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DETERMINATION OF THERAPEUTIC AND TOXIC CONCENTRATIONS OF DOXEPIN AND LOXAPINE USING GAS—LIQUID CHROMATOGRAPHY WITH A NITROGEN-SENSITIVE DETECTOR, AND GAS CHROMATOGRAPHY—MASS SPECTROMETRY OF LOXAPINE

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SUMMARY

A gas—liquid chromatographic procedure is presented for the determination of therapeutic and toxic serum levels of doxepin and loxapine, using a nitrogen—phosphorus-sensitive detector. Amitriptyline is used as the internal standard. The method is accurate, sensitive and specific with no derivatization required prior to analysis. An advantage of the procedure is the small serum sample size needed for analysis and the selectivity and sensitivity of the detector, with the limit of detection being 3 and 2 μg/l for doxepin and loxapine, respectively. Nine cases of doxepin and loxapine misuse are presented. Serum doxepin concentrations ranged from 113 to 439 μg/l, with a loxapine concentration of 192 μg/l observed in one patient. The presence of the tricyclics was identified and confirmed by gas chromatography—mass spectrometry and the mass spectrum of loxapine is reported.

INTRODUCTION

Loxapine succinate (Loxitane), a dibenzoxazepine, and doxepin hydrochloride (Sinequan), a dibenzoxepine, are tricyclic derivatives (Fig. 1) which like other tricyclics such as amitriptyline, nortriptyline, imipramine, desimipramine and protriptyline, have effective antidepressant and antianxiety activity [1,2]. Loxapine is especially effective in the treatment of schizophrenia [3,4]. An apparent correlation between circulating blood levels and therapeutic effect has been reported for doxepin and other tricyclic antidepressants [5—7]. A definite correlation between administered daily dosage and therapeutic effect has also been established for loxapine [2—4]. Recently an attempt was made to correlate circulating blood levels and therapeutic effect for loxapine [8].

The more common use of these and other tricyclic drugs has brought about an increase in tricyclic abuse and self-inflicted poisonings [9]. Patients may take the drugs for therapeutic purposes or suicide gestures. Because of car-

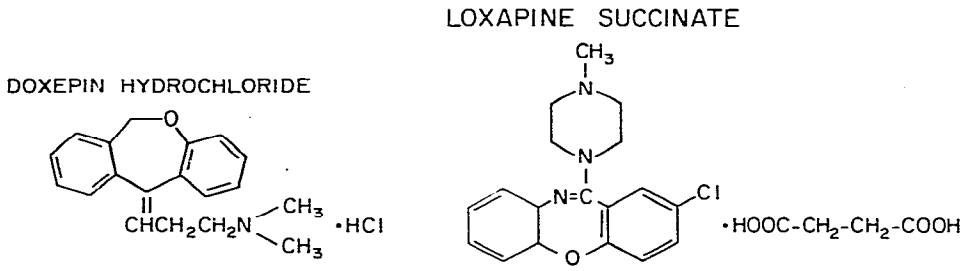

Fig. 1. Chemical structures of doxepin hydrochloride (Sinequan) and loxapine succinate (Loxitane).

diovascular and other complications, the identification and quantitation of doxepin and loxapine overdose is important.

A number of procedures have been reported for the determination of doxepin in serum [10—16]. Recently a procedure for the determination of loxapine and its metabolites has also been reported [8]. We report here a gas—liquid chromatographic (GLC) procedure using a nitrogen—phosphorus-sensitive detector for the simultaneous determination of doxepin and loxapine in serum. An advantage of this procedure is the small sample size used for analysis and the sensitivity and selectivity of the detector. No derivatization is required and quantitation is achieved from therapeutic to toxic levels. To illustrate the applicability of this procedure, eight representative patients involved in doxepin misuse and one patient involved in loxapine misuse as seen in the emergency room are presented.

EXPERIMENTAL

Reagents

All reagents used were spectral grade: heptane, chloroform, methanol, isobutanol. Anesthetic grade diethyl ether (J.T. Baker, Phillipsburg, N.J., U.S.A.) was used. Amitriptyline HCl (Merck, Sharp and Dohme, Rahway, N.J., U.S.A.), doxepin HCl (Sinequan; Pfizer, Brooklyn, N.Y., U.S.A.), and loxapine succinate (Loxitane; American Cyanamid Co., Pearl River, N.Y., U.S.A.) were used as the salts; however, all concentrations are expressed as the free base. A 1 g/l stock aqueous solution of doxepin, loxapine, and amitriptyline was prepared in deionized distilled water. A 10 mg/l working aqueous solution of the above was prepared in distilled deionized water. Serum standards were made up by the addition of small amounts of aqueous doxepin and loxapine 10 mg/l working standards to normal pooled human serum. An aqueous amitriptyline internal standard was added to the doxepin and loxapine standards and taken through the entire extraction procedure. A 0.5 *M* NaOH solution was prepared from solid sodium hydroxide, and a 0.1*M* HCl solution was prepared from concentrated hydrochloric acid.

