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Research Article

DEVELOPMENT OF ANALYTICAL METHOD FOR IDENTIFICATION OF SIBUTRAMINE HYDROCHLORIDE IN TRADITIONAL MEDICINE USING SOLID PHASE EXTRACTION: HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

Objective: The purpose of this study is to develop a rapid and sensitive method for identifying sibutramine hydrochloride (HCl).

Methods: Reversed-phase solid phase extraction (SPE) method with hydrophilic-lipophilic balance cartridge was selected to be developed and optimized. Optimization was done by optimizing solvent in the sample preparation and rinse solvent in the washing step. The extract of SPE was injected into reversed-phase high-performance liquid chromatography (HPLC) with C_{18} column with photodiode array detector. Furthermore, the optimized results were validated which included specificity and limit detection. Validated analytical method was used to analyze sibutramine HCl qualitatively in slimming herbal medicine which was obtained from the market.

Results: Analysis method optimization showed that 3% orthophosphoric acid was the optimum solvent to extract sibutramine HCl in SPE. Then, it was shaken for 30 minutes and filtered; the filtrate was put in SPE cartridge that had been conditioned using ethanol and water. After rinsing with ammonium hydroxide (NH_4OH) solution in water and NH_4OH solution in 80% ethanol, the analyte was eluted with acetonitrile. Identification of sibutramine HCl was done by HPLC at wavelength 254 nm. Sibutramine HCl gave maximum wavelength at 222 nm. Sibutramine HCl calibration curve gave quite linear results ranging from 0.10 to 0.50 mg/ml with R^2=0.9966. The limit of detection of this assay was 2.326 µg/ml. Specificity of this method is quite good. Simulation sample gave resolution for diethylpropion as 2.415; sibutramine HCl as 2.877, and amphetamine sulfate as 5.673. The recovery of this method was 38.0-45.0%.

Conclusion: This study showed faster and specific method for identifying sibutramine HCl in the traditional medicine.

Keywords: Sibutramine hydrochloride, Solid phase extraction, Hydrophilic-lipophilic balance, Slimming herbal medicine, High-performance liquid chromatography.

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INTRODUCTION

National Agency of Drug and Food Control (NADFC) has a vision to be an institution Food and Drug Administration which is innovative, reliable, and internationally recognized to protect the public. One of the NADFC duties is monitoring the utilization and distribution of traditional medicine in the Indonesian society. The regulation mentions that traditional medicine should not contain pure compound form of isolation or synthetic or semisynthetic which has effects as medicine or narcotic.

Indonesia is an agrarian country which has varied natural resources that have potential to produce traditional medicine. This condition encourages market business opportunities in traditional medicine industry. Sometimes, a few businesspeople improve the efficacy of their traditional medicine production by adding chemical drug. Chemical drug which is ever found in slimming herbal is sibutramine hydrochloride (HCl). This prohibition is based on the study of the sibutramine cardiovascular outcomes trial which showed an increasing risk of cardiovascular events.

The fast and sensitive method for detection of sibutramine HCl in herbal formulation using voltammetry of microparticles has been reported [1]. However, this method is expensive and less applicable. The usual existing methods for sibutramine HCl identification in slimming herbal medicine were using the liquid–liquid extraction and then continued with thin-layer chromatography (TLC). If the result is suspected positive, then it is followed by ultraviolet-visible (UV) spectrophotometry and confirmed by high-performance liquid chromatography (HPLC). This method is quite adequate in identifying sibutramine HCl in traditional medicine, but this method takes quite a long time and requires a lot of reagents. Sibutramine HCl is less soluble in organic solvent. Consequently, it needs a lot of sample amount to make spot in TLC. Therefore, it is necessary to develop method which is more reliable for identifying sibutramine HCl in slimming herbal medicine.

One of the extraction methods which are now developed to identify chemical drug is solid phase extraction (SPE). The SPE extract can be directly injected into the HPLC. This method has provided good sensitivity and selectivity for other chemical drugs identification, such as piroxicam, diclofenac sodium, ibuprofen, phenylbutazone, and mefenamic acid [2]. In addition, this method was done in shorter time and reduced reagent requirement [3]. In general, the combination of SPE and HPLC method is often used because it does not need to eliminate all of the impurities in the sample matrix so that the identification can be shortened [4].

MATERIALS AND METHODS

Materials

Reference standard of sibutramine HCl, amphetamine sulfate, and diethylpropion was obtained from NADFC. Acetonitrile and methanol HPLC grade were purchased from JT Baker. Ammonia solution,

ammonium acetate, formic acid, orthophosphoric acid, sulfuric acid, potassium hydroxide, sodium hydroxide, and ethanol were purchased from Merck-Germany. SPE cartridge (Oasis hydrophilic-lipophilic balance [HLB], 3 cc 60 mg) was purchased from Waters. Water was produced from distilled water and filtered by Millipore filter. Simulation samples of slimming herbal medicine such as *Zingiberis purpurei*, *Zingiberis aromaticae*, *Curcuma domestica* rhizomes, and *Guazuma ulmifolia* leaves were purchased from a traditional medicine store.

Instrumentation

Analytical balance (Mettler Toledo), pH-meter (Mettler Toledo), shaker (IKA HS501D), 100-1000 µl micropipette (Eppendorf), HPLC (Agilent) with photodiode array detector, m Bondapak™ C_{18} column (3.9×300 mm), particle size 10 µm 125A were the instrumentations used.

Methods

The SPE-HPLC method was developed for identifying of chemical in slimming herbal medicine using SPE-HPLC. This research began with the preparation of simulation sample. Then, some experiments were performed to develop analytical methods including selection of sample solvent, cartridges conditioning solution, SPE rinse solvent, and analyte elution solvent. After finding the appropriate SPE conditions, the method was optimized including sample solvent and rinse solvent [5].

Validation of this method will be performed using sensitivity and specificity test. The specificity test will be performed against sibutramine HCl and compounds which have similar physical and chemical properties with sibutramine HCl and were amphetamine sulfate and diethylpropion, while the sensitivity test will be expressed as limit of detection (LOD). Linearity curve was predicted by calculating five analyte concentrations in triplicate each. To ensure the detection limits, repeatability test was performed on that value. From the standard deviation result, LOD was recalculated.

