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High performance liquid chromatographic and thin layer densitometric methods for the determination of risperidone in the presence of its degradation products in bulk powder and in tablets

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Abstract

Two reproducible stability indicating methods were developed for the determination of risperidone (RISP) in presence of its degradation products in pure form and in tablets. The first method was based on reversed phase high performance liquid chromatography (HPLC), on Lichrosorb RP C 18 column (250 mm i.d., 4 mm, 10 μm), using methanol:0.05 M potassium dihydrogen phosphate pH 7 (65:35 (v/v)) as the mobile phase at a flow rate of 1 ml min^{-1} at ambient temperature. Quantification was achieved with UV detection at 280 nm over a concentration range of 25–500 μg ml^{-1} with mean percentage recovery of 99.87 ± 1.049. The method retained its accuracy in the presence of up to 90% of RISP degradation products. The second method was based on TLC separation of RISP from its degradation products followed by densitometric measurement of the intact drug spot at 280 nm. The separation was carried out on aluminum sheet of silica gel $60F_{254}$ using acetonitrile:methanol:propanol:triethanolamine (8.5:1.2:0.6:0.2 (v/v/v/v)), as the mobile phase, over a concentration range of 2–10 μg per spot and mean percentage recovery of 100.1 ± 1.18. The two methods were simple, precise, sensitive and could be successfully applied for the determination of pure, laboratory prepared mixtures and tablets. The results obtained were compared with the manufacturer's method.
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Keywords: Risperidone; Stability indicating methods; HPLC method; TLC densitometric method

1. Introduction

Risperidone (RISP), 3-[2-[4-(6-fluoro-1,2-benzisoxazol-3-yl)piperidin-1-yl]ethyl]-2-methyl-6,7,8,9-tetrahydro-4H-pyrido[1,2-a]pyrimidin-4-one [1,2], is a benzisoxazole antipsychotic, reported to be an antagonist to dopamine D2 and serotonin (5HT2), adrenergic, and histamine (H1) receptors [3].

It is described as an atypical antipsychotic. It is given by mouth for the treatment of schizophrenia and other psychoses. Several methods have been reported for the determination of risperidone in bulk powder, pharmaceutical formulations, and in biological fluids. HPLC methods for the determination of risperidone in bulk powder [4,5], in urine [6], in plasma and serum [6–8] in post mortem fluids [9] and in pharmaceutical formulations [10–12] have been reported. Risperidone was also determined together with its major metabolite 9-hydroxy risperidone, by HPLC in serum and plasma using different columns and different mobile phase mixtures [13–27]. Two electrospray ionization tandem MS methods for detection of risperidone were reported [28,29]. Resperidone was determined in blood by LC-ionspray tandem–MS using C18 column [30]. Methods like dual-plate overpressured layer chromatography [31], capillary gas chromatography [32], negative ion chemical ionization GC–MS [33], capillary zone electrophoresis [34,35] and sheathless capillary electrophoresis [36], have been published. None of the reported methods determined the drug in the presence of its degradation products, which may result from decomposition

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of RISP under inappropriate storage conditions. As there was no report yet on this aspect, it was felt necessary to develop chromatographic methods such as HPLC and TLC densitometric methods for the purity evaluation and quantitative determination of the drug. The two proposed methods are stability indicating and were used for the determination of RISP in commercial tablets without interference from the excipients normally used in tablet formulations. Although, the HPLC method is simple, accurate yet, the TLC method is much more sensitive since it allows the determination of the drug with quantification limit up to 629.85 ng per spot.

2. Experimental

2.1. Materials and chemicals

Risperidone standard and five risperidone degradation products were kindly supplied by Janssen (Egypt).

Risperdal tablets, of batch number 00HL111, was labeled to contain 2 mg of RISP per tablet.

HPLC grade methanol and acetonitrile were purchased from Merck (USA).

The water for HPLC was prepared by double glass distillation and filtration through Millipore 0.45 um, white nylon. HNWP 47 mm filters.

Propanol, triethanolamine, ammonium acetate and potassium dihydrogen phosphate were obtained from BDH chemicals (Pool, England) and were of analytical grade.

