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Mohamed Oraby: conceptualization, methodology, software, data curation, and writing the first draught.

Ahmed S. Ahmed: visualization, investigation.

Mohamed A. Abdel-Lateef: conceptualization and supervision.

Mahmoud A. H. Mostafa: writing, validation, and data curation.

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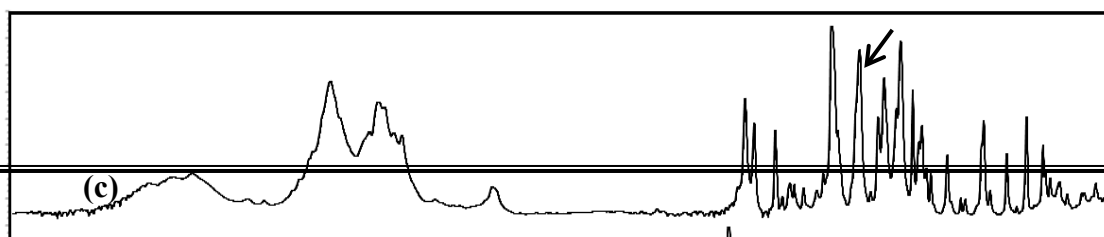Mohamed A. Abdel-Lateef.

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(c)

The FTIR spectra of Fampridine, Dexamethasone (b), and Fluoxetine (c) in the region of 4000-400 cm^{-1}.

Employ FTIR spectroscopic method for determination of certain Multiple Sclerosis medications in plasma and pharmaceutical formulations

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Abstract

A selective, simple, rapid, green, cost-effective, and non-destructive assay was proposed for the simultaneous quantitation of Fampridine (FPN), Dexamethasone (DMS), and Fluoxetine (FLX) in spiked human plasma and commercial dosage forms without interference from common drugs excipients. The solid state of a tertiary mixture of FPN, DMS, and FLX was determined by Fourier transformation infrared (FTIR) Spectroscopy. The calibration curves were linear in the ranges of 1.0-8.0, 0.9-8.0, and 1.2-10.0 µg/mg for FPN, DMS, and FLX, respectively. The limits of detection (LODs) were 0.34, 0.30, and 0.40, and the limits of quantitation (LOQs) were 1.0, 0.9, and 1.2 µg/mg for FPN, DMS, and FLX respectively. The suggested method was validated using the ICH guidelines. The presented assay was properly used for the analysis of the cited drugs in spiked human plasma and pharmaceutical formulations.

Keywords: Fampridine, Dexamethasone, fluoxetine, plasma, pharmaceutical formulations, FTIR.

1. Introduction

Multiple sclerosis (MS), also known as encephalomyelitis disseminate [1], is a widespread neurological disease that affects more than 2.3 million individuals worldwide. MS is a condition diagnosed by neurological inflammation of the Central Nervous System (CNS) [2, 3]. MS is a demyelinating disease in which nerve cells in the brain and spinal cord lose their protecting covers [1]. MS damages myelin and axons in the CNS to varying degrees, which impairs the ability of the nervous system to transmit signals, resulting in a variety of signs and symptoms including physical, emotional, double vision, blindness in one eye, muscle fatigue, and problems with sensation or balance [4, 5]. MS was first recognized as a disease in 1868 by Jean-Martin Charcot, a professor of Neurology at Paris University, who referred to the condition as sclérose en plaques. T-cells were identified to induce MS. The activity of B-cell-focused therapies defies the autoimmune dogma of T-cell [3]. Fampridine (FPN, Fig. 1a) is a symptomatic treatment for MS that enhances axonal conduction factor at demyelinated internodes by facilitating neural influx transmission through demyelinated axons, and blocking the K^+ channel at millimolar concentrations [6-9]. FPN whose clinical neurologic effects are similar to the K^+ channel molecular mechanism blockade, can increase cellular-level neuronal excitability. FPN can also improve synaptic and neuromuscular transmission by enhancing the conduction in demyelinated axons [10, 11]. In 2010, the Food and

