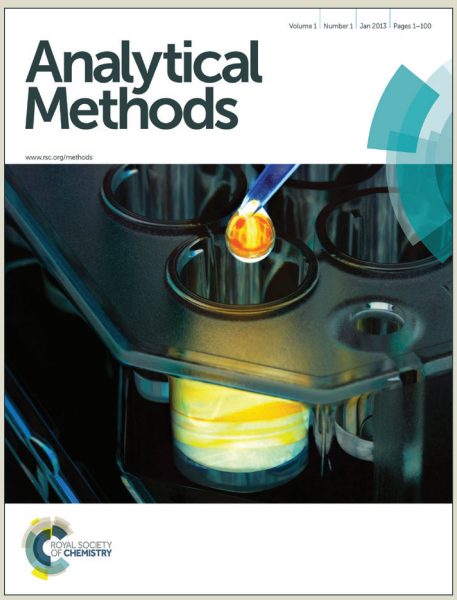
Analytical Methods

Accepted Manuscript



ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/methods

Reliable identification and quantification of three diethylphenethylamines in a *Dendrobium*-based dietary supplement.

Wahlström R, Styles C, Hägglund G[1]

Abstract

An analytical method has been developed for the identification and quantification of N,α-diethyl-phenylethylamine, N,N-diethyl-phenylethylamine and N,β-diethyl-phenylethylamine (N,α-DEPEA, N,N-DEPEA and N,β-DEPEA respectively) in a pre-workout dietary supplement containing extract from Orchids of the *Dendrobium* genus.

UHPLC-MS/MS (QQQ) technology was utilized for identification and quantitation using custom synthesized external standards as references. In less than 9 minutes the method successfully identifies N,α-DEPEA, N,N-DEPEA and N,β-DEPEA down to 0.1ng/ml and quantifies the same substances down to 0.3ng/ml. The method proved to be rapid, stable and reproducible with good separation and detection limits despite the substantial technical difficulties related to the structural similarity and significant difference in relative concentrations between the three analytes.

N,β-DEPEA proved difficult to separate from N,α-DEPEA both chromatographically and mass spectrometry-wise due to the notable differences in concentration and their structural similarity. The presence of N,β-DEPEA in the analyzed sample type has not been studied in previously published results, thus this study adds to the current scientific interest in characterizing the contents of certain pre-workout dietary supplements.

The amounts found in the products vary between batches, but were found to be around 2 mg/g for N,β-DEPEA, 0.3-4 µg/g for N,α-DEPEA and 60-230 ng/g for N,N-DEPEA.

Keywords: dietary supplement, phenethylamines, N,α-DEPEA, N,N-DEPEA, N,β-DEPEA, UHPLC-QQQ, UHPLC-MS/MS, Craze

Introduction

Phenethylamines (PEA, CAS No: 64-04-0, Figure 1) are a class of substances characterized by their phenethylamine-backbone onto which various substituents have been attached. The PEA-class has many members known for their stimulative or psychoactive properties such as methamphetamine (CAS No: 537-46-2, Figure 1) (1). N,α-DEPEA (CAS No: 119486-07-6, Figure 1) is another PEA which, based on its structure, has potential functionality as a stimulant (2). As many other PEA's, N,α-DEPEA is banned in competitions by WADA (3).

Certain dietary supplements meant to be ingested before workout (pre-work out, pwo) have been reported to contain N,α-DEPEA. Lee *et al.* (4) recently published a GC-MS and NMR method with which N,α-DEPEA was found in a dietary supplement named "Craze" marketed by Driven Sports Inc.

[1] All authors are employees of Q&Q Labs AB, Frans Perssons väg 6, 412 76 Göteborg, Sweden. Contact: info@qandqlabs.se. Tel: +46(0)72 72 74 684. www.qandqlabs.se.

Cohen *et al.* (5) developed two different LC-MS (QTOF/Orbitrap) methods for the analysis of N,α-DEPEA in the same product and have published data showing the detection of the analyte in samples using both methods. Cohen *et al.* also report in an online press release (6) on detection of N,α-DEPEA but have not published details of their methodology apart from the analysis being done using an LC-MS-system (Orbitrap).

Elsohly *et al.* (7) recently published a study on the presence of N,α-DEPEA, N,N-DEPEA and PEA in a larger number of pwo-products. The identification and quantification was based on custom synthesized standards and an LC-MS/MS (QTrap) method.

The studied pwo-products have a common ingredient in extracts from orchids (*Orchidaceae* family) of the *Dendrobium* genus, which according to traditional Chinese medicine are claimed to have medicinal properties (8). N,α-DEPEA is a relatively unstudied substance with few scientific references published regarding its properties. The substance was first described in a scientific paper in 1991 by Noggle *et al.* (2) but was allegedly patented in 1988 by Knoll Pharmaceuticals (4).

