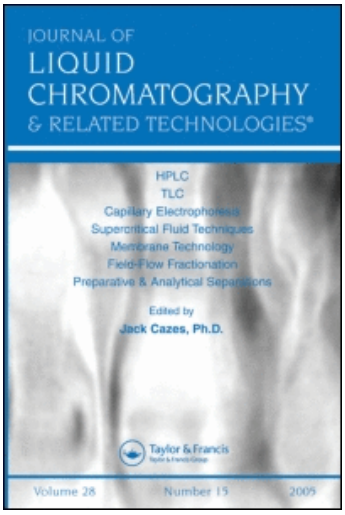

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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To cite this Article Ryan, Timothy W.(1993) 'Identification and Quantification of Tricyclic Antidepressants by Uv-Photodiode Array Detection with Multicomponent Analysis', Journal of Liquid Chromatography & Related Technologies, 16: 7, 1545 — 1560
To link to this Article: DOI: 10.1080/10826079308020972
URL: http://dx.doi.org/10.1080/10826079308020972

JOURNAL OF LIQUID CHROMATOGRAPHY, 16(7), 1545-1560 (1993)

IDENTIFICATION AND QUANTIFICATION OF TRICYCLIC ANTIDEPRESSANTS BY UV-PHOTODIODE ARRAY DETECTION WITH MULTICOMPONENT ANALYSIS

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ABSTRACT

The procedure described is a method for the identification and quantification of tricyclic antidepressants (TCA's) by HPLC UV-photodiode array detection. By incorporating both retention and spectral characteristics into analyses of known standards, ten different TCA's (amitriptyline, amoxapine, carbamazepine, cyclobenzaprine, desipramine, doxepin, imipramine, maprotiline, nortriptyline and protriptyline) were accurately identified and quantified at concentrations down to less than 1 μg/ml. In cases where chromatographic resolution was incomplete, decomposition of the composite spectra by multicomponent analysis (MCA) provided accurate identification and quantification of the individual constituents.

INTRODUCTION

Tricyclic antidepressants (TCA's) are commonly prescribed for disorders ranging from depression to grand mal seizures. There are many methods for the separation and identification of these compounds in a variety of sample types. Hughes and Osselton offer a comparison of techniques for the analysis of TCA's

in whole blood[1]. Dorey et al have evaluated HPLC vs enzyme immunoassay techniques[2]. Mazhar and Binder provide extensive data on an HPLC method for the analysis of TCA's and a variety of related materials[3], while Segatti et al and Lucarelli demonstrated simultaneous determination of desipramine, nortriptyline, imipramine, amitriptyline and clomipramine[4]. Ni et al discuss the automatic determination of imipramine and clomipramine by HPLC[5], and Lin and Frade provide a method for a number of TCA's by HPLC[6]. Because of the wide use of these drugs, a number of examples in the literature discuss sample preparation techniques. These examples are important because a variety of preparative techniques can be coupled to the numerous HPLC techniques in order to provide the analyst with the ability to tailor a specific extraction/preparation to separation regimen for a specific application. Matsumoto et al desribe a technique which employs column switching[7], while other articles discuss the use of solid phase extraction[3, 8-13]. In addition, Posluszny, Weinberger and Woolf have previously employed HPLC UV-photodiode array detection for identifying some TCA's (imipramine, desipramine, amitriptyline, nortriptyline and doxepin)[14].

While structurally very similar (Figure 1), the UV spectra of the TCA's are for the most part quite distinct (Figure 2). In those cases where the UV spectra are highly similar (such as amitriptyline and nortriptyline; desipramine and imipramine), retention time differences are sufficient to prevent peak misidentification. Although UV-photodiode array spectra do not contain the informational content provided by other spectral techniques (such as mass spec), it has been proven to be very accurate at distinguishing among even highly similar UV spectra at sample concentrations as low as 1 μg/ml[15,16]. These experiments describe a method for HPLC UV-photodiode array detection as a means of providing separation, identification and quantification of TCA's. Although this method does not achieve baseline chromatographic resolution of all ten TCA's simultaneously, by using the combination of retention time and spectral comparison to known standards, the drug substances can be accurately identified and quantitated. In cases where coelution can occur, available software provides for the decomposition of the composite spectra into its *individual* constituents for identification and quantification. Other spectroscopic techniques that employ multicomponent analysis (MCA), such as ICP-AES, use

