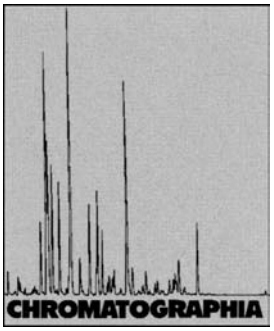
SPE–TLC Profiling of Impurities in 1-(3,4-Methylenedioxyphenyl)-2-nitropropene, an Intermediate in 3,4-Methylenedioxymethamphetamine (MDMA) Synthesis



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J. Kochana[1,✉], J. Wilamowski[2], A. Parczewski[1,3]

[1] Department of Analytical Chemistry, Faculty of Chemistry, Jagiellonian University, Ingardena 3, 30-060 Cracow, Poland; E-Mail: kochana@chemia.uj.edu.pl
[2] Department of Organic Chemistry, Faculty of Chemistry, Jagiellonian University, Ingardena 3, 30-060 Cracow, Poland
[3] Institute of Forensic Research, Westerplatte 9, 31-033 Cracow, Poland

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Abstract

Impurity profiling of 1–(3,4-methylenedioxyphenyl)-2-nitropropene, an intermediate in the synthesis of 3,4-methylenedioxymethamphetamine (MDMA), has been studied by SPE combined with TLC. Extraction of impurities was performed on C_{18} columns. TLC separation was performed on 0.2 mm silica gel 60 plates, with fluorescent indicator F_{254}, in a horizontal developing chamber. To improve the quality of the profile (increase the TLC sensitivity) SPE extracts were concentrated by evaporation in a stream of nitrogen. The usefulness of methanol, ethanol, ethyl acetate, chloroform, acetonitrile, and their mixtures as mobile phases was tested by use of Gibbs's triangle. Spots of the separated impurities were observed under UV light ($\lambda_{exc} = 254$ and 366 nm).

The proposed characteristics of profile quality (optimization criteria) are based on matrix presentation of the TLC pattern. They take into account, simultaneously, the number of spots revealed, differences between R_F values, and the intensity of fluorescence.

Keywords

Thin-layer chromatography
Solid phase extraction
Impurity profiling
3,4-Methylenedioxymethamphetamine intermediates

Introduction

Drug abuse remains a very serious social problem in Europe. According to the last annual report of the European Monitoring Centre for Drugs and Drug Addiction [1] amphetamine and MDMA, known as 'ecstasy', seizures and consumption have increased substantially over the last decade. Although use of recreational drugs (for example ecstasy) among the general population is low, use among people in nightlife settings is relatively high [2]. Rough estimation suggests that between 3 and 3.5 million adults in the EU have tried ecstasy at least once. Of these, about 500 000 have used it once a week or more over a period of time.

To control the production and abuse of drugs it is necessary to develop analytical procedures which provide the valuable information required for law-enforcement purposes. Drug profiling procedures seem to deliver useful information. The main aim of profiling the impurities in a drug is determination of chemical similarities between drug samples seized by police. Consequently, profiling enables differentiation between drug samples produced by different methods of synthesis, in different illicit laboratories, or under different experimental conditions.

Of the methods developed for profiling impurities in amphetamine and its derivatives, chromatographic methods, both GC and HPLC, are used most frequently [2–11]. Spectroscopic methods, e.g. MS, NMR, FTIR, UV, and NIR, have also been reported [8, 12, 13]. A few papers describe the profiling of MDMA impurities by gas chromatography [14–20].

The aim of our work was to establish a TLC method for screening profiling MDMA impurities. This paper focuses on SPE–TLC profiling of 1-(3,4-methylenedioxyphenyl)-2-nitropropene, an important intermediate in MDMA synthesis. It was obtained in our laboratory from piperonal, which is also used as substrate in a method used in clandestine laboratories. The intermediate can be assumed to contain impurities from the first steps of synthesis to the final product (MDMA). To improve profile quality (increase TLC sensitivity) the SPE extract was concentrated by evaporation under a stream of nitrogen. The usefulness of one-component and multicomponent mobile phases was examined. The com-

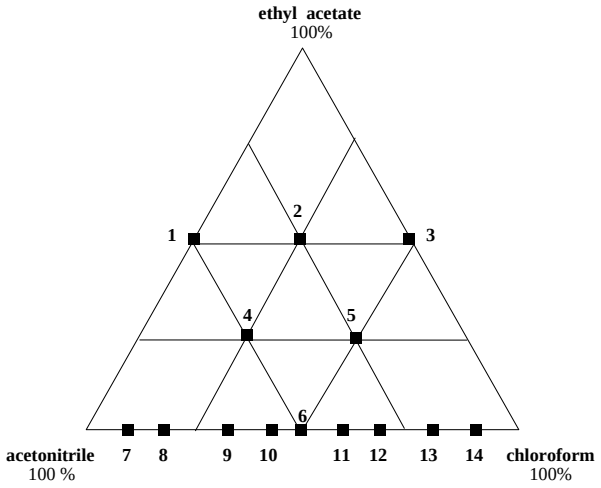

Fig. 1. Gibbs's triangle – the composition of two- and three-component mobile phases (Table 2)

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

Fig. 2. Matrix presentation of SPE–TLC profile quality. N is the number of spots revealed; a_{ij} and a_{ii} are explained in the text

position of the latter was varied in accordance with Gibbs's triangle. The quality of profiles was estimated by means of semiquantitative criteria based on matrix presentation of TLC patterns [21, 22]. These take into account, simultaneously, the number of spots revealed, differences between R_F values, and fluorescence intensity.

