

THE RAPID AND GREEN HAIR ANALYSIS METHOD DEVELOPMENT USING FTIR FOR PAPAVERINE HCL DETERMINATION

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ABSTRACT

Objective: This study was aimed to develop a rapid analysis using FTIR (Fourier Transform Infra-Red) for papaverine hydrochloride (HCl) determination in the hair sample, supported by a mathematical manipulation; which never been reported before in toxicology and forensic analysis.

Methods: Firstly, the method was checked its validity to ensure the feasibility for the quantitative purpose. The absorbance spectrums were collected by measure the drug, matrix, and its mixture. A spectra which showed the best specificity and linearity then was selected and derived. Afterwards, the area under the curve (AUC) was measured. A series of concentration was used for compose the calibration curve. Based on the result, some validation parameters were checked thoroughly. Further, for sample preparation, hair was collected non-invasively, then was decontaminated using soap. Next, it was immersed into a papaverine HCl solution at a concentration of 25 mg/ml along days. Finally, the amount of drugs absorbed were measured by the developed method using FTIR.

Results: Experimental data showed that all validation parameters could be fulfilled by the developed method. The selected spectra for the content determination was 1320-1230 cm^{-1} . Its linearity was represented by a correlation coefficient value (r) ≥ 0.9999 , variation coefficient (V_{x0}) $\leq 2.0\%$. The limit of detection (LOD) was 0.00618% w/w, meanwhile, the limit of quantitation (LOQ) was 0.02060% w/w, respectively. The percent recovery was in the range 97-103% with the relative standard deviation (RSD) was $\leq 2.0\%$. The drug has detected after 72 h immersion, moreover, after 192 h the concentration gained was 0.1594 \pm 0.0011% w/w.

Conclusion: As the conclusion, FTIR absorbance-derivative method is adequate as a rapid procedure for determine papaverine HCl in the hair sample. This method shows the appropriate of specificity, accuracy and precise. In addition, it shows the advantages of simplicity, green/eco-friendly, and cost-efficiency.

Keywords: Papaverine HCl, Hair analysis, Rapid method development, FTIR, Quantitative, Absorbance derivate, Mathematically manipulation, Direct measurement, Non-invasive, Toxicology, Forensic, Green analysis

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INTRODUCTION

Time by time, not only drug production developed into more advanced but also followed by the growth of distribution facilities. The development of drug distribution facilities, the spread becomes wider and can be easily obtained by various layers of society. Thus, there is a high probability of misuse of the effects given by the drug. One class of drugs that are often misused is psychoactive drugs. The compounds work in the central nervous system that can change consciousness, mood and mind. These effects on the central nervous system are generally expected by users to get pleasure and avoid pain. The change in brain function can cause dependence. These compounds affect the processes of perception, emotion, and motivation in the brain [1, 2]. One drug that has widely been misused for a long time is papaverine HCl, which belongs to the opium group.

With the increasing number of misuse of the use of papaverine HCl, a simple but accurate test method is needed to detect misuse. In this experiment, hair analysis was aimed to confirm its suitability to test this drug. The advantages of using hair samples for testing include fast, simple and non-invasive to collect. Besides, the sampling can be monitored easily, so that it can reduce fraud by donors. In addition, this specimen is very stable compared to other sample forms and can be storage in the ambient condition. Moreover, hair can save the drug, which was consumed a few months beforehand. As a comparison, urine is generally only 2-3 d for most drugs, meanwhile blood and saliva approximately one day merely. Last but not least, it also can be used to identify the long-term abuse, and make it possible to analyze abuse from month to month in more detail [3-7].

The initial hypothesis of this study was that there is a distribution of papaverine HCl compounds from the blood into hair and stored there "forever" [8-10]. The aim of this study was to detect and quantify the

psychoactive drug in hair samples using FTIR. Infrared analysis has been applied a long time to identification. In fact, some previous researches has proved that this method can be used for the quantitative purpose, in a simple and rapid way [11, 12]. Small compounds with high hydrophobicity and the presence of ionizable base groups tend to bind to hair structures [13, 14]. As a psychotropic agent, papaverine HCl is a hydrophobic substance which need an organic solvent to dissolve it [15]. The active compound has a minimal effect on the central nervous system, although large doses can cause sedation and drowsiness in some patients. Side effects that can be caused, includes depressant and sedation in the central nervous system. These side effects can be misused by the users [16].

