ORIGINAL ARTICLE

Development and Validation of Ultraviolet Spectrophotometric Method for Estimation of Frovatriptan Succinate Monohydrate in Bulk and Pharmaceutical Dosage form

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Abstract

Introduction: The research explains the simple, robust, and rapid spectrophotometric method for the estimation of frovatriptan succinate monohydrate (FSM) as API and in films forms. **Materials and Methods:** FSM was determined by ultraviolet-visible double-beam spectrophotometer at 244 nm as wavelength maxima in pH 6.8 simulated salivary fluid. The developed method was validated by taking parameters according to the ICH Q2 (R1) guidelines. **Results and Discussion:** Beer's law was found to be obeyed in the concentration range of 0.1–8 µg/ml with a correlation coefficient of 0.99. Percentage relative standard deviation for all validation parameters was found to be <2%. This analysis method was successfully applied for the determination of FSM in sublingual film dosage forms. **Conclusion:** The results demonstrate that the developed method is accurate, precise, robust, and reproducible, and hence, the developed spectrophotometric method can be used for analysis of FSM in bulk and other pharmaceutical dosage forms.

Key words: Frovatriptan succinate monohydrate, ICH, sublingual, simulated salivary fluid, spectrophotometric, validation

INTRODUCTION

Frovatriptan succinate monohydrate (FSM) is a potent antimigraine drug used for the treatment of acute migraine attacks, especially menstruation migraine. It belongs to the category of triptan which chiefly acts at 5-hydroxyl-tryptamine (5-HT) receptor, especially 5-HT_B and 5-HT_D receptors, and constricts the dilated extracerebral and intracranial arteries of the migraine patients, thus giving relief to intense pain suffered on account of dilated vessels in migraine patient. FSM is 3-methylamino-6-carboxamido-1, 2, 3, 4-tetrahydrocarbazole succinate monohydrate and its structure is presented in Figure 1.[1-3]

There are various analytical methods which had been developed for FSM using ultraviolet (UV) and high-performance liquid chromatographic (HPLC). Laughers *et al.*[4] have developed HPLC method for analysis of FSM in blood plasma. Literature review also revealed that various other HPLC methods developed for estimation of FSM.[5-8] There are also some UV spectroscopic methods developed among which one is developed by Acharjya *et al*., Acharjya *et al.*[10] Verma *et al*.[11] Based on our knowledge and review of literature analysis of FSM in simulated salivary fluid (SSF), pH 6.8 was not reported. The objective of the present work is to develop and validate a new simple UV method for the routine estimation of FSM

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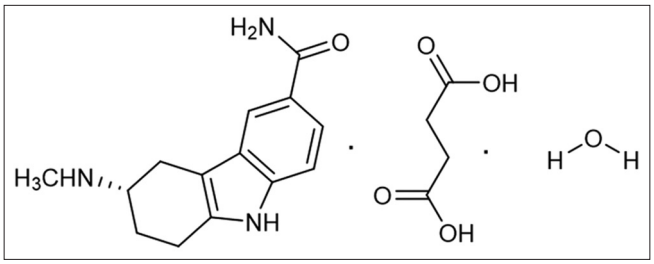

Figure 1: Frovatriptan succinate monohydrate structure

in pH 6.8 SSF which could have further applicability for the determination of FSM in *in vitro* dissolution studies of drug-loaded sublingual films developed by us Singh *et al*.[12] and other dosage forms. The developed method was validated as per the ICH Q2 (R1) guidelines.[13]

Experimental

Material and reagents

Frovatriptan succinate monohydrate (FSM) was obtained from Azakem Chemicals (Hyderabad, India). Potassium dihydrogen phosphate and disodium hydrogen phosphate were procured from Merck Specialties Pvt., Ltd., Mumbai, India, whereas sodium chloride and phosphoric acid were procured from Loba Chemie, Mumbai, India. All other chemicals and reagents used in the procedure were of analytical grade.

Instruments

Double-beam UV-visible spectrophotometer (UV-1800, Shimadzu, Japan) which is connected with computer having UV-Probe software was used.

