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GAS CHROMATOGRAPHIC ANALYSIS OF ENDOGENOUS CATECHOLAMINES, PHENOLIC AMINES AND DERIVED ISOQUINOLINES USING SHORT GLASS CAPILLARY COLUMNS AND ELECTRON-CAPTURE DETECTION

THOMAS C. ORIGITANO and MICHAEL A. COLLINS*

Department of Biochemistry and Biophysics, Loyola University of Chicago, Stritch School of Medicine, Maywood, IL 60153 (U.S.A.)

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SUMMARY

A gas chromatographic method is described for the concomitant separation and analysis of catecholamines, catecholamine or 3,4-dihydroxyphenylethylamine condensation products (tetrahydroisoquinolines), and their isomeric mono-O-methyl (phenolic) metabolites which may be present in neuronal tissues, utilizing short glass capillary columns and electron-capture detection. Isomeric phenolic amines that were not generally separable with conventional-packed gas chromatographic columns were rapidly resolved on the capillary system, and with their catecholamine or catechol isoquinoline precursors, quantitated with high sensitivity (0.25—7.0 pg) and reproducibility. Key steps in the approach with tissues include initial amine isolation with a weak cation-exchange resin (BioRex-70), fluoracyl derivative formation, and brief washing of the derivatives with ammonium phosphate buffer (pH 5.8) just prior to capillary analysis; overall recoveries of amines or alkaloids added to rat brain homogenates ranged from 79% to 89%. Application of the method is demonstrated in an assay of endogenous dopamine in rat corpus striatum and hypothalamus. This new procedure should complement and in some instances may be preferred over liquid chromatographic assays for catecholic and phenolic amines and isoquinolines, and ought to be applicable to mass spectrometric detectors as well.

INTRODUCTION

Gas chromatographic (GC) methodologies for the measurement of catecholamines (CAs) and their amine metabolites in tissues have been available for several years. Such assays generally have required the sensitive electron-capture cell or a mass spectrometer as a detector [1]. One often unacknowledged limiting factor with these similar GC-based assays has been the degree of resolu-

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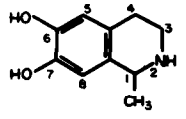
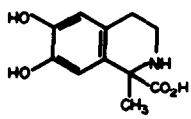
tion of geometric and structural neuroamine isomers in complex mixtures. In GC studies with CAs and their recently appreciated trace carbonyl condensation products, the 1,2,3,4-tetrahydroisoquinolines (TIQs), we frequently experienced difficulties separating certain isomeric O-methylated metabolites on conventional wide-bore (packed) GC columns. Bail et al. [2] first noted this problem with mono-O-methylated derivatives of simple TIQ condensation products of dopamine (DA).

To overcome resolution problems in GC analyses of neuroamines which employ the relatively accessible electron-capture detector [3], we explored the use of short capillary (open tubular) columns. There has been very little application of capillary methodology to assays of endogenous neurochemicals (see Discussion). With their high retention time stabilities and superior efficiencies (3000 plates per m), sufficiently inert capillary columns should be very appropriate for separations of geometric isomers of neuroamines. In this report, separation and quantitation of the CAs, O-methylated CA isomers, and a select number of simple catecholic and phenolic isoquinolines are demonstrated using short glass capillary columns, a state-of-the-art splitter injection mode and an autoinjector. Resolution far exceeded and detection limits easily equaled those obtained with conventional-packed GC columns coupled to an electron-capture detector or a mass spectrometer.

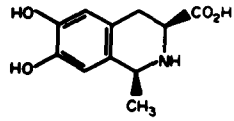
Isoquinolines and β-carbolines (indoleamine condensation products) constitute a relatively new group of potentially endogenous neuroamine derivatives (mammalian alkaloids) that could be of pathophysiological importance in certain disease conditions [4]. This capillary GC method, with its high sensitivity and reproducibility, can aid in determining precisely which alkaloids are in vivo substances. The overall procedure was used earlier [5] to clarify the apparent absence of the TIQs, salsolinol (SAL, Fig. 1) and its 7-O-methylated metabolite (7M-SAL), from corpus striatum of normal untreated rats. For the purpose of this report, an endogenous catecholamine, dopamine, is quantitated



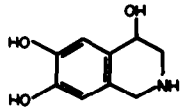
(d,l) Salsolinol (SAL)