The Craze-products label does not state that the product contains N,α-DEPEA, however N,N-DEPEA (Figure 1), a structural isomer of N,α-DEPEA, is stated as a constituent of the added *Dendrobium* extract. From preliminary (unpublished) results, a peak similar to N,α-DEPEA was detected in an analysis of the product Craze. The compound was theoretically suggested to be N,β-DEPEA (Figure 1) which is another structural isomer of N,α-DEPEA with less structural similarity to methamphetamine. The current paper verifies that the product Craze contains higher concentrations of N,β-DEPEA than N,α-DEPEA.

The same product analyzed by Lee *et al.* and Cohen *et al.* was used as the sample matrix in the currently described development of an analytical method for the reliable identification and quantification of three structural isomers N,α-DEPEA, N,N-DEPEA and N,β-DEPEA. The work was performed using external standards for all three substances an extensive method validation was carried out to ascertain the stability and reliability of the method.

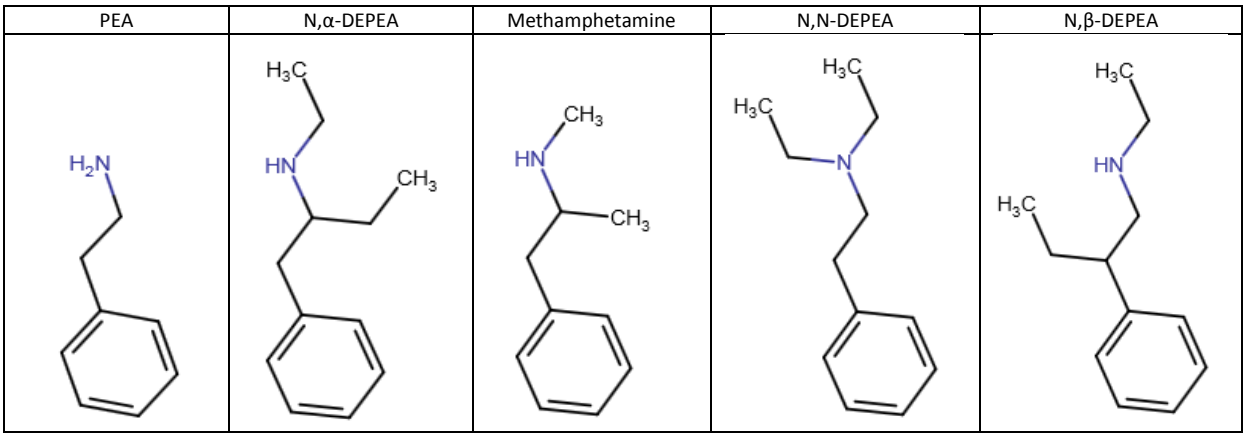


Figure 1. Chemical structures of the, in the current paper, discussed compounds. All are of the Phenethylamine-class with various levels of structural homology.

Experimental

Chemicals

N,α-DEPEA, N,N-DEPEA and N,β-DEPEA were custom synthesized by Red Glead Discovery (Lund, Sweden). The structures were verified by NMR and vial label compared to vial content was double checked by blind control as an unmarked sample was sent back to the producer for confirmation by NMR. To further verify the identity, N,α-DEPEA was purchased from a second supplier (Enamine, Kiev, Ukraine, prod.no. EN300-106805, lot no. T7604808). The two N,α-DEPEA substances were compared on UHPLC-MS/MS and proved identical based on fragmentation patterns and retention times.

NMR-spectra of the four standards purchased are displayed in Figure S1 through Figure S4 in the supplemental information. The spectrum in Figure S1 has lower resolution and was supplied lacking its y-axis which makes it difficult to compare to the spectrum in Figure S2. However, the large difference are most probably due to Figure S1 being free base dissolved in $CDCl_3$ while Figure S2 is a HCl salt dissolved in DMSO-d6.

Standards were dissolved and diluted in methanol to prepare calibration and spiking standards.

Methanol (HPLC grade), Formic acid (100%, p.a.) and Ammonium formate (p.a.) was obtained from Thermo Fisher Scientific (Waltham, MA, USA). Water was purified using a Millipore (Billerica, MA, USA) Milli-Q Integral 3 system.

Samples

Two samples of the dietary supplement “Craze” were obtained directly from Driven Sports Inc. (New York, NY, USA). The samples were of the flavors “Candy Grape” (batch.no: 1305323) and “Berry Lemonade” (batch. no: 1303298). These samples were used for method development and validation.

After method development and validation two new samples were acquired from an online retailer. The samples were of the flavors “Candy Grape” (batch.no: 1204135) and “Berry Lemonade” (batch. no: 1203079).

