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Separation of basic drug substances of very similar structure using micellar electrokinetic chromatography*

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Abstract: Small molecules having very similar molecular structure — some even with the same mass over charge — are not trivial to separate by capillary electrophoresis in free solution. However, addition of surfactants to the electrophoretic buffer and thus using the principle of micellar electrokinetic chromatography may provide separation with high resolution. The use of zwitterionic and nonionic surfactants is demonstrated and an example of how a developed system may be used for drug purity testing is given.

Keywords: *Micellar electrokinetic chromatography (MEKC); imipramine; amitriptyline; basic drugs; Tween 20, 3-(N,N-dimethylmyristylammonium) propanesulphonate.*

Introduction

Capillary electrophoresis is known as a highly efficient separation technique and its basic separation mechanism is based on rate processes where ions or particles with differences in mass over charge may be separated in an electrical field.

When solutes have very similar structure and thus exhibit similar or even equal mass over charge separation may be hard to obtain unless special techniques are used. Such a technique may be micellar electrokinetic chromatography (MEKC), where one or more surfactants are added to the electrophoresis buffer. In these systems a micellar, lipophilic pseudo-phase is formed in the buffer and ionic solutes having similar electrophoretic migration rates but different affinity for the micellar pseudo-phase may thus be separated. Also neutral solutes may be separated if the micelles have an electrophoretic mobility themselves. One may argue that the use of nonionic and zwitterionic surfactants will not lead to MEKC as neutral solutes may not be separated [1]. This may be true for the nonionic surfactants where the most significant effect is the lowering of the electroosmotic flow. However, it has been shown that neutral solutes may be separated when using zwitterionic surfactants [2] probably due to association of negative charges to the zwitterionic micelles.

When separating ionic solutes different distribution of the solutes to the micelles is definitely involved as part of the separation mechanism, and therefore we prefer to include the methods using nonionic and zwitterionic surfactants as MEKC techniques. The use of surfactants for alteration of the selectivity in the separation of basic drugs have only been described by a few workers [3–5] and only for solutes having fairly different structures.

In this paper we have studied the possibility to improve the separation of some basic drug substances with very similar structures by using MEKC. We have used two groups of test substances: the test solutes in the first group only differ in their amine function and in the second group the test solutes have identical mass over charge.

Experimental

Chemicals

6-Amino caproic acid (6-ACA) and polyoxyethylenesorbitan monolaurate (Tween 20)

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were obtained from Sigma Chemical Co. (St Louis, USA). 3-(N,N-Dimethylmyristylammonium) propanesulphonate (MAPS) was obtained from Fluka Chemie AG (Buchs, Switzerland). Acetic acid was obtained from Riedel-de Häen (Seelze, Germany).

Imipramine hydrochloride (IMI), desmethyl imipramine hydrochloride (DMI), imipramine N-oxide hydrochloride (DINO) and methyl imipraminium iodide (IMP-CH_3) were obtained from Dumex Ltd (Copenhagen, Denmark). Di-desmethyl imipramine hydrochloride (DDMI) was obtained from CIBA-GEIGY Ltd (Basel, Switzerland). Maprotilene hydrochloride (MAP), litracene hydrochloride (LIT), protriptylene hydrochloride (PRO), nortriptylene hydrochloride (NOR) and amitriptylene hydrochloride (AMI) were obtained from H. Lundbeck Ltd (Valby, Denmark).

Sample preparation

All samples were dissolved in distilled water (0.2 mg ml^{-1}). For testing of impurities in DINO 5.0 mg ml^{-1} of this substance was dissolved in 5% methanol. Standard addition was performed by adding 10 µl of each of 0.05 mg ml^{-1} solutions of IMP-CH_3, IMP, DMI and DDMI to 300 µl of the DINO test solution.