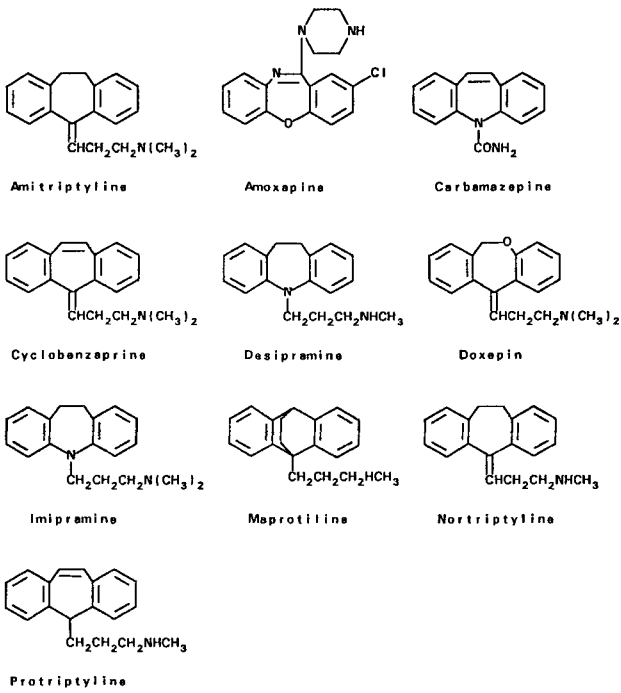


FIGURE 1.

TRICYCLIC ANTIDEPRESSANT STRUCTURES

Kalman filtering in order to provide composite spectral interpretation[17]. In the case of this photodiode array detector, the interpretation of multicomponent spectra is limited to an overdetermined system of linear equations, which is performed by a least squares method. Excoffier et al provide an extensive description on this technique as it applies to the interpretation of the multicomponent spectra encountered in these experiments[18].

MATERIALS AND METHODS

The equipment used included a Model 9010 pump, a Model 9095 autosampler and a Model 9065 photodiode array detector (all by Varian

