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Spectrofluorimetric and derivative absorption spectrophotometric techniques for the determination of loperamide hydrochloride in pharmaceutical formulations

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Abstract: Simple, sensitive and rapid spectrofluorimetric and derivative absorption spectrophotometric procedures are described for the accurate determination of loperamide hydrochloride. The spectrofluorimetric method involves the measurement of the fluorescence of the compound in an ethanol–sulphuric acid mixture (90:10, v/v) using either the direct or the synchronous modes of measurement. Optimum conditions for maximum fluorescence are described. The derivative spectrophotometric method involves the measurement of either the second derivative peak amplitude (crest to trough, i.e. maximum to minimum) between 258 and 263 nm or the second derivative peak height (i.e. maximum to zero line) at 224 nm of an ethanolic solution of the drug. The proposed methods have been used for the determination of loperamide in pharmaceutical formulations. Compared to the pharmacopoeial method, the proposed methods give equally accurate (*t*-test) and precise (*f*-test) results. The proposed methods have the advantages of being highly sensitive for the determination of small concentrations of loperamide, a weak UV-absorbing compound prescribed in low doses.

Keywords: *Loperamide hydrochloride; fluorescence; synchronous fluorescence; derivative spectrophotometry; derivative ratios.*

Introduction

Spectrofluorimetry has long been applied in the field of biomedical studies, including clinical, pharmacological and pharmaceutical analysis, because of the higher sensitivity and degree of specificity than is attainable in a corresponding absorption spectrophotometric assay. However, light scattering is one of a large number of experimental variables that affect fluorometric methods of analysis [1]. Synchronous fluorescence spectrophotometry has advantages over conventional spectrofluorimetry: the Rayleigh scattering is practically eliminated, the Raman scattering is broadened and the spectral bandwidth is reduced, thus giving rise to better defined spectra and increased selectivity [2, 3].

The application of derivative techniques to spectrophotometry has proved to be useful in resolving spectral overlap and matrix interference [4]. The technique has wide applications for the assay of single or multicomponent dosage forms [5, 6] and in stability studies for the determination of an intact drug in the presence of its degradation products [7].

Loperamide hydrochloride, 4-(*p*-chlorophenyl)-4-hydroxy-N,N-dimethyl-α,α-diphenyl-1-piperidinebutyramide monohydrochloride, is used for the control and symptomatic relief of acute non-specific diarrhoea and chronic diarrhoea associated with inflammatory bowel diseases [8]. The weak UV-absorption of loperamide hydrochloride (A 1%, 1 cm is 13.2 in ethanol) [9] in addition to the small dose (2 mg per capsule or 1 mg per 5 ml syrup) means that a direct spectrophotometric assay is susceptible to interference from the formulation excipients and this necessitates the development of alternative methods for the routine assay of the drug in pharmaceutical formulations. The official assay method for the determination of loperamide hydrochloride in pharmaceutical formulations is based on HPLC [10].

The present study deals with the development of a simple and sensitive spectrofluorimetric method and a second derivative spectrophotometric method for the determination of loperamide hydrochloride in low dose formulations.

Experimental

Materials

An authentic sample of loperamide hydro-

chloride (M.S. Chemicals, Italy) was gifted by the Alexandria Co. for Pharmaceutical and Chemical Industries. Also used were sulphuric acid (specific gravity 1.84, analytical reagent, BDH, UK), ethanol (analytical reagent, BDH, UK) and acetonitrile (HPLC grade, BDH, UK).

Reagent

Ethanolic sulphuric acid mixture was prepared by adding 900 ml of ethanol slowly to 100 ml of sulphuric acid (SG 1.84) with cooling.

Apparatus

A Perkin–Elmer Model 550S UV–vis spectrophotometer and Hitachi Model 561 recorder were used. The absorption and second derivative spectra of the reference and test solutions were recorded in 1-cm quartz cells over the range 320–210 nm. Suitable settings were: scan speed 120 nm min^{-1}, charge speed 60 nm min^{-1}, response time 6 s and slit width 2 nm.

A Perkin–Elmer Model 650-10S fluorescence spectrofluorimeter and Perkin–Elmer Model 56 recorder were used. Conventional fluorescence spectra (λ_{ex} 310 nm) and synchronous fluorescence spectra ($\Delta\lambda$ 55 nm) were recorded in 1-cm quartz cells over the range 320–420 nm. Suitable settings were: excitation and emission monochromator slit width, 10 nm; response, normal; sensitivity range, 0.3; scan speed, 120 nm min^{-1} and chart speed 60 nm min^{-1}.