2.2. Apparatus

(i) HPLC consists of Hewlett-Packard series 1100 was equipped with quaternary pump, diode array detector and a manual injector 20 μl loop. Column used was prepared Lichrosorb RP-18 (250 mm × 4 mm i.d., 10 μm particle size).
(ii) Ultrasonic bath used was J.P. Selecta, s-a, cd.300513 (Barcelona, Spain).
(iii) Shimadzu-dual wavelength lamp flying CS 9301densitometer (Tokyo, Japan).
(iv) Ultraviolet short wave length lamp (254 nm).
(v) TLC plates used was silica gel/TLC cards with fluorescent indicator 254 nm, layer thickness: 0.2 mm 20 × 20 cm aluminum cards Fluka packed in (Switzerland).

2.3. Chromatographic conditions

2.3.1. HPLC method

A repacked column (Lichrosorb RP-18250 × 4(10 μm)) was used at ambient temperature. The mobile phase consisted of methanol: 0.05 M potassium dihydrogen phosphate buffer pH 7 (65:35 (v/v)) was prepared and was pumped at a flow rate of 1 ml min^{-1}. The mobile phase was filtered through Millipore filter 0.45 μm, white nylon HNWP 47 mm and was degassed before use. The elution was monitored at 280 nm. The injection volume was 20 μl.

2.3.2. TLC densitometric method

For TLC with UV densitometric analysis, solutions of the tested substance were applied to silica gel 60 F_{254} TLC plates 20 × 20 using 20 μl pipette. The plate was placed in a chromatographic tank previously saturated for 45 min with developing mobile phase; acetontrile:methanol:propanol:triethanolamine (8.5:1.2:0.6:0.2 (v/v/v/v)).The plate was developed by normal vertical developing tank at ambient temperature for 16 cm distance. The spots were detected under a UV lamp (254 nm) and the drug was scanned densitometrically (in flying-spot mode) at 280 nm. A Shimadzu dual wavelength flying-spot scanner was used for densitometric evaluation of the plates at the following settings: photomode: reflection, lane: auto, zero set mode: at start, scan mode: linear, difference: off, lambda: single, trace: off.

2.4. Standard stock solution

(i) Standard risperidone stock (0.5 mg ml^{-1}): it was prepared in methanol and refrigerated (solution was stable for several weeks; when kept at room temperature away from direct sun light in tightly capped bottle).
(ii) Standard mixture of degradation products: accurately weighed quantities of the five degradation products were transferred to the same 50 ml volumetric flask, they were dissolved and made up to volume with methanol (0.1 mg ml^{-1} each) and refrigerated (solution was stable for several weeks; when kept at room temperature).

2.5. Calibration

2.5.1. For HPLC method

Accurately measured aliquots equivalent to 0.25–5.00 mg of RISP were transferred into a series of 10 ml volumetric flasks and completed to volume with methanol. Twenty microlitres of each solution was injected under operating chromatographic conditions described above. Calibration graph was constructed by plotting peak areas versus concentration of RISP and the regression equation was calculated.

2.5.2. For TLC densitometric method

Construction of calibration curve: accurately measured aliquots equivalent to 0.5–2.5 mg of RISP from its stock solution were transferred into a series of 5 ml volumetric flasks, completed to volume with methanol. Twenty microlitres of each solution was applied using 20 μl pipette. Spots were spaced 2 cm apart from each other and 1.5 cm from the bottom edge of the plate. Calibration curve was constructed by plotting peak areas versus concentration of RISP, and the regression equation was calculated.

2.6. Sample solution

2.6.1. Assay of laboratory prepared mixture

Accurately measured aliquots equivalent to 0.5–4.5 and 0.5–2.5 mg RISP from its stock solution were transferred to series of 10 and 5 ml volumetric flasks (for HPLC and TLC methods, respectively) and from 10–90% of the standard mixture of the degradation products using its stock solution and the volume was completed with methanol. The procedure was followed as under construction of calibration curve for HPLC and TLC densitometric methods; starting from "20 μl" of each solution and so on.

2.6.2. Assay of tablets

Twenty tablets were accurately weighed and finely powdered. An accurately weighed amount equivalent to 10 mg of RISP was transferred into 25 ml volumetric flask, dissolved in 20 ml of methanol and completed to volume with the same solvent. Mechanically shacked for few minutes, and filtered. Accurately measured aliquots equivalent to 0.5–3.5 mg and 0.5–2.0 mg of RISP (for HPLC and TLC methods, respectively) were transferred into 10 ml and 5ml volumetric flasks The volume was completed with methanol. The procedure was followed as under construction of calibration curve starting from "20 μl" of each solution and so on.