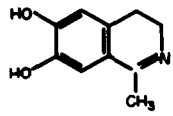
(d,l) SAL-1-Carboxylic Acid
SAL-1-CA



(cis) SAL-3-Carboxylic Acid
(SAL-3-CA)



(d,l)4-Hydroxyl-1-Desmethylsalsolinol
(4-HO-DSAL)



1,2-dehydro-SAL

Fig. 1. 6,7-Dihydroxy (catecholic) isoquinolines used in this study. The ten other isoquinolines in Table I are the respective 6-O-methyl and 7-O-methyl (phenolic) alkaloids related to these five substrates.

in two rat brain regions with the overall capillary GC procedure. In another report we show the applicability of this high-resolution capillary GC method using electron-capture detection (ECD) in studies of TIQ O-methylation patterns in rat brain [6].

MATERIALS AND METHODS

The capillary GC studies with the catecholic and phenolic amines were carried out with a Varian 3700 gas chromatograph equipped with dual ^{63}Ni electron-capture detectors (pulsed mode), a Varian 8000 automatic liquid sampler injector, and a polyphenylmethylsiloxane (OV-17) wall-coated open tubular (WCOT) glass capillary column (10 m × 0.25 mm; Alltech Assoc.). The split ratio was 10:1, and the head pressure of the carrier gas, oxygen-free nitrogen, was 0.8 kg/cm. The temperatures of the injector and detector were 250°C and 350°C, respectively, and column temperatures were as indicated in the figure legends or tables. Conventional columns (1.83 m × 6.35 mm, glass) packed with 3% OV-101, 3% OV-17, 5% SE-30 or 3% SE-54 on Gas Chrom Q (80—100 mesh) were coupled to the other electron-capture detector. Peak areas and retention times were obtained with a Varian CDS-111C computing integrator.

When confirmatory chromatography of the amines before derivatization was needed, an isocratic high-performance liquid chromatographic (HPLC) system consisting of a 25 cm × 4 mm BioSil (Bio-Rad Labs.) 10-μm C_{18} reversed-phase column and a BioAnalytical Systems LC-2A electrochemical detector (0.79 V) was utilized, generally with a mobile phase of 0.1 *M* sodium dihydrogen phosphate (pH range 4—5.5) containing 1 m*M* Na_2EDTA and varying percentages of methanol. A detailed account of the HPLC of simple and complex isoquinolines is in preparation. Thin-layer chromatography (TLC), when required to monitor synthetic reactions, was accomplished on 5 cm × 25 cm silica gel plates (Whatman) in *sec*-butanol—acetic acid—water (4:1:1, v/v/v) or on aluminum oxide plates (Macherey-Nagel) in chloroform—methanol—water (70:50:5, v/v/v). Compounds were visualized in an iodine chamber. The aluminum oxide plate was qualitatively useful for complete separation of catechols, which did not migrate in this case, from phenols, which did.

Chemicals and reagents

All of the isoquinolines used in this study have been characterized and reported in the literature. The TIQs *d,l*-salsolinol (HCl salt), *d,l*-salsolinol-1-carboxylic acid (*d,l*-SAL-1-CA) and *cis*-salsolinol-3-carboxylic acid (*cis*-SAL-3-CA) in Fig. 1, and their respective racemic mono-O-methyl derivatives (6M-SAL, 7M-SAL, 6M-SAL-1-CA, 7M-SAL-1-CA, 6M-SAL-3-CA and 7M-SAL-3-CA), were synthesized in yields ranging from 20% to 75% by aqueous (pH 4—5) condensation of the appropriate open-chain amine or amino acid with a three- to four-fold molar excess of acetaldehyde (Baker, analytical grade) or pyruvic acid (Sigma) at room temperature [7—9]. Elevated temperatures (60—70°C) were necessary to obtain sufficient (but low) yields of the 6-O-methylated TIQ isomers.

The 4-hydroxylated TIQs related to norepinephrine (NE) [*d,l*-4-hydroxyl-1-desmethylsalsolinol (*dl*-4-HO-DSAL) in Fig. 1 and its two mono-O-methyl

derivatives] were available as racemic HCl salts from earlier syntheses [10]. The three dihydroisoquinolines (DIQs) used in this study (1,2-dehydro-SAL in Fig. 1 and its respective 6- and 7-O-methyl analogues) were obtained as HBr salts via Bischler—Napieralski cyclizations of the appropriate mono- or dimethoxylated amide [11—13] followed by demethylation with refluxing 48% HBr where required. 3,4-Dihydroxyphenylethylamine (DOPA), the CAs, and their phenolic metabolites utilized as standards or synthetic reactants were purchased from Sigma, Aldrich or Regis.