Development of analytical method I

Simulation sample was diluted with ethanol, shaken for 30 minutes, and then filtered. The filtrate was divided into two parts, of which the first part acidified and the other part without acidified. SPE cartridge was conditioned successively with 1.5 ml of methanol and 1.5 ml of water. 800 µl of sample solution was loaded into the cartridge, allowed to drip slowly and helped by vacuum manifold, and then washed successively with the first rinse: Aqueous alkaline solution and the second rinse: 5% base solution in methanol. There were three kinds of alkaline solutions tested: 2.5% ammonium hydroxide (NH_4OH), KOH dilute solution, and NaOH dilute solution. Then, it was eluted with 800 µl of acetonitrile and transferred into autosampler vial. The extract was directly injected into the HPLC using a reversed-phase chromatography and isocratic mobile phase with the following conditions: Column m Bondapak™ C_{18} (3.9×300 mm), particle size 10 µm 125 A, room temperature, mobile phase ammonium acetate 0.05 M-acetonitrile (10: 90), flow rate 1.0 ml/minute, and detector spectrophotometer UV at wavelength 254 nm.

Development of analytical method II

Ethanol as solvent was replaced with acid solution to reduce matrix as carrier. The acid solutions used were sulfuric acid and orthophosphoric acid. In addition, it was also tested with base 2.5% NH_4OH and 2.5% formic acid. The filtrate was diluted with acid and then washed with NH_4OH solution in water and 2.5% NH_4OH in methanol. While for filtrate with 2.5% NH_4OH solvent, it was rinsed with 0.1 N HCl and 30% methanol.

Development of analytical method III

In purpose to develop an environmentally friendly analytical method, the methanol was replaced by ethanol. It was also tested phosphoric acid solution with various pH and orthophosphoric acid with various concentrations.

Optimization of the orthophosphoric acid concentration

Simulation sample was diluted in orthophosphoric acid at concentration of 2.0-4.0%. The filtrate was separated by solid phase extraction using 2.5% NH_4OH in water and 2.5% NH_4OH in 5% ethanol as rinse solvent. Then, it was eluted with acetonitrile. The result was injected further into the HPLC.

Optimization of the SPE rinsing solution

The simulation sample filtrate was extracted using SPE method. Cartridge which has been inserted by filtrate then rinsed with 2.5% NH_4OH in water and 2.5% NH_4OH solution in ethanol. The ethanol solution was prepared in concentration ranged from 10% to 90%.

Standard solution preparation

Sibutramine HCl (Fig. 1) standard solution was prepared in ethanol at concentration of 0.5%.

Spike solution series preparation for linearity curve

Spike solution series were done by preparing five simulation samples and each spiked sample was weighed 1 g, dissolved in 10 ml orthophosphoric acid solution, and then added with sibutramine HCl standard to obtain five different concentrations, namely 1.1692; 2.3615; 3.5147; 4.6790, and 5.8310 mg/g. Each concentration was performed in triplicate and then extracted using SPE method.

Validation of analytical method

Specificity test

Simulation sample was added with sibutramine HCl, amphetamine sulfate, and diethylpropion. The sample was mixed and grounded by mortar to obtain a homogeneous mixture of sample. The mixture was weighed 1 g and then continued by SPE. Analysis was done in triplicate.

LOD

LOD was predicted by calculating the standard deviation of linearity curve [6]. Determination of LOD was followed by injecting that concentration six times into HPLC. Standard deviation was used to calculate the next LOD. The obtained concentration was tested using SPE-HPLC.

Identification of sibutramine HCl in slimming herbal samples

The slimming herbal samples were taken from the market. Samples selected based on their track record showed positive sibutramine HCl. The samples were analyzed using the procedure that has been validated.

RESULTS

From Table 1, it can be seen that the recovery of the method development has not been quite good. Samples which were acidified first can give a better recovery.

Table 2 exposes that acidified with sulfuric acid is better than with orthophosphoric acid.

From Fig. 2, it can be seen that orthophosphoric acid was selected as a solvent in sample preparation which it can extract sibutramine HCl well and reduce the interference of other contaminants.

From Fig. 3, it can be seen that the replacement of methanol with ethanol did not give quite different results.

Fig. 4 reveals that the peak of sibutramine HCl using ethanol was greater than methanol.

From Table 3, it can be seen that the experiment with 3.0% orthophosphoric acid solvent gave the highest area of 334.0803.

From Table 4 and Fig. 6, it can be seen that the rinse solvent using a solution 2.5% NH_4OH in ethanol 80% gave the optimum result which showed that the largest peak area of sibutramine HCl with peak area of matrix was not too large.

Table 1: Comparison of SPE sibutramine HCl with and without acidification

Sample	Acidification	Rinse solvent	Eluent	RT (min)	AUC (mAu)
Sibutramine HCl				7.049	700
Sample A	-	NH_4OH (water+methanol)	Acetonitrile+ammonium acetate	7.159	10
Sample B	-	NH_4OH (water+methanol)	Acetonitrile	7.227	15
Sample C	-	Water+methanol	Acetonitrile+ammonium acetate	7.224	5
Sample D	-	Water+methanol	Acetonitrile	7.285	11
Sample E	Orthophosphoric acid	NH_4OH (water+methanol)	Acetonitrile+ammonium acetate	6.852	14
Sample F	Orthophosphoric acid	NH_4OH (water+methanol)	Acetonitrile	7.188	22
Sample G	Orthophosphoric acid	Water+methanol	Acetonitrile+ammonium acetate	6.786	13

Table 2: Comparison of SPE sibutramine HCl with acid and rinse solvent combination

Sample	Acidified	Rinse solvent	Eluent	RT (min)	AUC (mAU)
Sample IV A	Orthophosphoric acid	NH_4OH (water+methanol)	Acetonitrile	6.552	135.3798
Sample IV B	Orthophosphoric acid	KOH (water+methanol)	Acetonitrile	6.52	143.115
Sample IV C	Orthophosphoric acid	NaOH (water+methanol)	Acetonitrile	6.502	145.3207
Sample IV D	Sulfuric acid	NH_4OH (water+methanol)	Acetonitrile	6.498	148.6597
Sample IV E	Sulfuric acid	KOH (water+methanol)	Acetonitrile	6.483	160.4733
Sample IV F	Sulfuric acid	NaOH (water+methanol)	Acetonitrile	6.473	150.6062

