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Simultaneous determination of procaine and *para*-aminobenzoic acid by LC–MS/MS method

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Abstract

A sensitive high performance liquid chromatography tandem mass spectrometry (LC–MS/MS) method has been developed for simultaneous determination of procaine and its metabolite *p*-aminobenzoic acid (PABA). *N*-Acetylprocainamide (NAPA) was used as an internal standard for procaine and PABA analysis. This assay method has also been validated in terms of linearity, lower limit of detection, lower limit of quantitation, accuracy and precision as per ICH guidelines. Chromatography was carried out on an XTerra™ MS C_{18} column and mass spectrometric analysis was performed using a Quattro Micro™ mass spectrometer working with electro-spray ionization (ESI) source in the positive ion mode. Enhanced selectivity was achieved using multiple reaction monitoring (MRM) functions, m/z 237 → 100, m/z 138 → 120, and m/z 278 → 205 for procaine, PABA and NAPA, respectively. Retention times for PABA, procaine and NAPA were 4.0, 4.7 and 5.8 min, respectively. Linearity for each calibration curve was observed across a range from 100 nM to 5000 nM for PABA, and from 10 nM to 5000 nM for procaine. The intra- and inter-day relative standard deviations (RSD) were <5%.

Keywords: Esters; LC–MS/MS; Local anesthetics; NAPA; PABA; Procaine

1. Introduction

Procaine, 4-aminobenzoic acid 2-(diethylamino) ethyl ester, is a local anesthetic [1] which is marketed as its hydrochloride salt under a variety of names such as novocaine or neocaine. It is administered by injection because of its poor penetration of mucous membranes after which it is metabolized primarily by plasma butyrylcholinesterase and secondarily by liver esterases to produce *p*-aminobenzoic acid (PABA) and diethylaminoethanol [2]. Procaine hydrochloride is also the active substance in the Romanian drugs Gerovital H3 and Aslavital, both of which are used in the treatment of aging and trophy disturbances [1]. PABA is a key building block in the enzymatic synthesis of dihydrofolic acid [3–5] and is found in plant and animal tissues [3]. It has also been administered as a therapeutic, namely as a treatment for typhus and other rickettsial diseases [3], as well as used as a common ingredient in sunscreen agents [6].

The determination of PABA as an impurity in drugs containing procaine or procaine raw materials has been previously performed by using UV and HPLC methods [7,8]. Several other methods have also been reported in the literature for the determination of PABA based on colorimetric [9], spectrometric [10,11], chromatographic [12,13] and electrochemical [14,15] detection. Procaine has likewise been detected by various methods including polarimetry [16], HPLC [17,18], IR [19], fluorimetric [20] and Raman spectroscopy [21]. A few methods for the simultaneous detection of procaine and PABA have been reported in multi-target screening analysis using HPLC [22–24]. These methods require a long analysis time [23], lack high sensitivity in biological matrices [23], or require amine additives to improve peak shapes [24]. Finally, given the antiarrhythmic properties of *N*-acetylprocainamide (NAPA), methods for its determination have also been developed [25–27]. The chemical structures of procaine, PABA and NAPA are shown in Fig. 1.

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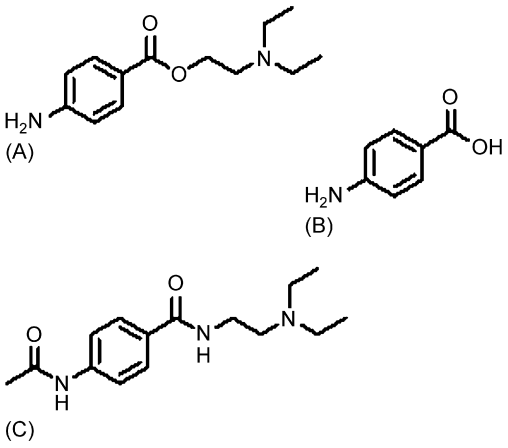

Fig. 1. Chemical structures of: (A) procaine; (B) PABA; and (C) NAPA.

