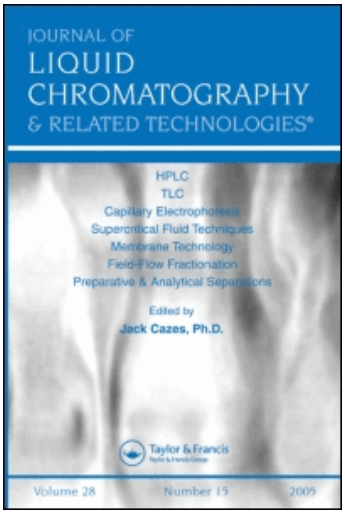

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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To cite this Article Wong, Steven H. Y. , Mchugh, Suzanne L. , Dolan, John and Cohen, Ken A.(1986) 'Tricyclic Antidepressant Analysis by Reversed Phase Liquid Chromato-Graphy Using Phenyl Columns', Journal of Liquid Chromatography & Related Technologies, 9: 11, 2511 — 2538

To link to this Article: DOI: 10.1080/01483918608076881

URL: http://dx.doi.org/10.1080/01483918608076881

JOURNAL OF LIQUID CHROMATOGRAPHY, 9(11), 2511-2538 (1986)

TRICYCLIC ANTIDEPRESSANT ANALYSIS BY REVERSED PHASE LIQUID CHROMATOGRAPHY USING PHENYL COLUMNS†

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ABSTRACT

Tricyclic antidepressant (TCA) analysis by reversed-phase liquid chromatography was achieved by a novel approach using two mini phenyl columns (4.5 x 50 mm). Analyses were organized into two drug groups: 1. n-desmethyl doxepin, doxepin, nortriptyline and amitriptyline, and 2. desipramine and imipramine. Sample preparation was modified from a published procedure (Wong and McCauley, J. Liq. Chromatogr., 4, 849 (1981)), using n-hexane/isoamyl alcohol (99:1) as the organic extractant, followed by back-extracting with dil. phosphoric acid. Both steps were carried out by using polypropylene tubes to minimize adsorptive

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†Presented in part at the Federation of Analytical Chemistry and Spectroscopy Society, September, 1982, Philadelphia and the Annual Meeting of the American Association for Clinical Chemistry, August, 1983, New York City.

 0148-3919/86/0911-2511$3.50/0

loss of drugs. Chromatographic parameters were: two mini phenyl columns; mobile phase 0.01 M n-nonylamine in 0.02 M NaH_2PO_4, pH = 3.0 /ACN (9:1); flow rate 3.0 ml/min.; ambient temperature; sample volume 50 ul, and detection wavelength 214 nm at 0.01 to 0.05 AUFS. Analysis time was about 8 minutes. Peak height ratios were linearly correlated to concentrations ranging from 25 to 800 µg/L. Day-to-day coefficients of variation ranged from 2.5 to 6.9% (n = 31 to 34). Recoveries were 71 to 86%. Fluphenazine and perphenazine did not interfere with the TCA peaks, while a thioridazine metabolite interfered with the internal standard. Within the past two and a half years, this procedure was used for clinical quantitation and/or confirmation purposes. Based on this experience, criteria are suggested for using this procedure as a viable alternative for TCAs monitoring.

INTRODUCTION

Monitoring of tricyclic antidepressants (TCAs) may serve as a useful adjunct to depression therapy (1). At present, nortriptyline's therapeutic range, 50-150 µg/L, is well established, while those of the other TCAs await further investigation. Pharmacokinetic and routine clinical laboratory analyses of TCA require simple and reliable procedures, possibly free from chromatographic interference by neuroleptics, such as fluphenazine, perphenazine and thioridazine which may be co-administered with TCAs. The present study was designed to meet the above needs. It included an initial evaluation of the phenyl column for TCA analyses, followed by a long-term (two and a half years) clinical study.

IBM Instruments Liquid Chromatograph has not been certified for clinical application.

Non-standard abbreviations - DES, desipramine; IMI, imipramine; NOR, nortriptyline; AMI, amitriptyline; ND-DOX, n-desmethyl doxepin; and DOX, doxepin.

Within the clinical laboratories engaging in TCAs monitoring, the predominant mode is reversed-phase liquid chromatography (RP-LC), followed by gas chromatography and radioimmunoassay. A "semi-quantitative" assay - Enzyme Multiplied Immunoassay Technique (EMIT) by Syva (Palo Alto, CA 94303) may be used to identify high concentration of TCA, and recently, a quantitative EMIT assay has been introduced, and its clinical efficacy awaits clinical evaluation.

Liquid chromatography in both the reversed-phase and normal phase modes has been used to analyze TCAs, a tetracyclic (maprotiline), and other atypical antidepressants such as trazodone. From the reviews of Scoggins, et al. (2) and Wong (1), the popular LC column choices include reversed-phase using C-18 and CN. Sutfin, D'Ambrosio and Jusko utilized a silica column and detector at either 214 or 254 nm for the determination of eight tri- and tetracyclic antidepressants and their major metabolites (3). McCormick, Nielsen and Jatlow described a RP-LC assay of alprazolam (a recently introduced antianxiety and antidepressant drug) by using a C-18 column and detection at 202 nm (4). Lensmeyer and Evenson analyzed TCAs using a cyanopropyl column with recycled solvent and suggested that the separation was due to the predominant reversed-phase mode (5). Visser, et al., reported simultaneous TCA assay using a CN-bonded phase column (6).

