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SEPARATION OF THE INDOLEALKYLAMINES AND THEIR ANALOGOUS TETRAHYDRO-β-CARBOLINES BY LIQUID CHROMATOGRAPHY

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SUMMARY

Liquid chromatographic procedures for the separation of the N-methylated and analogous tetrahydro-β-carboline derivatives of 5-hydroxytryptamine, 5-methoxytryptamine, and tryptamine are described. The methods involve the use of normal-phase chromatography and chromatography on both ODS and strong cation-exchange columns. The judicious use of these procedures allows the unique chromatographic characterisation of each of the compounds studied. When combined with fluorescence monitoring of the column eluents sub-nanogram quantities of both the indolealkylamines and tetrahydro-β-carbolines can be detected.

INTRODUCTION

Both the psychotomimetic indolealkylamines [1—4] and their structurally analogous tetrahydro-β-carbolines [5—9] have now been detected in mammalian body fluids and tissues including those of the human. Enzymatic mechanisms for the in vivo and in vitro synthesis of these compounds have also been described [10—13].

While liquid chromatographic procedures for the separation of the indole-

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amines and their β-carboline derivatives, prior to analysis, have previously been described [6, 14, 15] on-line detection has largely been confined to UV monitoring.

In a recent study [10] we presented a rapid liquid chromatographic procedure for the separation and detection of the products of rabbit lung indoleamine N-methyltransferase activity. In addition a preliminary study of the separation of tryptamine, its N-methylated derivatives and their β-carboline analogues was presented. We now report a detailed and systematic study of the separation and detection of the N-methylated derivatives and analogous β-carbolines of tryptamine, 5-hydroxytryptamine and 5-methoxytryptamine. Several chromatographic systems involving the use of strong cation-exchange, silica and ODS reversed-phase columns are described. The judicious use of these procedures may permit the unique chromatographic characterisation of each of these compounds. When combined with fluorometric detection sub-nanogram quantities of both the indolealkylamines and β-carbolines can be detected.

EXPERIMENTAL

Materials

L-Cysteine hydrochloride, N,N-dimethyltryptamine (DMT), N-methyltryptamine (NMT), 5-hydroxytryptamine (serotonin) creatinine sulphate (5HT), 5-hydroxy-N,N-dimethyltryptamine (bufotenin) oxalate (5OHDMT), 5-methoxytryptamine hydrochloride and 5-methoxy-N,N-dimethyltryptamine (5MeODMT) were purchased from Sigma (St. Louis, MO, U.S.A.); tryptamine hydrochloride from Calbiochem (Los Angeles, CA, U.S.A.); and N-methyl-5-hydroxytryptamine oxalate (NMS) from Aldrich (Milwaukee, WI, U.S.A.). 1,2,3,4-Tetrahydro-β-carboline (THBC), 2-methyl-1,2,3,4-tetrahydro-β-carboline (MTHBC), 6-hydroxy-1,2,3,4-tetrahydro-β-carboline (6OHTHBC), 6-methoxy-1,2,3,4-tetrahydro-β-carboline hydrochloride (6MeOTHBC), and 5-methoxy-N-methyltryptamine (5MeONMT) were kindly donated by Dr. S.A. Barker, University of Alabama.

All solvents used for chromatography were of analytical purity and glass distilled prior to use. The mobile phase for the silica column (anhydrous methanol containing 0.4% 5 *M* aqueous ammonia and 1 m*M* cysteine hydrochloride) was stored at room temperature for at least 24 h and any precipitate formed was removed by filtration prior to use of the mobile phase.

Instrumentation

Separation of the indoleamines and their analogous β-carbolines was achieved on a Perkin-Elmer Series 3B liquid chromatograph using a silica column (DuPont Zorbax, 25 cm × 4.6 mm I.D., 6 μm). The mobile phase was anhydrous methanol containing 0.4% aqueous ammonia and 1 m*M* cysteine hydrochloride. The flow-rate was 1.5 ml/min.