Apparatus

Analyses were performed on a Perkin Elmer 3920 gas chromatograph equipped with a flame ionization and a nitrogen—phosphorus-sensitive detector

(Perkin Elmer, Norwalk, Conn., U.S.A.). A 1.8 m × 2 mm I.D. round glass column packed with 3% OV-17 on 100—120 mesh Gas-Chrom Q (Applied Science Labs., State College, Pa., U.S.A.) was used to accomplish separation with the following gas chromatographic (GC) conditions: helium carrier gas with a flow-rate of 40 ml/min, injector temperature 275°, interface temperature 275°. The column temperature was programmed as follows: initial temperature was maintained at 235° for 6 min and then programmed to 280° at 32°/min and maintained at 280° for 4 min. Air-flow to detector was 40 p.s.i., hydrogen flow to detector 12 p.s.i. The detector voltage was a 5.5—5.8 V setting on the variable power source; detector voltage was increased with bead aging for sensitivity. A Perkin Elmer 26 recorder set at a range of 1 mV and a chart speed of 10 mm/min was used to record all chromatograms.

The detector used in the analysis was the nitrogen—phosphorus-sensitive detector purchased from Perkin Elmer. The principle of its operation has been described [17].

Gas chromatographic—mass spectrometric (GC—MS) analyses were performed on a Hewlett-Packard 5985A quadrupole GC—MS system (Hewlett-Packard, Palo Alto, Calif., U.S.A.) using the electron impact (EI) mode. The system consists of an HP 5840A gas chromatograph interfaced with the mass spectrometer, an HP 7900 disc drive, an HP 2109 computer and a Tektronix 4012 graphic display. A 1.2 m × 2 mm I.D. column packed with 2% SP-2250 on Chromosorb W HP 100—120 mesh was used for the GC—MS analysis.

Procedure

The procedure used is a modification of our previous procedure [9]. To a 50-ml glass stoppered centrifuge tube add 2 ml of serum, 1 ml of 0.5 *M* NaOH and 30 ml of 4% isobutanol in *n*-heptane. Shake for 5 min, centrifuge for 5 min and filter through phase separation paper. To the organic filtrate add 5 ml of 0.1 *M* HCl, shake for 5 min, centrifuge for 5 min, and discard the organic layer. Wash the aqueous layer with 30 ml of *n*-heptane, back extract in 10 ml of ether, and evaporate to dryness.

The residue was dissolved in 25 μl of absolute ethanol or chloroform—methanol (1:1) and subjected to analysis by injecting 1 μl of the reconstituted sample onto the column.

In some cases where the internal standard was not carried through the extraction procedure, the internal standard was added with the absolute ethanol or chloroform—methanol mixture.

Standard curves were obtained by analyzing serum standards containing known amounts of doxepin and loxapine. Serum standards containing 50, 100, 200, 300, 400, and 500 μg/l of doxepin or loxapine were used for patients with therapeutic levels; serum standards containing 0.5, 1, 2, 3, 4 and 5 mg/l of doxepin or loxapine were used for toxic levels. The concentration of amitriptyline internal standard used for the therapeutic range was 100 μg/l, and the concentration for toxic levels was 1 mg/l. Following chromatography, the ratio of drug to internal standard peak area or peak height was calculated and plotted against its concentration. The doxepin or loxapine concentration in patients was obtained from the curve and calculated using the nearest serum standard. No significant difference between the two methods of calculation was observed.

RESULTS

Chromatograms of serum blanks, and a serum standard containing doxepin, loxapine and amitriptyline as internal standard, taken through the extraction procedure under the described conditions of study are shown in Fig. 2. The retention times at 235° were 3.6 and 4.1 min for amitriptyline and doxepin, respectively, and 9.5 min for loxapine after programming to 280° at 32°/min. Fig. 2 also indicates that the detector was most sensitive to loxapine, followed by amitriptyline and then doxepin. Sensitivity was governed by the ability of these drugs to form cyan-free radicals. Blank serum samples assayed in the same manner as standards and patients gave no significant peaks on the chromatogram that might interfere with the analysis (Fig. 2). However, Fig. 2 shows an impurity peak which appeared in some extractions that interferes with desmethyldoxepin and protriptyline; it was attempted to use the latter as an internal standard instead of amitriptyline. This interference could be overcome by decreasing the oven temperature or carrier gas flow-rate, but this would have increased the loxapine retention time resulting in increased analysis time. Under the conditions of this study, nortriptyline interferes with doxepin, which is in agreement with other literature findings [12]. In addition, desmethyldoxepin will interfere with protriptyline.

