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A stir bar sorptive extraction device coupled with a gas chromatography flame ionization detector for the determination of abused prescription drugs in lean cocktail samples†

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A lean cocktail is a mixed drink for the non-medical use of prescription medications that has emerged in recent years as a drug of abuse and is related to drug-facilitated crimes. The determination of active ingredients in a lean cocktail is necessary for forensic investigations. This work presents an in-house developed stir bar sorptive extraction (SBSE) device with an XAD-2 adsorbent followed by analysis using GC-FID for the extraction and determination of the five main abused prescription drugs (diphenhydramine, tramadol, chlorpheniramine, dextromethorphan and promethazine) in lean cocktail samples. Under optimized conditions, the developed method provided linearity for 1.0–250 μg mL^{-1} of each of the five abused prescription drugs. The limits of detection and limits of quantitation were in the respective ranges of 0.25–0.5 μg mL^{-1} and 1.0–1.5 μg mL^{-1}. The percentage of extraction was 85.0–94.9%. The intra-day and inter-day precisions were 1.2–14.4% RSD and 1.4–15.8% RSD, respectively. Good relative recoveries in the range of 86.7–110.3% and 88.5–107.9% were obtained when the proposed method was applied for extraction and analysis of abused prescription drugs in five lean cocktail samples. The developed method can be a useful tool for measuring the levels of abused prescription drugs in a lean cocktail and the data could also be used as evidence in a forensic investigation.

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1. Introduction

Abused prescription drugs cause serious health and social problems.[1] The three classes of prescription drugs that are the most abused are opioids, central nervous system (CNS) depressants, and stimulants.[2] An emerging trend of prescription drug abuse is in the form of lean cocktails, also known as purple drank, sizzurp, the South Asian cocktail, and dirty sprite. The drinks can contain cough syrup containing codeine and promethazine, soft drinks, sweets, and alcohol beverages.[3] During the 1960s, the lean cocktail emerged in American hip-hop culture and its popularity expanded to Eastern countries, including Thailand. Some Thai teenagers and high school students use medicines for cough and cold, which are easy to purchase from a drug store, to mix with soft drinks. The drink mixture may contain tramadol, promethazine, chlorpheniramine, dextromethorphan and diphenhydramine (see chemical structures in Fig. 1(A)) which can produce an additive effect. Additionally, these types of drugs, especially when coupled with alcohol, produce depressant effects. Consequently, drinking lean cocktails creates euphoria, dizziness, and drowsiness.[4] Drug addiction may result from long-term use. An overdose may cause life threatening consequences including seizure, somnolence, respiratory and cardiac failure, coma, and death.[3] Owing to the threat of prescription drug misuse, detection of active

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ingredients in a lean cocktail and in biological fluids is necessary for forensic investigations.

Several methods for analysis of tramadol, promethazine, chlorpheniramine, dextromethorphan and diphenhydramine have been reported, using spectrophotometry,[5–9] high performance liquid chromatography (HPLC),[10] gas chromatography (GC),[11] capillary electrophoresis (CE),[12] and electrochemical techniques.[13] However, these reported techniques have some limitations. For example, spectrophotometry is not suitable for simultaneous detection of all of the compounds in the sample. Capillary electrophoresis requires time to condition the capillary tube to maintain adequate reproducibility of run-to-run injections. Limitations of electrochemical methods include their lack of specificity, formation of biofilms and fouling. Chromatographic analyses including GC and HPLC are the most commonly used techniques because they provide high sensitivity and selectivity. However, direct sample injection might not be suitable for quantifying the abused prescription drugs in lean cocktails or biological fluids (such as urine or whole blood) due to the following reasons: The concentrations of these compounds in the samples are relatively low or the matrices in the samples are often complex. Accordingly, sample preparation is required prior to analysis to pre-concentrate the analyte and to eliminate interferences from sample matrices. The development of sample preparation techniques has been reported for the pre-concentration and extraction of main prescription drugs and some additive drugs, such as the dilute-and-shoot procedure with an appropriate solvent,[14] liquid–liquid extraction (LLE),[11] liquid phase microextraction (LPME),[15] dispersive liquid–liquid microextraction (DLLME),[16] and solid-phase extraction (SPE).[17] Among these methods, stir bar sorptive extraction (SBSE) has attracted much attention for applications in extraction procedures, due to its high effectiveness in extracting non-polar and medium-polarity compounds from liquid samples.[18] SBSE is capable of extracting and enriching compounds from liquid matrices. Moreover, an SBSE bar can be reused after a simple washing operation. An SBSE device has been commonly prepared using polydimethylsiloxane (PDMS) coated onto a glass-enveloped magnet as the sorbent.[19] Nevertheless, an SBSE device coated with PDMS fails to extract highly polar compounds. To overcome this drawback, a new design of SBSE using a stainless-steel net containing a polymeric sorbent with Teflon caps in a dumbbell shape has been introduced for phthalate ester extraction from food samples.[20] The stainless steel net dumbbell-shaped stir-bar allows the flow of solution in every direction, even from the bottom of the bar, facilitating good interaction of samples with the sorbent packed inside the stir-bar. This type of the SBSE device is easy to use and operate and available for several sorbent materials. Additionally, the Amberlite XAD-2 adsorbent is a hydrophobic crosslinked polystyrene copolymer resin with a large surface area and a chemically homogeneous non-ionic structure that is widely used to extract acidic, neutral and basic drugs from biological samples.[21] This proposed polymeric material has high potential to be used as a sorbent for the extraction of five abused prescription drugs.

In this work, a dumbbell shaped SBSE device with an XAD-2 adsorbent was in-house constructed for sample preparation, followed by analysis using GC-FID for determination of the five abused prescription drugs. The experimental parameters that affect the extraction efficiency using SBSE with the XAD-2 adsorbent were investigated. The developed method was

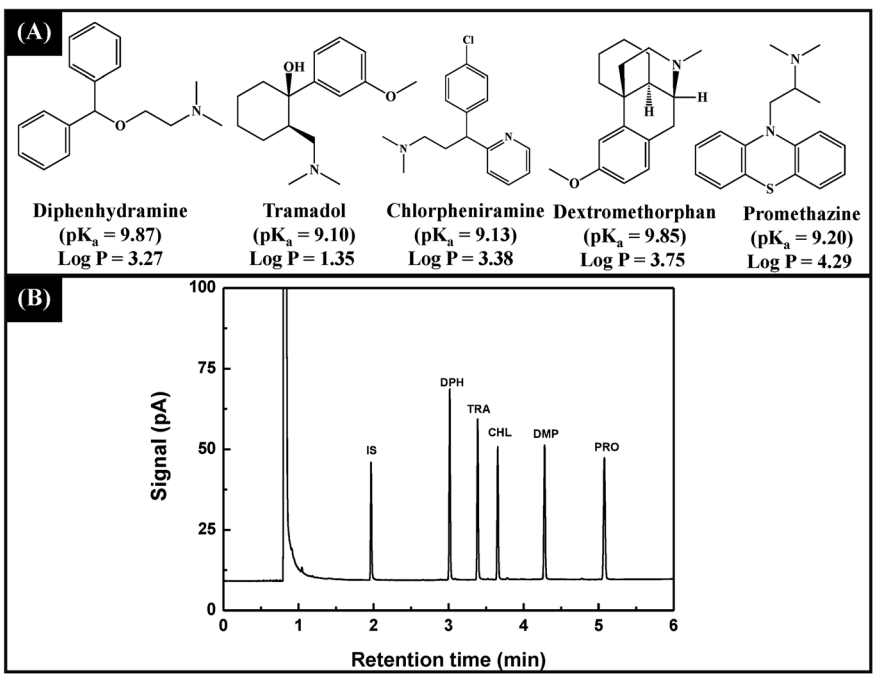

Fig. 1 (A) Chemical structures of diphenhydramine, tramadol, chlorpheniramine, dextromethorphan and promethazine. (B) Chromatographic separation of 50 μg mL^{-1} of a standard mixture of the internal standard (IS), diphenhydramine (DPH), tramadol (TRA), chlorpheniramine (CHL), dextromethorphan (DMP) and promethazine (PRO).

validated and applied to the determination of abused prescription drugs in lean cocktail samples.