3. Result and discussion

3.1. HPLC method

The developed HPLC method has been applied for the determination of RISP in presence of its five degradation products Fig. 1. To optimize the HPLC parameters, several mobile phases composition were tried. A satisfactory separation and peak symmetry for the drug and its degradation products were obtained with mobile phase consisting of methanol: 0.05 M potassium dihydrogen phosphate buffer pH 7 (65:35 (v/v)) at ambient temperature. The pH of the mobile phase was adjusted by varying the pH of the buffer used. Upper and lower pH of 7.0 ± 0.5 showed poor resolution of the drug from its five known degradation products. Variable columns were used but Lichrosorb RP C18 250 mm × 4 mm (10 μm) column gave the minimal elution time with good resolution. Quantitation was achieved with UV detection at 280 nm based on peak area.

A representative chromatogram is shown in Fig. 2. The retention time of RISP and degradation products I–V were 17.005, 2.038, 8.732, 4.570, 5.855 (degradation II & III are isomers). The peaks obtained were sharp and have a clear base line separation. Although the method does not separate the two isomers of the degradation products, yet it has the advantage of separating the intact molecule with R_t = 17.005 min from the five known degradation products; the nearest one of which has R_t of 8.732 min. Some trials to separate the two isomers were failed. It was found also, that when subjecting the intact molecule to acid hydrolysis, the same peaks with the exact retention times were obtained.

3.2. TLC densitometic method

A TLC densitometric technique is suggested for the determination of RISP in the presence of its degradation products based on the difference in R_f values. Several mobile phases were tried to accomplish complete separation of RISP from its degradation products. Using the mobile phase acetonitrile:methanol:propanol:triethanolamine (8.5:1.2:0.6:0.2 (v/v/v/v)) and silica gel/TLC cards with fluorescent indicator, 254 nm; layer thickness, 0.2 mm; 20 cm × 20 cm aluminum cards complete separation was attained where R_f values were 0.33 for RISP and 0.1, 1.28 and 0.24, for the degradation products I, IV and V, respectively. While degradation products II and III remain on the base line with R_f values of 0.

A wavelength of 280 nm was used for the quantification of the drug.

A representative chromatogram is shown in Fig. 3.

3.3. Validation of the proposed methods

3.3.1. Linearity

To determine the linearity of HPLC and TLC, response, calibration standard solutions of RISP were prepared as in the text. Linear correlation was obtained between peak areas and concentration of RISP in concentration range of 25–500 μg ml^{-1} and 2–10 μg per spot for HPLC and TLC, respectively.

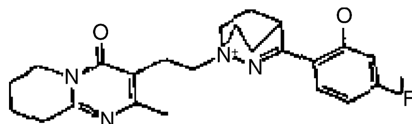
Characteristic parameters for regression equations and correlation coefficients were given in Table 1.The linearity of the calibration graphs were validated by the high value of correlation coefficients of the regression.

3.3.2. Accuracy and precision

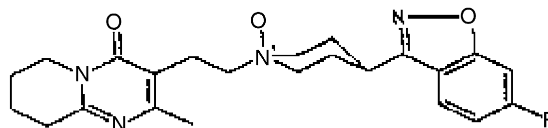
By applying the proposed methods, it was possible to determine RISP in its pure form with mean percentage recoveries of 99.86 ± 1.020 and 100.10 ± 1.001 by HPLC and TLC densitometric methods, respectively.

In the case of quantitative analysis of impurities, the accuracy should be assessed on samples of drug substances spiked with known amount of impurities (USPXXVI). So, the accuracy and precision of the proposed methods were also assessed using its impurities; the five known degradation products I–V were mixed with the intact drug in various ratios, and the recoveries were determined. The results shown indicate that the accuracy of the method are not affected by the presence of up to 90% of the degradation products with mean percentage recoveries of 99.79 ± 0.547% and 99.93 ± 0.919% for the HPLC and TLC methods, respectively. The mean and relative standard deviations for both methods are indicating good precision (less than 1%). The results are presented in Table 2.

