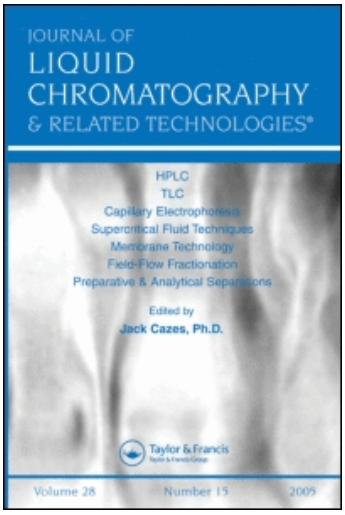

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TLC Profiling of Impurities of 1-(3,4-Methylenedioxyphenyl)-2-nitropropene an Intermediate in MDMA Synthesis. Influence of Sample Preparation Methods and Conditions

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ABSTRACT

Thin-layer chromatography (TLC) was employed for screening profiling of impurities of 1-(3,4-methylenedioxyphenyl)-2-nitropropene, an intermediate product of (3,4-methylenedioxy)methamphetamine (MDMA) synthesis (from piperonal). Liquid–liquid extraction (LLE) and solid phase extraction (SPE) were applied to isolate impurities from the drug matrix. The SPE extraction process was performed on C_8 columns and LLE was carried out using *n*-heptane. The influence of buffer solutions

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(pH 1, 4, 5, 6, 7, 8, 10, 11, 13, and 14) on the extraction efficiency was investigated. The chromatograms were developed with the use of chloroform : ethyl acetate (98 : 2) mixture. The spots of separated impurities were observed under UV light at $\lambda_{exc} = 254$ and 366 nm. The proposed semiquantitative criterion of profile (chromatogram) quality has been based on a matrix presentation of TLC patterns. It takes into account the number of spots revealed, differences between R_f values, and intensity of fluorescence, simultaneously.

Key Words: LLE/TLC profiling; SPE/TLC profiling; MDMA.

INTRODUCTION

In the 1980s, 3,4-(methylenedioxy)methamphetamine (MDMA), widely known as "ecstasy," appeared in parts of Europe and began to be used in the dance-scene culture known as "rave," "acid house," or "techno."[1] Presently, Europe remains an important area for the production and use of amphetamine and ecstasy.[2] Although there has been some fluctuation in recent years, both amphetamine and MDMA seizures (numbers and quantities) have increased substantially in Europe over the last decade. To control the production and abuse of drugs it is necessary to develop analytical procedures that could provide valuable information required for law enforcement purposes.

Each seized drug sample contains impurities, which depend on the method of synthesis, specific reaction conditions, substrates, intermediate products, side reaction, purification procedures, and diluents applied. The samples synthesised by the same method, in the same laboratory, belonging to the same bath, contain similar composition of impurities (a profile). On the other hand, any change in conditions of synthesis, the use of different sources of substrates, results in different profiles (chromatograms) of impurities. Consequently, profiling of drug impurities can provide useful information concerning the method of synthesis, the origin of a drug sample, connection between illicit drug laboratories, ways of distribution, and deliverers.

While there is a number of papers concerning amphetamine and methamphetamine profiling, only a few deal with profiling of MDMA impurities.[3–9] It is usually carried out by means of a GC method preceded by liquid–liquid extraction (LLE),[3–5] SPME,[6] or solid phase extraction (SPE).[8] Determination of "common-batch" members in a set of confiscated MDMA samples by determination of the natural isotope abundances has also been reported.[9] Usually, an MDMA sample (a powder) is dissolved in the acetate (pH = 5),[6] phosphate (pH = 9), or carbonate (pH = 10)[3] buffer solution. Also, solutions of pH = 11.5[5] and 12.8[4] were applied.

The aim of the present work was to study the influence of sample preparation methods, particularly pH of buffer solution used in sample dissolution, on the thin layer chromatography (TLC) profiling process. Also, the efficiency of LLE and SPE of drug impurities has been compared.