Separations were also achieved using a strong cation-exchange column (Whatman Partisil 10 SCX, 25 cm × 4.6 mm I.D., 10 μm). The mobile phase was methanol—0.053 *M* phosphoric acid/ammonia buffer, pH 4.0 (30:70) with a flow-rate of 1.5 ml/min. A reversed-phase chromatographic procedure

involving an ODS column (DuPont Zorbax 25 cm × 4.6 mm I.D., 6 μm) with a mobile phase consisting of acetonitrile—0.053 *M* phosphoric acid/ammonia buffer, pH 7.0 (70:30) at a flow-rate of 2 ml/min was also used.

The spectroscopic detectors used included a Perkin-Elmer 650-10S fluorescence spectrometer, a Perkin-Elmer 3000 fluorescence spectrometer and a Perkin-Elmer LC-75 variable-wavelength UV detector with an auto control scanning accessory.

Spectroscopic studies

The optimum wavelengths for detection by both fluorescence spectroscopy and UV absorption spectroscopy were determined for each species by halting the flow of the mobile phase as each component entered the appropriate detector flow cell. Excitation and emission spectra were recorded and the wavelengths corresponding to the maximum fluorescence of each indoleamine species were determined. The wavelengths of maximum UV absorption were obtained by scanning the UV spectra using the LC-75 auto control unit.

RESULTS

Spectroscopic detection

During the development of procedures for the separation of the indoleamines and their analogous β-carbolines on a silica column a number of param-

TABLE I

SPECTRAL CHARACTERISTICS OF INDOLEAMINES AND β-CARBOLINES CHROMATOGRAPHED ON SILICA, PARTISIL 10 SCX AND ZORBAX ODS COLUMNS

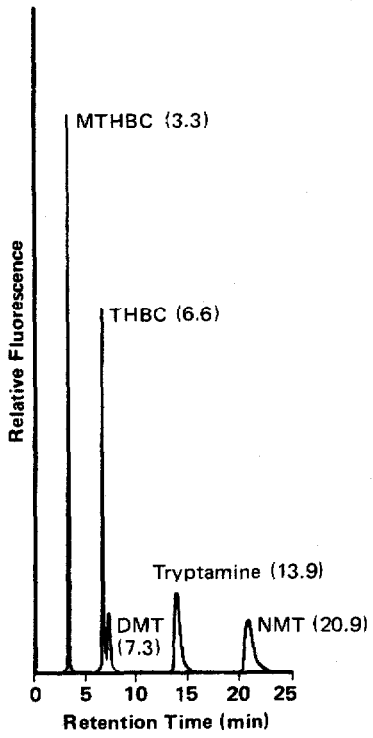
Indoleamines and β-carbolines (10 nmol) were chromatographed on a silica, SCX* or ODS** column and the UV absorption and fluorescence spectra recorded as described under Methods. Minimum detectable limits (MDL) (fluorescence) were estimated by analysis of peak heights obtained after chromatography of 0.5—5.0 pmol of each derivative. Each value represents the mean of three determinations. The coefficients of variation in peak heights were < 5%.

Compound	λ_{max} UV (nm)	λ_{ex} fluorescence (nm)	λ_{em} fluorescence (nm)	MDL fluorescence (pmol)
Tryptamine	220; 220**	278; 276**	348; 346**	0.16; 0.07**
NMT	220; 219**	279; 275**	348; 343**	1.2; 0.3**
DMT	221; 219**	278; 276**	347; 343**	0.4; 0.62**
5HT	222	276	338	0.11
NMS	220	276	337	0.82
5OHDMT	222	276	337	0.06
5-Methoxy-tryptamine	221; 220*; 220**	273; 270*; 274**	334; 335*; 335**	0.12; 0.09*; 0.03**
5MeONMT	220; 220*; 220**	276; 270*; 272**	335; 333*; 335**	0.36, 0.12*; 0.08**
5MeODMT	222; 220*; 220**	277; 270*; 271**	335; 336*; 334**	0.09; 0.24*; 0.08**
THBC	225; 220*; 220**	278; 265*; 269**	350; 345*; 345**	0.18; 0.1*; 0.12**
MTHBC	223; 220*; 219**	278; 265*; 271**	349; 343*; 346**	0.06; 0.3*; 0.19**
6OHTHBC	222; 220*	276; 269*	333; 334*	0.22; 0.07*
6MeOTHBC	225; 218*; 220**	277; 270*; 269**	333; 332*; 332**	0.23; 0.08*; 0.08**

