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High speed liquid chromatography of phenylethanolamines for the kinetic analysis of [^{11}C]-*meta*-hydroxyephedrine and metabolites in plasma

Jeanne M. Link[b], Robert E. Synovec[a,*], Kenneth A. Krohn[b], James H. Caldwell[c]

[a]*Department of Chemistry, Box 351700, University of Washington, Seattle, WA 98195-1700, USA*
[b]*Department of Radiology, Box 356004, University of Washington, Seattle, WA 98195-6004, USA*
[c]*Division of Cardiology, VA Medical Center, Seattle, WA 98108, USA*

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Abstract

A method is developed and described for analysis of [^{11}C]-*meta*-hydroxyephedrine, [^{11}C]MHED, a tracer of cardiac function, and its metabolites in plasma samples. The method combines on-column solid-phase extraction and separation on a single weak cation-exchange column. Phenylethanolamines were used to develop the separation method that concentrates the analytes on-column from physiological saline and then elutes them by changing to an acidic mobile phase. Hydrophobic interactions determine the selectivity, and elution order is the same as for reversed-phase liquid chromatography on a C_{18} stationary phase. The mechanism of separation is mixed mode, with ion-exchange coupled with a reversed-phase liquid chromatography mechanism. Each sample analysis requires only 10 min and does not require deproteinization or the use of organic solvents. In human samples, a single plasma metabolite of [^{11}C]MHED along with the parent compound were observed using this method. The method was sufficiently rapid so that in 70 min seven samples were assayed, providing a well-defined time course for MHED and its metabolites in blood. The metabolite concentration increased with time to ≈85% of the plasma activity 50 min after administration. The results with the developed method are comparable to those described for reversed-phase separations, with the advantage that our method does not require deproteinization, reducing sample analysis time by a factor of two.

Keywords: Phenylethanolamines; Hydroxyephedrine

1. Introduction

Carbon-11 labeled *meta*-hydroxyephedrine, [^{11}C]MHED, is an analog of the neurotransmitters norepinephrine and epinephrine and is used to probe, through positron emission tomographic (PET) imaging, cardiac neuronal function. PET images display radioactive decay events in anatomical regions during discrete time intervals. These images can be mathematically modeled to estimate regional biochemical rates of uptake, metabolism and clearance [1]. Part of this process involves correcting the imaging data for the various chemical forms of the radioactivity in the blood.

Blood samples are taken from patients at various times during the imaging experiment and analyzed. The metabolites of [^{11}C]MHED may be the same compounds as nonradioactive compounds occurring

*Corresponding author.

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naturally in the body. Because only the kinetics of the injected tracer and its metabolites are of interest, the radioactive rather than the stable compounds must be measured. The half-life of ^{11}C is 20.4 min, so blood analyses must be completed rapidly, preferably within 10 min of obtaining the sample. Furthermore, the total amount of ^{11}C decreases from about 10 000 to 15 disintegrations per second (dps) in a milliliter of blood over the course of the study. The result is that large volumes of plasma, ~0.5 ml, must be analyzed in order to have a detectable radiation signal.

These requirements are more stringent than typical catecholamine assays which involve two steps, separation from a biological matrix of blood or urine, after which reversed-phase high-performance liquid chromatography (HPLC) or gas chromatography (GC) are the separation methods of choice [2–8], although development of new techniques continues [9,10]. These separations are performed on too small a volume, a few microliters, or too long a time scale, greater than 30 min per sample, to be used for analysis of [^{11}C]MHED in blood [11].

The metabolism of ephedrine and metaraminol, compounds closely related to MHED have been reported. Routes of metabolism are *N*-demethylation or oxidation and then conjugation [12–15], leading to metabolites which are more hydrophilic than the parent compounds. There is also evidence of hydroxylation and formation of *O*-methylated catechols [16], a pathway that leads to more lipophilic metabolites than the parent compound.

1.1. PET metabolite analysis methods

PET metabolite analyses [17] use a variety of techniques, HPLC, thin layer chromatography (TLC) and solid-phase extraction (SPE), to separate radiolabeled metabolites. Deproteinization with organic extraction is most often used to prepare the whole blood samples. Two methods for analysis of metabolites of [^{11}C]MHED have been reported. Rosenspire et al. [18] measured radioactive metabolites in blood and several organs from a guinea pig. Samples were deproteinized using acid, centrifuged and the supernatant was analyzed using reversed-phase HPLC. At 5 min after injection, most radioactivity was unmetabolized [^{11}C]MHED. At 30 min after injection <50% of the ^{11}C in liver but >95% in heart was [^{11}C]MHED. The order of elution on reversed-phase adsorbents indicated that the metabolites were more hydrophilic than the parent compound.

