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Two-phase derivatization of pentazocine with chloroformate esters and determination by gas chromatography

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Abstract: Two-phase derivatization with chloroformate esters of tertiary amines that contain a phenol group has been studied. The derivatives were analysed by gas chromatography with sensitive and selective detectors. The N,O-bis derivative of pentazocine was formed using an aqueous phase at pH 9.8, with tetrabutylammonium ion for extraction of the phenolate ion as an ion-pair. The organic phase comprised methylene chloride with trichloroethyl chloroformate or ethyl chloroformate. At pH 10.7 the derivative was hydrolysed by hydroxide ions extracted into the organic phase. Hydrolysis also occurred when more lipophilic counter-ions such as tetrapentylammonium ions were used. Two-phase derivatization could also be achieved with toluene or ethyl acetate as the organic phase, although the reaction rate was considerably slower. The method was also applied to the assay of pentazocine in plasma. The best results were achieved after selective extraction of pentazocine from plasma, two-phase derivatization with ethyl chloroformate and determination by mass spectrometry or thermionic detection. The minimum detectable concentration of pentazocine was 10 ng/ml in a 1-ml plasma sample.

Keywords: *Pentazocine; gas chromatography; two-phase derivatization; phenolic tertiary amines.*

Introduction

Derivatization of tertiary amines with chloroformate esters produces carbamates with excellent gas chromatographic properties that are suitable for the analysis of low concentrations of the amines. By proper choice of the chloroformate ester, determination of the derivative can be performed with sensitive and selective methods of detection such as electron capture [1–3], thermionic techniques [4] or mass spectrometry (MS) [7]. The derivatization of tertiary amines was initially performed in an organic solvent at an elevated temperature [1]. Tertiary amines with an allylic or benzylic substituent could also be derivatized using a two-phase system after addition of iodide ion to the aqueous phase [2]. The choice of 2,4-dichlorophenyl- and pentafluorobenzyl

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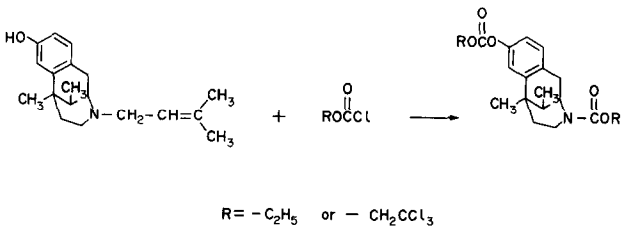
chloroformate as chloroformate esters has extended the application of the two-phase derivatization principle to N,N-dimethyl-substituted tertiary amines [5].

Phenolate ions form stable derivatives with chloroformate esters [6]. This reaction, which was most favourably performed in a two-phase system, was applied to the analysis of the analgesic drug ketobemidone and its N-demethylated metabolite in plasma [7]. This knowledge has been of value in the development of an analytical procedure for another aminophenol, the analgesic drug pentazocine. Previously pentazocine has been determined by electron capture gas chromatography, after selective extraction and derivatization by extractive alkylation with pentafluorobenzyl bromide [8]. Owing to the polar character of the underivatized tertiary amino group, severe adsorption losses were encountered during gas chromatography (GC). Priming of the column improved yield and precision, but the time-consuming work-up procedure limited the number of plasma samples which could be processed in one day.

Two-phase derivatization with chloroformate ester would therefore offer advantages by giving a N,O-bis derivative (Scheme 1) with good GC properties. The use of trichloroethyl chloroformate will result in a derivative that is detectable in low concentration by electron capture detection; reagents such as ethyl chloroformate will produce suitable derivatives for sensitive and selective detection with the thermionic detector or with MS and selective ion monitoring.



Scheme 1
Derivatization of pentazocine with chloroformate esters.

Experimental

Gas chromatography

A Pye GCV gas chromatograph with flame ionization, thermionic and electron capture detectors was used. The 1500 × 2-mm i.d. glass column was packed with 3% OV17 on Gas Chrom Q 100/120 mesh (Supelco Inc., Bellefonte, PA, USA). The column temperature was 280°C for the analysis of the trichloroethyl chloroformate derivative, and 250°C for the analysis of the ethyl chloroformate derivative of pentazocine. Injector and detector temperatures were 310°C. Flow rate of nitrogen carrier gas was 30 ml/min; flow rates of hydrogen and air were 30 ml/min and 300 ml/min, respectively.

