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Research Article

SIMULTANEOUS DETERMINATION OF DEXRABEPRAZOLE AND DOMPERIDONE IN CAPSULE FORM BY USING HPTLC AND MASS CONFIRMATION BY HPTLC-MS

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ABSTRACT

A rapid, quantitative and optimised high-performance thin-layer chromatographic method coupled to mass spectrometry using electro spray ionization (HPTLC/ESI-MS) for the separation and quantification of dexrabeprazole and domperidone in combined capsule dosage form has been developed and validated. The formulations were separated on silica gel 60 F254 plates using a saturated mixture of toluene: ethyl acetate: methanol (6:5:2 v/v). Densitometric quantification was performed at 285 nm by reflectance scanning and the retention factors for dexrabeprazole and domperidone were found to be 0.53±0.02 and 0.43±0.02. The detector response was linear in the concentration range of 50-500 ng/band for both dexrabeprazole and domperidone with a regression coefficient(r) of 0.9993 and 0.9990. Recoveries of spiked samples at three levels ranged from 99.55 to 101.8% dexrabeprazole and 99.37 to 100.8% for domperidone the validated lowest limit of detection and quantification were 21.22 ng/band and 16.95 ng/band and 70.73 ng/band and 56.51 ng/band for dexrabeprazole and domperidone respectively. The Selectivity was evaluated determining the peak purity by UV-spectrophotometry. Additionally the peak identities as well as the purities were confirmed by mass spectrometry. The ESI^+ mass spectra showed the $[M+H]^+$ ions for dexrabeprazole and domperidone detected at *m/z* 360.3 and 426.8 being acquired directly from the sample bands by an elution-based interface. This simple, yet a reliable planar chromatographic method with less elaborate sample preparation, enhanced separation efficiency with an online confirmation by MS offers a good alternative for the analysis of pharmaceutical formulations.

Keywords: Dexrabeprazole sodium; Domperidone; HPTLC; mass spectrometry.

1 INTRODUCTION

Dexrabeprazole sodium is R (+)-isomer of rabeprazole (2-[[[4-(3-methoxypropoxy)-3-methyl-2-pyridinyl]-methyl] sulfinyl] 1H-benzimidazole) (**Figure1 (a)**). It is a proton pump inhibitor that suppresses gastric acid secretion by specific inhibition of the gastric H+ K+ ATPase enzyme system at the secretory surface of the gastric parietal cell[1]. Chemically, domperidone is 5-chloro-1-[1-[3-(2-oxo-2, 3-dihydro-1H-benzimidazol-1-yl) propyl]-piperidin-4-yl]-1, 3-dihydro-2H-enzimidazol-2-one (**Figure 1(b)**). The drug is a dopamine receptor (D2) antagonist which is used as antiemetic drug and is official in British Pharmacopeia. It increases spontaneous gastric activity and antagonizes dopamine inhibition of gastric

emptying[2]. Domperidone alone or in combination with other drugs is reported to be estimated by high performance liquid chromatography (HPLC), spectrometry, high performance thin layer chromatography (HPTLC) and liquid chromatography-mass spectrometry (LC-MS)[3-17]. However, Sohan S.Chitlange et al.[18] developed a spectrophotometry method for the analysis of domperidone and dexrabeprazole in combine dosage form.

In pharmaceutical analysis, TLC methods were in general, substituted by HPLC methods and HPTLC has never been upgraded as a powerful separation tool. Therefore honest efforts were made to show that HPTLC, which stands for sophisticated instrumentation and enhanced separation efficiency based on the reduced particle size of the adsorbent used, could be an effective alternative, or a real complementary method to HPLC. Thus in the last decade the planar chromatographic system has continually been improved through full automatization of various analytical steps, including automated control of the plate activity and highly reproducible separation efficiency. This facilitates highly reliable quantitative analysis with novel features like multiple detection by UV-Vis and fluorescence, in situ post-chromatographic derivatization, and less elaborate sample preparation making planar chromatography an effective tool for high-throughput analysis. Further, interfacing HPTLC with MS has gained growing attention in the last decade and an overview is given by Morlock and Schwack[19]. Regarding interfacing MS, many interesting methods have been developed[20-24]. However HPTLC/UV coupled with MS detections are now widely used for the separation and quantitative determination of individual chemical entities .The first such attempt was accomplished by Anderson and Busch[25] who successfully designed an offline extraction probe to extract samples directly from HPTLC plates. Small dimension of the (0.5 mm) extraction capillary compared to the dimension of a HPTLC zone (5 mm) was a setback. However, Wachs and Henion[26] efficiently demonstrated a potential probe design for surface sampling /electro spray emitter system in the field of planar chromatography as an online method. Based on the fundamental research and uncompromising efforts of several authors, the research on HPTLC-MS hyphenated approach has reached a stage where it is today. This mainly includes elution based interfaces coupled to eletrospray ionization (ESI)[27-28] and the same has been utilized in the present study.

