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UV-spectrophotometry determination of taurine in energy drink mixtures

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Abstract: The aim of this study is developing and validation of UV-spectrophotometric method for determination of taurine in energy drink mixtures. The investigation includes validation of procedures for performance of the tests for identification, purity and assay. Analytical parameters precision, accuracy, selectivity, linearity, limit of detection and limit of quantitation were studied and compared. Developed method allows selectively determination of taurine in the present of caffeine at different conditions (algorithm, wavelength zones). The preferences are estimation the analytical parameters from the validation procedures. They let to compose the criteria in accordance with European Pharmacopoeia and EU regulates about the use of official analytical programs for quality control of supplements containing taurine. The method has been applied successfully in analysis of energy food drinks preparation in the appointed for each of their aspect of applications.

Keywords: Taurine, Energy Food Drinks, Ninhydrin Reaction, UV-VIS Spectrophotometry

1. Introduction

Taurine (2-aminoethanesulfonic acid) is an organic acid - derivative of cysteine which has a regular usage in energy food drinks. There are written many physiology effects of taurine which are explained with amino terminal group in the structure and sulfonic acid group moiety [1, 2]. Taurine crosses the blood-brain barrier and has been implicated in a wide array of physiological phenomena. It includes inhibitory neurotransmission, long-term potential in the striatum/hippocampus membrane stabilization, feedback inhibition of neutrophil/macrophage respiratory burst, adipose tissue regulation. Taurine takes part in the possible prevention of obesity, calcium homeostasis, recovery from osmotic shock, protection against glutamate citotoxicity and prevention of epileptic seizures. It also acts as an antioxidant and protects against toxicity of various substances (such as lead and cadmium). Additionally, supplementation with taurine has been shown to prevent oxidative stress induced by exercise. Taurine helps people with congestive heart failure by increasing the force and effectiveness of heart-muscle contractions. Studies have shown that taurine can influence defects in nerve blood flow, motor nerve conduction velocity, and nerve sensory thresholds in experimental diabetic neuropathic rats. Taurine is conjugated via its amino terminal group with chenodeoxycholic acid and cholic acid to form the bile salts sodium taurochenodeoxycholate and sodium taurocholate. The low pKa of taurine's sulfonic acid group ensures that this moiety is negatively charged in the pH ranges normally found in the intestinal tract and, thus, improves the surfactant properties of the cholic acid conjugate. Taurine is necessary for normal skeletal muscle functioning [2-4].

The wide-spread usage of energy drinks with taurine increases the cases with unwanted side effects and requires the development of an analytical program including identification tests and assays with accuracy, precision and selectivity for monitoring of number supplements especially for those which has free distribution on the market.

For determination of taurine several analytical methods are described: HPLC with pre- and post – column derivatization with different reagents and UV detection, HPLC with mass spectrometry; HILIC-chromatography with UV and ELSD detection, UV-spectrophotometric methods [5-18]. All this methods solve specific problems in rather different matrixes and for different aims.

Aim: The aim of this study is a developing and validation of UV-spectrophotometry method for identification and quantitation of taurine in supplement and drug mixtures with high selectivity and wide spectra of applications in the

compliance with European Pharmacopoeia regulates.

2. Experimental Methods

2.1. Reagents

Taurine reference substance (RS), Caffeine RS, ninhydrin, 70 vol. % ethanol, energy food drinks, containing 35 mg taurine, model mixtures from reference substances, containing 25, 35 and 50 mg taurine and 150 mg caffeine.

2.2. System

UV/VIS Spectrometer HP;
Diode array detector;
Wavelength Range – 190 – 820 nm;
Wavelenght Accuracy – $\pm$ 2 nm;
UV/VIS operating software.

2.2.1. Analytical Calculations

Analytical calculations are based on Single standard method at fixed conditions. Method's options are given on table 1.

Table 1. *Analytical calculations.*

Calculations	Method's options
Analysis	Single standard method (SCA)
Calibration Curve type	Beer's Law
Algorithm	Least Squares fit (LSQ)
Derivative order	0
Polynomial Degree	0
Smoothing Points	1
Data Interval	2 nm
Analytical Wavelength Zone	200 nm to 800 nm
Temperature	25 °C

2.3. Sample Preparation

2.3.1. Reference Solutions

Reference solutions containing taurine and caffeine were prepared by dissolving of accurately weighed 35 mg RS taurine and 50 mg RS caffeine with 10 ml 70 vol. % ethanol. Aliquot volumes from solutions were diluted with the same solvent to obtain solutions with appropriate known concentration.