2. Experimental

2.1 Chemicals and reagents

Tramadol hydrochloride (purity ≥99.0%) was purchased from Sigma-Aldrich (St. Louis, USA). Promethazine hydrochloride (purity ≥98.0%), chlorpheniramine maleate, (purity ≥99.0%) and diphenhydramine hydrochloride (purity ≥99.0%) were purchased from Tokyo Chemical Industry (Tokyo, Japan). Dextromethorphan hydrobromide monohydrate (purity ≥99.0%) was purchased from Sigma-Aldrich (St. Louis, USA). Diphenylamine (purity ≥99.0%) was purchased from Loba Chemie Pvt. Ltd (Mumbai, India). Analytical grade methanol and ethanol were purchased from Merck (Darmstadt, Germany). Acetonitrile was purchased from Loba Chemie Pvt. Ltd (Mumbai, India). Ethyl acetate was obtained from QRëC (New Zealand). Hydrochloric acid and sodium hydroxide were purchased from Merck (Darmstadt, Germany). A Teflon rod with a diameter of 6.0 mm was purchased from a local shop in Thailand. A metal rod with a strand diameter of 0.25 mm was obtained from Tianjin Xingang (Hebei, China). Amberlite XAD-2 adsorbent powder was purchased from Merck (Darmstadt, Germany). Ultrapure water (18.0 MΩ cm) was obtained from Merck Millipore Simplicity® Water Purification Systems (Darmstadt, Germany). Helium (UHP), nitrogen (UHP), air zero (HP) and hydrogen (HP) gases were supplied by TIG, Thailand.

2.2 Chromatographic conditions

A 6890 N gas chromatograph (GC) equipped with a split/splitless injector and a flame ionization detector was used to conduct the analyses with a liquid autosampler. A capillary HP-5 column (30 m × 0.25 mm id × 0.25 μm thickness) (Agilent Technologies, Palo Alto, CA, USA) was used for separation. The chromatographic conditions were modified from the previous report.[14] Helium (He) was used as the carrier gas with a flow rate of 1.0 mL min^{-1}. Nitrogen (N_2) was used as the makeup gas for the FID detector with a flow rate of 25 mL min^{-1}. Both hydrogen (H_2) and the oxidant gas (air zero) were controlled to the respective flow rates of 30 mL min^{-1} and 300 mL min^{-1}. The injection volume was fixed at 1 μL.

All the runs were operated in split injection mode (10 : 1 split ratio) with the injector temperature set at 260 °C. The oven temperature program was set at an initial temperature of 180 °C for 1 min, then ramped up at a rate of 20 °C min^{-1} to 240 °C that was held for 1 min. Finally, the temperature was increased to 280 °C with a temperature ramp rate of 20 °C min^{-1} and held at this temperature for 1 min before cooling down to the initial temperature for the next injection. The signals of separated target analytes were detected by FID at 280 °C. Agilent Chemstation 4.01 software (Agilent Technologies) was employed for both instrument operation and data analysis.

2.3 Preparation of standard solutions

Stock standard solutions (10 mg mL^{-1}) of diphenhydramine, tramadol, chlorpheniramine, dextromethorphan, promethazine and diphenylamine (internal standard) were prepared by dissolving the standard compounds in methanol. The stock solutions were further diluted with methanol to obtain working standard solutions at concentrations of 5 mg mL^{-1}. A mixture of standards (500 μg mL^{-1} of each compound) was prepared daily in methanol. The stock solution of the internal standard was also diluted with pure methanol to a concentration of 25 μg mL^{-1}. A mixture of standards was kept in a refrigerator at 4 °C until needed. Soft drink was used as a matrix of blank samples and of calibration standard mixtures including spiked samples which were used throughout the method development and validation.

2.4 In-house preparation of an SBSE device

An in-house made SBSE device was prepared following the procedure developed by Sukree and co-workers.[20] The preparation procedure is presented in Fig. S1.† Briefly, a square sheet of stainless steel net with dimensions of 1.8 cm × 1.8 cm was prepared and rolled up to produce a tube like shape (approximately 0.4 cm I.D.). To make caps, a Teflon rod was drilled into sections of 0.6 cm O.D., 0.4 cm I.D., and 0.2 cm depth. A metal rod (0.2 cm diameter and 1.8 cm length) was employed by inserting it in the middle to create a magnetic bar so that the device would stir when placed on a magnetic stirrer. Then, the Amberlite XAD-2 adsorbent (60 mg; 60 mesh size) was filled into the open end of the stainless steel tube before it was capped with another Teflon lid to secure the content. Finally, the in-house made stainless steel net dumbbell shaped stir-bar was obtained. Prior to use, the dumbbell-shaped-SBSE device was cleaned up and conditioned. For clean-up and conditioning, the SBSE device was used to stir 4.0 mL of acetone to eliminate any residues, followed by conditioning in 4.0 mL of ethanol and in 10.0 mL of ultrapure water to enhance the wettability of the adsorbent. Afterwards, the in-house made SBSE device was ready for use in the simultaneous extraction of the abused prescription drugs in lean cocktail samples.

2.5 Stir bar extraction procedure

The in-house made SBSE device was applied to the simultaneous extraction of abused prescription drugs (diphenhydramine, chlorpheniramine, tramadol, dextromethorphan, promethazine and an internal standard) in lean cocktail samples. The sample solution (2.0 mL) was adjusted to pH 12 with 1.0 M NaOH after the addition of 50 μL internal standard (final concentration 25 μg mL^{-1}). A 10% w/v of NaCl was added into the lean cocktail sample. The SBSE device was placed into the vial (20 mL) containing 2.0 mL of the sample solution. Then the sample was stirred at 750 rpm and room temperature for 30 min. Subsequently, the SBSE device was removed and dried using a lint-free tissue to remove water droplets. Analytes had been adsorbed into the Amberlite XAD-2 adsorbent in the stainless steel mesh tube. After extraction, the absorbed analytes were desorbed using 2.0 mL acetonitrile with stirring for 5 min. The desorption solvent was dried in a stream of nitrogen. The injection volume was fixed at 1 μL. The residue was reconstituted in 1.0 mL of methanol (see Fig. 2).

2.6 Method validation

To validate the method, the following analytical characteristics were evaluated: linearity and range, limit of detection, limit of quantitation, precision, accuracy, and selectivity. The validation was conducted in accordance with the International Conference on Harmonization (ICH) tripartite guideline validation of analytical procedures: text and methodology Q2(R1).[22] The reproducibility of in-house SBSE device preparations was evaluated based on the AOAC official methods of analysis, guidelines for standard method performance requirements, appendix F.[23]

The selectivity of the method was evaluated by checking for interference by commonly abused prescription drugs in lean cocktails. Lean cocktail samples spiked with five abused prescription drugs and an internal standard were subjected to sample preparation procedures. The concentrations of five abused prescription drugs and the internal standard were 50 μg mL^{-1} and 25 μg mL^{-1}, respectively. Each sample was analyzed to detect chromatographic interference.

