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A novel green HPTLC method for simultaneous analysis of four antipsychotics in their pharmaceutical formulations: Assessment by Eco-scale

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Abstract

Implementing green analytical techniques has been one of the main objectives of the analytical chemistry community for the past two decades. The aim of transitioning to green analytical chemistry is to establish new methods environmentally benign instead of traditional methods. According to WHO, 20 million people worldwide are affected by schizophrenia, and 45 million people worldwide are affected by bipolar disorders. For that, in this work we develop a green HPTLC method to quantify simultaneously the four antipsychotics chloropromazine HCl, asenapine maleate, quetiapine fumarate and aripiprazole in pure form and tablet formulations. In this chromatographic method, separation was performed using silica gel 60 F254 HPTLC plates, the mobile phase composed of binary mixture of green solvents (ethanol : water (9 : 1 v/v)) which gave compact band with R_f values ranged between 0.14 to 0.70 for the drugs. The method was validated for linearity, accuracy, precision, selectivity and robustness according to ICH guidelines. It was assessed for greenness by analytical Eco-scale and compared with reported HPTLC methods for single analysis of these antipsychotics.

Key words: antipsychotics; Analytical eco-scale; HPTLC.

1. Introduction

Antipsychotics are widely used for the treatment of psychotic symptoms. To reach to the adequate treatment in such pathologies, it is very difficult and a large percentage of patients who take antipsychotics, have to administer a combination of them. Antipsychotics is classified to typical or atypical. Typical antipsychotics, also called first-generation antipsychotics, are the older medications used to treat psychotic symptoms, from them is Chlorpromazine HCl (CH) which is chemically called, 2-Chloro-10-[3-(dimethylamino) propyl]-phenothiazine monohydrochloride.CH is widely prescribed for treatment of schizophrenia, and is also used to treat other diseases such as bipolar disorder (Wishart, et al,2018). The literature survey showed that CH was determined by several analytical methods include spectrophotometric (AL-Kaffiji, 2013), electrochemical (Jihad,2015; Zayed,2012), HPLC (Fadhel, 2018; Usha, 2014; Khelfi,.2018; Mahadik, 2002)and HPTLC (Davis ,1984). Atypical antipsychotics, the second-generation antipsychotics, are drugs that exhibit superior efficacy with fewer side effects compared to typical antipsychotics (Fragou,2012),from them

is Asenapine Maleate (AS),(3aRS,12bRS)-5-chloro-2-methyl-2,3,3a,12b-tetrahydro-1H-dibenzo [2,3:6,7] oxepino[4,5-c]pyrrole(2Z)-2-butenedioate(1:1). AS is indicated for the treatment of schizophrenia and acute mania or mixed episodes associated with bipolar disorder (Wishart, 2018). Literature survey revealed quantification of AS by different analytical techniques as spectrophotometric method (Gandhimathi, 2012; Surekha, 2013), HPLC (Kumar, 2017; Managuli, 2016; Chhalotiya, 2012; Govindarajan, 2014;Karaca, 2017) and HPTLC (Patel,2015). Quetiapine fumarate (QU), whose chemical name is bis[2-(2-[4-(dibenzo [b,f] [1,4]thiazepin-11-yl)] ethoxy) ethanol] fumarate, is atypical antipsychotic, very effective for negative and positive schizophrenia, as well as cognitive impairment (D. Wishart, et al,2018). Several approaches have been reported for the quantitative determination of QU in bulk, and pharmaceutical and biological samples which include spectrophotometric (Bagade , 2009; Vessalli, 2013; Lakshmi, 2009), electrochemical [Nigovic´, 2011;Nebsen, 2016), HPLC (Semin, 2015 ; Kumar, 2013; Venkata, 2013; Youssef, 2016) and HPTLC(Sathiyaa, 2010). Aripiprazole (AR),7-(4-[4-(2,3-dichlorophenyl)-1 piperazinyl] (butoxy)-3,4-dihydro-2(1H)-quinolinone is a novel, atypical antipsychotic drug for treatment of schizophrenia (D. Wishart, et al,2018). Several analytical methods were reported to quantify AR in different matrices, include spectrophotometric (Subbayamma, 2010),electrochemical (Merli, 2013), HPLC (Kalaichelvi, 2010); Djordjević Filijović, 2014; Srinivas, 2008 ; Ravinder, 2012; Soponar, 2014) and HPTLC (Patel ,2018; Tawale ,2018).The chosen drugs are from the most frequently prescribed antipsychotics to treat schizophrenia and bipolar disorders (Roberts, 2018; Bjornestad,2020).

