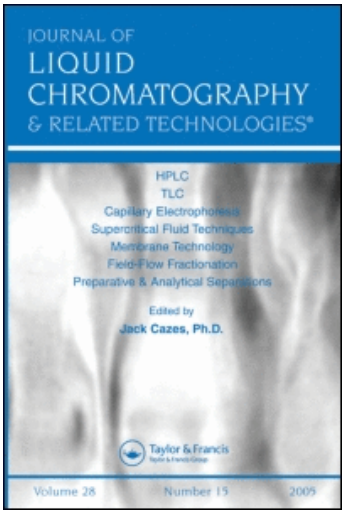

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

SPE/TLC Profiling of the Impurities of MDMA: The Influence of an Agglutinant, Diluents, and Adulterants

J. Kochana[a]; A. Parczewski[b]; J. Wilamowski[c]
[a] Department of Analytical Chemistry, Faculty of Chemistry, Jagiellonian University, Cracow, Poland [b] Institute of Forensic Research, Cracow, Poland [c] Department of Organic Chemistry, Faculty of Chemistry, Jagiellonian University, Cracow, Poland

To cite this Article Kochana, J. , Parczewski, A. and Wilamowski, J.(2006) 'SPE/TLC Profiling of the Impurities of MDMA: The Influence of an Agglutinant, Diluents, and Adulterants', Journal of Liquid Chromatography & Related Technologies, 29: 9, 1247 — 1256
To link to this Article: DOI: 10.1080/10826070600598894
URL: http://dx.doi.org/10.1080/10826070600598894

Journal of Liquid Chromatography & Related Technologies®, 29: 1247–1256, 2006

ISSN 1082-6076 print/1520-572X online
DOI: 10.1080/10826070600598894

SPE/TLC Profiling of the Impurities of MDMA: The Influence of an Agglutinant, Diluents, and Adulterants

J. Kochana
Department of Analytical Chemistry, Faculty of Chemistry, Jagiellonian University, Cracow, Poland

A. Parczewski
Institute of Forensic Research, Cracow, Poland

J. Wilamowski
Department of Organic Chemistry, Faculty of Chemistry, Jagiellonian University, Cracow, Poland

Abstract: The SPE/TLC method has been used in the screening profiling of impurities extracted (SPE) from MDMA (3,4-methylenedioxymethamphetamine), the main active component of Ecstasy tablets. Sought for, was the optimum buffer solution used in sample dissolution and optimum composition of eluent used in TLC separation. The spots of impurities were observed under UV light at $\lambda_{exc} = 366$ nm.

The influence of magnesium stearate, used as an agglutinant in preparation of drug tablets, as well as adulterants (aspirin, paracetamol and caffeine) and diluents (glucose and citric acid) on the TLC profile of impurities was examined. The composition of the above additives was varied in accordance with an unsymmetrical 3^2 factorial design.

The quality of TLC profiles was estimated by means of semiquantitative criteria, which are based on matrix presentation of TLC patterns. The number of spots revealed, differences between R_f values, and intensity of fluorescence are taken into account, simultaneously.

Keywords: MDMA, Profiling of impurities, SPE/TLC

Address correspondence to J. Kochana, Department of Analytical Chemistry, Faculty of Chemistry, Jagiellonian University, Ingardena 3, Cracow 30-060, Poland. E-mail: kochana@chemia.uj.edu.pl

INTRODUCTION

3,4-Methylenedioxymethamphetamine, MDMA, is a main active component of street drug tablets known as Ecstasy. In the past few years, Ecstasy has become one of the most popular, so called recreational drugs, which is used among young people in nightlife settings.[1] Rough estimation suggests that about 500,000 EU citizens have used it once a week or more over a period of time. To control the drug market and abuse of the drug, it is necessary to develop analytical procedures, which provide valuable information for police and law enforcement purposes.

Drug profiling consists of physical and chemical characterization of seized drug samples. The aim of profiling is to supply information about the method and experimental conditions of synthesis, and the origin of seized samples. It is useful in investigation aimed at searching for illicit drug laboratories, ways of drug distribution, and the net of deliverers.