The linearity and working range were evaluated by analyzing five replicates of calibration standard mixtures in the concentration range of 0.25–250 μg mL^{-1} for all abused prescription drugs. There were seven concentrations of each analyte compound. The signals of the analyte (y axis) were plotted against the known concentrations (x axis). The linear relationship was analyzed by the least squares method.

The limits of detection and quantitation were determined based on analysis of the signals from the samples spiked with known concentrations of the analyte along with those from blank samples, and an examination of the signal-to-noise ratio. The limits of detection and limits of quantitation were obtained for respective signal-to-noise ratios of 3 and 10.

The precision and accuracy of the proposed method for the extraction and analysis of abused prescription drugs were determined by examining the intra-day and inter-day reproducibility. The intra-day and inter-day precision were determined by extracting and analyzing standard quality control samples (LOQ, low, medium and high concentrations) at the following concentrations: diphenhydramine, tramadol, dextromethorphan, and promethazine each at 1.0, 2.5, 50, and 100 μg mL^{-1} and chlorpheniramine each at 1.5, 2.5, 50, and 100 μg mL^{-1} for lean cocktail samples.

The extraction and analysis were performed within one day using five replicates of each sample and over five consecutive days using five replicates of each sample per day. The precision of the developed method is expressed as relative standard deviation (% RSD). The intra-day and inter-day accuracy of the proposed method were also determined using the standard quality control samples with five replicates for each concentration. Accuracy is expressed in terms of relative recovery percentage, which was calculated from the following equation: relative recovery (%) = $(C_A - C_B)/C_C \times 100$, where C_A is the total concentration of the prescription drug found in the sample after the addition of the standard solution, C_B is the original concentration of the prescription drug in real samples, and C_C is the concentration of the prescription drug spiked in the sample.

2.7 Analysis of lean cocktail samples

Five lean cocktail samples were obtained from high schools located in the Songkhla province of Southern Thailand. The

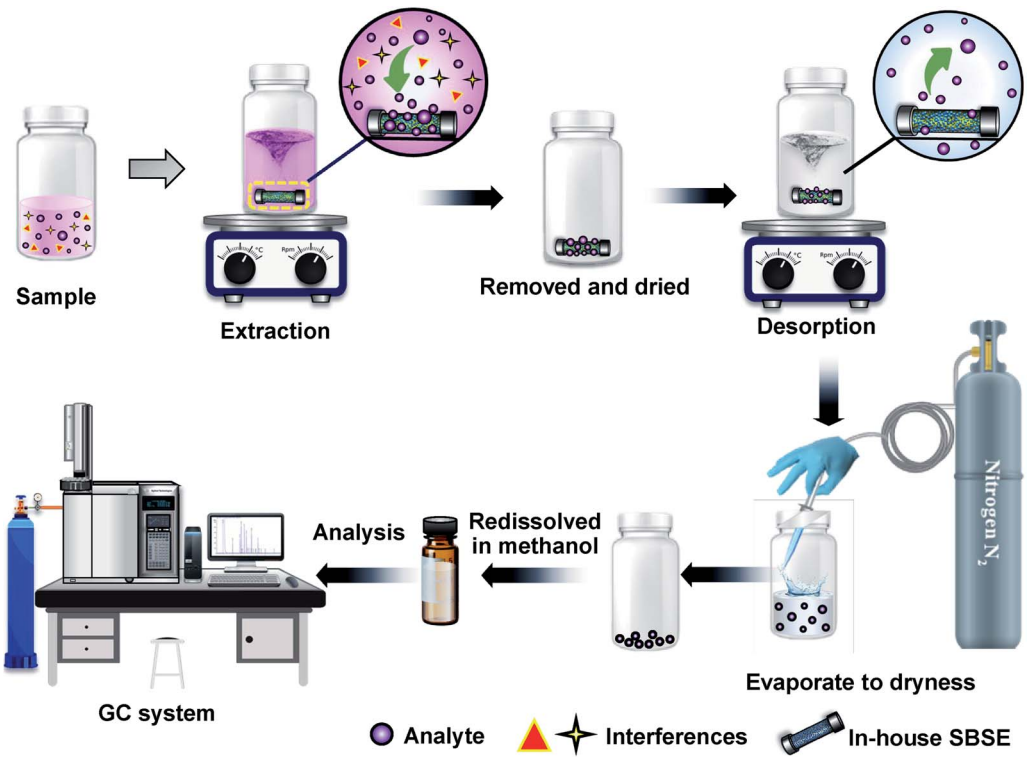

Fig. 2 A schematic diagram of the extraction of abused prescription drugs by using the in-house made SBSE device.

samples were kept at −4 °C until analysis. Prior to the analysis, each sample was defrosted at room temperature (*ca.* 25 °C) and three 5.0 mL aliquots were prepared. Each aliquot was degassed for 15 min and filtered through a filter disk (0.45 μm cellulose acetate) before extraction.

3. Results and discussion

3.1 GC-FID conditions

The chromatographic conditions employed in this work were modified from a previous report.[14] The GC-FID conditions for the determination of the five main abused prescription drugs (diphenhydramine, tramadol, chlorpheniramine, dextromethorphan and promethazine) were found to be flow rates of the carrier, make up, oxidant and fuel gases at 1.0, 25, 300 and 30 mL min^{-1}, respectively. All the runs were operated in split injection mode (10 : 1 split ratio). The injector and detector temperatures were 260 °C and 280 °C, respectively. A gradient temperature program was selected because it was more effective at simultaneously separating all of the abused prescription drugs than an isothermal system. The oven temperature program was set at an initial temperature of 180 °C for 1 min, then ramped up at a rate of 20 °C min^{-1} to 240 °C that was held for 1 min. Finally, the temperature was increased to 280 °C with a temperature ramp rate of 20 °C min^{-1} and held at this temperature for 1 min before cooling down to the initial temperature for the next injection. Under the optimal conditions, the chromatogram showed that all analytes were well separated with no interfering peaks (Fig. 1(B)). The analytes were sequentially eluted within 5.1 min in the order of first having the internal standard (t_R = 1.9 min), then diphenhydramine (t_R = 2.8 min), tramadol (t_R = 3.4 min), chlorpheniramine (t_R = 3.7 min), dextromethorphan (t_R = 4.3 min), and promethazine (t_R = 5.1 min). The total analysis time was less than 8.0 min. The resolution between two analyte peaks exceeded 1.5. The variation in the analysis precision of the peak area of each injection was less than 4.8%. The results indicated that our method produced acceptable resolution, and high efficiency and selectivity for the separation of abused prescription drugs in a lean cocktail.

3.2 Optimization of SBSE for extraction of abused prescription drugs

Various parameters that may influence the extraction efficiency were investigated to obtain the optimum sample preparation conditions. The parameters that were optimized were the amount of the XAD-2 adsorbent, the pH of the sample solution, type of desorption solvent, extraction time, stirring rate, salting out effect, desorption time, and desorption solvent volume. The initial extraction conditions were 2.0 mL of 50 μg mL^{-1} of diphenhydramine, tramadol, chlorpheniramine, dextromethorphan and promethazine with 25 μg mL^{-1} of internal standard solution (diphenylamine), 60 mg of the XAD-2 adsorbent, 30 min for the extraction time and 5 min for desorption time with a fixed stirring speed of 750 rpm and 2.0 mL of acetonitrile as desorption solvent. During the optimization study, the process was carried out by varying one variable being evaluated at a time, while the others were kept constant.