From the literature survey, there is no report for the simultaneous determination of these four antipsychotics in synthetic mixture nor tablet formulations using HPTLC method.

HPTLC is an analytical technique based on TLC, but with enhancements in order to increase the resolution of the analytes to be separated and to allow quantitative analysis of the compounds. HPTLC method, is known to be cheap, sensitive, robust, simple, rapid, and efficient tool in quantitative analysis of compounds,(Inamuddin, , 2014). Most of the routine analytical methods reported for the analysis of these antipsychotics in literatures used toxic solvents as the mobile phase. Moreover, a little attention had been paid towards the environmental effects of analytical methods. Nowadays, most of the research reports focus on clean analytical chemistry or environmentally-benign analytical methods (Haq, 2017).

The aim of this study is to develop a green HPTLC method for simultaneous identification and quantification of these antipsychotics in pharmaceutical forms to help pharmaceutical factories in keeping time and effort and keeping the environment clean. HPTLC method was optimized individually and it had good reproducibility, precision and accuracy with acceptable limits of detection and quantification.

In addition, the greenness of the developed method was assessed by analytical Eco-scale (Gałuszka, 2012) and was compared to four other previously reported analytical methods(Davis,1984; Patel, 2015; Sathiyaa,2010; Tawale,2018) for the single determination of these analytes.

2. Experimental

2.1 HPTLC Instruments

The HPTLC instrument consisted of a CAMAG (Muttenz,Switzerland) Linomat V sample applicator with a 100-µL applicator syringe (Hamilton, Bonadauz, Switzerland). Chromatography was performed on (20×10 cm2, pre-coated silica gel aluminium plates 60 F254). Twin- trough glass development chamber, 20×20 cm2 was used.A CAMAG TLC scanner was utilized for densitometric scanning of the developed chromatogram. All the drugs and chemicals were weighted on a Shimadzu electronic balance (AX 200, Shimadzu Corp., Kyoto, Japan).

2.2 Chemicals and Reagents

AR and QU were kindly given by Utopia Company, Egypt and certified to have 99.9% and 99.8% respectively. While, CH and AS were brought from Sigma-Aldrich and certified to have 98% and 99.5%. All solvents were from Sigma Aldrich. De-ionized water was produced in-laboratory by Millipore water purification system.

Asenapine® tablets (batch No.042) (E.G.P.I, Al obour City, Egypt), were labeled to have 10 mg asenapine maleate, Neurazine® tablets (batch No. 147070) (Misr Co. AL obour City, Egypt), were labeled to have 100 mg chlorpromazine hydrochloride,

aripiprazole® tablets (batch No. 121129) (Multi apex pharmaceutical company - Egypt), were labeled to have 10 mg aripiprazole, quitapex® tablets (batch No. AT07130820) (Inspire pharma, Egypt), were labeled to have 25 mg quetiapine fumarate.

2.3. HPTLC condition

Solutions of the samples and standards were applied to the HPTLC plates as bands rather than spots. The mobile phase consists of ethanol: water (9:1, v/v). The development chamber was saturated with mobile phase for 20 min. All HPTLC plates were first subjected to activation at 60◦C for 10 min before sample application. Bands were applied as 3 mm long and 8 mm intervals and 10 mm from the bottom of the plate. After developing over a distance of 8 cm, the HPTLC plate was air dried and scanned at 220 nm. The scan length and width were adjusted to cover the entire band.