The IR absorption spectrum obtained can be described as a characteristic of a chemical compound, because it can read almost all of the difference's vibration energy levels [17, 18]. Actually, some experiment of hair analysis has been reported using FTIR [19-21]. However, this method has the lack specificity due to many spectrum appearances. Even so, the mathematical manipulation by derivation of absorbance will be applied to improve its selectivity and sensitivity. Thereof, this method was expected can be utilized to detect and measure papaverine HCl in a rapid a green procedure, without any organic solvent used.

MATERIALS AND METHODS

Material and apparatus

The instrument and tool-using in this experiment were FTIR Jasco-4200 type A (Japan), a milligram electronic scales (Mettler Toledo, USA), small marble mortar, pestle, pellet pressing, oven, beaker, measuring flask. Meanwhile, the materials were papaverine HCl standard (USP), ethanol 95% (Merck, Germany), potassium bromide

(Merck, Germany), human hair samples (woman, black color without dying).

Methods

Present research was conducted in several stages. First step was sample preparation, the second was the validation of FTIR method, then the last was an application of it to analyze the drug concentration in the hair sample.

Development of the FTIR method

Firstly, standard of the drug was analyzed using FTIR to get the transmittance spectrum. Then, it was transformed to absorbance, next was derivated mathematically using the program included in the instrument. The spectra which shown clear and was not overlapped is the choice. The selected spectra then were investigated its linearity, detection and quantification limit, range of analysis, afterwards evaluated accuracy and precision of intra-and inter-day. Further, the hair sample was prepared to be analyzed. Some head hairs were soaked with the drug solution along eight days. Sampling was conducted every day and measured.

Measurement of papaverine HCl standard

Papaverine HCl was dispersed with potassium bromide in a series of the concentration of 0.25-1.5% w/w. A total of 10 mg of the mixture was taken and pressed. Then it was measured using FTIR Jasco-4200 type A in waves of 4000 to 400 cm^{-1} , three times. The spectrum was corrected against the baseline and converted into its derivatives so that it was clearer to increase the selectivity. The area under the curve was measured in several ranges of the wave numbers. Determination of the wave number range was done by plotting the concentration of papaverine HCl to the area under the curve (AUC) of the derivated absorbance-spectra. The range of the wave numbers which could produce a strong and consistent peak was observed and selected.

Validation method

Specificity test was performed by a measured spectrum of hair samples with and without papaverine HCl. Then, all resulted were overlaid. An independent spectra which were the cleanest and no interference of the matrix, was selected.

The standard concentrations of papaverine HCl in potassium bromide was prepared (0.25; 0.50; 0.75; 1.00; 1.25 and 1.50% w/w). For each concentration, three measurements were taken. The transmittance spectrum then was transformed to be absorption, next was derivated mathematically using the software included in the instrument. The area under the curve (AUC) was calculated, further was plotted against concentration. The curve yielded was observed its linearity. The acceptability criterion for linearity is the

correlation coefficient (r) ≥ 0.999 and the coefficient of variation from the regression function $\leq 2.0\%$ [22, 23].

The LOD and LOQ can be estimated from the standard curve produced by the following equation:

$$\text{LOD} = 3 \times \text{Sb}/b$$

$$\text{LOQ} = (10 \times \text{Sb})/b$$

With Sb is the standard deviation, and b is the slope of the curve obtained [23, 24].

The concentration range used for the linearity test, accuracy and precision were determined from the observations and the resulting calibration equations.

Accuracy test then was carried out by measuring the standard of papaverine HCl with concentrations of 80, 100 and 120%, three times for each concentration. Acceptance criteria are recovery of 97-103% [24, 25].

Precision test was carried out by measuring the concentration 100% of papaverine HCl, as much as six times a day, and measurements were taken in three different days. The acceptability criterion for precision is the relative standard deviation (RSD) $\leq 2\%$ [22, 24].

