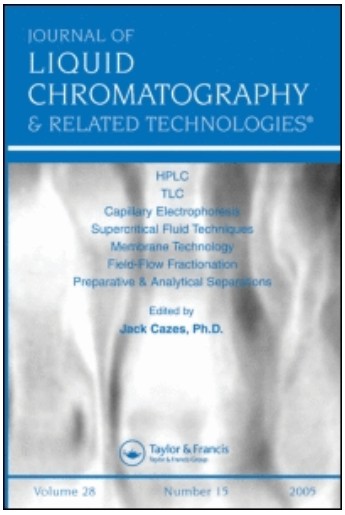

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Determination of Exogenously Administered Dopamine in Infant Plasma Using Liquid Chromatography with Electrochemical Detection

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To cite this Article Bhatt, Varsha and Nahata, Milap C.(1989) 'Determination of Exogenously Administered Dopamine in Infant Plasma Using Liquid Chromatography with Electrochemical Detection', Journal of Liquid Chromatography & Related Technologies, 12: 8, 1463 — 1471
To link to this Article: DOI: 10.1080/01483918908049517
URL: http://dx.doi.org/10.1080/01483918908049517

JOURNAL OF LIQUID CHROMATOGRAPHY, 12(8), 1463-1471 (1989)

DETERMINATION OF EXOGENOUSLY ADMINISTERED DOPAMINE IN INFANT PLASMA USING LIQUID CHROMATOGRAPHY WITH ELECTROCHEMICAL DETECTION

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ABSTRACT

A simple, rapid liquid chromatography method is described for the measurement of exogenously administered dopamine in infant plasma, using electrochemical detection and 3,4 dihydroxybenzylamine hydrobromide as the internal standard (IS).

The mobile phase consisted of a monochloroacetate buffer (pH=3.0) with sodium octyl sulfate as the ion-pairing agent, and 2.5% acetonitrile. A biophase II^{R} reverse phase column was used, and the electrochemical detector was set at +650 mV versus Ag/AgCl. The retention times of IS and dopamine were 3.3 and 6.5 minutes, respectively. The recovery of dopamine following an alumina extraction procedure ranged from 75 to 80%. The inter- and intra-day coefficients of variation using this method were less than 10% and less than 5%, respectively. The major advantages of this method include use of a very small volume of plasma sample (20 µl) and ability to determine very wide ranges of plasma concentrations of dopamine following its administration intravenously. The method was used to measure the plasma concentrations of dopamine in two newborn infants to demonstrate its clinical application.

INTRODUCTION

Dopamine hydrochloride (3-hydroxytyramine hydrochloride) is widely used to increase blood pressure, cardiac output, urine output and peripheral perfusion in critically ill newborn infants and children with shock and congestive heart failure.[1]

Current dosage guidelines for infants and children are not based on adequate pharmacokinetic studies. This is partly due to lack of an accurate, sensitive and specific analytical method for determination of dopamine in plasma following its administration. Methods such as gas chromatography with electron capture or mass spectrophotometric detection, radioenzymatic assay (REA) and liquid chromatography (HPLC) with fluorescence detection have all been used previously for determination of dopamine in plasma.[2] However, lack of specificity (REA), sensitivity (HPLC with fluorescence detection), the need for derivatization (fluorescence and REA) and specialized detection procedures (gas chromatography) make these methods cumbersome, expensive, time-consuming and hence less suitable for routine use.[2,3]

Liquid chromatography with electrochemical (EC) detection involves a simple liquid-solid extraction of catecholamines onto alumina, followed by their elution with a dilute acid.[2-5] The method is comparable to REA in terms of sensitivity.[6] However, it should be more specific than REA for the quantitation of catecholamines. Although HPLC with EC detection has been widely used for determination of endogenous catecholamines and their metabolites in plasma, tissue and urine, no information is available about its usefulness in measuring plasma catecholamines following exogenous administration. A large sample volume of 0.5 to 2 ml is required for endogenous catecholamine measurement, since these are present in relatively low amounts in plasma.[2,3] This makes the existing method less suitable for infants and children. Besides, there is no HPLC method available which has been validated for the measurement of high dopamine concentrations in plasma, which would be expected in pediatric patients receiving dopamine infusions.

