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Determination of antihyperglycemic drugs in nanomolar concentration levels by micellar electrokinetic chromatography with non-ionic surfactant

Vítězslav Maier*, Joanna Znaleziona, David Jirovský, Jana Skopalová, Jan Petr, Juraj Ševčík

Department of Analytical Chemistry, Faculty of Science, Palacký University, Tr. Svobody 8, CZ-77146 Olomouc, Czech Republic

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ABSTRACT

A method for the separation of six selected antihyperglycemic (antidiabetic) drugs (tolbutamide, gliclazide, glimepiride, glibenclamide, repaglinide, and glipizide) was developed with use of micellar electrokinetic chromatography. Two non-ionic poly(ethylene glycol)-based surfactants Genapol X-080 and Triton X-114 (reduced) were studied as neutral pseudostationary phases. High alkaline pH 10.0 was used to obtain negative charges of separated antidiabetic drugs and non-ionic surfactants were employed for selectivity alteration. Both non-ionic surfactants provided good selectivity at concentration 0.2% (v/v) in sodium borate buffer and the separation of six drugs was obtained within 5 min. An on-line preconcentration method based on reversed electrode polarity switching was employed for the determination of antihyperglycemic drugs in blood serum after acetonitrile protein precipitation. The limits of detection ranged from 20.8 nmol L^{-1} for tolbutamide to 6.5 nmol L^{-1} for glibenclamide, respectively.

1. Introduction

Electromigration techniques offer several benefits (high efficiency, resolution, and low sample consumption) for separation of low-molecular-weight drugs and pharmaceutical compounds. Capillary zone electrophoresis (CZE) allows separation of charged analytes only, whereas micellar electrokinetic chromatography (MEKC) and microemulsion electrokinetic chromatography (MEEKC) enable simultaneous separation of charged and neutral analytes with use of surfactant or microemulsion in running electrolyte. The benefit of MEKC and MEEKC compared to CZE is in combination of two separation mechanisms—migration of charged analytes according to their free solution mobilities and distribution of neutral and/or charged analytes between micelles or microemulsion drops and water based running electrolyte simultaneously. The selectivity manipulation can be higher in MEKC and MEEKC than in CZE owing to the interactions between analytes and micellar or microemulsion phases in the electrolyte. MEKC is more widely used than MEEKC, because MEKC is less demanding for method optimization and for prediction of migration behavior of analytes. Several review papers [1–6] dealing with the theory, optimization and discussion of migration behavior of drugs in MEKC have been published. The most frequently used surfactants are ionic surfactants [e.g. sodium dodecyl sulfate (SDS) and hexadecyltrimethylammonium bromide (CTAB)], but using of non-ionic surfactants in MEKC is possible, too. Non-ionic surfactants could be used for the selectivity alteration in MEKC [7,8] as the second surfactant for creating mixed micelles with ionic surfactants (typically mixture of SDS with Brij or Tween). Only a few papers discussed employing non-ionic surfactants separately as a micellar phase for separation of charged analytes [9–12]. Generally, MEKC with non-ionic surfactant requires charged analytes, because non-ionic surfactant does not have electric charge and separation of neutral analytes is impossible. However some publications [13–18] showed the very important separations of neutral analytes (e.g. aromatic hydrocarbons and their derivatives, acidic herbicides) with using *in situ* charged alkylglycoside surfactants micells. Alkylglycoside surfactants are dynamically charged by borate and alkaline pH of running electrolytes. The principle of charging of a neutral surfactant is based on com-

* Corresponding author. Tel.: +420 585634417; fax: +420 585634433.
E-mail address: vitezslav.maier@upol.cz (V. Maier).

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plexation of a diol group of a polyol surfactant with borate or boronate ion. This phenomenon was described for non-ionic surfactants with diol moieties such as alkyl-β-D-glucopyranosides, N-D-gluco-N-methylalkanamides, octyl-β-D-maltopyranoside and octanoylsucrose.

Molina and Silva [10] published MEKC separation of phosphorus-containing amino acid herbicides and aminoethylphosphonic acids applying neutral surfactant Triton X-100 and laser induced fluorescence (LIF) as a detection technique. Triton based surfactants have substituted benzene ring in their structures and thus limit the sensitivity of UV detection. The use of non-ionic surfactants in MEKC separation of charged analytes has some advantages such as no contribution to the electrical current and to the generation of Joule heating, which is limiting factor for ionic surfactants, for instance SDS. The separation voltage can be higher than in MEKC with ionic surfactant. The values of critical micellar concentrations (CMCs) of non-ionic surfactants [19] are lower than those of ionic surfactants, concerning that the amount of non-ionic surfactant necessary for formation of the micelles in the running electrolyte is lower, too. However, the main disadvantages of using non-ionic surfactants in MEKC are impossibility of separation of neutral compounds (except for the *in situ* derivatization of neutral surfactant mentioned above) and formation of dynamic coating of an inner capillary wall. Moreover some of non-ionic surfactants have lower cloud point temperature (CPT) [19]. Above CPT the phase separation of non-ionic surfactant in an electrolyte is occurred, and the usage of UV or LIF detection is impossible due to the beam scattering.