Gas chromatography–mass spectrometry

After derivatization pentazocine was analysed in biological samples using a Finnigan model 4000 gas chromatograph–mass spectrometer, interfaced to a Finnigan model 6100 data system (Finnigan, Sunnyvale, CA, USA). The mass spectrometer was operated in the electron impact mode with an ionization energy of 40 eV. Samples were introduced into the gas chromatograph by the falling needle technique. The 12 m × 0.3 mm i.d. glass capillary column (Ultrasep®, OY Separation Research, Turku, Finland) contained SE 30 as the stationary phase and was operated at 250°C. During selected ion monitoring the

instrument was adjusted to monitor the ions m/z = 361 for the N,O-bis (ethoxycarbonyl) derivative of pentazocine and m/z = 321 for the N,O-bis (ethoxycarbonyl) derivative of the internal standard, norketobemidone.

Reagents and chemicals

Trichloroethyl chloroformate was purchased from EGA Chemie (Steinheim bei Heidenheim, FRG) and ethyl chloroformate was from Fluka AG (Buchs, Switzerland). Pentazocine was supplied by Sterling Winthrop (New York, USA), naloxone chloride by Endo Laboratories Inc. (Garden City, NY, USA) and morphine chloride from Apoklesbobget, Sweden. Norketobemidone was prepared as described by On-ishi and May [9]. N,N-Dimethylethylene diamine and the organic solvents were obtained from E. Merck AG (Darmstadt, FRG). The solvents were used without further purification except heptane, which was distilled.

Tetrabutylammonium ion solutions were prepared by neutralization of the hydrogen sulphate (Labkemi AB, Stockholm, Sweden) and dilution to volume with buffer. Tetrapentylammonium ion solutions were made from the iodide salt (Eastman-Kodak, Rochester, NY, USA) by shaking overnight with an equal amount of silver oxide in water, followed by filtration and dilution to volume with water. Phosphate or carbonate buffers (1.0 M) were used in the study of derivatization conditions, whereas a saturated solution of sodium hydrogen carbonate was used in the extraction of pentazocine from plasma samples. Silver sulphate was prepared as a saturated solution in water.

In the evaluation of reaction conditions dotriacontane and hexatriacontane were used as internal markers. In the assay of pentazocine in plasma, norketobemidone (2 μg/ml) was used as the internal standard.

Identity of derivatives

The following prominent peaks were observed in the mass spectra of the derivatives after reaction with chloroformate ester: N,O-Bis-(ethoxycarbonyl) derivative of pentazocine: m/z (percentage relative abundance) 88 (100), 102 (52), 117 (50), 173 (26), 361 (M^+; m/e = 6). N,O-bis(trichloroethoxycarbonyl) derivative of pentazocine: m/z (percentage relative abundance): 72 (75), 88 (99), 95 (71), 131 (78), 173 (100), 204 (60), 219 (47), 343 (10), 347 (10) and 567 (M^+; m/e = 2). The mass spectra of the N,O-bis(ethoxycarbonyl) derivative of norketobemidone have been described previously [7]. No mass spectra were obtained from the derivatization of norketobemidone with trichloroethyl chloroformate owing to the low volatility of the derivative in the gas chromatographic system.

Methods

Evaluation of reaction conditions. Pentazocine lactate, morphine chloride or naloxone chloride was dissolved in water to give a concentration of 1 mg/ml (0.003 M) and an equal volume of 0.1 M tetra-alkylammonium ion in phosphate buffers (at various pH values) was added. In some instances 0.05 M potassium iodide was present. This aqueous phase in a series of tubes was shaken with an equal volume of organic phase containing various concentrations of trichloroethyl chloroformate or ethyl chloroformate and an internal marker (0.3 mg/ml). At certain times, the reaction was stopped by addition of 0.1 M sulphuric acid and the derivative was determined by GC using flame ionization detection. The peak–height ratio of the derivative:internal standard was calculated. Since it was not possible to obtain the derivatives in crystalline form, the

yields are based upon the maximum yield obtained using trichloroethyl chloroformate and ethyl chloroformate respectively.