HPTLC is very fast, convenient and versatile method for quantitative separations. In the past, unknown substances were scraped off from the TLC/HPTLC plate, eluted into a tube and transferred into the MS system. Now, a very convenient and universal TLC-MS Interface is available which can semi-automatically extract zones of interest and direct them online into any brand of HPLC-MS system. The interface is quickly and easily connected (by two fittings) to any LC-MS without any adjustments or modifications. Questioned substances are directly extracted from a TLC/HPTLC plate and sensitive mass spectrometric signals are obtained within a minute per substance zone. The interface extracts the complete sample zone with its depth profile and thus allows detections comparable to HPLC down to the pg/zone range. The present interface has been proven to be one of the most reliable and versatile interfaces for TLC/HPTLC-MS coupling.

Till date no HPTLC method has been reported for the simultaneous estimation of dexrabeprazole and domperidone in combination. Therefore, the objective of the present study is to develop a HPTLC-UV-MS method which could accomplish the simultaneous separation and detection of dexrabeprazole and domperidone in combined capsule dosage form whereby the satisfactory separation power of HPTLC could be well proven by purity calculation of UV-spectra and HPTLC/ESI-MS. The proposed method is optimized and validated according to the International Conference on Harmonization (ICH) guidelines[29]. Here in, we describe the details of our investigative study and the potential utility of the same.

2 EXPERIMENTAL

2.1 MATERIALS

Analytically pure samples of dexrabeprazole and domperidone were kind a gift from Sipra labs, Hyderabad, India, and were used without further purification. The pharmaceutical dosage form used in this study was DIRAB-D capsules (Hetero

Healthcare Ltd., Hyderabad, India) labeled to contain 10 mg of dexrabeprazole and 30 mg of domperidone and purchased from a local pharmacy. All chemicals and reagents used were of chromatographic grade and purchased from Merck Chemicals, India.

2.2 Standard Solution

Standard stock solutions of concentration 1 mg/mL (1000 ng/µL) of dexrabeprazole and 1 mg/mL (1000 ng/µL) of domperidone were prepared separately using methanol. From the standard stock solution, the mixed standard solution was prepared using methanol to contain 100 ng/µL of dexrabeprazole and 100 ng/µL of domperidone.

2.3 Sample Solution

The capsules (10) were weighed accurately and finely powdered. A quantity of powder equivalent to 10 mg dexrabeprazole (30 mg domperidone) was weighed and transferred to a 100 mL volumetric flask containing about 50 mL methanol. The solution was ultrasonicated for 5 min, and the volume was made up to the mark with methanol. The solution was filtered (Whatman No. 41) and made up to the volume.

2.4 HPTLC/ESI-MS

TLC was performed on 10×10 cm plates pre coated with 60F-254 (With 0.25mm thickness; Merck, Darmstadt, Germany) and the plates were washed with methanol before use. The sample 1µL (100 ng/band for dexrabeprazole and 300 ng/band for domperidone) and mixed standard solution 0.5 µL to 5 µL (50-500 ng / band dexrabeprazole and 50-500 ng /band domperidone) were applied by using Linomat V applicator (Muttenz, Switzerland, supplied by Anchrom technologists, Mumbai), with the following settings for eleven bands per plate: band length 4 mm, band distance 8 mm, application rate 6 µL/sec, application position x-axis 10.0 mm and y-axis 10.0 mm each. The mobile phase consisted of a saturated mixture of toluene: ethyl acetate: methanol (6:5:2 v/v) and chromatography was carried out using 10 mL of mobile phase in a 10 × 10 cm twin trough glass chamber with linear ascending development. The optimized chamber saturation time for mobile phase was 20 min. at room temperature. The length of chromatogram run was 8.5 cm and subsequent to the development, the TLC plates were dried in a current of air with the help of a dryer in wooden chamber with adequate ventilation. Densitometric scanning was performed with Camag TLC scanner III in the absorbance-reflectance mode at 254 nm with a slit dimension of 3.0 mm x 0.45 mm and a scanning speed of 20 mm/sec. All instruments were operated by win CATS software (v 143 CAMAG) resident in the system. The source of radiation utilized was deuterium lamp emitting a continuous UV spectrum between 200 and 400 nm and concentrations of the compound chromatographed were determined from the intensity of diffusely reflected light. Further, for digital documentation, the digistore 2 documentation system (CAMAG) consisting of illuminator Reprostar 3 and digital camera power shot G2 (Canon, Tokyo, Japan) was used. After scanning the plate, the exact position of the separated bands were marked with a pencil.

