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LC of pharmaceutically important halogenated 8-hydroxyquinolines after precolumn derivatization with Pd (II)

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Abstract

An accurate, sensitive, and selective reversed phase high performance liquid chromatographic (HPLC) method was developed for the analysis of two halogenated 8-hydroxyquinoline derivatives; clioquinol (CQN) and iodoquinol (IQN). The proposed method depends on the complexation ability of the studied compounds with Pd(II) ions. Reversed phase chromatography was conducted using a 300 × 3.9 mm i.d. stainless steel column packed with 10 μm Bondclone phenyl at ambient temperature. A solution containing 0.005% w/v of Pd(II)-chloride in a mixture of acetonitrile–methanol–water (3:3:4 v/v/v) of pH 3.7 as a mobile phase pumped at a flow rate of 0.75 ml min^{-1}. UV-detection was performed at 282 and 285 nm for CQN and IQN, respectively. The method showed excellent linearity in the range 0.05–1.8 and 0.1–3.0 μg ml^{-1} with limit of detection (S/N = 2) 4.8 ng ml^{-1} (1.57×10^{-8} M) and 6.4 ng ml^{-1} (1.61×10^{-8} M) for CQN and IQN, respectively. The suggested method was successfully applied for the analysis of the studied drugs in bulk with average% recoveries of 99.68 ± 0.44 for CQN and 99.65 ± 0.53 for IQN. The proposed method was successfully applied for the analysis of the studied drugs in single or combined dosage forms with average% recoveries of 99.41 ± 0.51–100.02 ± 0.63. The proposed method could be used successfully for the determination of the studied compounds in the presence of their degradation product as they could be eluted with different retention times. The presence of metronidazole (MNZ) or tolnaftate (TFT) with the studied drugs does not affect their accurate determination. The results obtained were favorably compared with those obtained by the reference method. The results were satisfactorily, accurate, and precise.

Keywords: HPLC; Halogenated 8-hydroxyquinoline; Pd(II)-complexes; Clioquinol; Iodoquinol; Dosage forms

1. Introduction

Halogenated derivatives of 8-hydroxyquinoline have been widely used in the treatment of various intestinal and vaginal infections. Greatest success was achieved with iodinated 8-hydroxyquinolines.

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Among the commonly used drugs are clioquinol (CQN) and iodoquinol (IQN). A comprehensive chemical study was reviewed earlier [1].

The literature revealed different techniques for the analysis of both drugs either per se or in dosage forms which includes; spectrophotometry [2–4], polarography [5,6], TLC [7,8], HPLC in pharmaceutical preparations [9,10] or in biological fluids [11–13].

The complexation ability of the studied quinolines with different metals such as Sn(IV) [14], Cu(II) [15], Co(II) [16], V [17], and Nb(V) [18], was investigated using different spectrophotometric techniques. Ni(II) complexes with 8-hydroxyquinolines were reported for colorimetric [19] and HPLC separation and determination of these compounds [20]. B.P. 98, recommended a reversed phase HPLC method for determination of CQN in CQN–betamethazone cream and ointment, where precolumn derivatization of CQN with Ni(II) was described [21]. Recently Pd(II) was reported to form binary and ternary complexes with many drugs [22–31]. In our proposed method a new approach for the analysis of these analytes via their complexation with Pd(II) by HPLC technique was investigated. Different chromatographic conditions were studied in an attempt to optimize a simple, sensitive, and selective method for the evaluation of the studied compounds in bulk and dosage forms.

The proposed method showed increased sensitivity over the reported LC methods. As for HPLC determination of the studied compounds after complexation with Ni(II) [20,21] showed lower sensitivity than the proposed method which includes complexation of the drugs with Pd(II).

2. Experimental

2.1. Materials and reagents

Clioquinol (CQN), *iodoquinol* (IQN), *metronidazole* (MNZ), and *tolnaftat* (TFT) were kindly provided by Memphis Chemical Co (Cairo, Egypt) and were used as received.

Clioquinol eardrops, (commercially, locacorten vioform eardrops 1 mg ml^{-1}) contains in addition to CQN, flumetason pivalic and polyethylene glycol.

Clioquinol cream, (commercially, vioderm hydrocortisone) contains in addition to CQN, hdyrocortisone in its cream base. Clioquinol eardrops and cream were obtained from commercial sources.

Clioquinol cream, (commercially, quadriderm cream 1%) contains in addition to CQN, betamethazone valerate, gentamicin sulphate and TFT in its cream base. Quadriderm cream was kindly provided by Memphis Chemical Co (Cairo, Egypt).