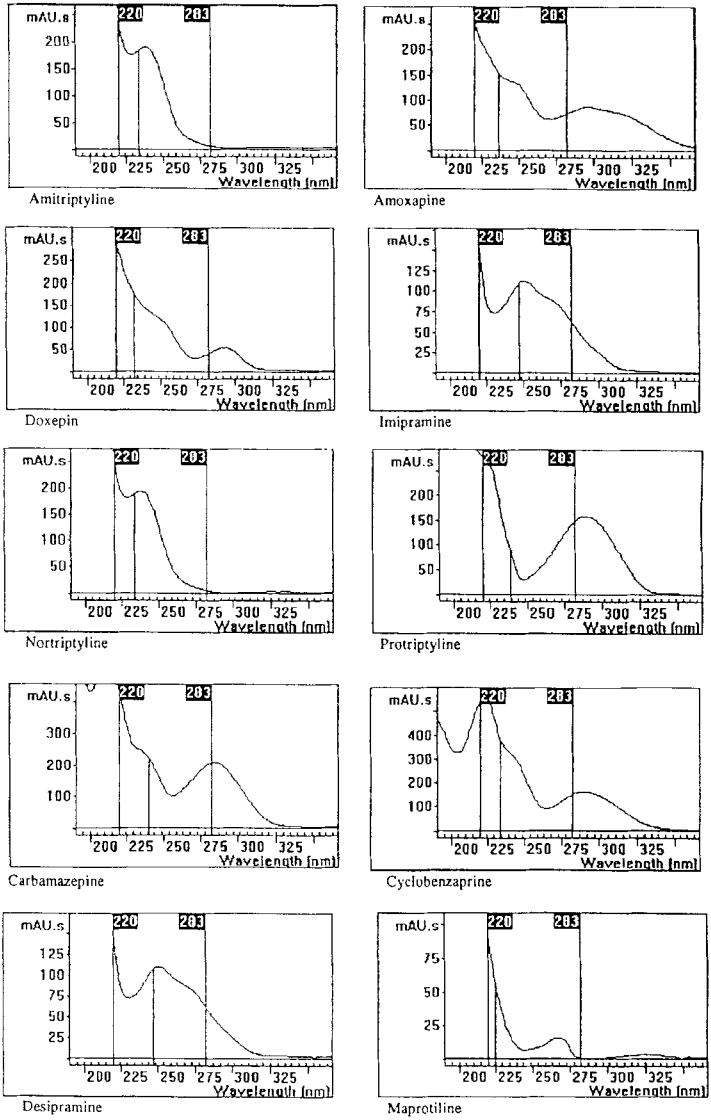


FIGURE 2

UV SPECTRA OF TRICYCLIC ANTIDEPRESSANTS

Instrument Division, Walnut Creek CA USA). System control was via the series 9020 LC Star Workstation, Revision C (Varian Instrument Division). Spectral manipulation and comparison were performed using PolyView™ version 2.02 (Varian Instrument Division). The LC control and spectral processing software function in a Windows™ environment (Windows Version 3.0, Microsoft Corporation, Redmond WA USA) on a 386 PC equipped with a math coprocessor. Separations were achived using a 250 x 4.6 mm Econosil C8 column (Alltech Associates, Inc., Deerfield IL USA). B&J Brand™ high purity acetonitrile was purchased from Burdick and Jackson (Baxter Healthcare Corporation, Muskegon MI USA). Distilled de-ionized water was provided by a NANOpure II water purification system (Barnstead Thermolyne, Dubuque IA USA). Samples of amoxapine, carbamazepine, desipramine, imipramine, maprotiline, protriptyline and nortriptyline were purchased from the United States Pharmacopeia (Rockville MD USA). Samples of amitriptyline, cyclobenzaprine, and doxepin were provide by Ganes Chemicals, Inc. (Pennsville NJ USA).

The mobile phase used was 70/30 0.020M KH_2PO_4 - 0.014M TEA pH 3.0 (H_3PO_4)/acetonitrile. After mixing, the mobile phase was filtered and degassed by sonication under vacuum. Stock solutions of the TCA's were prepared in mobile phase. These solutions were diluted to provide standard solutions for both retention and spectral information. Table 1 lists the retention times of the reference standards and the stock solution concentrations. Each sample mix dilution was analyzed three times (20 µl inj) in order to ascertain the accuracy and precision. The analyses of the samples were compared to the spectral library created by analysis of standard solutions of the TCA's for identification and quantification.

RESULTS

Figure 3 is an example of the analyses of the Mix 1 sample. This sample contained amoxapine, amitriptyline, nortriptyline, imipramine and carbamazepine. While amoxapine, amitriptyline and carbamazepine are well

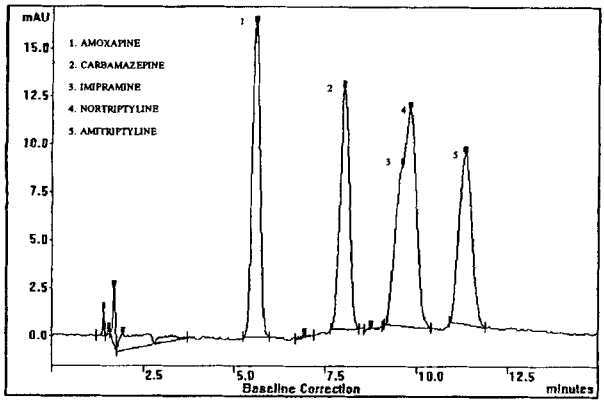
TABLE 1

TRICYCLIC ANTIDEPRESSANT STANDARDS

TCA	RETENTION TIME (min)	STOCK SOL'N CONC (μg/ml)
Amoxapine	5.505	505
Doxepin	6.402	414
Carbamazepine	7.287	398
Desipramine	7.311	400
Imipramine	8.343	404
Cyclobenzaprine	8.644	455
Protriptyline	8.786	370
Nortriptyline	9.861	413
Maprotiline	10.524	414
Amitriptyline	11.004	505

resolved, nortriptyline and imipramine are very close to coeluting and will require MCA for both positive identification and quantification.

