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Short communication

Development of a simple column-switching high-performance liquid chromatography (HPLC) method for rapid and simultaneous routine serum monitoring of lamotrigine, oxcarbazepine and 10-monohydroxycarbazepine (MHD)

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Abstract

Using isocratic column-switching high-performance liquid chromatography (HPLC) we established a group method for automated quantitative analysis of the antiepileptic drugs lamotrigine, oxcarbazepine and its metabolite 10-monohydroxycarbazepine (MHD) that are also used in psychiatry as mood stabilizers. Samples were cleaned from interfering proteins and lipids by transfer onto a pre-column, using a PerfectBond® C-8 material, with 8% acetonitrile in water as a pre-column eluent. Separation was performed by elution onto the analytical column (Betasil® C6 5 μm, 250 mm × 4.6 mm) at a flow rate of 1.0 ml/min with potassium dihydrogenphosphate buffer (20 mmol/l, pH3.0)/acetonitrile (70/30; v/v) as analytical eluent. UV-spectrophotometric detection was set to 215 nm for all three compounds. The analytical run was finished within 18 min. Detection limit was 30 ng/ml for lamotrigine, 35 ng/ml for oxcarbazepine and 25 ng/ml for 10-monohydroxycarbazepine. The method was found to be suitable for automated analysis of serum samples of patients treated with lamotrigine and oxcarbazepine.

Keywords: Column-switching; Lamotrigine; Oxcarbazepine; 10-monohydroxycarbazepine; Therapeutic drug monitoring

1. Introduction

Lamotrigine and oxcarbazepine are well known as antiepileptic therapeutics against (focal) epileptic seizures in children and adults. Oxcarbazepine (10,11-dihydro-10-oxo-5H-dibenz[b,f]azepine-5-carboxamide) structurally differs from its tricyclic analogue carbamazepine in a 10-keto group (Fig. 1). Oxcarbazepine has an elimination half-life of 1–5 h and is metabolised to the pharmacological active compound of 10-monohydroxycarbazepine (MHD, monohydroxy derivate), which is known to have the relevant activity [1]. Therefore, serum levels of oxcarbazepine are lower than those of its metabolite. The therapeutic reference range for MHD is proposed to be between 10 and 20 mg/l [2]. Oxcarbazepine turned out to have potency as mood stabilizer in bipolar disorders, in manic episodes as well as in acute depression, and is sometimes added to an antidepressant medication [3]. Lamotrigine is a phenyltriazine derivate (Fig. 1) that structurally differs from all other antiepileptic drugs. It undergoes glucuronidation by uridine-glucuronyl-transferase isoenzyme 1A4 (UGT1A4); the cytochrome – P450 – system is not involved. The 2N-glucuronide is not known to have any therapeutic activity [4]. Since 2003, lamotrigine is approved to the German market to prevent depressive episodes in patients with bipolar disorders. Lamotrigine inhibits voltage-activated sodium channels; the presynaptic release of the excitatory neurotransmitter glutamate is decreased. Therapeutic drug monitoring of lamotrigine is suggested in order to control patients with infrequent seizures [5]. For its use as mood stabilizer no recommendation is given [6]. A therapeutic reference range for lamotrigine has not yet been established, neither for the use as antiepileptic nor for the use in bipolar disorders; therefore,

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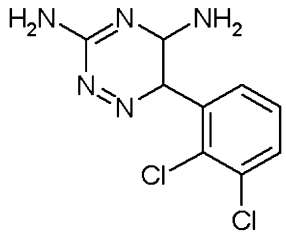
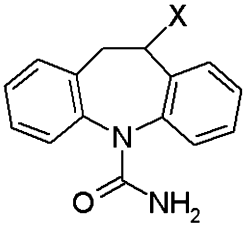
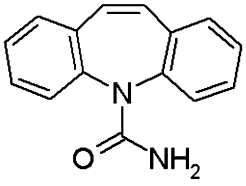


lamotrigine



X : =O, oxcarbazepine
X : -OH, 10 - monohydroxycarbazepine



carbamazepine

Fig. 1. Chemical structures of lamotrigine, oxcarbazepine, 10-monohydroxycarbazepine (MHD) and carbamazepine.

we decided on a tentative therapeutic range mentioned before [7].