SPE: Solid phase extraction

Table 3: Optimization of orthophosphoric acid concentration

Orthophosphoric acid (%)	Matrix		Sibutramine HCl	
	Retention time (minute)	Area (mAU)	Retention time (minute)	Area (mAU)
2.0	3.147	6213.0825	7.023	235.9797
2.5	3.156	6264.4951	6.944	320.3194
3.0	3.166	5853.4243	7.002	334.0803
3.5	3.155	4760.6875	6.996	268.5946
4.0	3.16	5601.2363	7.014	325.9615

Table 4: Optimization of solvent in washing step

2.5% NH_4OH dissolved in	Matrix		Sibutramine HCl	
	Retention time (min)	Area (mAU)	Retention time (minute)	Area (mAU)
Ethanol 10	3.148	5331.3794	6.733	329.6230
Ethanol 20	3.160	3956.1814	6.806	311.4466
Ethanol 30	3.152	3720.9368	6.794	351.0233
Ethanol 40	3.147	3197.1768	6.810	355.5089
Ethanol 50	3.153	1174.8364	6.824	249.5518
Ethanol 60	3.220	719.7321	6.825	377.0536
Ethanol 70	3.266	456.8080	6.836	381.6412
Ethanol 80	3.227	296.2206	6.841	418.3520
Ethanol 90	3.230	172.2773	6.893	93.4499

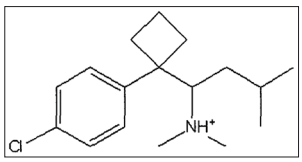

Fig. 1: Inorganic acid salt of sibutramine. X=HSO_4, Br, H_2PO_4 H_2O

Fig. 7 demonstrates that resolution of each drug chemicals was diethylpropion 2.41, sibutramine hydrochloride 2.88, and amphetamine sulfate 5.67.

Linearity curve of five different concentrations of sibutramine HCl is shown in Fig. 8.

From Table 5, it can be seen that the smallest LOD in SPE-HPLC of sibutramine HCl was 38.870 µg/ml.

DISCUSSION

Development of analytical method I

Reversed-phase SPE system was used in this study because sibutramine HCl is an organic compound with nonpolar dominant characteristic. Sorbent of HLB cartridge is a universal reversed-phase type sorbent. Most chemical drug as adulterant is organic compound, so this system is expected to able to identify other chemical drug also with minor modification. These cartridges were chosen based on consideration that little sample volume with low matrix concentration. The results can be seen in Table 1.

Standard solution which was directly injected without going through the SPE cartridge gave high peak of 700 mAu, while the high peak of SPE result only gave <22 mAu. The simulation samples which were diluted with ethanol enable the analyte breakthrough the sorbent. HLB cartridge contains two types of monomer, namely m-divinylbenzene which is lipophilic and n-vinylpyrrolidone which is hydrophilic. Polymer sorbent is more resistant against the effects of water which related with

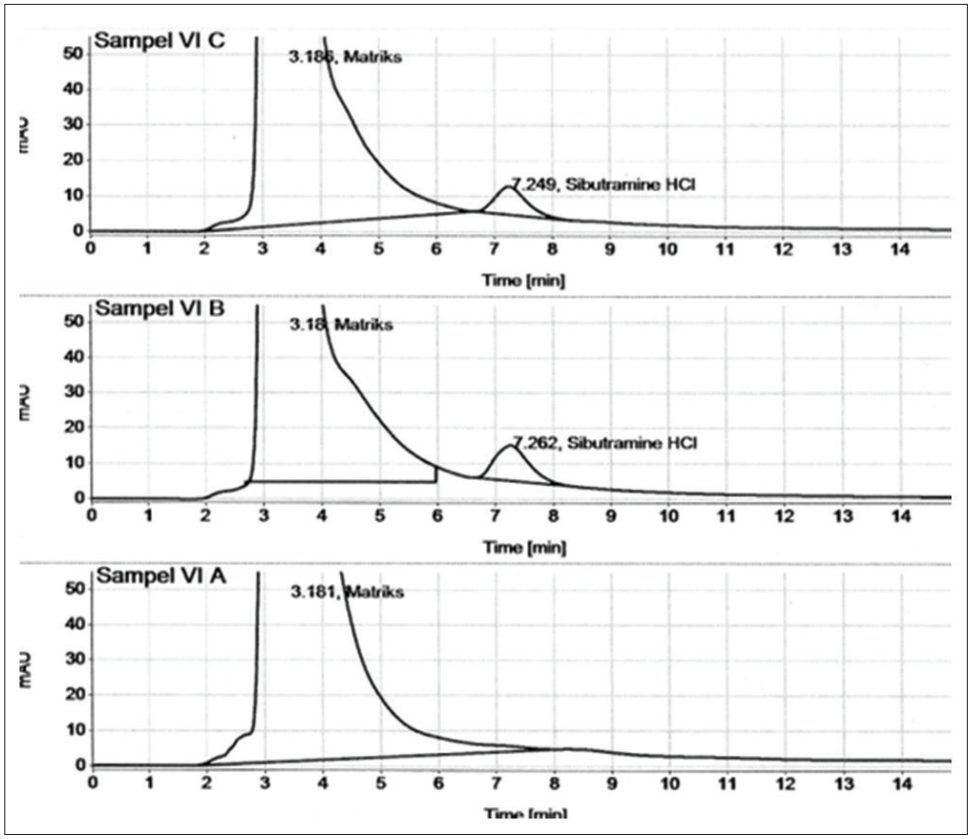

Fig. 2: Chromatogram of sibutramine hydrochloride with acid-base addition variation