All samples were checked for signs of forgery according to advice given on the manufacturers’ homepage and made sure to be unadulterated by verifying the integrity of the foil wraps and inner lid seals. Samples were stored at ambient temperature in a locked compartment. The reference standards were kept separate from the samples.

Instrumental and Analytical Conditions

A 1290 UHPLC connected to a 6460 QQQ mass spectrometer equipped with an AJS ESI (Agilent Jet Stream Electro Spray Ionization) ion source and a Zorbax Eclipse Plus C18 column (100x2.1mm, 1.8µm) (Agilent Technologies, Santa Clara, CA, USA) was used for the analysis of the samples.

A chromatographic method was developed and optimized in-house, where several compositions of gradient was tried and a slow increase of organic phase was found optimal for separation of the structurally similar N,α-DEPEA and N,β-DEPEA. The eluents found most suitable were 0.01M ammonium formate and 0.1% (v/v) formic acid in Milli-Q grade water (eluent A) and 0.01M ammonium formate and 0.1% (v/v) formic acid in methanol (eluent B). This eluent composition was

used for the gradient table below (Table 1) in conjunction with the MS-parameters listed in Table 2 and Table 3 below.

Table 1. LC parameters for the chromatographic method of separating N,α-DEPEA, N,N-DEPEA and N,β-DEPEA

Time (min)	Eluent A (%)	Eluent B (%)	Flow (ml/min)	Column temp (°C)
0	85	15	0.5	50
5.5	73	27	0.5	50
5.6	1	99	0.5	50
6.6	1	99	0.5	50
6.7	85	15	0.5	50
8.3	85	15	0.5	50

Table 2. Source parameters for the Mass Spectrometry analysis of N,α-DEPEA, N,N-DEPEA and N,β-DEPEA

Gas temp (°C)	350
Gas flow (l/min)	10
Nebulizer (psi)	35
Sheath gas heat (°C)	400
Sheath gas flow (l/min)	12
Capillary (V)	3000
VCharging	0

Table 3. Scan segments for the Mass Spectrometry analysis and retention time of N,α-DEPEA, N,N-DEPEA and N,β-DEPEA. Shaded lines are quantification ions.

Analyte	Precursor Ion (m/z)	Product Ion (m/z)	Ion Ratio (%)	Dwell (msek)	Fragmentor (V)	CE (V)	Cell Acc. (V)	Polarity	Retention time (sek)
N,β-DEPEA	178.2	91.1		200	70	45	4	pos	5.110
N,β-DEPEA	178.2	65.1	44.8	200	70	53	4	pos	5.110
N,α-DEPEA	178.2	91.1		200	60	17	4	pos	4.778
N,α-DEPEA	178.2	65.1	46.7	200	60	53	4	pos	4.778
N,N-DEPEA	178.2	105.1		200	70	21	4	pos	2.615
N,N-DEPEA	178.2	77.1	49.0	200	70	53	4	pos	2.615
N,N-DEPEA	178.2	51.1	31.2	200	70	77	4	pos	2.615

Extraction Procedure

An extraction method was developed in-house where 1g of each sample was weighed and dissolved in 100 ml methanol followed by extraction using ultrasonic bath (10min). Samples were centrifuged (21000g for 10min), the supernatant was decanted, diluted in 10x steps in methanol and 0.5µl was injected on UHPLC-MS/MS.

Results & Discussion

Method validation

The method validation strategy included the verification and determination of the following parameters: specificity, linearity/range (including LOD & LOQ), trueness, intermediate precision, repeatability, stability and measurement uncertainty. The method was validated according to ICH guidelines (9) and the work was performed according to ISO 17025 (10).

Specificity

The relative structural similarity of N,α-DEPEA and N,β-DEPEA meant a certain technical complexity in the separation part of the method development . Simultaneously, the significant concentration variance between N,α-DEPEA and N,β-DEPEA in the samples created an imposing requirement on good separation as the two substances have the same qual and quant transitions. Since the concentration of N,β-DEPEA was found to be in the mg/g range while N,α-DEPEA was found to be in the µg/g range the response peak of N,β-DEPEA would have easily obscured the N,α-DEPEA signal if sufficient separation was not obtained. Thus good chromatographic separation is extremely vital in

order to differentiate properly between N,α-DEPEA and N,β-DEPEA, in this UHPLC is a more useful tool than HPLC.

In addition, other unknown substances with the same transitions also appear in the chromatograms which potentially could create coelution issues. Figure 2 and Figure 3 below show unidentified compounds (RT 4.15min and 1.95min respectively) with the same transition as the analyte. These peaks could very well represent other PEAs. Since the relative concentration of N,β-DEPEA is so much higher than the other two analytes, neither the N,α-DEPEA peak or the unknown compound peak at RT 4.15min from Figure 2 can be seen in Figure 4, despite identical transitions.

