International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 8, Issue 8, 2016

Original Article

ASSAY OF FOUR PSYCHOTROPIC DRUGS CHLORPROMAZINE, CLOMIPRAMINE, AMITRIPTYLINE AND NORTRIPTYLINE IN TABLETS BY A SINGLE HPLC METHOD

SALEH TREFI

Quality Control and Pharmaceutical Chemistry Department-University of Aleppo-Syrian Arab Republic
Email: trefi76@yahoo.fr

Received: 12 Apr 2016 Revised and Accepted: 20 June 2016

ABSTRACT

Objective: The objective of the study was to develop and validate a novel and single HPLC method, which is a additional practical choice in quality control laboratories, in order to assay four psychotropic drugs chlorpromazine, clomipramine, amitriptyline and nortriptyline in tablets.

Methods: The chromatographic conditions comprised of a classical C_8-type stationary phase (250 × 4.6 mm, 5µ), with a mobile phase consisting of 3g of sodium lauryl sulfate in a mixture of 400 ml of deionized water and 600 ml of acetonitrile, then 0.5 g of ammonium nitrate was added and apparent pH of 3.0 was adjusted with glacial acetic acid. The flow rate was 1 ml/min; the detection wavelength was at 254 nm under a temperature of 22 °C.

Results: The method was validated for linearity with correlation coefficients very close to one, the accuracy with mean recovery values between 95.0-105.0%, precision with relative standard deviations of the calculated concentrations less than 5.0% and specificity in the presence of degradation products and excipients. Then it was used successfully to the assay of these drugs in tablets purchased from Syria. These analyzed tablets contained the active ingredient within 100±5% of stated concentration, within the limits specified by British Pharmacopeia.

Conclusion: The results presented in this paper showed that the developed method was simple and practical for the determination of the four drugs in tablets.

Keywords: HPLC, Chlorpromazine, Clomipramine, Amitriptyline, Nortriptyline, Tablets

INTRODUCTION

An antipsychotic drug (chlorpromazine) that ameliorate mental aberration and three of monoamine reuptake inhibitors (tricyclic antidepressants TCA), that are a group of closely related agents, were chosen for this study.

Chlorpromazine: the first phenothiazine compound introduced into therapy and still useful as an antipsychotic. Other uses are in nausea, vomiting and hiccough. Clomipramine: it is up to 50 times as potent as imipramine inhibitors in improving the depressant states. Amitriptyline is one of the most anticholinergic sedatives of the TCA. N-demethylation occurs, and nortriptyline is produced, which has a less anticholinergic sedative, and more stimulant action than amitriptyline (fig. 1) [1].

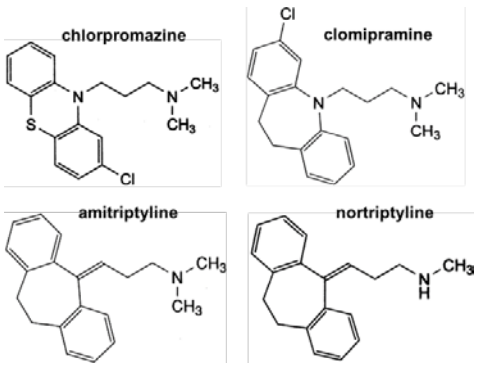

Fig. 1: Chemical structures of chlorpromazine, clomipramine, amitriptyline and nortriptyline

The recommended analytical methods of analyzing chlorpromazine, clomipramine, amitriptyline, nortriptyline and their related substances as raw materials by the British Pharmacopeia is HPLC with different conditions, especially the mobile phases, the used columns and other experimental parameters [2].

Other analytical methods for analyzing the psychiatric pharmaceuticals in formulations are reported in the bibliography. Many chromatographic methods such as classic HPLC [3-8], micellar LC and capillary electrophoresis [9-13] are reported. Electrochemical methods may be used for the same purpose [14, 15]. Many non-chromatographic methods also are reported [16-19].

As we observed that the previous HPLC methods are using different mobile phases with different parameters, for this reason, we decided to propose a single HPLC method with the same parameters. This proposed method may be applied to the four drugs with some advantages. The use of sodium lauryl sulfate in preparing the mobile phase improve its flow in the HPLC chain and reduce problems of precipitation of salts arising from the use of buffers and the C_8 column is also less expensive.

In addition, we must update the analytical methods in a regular manner in quality control laboratories, in order to choose the best method of assay for each drug.

Therefore, the objective of this work was to develop and validate an additional new simple and single HPLC method, for the assay of four psychotropic drugs chlorpromazine, clomipramine, amitriptyline and nortriptyline in tablets using the same experimental parameters.

