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Determination of some antihistaminic drugs by atomic absorption spectrometry and colorimetric methods

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Abstract

Atomic absorption spectrometry (AAS) and colourimetric methods have been developed for the determination of pizotifen (I), ketotifen (II) and loratadine (III). The first method depends on the reaction of the three drugs (I); (II) and (III) with cobalt thiocyanate reagent at pH 2 to give ternary complexes. These complexes are readily extracted with organic solvent and estimated by indirect atomic absorption method via the determination of the cobalt content in the formed complex after extraction in 0.1 M hydrochloric acid. It was found that the three drugs can be determined in the concentration ranges from 10 to 74, 12 to 95 and 10 to 93 μg ml^{-1} with mean percentage recovery of 99.71 ± 0.87, 99.70 ± 0.79 and 99.62 ± 0.75%, respectively. The second method is based on the formation of orange red ion pairs as a result of the reaction between (I); (II) and (III) and molybdenum thiocyanate with maximum absorption at 469.5 nm in dichloromethane. Appropriate conditions were established for the colour reaction. Under the proposed conditions linearity was obeyed in the concentration ranges 3.5–25, 5–37.5 and 2.5–22.5 μg ml^{-1} with mean percentage recovery of 99.60 ± 0.41, 100.11 ± 0.43 and 99.31 ± 0.47% for (I); (II) and (III), respectively. The third method depends on the formation of radical ion using 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ). The colour formed was measured at 588 nm for the three drugs (I); (II) and (III), respectively. The method is valid in concentration range 10–80 μg ml^{-1} with mean percentage recovery 99.75 ± 0.44, 99.94 ± 0.72 and 99.17 ± 0.36% for (I); (II) and (III), respectively. The proposed methods were applied to the analysis of pharmaceutical preparations. The results obtained were statistically analysed and compared with those obtained by applying the official and reference methods.

Keywords: Atomic absorption spectrometry; Colorimetry; Pizotifen; Ketotifen; Loratadine

1. Introduction

Pizotifen (I), ketotifen (II) and loratadine (III) are widely used as an antihistaminic. Several methods have been reported for the quantitative determination of (I); (II) and (III) including UV

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[1,2], colourimetry [3,4], differential pulse polarography [2,5], titrimetry [6,7], densitometry [8], HPLC [9–11] and GC [12–15] methods. The chemical structure of (I); (II) and (III) are shown in Fig. 1.

Reaction of the investigated drugs with cobalt and molybdenum thiocyanate have not been examined before and also no atomic absorption methods for the quantification of these drugs have been published. Although densitometric [8], HPLC [9–11] and GC [12–14] methods are reported for the determination of the cited drugs yet the proposed colourimetric and atomic absorption methods are less expensive, without loss of accuracy and hence more suitable for application in quality control laboratories in developing countries. In addition 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ) method is more simple since it is single step procedure. The sensitivity of molybdate method is comparable to that of the published densitometric [8] and HPLC [9] methods. Therefore the aim of the present work was to develop simple, sensitive and selective methods for the application of these techniques for routine quality control analysis.

2. Experimental

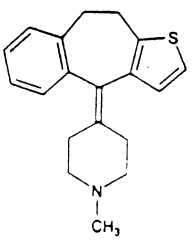
2.1. Apparatus

1. UV/Vis Spectrophotometer, SHIMADZU UV-160, using quartz cell ($1 \times 1 \times 3$ cm), slit width 2.0 nm.
2. Digital pH meter, PW 9409 Pye Unicum.
3. Atomic absorption spectrometer, spectra AA-20 Varian. Using acetylene air flame under these conditions, cobalt hollow cathode lamp; slit width 0.2 mm; wavelength, 240.7 nm; lamp current, 7 mA.

