FORENSIC SCIENCE
JOURNAL SINCE 2002

Forensic Science Journal
2002;1:47-53

# Taguchi's method in optimizing the experimental conditions of simultaneous supercritical fluid extraction and chemical derivatization for the gas chromatographic-mass spectrometric determination of amphetamine and methamphetamine in aqueous matrix

**Sheng-Meng Wang,[1,*] Ph.D.; Yun-Seng Giang,[1] Ph.D.; Yong-Chien Ling,[2] Ph.D.**

*[1] Department of Forensic Science, Central Police University, 56 Shu Jen Road, Taoyuan 333, Taiwan ROC.*
*[2] Department of Chemistry, National Tsing Hua University, Hsinchu 300, Taiwan ROC.*

Received: 12 September 2001/ Received in revised form: 9 December 2001 / Accepted: 21 December 2001

## ABSTRACT

With the aid of gas chromatography-mass spectrometry (GC-MS), a systematical and analytical evaluation method called Taguchi's quality engineering has been applied to the in-situ supercritical fluid extraction (SFE) and chemical derivatization (ChD) of amphetamines from water to afford the optimal experimental conditions and hence achieved the highest recoveries of the analytes (as their trifluoroacetyl derivatives) and the best robustness of the quatitation out of the least number of experimental runs. The optimal levels thus determined for the four influential factors are: pressure, 4000 psi; temperature, 90˚C; time for static SFE, 5 min; amount of TFA (ChD agent), 100 μL. Also, the mean recoveries achieved by simultaneous SFE-ChD, i.e., 95% (rsd = 3.8%) for AP and 89% (rsd = 4.0%) for MA, are significantly better than the corresponding overall recoveries obtained upon stepwise SFE-ChD, suggesting the unreacted TFA in the former procedure has strengthened the extracting power of $CO_2$ fluid as has been evidenced by a control test. The success of this study has significantly strengthened the base and broadened the usefulness of both simultaneous SFE-ChD and Taguchi's method.

**Keywords:** Drug urinalysis, Taguchi's method, Supercritical fluid extraction, Chemical derivatization, Amphetamine, Methamphetamine

## Introduction

The first idea of a "design of experiments" may be traced back to the 1920s when R.A. Fisher proposed a systematical and analytical approach to optimize his experimental conditions [1]. Since then all of the major enterprises in the world have paid most of their attention to the issue of quality control. Having kicked off its development in 1949, Taguchi's method, also known as Taguchi's quality engineering, earned its worldwide reputation mostly during 1980-82, at which time Dr. Taguchi worked for quite a few enterprises in Japan and the States including the AT & T Bell Laboratory as a visiting consultant [2,3]. To date, Taguchi's method has been one of the two most effective tools for improving quality, with the other being ISO-9000.

Compared to traditional or the American way of quality control, which places most of the emphasis on the so-called "system design" (USA, 70% vs. Japan, 40%), Taguchi's or the Japanese method while paying comparable attention to "tolerance design" (USA, 28% vs. Japan, 20%) lays considerably greater stress on "parameter design" (USA, 2% vs. Japan, 40% : ) [4]. This overturning strategy of Taguchi's method will not only keep a product's external specifications at a potential level but also significantly enhance its reliability, manufacturability, and broad-sense value. For a specific experiment or series of experiments whose quality or efficiency is of concern, Taguchi's parameter design usually starts with the recognition of so-called prominent or influential factors followed by a systematic evaluation based on an orthogonal table and a few response tables so as to afford the optimal yet simple experimental conditions and achieve the highest economy yet strongest robustness of the products as well as its manufacturing process. Meanwhile, Taguchi's tolerance design is usually conducted with the aid of an ANOVA, i.e., analysis of variance, while keeping quality and cost in balance.