Composition (content) of impurities contained in each drug sample makes up a so called profile of impurities. The profile of impurities depends on the synthesis method, specific reaction conditions, and substrates applied. Thus, the impurities contain intermediate and side reaction products. Purification procedures, the way the drug has been prepared for distribution, agglutinants, diluents, and adulterants, that are added to the drug before it is brought into the illegal market, usually influence the profile of impurities.

There are several reports in the literature concerning profiling of MDMA impurities. Generally, gas chromatography: GC/MS,[2–4] GC × GC/TOF-MS,[5] GC/FID[6,7] has been employed for this purpose. The spectroscopic method, Raman spectroscopy[8] and FTIR/ESI-MS[9] have also been reported. On the basis of nitrogen isotopic ratio measured by gas chromatography–combustion–isotope ratio mass spectrometry (GC-C-IRMS), Palhol et al.[10] establish links between Ecstasy tablets. Our previous papers[11,12] concern SPE/TLC profiling of impurities of 1-(3,4-methylenedioxyphenyl)-2-nitropropene, an intermediate product of MDMA synthesis (piperonal used as a substrate).

The main aim of this work was to develop a TLC method useful in screening profiling of the impurities extracted (SPE) from MDMA. The optimum buffer solution used in sample dissolution and composition of eluent for TLC separation were sought for. The influence of magnesium stearate, used as an agglutinant in preparation of drug tablets, as well as two component matrices containing an agglutinant and adulterant (aspirin, paracetamol and caffeine) or diluent (glucose and citric acid) on the profile was examined. The composition of additives in two component matrices was varied in accordance with an unsymmetrical 3^2 factorial design.

The proposed semiquantitative characteristics of profile quality (optimization criterions) are based on a matrix presentation of a TLC pattern.[13] They take into account, simultaneously, the number of spots revealed, differences between R_f values, and intensity of fluorescence.

EXPERIMENTAL

Chemicals and Reagents

MDMA (3,4-methylenedioxymethamphetamine, synthesised from isosafrole), acetonitrile, chloroform, acetone, methanol, were all gradient grade (Merck, Germany); buffer solutions: pH = 7 (30.5 mL 0.2 mol/L Na_2HPO_4, 19.5 mL 0.2 mol/L NaH_2PO_4, filled up by distilled water to 100 mL), pH = 11 (22.7 mL 0.1 mol/L NaOH mL, 50.0 mL 0.05 mol/L $NaHCO_3$, filled up by distilled water to 100 mL), pH = 14 (1 mol/L NaOH); caffeine (99%, Sigma-Aldrich); acetylsalicylic acid (aspirin, 99.5%, Sigma-Aldrich); citric acid (monohydrate), paracetamol, and glucose (all analytical grade, POCh Gliwice, Poland); magnesium stearate (analytical grade, POL-NIL, Poland).

Apparatus

Vac Elut (Varian, USA) with C_{18} (100 mg) extraction columns (Baker Bond) was used for SPE extraction of impurities from the drug matrices; Nanomat IV (Camag, Switzerland) application device and a horizontal developing chamber (Camag, Switzerland) were applied for separation on silica gel plates (0.2 mm) with fluorescent indicator $60F_{254}$ (Merck, Germany); UV lamp (Cobrabid, Poland) was used for detection of spots at $\lambda_{exc} = 366$ nm.

Sample Preparation and Profiling Procedure

A solution of 75 mg MDMA (hydrochloride) in 550 μL of buffer solution was prepared. Appropriate amounts of additives: an agglutinant (magnesium stearate), adulterants (aspirin, paracetamol and caffeine), and diluents (glucose and citric acid) were added to the MDMA solution to make several compositions of drug matrix, according to an unsymmetrical 3^2 factorial (see Figure 1). Factorial experiments are based on varying all factors simultaneously, at a limited number of factor levels.[14] Three level two factorial design, 3^2, means two factors: concentration of magnesium stearate and concentration of other additions, at a three concentration level. For magnesium stearate concentration, levels were 0%, 1%, and 5%; for glucose 0%, 10%, and 50%, for other additives 0%, 2%, and 10%, with respect to the mass of hypothetic Ecstasy tablets (150 mg). It was assumed that MDMA makes 50% of the Ecstasy tablet, and that each tablet contains magnesium stearate. The influence of magnesium stearate in the case of a one component matrix was tested in the concentration range 0%–5% (every 0.25%).

