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REVIEW

QUANTITATIVE ANALYSIS OF COMMON ANAESTHETIC AGENTS

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1. INTRODUCTION

Anaesthetics comprise a variety of drugs which differ in chemical structure. The chemical structure of a compound not only influences the analytical method best suited to its quantitation, but also its acid/base character and its extractability.

The dose administered, the bioavailability of the dosage form and the pharmacokinetic profile of the drug govern the circulating concentrations of either the parent drug and/or its metabolites present in vivo, and dictate the ultimate sensitivity required for the analytical method.

Drug monitoring in the field of anaesthetics may involve quantitation in the microgram (10^{-6} g or ppm) or nanogram (10^{-9} g or ppb) per millilitre concentration range. These concentrations are present in biological fluids or tissues — or in the case of pollution measurements in a multicomponent gas mixture — which present complex matrices from which the compounds of interest must be selectively extracted and cleaned up prior to quantitation.

It is unfortunate that so many of the publications on applications of the various analytical techniques to anaesthetic practice so far have contained insufficient experimental information (especially in the case of inhalational anaesthetics). The aim of this review is to provide a survey of advances in the methods of the quantitative analysis of anaesthetics in current use with special attention being paid to practical applications.

2. BIOLOGICAL IMPORTANCE OF MONITORING ANAESTHETIC AGENTS

2.1. Evaluation of the basic pharmacokinetic data

Understanding the pharmacokinetics of anaesthetic drugs is important for their sound practical use. It allows predictions about drug concentrations in the body as related to dosage, time, and physiological and pathological alterations in biological functions. It may also provide preliminary indications of the likelihood and types of drug interactions that may be encountered.

2.2. Altered pharmacokinetics

Kinetic data derived from animal studies, from in vitro investigations or in some cases from studies in healthy volunteers have to be associated with studies

conducted in the operating room during the actual use of the drugs. The interaction of anaesthetic drugs with premedication and other anaesthetic agents [1], altered pharmacokinetics in children [2] and the elderly [3], during pregnancy, diseases of the heart [4], liver [4—8] and kidney [4], during anaesthesia and long-term application have to be considered.

2.3. Therapeutic drug monitoring

Long-term application of some anaesthetic drugs (e.g. high-dose therapy with barbiturates for the control of elevated intracranial pressure in patients with severe acute head injury or metabolic brain disorders like Reye's syndrome) requires monitoring to make sure that the therapeutic range is quickly achieved and maintained, also to avoid severe toxicity in the treated patients who additionally often suffer from hepatic or renal failure. In addition to routine monitoring, emergency analysis of anaesthetic drugs must often be performed in cases of regimen failure or accidental overdose.

2.4. Drug abuse

Some of the anaesthetic compounds are used extensively in pharmaceutical preparations (barbiturates, benzodiazepines). They are also subject to abuse and are available on the illicit market. For this reason emergency drug analysis is required in cases where a drug or drugs of unknown origin have been taken and, therefore, a toxicological screen is necessary. If the procedure is both qualitative and quantitative, it can be used for assessing the nature and severity of the intoxication (so that management can match severity) and also for establishing a baseline by which the patient can be followed.

2.5. Pollution measurement

In the case of the inhalational anaesthetics monitoring of the operating room anaesthetic level and personnel exposure fulfills three objectives: (1) it determines the effectiveness of the leak-detecting and leak-proving programme, (2) it uncovers occult leaks of anaesthetic gases from unexpected sites, (3) it documents compliance with recommendations of target trace anaesthetic gas levels and presumably safer working conditions in the operating rooms.

3. GENERAL ANAESTHETIC AGENTS

3.1. Inhalational anaesthetics

Inhalational anaesthetic agents which were popular in the past like diethyl ether, ethyl chloride, vinyl ether, fluroxene, cyclopropane, ethylene, trichloroethylene and chloroform are now less useful because the first six agents are flammable and explosive at concentrations necessary for anaesthesia or because of their toxicity in the case of the three halogenated compounds.

The inhalational general anaesthetic agents in current use are nitrous oxide, halothane, enflurane, isoflurane and methoxyflurane. Nitrous oxide (N_2O) is a

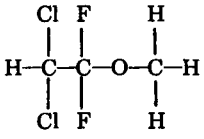
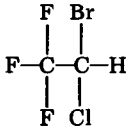
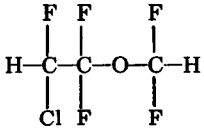
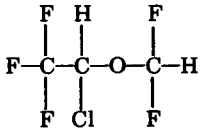
gas at normal ambient temperature, whereas the other four agents are volatile organic liquids: halothane (2-bromo-2-chloro-1,1,1-trifluoroethane), enflurane (2-chloro-1,1,2-trifluoroethyldifluoromethyl ether), isoflurane (1-chloro-2,2,2-trifluoroethyldifluoromethyl ether), methoxyflurane (2,2-dichloro-1,1-difluoroethylmethyl ether). Structures and some of their chemical properties are shown in Table 1 [9].

They are of different potency as expressed as the minimum alveolar concentrations (MAC) [10], which are a measure for the tension of partial pressure necessary in the brain for anaesthesia. A dose of 1 MAC will prevent movement in response to surgical incision in 50% of subjects. Therefore volatile anaesthetics are administered as a mixture in a carrier gas (usually 0.3—1.5% in oxygen or as mixtures of nitrous oxide—oxygen) for maintenance of anaesthesia.

The blood/gas partition coefficient is a measure of the quantity of vapour that must be transferred to the blood from the alveolar gas in order to achieve a given tension. The oil/gas partition coefficient gives information about how

TABLE 1

STRUCTURES AND SOME PROPERTIES OF INHALATIONAL ANAESTHETIC AGENTS [9]

Anaesthetic		MAC* (%)	Vapour pressure (Pa · 10^3 at 20°C)	Blood/gas partition coefficient (at 37°C)	Oil/gas partition coefficient (at 37°C)
	Methoxyflurane	0.16	3.0	12.0	970
	Halothane	0.75	32.4	2.3	224
	Enflurane	1.68	23.3	1.9	98
	Isoflurane	1.40	33.3	1.4	99
N_2O	Nitrous oxide	105	gas	0.47	1.4

*MAC = Minimum alveolar concentration [10]. MAC > 100% means that hyperbaric conditions would be required to reach 1 MAC.

rapid the agent achieves equilibrium in the fatty tissues and about the period of elimination after discontinuation of the anaesthetic. The volatility of these agents restrict the analytical possibilities for their estimation. For example: thin-layer chromatographic (TLC) or high-performance liquid chromatographic (HPLC) techniques are applicable only to a very small extent. On the other hand, various methods of dealing with the measurement of gases or compounds in gaseous condition, e.g. gas—liquid chromatography (GLC) or gas—solid chromatography (GSC), are the methods of choice for the monitoring of such compounds.

Monitoring levels of anaesthetic gases or volatiles is important in two different fields: the exposure of operating room personnel to anaesthetic agents (pollution measurements) and the dosage/uptake of these agents to/by the patient for the induction and maintenance of anaesthesia (non-invasive methods, invasive methods).

3.1.1. Pollution measurement

Since the mid-1960s, epidemiological studies have shown a wide range of possible hazardous effects associated with chronic exposure of operating room personnel to trace concentrations of anaesthetic gases and vapours [11, 12]. In 1977 a criteria document of the National Institute for Occupational Safety and Health (NIOSH) in the United States has recommended a standard of 25 ppm (parts per million) for nitrous oxide and 2 ppm for halogenated anaesthetics, including halothane and enflurane [13]. In this publication the carcinogenic, teratogenic, mutagenic as well as the hepatorenal toxicity risks of the different anaesthetic vapours are discussed.

Vapours of anaesthetic agents are discharged into operating theatre air not only from the patient's expired gases, but also from filling and draining vaporizers, whether or not spillage occurs, and they are also released from ill-fitting joints causing leakage.

Anaesthetic pollution not only occurs in the operating theatres. Significant levels of contamination have been found in locations where anaesthetics are not usually administered (e.g. corridors of an operating room suite, anaesthetic workrooms and recovery rooms) [14].

In order to determine the nature and extent of the risks of exposure and in order to test the effectiveness of contamination control systems, it is important to have reliable, precise and accurate data for the establishment of current normal contamination patterns in theatres of various design and of various work patterns.

For this purpose a number of different methods are in use. They are summarized in tabular form in a publication by Bencsáth et al. [15] and are of distinct usefulness according to selectivity, sensitivity, time spent and costs. On the other hand, GLC and infrared spectrophotometric methods have proved quite useful under these circumstances.

3.1.1.1. Gas—liquid chromatography. A fundamental publication on the study of the pollution in operating theatre atmospheres by volatile anaesthetic agents by Robinson et al. [16] described the GLC measurement of nitrous oxide, trichloroethylene and halothane after various sampling techniques with electron-capture detection (ECD). Disadvantages of earlier studies are discussed

together with ways in which they might be overcome. Special attention is paid to analysis, sensitivity, sampling standardization and data presentation. Using the method described, non-zero halothane concentrations were measured during a three-month period in the general working zone of an out-patient dental operating theatre (similarily conducted by Thompson et al. [17]), also the exposure of an anaesthetist to halothane during an operating session, and the inadvertant contamination of anaesthetic circuits with halothane by vaporizer leakage testing [18].

In addition, using the same GLC conditions, the authors measured blood halothane concentrations in anaesthetists, theatre nurses and dental students. Therefore 2-ml samples of blood were taken at the beginning and at the end of an operating session. Each sample (1 ml) was extracted using hexane (2 ml). Aliquots from the *n*-hexane extracts (1 μl) were injected into the gas chromatograph.

Paying particular attention to the evaluation of the most adequate sampling technique to determine individual occupational exposure to inhalational anaesthetics (e.g. spot sampling, integrated personal sampling, end-tidal and blood sampling), Davenport et al. [19] described a GLC method for measuring average concentrations of halothane and nitrous oxide after spot sampling and integrated personal sampling. Both sampling techniques are compared with one another.

Data produced using integrated personal sampling and the outlined GLC assay provide an overall picture of the ambient levels of the anaesthetics halothane and nitrous oxide in twenty hospitals [20]. This GLC assay was also used by Mehta et al. [21] for the monitoring of occupational exposure to nitrous oxide in non-ventilated operation rooms after different sampling techniques.

For examining the effectiveness of scavanging systems and the importance of maintenance programmes on the reduction of the exposure of operating room personnel to anaesthetic agents, Sass-Kortsak et al. [22] described a GLC method for the determination of halothane and enflurane concentrations after adsorption on activated charcoal. Glass tubes containing activated charcoal were connected to pumps calibrated to draw air through the tubes at approximately 200 ml/min. The exact volume of air drawn through the tube was determined from the calibrated flow-rated and the time over which the sample was obtained. Halothane and enflurane were desorbed from the charcoal by shaking it with 1 ml of carbon disulphide for 30 min. Aliquots of the solutions were injected into the gas chromatograph.

Pollution of delivery ward atmosphere by anaesthetic gases and vapours was investigated by Dahlgren et al. [23]. To measure methoxyflurane concentrations in the atmosphere a transportable gas chromatograph with a flame ionization detector was used. Sampling was done with an automatic sampling device equippped with 30 ml hypodermic syringes.

A rather instructive publication in the field of the detection of halothane in the air of operating rooms by Bencsáth et al. [15] in 1980 not only summarized the different methods for the measurement of halothane vapour, but also described a suitable GLC method for its estimation.

Using a special variant of the head-space technique after "personal air

sampling" (sample concentration by adsorption on activated charcoal), chromatography was performed on a column combination with effluent-splitting and flame ionization—electron-capture double detection. Sampling and preparation of the sample in detail: 7.5 or 15 l air (corresponding to a sampling period of 30 or 60 min) are drawn through a tube filled with 400 mg activated charcoal. At the end of the sampling period the charcoal is filled into an injection bottle (24 ml) which is closed and stored in a refrigerator. Prior to gas chromatographic (GC) analysis, 2 ml of benzyl alcohol including the internal standard (30 μg/ml 2,3-dimethylhexane) are added to the halothane loaded charcoal. After re-closing, the bottle is tempered in a water bath at 60°C. Injection volume with conventional head-space technique: 200—1000 μl. Due to the great number of samples an automatic sampler was used.

The simultaneous use of the two detectors extends the linear dynamic range for the detection of halothane from 1 ppb (parts per billion) to 300 ppm (related on 15 l sample volumes) (Fig. 1). Gas chromatograms of original air samples from an operating room are shown in Fig. 2. (GLC conditions of this chapter are listed in Table 2).

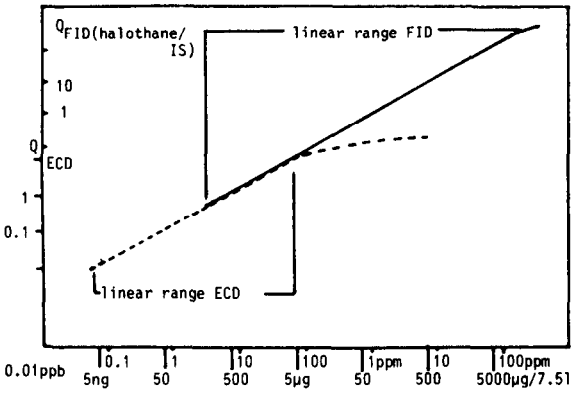


Fig. 1. GC calibration diagram for the determination of halothane with flame-ionization/electron-capture double detection [15].

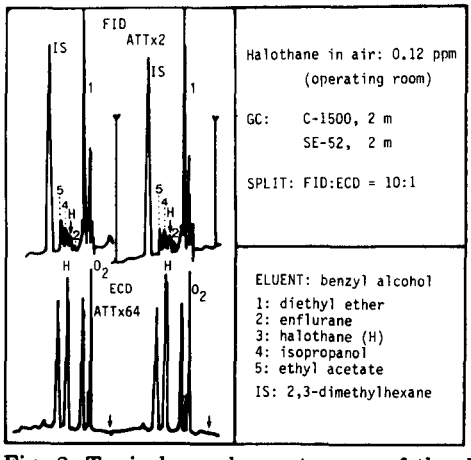


Fig. 2. Typical gas chromatogram of the determination of halothane in operating room air with flame-ionization/electron-capture double detection [15].