The optimization experiments were performed using spiked lean cocktail samples and the extraction efficiency was determined in terms of extraction recovery (% ER). The extraction recovery was calculated using the following formula:

$$\text{Extraction recovery (\% ER)} = [(C_f - C_u)/C_a] \times 100$$

where C_a is the calculated concentration of the analyte added to the tested sample, C_f is the concentration of the fortified and C_u is the concentration of the unfortified. The standard deviations of the recoveries are from three replicate experiments for each spiked sample.

3.2.1 Amount of the XAD-2 adsorbent. The effect of the amount of XAD-2 adsorbent filling in the stir bar was studied in the range from 30 to 60 mg. The results are presented in Fig. 3(A). The extraction efficiency increased with the amount of the XAD-2 adsorbent from 30 to 60 mg. The percentage recovery of the target analyte increased from 60.6 ± 4.0% to 80.6 ± 2.4% for diphenhydramine, from 55.1 ± 3.2% to 77.5 ± 2.0% for tramadol, from 64.1 ± 3.8% to 80.4 ± 2.5% for chlorpheniramine, from 56.0 ± 2.9% to 78.8 ± 2.4% for dextromethorphan, from 62.5 ± 3.6% to 77.0 ± 1.0% for promethazine and from 55.4 ± 4.2% to 75.4 ± 3.6% for the internal standard, on increasing the amount of the sorbent to 60 mg. The efficient adsorption of abused prescription drugs by the XAD-2 adsorbent was facilitated *via* π–π stacking and hydrophobic interactions. Increasing the amount of the adsorbent in the stir bar led to a higher surface area of the adsorbent, resulting in a higher extraction efficiency. Hence, 60 mg of the XAD-2 adsorbent was selected as the optimal amount, because it gave recoveries of all six compounds in the range from 75.4 ± 3.6 to 80.6 ± 2.4% with relative standard deviations below 5%.

3.2.2 pH of sample solution. The pH of sample solution is an important factor that could affect the extraction efficiency. Its influence was investigated by varying the sample solution pH among 4, 6, 8, 10, 12 and 14 by adjusting with HCl or NaOH. Fig. 3(B) shows that the extraction efficiency of the six target analytes increased with pH from 4 to 12. The sample pH had no obvious further effect when increased to 14. The maximum recoveries obtained at pH 12 were 76.8 ± 5.6% for diphenhydramine, 77.1 ± 2.8% for tramadol, 75.7 ± 2.8% for chlorpheniramine, 73.7 ± 1.6% for dextromethorphan, 76.7 ± 3.0% for promethazine and 73.6 ± 4.2% for the internal standard. This is possibly due to the pK_a values of target analytes. Diphenhydramine (pK_a, 8.87), tramadol (pK_a, 9.10), chlorpheniramine (pK_a, 9.13), dextromethorphan (pK_a, 9.85), promethazine (pK_a, 9.20) and the internal standard (pK_a, 1.00) are in unionized form in an alkaline environment. Therefore, such an environment facilitates the adsorption of all target analytes by the XAD-2 adsorbent *via* π–π stacking and hydrophobic interactions. The sample solution was adjusted to pH 12 in the subsequent experiments.

3.2.3 Type of desorption solvent. The adsorption of the abused prescription drugs onto the XAD-2 adsorbent is possibly based on π–π stacking and hydrophobic interactions. Therefore, desorption solvents with different polarities were

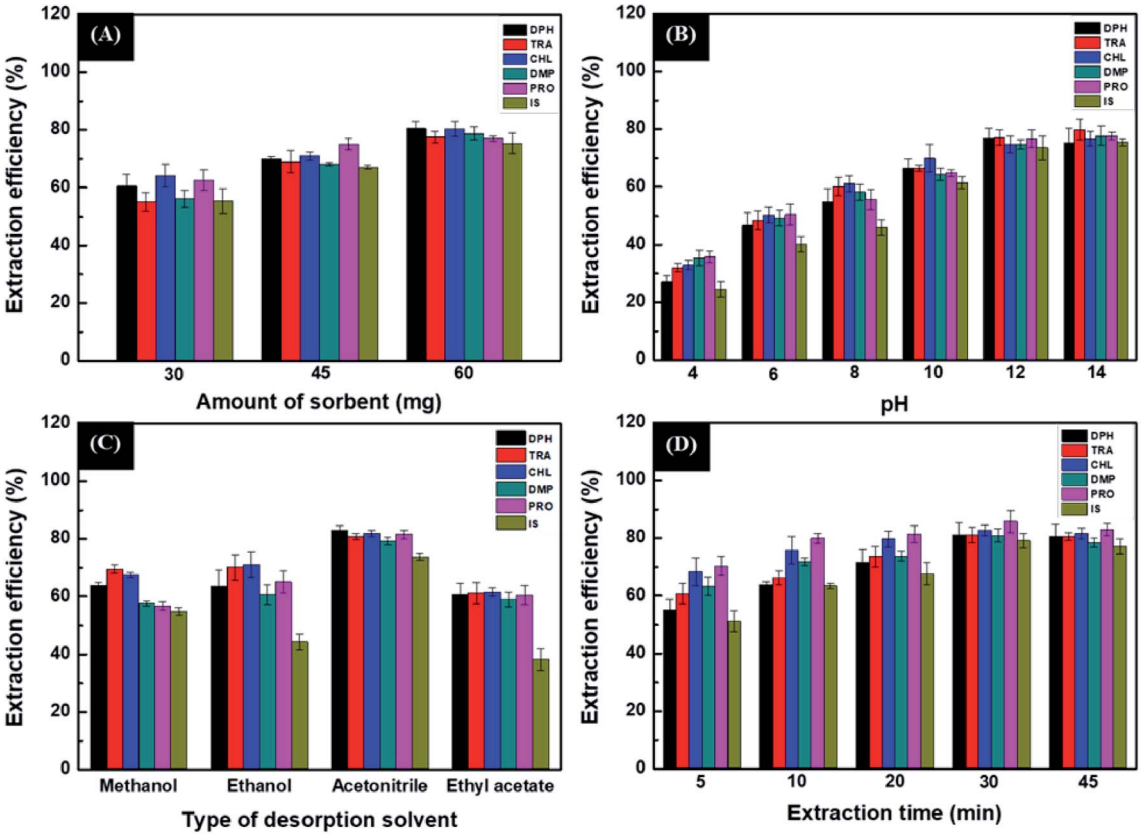

Fig. 3 Effects of (A) amount of the XAD-2 adsorbent, (B) pH of the sample, (C) type of desorption solvent, and (D) extraction time on the extraction efficiency of diphenhydramine (DPH), tramadol (TRA), chlorpheniramine (CHL), dextromethorphan (DMP), promethazine (PRO) and the internal standard (IS).

investigated including methanol (polarity index 5.1), ethanol (polarity index 5.2), acetonitrile (polarity index 5.8) and ethyl acetate (polarity index 4.4). The results showed that the highest extraction efficiency was obtained with acetonitrile because it can desorb all target analytes, with recoveries of 83.1 ± 1.5% for diphenhydramine, 80.8 ± 1.1% for tramadol, 82.0 ± 1.1% for chlorpheniramine, 79.2 ± 1.3% for dextromethorphan, 81.6 ± 0.5% for promethazine and 73.7 ± 1.2% for the internal standard (Fig. 3(C)). When methanol, ethanol or ethyl acetate was used as the elution solvent, the recoveries of all target analytes were in the range from 54.7 ± 1.0% to 69.5 ± 1.5%, from 44.4 ± 2.8% to 70.2 ± 4.0% and from 38.3 ± 3.8% to 61.5 ± 1.5%, respectively. Therefore, acetonitrile was selected as the desorption solvent for this work.

