High-Performance Thin-Layer Chromatography Method for the Simultaneous Determination of Itopride, Pantoprazole, and Mosapride in Their Formulations and Spiked Human Plasma

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Key Words:

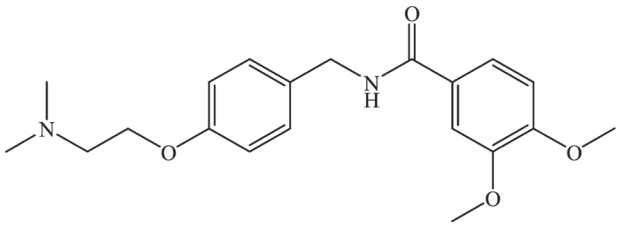
Itopride
Pantoprazole
Mosapride
High-performance thin-layer chromatography

Summary

The combination of itopride (ITP), pantoprazole (PAN), and mosapride (MS) is widely used in the treatment of many gastrointestinal tract (GIT) disorders. For that purpose, a new, simple, precise, accurate, and rapid high-performance thin-layer chromatography (HPTLC) method was developed and validated for the simultaneous determination of ITP, PAN, and MS in their pharmaceutical formulations. The method used Merck HPTLC aluminum plates precoated with silica gel 60 F_{254} as the stationary phase. The mobile phase consisted of methylene chloride–ethyl acetate–methanol–ammonia (25%) (12:2:0.8:0.2, *v*/*v*); this system was found to give compact spot of itopride (R_F value of 0.22 ± 0.008), pantoprazole (R_F value of 0.41 ± 0.006), and mosapride (R_F value of 0.62 ± 0.029). The wavelength of thin-layer chromatography (TLC) scanner was set at 289 nm for both detection and quantitation. The calibration curves were linear over the range of 100–1500 ng spot^{-1} for ITP and MS, and 70–1500 ng spot^{-1} for PAN. The detection limits were 32.5, 16.8, and 29.8 for ITP, PAN, and MS, and the quantitation limits were 98.5, 50.3, and 90.5 for ITP, PAN, and MS. The proposed analytical method was validated according to the International Conference on Harmonization (ICH) guidelines, and the results were acceptable. The proposed method has been successfully applied for the determination of the studied drugs in their pharmaceutical preparations as well as in spiked human plasma and it gave excellent percent of recovery. The results showed excellent agreement with the reported method with respect to precision and accuracy.

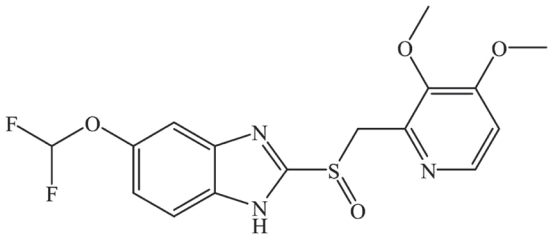
1 Introduction

Itopride hydrochloride (ITP, **Figure 1**), *N*-[4-(2-dimethylamino-ethoxy)-benzyl]-3,4-dimethoxy-benzamide, is a novel gastroprokinetic agent which enhances gastrointestinal motor activity through synergistic effects of dopamine D2 receptor blockade and acetylcholine esterase inhibition [1, 2]. ITP is prescribed for the treatment of gastrointestinal symptoms caused by reduced gastrointestinal motility, *e.g.,* a feeling of gastric fullness, upper abdominal pain, heartburn, vomiting, nausea, and anorexia produced from conditions like functional



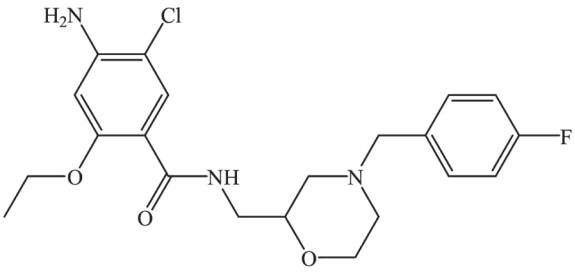
N-[4-(2-Dimethylamino-ethoxy)-benzyl]-3,4-dimethoxy-benzamide

Itopride



5-Difluoromethoxy-2-(3,4-dimethoxy-pyridin-2-ylmethanesulfinyl)-1*H*-benzoimidazole

Pantoprazole



4-Amino-5-chloro-2-ethoxy-*N*-[4-(4-fluoro-benzyl)-morpholin-2-ylmethyl]-benzamide

Mosapride

Figure 1

The chemical structures of the studied drugs.

