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# An evaporation-free solid-phase extraction method for rapid and accurate analysis of sumatriptan in human plasma by LC–MS/MS

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**Abstract**

An evaporation-free solid-phase extraction (SPE) method was developed and validated for sumatriptan. High organic washing (50% methanol) and low organic elution (20% methanol) were used and the recovery was greater than 92%. The eluate was injected into a C18 column without evaporation and reconstitution. Sumatriptan was monitored in positive ion mode with mass transition of *m/z* 296.4–58.1 amu. The calibration curve was 1–100 ng/mL ($r \geq 0.9923$). The inter-day and intra-day precisions ranged from 4.53 to 9.12% and 1.72 to 6.93%, respectively. This method features reduced cost and pollution, clean extract, high speed, and most importantly overall method reliability.


*Keywords:* Evaporation-free; Sumatriptan; Solid-phase extraction; LC–MS/MS; Human plasma

## 1. Introduction

A dilemma exists in developing bioanalytical method for highly hydrophilic drugs. Their high hydrophilicity causes problems during sample preparation and liquid-chromatographic separation.

For extraction, SPE is usually the method of choice for hydrophilic compounds as liquid–liquid extraction often results in less satisfactory recovery. In SPE, cartridges loaded with highly hydrophilic compounds cannot be washed with high organic washing solution. However, high organic and volatile elution solution, for instance 100% methanol, has to be used to facilitate the subsequent commonly used evaporation step.

In LC separation, very low percentage of organic content in mobile phase, e.g. 10% methanol, has to be used for hydrophilic compounds to obtain enough retention on reversed-phase columns, such as C18 column, to allow separation from solvent front. Accordingly, low organic reconstitution solution (usually the same as the mobile phase) is frequently used to reconstitute the dry extract after the evaporation step.

The large difference in organic content between the elution and reconstitution solutions can create some analytical problems. A lot of components from the matrix or from the reagents may co-elute with the analyte of interest, which may then either interfere with the separation and detection or cause matrix effect, such as ion suppression. In addition, some co-eluted components may not be soluble in reconstitution solution, which may clog the LC system, especially the column.

Sumatriptan, widely used in the treatment of migraine or related diseases, is an example of very hydrophilic drugs (Fig. 1). Several bioanalytical methods have been published for the determination of sumatriptan in human plasma [1–6] and most of them were based on SPE [2–6]. In these SPE methods, very low percentages of organic content, e.g. 5%, 10%, or 30%, were used in the washing of SPE cartridges.

In Anapharm Québec laboratory, an LC–MS/MS method with SPE was developed and validated for sumatriptan in human plasma. The dilemma mentioned above posed challenges. During SPE, only 10% methanol was used in the washing step to avoid analyte loss. The elution solution used was 99% acetonitrile while the reconstitution solution (mobile phase) was 40% methanol. After the evaporation and reconstitution, an extra filtration step had to be incorporated (Fig. 2a) to remove some undissolved co-extracted matrix components,

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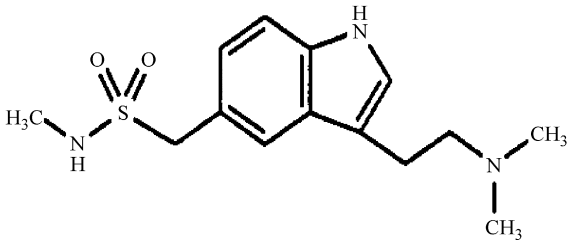

Fig. 1. Chemical structure of sumatriptan.

which not only cost more, but also increased sample-processing time.

To solve these problems, we propose an evaporation-free SPE method, using high organic washing solution and low organic elution solution and inject the eluate directly into the chromatographic system without evaporation and reconstitution steps. The evaporation-free extraction method introduces much desired benefits, for example, the reduced cost, pollution, and processing time. Most importantly, potential evaporative/adsorptive losses, chemical reaction/transformation, and contamination during evaporation, reconstitution, and sample transfer steps are avoided or reduced. Overall, it leads to a faster, economic and reliable bioanalytical method.