TABLE 2

GLC CONDITIONS FOR MEASUREMENT OF AMBIENT AIR CONTAMINATION BY VOLATILE ANAESTHETICS

Agents	Detection	Conditions*	Sensitivity**	Ref.
Halothane, trichloroethylene	ECD	5 m × 6 mm I.D. glass with 10% DC 560 silicone on universal support (80—100 mesh); CT: 90° C; DT: 200° C; Ar—5% CH_4 60 ml/min	Halothane 3—4 ng/l Trichloro-ethylene 1 μg/l	16
Nitrous oxide—halothane		1.53 m × 6 mm I.D. with Porapak Q; CT: 100° C or 170° C; DT: 200° C; Ar—5% CH_4	Nitrous oxide 10 ppm (18 μg/l)	
Halothane	FID	2.13 m × 3.18 mm I.D. glass with 10% OV-101 on Chromosorb W (100—120 mesh); CT: 80° C; DT: 250° C; He 20—25 ml/min	Range 0.3—2500 ppm	19
Nitrous oxide	ECD	3 m × 3.2 mm I.D. glass with Porapak Q (80—100 mesh); CT: 180° C; DT: 250° C; Ar, 25 ml/min	Range 25—1500 ppm	19
Halothane, enflurane	FID	Column combination: 0.92 m × 3.2 mm I.D. stainless steel with 20% FFAB on Chromosorb W (80—100 mesh) + 0.92 m × 3.2 mm I.D. stainless steel with 10% TCEP and 10% FFAB on Chromosorb W (80—100 mesh); CT: 50° C; N_2 30 ml/min	Halothane 5 μg Enflurane 6 μg	22
Methoxyflurane	FID	1 m × 2.2 mm I.D. with 2% Carbowax 400 on Chromosorb G (60—80 mesh); CT: 75°C	0.1 ± 0.05 ppm	23
Halothane	FID/ECD	Column combination: 2 m × 3.2 mm I.D. with 15% Carbowax 1500 on Chromosorb P, AW (80—100 mesh) + 2 m × 3.2 mm I.D. with 10% GE-SE-52 on Chromosorb P, AW DMCS (80—100 mesh); CT: 80—85° C; IT: 150° C; DT: 180° C; effluent split FID/ECD 10:1	1 ppm ± 4.7%	15

*Columns; CT = column temperature; DT = detector temperature; IT = injection port temperature; carrier gas flow.

**For the conversion of ppm or ppb to μg/ml see Robinson et al. [16].

3.1.1.2. Infrared spectrophotometric methods. For continuous measurement of inhalational anaesthetics in ambient air infrared analysers of different designs are currently in use. They are preferably used for the estimation of nitrous oxide concentrations [22—25] and provide valuable information about concentration peaks caused by typical handling in an operating room. In relation to the detection wavelength by the use of infrared analyser filters, it is also possible to detect halothane and enflurane by this method [15, 24—26].

To ensure accurate spectrophotometric readings, the instruments have to be checked frequently and calibrated after each survey. Isopropyl alcohol, ethyl alcohol, methyl alcohol, formaldehyde, carbon dioxide, water vapour and fluorocarbons are known to interfere with the infrared analysis of anaesthetic gases [13, 27]. In combination with a reference cell the selectivity of such analysers is sufficient in the range 1—1000 ppm for nitrous oxide and 0.5—400 ppm for halothane.

3.1.2. Patient drug level monitoring

3.1.2.1. Non-invasive methods (breath-to-breath monitoring). The control of the induction and maintenance of anaesthesia presents an interesting problem to both the practising anaesthetist and the systems engineer. Previous work in this field, as reviewed by Chilcoat in 1980 [28], has been mainly directed towards techniques in which the inspired concentration is either pre-programmed or controlled in order to regulate some measurable physiological variable (arterial pressure or electroencephalogram), which is assumed to be related to depth of anaesthesia. The construction of a rapid-response ultraviolet (UV) halothane meter [29] enables the development of a system controlling alveolar anaesthetic concentration, observed from the end-tidal measurement, of patients undergoing halothane, with or without nitrous oxide, anaesthesia under controlled ventilation [30, 31]. The halothane meter, which allows breath-by-breath halothane monitoring in clinical anaesthesia in adults and children (the volumes of the infant and adult cuvettes are 0.56 and 4.52 ml, respectively) by a safe non-invasive method, is rapid in response (about 40 msec) and its compact size allows positioning close to the subject. Zero drift is equivalent to less than 0.04—0.06% halothane after a warm-up period of 1 h. The lamp output energy was at 253.7 nm and all other mercury emission was eliminated by the use of narrow-band filters. None of the other respiratory gases and anaesthetic agents — oxygen, nitrogen, carbon dioxide, water vapour and nitrous oxide — would absorb measurable quantities of light of this wavelength.

Another instrument extremely useful for respiratory gas analysis of general purpose is the mass spectrometer. However, its sophistication, its expense and waiting time between analyses rendered it unsuitable for continuous use in operating rooms or intensive-care units [32, 33]. Nevertheless, after two years of experience Ozanne et al. [34] in 1981 described the use of a mass spectrometer for the continuous monitoring of nitrous oxide and the volatile anaesthetic agents (besides nitrogen, oxygen and carbon dioxide) in up to ten active rooms simultaneously to determine inspired and end-tidal concentrations of these gases. Gases were introduced to the ion source of the mass spectrometer — being located centrally — by a special sampling device, fitted with thirteen

three-way solenoid valves which were located at the end of 30 m × 1.07 mm I.D. nylon catheters and were attached to the patient's airways. The three volatile agents the examiners used were halothane, enflurane and isoflurane. These are partially fragmentated in the mass spectrometer ion source. Each gas has a major ion fragment at *m/z* 67 which is used for analysis. Another fragment appears at *m/z* 51. The ratio of 67/51 is used for identification, since it is a characteristic element of each agent (it is 7.5 for halothane, 1.45 for enflurane and 0.44 for isoflurane).

A computer program was developed to determine the inspired and end-tidal concentrations of each gas, timed from the carbon dioxide waveform, to generate individual graphic displays for each operating room terminal, to store the data permanently and to control the bank of thirteen solenoid valves. The monitoring facility was used in each room for an average of 5.5 h per day. Inherent problems have resulted in an inoperative time of less than 2%. In the author's opinion two years of experience suggest that the described multi-patient anaesthetic mass spectrometry (MS) system can facilitate detection of faulty technique and equipment, reduce cost of anaesthetic agents, increase patient safety, aid research and teaching and diminish exposure of operating room personnel to anaesthetics.

Another technique for the continuous monitoring of inspiratory and end-tidal anaesthetic vapour using a piezoelectric detector was tested by Hayes et al. [35]. In this study the accuracy, response time, gas interference and water vapour dependence of the relatively inexpensive compact and fast analyser were measured. Since water vapour interference resulted in a large offset, it seems that relative inspired — expired differences can be measured, but absolute end-tidal measurements will be in question.

3.1.2.2. Invasive methods (blood/plasma drug level monitoring). GLC proved quite suitable for the quantitative analysis of volatile anaesthetics in blood or other biological fluids (conditions are listed in Table 3). Procedures in this field are of two types: the indirect method in which the anaesthetic is separated from the biological fluid prior to injection (head-space methods, solvent extraction and distillation) and the direct method in which GC is performed on a sample of whole blood.

Reid et al. [36] described a direct injection GLC procedure suitable for the routine analysis of volatile halogenated anaesthetics in whole blood. Using an external injection port which allows the injection of a small sample of whole blood (4—40 μl) into the pre-heated carrier gas stream they avoided the problems due to contamination of the chromatographic column with non-volatile blood components. Flash evaporation within a changeable U-tube filled with a loose glass wool filter plug at 180°C removes the non-volatile components and only the volatile components enter the column. Changing the contaminated U-tube occupies not more than 60 sec. The external injection port — a heatable special valve system — is described in detail. Analysis of blood samples (450 μl) was performed on EDTA-anticoagulated blood using Reacti-vials each equipped with a magnetic stirring bar and a Mininert valve. Isobutanol was used as the internal standard. The vials were filled to the rim by adding water.

The outlined method permitted the accurate analysis of halothane, methoxy-

flurane, diethyl ether and ethanol over the range of approximately 10 μg/ml to 1 mg/ml. Using this analytical procedure, the distribution of halothane between the cells and plasma of human blood at 4°C was found to be 2.0 ± 0.2. In addition, the method was used for the measurement of halothane concentrations in a study about interactions of halothane with the five major constituents of human blood, namely haemoglobin, albumin, red cell membranes, triglycerides and gamma-globulin [37].

TABLE 3

GLC CONDITIONS FOR THE MEASUREMENT OF BLOOD CONCENTRATIONS OF VOLATILE ANAESTHETICS

Agents	Detection	Conditions*	Sensitivity**	Ref.
Halothane, methoxyflurane, diethyl ether, (ethanol), I.S.: isobutanol	FID	1.83 m × 2 mm I.D. glass with Chromosorb 101 (80—100 mesh); CT: 110—180°C at 6°C/min; IT: 200°C (external IT: 180°C); DT: 250°C; He or N_2 25 ml/min; direct	Range approximately 10 μg/ml to 1 mg/ml	36
Halothane, I.S.: trichloroethylene	ECD	1.53 m × 3 mm I.D. with 15% diethylhexylsebacate on Chromosorb WAW DCMS (80—100 mesh); CT: 90°C; IT: 175°C; DT: 175°C; N_2 20 ml/min; solvent extraction	Range 2—100 μg/ml	38
Enflurane (halothane)	TCD	1.83 m stainless steel with 5% SE-30 on Varaport 30 (100—120 mesh); CT: 80°C (2 min) to 200°C at 50°C/min; IT: 150°C; DT: 200°C; solvent extraction; headspace	Range 1.4—150 μg/ml	39, 40
Isoflurane	FID	Column with 0.8% SE-30 on Carbopack B; CT: 170°C; IT: 200°C; DT: 250°C; headspace		41
Halothane, enflurane, methoxyflurane, ethylene, N_2O, cyclopropane, trichloroethylene (I.S. method)	FID/ECD	2 m × 2 mm I.D. glass with 0.3% Carbowax 20M on Carbopack (80—100 mesh); CT: 35—175°C at 5°C/min (8 min held); IT: 275°C; DT: 275°C; N_2 30 ml/min; effluent split FID/ECD = 9:1; headspace		42
Volatile halocarbons	FID	55 m × 0.25 mm I.D. glass open-tubular coated with OV-101 (0.9 μm); CT: 35°C (5 min) to 175°C at 5°C/min; He 30 cm/sec; headspace	5—10 ppb	43

*Columns; CT = column temperature; DT = detector temperature; IT = injection port temperature; carrier gas flow; injection techniques.

**For the conversion of ppm or ppb values to μg/ml see Robinson et al. [16].

Tunstall and Hawksworth [38] reported a GLC assay of halothane after solvent extraction; 5 μl of whole blood were injected below the surface of 5 ml of toluene, containing 1.25 μg of trichloroethylene as internal standard. After mixing (30 sec) and centrifugation for 5 min at ca. 500 *g*, the sample could be stored in a refrigerator overnight and could be analysed the following day without loss of halothane. The calibration curve was non-linear owing to the non-linear response of the detector to high concentrations of halothane. The precision of the measurement was ± 4.6% (S.E.M., $n = 6$) at 2 μg/ml and ± 0.8% (S.E.M., $n = 6$) at 100 μg/ml.

For the determinaton of the blood/gas partition coefficient of enflurane in non-obese and morbidly obese patients, Borel et al. [39] used head-space and solvent extraction techniques in connection with GLC conditions described earlier by Miller and Gandolfi [40]. The rather simple and time-saving extraction of 1 ml of whole blood (immediately upon collection) with 2 ml of *n*-heptane for a period of 5 min and injection of an 100 μl aliquot of the *n*-heptane phase into the gas chromatograph allows the detection of enflurane at 1.4 μg/ml of whole blood with a thermal conductivity detector. Analysis of enflurane standards ranging in concentrations from 1.4 μg/ml to 1.4 mg/ml demonstrated linearity ($r = 0.9999$) and 98% extraction into hexane overall.

Using a multiple gas phase equilibration technique, Knill et al. [41] determined the blood/gas partition coefficient in blood samples of patients undergoing minor or major surgical procedures after isoflurane or isoflurane—nitrous oxide (50—70%) anaesthesia, by GLC with flame ionization detection (FID). After anaerobically sampling of blood (10 ml) in a gas-tight syringe the samples were sealed immediately and placed on ice until analysis. Analysis was performed by adding an equal volume of air (somewhat less than 10 ml) to the sample syringe. Then the syringe was transferred to a shaking water bath maintained at 37°C for 20 min. After an exact equalization of the volumes of the two phases and a re-equilibration time of 20 min, the portion of gas phase was injected into the gas chromatograph.

Although it was evaluated for the detection and identification of volatile organic compounds as an aid in the diagnosis of solvent abuse, Ramsey and Flanagan [42] reported a head-space GC method which also proved suitable for quantitative analysis of blood specimens containing the volatile anaesthetics (e.g. halothane, enflurane, methoxyflurane, ethylene, nitrous oxide, cyclopropane, trichloroethylene). After addition of an appropriate internal standard solution (e.g. in the case of halothane, bromodichloromethane) to the sample followed by equilibration lasting 30 min at 65°C, a portion of the head space is analysed. Calibration can be made in the usual manner.

A GLC assay with an open tubular column for the head-space analysis of halocarbons in water [43] also seems to be useful for the quantitation of the volatile anaesthetic halocarbons in blood or other biological fluids. The possibilities of a capillary system in the split injection mode with low split ratios (e.g. 1:20, 1:30) would permit the analysis of trace concentrations in connection with properly resolved chromatographic peaks.

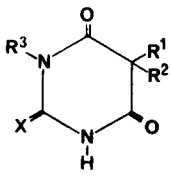
For further information on the head-space technique with special attention to pre-concentration of volatiles for trace organic analysis by GC see the review by Núñez et al. [44].

3.2. Intravenous anaesthetics

Intravenous anaesthetic agents are widely used for the induction of anaesthesia and as sole agents for short procedures.

3.2.1. Barbiturates

The barbiturates were introduced as intravenous anaesthetic agents by Lundy in 1935 (thiopental [45]). Thiopental [5-ethyl-5-(1′-methylbutyl)-2-thiobarbituric acid] and methohexital [1-methyl-5-allyl-5-(1′-methylpentyn-2′-yl)barbituric acid] are short-acting barbituric acids which are widely used. Considerable interest is currently being devoted to newer applications of these drugs and of two other barbiturates, pentobarbital [5-ethyl-5-(1′-methylbutyl)barbituric acid] and phenobarbital (5-ethyl-5-phenylbarbituric acid). Attention is being paid to their role in the management of cerebral hypoxia [46] and intracranial hypertension [47]. In these indications, large doses are given intravenously over a period of a few days and a control of plasma levels is necessary. Structures of these four barbituric acids are shown in Fig. 3.