Determinations of papaverine HCl level in the sample

The hair-sample was prepared by collecting 20 strands of human hair with the length of 10 cm. These were washed separately with 1 ml of liquid detergent to remove external contaminants and rinsed thoroughly with distilled water. Next, it be filled into and soaked in a papaverine HCl solution with a concentration of 250 mg/25 ml along eight days. The samples then were air-dried. Sampling was carried out every 24 h. In each measurement, one strand of hair was pulverized using a marble mortar. Further, the sample was added and dispersed by potassium bromide crystal then to be pressed to produce a pellet. Finally, it be analyzed using FTIR.

RESULTS AND DISCUSSION

Measurement of papaverine HCl standard

The development method was begun by quantifying the concentrations of papaverine HCl in potassium bromide. The spectrum would show the relationship between the papaverine HCl's concentrations and percent transmittance. The greater of concentration will produce the smaller of the percentage of transmittance. It due to the more lighting is absorbed as shown in fig. 1. It is in accordance with the Lambert-Beer law which states the concentration is proportionally direct to absorbance and inversely to the logarithmic transmittance.

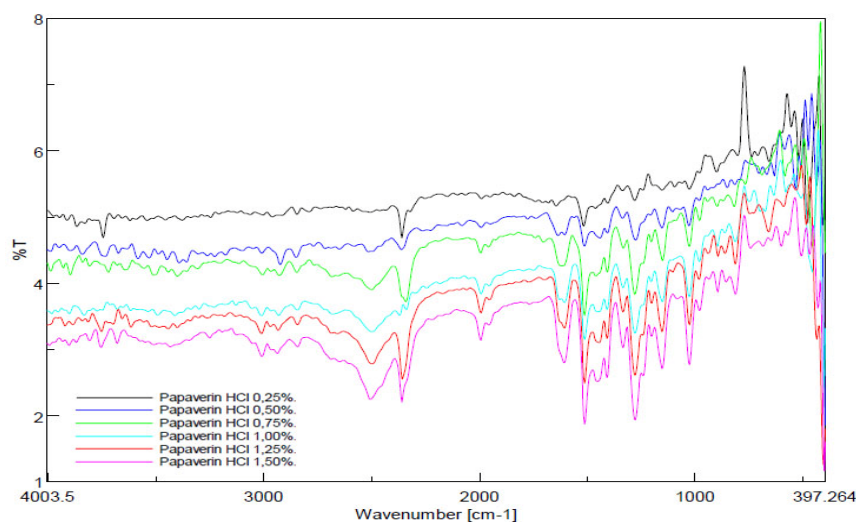


Fig. 1: The overlaid spectra of a series of papaverine HCl's concentration

Spectra derivatization

The FTIR spectrum obtained was next transformed to absorbance and afterwards was derivatized. Before derivatization was carried out, the baseline correction was performed. From the spectrum of

derivatization results obtained AUC. The AUC value at various wavenumbers that produces a strong and consistent peak is plotted against the concentration of papaverine HCl in potassium bromide (% w/w) and then determines its linearity. Fig. 2 shows the spectrums of derivatization of the standard of papaverine HCl.

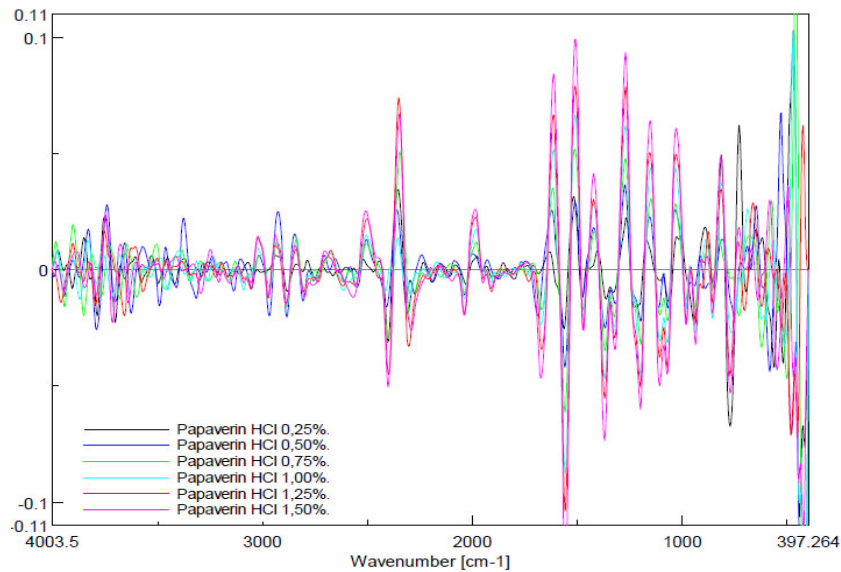


Fig. 2: Overlay of papaverine HCl absorbance spectra derivative in a varies concentration

Furthermore, the derivated spectrums are shown in fig. 3. Next, it was selected to be used to determine the range of wave numbers by

choice one spectra that has the best linearity and was not interfered by matrix.

