYDANTOIN) AND ITS DEMETHYLATED METABOLITE BY SELECTIVE ION MONITORING

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SUMMARY

Mephenytoin (3-methyl-5-ethyl-5-phenylhydantoin) and its demethylated metabolite Nirvanol (5-ethyl-5-phenylhydantoin) were measured by a selective ion monitoring technique. This method was used in the analysis of both compounds in plasma from a patient receiving 50 mg and 400 mg of mephenytoin in single oral doses. Both compounds were extracted from plasma and ethylated prior to analysis by electron-impact mass spectrometry. Alkylation, using ethyl iodide in 2-butanone, occurred in the N-1 and N-3 positions of the hydantoin ring when concentrated KOH was added to the reaction mixture. The lower limits of quantitation for mephenytoin and Nirvanol were 10 ng/ml and 50 ng/ml, respectively, and were found to be reproducible within 8% upon repeated quantification.

INTRODUCTION

Mephenytoin (3-methyl-5-ethyl-5-phenylhydantoin; Mesantoin) was introduced in 1945 for the treatment of a wide range of seizure types, including generalized tonic—clonic seizures, elementary partial seizures, and complex partial seizures, It has action against pentylenetetrazol convulsions and abolishes the tonic phase of maximal electroshock seizures in man and animals [1].

Butler [2, 3] and Butler and Waddell [4] showed that the primary metabolite of mephenytoin in dogs and man was the demethylated product, 5-ethyl-5-phenylhydantoin, Nirvanol (Fig. 1). The liver was found to be the principal organ of metabolism. Mephenytoin is converted to Nirvanol, which is subsequently converted to the primary urinary metabolite 5-ethyl-5-(*p*-hydroxyphenyl)hydantoin. Kupferberg and Yonekawa [5] found that the anticonvul-

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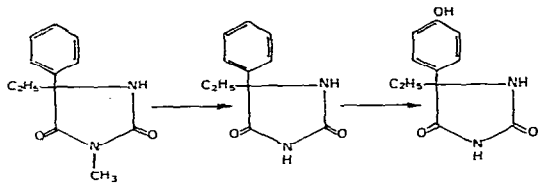

Mephenytoin — 5-ethyl-5-phenyl-hydantoin (Nirvanol) — 5-ethyl-5-(4-hydroxy-phenyl)-hydantoin

Fig. 1. The metabolic conversion of mephenytoin to Nirvanol and its hydroxylation to 5-ethyl-5-(4-hydroxyphenyl)hydantoin.

sant activity of mephenytoin in mice remained essentially unchanged during a 2-h period following a single intraperitoneal injection of mephenytoin. Mephenytoin levels in the plasma and brain rose quickly and fell at later times, whereas the Nirvanol levels rose slowly and reached their maximum at 2 h. The conclusion was that mephenytoin was responsible for the early activity and Nirvanol for the later activity.

Few methods for the simultaneous analysis of mephenytoin and Nirvanol have been reported. Friel and Troupin [6] have described a gas chromatographic method for the analysis of several anticonvulsant drugs, including mephenytoin and Nirvanol. Derivative formation was accomplished by "flash-heater" ethylation, using triethylphenylammonium hydroxide as the ethyl donor. The ethylated products were nearly resolved on an OV-1 column. The only interfering substance was the ethylated product of mephobarbital, which could not be resolved from Nirvanol. The levels of mephenytoin were found to be near the method's lower limits of detection in patients on chronic mephenytoin therapy. Kupferberg and Yonekawa [5] analyzed mephenytoin and Nirvanol as the trimethylsilyl derivatives. The lower limit of sensitivity was approximately 1 μg for each compound extracted from biological tissue. Baseline resolution was difficult despite the use of a variety of phases of differing polarity.

Quantitative selective ion monitoring (SIM) overcomes many of the above-mentioned problems. Low levels of drugs can be quantitated with an increased degree of specificity. Baseline resolution of closely eluting peaks of compounds differing in concentration by as much as twenty times can be quantitated by monitoring the different *m/e* of each compound.