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dyspepsia or chronic gastritis [1, 2]. Different analytical methods were reported for the determination of ITP in pharmaceutical preparations and human plasma, including spectrophotometry [3–5], electrochemical method [6], reversed-phase high-performance liquid chromatography (RP-HPLC) with ultraviolet detection [7–9], HPLC with ultraviolet detection [10, 11], HPLC with fluorescence detection [12, 13], HPLC coupled to tandem mass spectrophotometer detector [14, 15], HPLC with chemiluminescence detection [16], high-performance thin-layer chromatography (HPTLC) [17], and spectrofluorometry [18, 19]. Pantoprazole (PAN, Figure 1), 5-difluoromethoxy-2-(3,4-dimethoxypyridine-2-ylmethansulfinyl)-1*H*-benzoimidazole, a proton pump inhibitor inhibits gastric acid secretion *via* its effect on the gastric acid pump H^+, K^+ adenosine phosphatase of the parietal cell, leading to the block of the final step of acid secretion. PAN and other proton pump inhibitors decrease gastric acid secretion more than H_2 receptor blockers, so PAN is clinically used in the treatment of peptic ulcer and gastroesophageal reflux and is very effective in patients with Zollinger–Ellison syndrome [20].

Different analytical methods were reported for the determination of PAN in pharmaceutical preparations and human plasma, including spectrophotometry [21–23], HPLC [24–26], and liquid chromatography–mass spectrometry (LC–MS) [27].

Mosapride citrate (MS, Figure 1), 3,4,5-trimethoxy-benzoic acid 2-dimethylamino-2-phenyl-butyl ester, is a potent gastroprokinetic agent which is a selective serotonin 5-HT_4 agonist and is used in treating gastrointestinal motility dysfunction associated with non-ulcer dyspepsia and esophagitis and in improving esophageal motor function in patients with chronic gastroesophageal reflux disease [28]. Several analytical methods were reported for the determination of MS in both pharmaceutical preparations and biological fluids, including spectrophotometry [29, 30], electrochemical method [31], RP-HPLC with ultraviolet detection [30, 32], HPLC connected tandem mass spectrophotometer [33–36], and spectrofluorometry [37, 38].

HPLC method has been reported for the simultaneous determination of PAN and ITP [9], PAN and MS [39], and ITP and MS [40], but no analytical method has yet been reported for the simultaneous determination of ITP, PAN, and MS.

The main objective of this work was to create a simple, economic, accurate, and sensitive HPTLC for the simultaneous determination of ITP, PAN, and MS in their pharmaceutical preparations. The proposed method has an advantage of being very simple and rapid that can reduce the duration of analysis, so it is very suitable for the routine determination of the studied drugs. HPTLC, as a technique of analysis, uses a small quantity of mobile phase and sample and does not depend on critical or expensive chemicals unlike HPLC; thus, it reduces analysis time and cost per analysis.

2 Experimental

2.1 Instrumentation

An HPTLC system with the following specifications was used: CAMAG Linomat V sample applicator (CAMAG, Muttenz, Switzerland); CAMAG 100-mL sample syringe; band width, 4 mm; application rate, 15 s μL^{-1}; application volume, 3; slit dimension, 3 × 0.45 mm; and scanning speed, 20 mm s^{-1}. Densitometric scanning was performed using a CAMAG thin-layer chromatography (TLC) Scanner 3, operated by winCATS evaluation software (version 1.4.4.6337). Sample was applied on HPTLC aluminum plates precoated with silica gel 60 F_{254} (20 cm × 10 cm with 250 µm thickness; Merck, Darmstadt, Germany). The mobile phase consisted of methylene chloride–ethyl acetate–methanol–ammonia (25%) (12:2:0.8:0.2, *v*/*v*). The plates were activated at 60°C for 10 min before sample application. Samples were applied as bands of 3 mm long at 5 mm intervals under nitrogen stream. The sensitivity was kept at auto mode. Linear ascending chromatogram development to a distance of 9 cm was performed in 20 cm × 20 cm twin-trough TLC chamber (CAMAG) at room temperature, previously saturated for 30 min with the mobile phase before the development; TLC plates were dried well in a current of air using air dryer. The plates were subjected to densitometric scanning using a CAMAG TLC Scanner 3 in absorbance mode at 289 nm using a deuterium lamp as the source of radiation.

2.2 Materials and Reagents

All materials used in this study were of analytical grade. ITP with 99.9% purity was kindly provided by Borg Pharmaceutical Industries (Alexandria, Egypt). PAN with 99.8% purity was kindly provided by Sigma Pharmaceutical Industries (Quesna city, Egypt). MS with 99.9% purity was kindly provided by Marcyrl Pharmaceutical Industries (El Obour city, Cairo, Egypt). Methylene chloride, ethyl acetate, methanol, and ammonia (25%) of analytical grade were obtained from El Nasr Chemical Co. (Abo-Zaabal, Cairo, Egypt).

