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Short Communication

Improved method for capillary gas–liquid chromatography/nitrogen–phosphorus detection determination of pentacaine in serum

VLADIMÍR MARKO

Institute of Experimental Pharmacology, Slovak Academy of Sciences, CS-84216 Bratislava, Czechoslovakia

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Introduction

Pentacaine is a new cytoprotective agent with local anaesthetic activity which has recently been investigated as an anti-ulceric drug [1]

As the drug undergoes thermal degradation during gas chromatography [2], an on-column methylation method employing trimethylanilinium hydroxide (TMAH) was developed [3] and utilized for the determination of pentacaine and its structural analogues by capillary gas chromatography using the splitless injection mode The paper describing the method has been published [4]

A high molar ratio between TMAH and analytes in the solution injected was necessary for quantitative methylation In contrast to the packed-column determination, for which a molar ratio of 5 1 was sufficient [3], the molar excess of TMAH for capillary-column determination has to reach the level of some 100-fold This large amount of TMAH injected on the top of the capillary column (about 10 μg/injection) was found to result in the occasional loss of sensitivity during long-term studies on pentacaine determination Since the sensitivity was fully restored after cutting off the first 15 cm of the column, it was proposed that TMAH activated the top of the column, resulting in the adsorption of pentacaine Thus, a method with lower excess of TMAH and/or with different management of TMAH injected had to be developed

Direct injection of the sample presented one possible solution to the problem There were two reasons which favoured this approach Firstly, direct injection and capillary-column separation is similar to packed-column separation (no solvent effect or cold trapping), secondly, the volume of the inlet of the instrument used is, in the case of direct injection, about half that of the inlet for splitless injection (with twice the concentration of reactants)

Materials and Methods

Analyses were performed under isothermal conditions on a Model 5880A Hewlett–Packard gas chromatograph equipped with a thermionic selective nitrogen–phosphorus detector (NPD) A wide-bore fused silica column HP-1 (30 m, 0 53 mm, 1 d , film thickness 0 88 μm, Hewlett–Packard, Wien, Austria) was used The operating conditions were column temperature, 285°C, injector and detector temperatures, 300°C, nitrogen as a carrier gas 5 ml min^{-1} and as a make-up gas 15 ml min^{-1}, purge activation time 30 s Samples were injected with the HP 7673A autosampler

The selective solid-phase extraction method used in the author's earlier work [4] was modified only slightly by using Separcol SI C18 extraction columns (Centre of Chemical Research, Slovak Academy of Sciences, Bratislava, Czechoslovakia)

The on-column formation of *N*-methyl derivatives of pentacaine and of the internal standard (*O*-hexyl analogue of pentacaine [5]) was studied using solutions of 1 μg of both analytes and different amounts of TMAH (0 1 M in methanol, Fluka, Buchs, Switzerland) in 300 μl of ethyl acetate with the excess of TMAH in the range of 5 to 100

In the quantitative determination of pentacaine in real samples, 2 to 4 ml of dog or human serum samples were analysed To each sample, an aqueous solution of the internal standard was added to give a concentration of 200 ng ml^{-1} The samples were divided into two parts and carried through the extraction procedure described in earlier work [4] After elution of the analytes from extraction columns with methanol, the latter was evaporated to dryness and 250 μl of ethyl acetate was added The solution was placed in autosampler vials, 3 μl of TMAH was added and 3 μl of the solution was injected into the gas chromatograph The retention times of the *N*-methyl derivative of pentacaine and the internal standard were 5 5 and 6 5 min, respectively No interferences were observed from endogenous substances or metabolites

From this work it is apparent that, when using direct injection, the amount of TMAH required for quantitative derivatization of pentacaine and the internal standard is about 20 times less than that used with the splitless injection mode (about 0 5 μg/injection) Thus, by changing the injection mode protection of the column and maintenance of sensitivity during long-term studies was achieved Moreover, two additional advantages were noted for direct injection compared to the splitless injection mode The first is that the time taken for each determination falls from 20 to 7 5 min The second is improved precision Intra-day variation data at 10 and 100 ng ml^{-1} were 8 7 and 9 0%, respectively, using splitless injection mode [4] and 4 2 and 5 6%, respectively using the direct injection mode

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