The obtained suspension was mixed on a horizontal shaker (for 30 min), then centrifuged (13 000 rpm, 5 min), and 500 μL of the supernatant liquid

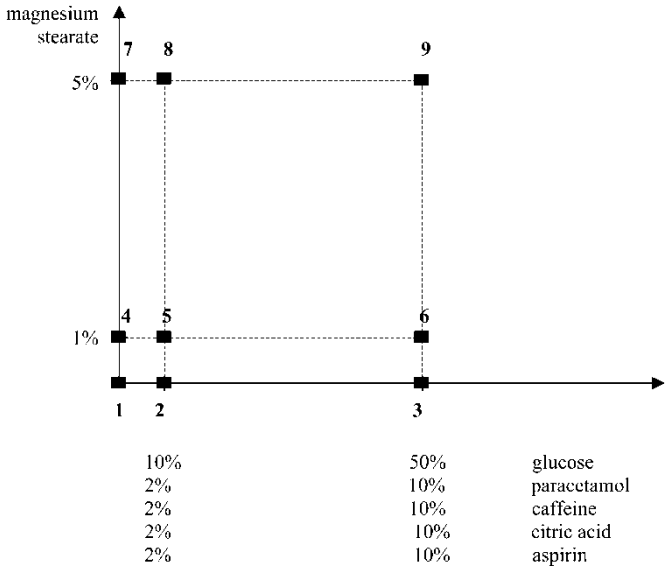

Figure 1. Composition of drug matrices according to an unsymmetrical 3^2 factorial.

was injected onto a C^{18} SPE column. Two portions of 1 mL of distilled water were used for matrix elution. The analytes were recovered by washing the column with 10 portions of methanol, each 50 μL. The volume of 6 μL of extracted impurities was placed onto a TLC plate. The separated spots were revealed under UV light.

Some additives: glucose, paracetamol, caffeine, and acetylsalicylic acid extinguished fluorescence of the TLC plate at 254 nm, but their spots do not interfere with spots of selected impurities, which were revealed at 366 nm.

Preliminary Experiments

The usefulness of buffer solutions pH = 7, 11, and 14 in drug dissolution was examined. The effectiveness of the following mixtures of organic solvents in TLC separation was investigated: acetonitrile : chloroform (1 : 1 v/v), chloroform : methanol (9 : 1 v/v), acetonitrile : chloroform : ammonia (2 : 8 : 1 v/v/v), chloroform : methanol : ammonia (9 : 1 : 1), chloroform : acetone : methanol : ammonia (10 : 8 : 1 : 1 v/v/v/v).

Methodology

Each TLC chromatogram obtained, was described using a symmetrical matrix presented in Figure 2. The values of matrix elements, a_{ij} and a_{ii}, were assumed in an arbitral way, which is explained in the caption to

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

Figure 2. Matrix presentation of a SPE/TLC profile quality; N = number of spots revealed: $a_{ij} = 1$ if spots i and j are completely separated (arbitrarily: if 100 ($R_{fi} - R_{fj}$) > 6); $a_{ij} = 0.5$ if spots i and j are partly separated; $a_{ij} = 0$ if spots overlap; $a_{ii} = 1$ if spot i fluoresces intensively; $a_{ii} = 0.5$ if spots exhibits clear fluorescence; $a_{ii} = 0.1$ in case of faint but still visible fluorescence; $a_{ii} = 0$ no fluorescence.