MATERIALS AND METHODS

Materials

Working standards of chlorpromazine, clomipramine, amitriptyline and nortriptyline were provided by Shifa Pharmaceutical Industries, Aleppo-Syria. The commercial tablets (brand name-Largactil 100, Anafranil 10, Shifatryptil 25 and Motival 10) were purchased from Syria; one commercial formulation was analyzed for each active

pharmaceutical ingredient. All samples, as received, were stored in the dark at ambient temperature and humidity. They were all analyzed within expiry dates. All the other used reagents were of HPLC grade: acetonitrile (Scharlau), glacial acetic acid (SCP), sodium lauryl sulfate (Roth), ammonium nitrate (Sigma), deionized Water for HPLC and syringe filters 0.45 µm.

Instrumentation

The HPLC instrument used was an Agilent 1260 infinity, equipped with a UV detector. The pH meter used was from Crison.

References preparation

A precise quantity of the working standards was accurately weighed, then dissolved in a sufficient volume of deionized water to obtain the starting standard solutions: chlorpromazine 1 mg/ml, clomipramine 1 mg/ml, amitriptyline 0.5 mg/ml and nortriptyline 0.4 mg/ml. These starting standard solutions were used for the preparation of the diluted solutions.

Method development and optimization of chromatographic conditions

Selection of detection wavelength

The common wavelength of the four medicaments utilized was at 254 nm because of the similar structures of these drugs that contain aromatic cycles.

Column selection

A Chrometisil120-5-C_8 SH reversed phase column, 250 x 4.6 mm was utilized.

Mobile phase preparation

The mobile phase is consisting of 3g of sodium lauryl sulfate in a mixture of 400 ml of deionized water, 600 ml of acetonitrile, then 0.5g of ammonium nitrate was added, and apparent pH of 3 was adjusted with glacial acetic acid.

Tablets solutions preparation

Ten tablets (Largactil-chlorpromazine 100 mg) were crushed and powdered, then transferred into a 1000 ml volumetric flask containing deionized water, the content was dispersed under magnetic stirring during 15 min and sonicated for 10 min, until the active pharmaceutical ingredient was well dissolved (C= 1 mg/ml).

Twenty-five tablets (Anafranil-clomipramine 10 mg) were taken instead of 10 because the dose of this active ingredient is 10 mg. They were crushed and powdered then, transferred into a 500 ml volumetric flask containing deionized water, the content was dispersed under magnetic stirring during 15 min and sonicated for 10 min, until the active pharmaceutical ingredient was well dissolved (C= 0.5 mg/ml).

Ten tablets (Shifatryptil-amitriptyline 25 mg) were crushed and powdered, then transferred into a 500 ml volumetric flask containing deionized water, the content was dispersed under magnetic stirring during 15 min and sonicated for 10 min, until the active pharmaceutical ingredient was well dissolved (C= 0.5 mg/ml).

Ten tablets (Motival-nortriptyline 10 mg) were crushed and powdered, then transferred into a 250 ml volumetric flask containing deionized water, the content was dispersed under magnetic stirring during 15 min and sonicated for 10 min, until the active pharmaceutical ingredient was well dissolved (C= 0.4 mg/ml).

Degraded tablets solutions

The tablets solutions prepared as mentioned above were standing at room temperature and sunlight for 7 d. Then, they were analyzed for specificity tests demonstration.

RESULTS AND DISCUSSION

HPLC analysis

The chromatographic conditions comprised of a classical C_8-type stationary phase (250 × 4.6 mm, 5µ), with a mobile phase consisting of 3 g of sodium lauryl sulfate in a mixture of 400 ml of water and 600 ml of acetonitrile, then 0.5 g of ammonium nitrate was added and apparent pH of 3.0 was adjusted with glacial acetic acid. The flow rate was 1 ml/min; the detection was at 254 nm under a temperature of 22 °C. The reference solutions were injected under the previous chromatographic conditions; the retention times were chlorpromazine 11 min, clomipramine 12 min, amitriptyline 10.2 min and nortriptyline 9.5 min. as showed in (fig. 2).

