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## SIMPLIFIED METHOD FOR DETERMINATION OF THE TETRACYCLIC ANTIDEPRESSANT MIANSERIN IN HUMAN PLASMA USING GAS CHROMATOGRAPHY WITH NITROGEN DETECTION

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### SUMMARY

A simplified gas chromatographic method for determination of the antidepressant drug mianserin in human plasma is described. Application of a nitrogen-sensitive detector reduces the assay procedure to extraction, concentration and gas chromatographic determination. The method is suitable to determine mianserin in human plasma at the 1 ng/ml level on a routine basis. At the 20 ng/ml level the deviation of the mean from the true value and the relative standard deviation amount to 1.0% and 6.8%, respectively.

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### INTRODUCTION

Until now, quantitation of mianserin (Fig. 1) in biological samples for clinical studies and animal experiments has been performed using mass fragmentography at our laboratories. Although the assay for mianserin [1, 2] has been gradually improved over the past five years, and the analyses have become more and more automated to handle a large number of samples per year [3], the use of mass fragmentography itself hampers the general applicability of the assay method. Gas chromatography (GC) using a specific nitrogen-sensitive detector is a possible alternative for mass fragmentography. However, the use of alkali flame ionization detectors (AFID) for gas chromatographic detection of nitrogen-containing compounds has never become wide-spread because of the signal instability observed during daily routine operation [4].

The introduction of a new generation of nitrogen/phosphorus-sensitive detectors (NPD) has dramatically changed the situation [5–7]. With these types of detector, also referred to as thermionic ionization detectors, signals

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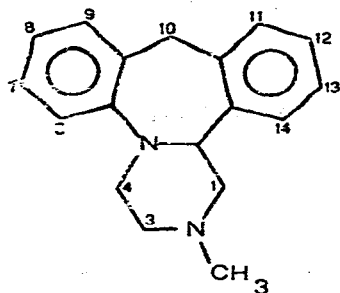


Fig. 1. Mianserin (1,2,3,4,10,14b-hexahydro-2-methyldibenzo[c,f]-pyrazino[1,2-a]-azepine). Also the lab code Org GB 94 is in use denoting the salt mianserin monohydrochloride.

of high stability during long-term operation are obtained. Although the detector design differs from manufacturer to manufacturer [5-7] and the principle of operation is still rather obscure, all these nitrogen-sensitive detectors have an independent electrically-heated bead as alkali source, which is operated in an air-hydrogen environment. The supply of hydrogen is not enough to provide a self-sustaining hydrogen-air flame, thereby generating a flame zone around the glowing alkali bead only. In the low-temperature plasma surrounding the bead, organic molecules are pyrolyzed; the highly reactive radicals formed are involved in reactions with alkali atoms, while the ions formed are responsible for the detector signals observed [5-12]. Regardless of the mystic surrounding the reaction mechanisms, the NPD has already found many applications in the field of drug research [13-25].

The sensitivity and specificity of the nitrogen-sensitive detector and the general accessibility of gas chromatography in other laboratories, forced us to investigate in detail the merits of the GC assay with nitrogen detection for determination of mianserin in plasma. In this paper, the assay method is described in detail. A comparative study of the present assay method and the previously used method employing mass fragmentography [1, 2] will be published elsewhere [26].

## MATERIALS AND METHODS

### *Solvents*

All solvents were purchased from Merck (Darmstadt, G.F.R.). Ammonia, *n*-hexane and methanol were of Suprapur, Uvasol and analytical-reagent grade quality, respectively.

### *Glassware*

The glassware was cleaned using normal laboratory washers (Miele type G19) and standard washing agents (Neodisher F, N and S, Chemische Fabrik Dr. Weigert, Hamburg, G.F.R.). After this washing procedure, the glassware was cleaned ultrasonically in a hydrochloric acidified water bath, rinsed with distilled water and dried. Before use, the glassware was rinsed thoroughly with the organic solvent to be used in the assay procedure.

### *Internal standard*

For the GC assay method, 7-methylmianserin was chosen as internal standard. Because of their structural relationship, mianserin and 7-methylmianserin behave chemically quite similarly during the assay procedure.

### *Gas chromatography with nitrogen detection*

A Hewlett-Packard gas chromatograph Model 5710A, equipped with a 3 m × 2 mm I.D. glass column packed with 1% OV-25 on Gas-Chrom Q (80–100 mesh) was used. The Hewlett-Packard N/P detector Model 1878C.A was operated at a voltage of 15–18 V for heating the bead. The temperatures of injector, column and detector oven were 300°, 240° and 300°, respectively. Nitrogen was used as a carrier gas at a flow-rate of 20 ml/min. The gas flow-rate for the N/P detector was 3 ml/min for hydrogen and 50 ml/min for air.