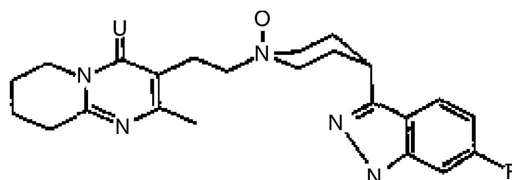
The proposed methods were successfully applied for the analysis of the drug in its tablets form, and the recovery ex-



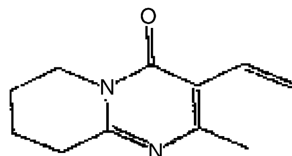
3-(4-Fluoro-2-hydroxy-phenyl)-6-[2-(2-methyl-4-oxo-6,7,8,9-terahydro-4H-pyrido[1,2-a]pyrimidin-3-y1)-ethyl]-2-aza-1-azonia-bicyclo[2.2.2]oct-2-ene.



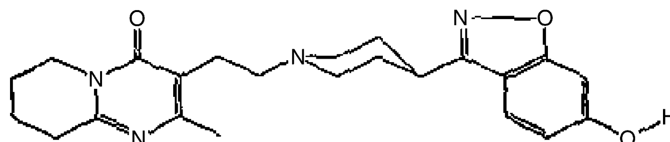
4-(6-Fluoro-benzo[d]isoxazol-3-y1)-1-hydroxy-1-[2-(2-methyl-4-oxo-6,7,8,9-tetrahydro-4H-pyrido[1,2-a]pyrimidin-3-y1)-ethy1]-piperidinium.



4-(6-Fluoro-benzo[d]isoxazol-3-y1)-1-hydroxy-1-[2-(2-methyl-4-oxo-6,7,8,9,-tetrahydro-4H-pyrido[1,2-a]pyrimidine-3-y1)-ethyl]-piperidinium.



3-ethenyl-6,7,8,8-tetrahydro-2-methyl-4H-pyrido[1,2-a]pyrimidin-4-one.



6,7,8,9-tetrahydro-3-[2-[4-(6-hydroxy-1,2-benzisoxazol-3-y1)-1-piperdinyl] ethyl]-2-methyl-4H-pyrido[1,2-a]pyrimidin-4-one.

Fig. 1. Chemical structure of RISP degradation products (I–V).

Table 1
Characteristic parameters

Parameter	HPLC method	TLC densitometric method
Concentration range	25–500 μg ml^{-1}	2–10 μg per spot
Detection limit [LOD]	3.00 μg ml^{-1}	249.69 ng per spot
Quantification limit [LOQ]	12.40 μg ml^{-1}	629.85 ng per spot
Regression equation (y)[a]		
Slope (b)	14.83079775	589.2268
Confidence limit[b] of slope	14.83 ± 0.039	589.227 ± 0.0219
Intercept (a)	16.1646146	51.1262
Confidence limit[b] of intercept	16.16 ± 0.059	51.1262 ± 0.039
Correlation coefficient (r)	0.9999	0.9998

[a] $Y = a + bc$, where c is the concentration.
[b] 95% confidence limit.