Iodoquinol tablets, were commercially found in single formulations under the name, iodoquinol tablet (was kindly provided by South Egypt Drugs Industries Company, SEDICO, 6 October City) and paramibe tablet. Also IQN was found in combined formulations under the name iodoquinol compound tablet which contains IQN and MNZ. Both paramibe and paramibe compound tablets were kindly provided by Chemical Industries Development (CID, Giza, Egypt). These formulations are listed in Table 2.

Palladium chloride (Merck), 0.1% solution was prepared by dissolving an accurately weighed 0.1 g in 5 ml deionized distilled water containing 0.1 ml of concentrated hydrochloric acid and warming the mixture in water-bath. The solution was cooled and diluted with water in 100 ml measuring flask.

Acetonitrile and *methanol*, HPLC grade, Hiper solv. (Merck).

Drug stock solutions, Stock solutions 0.2 mg ml^{-1} of CQN and IQN were prepared by dissolving 20 mg into 100 ml measuring flask with methanol. These solutions were found stable at least two weeks without alteration when kept in the dark.

Working standard solutions, standard solutions were prepared daily from the previous stock by serial dilutions with methanol to contain 0.5–18 and 1–30 μg ml^{-1} for CQN and IQN, respectively. However, these solutions remain stable during the actual time of the analysis in the same day.

2.2. Instrumentation and chromatographic conditions

Chromatographic analyses were carried out using LKB liquid chromatograph consists of (LKB-Produkter AB, Bromma, Weeden) Model 2150 solvent delivery pump, a Rheodyne (Berkeley, CA, USA) Model 7125–7150 μl loop and equipped with Knauer (Berlin, Germany) Model K-2500 variable wavelength detector, set at 282 nm for CQN and 285 nm for IQN. The study of the recommended procedure was performed on a stainless steel column (300 × 3.9 mm) packed with particles of silica the surface of which has been modified with chemically bonded phenyl groups (10 μm) (Bondclone phenyl column is suitable). The chromatograms were recorded and processed on Shimadzu (Kyoto, Japan) Model C-R6A Chromatopac integrator. The analysis was achieved isocratically using a solvent mixture of acetonitrile–methanol–H_2O (3:3:4 v/v/v) containing 0.005% w/v Pd(II) chloride and has pH 3.7 as the mobile phase, the eluent was filtered through 0.45 μm membrane filter (Gelman Instrument Co), degassed and pumped at a flow rate of 0.75 ml min^{-1} and ambient temperature.

2.3. Procedure for calibration curves

1 ml aliquot of the working solutions was transferred into 10 ml volumetric flasks, 0.5 ml of 0.1% Pd (II) solution was added and diluted with the mobile phase to the mark. 50 μl aliquots were injected (triplicate) and eluted with the mobile phase under the reported chromatographic conditions.

2.4. Procedure for dosage forms

2.4.1. Tablet formulations

Twenty tablets were weighed and pulverized. An accurately weighed amount of the powder equivalent to contain 10.0 mg of each drug was extracted with 3 × 25 ml of methanol by sonication for 5 min. The extracted solution was filtered into 100 ml measuring flask, completed to the mark with the same solvent and mixed well. A working solution of 10 μg ml^{-1} was prepared by suitable dilution. The procedure under Section 2.3 was followed.

2.4.2. Eardrops

One milliliter of eardrops containing 10 mg ml^{-1} of CQN was transferred into 100 ml measuring flask and completed to the mark with methanol. The solution was mixed well. A working solution of 10 μg ml^{-1} was prepared by dilution with methanol. The procedure under Section 2.3 was followed.

2.4.3. Cream

The contents of five containers were mixed and a sample equivalent to contain 10.0 mg of CQN was accurately weighed into 50 ml beaker. A 25 ml methanol was added, heated on water-bath for 5 min. The sample was extracted with sonication, the solution cooled in ice and filtered into 100 ml measuring flask, the extraction process was repeated twice. The filtrate and washings were completed to the mark with the same solvent and mixed well. A solution containing 10 μg ml^{-1} was prepared by dilution with methanol and the procedure under Section 2.3 was followed.

The concentration of CQN or IQN was calculated either from the prepared calibration curves or from the corresponding regression equations.