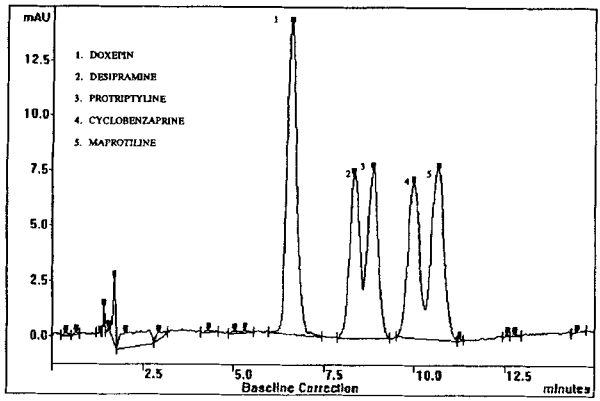
Figure 4 is an example of the analyses of the Mix 2 sample. In this sample the only baseline resolved peak is doxepin. The next two analytes, desipramine and protriptyline, are incompletely resolved from one another. Although the resolution is superior to that obtained for the nortriptyline - imipramine in Mix 1, since baseline resolution is not achieved MCA will also be used in the accurate identification and quantification of these materials. The same case is also true for the final pair of analytes, cyclobenzaprine and maprotiline.



Absorbance at 210 nm

FIGURE 3

HPLC ANALYSIS OF TCA MIX 1 SAMPLE



Absorbance at 210 nm

FIGURE 4

HPLC ANALYSIS OF TCA MIX 2 SAMPLE

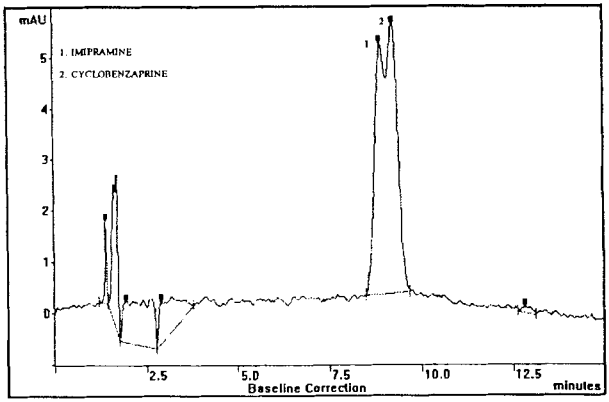


FIGURE 5

HPLC ANALYSIS OF TCA MIX 3 SAMPLE

Figure 5 is an example of the analyses of the Mix 3 sample. Only two components are present in this sample, cyclobenzaprine and imipramine. This sample was prepared to confirm the accuracy of the peak identification and quantification by MCA. Figure 6 is the MCA analysis report for the interpretation of the incompletely resolved peaks.

Figure 7 is an example of the analyses of the Mix 4 sample. This sample contained only carbamazepine and desipramine and represented the most challenging sample. The two analytes almost exactly coelute, hence interpretation of the composite spectra will be critical to the identification and quantification of the peaks. Figure 8 is the MCA analysis report for the interpretation of the composite spectra.

DISCUSSION

A summary of the results of the analyses is reported in Table 2. Triplicate injections of each sample and dilution were performed. The table