Figure 2. The quality of a chromatogram was characterized by the following optimisation parameters:[11–13]

$$Y_1 = \sum_{\substack{i,j=1 \\ i<j}}^{N} aij; \quad Y_2 = \sum_{i=1}^{N} aii \tag{1}$$

The dependence of criterions Y on experimental factors X's (concentrations of additives in drug matrix) was approximated with the use of polynomial models:[13,15]

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 \tag{2}$$

The regression coefficients, b, were calculated on the basis of the results of 2^2 factorial experiments. They correspond to the main, b_i, and interaction, b_{ij}, effects of factors X's.

All nine 2^2 factorials that can be extracted from the 3^2 experimental plan in Figure 1 were taken into account.

RESULTS AND DISCUSSION

Results of Preliminary Experiments

On the basis of TLC chromatograms obtained for MDMA dissolved in buffer solutions pH = 7, 11, and 14, values of Y's criteria were calculated (Eq. 1) and compared. The highest value of Y_1 and Y_2, 9.5 and 1.4, respectively, were obtained at pH = 11. For buffer at pH = 7, Y_1 equaled 4.5 and $Y_2 = 1.3$, while for pH = 14, $Y_1 = 0$ and $Y_2 = 1.0$.

From among solutions used as mobile phase in TLC, the best separations were obtained for chloroform:methanol (9 : 1 v/v) mixture. Good separation was observed for chloroform:methanol:ammonia (9 : 1 : 1 v/v/v) as well, but R_f values were not repeatable.

In further experiments, buffer solution at pH = 11 was used for dissolution of drug samples. A mixture of chloroform and methanol (9 : 1 v/v) was employed in TLC separations.

Repeatability of SPE/TLC Profiling of MDMA Impurities

On the TLC profile of extracted impurities of MDMA, five spots were revealed; their R_f values were: 0; 0.14; 0.17; 0.24 and 0.33. To verify the repeatability of the extraction of impurities and their TLC separation five profiling processes were performed, three separations for each extract. The errors in Y_1 and Y_2 within extraction estimated were 0.1 and 0.0, respectively. The errors between extractions were comparable with those obtained within extraction. Consequently, the repeatability of the whole profiling procedure was satisfied.

Influence of Magnesium Stearate

It was found that both quality parameters Y_1 and Y_2 (Eq. 1) did not significantly change with increasing concentration of magnesium stearate in a range of 0%–5% (every 0.25%). The values of criteria Y were calculated from the results of two extractions and three TLC separations of each extract, for each concentration of agglutinant. In two cases, for 1.75% and 3.5% of magnesium stearate, Y_1 noticeably decreased, which was as a result of the reduction of the number of spots observed. The quality criteria seem to be too sensitive to the number of spots revealed (for five completely separated spots $Y_1 = 10$, while for four spots $Y_1 = 6$).

Influence of Two Component Drug Matrices

Parameters of chromatogram quality, Y_1 and Y_2, were determined for each experimental point (compositions of drug) in a plan presented in Figure 1.

On the basis of the results of nine 2^2 factorials extracted from the plan presented in Figure 1, the main (b_1, b_2) and interaction (b_{12}) effects of the additives on parameters Y_1 and Y_2 were calculated.

The uncertainty (error) of determination of the effects b for Y_1 (Eq. 1) was roughly estimated (from the error propagation principle) as follows:[14]

$$(\Delta b)^2 = \frac{1}{4^2}\sum_{u=1}^{4}(\Delta Y)_u^2 = \frac{(\Delta Y)^2}{4} \tag{3}$$

Table 1. Main and interaction effects of concentration of additives (see Figure 1) on response Y_1 (see Eq. 1)