	R^1	R^2	R^3	X
thiopental	$-C_2H_5$	$-CH(CH_3)-CH_2-CH_2-CH_3$	-H	S
methohexital	$-CH_2-CH=CH_2$	$-CH(CH_3)-C\equiv C-CH_2-CH_3$	$-CH_3$	O
pentobarbital	$-C_2H_5$	$-CH(CH_3)-CH_2-CH_2-CH_3$	-H	O
phenobarbital	$-C_2H_5$	$-C_6H_5$	-H	O

Fig. 3. Chemical structures of the barbituric acids thiopental, methohexital, pentobarbital and phenobarbital.

3.2.1.1. Gas—liquid chromatography. The analysis of barbiturates by GLC was reviewed by Pillai and Dilli in 1981 [48]. These authors gave a comprehensive survey of the advances in the analytical chemistry of the barbiturates studied by the GLC technique in the late sixties and in the seventies. In the last few years some reports described the GC analysis of the barbiturates mentioned above in studies on their pharmacokinetics, on their monitoring in continuous application of high doses, and on toxicology.

A simple GC method for the quantitative determination and identification of commercially available barbiturates in serum (including pento- and phenobarbital) in the area of clinical toxicology is described by Külpmann [49]. After saturation of the serum sample with ammonium sulphate and extraction with chloroform, chromatography was performed on either 3% SP-2250, 3% CDMS or 3% OV-101 with FID. The specificity of the method was checked by comparing the results obtained before and after additional purification of the extract by TLC, and the retention times of about 100 drugs in the GC systems were determined. For quantitative analysis hexobarbital could be used as internal standard. The precision in the series was between 6% and 7%; the recovery for pentobarbital was 98.3% and for phenobarbital 96.1% at 10 μg/ml. The detection limit for pentobarbital was 0.8 μg/ml.

Within another report upon the identification of 5,5-disubstituted

barbiturates [50], which are subject to abuse, a GC—FID method is described which also seems to be suitable for the quantitative analysis of the barbiturates of interest. The use of a capillary vitreous silica column (50 m × 2 mm I.D.) with a bonded methyl silicone stationary phase resulted in a proper separation of pentobarbital, methohexital, phenobarbital (hexobarbital as internal standard, I.S.), and thiopental (I.S.: mephobarbital). The use of a shorter column (30 m) would reduce the analysis time without loss of chromatographic separation.

Other assays using FID of the underivatized barbituric acid after GLC separation in glass columns of different length and internal diameter (3.0—0.9 m, 4—2 mm I.D.) on different non-polar and medium-polar stationary phases were reported: Phenobarbital [51] and thiopental [52] from serum (10—100 μg/ml; 1—30 μg/ml) after acidic extraction with methylene chloride (I.S.: 5-methyl-5-phenylhydantoin) on a mixed stationary phase (2% SP-2110—1% SP-2510 DA) with a precision for phenobarbital of 2.6% within-day and 3.4% between-day at 24.2 μg/ml and a coefficient of variation (C.V.) obtained by extraction and analysis of six replicates of 15.5 μg/ml of 5.2% (7.3 μg/ml: C.V. = 6.1%) in the case of thiopental. The analytical recovery is 97% for phenobarbital and 95% for thiopental. Thiopental in plasma [53] after a time-consuming extraction method (I.S.: butethal) on 5% OV-1 with a sensitivity of 25 ng/ml and a concentration range of 0.1—30 μg/ml. The use of hexane reduces the in vitro desulphuration of thiopental to pentobarbital observed in solvents such as diethyl ether. However, the extraction of thiopental from biological fluids inherently results in a small amount of transformation.

Thiopental (and its metabolite pentobarbital) monitoring in serum (9.3—84 μg/ml) [54] after saturation with ammonium sulphate and extraction with chloroform (I.S.: cyclobarbital) on 2% SP-2510 DA. Imprecision from day to day: C.V. 7.7%; recovery 97%. The specificity was checked by comparing the retention time of this barbiturate with those of more than eighty drugs. Of all the checked substances, only doxepin can interfere with the quantitative determination. The therapeutic concentration of doxepin, however, is beyond the detectability of the method.

The determination of underivatized pentobarbital (5—50 μg/ml) and certain other barbiturates (e.g. phenobarbital) by capillary GLC—FID was reported by Wallace et al. [55]. After the extraction of plasma specimen (I.S.: thioseconal) with chloroform, the use of a 25 m × 0.31 mm I.D. SE-54 bonded-phase capillary column (column temperature 190°C) exhibited complete baseline resolution of the eight barbiturates which were examined. The limit of sensitivity for a 30:1 split was determined to be 0.5 μg/ml (n = 5) with a C.V. of 3.35%. Altering the split ratio from 30:1 to 5:1 significantly increases the sensitivity of the method and makes it suitable for monitoring paediatric patients. The recoveries of pentobarbital from plasma exceed 95% at the low and high concentrations. The within-day C.V. was 1.85% at 15 μg/ml (n = 6). Day-to-day C.V. was 4.5% at 15 μg/ml and 3.8% at 35 μg/ml. Twenty-three drugs of clinical importance were investigated for possible interference. Only acetaminophen chromatographed within the retention time range observed for the barbiturates but does not interfere with either pento- or phenobarbital.

Since GC of native barbiturates is critical because of peak tailing and low

sensitivity due to the interrelationship of the polar sides of the molecules with the active sites of the column packing or adsorption on glass surfaces, derivatization procedures for this group of compounds forming less polar derivatives are well known and used in over 75% of important publications on the GC of barbiturates [48]. Methylation appears to be the most satisfying derivatization procedure.

On-column methylation with 40% trimethylphenylammonium hydroxide (TMAH) in methanol following the acidic extraction was reported for the estimation of plasma levels of thiopental (I.S.: secobarbital) [56]. GLC on 3% SE-30 (FID) allowed the determination of thiopental down to 0.2 μg/ml.

Another publication dealing with the routine identification and quantitation of eleven barbiturates in biological samples [57] (also methohexital, phenobarbital, pentobarbital) on the stationary phases OV-17 or OV-101 in the concentration range 5—50 μg/ml described FID after methylation of the free acids with trimethylanilinium hydroxide 0.2 *M* in methanol. A rather troublesome extraction procedure and the length of one GLC run of about 70 min (retention time of phenobarbital 60 min) are the reasons for this procedure's inefficiency for routine drug level monitoring.

ECD of barbiturates has not become a standard method, perhaps due to the tedious work-up and derivatization procedures reported [48]. Smith et al. [58] compared measurement of thiopental in plasma (I.S.: thiamylal) on the liquid phases 3% OV-1, 3% SP-2100, 10% SP-2100 and 3% OV-17 with either ECD or flame photometric detection after methylation of the ethyl acetate extract with 0.2 *M* trimethylanilinium hydroxide in methanol for 15 min. The final choice for the qualitative analysis (concentration range 0.01—30 μg/ml) was 3% OV-17 and ECD, providing greater sensitivity and a broader linear range for the compounds of interest.

The GLC determination of methohexital in plasma or whole blood with sufficient sensitivity and selectivity for pharmacokinetic studies is reported by Björkman et al. [59] with ECD of the pentafluorobenzyl derivative, and by Heusler et al. [60] who used nitrogen-selective FID (N-FID) of the unchanged drug, both after chromatographic separation on 3% OV-17. Björkman et al. [59] used a rather time-consuming work-up procedure with extraction—re-extraction steps into toluene followed by the derivatization procedure, although for samples containing more than 1 μg/ml the re-extraction step can be omitted. The lowest plasma methohexital concentration which can be quantitated satisfactorily is around 0.1 μg/ml. Samples showing plasma concentration above 4 μg/ml should be re-run after dilution due to the non-linearity of the ECD response at higher methohexital concentrations.

A modification of an earlier published assay of methohexital [61] in plasma by Heusler et al. [60] allows the measurement of low concentrations (< 50 ng/ml) of the underivatized drug in plasma and whole blood after a single extraction step with a mixture of light petroleum—diethyl ether—propanol-2 (50:50:2). Further development of this method, using a capillary column (fused silica with chemically bonded phases like OV-1 or OV-17) instead of a packed glass column, splitless injection techniques and an automatic injection device, allows measurement of as many as 200 samples per day in a concentration range of 0.02—10 μg/ml. In all cases within-run and day-to-day imprecision

is not greater than 6%. The method also proves useful for the quantitative determination of pentobarbital and methohexital simultaneously in presence of lidocaine and its metabolites in plasma samples (Fig. 4). The use of a solid injection technique makes it feasible to measure methohexital in the breast milk of women after delivery down to 5 ng/ml without difficulties (Fig. 5).

Sensitive and — according to the selectivity of the N-FID — simple methods with minor work-up have also been reported for the analysis of thiopental [62] and pentobarbital [63] in plasma or serum. After the extraction with hexane—2-propanol, 4:1 (I.S.: phenobarbital) and methylation with iodomethane in refluxing acetone in the presence of sodium carbonate, GLC determination of thiopental was performed on 3% OV-17 within 2 min in the concentration range 25 ng/ml to 10 μg/ml. Analytical recovery was to be found in the range 80—90%. Diazepam, fentanyl, and morphine drugs frequently used in conjunction with thiopental are bases and are not extracted in this procedure. The sensitivity of this method (25 ng/ml) makes it possible to characterize the pharmacokinetics of thiopental completely after the usual induction doses of 3—4 mg/kg.

The GLC method for the determination of pentobarbital [54] using the

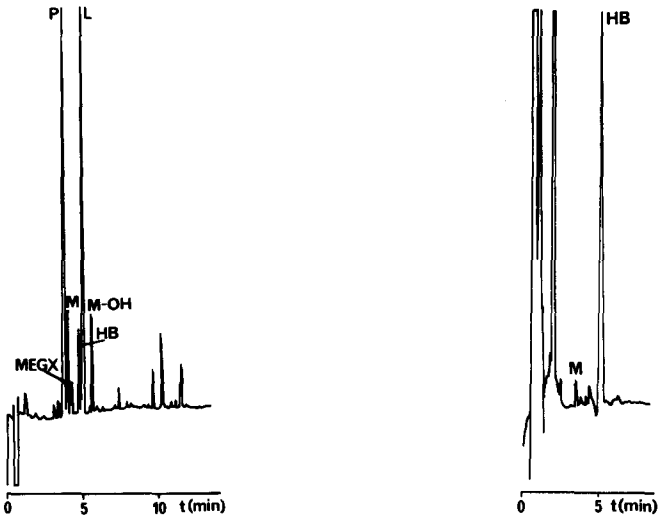


Fig. 4. Gas chromatogram of a plasma extract of a patient in the intensive care unit receiving lidocaine intravenously 2 g per day for antiarrhythmic therapy, pentobarbital intravenously 1 g per day by repeated bolus injections for sedation and 80 min after a single intravenous dose of 300 mg of methohexital. Results: pentobarbital (P) = 19.2 μg/ml; methohexital (M) = 0.77 μg/ml; lidocaine (L) = 1.24 μg/ml; hexobarbital, internal standard (HB) = 0.5 μg/ml (MEGX = monodesethylated metabolite of L; M-OH = 4'-hydroxy metabolite of M). GC conditions: capillary column fused-silica OV-101 (10 m × 0.2 mm I.D.); CT: 150° C (1 min) to 227° C (4 min) at 7° C/min, IT: 250° C, DT: 300° C; carrier gas He 0.8 ml/min; direct injection (1 μl); N-FID.

Fig. 5. Gas chromatogram of a 0.5-ml breast milk extract of a woman 13 h after a 120-mg methohexital anaesthetic induction dose. Result: methohexital (M) = 18 ng/ml; hexobarbital, internal standard (HB) = 0.5 μg/ml. GC conditions: capillary column SCOT OV-17 (10 m × 0.5 mm I.D.); CT: 180° C to 230° C at 6° C/min, IT: 250° C, DT: 300° C; carrier gas He 10 ml/min; solid injection; N-FID.

same methylation procedure after extraction with diethyl ether (I.S.: secobarbital; minimum detected concentration: 80 ng/ml) is sensitive enough for pharmacokinetic measurements. On account of the non-separation of thio- and pentobarbital on the OV-101 phase used, this assay, however, would only be practicable with the use of stationary phases like OV-17 or other phases of moderate polarity.

The sensitivity and selectivity in the qualitative analysis of pentobarbital in plasma or saliva is drastically increased by a GLC assay with MS detection in the multiple ion detection (MID) mode [64]. After acidic extraction and on column methylation with trimethylanilinium hydroxide (I.S.: butabarbital) 1 ng/ml could be quantified. Recovery of the barbiturate including all steps of the analytical procedure lies in the range 80 ± 2%. This procedure can also be used without modification for measuring the pharmacokinetics of a pulse dose of pentobarbital, labelled with stable isotopes, during uninterrupted treatment.

3.2.1.2. High-performance liquid chromatography. Hydrocarbonaceous bonded phases have been widely used in HPLC for the separation of small groups of barbiturates. The so-called reversed-phase HPLC is performed with aqueous eluents with different proportions of water-miscible organic solvents (preferable methanol or acetonitrile). Changing the pH of the eluent causes differences in selectivity and sensitivity. Octadecyl sulphate (ODS or C_{18}) phase on silica is the stationary phase of choice in the separation of barbiturates with HPLC. Reversed-phase HPLC on C_{18} columns for the quantitative analysis of the barbiturates of interest in biological fluids or tissues in the field of pharmacokinetic studies or in monitoring the levels after aggressive treatment with these drugs over a few days, is reported for methohexital [65—67], pentobarbital [65, 68—72], phenobarbital (65, 68] and thiopental [66, 67, 69—79].