2.3.2. Test Solutions

Model mixtures containing 35 mg taurine RS and 50 mg caffeine RS were dissolved with 70 vol. % ethanol in volumetric flask up to 10 ml. 1.0 ml from obtained solution was diluted to obtain solutions with appropriate concentration.

Test solutions of model mixtures containing 25, 35 and 50 mg taurine RS and 150 mg caffeine RS were prepared by the same manner.

Aliquot volume from energy food drink containing 35 mg taurine and 50 mg caffeine was evaporated to dryness and the residue was dissolved with 10 ml 70 vol. % ethanol and diluted with the same solvent to obtain a solution with necessary concentration.

2.3.3. Blank Solution

70 vol. % ethanol.

2.3.4. Procedure

5.0 ml from each of reference, test and blank solutions described above were heated with 5.0 ml 0.2 % solution of ninhydrine for 20 min at 70 °C. After cooling the obtained sample solutions were diluted up to necessary volume.

The prepared solutions were analyzed by normal spectrophotometry in VIS range at fixed wavelength 570 nm for model mixtures containing taurine-ninhydrin color compound against 70 vol. % ethanol matrix as blank solution. The investigations were carried out using single standard method.

3. Results and Discussion

In energy drink mixtures taurine usually is in concomitant composition with xanthine derivative caffeine which quantities vary from 50 to 150 mg. Caffeine shows intensive and specific absorption in UV range and at simultaneously UV-spectrophotometry determination with taurine it's UV spectra cover those of taurine in analytical zone from 190 to 400 nm (fig. 2, (1)). Due to this authors take advantage to the fact that taurine reacts selectively with ninhydrin after warming. The product of reaction is a color compound and it has absorption max at 570 nm in 70 vol. % ethanol matrix (fig. 2, (2)). Ninhydrin reacts with primary and secondary amines producing a blue or purple reaction product (diketohydrindylidene - diketohydrindamine)[19](fig. 1). The intensity of coloring is proportional to concentration of analyzed compounds (first order reaction).

The reaction was negative to caffeine, tertiary amines and amines with aromatic moiety.

Based on it UV-spectrophotometry method was developed and validated in respect of analytical parameters selectivity, precision, accuracy, linearity, limit of detection and limit of quantitation. The conditions were verified for taurine as reference substance and energy mixtures with caffeine with negative ninhydrin test.

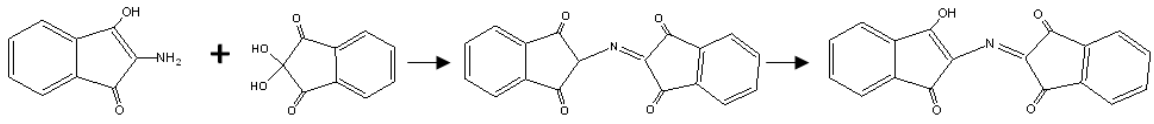

Fig 1. *Structure of blue reaction product.*

3.1. Validation of UV-Spectrophotometric Method

Selectivity:

Using SCA selectivity was achieved measuring by normal spectrophotometry at 570 nm in 70 vol. % ethanol matrix.