Osman et al. [19] used C_{18} reversed-phase HPLC to separate MHED and its metabolites in 0.5–1 ml samples of acid-deproteinized plasma from rats and humans. They found at least 3 metabolites in rat plasma but only one in humans. Thus, [^{11}C]MHED is metabolized differently between species and possibly with disease. The amount of metabolites changes with time so that blood must be sampled at several times [19].

Separation of catecholamines using weak cation-exchangers has been reported [20]. Cation-exchange stationary phases are now available with low hydrophobicity and improved efficiency for use in HPLC. These resins should allow proteins to pass through the column without altering the stationary phase or denaturing the proteins, both of which have interfered with analysis of plasma by reversed-phase HPLC [17]. The implication is that separation of biological amines might be accomplished rapidly in the presence of protein and without the use of organic solvents. This would simplify and shorten the analysis, essential to effectively follow the metabolite kinetics, and it would also minimize disposal of biohazardous human materials.

[^{11}C]MHED and its metabolites are amines which are protonated at physiological pH and are substrates for cation-exchange. Any *N*-demethylated metabolites would no longer contain ^{11}C and thus not be of interest; the radiocarbon would become volatile $^{11}CO_2$ [21]. The hydrophobicity of a resin-based cation-exchange stationary phase should increase the capacity of the resin for organic amines. The resin should initially concentrate the amines from the plasma, i.e., on-column solid-phase extraction, and provide separation due to both cation-exchange and hydrophobic interaction of molecules of different hydrophobicity. We have evaluated the feasibility of this approach by developing a cation-exchange HPLC method for separation of [^{11}C]MHED and its metabolites on a carboxylate-based cation-exchange resin. The separation has been evaluated with non-radioactive phenylethanolamine standards and plasma spiked with [^{11}C]MHED. This separation is compared with the separation using reversed-phase

HPLC. A kinetic study of metabolites in plasma samples from patients injected with [^{11}C]MHED is also presented.

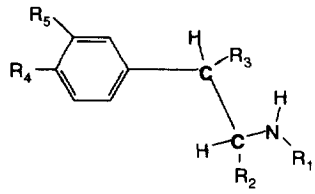
2. Experimental

2.1. Chemicals

In order to develop a method for separation of MHED from its metabolites, several test compounds (Table 1) were chosen. They are similar to MHED but have different functional groups which could be present as a result of metabolism. These compounds were obtained from Sigma (St. Louis, MO, USA) or from Research Biochemicals International (Natwick, MA, USA).

Sodium chloride and potassium chloride were reagent grade and obtained from J.T. Baker (Phillipsburg, NJ, USA). Ammonium chloride was reagent grade and obtained from Allied Chemical (Morristown, NJ, USA). Hydrochloric acid (0.1 molar reagent grade), methanesulfonic acid (99%) and ammonium formate (reagent grade) were obtained from Aldrich (Milwaukee, WI, USA). Benzenesulfonate and hexanesulfonate were obtained from Sigma. Human serum albumin (25%) was obtained from Armour (Kankakee, IL, USA). Saline solution (0.9%) was obtained from Baxter Healthcare (Deerfield, IL, USA). Solutions were made up in distilled deionized water from a 20 MΩ Millipore (Bedford, MA, USA) system.

Table 1
Test compounds



Compound	R1	R2	R3	R4	R5
Octopamine	H	H	OH	OH	H
Norepinephrine	H	H	OH	OH	OH
α-Methylnorepinephrine	H	CH_3	OH	OH	OH
Epinephrine	CH_3	H	OH	OH	OH
Phenylephrine	CH_3	H	OH	H	OH
Metaraminol	H	CH_3	OH	H	OH
Deoxyepinephrine	CH_3	H	H	OH	OH
MHED	CH_3	CH_3	OH	H	OH
[^{11}C]MHED	$^{11}CH_3$	CH_3	OH	H	OH
Ephedrine	CH_3	CH_3	OH	H	H

2.2. Radiosynthesis of [^{11}C]MHED

[^{11}C]MHED was synthesized [18] by bubbling [^{11}C]CH_3I into a vial containing metaraminol free base (<1 mg) in 210 μl of dimethylformamide and 70 μl of DMSO. The vial was capped and heated for 5 min at 100°C. The [^{11}C]MHED product was purified by semi-preparative HPLC using an Inertsil ODS-2 C_{18} column (Metachem, Torrance, CA, USA; 250×10 mm I.D., 5 μm particles), eluted with 0.15 *M* sodium acetate (pH 6)–ethanol (95:5, v/v). The retention volume for MHED was 55 ml (*k'* of 7.0). Chemical purity was assayed using an Inertsil ODS-2 column (Metachem; 250×4.6 mm I.D., 5 μm particles), with an ODS-2 guard column (10×4.6 mm, 5 μm particles), eluted with 0.2 *M* ammonium formate (pH 6)–acetonitrile (95:5, v/v) and UV absorbance detection at 272 nm. Retention was verified against standards of metaraminol and MHED (made according to the above procedure using nonradioactive CH_3I and structure confirmed using HPLC, NMR, mass spectrometry and polarimetry). Retention volumes for metaraminol and MHED were 7.4 and 10.7 ml, respectively. The freshly prepared [^{11}C]MHED showed radiochemical purity of >98%, and a specific activity of 4400 Ci/mmol (signal-to-noise ratio=9) at end of synthesis, 30 min after end of bombardment.