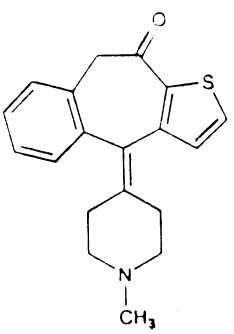
2.2. Materials

2.2.1. Pure samples

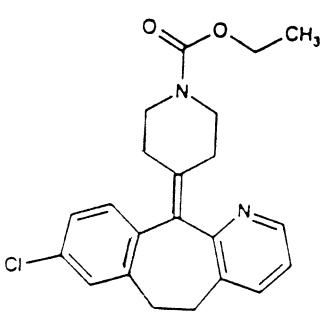
1. Pizotifen was kindly supplied by Swisspharma Egypt SAE, Cairo, Egypt. The purity of the sample was found to be $99.40 \pm 0.78\%$ according to B. P93 method [6].
2. Ketotifen was kindly supplied by Swisspharma Egypt SAE, Cairo, Egypt. The purity of the sample was found to be $100.07 \pm 0.94\%$ according to reference method [7].
3. Loratadine was kindly supplied by Medical Union Pharmaceutical, Cairo, Egypt. The purity of the sample was found to be $99.20 \pm 0.34\%$ according to the reference method [16].



Pizotifen



Ketotifen



Loratadine

Fig. 1. Structure of the three studied drugs.

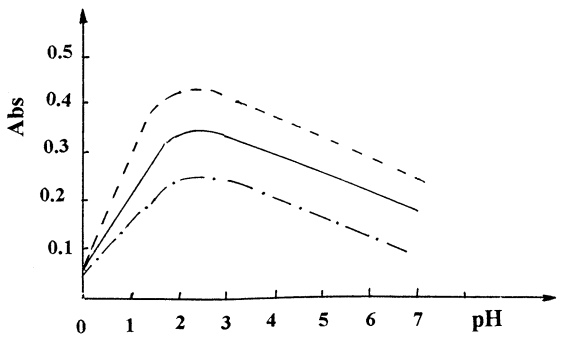


Fig. 2. Effect of pH in atomic absorption method (50 μg ml^{-1}) of pizotifen (-----), ketotifen (——) and loratidine (–·–·–·–).

2.2.2. Market samples

1. Mosegor tablets, batch no. 071. Each tablet was labelled to contain pizotifen 0.5 mg, maleic acid 0.23 mg, magnesium stearate 0.55mg, talc 13.62 mg, polyvinyl pyrrolidone 2.2 mg, maize starch 5.5 mg, lactose 45.47 mg, silicic acid 0.23 mg, acacia 0.85 mg, titanium dioxide 1.52 mg and sucrose 19.33 mg.
2. Mosegor syrup, batch no. 2664. Each 100 ml syrup was labelled to contain pizotifen 5 mg, disodium hydrogen phosphate 196 mg, citric acid anhydrous 127.2 mg, lemon essence no. 1 solution 80 mg, mandarin flavour 20 mg, nipacombine (nipasol and nipagen) 50 mg, propylene glycol 2 g, sorbitol 70% 5 g, sugar crystalline 3 g and demineralised water added to 100 ml.
3. Zaditen tablets, batch no. B232. Each tablet was labelled to contain ketotifen hydrogen fumarate 1 mg, lactose 1.38 mg, maize starch 26 mg and magnesium stearate 0.65 mg.
4. Zaditen syrup, batch no. G546. Each 100 ml syrup was labelled to contain ketotifen 27.6 mg, fumaric acid 7.6 mg, strawberry flavour 10.5 mg, propyl paraben 30 mg, methyl paraben 70 mg, citric acid anhydrous 256 mg, disodium hydrogen phosphate anhydrous 284 mg, Lycasine 80g and demineralised water 41.32 g.
5. Claritine tablets, batch no. 94660. Each tablet was labelled to contain loratadine 10 mg, lactose 71 mg, magnesium stearate 1 mg and corn starch 18 mg.