In HPLC of barbiturates on C_{18} columns two problems must be considered: (1) barbiturates gave a poor peak shape with silica when neutral or basic eluents were used, (2) the unionized forms of oxobarbiturates have very weak absorbing properties. For this reason non-specific detection in the far ultraviolet region at 195 nm was used to analyse pheno- and pentobarbital [59] and metho- and thiobarbital [66, 67] in serum, plasma or whole blood. The short wavelength precludes the use of alcohols in the mobile phase, and therefore phosphate buffer—acetonitrile mixtures in the pH range 4.2—4.6 were employed. 5-(4-Methylphenyl)-5-phenylhydantoin [68], hexobarbital [67] or thiopental in the case of methohexital or the reverse [66] were used as internal standards. Solvent extraction of the biological material with either hexane toluene or ethyl acetate is necessary to avoid interference with other drugs and endogenous substances and to increase the sensitivity for detecting the short-acting barbiturates in low therapeutic concentrations (methohexital 10 ng/ml to 25 μg/ml [66]; 60 ng/ml to 16 μg/ml [67]; pheno- and pentobarbital 0.5 μg/ml to 100 μg/ml [68]; and thiopental 0.25 μg/ml to 16 μg/ml). Analytical recoveries in most cases were between 80% and 100%. Possible interference with other drugs was tested [68].

Thiopental, which has a UV absorption maximum in the range 300—290 nm, can be detected more sensitively and more selectively at these longer wavelengths. Therefore many HPLC methods for the measurement of thiopental in

TABLE 4

CONDITIONS AND ANALYTICAL DATA FOR HPLC ASSAYS OF THIOPENTAL ON C_{18} COLUMNS AT HIGHER WAVELENGTHS

Sample	Sample preparation	I.S.	Detector wavelength (nm)	Mobile phase	Sensitivity, linearity, precision and analytical recovery	Ref.
Plasma Urine Breast milk	Direct (precolumn) Direct (precolumn) Alkaline pre-extraction with diethyl ether	Quinoline	290	0.1% sodium citrate buffer (pH 6.5)—methanol (45:55)	0.5—20 μg/ml	69, 73
Plasma	After acidic extraction with diethyl ether	Carbamazepine	290	0.2% ammonium phosphate buffer (pH 3.5)—methanol (60:40)	Precision at 5 μg/ml Within-run C.V.: 2.8% Within-day C.V.: 6.2% Day-to-day C.V.: 4.6% Recovery: 70%	76
Plasma	After acidic extraction with ethyl acetate	Carbamazepine	290	Methanol—water (60:40)	0.2—120 μg/ml C.V.: 5—6% Recovery: 95%	78
Blood, tissue homogenate	After extraction with dichloromethane, with back-extraction	Phenolphthalein	290	Methanol—water (60:40)	1—60 μg/ml Precision at 5 μg/ml Within-run C.V.: 3.9% Day-to-day C.V.: 8.2%	79
Serum	Direct after protein precipitation	Phenolphthalein	280	4×10^{-4} *M* phosphate buffer (pH 6.5)—methanol (52:48)	Up to 200 μg/ml Recovery: 99%	77
Plasma	Direct	Flufenamic acid	280	Ammonium phosphate buffer (pH 7.9)—methanol (42:58)	0.5—10 μg/ml 10—75 μg/ml precision at 34 μg/ml Day-to-day C.V.: 4.4%	72
Serum	Direct	—	280	0.02 *M* KCl solution (pH 2)—methanol (50:50)	0.09 μg/ml Range: 0.1—20 μg/ml Recovery: 101%	74

blood, urine, breast milk or tissues used variable-wavelength detectors or standard filters at 290 nm [69, 73, 76, 78, 79] and 280 nm [72, 74, 77] for UV detection. The choice of internal standards with sufficient UV absorbance in this range is a considerable problem in the development of such assays. Flufenamic acid [72], quinoline [69, 73], phenolphthalein [77, 79] and carbamazepine [76, 78] proved suitable for this purpose because of their spectrophotometric properties.

By using these longer wavelengths sample preparation is very simple; in some cases plasma and urine samples were only diluted with acetonitrile or methanol—acetonitrile to precipitate the protein [72, 77] and/or after a single centrifugation step [69, 73] an aliquot of the supernate was injected directly into the liquid chromatograph using short pre-columns to save the chromatographic column. The use of alcohol-containing mobile phases is possible. Further information about these HPLC assays is given in Table 4.

A micro-method for the determination of thiopental in plasma (50 μl) by HPLC using a C_6 column for separation is described by Premel-Cabic et al. [80]. After the addition of 200 μl of the acetonitrile solution of the internal standard, flunitrazepam, and a brief centrifugation, 25-μl aliquots were injected via an automatic sampler. The mobile phase was a mixture (70:30) of 0.01 *M* sodium acetate adjusted to pH 3.6 by concentrated acetic acid and acetonitrile (detection wavelength: 280 nm). The linearity of this method is guaranteed up to 100 μg/ml; the detection limit for plasma samples was 0.5 μg/ml. Within-run variability of this method is less than 2%.

Other reversed-phase HPLC assays with UV detection of thiopental in plasma [75] and thiopental, besides pentobarbital, in plasma and brain tissue [70] on C_{18} used 254 nm as detection wavelength after the extraction of the acidified specimen with diethyl ether or chloroform and the elution with the mobile phases methanol—water (50:50) or 0.01 *M* phosphate buffer—acetonitrile—tetrahydrofuran (78:22:4). Both assays are sensitive enough to quantitate plasma levels as needed to guide therapy in long-term application of these barbiturates.

The method of Sharman and Ahern [72] for the determination of thiopental in plasma can also be used to estimate pentobarbital in plasma at a detection wavelength of 254 nm or 220 nm. Furthermore, measurement of pentobarbital in plasma and urine is possible after an identical extraction procedure to that for the analysis of thiopental [69] except that 215 nm is used as detection wavelength, with a mobile phase of ammonium phosphate buffer, pH 5.5—isopropanol—methanol (65:30:5).

An HPLC method for the simultaneous analysis of thio- and pentobarbital on C_{18} with detection at 240 nm (detection limit 1 μg/ml) by Kelner and Bailey [71] requires a time-consuming extraction procedure before injection and is therefore not suitable for routine analysis. Reversed-phase HPLC from pH 1 to 13 is possible with the macroporous poly(styrene-divinylbenzene) adsorbent PRP 1. This allows the barbiturates to be chromatographed by reversed phase at an alkaline pH consistent with enhanced UV aborption and thus increased detectability at 240 nm. Gupta et al. [81] reported a liquid chromatographic (LC) procedure for the quantitative determination of pentobarbital (or phenobarbital) in plasma (5—80 μg/ml) on this PRP-1 XAD-2 resin. 5-Ethyl-5-*p*-

toluoylbarbituric acid served as internal standard. After extraction with chloroform and addition of sodium hydroxide solution, aliquots of 25 μl of the alkaline aqueous phase were injected. The mobile phase consists of methanol—acetonitrile—0.2 *M* sodium hydroxide in the ratio 10:3:87. The reported retention times are phenobarbital 2.5 min, I.S. 5.5 min and pentobarbital 7.5 min.

Improved detectability of barbiturates in HPLC by precolumn derivatization with 2-naphthacylbromide is described by Hulshoff et al. [82]. These derivatives are formed in acetone at 30°C within 30 min using caesium carbonate as a catalyst. The derivatives strongly absorb UV radiation at 254 nm. The method has excellent sensitivity. After chromatography on a C_{18} column (methanol—water, 80:20) it is possible to measure pheno- or pentobarbital levels of about 0.5 μg/ml in plasma or serum samples of 10 μl. Another barbiturate, such as hexo-, hepto- or butobarbital, could serve as internal standard.

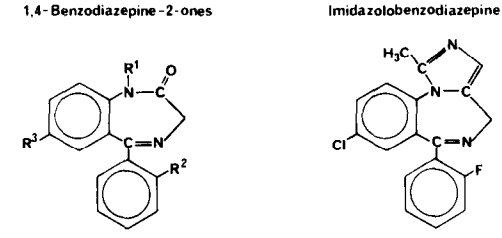
LC methods for the determination of antiepileptic drugs [83] or for their identification in combination with GLC and TLC in abuse (including barbiturates) [65] can also be applied to quantitative analysis of the barbiturates of interest.

3.2.1.3. Other assay techniques. Spectrofluorimetric [84, 85] or UV spectrophotometric methods [84, 86—88] are not specific for the quantitative analysis of barbiturates when contrasted to chromatographic methods and are, with some exceptions [87, 88], not suitable for measuring therapeutic concentrations of these drugs. TLC, which often offers the ability to distinguish one drug from another, is only suitable as a screening procedure for barbiturates [65] in toxicological or forensic analyses.

The commercially available enzyme-multiplied immunoassay technique (EMIT) for barbiturates was found to be useful in the measurement of pentobarbital [89]. The application of the method is limited to patients receiving pentobarbital as the only barbiturate. On account of its low sensitivity (10 μg/ml) the use of the technique in clinical laboratories is limited to monitoring patients undergoing high-dose pentobarbital therapy.

3.2.2. Benzodiazepines

The 1,4-benzodiazepine class of compounds has yielded several important drugs, the majority of which belong to the 1,4-benzodiazepine-2-ones, which



	R^1	R^2	R^3
Diazepam	CH_3	H	Cl
Flunitrazepam	CH_3	F	NO_2

Fig. 6. Chemical structures of the three major benzodiazepines used in anaesthesia.

are used as anxiolytic or anticonvulsant agents, hypnotics and muscle relaxants. Some of them (diazepam, flunitrazepam) and the imidazolobenzodiazepine derivative midazolam (Fig. 6) are currently used also for induction of anaesthesia when it is desirable to minimize cardiovascular effects.

Diazepam and flunitrazepam are insoluble in water and are supplied for injection dissolved in a mixture of organic solvents. Because of its imidazole ring midazolam is such a strong base that water-soluble salts are readily obtained and it is preferably administered as its maleate salt.

On account of the extensive biotransformation of the benzodiazepines in man resulting in the presence of one or more pharmacologically active metabolites, specificity of analysis is essential. Therapeutic doses for these compounds are usually low; intravenous doses for induction of anaesthesia are about 0.03 mg/kg (flunitrazepam), 0.2 mg/kg (midazolam) and 0.3—0.8 mg/kg (diazepam), hence the analytical method for their quantitation in biological media must be very sensitive, since the blood concentrations are located in the lower nanogram (10^{-9} g) per millilitre range.

Considering the representative and comprehensive reviews of De Silva [90, 91] in 1982 and 1983 on the determination of benzodiazepines in biological fluids, including GLC—ECD analysis of specific benzodiazepines, GC with chemical-ionization (CI)—MS, GC analysis with other ionization detectors (N-FID), HPLC, TLC, differential pulse polarography (DPP) [92] and radioimmunoassay (RIA), with special attention on biotransformation and sample preparation, only some newer sensitive assays for the determination of diazepam and midazolam are reported here.

On account of the high sensitivity, specificity, possible automation and relatively low cost, GLC—ECD is the most practicable method in this field. Greenblatt et al. [93] reported an automated GLC—ECD assay for pharmacokinetic studies of midazolam after a simple and rapid alkaline extraction procedure with benzene containing 1.5% isoamyl alcohol. Chromatography was performed on 3% SP-2550 isothermal at 265°C. The sensitivity limits of the method were approximately 2—3 ng/ml.

Other GLC—ECD assays for the determination of midazolam and its α-hydroxy metabolite in plasma [94, 95], after more or less time-consuming extraction procedures into diethyl ether at alkaline pH, are of similar sensitivity (4—5 ng/ml). The metabolite was silylated before chromatography on 5% OV-101 phases.

GC—CI-MS coupled with selected-ion monitoring (SIM) is the method of choice for the quantitation of very small amounts of drugs and metabolites in biological fluids due to its excellent sensitivity and specificity. Compounds containing an electrophore, e.g. Cl, F, $-NO_2$ groups attached to an aromatic ring (especially one which is in conjuction with a carbonyl or amide group as are the 1,4-benzodiazepin-2-ones), have a high probability of capturing a thermal electron to produce a negatively charged molecular ion $(M - H)^-$. Therefore the use of GC—NCI-MS in the analysis of benzodiazepines is highly recommended. Rubio et al. [96] reported the quantitative analysis of midazolam and two of its metabolites (α-hydroxymethyl- and N-desmethylmidazolam) in plasma by this technique. Stable isotope analogues were used as internal standards. Silylation is necessary before chromatography. For all

three compounds the limit of quantitation is 1 ng/ml with good precision. The sensitivity of GC with electron-impact (EI) MS in the SIM mode with positive-ion detection in the analysis of midazolam is limited to 5 ng/ml [97].

Although less sensitive than GLC—ECD or GC—MS techniques, GLC with nitrogen-selective detection (N-FID) is applied to the analysis of diazepam. The use of a wall-coated open tubular column instead of a packed column in combination with this selective detector enables the determination of 5 ng/ml midazolam in 1-ml plasma samples [98].

The use of bonded-phase extraction columns for rapid sample preparation in routine HPLC analysis of diazepam and its pharmacologically active metabolite N-desmethyldiazepam is described by Good and Andrews [99]. Quantitation was carried out by UV detection at 242 nm and remarkable sensitivity of 5—10 ng/ml for each drug after the work-up of 1—2 ml of serum samples has been achieved.

3.2.3. Etomidate

Etomidate [(*R*)-(+)-ethyl-1-(phenylethyl)-1H-imidazole-carboxylate] (Fig. 7) is a short-acting imidazole hypnotic which is commonly used for induction of anaesthesia. In Britain its use for sedation in the intensive-care unit has been suspended, because it seriously hampers adrenal cortical function [100].

For studying the pharmacokinetics of this drug associated with prolonged

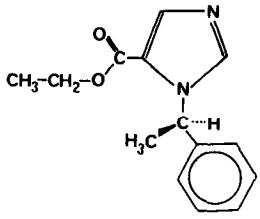


Fig. 7. Chemical structure of etomidate.