Apparatus

HP^{3D} Capillary Electrophoresis system (Hewlett–Packard, Waldbronn, Germany) equipped with an on-column diode array detector (DAD) was used. A detection wavelength of 214 nm, bandwith 16 with a reference wavelength of 350, bandwidth 20 was used for the application. The separation was performed in a fused-silica capillary (56 cm × 75 µm i.d.) (Polymicro Technologies, Phoenix, AZ, USA). The capillary was thermostated to 30°C by air. Samples were kept at ambient temperature in the autosampler and injected by applying pressure of 2 kPa (20 mbar) for 3 s. A voltage of 20 kV was applied during analysis.

Waters Quanta 4000 capillary electrophoresis system (Waters, Milford, MA, USA) was used for the studies with MAPS and Tween 20. On-column detection was performed by UV absorption at 214 nm. The separation was performed in a fused-silica capillary (56 cm × 75 µm i.d.).

Sample injection was accomplished by hydrostatic injection for 15 s. All analyses were performed using an applied voltage of 20 kV.

Collecting of data were performed using Turbochrom version 3.3 software (PE, NELSON, Cupertion, CA, USA).

6-ACA pH 4.0 (0.05 M, adjusted by acetic acid) was used as the background electrolyte.

Results and Discussion

The successful use of MEKC for testing of drug substances for content of related substances have already been demonstrated [6, 7]. As the impurities often possess a similar structure to that of the drug itself, high selectivities are required for sufficient separation of the impurities from the major drug peak.

Test solutes

We have used two groups of test substances. One group constitute a mixture of the drug imipramine and some of its analogues with identical carbon skeleton but differing in their amine functionality (Fig. 1). Thus, we are able to compare the separation of a mixture of a primary, a secondary and a tertiary amine as well as a quarternary ammonium compound and an amine-N-oxide.

The second group of solutes are all very similar in structure and, furthermore, three respectively two have the same mass over charge (Fig. 2).

Surfactants

Different types of surfactants (Table 1) may be used for MEKC. The use of quaternary ammonium compounds will reverse the osmotic flow and anionic surfactants like sodium dodecyl sulphate may often lead to undesired very strong electrostatic interactions with basic solutes [8]. Furthermore, the ionic surfactants contributes to the conductivity of the buffer and this may limit the use of higher voltages.

We, therefore, prefer to use either zwitterionic or nonionic surfactants which do not change the ionic strength of the buffer.

In the present study the zwitterionic surfactant MAPS and the nonionic surfactant Tween 20 have been investigated for their ability to enhance separation of basic drug substances (Fig. 3).

The most important parameter influencing the selectivity of the systems was found to be

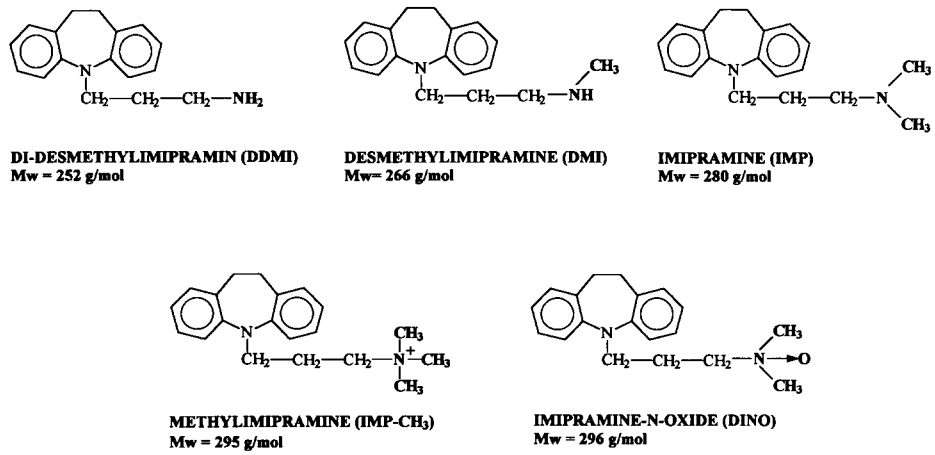


Figure 1
Structure of imipramine and four analogues.

