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Identification and quantification of vinpocetine and picamilon in dietary supplements sold in the United States

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Vinpocetine and picamilon are drugs prescribed in many countries to treat a variety of cerebrovascular disorders. In the United States, vinpocetine and picamilon have never been approved by the US Food and Drug Administration, but they are both available for sale directly to consumers as dietary supplements. We designed our study to determine the accuracy of supplement labels with regard to the presence and quantity of vinpocetine and picamilon. A validated ultra-high performance liquid chromatography-photodiode-array method was developed for the quantification of vinpocetine and picamilon. The separation was achieved using a reversed phase (C-18) column, photodiode array detection, and water/acetonitrile as the mobile phase. Vinpocetine and picamilon were detected at concentrations as low as 10 and 50 ng/mL, respectively. The presence of vinpocetine and picamilon was confirmed using reference standards. Twenty-three supplements labelled as containing vinpocetine were available for sale at two large supplement retail chains; 17 contained vinpocetine with quantities ranging from 0.3 to 32 mg per recommended daily serving. No vinpocetine was detected in six of the sampled supplements. The supplement label implied that vinpocetine was a constituent of lesser periwinkle in three of the supplements. Of the 31 picamilon supplements available for sale from a variety of retailers: 30 contained picamilon in quantities ranging from 2.7 to 721.5 mg per recommended daily serving. We found that consumers cannot obtain accurate information from supplement labels regarding the presence or quantity of vinpocetine and picamilon. Copyright © 2015 John Wiley & Sons, Ltd.

Keywords: lesser periwinkle; vinpocetine; picamilon; dietary supplements; UHPLC-PDA

Introduction

Dietary supplements in the United States (USA) are regulated as food, sold directly to consumers, and cannot be marketed to treat disease. Given this regulatory paradigm, it is surprising to find that hundreds of brands of dietary supplements are openly labelled as containing prescription drugs never approved by the US Food and Drug Administration (FDA). Vinpocetine and picamilon are two such drugs sold directly to consumers in dietary supplements.

Vinpocetine is prescribed in Germany, Russia, China and other countries at dosages from 5 to 40 mg for acute stroke and cognitive impairment.[1,2] Its neuroprotective effects have never been proven,[2,3] and it has displayed adverse effects including flushing, headaches, and decreased blood pressure.[4] More than 300 brands of supplements labelled as containing vinpocetine[5] are available for sale in the USA. Vinpocetine is not found in nature but is closely related to vincamine, a natural and major component of leaves of the lesser periwinkle plant (*Vinca minor* L).[6–9] Vinpocetine is a dehydrated derivative of vincamine in which the methyl ester is replaced by an ethyl ester (Figure 1) and several approaches to synthesizing vinpocetine have been described.[10–13]

Picamilon (also known as nicotinoyl-GABA, pycamilon, and pikamilon) was developed by Russian investigators in an effort to increase central nervous system (CNS) levels of γ-aminobutyric acid (GABA).[14,15] Drugs that can mimic or increase GABA activity in the brain have the potential to provide anti-anxiety and anti-convulsive effects.[16] GABA itself, when consumed orally, does not cross the blood-brain barrier.[17–19] Much research has, therefore, been devoted to developing orally active agents that have GABA-like effects. Both gabapentin (a GABA analogue) and picamilon (Figure 1) were designed to be orally administered drugs that cross the blood-brain barrier and provide inhibitory CNS effects. Picamilon, prescribed in dosages from 50 mg to 200 mg, is able to cross the blood-brain barrier in animal models and, once in the CNS, is hydrolyzed into GABA and nicotinic acid.[20] The released GABA could potentially have inhibitory properties including anti-anxiety and anti-convulsant effects.[16] Nicotinic acid would also be released, potentially leading to dilation of CNS blood vessels.[21] The FDA has never approved picamilon for use in the USA, but picamilon is used in Russia to treat various neurological conditions.[22]

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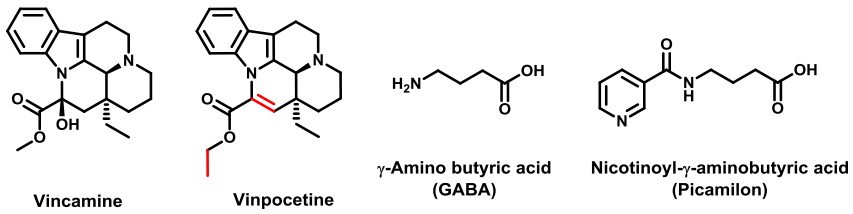

Figure 1. Chemical structures of vincamine, vinpocetine, GABA and picamilon.

While GABA and nicotinic acid are both found in nature, to our knowledge, picamilon has only been produced synthetically and has no known natural source. Picamilon has been synthesized using several methods, often beginning with either nicotinic acid hydrazide or nicotinic acid. The acid group in nicotinic acid is converted to an active ester and condensed with GABA or 2-pyrrolidinone to yield nicotinoyl-GABA (picamilon).[23–25]

To our knowledge, the accuracy of the information available on the label regarding the quantities of these two drugs in dietary supplements has not been previously studied. Spectrophotometric and high performance liquid chromatographic (HPLC) methods have previously been used to determine the quantity of vinpocetine in laboratory-prepared mixtures and pharmaceutical preparations,[9,26] HPLC methods have been applied to detecting vinpocetine in biological matrices including human and rat plasma[27,28] and gas chromatographic-mass spectroscopy (GC-MS) methods[29] have been used for biological matrices such as human plasma. In respect to picamilon, previous researchers used HPLC to determine the quantity of picamilon in biological matrices such as human plasma samples.[30] Only a few methods have been reported for the analysis of vinpocetine or picamilon. These methods required long run times, showed poor sensitivity or provided inadequate validation data.