The absolute percentage recovery of doxepin and loxapine by our method averaged 65 ± 10% for serum standards of 50, 100, 200, 300, 400, and 500 μg/l. The percentage recovery relative to serum standards averaged 98 ± 10%. This compares well with previously described procedures for other tricyclic antidepressants [9].

The linearity of the entire assay was demonstrated by extracting 2 ml of serum samples in duplicate, containing 50, 100, 200, 300, 400, and 500 μg/l of doxepin and loxapine with 100 μg/l of amitriptyline as internal standard.

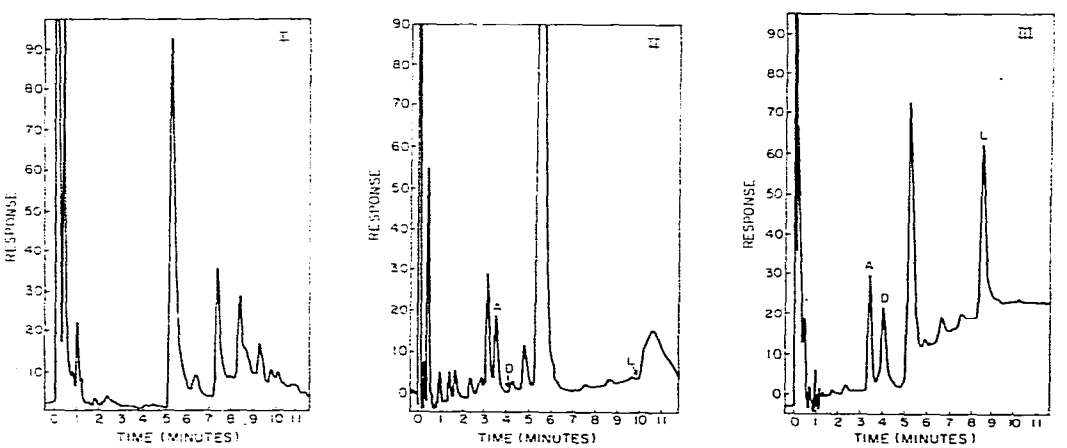

Fig. 2. Gas chromatograms of (I) serum blank, attenuation × 8; (II) serum blank with amitriptyline (A) as internal standard (100 μg/l), with D and L representing expected retention times for doxepin and loxapine, attenuation × 32; (III) serum standard containing 200 μg/l of doxepin (D), loxapine (L) and amitriptyline (A) as internal standard, attenuation × 4.

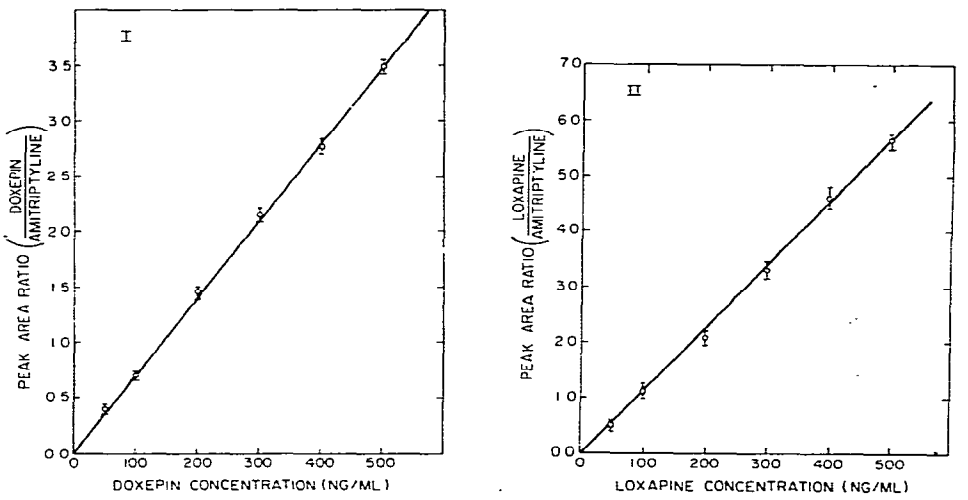

Fig. 3. Standard curves using serum standards containing 50, 100, 200, 300, 400 and 500 μg/l of doxepin (I) and loxapine (II). Plot of peak area ratio against concentration. Internal standard amitriptyline 100 μg/l. (I) Slope = 6.8×10^{-3}, y intercept = 5.3×10^{-2}, correlation (r) = 0.99, Syx = 0.03. (II) = 1.2×10^{-2}, y intercept = — 0.11, correlation (r) = 0.99, Syx = 0.06.

The extract was subjected to GC analysis under the described conditions, and the peak area ratio was plotted against concentration (Fig. 3). Linearity extended through the 500 μg/l standard for the lower concentration range of standards used and through the 5 mg/l standard for the higher concentration range. Aqueous standards of 10 μg/l of loxapine could easily be quantitated by this procedure.