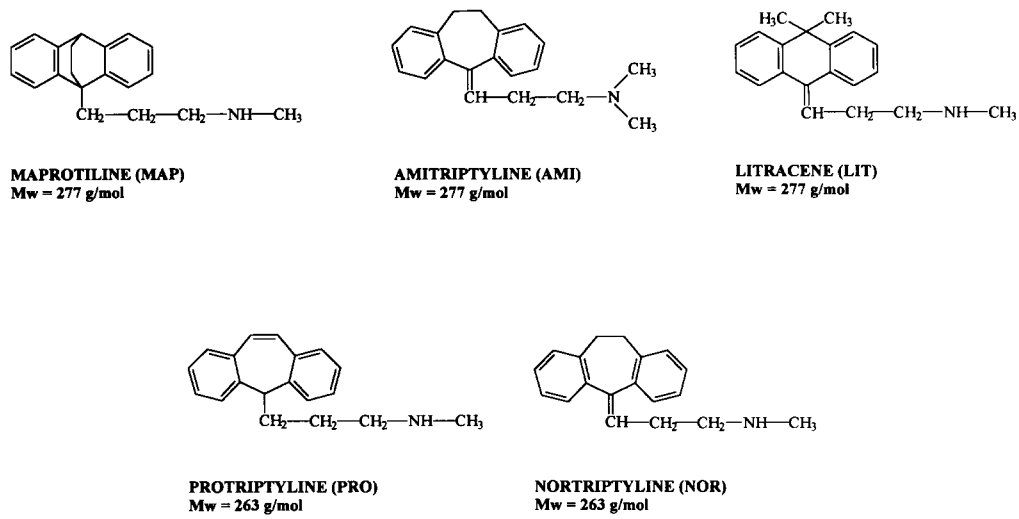