The aim of the present work was to develop a simple, precise, specific, accurate, and validated ultra-high performance liquid chromatography (UHPLC) method according to International Conference on Harmonisation (ICH) guidelines[31] for the estimation and routine analysis of vinpocetine and picamilon in dietary supplements.

Materials and methods

Materials

The standard compounds, vinpocetine and vincamine (Figure 1), were purchased from Sigma Aldrich Chemical Co. (>98% purity) (St Louis, MO, USA). Picamilon was purchased from BOC Sciences (>98% purity) (Shirley, NY, USA) .

Hundreds of supplements labelled as containing vinpocetine are available for sale in the USA. The study was limited to supplements listing vinpocetine as an ingredient sold on the websites of two of the largest retailers of supplements in the USA (i.e., GNC and Vitamin Shoppe). Twenty-three supplements labelled as containing vinpocetine were sold on these two retailers' websites. Supplements were purchased directly from the retailers' websites between May and June 2014. Authenticated dried leaves of *Vinca minor* L. (NCNPR accession # 3032) were obtained from the cultivated, living collection of the NCNPR Maynard W. Quimby Medicinal Plant Garden, University of Mississippi. Authenticated dried leaves of *Vinca minor* L. (NCNPR accession # 8978) were obtained from the Missouri Botanical Garden (St. Louis, Missouri, USA).

Picamilon is not sold in as many supplements as vinpocetine, and therefore we attempted to purchase as many supplements labelled as containing picamilon as possible. The supplements were identified as follows: (1) 27 supplements were identified by searching the National Institute of Health's Dietary Supplement Label Database for pikatropin or picamilon; (2) other supplements made by the manufacturers of these 27 supplements were examined to determine if other products listed picamilon or pikatropin; and (3) Google search engine was queried for 'supplements containing pikatropin' and 'supplements containing picamilon'. In total, 35 supplements listing picamilon or pikatropin were identified (henceforth referred to as picamilon supplements). We attempted to purchase one sample of each of the 35 picamilon supplements from online retailers in January 2015. Four picamilon supplements never arrived from the retailers, therefore we analyzed one sample of each of the 31 picamilon supplements received.

Preparation of standard solutions

An individual stock solution of the standard compounds was prepared at a concentration of 1 mg/mL in methanol. The calibration curves were prepared at seven different concentration levels. The range of the calibration curves was 0.1–100 μg/mL for vinpocetine and picamilon.

Sample preparation

For capsules/tablets/powders

Ten tablets were weighed and then pulverized in a mortar and pestle. For capsules, ten samples were weighed, opened, and the contents mixed and triturated in a mortar and pestle. For powder samples, one scoop was mixed and triturated in a mortar and pestle.

For solids

Either 500 mg or 10 mg were used for extraction. Five hundred mg were used for the dry plant samples and for all supplements that did not provide a specific quantity of vinpocetine or picamilon on the label. Ten mg were used for each supplement sample that did list a specific quantity of vinpocetine or picamilon on the label. All samples were then sonicated in 2.5 mL of methanol for 30 min followed by centrifugation for 10 min at 959 x g. The supernatant was transferred to a 10 mL volumetric flask. The procedure was repeated four more times with 2.0 mL methanol and the respective supernatants were combined. The final volume was adjusted to 10.0 mL with methanol and the resulting solution was mixed

thoroughly. Prior to injection, an adequate volume (ca. 2 mL) was passed through a 0.45 μm PTFE membrane filter. The first 1.0 mL was discarded and the remaining volume was collected in an LC sample vial.

For liquids

One mL of directly filtered samples was used for analysis and also separately about 120 mL of the liquid was lyophilized, weighed and dissolved in 10.0 mL of methanol. The mixture was vortexed for 30 seconds and sonicated for 10 min, vortexed for 30 s and centrifuge for 10 min at 959 x g. The clear supernatant solutions were directly filtered and lyophilized samples were used for analysis.

Validation procedure

The newly developed UHPLC method was validated in terms of precision, accuracy, and linearity according to ICH guidelines.[31] The limit of detection (LOD) and limit of quantification (LOQ) were determined by injecting a series of dilute solutions with known concentrations for each standard. LOD and LOQ were defined as the signal-to-noise ratio equal to 2 or 3 and 10, respectively. The accuracy of the assay method was evaluated in triplicate using three concentration levels of 1, 25, and 50 μg/mL. Intra- and inter-day variation of the assay was determined on 3 consecutive days with 3 repetitions each.

Instrumentation and experimental conditions

UHPLC-PDA analysis

All analyses were performed on a Waters Acquity UPLC™ H-Class system (Waters Corp., Milford, MA, USA) including quaternary solvent manager, sampler manager-flow through needle, column heater and photo-diode array (PDA) detector connected to Waters Empower 2 data station. An Acquity UPLC™ BEH 130 C18 column (50 mm × 2.1 mm I.D., 1.7 μm) also from Waters was used. The column and sample temperature were maintained at 40°C and 20°C, respectively. The column was equipped with an LC-18 guard column (Vanguard 2.1 x 5 mm, Waters Corp., Milford, MA, USA). The mobile phase for vinpocetine consisted of water (0.1% formic acid) (A), acetonitrile (B) (0.1% formic acid) at a flow rate of 0.23 mL/min, which were applied in the following linear gradient elution: 0 min, 90% A:10% B and ramped to 30% A:70% B within 10 min whereas the mobile phase used for picamilon consisted of water (0.1% formic acid) (A), acetonitrile (B) (0.1% formic acid) at a flow rate of 0.2 mL/min, which was applied in the following linear isocratic elution: 0 min, 95% A:5% B for 2 min. Separation was followed by a 2 min washing procedure with 100% B and re-equilibration period of 3.5 min. Strong needle wash solution (95/5; acetonitrile/water) and weak needle wash solution (10/90; acetonitrile/water) were used. All solutions were filtered via 0.45 μm membrane filters and degassed before their usage. The total run time for analysis was 10 min for vinpocetine and 2 min for picamilon. The injection volume was 10 μL. The absorption maxima were 222, 269 nm and 314 nm for vinpocetine whereas the absorption maxima were 213 nm and 261 nm for picamilon (Figure 1). Peaks were assigned by spiking the samples with standard compounds, UV spectra and comparison of retention times.