Determination of pentazocine in plasma. To a plasma sample (1.0–2.0 ml) that contained pentazocine was added 0.1 ml internal standard solution and saturated carbonate buffer to 5 ml. This aqueous phase was shaken for 15 min with 5 ml toluene–n-butanol (4:1 v/v). After centrifugation at 500 ***g*** for 10 min, the organic phase was transferred to another tube and extracted with 1 ml sulphuric acid (0.1 M). This aqueous phase was transferred to another tube and 1 ml phosphate buffer (pH 9.8) was added together with 1 ml 0.1 M tetrabutylammonium ion in 1.0 M phosphate buffer (pH 9.8) and 1 ml methylene chloride containing 1% of trichloroethyl chloroformate or 5% of ethyl chloroformate. This mixture was shaken for 45 min and after centrifugation the organic phase was transferred to a dry tube and evaporated under a stream of nitrogen. The residue was reconstituted in 100 μl toluene; the organic phase was washed with 1.0 ml saturated silver sulphate solution. The analysis was performed by GC with thermionic detection or GC–MS.

Analysis by electron capture gas chromatography could only be performed after removal of excess trichloroethyl chloroformate. After evaporation, the residue was dissolved in heptane and 2 μl N,N-dimethylethylene diamine was added. After 1 min the organic phase was washed with saturated silver sulphate solution. Thereafter, the organic phase was washed twice with 3.0 ml sulphuric acid (0.5 M) to remove the trichloroethyl carbamate and the excess of N,N-dimethylethylene diamine, and subsequently the organic phase was washed with 1.0 ml saturated silver sulphate solution. Two μl of the organic phase was injected into the gas chromatograph. Standard curves were prepared from the results of experiments in which known concentrations of pentazocine in blank plasma were treated according to the procedures described above.

Results and Discussion

Derivatization of pentazocine with trichloroethyl chloroformate

The phenolate ion readily reacts with chloroformate ester in buffered aqueous solution [6] and in a two-phase system [7]. Reaction in buffered aqueous solution did not give a quantitative yield of the N-O-bis(ethylcarbonyl) derivative of norketobemidone with ethyl chloroformate owing to rapid hydrolysis of reagent [6]. In a two-phase system, the phenolate ion was extracted as an ion-pair with tetrabutylammonium ion into the organic phase where the reaction took place. Quantitative formation of the N,O-bis(ethoxycarbonyl) derivative was achieved within a few minutes [7].

Effect of pH and tetraalkylammonium ion. Pentazocine forms the N,O-bis(trichloroethylcarbonyl) derivative with trichloroethyl chloroformate. The derivative is most rapidly formed in a two-phase system with methylene chloride as organic phase and with tetrabutylammonium ion as counter-ion in the aqueous phase at pH 9.8. Within 5 min, a yield of 67% of the bis derivative was obtained and the reaction was complete within 20 min (Fig. 1). Degradation of the derivative occurred where reaction times were longer than 2 h. Degradation is probably due to hydrolysis by hydroxyl ions extracted as an ion-pair into the organic phase. Even greater degradation was observed at a higher pH of the aqueous phase (Fig. 1). A more lipophilic counter-ion in the aqueous phase such as tetrapentylammonium ion also resulted in a low yield of derivative due to the same effect. At pH 7 more than 2 h was required for quantitative reaction (Fig. 1).

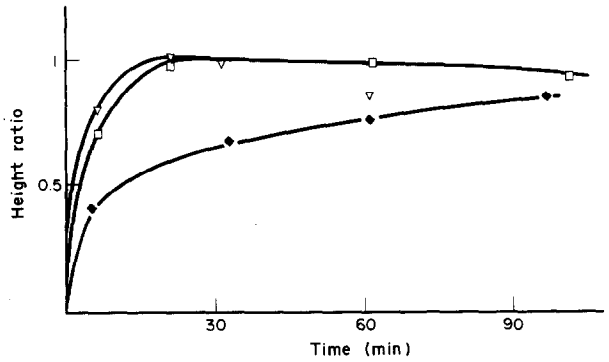


Figure 1
Influence of pH on the rate of formation of N,O-bis(trichloroethoxycarbonyl) derivative of pentazocine. Concentration of pentazocine: 0.003 M; organic phase: methylene chloride with 0.06 M trichloroethyl chloroformate, 1 ml; aqueous phase: 0.05 M tetrabutyl ammonium ion in 1 M phosphate buffer pH = 7.0 (◆—◆), 1 M carbonate buffer pH = 9.8 (□—□), 1 M phosphate buffer pH = 10.7 (▽—▽). The yield is based upon the maximum yield obtained using buffer (pH 9.8) with a reaction time of 60 min.

Reagent concentration. The reaction rate in the formation of the N,O-bis derivative of pentazocine increased with increasing reagent concentration. With 0.06 M trichloroethyl chloroformate a quantitative yield was obtained within 20 min, whereas 1 h was required with a reagent concentration of 0.006 M. However, with 0.3 M trichloroethyl chloroformate, quantitative derivatization was not obtained and the derivative was decomposed. The reason for the decomposition is not known. The reagent may react with the quaternary ammonium ion to give products which enhance hydrolysis of the derivative. This effect was even more pronounced when tetrapentylammonium ion was used as the counter-ion.