Drug Administration (FDA) approved the use of FPN in patients with MS to enhance their walking capacity. Because the effects of FPN are not limited to long motor pathways, patients may experience functional benefits in addition to increased walking speed [9]. FPN causes a slower increase to a lower peak concentration (C_{max}), 3–4 hours after administration, with no effect on the area under the curve (AUC). Also, there is a small increase to C_{max} and a small decrease to AUC when FPN is taken with food. FPN was fully and rapidly removed as unchanged medication by urinary excretion within 24 hours indicating that FPN does not undergo significant metabolic change. Various studies revealed that CYP2E1 was the main enzyme responsible for the hydroxylation of FPN. As a result, 3-Hydroxy-4-aminopyridine and 3-hydroxy-4-aminopyridine sulfate were FPN metabolites [12-14]. Fluoxetine hydrochloride (FLX, Fig. 1b) is an antidepressant, known as a selective serotonin-reuptake inhibitor (SSRI), used to treating depression and other psychiatric conditions [15]. FLX has been shown in rats with experimental autoimmune encephalitis and in patients with relapsing MS to minimize inflammatory responses. Many preclinical studies indicate that FLX has neuroprotective properties that could benefit patients with MS [16]. FLX increases the release of brain-derived neurotrophic factor from astrocytes and activates astrocytic glycogenolysis which is necessary for astrocyte absorption of sodium-dependent glutamate and lactate release which provides energy to axons. FLX can enhance the release of astrocytes from neurotrophic neuroprotective brain-derived factor, dilates cerebral arterioles, and increases cerebral blood flow independent of the endothelium [16-19]. After oral administration, FLX is well-absorbed from the gastrointestinal tract and the presence of food does not affect its bioavailability in both healthy volunteers and patients with depression. Demethylation of FLX in the liver produces norfluoxetine (NFLX), the only active metabolite. Electroencephalogram testing revealed that maximal CNS efficacy occurred between 8 and 10 hours post-dose. A delay in the formation of NFLX may explain the time difference between C_{max} and maximal pharmacodynamic effects [20]. Dexamethasone (DMS, Fig. 1c) is an anti-inflammatory corticosteroid can be taken orally or given as an injection to treating MS relapse [21]. DMS can enhance the resolution of lesional edema and blood reversal in MS. The FDA has only approved the generic brand of the drug for the treatment of MS. Three more

polar metabolites of DMS were discovered in urine. The identity of these metabolites has not been recognized, but their levels in urine increased after diphenylhydantoin induction of hepatic enzymes [22, 23]. DMS is well-known as a substrate and inducer of CYP3A. As a result, DMS C_{max} in nude mice may be attributed to its induction of CYP3A after several doses [24]. Because, these medications are used in combination to treating MS symptoms [21, 25], simultaneous quantitation of FPN, DMS, and FLX is highly important. Different articles for the determination of the cited drugs have been published, including; fluorometry [26, 27], LC-MS/MS [28-30], HPLC-DA [31-33], HPLC-UV [34], electrochemistry [35], spectrophotometry [36, 37], electrophoresis [8, 38, 39]. Green Chemistry (GC) is concerned with the application of methodologies and analytical tools to minimize or eliminate the use or production of chemicals which are hazardous to human health or the environment [40-42]. The development of alternative direct procedures that do not include organic solvents is one of the three ways to reduce the negative environmental effects of analytical procedures. Solid-phase extraction (SPE) is a useful technique for minimizing the use of large quantities of organic solvents in pre-concentration and extraction procedures. Molecularly imprinted polymers (MIPs) is also of interest in GC, as it can be used as SPE units. Liquid phase microextraction is also a new technique that uses a small amount of solvents [41]. As a result, incorporating GC concepts into the design of analytical methods is important for decreasing harmful environmental and human health negative impacts. Infrared has many benefits to qualify and quantify active principle ingredients (APIs) in semi-solid, solid-state, and biological matrices (whole blood, serum, plasma, urine, human milk, amniotic fluid cerebrospinal fluid, skin, hair, and tissues) [43-50]. Because of their versatility, speed, ease of use, and low solvent consumption, FTIR spectroscopic methods are used to monitor drug quality. For evaluating the studied drugs in their medicinal formulations, FTIR methods are frequently less time-consuming and non-destructive procedures. At the same time, common drug additives do not cause any considerable interference. As, FTIR methods do not need a large quantities of solvents, they help to reduce the environmental hazards associated with industrial chemical waste [47, 51, 52]. The presented work aims to develop a simple, non-destructive, echo-friendly FTIR assay for the quantitation of FPN, DMS, and FLX in