Taguchi's quality control is particularly well suited

* Corresponding author, e-mail:smwang@sun4.cpu.edu.tw

for those cases in which there exist considerable interactions among the influential factors. In this paper we shall take simultaneous supercritical fluid extraction and chemical derivatization (SFE-ChD) as an example. So far as SFE is concerned, over the past decade there has been a tremendous increase in the use of it as a sample preparation method. Its simplicity, speed, high selectivity, high efficiency, low cost, solvent saving, and being non-toxic have made this technique highly acclaimed for the recovery of drugs [5-14], explosives [15], pesticides [16-18], polychlorobiphenyls and dioxins [19-23], caffeine [24], fire accelerants [25,26], and many other organic and organomettallic compounds [27-31] from a variety of matrices, both biological and non-biological, and both solid and liquid. In a recent publication, we further reported the simultaneous processing of SFE and ChD for the gas chromatographic-mass spectrometric (GC-MS) determination of amphetamine and methamphetamine in urine, with the analytes being originally in their ionic forms [32]. In order to achieve an immunity to a wide variety of chemical and physical interferences and to improve the quantitative analytical quality, isotope-dilution method was employed in that study. Namely, known amounts of d8-labeled internal standards (I.S.s) were routinely added to the urine sample prior to performing the one-pot SFE-ChD. Nevertheless, since a forensic drug urinalysis of unquestionable quality relies on a sound sample pretreatment, an effective GC-MS analytical methodology, and a critical data evaluation process, the important role of the combined SFE-ChD with respect to its actual efficiency in the overall analytical scheme cannot be over-emphasized. Therefore, our previous simultaneous-SFE-ChD study was started with the optimization of its experimental conditions, which would be best realized by employing Taguchi's method and reported separately in the present paper.

## Experimental

*Materials*

Racemic *d,l*-amphetamine sulfate (AP · $H_2SO_4$) and d,l-methamphetamine hydrochloride (MA · HCl) both in methanol were purchased from Sigma Chemical Co., USA; ammonium hydroxide (28.0-30.0%) from J.T. Baker Inc., USA; trifluoroacetic anhydride (TFA) from Ferak Berlin Co., Germany; ethyl acetate (EA) from Fisher Chemical, USA; liquid carbon dioxide from Scott Specialty Gases, Inc., USA; celite from ISCO Co., USA; glass beads from LMS Co., Germany. All of the above agents/materials were directly used without further purification.

Trifluoroacetylamphetamine (AP-TFA) and trifluoroacetylmethamphetamine (MA-TFA) were used as the I.S.s for the calculation of: (1) simultaneous-SFE-ChD recoveries of MA.HCl and AP · $H_2SO_4$, respectively; (2) ChD yields of MA · HCl and AP · $H_2SO_4$, respectively; and (3) SFE recoveries of and MA-TFA AP-TFA, respectively. Authentic AP-TFA was prepared as follows: To a reaction vial containing 0.5 g of *l*-AP (neat liquid in free-base form; Sigma Chemical Co.) was added 3 mL of TFA. The reaction mixture in the sealed vial was incubated at 80˚C for 18 hrs and then cooled to room temperature. The crude TFA-dirivative was purified by purging with dry nitrogen followed by vacuuming to dryness. The resulting residue was identified as AP-TFA of 99.43 % purity based on its GC-MS total ion chromatogram. Likewise, authentic MA-TFA of 99.98 % purity was prepared using 0.5 g of *l*-MA (neat liquid in free-base form; Sigma Chemical Co.).

In performing simultaneous SFE-ChD for the title evaluation, deionized (D.I.) water instead of human urine was used as the sample matrix. So doing would help clarify the essentials of the evaluation methodology itself, but would generally not affect the effectiveness of the evaluation process when applied to other aqueous matrices such as urine, blood, etc.

*General procedure of simultaneous SFE-ChD for the title evaluation*

A 10-µL (the amounts used for establishing the method calibration curves are stated in the Experimental Section ) portion of AP · $H_2SO_4$ or MA · HCl (the two analytes were treated separately) in methanol was added to 1 mL of D.I. water. A ca. 200-µL portion of ammonium hydroxide was then added to alkalize the aqueous matrix (pH 10-12). The resulting solution was transferred to a 2.5-mL extraction cell that had previously been filled with celite and glass beads. The cell was subjected to vacuum to remove methanol and water. This drying step took about 3 hrs. To save time, it is advisable to run a number of samples at a time.

To each dry sample was added a tested amount of TFA. The mixture was subjected to simultaneous SFE-ChD. This step utilized an ISCO SFX$^{TM}$220 Supercritical Fluid Extraction System equipped with an SFX 220 extractor, an SFX 200 controller and a 260D syringe pump, and was performed at two stages. Stage 1: simultaneous static SFE-ChD under a tested pressure and at a tested temperature for a tested period of time. Stage 2: dynamic elution with 12.5 mL of supercritical carbon dioxide using a variable restrictor to keep the flow-rate at 1 mL/min. The eluent was trapped with a micro-concentration tube containing 5 mL of EA, fortified with 10 µL of authentic 1000-µg/mL MA-TFA (as I.S. for AP · $H_2SO_4$) or AP-TFA (as I.S. for MA · HCl) solution, and concentrated to 200 µL by purging with nitrogen gas. A 1-µL aliquot of this solution was injected for each GC-MS analysis.