The TLC-MS interface (CAMAG, Switzerland) extraction head contained two connections on the topside, one inlet and one outlet. On the bottom surface there was a cutting edge seal with a height of about the thickness of the platelayer. The extraction head was pressed onto the plate, (circular cutting edge seal of 4 mm i.d) into the adsorption layer to create a leakage free seal. With the help of a laser crosshair, the extractor head was easily positioned on a selected zone from bypass position (**Figure 2(a)**). After lowering the piston, the valve was switched to extraction position (**Figure 2 (b)**). Now, the solvent was pumped with a flow rate of 0.1 mL/min through the extraction head to elute the sample and transported it through the integrated frit to the MS-System. The elution was accomplished as circular zones of 4 mm diameter from the plate in about one minute. Methanol was used as the extraction solvent. The following MS parameters were optimized in ESI^+ mode: source temperature 325ºC, capillary voltage 1.22 kV, HV lens 3.5 kV, capillary current 17.09 nA, skimmer voltage 40 V, nitrogen as nebulizing (35 Psi) and drying gas at a flow rate of 5.0 mL/min.

3 RESULTS AND DISCUSSION

3.1 Method optimization and wavelength selection

Mobile phase optimization plays a major role in accomplishing the desired separation profiles. Thus, different mobile phases containing various ratios of toluene: methanol, acetone and ethyl acetate were examined. However, only toluene: ethyl acetate: methanol (6:5:2 v/v) offered the best separations with well resolved zones but methanol content was always crucial and any change in the ratio presented would drastically alter the chromatographic profiles. Chamber saturation for 20 min with the mobile phase facilitated the best chromatographic behavior with well defined bands with selective retention factors for dexrabeprazole (053±0.02) and domperidone (0.43±0.02) respectively as depicted in the chromatogram (**Figure 3)** and also as a video image (**Figure 4 (a)**). The optimum wavelength for detection and quantification was 284 nm when scanned at 254 nm and confirmed by UV spectra of dexrabeprazole and domperidone (**Figure 4 (c)**).

3.2 Experimental setup

To accomplish an accurate analytical investigation, the potential sources of error resulting from various experimental stages had to be reduced. Thus, the TLC sheets were prewshed, smaller band lengths were chosen, the chamber was adequately saturated with the mobile phase and proper fixation of the aluminum sheet under the extraction head was always ensured.

3.3 Analytical response

Based on the ICH guide lines [29], a calibration plot was arrived at with ten analyte levels in duplicate, applying different volumes of the mixed standard solution. The calibration data fit a linear regression model with a regression coefficient of 0.9993 and 0.9990 respectively **(Table 1)** over a concentration range of 50-500 ng/band. Precision was evaluated through repeatability and intermediate precision. Repeatability was calculated analyzing the same pharmaceutical sample (n=6) on the sample plate resulting in a relative standard deviation of <2% while intermediate precision was measured on different days using different plates with RSD of <2% **(Table 2)**. Further, the recovery studies were carried out by spiking standard drug solution to pre anlysed sample solutions at 3 different levels of concentration corresponding to 50%, 100%, and 150% of label claim. Chromatographic analysis was carried out in triplicate. Recovery at three levels ranged between 99.55-101.8% for dexrabeprazole with RSD values of 0.41-0.84,while for domperidone the recovery results ranged from 99.37 -100.8% with RSD of 0.39-0.72 % as presented in **Table 3**. Limit of Detection (LOD) and Quantification (LOQ) were measured using the formula LOD =3.3σ/S, LOQ=10 σ /S where σ is the standard deviation of the response and S is the slope of the calibration curve. Thus, the LOD and LOQ for dexrabeprazole were 21.22 ng/band and 70.73 ng/band and 16.95 ng/band and 56.51 ng/band for domperidone respectively **(Table 1)**.