As depicted in Figure 2 Figure 4**Error! Reference source not found.**through Figure 3 below, retention times and ratios between quant/qual transitions coincide perfectly with single standards. Though it should be noted that the software used for data analysis automatically normalize qual peaks against the quant peak. In the qual window (right pane in each figure) the actual ratio is the first number, the percentage is the normalized relationship which the trace has been increased to fit.

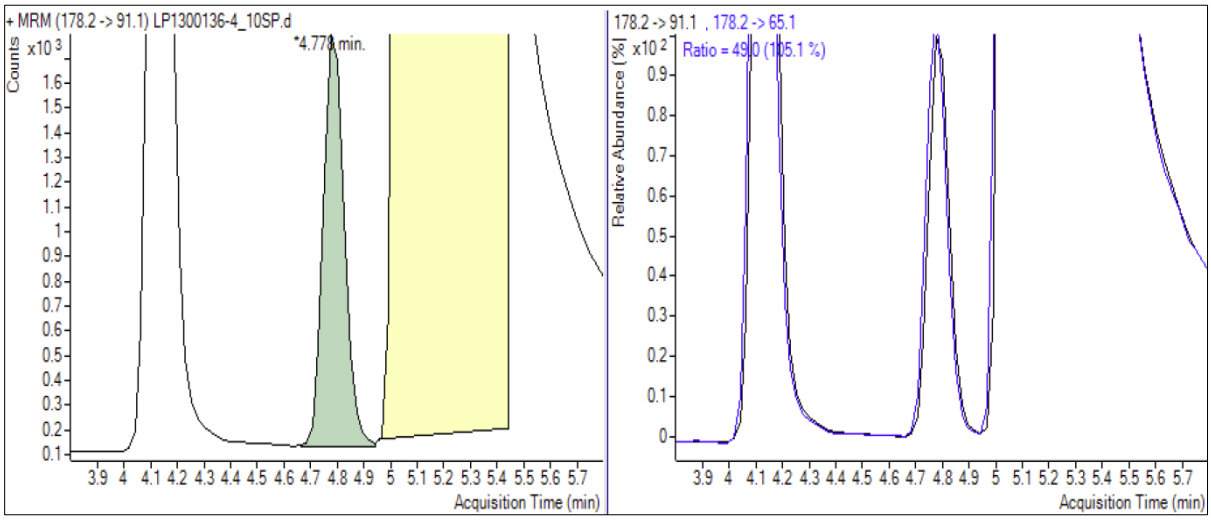


Figure 2. N,α-DEPEA (green) detected in the sample (lot no 1305323) at 10x dilution from the 1g/100ml initial dilution. The N,β-DEPEA peak is marked yellow and is visible due to it analogous transitions. The unmarked peak at RT 4.15 is an unidentified compound with the same transitions. The right chromatogram shows the ratio between the qual and quant transitions.

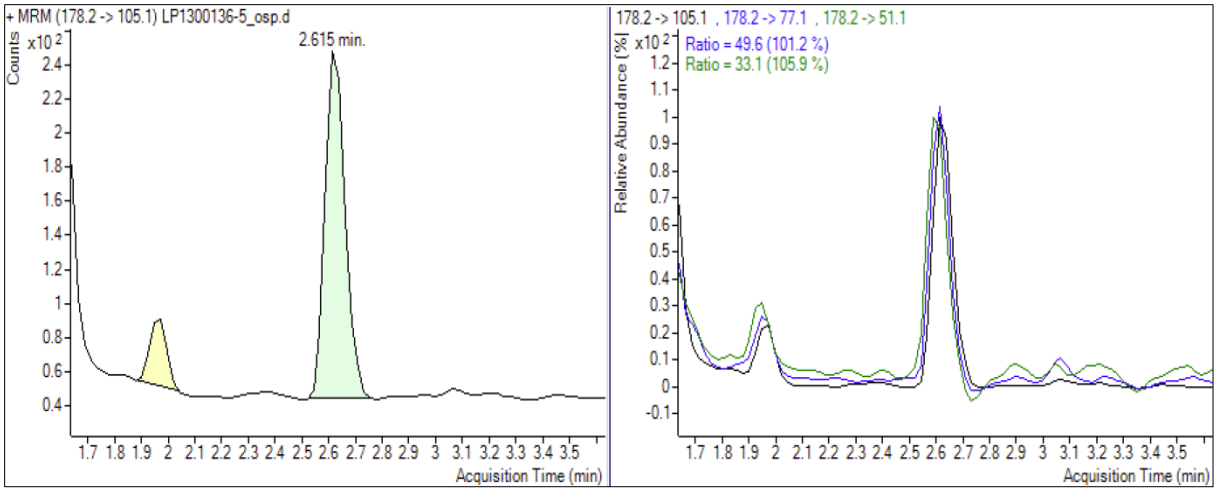


Figure 3. N,N-DEPEA (green) detected in the sample (lot no 1305323) at the 1g/100ml initial dilution. The yellow peak at RT 1.95 is an unidentified compound with the same transitions. The right chromatogram shows the ratio between the qual and quant transitions.