Our initial study was undertaken to explore alternatives to the published reversed-phase analyses for TCAs. An amine/phosphate buffer, based on the work of Melander, et al. (7), was used as the

mobile phase. Kabra and associates (8) showed that a small amount of competing base in the mobile phase eliminated tailing of basic compounds and permitted the use of mobile phases in the "safe" acidic pH range. In the present study, a mobile phase of n-nonylamine/phosphate/ acetonitrile was used to compare the selectivity of three bonded phase columns - phenyl, C-8, and CN - for the separation of seven TCAs. Based on this comparison, the phenyl column was chosen for further investigation. The application of the phenyl column for TCA analyses with fluorescence detection was previously reported by Reece, Zacest and Barrow (9). The present study differed from the above procedure in column configuration, mobile phase, and method of detection. Phenyl column analyses of other drugs such as theophylline and caffeine had been recently investigated by Wong, et al. (10).

In optimizing the analysis time in this study, the first approach was to divide the compounds into two groups rather than trying to achieve complete baseline resolution of all TCAs. Group selection, based on clinical drug administration practice, shortened the overall analysis time from 15 minutes to 8 minutes when using a 15 cm phenyl column. A second approach was the substitution of two 5 cm phenyl mini columns for the 15 cm analytical column. Two reasons were proposed for this column configuration. First, the overall shorter column length (10 cm) would result in faster analysis time, thus reducing solvent consumption. Second, the first mini column would serve as a guard column, and could be discarded when column performance

deteriorates. The second column would then replace the discarded column as a guard column, and a new column would be attached at its outlet, minimizing column replacement cost. Another time-saving measure was the modification of published sample preparation techniques (11), replacing silanized glass tubes with polypropylene tubes to minimize drug adsorptive loss. An additional modification of that procedure was the use of 800 ng clomipramine as the internal standard.

Preliminary data of column selectivity and comparison studies, presented earlier (12, 13), are summarized here. These experiences and the results of a long-term two and a half years' clinical evaluation indicate that the described procedure may be used alone or in complimentary fashion to a procedure utilizing straight-chain functional column such as C-18 for enhancing TCAs monitoring - especially for patient treatment in combination therapy with neuroleptics.

MATERIALS AND METHODS

Reagents

Acetonitrile (ACN), hexane and methanol, distilled in glass, ultra-violet grade, were purchased from Burdick and Jackson Labs. (Muskegon, Mich. 49442). Isoamyl alcohol, orthophosphoric acid, potassium dihydrogen phosphate and sodium dihydrogen phosphate were "Baker-Analyzed" Reagent Grade (Phillipsburg, N.J. 08865). N-nonylamine was purchased from Sigma Chemical Co. (St. Louis, MO 63178). Imipramine, desipramine, amitriptyline and nortriptyline

were obtained from drug companies as described previously (11). Doxepin and n-desmethyl doxepin were gifts of Pfizer Pharmaceutical (New York, NY 10017). Drug standards were also prepared as previously described (11).

Mobile Phase

For the preparation of mobile phase, 11.04 gm of $NaH_2PO_4.H_2O$ was dissolved in 4 liters of water and the pH was adjusted to 3.0 with ortho-phosphoric acid. Then, 7.3 ml of n-nonylamine was added, followed by stirring the soapy solution for at least one half-hour inside the hood for adequate ventilation. The solution was filtered and refrigerated until analysis. Then it was mixed with ACN (9:1) and degassed.

Instrumentation

Two separate chromatographs were used to carry out the TCAs analyses. For analysis with the uBondapak C-18 column (4.6 mm x 30 cm) (Waters Assoc., Milford, MA 01757), connected to a Bondapak/Porasil guard column, the chromatograph used was one previously described (11). For the other analysis with two mini phenyl columns (4.5 x 50 mm) (IBM Instruments), the chromatograph used was a ternary gradient liquid chromatograph, Model LC/9533, equipped with a Model LC/9523 variable wavelength detector, set at 214 nm (IBM Instruments).

Sampling

Patients were from the in-patient and out-patient clinics of our hospitals and other affiliated hospitals. Generally, monitoring was recommended after at least 2 weeks of therapy, and 1/2 hour

before, or 12 hours after ingestion. A syringe was used to draw blood, followed by emptying the content into an EDTA, silanized glass test tube. Alternatively, a venipuncture was performed and blood was collected in a Lavender Top Vacutainer Brand tube (Becton-Dickinson, NJ 07070). The clinical efficacy of this device for TCA sampling was established from a previous study (14). After centrifugation of these tubes, the plasma was transferred to a marked polypropylene tube for frozen storage until analysis.

Extraction

Extraction procedure was similar to the published procedures (11,14-16). Briefly, after the addition of 800 ng of clomipramine as the internal standard (note that 800 ng of NOR was used for the ND-DOX and DOX assay by the C-18 procedure) the drugs were extracted from alkalinized plasma with *n*-hexane/isoamyl alcohol (99:1) and back-extracted by dil. phosphoric acid. About 50 µl of the total 150 to 175 µl of TCA extracts was used for TCAs analyses by either the C-18 or phenyl column procedures. (It is important to note that at least two injections can be made using the manual injection technique.)