pure and medicinal dosage forms, in addition to the quantitation of a ternary mixture containing the

cited drugs. The ternary mixture analysis was performed to show that the proposed method is

capable of identifying the cited drugs in a combination. This is the first time a basic FTIR method

has been used to evaluate this ternary mixture in spiked human plasma, pure form, and

pharmaceutical formulations.

2. Experimental

2.1.1. Materials and Reagents

Potassium bromide (KBr, IR grade) and chloroform were obtained from Sigma Aldrich (Munich, Germany),

FPN was purchased from Acros Organics (Geel, Belgium). DMS and FLX were kindly supplied by Kahira

Pharmaceutical and Chemical Industries, Cairo, Egypt.

2.1.2. Standard powders preparation

Accurately weighed quantities equivalent to 50.0 mg of each of FPN, DMS, and FLX, were properly and

individually transported to a porcelain dish. The powder was mixed in 10.0 g KBr to achieve a final

concentration of 5.0 mg/g for all the cited drugs.

2.2. Instrumentations

A Nicolet 6700 FTIR Gold Spectrometer was used for all FTIR measurements and the data was processed

using OMNIC software version 8 (Madison, Wisconsin, US). All hydraulic presses were operated via a Perkin

Elmer die press (Fisher Scientific Instruments Corporation, Massachusetts, United States). KBr discs of FPN,

DMS, and FLX were prepared using the Qwik handi press. The resolution of spectra was obtained by taking

the average of 32 scans in the mid-IR region (4000-400 cm^{-1}). GRAMSAI was used to manage the FTIR

spectra which have been imported into the Galactic SPC format (Galactic Industries, Salem, NH, USA,

version 7.01).

2.3. Pharmaceutical formulations

Dalfarosis® tablets; defined to contain 10 mg FPN (B.N. # 190715) manufactured by Al-Andalus

Pharmaceutical Industries, Cairo, Egypt. Dexazone® tablets; defined to contain 0.5 mg DMS (B.N. #

20103334) manufactured by Kahira Pharmaceutical and Chemical Industries, Cairo, Egypt. Philozac®

capsules, defined to contain 20 mg FLX (B.N. # 196177) manufactured by Amoun Pharmaceutical Industries

Company, El Obour City, Cairo, Egypt.

2.4. Method procedure

Accurately weighed quantities of FPN, DMS, and FLX were applied to a 1.0 g KBr powder in a porcelain mortar, resulting in final concentrations of (1.0-5.0 g/mg), (1.0-10 g/mg), and (1.0-10 g/mg) for FPN, DMS, and FLX, respectively. The powders were thoroughly mixed using a 15 mm porcelain mortar and pestle. The cited drugs and their commercial preparations were scanned in the Mid-IR region (4000-400 cm^{-1}). By plotting the peak area of the selected FTIR band against drug concentration, the standard calibration curves were established.