3.2.4 Extraction time. The time required for the adsorption of the target analytes from the sample solution onto the SBSE device is also an important parameter that can affect the extraction efficiency. The effect of the extraction time was investigated from 5 to 45 min. The results are illustrated in Fig. 3(D). As can be seen, the extraction efficiency of all analytes increased rapidly when the extraction time was increased from 5 to 30 min and remained almost constant with a further increase of extraction time to 45 min. At 30 min the recoveries reached their maximum level of 83.1 ± 1.5% for diphenhydramine, 81.2 ± 4.2% for tramadol, 79.0 ± 2.6% for chlorpheniramine, 82.7 ± 1.9% for dextromethorphan, 81.0 ± 5.2% for promethazine and 79.2 ± 2.5% for the internal standard. Therefore, an extraction time of 30 min was selected for further experiments.

3.2.5 Stirring speed. The stirring speed of the stir bar can accelerate the mass transfer helping to shorten equilibration time during extraction/desorption steps of all target analytes from sample media to the XAD-2 adsorbent and *vice versa*. The effect of the stirring speed on the extraction efficiency in the range from 500 to 1000 rpm was investigated. The results shown in Fig. 4(A) present the recoveries of all target analytes that increased from 58.6 ± 1.6% to 82.0 ± 2.5% for diphenhydramine, from 58.4 ± 1.7% to 80.7 ± 2.0% for tramadol, from 63.1 ± 1.5% to 82.0 ± 2.5% for chlorpheniramine, from 57.8 ± 3.0% to 80.2 ± 2.3% for dextromethorphan, from 62.0 ± 3.9% to 81.6 ± 1.0% for promethazine and from 59.4 ± 2.8% to 74.7 ± 3.6% for the internal standard on increasing the stirring rate to 750 rpm, and then leveled off. Therefore, a stirring rate of 750 rpm was used in the next experiments.

3.2.6 Salting out effect. Salt was added to the aqueous sample to decrease the target analyte solubility and to increase the partition of analytes into the sorbent. The addition of NaCl was tested between 0 and 20% (w/v). The results in Fig. 4(B) indicate that the recovery percentages of the six target analytes increased with the concentration of NaCl from 0 to 10% (from

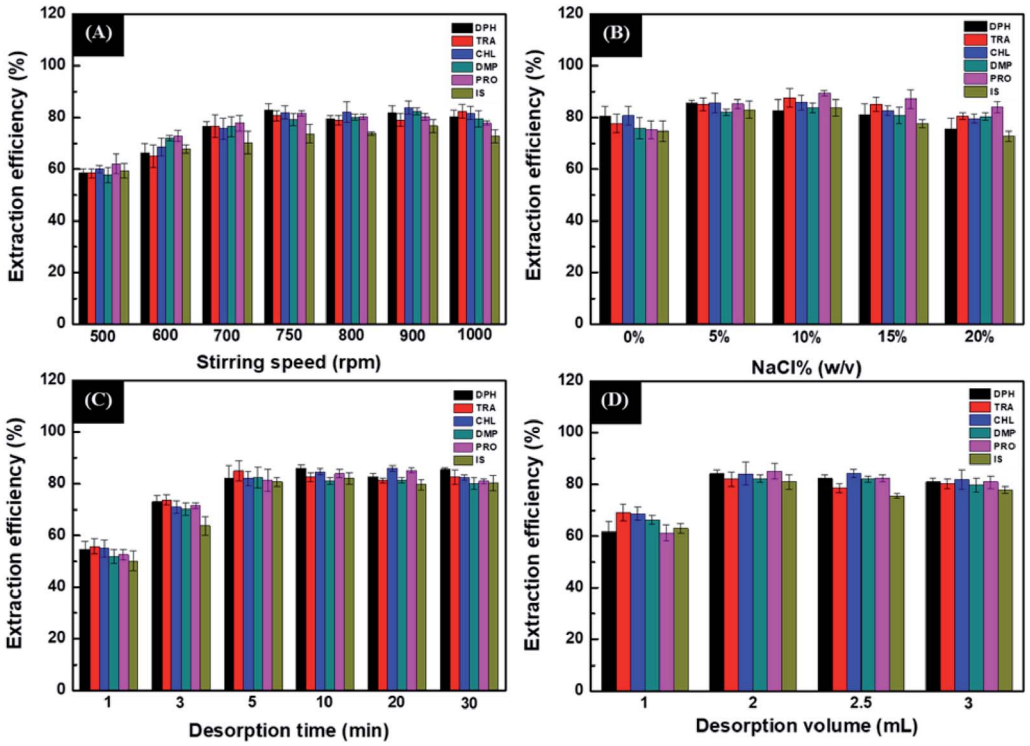

Fig. 4 Effects of (A) stirring speed, (B) salting out, (C) desorption time, and (D) desorption volume on the extraction efficiency of diphenhydramine (DPH), tramadol (TRA), chlorpheniramine (CHL), dextromethorphan (DMP), promethazine (PRO) and the internal standard (IS).

80.5 ± 5.7% to 82.6 ± 4.3% for diphenhydramine, from 77.7 ± 3.5% to 87.5 ± 3.6% for tramadol, from 80.7 ± 4.7% to 85.8 ± 2.7% for chlorpheniramine, from 75.9 ± 5.2% to 83.8 ± 1.0% for dextromethorphan, from 75.3 ± 3.94% to 89.4 ± 0.9% for promethazine and from 74.8 ± 5.1% to 83.7 ± 5.2% for the internal standard). The recoveries of the six target analytes decreased with the further addition of salt to 20%. This is probably due to a viscosity increase in the lean cocktail sample solution, which hindered adsorption of the target analytes.[24] Therefore, 10% w/v of NaCl was employed in the next experiments.

3.2.7 Desorption time. The effect of desorption time was also studied in the range from 1 to 30 min. The results are presented in Fig. 4(C). The recoveries of all target analytes increased with increasing desorption time from 1 to 5 min. The recoveries improved from 64.5 ± 3.1% to 82.3 ± 4.6% for diphenhydramine, from 65.7 ± 2.9% to 85.1 ± 3.9% for tramadol, from 64.9 ± 3.3% to 82.1 ± 2.9% for chlorpheniramine, from 61.9 ± 3.0% to 82.4 ± 3.9% for dextromethorphan, from 62.6 ± 1.9% to 83.3 ± 4.2% for promethazine and from 60.1 ± 3.8% to 80.1 ± 1.6% for the internal standard. On prolonging the time further, they remained constant. According to the results, 5 min was the shortest time that gave the highest recoveries and was, therefore, selected as the desorption time in further experiments.