## 2. Experimental

### 2.1. Chemicals and regents

Sumatriptan succinate and its internal standard (IS) were obtained from Euresian (Mumbai, India) and Cerilliant (Round Rock, USA), respectively. Acetonitrile and methanol (Omnisolv) were obtained from EMD (Toronto, Canada). Acetic acid (glacial, AnalaR), ammonium acetate (AnalaR), and hydrochloric acid (Assured) were purchased from EMD (Toronto, Canada). Ammonium hydroxide and Trizma® base were obtained from Sigma (Oakville, Canada). Human EDTA $K_3$ plasma was obtained from Vally Biomedical (Winchester, USA). Water was obtained form Milli-Q water system (Milford, USA). High-purity

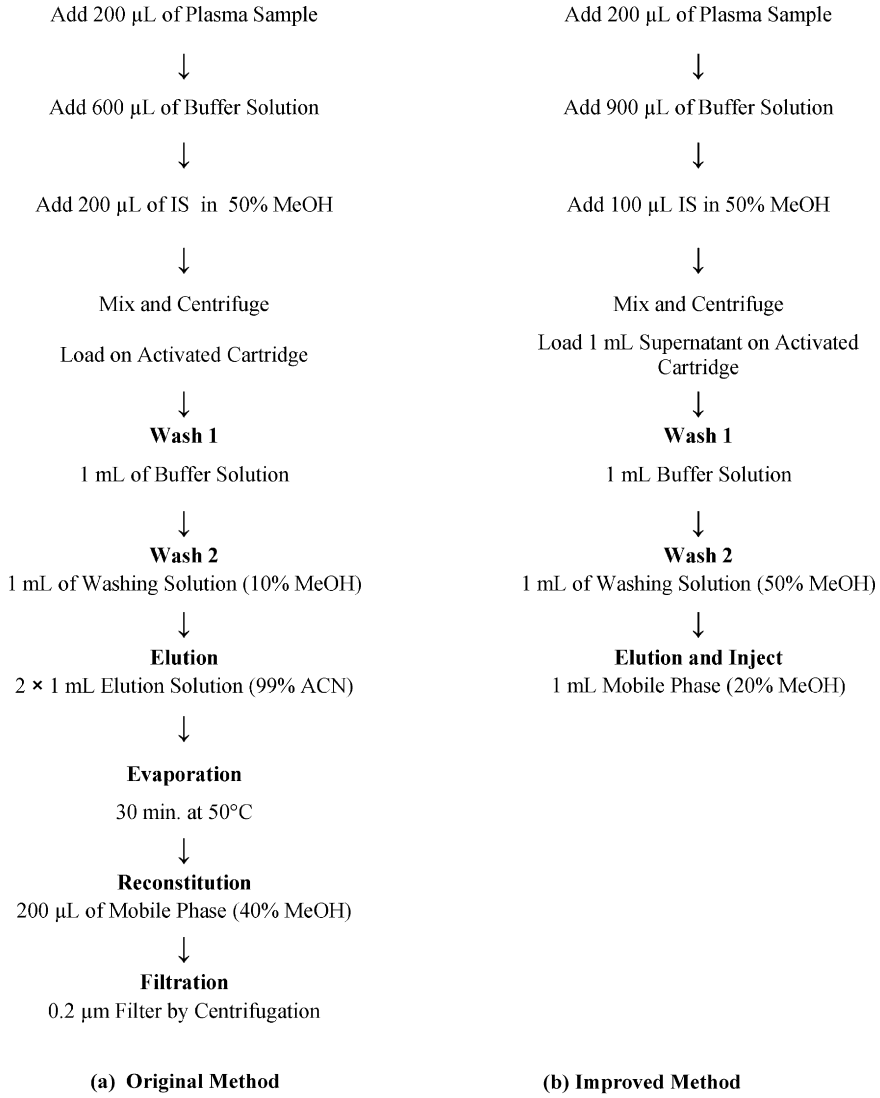


Fig. 2. Comparison of sample processing procedures between (a) the original and (b) the improved method. MeOH: methanol.

liquid nitrogen was supplied by Prodair (Mississauga, Canada).

### 2.2. Calibration standards and quality control samples

The stock solutions of sumatriptan were prepared in water at the concentration of 100 μg/mL. All intermediate and working solutions were prepared by successive dilution of the stock solutions with water. Calibration standards were prepared in control human EDTA $K_3$ plasma at concentrations of 0.99, 1.99, 9.94, 19.88, 39.76, 59.64, 79.52, and 99.40 ng/mL. Quality control samples were prepared at concentrations of 1.00, 3.01, 15.01, 30.12, 70.28, and 100.04 ng/mL.