The above procedure was used to determine serum doxepin and loxapine levels in patients seen in the emergency room and suspected of having ingested these drugs. A review of nine patients seen in the emergency room is summarized in Table I. Shown in Table I is the patient's alleged drug ingestion, clinical symptoms, and doxepin and loxapine serum levels. Some of the patients in Table I lack a serum concentration due to the unavailability of serum at the time of analysis. In all cases, doxepin and loxapine were identified and confirmed by three separate methods. Doxepin and loxapine were first identified in the urine and/or gastric samples of each patient by ultraviolet (UV) spectrophotometry, thin-layer chromatography (TLC), and GC as previously described [9]. Thin-layer plates were dried and sprayed with acidified iodoplatinate for visualization of the drugs and metabolites. Doxepin, loxapine, and possibly a doxepin metabolite in one of the samples (urine samples of patient No. 6) were observed. The TLC spots were scraped off and the drugs extracted for GLC analysis. The approximate concentrations of doxepin or loxapine in urine and/or gastric samples were calculated and are also given in Table I. The doxepin metabolite has a longer retention time than doxepin under the same GC conditions of study with a retention time of 1.37 relative to amitriptyline (Table II), and corresponds to that of desmethyldoxepin.

TABLE I

SUMMARY OF PATIENTS INVOLVED IN DOXEPIN AND LOXAPINE POISONING

Patient*	Drugs allegedly ingested	Clinical symptoms	UV spectra for		TLC**		Serum level (μg/l) of drug ingested (GLC)***
			Doxepin	Loxapine	Doxepin	Loxapine	
1. 27 years B-F	Sinequan unknown amount, 1/2 beer	Dizziness	+	—	+	—	Dox. 192
2. 25 years W-F	Sinequan or tricyclic subgroup, Triavil	Comatose, obtunded	+	—	+ G(811)	—	Dox. 431
3. 25 years W-F	Sinequan or tricyclic subgroup, Triavil	Semicomatose, pale and drousy but easily arousable	Unable to identify		—	+	Lox. 192
4. 31 years W-F	Sinequan 12/50 mg tablets	Lethargic but easily arousable	+	—	+ U(9.5)	—	Dox. Serum sample not available
5. 35 years W-M	Stelazine, Valium, and Sinequan		+	—	+	—	Dox. 112
6. 45 years W-M	Sinequan, suicide attempt		Unable to identify		+ U(14.2)	—	Serum sample not available
7. 59 years W-M	Librium, Sinequan and Valium	Depression, dizziness, slow in speech and thought	+	—	+ G(97.2) U(10.3)	—	Dox. 182
8.	Sinequan		+	—	+ U(5.2)	—	Dox. Serum sample not available
9. 38 years W-F	Sinequan, Omnipen, and Naprosyn		+	—	+ G(1.1)	—	Dox. Serum sample not available

*B = Black; w = white; f = female; m = male.
**G = Gastric sample; U = urine sample. Concentration in parentheses in mg/l; + = positive; — = negative.
***Values using peak area ratios for calculation. Single determinations.

TABLE II

RETENTION TIMES OF TRICYCLICS RELATIVE TO AMITRIPTYLINE ON 3% OV-17

Conditions: isothermal at 235° or programmed as in procedure.

Substance	Relative retention times			
	Ref. 9	Ref. 12	Ref.21	This work
Amitriptyline	1.00	1.00	1.00	1.00
Nortriptyline	1.18	1.12	1.16	1.14
Protriptyline	1.38	—	1.35	1.33
Imipramine	—	1.07	—	0.94
Desimipramine	—	1.24	—	1.14
Doxepin	—	1.13	—	1.17
Desmethyldoxepin	—	1.27	—	1.37
Loxapine	—	—		2.71*
SKF 525-A	—	—		1.32
Trihexyphenidyl	—	—		1.17

*Programmed to 280°.

Gas—liquid chromatographic analysis was performed on the serum to identify the drugs qualitatively and to quantitate them. Fig. 4 shows the chromatograms of two patients, one with a serum concentration of 192 μg/l of doxepin, and one with a serum concentration of 192 μg/l of loxapine. Good agreement was observed in the use of area or peak height ratios for calculating results. The within-run precision (C.V.) for $n = 5$, using a 200 μg/l serum control and using peak height, averaged 6% for doxepin and 5% for loxapine with a 97 ± 12% and 108 ± 10% recovery for doxepin and loxapine, respectively. The within-run precision at 1 mg/l averaged 3% and 5% for doxepin and loxapine, respectively.