eters for their detection and estimation both by fluorescence spectroscopy and UV absorption spectroscopy were examined.

In Table I the excitation and emission wavelengths for maximum fluorescence of the indoleamines and β-carbolines are presented together with the minimum detectable limits (defined as signal-to-noise ratio > 2) for fluorescence detection. The values of λ_{max} for the absorption spectra are also given in Table I.

In addition to silica, separations of the various indoleamines and β-carbolines were also achieved on Partisil 10 SCX and Zorbax ODS columns. The optimum wavelengths for detection by both fluorescence and UV absorption together with the minimum detectable limits for fluorescence detection for 5-methoxytryptamine, its N-methylated derivatives, and a number of β-carboline analogues of the N-methylated derivatives of 5HT, tryptamine and 5-methoxytryptamine, chromatographed on Partisil 10 SCX, are presented



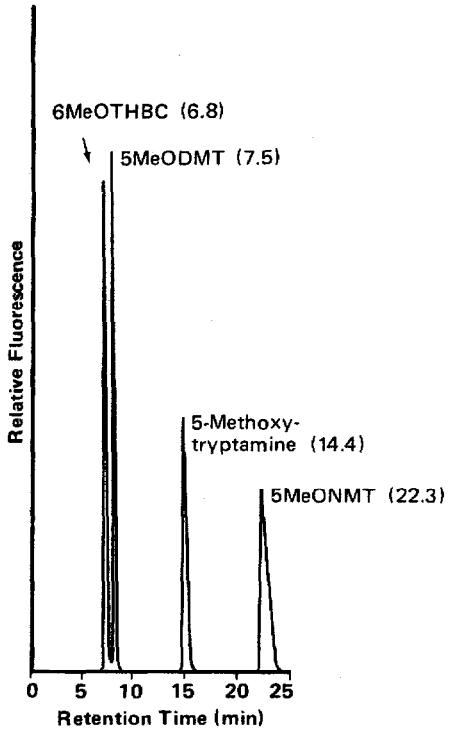


Fig. 1. Chromatogram of tryptamine, its N-methylated derivatives and their analogous β-carbolines. A mixture composed of 2 nmol of each derivative in 10 μl of methanol was applied to the DuPont silica column. The compounds were detected by fluorescence spectroscopy (λ_{ex} 278 nm, λ_{em} 347 nm). Retention times are indicated in parentheses.

Fig. 2. Chromatogram of 5-methoxytryptamine, its N-methylated derivatives and 6MeOTHBC. A mixture composed of 2 nmol of each derivative in 10 μl of methanol was applied to the silica column. The compounds were detected by fluorescence spectroscopy (λ_{ex} 277 nm, λ_{em} 335 nm).

in Table I. Similar data for tryptamine, 5-methoxytryptamine and their N-methylated derivatives and analogous β-carbolines separated on Zorbax ODS are also presented.

Chromatography

To achieve complete separation of tryptamine, 5HT, 5-methoxytryptamine and their various N-methylated derivatives and analogous β-carbolines a number of chromatographic procedures employing a silica column, a strong cation-exchange column and an ODS reversed-phase column were utilised.