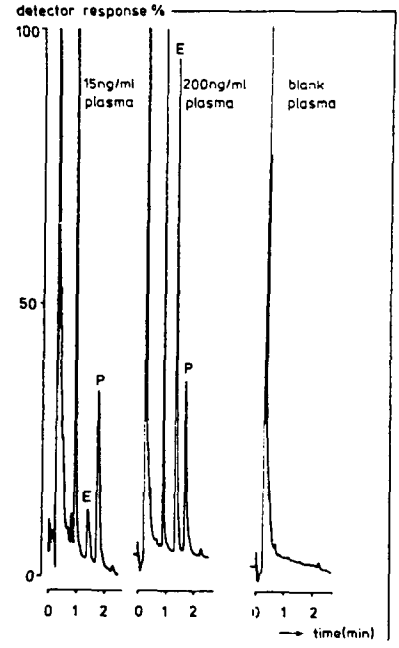


Fig. 8. Gas chromatogram of a plasma extract obtained from a male surgical patient immediately before (right), 8 min (centre; etomidate 200 ng/ml) and 240 min (left; etomidate 15 ng/ml) after receiving 17 mg etomidate intravenously. Peaks: E = etomidate; P = propoxate, I.S. (125 ng/ml). GC conditions: capillary column SCOT Carbowax 20M (10 m × 0.4 mm I.D.); CT: 170°C; IT: 200°C; DT: 300°C; carrier gas He 10 ml/min; solid injection; N-FID [104].

intravenous infusions GLC and HPLC assays are reported. Wynants et al. [101] reported a GLC assay of etomidate in human plasma after solvent extraction with hexane—diethyl ether (9:1), followed by back-extraction into 0.15 *M* sulphuric acid and re-extraction with methylene chloride. GLC with N-FID was performed on 3% OV-17 as the stationary phase at 200°C isothermal. The extraction recovery was approximately 74% and the detection limit 10 ng/ml (linear concentration range: 10 ng/ml to 10 μg/ml). The reproducibility was in the order of 5—10%.

Other methods described are slight modifications of this assay. They used single-step extraction and different amounts of the internal standard for low and high concentrations [102], or 3% Dexsil 300 as the liquid phase [103]. The detection limits were 30 or 5 ng/ml respectively. Propoxate [(+)-propyl-1-(1-phenylethyl)-1H-imidazole-5-carboxylate] was used as internal standard in all these GLC assays.

The assay of etomidate in plasma by capillary GC with nitrogen-selective detection by De Boer et al. [104] after a short extraction procedure (15 sec) with pentane permits the accurate and specific determination of underivatized etomidate and propoxate (I.S.) in relatively low concentrations. The detection limit lies at about 5 ng/ml, and the use of the 10-m support-coated open tubular (SCOT) Carbowax 20 M column allows chromatography within 2 min (Fig. 8). Solid injection is required in order to prevent deterioration of the column support caused by the organic solvents.

Mass fragmentographic determination of plasma etomidate concentrations is described by Van Hamme et al. [105] after single anaesthetic induction doses of 0.3 mg/kg intravenously for ear and eye surgery. GLC is performed on a glass column packed with Supelcoport (100—120 mesh) with 5% OV-17 at 230°C isothermal. At concentrations between 1 and 250 ng/ml plasma 4-ml samples were necessary which are extracted in a time-consuming procedure as a modification of that suggested by Wynants et al. [101]. Propoxate served as internal standard. The *m/z* 105 ion was monitored for the detection and quantitation of the drug and the internal standard. The practical limit of the assay was 1 ng/ml of plasma with a C.V. of 2.4%; the analytical recovery was 72 ± 3%. Using this very sensitive assay terminal phase plasma concentrations for more than 10 h after injection of a single dose can be detected [106].

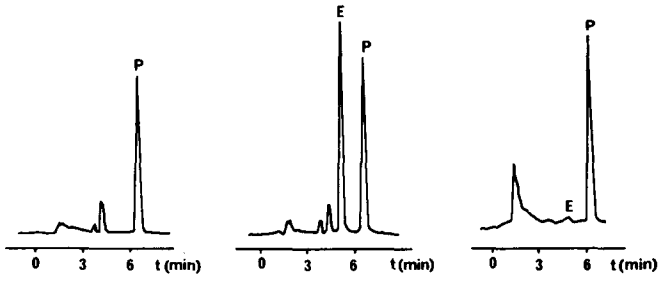
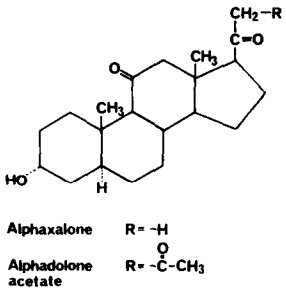


Fig. 9. Liquid chromatogram of a plasma extract before (left), 6 min after a bolus dose of 0.3 mg/kg followed by an infusion rate of 25 μg/kg/min etomidate (middle; etomidate 306 ng/ml) and 22 h after stopping the infusion (right; etomidate 0.4 ng/ml; 4 ml plasma extracted). Peaks: E = etomidate; P = propoxate, I.S. (800 ng/ml). HPLC conditions: column 25 cm × 4.6 mm I.D. octyl (C_8) 5 μm; mobile phase: acetonitrile—methanol—water (35:32.5:32.5); flow-rate 1.2 ml/min; UV detection wavelength 248 nm [107].

Monitoring plasma levels of etomidate by HLPC [107, 108] after a simple extraction technique and retention times for etomidate and propoxate (I.S.) of approximately 5 and 7 min is also suitable for routine measurement with high sensitivity (detection limit 8 ng/ml) and selectivity. Reversed-phase HPLC was performed on a C_8 column with a mixture of acetonitrile—methanol—water (35:32.5:32.5). The UV detection wavelength was 248 nm. The C.V. of this method was 2.8% at 500 ng/ml and 8.3% at 50 ng/ml (Fig. 9). The addition of potassium fluoride immediately after blood sampling to inhibit plasma esterase activity [105—107] is not necessary, because hydrolysis does not occur in human plasma unlike in rat plasma.

3.2.4. Althesin

Althesin is a mixture of the two pregnanedione steroids alphaxalone (3α-hydroxy-5α-pregnane-11,20-dione) and alphadolone acetate (21-acetoxy-3α-hydroxy-5-α-pregnane-11,20-dione) (Fig. 10) which have no hormonal activity. A non-ionic surface-active agent Cremopher EL (a poly-conjugated caster oil) was used to dissolve these compounds in a biologically acceptable medium (20%), in isotonic sodium chloride solution.



Alphaxalone R= -H

Alphadolone acetate R= -C-CH3 (O)

Fig. 10. Chemical structures of the steroids alphaxalone and alphadolone acetate.

Alphaxalone is the major and active constituent of this steroid anaesthetic agent [109]. It is rapidly metabolized in the body, and thus there is only little hang-over. There is some evidence suggesting the possibility of active metabolites and prolonged recovery times in patients with severe liver disease [110]. Only recently althesin was withdrawn as an intravenous hypnotic for sedation in intensive-care units in Britain due to anaphylactoid reactions to the solvent [100].

Sear and Prys-Roberts [111] measured plasma concentrations of alphaxalone during continuous infusion of althesin as a supplement to nitrous oxide—oxygen anaesthesia by GLC after trimethylsilylation. This publication was followed by a report [112] in which various GLC methods for the determination of this steroid in plasma are compared with each other. After alkaline extraction with light petroleum (80—100°C) and derivatization with either bis-trimethylsilyl acetamide in pyridine or methoxyamine hydrochloride in pyridine to form O-methoxime derivatives with or without further acetylation of the 3-OH function with acetic anhydride—pyridine, chromatography was performed on either OV-1, OV-17, QF 1 or Silar 10C (2.13-m columns) coated on to acid-washed, dimethyldichlorosilane-treated Celite 545 (80—100 mesh).

The internal standard used was either β-alphaxalone or the analogous Δ^{16}-unsaturated pregnene. FID and N-FID were compared with each other and the purity of the eluting compounds was checked by MS. Table 5 shows the relative retention times of alphaxalone, alphadolone acetate and related steroids in the different GLC systems. As the final result of this work, chromatography on OV-17 with N-FID after the formation of 20-O-methoxime-3-

TABLE 5

RELATIVE RETENTION TIMES OF ALPHAXALONE, ALPHADOLONE ACETATE AND RELATED STEROIDS IN SEVERAL DIFFERENT GLC SYSTEMS [112]

	Retention time relative to 5α-cholestane				
	OV-17 280°C	QF1 260°C	Silar 10C		OV-1 240°C
			240°C	260°C	
Pregnenolone					
Free	1.18	2.32		4.16	0.67
3-Trimethylsilyl ether	1.00	1.59	1.28		0.80
20-OMO*	1.44	1.42		2.00	0.80
20-OMO-3-acetate	1.74	2.06		1.85	1.10
3α-Alphaxalone					
Free	1.81	4.76		12.16	0.90
3-Trimethylsilyl ether	1.18	2.88	2.28		0.89
20-OMO	2.18	3.00		4.77	1.14
20-OMO-3-acetate	2.41	4.24		3.85	1.57
3β-Alphaxalone					
Free	1.92	5.46		14.62	0.94
3-Trimethylsilyl ether	1.52	3.70	3.46		1.14
20-OMO	2.18	3.36		5.54	1.14
20-OMO-3-acetate	2.92	4.65		4.31	1.57
3β-Δ^{16}-Alphaxalone					
Free	1.88	5.53		10.12	0.90
3-Trimethylsilyl ether	1.54	3.67	2.85		1.18
20-OMO	1.88	3.00		3.89	1.11
20-OMO-3-acetate	2.38	4.14		2.89	1.56
Alphadolone-21-acetate					
Free	4.59	12.30		43.08	2.34
3-Trimethylsilyl ether	2.92	7.42	8.19		2.15
20-OMO	4.52	8.18		13.39	2.22
20-OMO-3-acetate	5.04	11.65		9.70	2.58
Cholesterol					
Free	2.11	2.38		2.47	1.84
3-Trimethylsilyl ether	1.74	1.65	2.09		2.30
3-Acetate	2.67	3.59	3.19		2.73
Approximate retention time for 5α-cholestane (min)	4.5	3.5	2.0	1.5	6.0

*OMO = O-methyloxime.

acetates was the method of choice (11-oxo groups are sterically hindered and do not react). It allows detection of alphaxalone in plasma down to 5—10 ng/ml with a C.V. of 6.9% at 1 μg/ml (n = 10).

GLC—ECD on a 2-m column packed with 5% SE-30 coated Gas-Chrom Q was used by Frank et al. [113] for the estimation of alphaxalone plasma concentrations after total intravenous anaesthesia with repeated bolus doses of althesin. The steroid was extracted from plasma with diethyl ether, and a heptafluorobutyrate derivative formed to facilitate separation from endogenous compounds and quantitation by ECD. 3β-Alphaxalone was used as the internal standard.

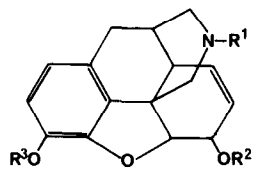
3.2.5. Opioid analgesics and neurolept—opioid combinations

Morphine, fentanyl, the new opiate analgesics, alfentanil and sufentanil, and other analgesics are frequently employed as supplements during anaesthesia with inhalational or intravenous agents. They are widely used to provide relief from pain during general anaesthesia of all types. For example the morphine—nitrous oxide technique has been utilized frequently for cardiac surgery.

When a potent narcotic analgesic such as fentanyl citrate is combined with droperidol, a neuroleptic compound, a state of neurolept analgesia is established, during which a variety of diagnostic and minor surgical procedures can be accomplished. Neurolept analgesia can be converted to neurolept anaesthesia by the concurrent administration of 65% nitrous oxide in oxygen.

3.2.5.1. Morphine. Assays with sufficient sensitivity to determine therapeutic concentrations of morphine (Fig. 11) (at ng/ml levels) in biological fluids used RIA [114], spectrofluorimetric [115, 116], HPLC [117—121], GLC [122—126], and TLC techniques [127].

RIA techniques for morphine are exquisitely sensitive (50 pg/ml of plasma),



R^1	R^2	R^3	
$-CH_3$	–H	–H	Morphine
$-CH_3$	–H	$-CH_3$	Codeine
–H	–H	–H	Normorphine
$-CH_2-CH=CH_2$	–H	–H	Nalorphine

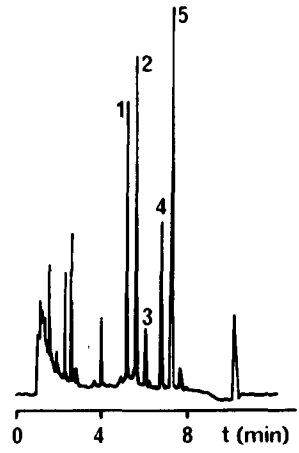


Fig. 11. Chemical structures of morphine and related opioids.

Fig. 12. Gas chromatogram obtained from a 1-ml serum standard containing 62 ng of morphine (1), 546 ng of codeine (2), 76 ng of normorphine (3), 189 ng of acetylmorphine (4) and 100 ng of nalorphine (5). GC conditions: capillary column SCOT OV-1 (25 m × 0.36 mm I.D.); CT: 220°C; IT: 250°C; DT: 300°C; carrier gas He 35 cm/sec; injection by falling needle; ECD [126].

but suffer from a potential lack of selectivity by measuring also metabolites and analogues. The major metabolite of morphine, morphine-3-glucuronide, has approximately 10% of the potency of the parent drug in displacing radiolabelled ligand from the antibody binding site. Fluorimetric methods are of high sensitivity, but lack specificity and have a high interference background.

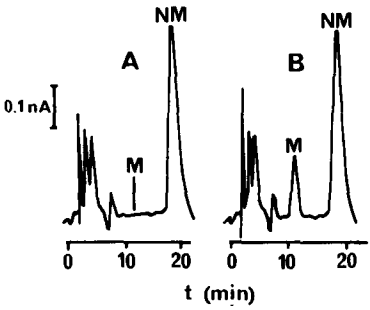
Chromatographic separation by TLC after radioisotopic derivatization with [^{3}H]Dns chloride to quantify morphine in biological fluids is reported by Garrett and Gurkan [127]. The spots on the plate (identified under UV light at 365 nm) were scraped off and the eluted radioactivity was determined by liquid scintillation. The standard deviations of this morphine assay were ± 18.6 ng/ml in 100 μl of plasma and ± 1.86 ng/ml in 1 ml of plasma. In addition, the TLC assay was compared with GLC—ECD analysis of pentafluoropropionated morphine in the range 0—5 ng of morphine per ml of plasma with a standard deviation of ± 0.46 ng/ml when 1 ml of plasma was taken. There were no significant differences among the two assays.

GLC assays with detection limits low enough for pharmacokinetic investigations, where only a few ng/ml are to be detected, are only possible with mass fragmentography [124, 125] or with ECD [122, 123, 126, 127]. In order to reduce the polarity of morphine and related compounds and to increase the sensitivity for ECD, acylation with fluorinated anhydrides and N-heptafluorobutyrylimidazole has been used.