This paper describes a quantitative SIM assay for mephenytoin and Nirvanol following plasma extraction and derivatization. Selected compounds of analogous structure which undergo a similar alkylation reaction were chosen as internal standards. The method was then applied to the quantitation of mephenytoin and Nirvanol in plasma from a patient receiving 50 mg and 400 mg of mephenytoin in single oral doses.

MATERIALS AND METHODS

Chemicals and reagents

Chloroform, hexane, and methanol "distilled in glass" were obtained from

Burdick & Jackson Labs. (Muskegon, Mich., U.S.A.). Ethyl iodide was obtained from Eastman Labs. (Rochester, N.Y., U.S.A.). 2-Butanone and 5-methyl-5-phenylhydantoin (MPH) were obtained from Aldrich (Milwaukee, Wisc., U.S.A.). Mephenytoin (MEPH) and Nirvanol (EPH) were supplied by Sandoz Pharmaceuticals (Hanover, N.J., U.S.A.). 3-Methyl-5-cyclopropyl-5-phenylhydantoin (MCPH) was supplied by Abbott Labs. (North Chicago, Ill., U.S.A.).

Extraction of mephenytoin and Nirvanol from plasma

A 1-ml volume of plasma, 1 ml of 0.25 *M* sodium phosphate buffer (pH 6.5), 8 ml of chloroform, and internal standards (see section on calibration curves) were combined in a 13-ml ground glass stoppered centrifuge tube. The mixture was shaken for 10 min, centrifuged, and the aqueous layer removed by aspiration. The chloroform was then transferred to a clean centrifuge tube and evaporated to dryness on a Buchler rotary evaporator under water vacuum. The residue was dissolved in a mixture of 3 ml methanol and 2 ml of 0.25 *M* HCl. The acidified methanol mixture was shaken with 5 ml of hexane and then centrifuged. The aqueous layer was then transferred to a new tube and equilibrated with 8 ml of chloroform by shaking for 10 min. The chloroform layer was then removed and dried under water vacuum.

Perethylation of mephenytoin and Nirvanol

The perethylation reaction involved the addition of 200 μl of 2-butanone, 5 μl of 10 *N* KOH, and 50 μl of ethyl iodide to the extracted mephenytoin and Nirvanol. The centrifuge tube was capped, sealed with Parafilm and placed in a water-bath at 65° for 90 min. The tubes were then removed, cooled, and 2 ml of 0.25 *M* HCl and 8 ml of chloroform were added. The tubes were shaken for 5 min and then centrifuged. The chloroform layer was removed and dried under water vacuum.

The dried residue was then dissolved in 50—100 μl of methanol, and 1—2 μl of this mixture were injected into the gas chromatograph—mass spectrometer for analysis by the electron impact (EI) and, in some cases, chemical ionization (CI) modes.

Apparatus

The instruments used in this study were a Hewlett-Packard 5982 mass spectrometer with a combined EI/CI source in line with a Hewlett-Packard 5933 Dual Disc Data System. A Hewlett-Packard 5700 series gas chromatograph fitted with 1.2 m × 2 mm I.D. column packed with 2% OV-101 on 80—100 mesh Chromosorb W HP was used to separate the compounds. The column temperature was 175°, while the temperatures of the injection port and glass-lined single-stage jet separator were 250° and 300°, respectively. Helium was used as a carrier at a flow-rate of 30 ml/min, giving a source pressure of 4×10^{-6} Torr.

The mass spectrometer was operated with an electron current of 250 μA, electron energy of 70 eV, and an ion source temperature of 190°.

The selective ion monitoring was carried out in the EI mode, monitoring *m/e* 217 for MEPH, 230 for MCPH, and 231 for MPH and EPH. The dwell time for

each mass was 300 msec. The results obtained by the EI mode were verified by the CI mode, monitoring *m/e* 249 for MEPH and MPH, 259 for MCPH, and 261 for EPH.