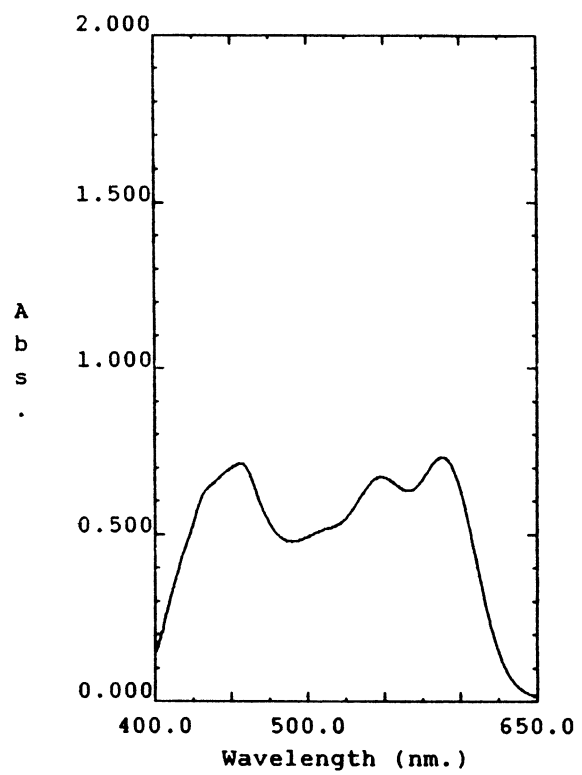


Fig. 3. Absorption spectra of loratadine–2,3-dichloro-5,6-dicyano-*p*-benzoquinone reaction product (58 μg ml^{-1}).

2.2.3. Reagents

1. Cobalt thiocyanate reagent: Dissolve 56.25 g ammonium thiocyanate and 23.8 g of cobalt chloride.$6H_2O$ in water to make 100 ml.
2. Citrate buffer [17] pH 2.
3. Hydrochloric acid, 0.1 M aqueous solutions.
4. Ammonium molybdate, 1×10^{-2} M aqueous solution containing 1% v/v concentrated ammonia solution.
5. Ammonium thiocyanate, 10% w/v aqueous solution.
6. Ascorbic acid, 10% w/v aqueous solution.
7. Sodium hydroxide, 1 M aqueous solution.
8. DDQ (Aldrich, Dorset, UK) 0.3% w/v in acetonitrile.

Table 1
Quantitative parameters for the determination of pizotifen, ketotifen and loratadine with atomic absorption spectrometry[a]

Compound	Linearity range (μg ml^{-1})	Intercept*	RSD of intercept	Slope*	RSD of slope	Correlation coefficient	LOD (μg ml^{-1})	LOQ (μg ml^{-1})
Pizotifen	10–74	0.014	5.6×10^{-2}	0.008	5.8×10^{-3}	0.9997	1.16	12.6
Ketotifen	12–95	0.027	7.4×10^{-3}	0.006	1.1×10^{-2}	0.9959	2.19	14.1
Loratadine	10–93	0.04	1.5×10^{-2}	0.004	7.0×10^{-3}	0.9969	1.85	12.6

[a] RSD, relative standard deviation; LOD, limit of detection; LOQ, limit of quantification.
* $n = 5$ in all instances.

2.2.4. Standard stock solutions

1. Pizotifen, 0.5 mg ml^{-1} in 60% ethyl alcohol and 0.5 mg ml^{-1} in acetonitrile, freshly prepared.
2. Ketotifen, 0.5 mg ml^{-1} in 60% ethyl alcohol and 0.5 mg ml^{-1} in acetonitrile, freshly prepared.
3. Loratadine, 0.5 mg ml^{-1} in 60% ethyl alcohol 0.5 mg ml^{-1} in methanol–acetonitrile (1:9), freshly prepared.