*Gas Chromatography-Mass spectrometry*

GC-MS analyses were carried out using a Hewlett-Packard HP-5890 Series II gas chromatograph coupled to an HP-5971 Series mass selective detector (MSD). The column used was a DB-5 capillary column (30 m × 0.25 mm I.D., 0.33 μm film thickness). The GC was operated in the splitless mode (i.e., purge off) while performing injection, but 1 min later the purge valve was turned on. The injector temperature was 250˚C. The column temperature was programmed from 100 to 280˚C at 10˚C/min, with the final temperature held for 12 min. Helium was used as the carrier gas at a flow-rate of 1 mL/min. Effluents from the GC column were transferred via a transfer line held at 280˚C to a 70-eV electron impact (EI) ionization source held at 180˚C. The GC-EIMS analyses of the relevant TFA-derivatives were performed in the SIM mode accompanied by extracted ion chromatograms (EIC) [32]. The three qualifier ions selected for AP-TFA were m/z 140, 118 and 91, and those for MA-TFA, m/z 154, 118 and 110. The quantifier ions used for AP-TFA and MA-TFA were m/z 140 and 154, respectively. The calibration curves were produced by plotting the peak-area ratio (analyte : I.S.) against the absolute weight of TFA-derivative of appropriate analyte in the serial standard (calibrator) solutions. The peak-area ratio used was the mean of triplicate analyses.

*Calibration curves and recovery calculations*

To calculate the respective recoveries of AP-TFA and MA-TFA achieved by simultaneous SFE-ChD as well as stepwise ChD and SFE, we need to first plot a couple of instrument calibration curves each showing the response factors (i.e., peak area ratios) of the calibrator vs. the I.S. at serial calibrator concentrations. The so-called method recoveries of AP·$H_2SO_4$ (in terms of AP-TFA), for instance, under various tested conditions are then obtained by dividing the regressed amount of the recovered AP-TFA by the originally spiked amount of AP-TFA or AP-TFA-equivalents.

For establishing the instrument calibration curve of AP-TFA, the serial calibrator solutions were prepared as follows: To a micro-concentration tube containing an appropriate amount of authentic AP-TFA in EA (1, 5, 10, 50, 100 μL of 1000-μg/mL solution) was added 10 μL of authentic 1000-μg/mL MA-TFA (as I.S.) solution. This would make the corresponding spikes contain 1, 5, 10, 50, and 100 μg, respectively, of AP-TFA, and 10 μg of MA-TFA. More EA was added to make up a 1-mL solution. A 1-μL aliquot of the resulting solution was injected onto the GC-MS for analysis. The instrument calibration curve of MA-TFA was produced separately but in the same way as for AP-TFA except that AP-TFA was used as the I.S.

To have a depictive and comparative look at the sensitivity of simultaneous SFE-ChD, a couple of the so-called method calibration curves with regard to the two analytes may be desirable. Taking AP·$H_2SO_4$ as an example, the serial calibrators used for producing the method calibration curves under various tested conditions were made by first adding 1, 5, 10, 50, 100 μL of authentic 1000-μg/mL solution of the salt (the direct comparison between the method calibration curve and the foregoing instrument calibration curve would be facilitated if the abscissa of method calibration curve shows the number of AP-TFA equivalents rather than the amount of AP·$H_2SO_4$ itself) in methanol to 1 mL of D.I. water, followed by the in-situ SFE-ChD described in the Experimental Section. A 1-μL aliquot of the resulting EA solution was injected for each GC/EIMS analysis. The method calibration curve of MA.HCl was produced separately but in the same way as for APAP·$H_2SO_4$ except that AP-TFA was used as the I.S.

The serial calibrator solutions used for regressing/calculating the ChD yields of AP-TFA from AP·$H_2SO_4$, for instance, under optimal conditions were prepared as follows: An appropriate amount of the salt in methanol (1, 5, 10, 50, 100 μL of 1000-μg/mL solution) was placed in an open ChD vial, and purged with nitrogen gas to dryness. To each residue was added 200 μL of TFA. The reaction mixture was sealed, incubated at 80˚C for 20 min, and then cooled to room temperature. The crude TFA-derivative was purified by purging with dry nitrogen to dryness. The fine residue was transferred to a micro-concentration tube by rinsing with EA. To the resulting solution was added 10 μL of authentic 1000-μg/mL MA-TFA (as I.S.). More EA was added to make up a 1-mL solution. A 1-μL aliquot of the resulting solution was injected for each GC-EIMS analysis. The MA·HCl counterpart was done in the same way except that AP-TFA was used as the I.S.