Our primary objective was to develop a simple, rapid, sensitive, and specific method for determination of high concentrations of exogenously administered dopamine in plasma using relatively small sample sizes (20-50 µl). The clinical application of this method was demonstrated by measurement of plasma dopamine concentrations in two newborn infants receiving dopamine infusion.

MATERIALS AND METHODS

Reagents

Pure dopamine hydrochloride powder, internal standard 3,4 dihydroxybenzylamine hydrobromide (DHBA HBr), trisma base, and octyl sulfate sodium (SOS) were purchased from Sigma Chemical Company (St. Louis, MO); monochloracetic acid (MCAA) and sodium hydroxide from Fisher Scientific (Fairlawn, NJ); sodium ethylenediamine tetracetate (Na_2EDTA) from Eastman Kodak Co. (Rochester, NY); perchloric acid (70% v/v) from GFS Chemicals (Columbus, OH); acid-washed aluminum oxide (AAO) from Bioanalytical Systems (BAS) Inc. (West Lafayette, IN); and monobasic potassium phosphate anhydrous, dibasic sodium phosphate anhydrous and concentrated hydrochloric acid (37% v/v) from Mallinckdrot, Inc. (Paris, KY). Triple distilled, deionized water was obtained from an in-house Milli-Q water system (Millipore, Bedford, MA).

Mobile phase, Standard Solutions and Buffers

The mobile phase was prepared using 0.15 mM MCAA buffer (pH= 3.0) with 1 mM SOS (ion-pairing agent) and 2.5% acetonitrile. Sodium ethylenediamine tetracetate, 0.5 mM was added as a chelating agent and the solution filtered through a 0.2 micron filter membrane under vacuum. Filtered mobile phase was stable for 4 weeks when stored in a refrigerator. The internal standard was prepared by dissolving 16 mg of DHBA HBr in 100 ml of 0.1 M perchloric acid and further diluted to a final concentration of 100 ng/ml. Dopamine hydrochloride (16.2 mg) was dissolved in 13.1 ml of 0.1 M perchloric acid to give a stock concentration of dopamine, 1 mg/ml. Portions of this solution were stored in disposable cryotubes (Internal Nunc,

Roskilde-Demark) at -70°C for three days. A fresh stock solution was made every three days. On the day of the experiment, this stock solution was further diluted with 0.1 M perchloric acid to obtain a range of concentrations from 25 to 2,500 pcg/20 µl. To prepare tris buffer, trisma base (45g) was combined with Na_2EDTA (5 gm) and dissolved in 200 ml of distilled water. The pH was adjusted to 8.6 using conc. hydrochloric acid. The final volume was adjusted to 250 ml. The buffer was filtered with a 0.2 µm membrane and stored in a refrigerator.

The phosphate buffer (0.1 M, pH=7) was prepared by combining anhydrous dibasic sodium phosphate and anhydrous monobasic potassium phosphate. Na_2EDTA (20 g/L) was added as a chelating agent. The solution was filtered and stored in a refrigerator.

Chromatographic Instrumentation

A BAS 200^R liquid chromatograph with a built-in EC detector was used for analysis. The solvent delivery system consisted of dual-piston chromatographic pump (isocratic or gradient mode), mobile phase manifold flasks, pulse dampner, a helium degassing system with continuous helium flow to deoxygenate the mobile phase, and a purge valve. A convection oven encased a Biophase II^R, reverse phase column (100 mm x 3.2 mm, 3 µm particle size; BAS, W. Lafayette, IN), a built-in rheodyne syringe sample injector (model 7125) and the EC detector. The EC detector system included a dual glassy carbon working electrode and a Ag/AgCl reference electrode. The entire system was controlled using BAS PC^R (BAS, W. Lafayette, IN) system control software, which was fully compatible with an IBM PC.