METHOD

Preparation of SSF (pH 6.8)

Simulated saliva fluid of pH 6.8 used in analysis was prepared as per composition described by Mashru *et al.* 2005.[14] It contains 2.38 g of disodium hydrogen phosphate (Na_2HPO_4), 0.19 g of potassium dihydrogen phosphate (KH_2PO_4), and 8.00 g of sodium chloride (NaCl) per 1000 ml of distilled water. The pH of solution was adjusted to 6.8 using orthophosphoric acid.

Finding of absorption maxima and construction of calibration curve for FSM in SSF (pH 6.8)

Stock solution of concentration 50 µg/ml of FSM was prepared by dissolving 156 mg of FSM (which is equivalent to 100 mg of free base) in 30 ml of pH 6.8 SSF present in volumetric flask whose volume was later made up to 100 ml by adding more SSF to obtain a concentration of 1 mg/ml. 5 ml of the resultant solution was withdrawn and transferred into 100 ml volumetric flask whose volume was made up to 100 ml with SSF (pH 6.8) so as to obtain stock concentration of 50 µg/ml. To obtain the wavelength maxima (λ_{max}), 4 µg/ml solution was prepared from the stock solution which was scanned on UV-visible spectrophotometer in range of 200–400 nm. Then, samples of different concentrations were prepared from the aliquots of stock solutions which were transferred into different precalibrated 10 ml volumetric flask whose volumes were then made up with SSF pH 6.8 so as to obtain solutions having different concentrations, namely, 1, 1.5, 2, 2.5, 3, 3.5, and 4 µg/ml. The prepared solutions of concentration from 1 to 4 µg/ml were analyzed at λ_{max} of 244 nm. Readings were taken in triplicate and average values were used for the construction of calibration plot. Linear regression equation and correlation coefficient were calculated from the calibration plot.

Analytical Method Development

Linearity and Range

Linearity had been accessed by calibration curve in which concentration solutions of FSM, namely, 1, 1.5, 2, 2.5, 3, 3.5, and 4 µg/ml, respectively, were prepared in triplicate and their absorbance was measured at 244 nm. The r^2 value was taken as measure of linearity. The range of developed UV method was calculated as interval between upper and lower concentration of FSM in the solution which obeys Beer's law.

Accuracy

Accuracy of the developed method was ascertained with the aid of three different concentration levels, namely, low concentration level (LCL) 1.0 µg/mL, intermediate concentration level (ICL) 2.0 µg/mL, and a higher concentration level (HCL) 4.0 µg/ml. Accuracy of the method was determined by calculation of percentage recovery at each level, percentage relative standard deviation (RSD) from each level, and standard deviation at each level. Finally, overall standard deviation, overall percentage RSD, and overall percentage recovery were determined taking into consideration all concentration levels chosen.[15]

Precision

Repeatability of the method was determined using different levels of drug concentrations as prepared in the accuracy studies. Interday and different analyst precision studies were also carried out to as part of intermediate precision to make sure that method is precise in nature. Drug concentrations (LCL, ICL, and HCL) in triplicates were prepared on three different days and studied for interday precision ($n = 27$). The same procedure was also used for different analyst precision

studies. The % mean recovery and % RSD were calculated were taken as precision measure.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

Empirical approach which involves subjecting minimum concentrations of calibration plot to series of dilutions whose absorbance values were determined through UV spectroscopy was adopted to calculate LOQ and LOD. The absorbance was measured in replicate of six till the value of RSD came as ≥10% and ≥30%, which signify LOQ and LOD, respectively, of the developed analytical method.

Robustness

Robustness of method is ability to remain unaffected by deliberate variations in method parameters. It was determined by three levels as were taken in accuracy studies whose pH was varied by ± 0.2 units by addition of orthophosphoric acid and sodium hydroxide solution. The absorbance measurements of different levels of FSM concentrations were done in triplicate ($n = 3$).