### *Assay procedure*

The internal standard, dissolved in water (40 ng/ml), was added to the plasma sample in approximately the same amount as the expected mianserin concentration, together with 100  $\mu$ l of concentrated ammonia to obtain pH > 11. After equilibration for at least 3 h, the plasma was extracted with 5 ml of *n*-hexane by thoroughly mixing and centrifuging for 5 min at 1500 *g*. The *n*-hexane extract was evaporated to dryness in a 10-ml conical tube at 45° under a gentle stream of nitrogen. The wall of the conical tube was rinsed thoroughly with 500  $\mu$ l of methanol, the methanolic solution was again evaporated to dryness at 45° under nitrogen, and the residue finally dissolved in 8–20  $\mu$ l of methanol. To avoid detector overloading and cross-over of previous injections, only an aliquot of 1–7  $\mu$ l from the methanolic solution was injected into the gas chromatograph for quantitation. The volume of sample injected was dependent on the expected drug plasma level; below 10 ng/ml the maximum solvent volume (7  $\mu$ l out of 8  $\mu$ l) was injected; for plasma levels within the range of 10–200 ng/ml 30% or less of the 20  $\mu$ l methanolic solution was used for injection.

### *Standardization and quality control on the results of analysis*

Standard solutions required for establishment of the calibration curve for the GC–NPD method contained mianserin and 7-methylmianserin in the concentration ratios 1:4; 1:2; 1:1; 2:1; and 4:1. The standard solutions were added to human plasma free of drug (blank) and analyzed by GC–NPD after sample processing as described previously in order to correct for the minor difference in yield of extraction from plasma between mianserin and its 7-methyl derivative.

On each day of measurement, plasma samples containing unknown concentrations of the drug, samples for standardization, blank human plasma samples and blank human plasma samples spiked with mianserin were analyzed randomly. The blank human plasma samples with and without mianserin added, served as controls for the overall analytical procedure.

### *Data processing*

For calculation of the calibration functions and mianserin levels, the measured peak heights of mianserin and internal standard together with required data on calibration mixtures, original sample volume and amount of internal standard added, were fed into a computer via a visual display terminal with keyboard connected to the DEC PDP-11 RSTS computer facilities of Akzo Pharma, Oss, The Netherlands.

## RESULTS AND DISCUSSION

### *Calibration curve*

The calibration curve for the GC-NPD method was calculated using polynomial regression analysis. A typical example of the calibration curve (peak height ratio  $y$  versus concentration ratio  $x$ ) is represented by  $y = -0.007x^2 + 1.400x - 0.025$  where each concentration ratio was measured in triplicate.

### *Detection limit*

Picogram amounts of pure mianserin can be detected with the N/P detector. However, the lower limit of detection for reliable mianserin determination in human plasma is not governed by the sensitivity for mianserin of the N/P detection alone. Also the work-up procedure and the endogenous components from the biological matrix could possibly contribute to the detector output signal. In these cases, the mianserin levels are influenced by the ratio of background signal to native response.

During the assay procedure, the total amount of mianserin injected into the GC-NPD system is determined by the yield of extraction (approx. 90%) and the solvent volume used for injection (maximum 7  $\mu$ l out of 8  $\mu$ l). Therefore, a maximum of approximately 80% of the amount of mianserin originally present in the plasma sample can be subjected to GC-NPD. The simplicity of the assay procedure, the high yield of extraction of mianserin from plasma and the possibility of efficient solvent transfer ensure that the loss of mianserin during the assay procedure is low. However, upon determination of mianserin levels below 1 ng/ml problems are encountered because at these levels the GC peak of mianserin is superimposed on the tails of the solvent and plasma impurity peaks. Consequently, the described assay method is sensitive enough to determine, on a routine basis, mianserin plasma levels above the 1 ng/ml level.

### *Specificity*

Gas chromatograms, which were obtained after injection of an extract from blank human plasma, blank human plasma spiked with a known amount of mianserin, and human plasma from a patient undergoing chronic mianserin treatment are shown in Fig. 2. No interference from endogenous plasma constituents eluting at the retention times of mianserin and the internal standard was found. Possible interference by the metabolites of mianserin was also investigated. The retention times of the major metabolites [27] relative to that of mianserin are presented in Table I. Only the mianserin N-oxide could possibly interfere with mianserin in the GC-NPD assay owing to its thermal