2.2.5. Preparation of test solution

2.2.5.1. Tablets. Weigh accurately an amount of the powdered tablet equivalent to 2 or 1 mg of each drug for atomic absorption spectrometry (AAS) and colourimetric methods, respectively, shake with 2×15 ml of the corresponding solvents used in the preparation of standard stock solution, filter and wash. Reduce the volume of the solvent to about 6 ml by evaporation. Transfer quantitatively into a 10 ml volumetric flask and complete to volume with the same solvent.

2.2.5.2. Syrups. Into separating funnel transfer accurately a measured volume of syrup equivalent to 2 or 1 mg of Pizotifen or ketotifen for AAS and colourimetric methods, respectively, render alkaline with 1 M sodium hydroxide (to pH 9–9.5) and extract with 3×25 ml chloroform. Filter the organic layer through cotton and evaporate on a water bath to dryness. Cool and transfer the residue with the aid of the corresponding solvents used in the preparation of standard stock solution into a 10 ml volumetric flask and complete to volume with the same solvent.

2.3. Procedures

2.3.1. Bulk powder

2.3.1.1. Atomic absorption spectrometry. Transfer accurately an aliquot of standard stock solution containing 1.5 mg of (I); (II) or (III), respectively, into a separating funnel, add 6 ml of cobalt thiocyanate reagent and complete to 15 ml with citrate buffer. Extract with 2×10 ml benzene, methylene chloride or chloroform, respectively, for 2 min and shake the organic layer with 0.1 M hydrochloric acid (2×10 ml).Transfer the acid extract to 25 ml volumetric flask and complete to volume with 0.1 M hydrochloric acid. Aspirate the acid extract directly in the atomic absorption spectrometer and measure the absorbance at 240.7 nm.

2.3.1.2. Molybdenum thiocyanate method. In 100 ml separating funnel mix 2 ml of ammonium molybdate, 8 ml of 5 M hydrochloric acid, 6 ml of each of ammonium thiocyanate and ascorbic acid and leave for 15 min. Add an aliquot of standard solution equivalent to 200 μg of the cited drug and dilute to 25 ml with water. After another 15 min, add accurately measured 10 ml of methylene chloride, shake vigorously for 1 min and allow to separate into two phases. Collect the organic layer and measure its absorbance at 469.5 nm against a reagent blank prepared similarly.

2.3.1.3. 2,3-dichloro-5,6-dicyano-p-benzoquinone method. Transfer an aliquot of standard stock solution equivalent to 0.5 mg of (I); (II), and (III)

into three separate 10 ml volumetric flasks. Add 5 ml of DDQ solution for (I) and 4 ml for (II) and (III). Complete to volume with acetonitrile and measure the absorbance at 588 nm against a reagent blank.

2.3.2. Assay of pharmaceutical formulations

Proceed as mentioned under bulk powder using an aliquot of test solution equivalent to 1 mg (AAS), 0.2 and 0.5 mg in molybdate and DDQ methods, respectively, for tablets and syrups.

3. Results and discussion

3.1. Atomic absorption spectrometry

Metal ions has been used by many researchers for the estimation of different pharmaceutical compounds applying AAS and colourimetric methods [18,19]. A survey of literature showed that the selected drugs have not been previously determined by using metal ions.

In this work the investigated drugs are found to react with cobalt (II) and thiocyanate ions to form stable ternary complexes. These complexes are insoluble in aqueous solution, but are readily extractable with organic solvents.

Determination of the cobalt content of the organic extract by AAS, provides an indirect determination of the investigated compounds. It was not possible to aspirate the organic solution of complexes in the atomic absorption spectrometer. Therefore, it was necessary to extract the cobalt contents from the organic solvent into dilute hydrochloric acid and measure its atomic absorption at 240.7 nm.

Optimum conditions of the reaction were carefully studied.

3.1.1. Effect of solvents

The weak complex cobalt thiocyanate ion is stabilised by the formation of an ion pair with organic bases. Several organic solvents were tried for extraction of ternary complexes, benzene, methylene chloride and chloroform were chosen for (I); (II) and (III), respectively. These solvents afforded maximum sensitivity and minimum blank reading.