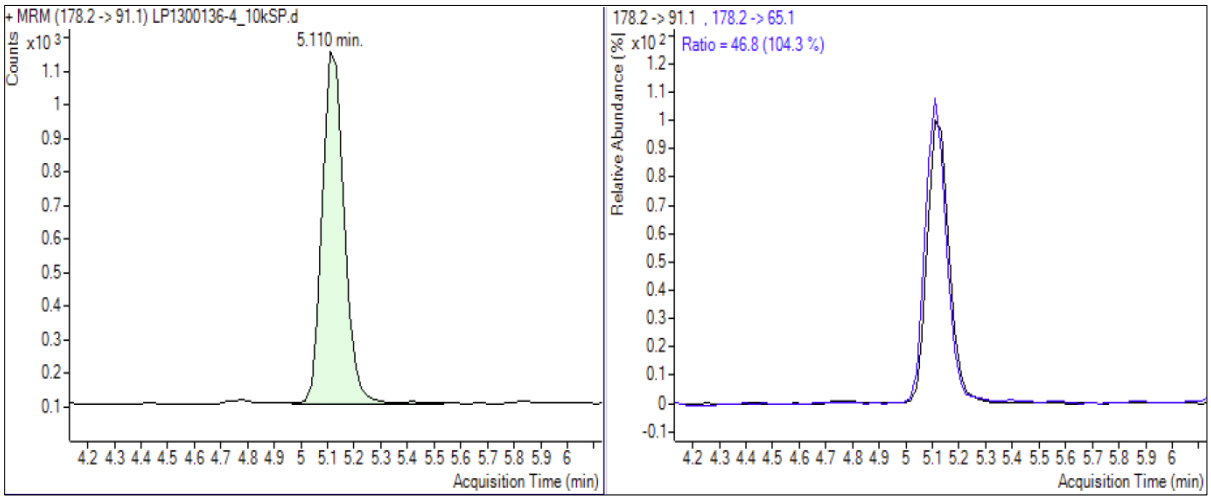


Figure 4. N,β-DEPEA detected in the sample (lot no 1305323) at 10,000x dilution from the 1g/100ml initial dilution. The N,α-DEPEA peak is not visible due to the low relative concentration. The right chromatogram shows the ratio between the qual and quant transitions.

The relatively large concentration differences of the analytes in the products posed a risk of cross-contaminations and/or carry over. In addition to method blanks (methanol), blanks were also taken from the dilution apparatus (MicroLab 600-series, Hamilton, Reno, NV, USA) before dilution of the samples and standards. Blank levels detected were determined to originate from carry over from the injection of the undiluted samples. N,β-DEPEA was the only analyte detected at levels above LOQ in all blanks on all days of validation. The detected blank levels were however deemed insignificant as they were 10,000 times lower than the N,β-DEPEA response of an undiluted sample. Due to the observed carry over contaminations in combination with the need to quantify samples at three different dilution levels (due to the large relative concentration differences), N,β-DEPEA was analyzed separately from N,α-DEPEA and N,N-DEPEA.

Linearity/Range

The linear range used for quantitative purposes was determined to 0.3-25 ng/ml for N,β-DEPEA and 0.3-8.3 ng/ml for N,α-DEPEA and N,N-DEPEA. Correlation coefficients (R^2) for all three substances for all three days were above 0.995, the collected linearity data are displayed in Table 4 below. The lowest standard point used for quantitation (LOQ) was 0.3 ng/ml where the signal to noise ratio was more than 20 for all three analytes. Limit of detection (LOD) was determined to be 0.1 ng/ml based on the signal to noise being more than 3 at this level (Figure 5).