2.3 Pharmaceutical Dosage Forms

The pharmaceutical dosage forms analyzed were as follows: Ganaton® tablets (batch No. 473037/3j), labeled to contain 50 mg of itopride/tablet, produced by Kahira Pharm. and Chem. Ind. Co., Cairo, Egypt; Itopride® tablets (batch No. 041027), labeled to contain 50 mg of itopride/tablet, produced by Borg Pharmaceutical Industries; Pantazole® tablets (batch No. 60457), labeled to contain 40 mg of PAN/tablet, produced by Sigma Pharmaceutical Industries; Protofix® tablets (batch No. 152261), labeled to contain 40 mg of PAN/tablet, produced by Tenth of Ramadan for Pharmaceutical Industries and Diagnostic Reagents (Rameda; 6th of October city, Egypt); Fluxopride® tablets (batch No. 1340444), labeled to contain 5 mg of MS/tablet, produced by Marcyrl Pharmaceutical Industries; Mosapride® tablets (batch No. 15423), labeled to contain 5 mg of MS/tablet, produced by Western Pharmaceutical Industries (El Obour city, Cairo, Egypt). All pharmaceutical dosage forms were purchased from a local pharmacy.

2.4 Calibration Curves

Stock standard solutions (100 µg mL^{-1}) of ITP, PAN, and MS were prepared by transferring accurately weighted amount of ITP, PAN, and MS powder equivalent to 10 mg of each drug to a 100-mL volumetric flask, diluted with methanol, dissolved well, and then completed to the mark with methanol; different volumes of stock standard solutions, 1–15 µL for ITP and MS and 0.7–15 µL for PAN, were spotted on the TLC plates, to give a final concentration range of 100–1500 ng $spot^{-1}$ for ITP and

MS and 70–1500 ng spot^{-1} for PAN. The calibration curves were obtained by plotting area under peak against corresponding drug concentration.

2.5 Procedure for Pharmaceutical Dosage Forms

Twenty tablets of Ganaton®, Itopride®, Pantazole®, Protofix®, Fluxopride®, and Mosapride® were weighted accurately, finely powdered, and mixed thoroughly. An accurate amount equivalent to 10 mg of ITP, PAN, and MS was weighted and transferred to a 100-mL volumetric flask, dissolved in about 50 mL of methanol. The contents of the flask were swirled, sonicated for 5 min, and then the volume was completed to the 100-mL mark with methanol. The flask contents were mixed well and filtered; the first portion of the filtrate was rejected. The obtained solutions were spotted on the TLC plates, with different volumes to give a final concentration within the concentration range of the calibration.

2.6 Procedure for Spiked Human Plasma

The plasma sample was kindly obtained from normal, healthy, male, human volunteers from Assiut University Hospital, Assiut, Egypt according to institutional guidelines. A sample of 5.0 mL of drug-free human blood sample was taken into a heparinized tube; the tube was vortex-mixed at 2000 rpm for 60 s and centrifuged at 4000 rpm for 30 min. Into a 10-mL stoppered calibrated tube, 1.0 mL of the drug-free plasma (supernatant) was spiked with 1 mL of stock standard solution. Two milliliters of acetonitrile as a precipitating agent for protein were diluted to the mark with distilled water and then centrifuged for about 15 min at 3500 rpm. A certain volume of the resulting supernatant was transferred to series of 10-mL volumetric flasks to obtain solutions within the concentration range of the studied drugs. Then, the general analytical procedure was followed. A blank experiment was carried out by treating the drug-free blood sample in the same manner without using the drug.

3 Results and Discussion

3.1 High-Performance Thin-Layer Chromatography

The combination of ITP, PAN, and MS is widely available as a medical prescription in the treatment of gastrointestinal tract (GIT) disorders especially in patients with combined symptoms of peptic ulcer, spasm, and vomiting. The present study provides a new, simple, sensitive, and economic HPTLC analytical method for the simultaneous determination of ITP, PAN, and MS. The R_F values of 0.22, 0.41, and 0.62 for ITP, PAN, and MS were obtained using the optimum mobile phase consisting of methylene chloride–ethyl acetate–methanol–ammonia (25%) (12:2:0.8:0.2, *v*/*v*). In order to determine the most suitable mobile phase for the separation of the three studied drugs, different solvent mixtures with different ratios were tested, for example, methylene chloride–methanol, chloroform–methanol, chloroform–methanol–formic acid, chloroform–ethyl acetate–ethanol, and methylene chloride–ethyl acetate–methanol in different ratios. It has been shown that using the mobile phase consisting of methylene chloride–ethyl acetate–methanol–ammonia (25%) (12:2:0.8:0.2, *v*/*v*) gave excellent resolution, sharp, compact, and symmetrical peak. Also, it was observed that the activation of the TLC plate at 60°C for 10 min and saturation of TLC tank with mobile phase for about 30 min before development improved the peak shape and the reproducibility of the method.

3.2 Validation of the Proposed Analytical Method

The proposed analytical method was validated according to the International Conference on Harmonization (ICH) guidelines [41] regarding linearity, limit of detection (LOD), limit of quantification (LOQ), precision, accuracy, robustness, and selectivity.

3.2.1 Linearity and Range

The linearity of the proposed HPTLC analytical method was evaluated by analyzing a serious concentration of the standard drugs solutions ranging between 100–1500 ng spot^{-1} for ITP and MS and 70–1500 ng spot^{-1} for PAN. Under the above described experimental conditions, the calibration curves were obtained by plotting area under peak against the corresponding drug concentration within a specific range. Each concentration was repeated three times.