Reactions were monitored initially by the TLC and HPLC techniques mentioned. Reaction products that failed to precipitate after several days were isolated by acidification (1 *M* hydrochloric acid), lyophilization and successive recrystallization from ethanol—ethyl acetate. Product purity was assessed by HPLC. Structures were proven by infrared (IR) and nuclear magnetic resonance (NMR) spectroscopy and by melting point comparisons with literature values.

Preparation of fluoracyl derivatives

Fluoracyl derivative preparation for capillary GC—ECD was achieved with the CAs, non-carboxylated TIQs and their mono-O-methylated analogues in a manner similar to a previously reported method [3]. To plastic-lined screw-top scintillation vials containing weighed amounts of the amine or amine salt, or to the lyophilized residues obtained from the ion-exchange column isolation (below) of aqueous extracts of tissue or standards, were added 500 μl of acetonitrile (Pierce) and 50 μl of heptafluorobutyryl anhydride (HFBA, Pierce). In some instances HFBA was replaced by pentafluoropropionyl anhydride (PFPA) or trifluoroacetyl anhydride (TFAA). After 30—40 min at room temperature, reactions were dried in a hood with a nitrogen stream. Samples or dried extracts containing 1- or 3-carboxylated TIQs were first treated with 200 μl of hexafluoroisopropanol (HFIP, Pierce) and 50 μl of fluoracyl agent, usually PFPA, and after 30 min at room temperature, were dried as above. The residue was further treated with PFPA (50 μl) and acetonitrile (500 μl) for 20 min, and blown dry with nitrogen. The final residues from fluoracylation or esterification—fluoracylation were dissolved in 1 ml sequanal-grade toluene (Pierce) and were transferred to glass centrifuge tubes. The toluene solutions were vortexed with 0.5 ml of 1 *M* ammonium phosphate (pH 5.8) for 60 sec and centrifuged briefly (3—4 min) in a clinical centrifuge. The top (organic) phases were carefully transferred to autoinjector vials and injected (1 μl) into the capillary GC system.

Animal experiments

Male Sprague—Dawley rats (100 ± 10 g) were killed by stunning and decapitation. Brains were removed and the cerebellar, corpus striatal and hypothalamic regions were taken according to Holman et al. [14], weighed and frozen on dry ice. Tissues were transferred to 7-ml plastic centrifuge tubes which contained fixed amounts (usually 75 or 150 ng) of 3,4-dihydroxybenzylamine (DHBA), and when required, varying amounts of the CAs, TIQs and/or the phenolic amines. Homogenization was done in ice-cold 75% ethanol (5 ml/g of tissue) utilizing a 20-sec burst of a Techmar Tissuemizer. The homogenizing blade was washed with 1 ml of fresh cold 75% ethanol after each sample, the

washings were added to the homogenates, and the combined fractions were centrifuged at 30,000 *g* (4°C) for 20 min.

In a modification of the weak cation-exchange isolation procedure of Holman et al. [14], supernatants from centrifugations were diluted two-fold with distilled water and applied to small columns (2.5 × 0.6 cm) of BioRex-70 resin (200—400 mesh; Bio-Rad Labs.). The resin was prepared by repeated stirring in distilled water and aspiration of suspended particles, followed by stirring in 3 *M* hydrochloric acid for 1 h, washing continuously with water until pH 5 was achieved, stirring in 3 *M* sodium hydroxide for 1 h and washing again to pH 5. It was then suspended in 0.1 *M* disodium hydrogen phosphate — sodium dihydrogen phosphate (pH 6.5) containing 10% Na_2EDTA. The effluents from the applied supernatants followed by 2 ml phosphate buffer (pH 6.5) and 2 ml distilled water were collected, combined and lyophilized. Assay by HPLC established that the amino acids and carboxylic acid metabolites were contained in these combined fractions. The columns were then treated with 2.5 ml of 1.0 *M* hydrochloric acid to elute amines and the effluent was frozen and lyophilized. The residues were treated as described under *Preparation of fluoracyl derivatives.*