2.3. Chromatography

Cation-exchange columns were obtained from Dionex (CG14, Sunnyvale, CA, USA). The CG14 stationary phase consists of 8 μm diameter particles of ethylvinylbenzene cross-linked with 55% divinylbenzene with 0.325 meq of carboxylic acid in columns that were 50×4 mm I.D. Reversed-phase HPLC separations were performed using an Inertsil ODS-2 column (Metachem, 250×4.6 mm I.D., 5 μm particles), with a 10 mm ODS-2, 5 μm particles, guard column.

A Constametric 4100 gradient pump (Thermo-

Separations, Riviera Beach, FL, USA) and Vici Valco (Houston, TX, USA) injector were used with either a Gilson Holochrome (Middleton, WI, USA) or Ocean Optics PC1000 (Dunedin, FL, USA) UV absorbance detector at 272 nm for the phenylethanolamine compounds and 254 or 310 nm for protein measurements. Injection volumes of the test compounds ranged from 10 to 1000 μl, whereas 0.5-ml injections were made for plasma separations.

In all studies radioactivity was measured by counting samples for 1 min each in a Packard Auto-Gamma 5000 scintillation detector (Downers Grove, IL, USA) with an efficiency of 22% for the 511 keV γ-radiation and a background of 0.6 to 1.0 counts per second (cps). Flow-through coincident 3″ NaI(Tl) detectors with an efficiency of 10% and a background of 0.1–0.2 cps were also used for the metabolite studies in sample volumes of either 0.3 or 1 ml.

2.4. Separation of [^{11}C]MHED and analogs

The CG14 cation-exchange column was evaluated for the separation of [^{11}C]MHED and metabolites from the vast excess of plasma proteins. This separation from plasma requires that the analytes "preconcentrate" on the column in the presence of physiological concentrations of both Na^+ and K^+ ions; 140 m*M* and 4 m*M*, respectively, in plasma [22], and that the analytes rapidly separate from each other and from the plasma proteins. In order to determine the best conditions to accomplish this, the separation mechanisms for the CG14 resin were examined.

Because the ability to retain amine cations in the presence of Na^+ and K^+ was critical to this work, the selectivity of the resin for the amine analytes in the presence solely of K^+, Na^+ and H^+ was evaluated by measuring the column retention of the test analytes while varying the eluent cation concentrations.

The selectivity for the test analytes using K^+ and Na^+ as eluent cations was reexamined in the presence of 5 m*M* MSA (pH 2.3) to reduce k' to a value where peak broadening didn't degrade the signal and obscure the peak retention time of the analyte. Retention of the test analytes was also measured as a function of eluent concentration using two acids, HCl and MSA, to determine if there was a difference in retention if an organic or inorganic acid was used for elution.

Ion pairing agents can be added to an eluent to modify the hydrophobicity of an analyte, by neutralizing the charge of the analyte. In order to evaluate the extent of hydrophobic interaction on CG14, the retention of the analytes was measured as a function of K^+ concentration with ion pairing reagents of different hydrophobicities added to the mobile phase. The ion pairing concentrations were: 5 m*M* MSA, 5 m*M* Na benzenesulfonate with 5 m*M* HCl, or 5 m*M* Na hexanesulfonate with 5 m*M* HCl. The addition of different ion pairing reagents should increase the hydrophobic column interactions with the analytes and increase k'.

2.5. Separation of [^{11}C]MHED from plasma

For testing of on-column protein retention, human serum albumin, the most abundant plasma protein, was injected on the CG14 column and eluted with 150 m*M* NaCl. The protein elution was monitored using UV absorbance spectroscopy as described above. To test whether [^{11}C]MHED could be extracted from plasma and retained on the CG14 column, 50 μl of 0.15 *M* sodium acetate–ethanol (95:5, v/v), containing a trace amount of [^{11}C]MHED, was mixed with 300 μl of human plasma and injected on the column with a mobile phase of 150 m*M* NaCl at 1.0 ml/min; the mobile phase was switched to 100 m*M* methanesulfonic acid (MSA) at 16 ml in order to elute the [^{11}C]MHED. Fractions (1 ml) of the effluent were collected and counted for radioactivity.