The statistical treatment of the data was carried out by using linear regression analysis, and the analytical parameters were calculated **(Table 1).** The correlation coefficients (r) for the studied drugs were 0.9994 for ITP and MS and 0.9998 for PAN, indicating excellent linearity.

Table 1

Analytical parameters for the analysis of ITP, PAN, and MS by the proposed HPTLC method.

Parameter	ITP	PAN	MS
Concentration range (ng/spot)	100–1500	70–1500	100–1500
Correlation coefficient (r)	0.9994	0.9998	0.9994
Determination coefficient (r^2)	0.9995	0.9998	0.9995
Slope	3.59	8.9	5.58
Intercept	932.5	1870.7	783.15
SD of the intercept (S_a)	35.4	44.8	50.5
SD of slope (S_b)	0.039	0.055	0.06
RSD of the slope (%)	1.08	0.618	1.075
LOD (ng mL^{-1})	33	17	30
LOQ (ng mL^{-1})	98	51	91

LOD: limit of detection, LOQ: limit of quantitation

3.2.2 Accuracy and Precision

The accuracy of the proposed HPTLC analytical method was evaluated at five concentrations with the specified concentration range of the studied drugs. Each concentration was replicated three times. The mean of the three measurements was calculated as found. The results of measurements are presented as percent recovery ± standard deviation **(Table 2).** The obtained results show the close agreement between the measured and

Table 2

Evaluation of the accuracy of the proposed HPTLC procedure for the determination of ITP, PAN, and MS at five concentration levels within the specified range.

Sample number	Conc. (ng spot^{-1})	ITP % Recovery[a)]		PAN % Recovery[a)]		MS % Recovery[a)]	
1	100	99.5	99.5	99.1	99.1	100.1	100.1
2	150	148.7	99.1	148.7	99.1	149.3	99.5
3	250	248.3	99.3	247.9	99.2	249.6	99.8
4	400	397.4	99.4	397	99.3	399.3	99.8
5	500	493.2	98.8	493.3	98.8	499.5	99.9
Mean		99.2		99.1		99.6	
SD		0.28		0.19		0.51	
RSD (%)		0.28		0.19		0.51	
RE		0.8		0.91		0.4	

SD: standard deviation, RSD: relative standard deviation, RE: relative error

[a)]Mean of three replicate measurements

true values, indicating a high accuracy of the proposed method. The intra-day precision was evaluated through replicate analysis of three concentrations of each drug on three successive days. The inter-day precision was also evaluated through replicate analysis of three concentrations of each drug over a period of 3 successive days. The results of intra-day and inter-day precision are summarized in **Table 3**. The calculated relative standard deviations of different measurements were below 2%, indicating the excellent precision of the proposed procedure at both levels of repeatability and intermediate precision.

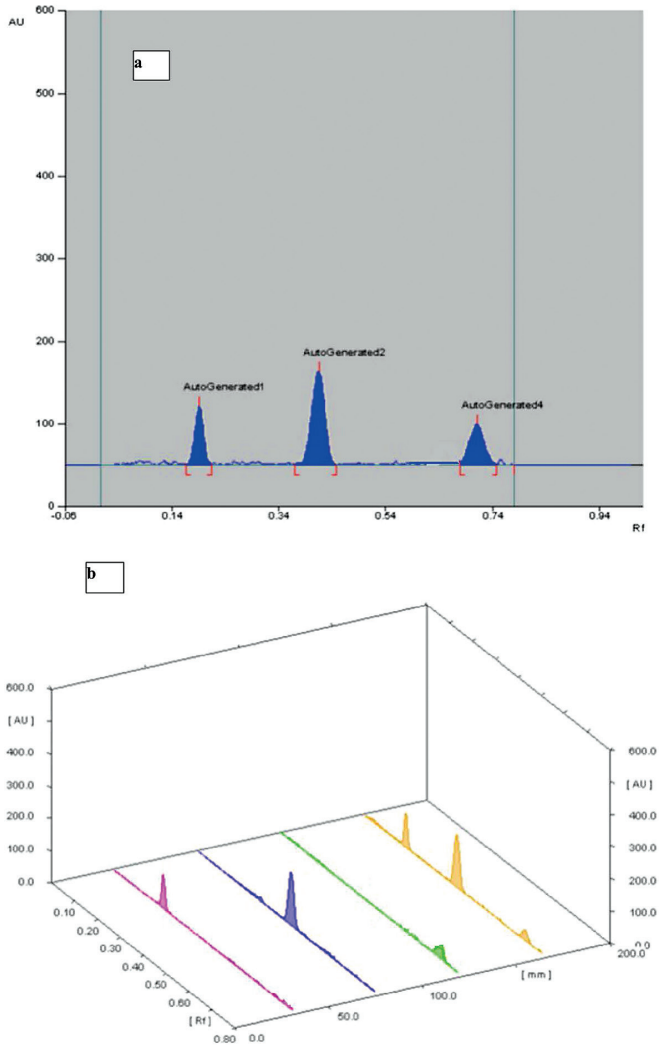

Figure 2

(a) Atypical HPTLC chromatogram of synthetic mixture of ITP (135 ng spot^{-1}), PAN (185 ng spot^{-1}), and MS (135 ng spot^{-1}). (b) Atypical 3D (HPTLC) chromatogram of ITP (135 ng spot^{-1}), PAN (185 ng spot^{-1}), and MS (135 ng spot^{-1}).