RESULTS

HFB versus other fluoracylated derivatives

Compared to their TFA derivatives, the HFB derivatives of amines and TIQs consistently gave greater ECD responses, more symmetrical peaks and better capillary separations of geometric isomers. The responses and peak symmetries for the PFP derivatives were equivalent to the HFB derivatives, but acceptable separations of the (PFP) O-methylated TIQs or of 3M-DA and 4M-DA were not always obtained. Satisfactory separations of the mono-O-methylated 1- or 3-carboxylated TIQs were achieved, however, when the carboxylated isomers were derivatized with HFIP—PFPA combinations (retention times are listed in Table I).

Effect of ammonium phosphate wash on capillary GC of HFB-derivatized amines

The capillary GC chromatograms of the HFB-derivatized TIQs or open-chain amines, prepared from standards in aqueous solutions or tissue extracts (but not from crystalline standards), showed unacceptably large solvent fronts. A 1-min extraction or wash of the toluene solution of HFB derivatives with 0.5 vol. of 1 *M* ammonium phosphate solution, pH 5.7—5.8, just prior to injection effectively reduced this solvent response (Fig. 2). This step was found necessary for capillary measurements of tissue NE, DA and SAL, or for any assay with DHBA as an internal standard. When HFB-derivatized amines in toluene were washed repeatedly (up to five times) with fresh ammonium phosphate aliquots and immediately injected, peak areas and retention times were not significantly affected. However, extended storage of the washed toluene solutions of the HFB derivatives in contact with the ammonium phosphate solution (pH 5.8) apparently resulted in complete loss of the derivatives. Additionally, when a saturated sodium borate solution or ammonium phosphate of

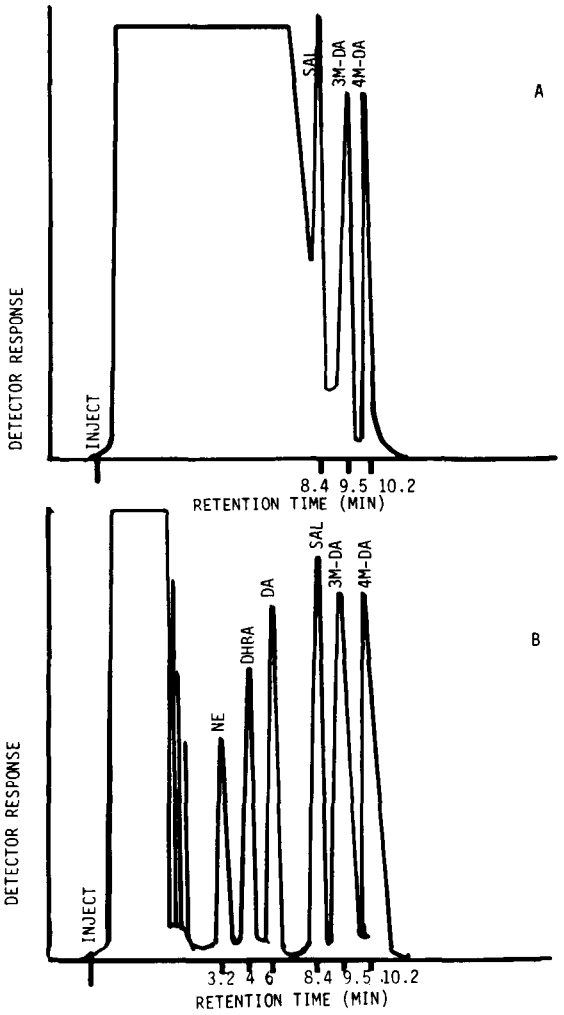


Fig. 2. The effect on the capillary GC—ECD chromatogram of a single ammonium phosphate buffer (pH 5.8) wash of a toluene solution containing a freshly derivatized (HFBA) mixture of NE, DHBA, DA, SAL, 3M-DA and 4M-DA. Chromatograms: (A) before wash; (B) immediately following wash. Procedure and chromatographic conditions are described in the text. Column temperature = 130°C.

pH 6 were employed as washes, recoveries of derivatized CAs or catechol TIQs were reduced to negligible values.