3.4 Selectivity

Selectivity was evaluated determining the peak purity and thus the compound identification was established with the help of R_f and UV spectra by comparison with the standards (**Figure 4 ((a), (b), (c))**. A further confirmation of adequate selectivity and separation power was demonstrated by MS. The sample bands were directly eluted from the plate in to the MS through elution based interface without any post chromatographic protocol .The mass spectrum obtained in the *m/z* range from 0-600 shows the ESI^+ mass spectra of dexrabeprazole and domperidone where the $[M+H]^+$ ions were detected at *m/z* 360.3 and 426.8 **(Figure 5)**. Thus, the identity of the bands as well as the adequate chromatographic selectivity was confirmed.

3.5 Sample Analysis

The results obtained for the amount of dexrabeprazole and domperidone in capsules as against the label claims were in good agreement suggesting that there is no interference from any of the excipients, which are generally present in capsules. It may therefore be inferred that dexrabeprazole and domperidone had no quantifiable additional impurities in the marketed formulations analyzed by use of this method. The good performance of the method was indicative of its suitability for routine analysis of dexrabeprazole and

domperidone in pharmaceutical dosage forms.

4 CONCLUSION

With the onset of stringent quality regulations for globalization, the pharmaceutical industry now requires sensitive and reliable analytical methods to ensure the product quality. In this context HPTLC-MS is a reliable alternative and complementary to other chromatographic methods. Therefore, this simple and rapid high through put HPTLC/ESI-MS method for the separation and determination of dexrabeprazole and domperidone in formulations has been developed validated and the potential utility of the same has been discussed. The presented HPTLC/ESI-MS method with an extraction head facilitated a quantitative extractability of both the drugs from silica gel phases with required analytical response and adequate sensitivity. The current study showed that the new hyphenation technique could be successfully employed not only for separation of drug molecules but also for the drug impurity profiling as well where the detection could be accomplished with comparable sensitives as in other methods like HPLC-MS. This new, convenient and universal HPTLC-MS interface, that facilitates complete substance zone extraction with its depth profile allows detections with contemporary relevance and finds great significance at a time when great importance is given for the quality of drugs specifically used for therapeutic intervention and life saving competency.

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Table 1: Linear regression data for the calibration curves (n=6)

Parameters	Dexrabeprazole	Domperidone
Linear range	50-500 ng/band	50-500 ng/band
Correlation coefficient (r) ±SD	0.9993±0.00072	00072 0.9990±0.00059
Slope±SD	5.4±0.51	7.3±0.86
Confidence limit of slope [a]	5.91-4.89	8.16-6.44
Intercept±SD	36.2±3.4	183.3± 4.7
Confidence limit of intercept [a]	39.6-32.8	188.0-178.6
LOD (ng/band)	21.22	16.95
LOQ (ng/band)	70.73	56.51
[a] 95% confidence limit		

Table 2: Precision studies (n=6)

Drug	Amount (ng/band)	Repeatability		Intermediate precision	
		Mean Area ((AU) ±SD)	%RSD	Mean Area ((AU) ±SD)	%RSD
DRA	50	588.8±8.20	1.39	565.40±6.30	1.11
	100	1150.9±14.1	1.22	1181.7±16.8	1.42
	150	1740.5± 18.9	1.08	1723.6±20.3	1.17
DOM	150	1454.1± 12.4	0.85	1484.6±13.8	0.92
	300	2531.8± 18.6	0.73	2380.5± 16.4	0.68
	450	3333.4± 21.7	0.65	3279.2±24.1	0.73

DRA = Dexrabeprazole, DOM = Domperidone

Table 3: Recovery studies (n=6)

Drug	Amount taken	Amount added	Total amount found	Recovery %	RSD %
DRA	100	100	203.70	101.8	0.56
	100	200	305.44	101.8	0.84
	100	300	398.22	99.55	0.41
DOM	200	100	298.13	99.37	0.39
	200	200	402.91	100.7	0.72
	200	300	504.18	100.8	0.46

