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UHPLC method development for the quantification of ropivacaine, adrenaline and clonidine in an anesthetic infusion

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A mixture of ropivacaine (5000 µg/ml), adrenaline tartrate (5 µg/ml) and clonidine (3.75 µg/ml) is commonly used in operating room for locoregional anesthesia. In order to assess the chemical stability of this infusion, an ultra-high-performance liquid chromatography (UHPLC) method was developed. The challenge of this development was to find a method allowing the three molecules quantification despite their important differences of both concentration and hydrophobicity. A reversed-phase column for polar compounds retention (ACQUITY UPLC HSS T3 1.8µm 2.1X150mm, Waters) was used: it was required for adrenaline (log P: -0.43) retention and it was also suitable for ropivacaine (log P: 4.07) and clonidine (log P: 2.49). A gradient separation was carried out with KH2PO4 25 mM pH 2.5 and acetonitrile. The run started with 2% of organic content for 1.7 minutes before being increased to 25% during 6.3 minutes. Re-equilibrating went on for two minutes. The column temperature was 30°C and the flow rate 0.4 ml/min. Due to the important difference of molecules concentrations, samples were injected times: a first time 0.1 µl to detect ropivacaine and the second time 3 µl to detect adrenaline and clonidine. Elution times were 5.6, 1.3 and 4.0 min respectively. The detections were performed with a photodiode array detector at 220 nm for ropivacaine and clonidine and at 280 nm for adrenaline. These wavelengths do not correspond to absorption maxima but they were chosen to avoid phosphate buffer and acetonitrile interferences. Finally, this method allows the quantifications of the three infusion components.

Biography

Marie-Lise Colsoul is graduated from the University of Louvain-la-Neuve as a Bioengineer. She is In-charge of the Development Department in the Chemistry Laboratory of the CHU UCL Namur hospital. She is involved in the Drug Stability Research Group (DSRG) which is a partnership between the hospital pharmacy, laboratory and scientific support unit to assess physical and chemical drugs stabilities.

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