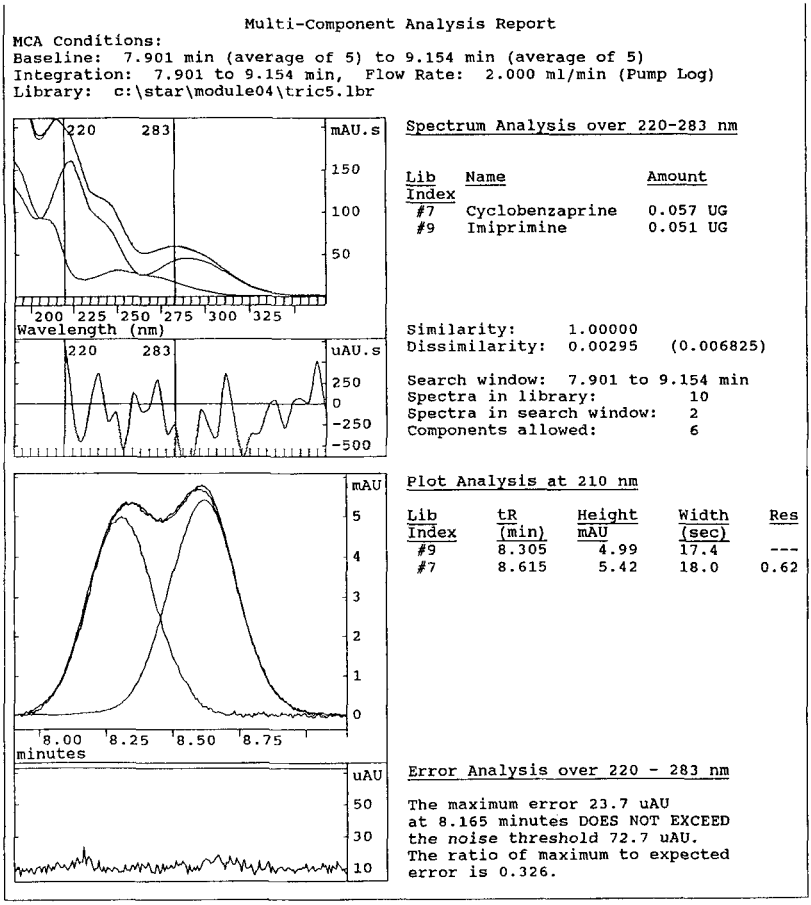


FIGURE 6

SPECTRA DECOMPOSITION BY MCA (MIX 3)

compares the amount of each compound as determined by quantitative analysis using the photodiode array detector to the calculated amount based on the concentrations of the stock solutions. In the Dilution #1 samples, 500 µl of each analyte stock solution was mixed with mobile phase in a 100 ml volumetric flask, in the Dilution #2 samples 200 µl was used and in the Dilution #3 samples 100 µl was used. Concentrations for the Dilution #3 samples ranged

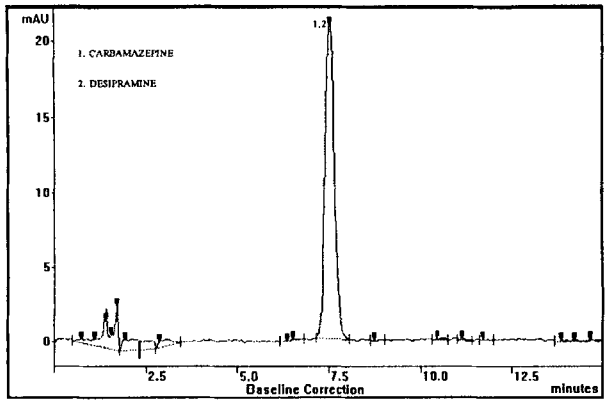


FIGURE 7

HPLC ANALYSIS OF TCA MIX 4 SAMPLE

from 0.75 to 1.0 μg/ml. In all samples and dilutions, the analytes were correctly identified.

In the cases of amoxapine, doxepin and amitriptyline the chromatographic resolution was sufficient to provide accurate identification. Because these compounds were also baseline resolved, quantification could be accomplished by the use of an external standard. In the course of these experiments, quantification of all sample components was performed by area spectrum analysis using the photodiode array detector. Area spectra are defined as the measurement of peak area in three dimensions, time (sec), amplitude (AU), and wavelength (nm). As with the two dimensional area encountered when using single wavelength UV detection (t vs abs), the three dimensional area spectrum is proportional to the concentration of the analyte(s) present. Adding the third dimension of λ is essential to MCA.