2.5 . KBr Disk Preparation

The 1.0 g KBr homogenized disks were made by mixing suitable amounts of FPN, DMS, FLX, ternary mixture (FPN, DMS, and FLX), and oven-drying was used to remove any remaining water vapors. The dried drugs were ground in a 15 mm porcelain mortar and pestle to decrease the particle size of the studied drugs after which subjected to a pressure of 5 tons for 5 minutes before FTIR measurements. For recording FTIR spectra, the studied drugs and their pharmaceutical preparations were scanned in the Mid-IR region (4000–400 cm^{-1}).

2.6. Applications

2.6.1. Analysis of FPN, DMS, and FLX in spiked human plasma

The blood samples were collected from healthy volunteers at the Hospital of Sohag University. All the volunteers gave written permission to use the samples obtained from them for research after being informed about the study's purpose and application. The protocol of plasma sampling from healthy volunteers followed the Declaration of Helsinki Recommendations [53] and the rules of Good Clinical Practice [54]. 5.0 mL of blood sample was drawn from healthy volunteers and transported to tubes containing EDTA. For isolation of plasma samples, 1.0 mL chloroform was added to the blood sample and centrifuged at 2000 rpm for 10 minutes to precipitate plasma proteins, after which 1.0 mL of the extracted plasma was spiked with 1.0 mL of the solution of the studied drug (2, 4, and 5 µg/mg of each) and centrifuged at 2000 rpm for 10 minutes [40].

After ensuring that the resulting supernatant was within the studied drugs concentration ranges, the general assay procedures were followed as mentioned under Section 2.4.

2.6.2. Pharmaceutical Preparations

For each pharmaceutical formulation containing FPN, FLX, or DMS, 20 tablets were precisely weighed, finely powdered in a mortar, and thoroughly blended. An amount of each cited drug equal to 10 mg was then transported to a 50 mL volumetric flask and dissolved in approximately 25 mL of chloroform. The flask's contents were swirled, rotated for 20 minutes, and thereafter filled up to the desired volume with the same solvent. The contents of the flasks were mixed, then filtered. The proposed assay was used to analyze different volumes of the diluted solutions.

3. Results and discussions

The absorbance mode of operation was used to record FTIR spectra of FPN, DMS, and FLX in the range of 4000-400 cm^{-1} using the absorbance mode of operation. These spectra demonstrate the complexity of the structural information that is obtained from the FTIR bands. Furthermore, scanning individual spectra of FPN co-formulated with DMS and FLX produced the following significant bands: 3175, 1705, and 1250 cm^{-1} for FPN, DMS, and FLX, respectively, which correspond to N-H, C=O, and C-F stretching band Fig. 2. The distinctive FTIR wavenumbers are listed in Table 1. Assignment of the prominent FTIR features was carried out in the light of their FTIR spectroscopic data previously reported [55-60].

3.1. Validation of the suggested method

The proposed procedures were validated using ICH guidelines to confirm that the developed method complies with the requirements of the cited analytical performance [61]. all validation experiments were examined through the established calibration range of the suggested method to confirm the validation of the suggested method.

3.1.1. Linearity & range

To minimize the relative error, calibration curves for FPN, DMS, and FLX were established by measuring a series of five concentrations for each studied drug and taking an average of three readings for each concentration. The regression equations were calculated using the least-squares method [62], and the corrected peak area intensity versus concentrations within the defined range was constructed and statistically handled.

The calibration curves were linear over the concentration ranges of 1.0-8.0, 0.9-8.0, and 1.2-10.0 for FPN,

DMS, and FLX, respectively. Different analytical parameters for FPN, DMS, and FLX were calculated

including determination coefficient (0.9649, 0.9894, and 0.9854), intercept (0.0559, 0.0056, and 0.0163),

slope (0.2262, 0.0745, and 0.1224), and the intercept standard deviation (0.0232, 0.0074, and 0.0185). The

results were summarised in Table 2.

3.1.2. Limits of detection (LOD) and limits of quantitation (LOQ)

According to the rules of ICH [61], LODs and LOQs for the cited drugs were determined applying the

following equations:

$$LOD = 3.3\sigma/S$$

$LOQ = 10\sigma/S$, where; S is the slope of the calibration curves and σ is the standard deviation of the intercept.