Chromatographic Conditions

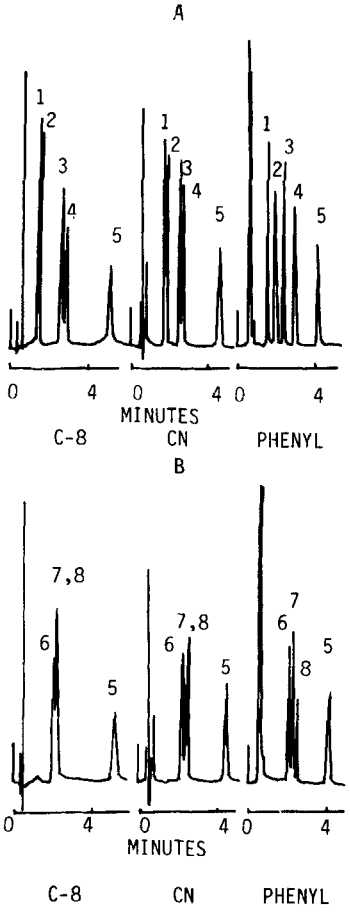
C-18 analysis was performed as described previously (11), except that the column temperature was 60°C for ND-DOX and DOX analyses and the mobile phase was phosphate/ACN (7:3). For the phenyl column, the flow rate = 3.0 ml/min., detection = 214 nm at 0.01 to 0.05 AUFS, and injection volume = 50 µl.

Quantitation and Statistical Analysis

Peak height ratios of standards were plotted against the drug concentrations. Quality control and patients' TCAs concentrations were estimated from these plots. Correlation of TCAs values from both procedures were evaluated by Linear Regression of the Advance Statistical Analysis (Radio Shack, Fort Worth, TX 76111).

RESULTS

Figures 1A and 1B show the chromatograms from preliminary studies using C-8, CN and phenyl columns. Using the stated chromatographic conditions, phenyl columns offered adequate resolution of six TCAs analyzed in two separate groups. Elution order for the first group was: ND-DOX, NOR, DOX, AMI, and I.S.; the second group: DES, protriptyline, IMI, and I.S. Since protriptyline is seldomly used, it was not included in the second group during the clinical study. As discussed previously in the introduction, the two phenyl "mini" column configuration was adapted. Mobile phase consisted of 10% instead of 20% ACN, so as to allow "added" resolution in order to compensate for column deterioration during the long-term clinical study. Consequently, the elution order of the first group was changed as follows: ND-DOX, DOX, NOR, AMI, and I.S. Figures 2,3 and 4 show the chromatographic analyses of extracts of "drug-free" and patients plasma with TCA present. Total analysis time was about 8 minutes. Table 1 lists the linear regression analysis of calibration (between 25 ug and 800 µg/L) and recovery data. Precision study results are summarized in Table 2.



Figures 1A & 1B - Selectivity studies of C-8, CN and phenyl columns with the following chromatographic parameters: column dimension: 4.5 mm x 150 cm. Peak identification: 1. desmethyldoxepin; 2. *nortriptyline*; 3. *doxepin*; 4. *amitriptyline*; 5. clomipramine; 6. desipramine; 7. protriptyline; and 8. imipramine.

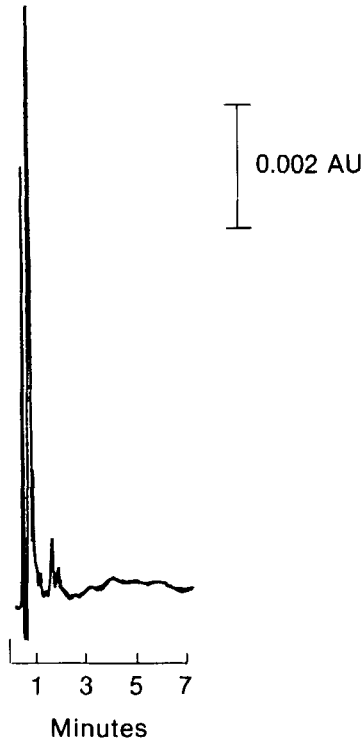


Figure 2 - Chromatogram of a "Drug Free" plasma extract, analyzed by the phenyl column.

Table 1

Linearity and Recovery Data

	r^2	Slope	y-intercept	Recovery
DES	0.998	3.68 X 10-3	-0.130	71%
IMI	0.999	2.91 X 10-3	-0.010	84%
NOR	0.999	4.52 X 10-3	-0.016	71%
AMI	0.999	3.23 X 10-3	-0.024	72%
ND-DOX	0.999	8.13 X 10-3	-0.069	86%
DOX	0.999	5.33 X 10-3	-0.035	84%

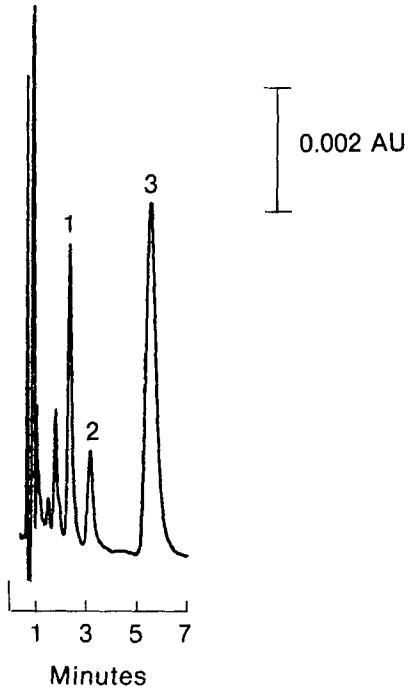


Figure 3 - Chromatogram of the patient plasma extract containing: 1. desipramine (119 ug/L); 2. imipramine (55 ug/L); and 3. clomipramine (internal standard), analyzed by the phenyl columns.