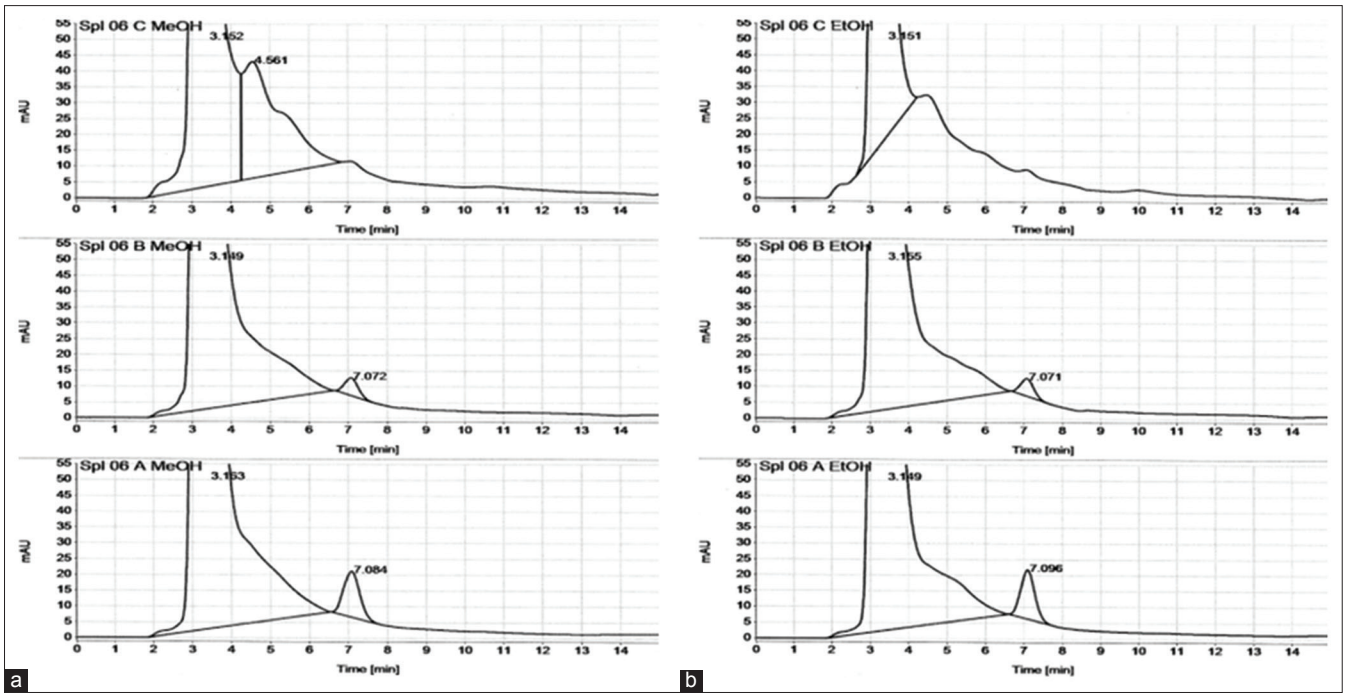

Fig. 3: Solid phase extraction of sibutramine hydrochloride with various solvent. (a) methanol solvent, (b) ethanol solvent, 06 A=pH 1.5, 06 B=pH 2, 06 C=pH 3

silanol activity on the silica-based sorbent. Ethanol is a good organic solvent for dissolving sibutramine HCl, but this solution would be like a semi-polar nature of ethanol, thus less retained on the sorbent of cartridge which is polar (hydrophilic) and non-polar (lipophilic). As a result, the interaction bonding between analyte and sorbent is not as strong as with analyte and solvent. Hence, most analytes were breakthrough sorbent.

Samples which were acidified first gave a better recovery. Sibutramine HCl is an alkaloid which is a weak base when added orthophosphoric