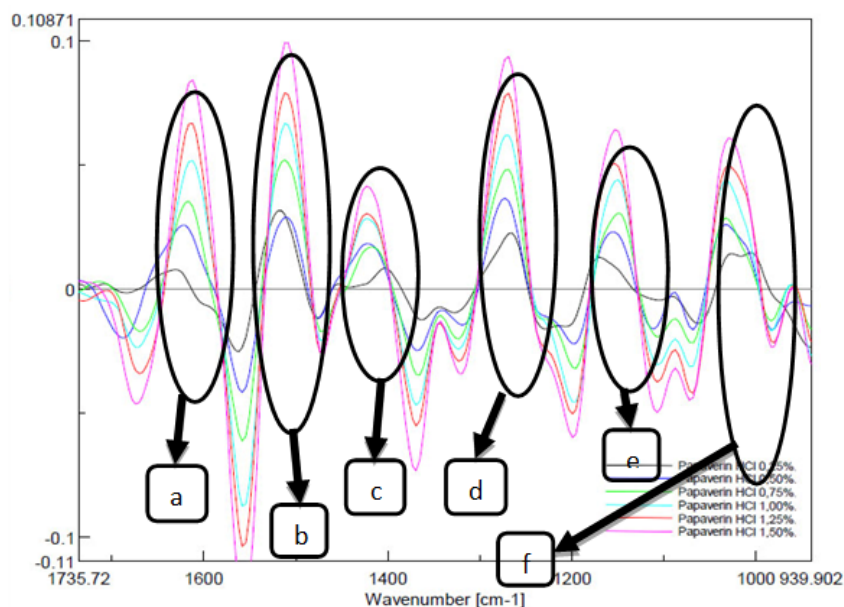


Fig. 3: Derivated absorbance-spectra of papaverin HCl standard at: a) 1670-1575 cm^{-1} , b) 1550-1475 cm^{-1} , c) 1480-1370 cm^{-1} , d) 1320-1230 cm^{-1} , e) 1200-1110 cm^{-1} , f) 1070-975 cm^{-1}

Spectra selection

Fig. 3 shows the spectrum of derivatization of raw papaverine HCl in a varies concentrations in several wavenumbers. Furthermore, the AUC for each derivated absorbance spectra in the range of wave numbers was determined. Then, each was plotted against the concentration of

papaverine HCl to test linearity. Table 1 shows the data collected and the coefficient of correlation (r) for each range of wave numbers. Spectra at 1320-1230 cm^{-1} had the highest correlation coefficient, with $r = 0.9999$. As noted, the calibration curve will be revealed in the sub-chapter of linearity. Therefore, this area was next used as the quantitative-based of the sample's assay method.

Table 1: AUC of absorbance-derivate spectra of papaverine HCl in a vary concentrations

C (% w/w)	AUC average±SD at the wavelength area (cm ⁻¹)*					
	1670-1575	1550-1475	1480-1370	1320-1230	1200-1110	1070-975
0.25	0.2639±0.0036	1.0160±0.0467	0.2371±0.0136	0.7277±0.0154	0.3995±0.0146	0.6449±0.0863
0.50	1.0672±0.0627	0.9718±0.1033	0.6925±0.0159	1.2182±0.0173	0.7508±0.0077	0.9976±0.0109
0.75	1.1677±0.0623	1.8649±0.0290	0.6358±0.0961	1.7452±0.0124	1.0326±0.0152	1.0540±0.1335
1.00	1.8239±0.0079	2.1602±0.0400	0.9707±0.0102	2.2810±0.0425	1.3988±0.0224	1.5202±0.1646
1.25	2.1619±0.0794	2.3989±0.1615	1.0533±0.0394	2.8059±0.0415	1.6939±0.0904	1.9301±0.0545
1.50	3.0038±0.2234	3.2052±0.0294	1.3813±0.1180	3.3229±0.1737	2.1592±0.1899	2.1666±0.0804
Coefficient correlation (r)	0.9684	0.9471	0.9312	0.9999	0.9952	0.974

