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SENSITIVE ASSAY OF METHADONE IN PLASMA BY USING CAPILLARY GAS CHROMATOGRAPHY WITH PHOTOIONIZATION DETECTION

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SUMMARY

A new gas chromatographic assay for methadone, utilizing a fused-silica capillary column, is presented Extreme sensitivity was reached, compared to nitrogen—phosphorus and mass spectrometry detection, by employing a photoionization detector Plasma concentrations of methadone as low as 1 ng/ml can easily be detected and, by further optimization, 0 1 ng/ml was reached The minimum detectable amount of methadone reaching the detector was 70 fg The results indicate that the photoionization detector has potential as a tool in drug monitoring

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INTRODUCTION

Methadone, a strong narcotic analgesic, is one of the drugs of choice for symptomatic treatment of severe pain, especially chronic agonizing pain in patients with malignant disease. The drug has, however, a rather long elimination half-life as compared to most other analgesics of the opoid type and must therefore be dosed with special care. Even slight increases in the dose may sometimes result in increased plasma concentration and signs of intoxication symptoms Basic pharmacokinetic properties of methadone have mainly been mapped out in drug addicts on maintenance treatment with the drug, but more studies in patients are needed, especially in patients with diseases that might interfere with the turnover of the drug. Furthermore, drug interactions with methadone have been reported and there is a need for more detailed studies of possible variations of plasma methadone concentrations during various pathological conditions. Literature within this field is rather sparse

A number of methods for the assay of methadone in plasma, based on conventional gas chromatography (GC) and utilizing different detectors, have been

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developed. With gas chromatography—mass spectrometry (GC—MS) methadone was measured with a sensitivity limit of 9 ng/ml [1] and by nitrogen—phosphorus detection (NPD) a sensitivity as low as 2—3 ng/ml has been reached [2]

Fused-silica capillary columns have up to now only been applied to the determination of methadone in urine [3], where high levels will be expected. Unfortunately, if a high throughput of samples is of significance, the sensitivity of the method is not satisfactory for the analysis of plasma A high throughput necessitates split injection and isothermal chromatography. Split injection, however, reduces the amount of sample reaching the detector, and this consequently increases the demand for detector sensitivity

The photoionization detector has a high response for most aromatic compounds [4, 5] and for certain structures the response is extremely good The mechanism behind this is, however, not fully understood

The use of the photoionization detector as a gas chromatographic detector for drug monitoring analysis seems to be an almost unexplored field. Jaramillo and Driscoll [6] report the sensitivity of the photoionization detector to be eight to sixteen times greater for barbiturates than that of the flame ionization detector.

Although no thorough study has been published, the photoionization detector would probably give a response superior also to the nitrogen—phosphorus detector for many aromatic nitrogen-containing drugs Our own studies of the detector, with a limited number of substances, support this statement.

According to our experience, methadone is one of those substances showing a remarkably high response to the photoionization detector. This paper describes a sensitive method, able to measure methadone in concentrations as low as 0 1 ng/ml of plasma or, which is perhaps more relevant, 1 ng/ml in only a 100-μl sample. The method also illustrates the potential of the photoionization detector as a tool in drug analysis

EXPERIMENTAL

Methadone hydrochloride was purchased from ACO (Stockholm, Sweden) The internal standards, 2-dimethylamino-4,4-diphenyl-5-nonanone (I.S A) and 2-dimethylamino-4,4-diphenyl-5-hexanone (I S B), were synthesized according to Attenburrow et al. [7]. Instead of ethyl iodide, butyl iodide and methyl iodide were used, which gave internal standards with two more or one less carbon atom, respectively, in the main chain as compared to methadone The structures of the internal standards were confirmed by NMR and MS.

The extraction solvents, heptane (Merck, Darmstadt, F.R G) and *tert.*-amylalcohol (Merck), were of analytical grade. Aqueous potassium carbonate and sulphuric acid were prepared from analytical-grade chemicals and distilled water. Culture tubes with screw caps (13 × 100 nm) (Corning Pyrex glassware) and 3-ml conical glass tubes (Werner-Glas, Stockholm, Sweden) were used in the extraction.