Specificity

The film dosage form containing 2.5 mg of FSM was placed in a beaker containing 100 ml simulated saliva (pH 6.8) and dispersed it into it by stirring over magnetic stirrer. The obtained dispersion was filtered through 0.45 µm nylon membrane filter and then suitably diluted again with simulated saliva (pH 6.8) to obtain a concentration of 4 µg/ml in triplicate. The obtained concentrations were then analyzed at 244 nm by UV spectrophotometer using simulated saliva (pH 6.8) as blank. Simulated saliva solution (pH 6.8) and placebo solution (containing excipients used in the preparation of film dosage form dissolved in pH 6.8 simulated saliva) were also prepared to check the interference of them with drug. Finally, pure drug stock solution of 4 µg/ml was prepared dissolving pure drug in simulated saliva (pH 6.8). Finally, all the solutions (i.e., simulated saliva solution, placebo solution, pure drug solutions, and film dispersed solution) were scanned individually in the range of 200–400 nm and analyzed for any change and shift in absorbance of drug by comparing it with simulated saliva solution and placebo solution. Finally, from the absorbance value of pure drug solution and film dosage form solution, percentage mean recovery and difference in concentration between drug film solution and pure drug solution was calculated.

Estimation of Percentage Cumulative Drug Dissolved from Developed Sublingual Film using Validated UV Method

The validated UV method was then used for *in vitro* dissolution study of the developed sublingual FSM film. FSM film from the batch used for specificity study was added into dissolution vessel of Type II dissolution apparatus (USP) containing 250 ml of pH 6.8 simulated saliva fluid kept at 37 ± 0.5°C at 50 rpm. 5 ml of sample was withdrawn and replaced with 5 ml of fresh media to maintain sink condition from the dissolution vessels at a various time interval, i.e., 1, 2, 5, 10, 15, 20, 25, and 30 minutes. The samples withdrawn were filtered through a 0.45 µm nylon filter and diluted suitably if required by pH 6.8 simulated saliva fluid. The samples were then analyzed at 244 nm by UV spectrophotometer using a dissolution medium as blank.[12]

RESULTS AND DISCUSSION

Finding of Absorption Maxima (λ_{max}) and Development of Calibration curve for FSM in pH 6.8 SSF (pH 6.8)

The absorption maxima of FSM were found to be 244 nm as shown in Figure 2. The linear regression equation of FSM in pH 6.8 SSF had shown regression coefficient (r^2) value near to 1 i.e., 0.9992,as shown in Figure 3. This r^2 value confirmed the high degree of positive correlation between the two variables, namely, absorbance and concentration. Moreover, low value of RSD below 2 for all concentration solution of FSM as showed in Table 1 further revealed reliability of the developed method.

Linearity and Range

Linearity of developed method in SSF (pH 6.8) was analyzed by means of regression coefficient value (r^2) over the absorbance range of 0.2–0.8. Value of regression coefficient was close to 1 as shown in Figure 3. The FSM concentrations from 0.1 to 8 µg/ml are the range of the developed method as that Beer–Lambert's law was obeyed at this concentration range with regression coefficient (r^2) near to 1, i.e., 0.99.

Accuracy

The accuracy data revealed that mean percentage recovery is 100.214 % (close to 100%) with overall percentage RSD value of 1.273 (<2), thus showing that developed method is highly accurate. Similar results for mean percentage recovery, standard deviation, and percentage RSD were obtained when different concentration levels (i.e., LCL, ICL level, and HCL) were analyzed as shown in Table 2. In each levels, RSD (%) was <2, percentage recovery close to 100%, and low value of standard deviation, i.e., in range of 0.264–0.610. From the results, it was proved that the method is accurate.

Precision

The results of repeatability and intermediate precision have been summarized in Table 3. There was minimal variation

Table 1: Absorbance value of different concentration of FSM solution at λ_{max} of 244 nm (*n*=3)

Concentration (µg/ml)	Absorbance	SD	Percent (%RSD)
0	0	0	0
1	0.213	0.003	1.242
1.5	0.330	0.005	1.553
2	0.445	0.005	1.061
2.5	0.547	0.006	1.116
3	0.667	0.005	0.708
3.5	0.756	0.005	0.596
4	0.886	0.004	0.397

RSD: Relative standard deviation, SD: Standard deviation, FSM: Frovatriptan succinate monohydrate

Table 2: Accuracy data of the various levels of FSM concentration (*n*=3)

Concentration of FSM taken (µg/ml)	% mean recovery	SD	% (RSD)
LCL	98.607	0.610	0.618
ICL	101.164	0.442	0.437
HCL	100.871	0.264	0.262

RSD: Relative standard deviation, SD: Standard deviation, LCL: Low concentration level, ICL: Intermediate concentration level, HCL: Higher concentration level, FSM: Frovatriptan succinate monohydrate