Calibration curves

Mephenytoin and Nirvanol calibration curves were produced by adding various amounts of each drug and the appropriate internal standard to human plasma and then extracting these compounds as described above. The range of amounts of mephenytoin and Nirvanol added to plasma was chosen to encompass the unknowns.

RESULTS AND DISCUSSION

The mass spectra of the four perethylated hydantoins are shown in Fig. 2. In all cases the EI spectra show that elimination of the alkyl group from the 5-position appears to be the prime reaction under 70 eV. Both Nirvanol and MPH have the same base *m/e*, 231, whereas MEPH and MCPH have a base *m/e* of 217. In all cases, the relative abundance of molecular ion of the perethylated

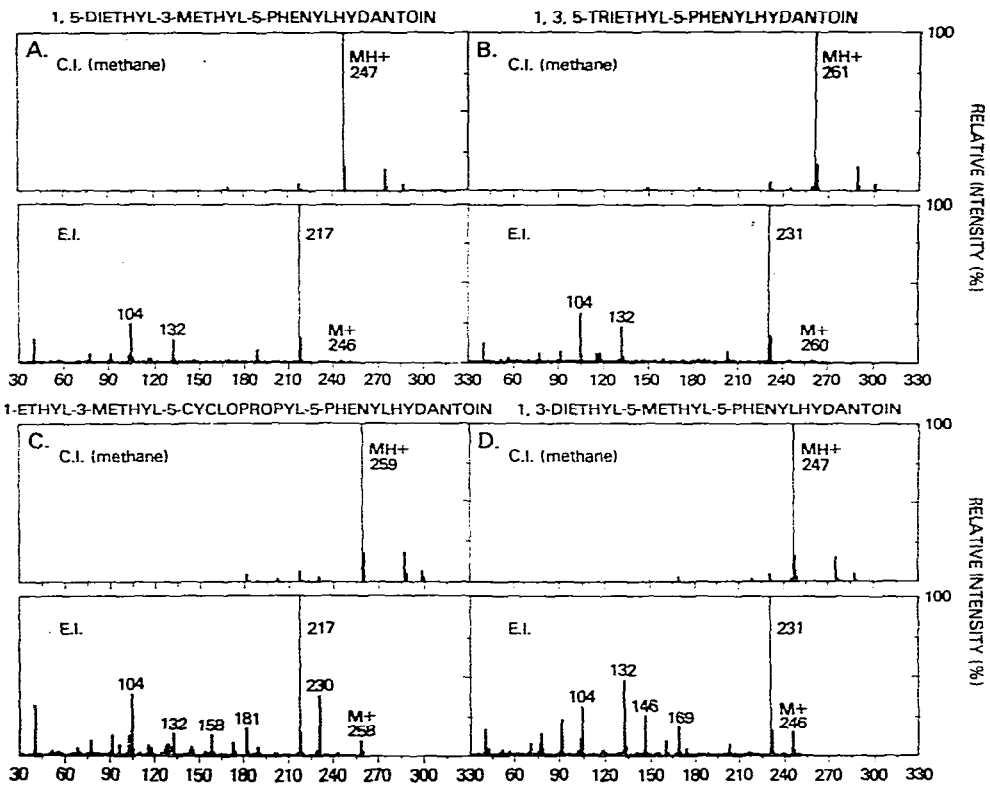

Fig. 2. Electron impact (EI) and chemical ionization (CI) (methane) mass spectra of the four perethylated hydantoins used in this study: (A) 1,5-diethyl-3-methyl-5-phenylhydantoin; (B) 1,3,5-triethyl-5-phenylhydantoin; (C) 1-ethyl-3-methyl-5-cyclopropyl-5-phenylhydantoin; (D) 1,3-diethyl-5-methyl-5-phenylhydantoin.

hydantoin is small. The CI spectra exhibit the characteristic quasimolecular ions, with little or no fragmentation of the molecule. The CI mode was applied to some of the initial samples to verify that the EI results were reproducible by a different means of quantification. In all cases the CI results were virtually identical to those of EI. Although the sensitivity of CI was greater than that of EI, the lower limits of sensitivity were not determined because the CI mode was used solely for verification.

