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A novel approach for HPLC determination of 2-cynaoacetamide using derivatization procedure with 2-hydroxyacetophenone as a new useful derivatization reagent

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ABSTRACT

A novel and sensitive derivatization procedure for the determination of 2-cynaoacetamide in pharmaceutical samples using liquid chromatography with the fluorescence detection was discovered. The method is based on derivatization of 2-cynaoacetamide using 2-hydroxyacetophenone as a new derivatization reagent. The product of derivatization reaction was isolated and characterized using spectroscopic techniques namely LC–MS, NMR and IR. The structure of 2-cyanoacetamide derivative was unambiguously assigned as a 2-amino-4-phenylfuran-3-carboxamide.

Two derivatization systems were optimized in terms of reaction temperature, reaction time, pH and concentration of 2-hydroxyacetophenone, and a new pre- and post-derivatization HPLC methods were developed. The separations on HPLC with pre-column derivatization were accomplished using stationary phase based on a XBridge C18 column (100 × 4.6, 3.5 μm) and isocratic elution using the mobile phase acetonitrile – 0.1% formic acid (30:70, v/v). The separations on the HPLC with post-column derivatization were performed on stationary phase on a TSKgel Amide-80 column (150 × 4.6 mm, 3 μm). The mobile phase was a mixture of acetonitrile, methanol and 10 mM sodium formate buffer at pH = 4.5 in ratio 3:2:95 (v/v).

Both HPLC methods were fully validated in terms of linearity, sensitivity (limit of detection and limit of quantification), accuracy and precision according to ICH guidelines. The pre-column derivatization method was linear in the range 1.1–2000 μg/l with method accuracy ≥ 98.2% and method precision RSD ≤ 4.8%. The post-column derivatization method was linear in the range 12–2000 μg/l. Method accuracy ≥ 96.3% and method precision RSD ≤ 3.5%. Proposed new methods were proved to be highly sensitive, simple and rapid, and were successfully applied to the determinations of 2-cynaoacetamide in pregabalin.
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1. Introduction

The drug pregabalin (PGB; (*S*)-3-(aminomethyl)-5methyl-hexanoic acid) is an antiepileptic and analgesic drug, which is structural analogue of γ-amino-butyric acid. PGB exhibits anti-seizure activity and is thought to be useful for treating, among other conditions, pain, physiological conditions associated with psychomotoric stimulants, inflammation, gastrointestinal damage, alcoholism, insomnia, and various psychiatric disorders, including mania and bipolar disorder [1,2].

The condensation of ketones with 2-cyanoacetamide in the presence of a condensing agent leaded to the formation of cis- and trans-additive products [3]. This reaction was used in the synthesis of PGB. The condensation of 3-methylbutanal with cyanoacetic acid ethyl ester or 2-cyanoacetamide in the presence of dipropylamine in refluxing hexane, followed by treatment with refluxing 6 M hydrochloric acid, furnished 3-isobutylglutaric acid. This compound was converted into the corresponding anhydride by treatment with acetic anhydride at reflux. The reaction of the anhydride with ammonium hydroxide afforded glutaramic amide, which was submitted to optical resolution with (*R*)-(+)-1-phenylethylamine, yielding the (*S*)-enantiomer of 3-carbamoylmethyl-5-methylhexanoic acid. Finally, this compound was submitted to a Hoffmann degradation with bromine and sodium hydroxide which provided PGB [4].

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Many reactions with 2-cynaoacetamide leading to fluorescent products are known, but the quantitative determination of 2-cynaoacetamide using separation methods with this type of derivatization reactions has not yet been published. The usefulness of 2-cyanoacetamide as a sensitive fluorogenic reagent for determination of reducing carbohydrates [5,6], catecholamines [7], uronic acids [8], aldoses [9], hexosamines [10], glycosaminoglycans (such as hyaluronic acid, chondroitin sulphate and dermatan sulphate) [11] and keratan sulfates [12] has been described. 2-Cyanoacetamide reacts with various classes carbohydrates in alkaline borate buffer at temperature of 100 °C to give highly intensive fluorescence products having various excitation and emission maxima [13]. The fluorescence reactions were applied to the detection of these analytes by HPLC after post-column derivatization. Finally, condensation reaction of 2-cyanoacetamide with compounds containing α-hydroxycarbonyl groups such as ascorbic acid [14] or 3,4-dihydroxyphenylalanine [15] was utilized for their fluorimetric determination.

The detailed mechanism of this reaction type has not been elucidated, although several compounds were isolated from the reaction mixture of glucose and 2-cynaoacetamide by combination of column and paper chromatography. On the basis of structural studies, the above mentioned isolated compounds were assigned be 3-cyano-2-pyridone and 3-cyano-2-pyrrolidone derivatives, and the latter compound was identified as a conjugated diene-diol [13].

Presented study was designed to find the optimal reaction conditions for pre- and post-column derivatization of 2-cyanoacetamide using with new derivatization agent such as 2-hydroxyacetophenone (2-HAP). A reasonable mechanism for this derivatization reaction was proposed. The other aim of the presented work was to develop a quick and sensitive quantitative chromatographic methods for the determination of 2-cyanoacetamide with specification limit 1000 ppm in PGB. The specification limit was set based on the requirements of ICH guidelines [16,17]. The applicability of new method was successfully verified by analysis of three batches of PGB.