Experimental points of 2^2 factorial (see Figure 1)	Caffeine			Citric acid			Aspirin		
	b_1	b_2	b_{12}	b_1	b_2	b_{12}	b_1	b_2	b_{12}
1,2,4,5	−0.10	0.15	0.05	−0.15	−1.75	0	0.88	−0.88	1.03
1,2,7,8	−0.20	0.05	−0.05	0.85	−0.75	1.00	0.88	−0.88	1.03
1,3,4,6	−0.08	2.63[a]	0.08	0.33	−2.83[a]	−0.18	−1.20	1.30	−1.05
1,3,7,9	−0.10	2.60[a]	0.05	−0.08	−2.58[a]	0.08	−0.08	2.43[a]	0.08
2,3,5,6	−0.03	2.48[a]	0.03	−0.33	−1.08	−0.18	−0.18	2.18[a]	2.08[a]
2,3,8,9	−0.15	2.55[a]	0.10	−0.93	−1.83	−0.93	0.95	3.30[a]	−0.95
5,6,8,9	−0.13	2.58[a]	0.08	1.25	2.00[a]	−0.75	−1.13	1.23	1.23
4,5,7,8	−0.10	0.10	−0.10	1.00	−0.75	1.00	0	0.15	0
4,6,7,9	−0.03	2.68[a]	−0.03	0.25	−2.75[a]	0.25	1.13	1.38	1.13

[a]Effects assumed to be significant (see text for explanation).

which is true if ΔY does not depend on u, where u numerates experimental points of factorial design. From (Eq. 1), it results that:

$$(\Delta Y)^2 = \sum \left(\frac{\partial Y}{\partial a_{ij}}\right)^2 (\Delta a)^2 = \sum (\Delta a)^2 = \frac{N(N-1)}{2}(\Delta a)^2$$

It was arbitrarily assumed that $\Delta a = 0.25$, then $(\Delta a)^2 = 1/16$ and $(\Delta Y)^2 = N(N-1)/32$. If $N = 5$ (number of spots observed) then from (Eq. 3) $\Delta b \cong 0.4$. In the case of Y_2 (see Eq. 1), $(\Delta Y)^2 = N\ (\Delta a)^2$. If $\Delta a = 0.25$ and the number of revealed spots equals $N = 5$, than, from (Eq. 3) $\Delta b \cong 0.3$. A main effect of a factor X, or an interaction effect, was assumed to be significant if $|b| \geq 5\ \Delta b$.

Only criterion Y_1 appeared sensitive to the changes in additives content in drugs mixtures. The main (b_1 and b_2) and interaction (b_{12}) effects of the additives on response Y_1 are presented in Table 1. They were determined on the basis of nine different 2^2 factorial experiments extracted from the plan presented in Figure 1. The 2^2 factorials are defined by experimental points given in the first column in Table 1. It is seen from Table 1, that in all cases tested, magnesium stearate did not affect the quality of TLC separation significantly. The effect of caffeine appeared significant only in the broader concentration intervals of the additive. An addition of caffeine changed the R_f's values of separated spots, also, an extra spot was revealed. No interaction effect of caffeine and magnesium stearate was detected. The similar effect was observed for aspirin, but there appeared a significant interaction effect detected within the 2^2 factorial: 2, 3, 5, 6 (see Figure 1). This indicates rather irregular dependence of Y_1 on magnesium stearate and aspirin concentrations. An addition of citric acid changed the R_f's values of separated spots as well, and also decreased the number of spots observed. No interaction effect of citric acid and magnesium stearate was detected. The remaining additives tested, glucose and paracetamol, did not affect the response Y_1 significantly.

CONCLUSION

To summarize, the proposed SPE/TLC method can be employed in the screening profiling of MDMA impurities. But, special attention should be paid to matrix composition. Our study showed that the presence in the drug matrix of an agglutinant (magnesium stearate) did not significantly change the TLC chromatogram. Also, glucose and paracetamol did not influence the TLC profile. An addition of caffeine, aspirin, or citric acid in a higher concentration range (2%–10%) changed the TLC profile. However, Ecstasy tablets do not often contain these additives at such a high concentration levels.

The method proposed proves to be a valuable tool for screening profiling of drug impurities before application of more sophisticated methods like GC/MS or HPLC appear to be necessary.

ACKNOWLEDGMENTS

This study was partly supported by grant OT 00C 01024 from the Committee for Scientific Research, Poland. The authors would like to thank Magdalena Kruszewska for help in the experimental part of this work.