DRA = Dexrabeprazole, DOM = Domperidone

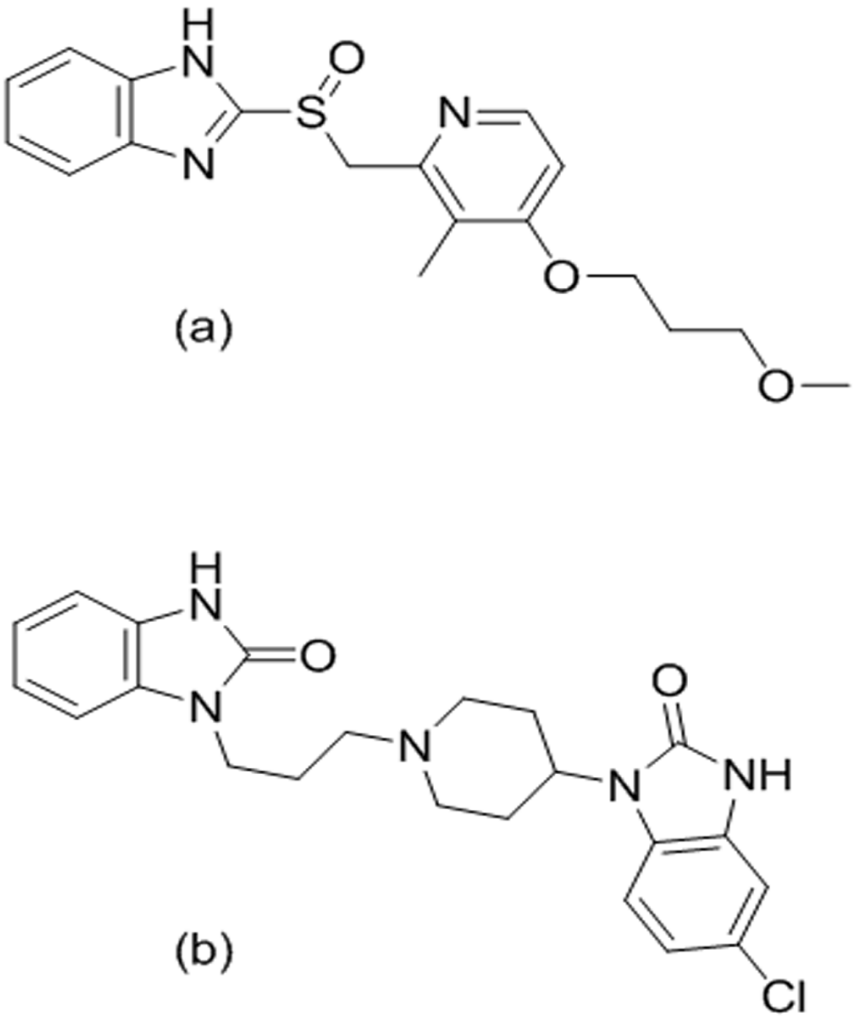


Fig. 1: Structure of dexrabeprazole (a) and domperidone (b)