The results of the separation mechanism studies were used to develop a gradient HPLC method for separation of MHED from the test compounds. Sodium chloride, 150 m*M*, was the mobile phase used for on-column preconcentration of the analytes. Sodium heparin (1 I.U. heparin/ml) obtained from SoloPak (Franklin Park, IL, USA) was added to saline to prevent clotting on the column. After the analytes were concentrated on the column, the mobile phase was changed to 20 m*M* MSA in 150 m*M* NaCl to elute the different analytes. The mobile phase was then switched to 100 m*M* MSA to ensure the column was fully eluted and then the column was

Table 2
Gradient HPLC method for separation of [^{11}C]MHED from its metabolites in plasma

Time (min)	Flow (ml/min)	Mobile phase
0–0.9	1.5	NaCl (150 m*M*)
0.9–1.0	1.5	Gradient from NaCl to 20 m*M* MSA in 150 m*M* NaCl
1.0–5.0	2.0	20 m*M* MSA in 150 m*M* NaCl
5.0–6.0	2.0	Gradient from 20 m*M* MSA to 100 m*M* MSA
6.0–7.5	2.0	100 m*M* MSA
7.5–7.6	2.0	Gradient from 100 m*M* MSA in 150 m*M* NaCl to 150 m*M* NaCl
7.6–9.6	2.0	NaCl (150 m*M*)

re-equilibrated with NaCl. The method is summarized in Table 2.

The hypothesis, phenylethanolamine analytes are concentrated on the top of the CG14 column matrix with a mobile phase containing NaCl, was tested by injecting different volumes – 10, 500 and 1000 μl of two test compounds, epinephrine and MHED – and using the gradient HPLC profile depicted in Table 2 for elution of the analytes.

2.6. Patient studies

Patients were injected with 15–20 mCi of [^{11}C]MHED and blood samples were collected at several times after injection. The samples were anticoagulated with lithium heparin, vortexed to mix any settled cells, and sampled for hematocrit determination, whole blood radioactivity measurement and for further analysis. The latter sample was centrifuged for 2 min at 4000 *g* on an Eppendorf (Madison, WI, USA) 5415 centrifuge. After centrifugation, the plasma supernatant was pipetted from the sedimented cells, an aliquot removed for radioactivity counting, and 500 μl injected through a 0.2-μm pore Acrodisc 13 nylon filter (Gelman Sciences, Ann Arbor, MI, USA) onto the CG14 cation-exchange column for analysis of [^{11}C]MHED and metabolites. The gradient HPLC method given in Table 2 was used for the separation.

3. Results and discussion

3.1. Separation of [^{11}C]MHED and analogs

The separation of [^{11}C]MHED and metabolites from plasma requires separation from a vast excess of plasma proteins in the presence of high salt concentrations and a variety of endogenous biochemicals including amines which compose plasma. The CG14 cation-exchange column was evaluated for the separation of [^{11}C]MHED and metabolites from the plasma matrix. This stationary phase is a weakly acidic carboxylate, cation-exchange resin with "low" hydrophobicity. This separation from the plasma requires that the analytes "preconcentrate" on the column in the presence of physiologic concentrations of both Na^+ and K^+ ions; 140 m*M* and 4 m*M*, respectively, in plasma [22], and that the analytes rapidly separate from each other and from the plasma proteins. Even with 100 m*M* solutions of NaCl or KCl at pH 7 as the mobile phase, capacity factors (k') were large, >30 for epinephrine, indicating the feasibility for concentrating amines directly on the CG14 resin from physiological saline samples.

The measured capacity factors using K^+ and Na^+ as eluent cations in the presence of 5 m*M* MSA (pH 2.3) are presented in Fig. 1 for MHED and in Table 3 for all of the compounds tested. Capacity factors for the phenylethanolamines on CG14 range from about two to several hundred. It is apparent from Fig. 1 and Table 3 that the selectivity coefficients for cations on CG14 are $H^+ > K^+ > Na^+$, i.e., H^+ being the strongest eluent for the amines. When the resin is in the Na^+ form, k' for the analytes is too great in order to achieve a sufficiently low analysis time. There was little or no difference in selectivity between the organic acid, MSA and HCl.