1-(3,4-Methylenedioxyphenyl)-2-nitropropene, an intermediate product of MDMA synthesis (piperonal used as a subtract) has been examined, as it may be supposed that this transfers the impurities from the first steps of synthesis to the final product (MDMA). On the other hand, the compound may be widely used as a model matrix for examination and development of profiling procedures because no special permission is necessary to deal with it.

The quality of TLC profiles was estimated by means of semiquantitative criterions based on matrix presentation of TLC patterns.[10,11] They take into account the number of spots revealed, differences between R_f values, and intensity of fluorescence, simultaneously. The approach was applied in SPE/TLC profiling of PMMA impurities.[10,11]

EXPERIMENTAL

Chemicals and Reagents

The following reagents were used: 1-(3,4-methylenedioxyphenyl)-2-nitropropene synthesised from piperonal,[7] chloroform (Merck, Germany, gradient grade), ethyl acetate (POCh, Poland, analytical grade), and buffer solutions: mixture of KCl and HCl (pH = 1), acetate buffer (pH = 4 and 5), phosphate buffer (pH = 6, 7, and 8), carbonate buffer (pH = 10 and 12), mixture of KCl and NaOH (pH = 13), 1 mol/L NaOH (pH = 14).

Profiling Procedure

The profiling procedure consisted of two steps: extraction of impurities and TLC separation.

SPE

The tested powered product (100 mg) was added to 1 mL of buffer solution, the suspension was mixed on a horizontal shaker (for 40 min), and then centrifuged. Supernatant liquid (500 μL) was injected into the C_8 (100 mg) extraction column (Baker Bond). VAC ELUT (Varian, USA) SPE set was used. The matrix was eluted with the use of 2 mL of distilled water. The analytes were recovered by washing the column with 5 portions of methanol, each 50 μL. The extract (8 μL) was taken for the TLC separation.

LLE

The tested powered product (200 mg) was added to 2 mL of buffer solution, the suspension was mixed on a horizontal shaker (for 40 min), 500 μL of *n*-heptane was added, and the mixture was shaken once again for 40 min. A portion of 4 μL of organic layer was taken for TLC separation.

TLC Separation

Silica gel plates (0.2 mm) with fluorescent indicator $60F_{254}$ (Merck, Germany), NANOMAT IV (CAMAG, Switzerland) application device, and a horizontal developing chamber (CAMAG, Switzerland) were applied. The chromatograms were developed with chloroform : ethyl acetate (98 : 2) mixture. The spots of separated impurities were observed under UV light at $\lambda_{exc} = 254$ and 366 nm.

Quality of the Profile

The proposed characteristics (criterions) of the profile quality are based on a matrix presentation of TLC pattern.[10,11] Dimension of a symmetrical matrix is equal to the number of spots revealed. It was assumed, arbitrarily, that an element a_{ij} of the matrix equals 1 if 100 $(R_{fi} - R_{fj}) > 6$. Then the spots i and j are completely separated. If the spots i and j are partly separated, but can be distinguished, a_{ij} equals 0.5. Otherwise $a_{ij} = 0$. The diagonal elements of the matrix, a_{ii}, equal 0 if the TLC plate is observed under $\lambda_{exc} = 254$ nm (spots extinguish the fluorescence of fluorescent indicator of plates). At $\lambda_{exc} = 366$ nm the spots fluorescence and the diagonal element $a_{ii} = 1$ if the spot i fluoresces intensively, $a_{ii} = 0.5$ in case of clear fluorescence, and $a_{ii} = 0.1$ in case of faint fluorescence. A scheme of matrix is shown in Fig. 1.