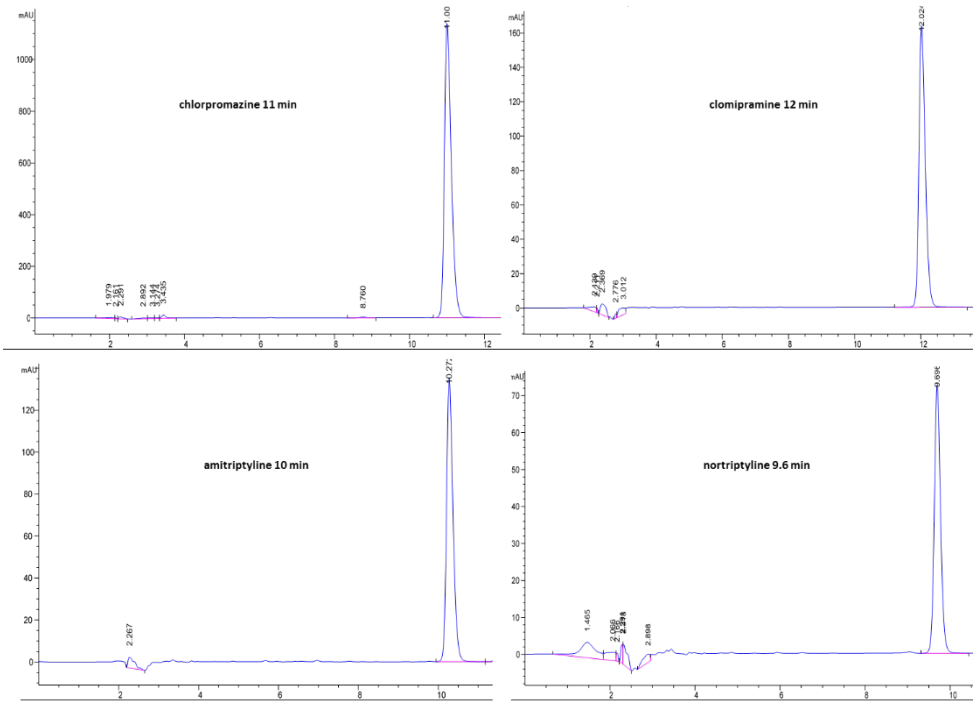

Fig. 2: Retention times of chlorpromazine, clomipramine, amitriptyline and nortriptyline

Analytical method validation

Method validation was performed under a variety of ICH and British Pharmacopeia recommended test conditions [2, 20].

Linearity

The linearity of analytical procedure: is its ability (within a given range) to obtain test results which are directly proportional to the concentration of an analyte in the sample [20].

The linearity was evaluated by linear regression analysis, which was calculated by the least square regression method. Five concentrations over the working range were prepared for each drug; this process was done three different times during three weeks (n=3). (fig. 3) showed the regression lines of chlorpromazine, clomipramine, amitriptyline and nortriptyline with the correlation coefficients (R^2) given in table 1. All the correlation coefficients were very close to one, so the developed method was linear for analyzing the four drugs.