A closer inspection of the data presented in Fig. 1 is needed in order to obtain proof of the supposed mixed-mode separation mechanism. For a purely cation-exchange retention mechanism, the capacity factors for monovalent cations are inversely propor-

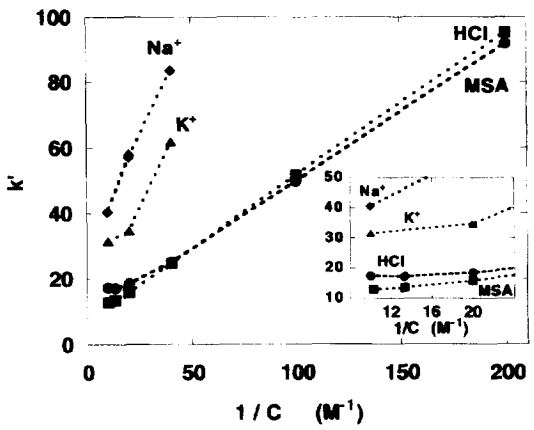


Fig. 1. Capacity factors for MHED vary linearly with the reciprocal of the cation eluent concentration (C) to ≈0.05 M. At cation concentrations >50 mM separation becomes independent of cation concentration. A CG14 column was used and eluted at a flow-rate of 3.0 ml/min. NaCl and KCl solutions containing 5 mM methanesulfonic acid (MSA) were used. The inset figure shows an expanded view of the values of k' at low $1/C$. (●) MSA; (■) HCl; (♦) NaCl; (▲) KCl.

tional to the eluent cation concentration, C, according to the following equation:

$$k' = K_c^a \frac{A}{C}$$

where k' is the capacity factor, K_c^a is the selectivity coefficient for the analyte, a, with respect to the eluent cation, c. The selectivity coefficient is constant for a given eluent. C is the concentration of the eluent ion, c, in the mobile phase, and A is a constant resulting from combining the resin ion capacity and the volume ratio of stationary phase to mobile phase. For all eluents, the relationship between k' and the reciprocal eluent cation concentration was nearly linear to about 50 mM eluent cation concentration, 20 M^{-1} on the x-axis of Fig. 1. At higher H^+ concentration, i.e., lower $1/C$, k' deviates from ion-exchange behavior and becomes independent of C, suggesting that retention is dominated by another mechanism at low pH. Weakly acidic cation-exchangers lose ion-exchange capacity when the pH is low enough to fully protonate the resin [23]. The 50 mM H^+ concentration required to render the k' invariant for the amines is consistent with a weak cation-exchange resin. This effect was evaluated further.

Fig. 2 shows the retention of the test analytes as a function of the MSA eluent concentration. The deviation from linearity at higher C is apparent, as the difference in retention between the test analytes becomes independent of the eluent concentration. When the k' values from Fig. 2 were "normalized" by dividing the k' values by the value of k' at 100 mM MSA, a concentration where retention is no longer a function of C, the normalized k' data for all of the model compounds (Fig. 3) produced nearly indistinguishable slopes between the phenylethanolamines tested. Although the CG14 resin is described as having a "low hydrophobicity", these results suggest that hydrophobic interactions dominate selectivity for the phenylethanolamines, as can be seen from the independence of the k' values for the analyte from the concentration C of the eluent cation at low pH and the marked slope of k' versus $1/C$ at higher pH, i.e., in the "ion-exchange region". The presentation of data in Figs. 2 and 3 suggests a marked superposition of the ion-exchange process, dominating at higher pH values, by an increasing contribution of hydrophobic interactions being most prominent at low pH.

In order to evaluate the extent of hydrophobic

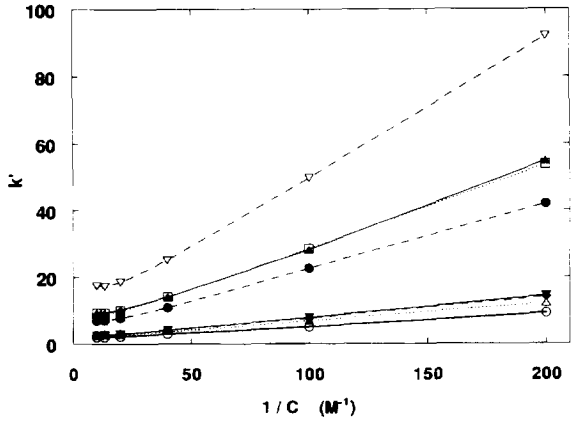


Fig. 2. Capacity factors for the phenylethanolamines vary linearly with the reciprocal of the MSA eluent concentration (C) to about 0.05 M. At cation concentrations >50 mM separation becomes independent of cation concentration. A CG14 column was used and eluted at a flow-rate of 3.0 ml/min. The average coefficient of variation of a k' value is 2%. (○) Norepinephrine; (▽) octopamine; (▲) metaraminol; (♦) epinephrine; (●) deoxy-epinephrine; (▽) MHED; (△) α-methyl norepinephrine; (□) phenylephrine.

