Development of an Analytical Assay for the Determination of Vinpocetine's Primary Metabolite, Apovincaminic Acid, in Human Plasma

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BACKGROUND

Vinpocetine (*VP*) is a derivative obtained from (+) vincamine, and is commonly extracted from periwinkle. It has been shown to alleviate risks associated with stroke by enhancing cerebral blood flow. *VP* is also useful for the treatment of epilepsy and for the prevention of dementia. Current research is examining whether VP's therapeutic effect is due to its active metabolite, *AVA*.

LABORATORY OBJECTIVES

I. EXPERIMENTAL AIMS

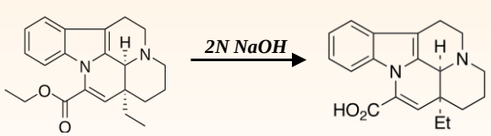
The aim of this UROP was to continue the development of an analytical method for the quantification of *VP* active metabolite, Apovincaminic Acid (*AVA*), in human plasma. Specifically, our project focused on the synthesis of *AVA* from *VP*, a standard-based extraction, and a qualitative determination of *AVA* via High Performance Liquid Chromatography-Mass Spectrometry (HPLC-MS).

II. SYNETHESIS OF AVA

The procedure employed for *AVA* synthesis was modeled after Christie and Rapoport. In summary, 2 N NaOH was added to a sample of *VP* and subsequently worked up in 1 M H_3PO_4.

Extraction of the organic layer, drying over anhydrous Na_2SO_4, vacuum filtration, and evaporation using a Rotovap yielded a crude residue of the *AVA* metabolite.

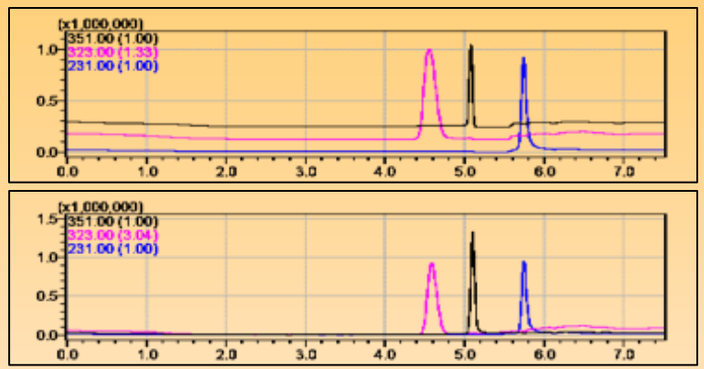
FIGURE 1: Synthesis of AVA from VP via Base-Catalyzed Esterfication



III. SOLID PHASE EXTRACTION

Extraction was performed on a standard solution containing known amounts of *VP*, *AVA*, and *S*-Naproxen (*Internal Standard, IS*). *Phenomenex Strata-X Solid Phase Extraction* (*SPE*) cartridges were used on a vacuum manifold (15 kPa). Following reconstitution of extracted compounds in mobile phase, samples were subjected to HPLC-MS in order to determine *% Recoveries*.

FIGURE 2: Chromatogram of Un-Extracted (TOP) and Extracted (BOTTOM) Samples



VP and *AVA* were separated by using the conditions listed in ***TABLE 2***. Samples were subjected to an organic gradient (20% - 70% HPLC Grade MeOH) over 7.5 minutes ***(FIGURE 4)***. *VP*, *AVA*, and *IS* standards were dried under N_2 gas and reconstituted in 80:20 DI H_2O:MeOH. Retention of the compounds remained consistent throughout experimentation with *AVA* eluting first (4.56 minutes), followed by *VP* and *IS* at 5.10 and 5.74 minutes, respectively.

TABLE 1:	**SPE CONDITIONS**
Cartridge Type	*Strata-X Polymeric Reversed Phase*
Sorbent Weight	*30 mg/mL*
Cartridge Volume	*1 mL*
Conditioning Solution	*1 mL MeOH; 1 mL H_2O*
Elution Solution	*0.5 mL H_2O*

TABLE 2:	**CHROMATOGRAPHIC CONDITIONS**
Aqueous Phase	*Ammonium Acetate : Methanol : Formic Acid (60 : 40 : 0.1, v/v/v)*
Organic Phase	*HPLC Grade Methanol (100)*
Gradient	*20% - 70%*
Flow Rate	*0.5 mL/min*
Column	*Reversed-Phase Luna C18 50 x 2.0 mm (Phenomenex)*

Referenced Literature

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2. Nyakas et al. 2009. Neuroprotective Effects of VP and its Major Metabolite Cis-AVA on NMDA-Induced Neurotoxicity in a Rat Entorhinal Cortex Lesion Model. CNS Neuroscience & Therapeutics 15 (2); MONTH; 89 – 99.
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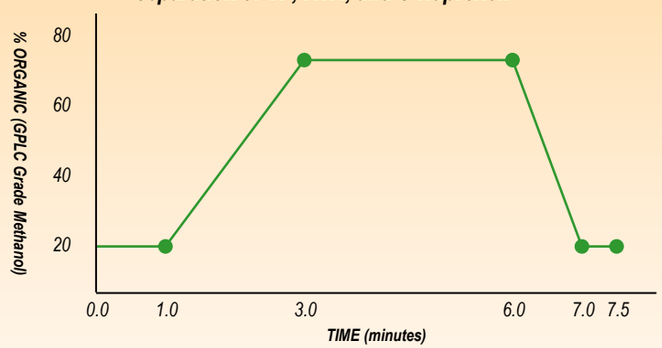
4. Xia et al. 2010. Determination of VP and its Primary Metabolite, AVA, in Rat Plasma by LCMS-MS. Journal of Chromatography B 22 (15); July; 1959 – 1966.
5. Christie and Rapoport 1984. Synthesis of Optically Pure Pipecolates from L-Asparagine. Application to the Total Synthesis of (+) – Apovicamine through Amino Acid Decarbonylation and Iminium Ion Cyclization. Journal of Organic Chemistry 50; MONTH; 1239 – 1246.

RESULTS

Chromatographic results of the product displayed substantial peaks at m/z 323.0, which correspond to *AVA*. In addition, *Thin Layer Chromatography* demonstrated that *VP* and the final product, *AVA*, were distinct yet similar in their movement; this was consistent with our expectations given the high degree of structural similarity between *VP* and *AVA*.

Analysis of chromatographic data was analyzed on *Shimadzu Scientific's LCMS Lab Solution* software. HPLC-MS peak intensities were integrated, and a *% Recovery* was determined. Recovery of *VP* ranged from 43 – 83%, while recovery of *AVA* ranged from 37 – 39%.

FIGURE 4: Chromatographic gradient used for the separation of VP, AVA, and S-Naproxen



CONCLUSIONS

The objective of developing a method for the synthesis of *AVA* from *VP* was fulfilled. A synthetic procedure was carried out, and the presence of *AVA* in the final product was qualitatively confirmed. Additionally, the HPLC-MS conditions necessary for separation of the main analytes were also achieved. The appropriate *SPE* cartridge and extraction procedure for our compounds of interest were determined, as well.

Future research should be directed towards optimizing the *SPE* procedure (from a plasma or serum matrix) and subsequent validation of the experimental method.

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