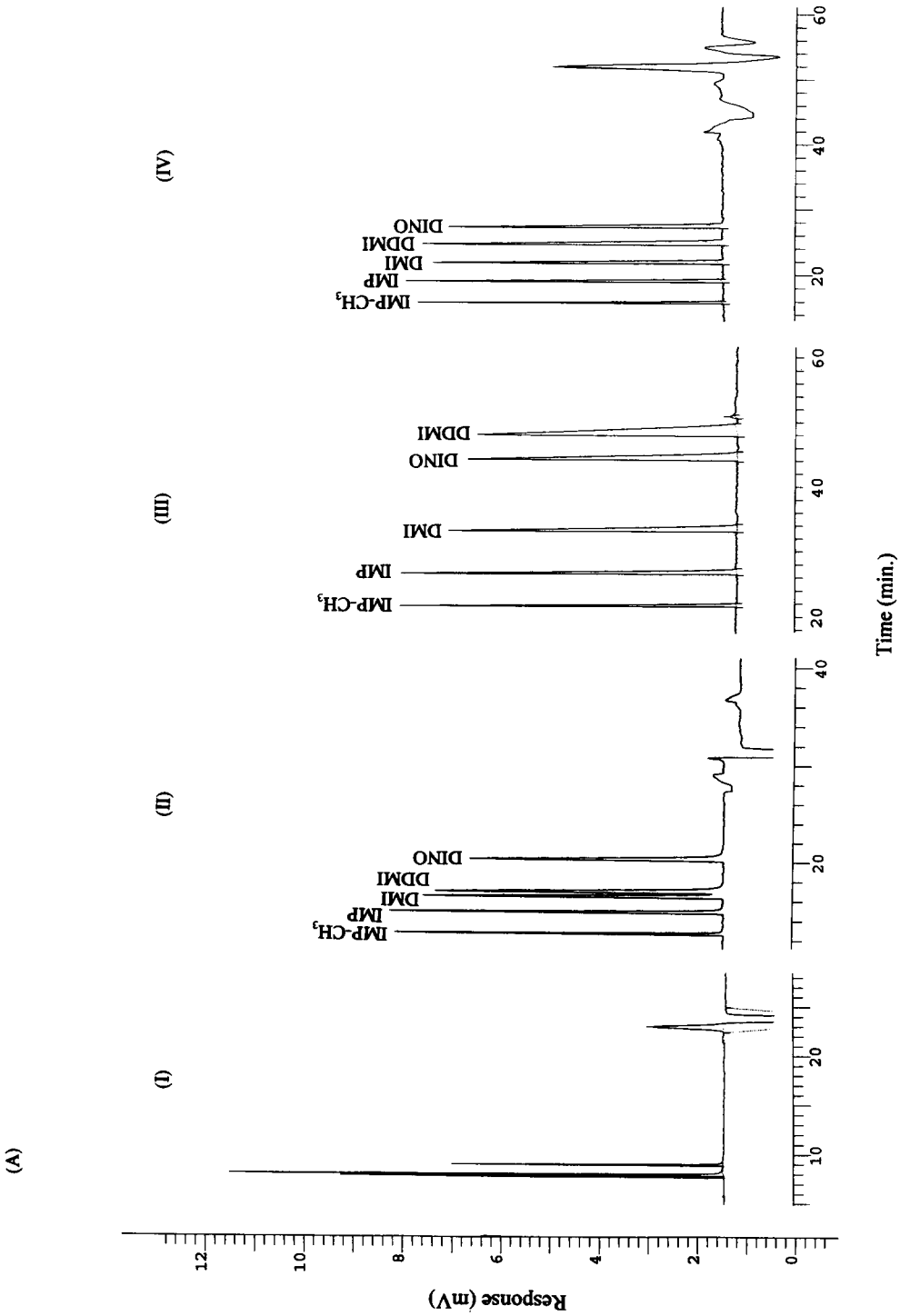