### 2.3. Sample processing

As shown in Fig. 2b, 200 μL of human EDTA $K_3$ plasma was aliquoted for sample processing. Later, 900 μL of 25 mM Trizma Base buffer and 100 μL of IS in 50% methanol were added and mixed. After centrifugation, 1 mL of the supernatant was loaded on Bond Elut C8 cartridge. The cartridge was washed with 1 mL each of the buffer solution and the washing solution (methanol and water (1:1, v/v, adjusted to a basic pH with ammonium hydroxide)). Then 1 mL of mobile phase was used for elution and the eluate was injected without evaporation and reconstitution into the chromatographic system.

### 2.4. LC–MS/MS conditions

The LC system consisted of a solvent delivery module (Hewlett-Packard series 1100 from Agilent, Montréal, Canada), an autosampler (PE series 200 of Perkin-Elmer, Toronto, Canada), and Zorbax SB-C18 column (50 × 4.6 mm, 5 μm, Agilent). The mobile phase was a mixture of methanol/water (20:80, v/v) with 5 mM ammonium acetate and the flow rate was 1 mL/min. The injection volume was 20 μL.

Mass spectrometric detection was carried out with a Sciex API 4000 equipped with a TurboIonSpray interface (MDS Sciex, Toronto, Canada). The ion source was operated in the positive mode. A transition of *m/z* 296.4 → 58.1 amu was monitored for sumatriptan with a dwell time of 500 ms. The Analyst™ software (version 1.4.1, MDS Sciex, Toronto, Canada) was used for data acquisition and processing. Calibration curves were constructed using sumatriptan and IS peak area ratios with a weighted ($1/C^2$) least-squares linear regression.

### 2.5. Recovery evaluation

The recovery of sumatriptan and IS was evaluated by comparing mean analyte or IS response of quality control samples with mean analyte or IS response of extracted control plasma spiked with appropriate amounts of the respective standard solutions.

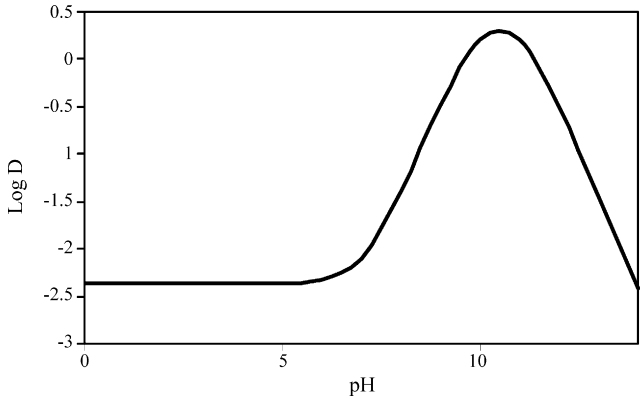


Fig. 3. Sumatriptan hydrophobicity vs. pH (log *D* values were determined by Pallas software, version 3.1, CompuDrug Chemistry, Ltd.).

## 3. Results and discussion

### 3.1. Method development

By studying the hydrophobicity change versus pH (Fig. 3), it is observed that sumatriptan can be more hydrophobic in basic media than in neutral or acidic media. Based on this characteristic, a new evaporation-free SPE method is designed. First, the organic content of washing solution is increased from 10% to 50% methanol. Since it is relatively more hydrophobic at the basic pH, sumatriptan will still be retained in SPE cartridges during washing. After the washing, the mobile phase (20% methanol) is used to elute sumatriptan. The eluate is then injected directly into the chromatographic system (Fig. 2b). Fig. 2 shows that the improved method significantly shortens the sample-processing time.

In order to maintain the high selectivity and sensitivity, tandem mass spectrometric detection was used for sumatriptan. In positive TurboIonSpray Q1 mass spectrum of sumatriptan, $[M+H]^+$ of sumatriptan was the predominant ion (*m/z* 296.4). As shown in the product ion spectrum of *m/z* 296.4 (Fig. 4), the most sensitive mass transition was from *m/z* 296.4 to *m/z* 58.1. Therefore, this transition was used for the quantitation of sumatriptan.