2.4. Standard antipsychotic solutions

Stock standard antipsychotic solutions in concentration 100 µg mL^{-1} for each drug were prepared. Different volumes of stock standard solutions ranging from 1 to 30 µL for CH, and AS, and from 0.5 to 20 µL for QU and AR, were spotted on the TLC plates, to give a final concentration range from 0.1 to 3 µg/band for CH, and AS, and from 0.05 to 2µg/band for QU and AR.

2.5. Sample preparation for the estimation of antipsychotics in their tablets

To prepare the samples, 10 tablets of each product were separately weighed and powdered. An amount of the powder equivalent to 10 mg for each drug was accurately weighed, placed in 100 mL volumetric flask and dissolved in ethanol for each drug except AS, which was dissolved in methanol by using the ultrasonic bath for 20 min and then cooled to room temperature. The solution was then diluted to volume with the same solvent and then filtered through 0.45 µm membrane filters. The first amount of the filtrate was rejected and the remainder was used as a stock sample solution. Various volumes of samples solutions were applied on plates.

The plates were developed in the previously described chromatographic conditions. The concentrations of each compound were determined by substituting in the regression equations.

3. Results and discussion

3.1. HPTLC method

For the first time, a simple, economic and environment friendly HPTLC method was developed for the concurrent determination of these antipsychotics. The mobile phase was prepared by simply mixing water and ethanol which are green solvents. This Eco-friendly HPTLC method will reduce the environmental toxicity caused by toxic solvents used in routine pharmaceutical analysis. Our research work gave a very good analytical technique for the estimation of the antipsychotics in tablet formulations. In this study, several modifications in the mobile phase composition were investigated. Among them (ethylacetate: ethanol, 9:1v/v), (chloroform:ethanol:water,1:5:4v/v/v),(ethanol: water, 7:3, v/v). All these tested mobile phases , resulted in bad separation and poor resolution between peaks. The most appropriate mobile phase was ethanol : water (9:1, v/v), it offered a compact, symmetrical and well resolved peak at R_f value were 0.14± 0.03 , 0.25± 0.03 ,0.55± 0.03 to 0.70± 0.03 for CH, AS, QU and AR, respectively, as demonstrated in Fig.1 and achieved satisfactory results for retardation, capacity, resolution and tailing factors for the four drugs (Table 1).The densitometric estimation of antipsychotics was carried out at 220 nm in the absorbance mode. The spectra of the bands were measured and maximum HPTLC response under absorbance mode was obtained at the wavelength of 220 nm.

Table (1) The system suitability test results of the developed HPTLC method for determination of the studied antipsychotics.

Parameters	CH	AS	QU	AR
Retardation factor R_f	0.14	0.25	0.55	0.7
Capacity factor(K')	6.14	3.00	0.82	0.43
Selectivity (α)[a]	2.05	3.67	1.91	
Resolution (R_s)[b]	2.20	6.00	3.00	
Tailing factor	0.98	0.96	1.04	1.05

[a] $\alpha = \mathbf{K'}_2 / \mathbf{K'}_1$, where $\mathbf{K'}$ is the capacity factor: $\mathbf{K'} = (1 - R_f)/R_f$.

[b] $Rs = [2 (R_{f2} - R_{f1})]/(W_1 + W_2)$, where R_f is retardation factor and W is peak width at 5% from the baseline of the peak height

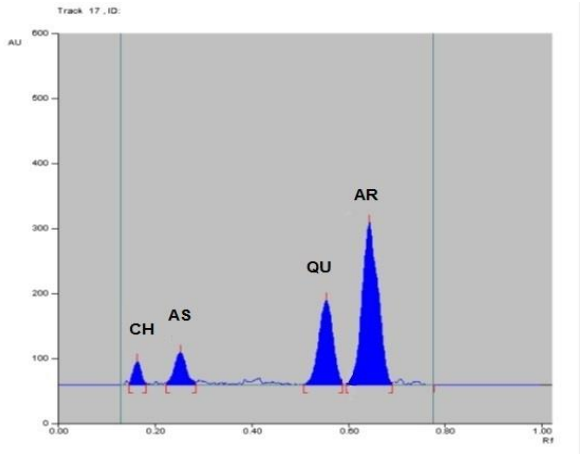

Fig.1 :HPTLC densitogram of synthetic mixture containing 0.25 µg /band of CH, 0.25 µg /band of AS, 0.7 µg /band of QU and 1 µg /band of AR.