Extraction

The samples were prepared for extraction by combining 50 mg AAO, 1 ml tris buffer, 2 ml phosphate buffer, 25 µl internal standard and 20 µl of dopamine standard solution or patient's plasma in a 5 ml conical reaction vial (Pierce Chemical Co., Rockford, IL). The mixture was immediately vortexed for 5 to 10 seconds and shaken

on a reciprocal mechanical shaker (Precision, GCA Corporation) for 5 minutes. The vial was then allowed to stand until the alumina settled to the bottom and the supernatant aspirated using a disposable pipette. The alumina was then washed twice with 1 ml distilled water and aspirated to near dryness each time. Care was taken not to lose any alumina in the aspirate. Distilled water, 0.5 to 1.0 ml was then added to the vial and resulting alumina slurry transferred to a microfilter (BAS W. Lafayette, IN) loaded with 0.2 µm nylon 66 microfilter membrane (BAS W. Lafayette, IN), using a disposable pipette. The microfilter was placed in a clinical centrifuge (Damon IEC Division Needham H&S MA) and the AAO spun to dryness at 2,000 rpm for 2 to 3 minutes. A new receiver tube was placed on the microfilter and 200 µl of 0.1 M perchloric added to the microfilter sample compartment. The microfilter was vortexed briefly (approximately 5 seconds), allowed to stand for 5 minutes and vortexed briefly (approximately 5 seconds) again. It was then centrifuged at 760 rpm for 2 minutes. The acidic extract in the receiver tube contained catecholamines ready for injection onto the column. Only one sample was extracted at a time and the injections were made manually.

Assay procedure

One hundred microliters of the extracted sample were loaded into the rheodyne injector containing a 200 µl capacity loop. Prior to sample analysis the column was allowed to equilibrate with the mobile phase overnight. When the column was not in use, it was flushed with 15 to 30 ml of mobile phase. The mobile phase was then recycled using the recycling facility of the BAS 200^{R} solvent delivery system. In this manner there was minimal mobile phase wastage and the column was always ready for analysis. The mobile phase (35°C), column (40°C) and the detector (41°C) were all temperature-controlled throughout the course of analysis. The mobile phase was deoxygenated using pure helium gas. The flow rate of the mobile phase was adjusted to 1 ml/min.

The EC detector was operated at an oxidation potential of +650 mV versus an Ag/AgCl reference electrode. The sensitivity (gain) of the detector was maintained between 10 to 50 nA full scale throughout the analysis period. The EC detector, once turned on, was allowed to stabilize overnight before analysis was started and never turned off unless it was planned not to conduct analysis for more than two weeks.

The chromatogram was recorded on an IBM PC compatible printer and the peaks were integrated using the BAS PC^R integration program. The peak heights and/or peak areas were used to determine concentrations.

To calibrate the instrument, specimens containing dopamine, 2,500 pcg/20 µl and internal standard (25 µl) were assayed in duplicate following extraction. To calculate the sample concentrations, peak height ratios (dopamine to internal standard) for unknown plasma samples were compared with ratios of known concentrations of the synthetic standard.

To determine linearity, a standard curve was run for concentrations ranging from 25 to 2,500 pcg/20 µl using 25 µl of the internal standard and the same extraction procedure as described earlier except that the 20 µl of plasma was replaced with 20 µl of standard dopamine solution in 0.1 M perchloric acid. A linear regression analysis gave an r value of 0.998 with a slope of 0.00015 and an intercept of 0.034.

The recovery of dopamine using our extraction procedure was determined by comparing the peak heights of extracted samples of standard dopamine and of plasma dopamine with peak heights of dopamine in aqueous solution injected onto the column without extraction. The recovery ranged from 75 to 80% for dopamine from both plasma and standard solution.

Clinical Application

Two newborn infants (postnatal age 16 hours and 55 hours) with suspected sepsis and presence of hypotension were studied following intravenous administration of dopamine, 10 µg/kg/min. A blood

sample was collected at steady-state for each infant. The blood was collected in a heparinized collection tube, transported to the laboratory on ice and the plasma separated immediately and stored at -70°C. Analysis was performed within two weeks of collecting the samples.

RESULTS AND DISCUSSION

A typical chromatogram for dopamine and the internal standard is shown in Figure 1. Under our assay conditions, each run lasted approximately 8 minutes. The retention times for the internal