Figure 2
Structure of three respectively two drug substances with similar mass over charge.

Table 1
Examples of different types of surfactants used in MEKC

Cationic:	Long chain trimethylammonium compounds, e.g. hexadecyl-trimethylammonium ions
Anionic:	Typically SDS = sodium dodecylsulphate
Zwitterionic:	Long chain dimethylammonium propanesulphonates, e.g. tetradecyl-dimethylammonium propanesulphonate
Non-ionic:	e.g. Tween 20, BRIJ 35


(A)
(I)
(II)
(III)
(IV)
IMP-CH3
IMP
DMI
DDMI
DINO
Response (mV)
Time (min.)

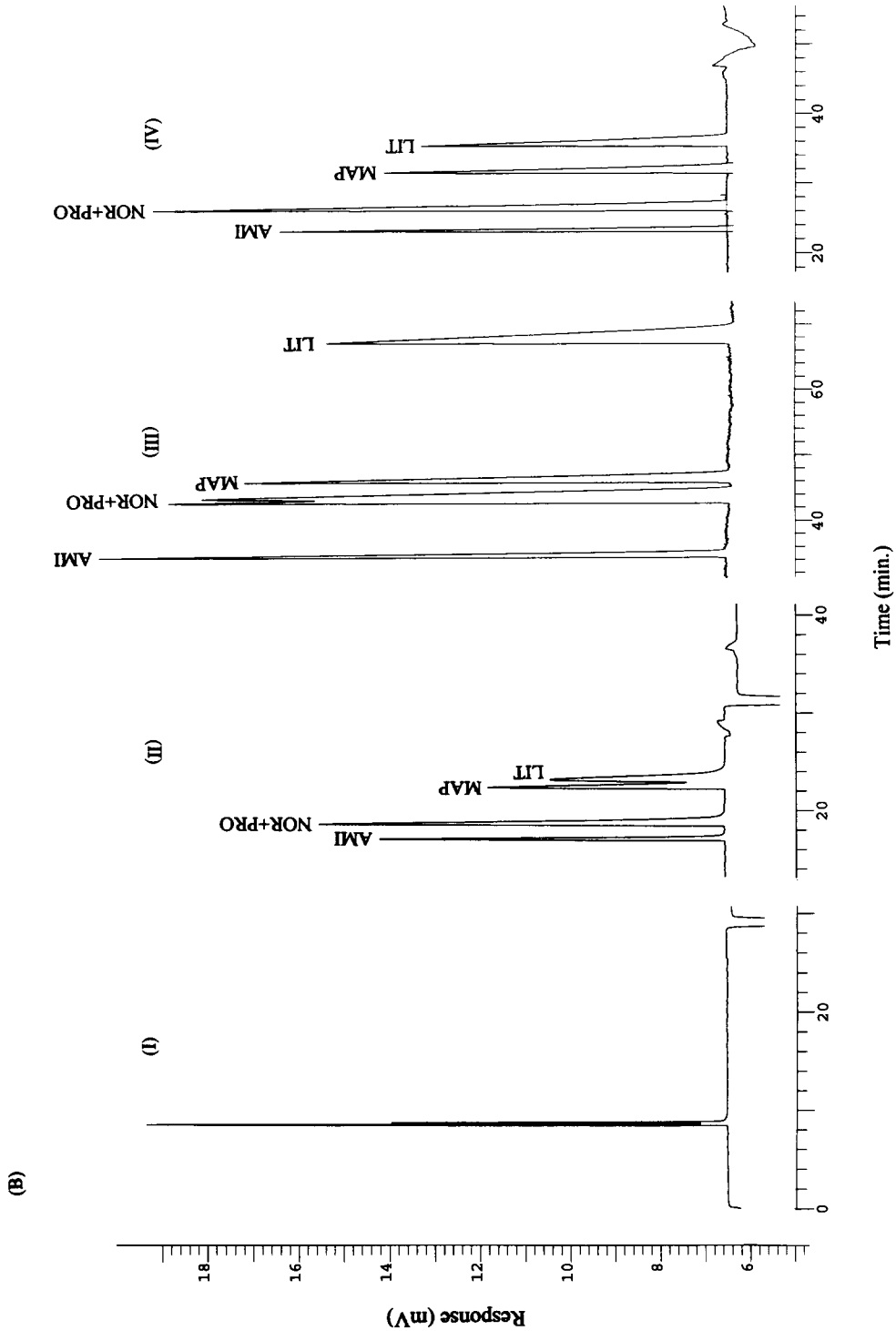


Figure 3
Electropherograms of (A) IMI, DMI, DDMI, IMP-CH_3, DINO and (B) MAP, LIT, PRO, NOR, AMI in (I) 0.05 M 6-ACA pH 4.0, (II) 0.05 M 6-ACA pH 4.0, 25 mM MAPS, (III) 0.05 M 6-ACA pH 4.0, 25 mM Tween 20 and (IV) 0.05 M 6-ACA pH 4.0, 25 mM MAPS, 15 mM Tween 20. Apparatus: Waters Quanta 4000. Conditions: Capillary is 56 cm to detector × 75 μm i.d.; temperature: 30°C; detection: UV 214 nm; buffer: 0.05 M 6-aminocaproic acid pH 4.0; voltage: 20 kV, current: 62 μA.