A GLC—ECD assay for the determination of opiates (morphine) in biological samples after derivatization with pentafluoropropionic anhydride and separation on a 25 m × 0.36 mm I.D. glass capillary column coated with OV-1 is described by Edlund [126]. The samples (1 ml of plasma) were buffered to pH 9 and extracted on silica columns, cleaned by extraction and finally derivatized. A solid injector with a falling glass needle was used due to its inertness and the possibility of injecting large fractions of the total sample. Nalorphine was used as the internal standard for the simultaneous determination of morphine, codeine, normorphine and 6-acetylmorphine at a column temperature of 220°C (Fig. 12). Separation, precision and sensitivity of the assay was excellent (detection limit of morphine is about 1 ng/ml).

HPLC methods require no derivatization step for either the chromatographic separation or the detection of morphine. However, methods utilizing UV detection are not sensitive enough. Alternatively HPLC methods with (amperometric) electrochemical detection (HPLC—ED) increase the sensitivity for morphine by 100-fold over UV detection techniques. By this method morphine was quantified amperometrically by the electrochemical oxidation of the phenolic hydroxyl groups [120]. After time-consuming extraction techniques and reversed-phase chromatography on C_{18} with either methanol—0.01 *M* potassium dihydrogen phosphate buffer (85:15) and nalorphine as internal standard [119] or methanol—water—ammonium hydroxide (50:50:0.1) and normorphine as internal standard [121], these analyses gave linear, quantitative results covering a wide range of morphine concentrations (1 to about 400 ng/ml plasma) with acceptable C.V. values. The method described by Owen and Sitar [121] used a somewhat simpler extraction technique which makes it more suitable for routine measurements. In addition, the HPLC—ED assay was compared with GLC—ECD, which was a modification of an earlier described

method by Dahlstrom et al. [122]. In GLC N-ethylnormorphine was used as the internal standard. The HPLC—ED analysis of morphine presents several advantages over GLC—ECD, because HPLC—ED combines increased sensitivity for morphine quantitation with a simplified extraction procedure of smaller (0.5 ml) samples and the absence of a derivatization process. Typical HPLC—ED profiles obtained from a blank and a spiked plasma sample are shown in Fig. 13.



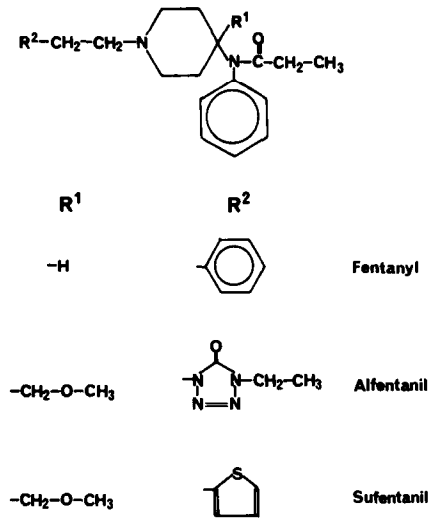


Fig. 13. Representative chromatograms for the elution of morphine (M) and normorphine (NM) by HPLC with electrochemical detection of a blank (A) and spiked (B; 15 ng of morphine) 0.5 ml plasma extract. Normorphine = internal standard = 200 ng. HPLC conditions: column 25 cm × 4 mm I.D. C_{18} 10 μm Bondapak; mobile phase: methanol—water—ammonium hydroxide (50:50:0.1); flow-rate 1.3 ml/min; glassy carbon working electrode +0.65 V, Ag/AgCl reference electrode [121].

Fig. 14. Chemical structures of fentanyl, alfentanil and sufentanil.

3.2.5.2. Fentanyl, alfentanil, sufentanil. Fentanyl, N-phenyl-N-[1-(2-phenylethyl)-4-piperidinyl] propionamide, is a very potent and fast-acting narcotic analgesic. This agent is combined with the neuroleptic drug droperidol or is administered as an adjunct to nitrous oxide for the induction and maintenance of anaesthesia in man. Due to its efficacy at low dosages, analytical methods for its quantification in biological fluids at therapeutic concentrations have to be very sensitive.

Alfentanil, N-phenyl-N-{1-[2-(4-ethyl-4,5-dihydro-5-oxo-1H-tetrazol-1-yl)-ethyl]-4-(methoxymethyl)-4-piperidinyl} propionamide, and sufentanil, N-phenyl-N-{4-(methoxymethyl)-1-[2-(2-thienyl)ethyl]-4-piperdinyl} propionamide, are two novel narcotic analgesics, with structures similar to that of fentanyl (Fig. 14). Both compounds are currently under clinical investigation in anaesthesiology.

To the present day, either radioassays [128—130], RIA [131—142, 148] or GLC [143—149] have been used to detect and measure the small concentrations of these narcotic analgesics in plasma, urine and tissues of man. Radioassays, although very sensitive, are not suitable for the routine monitoring

of these drugs in patients because they require the use of radioactively labelled drugs. The RIA methods are also very sensitive and sufficiently selective, but they are expensive, and commercial antiserum is not always available. The methods to measure fentanyl, alfentanil and sufentanil concentrations by RIA are quite similar. Using antisera to these drugs obtained form rabbits after repeated injections of the drug hapten (carboxy derivatives of the respective drug conjugated to bovine serum albumin), plasma, cerebrospinal fluid and urine levels less than 50 pg/ml can be detected. The RIA technique was used to evaluate the basic pharmacokinetic data of these drugs and to investigate the disposition of fentanyl and its analogues in patients who received these opioids during the course of clinical anaesthesia.

Other sensitive and selective assays for the determination of these very potent drugs in biological fluids after low doses (analgesic doses: fentanyl 4—8 μg/kg, alfentanil 50—125 μg/kg, sufentanil 0.5—5 μg/kg) and extensive biotransformation utilized either GLC—N-FID or GLC—MS.

Gillespie et al. [143] reported a GLC assay of fentanyl and its analogues with nitrogen-specific detection after chromatography on 3% OV-17. After extraction of the alkaline plasma sample (2 ml) with a mixture of hexane—ethanol (95:5), acidic back-extraction of the compounds of interest and final extraction at pH 13, plasma concentrations in the linear range from 0.25 to 64 ng/ml could be measured. Sensitivity was reported as 0.1 ng/ml fentanyl in plasma. The *p*-methoxyphenethyl derivative of fentanyl served as the internal standard. In the case of alfentanil the retention times (t_R) — at the isothermal conditions of 280°C — of this drug and the internal standard were quite similar [reported t_R (min): fentanyl 4.00; sufentanil 4.75; alfentanil 7.87; I.S. 7.95]. The analytical recovery was greater than 80% and the average C.V. values were less than 5%. Making use of rather similar GLC conditions, with additional FID, Phipps et al. [148] compared three methods (RIA, GLC—FID and GLC—N-FID) for the measurement of plasma fentanyl concentrations. Extraction before GLC assays was carried out from alkaline samples with benzene without acidic back-extraction with alfentanil as internal standard. Over the encountered range (0.2—100 ng/ml) there was no statistically significant difference between RIA and GLC using N-FID. Using the FID, GLC was less accurate than the other methods, particularly at low concentrations. In addition and in contrast to the RIA method, other analgesics which are chemically similar to fentanyl, may also be measured by the GLC—N-FID combination. Scepticism, however, concerning the measurement of such low concentrations (0.1 ng/ml [143] and 0.2 ng/ml [148]) of the drugs in practice by GLC—N-FID is certainly appropriate.

Van Rooy et al. [145] described the analysis of fentanyl and its metabolites in plasma of patients after the administration of high single doses (up to 60 μg/kg body weight), using GLC—N-FID on 3% OV-17 with a calculated detection limit of 3.3 ng/ml fentanyl in plasma. After repeated extractions with benzene from alkalinized plasma samples the metabolites were derivatized with acetic anhydride—pyridine (50:1) at 75°C for 2 h and identified by MS after chromatography on a capillary SCOT Carbowax 20 M 10-m column, namely as 1-(2-phenethyl)-4-N-anilinopiperidine (I) and 4-N-(N-propionyl-anilino)-piperidine (II). For mass fragmentography three *m/z* values were chosen: 245

for fentanyl, 231 for the acetylated derivative of I, and 231 and 274 for the derivative of II. Papaverine was used as internal standard.

The determination of alfentanil and sufentanil concentrations in plasma, urine and tissue samples by GLC—N-FID on OV-17 is reported by Woestenborghs et al. [146]. After the extraction of the alkaline samples with a mixture of hexane—isoamyl alcohol (98.5:1.5), acidic back-extraction into 0.05 *M* sulphuric acid and re-extraction at alkaline pH with the extraction mixture (in the case of tissue homogenate samples with an additional column extraction on Clin Elut^{M} prior to the re-extraction step) the residues after evaporation of the organic solvent mixture were reconstituted in 50 μl of methanol and 5 μl of this solution were injected into the gas chromatograph. At an isothermal column temperature of 290°C the retention times were 1.6, 2.7 and 3.6 min for sufentanil, alfentanil and the internal standard, respectively. The internal standard used was a research compound (R 38527), structurally analogous to alfentanil with a propyl bridge instead of the ethyl bridge between the heterocyclic ring systems of these compounds. The minimum detectable amount of alfentanil and sufentanil was 1 ng/ml of plasma and 2 ng/g of tissue. The recovery over the concentration range studied (1—1000 ng/ml or 2.5—500 ng/g) was 89 ± 4% (n = 6) and the precision was 3%. Using the same internal standard (R 38527), alfentanil and several deuterium-labelled analogues were measured quantitatively in plasma and urine by Gelijkens and Heykants [149] with combined capillary GLC—MS. The drugs and the internal standard were isolated from the biological material by an on-column extraction procedure on Bond Elut 1-ml cartridges. An aliquot of the methanolic extract was analysed by GLC—MS, using either EI (70 eV) or CI (isobutane). Calibration curves were constructed based on the peak area ratios of different ions monitored. A linear relationship was observed up to 10^3 ng/ml, while a detection limit of 0.5 ng/ml was attained. At the 10 ng/ml concentration level, a C.V. of 4% was observed.

GLC—CI (methane)-MS analysis of fentanyl in plasma [144] using stable-isotope-labelled fentanyl-d_3 as the internal standard after extraction into ethyl acetate, chromatography on 3% SE-30 and SIM at m/z 340.3 and m/z 337.3 for the internal standard and the drug resulted in a lowest measurable plasma fentanyl level of 0.5 ng/ml.

The application of HPLC—MS to the determination of fentanyl was described by Henion [150]. The CI micro-LC mass spectrum of fentanyl was obtained using micro-LC conditions of 40:60 acetonitrile—water at 8 μl/min on a 5 cm × 0.5 mm C_{18} ODS column. The abundant $(M + 1)^+$ ion at m/z 337 in combination with a fragment ion at m/z 150 allowed the specific SIM detection of trace levels of fentanyl.

3.2.5.3. Droperidol. Droperidol, 1{1-[4-*p*-fluorophenyl)-4-oxobutyl]-1,2,3,6-tetrahydro-4-pyridyl}-2-benzimidazolinone (Fig. 15), belongs to the butyrophenone class of drugs used in anaesthesiology and psychiatry. Droperidol is commonly used in conjunction with fentanyl in general anaesthesia and in the specific technique of neurolept anaesthesia.

Various methods of determining droperidol, such as colorimetry, spectrophotometry, fluorimetry and TLC, are only suitable for the determination of the drug in pharmaceutical preparations. An isocratic reversed-phase HPLC

method developed for the analysis of droperidol injection solutions by Doležalová [151] might be applied to the determination of this drug in biological samples. Optimum HPLC conditions found were the use of non-polar C_8 or C_{18} stationary phases and a mobile phase of methanol—0.02 *M* phosphate buffer, pH 6.8 (65:35) with a UV detection wavelength of 230 nm.

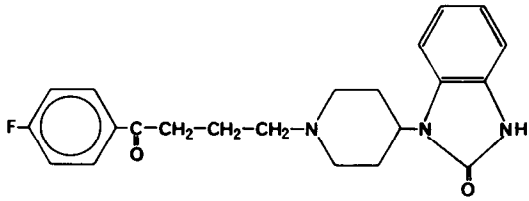


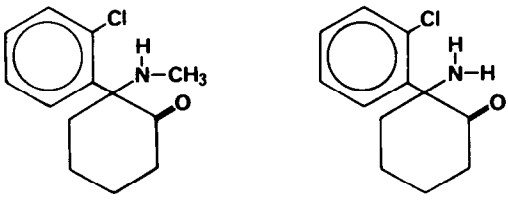
Fig. 15. Chemical structure of droperidol.

A GLC method for the analysis of butyrophenones (also droperidol) based on the Hofmann degradation reaction after quaternization of the pyrimidine nitrogen with methyl iodide and formation of the hydroxide with silver oxide in methanol is described by Rosenfeld et al. [152]. Analysis of the derivative of droperidol obtained by this reaction by GLC with FID on 3% OV-17 and identification of the eluting compounds characterized the degradation product suitable for quantification as the butyrophenone fragment. No underivatized droperidol was seen and each of the samples (n = 5) indicating total derivatization of the drug and completeness of the reaction.

Nevertheless, the major problem for the determination of droperidol concerning its instability, especially in very dilute solutions, remains. On the other hand, plasma concentrations of unchanged drug in man are lower than 100 ng/ml and decrease rapidly with a half-life of approximately 2 h. Therefore, therapeutic level monitoring or pharmacokinetic studies with the methods described above is hardly possible.

Only a RIA method developed by Hendriks et al. [153] proved suitable for the measurement of droperidol in the therapeutic concentration range. Using this method, the drug can be determined in amounts as low as 0.1 ng/ml. None of the known metabolites of droperidol interfered with this assay.

3.2.5.4. Ketamine. Ketamine, [2-(*o*-chlorophenyl)-2-(methylamino)] cyclohexanone (Fig. 16), is a member of the cyclohexylamine group of drugs which at therapeutic doses induces dissociative anaesthesia and is used as a pre-anaesthetic. The condition of dissociation from the environment as being experienced by the subject to whom such an agent is administered is similar to



Ketamine Norketamine

Fig. 16. Chemical structures of ketamine and its desmethylated metabolite, norketamine.

neurolept analgesia, but results from the administration of a single drug. Ketamine is a successor of the earlier drug phencyclidine — the first drug to be used for this purpose — but the frequent occurrence of unpleasant hallucinations and psychological problems after phencyclidine soon led to its abandonment. Ketamine is extensively metabolized in man, but of the metabolites norketamine is the only one with proven significant pharmacological activity.