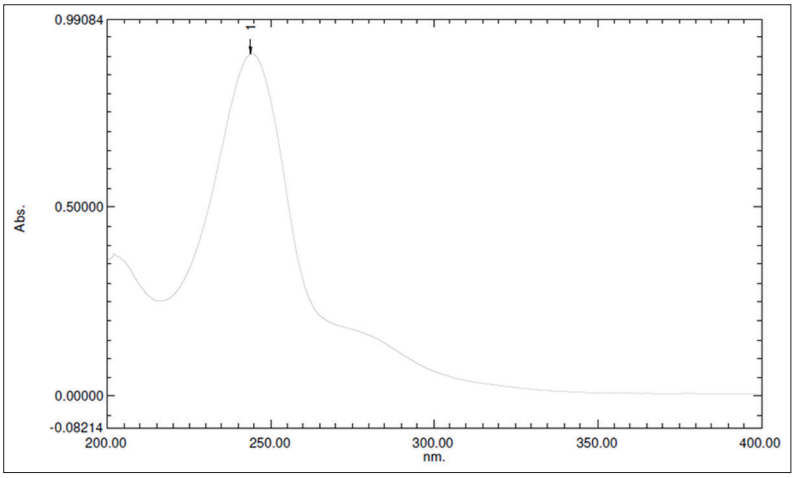

Figure 2: Frovatriptan succinate monohydrate solution (4 µg/ml) when scanned from 200 nm to 400 nm showing λ_{max} at 244 nm

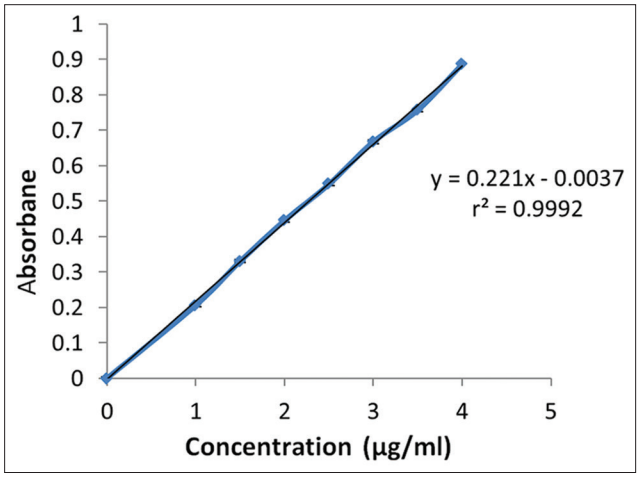

Figure 3: Calibration plot of frovatriptan succinate monohydrate in pH 6.8 simulated salivary fluid

in repeatability, intraday precision, and different analyst precision study at all levels of FSM concentration. The percentage mean recovery of repeatability was between 99.864 and 101.893, interday precision was 98.457–102.044, and different analyst precision was 99.361–102.069. The % RSD was found to not more than 2 for all types of conducted precision study which promises good precision of the developed UV method.

LOD and LOQ

The LOD and LOQ for the developed UV method were found to be 0.0125 µg/ml and 0.05 µg/ml, respectively, as per data of various FSM concentrations highlighted in Table 4. From the obtained results, it can be easily interpreted that this UV method is highly sensitive to analyze FSM.

Robustness

The results as depicted in Table 5 revealed that small variation by ± 0.2 in pH of the SSF did not change the results. For the various drug concentration levels at different pH, the results showed that RSD value was found to be below than 2 and percentage mean recovery was between 98.356 and 101.851 for all concentrations tested.

Specificity

The UV scan of pH 6.8 simulated saliva fluid and placebo solution does not show any absorbance as shown in Figure 4a and b. The UV spectrum of FSM from film solution as per Figure 4c was found not to be changed in the presence of excipients when compared with pure drug stock solution of Figure 4d, thus indicating no interaction between the drug and excipients. From Table 6, percentage mean recovery of drug from pure drug solution and FSM film solution was closed to 100% with low value of standard deviation, i.e. 1.41% and 1.23%, respectively, when analyzed by UV-visible spectrophotometric method. Therefore, the proposed analytical method shows specificity toward the drug.