Calibration points

Standard solutions of methadone (20 μg/ml) and internal standard (4 μg/ml)

were made up in 0 01 M hydrochloric acid Aliquots of the standard solution of methadone were diluted in 0.01 M hydrochloric acid to give standards with intermediate concentrations between 0 02 and 6 μg/ml. A 50-μl aliquot of each of the intermediate standards was added to 1 ml of plasma, giving ten final calibration points between 1 and 300 ng/ml

Extraction

Extraction was performed according to Peyton Jacobs, III et al [2] To 1 ml of plasma, 25 μl of internal standard (I.S. A, 4 μg/ml), 0.5 ml of 1 M potassium carbonate and 2 ml of heptane—*tert*-amylalcohol (9 1) solvent mixture were added The tubes were turned for 10 min and centrifuged The plasma phase was frozen in a dry-ice—acetone bath and the organic phase poured off into another tube. A 0 5-ml aliquot of 0 1 M sulphuric acid was added and methadone was re-extracted into the water phase After centrifugation, the water phase was frozen again and the organic phase discarded The water phase was thawed and washed with another 2 ml of the solvent mixture

After centrifugation and freezing, the organic phase was discarded, the thawed water phase was made alkaline with 1 ml of 1 M potassium carbonate and extracted with 100 μl of solvent mixture and then centrifuged The water phase was frozen again and the organic phase poured into a small conical glass-tube A 3-μl aliquot of the organic phase was injected into the capillary gas chromatograph with a split injector (split ratio 1 30)

Modified extraction procedure

Plasma (1 ml) mixed with 25 μl of internal standard (I S B, 20 ng/ml) and 0 5 ml of 1 M potassium carbonate was extracted with 2 ml of solvent mixture and centrifuged The plasma phase was frozen in a dry-ice—acetone bath and the organic phase was poured into another tube. A 0 5-ml aliquot of 0 1 M sulphuric acid was added and methadone was re-extracted into the water phase After centrifugation, the water phase was frozen and the organic phase discarded To the thawed water phase, 1 ml of 1 M potassium carbonate and 200 μl of chloroform were added After extraction and centrifugation, the water phase was removed and the chloroform phase was evaporated with nitrogen at room temperature. The residue was dissolved in 50 μl of acetonitrile All the glassware used in the modified procedure was silanized

Gas chromatography

The gas chromatographic analyses were performed on a Varian 3700 equipped with a photoionization detector (HNU Systems, Newton, MA, U S A.). The UV source used was a standard 10 2-eV lamp The chromatograms were recorded and integrated on a Chromatopac C-R1B (Shimadzu, Kyoto, Japan) Instead of using the original capillary insert to the photoionization detector, we modified a standard insert (03-908334-00) from Varian (Palo-Alto, CA, U.S.A), which made it easier to obtain a leak-tight connection between the column and the detector The columns (12 or 25 m × 0 2 mm I D) used were fused silica with a bonded 0 25-μm methylphenylsilicon phase (BP-5, SGE, Melbourne, Australia)

Helium, with a flow-rate of 40 cm/s, was used as the carrier gas and argon

(20 ml/min) as the make-up gas The temperature of the column was 240° C, of the injector 260° C and of the detector 280° C.

RESULTS AND DISCUSSION

Chromatography

Conversion from packed-column GC to fused-silica capillary chromatography, for the benefit of separation power, normally means compromising between speed and sensitivity. On one hand, split injection and isothermal chromatography maintain a high throughput of samples but only a small fraction of the injected material reaches the detector On the other hand, on-column injection offers high sensitivity but is accompanied by the need for temperature programming and tedious re-equilibration.

Detection

As steps to increase the concentration were already fully utilized during the extraction procedure, and an increase in the amount of sample taken to the analysis is undesirable, improvement in the sensitivity has to take place in the detector.

The nitrogen—phosphorus detector is sensitive enough in combination with packed-column GC [2], but in capillary chromatography, as outlined above, concentrations below 100 ng/ml of plasma would probably impose problems

Initial studies in our laboratory have shown that methadone elicits a very high response in the photoionization detector Nitrogen as well as argon were tested as the make-up gas. Senum [8] has, for some aromatic substances,

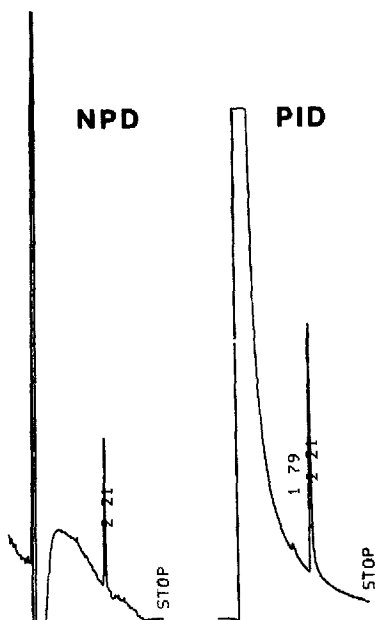

Fig 1 Chromatograms from two injections of 100 pg of methadone on the same capillary column, but detected with either the nitrogen–phosphorus detector (NPD) (attenuation 4) or the photoionization detector (PID) (attenuation 100)

shown that argon is superior to nitrogen and gives the highest sensitivity, our results indicate that this is also true for methadone. Possibly, the noble gases may shorten the life-time of the UV source [9], resulting in increased operative expenses, but no conclusive evidence that this should be the case has hitherto been presented.