The serial calibrator solutions used for regressing/calculating the SFE recoveries of AP-TFA, for instance, under optimal conditions were prepared as follows: An appropriate amount of AP-TFA in EA (1, 5, 10, 50, 100 μL of 1000-μg/mL solution) was further diluted with ca. 1 mL of EA. The resulting solution was transferred to a 2.5-mL extraction cell that had previously been filled with celite and glass beads. After removal of EA under vacuum, the cell was subjected to SFE as is described for simultaneous SFE-ChD in the Experimental Section. Also, a 1-μL aliquot of the resulting EA solution was injected for each GC/EIMS analysis.

*Taguchi's procedure for optimizing the experimental conditions of simultaneous SFE-ChD*

Taguchi's procedure followed in this study for the title evaluation is described as follows: (1) Select the

major influential factors involved in this study from both theoretical and empirical viewpoints, and set their respective levels (low, medium, and high) accordingly and appropriately. (2) Construct an appropriate orthogonal table. (3) Perform the respective simultaneous SFE-ChD experiments in triplicate under the various conditions listed in the orthogonal table (the two analytes were treated separately), and calculate the relevant recoveries and means. (4) Justify the above influential factors using an *F*-test. (5) Recognize the optimal experimental conditions (levels) that achieve the optimum recovery among those listed in the orthogonal table. (6) Further justify the above optimal levels by comparing the respective sums of "iso-level" recoveries. (7) Further justify the above optimal levels by comparing all the $\eta$ values resulting from various levels associated with the orthogonal table. [$\eta$ = S/N = $10 \times \log(1/\sigma^2)$, where S/N is signal-to-noise ratio, and $\sigma$ is standard deviation. Efforts were made to maximize the $\eta$ value and hence minimize the variance of recovery data.] (8) Under optimal experimental conditions, further validate the effectiveness of simultaneous SFE-ChD by comparing its resulting recovery as well as precision with those obtained from stepwise ChD and SFE.

## Results and discussion

*Calibration curves*

As has been shown in our previous work, simultaneous SFE-ChD is a both accurate and precise sample-preparation method [32]. In this study, the simultaneous-SFE-ChD (method) calibration curves (equations: y = 0.557x + 0.015 for MA; y = 0.014x + 0.018 for AP) plotted according to the procedures described in the Experimental Section show comparable slopes (i.e., sensitivities) to those of the instrument ones (equations: y = 0.577x + 0.012 for MA; y = 0.014x + 0.017 for AP) implying that simultaneous SFE-ChD does give high method recoveries. Meanwhile, all the calibration curves show excellent linearity within the range under testing, i.e., 1-100 μg/mL, with correlation coefficients typically exceeding 0.999. These should have strongly based the results of the title evaluation.

*Selection of influential factors and their respective levels*

Based on our previous experience in related work and those experimental conditions reported by other researchers for separate SFE and ChD [5-31], we chose a) pressure, b) temperature, c) time given for static SFE, and d) amount of TFA added as the four factors to be investigated. These factors reportedly have appreciable interaction with one another, and are only fit for Taguchi's optimization process. As an empirical rule suggested by many pioneer researchers, three levels, i.e., high, medium, and low levels, were exclusively set for each of the four factors. Since carbon dioxide's critical point lies at 31.3˚C/1070 psi and most of the previous studies on SFE had adopted a pressure no lower than 3000 psi and temperature no lower than 70˚C, we therefore set 3000, 4000, and 5000 psi as the low, medium, and high levels, respectively, for pressure and 70, 80, and 90˚C (in the order of low to high level) for temperature. It was also from our previous experience that we set (in the order of low to high level) 5, 12.5, and 20 min as the three levels for the time-length of static SFE and 5, 100, and 200 μL for the amount of TFA.