Peroral antidiabetics (PADs) are widely used for the treatment of diabetes disease (type II). Control of their concentration levels in biological fluids as well as pharmacokinetic studies requires fast, simple and sensitive method for their determination. Several methods of MEKC separation of PADs, utilizing the ionic surfactants, were recently published. Landers and co-workers [20,21] described separations of some PADs and their related main metabolites in urine by MEKC with addition of anionic surfactants SDS and cholic acid to the BGE. On-line concentration [22] based on solid phase extraction-capillary electrophoresis (SPE-CE) and MEKC separation of antidiabetic drugs in urine was reported, too. Moreover, Paroni et al. [23] compared the HPLC separation of PADs with MEKC separation. There are also several published methods for determination of antidiabetic drugs by LC [24–27], LC–MS [28–31], high performance thin layer chromatography [32–35] and GC [36] in urine or blood plasma. The limits of detection for antidiabetic drugs in published works were about 0.5 $\mu g\,L^{-1}$. Attia and co-workers [37] published the electrochemical determination of repaglinide in tablets and human serum on glassy carbon electrode and carbon paste electrode. The limit of detection of repaglinide on glassy carbon electrode was $1.062 \times 10^{-7}\,mol\,L^{-1}$.

The aim of this paper was to study the possibility of using two non-ionic surfactants Genapol X-080 and Triton X-114 (reduced) as micellar phases in MEKC separation of selected six PADs in sodium borate based running electrolyte. The structures of studied PADs are shown in Fig. 1. The studied PADs are acidic drugs, thus for their separation alkaline pH was chosen. An effect of pH, borate concentration and amount of non-ionic surfactants in the running electrolyte on separation of PADs and an influence of amount of surfactants on electroosmotic mobility were investigated. The presented method was applied on determination of PADs in blood serum after protein precipitation by acetonitrile. Because the therapeutic concentrations [38] of studied PADs in blood serum are

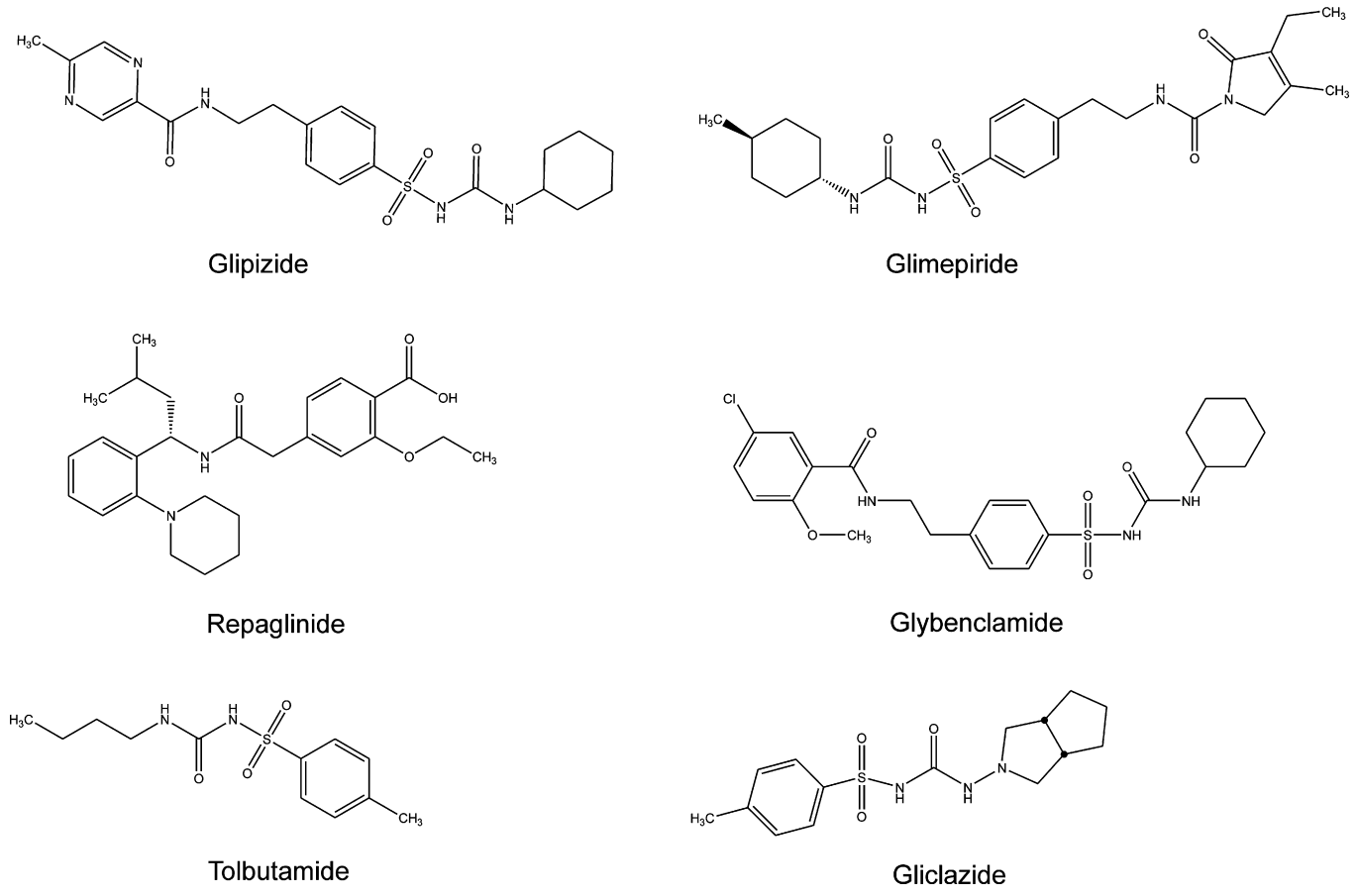

Fig. 1. The chemical structures of studied peroral antidiabetic drugs.