Capillary versus conventional GC separation of standard mixtures of CAs, TIQs and O-methyl derivatives

A comparison of the capillary GC versus conventional (packed) column GC separation of an HFB-derivatized mixture of DHBA, DA, SAL, 3M-DA, 4M-DA, 6M-SAL, and 7M-SAL is shown in Fig. 3. Complete separations of 3M-DA and 4M-DA and the two SAL isomers were readily obtained on the glass capillary column. As shown, a conventional-packed (1.83 m glass, 3% OV-17) column failed to separate the O-methylated DA TIQ isomers. Furthermore,

other packings referred to in Materials and methods in conventional columns were found unsuitable for the separation of the mono-O-methylated SAL isomers.

The 10-m glass capillary columns also were effective in separating mixtures (11—50 pg) of the three principal CAs (epinephrine, NE and DA) concurrent with their three main O-methylated amine metabolites in brain, normetanephrine, metanephrine and 3-O-methyl DA (chromatogram not shown). However, such low amounts of these catecholic and phenolic amines could not be chromatographed on longer (30—50 m) glass capillary columns of the same type and diameter. Fused-silica columns were not examined in this study.

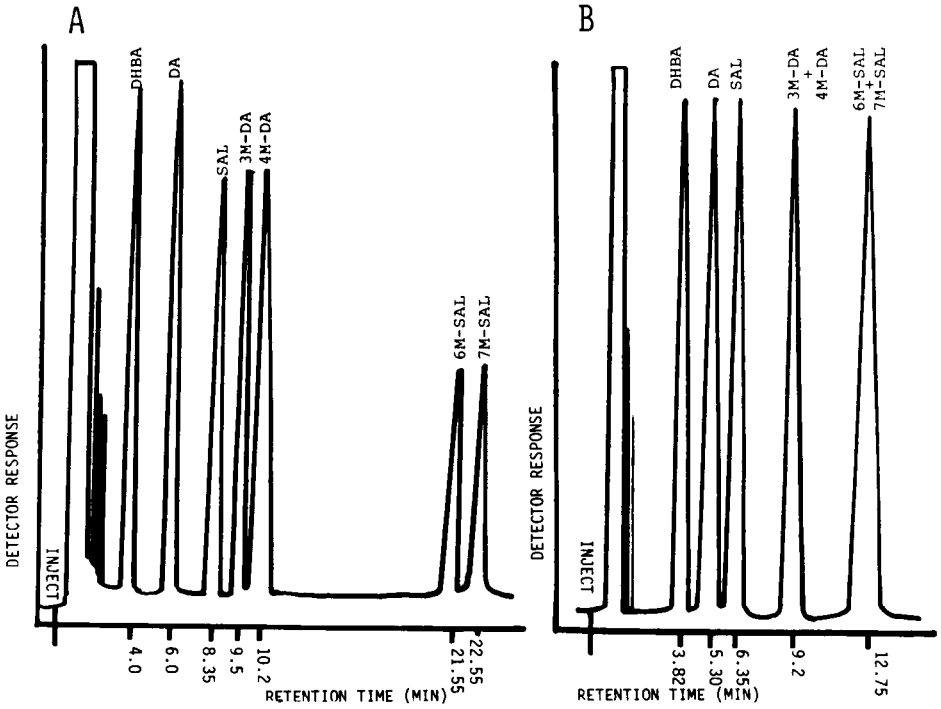


Fig. 3. GC comparison of an HFB-derivatized mixture of DHBA, DA, SAL, 3M-DA, 4M-DA, 6M-SAL and 7M-SAL chromatographed on (A) a 10-m WCOT OV-17 glass capillary column (130°C), versus (B) a conventional 1.83 m × 6.35 mm glass column packed with 3% OV-101 on Porapak Q (160°C).

Summary of capillary GC characteristics and recoveries for DA, representative isoquinolines, and their O-methyl derivatives

Table I is a summary of the relative retention times and minimum detectable quantities for DA, SAL, 4-HO-DSAL, SAL-1-CA, SAL-3-CA, 1,2-dehydro-SAL and their respective isomeric mono-O-methyl analogues following derivatization with either HFBA or PFPA—HFIP and capillary GC—ECD analysis at the indicated column temperatures. The overall recoveries (means of three to four samples) for these catecholic or phenolic compounds following addition to rat brain (cerebellar) tissue, aqueous ethanol extraction, isolation on BioRex-70, appropriate derivatization and capillary analysis are also shown. With this com-

TABLE I

CONDITIONS AND RECOVERIES OF STANDARDS FROM BRAIN TISSUE IN THE CAPILLARY GC ASSAY OF DAs AND TIQs

Chromatographic conditions: 10 m × 0.25 mm OV-17 glass WCOT column, injector temperature, 250°C, detector temperature, 350°C, attenuation range = 10^{-11} a.u.f.s.