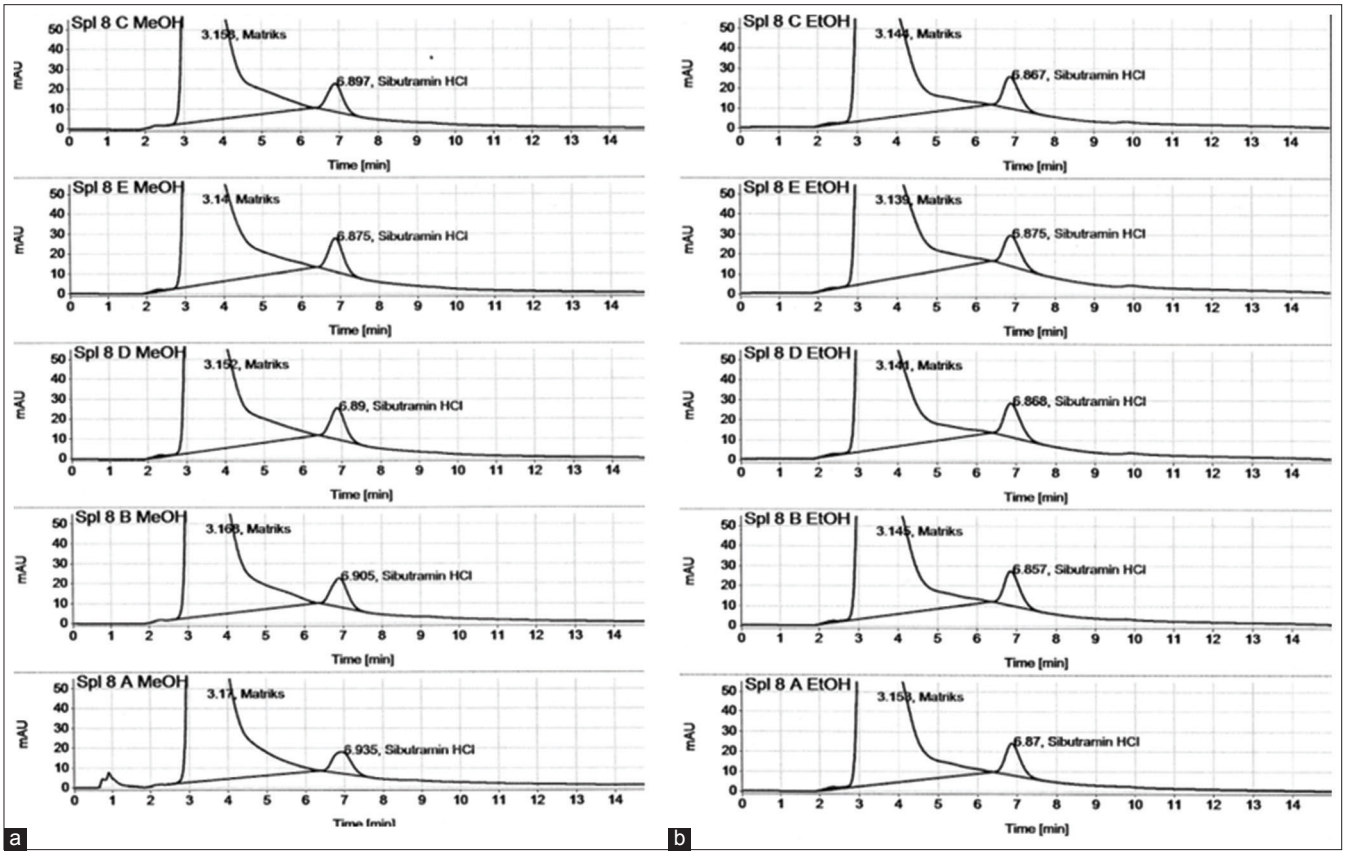

Fig. 4: Solid phase extraction of sibutramine hydrochloride with various orthophosphoric acid concentration. (a) methanol solvent, (b) ethanol solvent, A = orthophosphoric acid 1.5%, B = orthophosphoric acid 2.0%, C = orthophosphoric acid 2.5%, D = orthophosphoric acid 3.0%, E = orthophosphoric acid 3.5%

Table 5: Regression equation, correlation coefficient, and limit of detection of sibutramine HCl

Replication	Regression equation	R^2	LOD (µg/ml)
1	Y=2790.3X−92.824	0.9967	38.870
2	Y=2441.8X−25.12	0.9938	74.196
3	Y=2345.3X+3.3005	0.9888	62.334
Average		0.9931	58.466
Standard deviation		0.00399	17.978
Variance coefficient (%)		0.40240	30.749

HCL: Hydrochloride

acid will turn into alkyl ammonium salt. This substance is more soluble in water than its neutral form. Thus, the bonding between analytes and n-vinylpyrrolidone as hydrophilic monomer becomes stronger so that the amount of analyte that breakthrough will be reduced. The addition of NH_4OH as a weak base will turn a sibutramine salt into amine compound which is a weak base, so it will decrease analyte polarity and the solubility in water is also reduced. By the time the cartridge was eluted with acetonitrile as semipolar organic solvent, the bond of sorbent-analyte was not strong enough and the analyte was eluted. The purity of acetonitrile can affect elution strength so that 100% acetonitrile elute stronger than acetonitrile-ammonium acetate. The addition of orthophosphoric acid caused sibutramine HCl retained better by HLB cartridge.

Table 2 exposes that acidified with sulfuric acid was better than with orthophosphoric acid. Changing in base did not give significant effect to the result. This issues probably because it can maintain in same pH, but preparation sample which was dissolved in ethanol gave big peak of matrix and covered the peak of sibutramine HCl. Based on this result, it will be tried with another combination for sample preparation.

Development of analytical method II

Subsequently, in sample preparation, ethanol solvent was replaced with acid solution. Sibutramine HCl is soluble in water (2.9 mg/l at pH 5.2) and can be determined well in water using UV spectrophotometry with maximum wavelength at 223 nm [7]. Acid solutions compared between dilute orthophosphoric acid and dilute sulfuric acid, while the rinse solvent compared NaOH and NH_4OH. Besides the elution results, it was also seen the loading and washing results, which revealed that loading and washing did not give a sibutramine HCl peak. It means that analyte was retained in the sorbent and did not break through into the loading or washing solution. The sample which was dissolved in sulfuric acid was less stable compared to orthophosphoric acid. The sample which was extracted with sulfuric acid solution and washed with NH_4OH solution showed no peak of sibutramine HCl, but it would give peak of sibutramine HCl when rinsed using NaOH solution. While when using orthophosphoric acid solvent and rinsed by NH_4OH or NaOH solution showed sibutramine HCl peak.

Sulfuric acid as solvent will react with sibutramine HCl into alkyl ammonium salts, which was soluble in water and will be retained by hydrophilic monomer n-vinylpyrrolidone in sorbent and it needs a strong base (NaOH) to become sibutramine HCl. As a result, when washed with NH_4OH solution, it could not give perfect sibutramine HCl and did not provide a good spectrum. Based on the result,

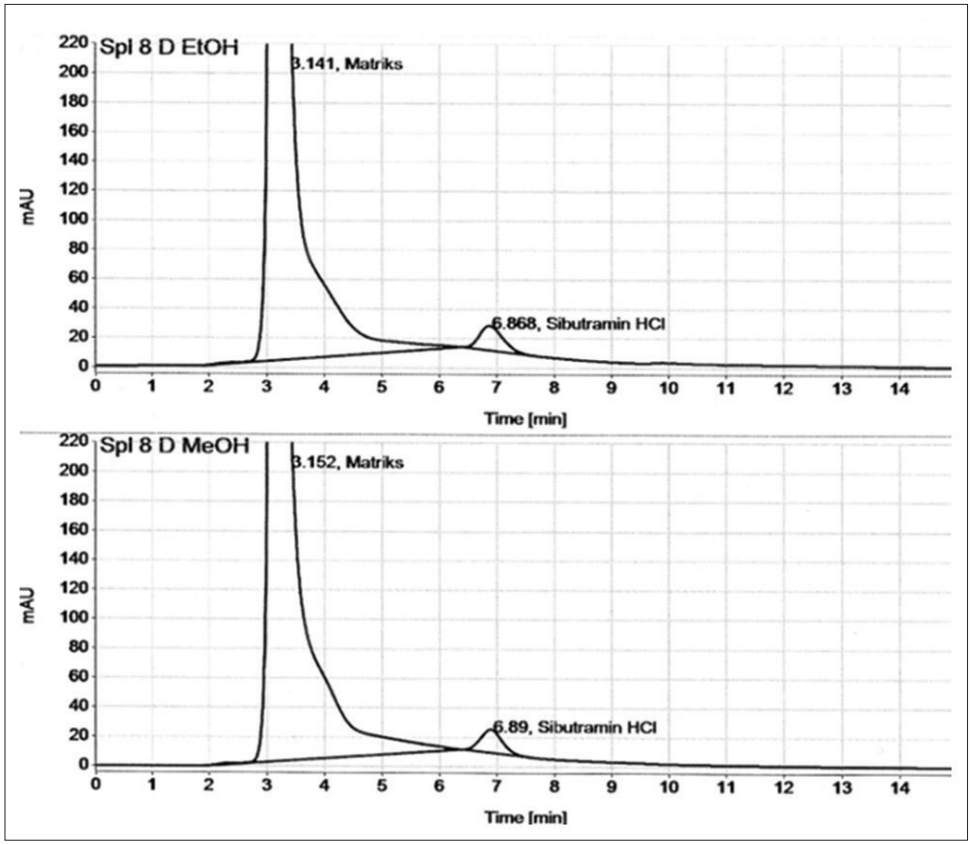

Fig. 5: Solid phase extraction of sibutramine hydrochloride with orthophosphoric acid 3.0% solvent