Table 4. Table showing the linearity data from each of the three analytes on each of the three validation days

	Validation day	Slope	Intercept	Determination coefficient (R^2)
N,β-DEPEA	1	2597	295	0.997
	2	2126	942	0.996
	3	807	196	0.999
N,α-DEPEA	1	2045	146	0.999
	2	1714	194	0.996
	3	628	61.5	0.998
N,N-DEPEA	1	1329	115	0.999
	2	942	178	0.996
	3	580	75.7	1.00

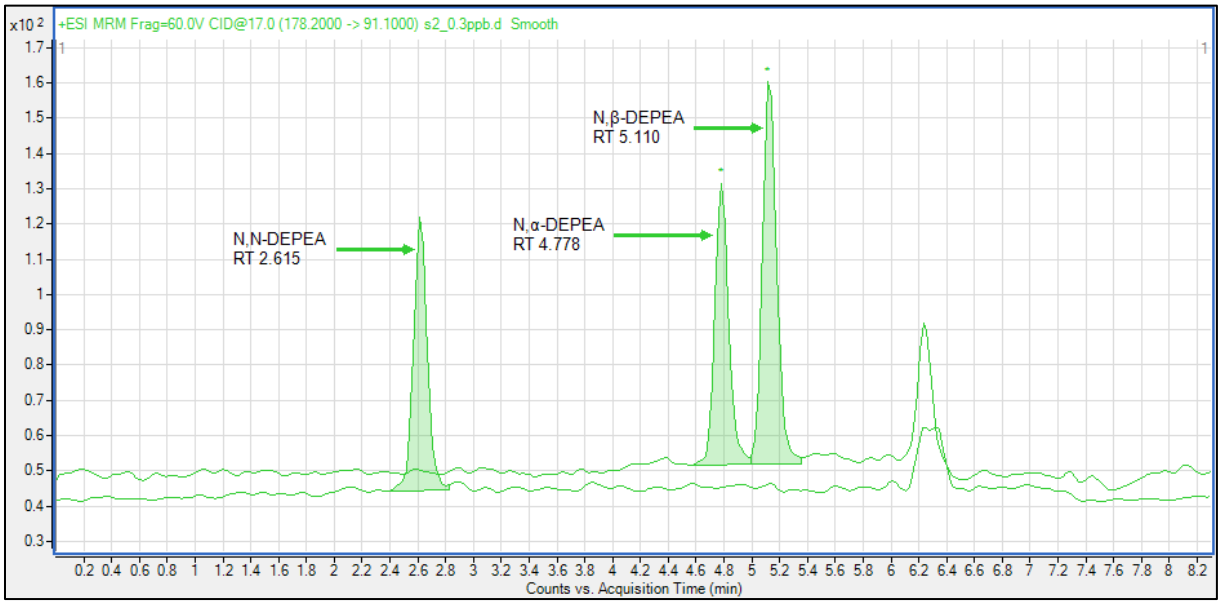


Figure 5. Chromatogram of mix standard at LOQ (0.3 ng/ml), all three analytes have signal-to-noise of more than 20.

Trueness

As all samples contained all three analytes, no blank samples were available and the contained amount thus had to be subtracted from found amounts in spiked samples. This fact also meant that evaluation of matrix effects was difficult. However, the recoveries of the spiked levels are acceptable and this indicates that matrix effects are low or insignificant.

Three samples of each of the two product samples were weighed in and extracted. All six samples were diluted to levels applicable for quantification of each analyte and were then spiked at levels 7.36, 7.38 and 7.44 µg/ml for N,β-DEPEA, N,α-DEPEA and N,N-DEPEA respectively. This corresponds to 7.5 mg of N,β-DEPEA, 7.5 µg of N,α-DEPEA and 755 ng of N,N-DEPEA per g of sample. Samples

were centrifuged, diluted and injected on the UHPLC-MS/MS. The results displayed in Table 5 below show that the method had good trueness and small variation.

Table 5. Average recovery and relative standard deviation (RSD) for six spiked samples (three of each product).

	N,β-DEPEA	N,α-DEPEA	N,N-DEPEA
Average recovery	109%	78%	91%
RSD	7.1%	8.4%	3.9%

Intermediate Precision

Three aliquots from each product were extracted and analyzed by two different analysts over three days (total of nine aliquots per product). The average amounts found in each product from the validation and the respective relative standard deviations (method repeatability) are displayed in Table 6.

Table 6. Amounts and relative standard deviation (RSD) for product with lot. no. 1303298 and 1305323

	Lot No	N,β-DEPEA	N,α-DEPEA	N,N-DEPEA
Average amount	1303298	2.01 mg/g	3.46 µg/g	55.7 ng/g
RSD		16%	6.6%	5.9%
Average amount	1305323	2.27 mg/g	4.21 µg/g	66.3 ng/g
RSD		12%	8.9%	6.8%

Instrument repeatability

A sample extract was injected 10 times to determine the instrument repeatability for each of the analytes. The relative standard deviation of the 10 injections was 2.5% for N,β-DEPEA, 2.1% for N,α-DEPEA and 4.0% for N,N-DEPEA which demonstrates acceptable instrument repeatability.