Table 3

Evaluation of the intra-day and inter-day precisions of the proposed HPTLC method for the determination of ITP, PAN, and MS in pure form.

Precision level	Conc. (ng spot^{-1})	MTC % Recovery[a)]	MTC ± SD	MTC RSD	ITP % Recovery[a)]	ITP ± SD	ITP RSD	MS % Recovery[a)]	MS ± SD	MS RSD
Intra-day	100	100.1	± 0.8	0.8	99.9	±0.38	0.38	100.1	±0.56	0.56
	150	99.7	± 0.75	0.75	100.2	±0.61	0.61	100.3	±0.8	0.8
	250	100.1	± 0.26	0.26	99.5	±0.44	0.44	100.4	±0.7	0.7
Inter-day	100	99.8	± 0.4	0.4	99.6	±0.78	0.78	99.8	±0.56	0.56
	150	99.5	± 0.7	0.7	99.3	±0.57	0.57	100.3	±1.2	1.2
	250	99.8	± 0.38	0.38	100.1	±0.3	0.3	99.6	±0.3	0.3

SD: standard deviation, RSD: relative standard deviation

[a)]Mean of three replicate measurement

Table 4

Determination of ITP, PAN, and MS in laboratory-prepared mixtures using the proposed HPTLC method.

Mix No.	Conc. (ng spot^{-1})			% Recovery[a)]		
	ITP	PAN	MS	ITP	PAN	MS
1	135	185	135	99.8	99.9	100.0
2	115	135	225	100.1	100.2	99.8
3	250	95	435	100.0	99.8	99.7
4	210	190	540	99.7	99.9	99.9
5	720	245	165	99.6	100.1	99.8
6	310	345	715	99.9	100.0	100.0
7	425	175	190	99.8	99.8	100.1
8	560	415	385	99.8	99.9	99.8
		Mean		99.84	99.95	99.89
		SD		0.16	0.14	0.135

SD: standard deviation
[a)]Mean of three replicate measurement

3.2.3 Limit of Detection and Limit of Quantitation [41]

The LOQ and LOD were determined based on the standard deviation of response and the slope of the calibration curve using the equations: LOD = 3.3 σ / S and LOQ = 10 σ / S, where S is the slope of the calibration curve and σ is the standard deviation of the intercept. The obtained results are presented in Table 1. The limits of detection were 33, 17, 30 ng spot^{-1} for ITP, PAN, and MS, while the limits of quantitation were 98.1, 51, and 91 ng spot^{-1} for ITP, PAN, and MS, which indicate a high sensitivity of the proposed HPTLC method compared with the reported spectrophotometric methods.

3.2.4 Selectivity

Method selectivity was achieved by preparing different mixtures of ITP, PAN, and MS within the linearity range concentration **(Figure 2).** The laboratory-prepared mixtures were analyzed according to the previous procedure described under the proposed HPTLC. Satisfactory results were obtained **(Table 4),** indicating the high selectivity of the proposed methods for the determination of ITP, PAN, and MS.

3.2.5 Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in the method parameters. To test the robustness of the proposed HPTLC analytical method, different compositions of the mobile phase were used **(Table 5).**

3.3 Application to Pharmaceutical Dosage Forms

The proposed method was successfully applied to the determination of the studied drugs in their pharmaceutical dosage forms. The selectivity of the method was studied by observing

Table 6

Comparison between the proposed HPTLC and reported methods for the determination of ITP, PAN, and MS in their pharmaceutical dosage forms.

Dosage form	% Recovery[a)] ± SD				*t*-Value[b)]	*f*-Value[b)]
	Proposed		Reported[c)]			
Ganaton® tablet 50 mg ITP/tablet	99.2	±0.66	98.1	±0.88	2.3	1.8
Itopride® tablet 50 mg ITP/tablet	98.9	±0.86	97.7	±0.87	2.2	1.1
Pantazole® tablet 40 mg PAN/tablet	99.8	±0.6	98.6	±0.73	2.8	1.5
Protofix® tablet 40 mg PAN/tablet	99.8	±0.7	98.4	±1.1	2.4	2.6
Fluxopride® tablet 10 mg MTC/tablet	99.3	±0.79	99.3	±0.6	0.05	1.7
Mosabride® tablet 10 mg MTC/tablet	99.6	±0.55	98.8	±0.71	1.97	1.68

[a)]The values are the mean of five determinations
[b)]The tabulated *t*- and *F*-values at 95% confidence limit are 2.78 and 6.39, respectively
[c)]Reported methods [3, 23, 29]

Table 5

Robustness study of the proposed HPTLC method for the determination of ITP, PAN, and MS (100, 200 ng spot^{-1}) in pure form.