Estimation of Percentage Drug Dissolved from Film Dosage form

Cumulative percentage of drug dissolved at various time intervals was then estimated from the sublingual film formulation containing FSM using above validated UV

Table 3: Data for various types of precision study (*n*=27)

Concentration of FSM taken (µg/ml)	Recovery level	Absorbance (Ab) of FSM observed Ab±S.D.	Mean concentration of FSM observed (µg/ml)	% Mean Recovery	% RSD
Repeatability					
1	LCL	0.207±0.002	0.999	99.864	0.906
2	ICL	0.447±0.003	2.038	101.893	0.714
4	HCL	0.887±0.007	4.029	100.720	0.748
Interday precision (intermediate precision)					
1	LCL	0.214±0.002	0.985	98.457	1.113
2	ICL	0.447±0.003	2.041	102.044	0.718
4	HCL	0.886±0.003	4.027	100.670	0.336
Different analyst precision (intermediate precision)					
1	LCL	0.216±0.003	0.994	99.361	1.412
2	ICL	0.447±0.007	2.041	102.069	1.475
4	HCL	0.887±0.007	4.031	100.770	0.748

RSD: Relative standard deviation, SD: Standard deviation, LCL: Low concentration level, ICL: Intermediate concentration level, HCL: Higher concentration level, FSM: Frovatriptan succinate monohydrate

Table 4: LOD and LOQ (*n*=6)

Concentration (µg/ml)	Absorbance						Average	S.D.	% RSD
	n1	n2	n3	n4	n5	n6			
0	0	0	0	0	0	0	0	0	0
1	0.202	0.202	0.202	0.201	0.200	0.200	0.201	0.001	0.489
0.9	0.194	0.192	0.196	0.195	0.196	0.193	0.194	0.002	0.840
0.8	0.171	0.174	0.173	0.169	0.168	0.172	0.171	0.002	1.353
0.7	0.145	0.150	0.153	0.151	0.148	0.148	0.149	0.003	1.868
0.6	0.129	0.126	0.126	0.131	0.125	0.124	0.127	0.003	2.081
0.5	0.108	0.105	0.104	0.110	0.107	0.105	0.107	0.002	2.120
0.4	0.085	0.083	0.087	0.088	0.086	0.084	0.086	0.002	2.188
0.3	0.062	0.067	0.068	0.064	0.061	0.066	0.065	0.003	4.337
0.2	0.040	0.046	0.044	0.041	0.047	0.046	0.044	0.003	6.587
0.1	0.019	0.018	0.017	0.021	0.020	0.022	0.020	0.002	9.594
0.05	0.006	0.007	0.008	0.006	0.007	0.008	0.007	0.001	12.778
0.025	0.003	0.002	0.004	0.003	0.003	0.002	0.003	0.001	26.568
0.0125	0.001	0.001	0.002	0.001	0.002	0.001	0.001	0.001	38.730

RSD: Relative standard deviation, SD: Standard deviation, LOD: Limit of detection, LOQ: Limit of quantification