Stability

Samples were stored on the instrument at approximately 17 ^{o}C for three days with pierced septas and then reinjected. T-test calculations for each analyte showed that the t-stat value was not greater than the t-critical two-tail value, thus the values are not significantly different at 95% confidence level after three days on the instrument.

For all injections over all days of validation using several different preparations of eluents the retention times of the analytes were observed to be very stable (<0.1 % RSD) which suggest good stability of the methodology.

Measurement Uncertainty

Due to the similarity of the products and the measured standard deviation for each isomer in each product (see Intermediate precision), the best estimate of standard deviation for the method was deemed an average of the respective standard deviations (rounded up). The expanded measurement uncertainty with a coverage factor of two standard deviations, which corresponds to approximately 95% confidence level, was calculated using the method standard deviation for each analyte and the standard deviation of the analytical instruments used (11). Thus the measurement uncertainty is determined to be 28%for N,β-DEPEA, 16% for N,α-DEPEA and 14% for N,N-DEPEA.

Application of Method

The validated method was successfully used to identify and quantify the N,α-DEPEA, N,β-DEPEA and N,N-DEPEA concentrations in two samples purchased from an online retailer. These samples however proved to contain concentrations dissimilar to the previously tested samples, though the relative concentration relationship (mg/g to µg/g to ng/g between the three analytes) was the same.

Duplicate extractions and analyses for each sample is in-house standard practice. Standard deviations are calculated to ascertain similarity between the measurements. The standard deviations were noted to be significantly larger for N,α-DEPEA and N,N-DEPEA in the new samples compared to the samples used for method development and validation.

To verify these measurements two additional duplicate samples were analyzed and the standard deviations were replicated. Since the initial standard deviation values for N,β-DEPEA were in the same range as during validation and N,β-DEPEA is analyzed separately from the other two analytes, the new duplicates were not analyzed for N,β-DEPEA. Thus the average amount and standard deviations in Table 7 and Table 8 are based on two measurements for N,β-DEPEA and four measurements for N,α-DEPEA and N,N-DEPEA.

Visual comparison between the four available product samples showed an elevated inhomogeneity in the new batches compared to the ones used for method development and validation. The effect of the inhomogeneity in the new product samples resulted in significantly more uncertainty than that caused by the measurement process.

Table 7. Amounts and standard deviation for product with lot. no. 1204135. The relative standard deviation (RSD) is calculated from two measurements for N,β-DEPEA and four measurements for N,α-DEPEA and N,N-DEPEA

Lot no 1204135	N,β-DEPEA	N,α-DEPEA	N,N-DEPEA
Average amount	2.4 mg/g	0.33 µg/g	230 ng/g
RSD	11 %	20 %	20 %

Table 8. Amounts and standard deviation for product with lot. no. 1203079. The relative standard deviation (RSD) is calculated from two measurements for N,β-DEPEA and four measurements for N,α-DEPEA and N,N-DEPEA

Lot no 1203079	N,β-DEPEA	N,α-DEPEA	N,N-DEPEA
Average amount	1.9 mg/g	0.26 µg/g	160 ng/g
RSD	9 %	67 %	6 %

Discussion

This paper describes the results from method development and validation of a multi-analyte method for the identification and quantification of N,α-DEPEA, N,N-DEPEA and N,β-DEPEA in a *Dendrobium*-based food supplement in powder form. The method is rapid, stable, sensitive and has good separation between the analytes and can thus be used to reliably identify and quantify N,α-DEPEA, N,N-DEPEA and N,β-DEPEA in the validated matrix.

The reason for the high standard deviation (*e.g.* 67%) is probably due to inhomogeneity of the product samples, despite being in the form of a fine powder. Future studies of the sample matrix will include tests with manual homogenization of the sample powder and larger sample amounts to investigate and minimize the large standard deviations between duplicate samples containing low levels of the analytes. Despite this, the relative concentration relationships (mg/g to µg/g to ng/g)

between the three analytes were observed in all samples. However, the level of each analyte varies significantly between samples.

When comparing the amounts of N,N-DEPEA and N,α-DEPEA found in the batches used for validation and the amounts of the same substances found in the tested samples, a batch-to-batch inconsistency is evident. Additional levels of standard additions will be tested in future studies to verify that recoveries at different levels are as good as the levels published here.

The detected unknown compounds with the same transitions as the analytes are also of interest for future studies. The use of High Resolution Mass Spectrometry (HRMS) would be the first approach to structural elucidation (12) followed by NMR. With HRMS it would be possible to determine the detected unknown compounds accurate mass which may be useful in structural elucidation. It would also be possible to investigate if any other unknown compounds with similar mass coelute with any of the analytes.