Because UV spectra are being evaluated, the MCA software is limited in its ability to interpret sample spectra that may be comprised of more than 1

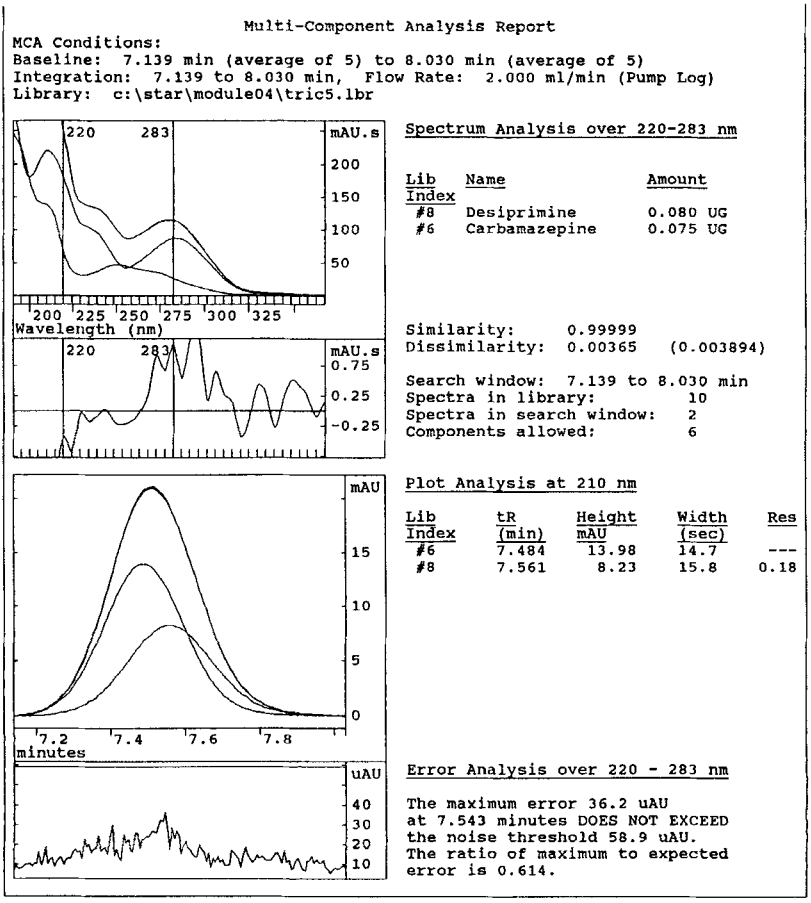


FIGURE 8

SPECTRA DECOMPOSITION BY MCA (MIX 4)

highly similar spectra. Therefore, the only separations that were absolutely critical were those that involved analytes with similar spectra i.e., amitriptyline from nortriptyline and desipramine from impramine (see Figure 2). Peak identification is therefore based on a combination of the analytes UV spectra and retention time. The UV absorbance is intrinsic to the specific compound. However, the retention time is a variable that depends on a number of

TABLE 2
QUANTITATIVE ANALYSIS BY UV-PHOTODIODE ARRAY

	Dilution #1 (µg)		Dilution #2 (µg)		Dilution #3 (µg)	
MIX 1	**PDA**	**CALC**	**PDA**	**CALC**	**PDA**	**CALC**
Amitriptyline	0.083 0.084 0.082	0.088	0.033 0.031 0.033	0.035	0.015 0.016 0.015	0.018
Amoxapine	0.104 0.104 0.105	0.101	0.042 0.041 0.042	0.040	0.020 0.021 0.021	0.020
Carbamazepine	0.076 0.075 0.076	0.080	0.030 0.030 0.030	0.032	0.014 0.014 0.015	0.016
Imipramine	0.076 0.076 0.076	0.081	0.029 0.029 0.031	0.032	0.013 0.012 0.016	0.016
Nortriptyline	0.077 0.077 0.078	0.083	0.030 0.032 0.031	0.033	0.015 0.015 0015	0.017
MIX 2						
Desipramine	0.078 0.078 0.077	0.080	0.030 0.031 0.030	0.032	0.015 0.013 0.014	0.016
Doxepin	0.079 0.079 0.079	0.083	0.033 0.032 0.032	0.033	0.015 0.015 0.015	0.017
Cyclobenzaprine	0.084 0.084 0.083	0.091	0.031 0.033 0.033	0.036	0.017 0.018 0.014	0.018
Maprotiline	0.075 0.078 0.079	0.083	0.028 0.033 0.036	0.033	0.014 0.016 0.016	0.017
Protriptyline	0.073 0.073 0.073	0.074	0.028 0.028 0.028	0.030	0.014 0.014 0.014	0.015
MIX 3						
Cyclobenzaprine	0.059 0.059 0.057	0.091	0.033 0.034 0.033	0.036	0.016 0.017 0.016	0.018
Imipramine	0.051 0.052 0.056	0.081	0.030 0.029 0.032	0.032	0.015 0.015 0.015	0.016
MIX 4						
Carbamazepine	0.075 0.076 0.078	0.080	0.031 0.031 0.031	0.032	0.015 0.015 0.015	0.016
Desipramine	0.080 0.081 0.081	0.080	0.031 0.031 0.030	0.032	0.015 0.015 0.014	0.016