Note: C: concentration of papaverine HCl, AUC: area under the curve of absorbance, SD: standard deviation. *All values are reported as mean±SD (n=3)

Validation of method

Specificity test

Specificity test was carried out by comparing FTIR spectra from hair, standard of papaverine HCl, and the mixture of both. Fig. 4 shows that the spectrum produced by papaverine HCl was almost the same as the mixed spectrum of hair-papaverine HCl. Nevertheless, there was a difference spectra at 3400 cm⁻¹, which indicated the presence

of an OH group produced by the mixture. The peak also belongs to the hair spectrum. At the wave-numbers 1500-1000 cm⁻¹, there was a sharp peak on the spectrum of papaverine HCl, and the spectrum produced by the hair-papaverine mixture. However, at the wave number, there is no peak spectrum of the hair.

This is showing that no interference of hair as the matrix towards papaverine HCl measurement. Then this spectra can be set as the specific for quantification.

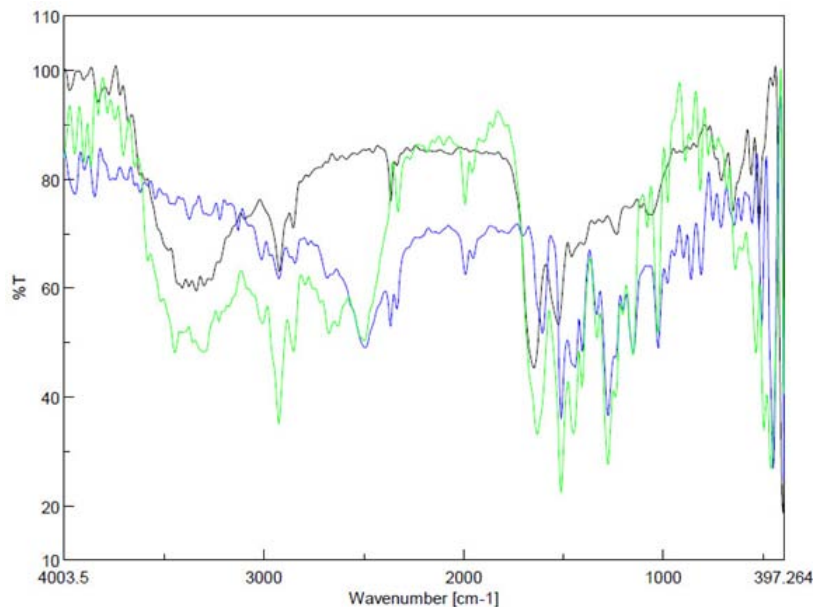


Fig. 4: Spectrum FTIR of: hair (black curve), papaverine HCl (blue curve) and the mixture of both matters (green curve)

Linearity test

The linearity test was carried out by plotting six concentrations of papaverine HCl in potassium bromide (0.25-1.5% w/w) against derivative AUC at wave numbers 1320-1230 cm⁻¹. Each

concentration was tested three times. Regression equations and correlation coefficients were obtained by plotting the concentration of the drug against AUC. Table 2 shows the AUC produced from the spectrum of raw derivatives of papaverine HCl for each concentration.

Table 2: Linearity data of papaverine HCl standard in the hair sample

C (% w/w)	AUC of derivated absorbance spectra*
0.25	0.7277±0.0154
0.50	1.2182±0.0173
0.75	1.7452±0.0124
1.00	2.2810±0.0425
1.25	2.8059±0.0415
1.50	3.3229±0.1737

Note: C: concentration of papaverine HCl, AUC: area under the curve of absorbance, *All values are reported as the mean of average±SD (n=3), from the data, a calibration curve was composed with the result is shown in fig. 5 as follows.