A chromatogram of a mixture containing tryptamine, NMT, DMT, THBC and MTHBC separated on a DuPont silica column is presented in Fig. 1. A similar chromatogram illustrating the complete separation of 5-methoxytryptamine, 5MeONMT, 5MeODMT and 6MeOTHBC is also presented (Fig. 2). During the chromatography of 5HT, NMS, 5OHDMT and 6OHTHBC the resolution of 5OHDMT and 6OHTHBC remained incomplete (Fig. 3). Our studies have indicated that complete separation of these two compounds can be achieved by reducing the concentration of aqueous ammonia in the mobile phase to 0.2% although this is accompanied by marked increases in the retention times of 5HT and NMS. While the silica column permits the complete separation of tryptamine, 5HT and 5-methoxytryptamine from

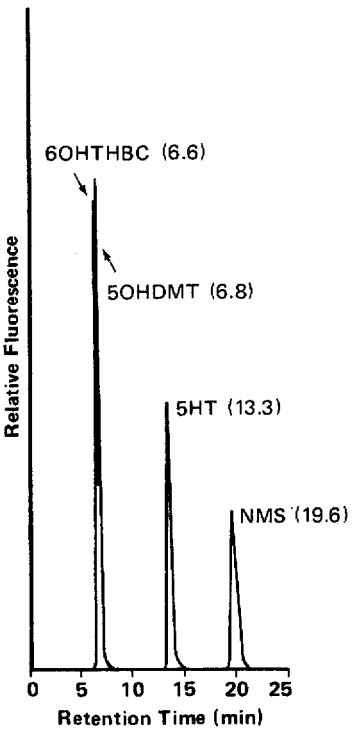


Fig. 3. Chromatogram of 5HT, its N-methylated derivatives and 6OHTHBC. A mixture composed of 2 nmol of each derivative in 10 μl of methanol was applied to the silica column. The compounds were detected by fluorescence spectroscopy (λ_{ex} 276 nm, λ_{em} 337 nm).

their respective N-methylated derivatives and β-carbolines, structurally analogous members of these three indoleamine series were found to display very similar chromatographic characteristics. To permit the separation and unique characterisation of these various derivatives, additional chromatographic procedures involving both a strong cation-exchange column and an ODS reversed-phase column were developed. Complete resolution of the mono- and N,N-dimethylated derivatives of 5HT and tryptamine (Fig. 4) and also those of 5-methoxytryptamine (Fig. 5) was achieved using a Partisil 10 SCX column. While the related β-carbolines 6OHTHBC, THBC, and MTHBC could also be completely separated from one another on the SCX column, in contrast to their behaviour on silica, they remain unresolved from their corresponding indolealkylamine analogues. While the Partisil 10 SCX column is invaluable in permitting the separation of the 5-hydroxylated indolealkylamines and β-carbolines from either the tryptamine or 5-methoxytryptamine series of analogues, members of the tryptamine and 5-methoxytryptamine series of derivatives could not be resolved from one another by this chromatographic system.

To achieve the desired separation of the mono- and N,N-methylated derivatives of tryptamine and 5-methoxytryptamine, chromatography on a Zorbax ODS column was employed. Under these conditions complete separation of