In the present investigations, the elements of the above mentioned matrix has been determined for each TLC separation. Next, values of the following optimization parameters (criterions of TLC profile quality) were calculated:

$$Y_1 = \sum_{\substack{i,j=1 \\ i<j}}^{N} a_{ij\,(254\,\mathrm{nm})}; \quad Y_2 = \sum_{\substack{i,j=1 \\ i<j}}^{N} a_{ij\,(366\,\mathrm{nm})}; \quad Y_3 = Y_1 + Y_2;$$

$$Y_4 = \sum_{i=1}^{N} a_{ii\,(366\,\mathrm{nm})} \tag{1}$$

$$\begin{array}{c} \\ 1 \\ 2 \\ 3 \\ 4 \\ \dots \\ N \end{array} \begin{array}{c} \begin{array}{cccccc} 1 & 2 & 3 & 4 & \dots & N \end{array} \\ \begin{bmatrix} a_{11} & a_{12} & a_{13} & a_{14} & \dots & a_{1N} \\ & a_{22} & a_{23} & a_{24} & \dots & a_{2N} \\ & & a_{33} & a_{34} & \dots & a_{3N} \\ & & & a_{44} & \dots & a_{4N} \\ & & & & a_{\dots} & a_{\dots N} \\ & & & & & a_{NN} \end{bmatrix} \end{array}$$

Figure 1. Matrix presentation of a SPE/TLC profile quality; N—number of spots revealed; a_{ij} and a_{ii} are explained in the text.

RESULTS AND DISCUSSION

On the basis of the results of TLC separations that were carried out after application of different buffer solutions and both extraction methods (LLE and SPE), values of the criterions Y_1, Y_2, Y_3, and Y_4 (Eq. (1)) were calculated. They are presented in Table 1. It is seen that the number of spots observed, as well as the Y values, strongly depends on pH of buffer solution in which samples were dissolved.

For LLE, the best profile quality was obtained at pH = 5. In these conditions, the numbers of spots revealed (influencing criterion Y_3) and the intensity of fluorescence (Y_4) are the highest. In the case of SPE, the best result was obtained when buffer of pH = 14 was used, that made possible the separation of eight clear fluoresced spots ($Y_3 = 19.5$ and $Y_4 = 3.0$).

Comparison of the data presented in Table 1 show that LLE is a more effective method of separation of characteristic drug impurities as compare to SPE. In most cases, the values of profile quality criterions, Y, appeared higher for LLE than for SPE.

CONCLUSIONS

It has been shown that buffer solutions (pH) used for preparation of drug samples strongly influences the impurity profile. The LLE proved to be the more effective (than SPE) method of isolation of characteristic impurities from the drug matrix.

Table 1. The influence of used buffer solution on the TLC profile.

Buffer solution (pH)	LLE						SPE					
	Number of spots revealed		Quality parameters[a]				Number of spots revealed		Quality parameters[a]			
	254 nm	366 nm	Y_1	Y_2	Y_3	Y_4	254 nm	366 nm	Y_1	Y_2	Y_3	Y_4
1	4	4	5.5	5.5	11.0	2.1	2	3	1.0	3.0	4.0	1.8
4	4	5	5.5	9.5	15.0	2.6	2	3	1.0	3.0	4.0	2.0
5	4	8	5.0	26.0	31.0	3.8	3	4	1.0	6.0	7.0	2.4
6	3	8	2.5	26.5	29.0	3.8	2	3	1.0	3.0	4.0	1.8
7	3	6	2.0	14.5	16.5	3.6	2	4	1.0	6.0	7.0	1.4
8	4	6	5.0	13.5	18.5	2.8	2	4	1.0	6.0	7.0	1.4
10	3	7	2.0	19.5	21.5	4.2	2	4	1.0	6.0	7.0	1.3
11	4	6	5.5	13.5	19.0	3.6	2	3	1.0	3.0	4.0	1.2
13	5	5	9.0	9.0	18.0	2.1	2	5	1.0	9.5	10.5	1.8
14	3	8	2.0	25.0	27.0	3.8	2	8	1.0	18.5	19.5	3.0

[a]Mean values calculated from the results of two experiments.

The studies on searching for optimum composition of TLC eluent have been presented elsewhere.[12] In addition to, the improvement of the quality profile (increase the TLC sensitivity) by thickening of SPE extract by evaporation in nitrogen stream has been tested.

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