3. Results and discussion

The formation of complexes between Pd(II) and the studied drugs was previously investigated in our laboratory [22]. UV-absorption spectra showed λ_{max} around 282–285 nm with appreciable values of ε. The complexes are reasonably stable for at least 1 h under the specified conditions, moreover the molar ratios indicate the expected stochiometric values. Due to the instantenous complexation of the studied drugs with Pd(II) ions, the oncolumn complexation without any hydrolysis was performed. This founding led to the highest sensitivity of the proposed method, as about 50 and 100 ng of CQN and IQN, respectively, can be determined quantitatively. Also the proposed LC method permits the separation and quantitation of CQN and TFT

in their co-formulations, while it was impossible in the UV-derivative method [22] where, the two troughs were overlapped. Furthermore the stability of the studied compounds had been investigated elsewhere [1]. The expected degraded product, mainly, 5-chloro-8-hydroxyquinoline, which still has the ability of complex formation, however, the formed complex will be eluted at different retention time. This proposal had been approved through the change of the polarity of the mobile phase where, only one peak corresponds to Pd-drug complex indicating the purity of the peaks. This founding is in favor of stability indicating method.

These potentiate the development of a pre-column derivatization LC method in an attempt to suggest a sensitive, selective and stability indicating method for the analysis of both drugs in bulk and dosage forms.

3.1. Chromatographic performances

A well-defined symmetrical peak was obtained upon measuring the response of the eluent under the performance parameters after thorough experimental trials, that could be summarized as follows:

Column, different columns were used for performance investigations including, Maxil 5 μ C18 column (250 × 4.6 mm), Luna 5 μ phenyl–hexyl (250 × 4.60 mm) and Bondclone 10 μ phenyl column (300 × 3.9 mm). The experimental studies revealed that, the first two types of columns eluted the complexed drugs at longer time exceeding 20 min. The third column was the most suitable since the retention time of Pd(II)–CQN was 11.875 min, and Pd(II)–IQN was 14.58 min, Fig. 1.

UV detection, the formed complexes were detected at 282 and 285 nm for CQN and IQN, respectively.

Mobile phase, different mobile phase systems were studied to achieve the best resolution and separation of the eluted peaks of Pd(II)–drug complex and Pd(II) which present in excess in the reaction mixture. A mobile phase of acetonitrile–methanol–water in different ratios were used. Standard solution of (CQN) as a model examplewas used to calculate the capacity factor (K') and

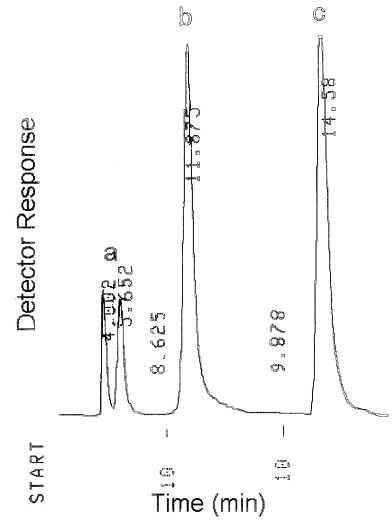

Fig. 1. Typical chromatograms of (a) Mobile phase containing Pd(II) (0.005% Pd(II), 4.002, 5.652 min); (b) Pd(II)–Clioquinol complex (1 μg ml^{-1} clioquinol, 11.875 min); (c) Pd(II)–Iodoquinol complex (2 μg ml^{-1} iodoqinol, 14.58 min).

tailing factor (T). These factors were calculated by application of the USP XXIV [32] procedure using the average of six injections. The values of K' and T were 1.83 ± 0.02 and 1.14 ± 0.11, respectively. A mobile phase containing acetonitrile and methanol 30% each in water (60% organic modifier) gave the highest values of N and lowest value of HETP in addition to the low values of K' and T, is appropriate for the good separation of the formed complexes, Fig. 2. Decreasing the percent of acetonitrile to 20 with 40% methanol leads to increasing the retention time > 20 min. On the other hand increasing the percent of acetonitrile up to 40 with 20% methanol leads to

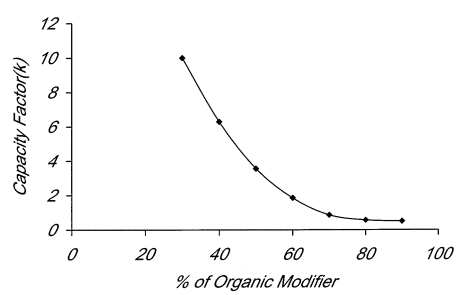

Fig. 2. Effect of percentage of organic modifier on capacity factor (K').

overlapping of the peaks of Pd(II)–drug, Pd(II) and solvent front. In the use of higher percentage of methanol a black precipitate was formed during sonication of the mobile phase which may be due to the reduction of Pd(II)–Pd. The effect of flow rate on the formation and separation of peaks of the formed complex and Pd(II)was studied and a flow rate of 0.75 ml min^{-1} was optimal for good separation in a reasonable time.