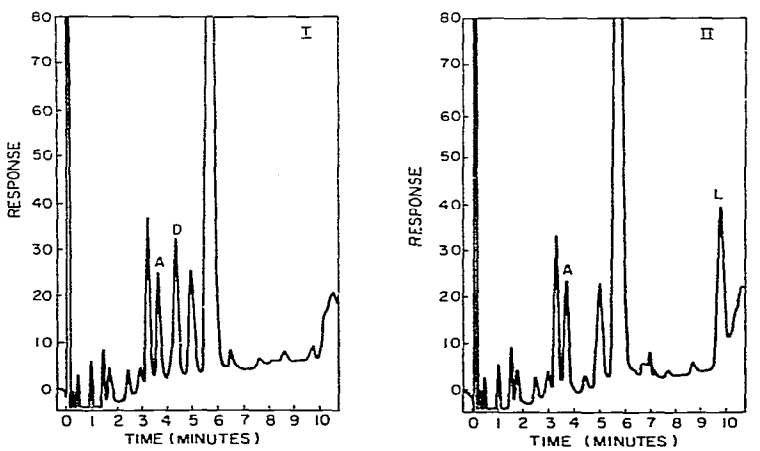

Fig. 4. (I) Gas chromatogram of a patient with a serum concentration of 192 μg/l of doxepin. Attenuation 1 × 32. A = Amitriptyline (100 μg/l), D = doxepin. (II) Gas chromatogram of a patient with a serum concentration of 192 μg/l of loxapine. Attenuation 1 × 32 for amitriptyline (A, 100 μg/l) and 1 × 64 for loxapine (L).

Serum levels quantitated by this method correlated well with the physical and clinical state of the patient, the higher the level the more obtunded and comatose the patient.

Gas chromatography—mass spectrometry

GC—MS was performed on the serum extracts to identify and confirm the presence of amitriptyline, doxepin, loxapine, and other possible substances present. A peak at *m/e* 58 with the same retention time as amitriptyline and doxepin confirmed the presence of these two compounds. The mass spectrum of loxapine is given in Fig. 5. Major peaks at *m/e* 83.2, 70.1, 257.1 and 193.0 were observed at the same retention times as loxapine which are indicative of the spectrum of loxapine (Fig. 5). During the mass spectral analysis, the presence of possible impurities present in the serum extracts was investigated. A number of impurities were looked for including tris(2-butoxyethyl) phosphate (B-D vacutainer plasticizer), and other plasticizers. The presence of a number of phthalates (*m/e* 149) at different retention times was observed, indicating contamination by various phthalate plasticizers.

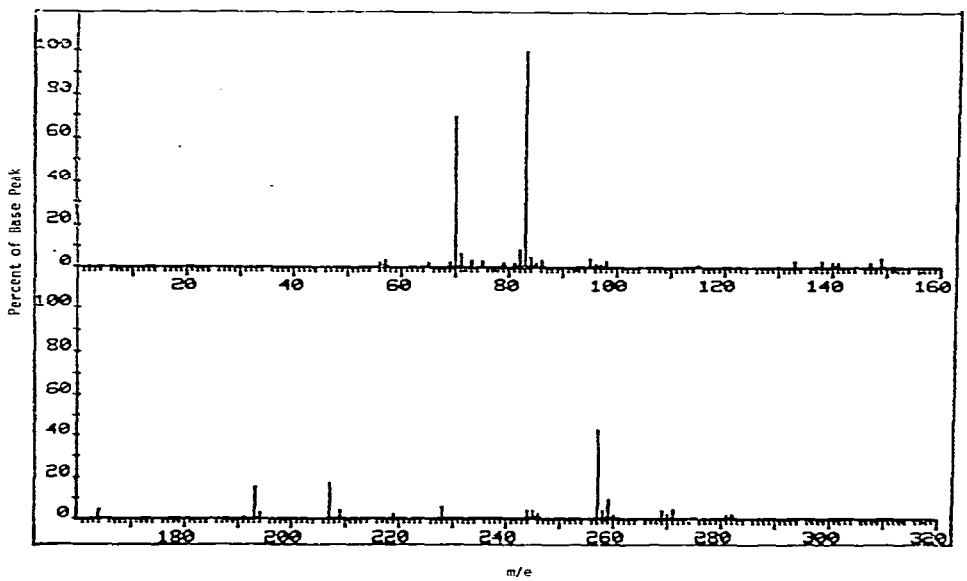

Fig. 5. GC—MS of loxapine using EI mode. Major peaks at *m/e* 83.2, 70.1, 257.1 and 193.0.

GC and GC—MS were also used for the identification and confirmation of loxapine and other tricyclics which were suspected in emergency room overdoses. Fig. 6 is the gas chromatogram of a urine extract of a patient seen in the emergency room who was suspected of having taken amitriptyline and loxapine. Fig. 7 gives the GC—MS total ion chromatogram of the same extract using *m/e* 70, 83, and 257 to monitor the presence of loxapine. The presence of amitriptyline was also confirmed by GC—MS by looking at the *m/e* 58 peak.