The approach has previously been employed for SPE–TLC profiling of impurities in PMMA (*p*-methoxymethamphetamine) [21, 22].

Experimental

Chemical and Reagents

1-(3,4-Methylenedioxyphenyl)-2-nitropropene was synthesised from piperonal [7]. Phosphate buffer, pH 7, and chloroform, methanol, and acetonitrile, all gradient grade, were from Merck (Darmstadt, Germany). Ethyl acetate and ethanol were from POCh (Gliwice, Poland) and were analytical grade.

Profiling Procedure

1-(3,4-Methylenedioxyphenyl)-2-nitropropene (100 mg) was added to 1 mL phosphate buffer (pH 7). The suspension was mixed on a horizontal vortex shaker, centrifuged, and 500 μL supernatant liquid was taken for solid-phase extraction (SPE). SPE was performed on 100-mg C_{18} columns (Baker Bond) with use of VacElut (Varian, USA) equipment. The sample matrix was washed from the column with distilled water (2 × 2 mL) and the impurities were removed with methanol (10 × 50 μL). The extract was evaporated to dryness under a stream of nitrogen, the residue was dissolved in 100 μL methanol, and the solution obtained was shaken on a horizontal shaker for 2 min.

A Nanomat IV (Camag, Switzerland) application device was used to apply 8 μL of the concentrated extract to silica gel 60 plates with fluorescent indicator F_{254} (Merck). TLC separations were conducted in a horizontal developing chamber (Camag). Spots of the separated impurities were observed under UV light ($\lambda_{exc} = 254$ and 366 nm).

Initial experiments were performed with one-component mobile phases—methanol, ethanol, ethyl acetate, chloroform, and acetonitrile. Multicomponent mobile phases were then used; the compositions of these were varied in accordance with Gibbs's triangle, as presented in Fig. 1.

Quality of Profile

The efficiency of SPE–TLC profiling, i.e. the quality of the chromatogram obtained, was characterized by use of a symmetrical matrix of dimension equal to the number (N) of spots revealed [21, 22]. It was assumed, arbitrarily, that an element a_{ij} of the matrix equals 1 if $100\,(R_{Fi} - R_{Fj}) > 6$, because spots i and j are then completely separated. If spots i and j are partly separated, but can be distinguished, $a_{ij} = 0.5$. Otherwise $a_{ij} = 0$. At $\lambda_{exc} = 366$ nm the spots fluoresce and the diagonal element $a_{ii} = 1$ if spot i fluoresces intensely, $a_{ii} = 0.5$ if fluorescence is clearly apparent, and $a_{ii} = 0.1$ if faint fluorescence is observed. A schematic diagram of the matrix is shown in Fig. 2.

The elements a_{ij} of this matrix were determined for each TLC separation and values of the following quality criteria were calculated:

$$Y_1 = \sum_{\substack{i,j=1 \\ i<j}}^{N} a_{ij(254\text{nm})};\ Y_2 = \sum_{\substack{i,j=1 \\ i<j}}^{N} a_{ij(366\text{nm})};$$
$$Y_3 = Y_1 + Y_2;\ Y_4 = \sum_{i=1}^{N} a_{ii(366\text{nm})} \quad (1)$$

where a_{ij} and a_{ii} are explained in Fig. 2 (the number of spots revealed, N, might be different for $\lambda_{exc} = 254$ and $\lambda_{exc} = 366$ nm).

Results and Discussion

One-Component Mobile Phases. Effect of Concentration of SPE Extract on Profile Quality

Values of criteria Y_1, Y_2, Y_3, and Y_4 (Eq. 1) calculated for TLC separations obtained by use of one-component mobile phases are presented in Table 1. The results of profiling without and with concentration of SPE extracts are compared. It is apparent that concentration of the extracts of the impurities resulted in significant improvement of profile quality for all mobile phases except chloroform. A possible explanation of this behaviour is that more volatile impurities could vaporized whereas the other contaminants, the concentrations of which increased as a result of evaporation of the solvent, could be separated with the use of chloroform and detected on TLC plate.