Compound	Derivative/ column temperature (°C)	Retention time (min)	Minimum detectable quantity (ng/ml)	Recovery from tissue* (%)
Dopamine (DA)	HFB/130	6.00	0.25	89
3M-DA	HFB/130	9.50	0.45	84
4M-DA	HFB/130	10.20	0.45	85
Salsolinol	HFB/130	8.40	0.25	88
6M-SAL	HFB/130	21.55	0.45	82
7M-SAL	HFB/130	22.55	0.45	82
4-HO-DSAL	HFB/130	12.05	2.00	85
6M-4-HO-DSAL	HFB/130	17.64	5.00	82
7M-4-HO-DSAL	HFB/130	18.80	7.00	82
SAL-1-CA	PFP—HFIP/125	6.45	1.00	83
6M-SAL-1-CA	PFP—HFIP/125	16.20	5.00	80
7M-SAL-1-CA	PFP—HFIP/125	18.16	5.00	80
SAL-3-CA	PFP—HFIP/155	5.40	1.00	85
6M-SAL-3-CA	PFP—HFIP/155	16.70	5.00	82
7M-SAL-3-CA	PFP—HFIP/155	19.25	5.00	82
1,2-Dehydro-SAL	HFB/145	4.55	1.00	79
6M-1,2-Dehydro-SAL	HFB/145	7.10	7.00	82
7M-1,2-Dehydro-SAL	HFB/145	8.50	2.00	84

*Mean of three to four samples of rat cerebellar tissue (100 mg per sample) containing added amines. Standard deviations varied between 3.5% and 7.5%.

plete procedure for the compounds investigated, recoveries varied from 79% to 89% with a standard deviation of less than 8%.

The cation-exchange (BioRex-70) isolation step was found necessary in this overall capillary GC procedure. Attempts to analyze the lyophilized ethanol extracts of TIQ-spiked tissues (after HFBA treatment and the buffer wash) gave unacceptably long (25 min) capillary solvent and reagent peaks. In using the BioRex columns with mixtures of DHBA, SAL, SAL-1-CA and their respective mono-O-methylated derivatives added to cerebellar tissue, 95—97% of the carboxylated TIQs (as determined by HPLC of the eluate on reversed-phase columns) were recovered in the primary elution, the 2 ml phosphate buffer and the 2 ml distilled water, with no detectable elution of CAs, non-carboxylated TIQs or DHBA. The amines (DHBA, SAL and O-methylated SAL isomers) then were eluted completely from the ion-exchange resin by 1 *M* hydrochloric acid, with no evidence of the presence of carboxylated TIQs.

The isolation of amine mixtures was also examined with the relatively strong cation-exchange resin, Dowex-AG50-WX. Removal of bound isoquinolines from columns (2.5 × 0.6 cm) of this resin required relatively stringent acid conditions (4 *M* hydrochloric acid—methanol, 1:1, v/v) and after lyophilization, only 20—40% of the amines was recovered, according to HPLC or capillary GC analysis.

Verification of the precision and linearity of the capillary GC method using SAL and DHBA

The precision of the capillary GC measurement was demonstrated with nine successive autosample injections of a mixture of the two (HFB-derivatized) catechol compounds, SAL (85 pg/ml) and internal standard, DHBA. The retention time and peak area for SAL varied by only 0.07% and 3.7%, respectively, and the mean area ratio (± S.D.) for SAL/DHBA was 0.891 ± 0.01, with a 1.2% relative deviation.

Linearity with standards was shown in experiments in which the area ratios for SAL/DHBA increased linearly from 4 to 83 ng/ml SAL in relation to a fixed (100 ng/ml) concentration of DHBA (internal standard). The correlation for the relationship was 0.9997. Similarly, the linearity of the overall procedure was confirmed in experiments in which SAL (from 10 to 100 ng/ml) and DHBA (100 ng/ml) were added to rat cerebellar tissue and carried through the extraction, derivatization, wash and analysis.