The LODs were 0.34, 0.30, and 0.40 while LOQs were 1.0, 0.9 and 1.2 µg/mg for FPN, DMS, and FLX

respectively. The results were presented in Table 2.

3.1.3. Precision and accuracy

The regression equations of FPN, DMS, and FLX were used to evaluate each of them at three

different concentration levels to assign recovery studies for the proposed FTIR method. The reasonable

accuracy of the proposed method was determined by the closeness of the obtained percent recovery values to

the true values, as shown in Table 3.

The observed relative standard deviations (RSDs) were less than 2.0%, indicating that the proposed method

was repeatable. The results were expressed in Table 2.

3.1.4. Ruggedness

It was tested to determine the studied drugs by using the same method procedures on two different apparatus

within two different labs and at different times. The data obtained show that the suggested method is

reproducible, the results were presented in Table 4.

4. Applications

4.1. Spiked human plasma with the studied drugs

The sensitivity of the suggested method permitted the analysis of FPN, DMS, and FLX in spiked human

plasma. The cited drugs concentrations were calculated using the regression equations for each drug. Table 5,

demonstrated the approximate mean recovery values. The obtained results revealed that the proposed assay is

appropriate for the detection of the cited drugs in spiked human plasma.

4.2. Pharmaceutical tablets

The proposed method has been used to monitor commercial tablets of the studied drugs and the obtained results were compared to those of published articles using Student's *t*- and F-tests at 95% confidence level [27, 36, 37]. It was observed that there was no significant difference between the calculated results of the proposed FTIR method and obtained results of the reported methods. This confirms the good precision and reliability of the proposed assay to quantify the studied drugs, the results were summarized in Table 6.

5. Conclusion

For the first time, a new selective FTIR spectroscopic method was proposed to determine FPN, DMS, and FLX in pure and a tertiary mixture. The advantages of this method include; sensitivity, cost-effectiveness, and environmental friendliness. As a result of these benefits, this technique is a simple, precise, and effective way to evaluate these drugs in commercial tablets with high accuracy and lower excipients interference. Because of its sensitivity, the suggested method allowed the analysis of the cited drugs in spiked human plasma.

Competing interest declaration

There are no known conflicting financial interests or personal relationships that could have influenced the work presented in this article.

The authors declare that there are no conflicts of interest.

Contribution to CRediT authorship statement

Mohamed Oraby*: was in charge of conceptualization, methodology, software, data curation, and writing the first draught. **Ahmed S. Ahmed:** was responsible for visualization, investigation. **Mohamed A. Abdel-Lateef:** was in charge of conceptualization and supervision. **Mahmoud A. H. Mostafa:** was responsible for writing, validation, and data curation. **Ahmed I. Hassan*:** was responsible for Methodology, writing analysis, and editing.

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Highlights:

- Multiple sclerosis is a neurological disease that affects more than 1 million individuals worldwide.

- Treatment of MS symptoms is based on the use of a combination of drugs.

- A simultaneous determination of concurrently administered drugs is required.

- The FTIR-Spectroscopic method is applied for the determination of Fampridine, Dexamethasone, and Fluoxetine in human plasma and pharmaceutical formulations.

- The proposed method is validated according to the international conference on harmonization (ICH).

Table 1: The distinctive FTIR wavenumbers (cm^{-1}) of the studied drugs.