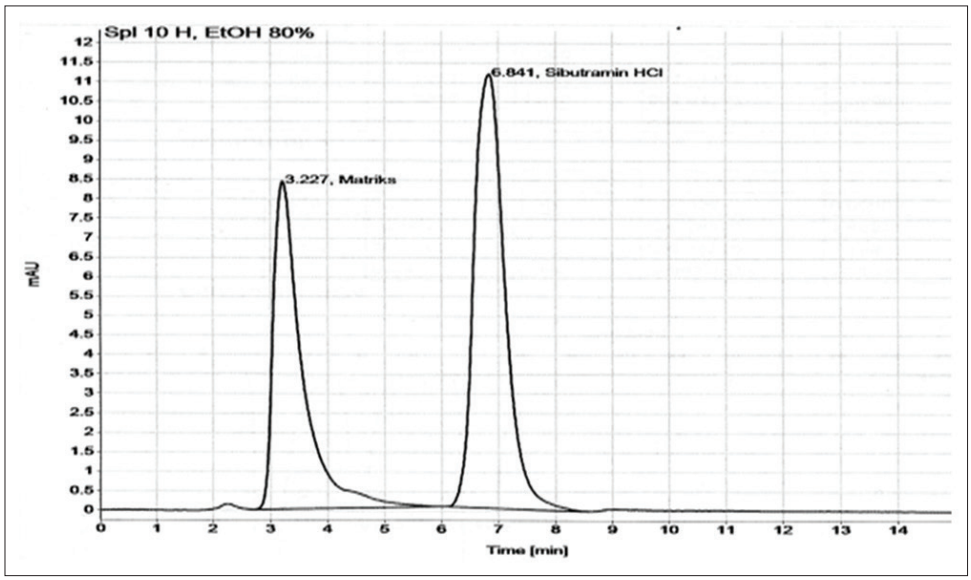

Fig. 6: Solid phase extraction of sibutramine hydrochloride with rinse solvent NH_4OH 2.5% solution in ethanol 80%

orthophosphoric acid was selected as sample solvent and NH_4OH solution as rinse solvent.

The next step of sample preparation was to test again sample with a different solvent, which was weak acid and weak base. The weak acids were 2.5% orthophosphoric acid and 2.5% formic acid, while the weak base was 2.5% NH_4OH. From Fig. 2, it can be seen that the dissolving samples in NH_4OH, could not show sibutramine HCl peak. This confirmed that the reaction between sibutramine HCl and acid plays an important role in the analytes extraction from traditional medicine sample. It was proven that both orthophosphoric acid and formic acid gave good peak of sibutramine HCl, but the peak with orthophosphoric acid solvent was greater than formic acid solvent. Besides that, the matrix peak with orthophosphoric acid solvent was smaller than with formic acid solvent. Based on the result, orthophosphoric acid was selected as a solvent in sample preparation; it can extract sibutramine HCl well and reduce the interference of other contaminants [8].

Development of analytical method III

Based on the previous result, it could be seen methanol was good solution as a conditioning solution for cartridge and rinse solvent, but actually methanol is harmful to humans. To develop analytical method

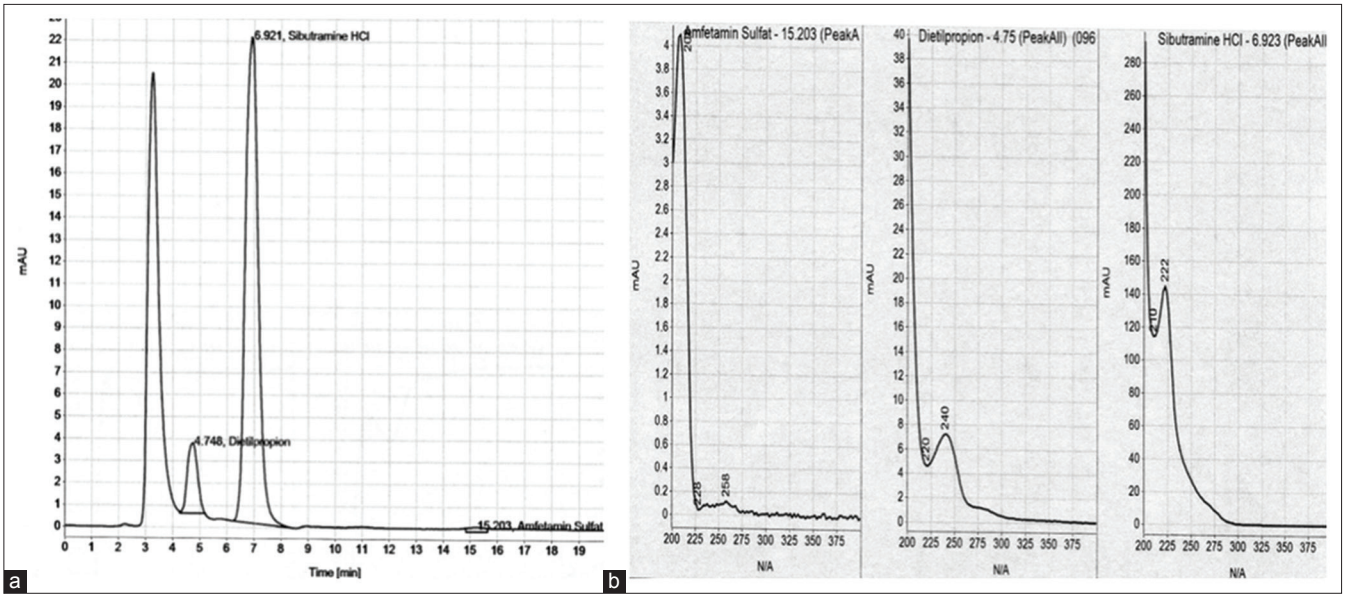

Fig. 7: The specificity test of solid phase extraction for sibutramine hydrochloride. (a) specificity chromatogram, (b) specificity spectrum