3.1.2. Effect of number of extraction, shaking time and stability

The study of number of extraction and shaking time showed that single extraction by shaking with 10 ml of the chosen organic solvent for only 2 min was sufficient. The colour obtained was stable for up to 2 h.

3.1.3. Optimum concentration of cobalt thiocyanate

It is revealed that 6 ml of the reagent is the optimum volume for the formation of ion pairs.

3.1.4. Effect of pH

Variation of the pH between 0 and 7.0 showed that extraction of the ternary complex is maximum at pH 2. At higher pH values a dissociation of the mixed ligand complex would occur resulting in the decrease of cobalt content as shown in Fig. 2.

Table 2
Quantitative parameters for the determination of pizotifen, ketotifen and loratadine with molybdenum thiocyanate method[a]

Compound	Linearity range (μg ml^{-1})	Intercept*	RSD of intercept	Slope*	RSD of slope	Correlation coefficient	LOD (μg ml^{-1})	LOQ (μg ml^{-1})
Pizotifen	3.5–25	0	2.5×10^{-2}	0.043	1.2×10^{-2}	1.00	0.31	7.9
Ketotifen	5–37.5	0.002	1.4×10^{-2}	0.028	8.8×10^{-3}	1.00	0.32	11.2
Loratadine	2.5–22.5	0.002	7.0×10^{-3}	0.047	1.6×10^{-2}	0.9999	0.27	7.9

[a] RSD, relative standard deviation; LOD, limit of detection; LOQ, limit of quantification.
* $n = 5$ in all instances.

Table 3
Quantitative parameters for the determination of pizotifen, ketotifen and loratadine with 2,3-dichloro-5,6-dicyano-*p*-benzoquinone method[a]

Compound	Linearity range (μg ml^{-1})	Intercept*	RSD of intercept	Slope*	RSD of slope	Correlation coefficient	LOD (μg ml^{-1})	LOQ (μg ml^{-1})
Pizotifen	10–80	−0.002	1.4×10^{-2}	0.013	2.3×10^{-2}	0.9999	1.00	12.6
Ketotifen	10–80	−0.004	1.2×10^{-2}	0.012	3.5×10^{-2}	0.9998	1.16	12.6
Loratadine	10–80	0.001	1.8×10^{-2}	0.0125	4.4×10^{-2}	0.9994	1.00	12.6

[a] RSD, relative standard deviation; LOD, limit of detection; LOQ, limit of quantification.
* $n = 5$ in all instances.

3.1.5. Aqueous to organic ratio

Experiments were performed in which volume of the organic solvent and the reagent were kept constant, while that of the aqueous phase was varied. The aqueous: organic ratio of choice was 3:2 in case of pizotifen and 1:1 in case of ketotifen and loratadine.

3.2. Molybdenum thiocyanate method

The organic positively charged investigated drugs form coloured ion pairs with the complex of SCN^- and molybdenum (V).This method is more selective, and sensitive for the determination of the cited compounds.

Molybdenum (V) formed by the reduction of molybdenum (VI) with ascorbic acid combines with ammonium thiocyanate to form a red binary molybdenum thiocyanate complex in 0.8–3.2 M hydrochloric acid solution [20] Pizotifen, ketotifen and loratadine react with the binary complex forming orange red ion pair which is highly soluble in dichloromethane while molybdenum (V) thiocyanate complex is not. The absorption spectra of the ion pairs show a maximum at 469.5 nm against a reagent blank.

3.2.1. Optimum conditions for molybdenum (V) ion pair

3.2.1.1. Effect of acidity. The optimum acidity range for extraction and maximum absorbances values was found to be 1–3.5 M hydrochloric acid. Different acids were tried include sulphuric, hydrochloric, phosphoric and nitric acid but the most suitable was found to be hydrochloric acid. This method was carried out in 1.6 M hydrochloric acid.