*Optimization of the experimental conditions of simultaneous SFE-ChD*

The three-level $L_9$ ($3^4$) orthogonal table used for the optimization process and the corresponding recoveries of AP-TFA and MA-TFA obtained under the nine candidate conditions are displayed in Table 1. Apparently, entry VI tops all the other eight entries with regard to the recoveries of the TFA derivatives, thus leading to the selection of entry VI's levels as optimal conditions for routine use. This manner of selection of optimal conditions and hence the achievement of optimum recovery was also justified by two preliminary processes. First, when the ANOVA (i.e., analysis of variance) was applied to the data, the *F*-test at 95% level of confidence indicated that the four factors stated above were all prominent; that is, there were always significant differences among the nine means of recoveries. As shown in Table 2, the most influential factor was the amount of TFA added, with *F* value being 268.5 for AP-TFA and 39.3 for MA-TFA, compared to $F_{0.05} = 3.55$. Furthermore, by "optimal quality of recovery data" we meant to maximize the appropriate recoveries. When the four factors were set at their respective optimal levels, it was found that each of the factors did result in the maximized sum of "iso-level" recoveries. Taking AP-TFA in Table 3 as an example, the four maximized sums are 435.19% for 4000 psi, 427.64% for 90˚C, 465.61% for 5 min, and 600.18% for 100 μL; all are far larger than their second largest counterparts. Here, once again, the amount of TFA added appears to be the most influential factor. As the second preliminary procedure to justify the selection of the above four optimal levels, the so-called "response tables" in terms of signal-to-noise ratios, i.e., the $\eta$ values, were set up in an effort to maximize the appropriate $\eta$ values and hence to minimize the variances of recovery data. Judging from the equation "$\eta$ = S/N = $10 \times \log(1/\sigma^2)$" already mentioned in Experimental Section, the smaller the variance, $\sigma^2$, the larger the $\eta$ value, and, also in Taguchi's words, the closer the mean of quality gets to the goal of quality. It turns out that all the factors and lev-

**Table 1** The $L_9$ ($3^4$) orthogonal table used for the optimization of the experimental conditions of simultaneous SFE-ChD (the left 5 columns[a]) and the respective recoveries[b,c] of AP-TFA and MA-TFA resulting from the nine candidate conditions.

| Entry | Pressure (psi) | Temperature (˚C) | Time for static SFE (min) | Amount of TFA (μL) | Recovery (%) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | AP-TFA | | | MA-TFA | | |
| | | | | | run 1 | run 2 | run 3 | run 1 | run 2 | run 3 |
| I | L (3000) | L (70) | L (5) | L (5) | 1.69 | 2.17 | 1.93 | 0.25 | 0.23 | 0.68 |
| II | L | M (80) | M (12.5) | M (100) | 40.07 | 25.83 | 25.10 | 9.20 | 14.79 | 16.08 |
| III | L | H (90) | H (20) | H (200) | 44.65 | 56.72 | 43.45 | 22.71 | 21.52 | 24.55 |
| VI | M (4000) | L | M | H | 42.48 | 58.17 | 46.34 | 12.49 | 26.69 | 54.43 |
| V | M | M | H | L | 2.41 | 1.69 | 2.90 | 0.20 | 0.38 | 0.27 |
| VI[d] | M | H | L | M | 92.93 | 95.10 | 93.17 | 99.74 | 91.40 | 82.86 |
| VII | H (5000) | L | H | M | 65.84 | 78.36 | 83.78 | 14.73 | 17.34 | 6.80 |
| VIII | M | M | L | H | 64.69 | 55.52 | 58.41 | 57.19 | 54.34 | 48.28 |
| IX | H | H | M | L | 0.60 | 0.50 | 0.52 | 0.33 | 0.18 | 0.34 |

[a] L: low level; M: medium level; H: high level.
[b] All recoveries were calculated based on using 10 μL of 1000-μg/mL sample solution and 10 μL of 100-μg/mL I.S. solution.
[c] Trifluoroacetylamphetamine (AP-TFA) was used as the I.S. for the recovery calculation of MA-TFA, and trifluoroacetylmethamphetamine (MA-TFA) as that of AP-TFA.
[d] The optimal experimental conditions.

**Table 2** The ANOVA table resulting from the simultaneous SFE-ChD of AP • $H_2SO_4$ and MA.HCl under various experimental conditions.

| Analyte | Factor | Sum square of inter-level deviations | Degrees of freedom | Mean square | *F* value | $F_{0.05}$ |
|---|---|---|---|---|---|---|
| AP • $H_2SO_4$ | Pressure | 2132.7 | 2 | 1066.3 | 28.7 | 3.55 |
| | Temperature | 1052.7 | 2 | 526.4 | 14.2 | |
| | Time for static SFE | 2440.0 | 2 | 1220 | 32.8 | |
| | Amount of TFA | 19954.5 | 2 | 9977.3 | 268.5 | |
| | Residual error | 668.8 | 18 | 37.2 | | |
| | Total | 26248.7 | 26 | | | |
| MA • HCl | Pressure | 3222.8 | 2 | 1611.4 | 16.2 | 3.55 |
| | Temperature | 2063.2 | 2 | 1031.6 | 10.4 | |
| | Time for static SFE | 6414.1 | 2 | 3207.1 | 32.2 | |
| | Amount of TFA | 7811.3 | 2 | 3905.7 | 39.3 | |
| | Residual error | 1790.5 | 18 | 99.5 | | |
| | Total | 21301.9 | 26 | | | |