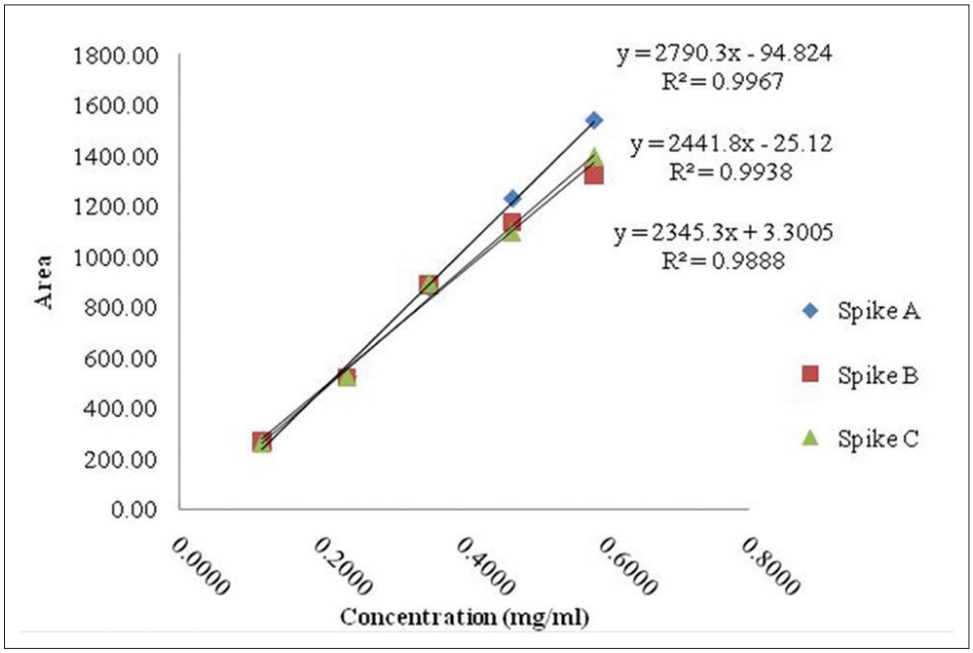

Fig. 8: Linearity curve of sibutramine hydrochloride

which safety for environment, the methanol was replaced with ethanol. The next step was to evaluate the effect of pH in sample preparation and the effect of ethanol in SPE treatment.

The literature data expressed that water-soluble sibutramine HCl at pH 5.2 [9], therefore, was carried out sample preparation by orthophosphoric acid solution with various pH. Variation of pH was done in two ways, namely orthophosphoric acid solution at pH 1.5, 2, 3, 4 and the use of phosphate buffer at pH 4.0, 5.06, 6.49. The result demonstrated that the higher pH gave the worst chromatogram. It was similar result to the use of phosphate buffer, and sibutramine HCl peak cannot be separated from the top of the matrix. Only the use of phosphate buffer at pH 4.0 gave two peaks, but sibutramine HCl showed the maximum wavelength at 226 nm and it was different from maximum wavelength of sibutramine HCl standard at 222 nm. It can be seen in Fig. 3 that the replacement of methanol with ethanol did not give quite different results. Changes in pH did not give good results; therefore, in the next step, it was performed by various concentration of orthophosphoric acid using methanol or ethanol. Orthophosphoric acid solution was prepared at concentration of 1.5%, 2.0%, 2.5%, 3.0%, and 3.5%. The higher orthophosphoric acid concentration gave the greater peak area of sibutramine HCl, which was given by 3.0% orthophosphoric acid. Figs 4 and 5 revealed that the peak of sibutramine HCl using ethanol was greater than methanol. Sibutramine solubility in ethanol is greater than in methanol [10]. Those solubility properties of sample can affect the retention of sorbent. Therefore, the next step was forwarded to use ethanol. The result was still fluctuating and then continued with using orthophosphoric acid with greater concentration.

In the next step, sample was prepared using orthophosphoric acid with concentration of 4.0%, 5.0%, 6.0%, 7.0%, 8.0% and ethanol in the SPE treatment. It revealed that the peak area which was obtained

did not provide significant difference. The use of orthophosphoric acid 4.0% showed decomposition of peak of sibutramine HCl and shift of retention time. Hence, it is necessary to ensure the orthophosphoric acid concentration which can give optimum result. Based on the overall study above, two variables were taken for optimizing development of analytical method that was variation of orthophosphoric acid concentration and ethanol concentration in the washing step.

Optimization of orthophosphoric acid concentration

The optimization results of orthophosphoric acid concentration in analytical method development can be seen in Table 3. From Table 3, it can be seen that the experiment with 3.0% orthophosphoric acid solvent gave the highest area of 334.0803. It was the same with the previous experiment in development of analytical method III which reported that 3.0% orthophosphoric acid concentration yielded significant result in SPE of sibutramine HCl.

Optimization of the SPE rinse solvent

Further optimization was performed by varying concentration of ethanol in washing step and sample preparation had been fixed using 3.0% orthophosphoric acid. The results of optimization of ethanol concentration can be seen in Table 4. From Table 4, it can be seen that the rinse solvent using a solution 2.5% NH_4OH in ethanol 80% gave the optimum result which showed the largest peak area of sibutramine HCl with peak area of matrix was not too large. SPE optimization by variation of the concentration of organic solvent in rinse solvent was also performed by Tak [11].

Analysis method validation

One method can be used in analysis method when the method has been validated. Based on USP 23, the parameters which should be validated were specificity and LOD.

