Food Chemistry 166 (2015) 150–157

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

Aqueous two phase system based on ionic liquid for isolation of quinine from human plasma sample

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ARTICLE INFO

Article history:
Received 18 August 2013
Received in revised form 24 February 2014
Accepted 8 June 2014
Available online 16 June 2014

Keywords:
Quinine
Human plasma
Ionic liquids
Chaotropic effect

ABSTRACT

Aqueous two phase system was applied for selective extraction of quinine from human plasma. Bi-phase was constructed from ionic liquid: butyl-methyl-imidazolium chloride after addition kosmotropic salts K_3PO_4 or KH_2PO_4. Quinine was determined in plasma samples after drinking of tonic containing quinine. Determination was performed by HPLC on 5-μm Zorbax SB-CN column and eluent containing 40% acetonitrile (v/v), 20 mM phosphate buffer at pH 3 and 40 mM $NaPF_6$ using external standard method. The spectrophotometric detection was set $\lambda = 214$ nm. Selective fluorescence detection was performed at excitation of 325 nm and emission of 375 nm. Proposed strategy provides suitable sample purification and gives extraction yields in the range of 89–106%. The determination coefficient (R^2) has a value $\geqslant 0.997$ in the range of 50–800 ng/ml quinine concentration. The limit of quantification was set at 27.9 ng/ml and the detection limit was found to be 8.4 ng/ml under fluorescence detection.

1. Introduction

Quinine (6′-methoxycinchonan-9-ol) belongs to β-carboline cinchona alkaloids. This naturally occurring alkaloid is extracted from the bark of cinchona tree growing in India, South America and Indonesia. Nowadays Java is considered as the main producer of this compound. Since anti-malaria properties of quinine were discovered it has been used in medicine for treatment of this parasitical disease for more than three centuries (White, 1992).

Due to its bitter taste, quinine is also commonly applied as an additive in different soft drinks as a flavouring agent. It should be emphasized that quinine was recognised as a potentially toxic compound due to its dangerous side effects, especially when it is abused. It has been reported that even excessive intake of tonic water containing quinine can be dangerous and can result in reversible toxicity. The most common 'cinchonism' syndromes cover among others: chest pain, asthma, disturbed vision, headache, fever, nausea, diarrhea, etc., but the most dangerous appear to be relaxation of muscles including muscles of the heart, which may end up as sudden death (Huston & Levinson, 2006; Samanidou, Evaggelopoulou, & Papadoyannis, 2005; White, 1992).

The pharmacological effect of quinine in human organism is related to its plasma concentration. So far, several analytical methods have been described for its determination in biological fluids. In all cases quantification is preceded by a isolation step including liquid–liquid extraction (LLE) Mirghani, Ericsson, Cook, Yu, & Gustafsson, 2001; Jansson, Gustafsson, & Mirghani, 2003 or solid-phase extraction (SPE) Samanidou et al., 2005 followed by normal or reversed-phase high-performance liquid chromatography (HPLC) with ultraviolet (UV) or fluorescence (FL) detection. Although the methods described possess enough sensitivity and precision, they require long analysis time, volatile organic solvents and additional instrumentation, for instance for SPE procedure. Mirghani et al. (2001) described quantification of quinine in plasma and urine samples on Zorbax XDB phenyl column under gradient elution mode and fluorescence detection. This method enabled detection of quinine after almost 20 min. Samples were prepared by protein precipitation with methanol. In turn Samanidou et al. (2005) proposed separation of quinine in biological fluids on a Kromasil C18 column and isocratic elution by the use of methanol–acetonitrile-0.1 ml/L ammonium acetate after its purification by SPE procedure. Quinine was detected after 6 min. However its peak was not fully resolved with another component of the analysed mixture-chloroquine.

In this paper, aqueous two phase system (ATPs) based on ionic liquid was elaborated with the aim to isolate quinine from human plasma. So far, only a few methods have been published describing ATP systems based on ionic liquids for isolation of alkaloids from biological fluids (Freire et al., 2010; Li, He, Liu, Li, & Liu, 2005). Considering quantitative analysis of trace-level of alkaloids as for drugs control or doping agents detection, ionic liquid based ATP's

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http://dx.doi.org/10.1016/j.foodchem.2014.06.037
0308-8146/

have been reported only for caffeine, nicotine, codeine and papaverine. Pioneering paper in this field was published in 2005 by Li et al. (2005). Authors have successfully used imidazolium based ionic liquids forming ATP in the presence of inorganic salt aqueous solution for quantification of opium alkaloids from *Pseudomonas papaveris*.

In this manuscript we developed the most efficient composition of ATP system for simple and rapid quinine isolation from human plasma sample after ingesting of tonic water. Quantification of quinine was further developed by elaborating on efficient reversed phase HPLC on Zorbax SB-CN column and eluent containing chaotropic additive for enhancing sensitivity of detection and elongation of quinine retention time. Photodiode (PAD) and fluorescence (FL) detection were compared accordingly to obtain the most advantageous sensitivity and selectivity of assay.