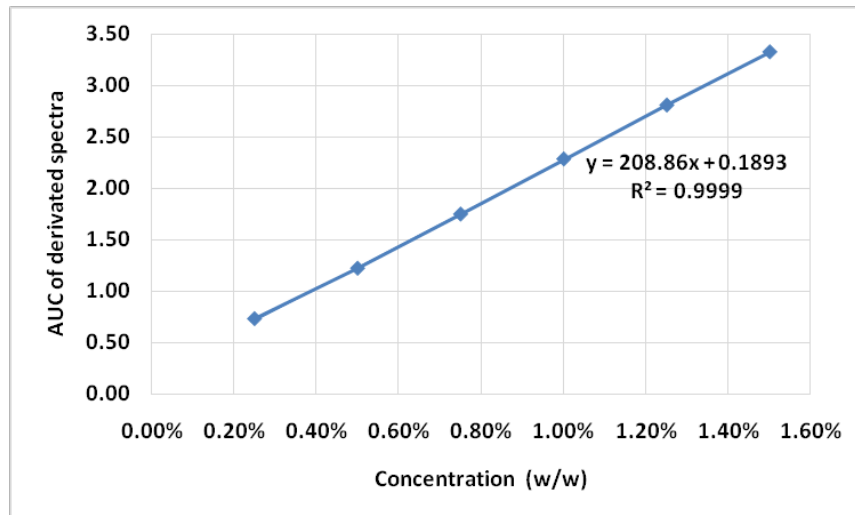


Fig. 5: The calibration curve from the linearity test

Fig. 5 shows that the regression equation obtained for range concentration of 0.25-1.50% (w/w) was: $y = 208.86x + 0.1893$. This result has a correlation coefficient (r) of 0.9999 and V_{x0} of 0.00206%. The value of r was obtained ≥ 0.999 with $V_{x0} \leq 2.0\%$, it indicates that the method used has met the acceptance of linearity [24].

Limit of detection and quantification

Detection and quantification limits were calculated based on data obtained from the linearity test, using the formula as follow:

$$S_{y/x} = \sqrt{\frac{\sum(y_1 - \bar{y}_1)^2}{n-2}} = 0.004303268$$

$$\text{Limit of detection (LOD)} = \frac{3 S_{y/x}}{b} = 0.00618\% \text{ w/w}$$

$$\text{Limit of quantification (LOQ)} = \frac{10 S_{y/x}}{b} = 0.02060\% \text{ w/w}$$

From the results above, the sensitivity of the instrument for the detection of samples with the smallest concentration was 0.00618% w/w. Next a concentration of 0.02060% w/w gave the accurate and precise results.

Range of analysis capacity

Based on the linearity test, in the concentration range of 0.25-1.50% w/w shows good linearity, accuracy, and precision. So, the concentration range can be used in this method.

Accuracy test

Accuracy test was carried out by measuring the concentrations of the standard were 80, 100 and 120% and measuring three times for each. Table 3 shows the AUC produced and percent recovery obtained for the concentrations.

Table 3: Accuracy data of papaverin HCl standard

C (%w/w)	AUC of derivated spectra	Average of AUC of derivated spectra*	% recovery
80	1.87247	1.84331±0.02527	98.99035
	1.82961		
	1.82785		
100	2.27627	2.29081±0.02127	100.618
	2.31522		
	2.28093		
120	2.69113	2.70452±0.01519	100.355
	2.72102		
	2.70140		

Note: C: concentration of papaverine HCl, AUC: area under the curve of absorbance, *All values are reported as mean of average±SD (n=3)

Table 3 reveals the percent of recovery for the concentrations of 80,100 and 120% w/w are in the range of 97-103% w/w. It means that the result meets the requirements for acceptance.

Precision test

The test was carried out by measuring the concentration of 100% papaverine HCl. It was conducted over three days, with each day was six measurements. Data was taken to obtain intra-day and inter-day precision.

Intra-day precision test

Table 4 shows the AUC resulted in measuring 100% papaverine HCl, repeated in three days. From table 4 above, the relative-standard-deviation of intra-day precision obtained from three-day measurement, all were less than 2.0% so that it meets the requirements for acceptance.

Inter-day precision

Table 5 shows the average of AUC of derivated-absorbance resulted from measuring 100% w/w papaverine HCl. This test also was carried out on three different days.

From table 5 above, the RSD of the inter-day precision test was less than 2.0%. It means that the result met the acceptance criteria [25].