Results and discussion

UHPLC-PDA analysis

The quantification of vinpocetine and picamilon was carried out using the UHPLC-PDA method at wavelength 269 nm and 261 nm, respectively. The UHPLC-PDA method described was tested with respect to sensitivity (LOD and LOQ), linearity, intra-day, and inter-day precision for three consecutive days, accuracy, specificity, stability, system suitability, and robustness. The actual amount of vinpocetine or picamilon consumed daily was calculated based on the recommended daily usage provided on the label. The estimated maximum daily intake (mg/day) was calculated by multiplying the weight of vinpocetine (mg) by the dilution factor by the suggested maximum daily intake in capsules or tablets/ weight (mg) of content in capsules or tablets.

Method validation

The validation study allowed the evaluation of the method for its suitability for routine analysis.

Linearity, range, LOD, and LOQ

The seven point calibration curve for vinpocetine and picamilon showed a linear correlation between concentration and peak area. Calibration data indicated the linearity (r^2>0.99) of the detector response for vinpocetine and picamilon were from 0.1–100 μg/mL. The limits of detection and limits of quantification were found to be 10 ng/mL and 25 ng/mL for vinpocetine and 50 ng/mL and 100 ng/mL for picamilon. All samples and standard were injected in triplicate.

Specificity

The specificity of the method was determined by injecting individual samples, wherein no interference was observed for any of the components. The chromatograms were visually checked for the appearance of any extra peaks. Peak purity and identity were verified by studying UV-spectrum data, as well as by spiking samples with the reference compound. Peak purity was confirmed by injecting concentrated standards (1 mg/mL) and detecting by PDA (at 269 nm for vinpocetine and 261 nm for picamilon). Peak purity was determined by checking the UV spectra of a peak at different points viz peak start, apex, and peak end. The UV spectra at all different points in a peak were the same. The purity of the chromatographic peaks was found to be satisfactory.

Stability of the solutions

The sample solutions (#16552 for vinpocetine; #17006 for picamilon) and standard solutions (10 μg/mL) were prepared as per the proposed method and subjected to stability studies at room temperature for 2 days. The sample solution was analyzed at initial and at different time intervals up to 48 h. No significant changes were observed in the concentrations of the components analyzed with respect to time.

System suitability

System suitability tests were used to ensure reproducibility by injecting 2 μL standard solutions of vinpocetine and picamilon at least six times. The % RSD ranged between 0.10% and 0.25% for vinpocetine and 0.05% and 0.13% for picamilon which was deemed acceptable.

Precision

Intra- and inter-day variation of the analysis was determined for 2 samples (#16552, #16574) of vinpocetine and 3 samples (#17006, #17024, #17033) of picamilon. It was performed three times on three different days and each run was repeated in triplicate. The intra-day RSD for the replicates were between 1.5 and 3.5% and RSD for the day to day replicates were between 2.5 and 4.5%.

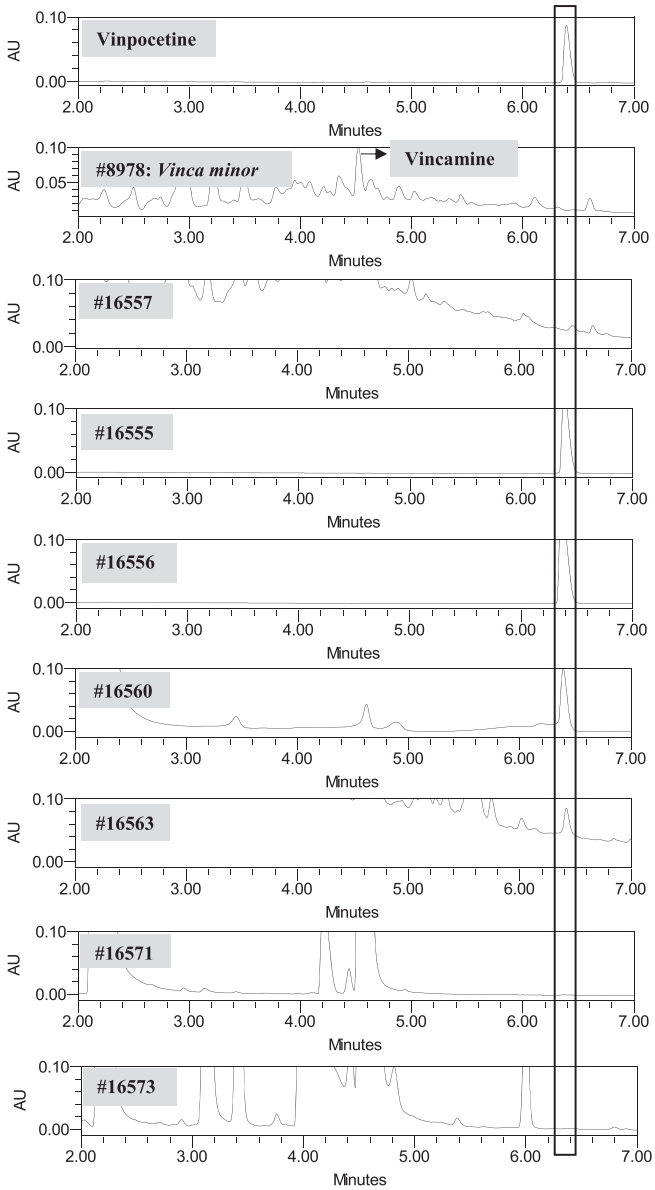

Figure 2. Ultra-high performance liquid chromatography-UV chromatograms of vinpocetine, plant samples and dietary supplements at 269 nm.