3.2. Method validation

HPTLC densitometric method was validated for "linearity, precision, accuracy, robustness, sensitivity and selectivity using the guidelines of ICH (ICH,2005).

3.2.1. Linearity

The linearities for the chosen antipsychotics were studied by plotting the concentration against measured peak area**.** The data for linear regression analysis of calibration curves of antipsychotics is tabulated in Table 2. The calibration curves were linear in the range of 0.1 to 3 µg/band for CH, and AS, and from 0.05 to 2µg/band for QU and AR.. Linear regression analysis showed good linear relationship. The correlation coefficient (R^2) was recorded as 0.9996 for AR, 0.9998 for QU,0.9998 for AS and 0.9998 for CH.

3.2.2.Detection and quantitation limits

Detection and quantitation limits were based on SD of the response and the slope of the calibration curve. The LOD and LOQ of the HPTLC technique were found to be 8.8 and 29.3 ng/band, 10.8 and 26.00 ng/band, 30.7and 102.3 ng/band and 26.2 and 87.33 ng/band for AR, QU, AS and CH, respectively. This observation suggested that the proposed HPTLC technique had good sensitivity which could be applied in wide range for detection and quantification of these antipsychotics effectively.

3.2.3. Selectivity

Method's selectivity was assessed by making eight mixtures of the studied analytes at different concentrations within the linearity range.

The laboratory-prepared mixtures were analyzed by the previous procedure presented. Acceptable recoveries ranging between 99.56% to 99.76% were achieved (Table 2) revealing the high selectivity of the method to determine AR, QU, AS and CH.

3.2.4. Accuracy

Pharmaceutical formulations consisting of these antipsychotics were subjected to the standard addition method. The mean percentage recoveries and their standard deviations were determined to each compound for six replicates (Table 2). The % recoveries of the added antipsychotics were between 99.57% and 100.32%.

3.2.5. Precision

For Repeatability, three chosen concentrations of the analytes (0.1,1,2 µg /band**)** were examined by the HPTLC methods, for three times at the same day. The results were listed in Table 2, where satisfactory values of relative standard deviation (RSD%) revealed good repeatability. For Intermediate precision, the same concentrations were determined at three days using the same procedures. The RSD% were within 2% and indicated satisfactory intermediate precision (Table 2).

3.2.6. Robustness

Two different minor changes in the composition of the mobile phase were tested; ethanol : water(9.5:0.5, v/v), ethanol : water (8.5:1.5, v/v), respectively. Results revealed that minor variations did not produce significant effect on separation efficiency and recovery percentage (Table 2).

3.2.7. Pharmaceutical formulations analysis

The HPTLC method was applied to determine CH, AS, QU and AR in Neurazine®, Asenapine®, Quitapex®, and Aripiprazole®tablets, respectively. Seven determinations were made. For each compound, results were acceptable and in good agreement with label statements (Table3, Fig.2). No methods were reported previously for the simultaneous determination of the studied antipsychotics using HPTLC. Therefore, the proposed green HPTLC method results were compared with the reported methods for the single determination of each drug (Davis,1984; Patel, 2015; Sathiyaa,2010; Tawale, 2018). Comparison between the results was performed statistically regarding accuracy and precision using Student's t-test and F-ratio at 95% confidence level (Table 3). No significant difference was observed between the results.

Table (2): Method validation parameters for determination of the studied antipsychotics using the developed green HPTLC method.