Choice of solvent. Several organic solvents were tested as reaction media in the two-phase derivatization of pentazocine with trichloroethyl chloroformate. The extent of extraction with tetrabutylammonium ion into diethyl ether, toluene and toluene–n-butanol was low, as shown by a low reaction rate. Where the counter-ion was substituted by a more lipophilic ion such as tetrapentylammonium ion, the reaction yield increased (Table 1). After a reaction time of 3 h, quantitative formation of the bis derivative only occurred with toluene.

Effect of potassium iodide. Tertiary amines form carbamates with trichloroethyl chloroformate [2]. The reaction proceeds through a reactive intermediate ion, which may be hydrolysed in the two-phase reaction [2]. In the presence of a strong nucleophilic agent such as iodide ion, the yield of carbamate was increased at the expense of the hydrolysis reaction. The favourable effect on the reaction rate after addition of iodide ion has been demonstrated in the derivatization of pethidine [10] and amitriptyline [5] with chloroformate ester. Iodide ion had no effect on the reaction rate in the two-phase derivatization of pentazocine. This is due to a low extraction of pentazocine as phenolate into the organic phase; the reaction rate was about the same with or without addition of iodide (cf. Table 2). However, an almost complete conversion to the N-trichloroethyl carbamate of pentazocine was obtained, whereas the yield of the bis derivative was low when the reaction was performed in the presence of iodide ion alone (0.1 M). This effect

Table 1
Yields* of the N,O-bis(trichloroethoxycarbonyl) derivative of pentazocine† with various organic phases

Solvent	Yield of N,O-bis(trichloroethoxycarbonyl) derivative of pentazocine (%)	
	Tetrabutylammonium ion	Tetrapentylammonium ion
Methylene chloride	85	50
Diethyl ether	0	10
Ethyl acetate	10	36
Toluene	3	45
Toluene–n-butanol (4:1 v/v)	5	20

* The yields are based upon the maximum yield obtained using methylene chloride with a reaction time of 60 min.

† Concentration of pentazocine, 0.003 M. Concentration of trichloroethyl chloroformate, 0.06 M. Aqueous phase: 1 M phosphate buffer (pH 9.8) with 0.05 M tetra-alkylammonium ion; volume, 2 ml. Reaction temperature 20°C and reaction time 10 min. Volume of organic phase, 1 ml.

Table 2
Yields* of the N,O-bis(trichloroethoxycarbonyl) derivative of pentazocine† with various additives to the aqueous phase

Additive to aqueous phase	Yield of N,O-bis(trichloroethoxycarbonyl) derivative of pentazocine (%) after:		
	5 min	20 min	60 min
—	4	10	38
0.05 M Potassium iodide	—	11	16
0.05 M Tetrabutylammonium ion	67	100	100
0.05 M Tetrabutylammonium iodide and potassium iodide	14	18	21

* The yields are based upon the maximum yield obtained using 0.05 M tetrabutylammonium ion with a reaction time of 60 min.

† Concentration of pentazocine, 0.003 M. Organic phase, methylene chloride with 0.06 M trichloroethyl chloroformate, 1 ml. Aqueous phase, 1 M phosphate buffer (pH 9.8).

of iodide ion on the chloroformate reaction has also been reported for azapetine [2]. Addition of potassium iodide together with tetrabutylammonium ion in the two-phase derivatization of pentazocine did not increase the reaction yield of the N,O-bis derivative (Table 2). This is most probably due to the competition of iodide ion with phenolate in the ion-pair extraction. The highest reaction rate was achieved in the presence of tetrabutyl ammonium ion.