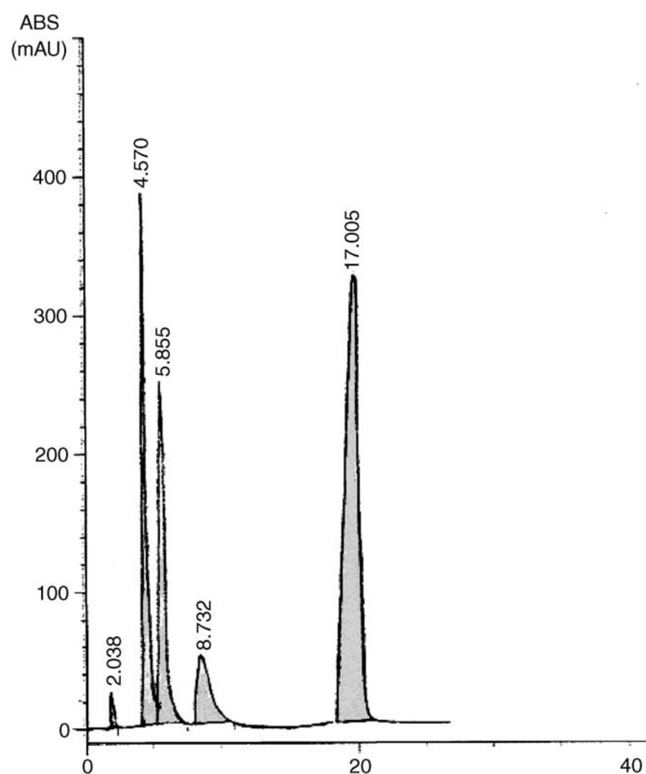


Fig. 2. A typical HPLC chromatogram of RISP and its degradation products; retention time of RISP is17.005 min, and that of the five degradation products are I: 2.038 min, II & III: 8.732 min (two isomers), IV: 4.570 min and V: 6.022 min, respectively. For chromatographic conditions, see Section 2.

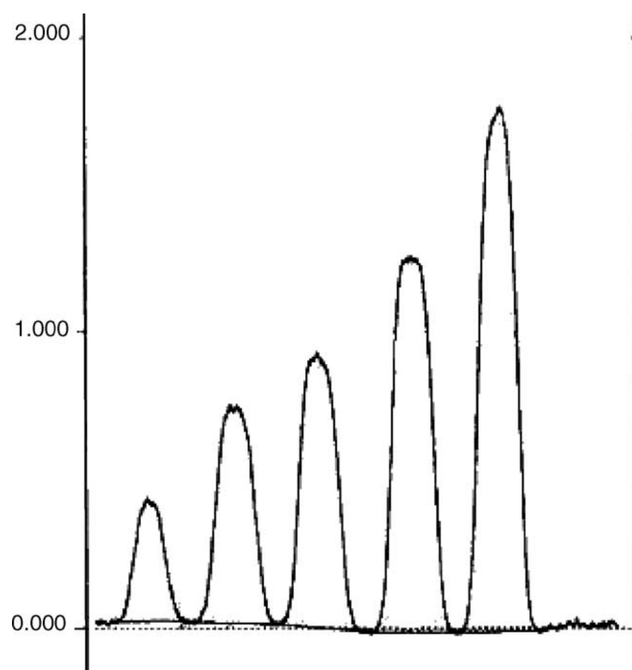


Fig. 3. TLC chromatogram of RISP at 280 nm.

Table 2
Results of the proposed chromatographic methods HPLC and TLC densitometric for the determination of RISP in the presence of its degradation products

Sample number	Degradation (%)	Recovery of the intact HPLC method[a]	Recovery of the intact TLC densitometric method[a]
1	10	100.50	98.50
2	30	99.60	99.20
3	50	99.10	100.10
4	70	99.30	101.00
5	80	100.20	100.30
6	90	100.05	100.50
Mean	–	99.79	99.93
R.S.D.[b]	–	0.547	0.919

[a] Each result is an average of three experiments.
[b] Relative standard deviation.

periments were carried out by spiking the already analyzed samples of the tablets with three different concentrations of standard RISP. The percent recoveries obtained were from 99.1 to 100.5% and from 98.5 to 101% by the HPLC and TLC densitometric methods, respectively. No interference of the excipients with the peaks of interest appeared; hence the proposed methods were applicable for the quantitative determination of RISP in pharmaceutical dosage form (Table 3).

Intermediate precision expresses within laboratory variations as on different days analysis or equipment within the same laboratory (USP26). Interday precision of the proposed methods were evaluated by assaying freshly prepared solutions in triplicates at three different concentrations. Intraday precision was evaluated by using freshly prepared solution in triplicates at three different days. These results are summarized in Table 4.

3.3.3. Limit of detection, limit of quantification

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were calculated using the following equations [37]:

$$\text{LOD} = 3 \times \frac{N}{B} \qquad \text{LOQ} = 10 \times \frac{N}{B}$$

where N is the standard deviation of the of the peak areas of the drug and B is the slope of the corresponding calibration curve. LOD for RISP in HPLC and TLC densitometric methods were found to be 2.96 μg ml^{-1} and 249.69 ng per spot, respectively. LOQ for RISP in HPLC and TLC densitometric methods were found to be 12.40 μg ml^{-1} and 629.85 ng per spot, with precision of 1.31 and 1.92 for the two methods, respectively.