Parameters	CH	AS	QU	AR
Calibration range (µg/ band)	0.1-3	0.1-3	0.05-2	0.05-2
Detection limit (ng/ band)	26.2	30.7	10.8	8.8
Quantitation limit (ng/ band)	87.33	102.30	36.00	29.33
Regression equation (Y)[a]: Slope (b)	20.7	20.6	53.6	79.5
Intercept (a)	0.62	0.86	1.99	2.1
Correlation coefficient (r)	0.9998	0.9998	0.9998	0.9996
Accuracy (mean% recovery of added amount ±SD)	99.57±1.11	98.98±1.14	100.32±1.20	99.70±1.17
Precision intra-day(RSD%)[b]	2.01-1.52-0.58	1.90-1.71-1.44	2.03-1.98-1.15	1.99-1.71-1.15
Precision inter-day(RSD%) [b]	1.85-1.00-1.72	1.57-1.74-0.87	2.02-1.96-0.87	2.01-1.52-1.72
Robustness (mean% recovery ±SD)[c]				
ethanol : water(9.5:0.5, v/v)	98.52±1.11	97.99±1.04	99.32±0.50	98.60±1.07
ethanol : water (8.5:1.5, v/v)	98.01±0.99	99.78±0.54	97.88±0.80	98.70±1.20
Selectivity (mean±SD)[d]	99.76±1.51	99.56±1.02	99.59±1.07	99.93±0.75

[a] $Y = a+bC$,where C is the concentration in µg ml^{-1} and Y is the peak area.
[b] three RSD% values , one for each concentration level .
[c] mean percentage recovery of three determinations.
[d] Average of determinations in eight laboratory prepared mixtures.

Table (3): Determination of AR, QU, AS and CH in tablet dosage forms using the proposed green HPTLC in comparison with HPTLC methods in the literature.

Dosage form	%Recovery [a] ± SD		t-value	F-value[b]
	Proposed	Reported		
Neurazine® tablets	99.01 ± 1.38	99.50 ± 0.64[c] (Davis ,1984)	0.85	0.99
Asenapine® tablets	98.99 ± 0.78	98.50 ± 1.14[c] (Patel,2015)	0.94	1.01
Quitapex® tablets	99.39±0.98	100.50 ± 1.05 [c] (Sathiyaa,2010)	2.04	0.98
Aripiprazole® tablets	98.24 ± 0.64	97.62 ±0.62 [c] (Tawale,2018)	1.84	0.97

[a] The values are mean of seven determinations.
[b] The tabulated t- and F-values at 95% confidence limit are 2.18 and 4.28, respectively.
[c] Reported HPTLC methods.