Specificity assay

Besides sibutramine HCl, the simulation sample was also added with diethylpropion and amphetamine sulfate because both chemicals are often added to slimming herbal medicine. Sibutramine is structurally related to amphetamine, but its mechanism of action is different. Sibutramine hydrochloride and amphetamine sulfate can also be analyzed by voltammetry method, but this method has difficulty in removing interferences of matrix [12]. From Fig. 7, it can be seen that the method quite specific, but it was not specific for amphetamine sulfate because it had very small peak. Sibutramine HCl had the highest peak compared to diethylpropion and amphetamine sulfate; therefore, it can be concluded that this method was selective for sibutramine HCl. From Fig. 7, it can be seen that resolution of each chemical drug was diethylpropion 2.41, sibutramine hydrochloride 2.88, and amphetamine sulfate 5.67. These results found the acceptance criteria for validation that is Rs <1.5.

Determination of detection limit

From five spiked samples with different concentration of sibutramine HCl, it was obtained linearity curve as Fig. 8. The correlation coefficient (R^2) obtained was not too good because R^2<0.999. From triplicate analysis, the highest correlation coefficient is 0.9967. It showed that this SPE method is more appropriate in qualitative analysis of sibutramine HCl than quantitative analysis. LOD can be predicted using regression equation and standard deviation.

By calculating and using data in Table 5, the smallest LOD in SPE-HPLC of sibutramine HCl was 38.870 µg/ml. Repeatability of standard deviation was used to calculate the smaller LOD. The LOD of this calculation was 3.30 µg/ml. Then, SPE-HPLC of sibutramine HCl was conducted using concentration of 37.22, 18.61, 9.305, 4.652, and 2.326 µg/ml to obtain lower LOD. The result showed that all of concentration gave small peak. Therefore, the LOD of this method was 2.326 µg/ml. However, the LOD of sibutramine HCl in this method was still bigger than the TLC-image analysis method that was 190 ng/spot [13].

The recovery of this method was also calculated, which obtained only 38.0-45.0%. The small recovery can be caused the high number of the LOD. The small recovery can be caused by few possibilities. First, there was analyte breakthrough the sorbent during washing step. The result of optimization of rinse solvent revealed that there was a little matrix at the end of extraction or the rinse solvent that can be used was not strong enough to pull the analyte from the sorbent. As a result, the analytes which were retained in the sorbent will be dropped in flushing step. Second, the elution solvent was not strong enough to pull all of the analytes in the sorbent. As long as contact, there will be interaction between analytes and the sorbent continuously and resuspended with the sorbent. As a result, not all of analytes will be dropped in elution step. Small amount of washing solvent which is retained in cartridge before elution also can recovery of sibutramine. Reconstitution step can be executed to increase the sensitivity of this method [14].

Identification of sibutramine HCl in slimming herbal sample

The validated method was used to test a sample from market which is suspected to contain sibutramine HCl. From Fig. 9, it can be seen that

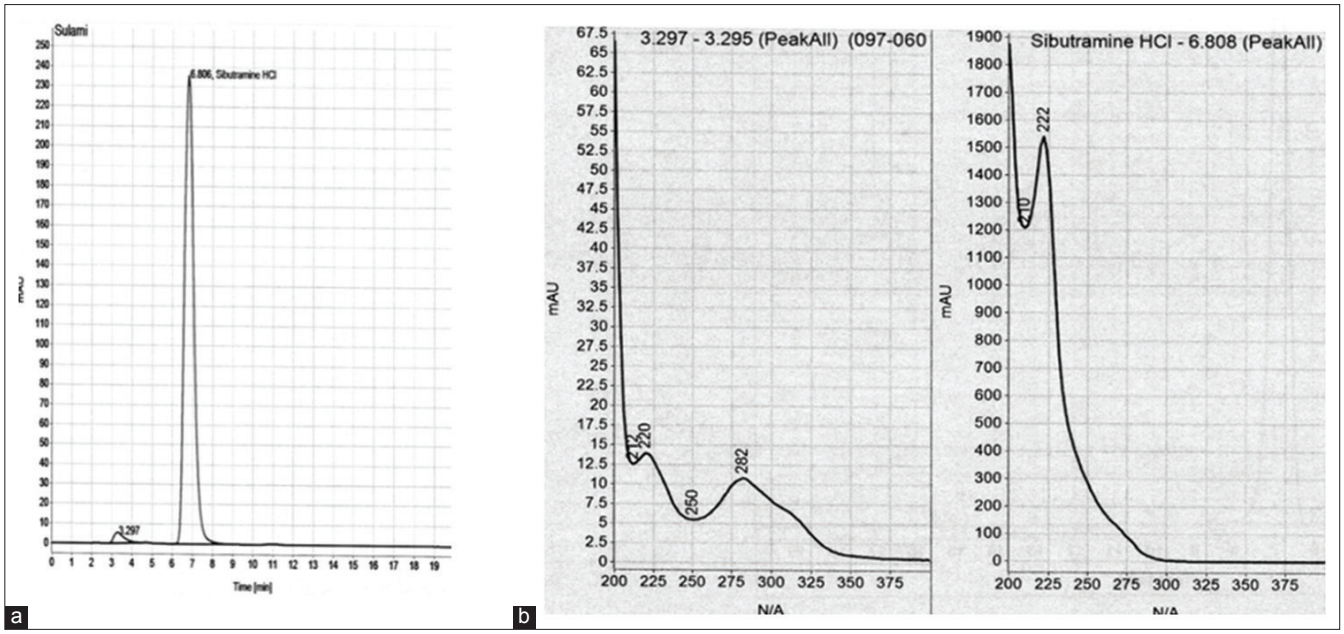

Fig. 9: Solid phase extraction result of sample from market. (a) chromatogram, (b) spectrum

this method is good enough and can be applied to test slimming herbal sample.

Based on Fig. 9, it is also seen that there was other peak beside sibutramine HCl peak and it provide a good spectrum. It showed that the SPE-HPLC method has good opportunity to be developed as an identification method of other chemical drugs. It would be great if this method could be used as a model to identify chemical drug compounds with specific properties but still provide great specificity.

CONCLUSIONS

Sibutramine HCl reaction with the acid solution in sample preparation gave more important role in development of analytical method than solubility sibutramine HCl in water. Ethanol could replace methanol in the analytical method development to identify sibutramine HCl. Optimum condition of SPE for sibutramine HCl was extracted using 3.0 % orthophosphoric acid and 2.5% NH_4OH in 80% ethanol as rinse solvent. This analysis method still has low recovery.

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