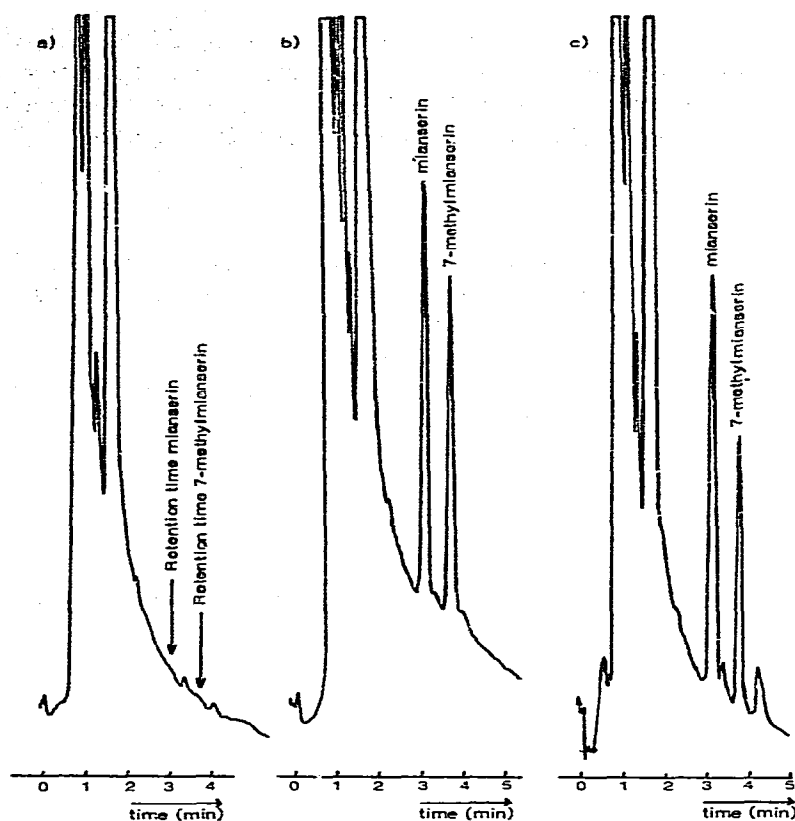


Fig. 2. Gas chromatograms after injection of a *n*-hexane extract of 1 ml human plasma samples: (a) blank; (b) blank, spiked with 10 ng of mianserin and 10 ng of the added internal standard, and (c) plasma from patients receiving mianserin treatment; to the sample 10 ng of the internal standard were added, the mianserin content was calculated to be 11.5 ng.

TABLE I

RETENTION TIMES OF THE MAJOR MIANSERIN METABOLITES RELATIVE TO MIANSERIN

Compound	Relative retention time
Mianserin	1.00
8-Hydroxymianserin	3.27
Desmethybmianserin	1.36
Mianserin N-oxide	1.00*
Internal standard (7-methylmianserin)	1.24

\*Decomposes thermally into mianserin as confirmed by GC-MS.

decomposition into mianserin. However, the N-oxide cannot be extracted from plasma under the assay conditions employed.

Other drugs concomitantly in use during mianserin therapy could possibly interfere with mianserin or the internal standard 7-methyl-mianserin. The as-

say procedure does, however, ensure a reasonable specificity; only drugs which can be extracted from basic plasma can appear in the *n*-hexane extract, most basic drugs can be separated from each other by GC [25] and only nitrogen- (or phosphorus)-containing compounds will be detected by the specific N/P detector.

#### *Accuracy and precision*

The deviation of the mean from the true value and the relative standard deviation as a measure for, respectively, the accuracy and precision of the GC-NPD assay method, were determined by analyzing blank plasma samples spiked with a known amount of mianserin. These spiked samples were analyzed intermittently over a period of three months during analyses of blank plasma samples and clinical samples. The accuracy and precision at the 20 ng/ml plasma level are shown in Table II.

TABLE II

GC-NPD ASSAY CHARACTERISTICS OF REFERENCES SAMPLES CONTAINING 20.0 NG MIANSERIN PER MILLILITRE OF PLASMA

Mean	(ng/ml)	19.80
Standard deviation	(ng/ml)	1.34
Deviation of the mean from the true value	(%)	— 1.0
Relative standard deviation	(%)	6.8
Number of data	(n)	41

#### CONCLUSIONS

The novel GC-NPD assay method for the determination of mianserin in human plasma offers ample sensitivity and specificity to determine mianserin levels as low as 1 ng per ml plasma during long-term daily operations. Compared with the previously described gas chromatographic-mass spectrometric (GC-MS) assay method [1, 2], the GC-NPD method involves only a simple extraction and concentration step, while the GC-MS method requires intermediate sample clean-up before introduction into the GC-MS system to enable daily routine analysis [2]. Furthermore, the GC-NPD assay method uses medium-priced, general accessible and reliable analytical instruments. Application of the GC-MS assay method requires the presence of sophisticated, costly analytical instruments together with highly-skilled personnel to operate them, while the equipment itself is subject to frequent breakdowns. Using packed GC columns, the specificity of the GC-NPD method can, however, not be compared with the specificity of a mass spectral method. Application of capillary GC columns can increase the specificity of the GC-NPD method, whenever problems are encountered owing to a lack of specificity. It has been demonstrated that GC-MS assay methods can be automated to a great extent [3, 28]. Using an autosampler for sample injection and a computer system for data acquisition, collection, processing and final drug level calculation, com-

plete automation will be easier with the GC-NPD method than with a GC-MS method.

The GC-NPD as well as the GC-MS assay method have been applied in our laboratory to plasma samples from depressed patients chronically treated with mianserin [29]. The results of the statistical evaluation of both assay methods with respect to intra-laboratory variation and (possible) systematic deviation between the results of analyses, are published separately [26].

#### ACKNOWLEDGEMENTS

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