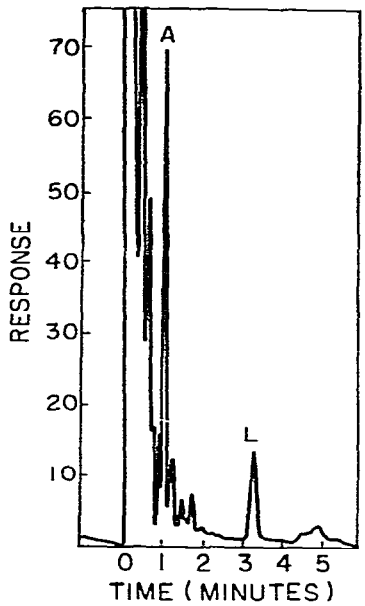

Fig. 6. Gas chromatogram of urine extract of a patient suspected of amitriptyline and loxapine misuse. Conditions: 280° isothermal. Attenuation 1 × 16. A = Amitriptyline, L = loxapine.

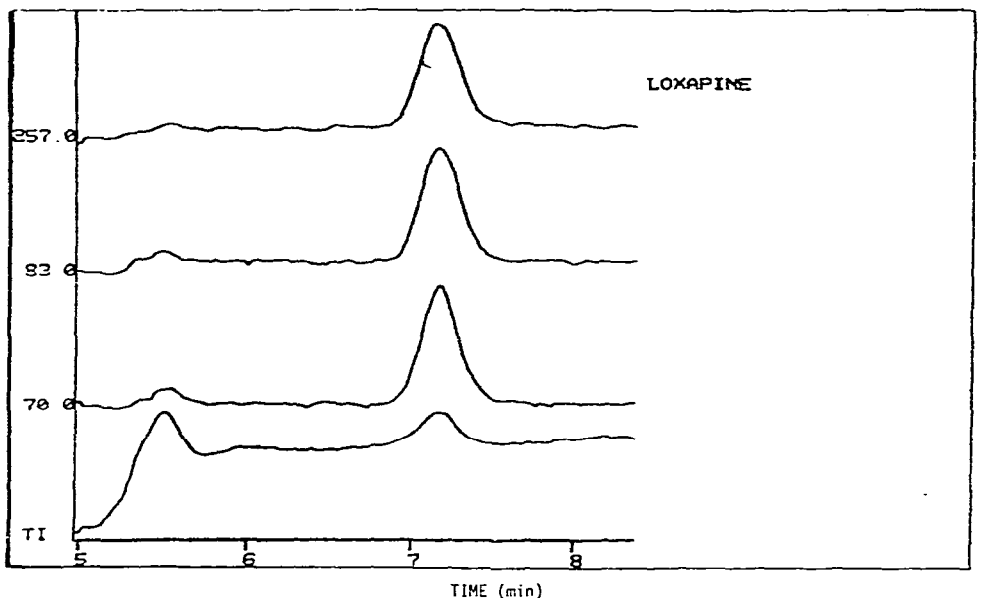

Fig. 7. Total ionization chromatogram of urine extract of patient (Fig. 6), using masses 70, 83, and 257 to monitor the presence of loxapine.

Solvent effects

The use of chloroform instead of ether in back-extracting the drugs from the 0.1 *N* HCl acidic solution and the use of ethanol instead of chloroform—methanol for redissolving the residue prior to GC were investigated. Serum blanks as well as sera containing 200 μg/l of doxepin and loxapine were extracted in the four different ways and chromatographed. Ether and ethanol were the preferred solvents to use for back-extracting and dissolving the final residue.

Internal standard

The use of different internal standards, SKF 525-A, trihexyphenidyl (Artane) and protriptyline, was investigated. Trihexyphenidyl could not be used since it has a retention time similar to that of doxepin under the conditions used in this study. SKF 525-A and protriptyline would interfere with the doxepin metabolite desmethyldoxepin which has a retention time similar to that of SKF 525-A and protriptyline [12]. Table II gives the GC retention times of all the tricyclics relative to amitriptyline and can be used to indicate the possible combinations of tricyclics that can be determined using the present procedure.

The effect of sample volume injected into the gas chromatograph on peak height ratio was also investigated. A definite decrease of the peak height ratio was observed with increased sample used for GC analysis. Doxepin with amitriptyline as the internal standard (n = 4) gave a 13% decrease in peak height ratio in going from 1 to 3 μl of sample injected onto the column. This is in agreement with the recently reported findings of decreased peak area ratios, in spite of the presence of an internal standard, with increasing sample volume in GC analysis [18]. Although a definite decrease in peak area ratio was reported between the ratio of analyte and internal standard with increasing sample

TABLE III

THIN-LAYER CHROMATOGRAPHIC DATA OF TRICYCLICS

Developing solvent: ethyl acetate—absolute ethanol—*n*-butanol—concentrated ammonium hydroxide (56:28:4:0.8). Positive acidic iodoplatinate reaction. R_F × 100 of amitriptyline = 62.