The perethylation procedure appears to reach equilibrium in 60—90 min. Small amounts of N,N-dimethylated products are formed which may be the result of methyl iodide contamination. There also appear to be small peaks eluting before the perethylated mephenytoin and Nirvanol peaks which show the same base *m/e* as the perethylated compounds and have similar fragmentation patterns. They are most likely either N,O or O,O isomers.

The ethylation of the N-1 position appears to be a much slower reaction than ethylation of N-3. The progression of the reaction was followed to equilibrium by monitoring the appearance of the 1,3-diethylated Nirvanol. MPH was chosen as the internal standard for Nirvanol because it undergoes a two-step alkylation, similar to Nirvanol. Likewise, MCPH was chosen as the internal standard for mephenytoin because of its alkylation of the N-1 position. In fact, either internal standard could be used in the quantitation. In the case of MCPH, the base *m/e* of 217 was not used in the analysis because the patient received phenobarbital; phenobarbital elutes close to MCPH and has an interfering *m/e* of 217. In this case, an *m/e* of 230 was used instead.

The base and solvent appear also to play an important role in the alkylation of 5-alkyl-5-phenylhydantoins. Results similar to those found by De Sagher et al. [7] were observed. When base of weaker concentration (for example, 0.5 *N*) was used, substitution occurred only in the 3-position. A concentration of 5—10 *N* was found to be maximal. Bases other than KOH were tried with little or no success in forming the perethylated product. These were: 0.1 *M* tetrabutylammonium hydroxide, 2 *M* tetramethylammonium hydroxide, and 0.2 *M* trimethylphenylammonium hydroxide. Extractive alkylation, using ethyl iodide and tetrabutylammonium hydrogen sulfate as the counter-ion according to the method of De Silva and Bekersky [8] produced 3-ethyl substitution.

Solvents such as methylene chloride, benzene—methylene chloride (9:1), and hexane—isopropyl alcohol (9:1) resulted in similar monosubstitution, with small but variable amounts of the 1,3-diethyl derivative.

The EI—SIM calibration curve for mephenytoin was obtained by plotting the ratio of the area of mephenytoin (*m/e* 217) to the area of MCPH internal standard (*m/e* 230) against the concentration of mephenytoin in human plasma. The results were linear over the concentration range 0.01—3.0 μg/ml, with a representative curve giving an r^2 value of 0.9991 and a *Y*-intercept of —0.0125. The Nirvanol calibration curve was obtained by plotting the ratio of the area of Nirvanol (*m/e* 231) to the area of MPH internal standard (*m/e* 231) against the concentration of Nirvanol in human plasma. The results were also linear over the concentration range of 0.05—4.0 μg/ml, with a typical curve giving an r^2 value of 0.9994 and a *Y*-intercept of —0.0171. The calibration curve standards were injected into the gas chromatograph—mass spectrometer at various times during the analysis of the unknowns. The peak area ratios for these standards injected several times did not vary by more than 6%.

The recoveries of mephenytoin and Nirvanol by the use of this extraction procedure were found to be 90% for mephenytoin and 85% for Nirvanol and were consistent over the range analyzed.

Fig. 3 shows representative SIM fragmentograms (EI and CI) of an extract of plasma obtained from a patient receiving a single 50 mg oral dose of mephenytoin. This patient was also treated chronically with phenobarbital and primidone for seizure control. As mentioned above, the perethylated product of phenobarbital yields an *m/e* of 217 on EI mass spectrometry, which precludes the use of MCPH as an internal standard. Primidone, phenylethylmalonamide, phenytoin, and carbamazepine do not yield interfering fragments.