Accuracy

The accuracy of the method was determined by spiking samples #16552, #16574) with a known amount of vinpocetine and samples #17006, #17024, #17033 with a known amount of picamilon. These samples were exhaustively extracted five times as discussed under 'Materials and methods' and dried then spiked with known amounts of the standard compound at three different concentrations, extracted again then analyzed under optimized conditions. The accuracy of the assay method was evaluated in triplicate at three concentration levels, 1, 25, and 50 μg/mL. The percentage recovery of these samples ranged from 98.0 to 102.1%.

Analysis of samples and dietary supplements

Vinpocetine analysis

The analysis was performed on 2 authenticated samples of *Vinca minor* and 23 dietary supplements. Vinpocetine was not detected in the analyzed *Vinca* plant material as expected but the leaves of *V. minor* did contain the major alkaloid vincamine, which is used in the pharmaceutical industry as a cerebral stimulant and vasodilator.[32] Figure 2 shows an example of the UHPLC-PDA chromatogram at 269 nm obtained for plant sample, and dietary supplements as compared to that of a vinpocetine standard at 10 μg/mL. Of 23 dietary supplements tested, 17 contained vinpocetine. No vinpocetine was detected in 26% (6/23) of the sampled supplements. Vinpocetine and vincamine were identified by their retention times at 6.4 min and 4.5 min and the UV spectra which were compared with the standard. Both analytes showed characteristic UV full scan spectra that could be easily differentiated (Figure 3). The unknown peaks closer to vincamine retention time (4.6 min) were compared with the UV spectra of standard vincamine as well as vincamine in *Vinca minor* plant. The UV spectra associated with the peaks eluting close to 4.6 min were inconsistent with vincamine based on differences in the UV spectra. The vincamine peak was also checked in all samples of dietary supplements at and around 4.5 min and no supplement contained vincamine based on the UV spectra and RT.

Vinpocetine was sold as if it were a constituent of lesser periwinkle in 3 supplements (13%; 3/23). Thirty-nine percent (9/23) of the vinpocetine supplements were misbranded because they either did not contain any vinpocetine or vinpocetine was represented as a natural constituent of lesser periwinkle. Seventy-four

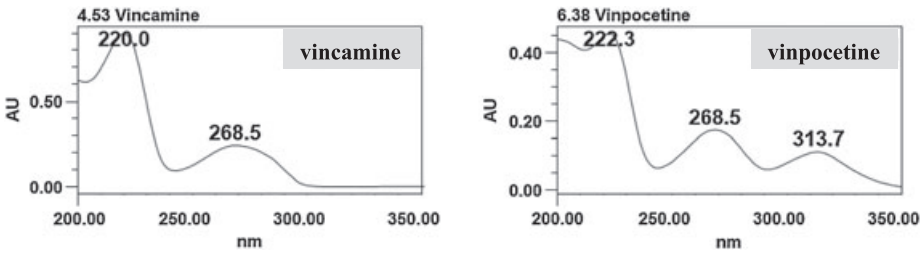

Figure 3. Ultra-violet spectrum of vincamine and vinpocetine.

Table 1. Vinpocetine content found in 23 dietary supplements and label claim information

#	Code #	Type of supplement	Label description of vinpocetine or periwinkle (labeled quantity of vinpocetine)	Amount/serving size of vinpocetine according to label	Serving size according to labeled directions	Maximum recommended daily dose	# of other ingredients listed on label	Vinpocetine in analyzed samples (mg/serving)	Calculated quantity of vinpocetine (mg) consumed based on maximum recommended daily servings
1	DS16552	-	vinpocetine	5 mg	1 capsule	3 servings	4	5.37	16.11
2	DS16553	memory booster	vinpocetine	10 mg	1 tablet	1 serving	-	10.1	10.1
3	DS16554	supports memory, focus & mood	vinpocetine	5 mg	1 capsule	2 servings	-	4.85	9.7
4	DS16555	liposome-enhanced	vinpocetine	5 mg	1 capsule	2 servings	-	4.79	9.58
5	DS16556	promotes cerebral metabolism	vinpocetine	10 mg	1 tablet	3 servings	-	10.59	31.77
6	DS16559	pre-workout, nitric oxide and energy enhancer	vinpocetine	-	1 scoop* (7.17 g)	1 serving	13	0.32	0.32
7	DS16560	pre-workout muscle formula	vinpocetine	-	1 scoop* (6 g)	1 serving	16	1.39	1.39
8	DS16561	pre-workout muscle formula	vinpocetine	-	1 scoop* (9 g)	1 serving	15	1.36	1.36
9	DS16562	muscle building, 3 in 1 metabolizing and cutting agent	vinpocetine	-	3 capsules	2 servings	21	5.85	11.7
10	DS16563	premium cutting and hardening compound	vinpocetine	-	3 Liqui-Capsules	2 servings	24	ND	ND
11	DS16564	brain health supplement	vinpocetine	-	4 tablets	2 servings	28	5.96	11.92
12	DS16565	brain enhancement supplement	vinpocetine	5 mg	2 capsules	1 serving	15	4.86	4.86
13	DS16566	memory cognitive enhancer	vinpocetine	-	3 capsules	1 serving	2	7.23	7.23
14	DS16567	neuro focusing agent	vinpocetine	-	2 tablets	2 servings	40	ND	ND
15	DS16568	fat metabolizer	vinpocetine	-	1 capsule	2 servings	21	1.05	2.10
16	DS16569	energy rush	vinpocetine	-	½ bottle	2 servings	19	ND	ND
17	DS16570	pre-training igniter advanced strength	lesser periwinkle (95% vinpocetine)	-	1 scoop* (22.5 g)	2 servings	41	0.29	0.58
18	DS16571	energy rush	vinpocetine	-	½ bottle	2 servings	19	ND	ND
19	DS16572	energy rush	vinpocetine	-	½ bottle	2 servings	15	ND	ND
20	DS16573	energy drink	vinpocetine	-	1 bottle	3 servings	17	ND	ND
21	DS16574	energy complex	vinpocetine	-	1 capsule	2 servings	31	1.04	2.08
22	DS16575	pre-training igniter advanced strength	lesser periwinkle (95% vinpocetine)	-	1 scoop* (22.5 g)	2 servings	41	0.21	0.42
23	DS16579	pre-training igniter advanced strength	lesser periwinkle (95% vinpocetine)	-	1 scoop* (22.5 g)	2 servings	41	0.28	0.56