Table 2

Precision Data

	Within-Run			Day-to-Day		
	Mean Conc. ug/L	CV	n	Mean Conc. ug/L	CV	n
DES	147.2	2.9%	6	151.8	6.6%	34
IMI	156.1	3.0%	6	161.5	4.0%	34
NOR	147.8	3.2%	6	153.4	6.2%	31
AMI	150.3	2.2%	6	151.1	2.5%	31
ND-DOX	152.9	1.9%	6	152.5	6.9%	31
DOX	151.4	1.8%	6	150.8	6.3%	31

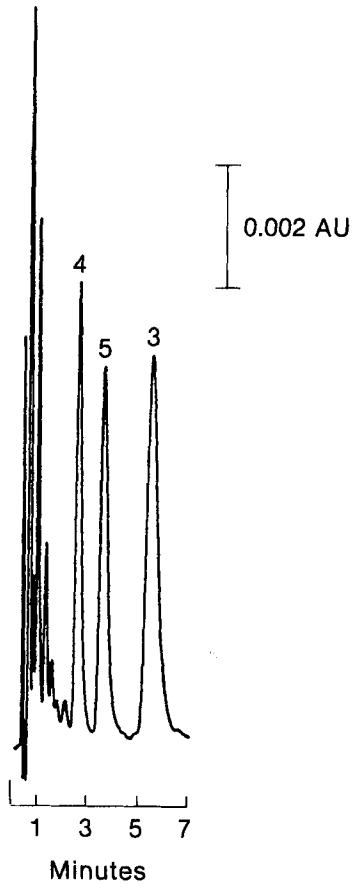


Figure 4 - Chromatogram of patient plasma extract containing: 4. nortriptyline (162 ug/L); 5. amitriptyline (197 ug/L); and 3. clomipramine (internal standard), analyzed by the phenyl columns.

Table 3

Capacity Factors, k', of Some Common Drugs

Barbital	1.7	7-OH-amoxapine	8.9
Acetaminophen	3.2	8-OH-amoxapine	8.9
Codeine	3.2	Trazodone	8.9
Meperidine	3.2	Desipramine	9.3
Chlordiazepoxide	3.5	Perphenazine	11.7
Phenobarbital	3.5	Flurazepam	11.7
Amobarbital	3.5	Phenytoin	11.7
Pentobarbital	4.4	Imipramine	12.0
Cimetidine	5.3	Nortriptyline	12.8
Secobarbital	5.3	Amoxapine	14.3
ND-Maprotiline	6.2	Chlorpromazine	16.2
ND-Doxepin	6.7	Amitriptyline	16.6
Maprotiline	7.1	Fluphenazine	17.1
Gluthethimide	8.0	Oxazepam	17.1
Propoxyphene	8.0	Lorazepam	22.5
Doxepin	8.9	Clomipramine	24.4
		Diazepam	27.9
		Thioridazine	50.8

Table 4

TCA Analysis by both methods for a six-month period

	IMI/DES	AMI/NOR	DOX/ND-DOX	TOTAL	% OF C-18 ANALYSES
1. Total C-18 Analyses	103	30	15	148	---
2. Confirmed Phenyl Col. Analyses	9	1	4	14	9.4%
3. Un-confirmed Phenyl col. Analyses	2	none	none	2	1.4%

Detection limits, based on S/N of 5, are about 1 ng. Table 3 lists the capacity factors of drugs checked for chromatographic interference. Figure 5 and 6 show the correlation plots of TCAs concentrations of patients, and patients and quality control, quantified by these two procedures. Figures 7 and 8 represent selected chromatograms, showing the selectivity of the phenyl column in resolving chromatographic interference peaks from other drugs, co-ingested by two patients treated with TCA, while Figure 9 shows the interference was not resolved for the analysis of another patient. Table 4 summarizes the result of TCA analyses by both methods within a six-month period.

DISCUSSION

With the emphasis of TCAs monitoring in the late 1970's, numerous articles have been published dealing with various aspects of clinical pharmacology and pharmacokinetics, sampling and quantitation methodologies. Reliability of clinical laboratory measurement, as shown by the American Association for Clinical Chemistry and the College of American Pathologists survey results, has markedly improved. However, with the exception of nortriptyline and imipramine, plasma levels of other TCAs do not correlate well with clinical response. Recently, the combination of plasma level and electrocardiogram QRS values has been proposed to be useful for providing rational antidepressant therapy (17-19).