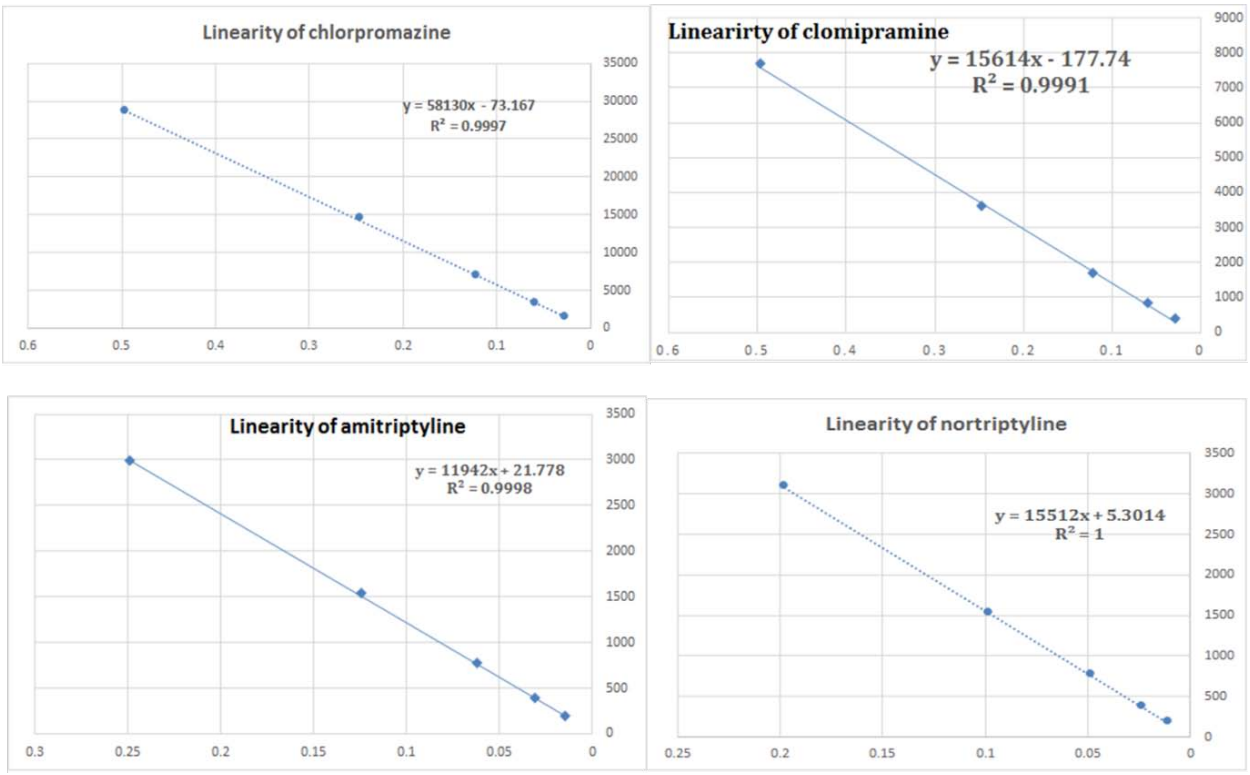

Fig. 3: Linearity lines of chlorpromazine, clomipramine, amitriptyline and nortriptyline

Table 1: Correlation coefficients of chlorpromazine, clomipramine, amitriptyline and nortriptyline

	Chlorpromazine	Clomipramine	Amitriptyline	Nortriptyline
Correlation coefficients[a] (R^2)	R^2 = 0.9997	R^2 = 0.9991	R^2 = 0.9998	R^2 = 1
Equation[a]	y = 58130x-73.167	y = 15614x-177.74	y = 11942x+21.778	y = 15512x+5.3014

[a]n = 3: five concentrations over the working range were prepared for each drug; this process was done three different times during three weeks.

Range

The linearity was demonstrated in the interval (0.03-0.5 mg/ml) for chlorpromazine, (0.03-0.5 mg/ml) clomipramine, (0.01-0.25 mg/ml) amitriptyline and (0.01-0.2 mg/ml) for nortriptyline.

Accuracy

The accuracy of an analytical procedure: expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. For the quantitative approaches, at least nine determinations across the specified range should be obtained [20].

Three concentration levels (0.0625, 0.125, and 0.25 mg/ml) have been used to study the accuracy of chlorpromazine. The results indicated that the individual recovery of chlorpromazine ranged from 98.1% to 102.1% with mean recovery of 100.1%, and relative standard deviation RSD of 2%. The recovery of chlorpromazine by the proposed method was accepted, as the mean recovery value was between 95.0-105.0% with RSD value, not more than 5.0%.

Three concentration levels (0.125, 0.25 and 0.5 mg/ml) have been used to study the accuracy of clomipramine. The individual recovery of clomipramine ranged from 96.4% to 100.9 %, with a mean recovery of 98.1 % and RSD of 2.5%. The recovery of clomipramine by the proposed method was accepted, as the mean recovery value was between 95.0-105.0% with RSD value, not more than 5.0%.

Three concentration levels (0.03, 0.06 and 0.125 mg/ml) have been used to study the accuracy of amitriptyline. The individual recovery of amitriptyline ranged from 99% to 101.4%, with a mean recovery of 100.5 % and RSD of 1.3%. The recovery of amitriptyline by the proposed method was accepted as the mean recovery value was between 95.0-105.0% with RSD value, not more than 5.0%.

Three concentration levels (0.03, 0.06and 0.125 mg/ml) have been used to study the accuracy of nortriptyline. The individual recovery of nortriptyline ranged from 99.5% to 100.3%, with mean recovery of 99.9 % and RSD of 0.4%. The recovery of nortriptyline by the proposed method was accepted as the mean recovery value was between 95.0-105.0% with RSD value, not more than 5.0%. table 2.

Table 2: Mean recoveries of assays of three concentration levels solutions of the four drugs

	Chlorpromazine	Clomipramine	Amitriptyline	Nortriptyline
Mean concentration level$_1$ %[a]	102.1±3.75	100.9±0.98	101.4±3.78	100.1±1.05
Mean concentration level$_2$ %[a]	99.9±3.85	96.9±3.37	101.1±1.58	99.5±3.23
Mean concentration level$_3$ %[a]	98.1±4.01	96.4±3.79	99.0±1.29	100.3±4.82
Mean recovery % (±) SD	100.1±2.0	98.1±2.4	100.5±1.3	99.9±0.4
RSD	2.0	2.5	1.3	0.4

[a]mean±SD, n = 3.

Precision

The precision of an analytical procedure: expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions [20].