Variation Effect of mobile phase composition	Conc. (ng spot^{-1})	ITP		PAN		MS	
		% Recovery[a)]	± SD	% Recovery[a)]	± SD	% Recovery[a)]	± SD
Methylene chloride–ethyl acetate–methanol–ammonia (25%) (12.5:1.5:0.8:0.2, *v*/*v*)	100	97	± 1.4	97.6	±1.4	97.6	±1.3
	200	97.8	± 1.04	97.04	±1.3	98.14	±0.6
Methylene chloride–ethyl acetate–methanol–ammonia (25%) (11.5:2.5:0.8:0.2, *v*/*v*)	100	97.3	± 1.5	96.3	±0.77	98	±0.9
	200	96.18	± 1.8	97.2	±1.2	98.3	±0.97

SD: standard deviation
[a)]Mean of three replicate measurement

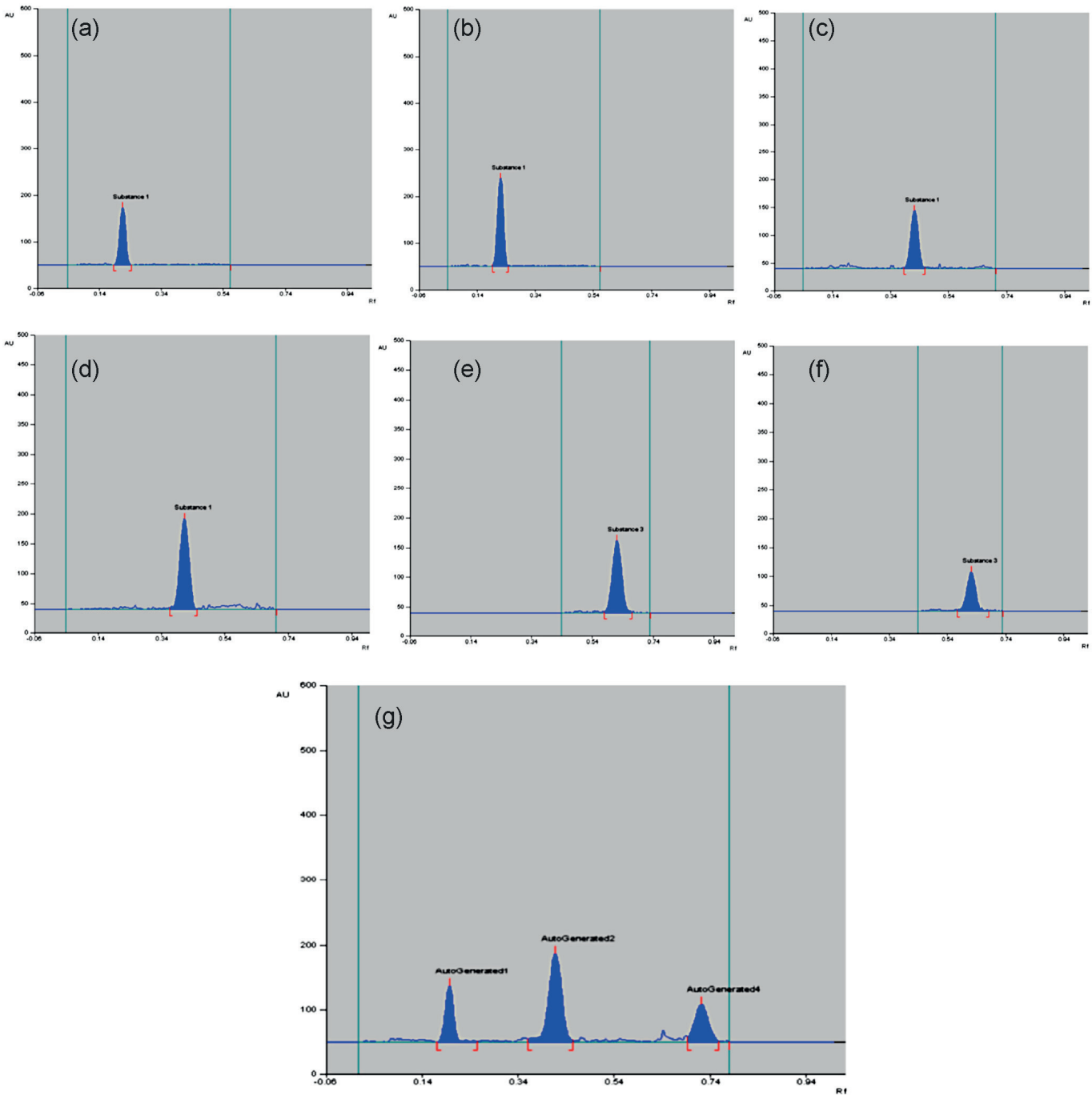

Figure 3

Atypical 2D chromatogram of (a) Ganaton® tablet (360 ng spot^{-1}); (b) Itopride® tablet (850 ng spot^{-1}); (c) Pantazole® tablet (80 ng spot^{-1}); (d) Protofix® tablet (250 ng spot^{-1}); (e) Fluxopride® tablet (500 ng spot^{-1}); (f) Mosabride® tablet (225 ng spot^{-1}); (g) mixture of Ganaton® 50 mg tablet (135 ng spot^{-1}), Pantazole® 40 mg tablet (190 ng spot^{-1}), and Fluxopride® 5 mg tablet (135 ng spot^{-1}).