Table 3
Capacity factors (k') for test compounds

Test compound	Mobile phase												
	KCl with 5 m*M* MSA	NaCl with 5 m*M* MSA					Methane sulfonic acid (MSA)						HCl
	25 m*M* KCl	25 m*M* NaCl	50 m*M* NaCl	100 m*M* NaCl	200 m*M* NaCl	400 m*M* NaCl	5 m*M* MSA	10 m*M* MSA	25 m*M* MSA	50 m*M* MSA	75 m*M* MSA	100 m*M* MSA	25 m*M* HCl
Norepinephrine	5.7	7.2	5.4	3.8	3.5	3.2	9.2	5.1	2.9	2.2	2.0	2.0	2.8
Epinephrine	8.6	11	8.3				14	7.7	4.1	3.0	2.8	2.8	4.1
α-Methylnorepinephrine	7.8	9.6	7.6	5.5	4.9	4.7	12	6.9	3.6	2.8	2.6	2.5	3.8
Octopamine	8.7	11	8.7	6.3	5.4	5.1	15	7.9	4.2	3.1	2.8	2.7	4.3
Deoxyepinephrine	25	33	26	18	16	16	42	23	11	7.8	7.1	7.0	11
Phenylephrine	36	42	32	24	21	21	54	29	14	10	9.2	9.2	14
Metaraminol bitartrate	37	47	34	24	23	21	55	28	14	10	9.3	9.3	14
MHED	50	83	57	40	41	39	92	50	25	19	17	17	25
Ephedrine	>250	>250	>250	>250	>250	>250	>250	>250	>250	>250	>250	>250	>250

The average coefficient of variation for k' is 2.0%

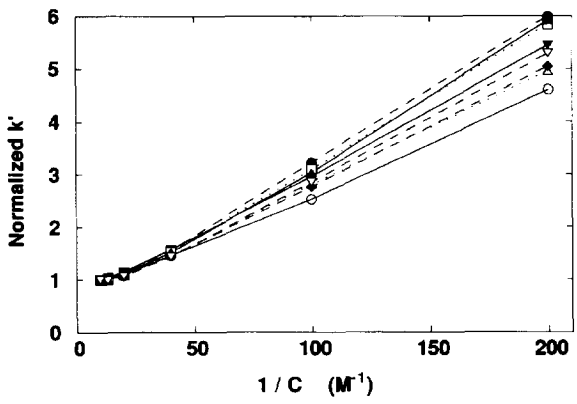


Fig. 3. Normalized capacity factors for the phenylethanolamines as described in Section 3.1 as a function of the reciprocal MSA eluent concentration (C). (○) Norepinephrine; (▽) octopamine; (▲) metaraminol; (♦) epinephrine; (●) deoxyepinephrine; (▽) MHED; (△) α-methyl norepinephrine; (□) phenylephrine.

interaction on CG14, the retention of the analytes was measured as a function of K^+ concentration with ion pairing reagents of different hydrophobicities added to the mobile phase. k' increases with the hydrophobicity of the ion pairing reagent for all of the test compounds (Fig. 4 for MHED).

Enhancement of the retention for cation-exchange resins by ion pairing reagents containing more or less extended hydrophobic substituents is widely exploited in liquid chromatographic separation processes. Although the mechanisms for the interaction are debated, the consensus for neutral compounds is that either hydrophobic interactions or Van der Waals interactions between the analyte and the resin contribute to the longer retention [24]. Small and Bremer [25] showed that ion pairing reagents in the eluent modify the hydrophobicity of an ion-exchanger by significantly enhancing partitioning of organic molecules in the resin, consistent with our findings. It is likely that the hydrophobic portion of the analytes are interacting with the resin stationary phase. Additionally, the test compounds were eluted using reversed-phase HPLC with a C_{18} stationary phase and the order of elution was the same as for cation-exchange on CG14. This evidence also supports the hypothesis that hydrophobic interaction dominates at low pH and thus greatly affects retention on the CG14 column. For this reason, the mechanism of separation is of mixed-mode character, combining ion-exchange and reversed-phase HPLC.

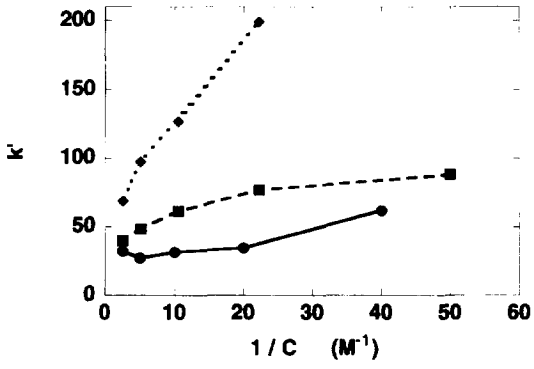


Fig. 4. Capacity factors for MHED on a CG14 column as a function of reciprocal K^+ concentration with either 5 mM MSA containing 5 mM HCl, benzenesulfonic acid containing 5 mM HCl, or hexanesulfonic acid containing 5 mM HCl added for ion pairing. The addition of ion pairing agents increases k' due to increased lipophilicity of the neutral amine complex. (●) MSA; (■) benzenesulfonic acid; (♦) hexanesulfonic acid.