Intermediate Precision: The solutions 0.5 mg/ml of chlorpromazine, 0.5 mg/ml of clomipramine, 0.06 mg/ml of amitriptyline and 0.2 mg/ml of nortriptyline have been prepared at three different times, by three analysts during three weeks, each solution was injected two times (N = 6). Relative standard deviations of the calculated concentrations (RSD) were given in table 3.

The RSD of chlorpromazine was 1.6%, 0.98% for clomipramine, 1.6% for amitriptyline and 1% for nortriptyline (not more than 5.0 %.). These results indicated that the intermediate Precision of this method was accepted for chlorpromazine, clomipramine, amitriptyline and nortriptyline.

Table 3: Relative standard deviation of the six assays of solutions of the four drugs

N	Chlorpromazine(0.5 mg/ml)	Clomipramine(0.5 mg/ml)	Amitriptyline(0.06 mg/ml)	Nortriptyline(0.2 mg/ml)
1	0.508	0.523	0.062	0.199
2	0.502	0.512	0.062	0.201
3	0.490	0.509	0.062	0.203
4	0.485	0.510	0.063	0.202
5	0.499	0.514	0.064	0.198
6	0.499	0.512	0.064	0.198
[a]Mean(±)SD	0.497±0.0082	0.513±0.0050	0.063±0.0009	0.200±0.002
RSD	1.66	0.98	1.56	1.05

[a]n = 6.

Limit of detection (LOD) and limit of quantitation (LOQ)

The calculated LODs and LOQs for chlorpromazine, clomipramine, amitriptyline and nortriptyline were mentioned in table 4. These results indicated that our method was less sensitive than many other methods mentioned in the bibliography. The limits of detection of T. A. Ivandini *et al.* method: 0.5 nM for clomipramine, 163 nM amitriptyline and 1080 nM for nortriptyline [21]. Marcelo Delmar Cantú *et al.* results were better than our results, the limits of quantification were between 30 and 50 ng ml^{-1}. These values were beyond the plasmatic therapeutic concentration [10]. Fardous A. Mohamed *et al.* developed a spectrofluorimetric method, which had limits of detection range 0.035–0.038 µg/ml and limits of quantitation range 0.116–0.125 µg/ml [17]. However, our method was not sufficiently sensitive for the assay of these antidepressants in biofluids at least at therapeutic doses [22]. On the other hand our method limits were sufficient to perform the quantitative analysis of these drugs in tablets.

Table 4: Limit of detection and quantification of the HPLC method

	Chlorpromazine(µg/ml)	Clomipramine(µg/ml)	Amitriptyline(µg/ml)	Nortriptyline(µg/ml)
Limit of detection	11.9	21.7	4.6	1.2
limit of quantitation	35.9	65.8	13.9	3.7

Specificity

Specificity is the ability to assess the analyte unequivocally in the presence of components, which may be expected to be present. Typically, these might include impurities, degradants, matrix, etc [20].

The chromatograms of the reference solutions indicated no additional peaks other than those of chlorpromazine 11 min, clomipramine 12 min, amitriptyline 10.2 min and nortriptyline 9.5 min (fig. 2).

In order to demonstrate the specificity of the method, tablets solutions were standing at room temperature and sunlight for 7 d. Then, they were injected.

The chromatogram of the standing tablets solution of chlorpromazine showed many additional peaks, but they were well resolved from the peak of chlorpromazine with a significant difference in the retention time (fig. 4).

The chromatogram of the standing tablets solution of clomipramine showed one additional peak 11.4 min, but it was separated from the peak of clomipramine (fig. 5).

The chromatograms of the standing tablets solutions of amitriptyline and nortriptyline showed no additional peaks, (fig. 6 and 7).

As a result, this method was well specific for the assay of chlorpromazine, clomipramine, amitriptyline and nortriptyline in the presence of their degradation products and excipients. On the other hand, tests for peak homogeneity were needed by using diode array detection or mass spectrometry, but we did not dispose of these techniques [20].

Tablets assay

Finally, we applied our method to analyze commercial tablets purchased from Syria, which contained the four psychoactive drugs. One formulation was analyzed for each drug. The data of tablets

contents were reported in table 5. It was reassuring that in this study all the formulations tested had concentrations measured within the specification of the British Pharmacopeia, which recommended that tablets should contain not less than 90% and not more than 110% of the labelled amount of the active pharmaceutical ingredient [2].