Pd (II) concentration and pH value, the effect of different concentrations of Pd(II) on the complete formation of the complex with the studied drugs was investigated. 0.5 ml of 0.1% solution of Pd (II) was optimal. Besides, the presence of the same percent of Pd(II) in the mobile phase was essential to prevent the hydrolysis of the formed complex. In the absence of Pd(II) in mobile phase a very small peak area was obtained, so a mobile phase containing 0.005% of Pd(II) in a mixture of acetonitrile–methanol–water (3:3:4 v/v/v) is the solvent of choice for good formation and separation of the formed complexes. The final pH of the mobile phase was found to be 3.7. Replacing water by phosphate or acetate buffer of pH 3.7 decreases the peak area of the formed complex.

3.2. Validation

3.2.1. Linearity

The calibration curves of Pd(II)–CQN complex and Pd(II)–IQN complex obtained by plotting the final concentration versus the peak area was found to be rectilinear over the range of 0.05–1.8 and 0.1–3.0 μg ml^{-1} for CQN and IQN, respectively. Linear regression analysis of the data gave the following equations:

$$P_{CQN} = -558.78 + 387194.2\ C_{CQN} \quad (r = 0.99998)$$
$$P_{IQN} = -1071.24 + 263542.2\ C_{IQN} \quad (r = 0.99998)$$

where C is the concentration of drug in μg ml^{-1} and P is the peak area.

Statistical analysis of the data gave small values of the standard deviations of the residuals, ($S_{y/x}$) 2.2×10^2 and 2.5×10^2, of slope, (S_b) 1.35×10^2 and 1.63×10^2, and of intercept, (S_a) 4.04×10^2 and 4.42×10^2 and the% relative error, (% Er) 0.167 and 0.201% for CQN and IQN, respectively.

Table 1
Accuracy and precision data for clioquinol and iodoquinol using the proposed method

Parameter	Clioquinol concentration (μg ml^{-1})			Iodoquinol concentration (μg ml^{-1})		
	0.5	1	1.5	0.5	1	1.5
Intra-day						
% recovery	99.84	99.60	98.85	100.48	99.75	98.90
	99.98	99.48	98.69	100.15	99.60	98.25
	100.12	98.94	99.45	99.98	99.86	99.25
	100.25	99.86	99.15	100.68	98.95	99.45
	100.59	99.92	99.98	100.85	99.89	99.85
Mean ($\bar{x}$)	100.16	99.56	99.22	100.43	99.61	99.14
±S.D	0.287	0.391	0.513	0.361	0.386	0.605
%R.S.D.	0.287	0.393	0.517	0.359	0.388	0.610
%Er	0.128	0.176	0.231	0.161	0.174	0.273
Inter-day						
% recovery	99.98	99.94	99.85	100.15	100.60	99.45
	99.65	99.48	100.00	99.92	99.98	98.85
	100.26	98.85	98.95	100.85	99.85	99.95
Mean ($\bar{x}$)	99.96	99.42	99.60	100.31	100.14	99.45
±S.D.	0.305	0.547	0.568	0.484	0.401	0.450
%R.S.D.	0.305	0.550	0.570	0.483	0.400	0.453
%Er	0.176	0.318	0.329	0.279	0.231	0.261

Table 2
Assay of clioquinol and iodoquinol in dosage forms using the proposed and reference method

Dosage forms	Clioquinol						Iodoquinol					
Parameters	Locacorten vioform ear drops 10 mg ml^{-1}[a]		Viodermhydrocortison cream 3%[b]		Quadriderm cream 1%[c]		Paramibe 250 mg per tablet[d]		Iodoquinole 250 mg per tablet[e]		Paramibe compound tablet 250 mg per tablet[f]	
% recovery	Proposed	Reference[g]	Proposed	Reference[g]	Proposed	Reference[g]	Proposed	Reference[g]	Proposed	Reference[g]	Proposed	Reference[g]
	99.80	99.94	100.36	101.10	100.14	100.58	98.69	100.21	98.92	100.87	98.75	98.38
	100.15	98.98	100.25	100.48	98.98	101.60	99.85	99.28	99.75	100.58	99.82	100.48
	100.45	99.69	99.94	99.98	100.58	99.21	99.76	100.86	100.28	99.95	99.35	99.50
	99.29	100.25	100.15	99.68	99.92	99.98	100.25	100.21	100.00	99.45	100.00	100.82
	99.18	99.48	98.85	100.28	100.36	99.87	100.8	98.99	100.12	98.85	99.15	101.04
Mean ($\bar{x}$)	99.77	99.67	99.99	100.30	100.00	100.25	99.75	99.91	99.81	99.94	99.41	100.04
Standard deviation ($\pm$S.D.)	0.545	0.480	0.612	0.532	0.619	0.808	0.626	0.763	0.536	0.822	0.506	1.101
Student's t-test[h]	0.308		1.069		0.512		0.363		0.296		1.357	
Variance ratio F-test[h]	1.29		1.29		2.06		1.48		2.36		1.49	