3.2.8 Desorption solvent volume. The volume of desorption solvent to remove the target analytes from the XAD-2 adsorbent was investigated in order to obtain the smallest volume of acetonitrile that provided the highest extraction efficiency. The volume of desorption solvent was varied from 1.0 to 3.0 mL. At 2.0 mL, the recoveries reached their maximum levels shown in Fig. 4(D) which are 84.2 ± 1.6% for diphenhydramine, 82.1 ± 2.7% for tramadol, 84.2 ± 4.5% for chlorpheniramine, 82.3 ± 1.4% for dextromethorphan, 85.1 ± 2.9% for promethazine and 81.1 ± 2.7% for the internal standard. Thus, a desorption solvent volume of 2.0 mL was adopted. All the experimental parameters optimized for the in-house made SBSE device are summarized in the ESI (Table S1†).

3.3 Method validation

3.3.1 Selectivity. Selectivity is the ability of an analytical method to differentiate and measure the analyte in the presence of potential interfering substances in the blank matrix. The chromatograms obtained in the analysis of the blank lean cocktail sample with extraction using the XAD-2 adsorbent, spiked lean cocktail sample (50 μg mL^{-1}) and spiked lean cocktail sample (50 μg mL^{-1}) without extraction are shown in Fig. 5. The results indicate that the developed SBSE coupled with the GC-FID method was selective because it was able to extract and determine all the analytes in spiked samples without any interfering peaks from the matrices. No significant response attributable to interfering components is observed at the retention time(s) of the abused prescription drugs or of the internal standard in the blank sample. The absence of interference signifies a complete extraction and separation of the analytes. It is evident that a high degree of clean-up was achieved by the proposed method.

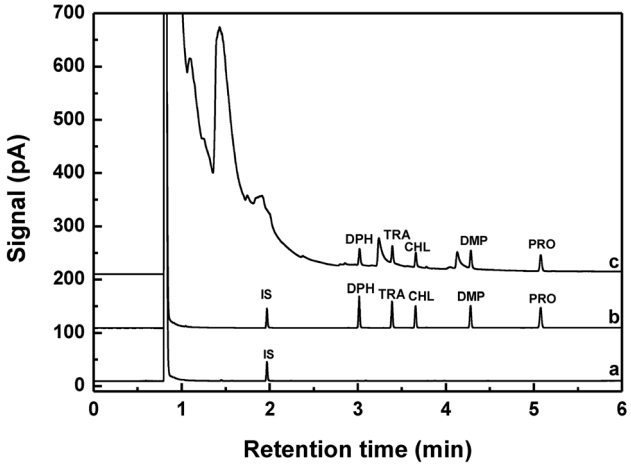

Fig. 5 GC-FID chromatograms of the five abused prescription drugs and internal standard pretreated without extraction and with extraction using SBSE. (a) Blank lean cocktail with extraction using SBSE, (b) spiked lean cocktail sample (50 μg mL^{-1}) with extraction using SBSE and (c) spiked lean cocktail sample (50 μg mL^{-1}) without SBSE. IS: internal standard, DPH: diphenhydramine, TRA: tramadol, CHL: chlorpheniramine, DMP: dextromethorphan and PRO: promethazine.

3.3.2 Linearity and sensitivity of the developed method. The linearity and sensitivity of the developed method were evaluated by GC-FID under the optimized conditions of extraction. Regression analysis shows linearity and ranges for all analytes (Table 1, see Fig. S2†). The linear concentration ranges were as follows: 1.0–250 μg mL^{-1} for all abused prescription drugs, except for chlorpheniramine (1.5–250 μg mL^{-1}), with linear equations of $y = (2.3 \times 10^{-2} \pm 8.5 \times 10^{-4})x + (0.101 \pm 0.096)$ for diphenhydramine, $y = (1.9 \times 10^{-2} \pm 7.9 \times 10^{-4})x + (0.122 \pm 0.090)$ for tramadol, $y = (1.7 \times 10^{-2} \pm 6.1 \times 10^{-4})x + (0.087 \pm 0.068)$ for chlorpheniramine, $y = (1.8 \times 10^{-2} \pm 6.5 \times 10^{-4})x + (0.090 \pm 0.074)$ for dextromethorphan, and $y = (1.9 \times 10^{-2} \pm 1.1 \times 10^{-4})x + (0.150 \pm 0.126)$ for promethazine. The coefficient of determination (r^2) was >0.99 (0.9900–0.9956) suggesting that the data were very well fit by linear regression. The method was sensitive, as shown by the limits of detection and limits of quantitation, which were determined based on signal-to-noise ratios of 3 and 10, respectively. The limits of detection and limits of quantification were reported as shown in Table 1.

3.3.3 Precision and accuracy of the developed method. To investigate the precision of the developed method, the extraction and analysis were conducted within the same day (intra-day precision) using five replicates of each sample and on five consecutive days (inter-day precision). The relative standard deviations (% RSD) were used to represent the method precision. Intra-day and inter-day precisions are shown in Table 2, and their ranges were 1.2–14.4% and 1.4–15.8%, respectively. Precision is the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous samples. All values met the acceptance criteria that precision should be better than (*i.e.* less than) 15%, except at the LOQ level, where it should not exceed 20%.[25] Intra-day and inter-day accuracy are shown in Table 2, and their ranges were 85.2–110.4% and 86.2–102.3%, respectively. All values met the acceptance criteria that accuracy should be better than (*i.e.* less than) ±15% of the nominal concentrations, except at the LOQ level, where it should not exceed 20%.[25] The results indicate that the method is precise and accurate, achieved using the in-house made SBSE device for extraction of abused prescription drugs and GC-FID for measurement of the extracts.

3.3.4 Reproducibility and lifetime of the prepared SBSE device. To determine the reproducibility of the in-house made SBSE devices, the stir-bars were prepared in sets of three bars each for six sets (18 stir-bars in total). Each of them was used for the extraction of 50 μg mL^{-1} of a spiked mixture of abused prescription drug standard solution and the internal standard (25 μg mL^{-1}) in lean cocktail samples. The relative standard deviations (% RSDs) were used to indicate reproducibility from each set of the prepared stir-bars. The obtained average recoveries of diphenhydramine, tramadol, chlorpheniramine, dextromethorphan, promethazine and the internal standard were 89.6 ± 4.3%, 85.0 ± 5.6%, 89.7 ± 3.2%, 94.9 ± 6.3% and 90.1 ± 2.1%, respectively. The percentage relative standard deviation (% RSD) was less than 7%. The results indicate that the developed in-house made SBSE device could be fabricated with good reproducibility.

To assess the lifetime and reusability of the prepared in-house made SBSE device, a spiked mixture of abused prescription drug standard solution (50 μg mL^{-1}) and internal standard solution (25 μg mL^{-1}) in soft drink was extracted using the proposed SBSE device. After desorption, the SBSE device was removed and each device was separately cleaned by stirring in 4.0 mL of acetone to eliminate any residues followed by conditioning in 4.0 mL of ethanol and 10.0 mL ultrapure water before it was used for the next extraction cycle. The extraction process was repeatedly performed for several cycles. The results

Table 1 Validation of the SBSE-GC-FID method for the determination of abused prescription drugs; the coefficient of determination (r^2), linear range, limit of detection (LOD), and limit of quantitation (LOQ)

Analyte	Coefficient of determination (r^2)	Linear range (μg mL^{-1})	LOD (μg mL^{-1})	LOQ (μg mL^{-1})
Diphenhydramine	0.9945	1.0–250	0.25	1.0
Tramadol	0.9929	1.0–250	0.25	1.0
Chlorpheniramine	0.9950	1.5–250	0.5	1.5
Dextromethorphan	0.9956	1.0–250	0.25	1.0
Promethazine	0.9900	1.0–250	0.25	1.0