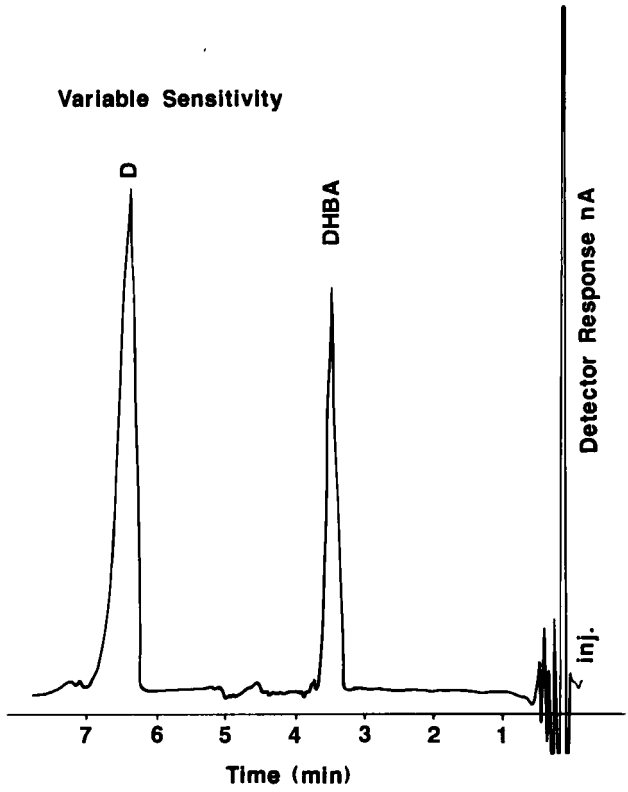

Figure: A typical chromatogram for dopamine (D) and internal standard (DHBA).

standard and dopamine were 3.3 minutes and 6.5 minutes, respectively. The inter- and intra-day coefficient of variation using this method was less than 10% and less than 5% respectively.

It was found more useful to inject extracted standards between patient samples rather than generate a 4 to 5 point standard curve at the beginning of the day since the former took into account the changing response of the electrochemical detector during the course of the day and hence provided more accurate results. At least two different concentrations were repeated twice a day, generally at the beginning of the day and after 5 to 7 patient samples had been analyzed. In our study, the plasma samples were stored at -70°C for a minimal of two weeks since no substantial loss of catecholamines has been reported during a two-week storage period.[7]

In two infants receiving dopamine at a dose of 10 µg/kg/min, the steady-state plasma concentrations were 0.06 and 0.065 µg/ml. Total clearance, calculated from dose divided by the steady-state plasma concentration was 10 and 9.2 L/kg/hr. These clearance values were substantially higher than those reported in infants using an REA method for the analysis of dopamine.[8] This suggests that REA may have overestimated the plasma concentration of dopamine due to poor specificity. In summary, we have described an HPLC method which is rapid, accurate, specific and suitable for clinical application for the quantitation of dopamine in plasma of seriously ill infants and children.

We acknowledge the assistance of Dr. Chester Duda, Ph.D. and Mr. Jan Allen Pursley of BAS, W. Lafayette, IN in the development of this assay.

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REFERENCES

1. Zoritsky A, Chernow B. Use of catecholamines in pediatrics. J Pediatr. 1984; 105:341-349.

2. Plasma catecholamines. LCEC application note no. 14. Bioanalytical systems Inc., West Lafayette, IN.

3 Hallman H, Farnebo L, Hamberger B and Jonsson G. A sensitive method for determination of plasma catecholamines using liquid chromatography with electrochemical detection. Life Sciences, 1978;23:1049-1052.

4. Plotsky M, Gibbs DM, Neill JD. Liquid chromatographic-electrochemical measurement of dopamine in hypophysical stalk blood of rats. Endocrinology. 1978;102:1887-1894.

5. Kotake C, Heffner T, Vosmer G, Seiden L. Determination of dopamine, norepirephine, scrotonin and their major metabolic products in rate brain by reverse-phase ion-pair high performance liquid chromatography with electrochemical detection. Pharmacol Biochem Behav, 1985;22:85-89

6. Goldstein DS, Feuerstein G, Izzo JL, Kopin IJ and Keiser HR. Validity and reliability of liquid chromatography with electrochemical detection for measuring plasma levels of norepinephine and epinephrine in man. Life Sciences, 1981;28:467-475.

7. Boudoux P, Perrett D, Besser AM. Methodological considerations in the determination of plasma catecholamines by high-performance liquid chromatography with electrochemical detection. Ann Clin Biochem, 1985;22:194-203.

8. Padbury JF, Agata Y, Baylen B, et al. Dopamine pharmacokinetics in critically ill newborn infants. J Pediatr, 1987,110:293-298.