3.2.1.2. Amount of ammonium molybdate. Two milliliters of 10^{-2} M ammonium molybdate in the final aqueous solution (25 ml) was required for the complete formation of the ion pairs to obtain maximum absorbance.

3.2.1.3. Amount of ammonium thiocyanate and ascorbic acid. It was found that 6 ml (10% w/v) of each in a final solution of 25 ml gave the most pronounced effect.

3.2.1.4. Effect of time of reaction. The complete formation of the ion pairs needs 30 min before extraction with dichloromethane at room temperature. The absorbance of molybdenum (V) thiocyanate binary complex was stable after 15 min and another 15 min was required to complete the formation of molybdenum (V) ion pairs.

3.2.1.5. Effect of other variables. Reproducible absorbance readings were obtained after a single extraction with 10 ml dichloromethane after a 1 min shaking time. Dichloromethane was selected as a solvent for the ion pairs as it selectively dissolves the ion pairs and not the binary molybdenum (V) thiocyanate complex. The stability of the colour was also studied and found that it is stable for more than 24 h at 25°C in the organic solvent.

Table 4
Determination of authentic samples of pizotifen, ketotifen and loratadine using the atomic absorption spectrometry (AAS), colourimetric and the reference methods[a,b]

Parameter	Pizotifen				Ketotifen				Loratadine			
	AAS method	Molybdenum method	DDQ method	Reference method [6]	AAS method	Molybdenum method	DDQ method	Reference method [7]	AAS method	Molybdenum method	DDQ method	Reference method [16]
%Mean	99.71	99.60	99.75	99.40	99.70	100.11	99.94	100.07	99.62	99.31	99.17	99.20
SD*	±0.87	±0.41	±0.44	±0.78	±0.79	±0.43	±0.72	±0.94	±0.75	±0.47	±0.36	±0.34
Variance	0.76	0.17	0.19	0.61	0.62	0.18	0.51	0.88	0.56	0.22	0.13	0.12
F calculated	1.24	3.59	3.21	—	1.42	4.98	1.73	—	4.69	1.83	1.08	—
t calculated	0.60	0.51	0.88	—	0.68	0.09	0.25	—	1.14	0.42	0.14	—

[a] DDQ, 2,3-dichloro-5,6-dicyano-*p*-benzoquinone.
[b] *F* tabulated (6.4); *t* tabulated (2.306); P = 0.05.
* $n = 5$

3.3. 2,3-dichloro-5,6-dicyano-p-benzoquinone method

The reaction of DDQ with basic drugs results in the formation of an intense red product which exhibits an absorption maxima at 460, 540 and 588 nm (Fig. 3) and the absorbance were measured at 588 nm.

In acetonitrile solutions of antihistaminic drugs and DDQ was found to yield an intense colour causing characteristic long wavelength absorption band with numerous vibration maxima in the electronic spectrum (Fig. 3).

The red radical anion DDQ was probably formed by the dissociation of an original donor–acceptor (DA) complex.

$$\ddot{D} + A \rightarrow (\ddot{D} - A) \xrightarrow{\text{polar solvent}} \dot{D} + \dot{A}^-$$

DA complex radical ions

The dissociation of the DA complex is promoted by the high ionising power of the solvent, acetonitrile [21].

As an assay solvent, acetonitrile afforded maximum sensitivity with DDQ, in addition, it is a good solvent for the reagent.

When various concentrations of DDQ were added to a fixed concentration of studied drugs, 4 ml of 0.3% solution of DDQ for (I); (III) and 5 ml for (II) was found to be sufficient for the production of maximum and reproducible colour intensity.

The optimum reaction time was determined by following the colour development at ambient temperature (20–25°C). The colour was developed immediately and remained stable for at least 30 min.