Table 2 Intra-day and inter-day precision and accuracy of the SBSE-GC-FID method for the determination of abused prescription drugs in the lean cocktail ($n = 5$)[a]

Analyte	Concentration ($\mu g\ mL^{-1}$)	Precision as the relative standard deviation (%)		Accuracy as the recovery (%)	
		Intra-day ($n = 5$)	Inter-day ($n = 5$)	Intra-day ($n = 5$)	Inter-day ($n = 5$)
Diphenhydramine	1.0	13.5	14.2	87.5	86.2
	2.5	12.7	14.6	105.5	90.4
	50	8.1	5.7	90.3	93.8
	100	1.7	3.0	92.8	91.8
Tramadol	1.0	8.3	12.9	90.1	90.4
	2.5	1.9	11.7	97.0	92.6
	50	8.4	5.2	91.1	95.8
	100	2.7	1.4	96.4	94.6
Chlorpheniramine	1.5	10.6	14.7	85.2	88.7
	2.5	6.3	11.3	93.0	91.4
	50	5.9	5.0	104.0	94.8
	100	1.2	3.4	95.2	92.0
Dextromethorphan	1.0	13.9	15.8	90.6	94.4
	2.5	8.5	14.2	96.3	98.7
	50	6.2	1.7	90.8	95.0
	100	1.5	3.7	101.6	98.1
Promethazine	1.0	14.4	13.1	110.4	93.2
	2.5	12.8	11.9	109.0	96.4
	50	4.8	5.5	92.5	96.0
	100	2.1	3.7	97.1	102.3

[a] % RSD: percentage of relative standard deviation.

show that the extraction efficiency remained higher than 80% over 10 cycles of extraction and the obtained average recoveries of diphenhydramine, tramadol, chlorpheniramine, dextromethorphan, promethazine and the internal standard were 82.8 ± 3.1%, 83.7 ± 2.4%, 86.8 ± 2.6%, 91.5 ± 4.7%, 86.1 ± 6.3% and 82.9 ± 4.3%, respectively. Hence, the presented SBSE device could be reused at least 10 times without any significant decrease in the extraction efficiency.

3.4 Real sample analysis

Under the optimum conditions, the developed method was used to determine the concentrations of abused prescription drugs in lean cocktail samples from high schools located in the Songkhla province of Southern Thailand. Matrix-matched calibration curves were used to determine the amounts of the abused prescription drugs present in the samples. GC chromatograms of lean cocktail samples with extraction using SBSE are shown in Fig. 6. The amounts of detected abused prescription drugs are summarized in Table 3. The results show that some lean cocktail samples contained either tramadol (no. L1; 244.6 $\mu g\ mL^{-1}$) or promethazine (no. L4; 108.9 $\mu g\ mL^{-1}$). Both tramadol and promethazine were detected in lean cocktail samples (no. L2 and L5 at 89.4–152.7 $\mu g\ mL^{-1}$ and 23.2–32.1 $\mu g\ mL^{-1}$, respectively). None of them contained diphenhydramine, chlorpheniramine and dextromethorphan. The results suggest that tramadol and promethazine may be the most widespread abused prescription drugs in the Songkhla province of Thailand. However, the type and amount of ingredients in a lean cocktail vary, probably depending on factors such as the preferred drug type of users and the degree of its effect, the environment of users, and the availability of the different drugs. The accuracy of the developed method was reported in terms of relative recovery, determined by spiking standard abused prescription drugs into real samples at 2.5, 50 and 100 $\mu g\ mL^{-1}$. The spiked samples were extracted and determined under the

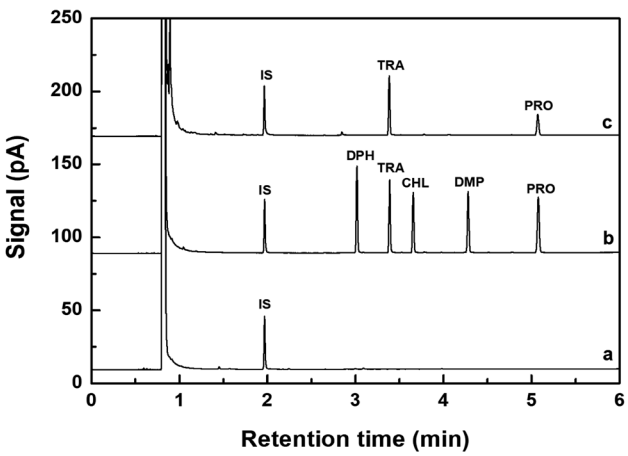

Fig. 6 GC-FID chromatograms of the five abused prescription drugs in lean cocktail samples obtained using the SBSE device: (a) blank lean cocktail sample, (b) lean cocktail spiked with a standard mixture of diphenhydramine (DPH), tramadol (TRA), chlorpheniramine (CHL), dextromethorphan (DMP), promethazine (PRO) (50 $\mu g\ mL^{-1}$) and the internal standard (25 $\mu g\ mL^{-1}$), and (c) selected lean cocktail sample (sample no. L2).

Table 3 Relative recovery of abused prescription drugs from spiked standard solution in lean cocktail samples and found concentrations of abused prescription drugs in lean cocktail samples[a]