REFERENCES

1. European monitoring centre for drugs and drugs addition, Recreational drug use—a key EU challenge. Drugs in Focus Nov–Dec, 2002.
2. Gimeno, P.; Besacier, F.; Chaudron-Thozet, H. Optimization of extraction parameters for chemical profiling of 3,4-methylenedioxymethamphetamine (MDMA) tablets. Foren. Sci. Int. **2003**, *132*, 182–194.
3. Gimeno, P.; Besacier, F.; Chaudron-Thozet, H.; Girard, J.; Lamotte, A. A contribution to the chemical profiling of 3,4-methylenedioxymethamphetamine (MDMA) tablets. Foren. Sci. Int. **2002**, *127*, 1–44.
4. Makino, Y.; Kurobane, S.; Miyasaka, K.; Nagano, K. Profiling of ecstasy tablets seized in Japan. Microgram J. **2003**, *1* (3–4), 169–176.
5. Song, S.M.; Marriott, P.; Kotsos, A.; Drummer, O.H.; Wynne, P. Comprehensive two-dimentional gas chromatography with time-of-flight mass spectrometry (GC × GC-TOFMS) for drug screening and confirmation. Foren. Sci. Int. **2004**, *143*, 87–101.
6. Rashed, A.M.; Anderson, R.A.; King, L.A. Solid-phase extraction for profiling of Ecstasy tablets. J. Foren. Sci. **2000**, *45* (2), 413–417.
7. Palhol, F.; Boyer, S.; Naulet, N.; Chabrillat, M. Impurity profiling of seized MDMA tablets by capillary gas chromatography. Anal. Bioanal. Chem. **2002**, *374*, 274–281.
8. Bell, S.E.J.; Barrett, L.J.; Burns, D.T.; Dennis, A.C.; Speers, S.J. Tracking the distribution of "ecstasy" tablets by Raman composition profiling: A large scale feasibility study. Analyst **2003**, *128* (11), 1331–1335.
9. Cheng, W.C.; Poon, N.L.; Chan, M.F. Chemical profiling of 3,4-methylenedioxymethamphetamine (MDMA) tablets seized in Hong Kong. J. Foren. Sci. **2003**, *48* (6).
10. Palhol, F.; Lamoureux, C.; Chabrillat, M.; Naulet, N. $^{15}N/^{14}N$ isotopic ratio and statistical analysis: an efficient way of linking Ecstasy tablets. Anal. Chim. Acta **2004**, *510*, 1–8.
11. Kochana, J.; Wilamowski, J.; Parczewski, A. TLC profiling of 1-(3,4-methylenedioxyphenyl)-2-nitropropene an intermediate in MDMA synthesis. Influence of sample preparation methods and conditions. J. Liq. Chromatogr. & Rel. Technol. **2004**, *27* (15), 2463–2470.
12. Kochana, J.; Wilamowski, J.; Parczewski, A. SPE-TLC profiling of impurities in 1-(3,4-methylenedioxyphenyl)-2-nitropropene, an intermediate in 3,4-methylenedioxymethamphetamine (MDMA) synthesis. Chromatographia **2004**, *60* (7/8), 481–484.
13. Kochana, J.; Wilamowski, J.; Parczewski, A. Profiling of impurities in *p*-methoxymethamphetamine (PMMA) by means of SPE/TLC method. Examination of the influence of experimental conditions according 2^4 factorial. Foren. Sci. Int. **2003**, *134*, 214–218.

14. Kellner, R.; Mermet, J.-M.; Otto, M.; Valcarcel, M.; Widmer, H.M. *Analytical Chemistry. A modern approach to Analytical Science*; Wiley-VCH Verlag GmbH & Co. KgaA: Germany, 2004, 151–158.
15. Kochana, J.; Wilamowski, J.; Parczewski, A.; Surma, M. Synthesis of standards of the most important markers of Leuckart *p*-methoxymethamphetamine (PMMA). Examination of the influence of experimental conditions and a drug diluent on SPE/TLC profiling. Foren. Sci. Int. **2003**, *134*, 207–213.

Received November 15, 2005
Accepted December 22, 2005
Manuscript 6782