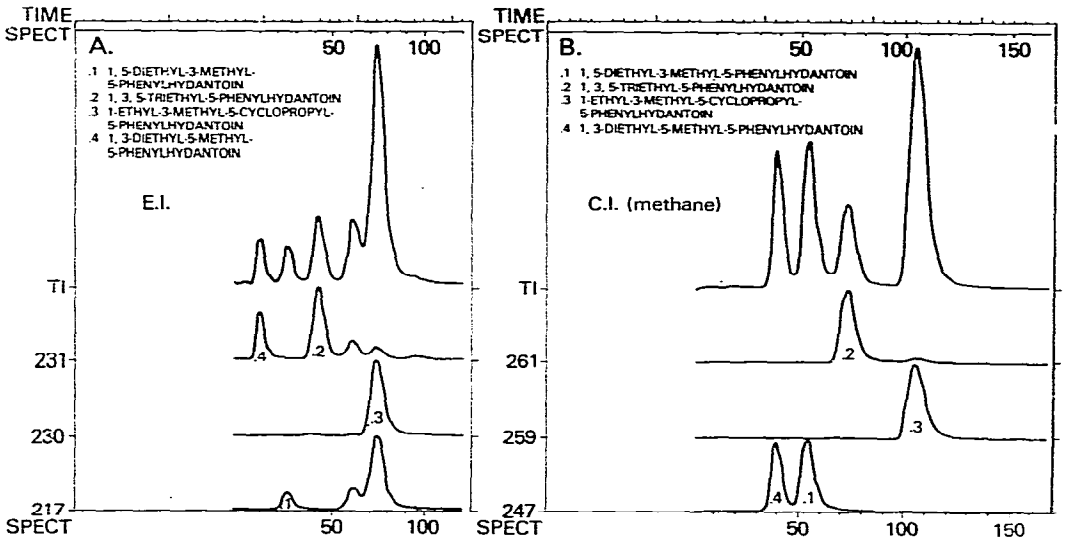

Fig. 3. Selective ion recordings of mephenytoin, Nirvanol, and their internal standards following extraction from plasma obtained from a patient receiving a single 50-mg oral dose of mephenytoin.

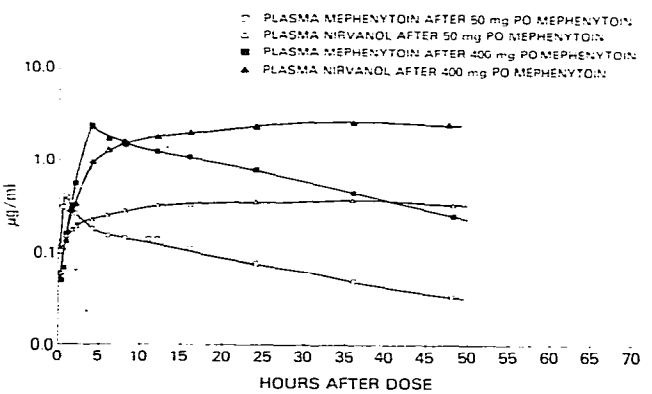

Fig. 4. The plasma concentrations of mephenytoin and Nirvanol in a patient after single oral doses of 50 mg and 400 mg of mephenytoin.

Fig. 4 shows the plasma levels of both mephenytoin and Nirvanol after the administration of 50 mg and 400 mg of mephenytoin in single oral doses. Mephenytoin was rapidly absorbed and disappeared from the plasma with a half-life of 12 h. Nirvanol slowly accumulated, reaching a maximum level in 24 h, and then slowly disappeared from the plasma. Nirvanol can be quantitated 144 h after the ingestion of a single 50-mg dose.

In conclusion, the gas chromatography—mass spectrometric selective ion monitoring technique can be used to measure submicrogram quantities of both mephenytoin and Nirvanol. Perethylation of these compounds produces stable derivatives and symmetrical chromatographic peaks. The choice of solvent and base is most important in determining the position of alkylation. The blood Nirvanol concentrations in patients on chronic mephenytoin therapy are high enough to be measured by standard chromatographic techniques because of the long half-life of Nirvanol. Selective ion monitoring analysis, however, can be most useful in determining the pharmacokinetic properties of mephenytoin and Nirvanol in epileptic patients.

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