GLC with either ECD [154—156], nitrogen-sensitive [157] or mass fragmentographic (158] detection is the method of choice for the determination of ketamine and its desmethylated metabolite in plasma and urine. All these methods use the 2-bromo analogue of ketamine as the internal standard. Extraction was carried out at alkaline pH of the samples with either chloroform [135], toluene [156], *n*-heptane [157] or benzene [158]. Ketamine and norketamine were analysed with [154—158] or without [155, 157] prior derivatization with heptafluorobutyric anhydride—pyridine or dimethylpyridine after chromatography on 3% OV-17 [154—158], Carbowax 20 M [157], or 2% SE-30 [157] at isothermal column temperatures of 180°C [157, 158] and 195°C [155, 156].

Concentrations of the drug and its metabolite down to 5 ng/ml are detectable with improved linearity of the N-FID assay up to 25 μg/ml. For urine the extract was purified on TLC before GC.

At abnormally high ketamine levels, for example in the case of intoxication or in vitro studies, FID techniques could also be utilized in the context of its speed (no derivatization necessary) [142].

A reported colorimetric assay [159], based on the formation of a methyl orange complex of ketamine in aqueous solutions at pH 4 is not sensitive (2—8 μg/ml) or selective (e.g. interference with atropine, chlorpromazine and chloroquine) enough to analyse therapeutic concentrations of ketamine.

4. LOCAL ANAESTHETIC AGENTS

Local anaesthetic drugs are weak bases, with a pK_a between 7.5 and 9.0, and cause loss of sensation without loss of consciousness. A wide variety of either amide-type (e.g. lidocaine, mepivacaine, etidocaine and bupivacaine) (Fig. 17) or ester-type (e.g. procaine, chloroprocaine, cocaine) drugs are used for this purpose [160]. Plasma or whole blood concentrations of local anaesthetics have been determined after various local and regional anaesthetic procedures (see review by Tucker and Mather [161]).

Among the common local anaesthetics used, lidocaine is probably the one that has been most closely examined, last but not least because of its additional use in the acute management of arrhythmias. Since in this application the margin of safety between the therapeutic (1—5 μg/ml) and toxic (> 5 μg/ml) plasma levels is small, a great number of assays have been reported dealing with the determination of concentrations of the drug and its active metabolites monoethylglycine xylidide (MEGX) and glycinexylidide (GX) in blood, urine and tissues.

Within the scope of this review it is impossible to summarize all publications on the field of the analysis of the various local anaesthetics or lidocaine alone. A summary of the GLC and GLC—MS assays for these agents is given by

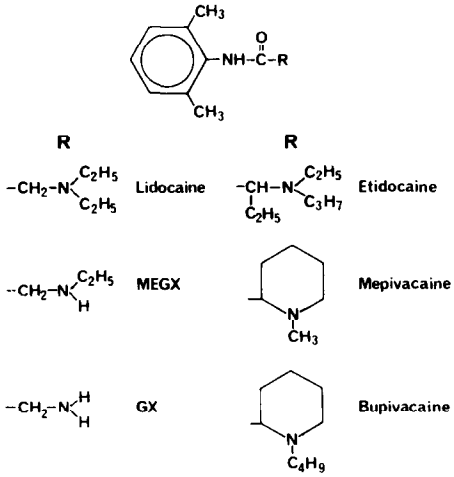


Fig. 17. Chemical structures of amide-type local anaesthetics.

Gudzinowicz and Gudzinowicz [125]. Here some recent studies on the quantitative analysis of lidocaine, its pharmacologically active metabolites MEGX and GX, and its structurally related compounds mepivacaine, etidocaine and bupivacaine — latter are becoming of increasing importance in spinal anaesthesia — are reported.

4.1. Lidocaine and structurally related amide-type local anaesthetics

4.1.1. Gas—liquid chromatography

GLC assays for lidocaine, its desethylated metabolites MEGX and GX, mepivacaine, etidocaine, bupivacaine and its desalkylated metabolite 2,6-pipecoloxylidine (PPX) on packed columns using N-FID [162—173], FID [174—187] and MS [164] are summarized, and details are given in Tables 6 and 7.

Assays measuring lidocaine in small plasma samples after a single extraction step in concentrations above 1 μg/ml are only suitable for therapeutic drug level monitoring to avoid toxic concentrations. In this case MEGX concentrations should also be determined, because this metabolite is about as pharmacologically active as the parent drug and is together with lidocaine at higher concentrations (> 6 μg/ml) responsible for the observed toxic side-effects. For pharmacokinetic studies only methods with practical sensitivities of less than 0.1 μg/ml are applicable.

The use of capillary columns in the GLC measurement of either lidocaine [188, 189] or bupivacaine [190, 191] and their desalkylated metabolites with selective detection by either mass fragmentography (SIM) [191] or N-FID [188—190] allows the determination of very low concentrations of these compounds in biological fluids. A high sensitivity is essential because plasma or blood levels after a local or regional anaesthetic procedure are usually very low, e.g. peak plasma levels after spinal anaesthesia with bupivacaine do not exceed 0.1 μg/ml.

Rosseel and Bogaert [188] reported an assay for lidocaine, MEGX and GX

TABLE 6

GLC OF LOCAL ANAESTHETICS ON PACKED GLASS COLUMNS WITH NITROGEN-SELECTIVE FLAME IONIZATION DETECTION

Agents	Sample	Conditions*	Solvent extraction	Sensitivity, linearity, precision and analytical recovery	Ref.
Lidocaine, MEGX, GX, I.S.: ethyl-methylglycine-xylidide	Plasma	3% SP-2250; CT: 200; IT: 310; DT: 275; He 30 ml/min	Alkaline (NaOH) with ethyl acetate	0.05 μg/ml each Within-run: at 3 μg/ml lidocaine C.V. 1.0%, GX C.V. 4.3%; MEGX C.V. 5.2% at 0.25 μg/ml lidocaine C.V. 2.6%, MEGX C.V. 5.9% GX C.V. 8.3% > 90% each	162
Lidocaine, (MEGX), I.S.: mepivacaine	Blood, serum, tissue	3% OV-101; CT: 200; IT: 225; DT: 300; He 30 ml/min	Alkaline (borate buffer pH 9.2) with toluene—hexane—isoamyl alcohol (78:20:2); for tissue homogenates with acidic back-extraction	0.1 μg/ml up to 13 μg/ml Within-day 4.6 μg/ml C.V. 2.9% Day-to-day 4.6 μg/ml C.V. 3.8%	163
Lidocaine, (MEGX), I.S.: SKF-525 2A	Post-mortem fluids and tissues	3% OV-1; CT: 223; IT: 250; DT: 300; He 30 ml/min Additional: MS, CT: 230; IT: 250; ST: 250; He 20 ml/min	15 ml samples 1. Alkaline ($NaHCO_3$) with diethyl ether 2. Alkaline (NaOH) 3. Neutral 4. Acidic back-extraction	0.1 μg/ml 1.5—100 μg/ml GC—MS C.V. < 6.5%	164
Lidocaine, MEGX, GX, I.S.: mepivacaine	Plasma	5% OV-101; CT: 190: IT: 310; DT: 270; He 30 ml/min	1. Alkaline (NaOH) with hexane—isoamyl alcohol (98:2) 2. Acidic back-extraction 3. Alkaline (carbonate—bicarbonate buffer (pH 9.8) with toluene—isoamyl alcohol (85:15)	2.5—500 ng/ml Within-day C.V. < 6.5% > 95%	165

Lidocaine, MEGX, I.S.: trimecaine	Plasma	2% Carbowax 20M—0.5% KOH; CT: 190; IT: 250; DT: 300; N_2 30 ml/min	Alkaline (borate buffer) with ethyl acetate	Lidocaine 0.3—75 μg/ml, MEGX 0.3—25 μg/ml C.V. < 8% Lidocaine 95% MEGX 99%	166
Lidocaine I.S.: 6′-chloro-α-methyl-1-pyrolidineaceto-o-toluidine	Plasma	3% OV-1; CT: 200; IT: 300; DT: 350; N_2 40 ml/min	100 μl alkaline (NaOH) with toluene	0.5—20 μg/ml C.V. < 7.5% 97.8% ± 7%	168
Lidocaine, I.S.: mepivacaine or ethylmethyl-glycinexylidide	Serum	3% OV-17; CT: 200; IT: 260; DT: 265; He 30 ml/min	Alkaline (NaOH) with benzene	0.1—9 μg/ml Within-run C.V. < 3% Between-run Lidocaine 98.6 ± 8.8% Mepivacaine 96.4 ± 7.3%	169, 170
Lidocaine, mepivacaine, bupivacaine, editocaine (tetracaine)	Plasma, cerebrospinal fluid, blood	3% OV-17; CT: 200; IT: 300; DT: 350; or 10% OV-1; CT: 250; IT: 300; DT: 350; He 30 ml/min	1. Extraction with diethyl ether 2. Acidic back-extraction 3. Alkaline (NaOH) with diethyl ether	2.5 ng/ml 20—500 ng/ml	171
Bupivacaine, 2,5-pipecoloxylidide, I.S.: lidocaine	Serum	3% OV-17; CT: 180—240 (8 min) at 20°C/min; IT: 260; DT: 260; N_2 16 ml/min	100 μl alkaline (Na_2CO_3 pH 11) with diethyl ether	5 ng/ml 0.1—1.6 μg/ml C.V. < 1% bupivacaine ~10% metabolite	172
Etidocaine	Plasma	3% SE-30; CT: 220; N_2 30 ml/min	1. Alkaline (Na_2HPO_4) with toluene—heptane—isoamyl alcohol (78.7:19.7:1.6) 2. Acidic back extraction 3. Alkaline (borate buffer pH 9.5)	50 ng/ml 0.1—50 μg/ml	173

*Stationary phase; CT = column temperature; IT = injection port temperature; DT = detector temperature; ST = separator temperature (°C); carrier gas flow.

TABLE 7

GLC OF LOCAL ANAESTHETICS ON PACKED GLASS COLUMNS WITH FLAME IONIZATION DETECTION

Agents	Sample	Conditions*	Solvent extraction	Sensitivity, linearity, precision and analytical recovery	Ref.
Lidocaine, I.S.: ethyl-methylglycine xylidide	Plasma	3% SP-2550; CT: 180: DT: 200; N_2 40 ml/min	Alkaline (NaOH) with dichloromethane	0.1 μg/ml C.V. 13%	174
Lidocaine, I.S.: mepivacaine	Plasma	3% OV-17; CT: 130 (1 min) to 260 (4 min) at 24/min IT: 200: DT: 280; N_2 45 ml/min	Alkaline (NaOH) with carbondisulphide after protein precipitation	0.04 μg/ml up to 8.0 μg/ml	175
Lidocaine, (tocainide), I.S.: *n*-eicosane (C_{20})	Plasma, serum	3% OV-101; CT: 230: DT: 250; N_2 40 ml/min	100 μl: extraction in Tris buffer with chloroform	0.2 μg/ml 0.2—10 μg/ml 2—10 μg/ml: C.V. < 5.2% 99.7 ± 7.3%	177, 178
Lidocaine, mepivacaine, bupivacaine, etidocaine, propitocaine	Blood, plasma	3% OV-17; CT: 250; IT: 270; N_2 40 ml/min	1. Alkaline (NaOH) with dichloroethylene 2. Acidic back-extraction 3. Alkaline (NaOH pH 10—11) with dichloro-methane	0.1—20 μg/ml at 0.5 μg/ml C.V. < 4.5% each lido. 92.6%, mepi. 91.1%, bupi. 71.1%, etido, 78.4%, propito. 95.4%	180
Lidocaine, I.S.: mepivacaine	Tissue	3% OV-17; CT: 235; IT: 285; N_2 68 ml/min	After repeated protein precipitation steps alkaline (NaOH) with chloroform	0.5—200 μg/g C.V. < 1% brain 98.6%, liver 99.8% muscle 89.1%	181
Bupivacaine, I.S.: cypro-heptadin	Plasma, cerebro-spinal fluid	3% OV-17; CT: 230 to 280 at 2° C/min; IT: 280; DT: 280; N_2 18 ml/min	1. Alkaline (NaOH) with diethyl ether 2. Acidic back-extraction 3. Alkaline (NaOH) with diethyl ether		182

Lidocaine, MEGX, GX, etidocaine, bupivacaine, I.S.: mepivacaine	Plasma	3% SE-30 or 3% OV-17; CT: 110; CT: 210; IT: 220—230	Alkaline (NaOH) with dichloromethane after protein precipitation	0.05 μg/ml 0.1—20 μg/ml average C.V.: 10.9%	183
Lidocaine, I.S.: etidocaine	Plasma	3% Carbowax 20M—5% KOH; CT: 165; IT: 250; DT: 250; N_2 27 ml/min	Alkaline (phosphate buffer pH 12) with chloroform	20 ng/ml 1—12 μg/ml C.V. < 5%	184
Lidocaine, bupivacaine I.S.: cyclizine	Plasma	5% OV-17; CT: 190 (6 min) to 250 at 8° C/min; IT: 250; DT: 265; N_2 23 ml/min	1. Alkaline (NaOH) with diethyl ether 2. Acidic back-extraction 3. Alkaline (NaOH) with dichloromethane	0.1 μg/ml 0.06—1 μg/ml 0.6—10 μg/ml Within-day: at 1.0 μg/ml lido. C.V. 3.9% bupi. C.V. 3.1% at 2.5 μg/ml lido. C.V. 5.2 % bupi. C.V. 3.7% lido. 90.8% bupi. 98.0% cyclizine 85.2%	185
Lidocaine, I.S.: mepivacaine	Plasma	3% OV-17; CT: 190: IT: 250; DT: 250; N_2 30 ml/min	Alkaline (NaOH) with dichloromethane	0.1 μg/ml (0.5 μg/ml) up to 50 μg/ml Within-day: at 5 μg/ml C.V. 1.8% at 10 μg/ml C.V. 1.9%	186
Lidocaine, I.S.: mepivacaine	Serum	2.5% SE-30; CT: 170 to 250 at 15° C/min; N_2 30 ml/min	1. Alkaline (NH_4Cl—NaOH buffer pH 9.3) with diethyl ether 2. Acidic back-extraction 3. Alkaline (NH_4Cl—NaOH buffer pH 9.3) with chloroform	0.3—11 μg/ml at 7.5 μg/ml Within-assay: C.V. 3.8% Between-assay: C.V. 5.4%	187

*Stationary phase; CT = column temperature; IT = injection port temperature; DT = detector temperature (° C); carrier gas flow.

in plasma after pre-extraction of the acidified sample with methylene chloride, followed by an alkaline extraction of the remaining aqueous phase with the same solvent. Trifluoroacetylation of the extract with trifluoroacetic anhydride in hexane resulted in derivatization of the metabolites to prevent peak tailing of these on the chromatographic capillary (20 m × 0.5 mm) glass column, wall coated with OV-17. All glassware was silanized prior to its use. GLC separation (trimecaine as internal standard) was performed within 8 min.