[a] Product of Novartis Pharma, Cairo, Egypt.
[b] Product of Cairo Pharmaceutical Co, Cairo, Egypt.
[c] Product of Memphis Chemical Co, Cairo, Egypt.
[d] Product of Chemical Industries Development, Giza, Egypt.
[e] Product of South Egypt Drug Industries Co, 6 October City, Egypt.
[f] Product of Chemical Industries Development, Giza, Egypt.
[g] Reference method [21].
[h] Tabulated t-and F-values at $P = 0.05$ [33] are: $t = 2.306$ and $F = 6.39$.

3.2.2. Limits of quantitation (LOQ) and limit of detection (LOD)

The limit of quantitation (LOQ) was determined by establishing the lowest concentration that can be measured with acceptable accuracy and precision; in this case CQN and IQN can be quantified under these conditions at concentration of 0.05 and 0.1 μg ml^{-1}, respectively. The limit of detection (LOD) was determined by establishing the minimum level at which the analyte can be reliably detected (S/N = 2) and it was found 4.8 ng ml^{-1} (1.57×10^{-8} M) and 6.4 ng ml^{-1} (1.61×10^{-8} M) for CQN and IQN, respectively.

3.2.3. Accuracy and precision

The proposed method was evaluated by studying the accuracy as percent relative error (%Er) and precision as percent relative standard deviation (%R.S.D) using three preparations with suitable concentrations Table 1.

The repeatability of the assay was found to be within 0.29–0.52 and 0.36–0.61% ($n = 5$) at 0.5, 1.0 and 1.5 μg ml^{-1} for CQN and IQN, respectively. The reproducibility of the assay at the same concentration levels was found to be within 0.30–0.57 and 0.40–0.48% ($n = 3$) for CQN and IQN, respectively. The intra-day ($n = 5$) inter-day ($n = 3$) accuracy calculated as % Er was found to be within 0.13–0.23 and 0.18–0.33% for CQN and 0.16–0.27 and 0.23–0.28% for IQN.

3.2.4. Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small but deliberate variation in method parameters. To optimize the assay parameters, the effect of organic modifier, pH and Pd(II) concentration on the capacity factor K', and T values were studied.

3.3. Dosage forms analysis

The proposed method was successfully applied to the assay of CQN and IQN in different pharmaceutical preparations including; tablets, eardrops and creams. The average percent recoveries of different concentrations were based on the average of five replicate determinations.

The results shown in Table 2 are in good agreement with those obtained with the reference method [21]. The complexation ability of Pd(II) was applied for the analysis of the studied compounds either single or co-formulated with other drugs such as metronidazole (MNZ), (Paramibe compound tablets) and Tolnaftate (TFT) (Quadriderm cream). As shown in Table 2, the presence of MNZ or TFT in combination with the studied drugs does not interfere in their accurate quantitative determination via their Pd(II) complexes since Pd(II)-MNZ complex and Pd(II)–TFT complex was completely separated from Pd (II) to drug peak.

4. Conclusion

Although the precolumn derivatization with Ni(II) was successfully applied in analysis of the studied compoundes, but the data obtained by the proposed method indicate high sensitivity as 50 and 100 ng ml^{-1} could be determined accurately with a minimum detectability (S/N = 2) of 4.8 ng ml^{-1} (1.57×10^{-8} M) and 6.4 ng ml^{-1} (1.6×10^{-8}M) for CQN and IQN, respectively. Also the method seems to be stability indicating since the Pd(II) complexes of the studied drugs could be eluted separately from Pd(II)-complexes of 8-hydroxy quinoline or 5-chloro-8-hydroxyqinoline which are precursors in the synthesis of bulk forms of the drugs. Furthermore the method seems to be selective since the studied compounds could be determined quantitatively without any interference from the co-formulated compounds in dosage forms.

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