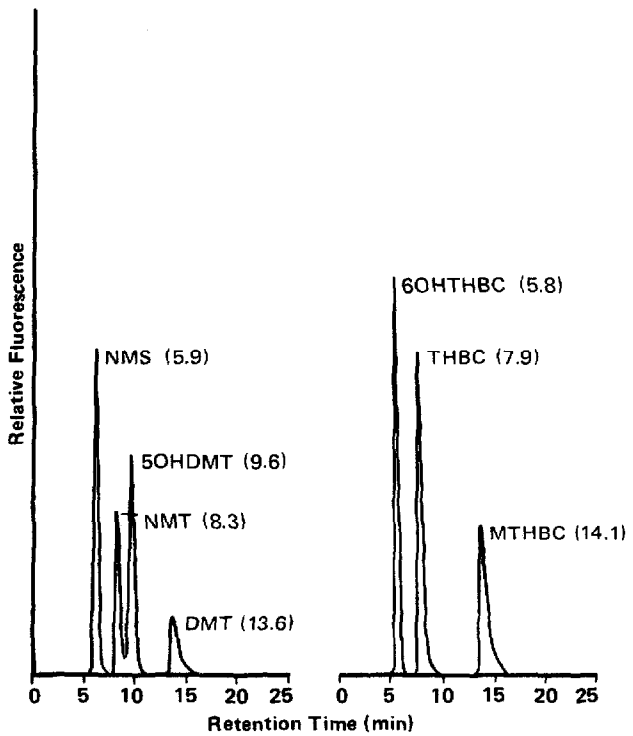


Fig. 4. Chromatograms of N-methylated derivatives of tryptamine and 5HT (left) and their analogous β-carbolines (right). A mixture composed of 2 nmol of each derivative in 10 μl of methanol was applied to a Partisil 10 SCX column. The compounds were detected by fluorescence spectroscopy (λ_{ex} 280 nm, λ_{em} 357 nm).

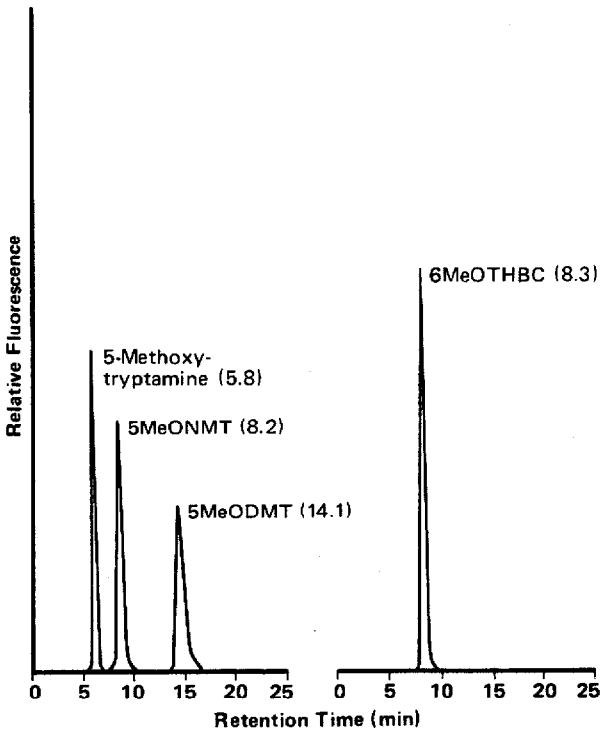


Fig. 5. Chromatograms of 5-methoxytryptamine its N-methylated derivatives (left) and 6MeOTHBC (right). A mixture composed of 2 nmol of each derivative in 10 μl of methanol was applied to a Partisil 10 SCX column. The compounds were detected by fluorescence spectroscopy (λ_{ex} 270 nm, λ_{em} 336 nm).