3.2. Separation of [^{11}C]MHED from plasma

The CG14 retention experiments indicated that the capacity factors were sufficient for complete separation of the test compounds in plasma containing approximately 140 mM NaCl. First, the plasma proteins leave the column unretained in the void volume, whereas MHED and its metabolites are concentrated on the top of the column matrix. These were then eluted by using a mobile phase containing either salt or salt and acid. When [^{11}C]MHED in human plasma was injected on the CG14 column with a mobile phase of 150 mM NaCl, no detectable radioactivity eluted in the first 16 ml, at which time the mobile phase was switched to 100 mM MSA, where 99.7±0.8% of the [^{11}C]MHED eluted at 23–25 ml. Thus, [^{11}C]MHED can be selectively retained on the CG14 resin and separated from plasma proteins.

Although the test compounds were separated by use of the developed HPLC method, the experimental data imply that the phenylethanolamine analytes are concentrated on the top of the column matrix with a mobile phase containing NaCl as the washing solvent for complete removal of plasma proteins.

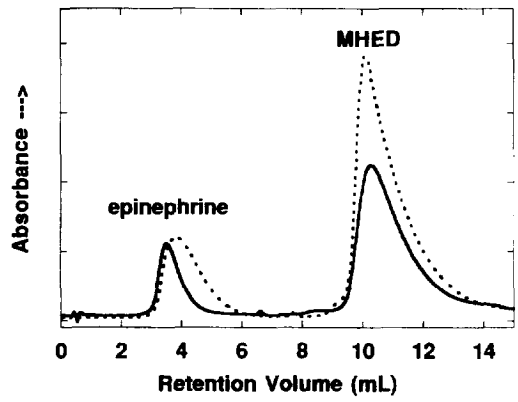


Fig. 5. Variation in retention profile for epinephrine and MHED for different injection volumes with a CG14 column. The number of theoretical plates, N, was measured for the two analytes for 10-μl and 1000-μl injections. N decreased for epinephrine by 50% for the 1000-μl injections relative to the 10-μl injection, but N did not change for MHED. The gradient method described in Table 2 was used for elution. The 10-μl injection contained 1.4 parts-per-thousand (ppt) and 2.8 ppt epinephrine and MHED, respectively, and the 1000-μl injection contained 30 parts-per-million (ppm) and 60 ppm of epinephrine and MHED, respectively. Absorbance is in arbitary units. (——) 10-μl injection; (· · ·) 1000-μl injection.

Subsequently, analyte elution from the column was effected by admixing of acid to the mobile phase. The results of injecting different volumes; 10, 500 and 1000 μl of two test compounds, epinephrine and MHED (Fig. 5), and using the gradient HPLC profile depicted in Table 2. The 500-μl data are not shown in Fig. 5 for clarity. The number of theoretical plates, N, [24] was used as a quantitative measure of the column performance with the different volume injections. The measured N decreased for epinephrine by 30% and 50% for the 500-μl and 1000-μl injections, respectively, relative to the 10-μl injection, but N did not change for MHED for the three volumes tested, indicating that the capacity for the amines is sufficient to allow negligible movement of the analytes through the column with NaCl as the mobile phase. Furthermore the solvent volume required to elute the MHED peak was approximately 1 ml and thus corresponding to the largest injection volume tested. This means that no dilution took place during separation as is only observed when the analyte is concentrated on the top of the column matrix [26].

3.3. Analysis of patient [^{11}C]MHED samples

The results of the various studies were exploited for establishment of the efficient gradient HPLC method described in Table 2. The separation of four test analytes using the gradient method developed for separation of [^{11}C]MHED from its metabolites, is shown in Fig. 6 for a 500-μl injection. Compounds differing in a single methyl group, MHED and metaraminol or norepinephrine and epinephrine, or a single hydroxyl group, MHED and epinephrine were resolved. Also shown is the elution profile for proteins in 0.5 ml of plasma detected by their absorbance at 310 nm.

A typical early 500-μl patient plasma sample analyzed by this method with integrated 20 s radioactivity sampling in a 1-ml flow-through radiation detector is also shown in Fig. 6. The samples obtained from patients show only two peaks, [^{11}C]MHED and an unidentified metabolite which increased proportionally in the plasma with time and which elutes at a retention between that of norepinephrine and epinephrine, consistent with being more hydrophilic than MHED.