ND = Not Detected

* Products measured with the scoops provided by the manufacturer

percent (17/23) of the supplement labels did not provide any information on the quantity of vinpocetine. For the 6 supplements that did list the quantity of vinpocetine on the label, the actual amount ranged from 95.6% to 107.4% of labelled content.

Total amounts of vinpocetine that would be consumed daily based on recommended serving sizes varied almost 100-fold from 0.32 mg/day (#DS 16559) to 32 mg/day (#DS 16556) (Table 1). These dosages range from trivial to prescription levels as vinpocetine is prescribed in dosages of 5 mg to 40 mg.

Picamilon analysis

The analyses were performed on one sample of each of the 31 dietary supplements labelled as containing picamilon or pikatropin. Figure 4 shows an example of the UHPLC-PDA chromatograms at 261 nm obtained for dietary supplements compared to a picamilon standard at 50 μg/mL. Of 31 dietary supplements tested, 30 contained picamilon. Picamilon was not detected in one supplement. The peak of picamilon in all samples was identified by comparing the RT and the UV spectrum obtained with the reference standard, which showed a characteristic spectrum with two maximum absorptions at (λ_{max}) 210 and 261 nm.

The contents varied greatly between products. The amount per serving of picamilon ranged from none to 249.1 mg (Table 2) compared to prescription dosages of 50 mg to 200 mg. In the 30 supplements that contained picamilon, the quantity of picamilon consumed by following the label's recommended maximum daily serving ranged from 2.7 mg/day to 721.5 mg/day (Table 2). These dosages range from trivial to exceeding prescription dosages.

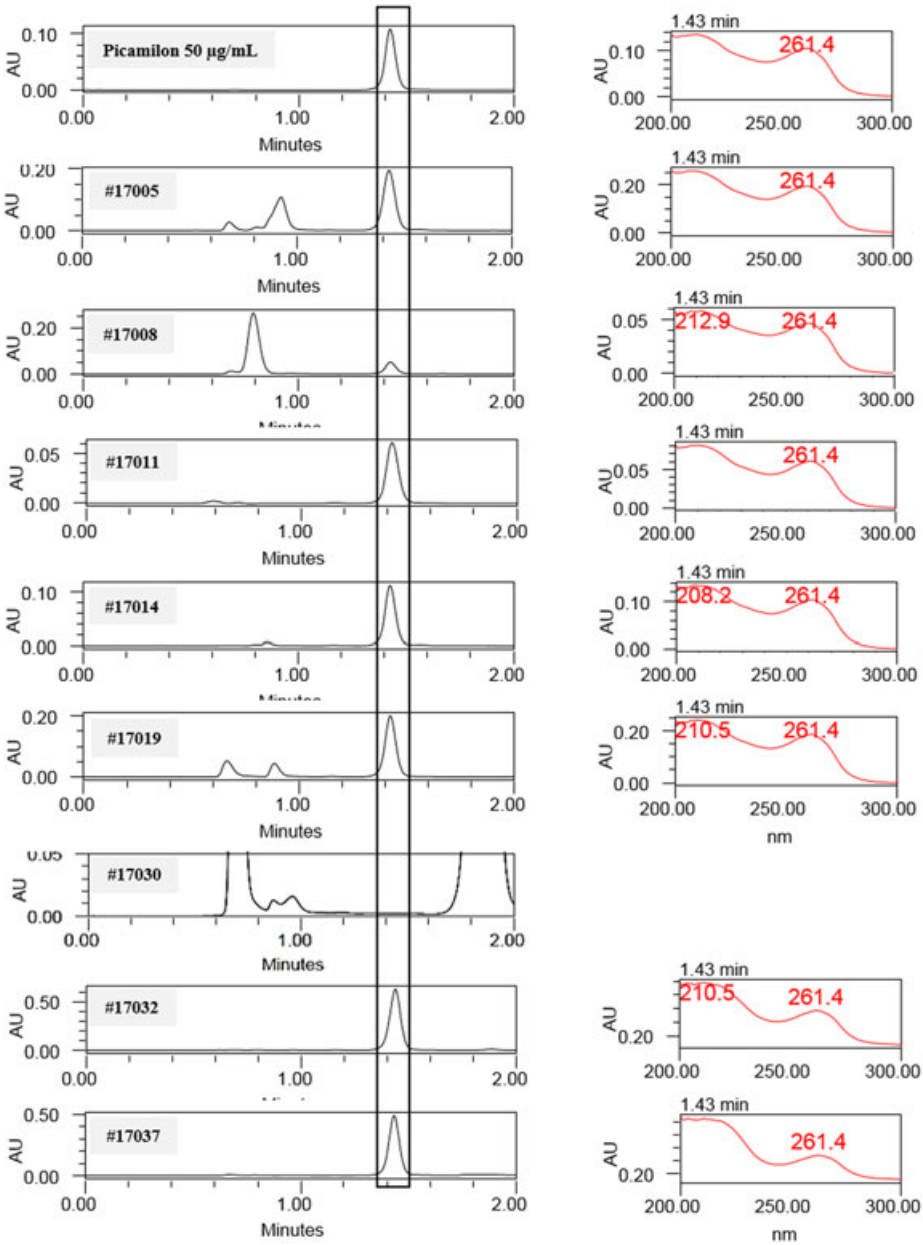

Figure 4. Ultra-high performance liquid chromatography-UV chromatograms of picamilon from dietary supplements at 261 nm.