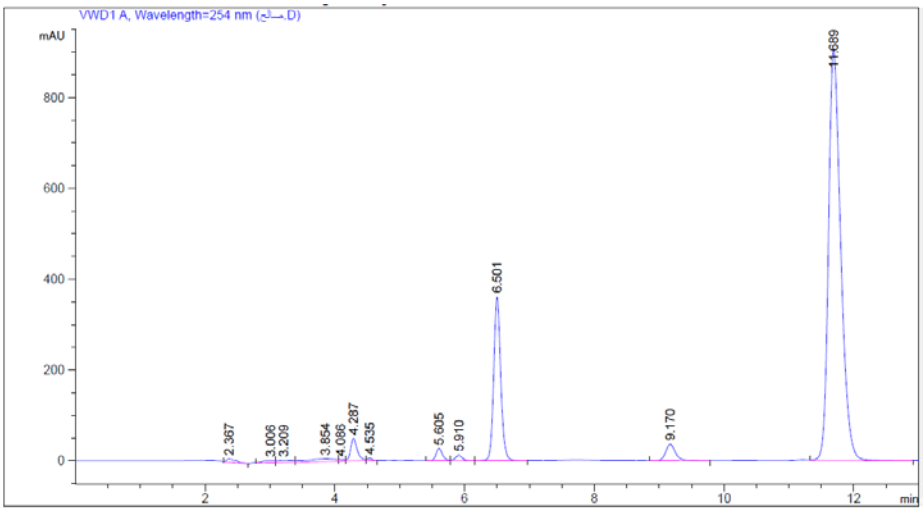

Fig. 4: Chromatogram of the standing tablets solution of chlorpromazine

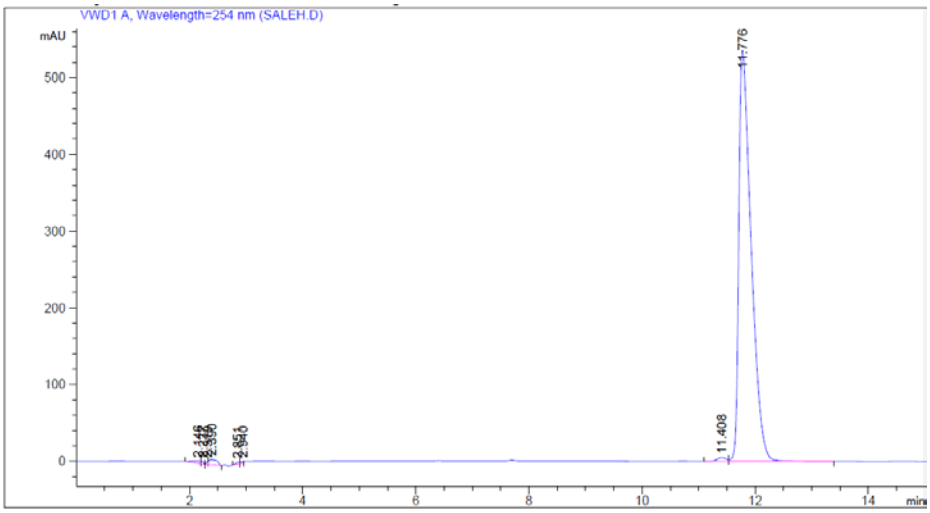

Fig. 5: Chromatogram of the standing tablets solution of clomipramine

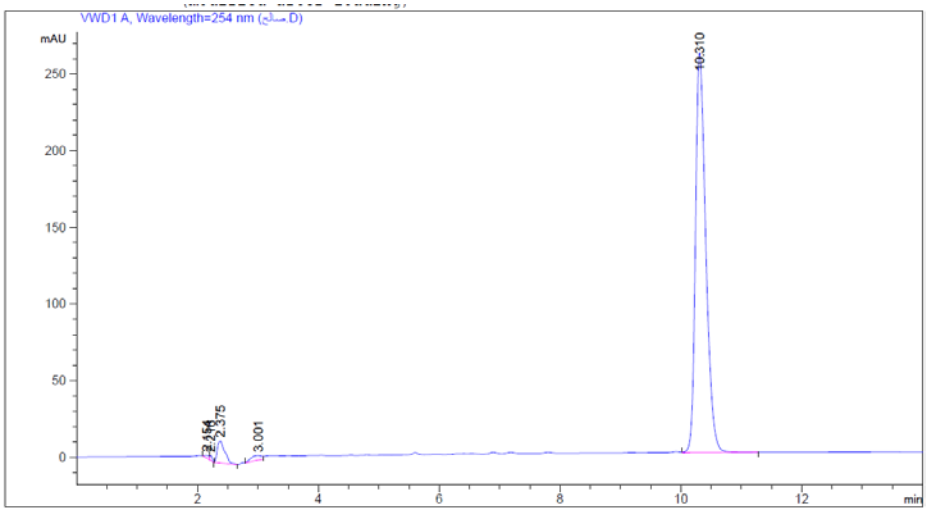

Fig. 6: Chromatogram of the standing tablets solution of amitriptyline

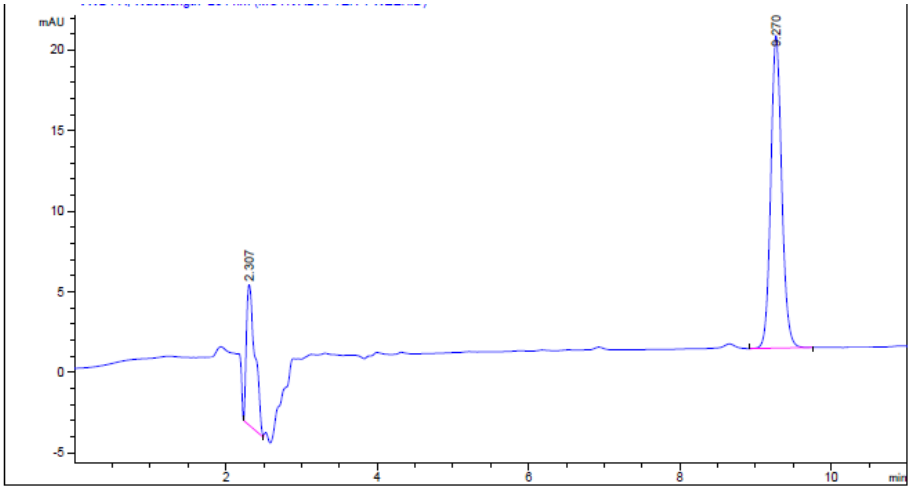

Fig. 7: Chromatogram of the standing tablets solution of nortriptyline

Table 5: Results of tablets assay by the developed HPLC method

Formulation name	Active ingredient and potency	Manufacturer name and country of production	Number of tablets	Drug content % (±) SD[a]	RSD
Largactil	chlorpromazine 100 mg	OUBARI (Syria)	10	101.45±2.7	2.67
Anafranil	clomipramine 10 mg	MPI (Syria)	25	100.57±2.37	2.36
Shifatryptil	amitriptyline 25 mg	Shifa (Syria)	10	99.63±2.83	2.85
Motival	Nortriptyline 10 mg	UNIPHARMA (Syria)	10	98.68±3.44	3.48

[a]mean%±SD, n = 3.

All the commercial tablets contained the active pharmaceutical ingredient, in the range 98.7-101.5% of the stated concentration with RSD not more than 5%. These data were in agreement with those reported by several authors who, for the validation of various analytical techniques, assayed the four drugs in pharmaceutical formulations from different countries and found contents between 90-110% of declared amount, within the limits specified by British Pharmacopeia. [3-5, 7-12, 17-19]. The results reported herein demonstrated that the quality of tablets of chlorpromazine, clomipramine, amitriptyline and nortriptyline sold in Syria was correct.

CONCLUSION

A simple, accurate and reproducible HPLC method has been developed for the determination of chlorpromazine, clomipramine, amitriptyline and nortriptyline contents in tablets. This method could be an additional analytical technique, particularly in quality control laboratories. The results reported herein demonstrated that the quality of tablets of chlorpromazine, clomipramine, amitriptyline and nortriptyline sold in Syria was correct. However, the method was not sufficiently sensitive for the assay of these antidepressants in biofluids at least at therapeutic doses.