Drug	R_F relative to amitriptyline
Amitriptyline	1.0
10-Hydroxyamitriptyline	0.83
Nortriptyline	0.61
10-Hydroxynortriptyline	0.35
Protriptyline	0.44
Imipramine	0.98
Desimipramine	0.50
Doxepin	1.04
Desmethyldoxepin	0.35
Loxitane	1.10

volume, the authors [18] did not investigate the same effect on peak height ratio.

DISCUSSION

In a limited study of aged patients given 50—300 mg of doxepin in a single daily dose and plasma analyzed 10—12 h after the bedtime dose, levels of doxepin and desmethyldoxepin of 9—131 μg/l were obtained. Therapeutic response was observed in patients with minimum plasma levels of 110 μg/l [5]. In another study, patients given 75—200 mg over a 3—37-week period attained plasma levels of doxepin and desmethyldoxepin of 57—252 μg/l. Desmethyldoxepin was not detected in 5 out of 7 of these patient's blood samples [11]. Fatal cases of doxepin overdose have been reported with blood levels of doxepin ranging from 0.7 to 29 mg/l [10, 19].

Very little information is available in the literature regarding serum levels of loxapine for optimum therapeutic effect [8]. However, this drug is being seen more and more in emergency room patients, and laboratories should be aware of its presence and be able to identify and determine its concentration.

Loxapine succinate has absorption maxima in 2 *M* H_2SO_4 at 253 nm (ϵ = 1.25×10^4) and 293 nm (ϵ = 1.08×10^4) with a slight shift of the absorption maxima to 250 and 296 nm in basic solution.

Using TLC, loxapine has an R_F value of 0.69 while doxepin has an R_F of 0.65 (Table III). The R_F values of the various tricyclics and their metabolites using TLC under the present conditions of study are given in Table III. Doxepin and loxapine are not well separated by TLC. However, using GC with a 3% OV-17 column, both drugs are very well separated for identification, quantitation, and confirmation by GC—MS in serum, urine and gastric contents (Figs. 2 and 4). Fig. 6 gives the gas chromatogram of a patient's urine analyzed for amitriptyline and loxapine isothermally at 280°. If only loxapine is to be determined, the analysis can be carried out at 280° using prazepam as internal standard. Prazepam has a retention time of 1.33 relative to loxapine under the present conditions of study and can readily be determined at a concentration of less than 1 μg/ml. At 280°, diazepam (Valium) has a retention time relative to loxapine (6.0 min) of 0.78 while chlordiazepoxide (Librium) has a relative retention time of 1.10. At 280° chlordiazepoxide is not well separated from loxapine and would be expected to interfere. However, both drugs can be analyzed by using a column temperature of 260°. The present procedure can be modified to determine diazepam and other benzodiazepines, which are commonly misused with tricyclics.

With the present procedure, serum concentrations of the parent drug can be determined over a wide range with a precision of 5—10% with close to 100% relative recovery using serum standards. The five patients who ingested doxepin had levels of 113—439 μg/l, which indicates that although some patients were toxic, none of the patients had concentrations in the lethal range of 10 mg/l [10, 19]. In some of the cases other drugs or alcohol were present, making the effect of doxepin or loxapine difficult to interpret in view of the multiple drug ingestion. Only doxepin was determined in the present cases, since the active metabolite desmethyldoxepin was not detected in our initial screen-

ing. An interference peak with the same retention time as desmethyldoxepin (Fig. 2) was also observed in some extractions, making the use of this procedure for the quantitative determination of desmethyldoxepin impossible at low concentrations. The desmethyl metabolite has a longer retention time than doxepin (Table II) and would be seen at high concentrations under the present conditions of study. A number of procedures, which have recently been reported, can be used for the direct determination of desmethyldoxepin [11, 12].

Of seven patients on long-term doxepin therapy only two had a measurable concentration of desmethyldoxepin [11]. In four fatal cases of doxepin poisoning of patients who were known to be on doxepin, the concentration of doxepin ranged from 0.7 to 29 mg/l with the desmethyldoxepin being 0.1—6.2 mg/l [19]. The desmethyl metabolite accounted for only 2—22% of the total drug found in blood. In a study with tricyclic overdose patients, no correlation was found between the state of unconsciousness and total tricyclic concentration present [16]. However, if one calculates the percentage of parent drug present, the cases with a higher percentage of the unmetabolized drug appear to have a higher degree of unconsciousness.