**Table 3** Sums of iso-level[a] recoveries of AP-TFA and MA-TFA calculated for the four influential factors under which the respective simultaneous SFE-ChD's of AP • $H_2SO_4$ and MA • HCl were carried out.

| Analyte | Pressure | | Temperature | | Time for static SFE | | Amount of TFA | |
|---|---|---|---|---|---|---|---|---|
| | Level | Sum of iso-level recoveries | Level | Sum of iso-level recoveries | Level | Sum of iso-level recoveries | Level | Sum of Iso-level recoveries |
| AP • $H_2SO_4$ | L | 241.6 | L | 380.8 | L | 465.6 | L | 14.4 |
| | M[b] | 435.2 | M | 276.6 | M | 239.6 | M | 600.2 |
| | H | 408.2 | H | 427.6 | H | 379.8 | H | 470.4 |
| MA • HCl | L | 110.0 | L | 133.6 | L | 435.0 | L | 2.9 |
| | M[b] | 368.5 | M | 200.7 | M | 134.5 | M | 352.9 |
| | H | 199.5 | H | 343.6 | H | 108.5 | H | 322.2 |

[a] L: low level; M: medium level; H: high level.
[b] The optimal combination of levels for both analytes: pressure, M (4000 psi); temperature (90˚C); time given for static SFE, L (5 min); amount of TFA added, M (100 μL).

**Table 4** The response table in terms of signal-to-noise ratios, i.e., the $\eta$ values, set up for the four influential factors under which the respective simultaneous SFE-ChD's of AP • $H_2SO_4$ and MA • HCl were carried out.

| Analyte | Factor | Level* | | | Spread of $\eta$ values | Optimal level based on this table |
|---|---|---|---|---|---|---|
| | | L | M | H | | |
| AP • $H_2SO_4$ | Pressure | 15.13 | 16.80 | 20.20 | 5.07 | H |
| | Temperature | 17.30 | 14.90 | 19.93 | 5.03 | H |
| | Time for static SFE | 21.17 | 15.57 | 15.40 | 5.77 | L |
| | Amount of TFA | 16.63 | 17.53 | 17.97 | 1.34 | H |
| MA • HCl | Pressure | 12.53 | 8.33 | 12.80 | 4.47 | H |
| | Temperature | 4.27 | 14.07 | 15.33 | 11.06 | H |
| | Time for static SFE | 12.37 | 7.83 | 13.47 | 5.64 | H |
| | Amount of TFA | 7.50 | 10.33 | 15.83 | 8.33 | H |

* L: low level; M: medium level; H: high level.

els showed only a minor effect on the variances of recoveries (Table 4). Therefore, it should still be acceptable if any of the final choices of the optimal levels gives only the second largest or even the smallest $\eta$ value.

*Validation of optimized simultaneous SFE-ChD*

At the optimal levels of the four factors, the effectiveness of the combined SFE-ChD was further validated by performing additional triplicate measurements and comparing the resulting recoveries and precisions with those obtained from two stepwise procedures (Table 5). Since the overall efficiency of stepwise ChD-SFE is equal to the yield of the ChD step times the recovery of the SFE step and our actually achieved TFA-derivatization yields were always nearly 100% (i.e., greater than 99%), it follows that simultaneous ChD-SFE (i.e., procedure A) typically gave higher recoveries than stepwise ChD-SFE (i.e., procedure B preceded by TFA-derivatization). The superiority of simultaneous ChD-SFE to stepwise ChD-SFE in efficiency is attributed to the unreacted TFA in the former procedure that considerably strengthens the extracting power of $CO_2$ fluid as was evidenced by the also better recoveries resulting from procedure C where another 5 µL of TFA (functioning as "modifier") was purposely added to the $CO_2$ fluid with other conditions being the same as those of procedure B. As to the precisions calculated for the recoveries of AP-TFA and MA-TFA after simultaneous SFE-ChD, the two relative standard deviations (RSDs) or coefficients of variance (CVs) listed in Table 5 for procedure A based upon triplicate analyses, 3.8% and 4.0%, are both indicative of the reliability of the proposed method.