2. Materials and methods

2.1. Chemicals

The compound: quinine hydrochloride was purchased from E.Merck (Darmstadt, Germany). HPLC grade acetonitrile (ACN) was obtained from E.Merck (Darmstadt, Germany). Orthophosphoric acid was purchased from P.O.Ch (Gliwice, Poland). Ionic liquid: 1-butyl-3-methyl-imidazolium chloride (BMIM Cl) were obtained from Fluka. Chaotropic salt: sodium hexafluorophosphate ($NaPF_6$), was obtained from Fluka, other salts: sodium phosphate and sodium hydrogen phosphate were obtained from P.O.Ch (Gliwice, Poland). HPLC water was obtained from Barnstead deionizing system (Dubuque, IA, USA). The phosphate buffer (pH = 3) was prepared by dissolving 0.5 ml 85% (m/m) orthophosphoric acid in 80 ml water and adjusting the pH appropriately with saturated sodium hydroxide solution.

2.2. Instrumentation

Experiments were performed using the LaChrom Elite HPLC Merck Hitachi (E.Merck, Darmstadt, Germany) model equipped with diode array detector, fluorescence detector, column oven L-7350 and solvent degasser L-761 and a Rheodyne injection valve with a 20-μL loop. The column (250 mm × 4.6 mm I.D.) was packed with 5-μm Zorbax SB-CN (pore size: 80 Å, surface area: 180 m^2/g) Sigma–Aldrich. The column was thermostated at 20 °C ± 0.1. Retention data were recorded at a flow-rate of 1 ml/min. Typical injection volumes were 20 μL. The detection of the compounds was set at appropriate wavelength chosen accordingly with the recorded spectra: λ = 214 nm. Fluorescence detection was performed at excitation 325 nm and emission 375 nm, respectively. The mobile phase contained 40% acetonitrile (v/v), 20 mM phosphate buffer at pH = 3 and 40 mM $NaPF_6$. The mobile phase was filtered using a filtration apparatus equipped with a Nylon 66 membrane filter (0.45 μm) Whatman (Maidstone, England).

2.3. Samples

For the validation procedure blank plasma samples spiked with quinine were used. Plasma samples were collected from a healthy volunteer before and 2 h after administration of a single dose of 2 L of water tonic Kinley (Coca-cola HBC, Poland). Venous blood was drawn into tubes containing heparin as anticoagulant. After centrifugation for 15 min at 1800×*g*, plasma was transferred to the test tube and frozen at −20 °C until analysis.

2.4. Aqueous two-phase system formation

To 100 μL aliquots of the plasma, 1 ml of an aqueous solutions of ionic liquid (BMIM Cl) in concentration ranging from 15 to 25 wt.%, 0.5 ml of quinine solution and 0.5 ml of water containing from 1.6 to 2.3 g of kosmotropic salts K_3PO_4 or K_2HPO_4 were added into 5 ml centrifugal tube. The mixture was mixed thoroughly, centrifuged and two clear phases were formed. The top phase was diluted to 1 ml with the mobile phase and directly injected into the HPLC system.

2.5. Analytical procedure

The analytical-method validation was carried out according to the ICH Q2 (R1) method-validation guidelines (ICH, 2005). The following validation parameters were established: selectivity, precision, linearity, limit of detection (LOD), limit of quantification (LOQ).

2.5.1. Linearity

Eight calibrator solutions at concentrations ranging from 50 to 800 ng/ml were prepared by supplementing 0.1 ml of filtered plasma. The mixtures were further analysed by procedure described above (Section 2.4).

The analyte peak area was plotted against the corresponding concentrations and the calibration curves were set up by means of the least-squares method. Limit of quantification and limit of detection values were determined as a signal to noise ratio of 10 or 3 for LOQ and LOD respectively.

2.5.2. Extraction yield and precision

Spiked blank samples were prepared according to procedure described in Section 2.4 and they were analysed by HPLC method described in Section 2.2. The percentage extraction yield was calculated for three concentration levels: 115, 230 and 650 ng/ml. The analysis was repeated six times giving intraday precision values and six times in another day giving intermediate precision values both expressed as percentage standard deviation value (SD).

2.5.3. Computations

All calculations were carried out using Microsoft Office Excel 2007. Linear regression analysis was performed with Microsoft Office Excel 2007.

3. Results and discussion

3.1. Chromatographic system

Quinine was separated and purified by chromatography on cyanopropyl column Zorbax SB-CN using 40% ACN/phosphate buffer at pH 3 containing 40 mM chaotropic additive $NaPF_6$. Chaotropic effect in HPLC has been studied in details by Kazakevich and Snow (2006), Kazakevich, LoBrutto, and Vivilecchia (2005), Jones, LoBrutto, and Kazakevich (2002), LoBrutto, Jones, Kazakevich, and McNair (2001), Pan, LoBrutto, Kazakevich, and Thompson (2004), LoBrutto, Jones, and Kazakevich (2001), Kazakevich, LoBrutto, Chan, and Patel (2001) and later on by others (Dai & Carr, 2005; Flieger, 2006, 2007; Pilorz & Choma, 2004). At pH lower than 4 quinine (Q) exists in fully protonated form of QH_2^{2+}. This statement is based on relationship between electrophoretic mobility (μ_{eff}) versus pH, indicating the presence of two different pK_a values. Two inflection points of this relationship were set at pH 4.34 and 8.51 (Pang, Kenseth, & Coliron, 2004). The structures of fully protonated and unprotonated forms are presented in Fig. 1. Enrichment of the mobile phase with anionic reagent causes ion-associated complex