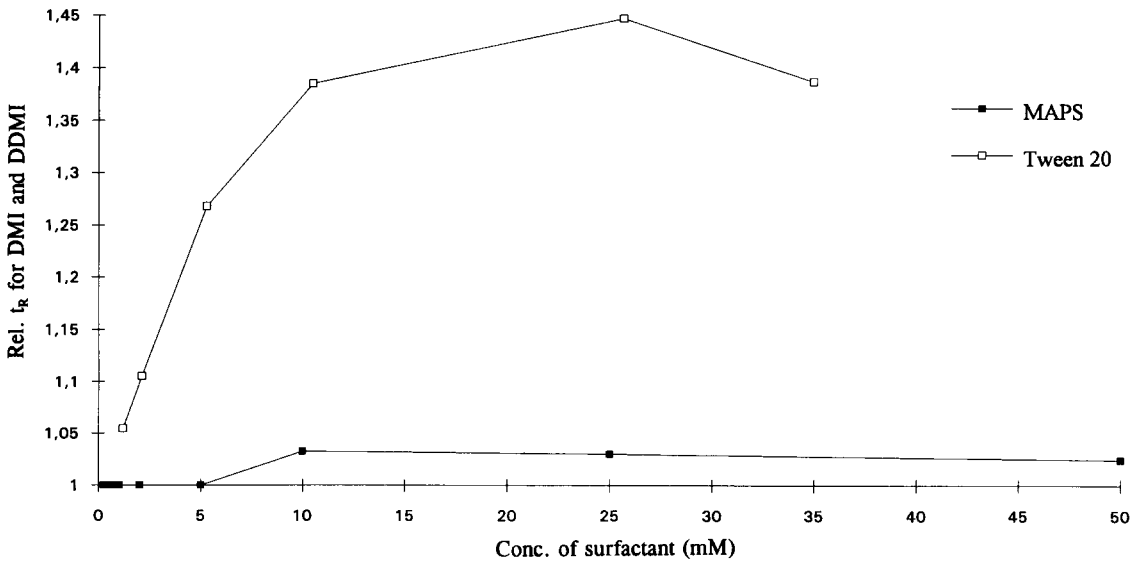


Figure 4
The relative migration times between DMI and DDMI as a function of the surfactant concentration.

the concentrations of the surfactants and in Fig. 4 the relative migration times between DMI and DDMI as a function of the concentrations are shown.

Substitution of MAPS for other long chain dimethylammonium propanesulphonates did not change the selectivity of the separation, just as the interchange between different surfactants of the Tween type neither changed this selectivity. It is interesting to note the difference between the influence of two surfactants on the relative mobility of the primary amine DDMI compared to the other solutes. Intermediate selectivity may be obtained using mixtures of the two surfactants.

Addition of the zwitterionic surfactant to the buffer decreases the observed mobility of the test solutes more than it decreases the electroosmotic flow. This is partly due to the fact that the zwitterionic micelles have a migration towards the anode [2]. The nonionic surfactant decreases the electroosmotic flow significantly and the separation of the test solutes is primarily a result of their electrophoretic mobility and their partition to the micellar pseudo-stationary phase.

Application

The method developed has been used for the investigation of related organic substances in the drug substance imipramine-N-oxide (DINO). Using a mixture of the two surfactants in the buffer four impurities was found (Fig. 5). Although the four impurities had mobilities close to those found for IMP-CH_3, IMP, DMI and DDMI the MEKC technique was not considered sufficiently robust that identification could be made by external standardization. Therefore, standard addition was applied for the identification. As expected small contents of IMP and DMI were found, but the two other impurities were not identified by this standard addition procedure. The limit of detection defined as twice the peak to peak noise was found to be 0.01% of impurity in DINO.

Conclusion

MEKC may be a very powerful tool to obtain separation of basic substances of very similar structure and the methods developed are suitable for testing of drug substances for content of related substances at a sufficiently low limit.