influencing factors. For this reason, great care must be taken to insure that the chromatographic system is thoroughly equilibrated before the analyses begin. If the chromatographic system is not equilibrated and significant retention time wandering occurs between samples and standards, peak misidentification (and inaccurate quantification) can result. In the case of extended analysis times and/or a large number of samples, the mobile phase container must be tightly sealed to prevent the evaporation of the organic or any volatile modifiers, the net result of which is to change the mobile phase composition and alter retention characteristics over time. An accurate deduction from these observations is that standards need to be run on a daily basis. Since retention time variations normally can occur as a result in minor fluctuations of chromatographic parameters, creation of a permanent quantitative spectral library is not feasible. However, since accurate quantitative analysis routinely requires a standard to be analyzed concurrently with the samples, the daily creation of quantitative spectral library does not represent a negative aspect of the technique.

In order to insure a high degree of accuracy for both identification and quantification, the "time window" for spectral evaluation was kept as narrow as possible. Each peak or composite peak was evaluted independently from the other analytes in the sample (when present). By doing this, only the minimum number of potential matches were considered for each inquiry.

In Table 2, a high degree of both accuracy and precision is observed for the PDA quantified values. Generally, all three analyses of each analyte in each sample dilution was within 10% of the calculated value. The only exception were the values observed for both cyclobenzaprine and imipramine in the Mix 3 Dilution #1 sample. For both compounds in this sample, the PDA determined value was only about 65% of the calculated value. Since the analyses of the other dilutions of this sample agree with the cyclobenzaprine values observed in Mix 2 (Dilutions #2 and #3) and the imipramine values observed in Mix 1 (Dilutions #2 and #3), it is likely that the aberration was due to an error in sample dilution. The results from the Mix 4 sample are particularly notable. Despite the fact that chromatographically these were the poorest resolved compounds, the PDA determined values agree strongly with the calculated values. This is also true for the imipramine and nortriptyline in Mix 1, which were also poorly resolved.

CONCLUSION

The technique of HPLC UV-photodiode array detection with multicomponent analysis (MCA) was successful in providing accurate identification and quantification of tricyclic antidepressants. High degrees of accuracy and precision were observed when comparing the PDA determined sample values with the calculated values. Primarily these experiments evaluate the use of photodiode array - MCA as a routine tool for HPLC analysis. Because complete chromatographic resolution of the sample peaks is not necessary when using MCA, method development can be greatly accelerated. When the coelution of analytes with similar spectra cannot be avoided, optimization of spectral discrimination by interpretation of a particular absorbance region where maximum spectral dissimilarity occurs can be used to provide accurate identification and quantification[19]. Such an enhancement of method development capabilities will benefit the many industries where HPLC analyses are common. It is of particular value in the chemical process environments such as bulk pharmaceutical manufacturing, where confirmation of product purity and the generation of accurate product purity profiles is of distinctive interest to the FDA[20].

ACKNOWLEDGEMENT

The author gratefully acknowledges the assistance of Terry L. Sheehan and Jean-Louis Excoffier in the preparation of this manuscript.

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Received: September 12, 1992
Accepted: September 21, 1992