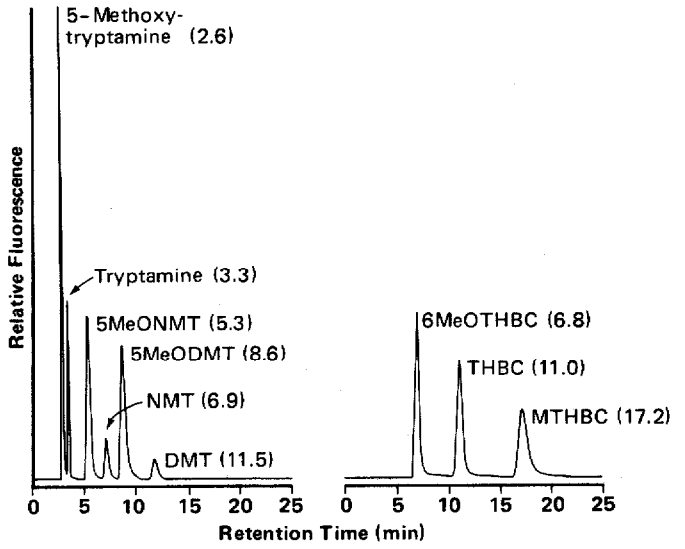


Fig. 6. Chromatograms of tryptamine, 5-methoxytryptamine their N-methylated derivatives (left) and analogous β-carbolines (right). A mixture composed of 1 nmol of each derivative in 10 μl of methanol was applied to a Zorbax ODS column. The compounds were detected by fluorescence spectroscopy (λ_{ex} 276 nm, λ_{em} 343 nm).

the methylated derivatives of both tryptamine and 5-methoxytryptamine could be achieved (Fig. 6). The β-carbolines 6MeOTHBC, THBC and MTHBC were also completely resolved from one another.

DISCUSSION

In a preliminary study [10] we reported that the mono- and N,N-dimethylated derivatives of tryptamine and their tetrahydro-β-carboline analogues can be separated by normal-phase liquid chromatography on a silica column. In this initial study, however, detection was limited to UV monitoring at 220 nm and indoleamines other than those of the tryptamine series were not examined.

Our current studies have shown that in addition to the separation of the tryptamine derivatives, the N-methylated and tetrahydro-β-carboline analogues of 5HT and 5-methoxytryptamine can also be resolved on silica. The chromatographic characteristics of analogous members of these three series of indoleamines were found to be very similar indicating that the substituent at the 5-position of the indole moiety (6-position of the β-carboline moiety) has only a minor influence on the chromatography of these compounds on silica. Although samples of 6-methoxy-2-methyl-tetrahydro-β-carboline and 6-hydroxy-2 methyl-tetrahydro-β-carboline were not available, by analogy with the behaviour of other derivatives in this series, their chromatographic characteristics are likely to be similar to those of MTHBC.

In contrast to normal-phase chromatography, during ion-exchange chromatography on Partisil 10 SCX the retention time characteristics of the indolealkylamines remain very similar to those of their respective analogous tetrahydro-β-carbolines. While chromatography on Zorbax ODS has the potential to resolve NMT, DMT and 5MeONMT from their individual tetrahydro-β-carboline analogues simultaneous separation of this series of compounds could not be achieved. These chromatographic systems however are invaluable for the separation of analogous members of the three indoleamine and tetrahydro-β-carboline series because of the differential influence of the hydroxy, methoxy and hydrogen substituents at the 5-position of the indole moiety and the 6-position of the β-carboline moiety on the chromatography of these compounds.

On-line detection of the indolealkylamines and tetrahydro-β-carbolines can be achieved by either UV absorption spectroscopy or by fluorescence spectroscopy. We have previously compared the use of both these techniques for the detection of 5HT, tryptamine and their mono- and N,N-dimethylated derivatives chromatographed on Partisil 10 SCX [10]. In addition to affording greater specificity the use of fluorescence spectroscopy greatly enhanced the detectability of these compounds. The minimum detectable amounts for fluorescence detection of the indolealkylamines and tetrahydro-β-carbolines chromatographed using the systems described above are within the sub-nanogram range.

The ability of normal-phase chromatography to group the various indoleamines and tetrahydro-β-carbolines according to their degree of N-methylation affords a unique method for the purification of specific classes of these

compounds. We have recently described the use of such procedures for the purification of 5OHDMT and DMT isolated from human urine [16]. When combined with analysis by reversed-phase or cation-exchange chromatography particularly in combination with fluorescence detection, such techniques may provide highly sensitive and specific methods for the detection and quantitation of indolealkylamines and tetrahydro-β-carbolines isolated from body fluids [16].

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