Derivatization of pentazocine with ethyl chloroformate

The reactivity of ethyl chloroformate in the formation of the N,O-bis(ethoxycarbonyl) derivative of pentazocine was considerably less than that of trichloroethyl chloroformate. Although the concentration of ethyl chloroformate was 10 times that of trichloroethyl chloroformate, the same reaction time was required for quantitative derivatization (Fig. 2). The positive effect of tetrabutylammonium ion was also demonstrated. In contrast to the N,O-bis(ethoxycarbonyl) derivative of norketobemidone which was quantitatively

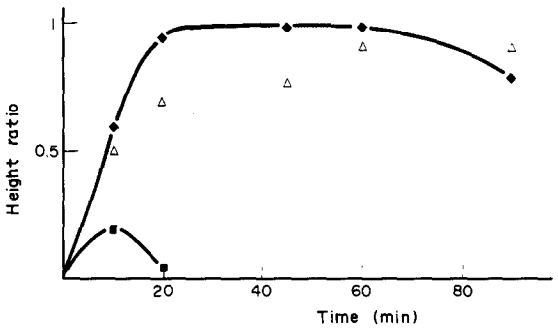


Figure 2
Time dependence of the two-phase derivatization of pentazocine with ethyl chloroformate. Organic phase: methylene chloride with 0.7 M ethyl chloroformate; aqueous phase (A): 0.05 M tetrabutyl ammonium ion in 1 M phosphate buffer (pH 9.8); ◆—◆, bis derivative of pentazocine; ■—■, mono derivative of pentazocine. Aqueous phase (B): 1 M phosphate buffer (pH 9.8) without tetrabutylammonium ion; △—△, bis derivative of pentazocine. The yield is based upon the maximum yield obtained of the bis derivative of pentazocine using buffer (pH 9.8) with a reation time of 60 min.

formed in 5 min [6], more than 20 min was required for the derivatization of pentazocine (Fig. 2). With a concentration of 0.3 M ethyl chloroformate, more than 1 h was required for the quantitative formation of the N,O-bis(ethoxycarbonyl) derivative of pentazocine.

Derivatization of alcoholic compounds with chloroformate esters

Efforts were made to apply the two-phase derivatization with chloroformate esters to other aminophenols containing an alcohol group. The alcohol rapidly reacted with trichloroethyl chloroformate but, in contact with the aqueous phase or on removal of excess reagent, the derivative rapidly decomposed.

Gas chromatographic analysis

Gas chromatographic analysis of the pentafluorobenzyl ether of pentazocine in the low concentration range was hampered due to severe adsorption losses [8]. Adsorption during GC was most probably caused by the polar tertiary amine group. This limitation was overcome in the present procedure in which the tertiary amine was converted to carbamate. The peak symmetry of the N,O-bis(ethoxycarbonyl) derivative of pentazocine was good when using packed columns with OV 17 as the stationary phase or with capillary columns with SE 30 as the stationary phase (Fig. 3).

Analysis of pentazocine in the low concentration range

Electron capture detection. Introduction of two trichloroethoxy carbonyl groups in pentazocine gave a derivative with good detectability by the electron capture detector. The minimum detectable quantity was 1.3×10^{-16} mol/s; this corresponds to a minimum detectable amount of 0.8 pg in an injected sample on a column with 2000 theoretical plates, with a retention time of 5 min and a signal-to-noise ratio of 3. Before analysis with electron capture GC, excess reagent has to be removed. It was not possible to destroy excess reagent with alcoholic alkali [1, 16], since this treatment caused rapid hydrolysis of the derivative. N,N-Dimethylethylene diamine forms carbamate with excess reagent; the product together with excess N,N-dimethylethylene diamine was easily extracted into the acidic aqueous phase [4]. However, N,N-dimethylethylene diamine caused hydrolysis of the derivative; this decreased the precision of the analytical procedure. Therefore,