2. Experimental

2.1. Chemicals and materials

Acetonitrile of HPLC grade and methanol HPLC grade (Merck, Czech Republic) and water purified on Milli-Q system (Millipore, USA) were used. Other chemicals were of analytical grade (Sigma, Czech Republic). Extraction solvent was prepared by mixing 50 ml water and 950 ml acetonitrile.

2.2. LC instrumentation and methods

Sample extraction was performed using an ultrasonic bath UCC4 (TESON, Slovakia). All chromatographic experiments were carried out using a liquid chromatograph system consisting of Alliance 2695 separation module and fluorescence detector W2475 (all Waters, USA). The system was controlled by data station using Empower software (Waters, USA).

A Pre-column derivatization. RP-HPLC separation was performed on a XBridge C18 column (100 × 4.6, 3.5 μm. Waters, Czech Republic) with isocratic elution using the mobile phase acetonitrile – 0.1% formic acid (30:70, v/v). The flow rate was set to 1.0 ml/min, the injection volume was 10 μl, the column was thermostated at 45 °C and the run time was 6 min. The fluorescence detection was carried out at excitation wavelength of 315 nm and emission wavelength of 383 nm.

B Post-column derivatization. Hydrophilic interaction chromatography (HILIC) was performed on a TSKgel Amide-80 column (150 × 4.6 mm, 3 μm; Sigma, Czech Republic). The mobile phase was a mixture of acetonitrile, methanol and 10 mM sodium formate buffer at pH = 4.5 in ratio 3:2:95 (v/v) and was delivered isocratically at a constant flow rate of 0.7 ml/min for 8 min. The injection volume was 10 μl, the column was maintained at 45 °C. Post-column addition of a derivatization mixture was performed in post-column reaction system PCRS-100 (volume 250 μl; ID 0.25 mm) (Science Instruments and Software, Czech Republic), placed between a chromatographic column and the detector and connected to the column with a low-dead-volume PEEK mixing tee (Valco Instruments Company, USA). The temperature of post-column reaction system was set up to 100 °C. As the post-column derivatization reagent, 5 mM 2-HAP in 10 mM sodium tetraborate pH 11 was used and delivered at a flow rate of 0.3 ml/min. The fluorescence detection was performed at an excitation wavelength of 356 nm and an emission wavelength of 445 nm.

2.3. LC–MS instrumentation and methods

High resolution/accurate mass (HRAM) MS experiments were performed on a LTQ XL Orbitrap Mass Spectrometer (Thermo, San Jose, USA) coupled to an HPLC HTS PAL system (CTC Analytics, Switzerland). LC separation was performed on a Kinetex C18, 150 × 3.0 mm, 2.6 μm (Phenomenex, Torrance, USA) column using 0.6 ml/min flow rate and mobile phase consisting of 10 mM ammonium formate (pH 6.3) and acetonitrile (gradient of acetonitrile ranging from 30% to 100% in 18 min). For an ionisation of the analytes an APCI ion source was operated in the positive ion mode (vaporizer temperature 400 °C, capillary temperature 300 °C, sheath gas flow: 35 arbitrary unit, aux gas flow: 5 arbitrary unit, sweep gas flow: 5 arbitrary unit, discharge current: 4 μA and tube lens voltage 40 V)

2.4. NMR, IR and other instruments and measurement conditions

Nuclear magnetic resonance (NMR) spectra was obtained using a Bruker Avance 500 (Bruker Biospin, Germany) at 500.13 MHz (1H) and 125.76 MHz, respectively. All NMR experiments were performed in dimethylsulfoxide at 298 K. At 500 MHz, standard 5 mm TBI (triple-broadband inverse) probe head equipped with z-gradient coils was employed for all measurements. FTIR spectra was measured by the single-reflection ATR (ZnSe) FTIR spectrometer Nicolet 6700 (Thermo, USA). Each spectrum was acquired by accumulation of 12 scans with 4 cm^{-1} resolution. FT-Raman spectra was measured on FT-Raman spectrometer RFS100/S (Bruker, Germany). Individual spectra was acquired by accumulation of 64 scans with 4 cm^{-1} resolution.

2.5. Standard and sample preparation

The standard of 2-cyanoacetamide (Sigma, Czech Republic, purity 99.1%) was dissolved in extraction solvent at a concentration of 100 mg/l to obtain the standard stock solution. The specification limit of 2-cyanoacetamide considered for validation studies was 1000 ppm with respect to PGB.

The sample preparation for post-column derivatization procedure was following: 10 mg of PGB was weighed into a 10 ml volumetric flask. 1 ml of 10 mM hydrochloric acid was added and sample was dissolved for 1 min in an ultrasonic bath. After cooling to the laboratory temperature, the flask was filled in by acetonitrile.

Pre-column derivatization procedure was accomplished by mixing appropriate volume of standard stock solution or 10 mg of PGB with 1 ml 0.1 M sodium tetraborate pH 11 and 0.75 ml of 5 mM 2-HAP in 10 ml reaction vial. 5 mM 2-HAP was prepared dissolving

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