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Development and validation of a GC–MS method for the determination of hydroxyzine and its active metabolite, cetirizine, in whole blood

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ABSTRACT

A simple, rapid, sensitive and accurate gas chromatography–mass spectrometric method was developed and validated for the simultaneous determination of hydroxyzine and cetirizine in whole blood. Solid-phase extraction procedure using Bond Elut LRC Certify II columns was used for the isolation of hydroxyzine and cetirizine from 1 mL whole blood followed by derivatization with a mixture of acetic anhydride:n-propanol (1:1, v/v). Limits of detection and quantification were 1.50 and 5.00 ng/mL, respectively. The assay was linear within the concentration range of 5.00–1000.0 ng/mL and the correlation coefficient was $R^2 \geq 0.993$ for both analytes. Absolute recovery was determined at three quality control concentration levels and was found to be at least 87.2% for both substances. Intra-day and inter-day accuracy values for both hydroxyzine and cetirizine were ranged from −1.2 to 3.8% and −2.7 to 2.0%, respectively, at the three concentration levels studied, whereas their respective intra-day and inter-day precision values were less than 9.9 and 6.5%, respectively, in terms of relative standard deviation (%RSD). The developed method was successfully applied for the quantification of hydroxyzine and cetirizine concentrations in whole blood, during the investigation of clinical cases where these two antihistamines were detected.

1. Introduction

Antihistamines or H_1-receptor antagonists are a class of drugs that inhibit the action of histamine and are used to alleviate symptoms associated with allergic reactions, while they also present anticholinergic, antiemetic, antiserotonergic, adrenergic or anti-adrenergic, and local anaesthetic actions, due to their additional interaction with the respective receptors [1].

Hydroxyzine, 2-[2-[4-[(4-chlorophenyl)-phenylmethyl]-piperazin-1-yl]-ethoxy]-ethanol, (Fig. 1) is a first generation piperazine H_1-antagonist that is commercially available as a hydrochloric salt in various forms and under various brand names the most common of which is ATARAX® (UCB) [2]. It is used as an antihistamine to the symptomatic treatment of pruritus, for the short-term symptomatic treatment of anxiety manifestations in adults, as a sedative during the pro-operative treatment and for the control of emesis [3]. In some cases, it has been also used to relieve symptoms of the opioid [4], benzodiazepine [5] and alcohol withdrawal syndrome [6]. As a first generation antihistamine, hydroxyzine possesses sedative properties that get enhanced when it is simultaneously administered with other central nervous system (CNS) depressants, such as alcohol, hypnotics, narcotics etc. Thus, it disrupts cognitive function and seriously affects the ability to drive and operate machines [7]. The therapeutic range for cetirizine is 0.11–0.36 μg/mL in plasma and the toxic concentrations are higher than 2.4 μg/mL [3], while a hydroxyzine-related fatal case reported a lethal concentration of 39 μg/mL [8].

Cetirizine, 2-[2-[4-chlorophenyl)-phenylmethyl]-piperazin-1-yl]-ethoxy]-acetic acid, (Fig. 1) is the major active carboxylic acid metabolite of hydroxyzine that accumulates in serum and possesses significant antihistaminic activity with no sedative effects. It is the racemic mixture of its two enantiomers (*R* and *S*) [9] that is also commercially available as its dihydrochloric salt in various forms and under various brand names, the most common of which is ZYRTEK® (UCB). Cetirizine is a second generation piperazine H_1-antagonist that is used to alleviate the symptoms

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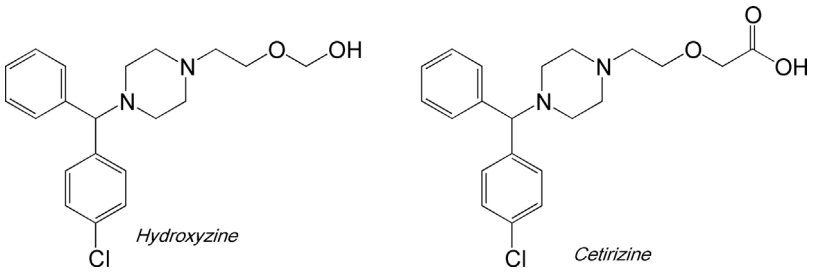

Fig. 1. Structures of hydroxyzine and cetirizine.

of seasonal and chronic allergic rhinitis, allergic conjunctivitis and chronic idiopathic urticaria [9]. Levocetirizine (Fig. 1) is the active *R*-enantiomer of cetirizine racemate and a third generation non sedative H_1-antihistaminic agent that was first marketed under the brand name XOZAL® (UCB) with the same indications as cetirizine [10]. Similarly to cetirizine, levocetirizine does not readily cross the blood-brain barrier and therefore exhibits no significant effects on the CNS in contrast to the first generation antihistamines. Thus, it is less possible to cause drowsiness of affect memory and impair cognitive function [11].