Conclusion

The developed method proved that the pwo-product “Craze” does contain N,β-DEPEA, N,α-DEPEA and N,N-DEPEA at measurable concentrations and that the product contains significantly more of N,β-DEPEA (which is lacking from the label) than N,N-DEPEA (which is on the label) and N,α-DEPEA (which is also not on the label). However, the product seems to have varying degrees of homogeneity and content of the analytes between lots.

The key technical feature of this paper is the achievement of the critical resolution needed to isolate and accurately identify and quantify two structurally similar isomers from each other and from other potential unknown coeluting compounds. Significant concentration differences in combination with identical transition patterns made this separation vital.

Comment on nomenclature

The three studied analytes have a plethora of IUPAC and common names in various literature. The authors suggest the abbreviations used throughout this paper for use in future publications in order for the research field to be more easily overviewed and searchable.

Other names used for N,α-DEPEA in other publications include but are not limited to; N,α-diethyl-phenylethylamine, NADEP, ETH, Ethyl(1-phenylbutan2-yl)amine and N,α-diethylbenzeneethanamine.

Other names used for N,β-DEPEA in other publications include but are not limited to; N,β-diethyl-phenylethylamine Ethyl(2-phenylbutyl)amine, N-ethyl-2-phenyl-1-butaneamine.

Other names used for N,N-DEPEA in other publications include but are not limited to; N,N-diethyl-phenylethylamine, N,N-Diethyl-β -Phenylethylamine, N,N-diethyl-benzeneethanamine & N,N-Diethyl-2-phenylethaneamine.

Disclaimer

The authors declare that they have no conflict of interest and no connection to the sports supplement market except as consultancy analytical laboratory. However, the current study has been made possible by support from Driven Sports Inc.

References

1. *Identification of N-ethyl-a-ethylphenethylamine in crystalline powder seized for suspected drug trafficking: a research chemical or a new designer drug?* **Jaesin Lee, Sanggil Choe, Hyeyoung Choi, Sewoong Heo, Eunmi Kim, Hyunju Kim, Eunjung Bang, Heesun Chung.** 2013, Forensic Toxicology, pp. 31:54–58.

2. **F. T. Noggle, JR., C. Randall Clark, Paula Pitts-Mink and Jack de Ruiter.** Liquid Chromatographic and Mass spectral analysis of 1-Phenyl-2-Butanamine: Homologues of the Amphetamines. *Journal of Liquid Chromatography.* 1991, pp. 1393-1408.

3. **Code, The World Anti-Doping.** *The 2014 prohibited list, International standard.* s.l. : World Anti-Doping Agency, 2014.

4. **Jaesin Lee, Bastiaan J. Venhuis, Sewoong Heo, Hyeyoung Choi, Ilung Seol, Eunmi Kim.** Identification and quantitation of N,a-diethylphenethylamine in preworkout supplements sold via the Internet. *Forensic Toxicol.* August 2013, pp. DOI 10.1007/s11419-013-0205-6.

5. **Pieter A. Cohen, John C. Travisb and Bastiaan J. Venhuisc.** A methamphetamine analog (N,α-diethylphenylethylamine) identified in a mainstream dietary supplement. *Drug Testing and Analysis.* October 2013, p. DOI 10.1002/dta.1578.

6. **Houlahan, Greta.** PRWeb. [Online] October 14, 2013. [Cited: February 19, 2014.] http://www.prweb.com/releases/2013/10/prweb11227052.htm.

7. **ElSohly, Mahmoud A. and Waseem Gul.** LC–MS-MS Analysis of Dietary Supplements for N-ethyl-a-ethyl-phenethylamine(ETH), N,N-diethylphenethylamine and Phenethylamine. *Journal of Analytical Toxicology.* March 38, 2014, pp. 63 –72.

8. **Wong, Ming.** *La Médecine Chinoise par les Plantes. Le Corps a Vivre series.* s.l. : Éditions Tcho, 1976.

9. **ICH Harmonized Tripartite Guideline.** *Validation of Analytical Procedures: Text and Methodology Q2(R1).* 2005.

10. **ISO/IEC 17025:2005/Cor 1:2006.** *General requirements for the competence of testing and calibration laboratories.* s.l. : Swedish Standards Institute, 2007.

11. **International Organization for Standardization, ISO.** *ISO/IEC Guide 98-3:2008, Guide to the expression of uncertainty in measurement (GUM:1995).* 1995.

12. **María Ibáñez, Juan V. Sancho, Lubertus Bijlsma, Alexander L.N. van Nuijs, Adrian Covaci, Félix Hernández.** Comprehensive analytical strategies based on high-resolution time-of-flight mass spectrometry to identify new psychoactive substances. *Trends in Analytical Chemistry.* 57, 2014, pp. 107-117.