CONFLICT OF INTERESTS

Declared none

REFERENCES

1. John H Block, John M Beale. Wilson and Gisvold's textbook of organic medicinal and pharmaceutical chemistry. 11th ed. Philadelphia: Lippincott Williams and Wilkins; 2004. p. 485-520.
2. British Pharmacopeia; 2013.
3. Suleiman Issa Sasa, Ibrahim Jalal. Determination of nortriptyline hydrochloride and fluphenazine hydrochloride in commercial tablets by reverse phase high-performance liquid chromatography. Microchem J 1988;38:181-7.
4. Safwan Ashour, Nuha Kattan. Simultaneous determination of nortriptyline hydrochloride and fluphenazine hydrochloride in microgram quantities from low dosage forms by liquid chromatography-UV detection. J Pharm Anal 2012;2:437-42.
5. MA Moreno, MP Ballesteros, P Frutos, JL Lastres, D-Castro. Comparison of UV spectrophotometric and LC methods for the determination of nortriptyline hydrochloride in polysorbate 80 based oil/water (o/w) microemulsions. J Pharm Biomed Anal 2000;22:287-94.
6. Fabrice Gritti, Georges Guiochon. Separation mechanism of nortriptyline and amitriptyline in RPLC. J Chromatogr A 2005;1090:39-57.
7. MA Raggi, G Casamenti, R Mandrioli, C Sabbioni, V Volterra. A rapid LC method for the identification and determination of CNS drugs in pharmaceutical formulations. J Pharm Biomed Anal 2000;23:161-7.
8. MJ Ruiz-Angel, S Carda-Broch EF. Simó-Alfonso MC Garcı, Alvarez-Coque. Optimized procedures for the reversed-phase liquid chromatographic analysis of formulations containing tricyclic antidepressants. J Pharm Biomed Anal 2003;32:71-84.
9. JJ Berzas Nevado, MJ Villaseñor Llerena, AM Contento Salcedo, E Aguas Nuevo. Assay validation for three antidepressants in pharmaceutical formulations: a Practical approach using capillary gas chromatography. J Pharm Biomed Anal 2005;38:52-9.
10. Marcelo Delmar Cantú, Sandro Hillebrand, Maria Eugênia Costa Queiroz, Fernando Mauro Lanças, Emanuel Carrilho. Validation of non-aqueous capillary electrophoresis for simultaneous determination of four tricyclic antidepressants in pharmaceutical formulations and plasma samples. J Chromatogr B 2004;799:127-32.
11. Hwang-Shang Kou, Cheng-Chung Chen, Yu-Hua Huang, Wei-Kung Ko, Hsin-Lung Wu, Shou-Mei Wu. A method for simultaneous determination of eight cyclic antidepressants by cyclodextrin-modified capillary zone electrophoresis: applications in pharmaceuticals. Anal Chim Acta 2004;525:23-30.
12. Vincenzo Pucci, Roberto Mandrioli, Anna Ferranti, Sandra Furlanetto, Maria Augusta Raggi. Quality control of commercial tablets containing the novel antipsychotic quetiapine. J Pharm Biomed Anal 2003;32:1037-44.
13. S Furlanetto, S Orlandini, B Pasquini, M Del Bubba, S Pinzauti. Quality by Design approach in the development of a solvent-modified micellar electrokinetic chromatography method:

finding the design space for the determination of amitriptyline and its impurities. Anal Chim Acta 2013;802:113-24.
14. M Goreti F Sales, José FC Tomás, Sandra R Lavandeira. Flow injection potentiometric determination of chlorpromazine. J Pharm Biomed Anal 2006;41:1280-6.
15. Eduardo Henrique Duarte, William Pereira dos Santos, Felipe Fantinato Hudari, José Luiz Bott Neto, Elen Romão Sartori, Luiz Henrique Dall'Antonia, *et al.* A highly improved method for sensitive determination of amitriptyline in pharmaceutical formulations using an unmodified carbon nanotube electrode in the presence of sulfuric acid. Talanta 2014;127:26-32.
16. Karine L Marques, João LM Santos, José LFC Lima. A multi commutated flow system for the chemiluminometric determination of clomipramine in pharmaceutical preparations. Anal Chim Acta 2004;518:31-6.
17. Fardous A Mohamed, Horria A Mohamed, Samiha A Hussein, Sameh A Ahmed. A validated spectrofluorimetric method for determination of some psychoactive drugs. J Pharm Biomed Anal 2005;39:139-46.
18. MEM Hassouna, AM Adawi, EA Ali. Extractive spectrophotometric determination of chlorpromazine and trifluoperazine hydrochloride in pharmaceutical preparations. Egyptian J Forensic Sci 2012;2:62–8.
19. Salah M Sultan, Mohammed O Hamad Al-Turabi, Jimmy S Hwang. Electron spin resonance for quantitative assay of chlorpromazine in drug formulations by oxidation with cerium (IV) in sulfuric acid media. Talanta 2000;51:327-31.
20. http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1__Guideline.pdf. [Last accessed on 10 Mar 2016].
21. TA Ivandini, BV Sarada, C Terashima, TN Rao, DA Tryk, H Ishiguro, *et al.* Electrochemical detection of tricyclic antidepressant drugs by HPLC using highly boron-doped diamond electrodes. J Electroanal Chem 2002;521:117-26.
22. Devasish Bose, Abhilasha Durgbanshi, Adrià Martinavarro-Domínguez, Maria-Elisa Capella-Peiró, Samuel Carda-Broch, Josep Esteve-Romero, *et al.* Amitriptyline and nortriptyline serum determination by micellar liquid chromatography. J Pharmacol Toxicol Methods 2005;52:323-9.

How to cite this article

- Saleh Trefi. Assay of four psychotropic drugs chlorpromazine, clomipramine, amitriptyline and nortriptyline in tablets by a single HPLC method. Int J Pharm Pharm Sci 2016;8(8):182-188.