any interference results from tablet excipients. It was shown that there is no interference from tablet excipients with the proposed method **(Figure 3).** The results obtained from the proposed method were compared with those obtained from reported methods using Student's *t*-test and *F*-test with respect to accuracy and precision. The results are presented in **Table 6.** It is clear from Table 6 that there is no significant difference between the results from the proposed method and reported methods [3, 23, 29] as indicated by Student's *t*-test and *F*-test, as the calculated values did not exceed the theoretical values at 95% confidence level. This indicates high accuracy and precision of the proposed method.

3.4 Application to Spiked Human Plasma

The proposed analytical method was successfully applied for the determination of the studied drugs in spiked human plasma. The concentration of each drug was computed from its corresponding regression equation. The studied drugs standard solutions were spiked to the plasma to give a final concentration of 100, 125, and 150 ng. The obtained results are presented in **Table 7.** The mean percents of recoveries of the concentrations of the three drugs in plasma were found to range from 97.5 to 98.7 with standard deviations ranging from 0.45 to 1.1. This indicates that the studied drugs can be successfully determined in spiked human plasma with a high degree of accuracy and

Table 7

Application of the proposed HPTLC method for the determination of ITP, PAN, and MS in spiked human plasma.

Added conc.	MTC			ITP			MS		
	Found conc.[a)]	% Recovery	± SD	Found conc.[a)]	% Recovery	± SD	Found conc.[a)]	% Recovery	± SD
100	98.5	98.5	±0.55	97.5	97.5	±0.74	97.5	97.5	±0.45
125	122.9	98.3	±0.74	123.2	98.7	±0.83	122.8	98.2	±0.99
150	147.8	98.5	±1.1	147	98	±0.6	148	98.6	±0.86

SD: standard deviation
[a)]Mean of five determination

precision without interference. These results suggest the possibility of this proposed analytical method to determine the concentration of the studied drugs in real human plasma samples after oral administration without significant matrix-related interference.

4 Conclusion

The present study described a simple, economic, highly sensitive (can determine the studied drugs in nanograms per spot), rapid, and less tedious method, which does not require any pre-treatment before analysis for the determination of ITP, PAN, and MS in their pharmaceutical dosage forms. The present study does not require tedious liquid–liquid extraction and, therefore, does not depend on expensive or critical chemical reagents or expensive instrumentation; this makes it more economic and simple which gives the advantage of applying the proposed method in the routine quality control analysis of these drugs. Also, the proposed method is considered environmentally friendly due to low concentrations of the organic solvents used in the mobile phase.

References

[1] *Y.S. Kim, T.H. Kim, C.S. Choi, Y.W. Shon, S.W. Kim, G.S. Seo, Y.H. Nah, M.G. Choi, S.C. Choi,* World J. Gastroenterol. **11** (2005) 4210–4214.

[2] *F. Katagiri, T. Shiga, S. Inoue, Y. Sato, H. Itoh, M. Takeyama,* Pharmacology **77** (2006) 115–121.

[3] *B. Choudhary, A. Goyal, S.L. Khokra,* Int. J. Pharm. Pharm. Sci. **1** (2009) 159–162.

[4] *Z. Santosh, K. Paresh, G. Jitendra, P. Anantwar, B. Sahebrao,* Int. J. Drug Deliv. **2** (2010) 340–343.

[5] *R.V. Heralgi, C.C. Simpi, N.V. Kalyane, S.R. Karajgi,* Asian J. Pharm. (AJP) **2** (2014) 159–165.

[6] *X. Hun, Z. Zhang,* Sens. Actuators B **131** (2008) 403–410.

[7] *A.I. Khan, Z. Khadra, I. Ahmad, L. Khan, A. Khan, M.I. Ullah, Z. Ismail,* J. Pharm. Biomed. Anal. **121** (2016) 6–12.

[8] *R.V. Thiruvengada, S.T.S. Mohamed, S. Ramkanth, M. Alagusundaram, M. Ganaprakash, K. Chetty, C. Madhusudhana,* J. Young Pharm. **2** (2010) 410–413.

[9] *K.R. Gupta, R.B. Chawala, S.G. Wadodkar,* Orbital: Electron. J. Chem. **2** (2010) 209–224.

[10] *V. Dighe, R. Sane, S. Menon, H. Tambe, S. Pillai,* J. Planar Chromatogr. **19** (2006) 319–323.

[11] *S.P. Sisinthy, S. Duraipandi, N.K. Rao, M.B. Rao,* Int. J. Pharm. Pharm. Sci. **7** (2015) 246–249.