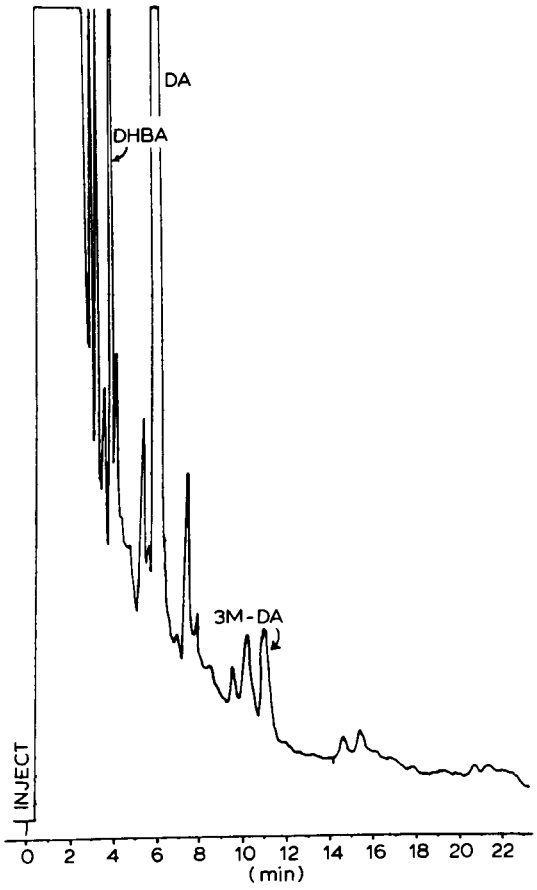


Fig. 4. Capillary GC—ECD chromatogram of HFB-derivatized amines from rat corpus striatum. Areas and retention times of DHBA and DA were obtained by computing integrator. Chromatographic conditions are described in text. Column temperature = 130°C. Attenuation setting of $2 \cdot 10^{-11}$ a.u.f.s. was used to demonstrate the absence of 4M-DA and the presence of 3M-DA, but it resulted in apparent solvent tailing and off-scale deflections of DA and DHBA.

Assay of endogenous dopamine in the rat corpus striatum and hypothalamus by short glass capillary GC—ECD

A representative chromatogram of rat corpus striatum extract (95 mg tissue) is shown in Fig. 4. Control chromatograms showed no interfering substances. The concentration of DA was quantitated with the use of the computing integrator. The striatal and hypothalamic means of twelve rats are shown in Table II, and are compared to reported DA values in the literature.

TABLE II

COMPARISON OF CAPILLARY GC—ECD ASSAY* OF DOPAMINE IN RAT STRIATUM AND HYPOTHALAMUS WITH VALUES IN THE LITERATURE OBTAINED BY HPLC AND FLUORIMETRY

Brain region	Dopamine (μg/g ± S.E.M.)	Method	Reference
Corpus striatum	9.50 ± 0.78 (n=12)	Capillary GC—ECD	This report
	8.80 ± 0.82	HPLC	[29]
	8.78 ± 0.89	Fluorimetry	[14]
Hypothalamus	1.90 ± 0.31	Capillary GC—ECD	This report
	2.10 ± 0.16	Fluorimetry	[14]

*Conditions were as described in the text and in Table I for dopamine.

DISCUSSION

To the best of our knowledge, the use of capillary columns has not been reported previously in GC analyses of endogenous CAs, either alone or simultaneously with their O-methylated metabolites. The TIQ alkaloids also had not been examined by capillary GC prior to this study. However, Martin et al. [15] provided a thorough study of the GC behavior of CAs and their O-methylated derivatives on 30-m fused-silica capillary columns using negative-ion detection, and subsequently determined the concentration of normetanephrine in human cerobrospinal fluid. Several other capillary methods have been reported for selected CA metabolites. LeGatt et al. [16] used capillary GC—ECD for the measurement of rat brain normetanephrine and 3-O-methyl DA. No attempts were made to demonstrate resolution of their potential 4-O-methylated isomers. With capillary GC—mass spectrometry (MS), oxidized CA derivatives were assayed in the urine by Muskiet et al. [17] and in the spinal fluid (homovanillic acid) by Vogt et al. [18]. Recent analytical GC studies have used capillary columns with series of amines which included the catecholamine compound isoproterenol [19, 20]. Overall, however, application of high-resolution capillary procedures in studies of catecholic and phenolic amines has been limited.