In carrying out TCA measurements, the majority of the procedures utilized reversed-phase C-18 or CN columns. In the present study, another column packing, phenyl, was used in

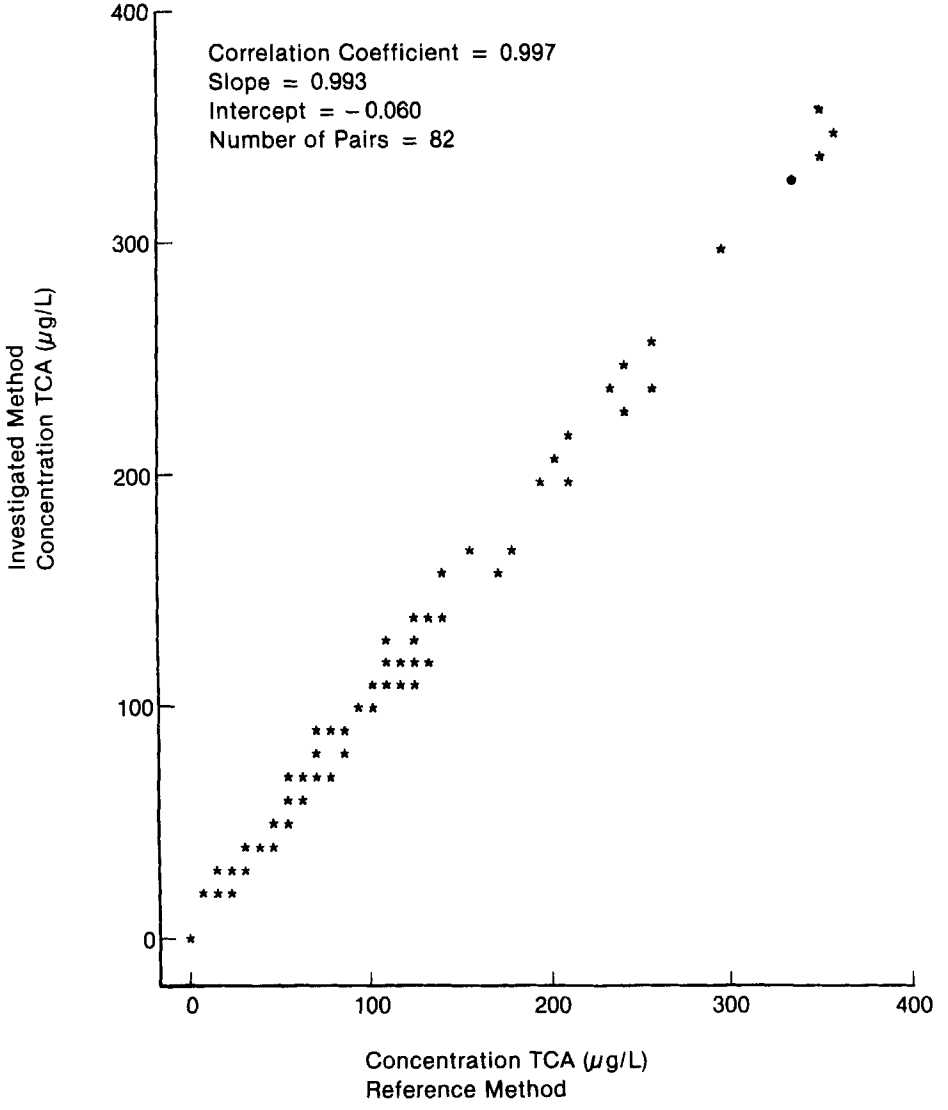


Figure 5 - Patient data. Comparison of plasma levels of TCAs obtained by two methods. Investigative method = phenyl column vs. reference method = C-18 column.