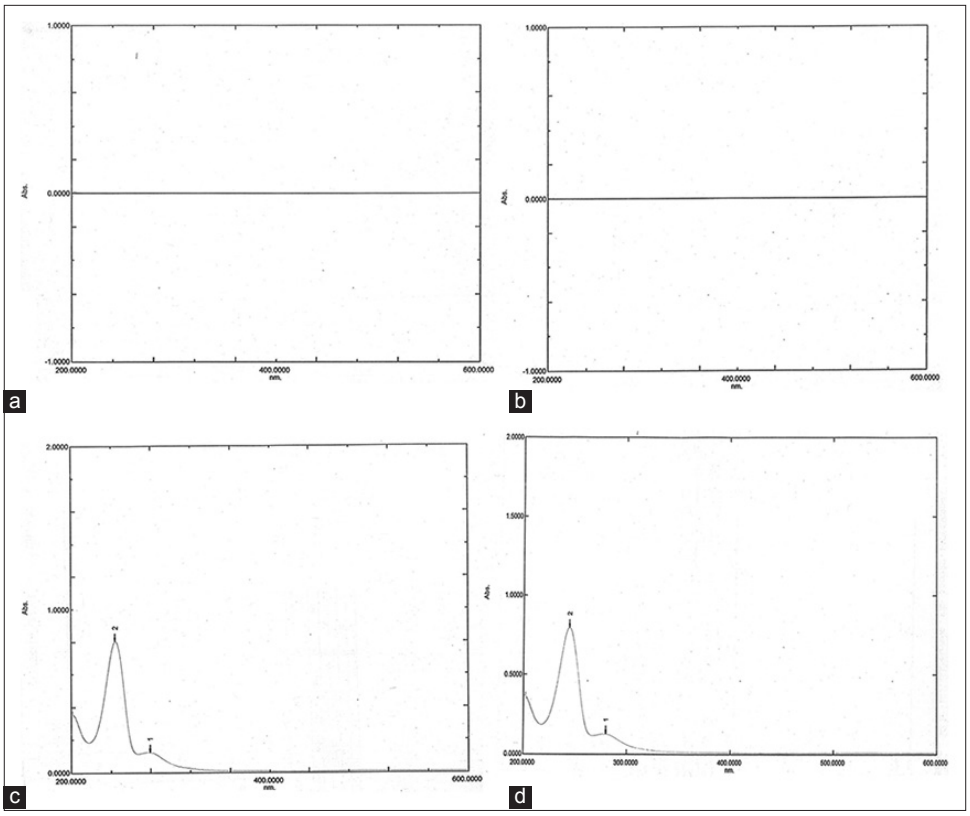

Figure 4: Ultraviolet scans of (a) Blank (pH 6.8 simulated saliva fluid, (b) Placebo solution, (c) drug solution obtained from film dosage form, (d) drug solution obtained from pure drug stock solution

Table 5: Robustness result of FSM validation study (*n*=3)

Concentration of FSM taken (µg/ml)	Recovery level	pH of SSF	Absorbance of FSM observed Ab±S.D	Mean concentration of FSM observed	% Mean recovery	% RSD
1	LCL	pH 6.6	0.214±0.002	0.984	98.356	0.958
1	LCL	pH 6.8	0.218±0.003	1.002	100.166	1.380
1	LCL	pH 7.0	0.219±0.003	1.009	100.920	1.128
2	ICL	pH 6.6	0.439±0.004	2.003	100.158	0.814
2	ICL	pH 6.8	0.444±0.004	2.026	101.290	0.893
2	ICL	pH 7.0	0.446±0.004	2.033	101.667	0.926
4	HCL	pH 6.6	0.877±0.005	3.987	99.664	0.536
4	HCL	pH 6.8	0.885±0.004	4.023	100.569	0.455
4	HCL	pH 7.0	0.897±0.004	4.074	101.851	0.420

RSD: Relative standard deviation, SD: Standard deviation, LCL: Low concentration level, ICL: Intermediate concentration level, HCL: Higher concentration level, FSM: Frovatriptan succinate monohydrate

Table 6: Results of the specificity study (*n*=6)

Mean concentration of FSM in film dosage form solution in µg/ml	Mean concentration of FSM in pure drug solution in µg/ml	Difference between pure drug and film concentration (µg/ml)	% Mean recovery of film dosage form solution containing FSM at 4 µg/ml	% Mean recovery of FSM pure drug solution at 4 µg/ml
3.990±0.05	4.021±0.06	0.031	99.75±1.23	100.525±1.41

FSM: Frovatriptan succinate monohydrate

spectrophotometric method. The results of the study are given in Table 7. From the result, it was found that the developed UV method was able to determine amount of FSM dissolved at various time intervals in simulated saliva fluid.

Table 7: *In vitro* dissolution of FSM-loaded film (*n*=3)

Time intervals (min)	Percentage drug dissolved (Mean±S.D.)
1	29.97±1.38
2	45.68±1.22
5	58.18±1.13
10	72.24±1.56
15	83.45±2.20
20	95.77±0.59
25	98.49±1.09
30	99.18±0.74

SD: Standard deviation, FSM: Frovatriptan succinate monohydrate

CONCLUSION

We have developed and validated UV spectrophotometric method as per the ICH Q2 (R1) guidelines. The method is found to be quite easy, fast, accurate, precise, robust, and economical for the routine analysis of FSM in bulk and other dosage forms, especially sublingual dosage forms in simulated saliva fluid of pH 6.8. Moreover, the present method provides a very good alternative for estimation of FSM by UV spectroscopy as compared to methods such as HPLC, and liquid chromatography-mass spectrometry which are expensive and require a high expertise to carry out these methods.

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