**Table 5** The recoveries[a,b] of AP-TFA and MA-TFA and the relevant precisions achieved by following three different procedures.

| Experimental procedure[c] | No. of run | AP-TFA (%) | MA-TFA (%) |
|---|---|---|---|
| Procedure A[d] | run 1[e] | 92 | 85 |
| | run 2[e] | 99 | 92 |
| | run 3[e] | 94 | 90 |
| | | $\overline{X}$ = 95 | $\overline{X}$ = 89 |
| | | sd = 3.6 | sd = 3.6 |
| | | rsd = 3.8 | rsd = 4.0 |
| Procedure B[f] | run 1 | 78 | 83 |
| | run 2 | 90 | 82 |
| | run 3 | 87 | 83 |
| | | $\overline{X}$ = 85 | $\overline{X}$ = 83 |
| | | sd = 6.2 | sd = 0.6 |
| | | rsd = 7.3 | rsd = 0.7 |
| Procedure C[g] | run 1 | 92 | 85 |
| | run 2 | 94 | 83 |
| | run 3 | 94 | 84 |
| | | $\overline{X}$ = 93 | $\overline{X}$ = 84 |
| | | sd = 1.2 | sd = 1.0 |
| | | rsd = 1.2 | rsd = 1.2 |

[a] All recoveries were calculated based on using 10 µL of 1000-µg/mL sample solution and 10 µL of 100-µg/mL I.S. solution.

[b] Trifluoroacetylamphetamine (AP-TFA) was used as the I.S. for the recovery calculation of MA-TFA, and trifluoroacetylmethamphetamine (MA-TFA) as that of AP-TFA.

[c] Only key conditions are described.

[d] Procedure A (the proposed procedure): (1) Add 100 µL of TFA to underivatized sample and perform simultaneous static SFE-ChD under 4000 psi at 90˚C for 5 min; (2) Add I.S.[a] prior to GC-MS analysis.

[e] These three runs were performed in addition to those three denoted "entry VI" in Table 1 and served as a confirmatory test, all six runs being under the optimal experimental conditions.

[f] Procedure B: (1) Perform static SFE on ready-made AP-TFA and MA-TFA under 4000 psi at 90˚C for 5 min; (2) Add I.S.[f] prior to GC-MS analysis.

[g] Procedure C: (1) Perform static SFE on ready-made AP-TFA and MA-TFA under 4000 psi at 90˚C for 5 min with another 5 µL of TFA added; (2) Add I.S.[f] prior to GC-MS analysis.

## Conclusions

Our previous report has demonstrated that simultaneous SFE and ChD followed by isotope dilution GC-MS is a sound analytical scheme for the determination of AP and MA in urine and meets the criteria adopted by the U.S. HHS and DoD (Department of Defense) drug testing programs. This paper by systematically and successfully applying Taguchi's quality optimization to the promising analytical scheme further strengthens the base and broadens the usefulness of both methods. Although for clarifying the questions of interest, D.I. water instead of human urine was used as the sample matrix at the evaluation stage, the title approach and its results apply to urinary matrix as has been demonstrated by the analyses of a number of real-case samples [32]. From the viewpoint of academic or basic research, it is hoped that Taguchi's method can be challenged with more forensic or non-forensic experiments and tested against more specimens with multiple types of analytes and matices.

## Acknowledgments

Financial support by the National Science Council of the Republic of China under grant No. NSC-88-2113-M-015-001 is gratefully acknowledged.

## References

1. Tsai M-S. *Design of Experiments*. Taipei, Taiwan, ROC. Hua-Tai Co. Ltd., 1991.
2. Taguchi G. *Introduction to Quality Engineering*. APO. 1986.
3. Lee Y-T. *Taguchi's Quality Engineering*. Taipei, Taiwan, ROC. Ko-Chien Co. Ltd., 1993.
4. Taguchi G. Off-line and on-line quality control systems. Tokyo, Japan. *Proc. Intern. Conf. on Quality Control*, 1978.
5. Messer DC, Taylor LT. Development of analytical SFE of a polar drug from an animal food matrix. *HRC* 1992;15:238-41.
6. Mulcahey LJ, Taylor LT. Collection efficiency of solid-surface and sorbent traps in supercritical fluid extraction with modified carbon-dioxide. *Anal Chem* 1992;64:981-4.
7. Edder P, Staub C, Veuthey JL, Haerdi W. Supercritical fluid extraction of opiates in hair of drug-addicts. *J Chromatogr B* 1994;658:75-86.
8. Scott KS, Oliver JS. Development of a supercritical-