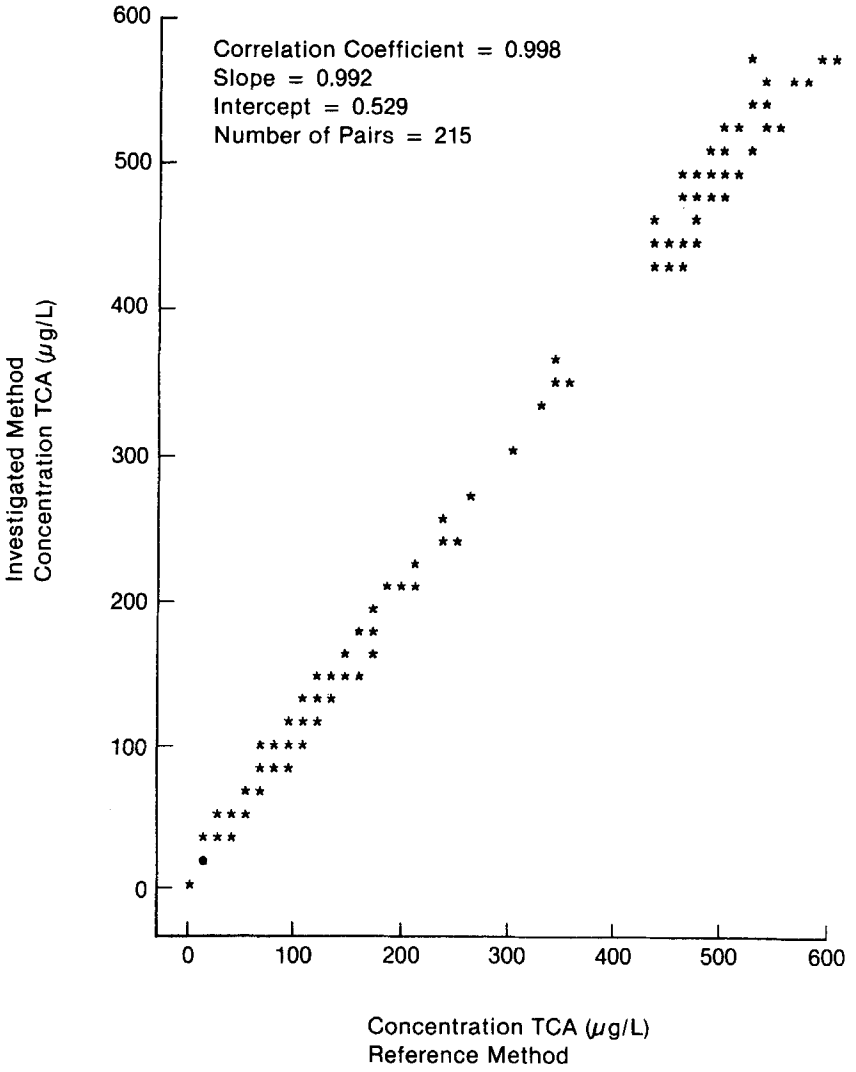


Figure 6 - Patient and quality control data. Comparison of plasma levels of TCAs obtained by two methods.

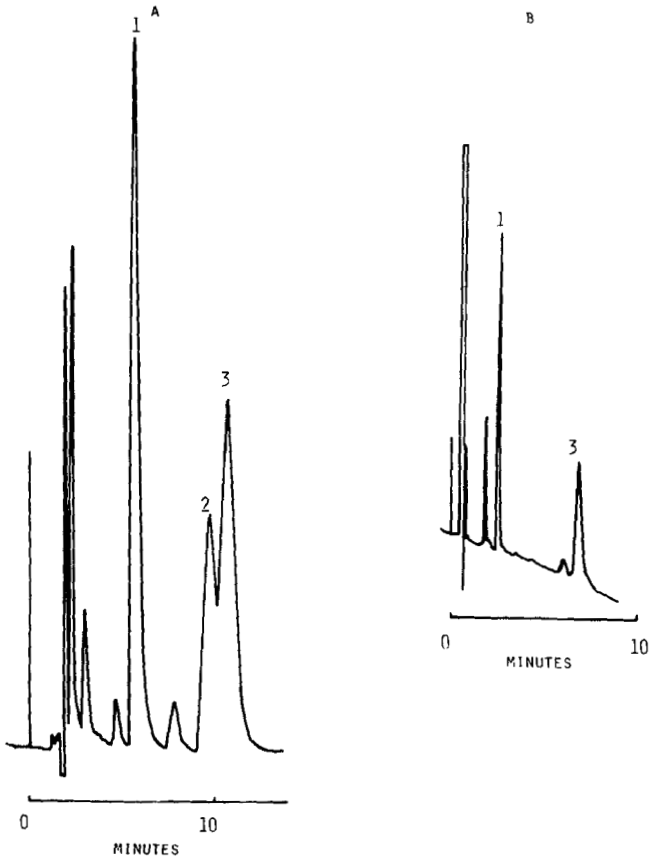


Figure 7 - Chromatograms of the plasma extract of a patient medicated with desipramine and other unidentified drug(s), (A) C-18 column, and (B) phenyl column. (Note that the interference was resolved by the phenyl column.) (Peak identification: 1. desipramine, 794 ug/L, 2. unidentified, and 3. I.S.)

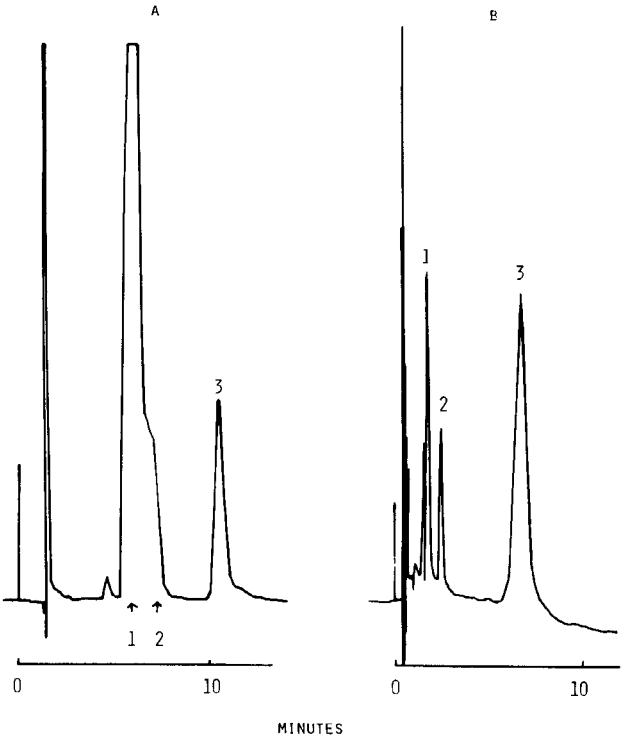


Figure 8 - Chromatogram of plasma extract of a patient medicated with doxepin and other drug(s), (A) C-18, and (B) phenyl. (Note that the interference was resolved by the phenyl column.) (Peak idenitfication: 1. N-DOX, 120 ug/L, 2. DOX, 113 ug/L, and 3. I.S. - NOR for Figure 8A and Clomipramine for Figure 8 B.)

combination with n-nonylamine/phosphate/acetonitrile as the mobile phase. The emphasis of this study was to investigate the different selectivity afforded by the phenyl ring structure as compared to that of the straight chain of the C-8, or the predominantly reversed phase character of the CN. Since TCAs and potentially interfering neuroleptics are heterocyclic and/or unsaturated compounds, the phenyl column may offer unique selectivity via