An HPLC system consisted of an Altex pump Model 100A (Altex, USA), a Rheodyne sample injection valve, an Altex variable-wavelength spectrophotometric detector Model 155 and an ODS (octadecylsilyl bonded to silica, C_{18}) column (Shandon, UK) was used.

Derivative spectrophotometric method

Standard solutions. A stock solution of loperamide hydrochloride was prepared by dissolving 100 mg, accurately weighed, in ethanol and diluting to 50 ml. A 1-ml aliquot of the stock solution was diluted to 100 ml with ethanol and the second derivative spectrum (^2D) was recorded (ordinate maximum and minimum ± 0.1) and the ^2D peak height (maximum to zero line) at 224 nm was measured. A second aliquot (10 ml) of the stock solution was diluted to 100 ml and the ^2D spectrum was recorded (ordinate maximum and minimum ± 0.0050) and the ^2D peak amplitude (maximum to minimum) between 258 and 263 nm was measured.

Capsules. An accurate weight of the weighed mixed powder of 20 capsules, equivalent to 20 mg of loperamide hydrochloride was extracted by shaking for 10 min with three portions of 50, 20 and 20 ml of ethanol. The extracts were filtered through No. 1 filter paper into a 100-ml volumetric flask and diluted to volume with ethanol. Two dilutions were prepared to contain 50 $\mu g\ ml^{-1}$ and 10 $\mu g\ ml^{-1}$ of loperamide hydrochloride in ethanol and their ^2D spectra were recorded as described for the standard solutions. The concentration of loperamide in the sample solution and hence in the capsules was calculated from the corresponding regression equation.

Drops. An aliquot of the drops equivalent to 20 mg was transferred to a 100 ml volumetric flask and diluted to volume with ethanol. The procedure was continued as described under the 'Capsules' section from the words 'Two dilutions were prepared to contain . . .'. The concentration of loperamide in the sample solution and consequently in the drops was calculated from the corresponding regression equation.

Syrup. An aliquot of the syrup equivalent to 10 mg of loperamide hydrochloride was basified with ammonia (5 ml of 33% solution) and extracted with chloroform (3 × 25 ml). The chloroform was evaporated and the residue was dissolved in ethanol (3 × 15 ml). The ethanolic solution was transferred to a 50-ml volumetric flask and diluted to volume with ethanol. The procedure was continued as described under the 'Capsules' section from the words 'Two dilutions were prepared to contain . . .'. The concentration of loperamide in the sample solution and hence in the syrup was calculated from the corresponding regression equation.

Spectrofluorimetric method

Standard solutions. A stock solution of loperamide hydrochloride was prepared by dissolving 100 mg, accurately weighed, in ethanol and diluting to 100 ml. A 10-ml aliquot of the stock solution was diluted with 30 ml of

water and basified with ammonia (5 ml of 33% solution). The solution was extracted with chloroform (3 × 25 ml) and the combined chloroformic extracts were evaporated to dryness under reduced pressure. The residue was extracted with ethanol–sulphuric acid mixture, and the extract was transferred to a 100-ml volumetric flask and completed to volume with the ethanol–sulphuric acid mixture. A 2-ml aliquot of this solution was diluted to 100 ml with ethanol–sulphuric acid mixture. The fluorescence spectrum (λ_{ex} 310 nm) and the synchronous fluorescence spectrum ($\Delta\lambda$ 55 nm) were recorded.

Capsules. An accurate weight of the weighed mixed contents of the 20 capsules equivalent to 20 mg of loperamide hydrochloride was extracted by shaking for 10 min with three portions of 50, 20 and 20 ml of ethanol. The extracts were filtered through No. 1 filter paper into a 100-ml volumetric flask and diluted to volume with ethanol. A 20-ml aliquot was diluted with 50 ml of water and basified with 5 ml of ammonia (33% solution) and the procedure was continued as described under the Standard solutions' section from the words 'The solution was extracted with chloroform (3 × 25 ml) and . . .' except that a 10-ml aliquot of the extract in ethanol–sulphuric acid was diluted to 20 ml with ethanol–sulphuric acid in the final stage. The concentration of loperamide in the sample and consequently in the capsules was calculated from the corresponding regression equation.