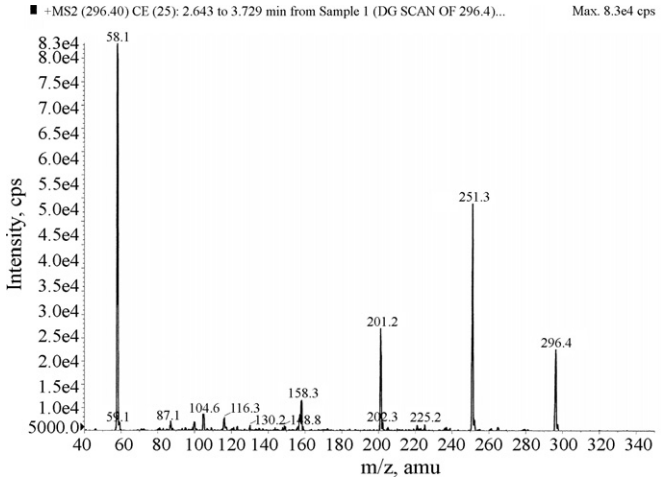


Fig. 4. Product ion mass spectrum of sumatriptan.

Table 1
Recovery of sumatriptan and its internal standard

| | Low QC | Medium QC | High QC |
|---|---|---|---|
| Original method | 85.89 | 85.26 | 81.94 |
| Improved method | 94.22 | 94.74 | 92.12 |

### 3.2. Recovery

In the original method (Fig. 2a), the washing solution contained very low percentage of organic solvent (10% methanol, v/v) while very high percentage of organic solvent (99% of acetonitrile, v/v) was used during elution to avoid analyte loss. However, in this improved method (Fig. 2b) the percentage of methanol (50%) in the washing solution is much higher than that in the elution solution, i.e. mobile phase (20%) and as such, one might think the recovery must be lower than that of the original method. On the contrary, as shown in Table 1, the recovery of the improved method is slightly higher than the recovery obtained with the original method. The reason might be that there are no extra losses associated with evaporation, reconstitution, high-speed centrifugation, and sample transfer steps.

### 3.3. Extract cleanliness

As mentioned earlier in this paper, a filtration step is necessary to remove the suspended extra matrix components in the original method (Fig. 2a). Despite this filtration step, components or particles smaller than 0.2 μm may remain with the analyte of interest. In the improved method, most of the matrix components are either washed down during the washing step or retained in the SPE cartridge. The extract obtained from the improved method is much cleaner than that from the original method.

Shown in Figs. 5 and 6 are the representative chromatograms of control plasma and the lower limit of quantitation samples obtained with the original and the improved methods. In the control plasma processed with the original method, there is an extra matrix component peak and the baseline is noisier. Even though there is a dilution factor of 5 in the improved method (analyte extracted from 0.2 mL sample to 1 mL of mobile phase), the signal in the improved method is at least two times higher (more accurate comparison is not possible owing to the difference in column and mobile phase used). The main reason for this might be that there is much less ion suppression from co-extracted matrix components in the improved method. A post-column infusion test proves that there is negligible ion suppression or no late eluter in the improved method (results not shown).