[12] *S.S. Singh, M. Jain, K. Sharma, B. Shah, M. Vyas, P. Thakkar, R. Shah, S. Singh, B. Lohray,* J. Chromatogr. B **818** (2005) 213–220.

[13] *P. Ptáček, S. Klíma, J. Macek,* J. Chromatogr. B **877** (2009) 842–846.

[14] *H-W. Lee, J-H. Seo, S-K. Choi, K-T. Lee,* Anal. Chim. Acta **583** (2007) 118–123.

[15] *A. Bose, U. Bhaumik, A. Ghosh, B. Chatterjee, U.S. Chakrabarty, A.K. Sarkar, T.K. Pal,* Chromatographia **69** (2009) 1233–1241.

[16] *Y. Sun, Z. Zhang, Z. Xi, Z. Shi, W. Tian,* Anal. Chim. Acta **648** (2009) 174–177.

[17] *A. Suganthi, S. John, T.K. Ravi,* Indian J. Pharm. Sci. **70** (2008) 366–368.

[18] *F. Ibrahim, J.J. Nasr,* Luminescence **31** (2015) 255–263.

[19] *M.I. Walash, F. Ibrahim, M.I. Eid, S.A.E. Abass,* J. Fluoresc. **23** (2013) 1293–1300.

[20] *G. Sachs,* Pharmacotherapy **17** (1997) 22–37.

[21] *K. Basavaiah, U.R.A. Kumar, T. Kalsang, K.B. Vinay,* Iranian J. Chem. Chem. Engineer. (IJCCE) **28** (2009) 31–36.

[22] *N.A.E.-R. Nesrin K. Ramadan, M.T. Ragab, B.A. El-Zeany,* Spectrochim. Acta A **137** (2015) 463–470.

[23] *K. Rajnish, S. Harinder, S. Pinderjit,* J. Chem. Pharm. Res. **3** (2011) 113–117.

[24] *M. Tanaka, H. Yamazaki,* Anal. Chem. **68** (1996) 1513–1516.

[25] *Q.B. Cassa, A.L.G. Degania, N.M. Cassianoa, Jr.J. Pedrazolli,* J. Chromatogr. B **766** (2002) 153–160.

[26] *A.M. Mansour, O.M. Sorour,* Chromatographia **53** (2001) S478–S479.

[27] *O. Peres, C.H. Oliveira, R.E. Barrientos-Astigarraga, V.M. Rezende, G.D. Mendes, D. de Nucci,* Arzneimittelforschung **54** (2004) 314–319.

[28] *R. Magnus, F. Caterina, C. Lars, L. Lars,* Eur. J. Gastroenterol. Hepatol. **15** (2003) 1115–1121.

[29] *S. Appala Raju, M. Shobha,* Asian J. Chem. **15** (2003) 529–531.

[30] *A.S. Birajdar, S.N. Meyyanathan, B. Suresh,* Int. J. Pharm. Stud. Res. **2** (2011) 29–36.

[31] *R. Jainz, K.R.N. Jadon,* J. Electrochem. Soc. **155** (2008) F104–F109.

[32] *Y.S. Krishnaiah, T.K. Murthy, D.G. Sankar, V. Satyanarayana,* Pharmazie **57** (2002) 814–816.

[33] *N.V.S. Ramakrishna, K.N. Vishwottam, S. Manoj, M. Koteshwara, J. Chidambara, D.P. Varma,* Biomed. Chromatogr. **19** (2005) 539–548.

[34] *Y.R. Kumar, J.M. Babu, M.S.P. Sarma, B. Seshidhar, S. Srinivasa Reddy, G. Sudarsan Reddy, K. Vyas,* J. Pharm. Biomed. Anal. **32** (2003) 361–368.

[35] *F. Qin, L.-Y. Chen, Y.-Y. Ma, D. Wang, J. Liu, X.-M. Lu, F. Li*, Yao xue xue bao (Acta Pharmaceutica Sinica) **42** (2007) 882–885.

[36] *Y. Aoki, H. Hakamata, Y. Igarashi, K. Uchida, H. Kobayashi, N. Hirayama, A. Kotani, F. Kusu,* J. Chromatogr. B **858** (2007) 135–142.

[37] *M.A. Hegazy, A.M. Yehia, A.A. Mostafa,* Drug Test. Anal. **4** (2012) 104–115.

[38] *D. Aleksandrova, Y. Scripinets, A. Yegorova,* Acta Pol. Pharm. Drug Res. **66** (2009) 605–610.

[39] *K.R. Gupta, R.B. Chawala, S.G. Wadodkar,* Orbital: Electron. J. Chem. **2** (2010) 209–224.

[40] *G. Darshan, B. Prachi, J. Reshma, R. Sadhana,* Int. J. Pharm. Sci. Res. **5** (2014) 907–912.

[41] International Conference on Harmonisation, Harmonised Tripartite Guideline: Validation of Analytical Procedures: Text and Methodology Q2(R1), Geneva, 2005.

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