Several thorough capillary GC studies are available with indoleamines and particularly their tetrahydro-β-carboline condensation products. Beck et al. [21] used 20-m glass capillary columns for MS studies on the occurrence of β-carbolines in tissues and dietary components. An earlier capillary report from the same laboratory focused on the indoleamine condensation products in urine and spinal fluid [22]. The MS of β-carbolines (following appropriate derivatization) was also investigated in detail on 30-m fused-silica capillary columns by Faull et al. [23]. Serotonin or its O-methylated and/or oxidized

metabolites have also been analyzed on glass capillary columns in GC—MS procedures [17, 24].

Concerning important steps in the methodology of this report, a substantial (and necessary) reduction in the solvent/reagent peak was brought about by the brief ammonium phosphate wash of freshly derivatized (fluoracylated) samples. As noted in Results, repeated washings of toluene solution of HFB-catecholamines with several (up to five were done) fresh ammonium phosphate aliquots, followed by immediate analysis, did not significantly change the peak areas. However, because of the susceptibility of fluoracylated derivatives to hydrolysis, the derivatized sample could not be stored for substantial lengths of time in contact with the aqueous wash. Other researchers using capillary GC—ECD have noted the necessity of a similar wash or clean-up step before injection. Davis et al. [25] washed PFP—HFIP-derivatized hydroxyphenylacetic acids with 1 *M* phosphate, pH 6 (cation not stated) prior to capillary GC—ECD analysis, and suggested that the wash removed excess and possibly hydrolyzed fluorinating agent which would otherwise saturate the electron-capture detector. Sodium borate buffer may have served a similar purpose in the *meta*-O-methyl CA procedure of LeGatt et al. [16] or in a serotonin assay by Calvery et al. [26]. For the derivatized CAs and catechol TIQs, saturated borate was unsuitable as a wash solution, perhaps because it promoted hydrolysis of the fluoracyl derivatives.

Another important consideration is our use of a weak cation-exchange resin such as BioRex-70 rather than a strong amine exchanger such as Dowex, or (for the catechol compounds) a binding material such as aluminum oxide. As indicated, the replacement of BioRex with Dowex required stronger acid eluents and resulted in substantially lower recoveries, probably because of compound degradation. The use of aluminum oxide in place of BioRex also gave lower recoveries of CAs, more contaminated chromatograms, and relatively large solvent fronts that could not be reduced effectively by the phosphate wash. Using the fluoracyl anhydrides as derivatizing agents, best results (maximal responses and essentially single derivatives) were obtained with HFBA, but the HFIP—PFPA combination pioneered by Watson et al. [27] for acid metabolites of CAs was also effective for carboxylated TIQs. Other fluoracylating agents such as fluoracyl imidazoles, found by Barker et al. [28] and Faull et al. [23] to be the best reagents for GC—MS studies with β-carboline condensation products, were not investigated in this study with CAs and TIQs. However, in previous GC—ECD work using conventional columns [3], fluoracyl imidazoles tended to produce multiple derivatives of the TIQs.

Contrary to results of other capillary applications with splitter injector modes, we found that the capillary system with an automatic injector provided a high degree of precision and reproducibility with derivatized mixtures of CAs or TIQs. Equally significant is that the autoinjector in combination with the capillary column permitted very accurate retention time values (0.1% deviation). As an indication of the method's practical applicability, the assay of rat striatal and hypothalamic DA gave values that compared reasonably well with results in the literature from HPLC and fluorimetry (Table II). Due to the extreme sensitivity and good linear range of new models of electron-capture detectors, not only DA but epinephrine or norepinephrine can be readily mea-

sured in the small brain tissue samples that are obtained, for example, by the punch technique.

The capillary GC—ECD procedure described could serve as an important adjunct to reversed-phase HPLC assay, which is now probably the most common means of CA and TIQ estimation. In many situations, when MS is not available or is cost-prohibitive, retention times and quantities of endogenous catecholic and phenolic amines could be confirmed rapidly by this highly specific and yet inexpensive technique. Notwithstanding its complementary role, we suggest that capillary GC would be of great value in determinations of those geometric isomers of mono-O-methylated CAs or TIQs which are not separated by conventional GC (as shown) or cation-exchange HPLC.

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