Table 2. Information regarding picamilon on label of 31 dietary supplements compared to quantity detected in analyzed samples of supplements

#	Code #	Type of supplement	Label description of picamilon	Amount/serving size of picamilon according to label	Serving size according to labeled directions	# of other ingredients listed on label	Picamilon in analyzed samples (mg/serving)	% actual picamilon compared to label amount	Calculated quantity of picamilon (mg) consumed based on maximum recommended daily servings
1	17005	-	pikatropin (pikamilon 98%)	-	1 scoop= 2400 mg*	8	28.8	-	57.6
2	17006	pre-training catalyst	pikatropin™ picamilon (N-nicotinoyl-R-aminobutyric acid)	25 mg	1 scoop= 7100 mg*	13	34.9	139.6	104.8
3	17007	ultimate pre-workout	pikatropin™	-	1 scoop= 6700 mg*	11	37.5	-	112.5
4	17008	pre-workout	pikatropin (picamilon)	-	1 scoop= 13000 mg*	18	85.8	-	257.4
5	17009	pre-workout amplifier	pikatropin™ (picamilon)	50 mg	1 scoop= 11600 mg*	10	52.0	104	104.0
6	17010	pre-workout	pikatropin™ (picamilon)	50 mg	1 scoop= 10100 mg*	11	58.3	116.6	116.6
7	17011	pre-porkout amplifier	pikatropin™ (picamilon)	50 mg	1 scoop= 11400 mg*	10	56.8	113.6	113.7
8	17012	pre-workout engine	pikatropin™ (picamilon)	-	1 scoop= 8400 mg*	15	46.2	-	138.6
9	17013	extreme performance and endurance	pikatropin (pikamilon 98%)	-	1 scoop= 5500 mg*	11	33	-	66
10	17014	-	pikatropin	-	3 capsules	7	68.7	-	68.7
11	17015	hi-energy fat burner	pikatropin™ (picamilon)	20 mg	2 caps	10	25	125	25.0
12	17016	intense energy pre-workout	pikatropin™ (picamilon)	50 mg	1 scoop= 7100 mg*	12	56.9	113.8	56.9
13	17017	pre-workout amplifier	pikatropin® (picamilon)	-	1 scoop= 6500 mg*	15	52.0	-	52.0
14	17019	high performance bioenergetic nitro-amine	pikatropin™ (picamilon)	-	1 scoop= 6700 mg*	13	67.0	-	67.0
15	17020	pre-workout	pikatropin® picamilon (N-nicotinoyl-R-aminobutyric acid)	-	1 scoop = 6800 mg*	16	32.6	-	97.8
16	17021	-	picamilon (nicotinoyl GABA)	250 mg	250 mg	-	249.1	99.6	498.3
17	17022	cognitive support	picamilon	100 mg	1 capsule	-	135.9	135.9	-
18	17023	-	picamilon (N-nicotinoyl GABA)	100 mg	1 capsule	-	120.2	120.2	721.5
19	17024	-	picamilon (nicotinoyl-γ-aminobutyric Acid)	150 mg	1 capsule	-	150.3	100.2	450.8

(Continues)

Table 2. (Continued)

#	Code #	Type of supplement	Label description of picamilon	Amount/serving size of picamilon according to label	Serving size according to labeled directions	# of other ingredients listed on label	Picamilon in analyzed samples (mg/serving)	% actual picamilon compared to label amount	Calculated quantity of picamilon (mg) consumed based on maximum recommended daily servings
20	17025	mental alertness, focus, relaxation, stress and anxiety support	picamilon (nicotinoyl-γ-aminobutyric Acid)	150 mg	1 capsule	-	236.9	157.9	473.8
21	17026	pre-workout	pikatropin™ (N-nicotinoyl-R-aminobutyric acid)	-	1 scoop = 13100mg*	13	26.0	-	26.0
22	17027	thermogenic and lypolytic	pikatropin™ picamilon (N-nicotinoyl-R-aminobutyric acid)	-	1 capsule	14	45.0	-	135.0
23	17028	ultra-concentrated pre-workout	picatropin (N-nicotinoyl GABA)	-	1 scoop= 3800mg*	9	2.7	-	2.7
24	17029	pre-workout	pikatropin™ (picamilon)	25 mg	1 scoop= 16000mg*	12	28.8	115.2	57.6
25	17030	thermogenic and lypolytic	pikatropin	-	1 capsule	5	ND	-	ND
26	17031	pre-training energy catalyst	pikatropin™ (picamilon)	-	1 scoop= 6300mg*	9	40.4	-	80.8
27	17032	fat metabolizer	pikatropin™ picamilon	-	1 capsule	18	34	-	68
28	17033	pre-workout	pikatro-pin(tma) (N-nicotinoyl-R-aminobutyric Acid)	-	1 scoop= 3700mg*	8	29.6	-	88.9
29	17035	ultra thermogenic	pikatropin	50 mg	1 capsule	20	49.9	99.8	99.8
30	17036	-	pikatropin	50 mg	1 capsule	20	53.5	107	107
31	17037	focal agonist	pikatropin™ picamilon	-	2 capsules	21	50.4	-	50.4

ND = Not Detected

* Products measured with scoops provided by the manufacturer

Fourteen supplements listed a specific amount of picamilon on the label (ranging from 25 mg to 250 mg); the actual amount of picamilon in these supplements varied from 99.6% to 157.9% of labelled claim.