Table 3
Application of the proposed methods to the determination of tablets

	Rispersal tablets recovery, % ± R.S.D.	Standard addition ± R.S.D.
TLC densitometric method	99.89 ± 0.390	100.480 ± 0.475
HPLC method	99.98 ± 0.930	100.020 ± 0.119

Table 4
Intra- and inter-day variation of RISP by the proposed methods

	HPLC method[a]			TLC densitometric method[a,b]		
Intra-day						
0 Day						
Concentration used ($\mu g\,ml^{-1}$)	50.00	450.00	250.00	4.00	6.00	10.00
Mean (%)	99.92	99.95	100.03	100.00	100.83	101.00
S.D.	0.15	0.19	0.15	0.10	0.13	0.02
R.S.D.	0.30	0.08	0.03	2.50	2.10	0.15
1 Day						
Concentration used ($\mu g\,ml^{-1}$)	50.00	450.00	250.00	4.00	6.00	10.00
Mean (%)	99.20	100.00	99.98	98.00	99.00	99.8
S.D.	0.56	0.20	0.20	0.08	0.06	0.10
R.S.D.	1.12	0.08	0.04	1.94	0.98	1.04
2 Day						
Concentration used ($\mu g\,ml^{-1}$)	50.00	250.00	450.00	4.00	6.00	10.00
Mean (%)	99.46	100.12	99.99	102.00	101.39	98.10
S.D.	0.36	0.21	0.21	0.08	0.07	0.12
R.S.D.	0.73	0.08	0.05	1.86	1.12	0.12
Inter-day						
Concentration used ($\mu g\,ml^{-1}$)	50.00	450.00	250.00	4.00	6.00	10.00
Mean (%)	99.56	100.00	99.99	100.00	100.33	99.63
S.D.	0.18	0.09	0.12	0.10	0.07	0.15
R.S.D.	0.36	0.04	0.03	2.50	1.23	1.47

[a] Eash result is an average of three results.
[b] Concentration in densitometric method is μg per spot.

Table 5
Comparison between the results obtained by the proposed methods and the manufacturer's method [38]

	HPLC method	TLC densitometric method	Manufacturer's method[a]
Concentration range	25–500 μg per ml	2–10 μg per spot	80–160 mg
Recovery (%)	99.87	100.081	99.46
Variance	1.098	1.383	0.726
R.S.D.[b]	1.046	1.175	0.857
N	5	5	5
F (6.39)[c]	1.451	1.905	–
T (2.31)[c]	0.690	0.0.958	–

[a] HPLC: using 5 μ Hypersil BDS C18, 250 mm × 4.6 mm column, acetonitrile: 0.5% ammonium acetate (40:60 (v/v)) as a mobile phase, flow rate of 1.2 ml min^{-1} and a UV detection at 280 nm.
[b] Relative standard deviation.
[c] Figures in parentheses represent corresponding to tabulated values for F and t at $P = 0.05$.

RISP was determined according to European Pharmacopoeia (2002) [28] by dissolving in anhydrous acetic acid and methyl ethyl ketone, using perchloric acid as titrant. The end point was determined potentiometrically. Therefore, the results obtained were compared preferably by that of the manufacturer's [38], which is an HPLC method. Table 5 shows that the calculated t- and F-values are less than the theoretical ones, confirming accuracy and precision at 95% confidence level. Moreover, the proposed methods were much more sensitive than the manufacturer's methods. Although, The proposed HPLC method is simple, accurate sensitive yet, the TLC method is much more sensitive than both the proposed and the manufacturer's since it allows the determination of the drug with quantification limit up to 629.85 ng per spot.

The methods demonstrated sufficient linearity, accuracy, precision and specificity to satisfy the corresponding criteria for stability indicating quality control procedure.

4. Conclusion

The goal of this work was achieved by separating and quantitating the new antipsychotic drug risperidone in presence of its degradation products in bulk powder and in tablets. HPLC and TLC densitometric methods have been developed and validated as described herein for the determination of the drug without any interferences from excipient, and in the presence of up to 90% of its degradation products. The concomitant analysis provides significant sensitivity (LOD were

2.96 μg ml^{-1} and 249.69 ng per spot for HPLC and TLC densitometric methods, respectively), as well as significant decrease in sample preparation, instrument run time over the other separation method.

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