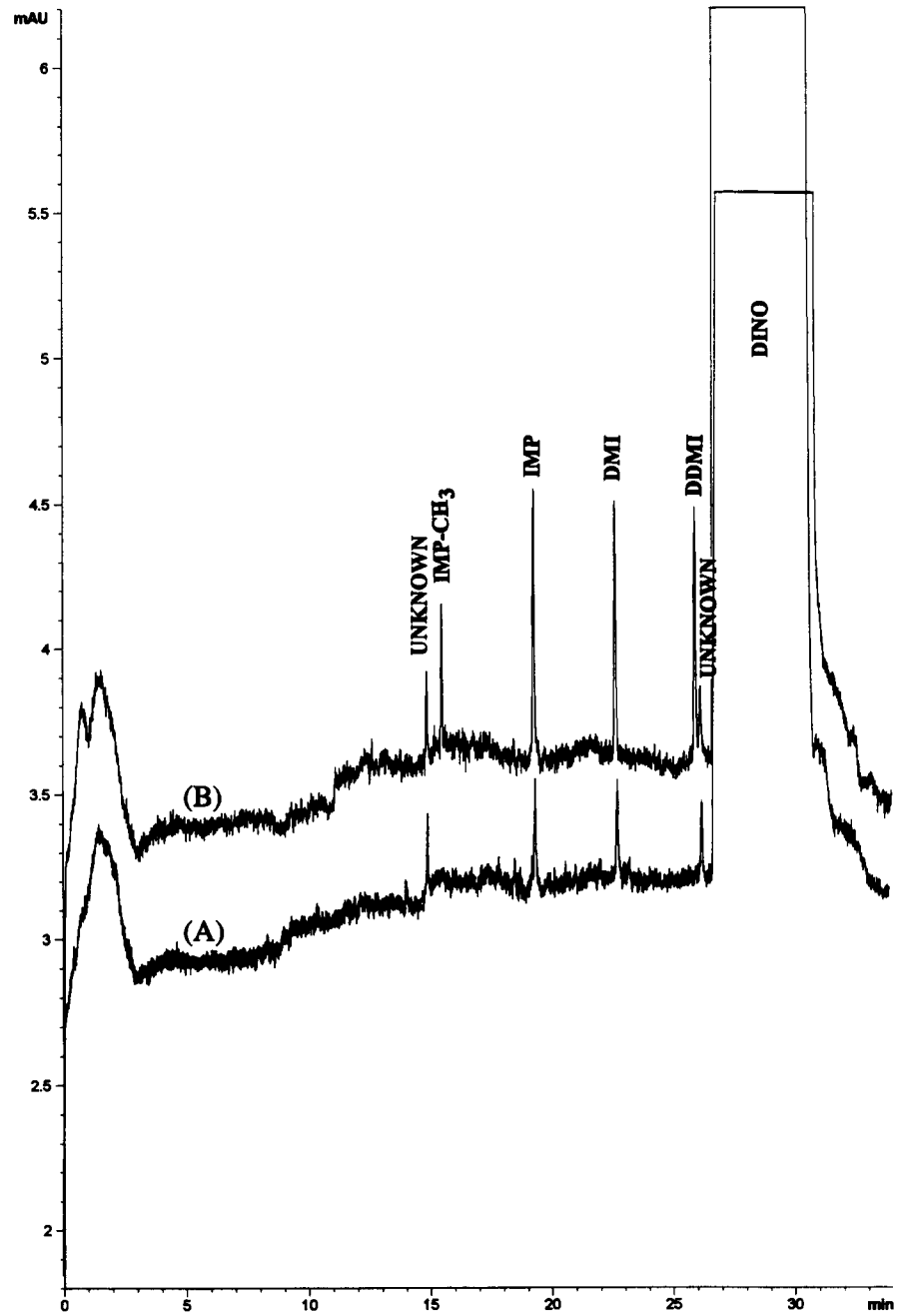


Figure 5
Electropherograms of (A) 5.0 mg ml^{-1} sample of DINO dissolved in 5% methanol and (B) standard addition of 10 µl 0.05 mg ml^{-1} IMP, DMI, DDMI and IMP-CH_3 dissolved in water to 300-µl DINO sample. Apparatus: Hewlett-Packard ^{3D}CD instrument. Conditions: as in Fig. 3A IV.

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