FTIR wavenumber			
FPN	DMS	FLX	Assignment*
3405		3420	v(NH) and v(NH$_2$)
3350			v(NH$_2$)

3175			u(NH)
	2950	2960	u(CH) and C-C ring modes
2925	2933		u(CH$_3$) and C-C ring modes
2855	2872		combination: (C=O) in-plane and C-C ring modes
	1705		u(C=O)
1618	1605	1616	u(C=C)
		1585	u(C-C) ring
		1517	$δ$(CCC)
	1461	1475	$δ$(CCH)
1455	1437	1455	$δ$(CH)
	1412	1425	$δ$(CH)
1366	1377		$δ$(CH$_2$)
1333		1355	u(C-N)
1270	1290		in-plane $δ$(CH)
		1250	u(C-F)
	1135	1122	in-plane $δ$(CH)
1055	1050	1050	in-plane $δ$(CH)
1022	1035	1025	in-plane $δ$(CH)
985	990	960	out-of-plane $δ$(C-H)
844	860	840	out-of-plane $δ$(C-H) ring
830		820	out-of-plane $δ$(C-H)
666		650	ring deformation
535		520	in-plane $δ$(CC)
443		475	out-of-plane $δ$(CCC)

*u and $δ$ stand for stretching and bending, respectively.

Table 2: The analytical parameters of FTIR spectroscopic method for assay of the cited drugs.

Parameter			
Linearity range (µg/mg)	1.0- 8.0	0.9-8.0	1.2-10.0
Determination coefficient (r^2)	0.9649	0.9894	0.9854
Regression equation	Y=0.0559+0.2262 X	Y=0.0056+0.0745 X	Y=0.0163+0.1224 X
Slope (b)±SD	0.2262 ± 0.0249	0.0745 ± 0.0029	0.1224 ± 0.0067
Intercept (a) ± SD	0.0559 ± 0.0232	0.0056 ± 0.0074	0.0163 ± 0.0185
LOD (µg/mg)	0.34	0.30	0.40
LOQ (µg/mg)	1.0	0.90	1.2

Table 3: Evaluation of accuracy and precision of FTIR spectroscopic method for assay of the cited drugs.

Parameter	FPN (%Found)[*]	DMS (%Found)[*]	FLX (%Found)[*]

	Taken	2.0	4.0	5.0	2.0	4.0	5.0	2.0	4.0	6.0

		2.0	4.0	5.0	2.0	4.0	5.0	2.0	4.0	6.0
Repeatability	1	98.6	99.4	98.7	99.5	97.9	99.4	96.8	97.8	96.9
	2	98.2	98.5	99.2	98.7	98.8	98.4	97	98.7	97.3
	3	97.8	100.1	98.3	98.6	99.6	98.0	98.1	99.5	98
	Mean	98.2	99.3	98.7	98.9	98.8	98.6	97.3	98.7	97.4
	SD	0.5	0.9	0.55	0.59	0.95	0.73	0.70	0.84	0.55
	%RSD	0.52	0.91	0.56	0.60	0.96	0.74	0.72	0.85	0.56
Intermediate precision	1	99.4	97.2	100	97.0	96.9	98.8	98.7	99.3	99.3
	2	98.8	96.9	98.9	97.4	97.1	99.4	99.3	98.3	98.5
	3							98.1	97.9	98.4
		99.7	97.9	98.3	98.1	98.2	98.2			
	Mean	99.3	97.3	99.0	97.5	97.4	98.8	98.7	98.5	98.7
	SD	0.56	0.61	0.96	0.66	0.80	0.72	0.60	0.72	0.48
	%RSD	0.56	0.62	0.97	0.67	0.82	0.74	0.61	0.73	0.50

* Average of three determinations.

Table 4: Ruggedness of the proposed FTIR spectroscopic method for assay of the cited drugs.

Parameter	% Recovery* ± SD		
	FPN (4.0 µg/mg)	DMS (4.0 µg/mg)	FLX (5.0 µg/mg)
1- Instrument			
Nicolet 6700 FTIR			
	99.7 ± 0.72	98.1 ± 1.38	100.2 ± 1.15
Jasco 6000 FTIR			
	99.3 ± 1.05	100.1 ± 1.04	99.7 ± 0.65
2- Inter-day variation			
1 day			
	99.7 ± 0.72	98.1 ± 1.38	100.2 ± 1.15
1 day			
	99.5 ± 0.98	97.3 ± 0.71	98.8 ± 0.54

*Average of three determinations.