De Boer et al. [189] described lidocaine analysis in plasma utilizing a capillary 10-m SCOT column coated with Carbowax 20 M—potassium hydroxide (column temperature: 160°C), a solid injection system and nitrogen-selective detection after extraction of the alkaline plasma sample with pentane. N-Methylhexobarbital was used as the internal standard. This assay technique also proves quite suitable to quantify bupivacaine in plasma [190] (I.S.: 1-pentyl-2,6-pipecoloxylidide; column temperature: 190°C). Linear concentration ranges tested were 3—100 ng/ml and 0.025—7 μg/ml. The C.V. reflecting both within-day and day-to-day variations, was 10% at a bupivacaine concentration of 3 ng/ml and did not exceed 6% at higher concentrations. The minimum detectable concentration was 1 ng/ml. The recovery of bupivacaine in the range 0.025—1.6 μg/ml was 73%.

We utilize this assay technique in our laboratory with minor modification to determine lidocaine, MEGX and GX in plasma and urine of patients to whom lidocaine was administered as cardiac antiarrhythmic agent. Modifications made are: lidocaine and its metabolites are extracted into chloroform; mepivacaine or bupivacaine are used as internal standards; the use of a fused-silica capillary column (15 or 7.5 m × 0.32 mm I.D.) with the chemically bonded phase DX 4 enables the direct injection of small volumes (1 μl) of a methanolic solution of the extract and therefore the use of an automatic sampler; column temperature programmed instead of isothermal conditions.

Chemically bonded phases are rather stable and solid injection to prevent the stripping-off of the stationary phase by the solvent is not necessary. This assay can also be applied to the analysis of other local anaesthetics and their metabolites after local or regional anaesthetic procedures. Chromatograms and conditions of the analysis of lidocaine and its desethylated metabolites in plasma and urine are shown in Figs. 18 and 19.

For studying the disposition of bupivacaine in mother, foetus and neonate following epidural anaesthesia for cesarean section, Kuhnert et al. [191] used an SIM technique after chromatographic separation on a short (1 m) column packed with 3% OV-17 on Gas-Chrom Q. Patients' plasma and urine samples (0.2—1 ml) were extracted using a modification described by Lesko et al. [172]. The modifications were: use of N-ethyl-N-*sec.*-butylglycinexylidide (W 12174), a lidocaine derivative, as the internal standard; saturation of the sodium carbonate solution with sodium chloride; use of benzene for the final solvent. Instrumental conditions were: carrier gas flow (He) 20 ml/min; oven temperature programmed from 200°C to 244°C at 16°C/min. The entire run time was 3.5 min. The ion intensities at *m/z* 84 for the desbutylated metabolite PPX, *m/z* 114 for the internal standard and *m/z* 140 for bupivacaine were monitored. Standard curves ranged from 300 to 2000 ng/ml for urine and from 4 to 1000 ng/ml for plasma and were linear. The relative standard deviation

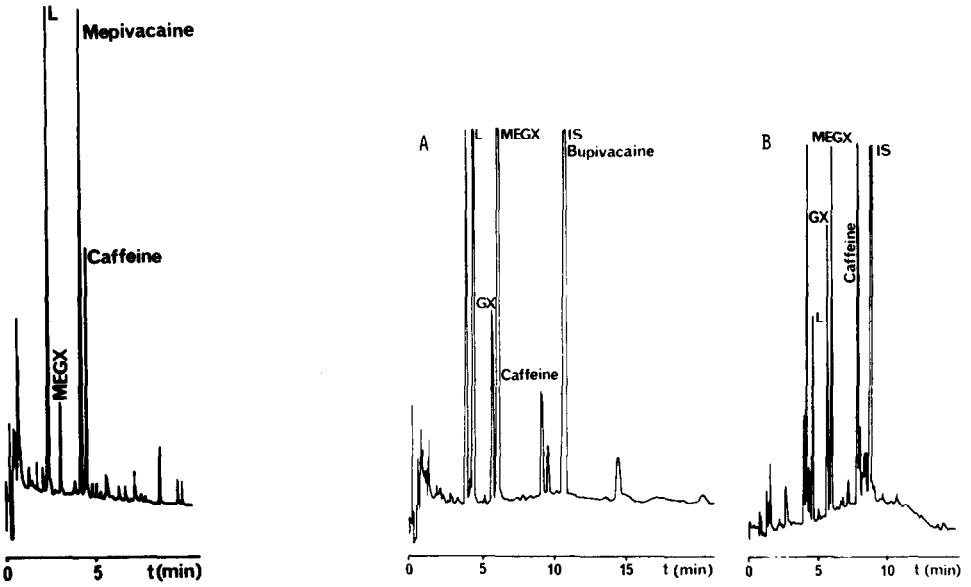


Fig. 18. Gas chromatogram of an extract of 1 ml of plasma of a patient who received a lidocaine infusion 2 g per day. Results: lidocaine (L) = 1.82 μg/ml; MEGX = 0.25 μg/ml; caffeine = 0.14 μg/ml; mepivacaine, internal standard = 5 μg/ml. GC conditions: capillary column fused-silica DX 4 (7.5 m × 0.32 mm I.D.); CT: 130° C (1 min) to 250° C at 12° C/min; IT: 250° C; DT: 300° C; carrier gas He 3 ml/min; direct injection 1 μl; N-FID.

Fig. 19. Gas chromatograms obtained from 0.5-ml urine extracts of patients receiving lidocaine infusion 2 g per day for antiarrhythmic therapy on different capillary columns. (A) Results: lidocaine (L) = 14.6 μg/ml; MEGX 19.7 μg/ml; GX = 4.5 μg/ml; (bupivacaine, internal standard (IS) = 8.2 μg/ml. GC conditions: capillary column SCOT carbowax 20M (10 m × 0.4 mm I.D.); CT: 190° C to 220° C at 3° C/min; IT: 250° C; DT: 300° C; carrier gas He 10 ml/min; solid injection; N-FID. (B) Results: L = 0.6 μg/ml; MEGX = 1.2 μg/ml; GX = 2.8 μg/ml, caffeine = 1.4 μg/ml; IS (bupivacaine) = 8.2 μg/ml. GC conditions: capillary column fused-silica DX 4 (15 m × 0.32 mm I.D.); CT: 160° C (1 min) to 237° C (3 min) at 7° C/min; IT: 250° C, DT: 300° C; carrier gas He 5 ml/min; direct injection (1 μl); N-FID.

(C.V.) for bupivacaine was 6.2% (15 ng) and 2.4% (500 ng) and for PPX 10.6% (15 ng) and 5.4% (500 ng).

4.1.2. High-performance liquid chromatography

Reversed-phase HPLC assays for lidocaine, besides other antiarrhythmic drugs [192, 193] and with simultaneous determination of the metabolites [194—196] in plasma or serum used UV detection at 205 nm [192—194], 200 nm [195] and 195 nm [196] after alkaline extraction of the sample [192, 193, 195, 196] or direct injection with the precolumn technique [194]. Conditions and analytical data are summarized in Table 8.

4.1.3. Mass spectrometry

Nelson et al. [197] reported the quantification of suspected metabolites of lidocaine in humans after using the direct insertion probe and CI (isobutane)-MS. Deuterated analogues of the metabolites of lidocaine were added to serial human plasma and urine samples and were used as the internal standards.

TABLE 8

CONDITIONS AND ANALYTICAL DATA FOR HPLC ASSAY OF LIDOCAINE AND ITS DESETHYLATED METABOLITES

Agents	Sample	Sample preparation	Conditions*	Analytical data**	Ref.
Lidocaine, (procainamide), I.S.: procainide	Serum	Alkaline (NH_4OH) + charcoal extraction of the charcoal with dichloromethane; after evaporation dissolved in methanol	(a) ODS Sil-X-I (b) 0.02 *M* phosphate buffer—acetonitrile (90:10) (c) UV 205 nm	0.1—20 μg/ml Day-to-day: 1 μg/ml C.V. 10.6% 20 μg/ml C.V. 5.6 % 1 μg/ml: 65 ± 3% 20 μg/ml: 63 ± 3%	192
Lidocaine, MEGX, GX	Plasma	Direct injection, 20 μl sample	(a) μBondapak C_{18} + precolumn Co: Pell ODS (b) 0.05 *M* phosphate buffer—acetonitrile (95:5) (c) UV 205 nm	1—10 μg/ml	194
Lidocaine, MEGX, GX I.S.: ethylmethylglycinexylidide	Plasma	0.5 ml alkaline (NaOH) with ethyl acetate; after evaporation dissolved in acidic aqueous phase	(a) μBondapak alkylphenyl (b) 0.006% phosphoric acid—acetonitrile (70:30) (c) UV 200 nm	20 ng/ml 0.1—10 μg/ml (non-linear) C.V. < 8%	195
Lidocaine, (disopyramide), (quinidine), I.S.: *p*-chlorodisopyramide	Serum	0.5 ml alkaline (NaOH) with dichloromethane; after evaporation dissolved in mobile phase	(a) μBondapak C_{18} (b) 0.03 *M* phosphate buffer—acetonitrile (72:28) pH 4.45 (c) UV 205 nm	1.0—5 μg/ml Within-day: C.V. 2.6% Day-to-day: C.V. 3.4%	193
Lidocaine, MEGX, GX, I.S.: ethylmethylglycinexylidide	Plasma	2 ml alkaline (NaOH) with dichloromethane; after evaporation dissolved in acidic aqueous phase	(a) RP-8 10 μm (b) 0.1 *M* phosphate buffer—acetonitrile (82:18) pH 3.2 (c) UV 195 nm	10 μg/ml Lido 0.1—5 μg/ml Metab. 0.04—2 μg/ml Day-to-day: C.V. < 7% Lido 94.3 ± 3.4%, MEGX 98.4 ± 4.1%, GX 77.2 ± 2.7%, mepi. 99.7 ± 2.1%	196

*(a) Stationary phase; (b) mobile phase; (c) detection wavelength.
**Detection limit; concentration range; precision; recovery.

The samples were extracted with either benzene (plasma) or methylene chloride (urine) at pH 8.5, and in the case of conjugates after enzymatic hydrolysis. The dried extracts were reconstituted in either ethanol (plasma) or methanolic hydrogen chloride (urine) and the CI mass spectra were determined at 120°C at an ion source chamber pressure of 0.5 Torr (66.7 Pa).

4.1.4. Enzyme-multiplied immunoassay technique

A commercially available homogeneous EMIT system for the determination of lidocaine in plasma was described by Walberg [198], and was compared to GLC techniques in its analytical capabilities [187, 198—200]. The EMIT assay used 50-μl aliquots of each sample mixed with EMIT reagent A which contains antibodies to lidocaine together with substrates for the enzyme glucose-6-phosphate dehydrogenase (G6PDH). Lidocaine in the plasma binds to this antibody. Then an enzyme-labelled lidocaine (G6PDH-labelled, EMIT) was added. The labelled lidocaine combines with any remaining unfilled antibody-binding sites and the enzyme activity is proportionally reduced. The residual enzymatic activity is directly related to the concentration of lidocaine present in the plasma. The unbound active enzyme converts NAD to NADH, resulting in an absorbance change. The enzyme activity was measured spectrophotometrically as the change in optical density at 340 nm. The EMIT assay is accurate and precise, requires only short preparation and analysis time and is extremely simple in its usage, but its intended range is lidocaine concentrations within the therapeutic window (1—12 μg/ml) as cardioactive drug. For pharmacological studies in which low levels of the drug may be encountered a minor modification in the procedure has to be made: samples < 1 μg/ml have to be measured without initial dilution, and thus the useful range of the technique to cover plasma concentrations of lidocaine between 50 and 2000 ng/ml [200] is extended.

Disadvantages in the use of this assay technique are the impossibility of measuring the active metabolite MEGX (which can produce significant toxic side-effects by accumulation in patients with renal failure) and possible cross-reactivity towards other structurally similar drugs (e.g. mepivacaine) [201].

5. CONCLUSIONS

Drug level monitoring of anaesthetic agents is helpful to obtain quantitative information about the absorption, distribution and elimination of these drugs, and the effect of disease states on these processes, as well as information concerning the correlation of efficacy and toxicity with their measured concentrations and their active metabolites in plasma and other biological material.

It is not a substitute for, but rather a supplement to, clinical monitoring and judgement. Additionally, in the case of the volatile anaesthetics, measurement of the ambient air contamination with these agents and, connected with the exposure of human subjects to them, may result in safer working conditions for the surgical staff.

The purpose of this review is to present a summary of analytical techniques suitable for the determination of the most popular anaesthetic drugs which are in practice currently.

Chromatographic methods like GLC or HPLC are superior to non-chromatographic assays like spectrophotometry, fluorimetry or colorimetry because of their selectivity. The very sensitive and mostly simple immunoassays, if available, are quite suitable for measuring low concentrations of these drugs if specificity is guaranteed.

The evaluation of newer column technologies for GLC (capillary columns with chemically bonded phases) and HPLC (reversed-phase packings with regular micrometre particles) in connection with the use of specific detection like N-FID or SIM-MS in GLC and electrochemical, specific ultraviolet, or mass spectrometric detection in HPLC, enables the analyst to perform quantitative analysis of the compounds of interest after relatively simple work-up prcedures with high sensitivity and selectivity.

6. ACKNOWLEDGEMENTS

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7. SUMMARY

A summary of chromatographic (e.g. gas chromatography, high-performance liquid chromatography) and non-chromatographic (e.g. radioimmunoassay, enzyme-multiplied immunoassay) analytical techniques suitable for the quantitative analysis of the most popular inhalational (halothane, methoxyflurane, enflurane, isoflurane and nitrous oxide), intravenous (barbiturate, benzodiazepines, etomidate, althesin, morphine, fentanyl, alfentanil, sufentanil, droperidol and ketamine) general and amide-type local (lidocaine, mepivacaine, etidocaine and bupivacaine) anaesthetic agents and some of their metabolites, in biological material, is described. In the case of inhalational anaesthetics attention is also payed to pollution measurement and breath-to-breath monitoring.

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