Mass spectrometry has been increasingly perceived to be an essential tool in the drug discovery process including lead identification, assessment of compound purity, quality control of bulk drug substance, and toxicology and pharmacokinetics [28]. Advantages of using mass spectrometry for detection includes selectivity and sensitivity, especially when combined with separation techniques such as gel electrophoresis, gas chromatography (GC) or liquid chromatography (LC). PABA has been detected as its ethyl-esterified derivatives by using gas chromatography–mass spectrometry [29–32], these methods also being applicable to analyses of folates from human whole blood [29–32]. While methods for detection of PABA using a nitrogen-phosphorus detector with an open tubular column have been met with difficulty [33], detection of procaine and other local anesthetics from human plasma and urine have been successfully reported by using GC–MS with electron impact ionization [34–36].

In comparison to GC methods, LC methods have advantages such as reduced sample preparation time because there is typically no need for derivatization [32]. Liquid chromatography coupled with single quadrupole mass spectrometry (LC–MS) offers high sensitivity. However, insufficient selectivity often complicates the unequivocal determination of analytes from biological matrices, sewages [37] and plant crude extracts [38]. Liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS) offers both high sensitivity and selectivity for the unambiguous determinations of trace-level concentrations of analytes even in complex matrices. LC–MS/MS detection of PABA has been reported from plants [38] but, in this case, a long analysis time was required. The closest report to the present study pertains to a LC–MS/MS method for determination of *p*-amino methyl benzoate from human whole blood [32]. Procaine has also been detected using LC–MS/MS methods in solutions [39–41] and from biological matrices [42,43] as one of the drugs in multi-target analysis. However, in these cases, PABA, the metabolite was not detected.

From this backdrop, it was interesting to find that no LC–MS/MS method appears to be available for simultaneous detection of procaine and PABA in a single run. Thus, there remains a need for an analytical method capable of selective, sensitive, rapid and reliable simultaneous determination of procaine and PABA. Such a method would particularly be useful for the determination of PABA as an impurity in drugs containing procaine and procaine raw materials, quality control studies, and in vitro enzyme assays where procaine and PABA need to be monitored simultaneously. We have developed such a method and, with minor modification, it can also be deployed across other anesthetics that fall within this category. To our knowledge, this is the first study for the simultaneous detection and quantitation of procaine and PABA by using an LC–MS/MS method. Linearity, lower limit of detection, lower limit of quantitation, precision and accuracy were assessed according to ICH guidelines [44].

2. Experimental

2.1. Materials

Procaine (>97% purity; lot #114K0569), *p*-aminobenzoic acid (99% purity; lot #03711DO), *N*-acetyl-procainamide (>99% purity; lot #10209JQ) and ammonium acetate (98% purity; lot #044K3443) were purchased from Sigma–Aldrich chemicals (St. Louis, MO). Assessment of the procaine impurities by LC–MS showed that they were not related to PABA and that they did not interfere with the determinations of either procaine or PABA. Methanol, acetonitrile and formic acid (FA) were of HPLC grade and were purchased from Fisher Scientific (New Jersey, USA). HPLC grade water was obtained using a Milli Q system. A 10 mM solution of ammonium acetate was prepared using HPLC water and the pH was adjusted to 4.0 using acetic acid. Nitrogen gas was produced from liquid nitrogen by Dewar. Liquid nitrogen and argon gas were purchased in high purity (99.998%) from Linde gas (Toledo, OH, USA).

2.2. Instrumentation

An Alliance® HT liquid chromatograph (model 2795) equipped with a quaternary pump, a degasser, an auto sampler/injector (syringe volume = 250 μl) and a column oven from Waters corporation (Milford, MA, USA) were used. Mass spectrometric analysis was performed using a Quattro Micro™ (triple–quadrupole) instrument from Micromass (Manchester, UK) equipped with an ESCi™ Multi mode ionization source. MassLynx (version 4.01) software from Micromass was used for data acquisition and handling.

2.3. LC conditions

An XTerra™ MS C_{18} analytical column (2.1 mm × 150 mm, 5 μm) and a guard column (2.1 mm × 10 mm, 5 μm) from Waters Corporation (Milford, MA, USA) were used for the chromatographic separation of procaine, PABA and NAPA. A security guard column (filter size: 0.2 μm) from MAC MOD (Chadds Ford, PA, USA) was also used for each analytical run. Chromatography was carried out via a gradient system with a flow rate of 400 μl/min after an injection volume of 10 μl. The mobile phase consisted of eluent A, 10 mM ammonium acetate