Table 5: Application of the proposed FTIR spectroscopic method for assay of the cited drugs in spiked human plasma.

Drug	Add conc. (μg/mg)	Found conc. (μg/mg)	%Recovery* ± SD
	2.0		97.5 ± 0.51
FPN	4.0	3.88	97.1 ± 0.28
	5.0	4.90	98.0 ± 1.03
	2.0	1.96	98.2 ± 0.74
DMS	4.0	3.92	98.1 ± 0.67
	5.0	4.86	97.5 ± 0.88
	2.0	1.95	97.3 ± 0.36
FLX	4.0	3.90	97.6 ± 0.45
	5.0	4.92	98.3 ± 0.97

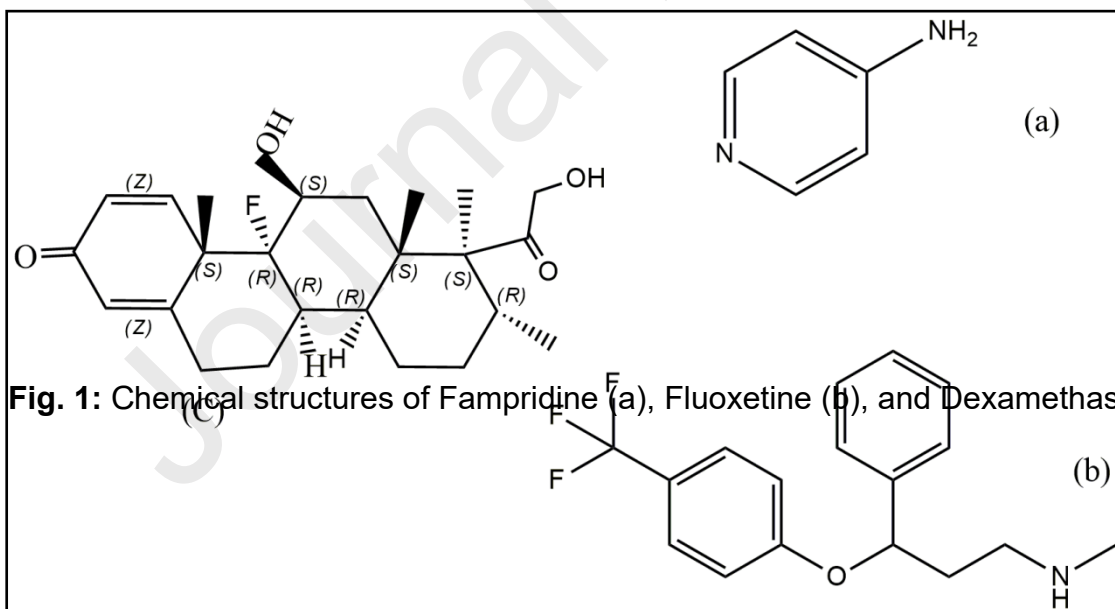* Average of three determinations

Table 6: Comparison between the proposed FTIR spectroscopic assay and the reported methods for determination of the selected drugs in their tablet dosage forms.

Pharmaceutical formulations	% Recovery ± SD[a]		t-value[b]	F-value[b]
	Proposed methods	Reported methods		

Dalfarosis®	102.5 ± 0.79	101.5 ± 0.57	1.88	1.62
(10 mg FPN/ Tablet)				
Dexazone®	100.3 ± 1.33	98.1 ± 1.25	1.13	2.36
(0.5 mg DMS/ Tablet)				
Philozac®	99.5 ± 1.12	97.3 ± 0.79	2.00	2.53
(20 mg FLX/ Capsule)				

[a] Average of three determinations.

[b] Tabulated values at 95% confidence limit are t =2.306, F= 6.338.

Fig. 1: Chemical structures of Fampridine (a), Fluoxetine (b), and Dexamethasone (C).

-400 cm⁻¹.