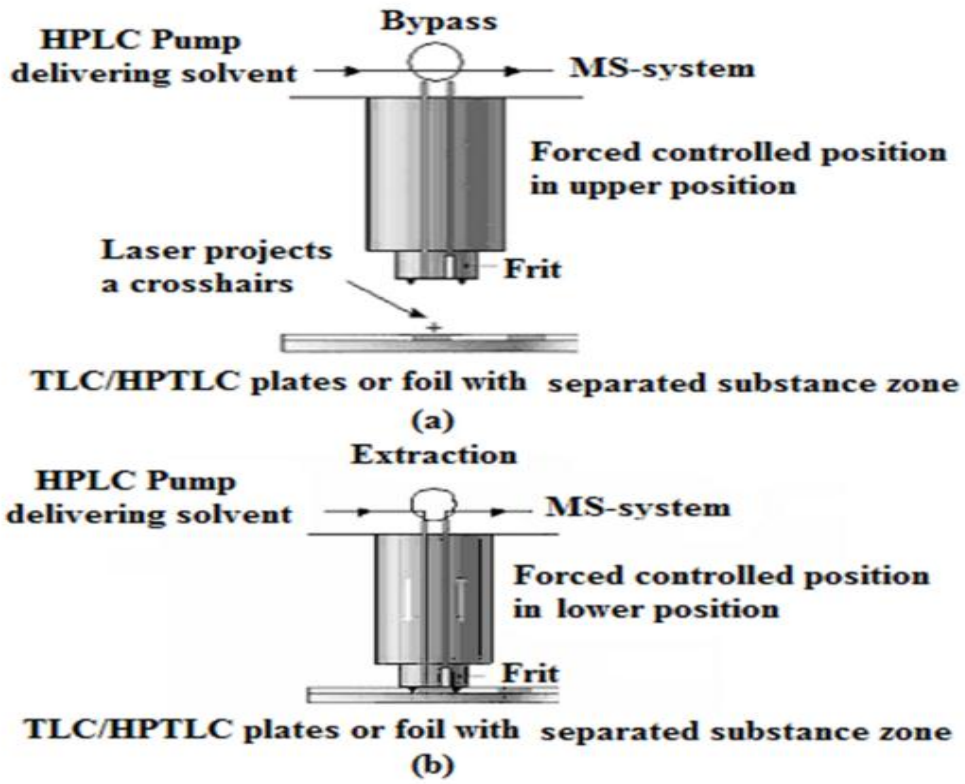


Fig. 2: (a) TLC-MS interface in bypass position, (b) TLC-MS interface in extraction position

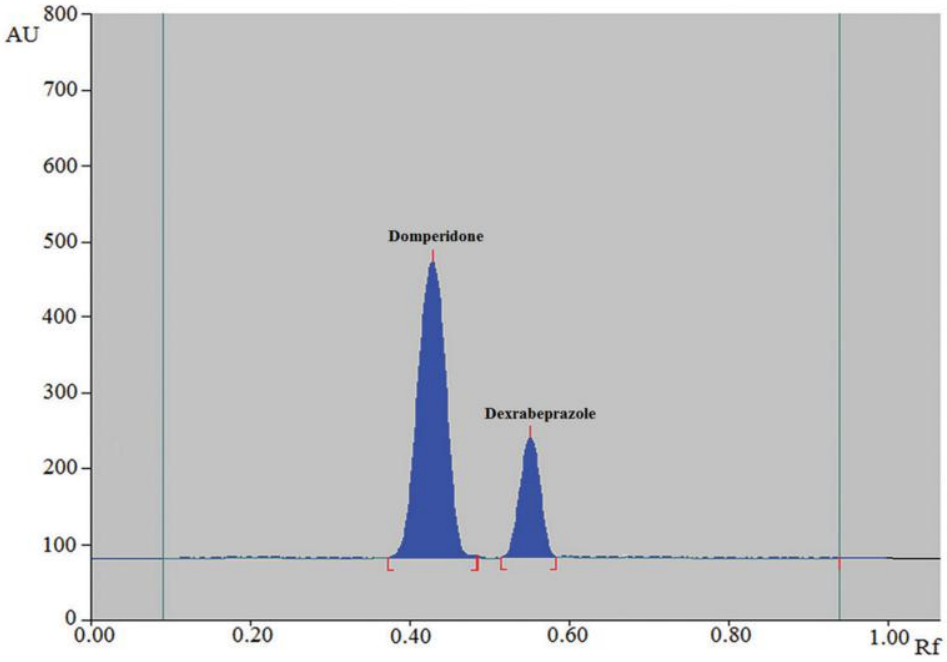


Fig. 3: A typical densitogram of domperidone (R_f 0.43±0.02) and dexrabeprazole (R_f 0.53±0.02) of formulation (DIRAB-D) showing no interference of excipients analysis