Drops and syrups. An aliquot of the drops or syrup equivalent to 4 mg of loperamide hydrochloride was diluted with 50 ml of water and rendered alkaline with ammonia (5 ml of 33% solution) and the procedure was continued as described under the 'Capsules' section from the words 'The solution was extracted with chloroform (3 × 25 ml) and . . .'. The concentrations of loperamide in the sample solutions and hence in the drops and syrups were calculated from the corresponding regression equation.

Results and Discussion

Development and validation of the fluorimetric method

A preliminary investigation of the fluorescence characteristics of loperamide showed that the compound is almost non-fluorescent in alkaline or neutral solutions. In acidic solution, 0.1–1 M sulphuric acid, the compound possesses very weak fluorescence that is independent of the strength of the acid. A solution of the drug in methanol or ethanol, exhibits maximum fluorescence at 370 nm (wavelength of excitation 310 nm) which could be enhanced by sulphuric acid (specific gravity 1.84). Figure 1 shows the fluorescence excitation and emission spectra of loperamide in ethanol containing 10%, v/v sulphuric acid (specific gravity 1.84) with maxima at 310 and

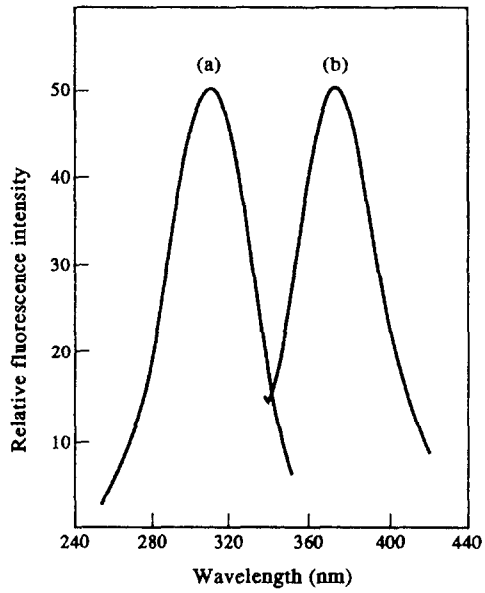

Figure 1
Fluorescence (a) excitation (λ_{em} = 370 nm) and (b) emission (λ_{ex} = 310 nm) spectra of loperamide (4 μg ml^{-1}) in ethanol containing 10%, v/v sulphuric acid (specific gravity, 1.84).

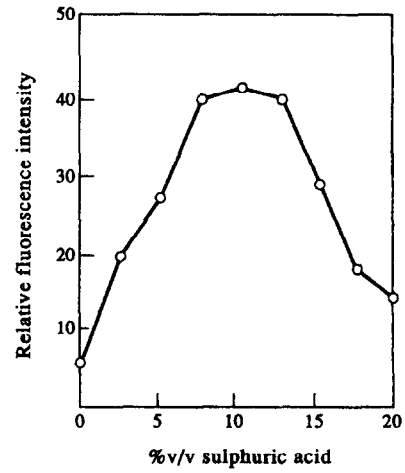

Figure 2
Effect of sulphuric acid (specific gravity, 1.84) on the fluorescence of loperamide (3.35 μg ml^{-1}) in ethanol.

Table 1
Regression equations and linearity ranges for the proposed methods for the determination of loperamide hydrochloride

Method	Conc. range ($\mu g\ ml^{-1}$)	Regression equation	r^*	$n\dagger$
Direct fluorimetry (F)	1–10	F (mm) = 12.52 C − 0.050	0.9996	7
Synchronous fluorimetry (SF)	0.25–5.0	SF (mm) = 30.9 C − 0.049	0.9997	8
Second derivative spectrophotometry $^2D_{224}$	5–25	$^2D_{224}$ (mm) = 34.22 C − 0.051	0.9999	6
Second derivative spectrophotometry $^2D_{258/263}$	40–200	$^2D_{258/263}$ (mm) = 4.67 C − 0.033	0.9998	6

*r: Correlation coefficient.
†n: Number of points used in regression.