fluid extraction method for the determination of temazepam in whole-blood. *J Anal Toxicol* 1997;21: 297-300.
9. Simmons BR, Stewart JT. Supercritical-fluid extraction of selected pharmaceuticals from water and serum. *J Chromatogr B* 1997;688:291-302.
10. Hartonen K, Riekkola ML. Detection of beta-blockers in urine by solid-phase extraction-supercritical fluid extraction and gas-chromatography mass-spectrometry. *J Chromatogr B* 1996;676:45-52.
11. Allen DL, Scott KS, Oliver JS. Comparison of solid-phase extraction and supercritical fluid extraction for the analysis of morphine in whole blood. J Anal Toxicol 1999;23:216-8.
12. Cross RF, Ezzell JL, Richter BE. The supercritical-fluid extraction of polar drugs (sulfonamides) from inert matrices and meat animal products. *J Chromatogr Sci* 1993;31:162-9.
13. McAvoy Y, Dost K, Jones DC, Cole MD, Geroge MW, Davidson G. Analysis of amphetamines by supercritical fluid chromatography, high-performance liquid chromatography, gas chromatography and capillary zone electrophoresis; a preliminary comparison. *Forensic Sci Int* 1999;99:13-22.
14. Slack C, McNair HM, Wasserzug L. Characterization of Semtex by supercritical fluid extraction and off-Line GC-ECD and GC-MS. *HRC* 1992;15:102-4.
15. Yinon J, Zitrin S. *Modern Methods and Applications in Analysis of Explosives*. UK.: John Wiley, Chichester, 1993.
16. Barnabas IJ, Dean JR, Hitchen SM, Owen SP. Supercritical-fluid extraction of organochlorine pesticides from an aqueous matrix. *J Chromatogr A* 1994;665:307-15.
17. Lopez-Avila V, Dodhiwala NS, Beckert WF. Supercritical fluid extraction and its application to environmental analysis. *J Chromatogr Sci* 1990;8: 468-76.
18. Howard AL, Taylor LT. Quantitative supercritical fluid extraction of sulfonyl urea herbicides from aqueous matrices via solid-phase extraction disks. *J Chromatogr Sci* 1992;30:374-82.
19. Langenfeld JJ, Hawthorne SB, Miller DJ, Pawliszyn J. Effects of temperature and pressure on supercritical fluid extraction efficiencies of polycyclic aromatic-hydrocarbons and polychlorinated-biphenyls. *Anal Chem* 1993;65:338-44.
20. Cammann K, Kleiböhmer W. Supercritical fluid chromatographic- separation of polychlorinated-biphenyls (PCBs) using a negative temperature-gradient for density programming. *HRC* 1991;14:327-9.
21. Onuska FI, Terry KA. Supercritical fluid extraction of 2,3,7,8-tetrachlorodibenzo-para-dioxin from sediment samples. *HRC* 1989;12:357-61.
22. Onuska FI, Terry KA. Supercritical fluid extraction of PCBs in tandem with high-resolution gas-chromatography in environmental analysis. HRC 1989; 12:527-31.
23. Hawthorne SB, Krieger MS, Miller DJ. Supercritical carbon-dioxide extraction of polychlorinated-biphenyls, polycyclic aromatic-hydrocarbons, heteroatom-containing polycyclic aromatic-hydrocarbons, and *n*-alkanes from polyurethane foam sorbents. *Anal Chem* 1989;61:736-40.
24. Hills JW, Hill Jr. HH, Maeda T. Simultaneous supercritical fluid derivatization and extraction. *Anal Chem* 1991;63:2152-5.
25. Bertsch W, Zhang QW. Sample preparation for the chemical analysis of debris in suspect arson cases. *Anal Chem Acta* 1990;236:183-95.
26. Vella AJ. Arson investigation using the ion trap detector. *J Forensic Sci Soc* 1992;32:131-42.
27. Lopez-Avila V, Benedicto J, Dodhiwala NS, Young R, Beckert WF. Development of an off-line SFE-IR method for petroleum-hydrocarbons in soils. *J Chromatogr Sci* 1992;30:335-43.
28. Emery AP, Chesler SN, MacCrehan WA. Recovery of diesel fuel from clays by supercritical fluid extraction gas-chromatography. *J Chromatogr* 1992;606: 221-8.
29. Hawthorne SB. Analytical-scale supercritical fluid extraction. *Anal Chem* 1990;62:633A.
30. Lin Y, Wai CM, Jean FM, Brauer RD. Supercritical-fluid extraction of thorium and uranium ions from solid and liquid materials with fluorinated beta-diketones and tributyl-phosphate. *Environ Sci Technol* 1994;28:1190-3.
31. Lin Y, Brauer RD, Laintz KE, Wai CM. Supercritical-fluid extraction of lanthanides and actinides from solid materials with a fluorinated beta-diketone. *Anal Chem* 1993;65:2549-51.
32. Wang S-M, Giang Y-S, Ling Y-C. Simultaneous supercritical fluid extraction and chemical derivatization for the gas chromatographic-isotope dilution mass spectrometric determination of amphetamine and methamphetamine in urine. *J Chromatogr B* 2001;759:17-26.