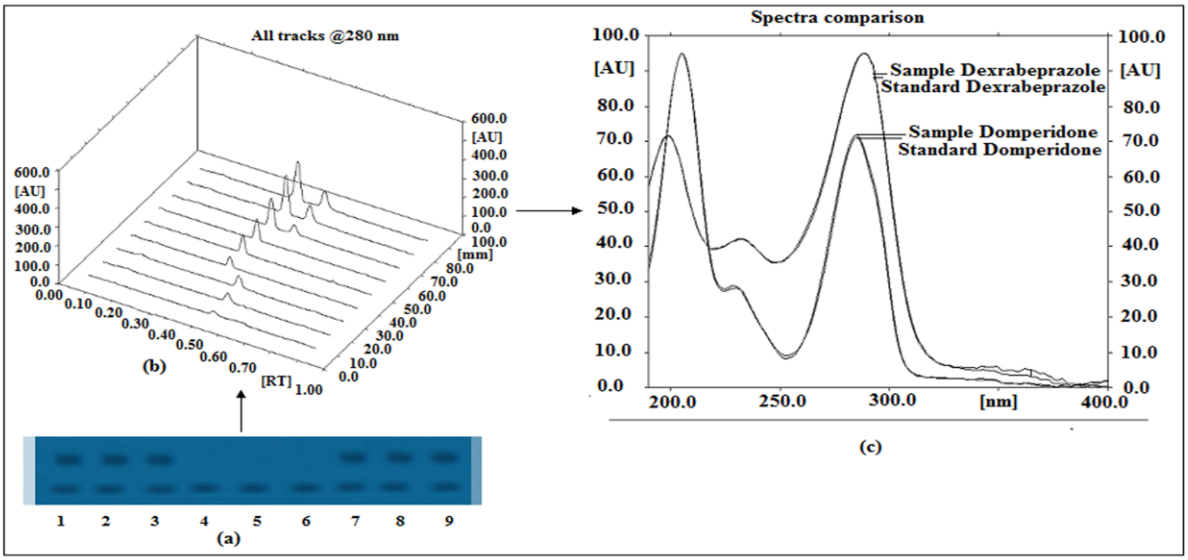
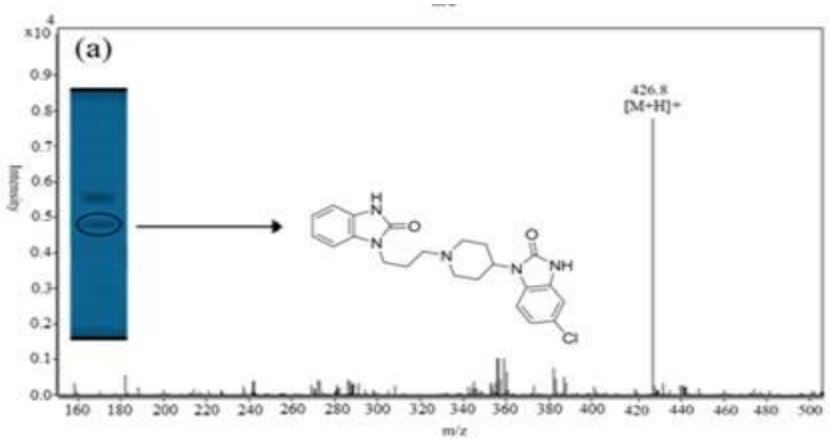


Fig. 4: (a) Video image of dexrabeprazole standard (1, 2, 3), domperidone standard (4, 5, 6), mixed standard (7) and sample (8, 9). (b) Densitogram of dexrabeprazole (R_f 0.53±0.02), domperidone (R_f 0.43±0.02) in standard and sample. (c) Insitu UV spectra of dexrabeprazole, domperidone standard and sample



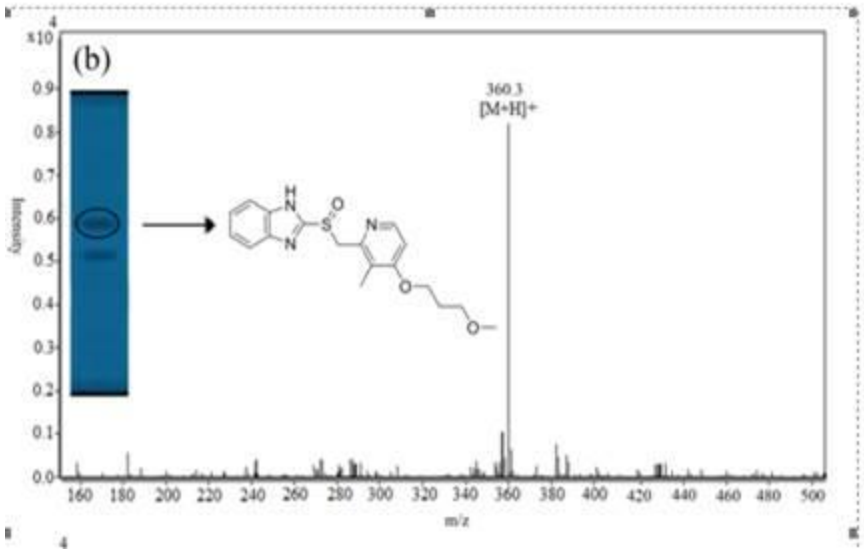


Fig. 5: Mass spectra of domperidone sample zone (50ng) (a) and dexrabeprazole sample zone (50ng) (b) obtained by HPTLC/ESI–MS