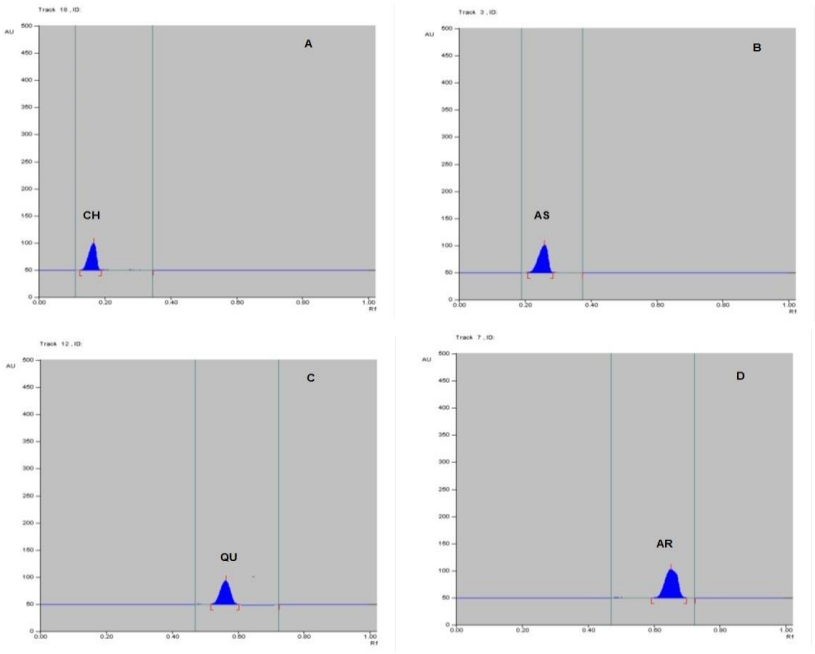

Fig.2: HPTLC densitogram of :a) Neurazine® tablets sample containing 0.3 µg /band of CH b) Asenapine® tablets sample containing 0.2 µg /band of AS, C) Quitapex® tablets sample containing 0.1 µg /band of QU ,c) Aripiprazole® tablets sample containing 0.1 µg /band of AR using the proposed HPTLC method.

Table (4): Penalty points of the analytical eco-scale for the determination of the studied compounds by the proposed HPTLC method and the reported HPTLC methods.

	Penalty points				
Parameters	**Proposed HPTLC method**	**Reported method for CH** (Davis ,1984)	**Reported method for AS** (Patel,2015)	**Reported method for QU** (Sathiyaa,2010)	**Reported method for AR** (Tawale,2018)
1-Reagents					
ethanol	**2**	**-**	**-**	**-**	**-**
water	**0**	**-**	**-**	**-**	**-**
Toluene	**-**	**6**	**-**	**6**	**6**
Methanol	**-**	**-**	**6**	**-**	**6**
Ethyl acetate	**-**	**-**	**-**	**4**	**-**
Diethyl amine	**-**	**-**	**-**	**6**	**-**
acetone	**-**	**4**	**-**	**-**	**-**
Ammonia	**-**	**6**	**-**	**-**	
2-Energy consumption	**1**	**1**	**1**	**1**	**1**
3-Occupational hazard	**0**	**0**	**0**	**0**	**0**
4- Waste	**3**	**3**	**3**	**3**	**3**
Total penalty points	**6**	**20**	**10**	**20**	**18**
Analytical eco-scale	**94**	**80**	**90**	**80**	**82**
Comment	**Highest value eco-scale**				

3.3. Assessment of greenness by Analytical Eco-scale

Analytical Eco-scale is a semi-quantitative assessment tool, used to examine the greenness of analytical methods by a comparative manner. It is based on calculating a numerical score, penalty points, for every step in the whole analytical method that may affect the green system, like solvents, their amounts, energy consumption, occupational hazard and waste production (Gałuszka,2012). The analytical Eco-scale total score is determined by subtracting all of these penalty points from 100 (which is the score of an ideal green method). A score of value greater than 75 means excellent green analysis, from 75 to 50 means acceptable green analysis and lower than 50 means inadequate green analysis. In addition, a method with a higher Eco-scale score is greener and more economical. Table 4 summarizes the results of the proposed HPTLC method, where the method achieved an excellent green analysis method with a score of 94 which is the highest score when compared with the reported methods (Davis ,1984; Patel,2015; Sathiyaa,2010; Tawale,2018) and moreover our proposed method allowed the simultaneous determination of these four antipsychotics.

4. Conclusion

The proposed HPTLC methodology is the first validated technique for the simultaneous analysis of chloropromazine HCl, asenapine maleate, quetiapine fumarate and aripiprazole in pure and tablet formulations using silica gel F254 plates and a simple mobile phase composed of water and ethanol only. It is accurate, precise, robust, sensitive and specific. The study was also directed to the implementation of green chemistry by replacing conventional solvents with greener ones without affecting method performance. It was assessed by analytical Eco-scale and found to be friendly to the environment due to greener solvent usage, low solvent consumption, short time of analysis. The proposed HPTLC technique could be efficiently utilized for routine screening of these antipsychotics in laboratories.

5. Conflict of interest

The authors declare no conflict of interest.

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