The time course for ^{11}C plasma radioactivity with respect to either drug or metabolite is presented in

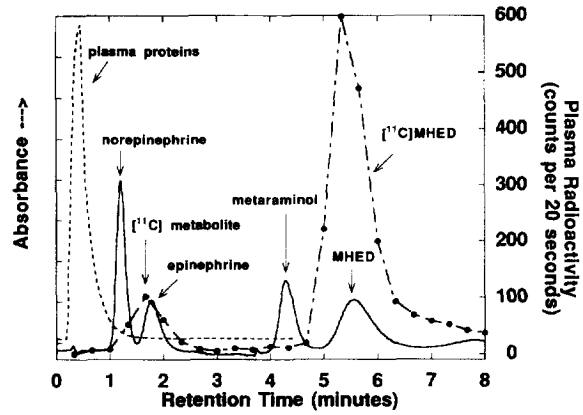


Fig. 6. Separation of 500-μl injections of standards; norepinephrine, epinephrine, metaraminol, MHED, patient plasma on a CG14 column by gradient method (Table 2). UV absorbance at 272 nm (arbitary units) for the standards and 310 nm for the proteins, data sampling rate 1 Hz. Monitoring of the profile of radioactivity with a 1-ml flow-through loop and counts integrated for 20 s per sampling point. (——) Standards; (– · –) plasma radioactivity; (· · ·) 500-μl plasma proteins.

Fig. 7. The metabolite is detected in plasma as early as 5 min after injection and the fraction of the ^{11}C which is in the metabolite peak increases with time. Very little ^{11}C is protein bound or in the form of bicarbonate, both of which would be eluted in the void volume. Our results from the patient study are consistent with the findings of Osman et al. [19], who also found a single metabolite in plasma which increased over time as the fraction of [^{11}C]MHED decreased. The advantage of our method is that the tedious step of deproteinization is not required prior to injection, reducing total analysis time by a factor of two. Also, the chromatographic analysis is fast and requires less volume of mobile phase; elution of MHED is at 5.5 min (10.5 ml) versus 10 min (30 ml) [19]. The total separation time, including gradient recycling, for our method is 9.6 min. The rapid separation results in a higher radioactivity count rate and smaller counting errors, which in combination markedly improve either accuracy or precision of the blood metabolite curve as shown in Fig. 7. A single guard column has been used for the injection of seven sequential samples, a complete patient study, with no change in pressure or retention over seven injections when an in-line filter preceded the column. Another advantage of this method is that the aqueous biohazard effluent can be disinfected with bleach and disposed through the sewer, thus avoiding the creation of mixed hazardous waste.

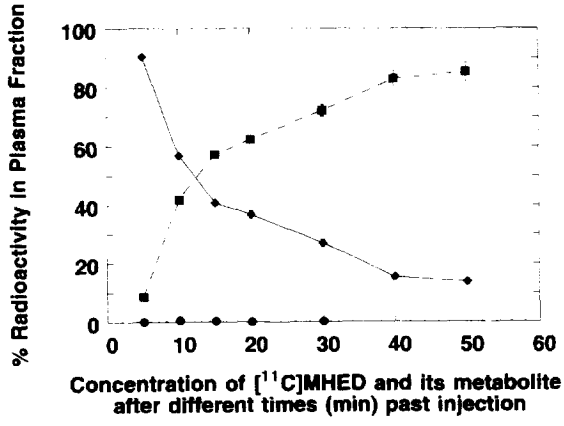


Fig. 7. The fraction of ^{11}C radioactivity in individual components of patient plasma samples varies with time after administration of [^{11}C]MHED. Separation of 0.5 ml of patient plasma using the CG14 column gradient method described in Table 2. Error bars are 1 S.D. propagated counting error and when not visible are smaller then the data point. (– ● –) C-11 bound to protein; (– ■ –) MHED metabolite: (– ♦ –) MHED.

4. Conclusion

This work shows that phenylethanolamines can be concentrated on-column from plasma using the CG14 cation-exchange column and eluted using an acidic mobile phase. Ion-exchange mechanisms determine retention but hydrophobic interactions determine selectivity. This mixed-mode ion-exchange/reversed-phase separation using an aqueous mobile phase is a methodology which might be exploited for studies of other biologically active amines, i.e., naturally occuring ones as well as xenobiotics and their corresponding metabolites. The separation of MHED from its metabolite via the mixed-mode separation mechanism was chosen because it is rapid and thus reduces analysis times to less than 10 min, which will not be achieved by "traditional" RP-HPLC. Further advantages of the weak cation-exchange matrix over the preponderantly used reversed-phase materials are that plasma proteins leave the column unretained and that no poisonous and biohazardous organic solvents are required.

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