Our study has several limitations. We analyzed only one sample of each supplement. In addition, we did not analyze supplements if they were not labelled as containing either vinpocetine or picamilon; therefore, we do not know if vinpocetine and picamilon are unlisted ingredients in other brands of supplements. Lastly, we only analyzed supplements for the presence and quantity of vinpocetine or picamilon. Many of these supplements listed more than eight other ingredients on the label. The accuracy of supplement label information regarding these other ingredients remains unknown.

Our study demonstrates that some pharmaceutical drugs prescribed in other countries are sold openly in dietary supplements in the USA. The first step to sell a new ingredient in dietary supplements in the USA is to notify the FDA by submitting a New Dietary Ingredient notification.[33,34] It is interesting to note that a notification for vinpocetine to be sold as a new ingredient in supplements was submitted to the FDA as early as 1997,[35,36] but we are not aware of a similar notification for picamilon having been submitted to the FDA.

Conclusions

The newly developed UHPLC-PDA method facilitated the detection of vinpocetine and picamilon. This method exhibited excellent performance in terms of sensitivity and is a suitable method for rapid analysis of vinpocetine and picamilon in dietary supplements. The developed method was validated for all the parameters tested and successfully applied to the identification of vinpocetine in authenticated plant samples and dietary supplements.

As expected, vinpocetine was not detected in 2 authenticated samples of *Vinca minor*. Of the 23 vinpocetine dietary supplements tested, 17 contained vinpocetine and the quantity of vinpocetine in these supplements ranged from 0.3 to 32 mg per recommended daily serving. No vinpocetine was detected in 26% (6/23) of the sampled supplements. Vinpocetine was sold as if it were a constituent of lesser periwinkle in 13% (3/23) of the supplements. In summary, 39% (9/23) of the vinpocetine supplements were misbranded, and 74% (17/23) of vinpocetine supplement labels did not provide any information on the quantity of vinpocetine.

Of the 31 picamilon supplements tested, 30 contained picamilon and the quantity of picamilon ranged from 2.7 to 721.5 mg per maximum recommended daily serving. In the supplements providing a specific quantity of picamilon on the label, actual quantity of picamilon ranged from 99.6 to 157.9% of labelled quantity.

Disclosures

B. Avula, A.G. Chittiboyina, S. Sagi, Y-H. Wang, M. Wang and P.A. Cohen do not have any conflicts of interest to declare. I. Khan reported receiving grants from the US Food and Drug Administration. I. Khan is also the coordinator of the International Conference on the Science of Botanicals which receives support for conference-related expenses from multiple supplement-related companies.

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References

[1] D. Bereczki, I. Fekete. Vinpocetine for acute ischaemic stroke. Cochrane Database of Systematic Reviews **2008**, Issue 1. Art. No.: CD000480.
[2] D. Bereczki, I. Fekete. A systematic review of vinpocetine therapy in acute ischaemic stroke. *Eur. J. Clin. Pharmacol.* **1999**, *55*, 349.
[3] S. Szatmári, P. Whitehouse. Vinpocetine for cognitive impairment and dementia. Cochrane Database of Systematic Reviews **2003**, Issue 1. Art. No.: CD003119.
[4] National Toxicology Program. Chemical information review document for vinpocetine. CAS No. 42971-09-5, September **2013**; Available at: http://ntp.niehs.nih.gov/ntp/htdocs/chem_background/exsumpdf/vinpocetine091613_508.pdf [12 August 2015]
[5] Natural Medicines Comprehensive Database. **2014**. Available at: http://www.naturaldatabase.com [12 August 2015]
[6] P. Bonoczk, B. Gulyas, V. Adam-Vizi, A. Nemes, E. Karpati, B. Kiss, M. Kapas, C. Szantay, I. Koncz, T. Zelles, A. Vas. Role of sodium channel inhibition in neuroprotection: Effect of vinpocetine. *Brain Res. Bull.* **2000**, *53*, 245.
[7] C. Lorincz, K. Szasz, L. Kisfaludy. The synthesis of ethyl apovincaminate. *Arzneimittelforschung*. **1976**, *26*, 1907.
[8] B. S. Nayak, L. M. P. Pereira. *Catharanthus roseus* flower extract has wound-healing activity in Sprague Dawley rats. *BMC Complement. Altern. Med.* **2006**, *6*, 41.
[9] S. Bhadra, S. Chandra Das, S. Roy, S. Arefeen, A. S. S. Rouf. Development and validation of RP-HPLC method for quantitative estimation of vinpocetine in pure and pharmaceutical dosage forms. *Chromatogr. Res. Int.* **2011**Article ID 801656. DOI:10.4061/2011/801656.
[10] Y. Kuge, H. Nakazawa, T. Kometani, T. Sugaya, K. Mochida, S. Tomioka. A facile one-pot synthesis of vinpocetine. *Synth. Commun.* **1994**, *24*, 759.
[11] L. Szabo, G. Kalaus, C. Szantay. Synthesis of Vinca Alkaloids and Related Compounds, XV[1)] A New Synthetic Route to (+)-Vincaminic and (+)-Apovincaminic Esters. *Arch. Pharm.* **1983**, *316*, 629.
[12] L. Szabo, J. Sapi, G. Kalaus, G. Argay, A. Kalman, E. Baitz-gacs, J. Tamas, C. Szantay. Synthesis of vinca alkaloids and related compounds—XVI: New route to the stereoselective synthesis of (+)-vincamine, (-)-vincamone and (+)-apovincaminic acid esters. *Tetrahedron*. **1983**, *39*, 3737.
[13] A. Nemes, L. Czibula, G. Visky, M. Farkas, L. Kreidl. Alternative routes to vincamine. *Heterocycles*. **1991**, *32*, 2329.
[14] É. A. Bendikov, L. M. Shmuilovich, V. M. Kopelevich. Effect of γ-aminobutyric acid, nicotinoyl-γ-aminobutyric acid, and its ethyl ester on central vasomotor reflex formation. *Bull. Exp. Biol. Med.* **1972**, *73*, 67.
[15] V. M. Kopelevich, V. I. Gunar. Some approaches to the directed search for new drugs based on nicotinic acid. *Pharm. Chem. J.* **1999**, *33*, 177.
[16] R. A. Shephard. Behavioral effects of GABA agonists in relation to anxiety and benzodiazepine action. *Life Sci.* **1987**, *40*, 2429.
[17] P. Krogsgaard-Larsen, P. Lenicque, P. Jacobsen. In CRC Handbook of Stereoisomers: Drugs in Psychopharmacology; D. F. Smith, Ed.; CRC Press: Boca Raton, FL, **1984**. 369.
[18] M. Desarmennieni, P. Feltz, P. M. Headley, F. Santangelo. SL 75 102 AS A γ-Aminobutyric acid agonist: Experiments on Dorsal Root Ganglion Neurones in Vitro. *Br. J. Pharmacol.* **1981**, *72*, 355.
[19] M. P. Remler, W. H. Marcussen. A GABA-EEG test of the blood-brain barrier near epileptic foci. *Appl. Neurophysiol.* **1983**, *46*, 276.
[20] B. F. Dorofeev, L. E. Kholodov. Pikamilon pharmacokinetics in animals. *Farmakologiia i toksikologiia (in Russian)*. **1991**, *54*, 66.
[21] A. Gille, E. T. Bodor, K. Ahmed, S. Offermanns. Nicotinic acid: pharmacological effects and mechanisms of action. *Annu. Rev. Pharmacol. Toxicol.* **2008**, *48*, 79.
[22] R. S. Mirzoian, T. S. Gan'shina. The new cerebrovascular preparation pikamilon. *Farmakologiia i toksikologiia (in Russian)*. **1989**, *52*, 23.
[23] K. Matsuyama, C. Yamashita, A. Noda, S. Goto, H. Noda, Y. Ichimaru, Y. Gomita. Evaluation of isonicotinoyl-gamma-aminobutyric acid (GABA) and nicotinoyl-GABA as pro-drugs of GABA. *Chem. Pharm. Bull.* **1984**, *32*, 4089.
[24] I.G. Gitlin, A.I. Dorozhko, N.T. Ioffe, V.A. Serebryanyi, S.I. Fokin, A. A. Shereshevskii. Process for preparation of N-nicotinoyl-γ-aminobutyric acid by ring opening of 2-pyrrolidone and subsequent reaction with ethyl nicotinate. RU2400476C2, **2010**.