370 nm, respectively. Figure 2 shows the effect of added sulphuric acid (specific gravity 1.84) on the fluorescence intensity. Maximum fluorescence was obtained with a concentration of sulphuric acid of 10%, v/v. A proportional relationship exists between the measured fluorescence and the concentration of the drug in the range 1–10 $\mu g\ ml^{-1}$ (Table 1).

A second technique, based on measuring the synchronous fluorescence of the compound in order to obtain a sharper fluorescence band and to minimize or avoid light scattering effects, was developed. To optimize the method, the effect of varying the wavelength interval between the excitation monochromator and the emission monochromator ($\Delta\lambda$) on the synchronous fluorescence of the compound was investigated. Figure 3 shows that maximum fluorescence occurs at $\Delta\lambda$ around 55 nm. Figure 4 shows the synchronous fluorescence spectrum of loperamide at $\Delta\lambda$ equal to 55 nm. A linear relationship exists between the measured synchronous fluorescence intensity and the concentration of the drug in the range 09.25–5.0 $\mu g\ ml^{-1}$ (Table 1).

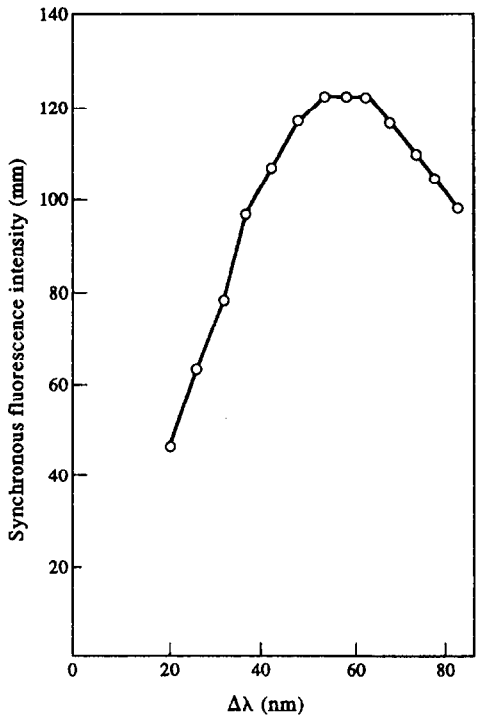

Figure 3
Effect of wavelength interval ($\Delta\lambda$) on the synchronous fluorescence of loperamide (4 $\mu g\ ml^{-1}$) in ethanol containing 10%, v/v sulphuric acid (specific gravity, 1.84) using excitation and emission spectral slitwidths of 10 nm.

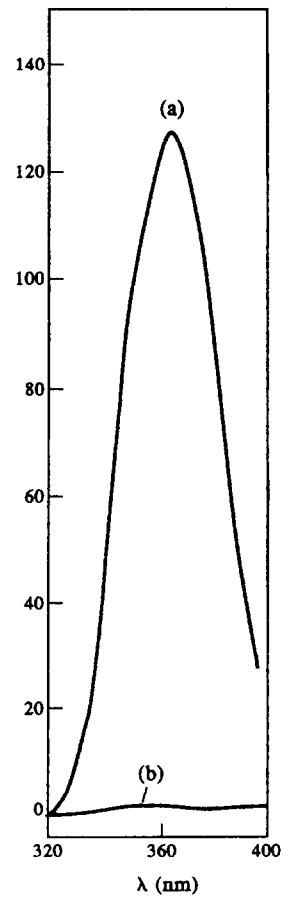

Figure 4
Synchronous fluorescence spectra of (a) loperamide (4 $\mu g\ ml^{-1}$) in ethanol containing 10%, v/v sulphuric acid (specific gravity, 1.84) and (b) solvent blank. $\Delta\lambda$ = 55 nm. Excitation and emission spectral slitwidth = 10 nm.

The reproducibility of both fluorescence techniques was determined by analysing a sample of loperamide 10 times. The results are reproducible with mean percentage recoveries of 100.2 and 100.0 for the direct and synchronous techniques, respectively. The relative standard deviations were 0.67 and 0.43% respectively, indicating that both techniques have good precision.