The response in the photoionization detector is concentration-dependent and thus the make-up flow will influence the peak shape and the response A decrease in the make-up flow increases the peak height but unfortunately also the peak width An optimal make-up flow was found between 15 and 20 ml/min

Fig 1 shows two chromatograms of 100 pg of methadone on the same 12-m BP-5 capillary column, one detected with the nitrogen- phosphorus detector and the other with the photoionization detector The attenuation of the nitrogen—phosphorus detector is 4 and of the photoionization detector 100, which means that the sensitivity ratio, based on peak height, is ca. 40 in favour of the photoionization detector. Calculated from the peak areas in Fig 1, the sensitivity ratio is ca 75

Extraction

The extraction of bases, in low concentrations, very often presents great problems, mainly because of adsorption of the base to, for example, glass walls Different methods were tested for the extraction of methadone. Variable drug recovery through evaporation losses was also a problem The method described by Peyton Jacob, III et al. [2], however, showed a high extraction recovery and few interfering peaks down to extreme low concentrations In this method, the organic and plasma (water) phases are separated by freezing the plasma (water) phase in a dry-ice—acetone bath and, most important, no evaporation step is included.

Quantitation

The standard curves show a good linearity for 1—300 ng/ml of plasma, with correlation coefficients normally between 0.995 and 0.999. Eventually, a tendency to curve-linearity can be detected above 200 ng/ml. This tendency seems to be a result of the chromatographic process, as the same curve-linearity is found after injection of simulated standards (not preceded by extraction) in the solvent mixture. The exact location of this minor chromatographic trouble is not yet clear.

The intra-assay precision of the method was tested by analysing ten replicates of plasma samples spiked with methadone at three different levels· 1, 10 and 100 ng/ml The coefficients of variation were 9 80, 2 92 and 2 29%, respectively

Application

The method outlined above has been used to measure methadone concentrations in plasma samples from patients treated for severe pain Samples were taken during steady-state conditions and at varying time intervals after intake of dose (ranging from 20 to 200 mg per 24 h). Plasma concentrations ranging from 59 to 1675 ng/ml have been found, which is well above the detection limit.

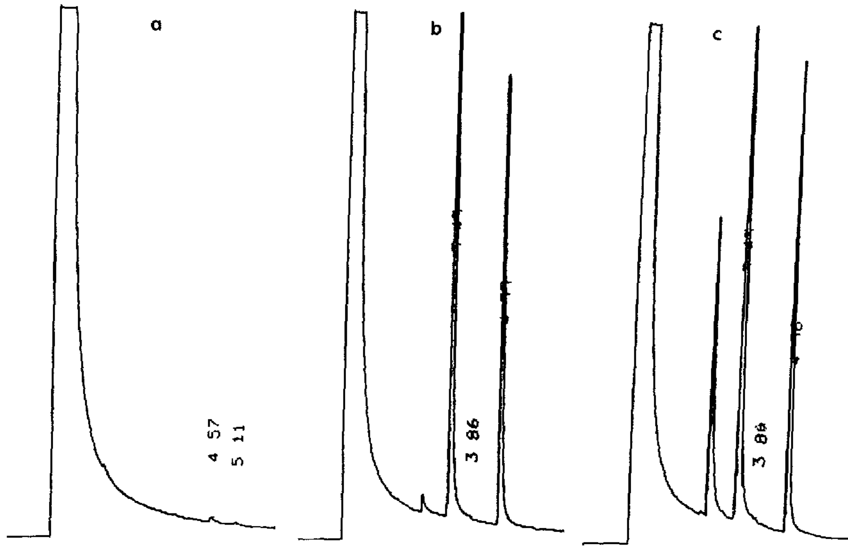

Fig 2 Chromatograms obtained in the assay of methadone in plasma The retention times of methadone and the internal standard were ca 3 4 and 4 6 min, respectively, when the 25-m capillary column was used (a) Blank plasma, (b) blank plasma spiked with 150 ng/ml methadone and 100 ng/ml internal standard, (c) plasma sample from patient treated with methadone (60 mg per 24 h) Measured concentration was 173 ng/ml