[25] Z.-Z. Qin, F.-B. Zhang, T.-N. Jiang, M.-J. Li, R.-X. Wang, Y.-I. Li. Synthesis and evaluation of nicotinoylamino acid compounds as radiosensitizer. *Chem. Res. Chin. Univ.* **2008**, *24*, 496.
[26] A. El-Gindy, S. Emara, M. K. Mesbah, G. M. Hadad. Spectrophotometric and liquid chromatographic determination of fenofibrate and vinpocetine and their hydrolysis products. *Il Farmaco.* **2005**, *60*, 425.
[27] A. Abd Elbary, N. Foda, O. El-Gazayerly, M. El Khatib. Reversed Phase Liquid Chromatographic Determination of Vinpocetine In Human Plasma and Its Pharmacokinetic Application. *Anal. Lett.* **2002**, *35*, 1041.
[28] H.-M. Xia, L.-N. Su, J.-W. Guo, G.-M. Liu, Z.-Q. Pang, X.-G. Jiang, J. Chen. Determination of vinpocetine and its primary metabolite, apovincaminic acid, in rat plasma by liquid chromatography-tandem mass spectrometry. *J. Chromatogr. B.* **2010**, *878*, 1959.
[29] M. Vatsova, S. Tzvetanov, A. Drenska, J. Goranscheva, N. Tyutyulkova. Improved gas chromatographic-mass spectrometric method for the quantitative determination of vinpocetine in human plasma. *J. Chromatogr. B Biomed. Sci. Appl.* **1997**, *702*, 221.
[30] W. Cuia, X. Chen, Y. Zhan, Z. Zhang, Y. Zhang, D. Zhong. Determination of picamilon concentration in human plasma by liquid chromatography–tandem mass spectrometry. *J. Chromatogr. B.* **2010**, *878*, 1181.
[31] ICH. Validation of Analytical Procedures: Text and Methodology. ICH Harmonized Tripartite Guidelines Available at: http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1__Guideline.pdf [12 August 2015]
[32] H. Yin, Y.-H. Sun. Vincamine-producing endophytic fungus isolated from *Vinca minor*. *Phytomed.* **2011**, *18*, 802.
[33] P. A. Cohen. Assessing supplement safety-the FDA's controversial proposal. *New Engl. J. Med.* **2012**, *366*, 389.
[34] Food and Drug Administration. Draft Guidance for Industry: dietary supplements: new dietary ingredient notifications and related issues. Available at: http://www.fda.gov/regulatoryinformation/guidances/ucm257563.htm [3 September 2015].
[35] J. Tanner. New dietary ingredient: vinpocetine. Available at: http://www.fda.gov/ohrms/dockets/dockets/95s0316/rpt0012_01.pdf [29 August 1997].
[36] P. A. Cohen. Vinpocetine: an unapproved drug sold as a dietary supplement. Mayo Clin. Proc. **2015**, DOI: 10.1016/j.mayocp.2015.07.008.