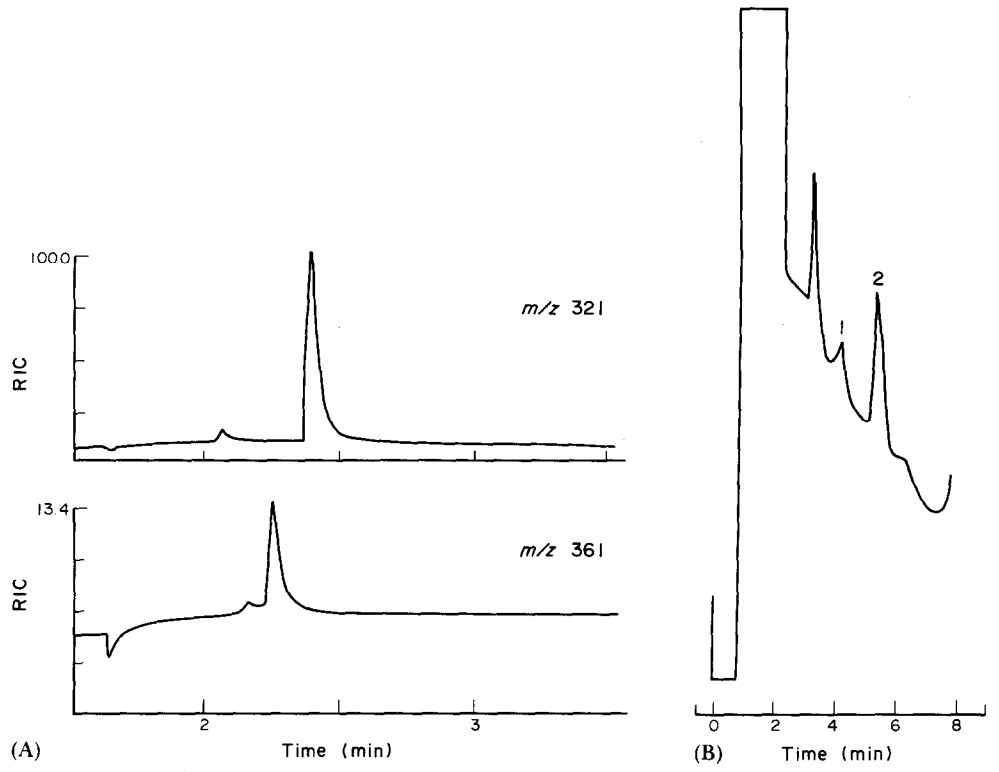


Figure 3
(A) Selected-ion recording from an analysis of a plasma sample spiked with 50 ng pentazocine in 1 ml plasma (lower panel) and 100 ng norketobemidone (upper panel). (B) Chromatogram from an analysis of pentazocine from 1 ml plasma. Concentration of pentazocine 50 ng/ml (1) and of norketobemidone (internal standard) 200 ng/ml (2).

it is not possible to use electron capture GC for the determination of pentazocine in low concentration after conversion to the N,O-bis(ethoxycarbonyl) derivative.

Thermionic detection. Ethyl chloroformate does not interfere with the thermionic detector and analysis by GC can be performed after evaporation of the reaction mixture to dryness, followed by reconstitution in a small volume of toluene and removal of tetrabutylammonium iodide by silver sulphate. The minimum detectable concentration was 5×10^{-13} mol/s corresponding to about 1 ng in an injected sample with the same GC conditions as described above. The detection limit is most probably determined by interference by the reagent. The lower limit of detection of pentazocine in a 1-ml plasma sample was estimated to be 20 ng/ml.

Mass spectrometric detection. The prominent MS peaks of pentazocine as the N,O-bis(ethoxycarbonyl) derivative have been described in the experimental section. The molecular ion (m/z = 361) was the only important peak in the high mass range. This mass number was chosen for selected ion monitoring. Owing to the low relative abundance of the molecular ion, the drug could not be detected by MS at concentrations lower than about 10 ng/ml of pentazocine.

Application to plasma samples

The principle of two-phase derivatization of pentazocine with chloroformate ester has also been applied to spiked plasma samples. The drug was determined by GC with MS or thermionic detection. Before derivatization it was necessary to selectively isolate pentazocine and the internal standard, norketobemidone, owing to the low yield and to disturbances caused by the plasma matrix on direct derivatization in the plasma sample. The method of isolation was as described previously [7], but use of saturated sodium hydrogen carbonate solution was found to increase the yield in isolation of the drug from plasma.

Pentazocine could be detected in a plasma sample in concentrations as low as 10 and 20 ng/ml with MS and thermionic detection, respectively. Mass spectrometric detection is more suitable for pharmacokinetic studies owing to its better sensitivity and fewer interferences in the chromatogram. The RSD in the analysis by GC with thermionic detection of 100 ng/ml of pentazocine was 7.2% (n = 6). Rectilinear standard curves were obtained in the range 50–5000 ng/ml of pentazocine with both thermionic (r^2 = 0.96–0.99) and MS detection (r = 0.99). The regression equation for thermionic detection was: $y = 0.0024x + 0.045$ (n = 4); for GC–MS analysis with SIM the regression was: $y = 0.00336x + 0.062$ (n = 4); range of pentazocine concentrations, 20–500 ng/ml.

The recoveries from aqueous and plasma samples were of the same order. Metabolites of pentazocine are not likely to interfere in the derivatization step; during metabolism, pentazocine is mainly converted to conjugated metabolites [12] which are not extracted from plasma, owing to their polar character; an alcohol and an acid side-chain metabolite have been traced in minor amounts in plasma [13], but will not be co-determined by the present method. Norpentazocine, which will form the same derivative as pentazocine, has not been traced in human plasma [14].

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