REFERENCES

1. Bodhankar SL, Jain BB, Ahire BP, Daude RB and Shitole PP. Indian J Pharmacol. 2006; 38:357-358.
2. British Pharmacopoeia. British Pharmacopoeia Commission Office, London: U.K. 1 2008;752-756.
3. Sivasubramanian L and Anilkumar V. Indian J Pharm Sci. 2007;69:674-676.
4. Patel B, Dedania Z, Dedania R, Chetan R, Vidyasagar G and Mehta RS. Asian J Research Chem. 2009;2:210-212.
5. Sivakumar T, Manavalan R and Valliappan K. Acta Chromatographica. 2008;20:549-562.
6. Park CW, Rhee YS, Go BW, Kam SH, Lee KH, Lee HS and Park ES. Arch Pharm Res. 2008;31:1195-1199.
7. Karthik A, Subramanian G, Ranjith Kumar A and Udupa N. Indian J Pharm Sci. 2007;69: 142-144.
8. Patel BH, Patel MM, Patel JR and Suhagia BN. J Liq Chromatogr Rel Technol. 2007;30: 439 – 445.
9. Gandhi SV, Sabnis SS, Dhavale ND and Jadhav VY. J AOAC Int. 2008;91:344-348.
10. Argekar AP and Shah SJ. J Pharm Biomed Anal. 1999;19:813-817.
11. Patel BH, Suhagia BN, Patel MM and Patel JR. Chromatographia. 2007;65:743- 748.
12. Kalra K, Naik S, Jarmal G and Mishra N. Asian J Research Chem. 2009;2:112-115.
13. Rajendraprasad Y, Rajasekhar K, Shankarananth V, Yaminikrishna HV, Saikumar S and Venkata Raghav reddy P. J Pharm Res. 2009;2:1593-1594.
14. Charde M, Walode S, Tajne M and Kasture A. Indian J Pharm Sci. 2006;68:658-659.
15. Amin AS and Ragab GH. Anal Sci. 2003;19:747-51.
16. Gandhi SV, Khan SL, Jadhav RT, Jadhav SS and Jadhav GA. J AOAC Int. 2009;92:1064-1067.
17. Zhan Li, Jing Yao, Ziqiang Zhang and Luyong Zhang. J Chromatogr Sci. 2009;47:881-884.
18. Chitlange SS, Mulla AL, Pawbake GR and Wankhede SB. Int J Pharm Qual Assur. 2010;2:31-34.
19. Morlock G, Schwack W and TrAC. Anal Chem. 2009;81:10275-10284.
20. Tames F, Watson ID, Morden W and Wilson ID. J Chromatogr B. 1999;729:341–346.
21. Parent AA, Anderson TM, Michaelis DJ, Jiang GL, Savage PB and

Linford MR. Appl Surf Sci. 2006;252:6746–6749.
22. Nakamura K, Suzuki Y, Goto-Inoue N, Yoshida-Noro C and Suzuki A. Anal Chem. 2006;78:5736–5743.
23. Fuchs B, Schiller J, Suss R, Nimptsch A, Schurenberg M, Suckau D. J Planar Chromatogr. 2009;22:35–42.
24. Meisen I, Distler U, Muthing J, Berkenkamp S, Dreisewerd K, Mathys W, Karch H and Mormann M. Anal Chem. 2009;81:3858–3866.
25. Anderson RM and Busch KL. J Planar Chromatogr. 1998;11:336-342.
26. Wachs T and Henion J. Anal Chem. 2001;73:632-638
27. Luftmann H. Anal Bioanal Chem. 2004;378:964–968.
28. Aranda M and Morlock G. Rapid Commun Mass Spectrom. 2007;21:1297–1303.
29. ICH Harmonised Tripartite Guideline, Validation of Analytical Procedures: Text and Methodology, Q2 (R1) Geneva, 2005.