Development and validation of the derivative spectrophotometric method

Figure 5(a) shows the UV-absorption spectrum of loperamide hydrochloride and its first derivative spectrum in ethanol. The compound possesses the chracteristic weak and sharp UV-absorption bands of benzenoid drugs [11] with maxima at 252, 258 and 264 nm and a non-characteristic strong absorption below 240 nm. The first derivative spectrum shows a maximum at 218 nm. The measurement at such a short wavelength is not favourable in quantitative analysis [11] because many compounds exhibit absorption in this range. Furthermore, the UV-absorption spectra of the adjuvants (Fig. 5(b)) show linear absorption increasing towards shorter wavelengths, which could not be corrected by the use of the first derivative technique. Consequently, measurement of the first derivative peak height at 218 nm would give erroneous results.

The second derivative UV absorption spectrum of the compound shows sharp peaks (ab, bc, dc and Fo, Fig. 5(c)). The second derivative spectra of the formulation adjuvants are superimposed on the zero line (Fig. 5(b)) indicating the elimination of their interference. To confirm that complete elimination of interference from the formulation adjuvants using the proposed derivative technique had

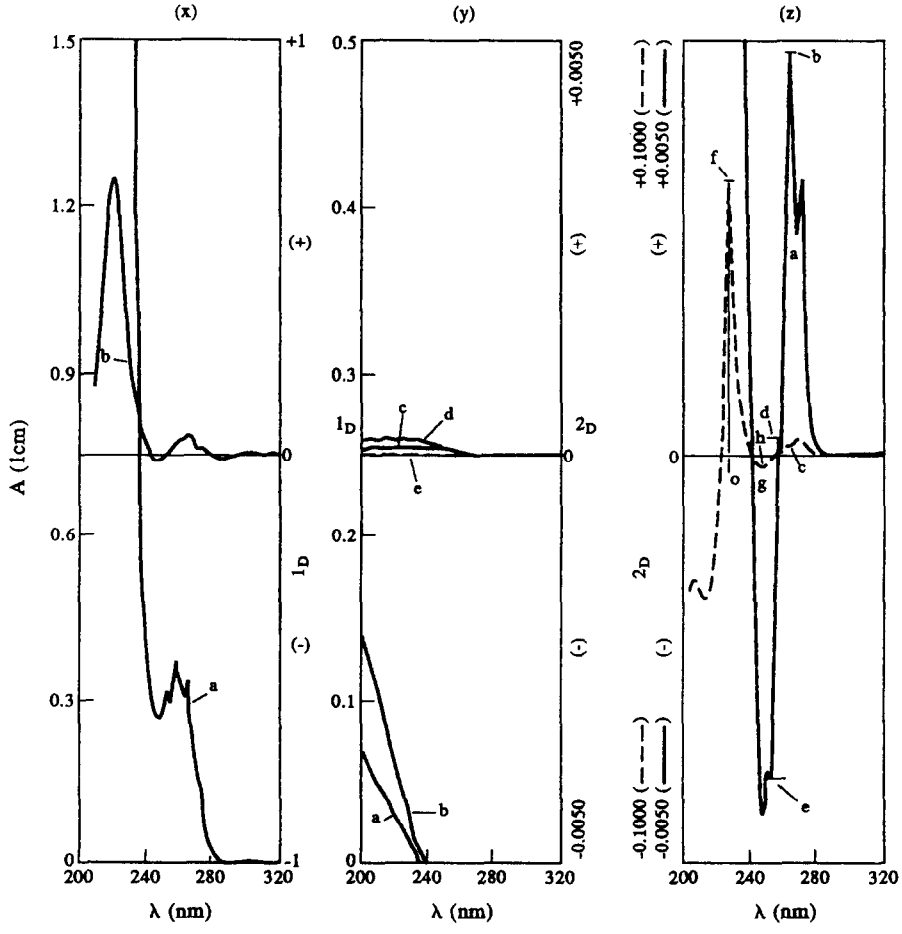

Figure 5
(a) UV absorption spectrum of 200 μg ml^{-1} of loperamide hydrochloride in ethanol (**a**) and its ^{1}D spectrum (**b**). (b) UV absorption spectra of capsules excipients (**a**) and drops excipients (**b**) in ethanol and their ^{1}D spectra (**c**) and (**d**), respectively and ^{2}D spectra (e). (c) ^{2}D spectra of loperamide hydrochloride in ethanol, (——) 200 μg ml^{-1} and (----) 20 μg ml^{-1}.

occurred, the second derivative amplitudes ratios, $^2D_{267-263}/^2D_{263-260}$ (ab/bc, Fig. 5c), $^2D_{263-260}/^2D_{257-252}$ (bc/de, Fig. 5c), $^2D_{267-263}/^2D_{257-252}$ (ab/de, Fig. 5(c)) and $^2D_{224}/^2D_{248}$ (fo/hg, Fig. 5c) were calculated for both the standard drug solution and the sample solutions of the different pharmaceutical dosage forms of the drug. The results (Table 2) show that the ratios of the sample solution are within 1% of those of the standard drug solution and that complete elimination of the interference had occurred. Consequently, the suggested derivative ratios technique could be used generally as a proof of complete elimination of interference prior to application of derivative spectrophotometry: most reported applications [4–7] lack this proof.