Table 1. Values of the quality criteria, Y, obtained by use of one-component mobile phases without and with concentration of the SPE extract

Mobile phase	Without concentration						With concentration					
	Number of spots revealed*		Quality criteria**				Number of spots revealed*		Quality criteria**			
	254 nm	366 nm	Y_1	Y_2	Y_3	Y_4	254 nm	366 nm	Y_1	Y_2	Y_3	Y_4
Acetonitrile	1;1	1;1	0	0	0	1.00	1;2	3;3	0.50	2.75	3.25	1.35
Chloroform	1;1	4;4	0	5.50	5.50	0.90	3;4	1;1	4.50	0	4.50	0.10
Ethanol	1;1	1;2	0	0.50	0.50	0.55	1;1	2;3	0	2.00	2.00	0.85
Methanol	1;1	1;1	0	0	0	0.10	1;1	2;3	0	1.75	1.75	0.85
Ethyl acetate	2;2	2;2	1	1	2.00	1.30	1;1	3;4	0	4.25	4.25	1.35

*In duplicate SPE–TLC procedures.
**Mean values calculated from the results of two extractions and two TLC separations of each extract.

On the basis of results presented in Table 1 subsequent experiments were performed with the mobile phases characterized by the highest values of Y_3 and Y_4 (acetonitrile, chloroform, and ethyl acetate) and the SPE extracts were concentrated by evaporation of the solvent before TLC separation of the impurities.

Multicomponent Mobile Phases

Quality data calculated for multicomponent mobile phases are compared in Table 2. The compositions of the mobile phases applied are presented on the Gibbs triangle in Fig. 1. From among mobile phases 1–6 the highest values of Y_3 and Y_4 were obtained for 1:1 acetonitrile–chloroform. Several acetonitrile–chloroform mixtures were then investigated (mobile phases 7–14 in Table 2); the best profile quality was obtained by use of 2:8 acetonitrile–chloroform.

Table 2. Values of the quality criteria, Y, obtained by use of multi-component mobile phases in accordance with the Gibbs triangle (with concentration of the SPE extract)

Mobile phase (see Fig. 1)		Proportions	Quality criteria*			
No.	Components		Y_1	Y_2	Y_3	Y_4
1	Acetonitrile–ethyl acetate	1:1	0	5.13	5.13	2.00
2	Acetonitrile–chloroform–ethyl acetate	1:1:2	0.50	5.50	6.00	1.60
3	Chloroform–ethyl acetate	1:1	0.88	3.00	3.38	1.50
4	Acetonitrile–chloroform–ethyl acetate	1:2:1	0.75	5.75	6.50	1.60
5	Acetonitrile–chloroform–ethyl acetate	2:1:1	0.50	2.75	3.25	1.50
6	Acetonitrile–chloroform	1:1	0.63	6.00	6.63	1.90
7	Acetonitrile–chloroform	9:1	0.56	3.81	4.37	1.20
8	Acetonitrile–chloroform	8:2	0.50	1.75	2.25	1.35
9	Acetonitrile–chloroform	7:3	0.50	2.56	3.06	1.50
10	Acetonitrile–chloroform	6:4	0	5.38	5.38	1.73
11	Acetonitrile–chloroform	4:6	0.50	4.38	4.88	1.71
12	Acetonitrile–chloroform	3:7	1.00	6.25	7.25	1.20
13	Acetonitrile–chloroform	2:8	1.00	9.64	10.64	1.27
14	Acetonitrile–chloroform	1:9	1.00	3.00	4.00	0.56

* Mean values calculated from the results of two extractions and two TLC separations of each extract.

Repeatability of SPE–TLC Profiling

To verify the repeatability of extraction of impurities and their TLC separation five extractions and five TLC separations (for each extract) were performed with 2:8 acetonitrile–chloroform as the best mobile phase.

Repeatability Within Extraction

The errors in Y_2, Y_3, and Y_4, estimated on the basis of experimental results from TLC separations performed on the same extract, were 0.27, 0.27, and 0.11, respectively (there was no reason to estimate ΔY_1 because $N = 1$).

Repeatability Between Extractions

For five extractions of the same sample the errors in the Y criteria estimated on the basis of the experiments were comparable with those obtained within extraction, except for Y_4. The relatively high value of ΔY_4 can be explained by the ambiguity of visual estimation of fluorescence intensity and the arbitrary scale assumed for a_{ii} (Fig. 1). It is expected that objective (e.g. densitometric) measurement of TLC spot 'intensities' will result in significant reduction of *RSD* for Y_4. The repeatability between extractions represents the repeatability of the profiling process used.

Conclusion

This work has demonstrated that profiling of impurities in an intermediate in MDMA synthesis can be achieved effectively by SPE–TLC. A 2:8 acetonitrile–chloroform mixture seemed to be the best mobile phase for TLC separation and gave a contaminant profile containing several clearly fluorescing spots. The repeatability of the method proved to be satisfactory.

Acknowledgement

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