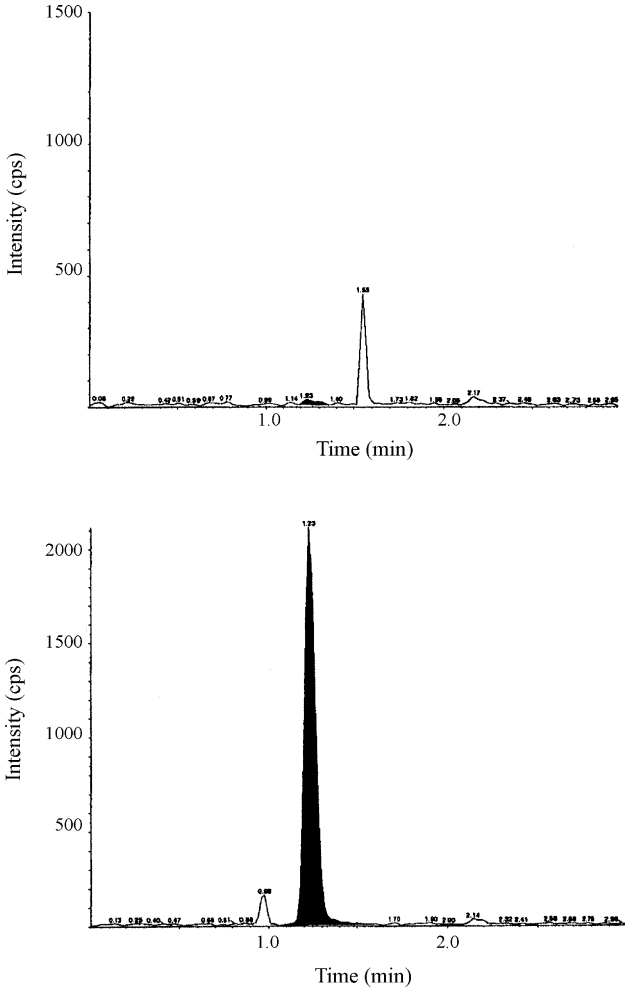


Fig. 5. Representative chromatograms of a processed control human EDTA $K_3$ plasma (upper panel) and the lower limit of quantitation sample containing sumatriptan (1 ng/mL) in human EDTA $K_3$ plasma (lower panel) using the original method (column: Betabasic C18, 100 × 4.6 mm, 3 μm; mobile phase: water/methanol (60/40), 5 mM ammonium acetate; Detector: API 4000 with mass transition of *m/z* 296.2 to 58.2 amu).

### 3.4. Assay precision and accuracy

Nine calibration curves were constructed and the correlation coefficient ranged from 0.9923 to 0.9992.

Table 2
Precision and accuracy of back-calculated standard concentrations

| | Nominal concentrations (ng/mL) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0.99 | 1.99 | 9.94 | 19.88 | 39.76 | 59.64 | 79.52 | 99.40 |
| *N* | 18 | 16 | 18 | 16 | 16 | 18 | 17 | 17 |
| Mean | 1.021 | 1.886 | 9.413 | 19.357 | 39.451 | 61.936 | 83.126 | 100.694 |
| SD (±) | 0.0397 | 0.1000 | 0.7639 | 1.2856 | 1.8742 | 2.8430 | 4.0166 | 8.2751 |
| CV (%) | 3.89 | 5.30 | 8.12 | 6.64 | 4.75 | 4.59 | 4.83 | 8.22 |
| % Nominal concentration | 103.13 | 94.77 | 94.70 | 97.37 | 99.22 | 103.85 | 104.53 | 101.30 |

Table 3
Inter-day (between-run)/intra-day (within-run) accuracy and precision of quality controls samples

| | Nominal concentrations (ng/mL) | | | | |
|---|---|---|---|---|---|
| | 1.00 | 3.01 | 30.12 | 70.28 | 100.04 |
| Between-run (inter-day) | | | | | |
| *N* | – | 52 | 50 | 53 | – |
| Mean | – | 3.017 | 28.207 | 70.394 | – |
| SD (±) | – | 0.1579 | 2.5734 | 3.1916 | – |
| CV (%) | – | 5.23 | 9.12 | 4.53 | – |
| % Nominal Conc. | – | 100.22 | 93.65 | 100.16 | – |
| Within-run (intra-day) | | | | | |
| *N* | 6 | 6 | 6 | 6 | 6 |
| Mean | 0.907 | 2.878 | 30.560 | 71.163 | 102.817 |
| SD (±) | 0.0592 | 0.1995 | 1.7547 | 4.1355 | 1.7694 |
| CV (%) | 6.53 | 6.93 | 5.71 | 5.81 | 1.72 |
| % Nominal Conc. | 90.67 | 95.63 | 101.46 | 101.26 | 102.78 |