Fig. 2 exhibits chromatograms from some determinations of methadone in plasma. No interfering peaks were observed on the detector attenuation used (X 80)

The ability of the photoionization detector

To investigate the potential of the photoionization detector, and especially the limit of determination for a substance eliciting very high response, plasma was spiked with methadone to concentrations from 0 1 to 1 0 ng/ml and analysed with a modified extraction procedure The major difference is that acetonitrile was used as the solvent because its ionization potential (12 2 eV) is two units higher than the fixed ionization potential of the UV source in the photoionization detector This means that the detector has a low response for this solvent, thus permitting a higher gain compared to when heptane—*tert*-amylalcohol is used

In Fig. 3, the chromatograms from the injections of blank, 0 1 and 0.8 ng/ml methadone are presented Calculated from the peak-height ratios, a correlation coefficient of 0 995 reveals the possibility of analysing methadone even at this very low level Calculations based on peak areas were less accurate, due to interfering peaks. Optimizing the extraction procedure will hopefully reduce the number and the size of the matrix peaks, which undoubtedly impose a problem at this level Replacement of the internal standard is probably also necessary. As far as methadone is concerned, this sensitivity is not needed and it is therefore more relevant to exploit the sensitivity by measuring concentration down to 1 ng/ml in only 0 1 ml of plasma than to measure 0 1 ng/ml in 1 0 ml of plasma This gives a reduction of the plasma interferences by ca 90% and, furthermore, the tiny volumes of capillary blood samples become feasible.

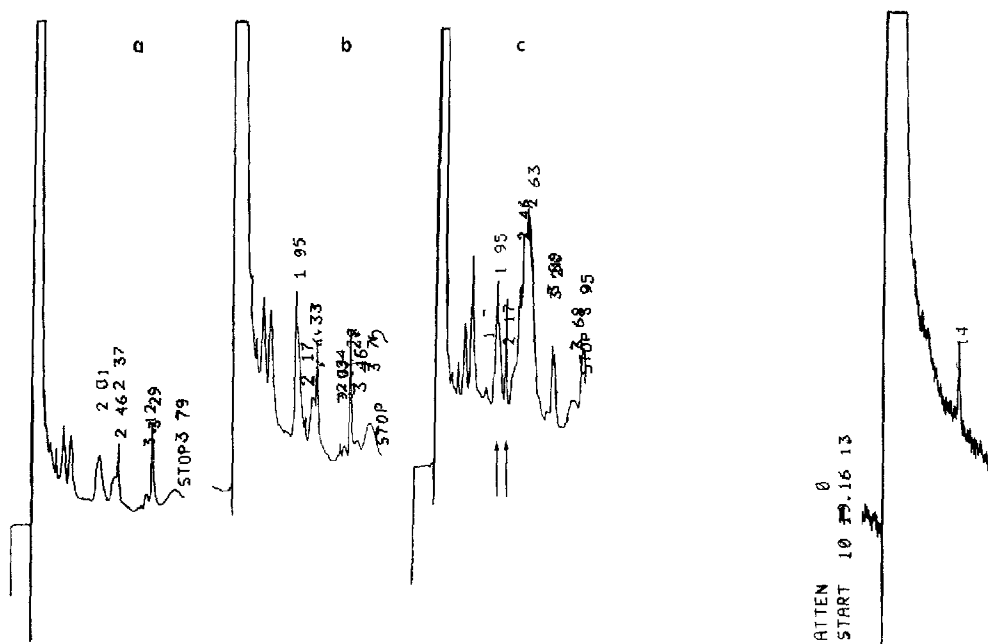

Fig 3 Chromatograms from the injection of (a) blank, (b) 0 1 ng/ml and (c) 0 8 ng/ml methadone in plasma taken through the modified extraction procedure The arrows indicate the retention times, on the 12-m capillary column, of the internal standard (on top of the interfering peak) and of methadone

Fig 4 Chromatogram from the injection of 6 pg methadone (split setting of 1 30) A 200-fg amount of methadone reached the photoionization detector, at an attenuation of 1

The limit of detection for methadone, calculated from the chromatogram shown in Fig. 4 (based on a signal-to-noise ratio of 3) was ca 70 fg.

The extremely high response for methadone is, however, not unique for that structure Several completely different aromatic structures have been found to elicit comparable responses in the photoionization detector and studies of these compounds are in progress

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