All validation parameters, namely specificity, linearity, limit of detection, limit of quantification, ranges, accuracy, and precision have been ensured. Furthermore, this method can be used to determine the levels of papaverine HCl in hair samples, directly without any extraction.

Determination of papaverine HCl levels in hair samples using FTIR

The content determination was done on hair that has been soaked in papaverine HCl solution. Sampling was carried out

every 24 h. Hairs were cut into very small pieces then dispersed in potassium bromide. The assay was conducted using a

validated FTIR method. The results of the analysis are shown in table 6 below.

Table 4: Intra-day precision test result

No.	AUC of the derivated spectra on the day-*		
	1	2	3
1	2.27627	2.18784	2.23709
2	2.31522	2.22518	2.26770
3	2.28093	2.22646	2.26547
4	2.23709	2.20305	2.24778
5	2.32047	2.23113	2.27357
6	2.36127	2.25550	2.25893
Average of AUC	2.29854	2.22153	2.27753
SD	0.04307	0.02350	0.03740
RSD (%)	1.87392	1.05764	1.64203

Note: AUC: area under the curve of absorbance, SD: standard deviation, RSD: relative standard of deviation., *All values are reported as one measurement.

Table 5: Inter-day precision data

The day-	Average of AUC absorbance's derivate*
1	2.29854±0.04307
2	2.22153±0.02350
3	2.27753±0.03740
Average of AUC	2.29854
SD	0.03981
RSD (%)	1.75695

Note: AUC: area under the curve of absorbance, SD: standard deviation, RSD: a relative of standard deviation, *All values are reported as mean of average±standard deviation with n = 3.

Table 6: Papaverine HCl content of the hair sample

The h of-	Average of AUC	Papaverin HCl level (% w/w)*
72	0.238223±0.000677	0.0234±0.0003
96	0.243545±0.003363	0.0260±0.0016
120	0.305179±0.003702	0.0555±0.0018
144	0.387432±0.002576	0.0949±0.0012
168	0.444529±0.001718	0.1222±0.0017
192	0.522187±0.002278	0.1594±0.0011

Note: AUC: area under the curve of absorbance, *All values are reported as mean of average±standard deviation with n = 3.

As sample preparation, papaverine HCl in this analysis simulation was entered into the hair externally. Surely the compound also can be entrapped internally, because hair is continually fed by blood, which contains everything that entered the body [3-7]. For the real and factual condition, hair sample should be taken after one-month usage, in an assumption that hair growing 1 cm/month [26-29].

As explained before, the method has the limit of detection and quantification as 0.00618% w/w and 0.02060% w/w. Data showed, before 72 h (three days) the drug can't be determined quantitatively, because the response was smaller than the quantification limit. After the time, papaverine HCl level increasing and was over the limit of quantification. The response clear yet and can be analyzed accurate and precisely. Therefore, the method was applicable to determine papaverine HCl in hair using FTIR, without any extraction and dilution.

Nothing perfect, on the other hand, this method has disadvantages too. Mainly are the lower of specificity and sensitivity than other instrumentations. Nevertheless, the derivation as a mathematical-manipulation has been proven can increase the specificity. As comparison, the sensitivities of FTIR methods calculated as approximately are 100-200 times lower than HPLC. However, it is almost equal compared to spectrophotometry uv-visible [9, 30, 31]. Fortunately, this method in the next development, still can be improved by the standard addition method. This way is commonly used to increase the response besides the derivation mathematically

support [32]. On the future, this should be attempted to support and improve the sensitivity [32]. Furthermore, based on over experience, FTIR's procedure was shown very easy and simple. Moreover, due to the free of organic solvent, this method can be stated as the greener, more economic and efficient. Briefly, it is suitable to be used as a rapid method for papaverine HCl quantification in the hair sample.

CONCLUSION

FTIR has been proven to be adequate and suitable for determining the levels of papaverine HCl in hair samples. The specific spectra lays at 1320-1230 cm^{-1} , with the detection and quantitation limits are 0.00618 and 0.02060% w/w respectively. This method showed advantages on its simplicity and efficiency due to not need any extraction. Therefore, it can be stated as the greener, rapid and cost-efficient.

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AUTHORS CONTRIBUTIONS

All the author have contributed equally

CONFLICT OF INTERESTS

Declared none

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