		% Relative recovery (%RSD)					Detected concentration (μg mL^{-1} ± SD)				
Sample no.	Spiked level (μg mL^{-1})	DPH	TRA	CHL	DMP	PRO	DPH	TRA	CHL	DMP	PRO
L1	0	—	—	—	—	—	ND	244.6 ± 0.1	ND	ND	ND
	2.5	109.3 (6.2)	90.5 (7.6)	91.3 (8.8)	90.2 (9.1)	102.3 (7.7)	2.8 ± 0.2	2.3 ± 0.2	2.3 ± 0.2	2.3 ± 0.2	2.6 ± 0.2
	50	90.4 (3.2)	87.9 (6.5)	90.6 (4.3)	93.6 (6.7)	110.1 (3.8)	45.2 ± 1.4	43.9 ± 2.8	45.3 ± 2.2	46.6 ± 3.1	55.0 ± 1.9
	100	92.4 (3.2)	90.5 (5.0)	91.1 (4.9)	110.3 (5.9)	91.9 (2.7)	92.4 ± 2.8	90.5 ± 4.6	91.1 ± 4.5	110.3 ± 6.6	91.9 ± 2.5
L2	0	—	—	—	—	—	ND	152.7 ± 0.1	ND	ND	32.1 ± 0.1
	2.5	86.7 (5.8)	90.7 (7.4)	86.9 (6.1)	90.6 (5.8)	91.0 (7.3)	2.2 ± 0.1	2.3 ± 0.2	2.2 ± 0.1	2.3 ± 0.1	2.3 ± 0.2
	50	90.4 (3.2)	91.0 (4.4)	90.6 (4.8)	91.0 (8.6)	93.4 (6.6)	45.2 ± 1.4	45.5 ± 2.0	45.3 ± 2.3	45.5 ± 3.9	46.7 ± 3.1
	100	93.1 (3.0)	100.4 (3.9)	94.1 (3.2)	105.7 (4.9)	95.6 (3.5)	93.1 ± 2.6	100.4 ± 3.9	94.1 ± 3.0	105.7 ± 5.2	95.6 ± 3.4
L3	0	—	—	—	—	—	ND	ND	ND	ND	108.9 ± 0.1
	2.5	88.7 (10.1)	91.3 (7.2)	90.7 (8.5)	102.7 (9.7)	91.7 (8.1)	2.2 ± 0.2	2.3 ± 0.1	2.2 ± 0.1	2.6 ± 0.3	2.3 ± 0.2
	50	93.8 (6.7)	92.1 (3.5)	89.3 (5.4)	95.5 (4.8)	92.0 (3.3)	46.9 ± 3.1	46.0 ± 1.6	44.6 ± 2.4	47.8 ± 2.3	46.0 ± 1.5
	100	97.7 (3.4)	97.4 (4.1)	97.1 (1.5)	91.4 (4.6)	95.6 (3.5)	97.6 ± 3.3	97.4 ± 4.0	97.1 ± 1.5	96.1 ± 4.6	95.6 ± 3.4
L4	0	—	—	—	—	—	ND	ND	ND	ND	ND
	2.5	90.7 (9.1)	90.4 (7.1)	87.6 (6.6)	93.6 (5.0)	90.1 (8.5)	2.3 ± 0.2	2.3 ± 0.1	2.2 ± 0.1	2.3 ± 0.1	2.3 ± 0.2
	50	94.2 (6.3)	95.0 (5.8)	90.9 (4.5)	90.3 (5.8)	91.6 (2.7)	47.1 ± 2.9	47.5 ± 2.7	45.4 ± 2.1	45.1 ± 2.6	45.8 ± 1.2
	100	107.3 (3.5)	103.2 (8.6)	92.0 (3.8)	94.4 (4.2)	95.6 (3.5)	107.3 ± 3.8	103.2 ± 8.8	92.0 ± 3.5	94.4 ± 3.9	95.6 ± 3.4
L5	0	—	—	—	—	—	ND	89.4 ± 0.1	ND	ND	23.2 ± 0.3
	2.5	97.1 (5.2)	91.9 (10.4)	90.4 (9.1)	88.9 (7.5)	95.9 (7.4)	2.4 ± 0.1	2.3 ± 0.2	2.3 ± 0.2	2.3 ± 0.2	2.4 ± 0.2
	50	92.7 (6.2)	96.2 (6.8)	91.1 (5.1)	93.8 (8.0)	96.6 (3.7)	46.3 ± 2.9	48.1 ± 6.9	45.6 ± 2.3	46.9 ± 3.8	48.3 ± 1.8
	100	102.7 (6.8)	99.2 (6.7)	96.3 (5.6)	103.1 (4.3)	93.6 (2.3)	102.7 ± 4.2	99.2 ± 6.6	96.3 ± 5.4	103.1 ± 4.4	93.6 ± 2.1

[a] ND: not detected.

Table 4 Comparison of the developed method with other methods for the determination of abused prescription drugs in pharmaceutical formulations, lean cocktails, and biological samples[a]

Analytical instrument	Extraction method	Sample type (sample volume; mL)	Organic solvent expended (mL)	Extraction time (min)	Extraction efficiency	LOD ($\mu g\ mL^{-1}$)	Ref.
HPLC-UV	LLE	Cold-cough syrups (10.0 mL)	250	20	NR	0.30–4.70	26
GC-FID	HS-SPME	Blood and urine (0.5 mL)	0.5	20	7.3–23.9%	0.62–1.35	30
GC-MS	LLE	Blood (2.0 mL)	18	45	NR	0.025–0.05	28
GC-MS	LLE	Urine (1.0 mL)	8.0	NR	80.0–90.0%	0.05	29
GC-MS	Pipette tip-SPE	Plasma (0.1 mL)	0.5	10	73.0–100.0%	0.002–0.007	32
HPLC-UV	VS-GO-D-μ-SPE	Plasma and urine (10.0 mL)	NR	13	NR	0.16	27
GC-MS	PDMS coated SBSE	Blood, urine, and tissue (3.0 mL)	NR	960	38.0–98.9%	NR	31
GC-MS	LLE	Dirty sprite (1.0 mL)	1.0	15	93.0–109%	0.3	33
GC-FID	SBSE	Lean cocktail (2.0 mL)	2.0	35	85.0–94.9%	0.25–0.5	This work

[a] GC-FID: gas chromatography-flame ionization detector; GC-MS: gas chromatography-mass spectrometry; HPLC-UV: high performance liquid chromatography-ultraviolet detector; LLE: liquid–liquid extraction; HS-SPME: headspace-solid phase microextraction; pipette tip-SPE; pipette tip-solid phase extraction; VS-GO-D-μ-SPE: vortex-assisted graphene oxide nanosheet dispersive micro-solid phase extraction; PDMS coated SBSE: polydimethylsiloxane coated stir bar sorptive extraction. NR: not reported.

optimum conditions. Satisfactory relative recoveries were achieved in the range from 86.7 to 110.3% (Table 3). These results indicate that the developed method is efficient enough to be used for the extraction and detection of abused prescription drugs in lean cocktail samples.

3.5 Comparison with other methods

The developed method was compared with previously reported methods with regard to the analytes of interest, organic solvent consumption, time required for sample preparation, extraction efficiency, and the limit of detection (Table 4). The present SBSE-GC-FID method is simple and reliable without the need for derivatization. The developed method uses a small volume of the sample, only 2.0 mL, which is less than those in previous studies.[26,27] The extraction time of the proposed method is reasonable and less than those in previous studies.[28,31] The proposed method also requires a minimal volume of organic solvent (2.0 mL) and produces a smaller amount of waste compared with a high performance liquid chromatography-ultraviolet-visible detector (HPLC-UV).[26] In addition, the proposed extraction method used a small volume of organic solvent which is also less than those of other reports.[26,28,29] The total extraction time of our method was 35 min, which is longer than that required in liquid–liquid extraction (LLE) and headspace-solid phase microextraction (HS-SPME) as reported by Njuguna *et al.*,[26] Nishikawa *et al.*[30] and Rosenberger *et al.*[33] However, an emulsion is obtained by the liquid–liquid extraction (LLE) method. The extraction time of the proposed SBSE method is faster than PDMS coated SBSE as reported by Crifasi *et al.*[31] The extraction time of our method (35 min) was nonetheless sufficient to simultaneously extract all the analytes of interest. The extraction efficiency of the developed method (85.0–94.9%) is in a similar range or better than that of the other methods. The limit of detection was either comparable to that reported by Juhascik *et al.*,[28] Hamid *et al.*[27] and Rosenberger *et al.*[33] or better than that reported by Njuguna *et al.*[26] and Nishikawa *et al.*[30] from previous reports. Additionally, the extensive linear range for each analyte indicates the feasibility of analyzing lean cocktail samples. For this reason, the proposed SBSE-GC-FID can be applied as an alternative analytical method for the determination of abused prescription drugs in suspected lean cocktail samples and in the urine of drug abuse suspects.

4. Conclusion

Stir bar sorptive extraction (SBSE) with the XAD-2 adsorbent coupled with GC-FID analysis was successfully developed and applied to the extraction and determination of the abused prescription drugs in lean cocktail samples. The proposed method showed high extraction efficiency with good precision. The in-house SBSE device is simple to prepare, easy and convenient to use, and cost-effective. The method provided sufficiently good linearity and sufficiently low limits of detection. The developed method is more cost-effective than GC-MS techniques. Therefore, it can be beneficial for routine screening and determinations of lean cocktail samples from drug abuse suspects, in forensic laboratories.

Conflicts of interest

All authors declare that there are no conflicts of interest.

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