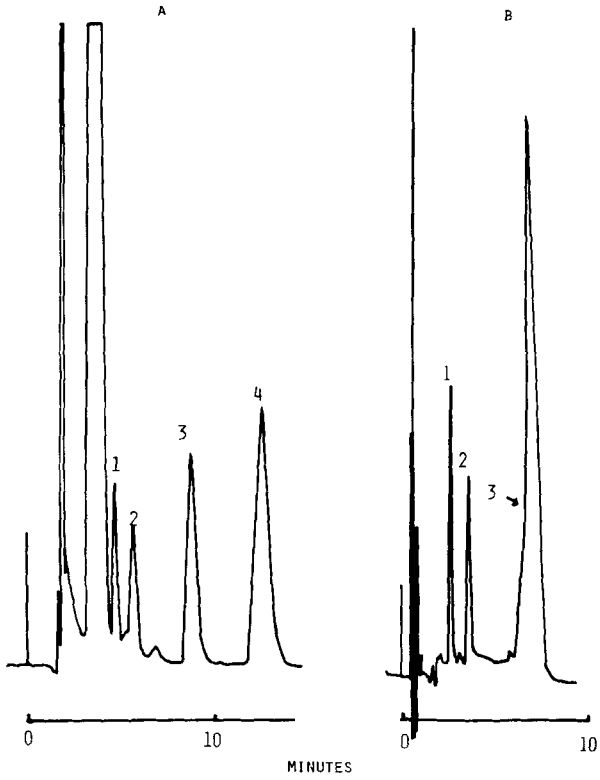


Figure 9 - Chromatograms of plasma extract of a patient medicated with imipramine and thioridazine, (A) C-18; (B) Phenyl (Note that the interference was not resolved by the phenyl column.) Peak identification: 1. DES, 121 ug/L, 2. IMI, 126 ug/L, 3. I.S., and 4. thioridazine.

mechanisms of cyclic interactions (20), π-π interactions (21), and/or aromatic stacking interactions (22). This would be expected to enhance the resolution of interfering drugs and metabolites from the TCAs studied. Although such an approach has not proven to be totally successful, as shown by later patient studies, the concept of using the phenyl column for general drug analysis merits further

investigation. This is based upon the heterocyclic structure of a vast number of drugs from antiasthmatics - theophylline; anticonvulsants - phenobarbital; antiarrhythmics - procainamide, and others. Indeed, the phenyl column could be used for theophylline and caffeine analysis (10).

Figures 1A and 1B show the results of preliminary comparisons of the phenyl, C-8, and CN columns. Under the same conditions of flow rate and mobile phase, phenyl offered better resolution than either CN or C-8. (However, by changing conditions, adequate resolution could be obtained with a CN column (prepared by another manufacturer), as demonstrated by previous studies (5,15,16) for TCA monitoring.) A proposed mechanism of molecular interaction between components of the stationary phase and the mobile phase is that the base (nonylamine) competes with the analytes for unreacted silanol sites of the silica (7). This competition minimizes the peak tailing associated with analyzing basic compounds in the reversed-phase mode. After initial equilibration time (1 hr. for both phenyl and CN, and over 24 hr. for C-8), silanol sites are effectively masked and run times would stabilize. This difference in the initial equilibration times may substantiate the competing base theory. The short chain length of the chemically bonded cyano group permits ready access of nonylamine to silanol sites. For the phenyl column, steric hinderance does not permit the phenyl rings to cluster as closely together as the straight chains of the octyl column, allowing nonylamine of the mobile phase to interact more rapidly with silica backbone of the phenyl phase. The longer

equilibration time noted for the octyl column most likely results from the tightly-packed, straight carbon chains of the octyl moiety, hindering ready access of nonylamine to the unreacted silanol sites.

After selecting the phenyl column, a "weaker" mobile phase of 10% of ACN instead of 20% was used to afford greater resolution, allowing for the resolution loss as the column deteriorates during long-term clinical use. As noted in RESULTS, the order of elution for the first group is ND-DOX, DOX, NOR, AMI, and I.S. Preliminary studies showed calibration was linear, and recovery ranged from 71 to 86%, comparable to the previously published procedure (11) and to the range cited by Scoggins, et al.(2). With acceptable coefficients of variations in the precision studies, preliminary comparisons of patient and quality control sample results showed that the clinical efficacy was comparable to a previously published TCA analysis using C-18 packing. The procedure was then evaluated for long-term studies to investigate two areas: first, the long term stability of the dual phenyl mini column configuration, and second, the ability to resolve interfering neuroleptics and their metabolites. The available data showed the first set of columns was stable up to 300 injections, while the second set, consisting of the second column of the first set and a new column, was stable up to 400 injections.

Due to the possible co-administration of TCAs with neuroleptics or other drugs, their chromatographic interference had occasionally prevented accurate estimation by the C-18 procedure.

with the different selectivity of the phenyl column, such interference was successfully resolved as shown by Figures 7B and 8B, but not resolved as shown by Figure 9B. The phenyl column was successfully used to resolve an interference peak with the internal standard as follows. Due to the presence of unknown drug(s), the C-18 analysis (Fig. 7A) shows that desipramine (1) and internal standard (3), elute at about 7 and 11 minutes, respectively, with an overlapping peak (2) at 10 minutes. Further inquiry into the patient history did not reveal any other medication. However, the possibility of other drug(s) could not be totally excluded. Due to the interference peak contributing to the internal standard peak height, an accurate estimation of peak height ratio and hence the patients' desipramine concentration could not be achieved. It may be possible to resolve these peaks by programming an integrator or computer, or to analyze by an alternate method - the phenyl column using the balance of the same drug extracts.