3.3.1. Calibration graphs and statistical parameters

The regression equations calculated with the methods described above are assembled in Tables 1–3, together with relative standard deviation (RSD) of intercept and slope at $P = 0.05$. Five samples of pure pizotifen, ketotifin and loratidine ranging from 10 to 95 μg ml^{-1} in AAS and DDQ methods and from 2.5 to 37.5 μg ml^{-1} in molybdenum thiocyanate method were used for calibration. The limits of detection (LOD) were calculated by means of a statistical treatment of calibration data [22]. The limit of quantification (LOQ) for each method was also calculated [23] and results are represented in Tables 1–3.

The proposed methods were applied for the analysis of pure drug samples Table 4 and the pharmaceutical preparations Table 5. The data shows that there is no significant difference between the proposed and the reference methods [6,7,16]. The validity of the proposed methods were assessed by applying the standard addition technique. The results are shown in Table 5.

Table 5
Determination of pizotifen, ketotifen and loratadine in pharmaceutical preparations by the proposed methods and the reference methods[a]

Preparations	Recovery*% ± SD			Reference method found % ± SD
	AAS methods	Molybdenum thiocyanate method	DDQ Method	
Mosegor tablet [24][b]	100.30 ± 0.47	99.21 ± 0.39	99.52 ± 0.59	99.70 ± 0.61
Mosegor syrup [24][b]	99.81 ± 0.45	99.10 ± 0.80	100.30 ± 0.91	99.61 ± 0.72
Zaditen tablet [7][c]	100.30 ± 0.26	99.31 ± 0.35	99.22 ± 0.38	99.61 ± 0.26
Zaditen syrup [7][c]	98.77 ± 0.76	99.11 ± 0.64	99.41 ± 0.87	98.82 ± 0.91
Claritine tablet [16][d]	99.43 ± 0.24	100.20 ± 0.44	99.90 ± 0.44	99.51 ± 0.54

[a] AAS, atomic absorption spectrometry; DDQ, 2,3-dichloro-5,6-dicyano-*p*-benzoquinone.
[b] Separation by thin layer chromatography, scratch, dissolve in methanol and measure the absorbance at 255 nm.
[c] UV spectrophotometric determination at 297 nm in methanol.
[d] HPLC method, UV detection at 254 nm.
* All the above results are the average of five determinations.

The proposed colourimetric methods are more sensitive or have the same sensitivity as the published spectrophotometric [2,4], polarographic [2,5], thin layer chromatography (TLC) densitometric [8] and HPLC [9] methods, they also have the same accuracy and precision. To the best of our knowledge no atomic absorption methods for the investigated drugs quantification have been reported. The proposed atomic absorption method was compared with published polarographic method [2] with respect to sensitivity and it was observed that they are equally sensitive.

4. Conclusion

No substantial differences among the three proposed methods arose from an analysis of the experimental results. Molybdate method is the more sensitive as it determines up to 2.5–5 μg ml^{-1} of the cited drugs, while the AAS and charge transfer DDQ methods have the same sensitivity and determine up to 10–12 μg ml^{-1} of I, II, or III. DDQ method being single step is more simple than the others, moreover the recovery of cited drugs by applying molybdate method is generally a little more accurate and precise as the variances range from 0.18 to 0.22 while in AAS and DDQ methods variances range from 0.56 to 0.76 and 0.13 to 0.51, respectively. LOD for AAS, molybdenum thiocyanate and DDQ methods ranges from 1.16 to 2.19, 0.27 to 0.32 and 1.00 to 1.16 μg ml^{-1}, respectively.

The proposed methods are highly sensitive more over they could be used for routine analysis of the studied drugs in raw materials and pharmaceutical formulations.

Spectral overlap and nonspecific relevant absorption from diluents and excepients adversely affect the determination of a drug in formulations particularly when an active ingredient is present at low concentration. The proposed AAS and colourimetric methods are selective and no interference from excepients and preservatives were observed.

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