Desmethyldoxepin's pharmacologic activity differs from that of the parent drug, the half-lives of doxepin and desmethyldoxepin being 16.8 and 51.3 h, respectively [20]. The present procedure can be used to study acute drug ingestions where metabolites have not accumulated to a large extent. For therapeutic monitoring of doxepin and loxapine, the present procedure could not be used to monitor the presence of metabolites. However, the determination of the parent drug alone may be of clinical usefulness.

GC—MS was used to confirm the presence of doxepin and loxapine in serum, gastric contents or urine, and to look at some of the possible interferences present. Looking at some of the extractions using this procedure (Figs. 2 and 4) by GC—MS, it became obvious that a contamination problem had occurred. After looking at these contaminants by GC—MS, it was realized that this was a phthalate contaminant as evidenced by the mass at *m/e* 149. After making new standards and changing solvents, many of the phthalate impurities disappeared. Although the phthalates did not interfere with the present analysis, possible interference could result from the use of this procedure for other tricyclics, especially in the early part of the chromatogram. GC—MS analysis of the various serum extracts indicated that substances were still present in the sera with an *m/e* of 149 mass units even after careful decontamination precautions. These residual peaks are due to phthalate plasticizers such as dioctylphthalate, dibutylphthalate and butylbutoxyphthalate. The large interfering peak after doxepin appears to be dioctylphthalate and does not appear to be tris(2-butoxyethyl) phosphate. To prove this, a red stoppered B-D vacutainer was placed in methanol and the dissolved plasticizers were analyzed by GC—MS. The spectrum of the serum impurity after doxepin (Fig. 4) was not consistent with the spectrum of tris(2-butoxyethyl) phosphate, but is similar to that of dioctylphthalate [21—23]. The stopper extract showed a number of plasticizers containing phthalates (*m/e* 149) including the tris(2-butoxyethyl) phosphate impurity (*m/e* 57, 85.1, 125, 100, 101) which did not appear in high concentration in the serum extracts.

Studies using chloroform or ether for back-extracting the drugs from the HCl aqueous layer indicate that ether is a better solvent to use. Chloroform is a less desirable solvent to use since it extracts a higher concentration of interfering serum components which are not seen in ether extracts. The use of ethanol for redissolving the evaporated extract prior to analysis is also preferred over chloroform because of the interference by chloride with the rubidium bead. Using chloroform, an abnormal chromatogram is observed due to the binding of chloride with the rubidium, resulting in a non-equilibrium state of rubidium atoms around the bead [17]. Using ethanol or some nonhalogenated solvent seems to correct the whole problem of baseline shifts.

A column packed with 3% SE-52 for analysis was also investigated. Although the column was appropriate for single drug analysis, it could not separate amitriptyline from doxepin and could not, therefore, be used for this analysis.

The use of different internal standards confirmed our initial belief, and that of others [12], that amitriptyline should be used as the internal standard. Although the possibility exists that amitriptyline ingestion may interfere with the amitriptyline internal standard used, it seems unlikely since in most cases it will be identified during the initial screen. If amitriptyline is suspected, then another internal standard (such as prazepam) will have to be used, or the analysis may have to be run without an internal standard, being especially careful that the injected volume is kept constant. Peak areas would be used for quantitation.

Using an internal standard to correct for sampling errors due to volume changes has recently been reported to be inaccurate [18]. Thus, one must maintain a constant volume of injection and a constant concentration of internal standard for accurate results. In addition, the concentration of the standard must be close to the concentration of the unknown. Using an internal standard and calculating peak height ratio, we also confirmed the above observation that a decrease in peak height ratio of standard to internal standard occurs with an increase in volume injected. We observed this decrease in peak height ratio, using a nitrogen-selective detector. In the previous study [18], a thermal conductivity detector and a flame ionization detector were used. Thus, for accurate results the volume injected must be kept constant, and the concentration of standard used must be close to the unknown concentration. Using peak areas with an internal standard, an error of 15% was observed in going from 1 to 2 μl of sample injected. We observed an error of 13% using peak height ratios upon changing the volume by 1 μl, which is in agreement with the peak area studies [18].

The procedure presented is a sensitive and selective direct method for the determination of doxepin and loxapine in serum. For best results, a constant volume of extract must be injected, and the use of nonchlorinated solvents is recommended for greater baseline stability using a nitrogen-sensitive detector. Low levels can be quantitated with the lower limit of detection of doxepin and loxapine being 3 and 2 μg/l, respectively. No derivatization is necessary, only a simple extraction of the pure drug. In addition, doxepin, loxapine and amitriptyline are eluted from the column after a steady baseline has been obtained. No solvent front of significant length is observed. Quantitation of drug levels can be easily accomplished by using peak height ratios instead of

peak area ratios. Other drugs in biological materials may be determined by this procedure or by a minor modification of it.

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