High-performance liquid chromatography (HPLC) methods for the three analytes have already been published [8–11]. The benefit of the method described in this paper is a simplification by switching from off-line to on-line drug – extraction using HPLC column-switching, which saves time, manpower and amount of serum that can be reduced to 100 μl.

2. Material and methods

2.1. Chemicals

Lamotrigine (CAS number 84057-84-1) was kindly supplied by Glaxo Smith Kline (Durham, Great Britain). Oxcarbazepine (CAS number 28721-07-5) and its main metabolite 10-monohydroxycarbazepine were offered by Novartis (Basel, Switzerland). Structures of the substances are shown in Fig. 1.

Additional chemicals used for the preparation of the eluents were of HPLC or analytical grade.

Potassium dihydrogenphosphate and ortho-phosphoric acid, Suprapur® 85% forming the buffer solution, were obtained from Merck KGaA (Darmstadt, Germany). Acetonitrile Ultra Gradient HPLC grade was obtained from J.T. Baker (Mallinckrodt Baker B.V., Deventer, Holland).

Water was deionized and filtered by means of a Millipore system by Millipore GmbH (Eschborn, Germany).

Human drug-free serum was prepared from whole blood drawn from healthy volunteers (Bezirksklinikum Regensburg, Germany).

2.2. Sample preparation

For validation experiments, stock solutions of lamotrigine, oxcarbazepine and 10-monohydroxycarbazepine were prepared by solubilising 5 mg of lamotrigine and 10-monohydroxycarbazepine and 1.6 mg oxcarbazepine in 5 ml of ethanol each. Oxcarbazepine was diluted by deionized water to 200 μg/ml. Dilution of the serum matrix with aqueous solution was below 10%. A calibration series of five concentrations were used for lamotrigine and four concentration levels for oxcarbazepine and for 10-monohydroxycarbazepine, respectively. Quality control samples containing the analytes were prepared in the same way as the calibration samples.

Stock solutions were stored at −20 °C.

The pre-column eluent – 8% acetonitrile in water – was set up by adding 160 ml acetonitrile to deionized water to obtain a total volume of 2000 ml. The mixture was degassed in an ultrasonic bath for 5 min. Pre-column eluent was also used to rinse the injection needle of the autosampler between injections in order to avoid carry-over effects by adhering proteins or drug remnants.

The isocratic analytical mobile phase consisted of 20 mmol/l phosphate buffer (5.44 g potassium dihydrogenphosphate in 2000 ml deionized water) and was supplemented with acetonitrile (70/30, %v/v). The pH-value was adjusted to 3.0 using ortho-phosphoric acid.

Serum samples of patients treated with lamotrigine and oxcarbazepine were sent to an external laboratory, while a total of 500 μl of each serum specimen was taken off for method development. Samples were centrifuged directly after delivery for 10 min at 4000 U/min and either rapidly analysed or stored at −20 °C.

2.3. Instrumentation

A Perfect Bond® C-8 material 20 μm, 20 mm × 2.1 mm (MZ-Analysentechnik, Mainz, Germany) served as pre-column, which was protected by an inline filter (RECIPE GmbH, Munich, Germany). The analytical column was a Betasil® C-6 column, 250 mm × 4.6 mm, 5 μm (Thermo Electron Corporation, Dreieich, Germany).

Analysis of the specimens was carried out on a Dionex HPLC system (Dionex GmbH, Idstein, Germany) consisting of a GINA 50 autosampler, a dual ternary low-pressure gradient pump P680 for delivering the pre-column eluent and the analytical eluent at the same time at a flow rate of 1.3 ml/min each. The process of switching from the pre-column to the analytical column was executed by an electric 10-port valve incorporated in a thermostatted column compartment, set to 25 °C. At 0–5 min, serum samples were delivered to the pre-column by pre-column eluent