A number of methods have been reported for the determination of hydroxyzine and (levo)cetirizine in biological fluids, separately or simultaneously with other drugs [12], including gas chromatography (GC) with flame ionization/electron capture detection (FID/ECD) [13,14], mass spectrometry (MS) [15,16], nitrogen-phosphorus detection [14,15,17]. High performance liquid chromatography (HPLC) techniques in combination with ultraviolet (UV) [18], diode-array (DAD) [19], fluorescence (FLD) [20] and MS detection [21,22], and more recently with tandem MS (MS/MS) [23,24] have also been used. To the best of our knowledge, there is only one published method for the simultaneous determination of hydroxyzine and cetirizine in human serum using HPLC–FLD [20], while there are only three other GC–MS methods that determine hydroxyzine alone in various biological fluids or gastric content [15,16]. There are no published methods for the determination of cetirizine with GC–MS.

To the best of our knowledge, there is no published GC–MS method for the simultaneous determination of these two analytes in blood. Thus, a GC–MS method was developed, optimized and validated for the simultaneous determination of hydroxyzine and cetirizine in whole blood samples. The developed method was successfully applied during the investigation of some clinical cases where these two antihistamine drugs were involved.

2. Materials and methods

2.1. Chemical and reagents

Hydroxyzine dihydrochloride (99.8%), cetirizine dihydrochloride (99.8%), hydroxyzine-d_4 (98.54%) dihydrochloride and cetirizine-d_8 (98.54%) were purchased from Clearsynth Labs Limited (Hyderabad, India). All solvents used (acetonitrile, dichloromethane, ethyl acetate, *n*-hexane and isopropanol) were of HPLC grade and were purchased from Merck (Darmstadt, Germany). Analytical reagents were purchased as follows: *N*,*O*-bis(trimethylsilyl)-trifluoracetamide (BSTFA) with 1% trimethylchlorsilane (TMCS) from Sigma-Aldrich (Steinheim, Germany), *N*-mehtyl-*N*-*tert*-butyldimethylsilyl-trifluoroacetamide (MTBSTFA) with 1% *tert*-butyldimethylsilylchloride (TBDMSCl) from Fluka (Steinheim, Germany), acetic anhydride 99%, 2,2,3,3,3-pentafluoropropionic anhydride 99% (PFPA) and 2,2,3,3,3-pentafluoro-n-propanol 97% (PFPrOH) from Sigma-Aldrich (Steinheim, Germany), trifluoroacetic anhydride 99% (TFAA) and heptafluorobutyric anhydride 99% (HFBA) from Fluka (Steinheim, Germany), n-propanol (HPLC grade) from LabScan (Dublin, Ireland). Glacial acetic acid (98%) and ammonium hydroxide 25% were of HPLC grade and obtained from Merck (Darmstadt, Germany). In this study, three different types of solid-phase extraction (SPE) columns (Bond Elut LRC Certify, Bond Elut LRC Certify II and HF Bond Elut LRC-C18) were used and were all obtained from Agilent Technologies (Lake Forest, CA, USA). Human blood was obtained, after informed consent, from healthy donors. Before its use it was screened negative for the presence of drugs by GC–MS, and then, it was pooled.

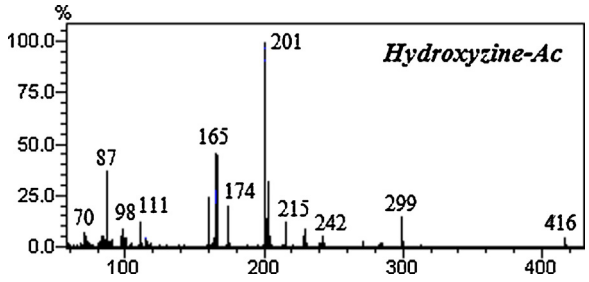

Fig. 2. Mass spectrum of acetylated hydroxyzine.

2.2. Instrumentation and other apparatus

The GC–MS analysis was performed using a Shimadzu GC-2010 equipped with a Shimadzu AOC-20i auto sampler system and interfaced with a Shimadzu QP 2010S mass spectrometer (Shimadzu, Tokyo, Japan). The separation of analytes was carried out using a cross-linked DB-1MS (12 m × 0.20 mm i.d., 0.33 μm) supplied by Agilent Technologies (IL, USA).

An MT 19 vortex (CHILTREN, London, UK), a 691 digital pH-meter with a glass electrode (METROHM, Herisau, Switzerland), an evaporating unit using nitrogen Reacti-Vap Model 18780 (PIERCE, Rockford, IL, USA) and a cooling centrifuge (ALSERA, Spain) were also used.

2.3. GC–MS analysis

The optimized GC conditions were as follows; initial oven temperature of 70 °C was held for 1 min, and then was increased to 130 °C at a rate of 10 °C/min followed by an increase to 300 °C at a rate of 30 °C/min, where it was held for 4 min (total run time = 16.67 min). The optimized temperatures of injection port, ion source and interface were 240, 200 and 300 °C, respectively. The carrier gas was helium at a flow rate of 1.0 mL/min. The mass spectrometer (MS) was operated in electron impact ionization/selective ion monitoring (EI/SIM) mode. The preliminary mass spectra of the derivatized compounds were obtained, for each analyte separately, in SCAN mode from *m*/*z* 50 to 600 amu. The mass spectra of the derivatized hydroxyzine and cetirizine are shown in Figs. 2 and 3.