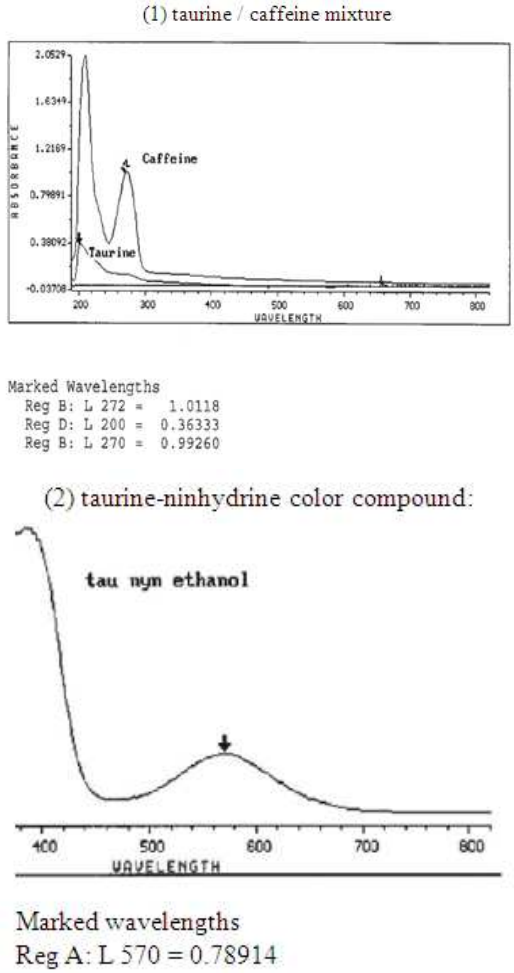

Fig 2. *UV spectra of taurine / caffeine mixture (1) and taurine-ninhydrine color compound (2) in 70 vol. % ethanol matrix.*

Precision:

Six (6) equal solutions from homogenous samples containing 35 mg taurine were analyzed by UV-spectrophotometric method. Standard deviation (SD = 0.00534 AU) and relative SD (RSD = +/- 1.44 %) were found based on obtained absorption values. The results are presented on Table 2.

Table 2. *Precision of samples containing taurine.*

Samples (n)	Obtained A (AU)	X_{mean}	SD	RSD (%)
1	0.376			
2	0.361			
3	0.373	0.371	0.0053	±1.44 %
4	0.370			
5	0.374			
6	0.373			

Accuracy:

Model mixtures of solutions containing 25, 35 and 50 mg taurine in concentration ratio 50 – 150 % of theoretical calculated quantity were prepared and analyzed three times each. The results are shown on Tables 3. They are presented as % recovery. At fixed analytical parameters all studied combinations with ratio 25 / 35 / 50 mg taurine : 50 mg caffeine responds to ICH and Pharmacopoeia requirements about accuracy tolerance.

Table 3. *Accuracy of model mixtures 1, 2 and 3 containing 25, 35 and 50 mg taurine respectively.*

Model mixtures (n)	Putted amount of taurine (g)	Obtained results for A (AU)	RSD (%)
1	0.025	0.183 0.180 0.174	± 2.56
2	0.035	0.202 0.213 0.206	± 2.68
3	0.050	0.370 0.374 0.373	± 0.55

Limit of detection:

140 µg for taurine, established on the base of ratio noise – signal – 1:3. Results are shown on fig. 3.

Limit of quantitation:

1400 µg for taurine, established on the base of ratio noise – signal – 1:10.

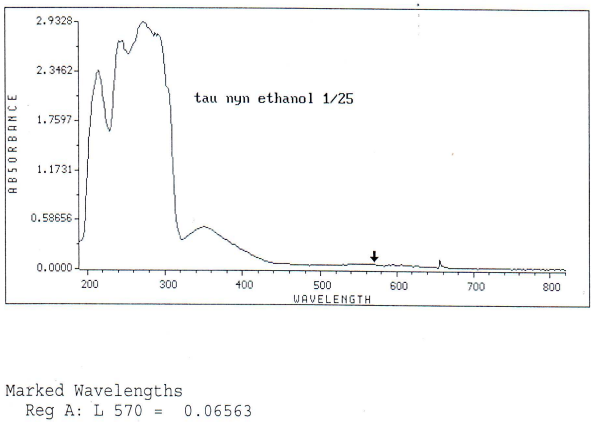

Fig 3. *UV-spectra of taurine-ninhydrin color compound in concentration ratio 1: 25 v/v.*

Linearity:

The analytical parameter linearity was studied in concentration ratio 0.1 – 0.004 g. The accordance between the absorption, measured in absorption units (AU) and concentrations in g/ml is proportional in the intervals. The correlation coefficients were found to be about 1 – 0.99692 at SD = +/- 0.02151 AU, N = 7 and P < 0.0001. Results for linearity of color compound in ethanol matrix are shown on table 4.

Table 4. *Linearity parameters of taurine-ninhydrine color compound in 70 vol. % ethanol matrix.*

Y = A + B * X		
Parameter	**Value**	**Error**
A	0.06108	0.01136
B	72.20057	2.53899
R	N	P
0.99692	7	<0.0001

4. Conclusion

The conditions of novel UV-spectrophotometry method for determination of taurine in supplement and drug energy mixtures were performed. The method was validated in complains with European Pharmacopoeia criteria and it distinctive properties are high selectivity, optimal values of analytical parameters and wide spectra of applications for purposes of pharmaceutical and toxicological practices.

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