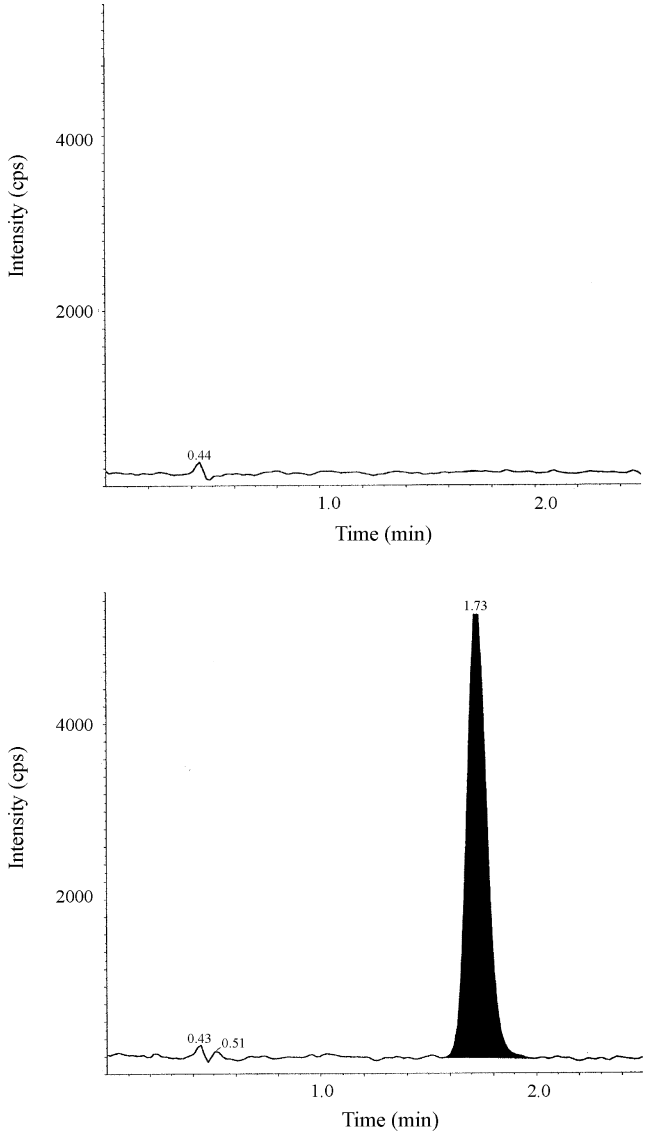


Fig. 6. Representative chromatograms of a processed control human EDTA $K_3$ plasma (upper panel) and the lower limit of quantitation sample containing sumatriptan (1 ng/mL) in human EDTA $K_3$ plasma (lower panel) using the improved method.

Table 4
Summary of incurred sample analysis

| | |
|---|---|
| Number of incurred samples | 1410 |
| Number of analytical runs | 18 |
| Reassay rate (%) | 2.27 |
| Reassay rate excluding diluted samples (%) | 0.99 |
| Run rejection rate (%) | 0.00 |

The accuracy and precision of back-calculated calibration standard concentrations are shown in Table 2. The inter-day (between-run) and intra-day (within-run) accuracy and precision of quality control samples are shown in Table 3. The results prove that the improved method is accurate and reproducible.

### 3.5. Application to bioequivalence study

The improved method was applied to analyze incurred samples from a bioequivalence study at Anapharm Richmond Hill laboratory. Table 4 summarizes the analytical method performance. All runs met the acceptance criteria and the reassay rate is as low as 0.99% (not including dilution reassays). These results show the robustness of the improved method. The better performance is mainly attributed to the fewer number of extraction steps and the isolation of cleaner extracts. Fewer steps can also minimise errors and potential contamination. Cleaner extracts can be translated to less possibility of interference and higher signal-to-noise (S/N) ratio.

### 3.6. Speed and cost

Since several time-consuming steps, e.g. evaporation, reconstitution, filtration, and sample transfers were removed, the processing time with the improved method was significantly reduced. For a typical analytical run with 120–150 samples, 2–3 h can be saved. In addition, the cost is also reduced owing to fewer materials used.

## 4. Conclusions

By taking advantages of sumatriptan hydrophobicity change versus pH, high organic washing and low organic elution steps are used to improve an originally validated in-house SPE method. The new evaporation-free SPE method significantly improved the quantitation of sumatriptan in terms of throughput, reliability, sensitivity, and cost. By removing evaporation and reconstitution steps, many potential problems associated with these steps are reduced or eliminated. To the best of our knowledge, this is the first report of an evaporation-free SPE method where the percentage of organic content in washing solution is higher than that used for elution.

We conclude that the evaporation-free SPE method proposed in this paper possesses a great potential in bioanalytical method development for hydrophilic compounds similar to sumatriptan.

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