The peak amplitude between 258 and 263 nm (bc, Fig. 5(c)) was chosen for quantitative analysis as it shows the largest amplitude. The peak height at 224 nm was also used to quantitate the compound as it provides a more sensitive measurement. A rectilinear relationship exists between the concentration of the drug in the range 40–200 μg ml^{-1} and the peak amplitude at the chosen wavelengths (Table 1).

The reproducibility of the derivative spectrophotometric method was investigated by analysing a sample of loperamide hydrochloride 10 times using the proposed procedure. The results were reproducible, with mean percentage recoveries of 100.2 and 100.2 by measuring the peak height at 224 nm and the peak amplitude between 258 and 263 nm, respectively. The relative standard deviations for both were less than 1%, indicating good precision.

Table 2
Ratios of second derivative amplitudes of the standard solution and different dosage forms of loperamide hydrochloride

Drug form	Second derivative ratio			
	a	*b*	*c*	*d*
Authentic drug	0.466	1.197	0.557	14.571
Capsules	0.462	1.199	0.550	14.574
	(0.400)	(0.200)	(0.700)	(0.300)
Syrups	0.473	1.203	0.566	14.580
	(0.400)	(0.600)	(0.900)	(0.900)
Drops	0.472	1.189	0.563	14.570
	(0.600)	(0.800)	(0.600)	(0.100)

a: The second derivative ratio of the amplitudes between 267–263 nm/263–260 nm. *b*: The second derivative ratio of the amplitudes between 263–260 nm/257–252 nm. *c*: The second derivative ratio of the amplitudes between 276–263/257–252 nm. *d*: The second derivative ratio of the peaks heights at 224/248 nm.

The figures in parentheses are the percentage difference between the ratios of the authentic drug and those of dosage form.

Table 3
Assay results

Pharmaceutical formulation	Found (as % of declared amount) and RSD (%)				
	$^2D_{224}$ method	$^2D_{258/263}$ method	CF method	SF method	Official method
Capsules	100.05 & 0.31	99.80 & 0.41	100.61 & 0.21	100.19 & 0.33	100.12 & 0.29
	(0.37)	(1.43)	(1.66)	(0.21)	
	1.14	2.00	1.91	1.29	
Drops	100.32 & 0.49	100.61 & 0.31	100.19 & 0.56	100.66 & 0.29	100.51 & 0.36
	(0.70)	(0.24)	(1.08)	(1.27)	
	1.85	1.35	2.42	1.54	
Syrups	101.50 & 0.29	101.42 & 0.48	101.49 & 0.32	101.36 & 0.41	101.29 & 0.45
	(0.45)	(0.43)	(0.86)	(0.25)	
	2.52	1.13	2.07	1.25	

RSD = relative standard deviation ($n = 5$).

The figures in parentheses are the calculated *t*-values, for which the corresponding theoretical value ($P = 0.95$) is 2.31 for $n_1 = n_2 = 5$.

The underlined figures are the calculated *f*-values, for which the corresponding theoretical value ($P = 0.95$) is 6.39 for $n_1 = n_2 = 5$.

CF: Conventional fluorescence; SF: synchronous fluorescence.

Analysis of pharmaceutical formulations

A number of formulations containing loperamide were assayed by the proposed methods. For comparison, the formulations were also assayed by the official HPLC method [10]. The results (Table 3) show no significant difference between the reproducibility (*f*-test) and the accuracy (*t*-test) of the proposed methods and the official method as the calculated values did not exceed the theoretical values.

Being direct, of good accuracy and reproducibility, simpler than the official HPLC method and not requiring complicated expensive instruments, the proposed methods are recommended for routine analysis and quality control of loperamide in its pharmaceutical formulations.

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