As noted previously, the total volume of TCA/phosphoric acid extract ranged from 150 to 175 μL. Having used about 50 μL for the C-18 column analyses, the balance, 100 to 125 μL would be sufficient for the phenyl column analysis. Further, these extracts, when refrigerated, were stable up to 24 to 36 hours, so that the second phenyl column analysis may be performed on the following day, allowing flexibility in scheduling. Indeed, when this extract was thus re-analyzed, as shown by Figure 7B, the internal standard (3) eluted at 8 minutes with complete resolution, allowing an accurate estimation of desipramine (794 μg/L).

In addition to resolving this interference peak with internal standard, the phenyl column was useful in resolving an interference peak during a doxepin analysis. Figure 8A shows that n-desmethyl doxepin (1) and doxepin (2) were "covered" by a large interference peak, which did not affect the internal standard nortriptyline (3). Again, patient history did not reveal other drugs. Phenyl column analysis (Figure 8B) shows n-DOX and DOX eluted completely free of interference.

However, for monitoring TCA plasma concentration of the patient medicated with thioridazine, the phenyl column procedure suffered from interference of the I.S. Figure 9A shows the C-18 analysis of a patient medicated with imipramine and thioridazine, a neuroleptic. DES, a metabolite of IMI, eluted on the "trailing edge" of a large unknown peak, and thioridazine (4) eluted at 13 minutes (after the internal standard). Since thioridazine is metabolized to 6 to 7 metabolites, the large unknown peak, possibly one or several of thioridazine metabolites could affect DES measurement. Thus, the phenyl column was used. As shown by Figure 9B, both DES (1) and IMI (2) eluted with complete resolution. However, the internal standard, indicated by arrow (3), co-eluted with an unidentified peak, possibly one of thioridazine's metabolites but not thioridazine, since the thioridazine eluted with $k' = 50.8$, much larger than that of I.S. ($k' = 24.4$). An alternative would be to use another TCA, such as amoxapine ($k' = 14.3$), as the internal standard.

As a result of this experience, our laboratory has adopted the following criteria for confirmation by the phenyl column procedure:

1. Patient History - If neuroleptics such as fluphenazine and perphenazine are co-administered with the chosen TCAs, as indicated by the patient's treatment protocol, phenyl column analysis would be used to confirm the result. For patients with thioridazine, phenyl column analyses with another I.S. such as amoxapine should be attempted.

2. Chromatographic interference - This may be due to known or unknown drugs and/or metabolites. Phenyl column analysis should be attempted as soon as possible.

3. High TCAs plasma concentration - For the concentration exceeding 500 µg/L, phenyl analysis would be needed to confirm for the lack of potential interference, which could contribute to the peak height, resulting in an erroneously high value.

4. Patients showing cardiotoxicity/seizures side effects - For these patients, phenyl column analysis would be useful to confirm that cardiotoxicity/seizure may be due to high plasma TCA concentrations.

Inherent with following the above four criteria, the laboratory would require a thorough patient history, the latest clinical manifestations, and performing the phenyl analysis as soon as technically feasible.

Using the above criteria for a six-month period, the result of using the phenyl column analysis for confirmation is outlined in Table 4. Of a total of 148 patient TCA analyses using the C-18 procedure, 16 (10.8%) required confirmation. Within this group, 14 (9.4%) were "confirmed", while 2 (1.4%) were "unconfirmed" and

exclusively for the IMI/DES group. This experience was typical of the two and one-half years long-term study. In this time period, the total number of patient's TCA analyses by C-18 procedure was 893, indicating that about 96 confirmations by phenyl analysis were performed.

A technical consideration for using the phenyl column is the need for column conditioning. Since the phenyl columns initially used for this study were packed under normal phase condition, isopropanol was pumped prior to use in reversed-phase analyses. Recently, the manufacturer has introduced phenyl columns packed in reversed-phase solvents that do not require this conditioning step.

In comparison to the previously reported C-18 column analysis, several advantages of the phenyl column are apparent. The phenyl analysis is performed at ambient temperature, whereas the C-18 column analysis requires elevated temperature to suppress peak tailing. For this reason, the phenyl column method uses instrumentation readily available in most clinical laboratories. This study also shows that the use of polypropylene tubes is acceptable for clinical analyses of TCAs. Some disadvantages of this reported phenyl column method are the short column life, and the need to organize the TCAs into two groups, rather than analyzing all TCAs simultaneously.

From our long-term study, the phenyl column has been shown to equilibrate quickly and reliably. Due to its ease and ability to resolve interference and the possible adaptation of the novel two mini column approach, the phenyl columns may be readily used as a

routine and/or confirmation procedure for TCA monitoring, providing a valuable alternative to the straight-chain C-18 or CN column.

ACKNOWLEDGEMENT

This study was supported partially by a grant from IBM Instruments. The authors also expressed their sincere appreciation to Ron Lewis for his support, to Sandy DellaFera, Rosinda Fernandes, and David Bogdan, for their superb technical assistance, and to Mary Gagnon for preparing the manuscript.

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